بسم الله الرحمن الرحيم

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The potential use of Baobab (*Adansonia digitata*) Fruit Pulp in Formulation of Functional Food

إمكانية إستخدام لب ثمار التبلدي لتجهيز غذاء وظيفي

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By:

Salma Elzen Ibraheem Elhassan

M. Sc. in Sudan University of science and Technology (2015)

B. Sc. of Science (Honors) in food Engineering and Technology

From Gezira University (2006)

Supervisors:

Professor Dr. Yousif Mohamed Ahmed Idris

Associate Professor Dr. Barka Mohammed Kabier Barka

Dr. Salma Elghali Mustafa

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الآيـة

قال تعالي :

(فَتَعَالَى اللَّهُ الْمَلِكُ الْحَقُّ وَلَا تَعْجَلْ بِالْقُرْآنِ مِنْ قَبْلِ أَنْ يُقْضَى إِلَيْكَ وَحْيُهُ وَقُلْ رَبِّ زِدْنِي عِلْمًا).

صدق الله العظيم

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Dedication

I dedicate this Thesis

To my great parents Rahma and Elzen.

To my sisters, brothers, aunts, uncle and all members of

my big family for their kind helps and support.

It also goes to teachers, scientists and researchers

Acknowledgement

Alhamdulillah, I finished my thesis with help and full support of my lord ALLAH, the guidance of my supervisors, cooperation of friends and family. I would like to express my deepest gratitude to my supervisory team: Professor. Yousif Mohamed Ahmed Idris, associate Professor Dr. Barka Mohammad Kabeir and Dr. Salma Elghali Mustafa for their excellent guidance, patience and valuable advice to complete this research. I would like to thank all staff at the Department of Food Science and Technology (College of Agricultural Studies, SUST) for this kind cooperation.

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List of Abbreviations

mg	Milligram
G	Gram
RDI	Recommended daily intake
Dw	Dry weight
Kg	kilogram
OH^-	hydroxyl radicals
DNA	Deoxyribonucleic acid
ROS	Reactive oxygen species
RNS	Reactive nitrogen species
GIT	Gastrointestinal Tract
FOS	Fructo oligosaccharides
F6PPK	Fructos-6-phosphate phosphoketolase
DPPH	1,1-dipheny1-2-picrylhydrazyl radical
TPTZ	2,4,6-tris(pyridin-2-y1)-1,3,5-triazine
TSS	Total soluble solids
AOAC	Association of Official Analytical Chemists
GAE	Gallic acid equivalent
CEQ	Catechin equivalents
FRAP	ferric reducing antioxidant power
Mmol	mill mole
HPLC	High-performance liquid chromatography
LC-MS/MS	liquid chromatography mass spectrometry
EPI	Enhanced Product Ion
TPC	Total phenolics content
TIC	Total ion chromatograms
NMR	Nuclear magnetic resonance
PNPG	p-nitrophenyl-α-Dglucopyranose
DMSO	dimethylsulfoxide
CFU	Colony forming Unit

TBC	Total bacterial count
K3 EDTA	Tripotassium Ethylene diamine tetra acetic acid
HGB	Hemoglobin concentration
RBC	red blood cell
WBC	white blood cell
AST	aspartate transferase
ALT	Alanine transferase
ALP	alkaline phosphatase
TP	total protein
Alb	albumin
Glob	globulin
LDL	Low density lipoprotein
HDL	High density lipoprotein
Log	Logarithm
N	Normality
(%)	Percentage
H	Hour
MRS	de mann- Rogosa –Sharpe
L	Liter
c^o	Degree Celsius
et al	Et Cetera(and company)
ANOVA	Analysis of Variance
Ml	Milliliter

Abstract

This study aimed to determine the physical characteristics of baobab fruit, phytochemical profile, bioactive components, biological activities**,** nutritional composition, antioxidant activity and pectin of baobab (*Adansonia digitata*) fruit pulp and to formulate Baobab fruit pulp based cream cheese as a functional food. In addition the effect of baobab cream cheese on general health of the rats was investigated. Baobab fruits were collected from four locations in Sudan (El Obeid, Um Ruwaba, Nyala and Damazin). The fruit pulp was obtained from baobab pods, then the seeds were removed and pulp powder was sieved using appropriate mesh. The length, width, thickness of fruit , weight of pulp, weight of seed, number of seed, weight of red fiber and weight of epicarp of baobab samples were determined. The proximate composition (moisture, protein, oil, fiber, ash and carbohydrate) of the pulp was analyzed according to AOAC methods, ascorbic acid content was determined using titration method and water based extraction method was used for pectin extraction. Aqueous, ethanolic and methanolic baobab fruit pulp extracts were used to determine the antioxidant activity using DPPH and FRAP methods. Phenolics and flavonoids contents of the extracts were determined spectrophotometrically using Folin–Ciocalteu reagent and aluminium chloride, respectively. Flavonoids and Phenolic profiles of pulp extracts were analyzed by LC-MS/MS. The methanolic extract of baobab pulp was subjected to analysis for alpha-glucosidase, nitric oxide inhibitors. ^{1}H NMR based metabolomics was used to investigate phytochemical constituents. Baobab fruit pulp with the highest content of phytochemical components was used to formulate a novel functional cream cheese supplemented with Bifidobacterium *longum* BB536. Cream cheese with different concentration of pulp (A: 10%, B: 15% and C: 20%) was prepared using cow milk and analysed for chemical composition, safety, growth of the BB536, and sensory properties .The effect of the formulated

baobab cream cheese on intestinal microflora and general health including hematology and blood biochemistry of fed on male Albino rats was investigated. Thirty rats were acclimatized to the experimental condition for fifteen days and randomly distributed into five different groups and received feeding for a period of 30 days. The results of physical characterization, morphological characteristics, approximate composition, sugars (total, reducing, none-reducing), specific sugars (Glucose, fructose, sucrose), and pectin showed highly significant variances ($P<0.05$) between fruits. Antioxidant activity of baobab pulp was significantly different ($P<0.05$) between different locations. There were significant differences ($P<0.05$) in total phenolic and flavonoids contents between extracts from different locations in Sudan. The concentrations of total phenolics ranged from 15.50 to 99.66 mg GA/g of Gallic acid equivalent (GAE) /g and flavonoids contents ranged from 1.03 to 21.53 mg of Catechin CA/g. Phytochemical profiles of baobab pulp was exhibited 22 phenolics, 47 flavonoids, 4 lipids, sugars, organic acid , iridoids , Stilbenes resveratrol derivate, octadecanoic acid and other compounds. Baobab pulp extract showed greater capability in inhibiting the generation of nitric oxide (NO) from the stimulated RAW264.7 cells at 98.45% inhibition (IC50 of 36.55µg/ml) and α-glucosidase enzyme activity (97.94% inhibition, IC50 of 58.59 µg /mL).Metabolites by NMR of baobab pulp extract were showed the abundance of 21 components including carbohydrates, organic acids, vitamin, amino acids, phenolic compounds, alkaloids and fatty acids.

Baobab fruit from Damazin has the highest contain of phytochemical components compared with other samples was used for formulation of baobab cream cheese. The nutritional composition of cream cheese was positively affected by addition of baobab pulp. The results obtained on *B. longum* BB536 viable count revealed significant ($P < 0.05$) increase in the 15 days of the refrigeration storage; however, the growth decreased at 30 days for all

cream cheese samples. All formulated cheese samples were safe, and E. *coli*, *Staphylococcus aurous*, salmonella were not detected. Moreover, formulated cream cheese was accepted by consumer panelists.

In vivo experimental rat fed baobab cream cheese did not reveal any side effect on general health, hematology, serum electrolytes and blood biochemistry parameters. Feeding also increased *bifidobacteria* and *lactobacillus* of colon but decreased total anaerobes, pathogens *staphylococcus*, *salmonella*, and *coliform.* In conclusion, Sudanese baobab fruit pulp is rich in phenolics, flavonoids, and a potential source of natural antioxidant to benefit health of consumer. Baobab cream cheese exhibited several beneficial positive effects on general health of rats and could have positive effect on general health of humans.

ملخص البحث

هدفت هذه الدراسة إلى تحديد الخصائص الفيزيائية لثمار الباوباب (*Adansonia digitata*) والمكونات الكيميائية النباتية ، والمكونات النشطة حيويا ، والأنشطة البيولوجية ، والتركيب الغذائي ، ونشاط مضادات الأكسدة والبكتين للب ثمار الباوباب وإنتاج جبنة كريمي بإستخدام لب الباوباب كغذاء وظيفي. بالإضافة إلى ذلك تمت دراسة تأثير الجبن الكريمي على الصحة العامة للفئران .جمعت ثمار الباوباب من أربعة مواقع في السودان (الأبيض، وأم روابه ،نيالا والدمازين) .تم الحصول على لب الفاكهة من حبوب الباوباب ، ثم تمت إزالة البذور و مسحوق اللب باستخدام غرابيل مناسبة .أولاً ، تم تحديد طول ، عرض ، سمك الفاكهة ، وزن اللب ، وزن البذور ، عدد البذور ، وزن الألياف الحمراء ووزن القشرة الخارجية لعينات الباوباب .ثم تم تحليل التركيب الغذائي (الرطوبة والبروتين والزيت والألياف والرماد والكربوهيدرات) للب باستخدام طريقة AOAC .علاوة على ذلك ، تم تحديد محتوى حمض الأسكوربيك باستخدام طريقة المعايرة .إستخدامت طريقة الإستخلاص بالمياه لإستخراج البكتين . أستخدمت مستخلصات لب الباوباب المائي ، إيثانوليك وميثانوليك لتحديد نشاط مضادات الأكسدة باستخدام طرق DPPH و FRAP ، تم تحديد المحتوي الفينولي و الفلافونويد من المستخلصات بإستخدام جهاز الطيف الضوئي وكاشف الفولين وكلوريد الألمنيوم ، على التوالي . وحللت مركبات الفلافونويد والفينوليك في المستخلصات بواسطة LC-MS / MS. ايضا تم إستخدام مستخلص لب الباوباب الميثانولي لتقدير تثبيط انزيم الألفا جليكوسيديز و أكسيد النيتريك.

تم إستخدام لب الفاكهة المحتوية علي أعلى محتوى من المكونات الكيميائية النباتية لإنتاج جبن كريمي وظيفي مخترع مدعم بالبكتريا. *Bifidobacterium longum* BB536. تم تحضير الجبن باستخدام حليب البقر مضاف الية تراكيز مختلفة من لب التبلدي (A: 10) ٪ ، B: 15 ٪ .C: 20. بعد ذلك إجريت التحاليل المختلفة على الجبن باوباب الكريمي مثل التركيب الكيميائي والسلامة ونمو BB536 ، و الإختبارات الحسية .أيضا تم إستخدام الجبن الكريمي المدعم لتغذية ذكور الفئران البيضاء،تم التحقق من تأثير التغذية على الأمعاء الدقيقة والصحة العامة بما في ذلك أمراض الدم والتحليل الكيميائي للدم للفئران . ثلاثون فارا إستخدمت للتجربة وتركت لمدة خمسة عشر يومًا للتأقلم ثم وزعت عشوائياً في خمسة مجموعات مختلفة وأعطيت التغذية لمدة 30 يومًا.

أظهرت نتائج الخصائص الفيزيائية ، والخصائيص المورفولوجية ، والتركيب التقريبي ، والسكريات (الكلية ، المختزلة، غير المختزلة، الجلوكوز ، الفركتوز ، السكروز) ، والبكتين وجود تباينات كبيرة للغاية ($P < 0.05$) بين ثمار التبلدي .واوضح نشاط مضادات الأكسدة في لب التبلدي اختلافًا كبيرًا بين المواقع المختلفة .بالإضافة إلى ذلك ، كانت هناك فروق معنوية واضحة في محتويات الفينول والفلافونيد بين مستخلصات المحاليل للتبلدى و المواقع المختلفة في

السودان.تراوحت تراكيز الفينولات الكلية من 15.50 إلى 99.66 مليجرام لكل جرام من مكافئ حمض الجاليك ومحتويات الفلافونويد تراوحت بين 1.03 إلى 21.53 مليجرام لكل جرام من *الكاتيكين*. اظهرت ثمار التبلدي احتوائها علي عدد من المركبات من ضمنها (22) الفينول ، 47 الفلافونويد ، 4الدهون ، والسكريات ، الأحماض العضوية ، و iridoids ، Stilbenes ، resveratrol derivate ، octadecanoic acid وغيرها من المركبات.

أظهرت نتائج أكسيد النيتريك (NO) أن مستخلص لب التبلدي قد حقق نجاحًا كبيرا في تثبيط توليد أكسيد النيتريك (NO) من خلايا RAW264.7 المحفزة بنسبة تثبيط (98.45٪) وIC50 بنسبة (36.55ميكروجرام / مل) وتثبيط نشاط الألفاجليكوسيديز بنسبة 97.94 ٪ و IC50 بنسبة (58.59 ميكروغرام / مل). وأظهرت عمليات الأيض باستخدام الرنين المغناطيسي النووي لمستخلص لب التبلدي وجود وفرة من المركبات (واحد وعشرون مركب) بما في ذلك الكربوهيدرات والأحماض العضوية ، والفيتامينات ، والأحماض الأمينية ، والمركبات الفينولية ، وأشباه القلويات والأحماض الدهنية.احتوت ثمار التبلدي التي جمعت من منطقة الدمازين علي أعلي نسب من المكونات الكيميائية مقارنة بالعينات الأخري لذلك أستخدمت لإنتاج الجبنة الكريمي باستخدام نسب مختلفة.أوضحت إضافة لب التبلدي تاثيرا إيجابيا في القيمة التغذوية للجبن الكريمي.

أظهرت النتائج التي تم الحصول عليها للعد البكتيري للبفيدو BB536 زيادة معنوية ($P < 0.05$) خلال الخمسة عشر يومًا تحت التخزين في الثلاجة ومع ذلك إنخفض النمو في الثلاثون يوما خلال التخزين لجميع أنواع الجبن الكريمي.

جميع أنواع الجبن الكريمي كانت أمنة للإستخدام حيث لم يتم الكشف عن وجود أي نوع من بكتريا إي .كولاي ، المكورات العنقودية والسالمونيلا. خلال فترة التخزين وعلاوة على ذلك ، تم قبول جميع أنواع الجبن الكريمي المصنع بإستخدام لب التبلدي من ناحية التقيم الحسي.

في جانب التجارب الحيوانية ، لم تكشف الفئران التجريبية التي تغذت على الجبن المصنع من التبلدي أي تأثير جانبي على الصحة العامة ، أمراض الدم ، المعادن و التحاليل الكيميائية للدم. أيضا أثرت التغذية علي زيادة بكتريا البفيدو والاكتيك في القولون وأدت إلى إنخفاض في مجموع البكتريا اللاهوائية والإستافيلوكوكس والسالمونيلا والكوليفورم. في الختام فاكهة ثمار الباوباب السودانية غنية بالفينوليك ، الفلافونويد ، وتعتبر مصدر طبيعي لمضادات الأكسدة الطبيعية لفائدة صحة المستهلك. أظهرت جبنة التبلدي الكريمي عدة آثار إيجابية مفيدة على الصحة العامة للفئران ويمكن أن يكون لها تأثير إيجابي على الصحة العامة للإنسان.

CHAPTER ONE

INTRODUCTION

Baobab (*Adansonia digitata*) belongs to the Malvaceae family and is a deciduous tree native to arid Central Africa (Yazzie *et al*., 1994). Its distribution area is large and this species can be found in most of Sub-Sahara Africa's semi-arid and sub-humid regions as well as in eastern Madagascar (Diop *et al*., 2005). In Sudan, the baobab is most frequently found on sandy soils and by seasonal streams `khors' in short grass savannas. It forms belts in Kordofan, Darfur, and Blue Nile (El Amin, 1990). The baobab has an extensive root system and high water holding capacity. It survives well in dry climates and is resistant to fire. The different plant parts are widely used as foods, medicines; the bark fibers are also used. The tree provides food, shelter, clothing as well as material for hunting and fishing (Gebauer *et al*., 2002, Sidibe and Williams, 2002).

In Sudan, ecotypes from different areas of the Sudan have different fruits in terms of size, shape and sweetness. Gebauer and luedeleng (2013) reported variations in baobab fruit phenotypes and percent of fruit pulp with fruit phenotype in Kordofan. The baobab fruit pulp is probably the most important foodstuff. It can be dissolved in water or milk. The liquid is then used as a drink, a sauce for food, a fermenting agent in local brewing, or as a substitute for cream of tartar in baking (Sidibe and Williams, 2002).

The dry baobab fruit pulp has a slightly tart, refreshing taste and is very nutritious, with particularly high values for carbohydrates, energy, calcium, potassium, thiamine, nicotinic acid and vitamin C (Arnold *et al*., 1985). Vitamin C content in the fruit pulp is stable, ranging from 1500-5000 mg/kg (Chadare and Linnemann., 2009; Scheuring *et al*. 1999).

The consumption of 40 g of baobab pulp for children is enough to cover 41.5% of the daily ready intake for iron, 25.4% of the recommended daily

intake (RDI) for zinc, and 35% for the RDI for calcium. For pregnant women, the consumption of 60 g and 100 g would cover 23.1% and 38.4% of the DRI for iron, 17.3% and 28.7% for the RDI for zinc, and 42.1% and 70.1% for the RDI for calcium, respectively. Baobab fruit contains detectable levels of α-carotene (0.17 .mg/g dry weight) and lutein (1.53 .mg/g dry weights). On the other hand, the fruit pulp can be considered as a rich source of amino acids and linoleic acid (Sena *et al*., 1998).

Dietary antioxidants, including polyphenolics compounds, vitamins E and C, and carotenoid, are believed to be effective nutrients in the prevention of oxidative stress related diseases) such as inflammation, cardiovascular disease, cancer and aging related disorders (Kaur and Kapoor 2001, Willet, 2001). The high antioxidant capacity of products deriving from *Adansonia digitata* show their therapeutical, nutraceutical and cosmoceutical potential (Vertuani *et al*., 2002; Besco *et al*., 2007). Red fiber of baobab fruit pulp is proposed as a new value-added ingredient for food preparation and nutraceutical application in the promotion of health. Due to its potential health benefits, Baobab fruit pulp is granted status in the European Union which will pave the way for its incorporation into several foods. Functional foods can be formulated using potential plant sources with and without probiotics; however, probiotics will enhance the value of the functional food because they aid the existing flora, or help repopulate the colon when bacteria levels are reduced by antibiotics, chemotherapy or disease. Probiotics are "living microorganisms which when administered in adequate amounts confer a health benefit on the host" though, this should also specify genus, species and strain level, as well as a safety assessment. Most of probiotic foods generate fatty acids, vitamins and other vital nutrients that improve the body's resistance against pathogens microorganisms (FAO/WHO, 2001; Abd El-Salam *et al*., 2012). Dairy products show the most adequate matrix for supplementation with probiotic cultures and prebiotic ingredients due to their

positive status in the mind of the consumers (Granato *et al*., 2010) .Knowledge about the nutritional value and use of baobab fruit could raise nutritional standards and also stimulate the market availability and trade of baobab powder (Sidibe *et al*., 1996).

Although Baobab fruit pulp is a valuable nutritional source, its phenolics and flavonoids profile have not been investigated and to our knowledge no baobab based functional food has been formulated. In addition, utilization of fruit in Sudan did not go beyond preparation for intake as traditional beverages. It is for this reason that greater attention should be given to this underutilized fruit.

General objective:

To evaluate the phytochemicals and nutritional value of baobab fruit pulp and formulated based functional food.

Specific objectives are to:

1-Analyze the chemical composition, sugars, pectin, vitamin C, and mineral contents (macro and micro, K, P, Fe, Ca, Mg, Na) and physical characterizations of baobab fruit pulp.

2-Determine phenolic contents, phenolic profile, flavonoids content and flavonoids profile for baobab fruit pulp

3- Determine the antioxidant activity, nitric oxide and alpha glycoside inhibitor for extracts of baobab fruit pulp.

4- Formulate cream cheese based functional food using baobab fruit pulp.

5- Evaluate the sensory properties and the shelf life of the baobab cream cheese.

6-Examine the health benefit on rats fed with the baobab cream cheese supplemented with bifidobacterium.

CHAPTER TWO

LITERATURE REVIEW

2.1Background of baobab (*Adansonia digitata*)

The African baobab and its related species have a place with the family bombacaceae and the genus *Adansonia*. The genus is a member of the tribe *Adansoniea*, or bombaceae. The family includes around 30 genera, six tribes and 250 species. Some of these species are utilized locally for wood, fruits, seeds or gum, however few are economically important. *Adansonia* forms an exceptional fruit product sort with a woody pericarp a spongy pulp with uniform seeds (Sidibe and Williams, 2002).

The baobab tree, *Adansonia digitata*, primarily grows naturally throughout semi-arid tropical Africa, yet has likewise been effectively acquainted with different locales, including Madagascar and India. The tree is fast-growing when young, some species can be very long lived; the oldest recorded tree was radiocarbon dated to 1,275 years old (Patrut *et al*., 2007). It holds great cultural significance to local people, felling of the baobab tree is generally dissuaded due to traditional believes. Legends about the baobab tree are common; it regularly appears on postage stamps and is the national tree of Senegal and Madagascar. The value of the baobab tree is no recent occurrence; baobab fruit was known to the ancient Egyptians, and records show that baobab products were traded in the markets of Cairo during the 16th Century (Wickens, 1980).

An imposing tree, *Adansonia digitata*can reach 25 meters in height and the trunks can surpass 20 meters in circumference. The wood is light and the giant trunks are often hollowed for a variety of uses. Hollowed baobabs have been utilized as stables, stores, bus shelters and water repositories (Sidibe and Williams, 2002; Wickens, 1980). It is commonly referred to as the ‘Tree of Life’, a use for almost every part of the tree has been recorded. Fibers are woven into ropes, basket nets and fishing lines .In East Africa the roots make

a red dye; the hard fruit shells are also used as pots for food (Sidibe and Williams, 2002). The leaves, particularly when young, are utilized as a part of a comparable approach to spinach, or can be dried, powdered and utilized as a part of soups. However, the pulp of the baobab fruit is the most important resource.

2.2 Taxonomy of baobab

Family**: Bombacaceae**
Tribe: **Adansonieae**
Genus: **Adansonia**
Species**: Digitata**
Scientific name**: *Adansonia digitata***

2.3 Nomenclature of baobab

The history of known references to African baobab is well documented in Baum (1995).The binomial *Adansonia digitata* was given by Linnaeus, the common name honoring Michel adanson who had been to Senegal in the 18th century and described baobab adanson. African baobab is a very Long-lived tree with multi-purpose uses. It is thought some trees are over 1000 years old.

2.4 Botanical description of baobab tree

The African baobab (*Adansonia digitata*) is an enormous deciduous tree, 20-25 m high with a swollen trunk, more or less fibrous, and aspongy inside. Together with similar species, such as the Indian Kapok (Ceiba pentandara), it forms part of a small pan-tropical family of the Bombacaceae. The trunk may be conical, cylindrical, bottle-shaped or short and stubby and can reach 10-20 m in diameter. Its crown and especially the branches resemble large roots when the tree has shed its leaves during the dry season (Sidibé and Williams, 2002). The bark is smooth, silver grey in colour with variations from brown to purple, and it may grow up to 10cm thick, with girth of more than 20cm. The bark fibers are normally stripped from the lower layers of the trunk, even though this murderous technique is fatal for other trees.

Leaves of young tree or first leaves of the season are for the most part basic; develop gave long petioles, palmate leaves with 3 –7 lanceolate pamphlets splendid above and pubescent underneath, when young. The petiole is up to 15 cm long and thickly pubescent when young. Single handouts may gauge 5-15 cm or more (Sidibé and Williams, 2002). The blossoms as reported (Dalzeil, 1948) are white, huge, and pendulous on long stalks. Calyx profoundly - 5-lobed, exceptionally hirsute tomentose on both surfaces, tomentose on both surfaces, petals settees outside, the blooming season is amongst June and September. Flowers bloom for around 24 hours and turn chestnut when old. Pollinationis done by bats, other small mammals and possibly by wind.

Fruit ripen from October to January, differ in shape among the varieties from baloney and cylinder to ovoid, measuring 12–40 cm length, 7–17 cm in diameter, averted brownish green momentum covers their husk, the shell of the husk is approximately 0.5–1 cm thick. Loaded with a white to roseate, floury acidic pulp, divided longitudinally by fibrous septa into about l0 chambers, containing a great number of kidney–shaped, black brownish to red brown incrusted, hard seeds with the size of small beans (Von May dell, 1990).Seeds which are up to 10 mm long, arrange in raw in 2-8globules per fruit. The seeds are attached to fibrous stands from the wall of the fruit (Nour *et al*., 1980).

2.5 Baobab distribution

The baobab tree, *Adansonia digitata* develops generally in semi-parched Africa, from Senegal eastwards to Kenya, and all through southern Africa and in Madgashgar (Sidibe *et al*., 1998). African baobab occurs naturally in most countries, south of Sahara with prominent nonappearance in Liberia, Uganda, Djibouti and Burundi. Basically, baobab is connected with the savanna, particularly the drier parts. Be that as it may, there are augmentations of the dissemination into timberland zone, presumably connected with human

residence. However, there are extensions of the distribution into forest area, probably associated with human habitation. In Sudan, the tree is found in the central regions in the places with rainfall between 600-1000 mm in sandy soil, in mountains and in short grass savanna. It forms belt in central Sudan, in Kordofan, Darfur, Blue Nile, Upper Nile and Bahr Elgazal State, but the best quality is found in Southern Kordofan State(Kheiri, 1996).

2.6 Uses of baobab in food

2.6.1 Fruits pulp

The baobab fruit pulp is probably the most important foodstuff. It can be dissolved in water or milk. The liquid is then used as a drink, a sauce for food, a fermenting agent in local brewing, or as a substitute for cream of tartar in baking (Sidibe and Williams, 2002).

The pulp has recently become a popular ingredient in ice products in urban areas (Scheuring *et al*., 1999; Sidibe *et al*.,1998) cited in (Gebauer *et al*., 2002), in various types of juices and jams. The pulp is never cooked as the hot beverages are being readied; rather it is added at the end of the preparation process after the drinks are allowed to cool (Sidibe *et al*., 1996). Fruit pulp is important in local diets as a seasoning component and appetizer. When the pulp is soaked in water, it produces a milky solution, which can be consumed as a milk substitute. The baobab fruit pods are also good for burning and a potash-rich vegetable salt may be obtained from this ash for making soap (Ajayi *et al*., 2003).

2.6.2 Seeds

Baobab seeds can be eaten fresh, or they might be dried and ground into flour which can either be added to soups and stews as a thickener, or roasted and ground into a paste, or boiled for a long time, fermented and then dried for use (Sidibe and Williams, 2002; FAO, 1988).

2.6.3 Leaves

The leaves of the baobab tree are a staple for some populaces in Africa, especially the central region of the continent (Yazzie *et al*., 1994; Gebauer *et al*., 2002). During the rainy season when the baobab leaves is delicate, tender, people harvest the leaves fresh. During the last month of the rainy season, leaves are harvested in great abundance and are dried for domestic use and for marketing during the dry season. The leaves are commonly sun-dried and either put away as entire leaved or beat and sieved into a fine powder (Sidibe *et al*., 1998). Young leaves are generally utilized, cooked as spinach, and every now and again dried, frequently powdered and utilized for sauces over porridges, thick gruels of grains, or boiled rice (Sidibe and Williams, 2002).

2.7. Medicinal uses of baobab

2.7.1 Traditional uses

The traditional medicinal uses of baobab were described by Sidibe and Williams (2002) as follows: baobab is utilized as a part of society solution as an antipyretic or febrifuge to defeat fever. Both leaves and fruit pulp are used for this purpose. Fruit pulp and powdered seeds are utilized as a part of instances of looseness of the bowels and advance sweat (i.e. diaphoretic). Powdered leaves can be utilized as an against asthmatic and they are known to have antihistamine and hostile to strain properties. They are variously used to treat fatigue, as a tonic and for insect bets, guinea worm and internal pains and to treat dysentery.

Leaves are used for many other conditions, diseases of the urinary tract, ophthalmic and otitis. Seeds are used in cases of diarrhea and severe cough. Oil extracted from seeds is used for inflamed gums and to ease diseased teeth. The widest use may be in folk medicine is the use of bark as a substitute for quinine in case of fever or as prophylactic.

2.7.2 Cosmetics uses

An infusion of roots in Zimbabwe to shower bodies was to advance smooth skin, (Wickens, 1982). Since the seeds oil is utilized to treat skin grumblings to a degree it is utilized cosmetically (Sidibe and Williams, 2002).

2.8 Chemical composition of fruit pulp

The natural product pulp contains a high measure of starch, low protein, and to a great degree low fat (Osman, 2004). As indicated by Murray *et al*. (2001), basic sugars in baobab pulp account for about 35.6% of the aggregate starch content. This clarifies the detectable sweet taste of the pulp. However, the sweetness may change for various sorts of pulp (Chadare and Linnemann., 2009).The low water content, strong acidity and high sugar content was confirmed by Cisse *et al*. (2009).

2.8.1 Amino acid profile

Protein represents around one-fifth of dry matter in baobab fruit pulp (17%), thus can be considered a rich source of amino acids. The baobab fruit pulp is particularly high in valine, tryptophan and phenylalanine + tyrosine. Comparing to baobab leaves, baobab fruit pulp is inferior in terms of overall protein quality (Sena *et al.*, 1998). There is a large variability in the reported amino acid contents of baobab fruit pulp, despite the fact that the authors use similar methods for determination (Chadare and Linnemann, 2009).

2.8.2 Fatty acid profile

Most fatty acids in the pulp don't achieve perceivable levels. Thus to the fatty acids, the fluctuation in the reported values is high, despite the fact that the utilization of indistinguishable techniques by the researchers (Chadare and Linnemann, 2009). Glew *et al*. (1997) recorded aggregate lipid substance of 155 mg/g dry weight. Also points baobab fruit as a rich source of linoleic acid, 27 mg/g dry weight, whereas it contains a very low amount of α-linolenic acid (<1 mg/g) .The two fatty acids which are essential for human

nutrition are linoleic acid (18:2n-6) and α-linolenic acid (18:3n-3) (Sena *et al.*, 1998).

2.8.3 Mineral composition

Baobab fruit pulp contains very little iron and is a relatively poor source of manganese, but contains exceptionally high calcium content (Sena *et al.*, 1998; Osman, 2004).The high calcium contents of the fruit pulp make baobab fruits attractive as a natural source of calcium supplementation for pregnant and lactating women, as well as for children and the elderly (Osman, 2004; Prentice *et al.*, 1993).

Chadare and Linnemann (2009) assessed the commitment of baobab pulp to the suggested day by day allow recommended daily intake (RDI) for iron, zinc and calcium for children and pregnant women. The scope of those minerals is conceivable just when the most noteworthy reported qualities are considered for the pulp. Hence, considering the most elevated reported qualities, the utilization of 40 g of baobab mash for youngsters is sufficient to cover 41.5% of the DRI for iron, 25.4% of the RDI for zinc, and 35% for the RDI for calcium. For pregnant women, the consumption of60 g and 100 g would cover 23.1% and 38.4% of the DRI for iron, 17.3% and 28.7% for the RDI for zinc, and 42.1% and 70.1% for the RDI for calcium, respectively.

2.8.4 Vitamins

Authors have investigated mainly vitamin C (Chadare and Linnemann, 2009). Baobab fruit pulp has among the most noteworthy vitamin C or ascorbic content found in any fruit (Carr, 1955; Nicol, 1957). Nnam and Obiakor, (2003) reports 373 mg/100 g wet weight, which is more than six times the level of vitamin C in citrus organic products (30-50 mg/100 g wet weight) and the most noteworthy known in regular natural products. Besco *et al*. (2007) recorded an ascorbic content in baobab fruit pulp going from 150-499 mg/100 g of product. Scheuring *et al*. (1999) uncovered a noteworthy baobab tree-to-tree inconstancy for vitamin C content in the baobab fruit pulp, ranging from

1500-5000 mg/kg and reported that it was entirely steady starting with one year then onto the next. The correct vitamin C content relies on upon the individual tree (Sidibe *et al*., 1996). With baobab fruit powder, a drink with a vitamin C content equal to that of orange juice is easily obtained. However, to retain vitamin C in soft drinks it is important not to boil the pulp but rather to add the powder to previously boiled water (Sidibe *et al*., 1996). Chadare and Linnemann (2009) estimated the contribution of baobab pulp to the recommended daily intake (RDI) for vitamin C for children and pregnant women. The consumption of 13.9 g of pulp with the lowest reported vitamin C content and 8.3 g of pulp with the highest reported vitamin C content is enough to cover the RDI for a child.

The consumption of 40 g of pulp by a pregnant woman will cover 84 to 141% of her RDI of vitamin C, considering the lowest and the highest vitamin C content of the pulp reported by authors. Baobab fruit contains detectable levels of α-carotene (0.17 μg/g dry weight) and lutein (1.53 μg/g dry weight) (Sena *et al*., 1998). Nour *et al*., 1980), point out baobab pulp as a valuable source of thiamine. Becker (1983) reported thiamine, riboflavin, and niacin content of 0.04, 0.07 and 2.16 mg/100g dw, respectively.

2.8.5 Pectin content

Baobab fruit pulp is rich in pectin, the vast majority of it being water-solvent with low substance of protopectin. A characteristic thickness value of water-solvent pectin are around 1/5 of those of commercial apple pectin and hence does not give a good jelly of high solid content because it tends to precipitate rapidly in acid media to form irregular gels (Nour *et al,* 1980).

2.8.6 Bioactive compounds and nutritional attributes from baobab fruit

Bioactive compounds are components of foods that influence physiological or cellular activities resulting in a beneficial health effect (Kris-Etherton *et al*., 2004). Unlike nutrients which prevent deficiency diseases, bioactive

compounds may reduce the risk of chronic diseases such as cardiovascular disease and cancer. Examples of bioactive compounds include carotenoids, proanthocyanidins and phenolic acids which are widely available in fruits and vegetables, cereals, tea and coffee. Baobab pulp contains very high levels of minerals (calcium, iron, magnesium, and sodium), vitamin C, organic acids, essential amino acids, sugars, sterols, saponins, triterpenes, flavonoids (high levels of procyanidin B2 and (-)-epicatechin), cellulose, fibres and tannins which may be responsible for its antioxidant, anti-inflammatory, antipyretic, analgesic, hepatoprotective, antimicrobial, antiviral, anti-trypanosoma, antidiarhoea properties and healing effects (Kaboré *et al*., 2011).

The presence of flavonoids and vitamins in most indigenous fruits provide evidence of antioxidant properties. For instance because of high natural vitamin C content, baobab fruit pulp has a well-documented antioxidant capability and may have a role in the extension of shelf-life for derived foods and beverages as well as cosmetics (Vermaak *et al*., 2011). The food/beverage industry could introduce baobab fruit pulp into food in order to act as a preserving ingredient by preventing oxidation of lipids in the food (Afolabi and Popoola, 2005). Baobab aqueous extract (800 mg/kg) gave comparable antiinflammatory effects to phenylbutazone (15 mg/kg), a classical drug used as painkiller (Kaboré *et al*., 2011). Baobab extract showed high polyphenol content and total antioxidant activity in vitro and reduced starch digestion and glycemic response (GR) in humans (Coe *et al*., 2013)

2.9 Vitamin C content

The baobab capsule pulp is reported to have a high content of vitamin C. one source reported that the content of vitamin C in the baobab fruit is 1690 mg/kg compared with 1060 mg/kg for fresh hot pepper, which is very famous for high content of vitamin C– that is why it is known as homegrown vitamin C source. (Agbessi dos-sintos and Tom, 1996).

The high convergence of vitamin C in baobab fruit pulp prompted to across the board dispersion of the tree in semi-dry parts of Africa. Sauberlich, (1994) abridged the state–of-the-craftsmanship data on the nutritious and clinical employments of vitamin C, otherwise called ascorbic acid .it is an effective hostile to oxidant and critical in human sustenance, vitamin. C has been appeared to be identified with low pulse, improved immunity against numerous tropical; illnesses bring down frequency of waterfall advancement and lower occurrence of coronary sicknesses. The day by day prescribed admission for solid, non-smoking grown-up is 65 mg; smokers require more vitamin. C than non –smoker coronary. While 65 mg a day is the minimum recommended intake, a full saturation of the total pool of vitamin. C in the body is about 140 mg a day. Convalescents recouping from irresistible ailments or nursing mother advantage fundamentally from day by day admissions surpassing, 250 mg, Sidibe *et al.* (1996). Utilizing the normal vitamin .C content in baobab fruit pulp, 2800 mg/kg these proposals can be changed over into measure of baobab powder. The day by day suggested measurements of vitamin c can be gotten from 23 g of baobab powder. The everyday immersion of vitamin .C pool in the body requires 50g of powder, the uncommon measurements of convalescents is 90 g. There are sufficient baobab trees that give these measures of vitamin. C to the a great many individuals in semi parched west Africa, (Sidibe *et al.*, 1996).with baobab organic product powder, one can without much of a stretch get a drink with vitamin C content equivalent to that of orange juices.

2.9.1 Importance of vitamin. C

Vitamin C, otherwise called ascorbic acid, unlike most mammals and other animals, humans do not have the ability to make their own vitamin C. Therefore, we must obtain vitamin C through our diet.

Vitamin C is required for the combination of collagen, a vital auxiliary part of veins, ligaments, tendons, and bone. Vitamin C also plays an important role in

the synthesis of the neurotransmitter, nor epinephrine. Neurotransmitters are critical to brain function and are known to affect mood. In addition, vitamin C is required for the synthesis of carnitine, a small molecule that is essential for the transport of fat into cellular organelles called mitochondria, where the fat is converted to energy (Carr and Frei, 1999). Research also suggests that vitamin C is involved in the metabolism of cholesterol to bile acids, which may have implications for blood cholesterol levels and the incidence of gallstones (Simon and Hudes, 2000).Vitamin C is likewise specially compelling cell support. Indeed, even in little sums vitamin C can ensure vital particles in the body, for example, proteins, lipids, sugars, and nucleic acids, from harm by free radicals and responsive oxygen species that can be created amid typical digestion system and also through presentation to poisons and contaminations. Vitamin C may likewise have the capacity to recover different cancer prevention agents, for example, vitamin E. One late investigation of cigarette smokers found that vitamin C recovered vitamin E from its oxidized shape (Bruno *et al*., 2006).

2.10Free radicals and antioxidants

2.10.1 Free radicals

2.10.1.1 Definition and formation of free radicals

A free radical is an atom or group of atoms that has one or more unpaired electrons and can have positive, negative or neutral charge. Free radicals are formed as necessary intermediates in a variety of normal biochemical reactions. When generated in excess or not appropriately controlled, free radicals can cause damage to a wide range of macromolecules. A prominent feature of free radicals is that they have extremely high chemical reactivity, which explains not only their normal biological activities, but how they cause damage on cells (Becker *et al.*, 2004).

Free radicals have been implicated in many disease conditions in humans, including arthritis, hemorrhagic shock, atherosclerosis, ischemia and

reperfusion injury of many organs, Alzheimer'sand Parkinson's disease, gastrointestinal dysfunctions, tumor promotion and carcinogenesis (Bagchi *et al*., 2000).

Oxygen has two unpaired electrons in separate orbital in its outer shell. The unpaired electrons make oxygen susceptible to the formation of radicals. Sequential reduction of molecular oxygen, equivalent to sequential addition of electrons, leads to formation of a group of reactive oxygen species including the superoxide anion, peroxide anion, hydroxyl radical anion along with the notation used to denote the structures (Becker *et al*., 2004).

2.10.1.2 Exogenous sources of free radicals

Exposure to radiation from the environment and man-made sources causes formation of free radicals. Low-wavelength electromagnetic radiation such as gamma rays splits water in the body to generate hydroxyl radicals (OH^-). The highly reactive OH^- thus formed begins to react vigorously with the nearby cells (Halliwell, 1994).It has been estimated that 1 to 3% of the oxygen we breathe in is used to make superoxide radicals (O_2-). Over 2 kg of O_2- is made in the human body every year and people with chronic inflammations may make much more to combat infection (Halliwell, 1994). The oxidants generated damage cellular macromolecules, including DNA, proteins and lipids and accumulation of such damage may contribute to ageing and age related diseases (Fraga *et al*., 1990).

2.10.1.3 Endogenous sources of free radicals

Enzymatically or non-enzymatically mediated electron transfer reactions are the source of free radicals produced in the cells. Electron leakage that occurs from electron transport chains, such as those in the mitochondria and endoplasmic reticulum, to molecular oxygen are the major source of free radicals (Fridovich, 1986). Free radicals are formed in cells of our body mainly from:

• Consumption of O_2 by mitochondria during normal aerobic respiration to produce H_2O Oxidants such as oxygen free radical, H_2O_2 and hydroxyl radical are the by- products of this process

• Destroying of bacteria and virus infected cells by phagogytic cells releases nitric oxide, hydrogen peroxide and oxygen free radical

• Degradation of fatty acids and other molecules by peroxisomes, the peroxisomes produce hydrogen peroxide as a byproduct, which is then degraded by catalyze.

2.10.1.4 Effects of free radicals on biological system

Numerous sorts of free radicals exist, however those of most worry in biological system, are derived from oxygen, and known collectively as reactive oxygen species (ROS),such as Nitric oxide (NO) is a free radical delivered from L-arginine by the movement of nitric oxide synthase. NO, a pro-inflammatory go-between plays a crucial part within the regulation of immune functions, neurotransmission, and vasodilatation. Nevertheless, NO overproduction happens in irregular physiological conditions and results in different inflammatory and neurodegenerative illnesses through tissue harm. Therefore, the inhibition of NO is an essential factor for the prevention and additionally treatment of numerous provocative sicknesses (Sharma *et al*., 2007). With the exception of reactive Oxygen species (ROS), the overrun of reactive nitrogen species (RNS) has conjointly been related to oxidative stress and with chronic inflammation, that were concerned within the pathophysiology of assorted diseases like inflammatory disease, diabetes, atherosclerosis, and carcinogenesis (Moncada *et al*., 1991). The damage from NO throughout the inflammation or carcinogenic method may well be reduced by the NO scavengers and NOS enzyme inhibitors. Variety of phytochemicals, like quercetin, tocopherol, and catechins are found to inhibit the harm of NO (Arroyo *et al.*, 1992; Kawada *et al*., 1998). Therefore, how to utilize the natural antioxidants in dietary plants to stop or improve RNS-mediated injury becomes vital. Reported by (Halliwell, 1997; Vendemiale *et*

al., 1999 .explained the natural antioxidants to inhibit lipid peroxidation or to protect the damage of free radicals (Halliwell, 1997; Vendemiale *et al*., 1999).The protection that fruits, greens, medicinal plant, and herbal plants provide in opposition to illnesses has been attributed to various antioxidant phytonutrients, which includes flavonoids, phenylpropanoids, phenolic acid, ascorbic acid, tocopherol, and carotenoids contained in these plant. Numerous studies have proven that accelerated nutritional consumption of polyphenol and flavonoids correlates with decreased oxidative stress, infection, tumor, and coronary heart disease (Laranjinha *et al*., 1994; Meng *et al*., 1999).

Diabetes mellitus is a metabolic illness in which the patient has high level of blood glucose. For the most part, there are two kinds of diabetes mellitus which are type 1 and type 2. Type 1diabetes is insulin-ward and occurs because of the insufficient insulin generation, which emanated from abandons in the insulin quality. Type 2 diabetes occurs when the body's cells have become insulin-resistant or when the pancreas no longer makes adequate insulin. Free radicals have a notable influence in the advancement of diabetes mellitus. It is as of now estimated that oxidative pressure is the normal pathogenic factor adding to insulin obstruction, β-cell brokenness, and impeded glucose resilience and at last to type 2 diabetes (Ceriello and Motz, 2004).

Alpha-Glucosidase is an intestinal enzyme that catalyzes the break of α-1.4-glycosidic bond in oligosaccharides into α-glucose molecules, which can be consumed by the digestive system (Gao *et al* , 2008) and reason the raised postprandial blood glucose level. Henceforth, the hindrance of the movement of this protein can successfully diminish the postprandial blood glucose level. Aalpha -glucosidase inhibitors consolidate with the intestinal α-glucosidase and hinder the arrival of glucose from the sugar and consequently restrain the take-up of postprandial blood glucose. There are numerous manufactured medications accessible to avert or regard diabetes, for example, acarbose,

vogibose and miglitol. Be that as it may, they may normally cause hepatic issue and other negative gastrointestinal side effects (Murai *et al*., 2002).Hence, cancer prevention agent and α-glucosidase inhibitors from normal source have turned out to be more favored decision to anticipate or treat diabetes.

2.10.2 Antioxidants

2.11.2.1Definition of antioxidants

An Antioxidant is defined as a substance that when present in low concentrations compared to those of an oxidizable substrate significantly delays or prevents oxidation of that substance (Halliwell and Guteridge, 1989). For the in vivo situation the idea of cell reinforcements incorporates cancer prevention agent catalysts, press official and transport proteins and different mixes influencing signal transduction and quality expression (Guteridge, 1989).

2.11.2..2Classes of antioxidants

Antioxidants are divided into two major classes, namely endogenous antioxidants and exogenous antioxidants.

2.11.2.3Endogenous antioxidants

Three groups of enzymes play important roles in protecting cells from oxidative stress (Becker *et al*., 2004). Firstly, superoxide dismutases (SOD) are enzymes that catalyze the conversion of two superoxides to hydrogen peroxide and oxygen. Hydrogen peroxide is substantially less toxic than superoxide. The detoxifying reaction catalyzed by SOD is ten thousand times faster than the un catalyzed reaction (Becker *et al*., 2004).

SODS are metal-containing enzymes that depend on bound manganese, copper or zinc ion for their antioxidant activity. In mammals, the manganese-containing enzyme is most abundant in mitochondria, while the zinc or copper forms are predominant in cytoplasm. SODs are inducible enzymes, with exposure of bacteria or vertebrate cells to higher concentrations of oxygen

resulting in rapid increases in the concentration of SOD. Secondly, catalyses, found in peroxisomes ineukaryotic cells, degrade hydrogen peroxide to water and oxygen, and hence complete the detoxification reaction started by SOD. Finally, glutathione peroxidases, a group of enzymes which are the most abundant contain selenium and like catalyses, degrade hydrogen peroxide. Glutathione is the most important intracellular defense against damage by reactive oxygen species. The cysteine on the glutathione molecule provides an exposed free sulphydryl group that is very reactive, providing an abundant target for radical attack. Reaction with radicals oxidizes glutathione but the reduced form is regenerated in a redox cycle that involves glutathione reductase and the electron acceptor (Baydar *et al*., 2007).

In addition to the three enzymes above, glutathione transferees, ceruloplasmin, hem oxygenase may participate in enzymatic control of oxygen radicals and their products.

2.11.2.4 Exogenous antioxidants

The three commonexogenous antioxidants prevention agents are vitamin E, vitamin C/ascorbic acid and glutathione. Vitamin E is the significant lipid-dissolvable antioxidant and plays an important role in protecting membranes from oxidative damage. The essential movement of vitamin E is to trap intermediary radicals in cellular membranes and consequently prevent lipid peroxidation of the membranes (Baydar *et al*., 2007).

Vitamin C is a water-solvent antioxidant that can reduce radicals from variety of sources. Vitamin C participates in recycling vitamin E radicals. Vitamin E radicals are created when the vitamin trap peroxy radicals in cellular membranes. Vitamin C likewise works as a professional oxidant in specific situations and now and then creates oxygen by-results of digestion system that can cause damage to cells (Coinu *et al*., 2007).In addition to vitamin E and vitamin C, phenolic compounds can function as antioxidants. The antioxidant properties of some plant extracts have been attributed partially to their phenolic compound contents (Coinu *et al*., 2007).

2.11.2.5 Phenolic compounds

2.11.2.5.1 Definition of phenolic compounds

Phenolic compounds are the most abundant secondary metabolites of plants. Chemically they are defined as substances possessing an aromatic ring with one or more hydroxyl substituent, although a more precise definition is based on their metabolic origin and defines them as substances derived from the shiikimate pathway and phenylpropanoid metabolism, in detail presented elsewhere (Ryan and Robards, 2002a; Ryan *et al*., 1998).

2.11.2.5. 2Classification of Phenolic Compounds

Classification of phenols is a very complex task, but a convenient one for the plant phenolic compounds is the one that distinguishes the number of constitutive carbon atoms in conjunction with the structure of the basic phenolic skeleton. The range of phenols is thus vast and currently comprises more than 8000 representatives of known structures, ranging from simple phenols (e.g. phenolic acids) up to a highly polymerized substances like tannins (Antolovich *et al*., 2000). (Bravo, 1998; Manach *et al*., 2004) as shown in Table 1.

2.11.2.5.3 Functions of phenolic compounds

Plant phenols embrace a considerable range of functions that could easily fall into two broad categories, reflecting the focus on activity in plants or bioactivity in humans via food consumption. Numerous surveys and monographs have been distributed portraying both, however here; just two have been given modifying their capacities in man (Fraga , 2010). Some of the most–in plants (Lattanzio *et al*., 2006) and vital are briefly presented below to elucidate the scientific importance of plant phenols research.

Table 1: Phenolic classes in plants

Phenolic class	**Carbon skeleton**
simple phenols	C6
hydroxybenzoic acids	C6-C1
phenylacetic acids	C6-C2
hydroxycinnamic acids, coumarins	C6-C3
naphthoquinones	C6-C4
Xanthones	C6-C1-C6
stilbenes, anthraquinones	C6-C2-C6
flavonoids	C6-C3-C6
tannins	(C6-C1)n, (C6-C3-C6)n
biflavonoids	(C6-C3-C6)2
lignans	(C6-C3)2
lignins	(C6-C3)n

2.11.2.5.3.1Functions of phenolic compounds in plants

Plant phenols have been associated with several roles. They can act as sun-screeners by absorbing the damaging UV light prior the photo-oxidation of cell constituents occurs (e.g. flavonoids), as signalling molecules enabling communication of plant with its environment or as visual signals for the pollinators and seed-dispersing animals (e.g. anthocyanins).They additionally have an Important part in the plant's safeguard component and in their physiological procedures; for example, they are involved in the synthesis of structural polymers (e.g. ferulic acid in lignification), they can affect seed germination and dormancy (e.g. ferulic acid) or can directly regulate the growth of plant via control of auxins biosynthesis (e.g. some flavonoids) (Lattanzio *et al*.,2006).

2.11.2.5.3.2 Functions of phenolic compounds in humans

The putative medical advantages of plant phenols have been for the most part connected with their antioxidant activities and their protective effects toward some of the major diseases such as cancer, cardiovascular, neurodegenerative and others. Hydrogen donators. Antiradical antioxidant prevention agents can give hydrogen particles to the free radicals and stop the oxidation chain responses connected with various sorts of cell oxidative harms and different obsessive conditions (Fraga , 2010). Enzyme inhibitors. Some plant phenols have the ability to suppress the free radical formation by inhibiting enzymes involved in their generation such as cytochrome P450 isoforms, lipoxygenases, cyclooxygenases and xanthine oxidases (Fraga, 2010). Metal chelators. The antioxidant activity of plant phenols may also be utilized via capability to chelate metal ions involved in the production of free radicals, though an opposite behavior was also observed (Fraga , 2010). Anticancer, anticardiovascular, antineurodegenerative and other actions. In addition to antioxidant activities, some plant phenols are thought to exert also others via interference with molecular processes. For example, they appeared to be

involved in the neuroprotection (Ramassamy, 2006), cardiovascular protection (Manach *et al*., 2005), menopause and osteoporosis prevention (Cornwell *et al*., 2004). Another emerging role of plant phenols is their protective role in some types of cancers, which may be exert via removal of carcinogenic agents, modulation of cancer cell signalling and antioxidant enzymatic activities as well as through the induction of apoptosis and cell cycle arrest (Hu, 2011).

2.11.2.5.1Flavonoids

Flavonoids are naturally occurring in plants and are thought to have positive effects on human health. Studies on flavonoids derivatives have shown a wide range of antibacterial, antiviral, anti-inflammatory, anticancer, and anti-allergic activities (Di Carlo *et al*., 1999; Montoro *et al.*, 2005).With their biological activity; flavonoids are important components of the human diet, although they are generally considered as non-nutrients. Sources of flavonoids are foods, beverages, different herbal drugs, and related phytomedicines (Montoro *et al*., 2005). Flavonoids are an important class of phenolic compounds, and have potent Antioxidant activity. The antioxidant property of flavonoids was the first mechanism of action studied with regard to their protective effect against cardiovascular diseases. Flavonoids have been shown to be highly effective scavengers of most oxidizing molecules, including singlet oxygen, and various free radicals (Bravo, 1998) implicated in several diseases.

2.12 Functional foods

2.12.1History of functional foods

The term useful functional foods was initially presented in Japan in the mid-1980s and refer to processed foods containing ingredients that aid specific bodily functions in addition to being nutritious. To date, Japan is the only country that has formulated a specific regulatory approval process for

functional foods, known as Foods for Specified Health Use (FOSHU), these foods are eligible to bear a seal of approval from the Japanese Ministry of Health and benefit (Arai, 1996). The Institute of Medicine's Food and Nutrition Board (IOM/FNB, 1994) found 100 products are licensed as FOSHU foods in Japan. In the United States, the functional foods category is not recognized legally. Irrespective of this, many organizations have proposed definitions for this new and emerging area of the food and nutrition sciences. The Institute of Medicine's Food and Nutrition Board (IOM/FNB, 1994) defined functional foods as "any food or food ingredient that may provide a health benefit beyond the traditional nutrients it contains." Health-conscious baby boomers have made functional foods the leading trend in the U.S food industry (Meyer, 1998) Estimates, however, of the magnitude of this market vary significantly, as there is no consensus on what constitutes a functional food. Decision Resources, (Waltham, 1998) estimates the market value of functional foods at $28.9 billion. More significant, maybe, is the potential of functional foods to mitigate disease, promote health, and reduce health care costs.

2.12.2 Definition of functional foods

Functional foods are normal foods and parts of the daily diet, but they contain a component that benefits some particular physiological function and reduce the risk of diseases (Salovaaro, 1999). The wide applications of functional food are in form containing probiotics and non-digestible carbohydrate known as prebiotics (Fuller and Gibson, 1997).

2.12.3Classification of functional foods

Functional food can be classified according to several principles, namely the food group it belongs to (e.g. dairy products, beverages, cereal products, confectionary, oils and fats), the diseases it is expected to prevent or alleviate (e.g. diabetes, osteoporosis, colon cancer), its physiological effects (e.g. immunology, digestibility, anti-tumour activity), the category of its specific

biologically active ingredients (e.g. minerals, antioxidants, lipids, probiotics), its physico-chemical and organoleptic properties (e.g. color, solubility, texture), or the processes that are used in its production (e.g. chromatography, encapsulation, freezing) (Juvan *et al.* 2005). literature various of these topical groups are used to classify functional foods, in the preparatory work for this report food groups were used for classification where possible and appropriate (e.g. beverages, dairy products, cereal products, oils and fats, convenience food, confectionery, bakery products, fresh produce, miscellaneous), although there is no unified classification of food, either (Eurostat,2007).The second level classification groups the functional food products within each food category by the biologically active ingredient (e.g. phenols, terpenoids, saccharide, lipids, peptides, fibers, plant extracts, bacteria cultures).

2.13 Probiotic concepts

2.13.1History of probiotics

The idea that some bacteria contained in our food may have beneficial effects is much older than the term probiotic. At the beginning of the 20th century, the Russian Nobel Prize Laureate Elie Metchnikoff associated the observed longevity of Bulgarian peasants with their high consumption of live microbes in fermented milk products, as he reported in his book.The prolongation of life (Metchnikoff, 1907). In1930, the Japanese scientist Minoru Shirota isolated a lactic acid bacterium from the feces of a healthy infant. Five years later, one of the first fermented milk drinks thought to support intestinal health was produced with the strain he developed and was named "Yakult". (Metchnikoff, 1907).

2.13.2 Definition of probiotics

The word ‘probiotic’, derived from the Greek language, means ‘for life’ (Fuller, 1989) and has had many definitions in the past. Definitions such as ‘substances produced by protozoa that stimulate the growth of another’ or ‘organisms and substances that have a beneficial effect on the host animal by

contributing to its intestinal microbial balance' were used. These general definitions were unsatisfactory because 'substances' include chemicals such as antibiotics. The definition of probiotics has since then been expanded to stress the importance of live cells as an essential component of an effective probiotic. Most recently, Huis- Veld and Havenaar (1991) broadened the definition of probiotics as being 'a mono- or mixed culture of live microorganisms which, applied to man or animal (e.g. as dried cells or as a fermented product), beneficially effects the host by improving the properties of the indigenous micro flora. This definition implies that probiotic products, for example fermented milk, contain live microorganisms and improve the health status of the host by exerting beneficial effects in the gastrointestinal tract.

2.13.3 Probiotic strains

Probiotic cultures naturally occur in certain fermented foods according to (Rosander *et al*., 2008). Below is a list of different strains of probiotic bacteria.

- Bacillus coagulans GBI-30, 6086
- Bifidobacterium animalis subscp. lactis BB-12
- Bifidobacterium longum subsp. BB536
- Lactobacillus acidophilus NCFM
- Lactobacillus paracasei St11
- Lactobacillus johnsonii La1
- Lactobacillus plantarum 299v
- Lactobacillus reuteri ATCC
- Lactobacillus reuteri Protectis.

2.13.4 Characteristics of probiotics microorganism

Characteristics of successful probiotics determine their ability to survive the upper digestive tract and to colonize in the intestinal lumen and colon for an undefined time period. Probiotics are safe for human consumption and no reports have found on any harmfulness or production of any specific toxins by these strains (Von Wright and Axelsson 2000., Salminen *et al.,* 1998).In addition, some probiotics could produce antimicrobial substances like bacteriocins. Therefore, the potential health benefit will depend on the characteristic profile of the probiotics. Some probiotic strains can reduce intestinal transit time, improve the quality of migrating motor complexes,(Husebye *etal*., 2001) and temporarily increase the rate of mitosis in enterocytes (Banasaz *et al*., 2002, Halvorsen *et al*., 2000).

The most common probiotics are Lactobacillus and Bifidobacterium. In general most probiotics are gram-positive, usually catalase-negative, rods with rounded ends, and occur in pairs, short, or long chains (Von Wright and Axelsson ,2000).They are non-flagellated, non-motile and non-spore-forming, and are intolerant to salt. Optimum growth temperature for most probiotics is 37°C but some strains such as L. casei prefer 30 °C and the optimum pH for initial growth is 6.5-7.0 (Von Wright and Axelsson, 2000). L. acidophilus is microaerophilic with anaerobic referencing and capability of aerobic growth.

Bifidobacterium are anaerobic but some species are aero-tolerant. Most probiotics bacteria are fastidious in their nutritional requirements (Desmazeaud, 1983; Marshall and Law, 1984). With regard to fermentation probiotics are either obligate homofermentative (ex. *L. acidophilus*, L. helvelicas), obligate heterofermentative (ex. L. *brevis*, L. *reuteri*), or facultative heterofermentative (ex. L. *casei*, L. plantarum), (Barrangou, 2011). Additionally, probiotics produce a variety of beneficial compounds such as

antimicrobials, lactic acid, hydrogen peroxide, and variety of bacteriocins (Gorbach *et al*., 2002).

Probiotics should have the ability to interact with the host micro flora and competitive with microbial pathogens, bacterial, viral, and fungal (Gorbach, 2002).

2.13.5 Criteria of selection of appropriate probiotic

Different aspects have to be considered in probiotic selection Safety criteria for any successful probiotic have been defined in several reviews (Lee and Salminen, 1995, Adams, 1999) include the following specifications:

1. Strains uses are preferably of human origin.
2. They are isolated from healthy human GI tract.
3. They have a history of being non-pathogenic.
4. They have no history of association with diseases such as infective endocarditic or GI disorders.
5. They do not deconjugate bile salts (bile salt deconjucation or dehydroxylation would be a negative trait in the small bowel (Marteau *et al*., 1995).
6. They do not carry transmissible antibiotic resistance genes.

While in selecting a preferable probiotic strain several aspects of functionality have to be considered:

1. Acid tolerance and tolerance to human gastric juice.
2. Bile tolerance (an important property for survival in the small bowel).
3. Adherence to epithelial surfaces and persistence in the human GI-tract.
4. Immunostimulation, but no pro-inflammatory effect.
5. Antagonistic activity against pathogens such as *Helicobacter pylori*, *Salmonella sp., Listeria monocytogenes* and *Clostridium.*
6. Antimutagenic and antigarcinogenic properties.

Feeding trials with different probiotic strains have shown that the probiotic strain usually disappears from the GI-tract within a couple of weeks after the

ingestion is discontinued (Fukushima *et al.,* 1998; Johansson *et al.,* 1998; Alander *et al.;* 1999; Donnet-Hughes *et al.,* 1999). The role of the probiotic persistence in the human GI-tract has therefore been questioned. However, even temporary persistence, which has been noted for several ingested probiotic strains, may enhance their chances for beneficial functions in the GI-tract, and is therefore considered a desirable trait. Necessary safety and functional criteria the aspects related to probiotic production and processing are also of utmost importance, such as:

1. Good sensory properties.
2. Phage resistance.
3. Viability during processing.
4. Stability in the product and during storage.

Good viability and activity of probiotics are considered prerequisites for optimal functionality. However, several studies have shown that non-viable probiotics can have beneficial effects such as immune modulation and carcinogen binding in the host (Salminen *et al*.; 1999).

2. 13.6*Bifidobacterium* as probiotics

Bifidobacteria is the predominant species of human colonic and faucal micro biota .It has been extensively introduced in the food industry and pharmaceutical applications (Guarner and Malagelada,2003). The prevalence of bifidobacteria in the feces of breast fed infant may have been a major reason for selecting strains of this group for use as probiotics (Lilly and Stillwell, 1965).

2.13.6.1Species of *Bifidobacterium*

B. angulatum; B. animalis; B. asteroides; B. bifidum; B. boum; B. breve; B. catenulatum; B. choerinum; B. coryneforme; B. cuniculi; B. dentium; B. gallicum; B. gallinarum; B indicum; B. longum; B. magnum; B. merycicum; B. minimum; B. pseudocatenulatum; B. pseudolongum; B. psychraerophilum; B. pullorum; B. ruminantium; B. saeculare; B. scardovii; B. simiae; B.

subtile; B. thermacidophilum; B. thermophilum; B. urinalis; B. sp. (Holzapfel *et al*., 1995).

2.13.6.2 *Bifidobacterium longum BB536*

Bifidobacterium longum is one of the *bifidobacterium* species found mainly in human faeces and it may be considered as the most common species of *bifidobacterium*, being found both in infant and adult. Potential benefits from consumption of *B. longum* include: antagonistic action toward intestinal pathogens, improved lactose utilization, anticarcinogenic action and control of serum cholesterol levels. Scientific studies showed the benefits offered by *Bifidobacteriumlongum BB536* (Kojima *et al*., 1996; Namba *et al*., 2003). Thus there is considerable interest in incorporating these healths's promoting *bifidobacterium* into food. Nevertheless, probiotic strains, particularly *bifidobacterium* are rarely used outside the diary based industry. The scarcity of animal milk in many countries makes it difficult to provide adequate *bifidobacterium* intake.

2.13.7 Effect of different nutrients on bifidobacteria growth and survival

Maintaining the growth and viability of bifidobacteria in dairy products has been a major challenge to the fermented dairy producers because of the inability of bifidobacteria to grow in milk. Therefore, there were a lot of suggestion to use a lot of nutrients to enhance the growth and survival of different strain of probiotic strains. This nutrient has termed as Bifidogenic Factors and defined by Modler (1994) as following Compound that survive direct metabolism by the host, and reach the large bowel or cecum for preferential metabolism by bifidobacterial. It has found that the growth and viability of probiotic in vitro were dependent on prebiotics as well as strain. However, using prebiotics with probiotic in milk has found to enhance the growth and survival of probiotic bacteria in fermented dairy products (Desai *et al*., 2004). Bifidobacterium animals showed good stability in yogurt in present of FOS and it was better than *B. longum* in yogurt contain FOS which

remained above 10^6CFU/g (Akalin *et al*., 2004; Akalin *et al*., 2007). Zhao *et al*. (2006) have found that casein hydrolysates shorten the fermentation time of yogurts and increase the probiotic count but the total number in the storage period has decreased, which related by the hydrolysates. FOS and lactulose were used in human diets to support the growth of bifidobacteria (Modler *et al*., 1990; Modler, 1994). Where it found that such prebiotics have potential to increase bifidobacteria level in a symbiotic yogurt products during the shelf life of the dairy products (Crittenden *et al*., 2001). Using the prebiotics and probiotics in combination coined as synbiosis (Roberfroid, 1998). Additions of Raffinose family oligosaccharides (RFOs) have beneficial effects on the survival of *Bifidobacterium Lactis Bb12* and *L. acidophilus* in dairy products (MartinezVillaluenga *et al*., 2006). Dave and Shah (1997) and Guler- Akin and Akin (2007) suggested adding cysteine to enhance the viability of probiotic bacteria in bio-yogurt. Others found that using of number of growth-promoting substances can improve the viability of bifidobacteria such as cysteine, acid hydrolysates, and tryptone (Dave and Shah, 1998). Bifidobacteria require carbonate or bicarbonate (or CO2 gas) as carbon sources and to help tolerant oxygen present, but it can‘t use fatty acids or organic acid as carbon source. Where it has established that cysteine or cystine is an essential nitrogen sources (Shah, 1997). Bouhallab *et al*. (1993) found that using tryptic digest of caseinomacropeptide enhanced the growth bifidobacteria in skim milk. Janer *et al*. (2004b) have used supplementation of 2% Caseinomacropeptide or whey protein in milk to enhance *B. lactis* growth. Klaver *et al*. (1993) and Dave and Shah (1998) stated that the amino acids and peptides of acid hydrolysis of casein improved the viability of probiotic bacteria. A previous study by Poch and Bezkorovainy (1988) has indicated that only a limited number of bifidobacteria will grow optimally in synthetic medium contain vitamins, nitrogen and carbon, but bovine casein hydrolysed and yeast extract have a promoting activity on bifidobacteria, and they have reported that κ–casein is the main growth promoter for bifidobacteria in

bovine milk (Azuma *et al*., 1984). In the same subject Saxelin *et al*. (1999) Indicate that the addition of glucose, yeast extract or milk protein fractions enhanced the growth of the most probiotic strains and co-combined with yogurt culture enhanced the survival of all the probiotic strains. The idea of mix probiotic, which is with low proteolytic activity with high proteolytic organisms such as Lactic Acid Bacteria or S. thermophiles, will enhance the growth and survival of these probiotic bacteria (Klaver *et al*., 1993; Shihata and Shah, 2000; Champagne *et al*., 2005). In other hand, the addition of casein hydrolysates was suggested to recover the slow proteolytic activity of some probiotic strains and enhance their growth and survival in dairy products (Lourens-Hattingh and Viljoen, 2001; Warminska-Radyko *et al*., 2002). In vitro study Ustunol and Gandhi (2001) have shown the great enhancement occur of using honey on the growth and survival of *Bifidobacterium bifidum* in Honey-Sweetened skim milk.

2. 13.8 Functional properties of probiotics

In spite of research progress in recent years can understand of gut of ecosystem is still fragmentary and consequently limits our comprehension of a normal or balanced microbial population. Thus, the impact of a functional property of the strain on composition and function of the intestinal population is still difficult to a certain (Holzapfel *et al*., 1995; Mercenier and Pvan, 2002).Never the less; (Kullisaar*et al.*, 2002). Numerous beneficial functions have been suggested for probiotic bacteria:

- Nutritional benefits of probiotics includes:

- Vitamin production, availability of minerals and trace elements.

- Production of important digestive enzymes. Such as- Production of β-glycosidase of alleviation of factors in tolerance of lactose.

- Barrier , restoration , antagonistic effects against:

- Infectious diarrhea.

- Antibiotic –associated diarrhea, irradiation –associated diarrhea.
- Cholesterol - lowering effect.
- Stimulation and improvement of the immune system.
- Enhancement of bowel motility, relief from constipation.
- Anti-carcinogenic effects in the colon.
- Maintenance of mucosal integrity.
- Reduction of inflammatory allergic reactions.
- Adherence and colonization resistance.
- Ant oxidative activities.

2.13.9Probiotic bacteria and functional foods

The growing understanding of the relationship between diet and health increased the demand for food with specific benefit beyond their basic nutrition such as improving the health and well being of human. This food is called Functional Food. However, Functional food has defined as one, which provides a specific health benefit over and above its normal nutritional status (Gibson and Rastall, 2004). Moreover, the functional foods must remain as foods (not capsules, etc.) and they must also reveal their effects in amount that can usually be expected to be consumed in the diet. It has been suggested that food will use as functional when it has shown beneficial effect on one or more target in the body and that beside their nutritional effects such as well-being and health of the host (Isolauri *et al*., 2002). The old generation of functional foods indicates of using supplements to the food to increase their nutrition and health effects such as vitamins and micronutrient. However, in the new concept of functional foods there was more interest in the gastrointestinal interactions (Salminen *et al*., 1998a), that led for more interest in the dominated organisms in the gastrointestinal tract (indigenous microflora) which found to beneficially effect human health, which known as probiotic bacteria. Therefore, the use of probiotic micro-flora was one of the most promising areas for the development of functional foods in the recent

studies (Gibson and Roberfroid, 1999) because of what probiotics has established a great benefit to human health. Bifidobacteria were the most dominated organisms in the gastrointestinal tract and their viability and metabolic activity have shown very beneficial effects on the health of the gastrointestinal tract (Gibson and Roberfroid, 1995) and that always related to the presence of a suitable environment and nutrients, which are very important for the viability and activity, for bifidobacteria to use it in the bowel as carbon and energy source, these compound were referred to as bifidogenic factors (O'Sullivan, 1996). At present, probiotics products and especially probiotics dairy foods are marketed successfully all over the world because of their acceptance of consumer and the awareness about their positive aspect for the health benefits.

2.13. 10 Health benefits of probiotic in human

Bacteria have a reputation for causing disease, but a growing body of scientific evidence suggests that you can treat and even prevent some illnesses with foods and supplements containing certain kinds of live bacteria. For example, Metchnik off related the longevity of Bulgarians to the present of *Lactobacillus bulgaricus* in the souring milk ―yogurt‖ . Moreover, in the Northern Europeans consume a lot of these beneficial microorganisms in the dairy products, because of their tradition of eating foods fermented with bacteria, such as yogurt and cheese, these beneficial bacteria have named later as probiotics. Probiotic microflora displays numerous health benefits beyond providing basic nutritional value. The health benefit reported of probiotics is the improvement in gut health and the prevention of intestinal infections and stimulating the immune system (Kailasapathy and Chin, 2000; Salminen and Gueimonde, 2004). Infection prevention is increasingly preferred over using the traditional action by chemotherapy with antibiotics, that rise the concern over development of antibiotic resistance has placed probiotics at the fore. The principal mechanism for this action is known as competitive colonization

or competitive inhibition (Alander *et al*., 1999; Chen *et al*., 1999; Naidu *et al*., 1999; Leahy *et al*., 2005). This is described as the creation of probiotic bacteria in the human intestine, which acts as a vital barrier to invasion by pathogens in the gastrointestinal tract of the human host. Over 90% of the total cells in the body are present as bacteria in the colon, getting 10^{12} CFU (Colony Format Unite) for every gram of large intestinal contents (Bourlioux *et al*., 2003; Anuradha and Rajeshwari, 2005). Under natural conditions, a protective gut microflora develops and there is no need for a bacterial supplement (Shanta-Retelny, 2005) but the changing food habits and lifestyle force us to take processed food, which affects our access to, and colonization, by probiotics. Moreover, we also consume antibacterial substances ranging from vinegar to antibiotics. To reside in the gut, When ingested, probiotic bacteria are resistant to gastric acidity and bile salts (Rinkinen *et al*, 2003; Minellia *et al*., 2004) and therefore pass through the upper gastrointestinal tract and have the ability to adhere to the intestinal mucosa. Further, the probiotics‘secretion of by products such as lactic acid and acetic acid lower the pH in the intestine and producing hydrogen peroxide inhibiting the growth of pathogens and helped to speed pathogens through the intestines (Laroia and Martin, 1991; Mishra and Lambert, 1996). The enhancement of the immune system is another reported health benefit of probiotics (Saarela *et al*., 2000; Perdigon *et al*., 2003), as it appears that this effect by balancing control of pro-inflammatory and anti-inflammatory cytokines (Pessi *et al*., 2000, Shah, 2001) and therefore probiotics are considered as immune-stimulatory agent and an important tools to prevent intestinal inflammation, stop gut mucosal dysfunction and decrease hypersensitivity (Isolauri, 2001). Recently, there is a relatively large volume of scientific literature basis supporting the use of probiotics for diarrhoea has started to become established. Probiotics have been shown effectiveness in the prevention of several types of diarrhea, including antibiotic-associated diarrhea, bacterial and viral diarrhea (including travelers‘diarrhea), as well as that caused by lactose intolerance (Rolfe, 2000).

It is thought that this action is due to the secretion of antimicrobial polypeptides known as bacteriocins and that by; reduction in gastrointestinal pH through stimulation of lactic acid-producing bacteria; a direct antagonistic action on gastrointestinal pathogens; competition with pathogens for binding and receptor sites; improved immune function; and competition for limited nutrients (Collins and Gibson, 1999). It seems that the effect of probiotics on travellers‘diarrhea depends on the bacterial strain used and the destination of the travelers (De Roos and Katan, 2000). Observational data suggest that consumption of fermented dairy products is associated with a lower prevalence of colon cancer, which is suggested that probiotics are capable of decreasing the risk of cancer (Mital and Garg, 1995). The Mechanisms described by Inhibition of carcinogens and pro-carcinogens, Inhibition of bacteria capable of converting pro-carcinogens to carcinogens (McIntosh, 1996). Moreover, probiotic and especially bifidobacteria has shown to increase the α- and β-galactosidase activities in the faecal samples after feeding with the fermented milk containing probiotics, which is considered to be an important probiotic quality, as it supports lactose digestion in the intestine and compensates for lactase-deficiency (Shah, 2001; De Vrese *et al*., 2001; LourensHattingh and Viljoen, 2001). Playne (2002) suggested that Health benefits imparted by probiotic bacteria are strain-specific and not species- or genus- specific. For example the strains Lactobacillus rhamnosus,*Lactobacillus* paracasei Shirota (Yakult), and *Bifidobacterium Lactis*Bb12, L. *acidophilus* La5 have the strongest human health efficacy data, against some or all of: lactose intolerance; rotavirus diarrhea; antibiotic-associated diarrhea and some other bacterial diarrheas and infections. B. animals found to stimulate the immune response in vivo (Sanders, 2000; Perdigon *et al*., 2003). Moreover, it has improve in control study the useful of *Bifidobacterium lactis* strain BB12 in prevention of acute diarrhea in infants (Chouraqui *et al*., 2004; Larsen *et al*., 2006; Mohan *et al*., 2006) and same by Lactobacillus (Jain *et al*., 2004). Bifidobacterium lactis *Bb12* well known in

their ability to improve the growth of children when supplement in their formula (Nopchinda *et al*., 2002). Candida can also be suppressed or controlled by viable L. acidophilus. It is also thought that non-viable forms exert such control but to a lesser extent (Ouwenhand and Salminen, 1999).

Probiotics, naturally found in the mouth, lower intestine and vagina of healthy individuals, help defend the body against invading pathogenic bacteria. Due to the dominance of common antibiotic treatment (which kills the beneficial organism as well as the harmful bacteria), many people are lacking healthy intestinal flora. The composition of the intestinal flora is relatively stable in healthy human beings between harmful and beneficial or natural bacteria. Among the beneficial bacteria are *Lactobacillus* spp. and *Bifidobacterium* spp. which play a useful role in the production of vitamins, organic acids and antimicrobial factors to inhibit pathogens. Any interactions of this balance in the gut microflora lead to make the harmful bacteria dominate the intestinal flora, which effect produce essential nutrients and increase the level of damaging substances, including carcinogens, putrefactive products and toxins (Mitsuoka, 1996; Salminen and Gueimonde, 2004). However, this balance could affect by the age, diet, travel and stress, which may lead to disease and can occur when toxins are secreted by pathogens in the intestinal mucosal barrier and other bacteria. Therefore, to maintain a well-balanced microflora in the gastrointestinal tract it has suggested introducing live bacteria or stimulating growth of beneficial 18 bacterial population groups which prevent harmful effects and promote beneficial actions of the intestinal microflora (Salminen *et al*., 1996; shah, 2000). Consuming probiotics with dairy foods buffers stomach acid and increases the likelihood that the bacteria will survive into the intestine. Dairy products containing probiotics also provide a number of essential nutrients including calcium and protein (Stanton *et al*., 2003).

2.13.11 Application of probiotic culture into food

Probiotic bacteria are applied in many different products worldwide. In addition to food products, probiotic cultures are also used in pharmaceuticals and animals feed. Most definitions of probiotics are based on live bacteria that confer a health benefit for consumer. The application of probiotics in food products depends on factors like water activity, processing and storage temperature, shelf life, oxygen content, pH and mechanical stress, salt content and content of the other harmful or essential ingredient (Goktepe *et al* ., 2006). Probiotic bacteria have been applied in fermented dairy products for many years, fruits juices have been shown to be suitable carriers for probiotics there is growing interest in applying probiotics to fermented meat products, vegetable based probiotic products, probiotics bacteria are also applied to infant nutrition powder and powdered milk drinks (Goktepe *et al* ., 2006).

2.13.12 Biochemistry of the fermentation process of bifidobacteria in milk

The common result of the bacterial fermentation process is lactate. It is referred to the bacteria, which produce lactate in their fermentation as Lactic Acid Bacteria. Some of these organisms require very complex nutrition, which is related to their environments such as plant, milk and intestinal of animals or humans. Lactic Acid Bacteria are strictly fermentative and some are oxygen tolerant, such as Streptococcus. Others are obligate anaerobes such as Bifidobacteria spp. but some can tolerant oxygen in the presence of carbon dioxide (CO_2) (Shah, 1997). Bifidobacteria naturally inhabitants the gut of animals and humans and their sensitive to oxygen is strain related and the less sensitive strain appears to possess weak catalyses activity that removes $H2O_2$. Hydrogen peroxide inactivates Fructos-6-phosphate phosphoketolase F6PPK (Shah, 1997), a key enzyme of bifidobacteria in carbohydrate metabolism (Fandi *et al*., 2001; Marks, 2004). Bifidobacteria do

not produce CO_2, Butyric or propionic acid (Modler *et al*., 1990). Their optimum growth temperature is 37 Cº to 43 Cº, and the optimum pH is 6.5 to 7.0 (Shah, 1997).

Bifidobacteria also produce thiamine, riboflavin, and vitamin B and K (Deguchi *et al*., 1985; Morishita *et al*., 1999; Hou *et al*., 2000; Sybesmaa *et al*., 2004).These genera are also unique by producing the lactic acid in the form L (+)- lactic acid that easier to metabolize by infants in compare with the D (-)- lactic acid form which produced by *L. acidophilus* and L. *bulgaricus* (Marks, 2004). There are three pathways have been suggested for the fermentation process of LAB of the carbohydrates to lactate. The homofermentative pathway, which yields 2 mol of lactate per mol of glucose:

Homofermentative pathway

Glucose ⟹ **2 lactate**

The heterofermentative pathway, which yields 1 mol of each lactate, ethanol, and CO2 per mol of glucose:

Heterofermentative pathway

Glucose ⟹ **lactate + ethanol + CO2**

The bifidum pathway, which yields acetate and lactate in ratio of 3 to 2 respectively:

Bifidum pathway

Glucose ⟹ **2 lactate + 3 acetate**

Bifidobacteria metabolized carbohydrate as main carbon source is through fructose-6-phosphate shunt by using Fructos-6-phosphate phosphoketolase (F6PPK), which is distinguishing bifidobacteria from lactobacilli. This

pathway produces L (+) lactic acid and acetic acid in ratio 3:2, some species produce formic acid and ethanol as well. Differs in the process of carbohydrates fermentation among homo-, heterofermentative bacteria and Bifidobacteria spp.Bifidobacteria utilize lactose, galactose and fructose beside glucose, and has the ability to metabolize oligosaccharides beside the simple sugars such as Inulin, FOS etc. This ability is strain related, for example it has found that Bifidobacterium *Lactis Bb12* possessed the enzymes required to utilize some kind of sugar such as raffinose family and lactose which are unable to metabolism by other strain of *bifidobacteria* (Martınez-Villaluenga and Gomez, 2007). These enzymes which were found in most strains of bifidobacteria and not found in the lactic acid bacteria (Desjardins *et al*., 1990), *Bifidobacterium Lactis Bb12* were found to possess the highest activity of such like enzymes which found with low activity in other strains, these enzymes such as *β*–glucosidase, *α*– glucosidase, D-glucosaminidase and *β*–galactosidase, which are very important in the fermentation process with bifidobacteria (Semjonovs *et al*., 2004; MartınezVillaluenga and Gomez, 2007). Bifidobacterium Lactis BB12 has the ability to grow faster in milk because of possessing the highest activity of leucine aminopeptidase to help the hydrolysis of milk proteins, which stimulate their growth (Desjardins et al., 1990; Martınez-Villaluenga and Gomez, 2007) .Janer *et al*. (2004a) has found the ability of *Bifidobacterium Lactis* Bb12 to cleave fructooligosaccharides (FOS), which has the β (2-1) linkage related to possess β- fructofuranosidase.

2.14 Cream cheese as aprobiotic product

Dairy products show the most adequate matrix for supplementation with probiotic cultures and prebiotic ingredients due to their positive status in the mind of the consumers (Granato *et al*., 2010). Cheese shows to be a high-quality substrate for the development of new probiotic foods, mainly some types of fresh cheeses, which, due to their technological characteristics, have

a series of advantages over other products (Roupas and Williams 2007; Ribeiro *et al*, 2009), such as: pH values that do not inhibitprobiotic multiplication and relatively high water activity and fat content (Stanton *et al*., 1998). Different types of cheeses have been successfully tested as mediums for probiotic strains of *Lactobacillus* and *Bifidobacterium*:Cheddar (Sharp *et al*.,2008), Cottage (Blanchette *et al* .,1996), Argentinian fresh cheese(Vinderola *et al* .,2009), Turkish Beyaz cheese (Kilic *et al* .,2009) and goat cheese (Gomes and Malcata 1998).

Cheeses show some technological advantages with respect to their supplementation with probiotic cultures and prebiotic ingredients.

Cream cheese is an un-ripened cheese has soft, rich, diacetyl flavor with creamy white color and a smooth consistency.It is highly nutritious being rich in fat and milk proteins (casein and whey) Proteins form network which includes fat droplets (Coutouly *et al.*, 2014). Traditionally, cream cheese made from cream or from a mixture of cream and milk or skim milk .According to United States, the food and drug Administration regulations cream cheese must have at least 33% fat but the moisture contents should not be more than 55% (Phadungath,2005).

2.14.1 Kinds of cream cheese

There are two main kinds of cream cheese based on fat contents in the initial and final composition. One is single cream cheese and other is double cream cheese. Double cream cheese having at least 9-11% fat content in the initial mix, while single-cream cheese having 4.5-5% fat content in the initial mix (Guinee *et al*.,1993). One type of cream cheese is triple cream cheese that has 75% fat in dry matter contents (Sanchez et al., 1996).

2.14.2. Defect of cream cheese

Defects in cream cheese can occur depending on the final pH of the cheese. The texture of the cheese will be soft, and the cheese will lack flavor, if the pH of the cheese is too high (>4.7).If the pH of the cheese is too low (< 4.6), the texture may be too grainy, and the flavor will be too acidic. In addition, cream cheese defects include whey separation from the product during storage and a grainy, sandy, or chalky texture, especially in the lower-fat types (Lucey, 2003).

CHAPTER THREE

MATERIALS AND METHOS

3.1. Materials

Baobab fruits were obtained from three different locations in Sudan: Northern Kordofan State (ElObeid, Umm Ruwaba) Southern Darfur State (Nyala), and Blue Nile State (Damazin). Fresh cow milk was obtained from Animal Production Department dairy farm, Sudan University of Science and Technology (Shambat).Strain culture (*Bifidobacterium* strains) was obtained from the stock culture of microbiology laboratory (Department of Food Science and Technology, College of Agricultural, Studies, Sudan University of Science and Technology).

The Folin- Ciocalteu's phenol reagent, Ascorbic acid, 2,2-diphenly-1-picrylhydrazyl (DPPH),,$FeC1_36H_2O$, KH_2PO_4 **,**Griess reagent, curcumin, Lipopoly saccharide (LPS), phosphate buffered saline (PBS), 2,4,6-tris(pyridin-2-y1)-1,3,5-triazine (TPTZ)recombinant murine IFN-γ, dimethylsulfoxide (DMSO), phosphate buffer, α-glucosidase enzyme, p-nitrophenyl-α-Dglucopyranose(PNPG), glycine, and quercetin were purchased from Sigma-Aldrich (Hamburg, Germany). Cell culture media, Dulbecco's Modified Eagle's Medium (DMEM) containing HEPES and L-glutamine for both, with and without phenol red, Penicillin–Streptomycin antibiotic solution, fetal bovine serum (FBS), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide(MTT) and triple Express enzyme were purchased from Gibco/BRL Life Technologies Inc. (Eggenstein, Germany).

For NMR analysis, deuterated methanol-d4 (CH_3OH-d_4) and trimethylsilyl propionic (TSP) acid-d4 sodium salt were supplied by Merck (Darmstadt, Germany). For LCMS analysis, Optima^{TM} LCMS grade solvents including: water, acetonitrile (ACN), methanol (MeOH), formic acid, and ammonium

formate were purchased from Fisher Scientific (Geel, Belgium). . All other reagents used were purchased from local company in Khartoum

3.1.1 Preparation of baobab fruit pulp

Baobab fruit pulp was obtained by breaking the capsules manually, then the seeds were removed and pulp powder was saved using appropriate mesh. The resulting fruit pulp was stored in a dark polyethylene bag in the freezer at (-15ºC) until used.

3.2 Methods

3.2.1 Physical characteristics of baobab fruit from different locations in Sudan

Fruits physical characteristics including fruit length, width and thickness of the wall, were measured using a Vernier Caliper. The weight of fruit pulp, seeds, red fiber and epicarp were determined using a sensitive balance. The number of seeds in baobab fruit was counted manually.

3.2.2 Physico-chemical methods

3.2.2.1 Total soluble solids

The total soluble solids as a percent (TSS %) of the different samples were measured as described by Ranganna (2001**)**.

Principle: The index of refraction of a substance is a ratio of light velocity under vacuum to its velocity in the substance which is largely dependent on the composition, concentration and temperature of the sample solution.

Procedure: After the adjustment of the Hand-Refractometer (No.002603, BS-eclipse, UK) with distilled water, the sample was placed on the surface of the Refractometer prism, the prism was closed and the reading was recorded to the nearest 0.01 as TSS %.

3.2.2.2 Hydrogen ion concentration

The pH of the different samples was determined as described by Ranganna (2001).

Principle: The pH value of the different samples was measured with pH-meter. After standardization of the pH-meter electrodes with a buffer solution, the reading of the sample is recorded as pH value.

Procedure: After standardization of the pH-meter (model HI 8521 microprocessor bench PH/MV/C˚ meter. Romania) with buffer solutions (pH 4.01 and 7.0), the electrode of the pH-meter was rinsed with distilled water, immersed in the sample and left to stand until a staple reading was achieved.

3.2.1.3 Titerable acidity

Ten grams of the pulp sample was diluted with distilled water (150ml) stirred for 15 minutes, then filtered. Ten ml of the pulp preparation was titrated against 0.1N NaOH using phenolphthalein as indicator. Total acidity (mg/100g) expressed as citric acid according to Ranganna(1979).

$$\text{Acidity (mg/100g)} = \frac{\text{Titre} * \text{N(NaOH)} * \text{dilution factor} * 100 * \text{eq.wt}}{\text{weight of sample} * 1000 * \text{vol.titre}}$$

Where:

eq.wt = equivalent weight of citric acid

3.2.2Proximate analyses

3.2.2.1 Moisture content

The moisture content was determined according to the standard methods of the Association of Official Analytical Chemists **(**AOAC, 2003**).**

Principal: The moisture content in, a weighed sample is removed by

heating the sample in an oven (under atmospheric pressure) at 105 °C. Then, the difference in weight before and after drying is calculated as a percentage of the initial weight.

Procedure: A sample of 2 g ±1 mg was weighed into a dish. Then, the sample was placed into an oven (No.03-822, FN 400, Turkey) at 105 ± 1 °C until a constant weight was obtained. After drying, the covered sample was transferred to desiccators and cooled to room temperature before reweighing. Triplicate results were obtained for each sample and the mean value was reported to two decimal points according to the following formula:

Calculation:

$$\text{Moisture content (\%)} = \frac{(Ws - Wd)}{\text{Sample weight (g)}} \times 100\%$$

Where:

Ws = weight of sample before drying.

Wd = weight of sample after drying.

3.2.2.2 Ash content

The ash content was determined according to the method described by theAOAC (2003).

Principle: The inorganic materials which are varying in concentration and composition are customary determined as a residue after being ignited at a specified heat degree.

Procedure: A sample of 5g ±1 mg was weighed into a pre-heated, cooled, weighed and tarred porcelain crucible and placed into a Muffle furnace (No.20. 301870, Carbolite, England) at 550°C until a white, gray ash was obtained. The crucible was transferred todesiccators, allowed to cool at room temperature and weighed. After that, the ash content was calculated

as a percentage based on the initial weight of the sample.

Calculation:

Ash (%) = [(Wt of crucible +Ash) - (Wt of empty crucible)] x 100 %

Initial weight (Wt)

3.2.2.3 Oil content

Oil content was determined according to the official method of the **AOAC (**2003).

Principle: The method determines the substances which-are soluble in petroleum ether (65-70 °C) and extractable under the specific conditions of Soxhlet extraction method. Then, the dried ether extract (oil content) is weighed and reported as a percentage based on the initial weight of the sample.

Procedure: A sample of 5g ± l mg was weighed into an extraction thimble and covered with cotton that previously extracted with hexane (No.9-16-24/25-29-51, LOBA Cheme, India). Then, the sample and a pre-dried and weighed extraction flask containing about 100 ml hexanes were attached to the extraction unit(Electrothermal, England) and the extraction process was conducted for 6 hour. At the end of the extraction period, the flask was disconnected from the unit and the solvent was re distilled. Later, the flask with the remaining crude hexane extract was put in an oven at 70 °C for 3 hrs, cooled to room temperature in a desiccators, reweighed and the dried extract was registered as oil content according to the following formula;

Calculation:

Oil content (%) = $\frac{(W2 - W1)}{W3} \times 100\ \%$

Where:

W_2 =Weight of the flask and hexane extract

W_1 =Weight of the empty flask

W_3=initial weight of the sample

3.2.2.4 Crude protein content

The protein content was determined in all samples by micro-Kjeldhal method using a copper sulfate-sodium sulfate catalyst according to the official method of the AOAC (2003).

Principle: The method consists of sample oxidation and conversion of its nitrogen to ammonia, which reacts with the excess amount of sulphuric acid forming ammonium sulphate. After that, the solution was made alkaline and the ammonia was distilled into a standard solution of boric acid (2%) to form the ammonia-boric acid complex. Which is titrated against a standard solution of HC1 (0.1N) .The protein content is calculated by multiplying the total N % by 6.25 as a conversion factor for protein.

Procedure: A sample of two grams (2 gm) was accurately weighed and transferred together with, 4g Na_2SO4 of Kjeldhal catalysts (No. 0665, Scharlauchemie, Spain) and 25 m1 of concentrated sulfuric acid (No.0548111, HDWIC, India) into a Kjeldhal digestion flask. After that, the flask was placed into a Kjeldhal digestion unit (No.4071477, type KI 26, Gerhardt, Germany) for about 2 hours until a colourless digest was obtained and the flask was left to cool at room temperature (25˚C).

The distillation of ammonia was carried out into 25m1 boric acid (2%) by using 20 ml sodium hydroxide solution (45%).

Finally, the distillate was titrated with standard solution of HC1 (0.1N) in the presence of 2-3 drops of bromocreasol green and methyl red as an indicator until a brown reddish colour was observed.

Calculation:

$$\text{Crude Protein (\%)} = \frac{(\text{ml Hcl sample} - \text{ml Hcl blank}) \times N \times 14.00 \times F}{\text{Sample weight (g)} \times 1000} \times 100\%$$

Where:

N: normality of HCl.

F: protein conversion factor = 6.25

3.2.2.5 Crude fiber content

The crude fiber was determined according to the official method of the AOAC (2003).

Principle: The crude fiber is determined gravimetrically after the sample is being chemically digested in chemical solutions. The weight of the residue after ignition is then corrected for ash content and is considered as a crude fiber.

Procedure: About 2g ± 1 mg of a defatted sample was placed into a conical flask containing 200 m1 of H_2SO_4 (0.26 N). The flask was then, fitted to a condenser and allowed to boil for 30 minutes. At the end of the digestion period, the flask was removed and the digest was filtered (under vacuum) through a porclain filter crucible (No.3). After that, the precipitate was repeatedly rinsed with distilled boiled water followed by boiling in 200 ml NaOH (0.23 N) solution for 30 minutes under reflux condenser and the precipitate was filtered, rinsed with hot distilled water, 20m1 ethyl alcohol (96%) and 20 ml diethyl ether.

Finally, the crucible was dried at 105 °C (overnight) to a constant weight, cooled , weighed, a shed in a Muffle furnace (No.20. 301870, Carbolite, England) at 550-600 °C until a constant weight was obtained and the difference in weight was considered as crude fiber.

Calculation:

$$\text{Crude fiber (\%)} = \frac{(W1 - W2) \times 100\%}{\text{Sample weight (g)}}$$

Where: W1 = weight of sample before ignition (g).

W2 = weight of sample after ignition (g).

3.2.2.6 Minerals content

Potassium (K), (Na) and calcium (Ca) were determined by flame photometer (Sherwood Flame Photometer i410, Sherwood Scientific Ltd. Cambridge, UK) according to procedure of AOAC (1990). The knob of flame photometer was adjusted to potassium, sodium and calcium respectively and reading was set to zero using deionized water. Blank solution was run and reading was again set to zero. Standard solution of each mineral was run and recorded the reading of flame photometer. The reading of potassium, sodium and calcium in each sample was taken by running the sample one by one. Standard solution was run after every sample .The standard curves (appendix I, II, III) were obtained by plotting absorbance values of standards against appropriate concentrations of these three elements. One gram of dried beverage samples was subjected to wet digestion method as described by Richards (1968). Then analysis was conducted through absorption spectrophotometer (Varian AA 240, Victoria, Australia) for determination of minerals (Mg and Fe) using standard curve. To determine phosphorus content in beverage samples, colorimetric estimation method was used as described by Kitson and Mellon (1944).

3.2.2.7 Total and reducing sugars

They were determined according to Lane and Eynon methods (AOAC, 1984). Twenty-five grammes of the pulps were filtered through a Whatman filter paper (No. 4) .then transferred to a 250 ml volumetric flask. 100 ml of distilled water was carefully added and then neutralized with 1.0 N NaOH to a pH 7.5-8.0. About 2ml of lead acetate were added and the flask was then shake, and left to stand for 10 minutes. Then 2 grams of sodium oxalate were added to remove the excess lead. Distilled water was again added to make the volume to mark (250ml). The solution then filtered and 50 ml of the filtrated were pipatted into a 250ml volumetric flask. 50g citric acid and 50 ml distilled water were added slowly to the new mixture. The contents of the

flask were boiled gently for 10 minutes to invert the sucrose, and when cooled a few drops of phenolphthalein were added. In order to neutralize the mixture, a 20% NaOH solution was continuously added until the colour of the mixture disappeared, and the volume was made to mark before titration.

Standard method of Titration: Ten ml of a mixed solution of Fehling (A) and (B) were pipatted into a conical flask. A burette was filled with a clarified sugar solution and the whole volume required reducing the Fehling's solution was run so that 0.5-1.0 ml was still required to complete the titration performed. The contents of the flask were mixed and heated to boiling for 2 minutes. Three drops of methylene blue indicator were added. Then the titration was completed until the colour has completely disappeared.

Mg total sugar in 100 ml = $\frac{\text{Factor} \times 100}{\text{Titre}}$

a- Total sugar %=

$$\frac{\text{Mg/100g} \times \text{ dilution } \times 100}{1000 \times \text{wt. taken.}}$$

The filtrate can be used directly for titration was according to Lane and Eynon (1984) using the following equation for calculation:

b- Reducing Sugars:

Reducing sugar % = $\frac{\text{Mg/100g} \times \text{dilution} \times 100}{1000 \times \text{wt.taken}}$

3.2.2.7.1 Non reducing sugars

Non reducing sugars = Total sugars – reducing sugars.

3.2.2.7.2 Determination of some sugars

The sugars profile of baobab fruit pulp was determined using HPLC as described by Legua *et al*. (2012), with some modification. HPLC used for analysis consisted of an autosampler (SIL –Method of sugar analysis by HPLC) 10 ADvp, SHIMADZU, KYOTO, JAPAN), binary pump system (LC-10ADvp solvent delivery module, SHIMADZU,KYOYO, JAPAN),colum oven(CTO-10 ADvp, SHIMADZU, KYOTO, JAPAN), 20micro liter of sample were injected onto normal –phase column (shim-pack clc-NH2(4.6mm i.d *15 cm,5µl practical size SHIMADZU, KYOTO, JAPAN) equipped with a guard column of the same material.

Preparation of sample solution: 5 gm of sample was transferred into 50 ml Volumetric flask by 20ml of water and followed by 12.5 ml ethanol; the volume was completed by water to the mark .Then the final solution was filtered by filter paper (Whatman International Limited, Kent, England) and followed by syringe filter (0.2 µm (Germany)into a sample vial and 20µl was injected into the column .Sugar was eluted andelution was carried out with isocratic mobile phase consisting of 80:20 acetonitrile /water at 30 ˚C and a flow rate of 1ml/min, the retention times of fructose, glucose, sucrose and maltose sugar are monitored using the refractive index detector (RID-10A.SHIMADZU.Kyto,JAPAN); the retention time obtained was compared to that determined using standards of these sugars .

The concentration of these sugars in the sample was determined by the following:-

Preparation of a mixture of these standards: - by dissolving 0.3051, 0.2561, 0.1523 and 0.1gm from each standard in a 25ml volumetric flask with water, 20µl from the standard solution were injected and the retention time peak area of each standard was recorded to compute the mass of the sugar in unknown sample

3.2.2.8 Calculation of carbohydrates

Carbohydrates were calculated by difference according to the following:

Total carbohydrates = 100% - [Moisture (%) + Protein (%) +Fat (%) + fiber (%) and Ash (%)]

3.2.2.9 Ascorbic acid

Determination of ascorbic acid was done using 2-6- Dichlorophenol Indophenol reagent according to AOAC (1990). This reagent is reduced by ascorbic acid to become colorless. It is prepared as follows: 0.2 g of 2-6-Dichlorophenol Indophenol dye was dissolved in 200 ml distilled water, and then filtered through Whatman filter paper (No. 2) into 500 ml Volumetric Flask and made up to volume with distilled water. The dye was standardized as follows: 50 g of standard ascorbic acid was weighed and made up to volume by distilled water in 250mL volumetric flask and 5ml aliquot were diluted with 5ml oxalic acid 10 % and titrated with the dye solution to a pink end point. One kg of ascorbic acid is equivalent to one ml of the dye used. Thus: strength of the dye =1/titre.

Procedure

Thirty grams of the baobab fruit pulp were blended with about 100 ml of 0.4% oxalic acid for two minutes in a blender. The blended mixture was made up to 500ml in a volumetric flask with 0.4% oxalic acid and filtered with Whatman filter paper No. 4. The ascorbic acid in the filtrate was titrated against standard 2-6 Dichlorophenol Indophenol and was calculated as follows:

$$\text{Ascorbic acid (mg/100g)} = \frac{\text{Titre} \times \text{dye strength} \times 100}{\text{Factor}}$$

$$\text{Factor} = \frac{\text{Sample wt.} \times \text{Sample volume for titration}}{\text{Total volumeof sample}}$$

3.2.2.10 Pectin extraction

3.2.2.10.1 Water based extraction of pectin

Pectin was extracted by the method of Malviya *et al*.(2010).The conventional water based extraction involves extracting the pectin using acidified water (pH 2) at temperature 70˚C for 4h.The hot acid extract was pressed in cheese clothand the cake was cooled to 4 ˚C. Pectin was precipitated by alcohol (ethanol) and water of ratio of 2:1 (v/v) followed by continuous stirring for 15 min .The mixture was further allowed to stand for twoh for better pectin precipitation. The allowed filtering the pectin substances which remained floating at the surface of alcohol-water mixture. Suspended pectin coagulate was filtered through cheesecloth, cleaned with alcohol (95%) and pressed. Pressed pectin was further dried to a constant weight at 35–45ºC in a hot air oven. The flowing equation calculated pectin yield:

$$\text{Pectin yield\%} = \frac{\text{weight of extracted pectin} \quad X100}{\text{Weight of dried sample}}$$

3.2.2.11Preparation of fruit pulp extracts

Baobab fruit pulp aqueous, methanolic and ethanolic extracts were prepared by the method of Kim and Lee (2002) with slight modification. Twenty gram of the sample from each was extracted separately with 80 ml of three different solvents: methanol/water (4:1) v/v, ethanol/water (4:1) v/v and water at room temperature for five hour using an orbital shaker (Germany, serial No.071006060) at 200 rpm and temperature at 30 °C. Then, the homogenate was centrifuged for 10 min at 3600 rpm (Eppendorf Centrifuge 5804, Hamburg, Germany) and the supernatant was removed. The residue was extracted once again at the same conditions. Then, both supernatants were filtered through Whatman No.4 filter paper (Whatman International Limited,

Kent, England) using a chilled Buchner funnel .The filtrate was transferred into evaporating flask with an additional 50ml 80% (aqueous ethanol) and were concentrated in a rotary evaporator (BüchíRotavapor, Switzerland) at 45°C. The resulting concentrates were then mixed with 15 ml of deionised (DI) water (NANO pure water system, Barnstead, Dubuque, Iowa, USA). The concentrates were then freeze-dried (freeze-drier Serial No. K12173-5 LTE SCINTIFIC LTD Great Britain.)to obtain crude extracts powder and kept in dark glass bottles at -18°C until used for analysis.

The residue of samples extract was calculated using the following equation.

$$\text{Yields extract \%:} \frac{\text{w2(weight of residue extract}}{\text{w1(weight of sample taken}}) * 100$$

3.2.2.12. Determination of the total phenolics content

Total phenolics content (TPC) was determined spectrophotometrically using Folin–Ciocalteu reagent according to the method of Tuberoso *et al*. (2010) with some modifications. One hundred µl of the sample (5mg/ml) were diluted with different solvents and added to 0.5 ml of 10% Folin–Ciocalteau's phenol reagent. After 5 min, 3 ml of 10% Na_2CO_3 (w/v) were added, the mixture was shaken, and then diluted with water to a final volume of 10 ml. After a 90 min incubation period at room temperature, the absorbance was read at 725 nm on a 10 mm quartz cuvette using a spectrophotometer (UV - 1800, serial No.A11454805048CD, SHIMADZU, Japan), against a blank. The total polyphenol content results, of the samples were expressed as the Gallic acid as mg/g of Gallic acid equivalent (GAE), using a calibration curve of a freshly prepared Gallic acid standard solution (10-100 mg/ml). All the samples were analyzed in four replicates and the mean values were calculated.

3.2.2.11 Total flavonoids

Total flavonoids content was determined spectrophotometerically using aluminum chloride by the method of Kim *et al*., (2003) .Four ml of distilled water were added to 1 ml of the fruit pulp extracts (5mg/ml). Then, 5%

sodium nitrite solution (0.3 ml) was added, followed by addition of 10% aluminum chloride solution (0.3 ml). Test tubes were incubated at room temperature (25-30 °C) for 5 min, and 2 ml of 1M sodium hydroxide was added to the mixture and the volume of reaction mixture was completed to 10 ml with distilled water. The mixture was thoroughly vortexed and the absorbance of the pink color developed was read at 510 nm. A calibration curve was prepared by Catechin (10-100 mg/ml) and the results were expressed as mg Catechin equivalents (CEQ) /g. All the samples were analyzed in four replicates and the mean values were calculated.

3.2.2.12Antioxidant activity assays

Antioxidant activity of the extracts was evaluated using DPPH (1, 1-dipheny1-2-picrylhydrazyl radical) and FRAP (ferric reducing antioxidant power) according to the method described by Tuberoso *et al*. (2010) with some modifications**.**

3.2.2.12.1DPPH assay

Principle

The scavenging reaction between (DPPH) and an antioxidant (H-A) can be written as:

(DPPH) + (H-A) ⟶ **DPPH-H+ (A)**

(Purple) (Yellow)

Antioxidants react with DPPH, which is a stable free radical and is reduced to the DPPHH and as consequence the absorbance's decreased from the DPPH radical to the DPPH-H form. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability (Oktay *et al*., 2003).

Procedure:

DPPH test was determined spectrophotometrically. This assay depends on

the limit of the capacity of the antioxidant to scavenge the radical cation 1, 1-dipheny1-2-picrylhydrazyl radical (DPPH). Fifty microlitres of diluted sample (5mg /ml, with solvent) were dissolved in 2 mL of 0.04 mmol/L DPPH in methanol. A calibration curve in the range 0.05-1.0 mmol/L was used for the Ascorbic acid, and data were expressed as Ascorbic acid equivalent antioxidant capacity (ASAC, mmol/g). Spectrophotometric readings were carried out at 517 nm, using plastic cuvette (10 mm) after an incubation period of 60 min in the dark.Samples were analyzed in four replicates and the mean values were calculated.

3.2.12.2 FRAP assay

Principle: Reducing power assay is based on the potential of phenolic compounds to donate their electrons to an oxidized substrate. The ferric state will be reduced to a ferrous state. The disadvantage of this assay is that other reducing agents in the extract such as ascorbic acid can also reduce the ferric state iron and hence the reducing power is cumulative activity of all reducing agents in the extract (Becker *et al*., 2004).

Procedure:

Ferric reducing antioxidant power evaluates antioxidants as reductants of Fe3+to Fe2+, which is chelated by 2,4,6-tris(pyridin-2-y1)-1,3,5-triazine (TPTZ) to form a Fe2+– TPTZ complex absorbing at 593 nm . The FRAP test was done by preparing a ferric complex TPTZ and Fe3+ (0.3123 g TPTZ, 0.5406 g FeC136H2O in 100 ml acetate buffer pH 3.6). Fifty microlitres of diluted sample (5mg/ml, with solvent), was dissolved in 2 ml of ferric compound and, followed by an incubation period of 4 min in the dark, absorbance at 593 nm was measured with a spectrophotometer. Quantitative analysis was performed according to the external standard method ($FeSO_4$, 0.1-2.00 mmol/L).Correlation of the absorbance with the concentration was expressed as mmol/g of Fe^{2+} Samples were analyzed in four replicates and

the mean values were calculated.

3.2.2.13 Identification of phenolic compounds by LC-MS/MS

The samples were analyzed according to the method described by Nowacka *et al*. (2014) with some modifications. Electro-spray ionization mass spectrometry (LC-EPI-MS/MS) was used to identify phenolic acids and flavonoids profile. UHPLC software Application Flexar FX-15 Pump, equipped with a binary gradient solvent pump, a degasser, and an auto sampler, column oven connected to 3200 QTRAP Mass spectrometer (AB Sciex, USA) was used for this purpose. Chromatographic separations were carried out at 25ºC, on column Phenomenex Synergi Fusion 100mm X 2.1mm, 5uM ,with a mobile phase consisting of water containing 5mM ammonium and 0.1% formic acid (solvent A) and acetonitrile containing 0.1% formic acid and 5mM ammonium (solvent B), the flow rate was 0.2 mL/min and 250 µL of samples were injected. The QTRAP-MS system was equipped with Enhanced Product Ion (EPI) operated in the negative-ion mode. EPI worked at the following setting: capillary temperature 500 °C, curtain gas at 10 psi, and negative ionization mode for a mass range from m/z 100 to 1500. The data was acquired and processed using Analyst Software Version 1.5.2. Injections were made for each sample. The analytes were identified by comparing retention time and m/z values obtained by MS and MS2 with the mass spectra identified according to the corresponding spectral characteristics: mass spectra, accurate mass, characteristic fragmentation and characteristic retention time, the Internet database of accurate mass spectrometry data (www.chemspider.com), mass spectra, mass bank and database: *http*://www.phenol-explorer.eu
were used for their identification.

3.2.2.14 Ultra performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) analysis

The sample for analysis was prepared by dissolving a 2 mg of methanolic extract in 1 ml of MeOH (LCMS grade) and then filtered it with 0.22 µm membrane filter. The UHPLC-MS/MS analysis was carried out using a Thermo Scientific Q Exactive^{TM}Focus Quadrupole-Orbitrap mass spectrometer with heated electrospray ionization(HESI-II) source coupled to a Surveyor UHPLC binary pump and auto-sampler (Thermo Fisher Scientific, Bremen, Germany). Liquid chromatography was performed using a C18 column (ACQUITY UPLC HSS-T3, 1.8 µm, 2.1 × 100 mm, Waters, Milford, MA, USA). A gradient mobile phase consisting of water (containing 0.1% formic acid and 10nm of ammonium format; solvent A) and acetonitrile(containing 0.1% formic acid; solvent B)was used for the separation of the analyte. The gradient program started with 5 to 100% solvent B from 0 to 20 min. The injection volume was set to 5 µL and flow rate was 0.25 mL/min.The MS parameters were adjusted as follows, spray volt-pressure -3.5kV, capillary temperature 320°C, sheath and auxiliary nitrogen gas flow 50 and 10 arbitrary units. The mass resolution was set at 70000 FWHM with collision-induced dissociation (CID) energy was adjusted to 30%. The UHPLC-MS/MS analysis was performed in negative ion mode and the total ion chromatograms (TIC) were recorded for m/z 100–1500 m/z. The spectrum preprocessing was using Thermo Xcalibur 2.2 (Thermo Fisher Scientific, Bremen, Germany) and the identification of the compounds was supported by its MS/MS data and comparison with online database (http://metabolomicsworkbench.org).

3.2.2.15 NMR measurement

About 30 mg of the methanolic extract was dissolved in 700 µl of CH_3OH-d_4 containing 0.1% TSP. The sample solution was then subjected to sonication for 30 min and centrifuged at 13000 rpm for 10 min to separate the

supernatant from the residue. Following this, 600µl of the supernatant was transferred to an NMR tube for ^{1}H NMR analysis. The ^{1}H NMR measurement was carried out as previously described (Kim *et al.*, 2010). The ^{1}H-NMR data of the extract obtained from a 500 MHz Varian INOVA NMR spectrometer (Varian Inc., California, USA), functioning at a frequency of 499.887 MHz at room temperature (25 °C). The acquisition time for ^{1}H NMR spectrum was 3.54 min, and 64 scans were performed. A PRESAT sequence was once used to suppress residual water signal with low energy selective irradiation. The acquired NMR spectrum was manually phased, baseline corrected and referenced to TSP as an internal standard using MestReNova software version 6.0.2 (Mestrelab Research S.L., A Coruña, Spain).

3.2.2.16 *In vitro* α-glucosidase inhibitor activity assay

The α-glucosidase inhibition activity was carried out according to the protocols described by Pramai *et al*.(2018).The p-nitrophenyl-*α*-D-glucopyranose (PNPG) which acted as a substrate was dissolved in 50mM phosphate buffer (pH 6.5). The prepared extracts were pipette into the wells of 96-well micro plate and incubated at room temperature for 5 min. Following this, 75 µL of the PNPG was loaded to every well containing the sample, blank substrate, negative control, and positive control, where by the rest of the wells 75 µL KH_2PO_4 buffer (30mM) were added. The incubation time of 15 min was then commenced. The reactions for the sample, blank, substrate, and negative control were inhibited by the addition of 50 µL 2M glycine (pH 10). In the meantime, 50 µL of deionized water was pipetted into the remaining wells of 96 well microplates. The absorbance was read at 405 nm using microplate reader (SPECTRAmax PLUS; Molecular devices, LLC, Sunnyvale, CA, USA). Quercetin acted as positive control. The α-glucosidase inhibition activity of the baobab extract was calculated as percentage inhibition using the following formula = $[(a_n - a_s) / a_n] \times 100$, whereby $a_n$refers to the absorbance difference between the negative control and the blank

substrate whereas $a_s$was the variation between the absorbance of the sample and the blank sample.

IC50 value denotes the concentration of sample required to inhibit 50% α-glucosidase activity.

3.2.2.17 *In vitro* Nitric oxide inhibitory activity

The NO inhibitory activity test and cell viability was assessed using the protocols reported by Abas *et al*. (2006) with some modifications. The RAW264.7 cells, the murine macrophage cell line were acquired from American Type Culture Collection (ATCC). Briefly, the RAW 264.7 cells were seeded into the wells of 96 well tissue culture plates and were stimulated with the inducer consisting of 200 U/ml of recombine antmurine IFN-γ and 10µg/ml lipopolysaccharide. A volume of 50 µL of the prepared extracts were pipetted into every well of cell plates with a final concentration of dimethylsulfoxide (DMSO) at 0.4% per well. The cells were incubated for 17 h at 37 ˚C, 5% CO_2 in humidified incubator. The amount of nitrite (NO_2^-) was determined with the freshly prepared Griess reagent (1% sulfanilamide, 0.1% N-(1-naphthyl)-ethylene diamine dihydrochloride, 2.5% H_3PO_4) at room temperature. Curcumin served as the positive control. The absorbance was read at 550 nm using Spectramax Plus (Molecular Devices) UV/Vismicroplate reader.

3.3 Production of probiotic baobab fruit pulp cream cheese

3.3.1. Preparation of fermentation inoculums

B.longumBB536 was obtained from the stock culture of microbiology laboratory (Department of Food Science and Technology, College of Agricultural Studies, SUST. The strain was maintained at -20 ˚C in 20% glycerol solution. Stock culture was prepared by activation of the strain in skim milk, incubate an aerobically at 37 ˚C for 24h. The obtained culture was reactivated again under the same conditions to prepare enough stock for the

experiment. The working culture was prepared by twice successive transformation in 10% sterilized skim milk (121˚C for 15 min) and incubation at 37 ˚C for 24h.

3.3.2 Production of baobab cream cheese

Fresh raw cow's milk was heated to 65ºC for 30 minutes and then cooled to 37 ºC, following this 2% of starter culture *BB536* was added to the milk and incubated at 37 ºC for 20 min divided in to three parts and treatments with (10%:A, 15%:B and 20%:C) of baobab fruit pulp powder. Next, the curd was homogenized with 25% oil Sun flower (Shams –Savola company ,Khartoum Sudan) ,0.50% gum Arabic and 1.0% sodium chloride, using mixer the resulting cream cheese were filled in to plastic containers and stored under refrigeration (4˚C) for up to 30 days. Subsequent physicochemical and microbiological analyses at storage were performed at 0, 15, 30 and 30 days.

3.3.4. Chemical analysis of milk and baobab cream cheese

3.3.4.1. Protein content

The crude protein was determined by the micro-Kjeldahl method according to AOAC (2003) as follows:

Digestion

Two gram of sample was weighed and placed in small digestion flask (50ml), about 0.4 gram catalyst mixture (96% anhydrous sodium sulphate and3.5% copper sulphate) was added and 3.5ml of approximately 98%of $H_2SO_4$was added. The contents of the flask were then heated on an electrical heater for 2hours till the colour changed to blue-green. The tubes were then removedfrom digester and allowed to cool.

Distillation

The digested sample was transferred to the distillation unit and 20ml of 40%sodium hydroxide were added. The result as ammonia was received in 100ml of 2%boric acid plus 3-4 drops of methyl red indicator. The distillation

was continued until the volume reached 50ml.

Titration

The content of the flask were titrated against 0.02 N HCL. The titrationreading was recorded. The crude protein was calculated using the following:

equation (calculated on dry matter basis):

$$\mathbf{N\%} = \frac{\boldsymbol{mlHCL \times Normality of HCL}(\mathbf{0.1}) \times \mathbf{0.014} \times \mathbf{100}}{\mathbf{Sample weight}}$$

Protein (%) = (N %) × 6.25

Where N = Nitrogen content.

0.014=molecular weight of nitrogen/1000

3.3.4.2. Determination of fat content

The fat content was determined by Gerber method according to AOAC (2003) as follows:

In a clean dry Gerber tube, 10 ml of sulfuric acid (density 1.815 gm/ml at 20ºC) were poured, and then 10.94 ml of milk sample and three grams of cream cheesesamples were added. Amy1 alcohol 1.0 ml (density 0.815) was added to themixture followed by addition of water to raise the level of fat in the column.The contents were thoroughly mixed till no white particles could be seen. TheGerber tubes were centrifuged at 1100 revolutions per minute (rpm) for 4-5minutes and the tubes were then transferred to a water bath at 65 ˚C for threeminutes. The fat percentage was then read out directly from the fat column.

3.3.4.3. Ash content

The ash content was determined according to the method described in AOAC (2003). Ten millimeters of milk samples and two g of cream cheese samples were weighed in a suitable crucible and evaporated to dryness on a steam bath. Thesample was placed in a muffle furnace (550ºC) for 1.5 hours, then cooled indesiccators and weighed. The ash content was calculated using the

following equation:

$$\text{Ash (\%)} = \frac{w1}{w2} * 100$$

Where:

W1= weight of ash

W2 = weight of sample

3.3.4.4 Determination of crude fiber

Fiber was determined according to official method of AOAC(2003) .About 2g of a sample was placed into a conical flask containing 200ml of H_2SO_4(0.26N). The flask was fitted to a condenser and allowed to boil for 30 minutes. At the end of the digestion period, the flask was removed and the digest was filtered through a proclaim filter crucible (No.3). After that, the precipitate was repeatedly rinsed with distilled boiled water followed by boiling in 200ml NaOH (0.23N) solution for 30 min under reflux condenser and the precipitate was filtered. Rinsed with hot distilled water, 20 ml ethyl alcohol (96%) and 20ml diethyl ether.Finally, the crucible was dried at 105 ˚C until a constant weight was obtained and the difference in weight was considered a crude fiber.

Crude fiber % =

$$\frac{[(\text{Dry residue + crucible(g)- (ignited residue +crucible (g))}]}{\text{Sample weight}} \times 100$$

3.3.4.5 Calculation of carbohydrates

Carbohydrates were calculated by difference according to the following:

Total carbohydrates = 100% - [Moisture (%) + Protein (%) +Fat (%) + fiber (%) and Ash (%)].

3.3.4.6 Determination of pH value

The pH value of the cream cheese and milk were determined using a pH-meter (model HI 8521 microprocessor bench PH/MV/C meter. Romania). Two standard buffer solution of pH 4.00 and 7.00 were used for calibration of the pH meter at room temperature. The pH meter was allowed to stabilize for one minute and then the pH of the different cream cheese and milk were directly measured.

3.3.5 Microbiological analysis of cream cheese

3.3.5.1. Preparation of equipments and media

Glass ware such as test tubes, flasks, Petri dishes and pipettes were sterilized by heating in an oven at 160 ˚C for 2 hours. Media, dilutions (distilled water), and tips were sterilized by autoclaving at 121˚C for 15 minutes. The media were then cooled to 45 ˚C (Barrow and Feltham, 1993).

3.3.5.2 Preparation of the samples

Different types of cream cheese samples stored under the similar conditions were taken in sterile plastic containers. Fifty grams of cream cheese were taken for microbiological examination. Samples were taken aseptically from containers. Ten grams of cheese were added to 90 ml of distill water in a flask and shaken well to complete mixing after that to make 10^{-2} dilution then 1 ml from the above mentioned dilution (10^{-1}) was aseptically transferred to 9 ml sterile peptone water. This procedure was repeated to make serial dilutions of 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}

from suitable dilutions , 1 ml was transferred to Petri-dishes (duplicate) followed by addition of 18-20 ml the culture medium was poured aseptically into each Petri- dish , mixed gently , left to solidity and incubated (in an inverted position) (Houghtby *et al*., 1992).

3.3.5.3 Enumeration of viable cell of *B. longumBB536*

MRS medium was used to enumerate *B. longumBB536* of different cream cheese using the plate count technique. Samples were drawn at one day and every 15days intervals during storages (45 days). One gram of cream cheese was diluted in peptone water, followed by plating on Rogosa agar (MRS) supplement with 0.05% L- cystiene. The plates were incubated an aerobically at 37 ˚C for 48 h. The growth was calculated as Colony Forming Unit per g (CFU/g).

Colony counters where:

Colony forming units/gm (CFU/gm) = Total number of colonies in the dilution multiplied by the reciprocal of dilution (Houghtby *et al*., 1992).

3.3.5.4 Total bacterial count (TBC)

Total bacterial count using nutrient agar medium was determined according to the method by Houghtby *et al*. (1993).

3.3.5.4. 1Preparation of the media

The medium was prepared according to manufacturer's instructions by dissolving 23.5 gram of powder in 1000 ml of distilled water, heated to boiling point and then sterilized in an autoclave at 121 ^{o}C for 15 minutes (Frank *et al*., 1993).

3.3.5.4. 2 Plating

From each selected dilution 1 ml was transferred into sterile Petri dishes (duplicate) followed by addition of 15 – 18 ml melted, cooled (45 ^{o}C) nutrient agar was mixed thoroughly by rotating the dishes first in one direction and then in the opposite direction. When medium was solidified, the dishes were incubated in an inverted position at 35 ± 2 °C for 24 hours.

3.3.5.4.3 Counting

Plates contain 25 – 250 colony were selected and counted using colony counter. The number of colony forming units (cfu) in the dilution was obtained by multiplying the reciprocal of the dilution.

3.3.5.5 *E.coli* counts

The count was performed according to William and Dennis (1998) using MaConkey agar media and Eosin methylene blue agar (EMB) for identification.

3.3.5.5.1 Preparation of the media

The manufacturer's instructions were followed by dissolving 55 grams of powder in 1000 ml of distilled water, heated to boiling point and sterilized in an autoclave at 121 ^{o}C for 15 minutes, (Christen *et al*., 1992).

3.3.5.5.2 Plating and counting

One ml amounts of each sample dilutions were streaked in dried plate of Maconkey agar media. The culture was incubated at 35 °C for 24 hours, and then colonies were used for further confirmation of the presence of *E. coli* by streaking a loop from each colony on Eosin methylene blue agar (EMB) for identification of colonies which show brilliant green, the characteristic features of growth of *E. Coli* in the medium. The isolates were further characterized by biochemical tests according to Barrow and Feltham (1993) .Special attention was paid to the pattern of reactions of the organism in IMVIC tests, and the positive tests were recorded for a positive E. coli count.

3.3.5.6 *Staphylococcus aureus* counts

The count was achieved according to method by Christen *et al*., (1992) using Mannitol salt agar.

3.3.5.6.1 Preparation of the media

The manufacturer's instructions were followed by dissolving 111 grams of powder in 1000 ml of distilled water, heated to boiling point and sterilized in an autoclave at 121 ^{o}C for 15 minutes (Christen *et al*., 1992).

3.3.5.6.2 Plating and counting

One ml quantities of each sample dilutions 10^{2}-10^{6} was transferred into sterile Petri dishes followed by addition of 15 – 18 ml melted media, cooled (45 ^{o}C) Petri dishes with Mannitol salt agar was mixed thoroughly by rotating the dishes first in one direction and then in the opposite direction. When medium was solidified, the culture was incubated at 35 ± 2 °C for 24hours where colonies of *Staphylococcus aureus* were recognized by bright yellow zones formation in Mannitol salt agar (Jawez and Adel, 1990) and then colonies were counted by colony counter.

3.3.5.7 Yeast and molds count

The yeast and molds count were determined according to Harrigan and McCance (1976).

3.3.5.7.1Preparation of the media

Media was prepared according to manufacture instructions by suspending 39 grams of Potatoes dextrose agar in one liter distilled water and boiled until it dissolved completely, then it was sterilized by autoclaving at 121ºC for 15minuts.

3.3.5.7.2 Plating and counting

One ml from suitable dilutions was transferred into sterile Petri dishes followed by addition of 15 – 18 ml potato dextrose agar. The plates were incubated at 25°C for up to 72 hours. And then colonies were counted by colony counter.

3.3.5.8. *Salmonella*

The count was achieved according to method by (Liong and Shah, 2006) using Brilliant green agar

3.3.5.8.1 Preparation of the media

Media was used according to manufacture instructions by suspending 58.09 grams of Brilliant green agar in one of liter distilled water , boiled until dissolved completely and then it was sterilized by autoclaving at 121ºC for 15 minutes.

3.3.5.8.2 Plating and counting

One ml quantity of each sample dilution was transferred into sterile Petri dishes (duplicate) followed by addition of 15 – 18 ml melted media, cooled to45^{o}C.Petridishes with Brilliant green agar was mixed thoroughly by rotating the dishes first in one direction and then in the opposite direction. Then media was solidified. The culture was incubated at 35 ± 2 °C for 24hours.

3.3.5.8.3 Counting

Plates contain 30 – 300 colony were selected and counted using colony counter. The number of colony forming units (CFU) in the dilution was obtained by multiplying the reciprocal of the dilution.

3.3.5.9 Sensory evaluation

Different baobab cream cheeses samples were subjected to sensory evaluation using trained panelists according to Narvhus *et al*. (1998). The samples were assessed for texture, color, flavor, and overall acceptability

3.3.6 Experimental rat's *in vivo*

3.3.6.1 Animals and Experimental design

3.3.6.1.1 Animals

Six weeks old male albino rats with an average initial weight of (95g) were purchased from College of pharmacy, Ahfad University for Women. They were housed six per cage. A 12 h light dark cycle and a controlled atmosphere (22.11±2.36°C) were maintained throughout the study. After fifteen days acclimatization period under experimental condition, rats were randomly assigned into five different groups (n = 6) at random and treated for thirty days. During the thirty days trial, the rats were offered water and fed adlibitum on all groups. The bedcovers in cages was changed twice a week. Individual body weight of every rat was measured at ten days intervals.

3.3.6.1.2 Experimental design

3.3.6.1.2.1 Chemical and apparatus

All chemicals and kits were purchase from Biosystems (Spain) and were provided by local company in Khartoum. Test tubes, (K3 EDTA) and sterile containers were obtained from local company in Khartoum.

3.3.6.1.2.2 Experimental design feed

All experimental designs were shown in table (2).

3.6.2.2 Blood sampling

Blood samples for three rats of each group were collected at the end of the experiment period (30 days)from vein plexus eye in sterile tubes containing EDTA and kept at 4 ºC for hematology analysis, after that the rats were sacrificed under anesthetize and the blood was collected in clean test tubes. Serum was separated by centrifugation at 1500 rpm for 20min to investigate the biochemistry parameter.

Table (2): The experimental rat groups and their diets

Group	Experimental diets (per 6 rats)
Control(C)	Normal diet + sterile water
Treatment A	Normal diet + sterile water+2.5ml orally daily of baobab cream cheese without added *Bifidobacterium longum 536*
Treatment B	Normal diet + sterile water+2.5ml orally daily of baobab cream cheese supplemented with 2% *BB536*
Treatment C	Normal diet + sterile water+5ml orally daily of baobab cream cheese supplemented with 2% *BB536*
Treatment D	Normal diet + sterile water+2.5ml orally daily of cream cheese spread produced by premier food using rennet

3.6.2.4 Hematology

The procedure for complete blood profile including: hemoglobin concentration (HGB), red blood cell (RBC), white blood cell (WBC) and their respective differentials were preformed with automated analyzer (Mindry-BC 3000 plus).

3.6.2.5 Biochemistry

3.6.2.5.1Serum enzymes andminerals

Serum enzyme [aspartate transferase (AST), alanine transferase (ALT) and alkaline phosphatase (ALP)] activities and total protein (TP), albumin (Alb), globulin (Glob) content and uric acid of the blood were estimated according to methods based on the enzymatic colorimetric test method also Na, ca, k, P were determined using kits method obtained from **BioSystems (Spain).**

3.6.2.5.2 Glucose

Measurement was based on the colorimetric end point test method.

Principle

Glucose is determined after enzymatic oxidation in the presence of glucose oxidase. The hydrogen peroxide formed reacts, under catalysis of peroxidase, with phenol and 4-aminophenazone to form a red-violet quinoneimine product.

Protocol

Test tubes the following were performed:

	Blank	Standard	Sample
Sample	-	-	10 µL
Standard	-	10 µL	-
Enzyme reagent	1000 µL	1000 µL	1000 µL

The reaction mixture was mixed well and incubated for 5 minutes at 37^{o}C. The absorbance of sample and standard was measured against reagent blank at 505 nm. The concentration of the standard was then multiplied by the product of the sample absorbance/standard absorbance.

3.6.2.5.3 Direct bilirubin

Principle

Direct bilirubin (conjugated) reacts in acid environment with diazotized sulphalinic acid. The formed coloured azobilirubin is measured photometrically at 546 nm.

Protocol

Test tubes the following were performed:

	Sample	**Blank**
Direct bilirubin reagent	1000 µL	1000 µL
Nitrite reagent	20 µL	-
Sample	50 µL	50 µL

The reaction mixture was mixed well and incubated in the dark at room temperature for 5 minutes. The absorbance of the sample was measured against respective sample blank within 8 minutes at 546 nm. The concentration wascalculated by subtracting the blank absorbance from the sample absorbance and multiplied by the factor.

3.6.2.5.4 Urea

Measurement was based on the colorimetric end point test method.

Principle

The Berthelot reaction has long been used for the measurement of urea and ammonia. The present method is a modified Berthelot method. The urea colorimetric procedure is a modification of the Berthelot reaction. Urea is converted to ammonia by the use of urease. Ammonium ions then react with a mixture of salicylate, sodium nitroprusside and hypochlorite to yield a blue-green chromophore. The intensity of the color formed is proportional to the urea concentration in the sample.

Protocol

	Blank	Standard	Sample
Urea buffer	1000 µL	1000 µL	1000 µL
Urea enzyme reagent	100 µL	100 µL	100 µL
Standard	-	10 µL	-
Sample	-	-	10 µL
Mixed well and incubated for 5 minutes at 37^{o}C.			
Urea colour developer	1000 µL	1000 µL	1000 µL
Mixed well and incubated for 5 minutes at 37^{o}C.			

The absorbance of sample and standard was measured against reagent blank at 578 nm. The concentration of the standard was then multiplied by the product of sample absorbance and divided by the standard absorbance.

3.6.2.5.5 Creatinine

Measurement was based on the colorimetric kinetic test method developed by Jaffe reaction.

Principle

Creatinine in alkaline solution reacts with picrate to form a coloured complex which absorbs at 500-520 nm. The amount of complex formed is directly proportional to the creatinine concentration.

Protocol

	Blank	Standard	Sample
Sample	-	-	100 μL
Standard	-	100 μL	-
Picrate and buffer reagents	1000 μL	1000 μL	1000 μL

The reaction mixture was mixed well and after 30 seconds at room temperature the initial absorbance was read and read again after 1 minute. The change in absorbance in a minute of the standard and sample were measured against the reagent blank at 500 nm. The concentration of the standard was then multiplied by the product of sample absorbance/standard absorbance.

3.6.2.5.6 Cholesterol

Measurement was based on the enzymatic colorimetric test method.

Principle

Cholesterol esters are hydrolysed to produce cholesterol. Hydrogen peroxide is then produced from oxidation of cholesterol by cholesterol oxidase. The indicator quinoneimine is formed from hydrogen peroxide and 4-aminoantypyrine in the presence of phenol and peroxide. The absorption of the red quinoneimine dye is proportional to the concentration of cholesterol in the sample.

Protocol

To test tubes the following was performed:

	Blank	Standard	Sample
Sample	-	-	10 µL
Standard	-	10 µL	-
Enzyme reagent	1000 µL	1000 µL	1000 µL

The reaction mixture was mixed well and incubated for 5 minutes at 37^{o}C. The absorbance of sample and standard was measured against reagent blank at 505 nm. The concentration of the standard was then multiplied by the product of sample absorbance and divided by the standard absorbance.

3.6.2.5.7 High density lipoprotein (HDL)

Measurement was based on the CHOD-PAP tests method

Principle

Low density lipoproteins (LDL and VLDL) and chylomicron fractions are precipitated by the precipitating reagent. After centrifugation, the cholesterol concentration in the HDL (high density lipoprotein) fraction remains in the supernatant in this phase and is determined by an enzymatic (CHOD-PAP) method.

Protocol

Precipitation step

Test tubes were contained 500 µL of each sample then added 500 µL Precipitating reagent was mixed and allows standing for 5 minutes.

Centrifuge for 10 minutes at 3000 rpm and determining the cholesterol content by the CHOD-PAP method. Only clear supernatant must be used.

Assay step: test tubes the following were performed

	Blank	**Standard**	**Sample**
Sample	-	-	50 µL
Standard	-	50 µL	-
Enzyme reagent	1000 µL	1000 µL	1000 µL

The reaction mixture was mixed well and incubated for 5 minutes at 37^{o}C. The absorbance of sample and standard was measured against reagent blank at 520 nm. The concentration of the standard was then multiplied by the product of sample absorbance and divided by the standard absorbance and a serum dilution factor.

3.6.2.5.8 Triglycerides

Measurement was based on the enzymatic colorimetric test method

Principle

Triglycerides are determined after enzymatic hydrolysis with lipases. The quinoneimine indicator if formed from hydrogen peroxide, 4-aminophenazone and 4-chorophenol under the catalytic influence of peroxidase.

Protocol

	Blank	Standard	Sample
Sample	-	-	10 µL
Standard	-	10 µL	-
Enzyme reagent	1000 µL	1000 µL	1000 µL

The reaction mixture was mixed well and incubated for 5 minutes at 37^{o}C. The absorbance of sample and standard was measured against reagent blank at 505 nm. The concentration of the standard was then multiplied by the product of sample absorbance and divided by the standard absorbance.

3.6.2.6 Bacteriological enumeration in colon contents

Three rats from each group were used for bacteriological enumeration in colon content were collected in severe aseptic conditions in sterile bottle to avoid any cross contamination. The content was homogenized inside a cabinet and serially diluted prior to plating on different agar plates.

Media used for total aerobe, total anaerobe and *enterobacteriacae* ,*bifidobacteria* and *salmonella* according to reported by (Santos *et al*., 2006).*Staphylococcus*, *lactobacillus* and coliform enumerated following Liong and Shah (2006) method. Incubation environment of media used for enumerations are shown in Table (3).

3.7 Statistical analysis

One- way ANOVA and two sample paired test were performed to examine significant differences between normally distributed data of replicated measurement. Probability level of less than 0.05 was considered significant ($p<0.05$). All data were analyzed using vision 16 MINITAB statistical software for windows (2006).

Table3: Enumeration media and incubation environments of different microbiota groups in colon of rats fed different cream cheeses.

Bacterial group	Type of media	Incubation
Total aerobe*	Nutrient agar	Aerobic
Total anaerobe**	Brain heart infusion agar	Anaerobic****
Coliform**	Macconky agar	Anaerobic****
Salmonella**	Brilliant green agar	Aerobic
Staphylococcus**	Mannitol salt agar	Aerobic
Lactobacillus**	De Man Rogosa Sharpe agar	Anaerobic****
Bifidobacteria**	De Man Rogosa Sharpe agar+ L- cystiene	Anaerobic****

[a]All samples were incubated at 37 ºC.

* Incubation for one day.

** Incubation for two days

** Incubation for three days

****Anaerobic condition was created in anaerobic jars

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Physical characteristics of baobab fruit from different locations in Sudan

Results in Table4, indicate high significant differences in physical characteristics of fruit collected from different locations. Eight distinct fruit shapes were observed and were described through visual examination: Oblong pointed, High – Spheroid, Ovate, Ellipsoid pointed, Clavate, Crescent, Ellipsoid and Fusiform shaped, (Fig1). Among them clavate and crescent shapes baobab were found only in El Obeid and Umm Ruwaba (North Kordofan State). However ovate baobab was found only in El Obeid (Kordofan).

Moreover, the physical attributes of baobab fruit from different locations namely: length, width, weight, the weight of pulp, the weight of seed, number of seed,the weight of red fiber and weight of epicarp showed significant ($p<0.05$) difference. As in Table 4, Oblong pointed baobab fruit showed length range of 16.33 -26.30, width range 5.55-7.55, weight of fruit 106.70 -255.07g, weight of seed 70.13 -123.03 g, weight of pulp 19.87-55.58g, number of seeds162.50-201.00seed , width of wall 5.05-6.10 mm, weight of red fiber 3.9-4.17g and weight of epicarp10.93-72.21g.

High – Spheroid baobab fruit showeda rangeof length from 12.51-16.72cm, width 8.60-11.48cm, the weight of fruit 182.57-200.62g, the weight of seeds 70.13 -123.03 g, weight of pulp 50.22 -68.60g, numberof seeds 146.50-203.50, the width of wall 3.85-4.00 mm, the weight of red fiber3.10 -3.92g and weight of epicarp46.56-61.09g.

Ellipsoid pointed baobab fruits showedarrangeof length from 15.15-19.88cm, width 7.05-9.30cm, the weight of fruit 182.57-200.62g, the weight of

seed49.41-97.85g, the weight of pulp25.22-47.17g, number of seed125.50 - 182.00, the width of the wall(3.25-4.15 mm), the weight of red fiber3.10 - 3.75g and weight of epicarp51.76 -82.50g.

Clavate shape was found in both baobab fruit pulp from El Obeid and Umm Ruwaba as showed measured of length of 9.25and21.7cm, width 5.00and10.43cm, weight of fruit 97.34 and185.99g, weight of seed 38.41and 79.62, weight of pulp20.17and33.26g, number of seed79.00 and103.50, width of wall 5.00and6.00 mm, weight of red fiber2.00and5.77g and weight of epicarp37.06and 67.57g, respectively.

Crescent shape was found only in a sample from Umm Ruwaba as showed of length22.80cm, width 5.50mm, the weight of fruit 103g, the weight of seed30.48g, the weight of pulp15.95g, number of seed 60.0the width of wall 5.40mm, the weight of red fiber2.02g and weight of epicarp55.70g.

Ellipsoid baobab fruit showed arrange of length from 17.20-20.20cm, width 8.07-9.27cm, the weight of fruit 149.50-256.09g, the weight of seeds 67.55 - 130.50g, the weight of pulp 24.71-34.45g,a number of seed 130.0 -255.50, the width of wall 3.00-3.45mm, the weight of red fiber 3.16 -6.78g and weight of epicarp48.47-84.37g.

Fusiform baobab fruit was showed no significant different ($p>0.05$) in mustered of length from range20.38 -21.64cm),while significant ($p<0.05$) different in width 21.64-21.64cm, weight of fruit 149.05 -154.38g, weight of seed 46.34 -51.32g,weight of pulp 21.73-35.58g, number of seed 84.00 - 132.50, width of wall 4.90-6.40mm, weight of red fiber 3.20 -3.88g and weight of epicarp63.30-76.70g.

Ovate baobab was found only in a sample from El Obeid as showed a rangeof length 9.357.80cm, width 7.80mm, the weight of fruit 147.8g, the weight of seeds 61.37g, the weight of pulp31.41g, number of seed 113.00, the width of wall4.9mm, the weight of the red fiber2.85gand weight of

epicarp52.79g.These results of measured parameter (Fruit length, Fruit width, Fruit weight) were slightly different from values(length14.32cm, width 7.78 and Fruit weight 149.75 g) reported by Nasreldin *et al*.,(2014) in morphological variation in fruit shapes of *Adansonia digitata* L. FromBlue Nile and North Kordofan states in Sudan, also the weight of pulp, the weight of seed,number of seed was showed different variation compared that reported by Munthali *et al*.(2012). Numerously published literature on baobab fruit phenotypic variations: (De Smedt *et al*., 2011; Barwick, 2004; Gebauer *et al*., 2002; Gruenwald and Galiza 2005; Nour *et al*. 1980). Gruenwald and Galizia (2005) reported that the percentage of fruit pulp varies according to the provenances. In southern Africa, fruit pulp comprises 16.5% of the fruit weight and seed weight 38% while in Senegal fruit pulp is about 12%.Moreover, theweight of red fiber and weight of epicarp were investigated for the first time in this study.These findings showed a significant variation of quantifiable characters of fruits among and within the studied states could be attributed to the different climatic gradient and environmental factors. Katsvanga *et al*., (2007) also, fruit characteristics are greatly affected by environmental factors, cultural factors in addition to genetic effects.

Table (4): Fruit physical characteristics of *Adansonia digitata,* from North Kordofan (El Obeid and Umm Ruwaba) ,Blue Nile (Damazin) and Southern Darfur (Nyala) States

Fruits shape	Sample	Length (cm)	Width (cm)	Weight of fruit (g)	Weight of seed(g)	Weight of pulp(g)	Number of seed	Width of Wall mm	Weight of red fiber(g)	Weight of epicarp (g)
Oblong pointed	El Obeid	17.32±1.40^{b}	6.11± 0.10^{a}	112.69 ±3.51^{c}	73.07 ± 3.62^{c}	19.87 ± 1.08^{b}	168.50 ± 9.19^{b}	5.48 ± 0.68^{a}	4.17± 0.23^{a}	15.53 ± 1.46^{b}
	Umm Ruwaba	6.30 ± 0.37^{a}	7.38± 0.55^{a}	255.07 ± 0.74^{a}	123.03 ±3.57^{a}	55.58 ± 5.54^{a}	162.50 ± 3.54^{a}	6.10 ± 0.14^{a}	4.10± 0.14^{a}	72.21 ± 5.12^{a}
	Damazin	5.30 ±0.12^{a}	7.55±0.06^{a}	223.13 ± 3.12^{b}	110.93 ± 0.39^{b}	50.94 ±3.22^{a}	190.00±1.41^{a}	5.35 ± 0.35^{a}	4.15± 0.21^{a}	56.78 ± 6.63^{a}
	Nyala	6.33 ± 0.31^{b}	5.55± 0.16^{a}	106.70 ± 9.18^{c}	70.13 ± 2.84^{c}	21.55 ±1.41^{b}	201.00±2.83^{b}	5.05 ± 0.07^{a}	3.90 ± 0.14^{a}	10.93 ± 4.50^{b}
High - Spheroid	El Obeid	6.72 ± 0.54^{a}	11.48 ±0.53^{a}	182.57± 3.48^{b}	79.08± 2.02$^{a b}$	50.22 ± 0.16^{b}	157.50± 3.54^{b}	4.00± 0.00^{a}	3.75± 0.21$^{a b}$	49.73 ± 1.80^{b}
	Umm Ruwaba	5.57 ±0.75^{ab}	10.85± 0.42^{a}	188.00 ± 3.39^{b}	73.16 ± 2.18^{b}	50.31 ± 2.81^{b}	46.50± 7.78^{a}	4.00± 0.00^{a}	3.92 ± 0.11^{a}	61.09± 1.55^{a}
	Damazin	12.48 ± 0.53^{b}	9.03 ± 0.11^{b}	200.62± 2.14^{a}	83.03 ± 0.98^{a}	68.60 ± 4.31^{a}	03.50± 4.95^{a}	3.85± 0.35^{a}	3.10 ±0.14^{b}	46.56± 2.06^{b}
	Nyala	12.51± 0.56^{b}	8.60± 0.14^{b}	199.72 ± 0.55^{a}	79.11± 0.96$^{a b}$	68.00± 0.00^{a}	191.00± 1.41^{b}	4.00± 0.00^{a}	3.40 ± 0.28$^{a b}$	48.81± 2.14

Cont:

Fruits shape	Sample	Length (cm)	Width (cm)	Weight of fruit (g)	Weight of seed(g)	Weight of pulp(g)	Number of seed	Width of Wall mm	Weight of red fiber(g)	Weight of epicarp (g)
Ellipsoid pointed	El Obeid	19.65± 1.91[a]	8.90± 0.85 [a b]	219.38 ±5.42 [a]	86.52± 2.83 [a]	47.17± 2.61[a]	157.50± 3.54 [a]	4.00± 0.00 [a]	3.10 ± 0.14 [a]	82.50 ± 5.66[a]
	Umm Ruwaba	19.88 ± 1.87[a]	9.30± 0.28[a]	203.78± 4.56[a]	97.85 ± 3.03[a]	37.69± 3.34[a]	182.00± 0.90[a]	4.15± 0.07[a]	3.75 ± 0.35[a]	64.73 ± 1.03[b]
	Damazin	17.09± 0.59[a]	7.57± 0.33 [a b]	151.54± 3.59[b]	49.41± 1.53[c]	26.82± 2.29[b]	128.00± 0.83[b]	4.00± 0.00 [a]	3.70 ± 0.28[a]	72.56± 0.21[ab]
	Nyala	15.15± 1.20[a]	7.05± 0.07 [b]	148.61± 4.24[b]	68.38± 4.16[b]	25.22± 0.31[b]	125.50± .36[b]	3.25± 0.35 [b]	3.26± 0.37[a]	51.76± 0.77[c]
Clavate	El Obeid	9.25± 0.35[b]	5.00± 0.00 [b]	97.34 ± 2.68[b]	38.41± 1.28[b]	20.17± 0.23[b]	79.00± 4.24 [b]	6.00± 0.00[a]	2.00± 0.00[b]	37.06± 4.15[b]
	Umm Ruwaba	21.79± 0.65[a]	10.43± 0.60[a]	185.99 ± 1.58[a]	79.62 ± 0.86[a]	33.26± 2.88[a]	103.50± 6.36 [a]	5.00± 0.00[b]	5.77 ± 0.33[a]	67.57± 3.63[a]
ovate	El Obeid	9.35±0.35	7.80±0.28	147.8±3.45	61.37±1.16	31.41±1.70	113.00±7.08	4.9±0.14	2.85±0.21	52.79±4.54
Crescent	Umm Ruwaba	22.80±3.39	5.50±0.28	103.80±4.50	30.48±2.23	15.95±0.88	60.00 ± 5.66	5.40±0.14	2.02±0.01	55.70 ±1.83

Fruits shape	Sample	Length (cm)	Width (cm)	Weight of fruit (g)	Weight of seed(g)	Weight of pulp(g)	Number of seed	Width of Wall mm	Weight of red fiber(g)	Weight of epicarp (g)
Ellipsoid	El Obeid	17.25± 0.35 [b]	9.27± 0.09[b]	162.83± 0.65[bc]	86.60 ± 2.26 [a]	24.71± 1.68[b]	210.50± 13.44[b]	3.15± 0.07[b]	6.50 ± 0.23 [a]	48.47± 2.47[b]
	Umm Ruwaba	17.20± 0.00[b]	10.42± 0.12 [a]	256.09± 7.68[a]	130.50± 3.54[b]	34.45± 1.07[a]	255.50± 7.78[a]	3.00± 0.00 [b]	6.78 ± 0.32[a]	84.37± 11.96[a]
	Damazin	17.30± 0.28[b]	8.07± 0.09[c]	174.47± 8.68[b]	91.94± 2.12[b]	28.71± 1.97[ab]	148.00± 9.90[c]	3.45± 0.07 [a]	3.88 ± 0.04 [b]	49.74 ± 4.83[b]
	Nyala	20.20± 0.14[a]	8.45± 0.35[c]	149.50± 2.12[c]	67.55 ± 3.61[c]	25.18 ± 0.77 [b]	130.0 ± 0.00 [c]	3.00± 0.00[b]	3.16 ±0.06 [b]	53.59± 2.24[b]
Fusiform	El Obeid	20.38 ± 0.88 [a]	8.05± 0.07 [a]	154.38± 1.17 [a]	47.92± 0.59 [a b]	33.59± 2.74[a]	104.0 ± 14.1 [a b]	4.90± 0.14 [a]	3.20 ± 0.00 [b]	73.10± 0.69[a]
	Umm Ruwaba	1.64± 1.64[a]	21.64± 0.23[b]	149.05 ± 2.89[a]	46.34 ± 0.97 [b]	21.73± 2.27 [b]	84.00 ± 2.83[b]	6.40± 0.57[a]	3.88 ± 0.18[a]	76.70± 0.99[a]
	Damazin	21.48± 0.39[a]	7.66± 0.21[ab]	152.92± 13.31[a]	51.32± 1.58[a]	35.58 ± 2.79 [a]	132.50± 3.54[a]	5.43± 0.33 [a]	3.68±0.035[a b]	63.30± 9.77[a]

Each values of means ±Stander deviation

Values that a bear different superscript that letter in the same Column are significantly differently at $p < 0.05$

Oblong pointed

Ellipsoid pointed

Fusiform

Ellipsoied

Clavate

Ovate

High – Spheroid **Crescent shaped**

Figure.1. Shapes of Bobab fruit (*Adansonia digitata L.*) in Sudan from North Kordofan state (El Obeid and Umm Ruwaba), Southern Darfur State (Nyala) and Blue Nile (Damazin) sates.

4.2 Physico-chemical properties of baobab fruit pulp from different locations in Sudan

4.2.1 pH **values**

Values of pH for different baobab fruit pulp samples are displayed in Table (5). Baobab pulp obtained from Nyala, had a pH value of 3.19, which is significantly (P≤ 0.05) higher as compared with values of 3.04, 3.03and3.02 pH for baobab from Damazin, El Obeid and Umm Ruwaba, respectively. This result was slightly lower as compared to findings by Ndabikunze *et al.* (2011) who reported a pH value of 3.4. These variations may be due to variations inorganic acids content of baobab fruit pulp.

4.2.2 Titratable acidity (TA)

Table (5) shows the titratable acidity of baobab fruit pulp from different locations in Sudan. There is significant (P≤ 0.05) difference (TA) between baobab fruits from different locations. Titratable acidity values recorded for baobab fruit were 2.37, 2.36, 2.67 and2.06 (mg/100g) El Obeid (Kordofan), Umm Ruwaba **(**Kordofan), Damazin (Blue Nile**)** and Nyala (Darfur), respectively. Furtherthere was no significant difference in titratable acidity of baobab fruit pulp from El Obeid and Umm Ruwaba could be attributed to the fact they are of the same ecotypes growing in soilswith similar chemical composition.

4.2.3 Moisture content

The Moisture content of baobab fruit pulp from different regions is shown in Table (6).It was found to be 6.52, 6.40, 6.95 and 6.79% for baobab fruit from Kordofan (El Obeid), Kordofan (Umm Ruwaba), Blue Nile (Damazin) and Darfur (Nyala), respectively. There is significantly different in moisture content (P≤ 0.05) .These values were inagreement with the value of 6.7% reported by Nour *et al*. (1980), but lesser than the range of 11.1-13.6% reported by Gaydou *et al*.(1982) . Value of moisture in Table 1 is also lower than the value of 10.4% reported by Osman (2004).

4.2.4 Protein content

The Protein content of baobab fruit pulp is shown in Table (6). Protein values of 5.44, 5.34, 5.56 and 5.11%were a found for baobab from Kordofan (El Obeid),Kordofan (Umm Ruwaba**),** Blue Nile (Damazin)and Darfur (Nyala),respectively .There is significant difference (P≤ 0.05) in protein amid the four baobab fruitpulps. Results in Table (1) were slightlylowerthan the value of 6.2% reported by Nour *et al*.(1980) for Sudanese baobab, but higher content than 2.3% concentration reported by Manferdini *et al*. (2002).

4.2.5 Oil content

As showed in Table (6), oil values in baobab were 0.70 %for Kordofan (Umm Ruwaba) and 0.64% for Blue Nile (Damazin) with no significant differences (P≤ 0.05).while oil values were 0.83% for Kordofan (El Obeid) and 1.20% for Darfur (Nyala) baobab fruits which is significantly different (P≤ 0.05).These results were noticeably higher compared to the value of 0.3%given by Osman (2004).

4.2.6 Fiber content

Table (6), displayed the fiber contents of baobab fruits pulp from Kordofan (El Obeid),Kordofan (Umm Ruwaba**),** Blue Nile (Damazin**)** and Darfur Nyala were 8.10, 6.83, 8.29 and 11.21%, respectively. Baobab fruit from Darfur showed significantly higher difference (P≤ 0.05) in fiber content. Result obtained for baobab fruit from Darfur was higher as compared to the values between 5.4 to 9% reported previous by Nour *et al*. (1980); Arnold *et al*. (1985) and Osman (2004).

4.2.7 Ash content

As shown in Table (6), ash content was 5.02% of baobab fruits from El Obeid (Kordofan), 4.85 %for Umm Ruwaba (Kordofan) and 5.01 %for baobab fruits

pulp from Blue Nile. Results showed no significant (P ≥ 0.05) difference in ash among three baobab fruits pulp. While that of Darfur (Nyala) has significantly (P≤ 0.05) higher ash content to 5.53%. Present values in Table 1were lower than the value of 7% reported by Okoho (1984).

4.2.8 Total carbohydrates

As shown in Table (6) the carbohydrates of baobab fruit pulp from El Obeid (Kordofan),Umm Ruwaba(Kordofan),Damazin (Blue Nile**)** and Nyala(Darfur) were 74.09, 75.31, 73.54 and 70.16 %, respectively. Results displayed no significant difference (P≥ 0.05) between baobab fruits pulp from El Obeid (Kordofan), Umm Ruwaba (Kordofan),and Damazin (Blue Nile) in carbohydrates .While baobab fruit from Darfur(Nyala)has significantly (P≤ 0.05) lower carbohydrates content than that of other samples. Data were in agreement with values of 73.7 to 81%reported by Gaydou *et al*. (1982); Okoho, (1984); Arnold *et al*. (1985) , Osman, (2004).

4.2.9 Total sugars

Total sugars were presented in Table (7), values obtained were 38.55,37.98 , 28.96 and 27.72 mg/100g baobab fruit from El Obeid(Kordofan),Umm Ruwaba(Kordofan), Damazin(Blue Nile**)** and Nyala(Darfur) respectively, which were slightly higher than the range of 16.9-25.3% reported by aydou *et al*., (1982). No significant differences (P≥ 0.05) were observed among the four samples.

4.2.10 Reducing sugars and non reducing sugars

Results of reducing sugars are shown in Table 7. Fruit pulps of Kordofan (Umm Ruwaba) and Blue Nile (Damazin) baobab were found to be similar in their reducing sugars content (13.16 and 13.13mg/100g respectively).

Table (5): Titratable acidity and pH value of baobab fruit pulp from different locations in Sudan

locations of samples	pH	Tetratable Acidity
El Obeid	3.03 ± 0.01^{bc}	2.37 ± 0.06^{b}
Umm Ruwaba	3.02 ± 0.01^{b}	2.36 ± 0.05^{b}
Blue Nile	3.04 ± 0.01^{b}	2.67 ± 0.12^{a}
Nyala	3.19 ± 0.01^{a}	2.06 ± 0.01^{c}

* Values are mean ± SD for replicate independent runs.

**Values that bear different superscript letter in the same Column are significantly different at $p<0.05$.

Table (6): Chemical composition (%) of baobab pulp from different locations in Sudan

Locations of samples	Moisture	Protein	Oil	Fiber	Ash	Carbohydrate
El Obeid	6.52 ± 0.00 ^{c}	5.44 ± 0.05^{ab}	0.83 ± 0.00 ^{b}	8.10 ± 1.61^{b}	5.02 ± 0.03^{b}	74.09 ±1.56^{a}
Kordofan	6.40 ± 0.00^{d}	5.34 ± 0.06 ^{b}	0.70 ± 0.01 ^{c}	6.83 ± 0.90^{b}	4.85 ± 0.13^{b}	75.31 ± 0.50^{a}
Damazin	6.95 ± 0.05 ^{a}	5.56± 0.04 ^{a}	0.64 ±0.00 ^{c}	8.29 ± 0.0^{7b}	5.013 ±0.01^{b}	73.54 ±0.06^{a}
Nyala	6.79 ± 0.07^{b}	5.11 ± 0.05 ^{c}	1.20 ±0.06^{a}	11.21 ± 0.46^{a}	5.53 ± 0.05 ^{a}	70.16 ± 0.56^{b}

* Each value is mean of three replicates expressed on wet matter basis.

Values are (mean) ± standard deviation.

** Values that bear different superscript letter in the same Column are significantly different at $p<0.05$.

However, Kordofan (El Obeid), and Darfur (Nyala) baobab fruit pulp showed significantly (P≤ 0.05) lower values (10.88 and 10.51 respectively).Also in Table(7) the fruit pulp from Kordofan (El Obeid),Kordofan (Umm Ruwaba), Blue Nile (Damazin) and Darfur Nyala contained non reducing sugars concentrations of 27.68 , 24.82 , 15.83 and17.14mg/100g, respectively. These differences in sugar (reducing and non-reducing) content may be attributed to climatic reasons and soil factors difference between the study locations.

4.2.11 some sugars of baobab fruit pulp from different locations in Sudan

As presented in Table (8) fructose, glucose and sucrose were detected in all baobab fruit samples. The highest sucrose level was found in baobab fruit pulp from (El obied) 19.44mg/100g. Fructose content among differentsample were 5.07, 4.83, 4.44and 2.33mg/100g in baobab fruit pulp from El Obeid, Umm Ruwaba, Damazin and Nyala, respectively. Glucose values ranged between 1.32-5.32 mg/100g in different baobab fruits. The results showed significant (P< 0.05) difference in sugars profile aboutthe source of baobab fruit. However these results were in disagreement with findings of Ibrahima *et al.*,(2013). They reported 7.9 g/100g, 7.0 and 1.7 g/100g in baobab fruit forglucose, fructose and sucrose, respectively. The variations in sugar profile betweenbaobabspulps mightare affected by variations in the soil related to different locations in Sudan as well as a probable genetic variation.

4.2.12 Ascorbic acid (vitamin C)

As presented in Table (9), the results showed significant difference (P≤ 0.05) in ascorbic acid content of baobab fruit pulp. The sample obtained from Damazin had the highest ascorbic acid content (372.52mg/100g) followed by El Obeid and Umm Ruwaba samples (355.97 mg/100g and 354.13), while that from Nyala showed the lowest level (345.82 mg/100g). These values were within the range reported by Besco *et al*.(2007) from 150-499 mg/100,

higher than the result found by Nour *et al.* (1980)which was 300.00 mg/100 g and lower than the result found by Nnam and Obiakor (2003)which was 373mg/100 g.

4.2.13 Pectin contents of baobab fruit pulp

The result of pectin content presented in Table 10, showed significant ($P \leq 0.05$) difference in baobab fruit pulp from El Obeid, UmmRuwaba, Damazin and Nyala which were found to contain 43.93%, 46.65%, 54.99% and 40.75% respectively. The highest content of pectin was recorded in baobab fruit from Damazin. This variationsin pectin content might be attributed to the origin of baobab, the storage environments, the processing techniques, a probable genetic distinction, ripening age difference as reported by Fagbohun *et al*.(2012).

Table (7): Total, reducing and non reducing sugars mg/100g of baobab fruit pulp from different locations in Sudan

Locations of samples	Total Sugars	Reducing Sugars	Non Reducing Sugars
El Obeid	38.55 ± 9.43[b]	10.88 ± 0.55[a]	27.68 ±8.88[a]
Umm Ruwaba	37.98 ± 5.69 [b]	13.16 ± 0.81[a]	24.82 ± 4.87[a]
Damazin	28.96 ± 1.3[a]	13.13 ± 1.68[a]	15.83± 2.99[a]
Nyala	27.72 ± 2.39[a]	10.51 ± 0.87[a]	17.14 ± 1.58[a]

* Each value is mean of three replicates expressed on wet matter basis
Values are (mean) ± standard deviation.

** Values that bear different superscript letter in the same Column are significantly different at $p<0.05$.

Table (8): Sugar content (mg/100g) of baobab fruit pulp from different locations in Sudan

Sample	Fructose	Glucose	Sucrose
Umm Ruwaba	4.83± 0.03[b]	3.49± 0.01[b]	18.64± 0.27[a]
El Obeid	5.07± 0.05[a]	5.32±0.02[a]	19.44± 0.65[a]
Damazin	4.44± 0.02[c]	1.32± 0.01[c]	13.34± 0.05[b]
Nyala	2.33± 0.01[d]	1.77± 0.02[d]	13.00± 0.05[b]

Each value in the mean of three replicates

Values are Mean ±Standard deviation

Values that a bear different superscript letter in the same Column are significantly different at $p<0.05$.

Table (9): Ascorbic acid content (mg/100g) of baobab fruit pulp

Sample	Ascorbic acid
El Obeid	355.97 ± 4.17[b]
Umm Ruwaba	354.13 ± 2.59[b]
Damazin	372.52 ± 2.00[a]
Nyala	345.82 ± 1.15[c]

Each value is mean ± standard deviation of three replicates expressed on dry matter basis. Values that bear different superscript letter in the same Colum are significantly different at $p<0.05$.

Table (10): Pectin contents (%) of baobab fruit pulp from different locations in Sudan

Sample	Pectin (%)
El Obeid	43.93 ± 3.96^{bc}
Umm Ruwaba	46.65 ± 0.61 ^{b}
Damazin	54.99 ± 0.10^{a}
Nyala	40.75 ± 1.18^{c}

* Each value is a mean of three replicates ± standard deviation.

** Values that bear different superscript letter in the column are significantly different at $p<0.05$.

4.2.14 Minerals

Table11. Shows minerals content (Ca, Na, K, Mg, P and Fe) of baobab fruit pulp from different locations in Sudan.

4.2.14.1Sodium content

Chemical analysis exposed significant (P≤ 0.05) difference in Na contents of the four baobab fruits pulp. Values obtained were found to 89.67 mg/100g from El Obeid (Kordofan) , 75.08 mg/100g from Umm Ruwaba(Kordofan) **()**, 33.99 mg/100g from Damazin (Blue Nile**)** and 37.59 mg/100g from Nyala(Darfur) , this value was higher as compared with27.8 mg/100g which was reported for baobab fruit pulp byOsman, (2004). The variation of the Na contents of the pulp between different locations may be explained by the environmental factors and the effect of soil type on fruit compositions.

4.2.14.2 Potassium content

Potassium values obtained were 16.68, 18.74, 37.59 and 28.71 mg/100g of baobab fruits pulp from El Obeid (Kordofan), Umm Ruwaba (Kordofan), Damazin (Blue Nile**)** andNyala (Darfur), respectively. The baobab fruits pulp from different locations were significantly (P≤ 0.05) different in their K content.

4.2.14.3 Calcium content

Calcium content of baobab fruit pulp ranged from 308.98 to398.20 mg/100g with average value of 358.67 mg/100g.There is significant differences (p≤0.05) in Ca among the four baobab fruit samples.Nour *et al*. (1980) and Manfredini *et al.* (2002) reported higher values of 655 and 670 mg/100g, respectively, however Osman, (2004) obtained a lower value of 295 mg/100g for baobab fruit.

4.2.14.4 Phosphorus content

Phosphorus content in baobab fruit pulp from EL Obeid and Umm Ruwaba (Kordofan**)** were found to have almost the same level of P (51.80 -51.57 mg/100g) with a mean value of 51 mg/100g,these values are similar to the value of 50.8 mg/100g reported by Nour *et al*, (1980). Phosphorus content in baobab fruit pulp fromDamazin (Blue Nile**)** andNyala (Darfur) were found to (81.71 -87.62 mg/100g), which were lower than the range of 96-118mg/100g of Baobab Fruit Company, (2002). In all samples the Phosphorus content were significantly ($P \leq 0.05$) different.

4.2.14.5 Magnesium content

Magnesium values obtained were 104.10, 70.95, 142.10 and 186.68 mg/100g for El Obeid (Kordofan), Umm Ruwaba(Kordofan),Damazin (Blue Nile) and Darfur (Nyala) baobab fruits, respectively. The all baobab fruits pulp were significantly ($P \leq 0.05$) different in their Mg content.

4.2.14.6 Iron content

Investigation showed that Fe content of baobab fruit was7.02 mg/100g for El Obeid (Kordofan) sample, 6.81 mg/100g for Umm Ruwaba (Kordofan**)**, 17.21mg/100g forDamazin (Blue Nile**)** and 27.21 mg/100g for Nyala (Darfur). Iron content was significantly different ($P \leq 0.05$) among baobab fruits pulp from different locations. The results from El Obeid (Kordofan) andUmm Ruwaba (Kordofan) are within the range reported by Nour *et al*. (1980) and Osman, (2004), who reported iron value of 8.6 and 9.3 mg/100g, respectively.

All mineral levels in this study differ with the locations of baobab in Sudan. This indicated thatmineral composition of the fruit pulp is significantly influenced by the location of origin which is consistent with that reported by Parkouda *et al*. (2007) for baobab in Mali and Burkina Faso. One probable

explanation clarification for the differences is that fruit composition is a disclosure of the soil characteristic of the microsite of each plant (Izhaki *et al*, 2002). It is claimed that the accessibility of nutrients in soil is very heterogeneous, demonstrating a strong spatial and temporal difference that is frequently linked to seasonal and climatic (Monokrousos *et al*., 2004).

4.2.15 Extraction yield

The yield of extracts obtained from 20 g of different Sudanese baobab fruit pulp using different solvents were shown in Table (12). The highest yield percentages of solid remains was obtained using water as extraction solvent for baobab fruit pulp from Nyala (72.85%) followed by Umm Ruwaba (71.50%) and then Damazin (63.80%) but the lowest yield was that from El Obeid (47.55%). Our results show that the extract yields vary depending on the origin region of baobab fruit and solvent used for the extraction.

4.2.16 Total phenolic contents

The total phenolic contents of different Baobab fruit pulp from three different states: El Obeid and Umm Ruwaba (Northern Kordofan State), Nyala Southern Darfur State) and Damazin (Blue Nile State) extracts were determined using the Folin- Ciocalteu'sreagent is expressed in terms of gallic acid equivalent (the standard curve equation:y= 0.00073x+0.0503, r2 = 0.997).The values obtained for the concentration of total phenols are expressed as mg of GA/g of extract.Table 13, showed significant difference ($P \leq 0.05$) in total phenolic contents in the examined extracts ;which ranged from 15.50 to 99.66mg GA/g. Those values were higher when compared to result by Lamien–Meda *et al*. (2008) .They reported value of 35.18mg /g while Salihand Yahia(2015)phenolic content were 13.92 mg GAE/g for baobabpulp.

Table (11): Mineral contents (mg/100g) of baobab fruit pulp

Locations of samples	Na	K	Ca	P	Mg	Fe
El Obeid	89.67 ± 0.62[a]	16.68 ± 0.51[c]	398.20 ± 1.8[a]	51.80 ± 1.09[c]	104.10 ± 0.99[c]	7.02 ± 0.03 [c]
Umm Ruwaba	75.08 ± 0.60[b]	18.74 ± 0.55[c]	391.82 ± 0.54[a]	51.57 ± 0.51[c]	70.95 ± 1.02[d]	6.81 ± 0.05[c]
Damazin	33.99 ± 0.14[d]	37.59 ± 0.64[a]	308.98 ± 7.02[c]	81.71 ± 1.59[b]	142.10 ± 2.94[b]	17.21± 0.13[b]
Nyala	37.59 ± 0.64[c]	28.71 ± 2.07[b]	335.69 ± 1.95[b]	87.62 ± 1.68[a]	186.68 ± 3.10[a]	27.21± 0.93[a]

* Each value is mean of three replicates.

Values are (mean) ± standard deviation.

** Values that bear different superscript letter in the same Column are significantly different at $p<0.05$.

The highest concentration of phenolics for all samples was measured in methanolic solvent followed by ethanolic and aqueous solvents extracts. That may be due to effect of polarity. High solubility of phenolics in polar solvents affords high concentration of these compounds in the extracts obtained using polar solvents for the extraction (Mohsen and Ammar, 2008). No significant difference between Nyala and Damazin samples in phenolics contents of the ethanolic extracts.

4.2.17 Total flavonoids contents

The concentration of flavonoids in different baobab fruit pulp extracts from three different Sudan states: El Obeid and Umm Ruwaba (Northern Kordofan State), Nyala (Southern Darfur State) and Damazin (Blue Nile State) was determined using spectrophotometric method with aluminum chloride. The content of flavonoids was expressed in terms of catechin equivalent (the standard curve equation: $Y=0.005x+0.147$, $r^2 = 1.00$), mg of CA/g of extracts (Table 13) showed significant difference ($P\leq 0.05$). The concentration of flavonoids in the examined extracts ranged from 1.03 to 21.53 mg of CA/g. Those values were higher when compared to result reported by Lamien–Meda *et al*. (2008). They reported value of 35.18mg GAE /g .The highest concentration of favnoids for all samples was measured in methanolic solvent followed by ethanolic and aqueous solvents extracts in baobab fruit pulp from Damazin followed by El Obeid ,Nyala and Umm Ruwaba in extract by methanolic. The concentration of flavonoids in fruit pulp extracts depends on the polarity of solvents used in the extract preparation (Min and Chun-Zhao, 2005).

4.2.18 Antioxidant activity assays

4.2.18.1 DPPH and FRAP assays

The DPPH and FRAP antioxidant capacity results are shown in Table 2. DPPH assay and FRAP results of the antioxidant capacities showed significant difference ($p \leq 0.05$) between the baobabs fruit pulp extracts. Moreover, FRAP antioxidant activity of the aqueous extracts revealed that baobab fruit from Damazin was significantly different ($p \leq 0.05$) from that of the other three geographical regions. These differences could be due to the fact that Damazin area in which baobab trees grow in mountainous with heavy rains, resulting in different characteristics of baobab fruits as compared to that from other geographical regions in Sudan. The environmental conditions may have also affected types of phenolic compound, as the environment could contribute in the selection of phenolic biosynthetic pathways. It is also probable that different baobab ecotypes in these regions are partially responsible for these variations.

The DPPH test is the oldest indirect method for determining the antioxidant activity which is based on the ability of the stable free radical 2,2-diphenyl-1-picrylhydrazyl to react with hydrogen donors including phenols (Roginsky and Lissi, 2005). The bleaching of DPPH solution increases regularly with increasing amount of fruit in a given volume. The bleaching action is mainly attributed to the presence of antioxidant compounds like polyphenols in the solution. The antioxidant capacity was evaluated by DPPH assay in aqueous and ethanolic extract compounds of the baobab fruit pulp from different Geographical Regions in Sudan (El Obeid, Umm Ruwaba, Nyala and Damazin). The antioxidant capacity evaluated by DPPH radical scavenging using ascorbic acid as standard ($R2 = 0.9866$) was found to be 227.92, 205.87, 201.10and 183.50 mg AEAC/g for ethanolic extract from Damazin, Nyala,El Obeid and Umm Ruwaba respectively.While in methanolic extract was found to be 232.70, 229.22, 223.20 and 211.22 from Damazin, Nyala, El Obeid and Umm Ruwaba respectively.

Table (12): The yields (%) of solid remains after extraction and evaporationfrom 20 g baobab fruit pulp

Sample	Aqueous extract	Ehanolic extract	Methanolic extract
El Obeid	47.55 ± 0.05c	22.95± 0.04a	15.15 ± 0.06b
Umm Ruwaba	71.50 ± 0.27a	18.95± 0.04d	11.85 ± 0.08c
Nyala	72.85 ± 0.05a	21.85± 0.04b	17.25 ± 0.05a
Damazin	63.80 ± 0.28b	20.00± 0.10c	17.45 ± 0.07a

Each value is mean ± standard deviation of four replicates expressed on dry matter basis. Values that bear different superscript letter in the same column are significantly different at $p<0.05$.

Table (13): Total phenols and flavonoids contents of baobab fruit pulp (mg/g) equivalents of gallic acid and catechin

	Total phenoicls			Total flavonoids		
Sample	**Aqueous extract**	**Ethanolic extract**	**Methanolic extract**	**Aqueousextract**	**Ethanolic extract**	**Methanolic extract**
El Obeid	21.38±0.20^{b}	42.29± 0.24^{b}	84.49 ± 0.34^{b}	1.03± 0.08^{c}	10.77± 0.23^{b}	11.66± 0.10^{b}
Umm Ruwaba	15.50 ±0.30^{d}	58.46 ± 0.22 ^{a}	99.43 ± 0.80^{a}	2.00 ± 0.10^{a}	15.76± 0.67^{a}	4.07± 0.11^{d}
Nyala	17.16 ± 0.05^{c}	35.36 ± 0.34^{c}	41.37 ± 0.13^{c}	1.62 ± 0.02^{b}	5.77 ± 0.07^{c}	4.62 ± 0.07^{c}
Damazin	22.62 ± 0.07^{a}	58.93 ± 0.22^{a}	99.66 ± 0.18^{a}	1.46 ± 0.02^{b}	16.06 ± 0.15^{a}	21.53 ± 0.12^{a}

Values are means ±SD (n=4); means with different superscripts in the same column are significantly different ($p \leq 0.05$).

Moreover, for aqueous extracts antioxidant capacity was found to be 221.30mg AEAC/g for Umm Ruwaba, 200.17mg AEAC/g, Damazin 193.67mg AEAC/g El Obeid and 184.70mg AEAC /g for Nyala.

DPPH assays free radical scavengers to a certain extent, however, the free radical scavenging efficiency of each species differs depending on the solvents used and the varieties studied as suggested previously by Hamid *et al*. (2002). Polar solvents are commonly used for extraction of antioxidants, therefore generally higher antioxidant activity could be observed in samples that were extracted with ethanol than in aqueous extracts. The highest antioxidant activity was observed in the ethanolic extracts of baobab fruit pulp from Damazin than those of the other geographical regions in Sudan.

In the aqueous extracts the highest antioxidant activity was observed in baobab fruit pulp from Umm Ruwaba. This might suggest that most of the antioxidants present were less polar and could be extracted by less polar solvent like water. Also, the variation of antioxidant activity obtained from baobab fruit pulp from different geographical regions in Sudan with different solvents systems in baobab fruit pulp might be affected by types and structures of phenolic compound. It had been reported that antioxidant activity is greatly influenced by number and configuration of hydroxyl group, as well as gylcosylation (Cai *et al*., 2004 , Zin *et al*., 2006), for instance, flavonoids without hydroxyl group (e.g. isoflavone, flavanone) tend to have reduced antioxidant activity.

The FRAP assay measured the ability of antioxidant to reduce Fe (3+) to Fe (2+). The results of the FRAP obtained were slightly lower than these of DPPH method. The FRAP values ranged from 203.90 to 222.28 for methanolic extracts , 217.04 to 209.33mmol /g of Fe+2 for ethanolic extracts and 205.80 to 191.64 for aqueous extracts (Table 14), using FeSO4 .7H2O as standard (R2=0.999). In this assay, significant difference was observed between FRAP values of the two types of extracts from different baobab fruit

pulp samples. Moreover, FRAP antioxidant activity of the aqueous extracts revealed that of Damazin baobab fruit pulp was significantly different ($p \leq 0.05$) from that of samples from other geographical regions in Sudan. These differences could be due to the fact that baobab trees grow in mountainous area with heavy rain fall in Damazin as compared to that from other geographical regions in Sudan and contained different components of phenolic acids and flavonoids profile.

A study by Wiehle *et al*., (2014) of baobabs from several locations in the Nuba Mountains, Sudan, reported a substantial genetic variation in which two distinct gene pools appeared. The variations in phenolics, flavonoids profile and antioxidant activity of baobab samples in this study, could probably be due to genetic, environmental factors or both, as well as soil chemical composition.

4.2.19 Phenolic compounds identified in extracts of baobab fruit pulp from different geographical regions in Sudan

The results of phenolic compounds in baobab fruit pulp extracts are shown in Table 15. The molecular weights of these compounds are between 137 and 1097 Da, suggesting that they belong to the class of simple and complex phenolic and flavonoids compounds. These compounds were fragmented to full scan spectra from 100 to 1500 Da by MS/MSon negative mode .The results of these analyses were compared to the fragments of mass spectra, mass bank and database: *http*://www. Phenol- explorer.eu was used for their identification. In Aqueous extract the phenolics and flavonoids profiles of baobab fruit pulp from different geographical regionsin Sudan were found to be composed of different components. The results of an aqueous extracts of baobab fruit pulp from El Obeid are shown in Figure2 (A). As shown in the figure, the extract contains one phenolic acid namely Ferulic acid derivative and 12 flavonoids component namely Cyanidin-3-glucoside, Kaempferol-3-Glucoside, Procyanidin trimer, Cyanidin 3-O-xylosyl- rutinoside, Ipriflavone

derivate , Pinocembrin derivate, Kaempferol glycoside derivate, Kaempferol glycoside derivate isomer, Delphinidin 3-[6-O-(p-coumaroyl)-β-D-glucopyranoside]-5-O-[4-O-acetyl-6-Omalonyl-β-D-glucopyranoside, Malvidin3-O-(6-O-(4-O-malonyl-α-rhamnopyranosyl)-β-glucopyranoside)-5-O-β-glucopyranoside and Kaempferol3-isorhamninoside-7-rhamnoside. Figure 2(B) shows the results of an aqueous extracts of baobab fruit pulp from Umm Ruwaba. It contains four phenolic acids namely ,(+)-(E)-caffeoyl-L-malic acid , Ferulic- caffeoyl glucose derivative, p-Hydroxybenzoic acid and Caffeoyl aspartic acid and eight flavonoids component namely, Procyanidin C_1, Cyanidin 3-O-xylosyl- rutinoside, Ipriflavone derivative, Ipriflavone derivative, Quercetin 3,4’-di-O-β-glucopyranoside, Methyl-myricetin-trihexoside (B), Petunidin 3-glucoside-5-(6''-acetylglucoside) and Theaflavin 3,3'-O-igallate.

Figure 2(C) shows the results of an aqueous extracts of baobab fruit pulp from Nyala, it has two phenolic acids (caffeoyl glucose derivative and Galloylshikimic acid) and five flavonoids components namely Cyanidin 3-glucoside-5,3'-di-(caffeoyl glucoside), petunidin3-glucoside-5-(6''-acetylglucoside),Malvidin3,5-O-diglucoside,Quercetin3,4’-di-O-β-glucopyranoside andQuercetin 3-(6'''-sinapylglucosyl)(1->2)-galactoside. The results of an aqueous extract of baobab fruit pulp from Damazin are shown in Table 15Figure 2(D). It has two phenolic acids, namely caffeoyl glucose and p-Coumaroyl glycolic acid and 11 flavonoids component, namely Robinetinidol-(4α->8)-catechin-(6->4α)-robinetinidol , Delphinidin 3-glucoside-5-(6- caffeoylglucoside, Kaempferol glycoside, Procyanidin C_1, Procyanidin B1, Pinocembrin derivative, Malvidin 3-O-(6''-p-coumaroyl-glucoside), Pelargonidin 3-(6''-malonylglucoside)-5-glucoside, Kaempferol glycoside derivative and Delphinidin3-glucoside-5-(6-acetylglucoside) .

Table (14): Antioxidant capacity, corresponding to the activity expressed as mg /g equivalents of ascorbic acid and mmol/g Feso4 .7H_2 O for each gram of samples (the value is the mean of 4 measures ± SD)

Extracts Samples	Methanolic DPPH mg/g	Methanolic FR AP mmol/g	Ethanolic DPPH mg/g	Ethanolic FRAP mmol/g	aqueous DPPH mg/g	aqueous FRAP mmol/g
El Obeid	223.20±0.99[a]	215.10±0.00[c]	201.1±0.00[c]	211.03±0.02[b]	193.67±0.04[c]	209.50±0.00[a]
Nyala	229.22±0.01[a]	217.24±0.01[b]	205.87±0.01[b]	210.99±0.03[b]	184.70±0.00[d]	206.83±0.01[a]
Umm Ruwaba	211.22±0.03[b]	203.90±0.00[d]	183.50±0.00[d]	209.33±0.02[c]	221.30±0.00[a]	205.80±0.00[a]
Damazin	232.70±0.00[b]	222.28±0.04[a]	227.92±0.00[a]	217.04±0.01[a]	200.17±0.03[b]	191.64±0.70[b]

* Values that bear different superscript letter in the same Colum are significantly different at $p<0.05$.

The aqueous extracts of baobab fruit pulp from different geographical regions in Sudan contain various components of phenolic acids and flavonoids. Phenolic acid (Ferulic acid) was found in baobab fruit pulp from El Obeidand Umm Ruwaba.While caffeoyl glucose derivative was found in Nyala and Damazin baobab fruit.On the other hand the flavonoids component Procyanidin C_1 exists in all of baobab fruit pulp samples except the sample from Nyala , Cyanidin 3-O-xylosyl- rutinoside and Ipriflavone derivative were found in the samples from El Obeid and Umm Ruwaba. Kaempferol glycoside was found in baobab fruit from ElObeid and Damazin, while Quercetin 3-(6'''-inapylglucosyl) was found in baobab fruit from Umm Ruwabaand Nyala.

The results of ethanolic extracts are shown in Figure 3. As shown in Figure 3 (A) the ethanolic extract of baobab fruit pulp from El Obeid contains one phenolic acid (Vanillic acid derivative) and six flavonoids components (Procyanidin trimmer , Kaempferol glycoside derivative, Malvidin 3-glucoside , Pelargonidin 3-O-[2-O-(β-D-xylopyranosyl)-6-O-(methyl-malonyl)-bata-D-alactopyranoside, Pelargonidin3-(6''-malonylglucoside)-7-(6'''-caffeylglucoside) and Cyanidin 3-(6''-(E)-p-coumaryl sambubioside)-5-glucoside.

Figure 3(B) shows the results of ethanolic extract of baobab fruit pulp from Umm Ruwaba which contains one phenolic acid (4,6-Dimethyl-3(4'-hydroxyphenyl)coumarins) and seven flavonoids components (epi- Catechin, Adenosine, Pinocembrin derivative, 7,8,3',4',5'-Pentamethoxy-6'',6''-dimethylpyrano[2'',3'':5,6]flavones, Kaempferol glycoside derivate, Isomer Kaempferol glycoside derivative and Petunidin 3-glucoside-5-(6''-acetylglucoside).

Figure 3(C) shows the results of ethanolic extract of baobab fruit pulp from Nyala, it has one phenolic acid (Ferulic acid derivative)and four flavonoids component (Petunidin 3-glucoside-5-(6''-acetylglucoside), Isomer Petunidin 3-

glucoside-5-(6"-acetylglucoside), Procyanidin dimer digallate (A-type) and Isomer Procyanidin dimer digallate (A-type).

Figure 3(D) shows the results of ethanolic extract of baobab fruit pulp from Damazin, it has two phenolic acids (Caffeoyl aspartic acid and Ferulic acid derivative) and eight flavonoids components (Kaempferol-3-rutinoside , Epicatechin 3,5-di-O-gallate, Quercetin-3-Arabinoside, 3-Hydroxyphloretin 2'-O-glucoside, Cyanidin 3-O-sambubioside, Pelargonidin 3-(6"-malonylglucoside)-5-glucoside, Delphinidin3-(6-malonylglucoside)-5-glucoside and Isomer Delphinidin 3-(6-malonylglucoside)-5-glucoside.

In ethanolic extracts of baobab fruit pulp form different geographical regions in Sudan various component of phenolics and flavonoids were found, The same phenolic acid (Ferulic acid derivative) was in baobab fruit pulp from Nyala and Damazin, while Kaempferol glycoside was found in baobab fruit pulp from El Obeid and Umm Ruwaba, Pelargonidin 3-(6"-malonylglucoside) was found in baobab fruit pulp from Obeid and Damazin. Petunidin 3-glucoside-5-(6"-acetylglucoside) was found in baobab fruit pulp from Umm Ruwaba and Nyala. The variation in phenolic acids and flavonoids profiles may be due to the differences in soil of the regions in Sudan and could be attributed to the climatic and geographical characteristics of the regions studied. The effects of these variables on the phenolic composition have also been mentioned by other authors (Baiano, *et al*. 2013; Taamalli *et al*., 2010). Few researchers have reported the presence of polyphenols including procyanidins (Coe *et al*., 2013; Kamatou *et al*., 2011; Shahat, 2006), procyanidin B2, (-)-epicatechin, (-)-epigallocatechin-3-O-gallate and gallic acid (Tembo *et al*., 2017) in baobab fruit pulp.

This investigation is the first report of phenolcis and flavonoids profile of Sudanese baobab fruit pulp.These results suggests that Sudanese baobab fruit pulp is a promising functional food ingredient.

4.3 Other phenolic and unidentified compounds

Several compounds are found in the negative ion mode. As shown in table (15), the molecular ion m/z (571) base fragment 228 indicates the Stilbenes Resveratrol derivate it was found in all baobab fruit pulp extracted by ethanolic except forthe baobabfruit from Damazin. m/z (517) Citric acid derivate found in baobab fruit from Nyala and Damazin in ethanolic extracts but in an aqueous extract it was found in only in the sample from El Obeid . M/z 191Citric acid was found in ethanolic extract for the baobabfruit from El Obeid. m/z 311(15,16-dihydroxy- 9Z,12Z-octadecadienoic acid) was found in baobab fruit from Umm Ruwaba in ehanolic extracts,m/z 297 (Oxooctadecanoic acid) it was found in baobab fruit from Nyala in an aqueous extract . Unidentified compounds in pulp extracts were indicated by molecular ion [M-H] *m/z* 1042,473,851,897,485,719 fragment ions of compounds are shown in (Table 15).

4.3 Phytochemicals identified, metabolic and biological activity

Baobab fruit pulp from Damazin which is high contain of phenol ,flavonoids, ascorbic acid was used to analyzed for Phytochemical constituents of the methanolic extract of baobab pulp were determined by ^{1}H NMR based metabolomics and LC –MS, alpha-glycosidase and nitric oxide inhibitors.

Table (15): Compounds identified by LC-EPI-MS/MS in extracts of baobab fruit pulp sample

			Aqueous Extracts	
Different locations of Sample	**RT**	**$[M-H]^-$ (m/z)**	**Fragment MS-MS ions (*m/z*)**	**Compound name**
El Obeid	1.32	897	351.03 ,527.04,175.01 ,545.11, 721.07	Cyaniding-3-glycoside
	1.92	515.12	110, 86 ,172	Citric acid derivate
	3.37	952	892.22 , 611.19, 674.16 , 820.25	Granatin B dehydrohexahydroxydiphenoyl
	4.21	407	255.03 ,407.03 , 227.01 ,211.01 243.03 ,283.03	Kaempferol-3-Glucoside
	4.45	687	289.06 ,397.04,245.06, 235.04	Procyanidin trimer
	7.11	293	221.15, 205.14, 148.08, 177.12 192.14, 236.09	Ferulic acid derivative
	8.07	727.42	653.40	Cyanidin 3-O-xylosyl- rutinoside

	11.85	901	842	Delphinidin 3-O-[6-O-(p-coumaroyl)-β-D-glucopyranoside]-5-O-[4-O-acetyl-6-O-malonyl-β-D-glucopyranoside
	12.67	887	826.68 , 854.64	Malvidin 3-O-(6-O-(4-O-malonyl-α-rhamnopyranosyl)-β-glucopyranoside)-5-O-β-glucopyranoside
	13.62	886	825.68,853.62	Kaempferol 3-isorhamninoside-7-rhamnoside
umm Ruwaba	0.60	295	295.06, 176.97,234.97,178.96	(+)-(E)-caffeoyl-L-malic acid
	1.21	1042	527.11, 175.00 ,351.01	Unknown
	1.44	583.03	193, 341 .449	Ferulic- caffeoyl glucose derivative
	4.09	865	289.06 , 407.04, 245.05, 577.11	Procyanidin timer

	5.42	277	116.99 ,145.06	Caffeoyl aspartic acid
	9.63	729	729	Cyanidin 3-O-xylosyl-rutinoside
	9.99	815	410.35, 279.23, 764.48	Ipriflavone derivate
	10.722	851	814.57, 410.35	Unknown
	11.08	642	625.53, 387	Quercetin 3,4’-di-O-β-glucopyranoside
	11.68	817	493.22,449.25,279	Methyl-myricetin-trihexoside (B)
	12.41	681	337.36 ,383.36,426.39 253.23, 223.22 ,365.36 ,408.38 644.59 , 662.60	Petunidin 3-glucoside-5-(6''-acetylglucoside)
	12.41	681	337.36 ,383.36,426.39 253.23, 223.22 ,365.36 ,408.38 644.59 , 662.60	Petunidin 3-glucoside-5-(6''-acetylglucoside)
	12.78	869	519.20, 504.24	Theaflavin 3,3'-O-digallate
Nyala	1.31	387	341	caffeoyl glucose derivative
	1.44	897	351.03 ,527.04,175.01 ,545.11, 721.07	Unknown

	3.12	1097	1079.36,615	Cyanidin 3-glucoside-5,3'-di-(caffeoylglucoside)
	7.81	297	183.00,197.02	Oxooctadecanoic acid
	8.44	325	183,169.99	Galloylshikimic acid
	9.91	680	337.35 ,383.34, 426.37	Petunidin 3-glucoside-5-(6''-acetylglucoside)
	10.48	655	309.33, 355.32	Malvidin 3,5-O-diglucoside
	11.66	642	625.56,387.29	Quercetin 3,4'-di-O-β-glucopyranoside
	12.86	832	796.59 , 634.6, 616.6,	Quercetin 3-(6'''-sinapylglucosyl)(1->2)-galactoside
Damazin	1.19	866	175.00, , 527.09, 351.02	Robinetinidol-(4α->8)-catechin-(6->4α)-robinetinidol
	1.55	431	179.06,59.02, 112.99	caffeoyl glucose

	3.24	1097	1079, 1037.28	Delphinidin 3-glucoside-5-(6-caffeoylglucoside
	4.09	407	255.02, 283.01, 227.05	Kaempferol glycoside
	4.45	687	669.03, 407.07, 397.07, 289.07 245.05	Procyanidin C1
	4.81	577	289.07, 407.05, 245.05, 161.02	Procyanidin B1
	7.29	222	168.84, 184.82	p-Coumaroyl glycolic acid
	9.39	540	255.22, 480.26	Pinocembrin detivate
	10.11	639	620.63, 602.54,572.51, ,382.35, 394.34, 364.33, 338.34	Malvidin 3-O-(6"-p-coumaroyl-glucoside)
	10.84	639	620.63, 602.54,572.51, ,382.35, 394.34, 364.33, 338.34	Isomer Malvidin 3-O-(6"-p-coumaroyl-glucoside)
	10.59	681	337.32, 383.34	Pelargonidin 3-(6"-malonylglucoside)-5-glucoside
	11.69	750	255.23, 283.27	Kaempferol glycoside derivate
	13.38	669	650.00, 323.38 369.37	Delphinidin 3-glucoside-5-(6-acetylglucoside)

Ethanolic Extracts				
Different locations of Sample	**RT**	**[M-H]⁻ (m/z)**	**Fragment MS-MS ions (*m/z*)**	**Compound name**
El Obeid	1.43	191.00	110.97, 86.97	Citric acid
	1.63	571	228.95, 166.97, 86.99	Resveratrol derivate
	4.10	689	397.07, 289.07, 245.05, 535.13	Procyanidin trimer T
	5.27	220	166.85, 184.87, 201.80	vanillic acid derivate
	10.69	749	255.22, 283.27	Kaempferol glycoside derivate
	10.93	735	698.57, 374.28	Malvidin 3-glucoside-
umm Ruwaba	1.67	571	228.95, 166.97, 86.99	Resveratrol derivate
	4.19	245	159.02, 173.03, 187.03	(epi)Catechin
	7.32	265	96.93,79,95	Adenosine

	8.52	452	255.22	Pinocembrin derivate
	9.73	473	392.31,452.29	Unknown
	10.57	454	454	7,8,3',4',5'-Pentamethoxy-6",6"-dimethylpyrano[2",3":5,6]flavones
	11.30	749	255.24, 283.26,227	Kaempferol glycoside derivate
	12.02	752	255.24 , 283.26,227.00	Isomer Kaempferol glycoside derivate
	12.63	719	682.61, 383.38, 337.37, 426.39	Unknown
	13.24	683	337.48 ,383.45, 426.45	Petunidin 3-glucoside-5-(6"-acetylglucoside)
Nyala	1.43	517	110.97, 86.99	Citric Acid derivate
	1.67	571	228.95, 166.97, 86.99	Resveratrol derivate
	7.07	293	221.14,205.11,236.09	Ferulic acid derivative
	8.88	485	485	Unknown
	9.72	683	664.00,337.36 ,383.32 664	Petunidin 3-glucoside-5-(6"-acetylglucoside)
	11.05	682	664.00, 337.44 ,383.41,	Isomer

			426.43	Petunidin 3-glucoside-5-(6"-acetylglucoside)
	11.65	719	682.64, 383.40, 337.39	Unknown
	12.50	879	842.61, 438.36, 680.68	Procyanidin dimer digallate (A-type)
	13.11	879	842.64, 438.36, 680.76	Isomer Procyanidin dimer digallate (A-type
Damazin	0.59	295	277.06, 177.01 , 235.02	Caffeoyl aspartic acid
	1.43	517	110.96, 173.01 ,86.98	Citric Acid derivate
	5.87	593	284.01, 255.02, 227.05, 145.06	Kaempferol-3-rutinoside
	7.19	293	221.15, 205.11, 148.04,177.08	Ferulic acid derivative
	7.92	593	277.21, 152.98, 241.00, 315.03	Epicatechin 3,5-di-O-gallate

	9.72	433	152.99, 170.97 ,279.27	Quercetin-3-Arabinoside
	8.88	452	255.22, 196.042, 78.96	3-Hydroxyphloretin 2'-O-glucoside
	10.81	616	162.83, 581.23	Cyanidin 3-O-sambubioside
	11.65	861	824.60, 644.59, 662.65	Pelargonidin 3-(6"-malonylglucoside)-5-glucoside
	11.90	713	666.59, 410.38, 267.25 367.37,255.00	Delphinidin 3-(6-malonylglucoside)-5-glucoside
	12.99	713	666.61, 410.44 422.42, 267.32,255.00	Isomer Delphinidin 3-(6-malonylglucoside)-5-glucoside

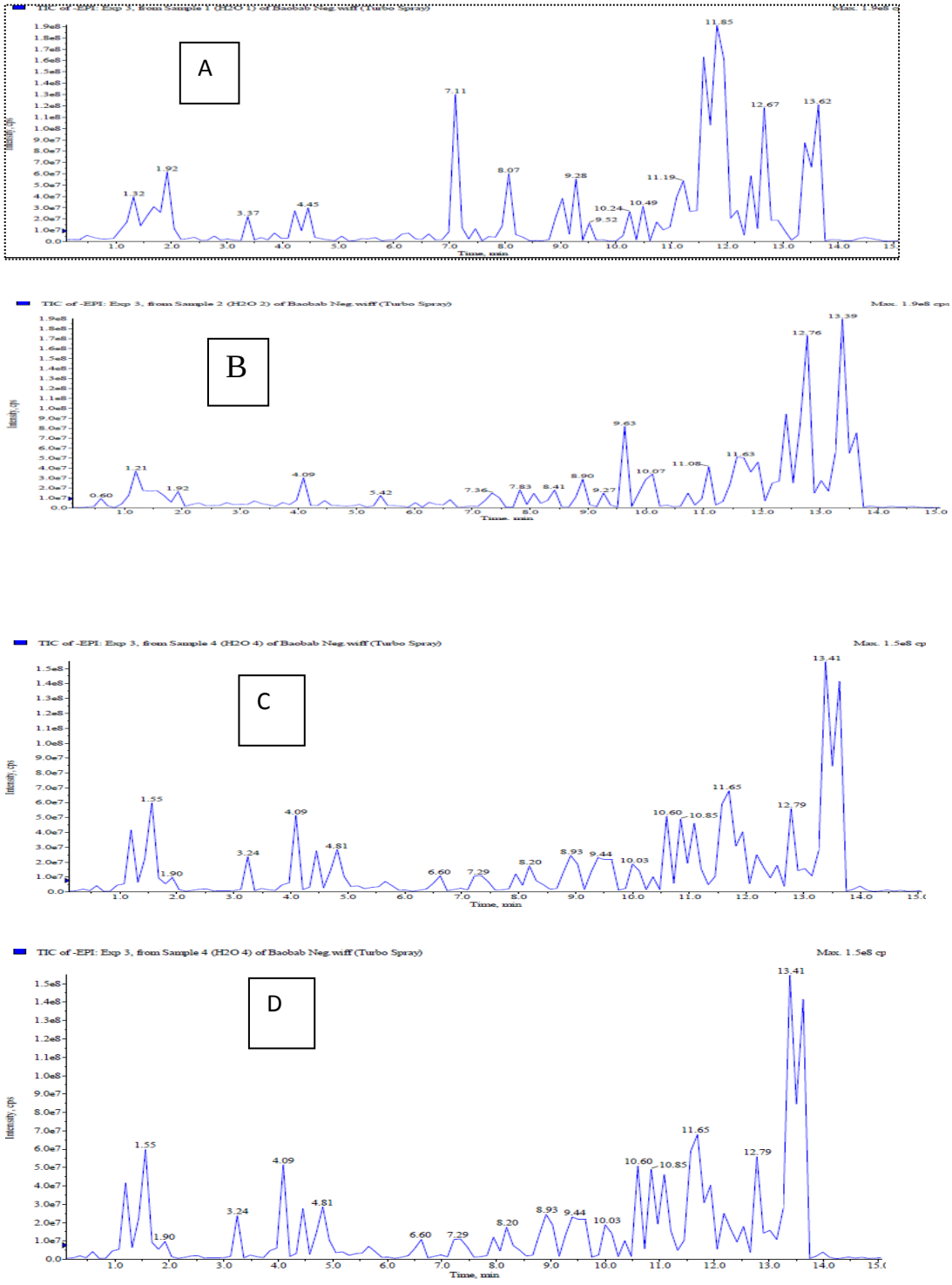

Fig .(2): Full spectrum LC- MS/ MS using aqueous extract of baobab fruit pulp from El Obeid (A) , Umm Ruwaba (B), Nyala (C) and Damazin (D).

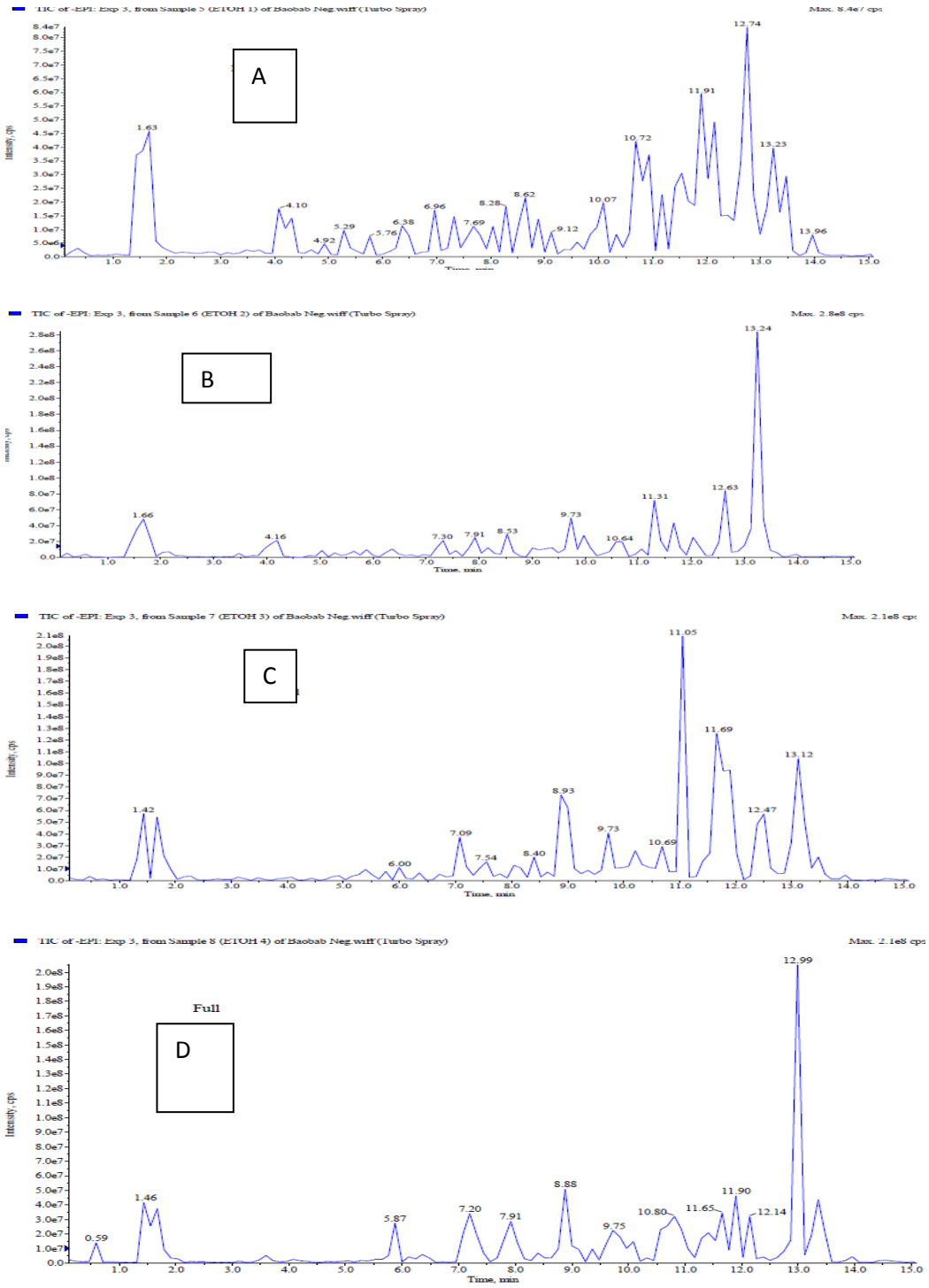

Fig .(3): Full spectrum LC- MS/ MS using ethanolic extract of baobab fruit pulp from El Obeid (A) , Umm Ruwaba (B), Nyala (C) and Damazin (D).

4.3.1LC-MS spectrometry analysis of phytochemical profiles

Total ion chromatogram (TIC) profiles exhibited that most of the major peaks detected in methanolic baobab fruit pulp extract were attributable to the presence of phenolic acids, flavonoids,Sugars, organic acid, lipids, iridoid glycosides and other, (Fig. 4). Summarizes the retention times (RT), m/z and MS/MS data of the detected metabolites in Table (16). Few researchers have reported the presence of polyphenols including procyanidins (Coe *et al*., 2013; Kamatou *et al*., 2011; Shahat, 2006), procyanidin B2, (-)-epicatechin, (-)-epigallocatechin-3-O-gallate and gallic acid (Tembo *et al*., 2017) in baobab fruit pulp.This investigation is the first report of phenolcis and flavonoids profile of methanolic extract of Sudanese baobab fruit pulp.

4.3.1.1 Identification of phenolic acids

baobab fruit pulp included several phenolic acids components as maybe chemical displays was presented based on the negative ion MS/MS data (Table 16), (m/z 405.0285) peak (5) at the retention time (RT) of 1.76min with fragment ions at m/z 191for the characteristic quinic, suggesting a molecular weight of caffeoyl quinic derivative ,Coumaric acid 2-glucoside (m/z 325, peak 11, RT 5.43), coumaric acid(m/z163,peak 28 ,at RT8.03),Feruloyl-β-D-glucose(m/z355,peak16, RT 6.33)and Homovanillic acid Peaks7,8,9and10 showed similar molecular ion peaks at m/z 341 indicating that they are probably isomers, tentatively identified as1-O-caffeoyl-hexose,1-O-caffeoyl-β-D-glucose,6-O-caffeoyl-β-D-glucose and 6-O-caffeoyl-α-D-glucose based on comparison with previous reports (Schutz *et al*., 2004). Sudanese baobab fruit was showed contain several components of phenolic. Although a few researchers have reported the presence of polyphenols including procyanidins and gallic acid (Tembo *et al.,* 2017,Coe *et al*., 2013; Kamatou *et al*., 2011; Shahat, 2006) .

4.3.1.2 Identification of flavonoids

Methanolic baobab fruit pulp extract contained several flavonoids glycosides components as probably chemical indicators was presented in table (16)(Fig.4).Epicatechin was the most abundant of flavonoids detect followed (-)-Epicatechin 3-O-gallate (peak 14) was identified by the deprotonated molecular ion at m/z 440.8891 and the fragment ions at m/z 305 and 190 also peaks 15,19,20, 22, 32 and 37 extract (Fig.) which showed characteristic ions at m/z 289, 865,289,304,440, and 593 (Table 1) attributable to(+)-Epicatechin isomer, Procyanidin C1, (+)-picatechin, (-)-gallocatechin, Catechin 3-O-gallate and Gallocatechin-(4alpha->8)-epicatechin. Peak18 (RT=6.2min) was identified asOsajin. Peak 21, 30 and 33(RT=7.3, 8.24 and 8.44mins**)** were presented as Kaempferol-3-O-glucoside, Kaempferide 3-rhamnoside-7-(6''-succinylglucoside) and Kaempferol-3-O-rutinoside respectively. Peak41 (RT = 10.46min) was identified as Pinobanksin 5-galactosyl-(1->4)-glucoside based on the deprotonated molecular ion at m/z 595, and fragment ions at m/z 433and279. Further MS/MS analysis gave Fustin 7-rhamnoside a fragment ion at m/z 433 and fragment ions at m/z 289and279. Peak 34and35 (RT=8.47and 8.78 mins) were identified as Chrysosplenetin and isomer Chrysosplenetin. Peak 44(RT = 11.75 min) with a deprotonated molecular ion peak at m/z 316 was tentatively assigned as Myricetin. Peak 46(RT=12.21min) as Paratocarpin D. Peak48 (RT = 12.31 min) was identified as quercetin based on the deprotonated molecular ion at m/z 301.

4.3.1.3 Identification of lipids

glycero-3-phosphocholine derivative,1,2-didodecanoyl-sn-glycero-3-phospho-(1'-sn-glycerol),1-pentadecanoyl-2-(13Z,16Z-docosadienoyl)-glycero-3-phosphate,glycero-3-phosphocholine derivative.

4.3.1.4 Identification of sugars and organic acids

Fig (4) exposed that (R)-3,3-dimethylmalic acid,citric acid, D-ascorbic acid, Dehydroascorbic acid, Bassic acid, Cinnamic acid and 2-oxopimelic acid were found in methanolic baobab pulp extract. Peak 1(RT=1.15min), peak 2(RT=1.28 min) and peak3 (RT=1.61min) were identified sugars compounds as Maltotriose, Maltose and Sucrose respectively.

4.3.1.5 Identification of iridoids and other components

Iridoids exhibit a wide range of bioactivity: antitumor, antinflammatory, neuroprotective, antioxidant, cardiovascular, hypoglycaemic, hypolipidemic, antispasmodic, antiviral, antimicrobial, immunomodulator and antiallergic effects (Bas *et al* .,2007).Iridous were detected in baobab fruit pulp extract Fig (4) peak 43, 47 and 52 were identified welloside,Feruloylcatalpol and 8-O-caffeoyl-6'-O-caffeoylajugol.while other compounds were identified in methanolic extract such as scopoletin, taraxerone,adenosine,(-)- asbestinine 2 and Decyl beta-D-maltopyranoside

4.3.2 ^{1}H NMR metabolites in methanolic extract of baobab fruit pulp

Several beneficial health and medicine effects of baobab fruit have been reported (Lamien-Meda *et al*., 2008; Wickens and Lowe, 2008).Nevertheless, there has not been any scientific identification that focused on the relationship of metabolite content with the evaluations of baobab fruit. In this study methanolic extract of baobab fruit was subjected to NMR analyses. Identified metabolites in baobab fruit extract were shown in table (17) and representative ^{1}H-NMR spectrumin Figure (5). Carbohydrates, Maltotriose, Sucrose, Maltose, Arabinose, Glucose, and Xylose. Aside of the carbohydrates organic acids and vitamin were detected such as Citric acid and Ascorbic acid, also amino acids Glycine, Aspartic acid, Glutamic acid, and important amino acid gamma- Amino butyric acid (GABA) were found.

Table (16): Compounds identified by LC MS2 in methanolic extract of baobab fruit pulp

No	RT (min)	m/z [M-H$^-$]	m/z MS2	compound
1	1.15	503.1619	341, 161, 143	Maltotriose
2	1.28	683.2252 [2M-H]$^-$	341, 161, 143	Maltose
3	1.61	683.2252 [2M-H]$^-$	341, 161, 143	Sucrose
4	1.62	161.0445	118, 105, 73, 71, 68, 59	(R)-3,3-dimethylmalic acid
5	1.76	405.0285	354, 191, 173, 155, 147, 129, 111, 87	Caffeoyl quinic derivative
6	2.33	191.0190	147, 103, 87	citric acid
7	4.8	341.1091	204, 179, 161	1-*O*-caffeoyl-hexose
8	4.96	341.1090	179, 161, 133	1-*O*-caffeoyl-β-D-glucose
9	5.11	341.1090	179, 161, 133	6-*O*-caffeoyl-β-D-glucose
10	5.21	341.0879	221, 203, 179, 161, 133	6-*O*-caffeoyl-α-D-glucose
11	5.43	325.0930	187, 163, 145	Coumaric acid 2-glucoside
12	5.51	174.9552	156, 119, 106, 91	D-ascorbic acid
13	5.67	481.1538	407, 345, 362, 190, 170, 113, 69	Decyl beta-D-maltopyranoside
14	5.92	440.8891	305, 190, 175, 169, 147	(-)-Epicatechin 3-O-gallate
15	6.08	289.0719	274, 203, 188, 165, 161, 151, 147, 137, 123, 109, 97	(+)-Epicatechin isomer
16	6.33	355.1036	217, 193, 175, 161	Feruloyl-β-D-glucose
17	6.49	172.957	155, 137, 114, 95	Dehydroascorbic acid
18	6.5	403.0648	318, 290, 245, 179, 137, 125, 113	Osajin
19	6.54	865.199	739, 713, 695, 577,	Procyanidin C1

			289, 273, 245, 137	
20	6.56	289.0718	274, 203, 188, 165, 161, 151, 147, 137, 123, 109, 97	(+)-Epicatechin
21	7.3	447.0935	285, 162, 151	Kaempferol-3-O-glucoside
22	7.4	304.9141	175, 147, 119	(-)-gallocatechin
23	7.51	713.4742	678, 418, 394, 207	1-pentadecanoyl-2-(13Z,16Z-docosadienoyl)-glycero-3-phosphate
24	7.51	836.5875	791, 585, 524, 389, 218, 207	glycero-3-phosphocholine derivative
25	7.61	826.5587	791, 325, 178	glycero-3-phosphocholine derivative
26	7.69	485.3344	439, 421, 370, 337, 242, 224, 195, 161	Bassic acid
27	7.7	475.3056	339, 289, 190, 175, 137	(-)-Asbestinine 2
28	8.03	163.0391	119, 93	coumaric acid
29	8.21	180.9724	168, 153, 141, 93	Homovanillic acid

Table (16): continuation

30	8.24	707.1233	593 (loss of succinate	Kaempferide 3-
31	8.37	146.9602	114), 447, 285	rhamnoside-7-(6"-
			104, 91	succinylglucoside)
				Cinnamic acid
32	8.4	440.889	463, 305, 277, 249,	Catechin 3-O-gallate
			195, 175, 147	
33	8.44	593.1307	447, 285, 151, 145	Kaempferol-3-O-
				rutinoside
34	8.47	372.9015	267, 249, 229, 209,	Chrysosplenetin
			191, 189, 175, 147,	
			119, 94	
35	8.78	372.9017	267, 249, 229, 217,	Chrysosplenetin
			199, 189, 175, 147,	isomer
			119, 97	
36	9.72	310.9312	205, 183, 175, 147,	3(3',4'-
			119	Dimethoxyphenyl)-7-
				methoxycoumarin
37	10.05	593.2734	315, 294, 241, 209,	Gallocatechin-(4alpha-
			165, 153	>8)-epicatechin
38	10.17	423.134	367, 337, 323	Taraxerone
39	10.27	191.1071	175, 124, 118, 93	Scopoletin
40	10.45	265.1479	150, 97, 80	Adenosine
41	10.46	595.2891	433, 279, 153	Pinobanksin 5-
				galactosyl-(1->4)-
				glucoside
42	11.06	433.2361	289, 279, 171, 153	Fustin 7-rhamnoside
43	11.26	699.3816	415, 397, 287, 263,	Welloside
			255, 217, 179, 161	
44	11.75	316.9479	289, 271, 141, 113	Myricetin
45	12.17	172.9571	155, 137	2-oxopimelic acid
46	12.21	407.1875	339, 308, 289, 255,	Paratocarpin D or 3'-
			183	Prenyl-3-(2-hydroxy-
				3-methylbutyl-3-enyl)-
				4,2',4'-
				trihydroxychalcone
47	12.22	537.3284	339, 255, 179, 161	Feruloylcatalpol

48	12.31	301.1662	285, 217, 151, 135	Quercetin
49	12.34	339.2000	310, 279, 216, 183	3'-(2-Hydroxy-3-methylbut-3-enyl)-4,2',4'-trihydroxychalcone
50	12.45	480.3098	461, 435, 413, 345, 283, 196	1-octadecanoyl-sn-glycero-3-phosphoethanolamine
51	12.75	609.2584	552, 549, 515, 473, 405, 181	1,2-didodecanoyl-sn-glycero-3-phospho-(1'-sn-glycerol)
52	14.23	671.2682	489, 421, 331, 179	8-O-caffeoyl-6'-O-caffeoylajugol

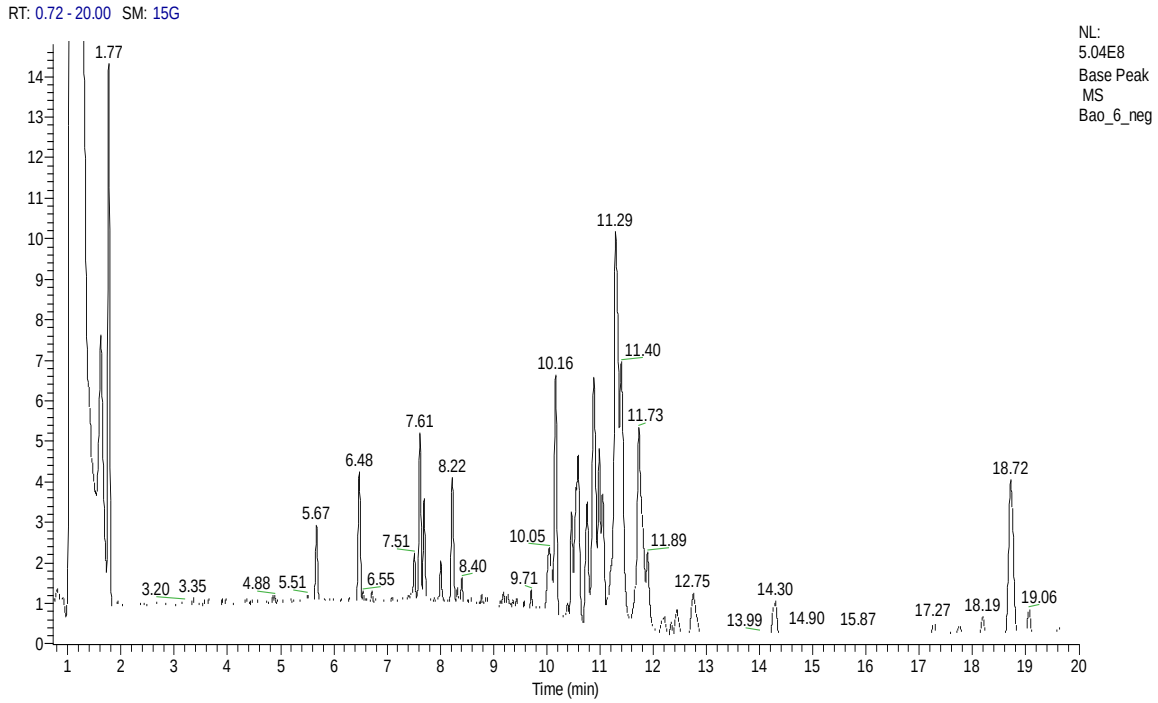

Fig.4. Total ion chromatogram of baobab extract in negative ion mode for peak assignments, see Table 1.

Several components of phenolics are identified in methanolic extract comprising Protocathecuic acid, Caffeoyl-α-D-glucose, Feruloylcatalpol, Caffeoylajugol and Methylgallic acid. Other metabolites like Alkaloids, 3-Methylxanthine, 1-Methylnicotinamide, β-Sitosterol and Fatty acids are also identified. These results are coordinated with previous results in which metabolites, such as specific (Methylgallic acid, Sucrose, Glucose, Ascorbic acid, Citric acid) were readily identified from baobab juice extracted by aqueous (Tembo *et al*., 2017) using NMR spectroscopy.

4.3.3 Nitric oxide inhibitory activity

Nitric oxide is a free radical product in mammalian cells, involved in the regulation of various physiological processes similar to regulation of vascular tone, inhibition of together platelet and leukocyte aggregation and adhesion, and inhibition of cell proliferation (Bredt and Snyder, 1990). However, excess production of nitric oxide (NO) is associated with inflammation (Komatsu *et al*., 2003). Table (18) show the Nitric oxide inhibitory activity of methanol extract of baobab fruit pulp. Methanol extract have Nitric oxide inhibitory activity, more than 90%, with IC50 of 36.55µg /mL.This information is interrelated to the several phenolic components in the methanolic baobab pulp extract (see tables 16 and17). The phenolic compounds have long been believed to have the possible to inhibit NO and peroxynitrite production (Conforti and Menichini, 2011).Also Flavonoids such as apigenin, wogonin, luteolin, tectorigenin and quercetin inhibited NO production the most active among 26 flavonoids derivatives tested by (Kim *et al*.1999) indicating that the presence of antioxidant molecules in all the methanolic baobab pulp extract is responsible for their inhibitory effect. Previous epidemiological studies have shown an inverse relationship between the consumption of antioxidant-rich food and the decrease of risk factors for some human diseases (Udenigwe *et al*., 2009). In addition, the secondary metabolite of plants has also been

reported to act as an excellent anti-inflammatory agent in oxidative stress and inflammation (Sheu *et al*., 2001).

4.3.4 α-glucosidase inhibitor activity assay

Diabetes mellitus is a chronic disorder reasoned by changed metabolism of carbohydrate, and thus creating the hyperglycemic state (Zheng *et al*., 2007). One therapeutic approach in the early stage diabetes is to decrease post-prandial hyperglycemia. It is famous that complex polysaccharides are hydrolyzed by amylases to dextrins which are further hydrolyzed to glucose by intestinal a-glucosidase before entering blood circulation through intestinal epithelium absorption. For this reason, inhibition of these enzymes could reduce the high post-prandial blood glucose levels in diabetics (Tundis *et al*., 2007). The enzyme reaction was performed under in vitro usingp-nitrophenyl-a-D-glucopyranoside (pNPG) as the substrate, which was hydrolyzed by a-glucosidase to release p-nitrophenol, a color agent that can be monitored at 405 nm (Babu *et al*., 2004). In vitro studies had shown that baobab fruit extracts interfere with starch degradation and reduce sugar release from starch-rich foods as bread (Coe *et al*., 2013).However; there are no study that established inhibition of these enzymes by Sudanese baobab fruit extracts. A. digitata fruit extract on the in vitro α-glucosidase inhibitory activity.Table (18) show the α-glucosidase inhibition activity of methanol extract of baobab fruit pulp. Methanol extract have high α-glucosidase inhibition activity, more than 90%, with IC50 of 58.59 µg /mL.Recently it has been confirmed that proanthocyanidins are potential natural α-glucosidase inhibitors (Hsu *et al* .,2018). Furthermore, flavonoid derivatives have been also established to be active as α-glucosidase inhibitors (Silva *et al*., 2016.).Numerous identified compounds (see table 16) have been previously established to be able to inhibit **α**-glucosidase, explaining the most of extracts activity.

Table (17): ^{1}H NMR (in CD3OD+0.1%TSP) characteristics of the identified metabolites in baobab fruit methanolic extract

No.	Tentative compound	δH (ppm)
1.	1-Methylnicotinamide	9.24 (s), 8.92 (d, J= 6.0 Hz), 8.86 (d, J= 8.0 Hz), 8.19 (m), 4.47 (br s, overlapped)
2.	3-Methylxanthine	8.03 (s)
3.	Protocathecuic acid	7.41 (d, overlapped), 7.36 (dd, overlapped), 6.92 (d, overlapped)
4.	Caffeoyl-α-$_{D}$-glucose	7.10 (br s), 6.80 (d, overlapped), 6.97 (m), 5.56 (d, J= 3.5 Hz), 4.46 (dd, overlapped), 4.32 (m)
5.	Feruloylcatalpol	5.18 (d, J= 8.0 Hz), 6.33 (d, J= 6.5 Hz), 5.096 (d, J= 8.0 Hz), (6.83 (d, overlapped, 7.16 (dd, J= 7.5, 1.8 Hz), 3.85 (s)
6.	Caffeoylajugol	5.56 (d, J= 3.5 Hz), 7.10 (br s), 6.78 (d, J= 8.0 Hz), 6.95 (m), 7.57 (d, J= 15.5 Hz), 6.30 (d, J= 15.5 Hz), 1.33 (s), 3.39 (t, J= 9.0 Hz), 4.65 J= 7.5 Hz), 2.36 (dd, J= 13.0, 6.5 Hz), 1.99 (m),
7.	Methylgallic acid	6.97 (s), 3.76 (s)
8.	Maltotriose	5.40 (d, J= 4.0 Hz), 5.11 (d, J= 3.5 Hz), 4.48 (4.18 (d, J= 8.0 Hz), 4.04 (m), 3.28-3.83 (m, overlapped), 3.13 (t, J= 9.0 Hz)
9.	Sucrose	5.40 (d, overlapped), 4.18 (d, J= 7.5 Hz), 4.06 (m, overlapped), 3.42-3.87 (m, overlapped),
10.	Maltose	5.40 (d, overlapped), 5.38 (d, overlapped), 4.67 (d, overlapped), 3.96 (m, overlapped), 3.42-3.87 (m, overlapped), 3.22 (t, overlapped)
11.	β-Sitosterol	5.35 (t, J= 5.0 Hz), 3.58 (m, overlapped), 0.72 (s), 1.02 (s), 0.97 (d, J= 7.0 Hz), 0.86 (d, J= 7.0 Hz), 0.84 (d, J= 7.0 Hz), 0.87 (t, J= 7.0 Hz, overlapped)
12.	Arabinose	5.29 (d, overlapped), 5.28 (d, J= 4.0 Hz), 4.52 (d, overlapped), 3.50-4.11 (m, overlapped)

13.	Glucose	5.22 (d, J= 4.0 Hz), 4.64 (d, J= 8.0 Hz), 3.68-3.86 (m), 3.34-3.45 (m), 3.18 (t, overlapped)
14.	Xylose	5.20 (d, J= 4.0 Hz), 4.57 (d, overlapped)
15.	Ascorbic acid	4.53 (d), 4.01 (m, overlapped), 3.75 (m, overlapped)
16.	Glycine	3.57 (s)
17.	Citric acid	2.90 (d, *J*=15.0 Hz), 2.79 (d, *J*=15.0 Hz)
18.	Aspartic acid	3.88 (m, overlapped), 2.82 (dd, overlapped), 2.65 (dd, *J*=16.0, 8.0 Hz)
19.	GABA	3.00 (t, *J*=7.0 Hz), 2.28 (t, *J*=7.5 Hz), 1.87 (m)
20.	Glutamic acid	2.35 (m), 2.14 (m), 2.07 (m)
21.	Fatty acids	1.28-1.32 (m)

s = singlet, dd = doublet of doublets, d = doublet, dt = doublet of triplets, t = triplet, m = multiplet

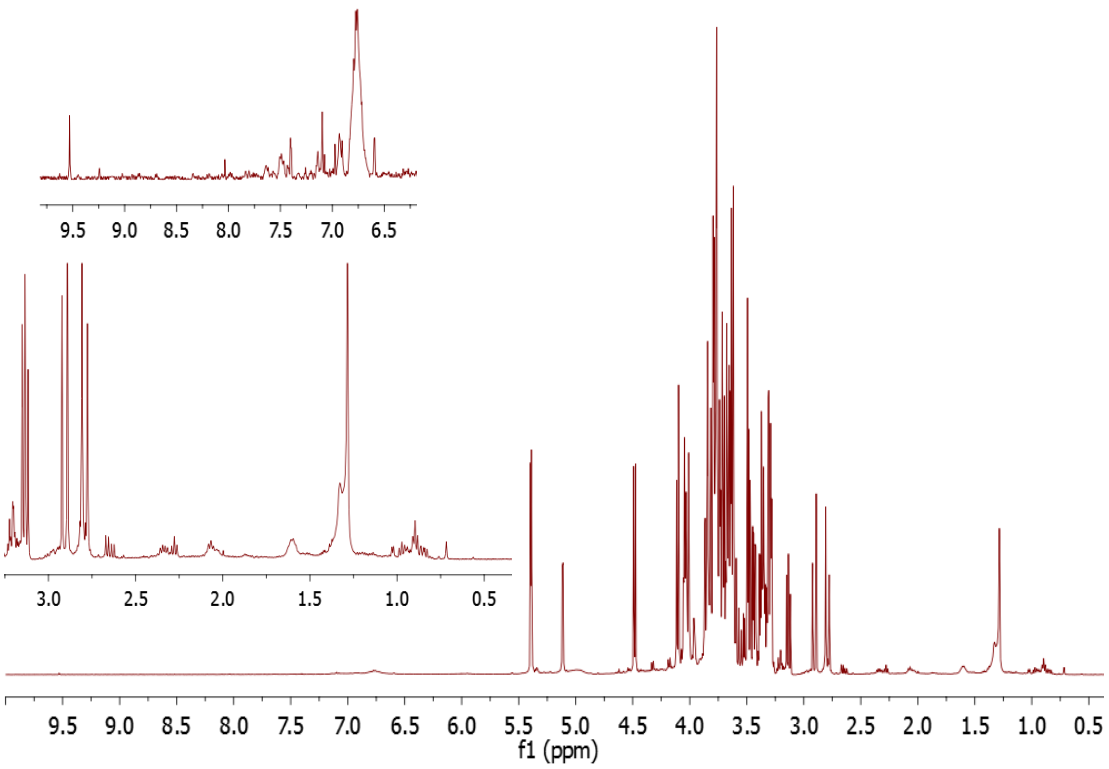

Fig 5: Representative ^{1}H-NMR spectrum identified metabolites in methanolic extract of baobab fruit pulp

Table (18): Nitric oxide and α-Glucosidase inhibitory activity in methanolic baobab fruit pulp extract

α-Glucosidase		**Nitric oxide**	
%inhibition (at 1000 µg/ml)	IC_{50} (µg/mL)	%inhibition (at 500 µg/ml)	IC_{50} (µg/mL)
97.94±0.30	58.59±4.56	98.45±0.59	36.55±1.79

Values are mean ± SD for three replicate independent runs

4.4 Formulation of baobab cream cheese supplemented with *Bifidobacteriumlongum BB53*

According to results after screening of baobab fruit pulp from different locations in Sudan, the sample of baobab fruit from Blue Nile was chosen to prepare for formulating the baobab cream cheese and the analysis of the product were conducted as follow:

4.4.1 Milk composition

Results of the proximate composition and pH of raw cow milk were presented in table (19), the levels of total solids, ash, fat, protein and pH were 10.10, 0.73, 3.26, 3.02 and 6.61, respectively.

4.4.2 Chemical composition of baobab fruit cream cheese

The result presented in table (20) shows the effected of different levels of baobab fruit pulp on chemical composition of cream cheese inculcated with *Bifidobacterium longum BB536.* Moisture content of cream cheese was significantly different (P< 0.05).The highest moisture content in cheese made with addition of10% baobab fruit pulp (A) compared to those made with 15%(B) and 20%(C) of baobab pulp .The value of moisture content ranged from 55.13 -60.35 %. This increase in moisture in cream cheese (A) may be due to the low contain of pulp fruit and low contain of hydrocolloid.

The fat content of cream cheese was also significantly (P<0.05) different. The highest fat content was in cream cheese made with 20 %(C) baobab pulp (25.27%) as compared to (22.25 %) and (21.61 %) in cheese made of 15 %(B) and 10%(A) of baobab pulp, respectively (table20).The fat content was trend to increaseswith an increase baobab fruitpulp. This might be attributed to aspercentage as low moisture content compared with the sample contains low of baobab pulp. According to (Van Boekel, 1993) the fat and protein ratios are interlinked as they form a major fraction of the total solids.

The protein content was significantly (P< 0.05) affected by the addition of baobab fruit pulp table (20).The highest protein content was in cheese made with 10% (A) of baobab fruit pulp in comparison to those made with 15%(B) and 20%(C) of baobab pulp. The value of protein content ranged from 8.25 - 9.21%. This increase in protein in cream cheese (A) may be due to the high level of *bifidobacterium*as protein source amino acid. Another important effect on the protein content was that of the fat and fruit pulp interaction where the protein content showed a slight decrease with an increase in the contraction of the added baobab pulp in the cream cheese types (10%.15%, 20%). This could be due to the fact baobab fruit pulp contains high including fat but low protein level than milk. On the other hand, it is obvious that the protein /fat components which form a major fraction of the total solids are linked where the higher percentage of the one component results in lower percentage of the other. The higher protein content and the lower fat content of the cream cheese contributed to its crumbly and firm texture (Paz *et al*., 1998).

Ash content in cream cheese was affected significant different (P<0.05) by addition of baobab fruit pulp. Ash content of cream cheese was significantly (P< 0.05) higher in cream cheese made with 20 %(C) baobab pulp (1.69 %), than in cream cheese made with15 %(B) 1.6%ash and10%(A) (1.39%ash)of baobab pulp, table(20). The ash content of the samples (B and C) increased with an increase in the % baobab pulp added. This can be explained by the truth that baobab powder has a high ash and mineral contents than in milk.

The baobab powder contains fiber (insoluble and soluble) which are indigestible polysaccharides that could be motive for the increase of crude fiber due to increased baobab pulp, in addition to helping as prebiotics. According to Igbabul *et al*.,(2014) fibers contributes to the health of the gastrointestinal system and metabolic system in human.

The fiber contents of cream cheese were significantly (P< 0.05) increased by different levels of baobab fruit pulp and presence *bifidobacterim BB536* table (20).The highest fiber levelwasin creamcheese made with 15% (C)of baobab fruit pulp. The value of fiber content ranged from 2.35 -3.54 %. This increase in fiber in cream cheese (C) may be due to the high level of fiberin baobab fruit pulp.

Carbohydrates content of cream cheese was significantly (P< 0.05) higher in cream cheese made with 20 %(C) of baobab pulp (6.12 %),as in table (20).Indicates that baobab fruit pulp is rich source of carbohydrate.

4.4.3 Effect of coagulant percentage on vitamin c content and pH values in baobab cream cheese

Table (21) shows the effect of coagulant percentage on vitamin (c). Content and pH values of baobab cream cheese .Vitamin. C content of cream cheese was significantly (P<0.05) different due different levels of baobab fruit pulp. The highest of vitamin C level was in cream cheese made with 20 %(C) of baobab pulp (74.37mg/g), as in table (21) the increases in (Vitamin C) of cream cheese may be due to the highest level of baobab pulp, as it contain the richest source of vitamin c .

The pH values of cream cheese was significantly (P< 0.05) decreased by increased in levels of baobab fruit pulp (table21).This decrease in pH in cream cheese by baobab fruit pulpmight be due to the high contain of ascorbic acid, other organic acids of baobab pulp and organic acids produced by BB536.

4.4.5 The growth of *Bifidobacterium longum* BB536 in different baobab fruit cream cheeses

Comparative growth of *Bifidobacterium longum BB536* in different of baobab cream cheeses (10%A, 15%B and 20%C) is shown in table (22). There were

significant ($p<0.05$) increases in *B. longum BB536* viable count in baobab cream cheese made with 15% (B) of baobab fruit as compared to other samples. The highest *B. longum BB536*growth was 9.05cfu/g in cream cheese made with 15% baobab fruit pulp. These differences in growth result of *B. longum BB536* could be attributed to variances in accessibility and containing level of nutrients required for growth in cream cheeses. Variation percentage of coagulation of baobab cream cheeses could complement the nutrient component of growth medium and increase viscosity However, the growth of strain *B. longum BB536* was affected by addition of different levels of baobab fruit pulp (table). Although of the declining in viable count of B. *longum BB536* in (B) and (C), the count still above the number required to presence in probiotic food which is at least 6 log cfu/g fermented foods (Viderola *et al*., 2000).

4.4.6 Microbiological quality of baobab cream cheese

The Total bacterial count of the different cream cheese types was significantly ($p<0.05$) affected by other components in cream cheese table (23).

The high contain of total bacterial count was found in cream cheese (A) followed by (B) and then cream cheese (C).*E. coli, Staphylococcus aureus, Salmonella,* Yeast and molds counts were not found on detected in all the types of cream cheese table (21).The absence of pathogenic might be affected by high pH and antioxidant properties in baobab fruit pulp. These results is in agreement facts of result polyphenols in pomegranate juice were most likely tannins, which have been shown to inhibit the growth of certain intestinal bifidobacteria, as well as pathogenic *Clostridium* and *Staphyloccocus aureus* (Bialonska *et al* ., 2009).

Table (19): Chemical composition and pH of raw cow milk

Composition andpH	Cow milk
Total solids (%)	10.1±0.10
Ash (%)	0.73±0.03
Fat (%)	3.26±0.06
Proteins (%)	3.02±0.01
pH	6.61±0.01

Values are mean ± SD of three replicates.

Table (20): Chemical composition (%) of three different baobab fruit cream cheeses supplemented with *Bifidobacteriumlongum BB536*

Component	A	B	C
Moisture	60.35 ± 0.14^{a}	58.16 ± 0.22^{b}	55.13 ± 0.46^{c}
Fat content	21.61 ± 0.28^{c}	22.25 ± 0.02^{b}	25.27 ± 0.08^{a}
Protein content	9.21 ± 0.02^{a}	8.95 ± 0.23^{a}	8.25 ± 0.05^{b}
Ash content	1.39 ± 0.01^{c}	1.60 ± 0.05^{b}	1.69 ± 0.04^{a}
Fiber	2.35 ± 0.05^{c}	3.05 ± 0.09^{b}	3.54 ± 0.32^{a}
Carbohydrates	5.10 ± 0.11^{b}	6.10 ± 0.15^{a}	6.12 ± 0.03^{a}

Values are means ±SD (n=3); means with different superscripts in the same row are significantly different ($p < 0.05$).

A: Baobab cream cheese prepared using 10% baobab fruit pulp

B: Baobab cream cheese prepared using 15% baobab fruit pulp

C: Baobab cream cheese prepared using 20% baobab fruit pulp

Table (21): Vitamin c. contents (mg/100g) and pH values of three different baobab fruit cream cheeses supplemented with *Bifido bacteriumlongum BB53*

sample	Vitamin .C	pH
A	47.19± 0.51^{c}	4.39± 0.02^{a}
B	55.29± 0.06^{b}	4.29± 0.02^{b}
C	74.37±0.04^{a}	4.11± 0.00^{c}

Values are means ±SD (n=3); means with different superscripts in the same row are significantly different ($p< 0.05$).

A: Baobab cream cheese prepared using 10% baobab fruit pulp

B: Baobab cream cheese prepared using 15% baobab fruit pulp

C: Baobab cream cheese prepared using 20% baobab fruit pulp

Table (22): The growth of *Bifidobacterium longum* BB536 in three different baobab fruit cream cheeses

Sample	*Bifidobacterium longum BB536* growth in baobab fruit cream cheese (cfu/g)
A	8.60 ± 0.49^{ab}
B	9.05 ± 0.03^{a}
C	8.10 ± 0.03^{b}

Values are means ±SD (n=3); means with different superscripts in the same row are significantly different ($p < 0.05$).

A: Baobab cream cheese prepared using 10% baobab fruit pulp

B: Baobab cream cheese prepared using 15% baobab fruit pulp

C: Baobab cream cheese prepared using 20% baobab fruit pulp

4.4.7 Sensory characteristics of baobab fruit pulp cream cheese

Table (24) shows the sensory characteristics of different cream cheese. All sensory characteristics of cream cheese demonstrated high significant ($P<0.01$) differences except flavor was no significant ($P>0.01$). The values of color scores were 1.53, 2.20 and 2.27 in baobab cream cheese A, B and C, respectively. The high score was found in sample (A) contained lower in baobab fruit pulp compared with other. The score of flavour of cream cheese was no significant ($P>0.01$) differences (table24). The texture of cream cheese was significantly ($P< 0.01$) higher in cream cheese C due to high level of baobab fruit pulp. The values of Saltiness scores were 2.13, 2.53 and3.13 in baobab cream cheese A, B and C respectively. The result indicated that saltines increased by increase level of baobab fruit pulp of cream cheese. The overall acceptability of the baobab cream cheeses were significantly ($P<0.01$) affected by baobab fruit pulp (table24).

4.4.8 Chemical composition of baobab cream cheese during storage

coagulant percentage (baobab fruit) and storage period showed were significant different ($P< 0.05$) effect on moisture, protein,fiber and Carbohydrates and no significant different ($P> 0.05$) were found on ash in all baobab cream cheeses and fat in all baobab cream cheeses except baobab cream cheese (C),Table(25).

As presented in table (25), the highest moisture content of (60.35%) was in baobab cream cheese made using 10% baobab pulp (A) at zero time of storage period. At 15 days and 30 days storage period the highest moisture content of 60.87and 59.67% were in (A) and (B) cream cheese, respectively, the lowest moisture of 58.37and 55.47% were in cream cheese (B) and (C), respectivelyat 30 days of storage period.

Table (23): Microbial load (CFU/g) of different baobab fruit cream cheeses supplemented with *Bifidobacteriumlongum BB536*

Parameters	**A**	**B**	**C**
TBC	4.16 ± 0.00^{a}	4.14 ± 0.015^{a}	4.05 ± 0.05^{b}
E. coli	nil	nil	nil
staphylocoous	nil	nil	nil
Salmonella	nil	nil	nil
Yeast	nil	nil	nil
molds	nil	nil	nil

Values are means ±SD (n=3); means with different superscripts in the same row are significantly different ($p< 0.05$).

A: Baobab cream cheese prepared using 10% baobab fruit pulp

B: Baobab cream cheese prepared using 15% baobab fruit pulp

C: Baobab cream cheese prepared using 20% baobab fruit pulp

Table (24): Effect of baobab pulp on sensory characteristics of cream cheese

samples	Color	Flavour	Texture	Saltiness	Overall acceptability
A	1.53± 0.64^{b}	2.40± 1.06^{a}	2.93± 0.80^{a}	2.13±0.64^{b}	3.00± 0.84^{a}
B	2.20± 0.56^{a}	2.20± 0.86^{a}	2.00± 0.66^{b}	2.53±0.83^{ab}	2.67± 0.62^{ab}
C	2.27± 0.88^{a}	1.73± 0.884^{a}	1.47± 0.64^{b}	3.13± 0.74^{a}	2.00± 0.93^{b}

Values are means ±SD (n=15); means with different superscripts in the same column are significantly different ($p< 0.05$).

A: Baobab cream cheese prepared using 10% baobab fruit pulp

B: Baobab cream chee se prepared using 15% baobab fruit pulp

C: Baobab cream cheese prepared using 20% baobab fruit pulp

These results were similar to that reported by Nabila *et al*., (2015) significant differences on the chemical composition of cream cheese was presented as Influenced by Hydrocolloids during storages. Protein contents of baobab cream cheese were significantly (P<0.05) affected by storage period table (25). The lowest protein contents of cream cheese were 8.09% in baobab cream cheese (C) at 15 days and 8.25% in baobab cream cheese (C) at zero time and the highest values was 10.34% in baobab cream cheese (A) at zero time and 9.99% in baobab cream cheese (B) at 15 days .

The fiber of baobab cream cheese was significantly (P<0.05) affected by storage period table (25). The fiber of the cream cheese made with 20 % of baobab pulp was 3.54% at zero time and decreased to 2.95% at 30days storage. The lowcontain fiber in all baobab fruit cream cheeses during the storage might be due to the consumption of fiber by *bifidobacterium BB536*.

Carbohydrates contents of baobab cream cheese were significantly (P<0.05) affected by addition of percentage of coagulant during storage period table (25). The lowest Carbohydrates contents of cheese was 4.39% in sample (A) at 30 days and the highest values were 6.12% in sample (C) at zero time and 5.69in sample (C) at 15 days in baobab cream cheese. The Carbohydrates contents were affected by growth of *BB536*.

Ash contents of baobab cream cheese showed no significant difference (P> 0.05) during storage period.

4.4.9 Microbiological quality of baobab cream cheese

4.4.9.1Viable growth of *Bifidobacterium longumBB536* in baobab cream cheeses during refrigerated storage

Table (26) shows the viable counts of *B. longum* BB536during refrigeration storage of different baobab cream cheeses. There were significant (p<0.05) increase in *B. longum BB536* viable count in all baobab cream cheeses. The rate of increase in the 15 days of the refrigeration storage were 1.18, 1.39 and

1.41CFU/g in baobab cream cheese (A), (B) and (C) respectively .While in the 30 days the reductions at the refrigeration storage in all cream cheeses compared with growth in 15 days were 0.36, 1.03and 0.64 in the baobab cream cheese (A),(B)and (C)respectively, these decrease may be due to inhibit of growth bacteria because of high acidic and found phenol in baobab fruit pulp in different cream cheeses .

4.4.9.2Microbial loads in different baobab fruit cream cheeses during refrigerated storage

Table (27) shows the total bacterial counts during refrigeration storage of different baobab cream cheeses. There were significant ($p<0.05$) decrease in total bacterial counts in all the baobab cream cheese samples. The rates of decrease in the 30 days of the refrigeration storage were 0.64, 0.65 and 0.64 CFU/g in baobab cream cheese (A), (B) and (C), respectively. This result may be affected by low moisture content and high fat during storage period in cream cheese these reason agreement result by (Varnam and Sutherland, 1994) the high moisture and low fat rapidly growing microbes. *E. coli*, *Staphylococcus aureus*, *Salmonella*, Yeast and molds counts were not found at zero time and were not detected in all the cream cheese samples during the storage periods.

Table (25): Chemical composition of baobab cream cheese during the storage

component	Storage period /days								
	0			15			30		
	A	B	C	A	B	C	A	B	C
Moisture	60.35±0.14^{a}	58.16±0.22^{b}	55.13±0.46^{c}	60.87±0.29^{a}	58.66±0 .22^{b}	56.40±0.17^{c}	59.67± 0.48^{a}	58.36±0.16 ^{b}	55.47 ±0.34 ^{c}
Fat	21.61± 0.28^{c}	22.25± 0.02^{b}	25.27± .08^{a}	21.34± 0.32^{c}	22.16± 0.04^{b}	25.11±0.03^{a}	22.00±0 .80^{b}	22.16±0 .04^{b}	25.11± 0.03^{a}
Protein	9.21 ± 0.02^{a}	8.95 ± 0.23^{a}	8.25 ± 0.05^{b}	9.27± 0.04^{a}	9.10± 0.24^{a}	8.09± 0.08^{b}	10.34 ± 0.06^{a}	10.10±0.24^{a}	9.23± 0.23^{b}
Ash	1.39 ± 0.01^{c}	1.60 ± 0 .05^{b}	1.69 ±0.04^{a}	1.37± 0.02^{b}	1.59± 0.06^{a}	1.67± 0.02^{a}	1.37± 0.02^{b}	1.59± 0.06^{a}	1.67± 0.02^{a}
Fiber	2.35 ± 0.05^{c}	3.05 ± 0.09^{b}	3.54 ± 0.32^{a}	2.25± 0.05^{b}	2.93± 0.08^{a}	3.04± 0.06^{a}	2.23±0.04 ^{b}	2.86 ± 0.06^{a}	2.95±0.05^{a}
Carbohydr -ates	5.10 ± .11^{b}	5.99 ± 0.15^{a}	6.12 ± 0.03^{a}	4.91± 0.06^{b}	5.56± 0.16^{a}	5.91± 0.07^{a}	4.39± 0.46^{b}	4.93± 0.13^{ab}	5.57± 0.34^{a}

Values are means ±SD (n=3); means with different superscripts in the same row are significantly different ($p < 0.05$).

A: Baobab cream cheese prepared using 10% baobab fruit pulp, B: Baobab cream cheese prepared using 15% baobab fruit pulp

C: Baobab cream cheese prepared using 20% baobab fruit pulp

Table (26): The growth of *Bifidobacterium longum* BB536 in different baobab fruit cream cheeses during refrigerated storage

Storage period (days)	Growth of *Bifidobacterium longum BB536* log cfu /g		
	A	B	C
0	9.05 ± 0.03^{a}	8.60 ± 0.49^{ab}	8.10 ± 0.03^{b}
15	10.23 ± 0.03^{a}	9.99 ± 0.02^{ab}	9.51 ± 0.54^{b}
30	9.87 ± 0.04^{a}	8.96 ± 0.02^{b}	8.87 ± 0.01^{c}

Values are means ±SD (n=3); means with different superscripts in the same row are significantly different ($p< 0.05$).

A: Baobab cream cheese prepared using 10% baobab fruit pulp

B: Baobab cream cheese prepared using 15% baobab fruit pulp

C: Baobab cream cheese prepared using 20% baobab fruit pulp

Table (27): Microbial loads in different baobab fruit cream cheeses during refrigerated storage

Parameters (Log CFU/g)	Storages period/ days					
	0			30		
	A	**B**	**C**	**A**	**B**	**C**
TBC	4.16± 0.00 [a]	4.14 ± 0.015[a]	4.05± 0.05[b]	3.52±0.01[a]	3.49±0.01[b]	3.41± 0.02[c]
E. coli	nil	nil	nil	nil	nil	nil
staphylocoous	nil	nil	nil	nil	nil	nil
salmonella	nil	nil	nil	nil	nil	nil
Yeast	nil	nil	nil	nil	nil	nil
Mold	nil	nil	nil	nil	nil	nil

Values are means ±SD (n=3); means with different superscripts in the same row are significantly different ($p < 0.05$).

A: Baobab cream cheese prepared using 10% baobab fruit pulp

B: Baobab cream cheese prepared using 15% baobab fruit pulp

C: Baobab cream cheese prepared using 20% baobab fruit pulp

4.5 Experimental animals

4.5.1Effect of oral feeding on different baobab cream cheeses (Animal assay)

Safety of different baobab cream cheeses in feed rats was assessed by body weight gain, blood biochemistry, blood hematology analysis and microbial groups in colon. Chemical compositions of rats feeding were shown in Table 28.

4.5.2Effect of oral feeding on body weights of rat

The mean values of body weights of rats at initial up to 30 days of study are shown in Table (29). The initial weight was measured before starting treatment and there was no significant difference between the five groups of rats. The highest increase in body weight at30 days of the experiment was observed for groups fed with the cream cheese spreadas compared to the control and other groups. All groups of rats have showed an equal growth pattern with variances in weight gain ranged between 66.17and 94.50 g at the end of experimental treatment period. Reduction in actual weight gain were not observed during the study , this preliminary results point toward the safe profiles of baobab cream cheese fed to the rats. Commonly in animal model studies, losses of weights are signs of negative health effects. The losses could take place in case of illnesses or toxicity (Abdo *et al.*, 2001). Potential interpretation of this observation may be the improved physiological condition of the gut by probiotics and fiber that improved metabolic activity and absorption of nutrients.

4.5.3 Feeding and water consumption intake of rats orally fed with different treatments

The results in Table (30) showed that there were no significant differences ($p > 0.05$) between five different rats groups in feed consumption. The lowest

feed consumption was in the group (B) fed on normal rats feed and given orally baobab cream cheese supplemented with 2% BB536.Moeover,the highest feed consumption was recorded for group (D) fed on normal rats feed and given orally cream cheese spread . But significant differences ($p < 0.05$) in water intake consumption that may be affected by nutrients composition of treatments fed.

4.5.4 Blood hematology and biochemistry of blood

Hematological parameters of the rats were examined as shown in Table (31).There are no significant differences ($p>0.05$) in some results obtained on complete blood count (CBC) analysis. While there were significant differences ($P\leq 0.05$) increases in the (HGB and Lymph) in all treatments except group (A), Neutr, and platelet count wereincreased in all groups as compared with control. Treatment with baobab cream cheese did not produce any harmful effect on haematological parameters but it improved haematological parameter.The increase in the HGB of rats treated by baobab cream cheese might be due to the fact that the baobab fruit is rich in vitamin C that could enhance the synthesizes of red blood cells by increased absorption of iron in blood.Therefore increased in the concentrationof lymphocytes is an indication that baobab cream cheese contains bioactive compounds result in improved immune system.While the reduced monocytes suggest absence of contamination.In general there are good indications of absence in inflammation and toxicity. This result is agreement with previouse reported on lymphocyte proliferation and immune modulatory properties of the spices by Yiming *et al*., (2012). Moreover, all the changes observed in blood hematologywere within normal ranges of rats. Thus indicating absence of malnutrition state deficiency in all treated of groups rats.

4.5.5 Biochemistry parameters of rats orally fed with different cream cheeses

As presented in Table (32) blood glucose decrease in all groups received baobab cream cheese as compared to the control .However all levels of glucose were within the normal range of rats. Baobab fruit pulp is rich source of phenol, flavonoids and fiber, also baobab fruit was inhibition of alpha glycosidase. Therefore affected levels of glucose in blood of treatment rats after. This finding is in agreement with reported by Kim *et al*. (2011) and Rojo *et al*. (2012).They found that Polyphenols may also reduce the glycemic response (GR) in in vivo. Also baobab fruit was found to reduce starch digestion in in vitro and in vivo experiment thus reduced the glycemic response (Coe *et al*., 2013).

Total protein level was higher ($p<0.05$) in (B) followed by D, A, C groups of treatment rats but the lowers level was in control group. The concentration of globulin and albumin were not significantly difference ($p< 0.05$) between rats groups. Their highest levels groups received the treatment than in the control group. Electrolytes play vital role in keeping body fluid pH and the dynamic equilibrium of water (Radostits *et al.*, 2007). Effect of oral Administration of baobab cream cheese on serum electrolytes Levels were show in Table (32).Sodium, potassium, calcium, phosphate and urea concentrations in the blood of treated rats groups were significantly ($P< 0.05$) differences from that of the control group.

Table (28): Chemical composition (%) of different experimental diets

Component	Constituents (%)			
	Control	Baobab cream cheese	Baobab cream cheese supplemented by BB536	Cream cheese spread
Moisture	6.09±0.08	55.34±0.08	55.13± 0.46	54.88±0.29
Fat content	8.02±0.08	25.10±0.03	25.27± 0.08	22.56±0.26
Protein content	15.62±0.35	14.15±0.18	8.25 ± 0.05	7.57±0.03
Ash content	1.87±0.18	1.69±0.04	1.69 ±0.04	0.93±0.05
Fiber	2.11±0.11	4.25±0.06	3.54 ± 0.32	0.00±0.00
Carbohydrates	66.15±0.51	1.16±0.29	6.12 ± 0.03	14.98±0.24

Values are mean ± SD of three replicates.

Table (29): Weight per g of different groups of rats on oral feeding by different treatments up to 30 days

Parameter	Oral feeding groups				
	Control	**A**	**B**	**C**	**D**
Initial body weight(g)	95.00±17.60[a]	98.00 ±10.66[a]	95.67±17.76[a]	99.50± 8.02[a]	99.50± 18.83[a]
Final body weight(g)	182.30± 25.5[a]	181.17±15.89[a]	177.67± 12.42[a]	165.67± 14.60[a]	194.00±18.75[a]
Weight gained (g)	87.33± 3.95[ab]	83.17± 6.18[ab]	82.00± 16.38[ab]	66.17± 14.16[b]	94.50± 15.49[a]

Values are means ±SD (n=6); means with different superscripts in the same row are significantly different ($p \leq 0.05$).

All groups feed on normal diets feed and oral of 2.5 ml of different diets treatment

Control group: orally fed sterile water,A: orally fed baobab cream cheese without added *Bifidobacterium longum 536*.

B: orally fed baobab cream cheese supplemented with 2% *BB536.*

C: orally feed duple dose of baobab cream cheese supplemented with 2% *BB536*.

D: orally feed cream cheese spread.

Table (30): Feeding intake and water consumption

Parameter	Oral feeding groups				
	Control	**A**	**B**	**C**	**D**
Food Intake(g/day)	110.61± 34.97[a]	104.78±36.06[a]	95.39± 44.44[a]	104.78± 6.06[a]	108.30±37.59[a]
Water consumption (ml/day)	214.35± 44.50[a]	167.70± 43.74[b]	198.6± 56.8[ab]	175.65± 38.12[b]	175.30± 35.64[b]

Values are means ±SD (n=6); means with different superscripts in the same row are significantly different ($p \leq 0.05$).

All groups feed on normal diets feed and orall of 2.5 ml of different diets treatment

Control group: orally fed sterile water

A: orally fed baobab cream cheese without added *Bifidobacterium longum 536.*

B: orally fed baobab cream cheese supplemented with 2% *BB536.*

C: orally feed duple dose of baobab cream cheese supplemented with 2% *BB536.*

D: orally feed cream cheese spread.

Table (31): Hematology parameters of rats orally fed with different cream cheeses for a period of 30 days

Parameter	Treatments				
	Control	**A**	**B**	**C**	**D**
WBCs /ʟ	8.20±2.04 [a]	6.23 ±0.23[a]	7.27±1.19[a]	6.16± 0.51a	8.10± 1.90a
HGB g/dl	14.33± 0.49ab	15.30±0.27a	14.80±0.20ab	14.20±0.36b	13.87± 0.55b
RBCs/ʟ	8.03± 0.15a	8.14 ±0.40 a	8.44± 0.19a	6.99± 1.54a	7.90 ±0.33a
HCT %	47.13 ±1.79a	46.20 ±3.06 a	47.23± 0.98 a	46.07± 1.99a	44.60± 1.87a
MCV/fl	58.77 ± 2.39a	56.80±1.39a	56.00± 0.27a	56.17± 1.67a	56.50± 1.25a
MCH/pg	17.83± 0.60a	17.93± 0.50a	17.50 ±0.27 a	17.27± 0.85a	17.50± 0.78a
MCHC/g/dl	30.37 ± 0.31a	31.60± 0.56a	31.2± 0.35a	30.83± 0.68a	31.03± 0.74a
RWD Cv/%	16.30 ± 1.04[a]	15.27± 0.30[a]	15.17 ±0.75[a]	16.13± 0.3[a]	16.30± 0.87[a]
RDW-SD/%	33.97 ± 1.80a	31.33± 1.50a	30.80± 0.52a	31.97± 0.98a	32.53± 0.98a
PLT/ʟ	717.67±13.58 b	733.7± 2.30ab	763.70±54.40ab	826.70± 32.3a	759.70 ±36.10ab
MPV/fl	6.70 ± 0.44a	6.63± 0.30a	6.80± 0.30 a	6.60± 0.36a	7.10 ±0.46a
PCT%	0.48 ± 0.04a	0.53± 0.09a	0.52± 0.05a	0.45± 0.15a	0.56 ± 0.08a
Neutr%	47.00± 0.61ab	47.00± 3.61ab	41.33 ±2.08b	48.67± 6.11ab	52.67 ±1.53a
Lymph%	42.00± 0.57ab	43.67± 4.04ab	49.33± 3.21a	42.00± 4.58ab	36.67 ± 2.31b

Cont:

Monocy%	8.00 ±1.73[a]	7.67 ± 2.08[a]	7.33± 0.58[a]	7.00± 1.00[a]	7.33 ±1.53[a]
Eosinoph%	3.00 ± 1.00[a]	1.67 ±0.58[a]	2.00± 1.00[a]	2.00± 0.00[a]	3.33 ± 2.31[a]
Bosinoph%	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]

Values are means ±SD (n=3); means with different superscripts in the same row are significantly different ($p \leq 0.05$).All groups feed on normal diets feed and orall of 2.5 ml of different diets treatment. Control group: orally fed sterile water

While creatinine and uric acid did not show significant ($p>0.05$) differences between all treated groups of rats. Creatinine, potassium ion and sodium ion high level in blood indicate kidney damage. They supply as markers of renal function. Urea, however, is formed at variable rates perhaps at increased cases of high protein diet, Gastro intestinal bleed and excretion which also depend upon extent of water reabsorption (Jozef *et al*., 2002). In this study, serum electrolytes in all treated rats group were showed significantly ($P< 0.05$) differences as compared with controls groups. Nevertheless, the values were within the normal ranges of rats. Thus, these findings suggest that baobab cream cheese may not affect the normal kidney function.

Evaluation of liver functions is very important in toxicity assessment because organs are necessary for the endurance and welfare of an organism. Referring to Table (32); AST and ALT did not differ significantly ($P>0.05$) as compared to the control. Also D.Billi was not significantly differences between groups in all treated, but ALP was significant different . Due to its distinctive abundance in the cytoplasm of liver cells, ALT has been commonly used as a marker to quantify suspected liver cell damage (Giannini *et al*., 2005). AST is more ubiquitous in nature. Moreover, it makes up to 80% and 20% of the total intracellular enzymes in hepatic mitochondria and cytoplasm, respectively, it is generally found in the heart, skeletal muscle, kidneys, brain, pancreas and blood cells (Sherlock ,2008; Mistry,2013). Any decreases in the level of serum ALT, AST, and ALP activity are indication of hepatic disease (Henry,1984).On the other hand the increase of ALP activity in serum is mainly due to the leakage of enzymes from the liver cells cytosol into the blood stream, which gives an indication of hepatotoxicity (Mansour *et al.* ,2002). Commonly, the serum ALP level increase a result of metabolic changes in the liver, such as administration of toxin, cirrhosis of the liver, hepatitis, and liver cancer (Chalasani *et al* .,2004) in this study all levels of enzymes were within the normal range of serum activity of rats. All groups

treated with baobab cream cheese showed the best result in serum activity.Could be explaned by the high antioxidant activity, vitamin. C, anti-inflammatory.Thus, a-myrin exhibit protective activities against liver damage in agreement with finding Al-Qarawi *et al*. (2003).

Table (32) shows the changes in plasma Triglyceride TC, HDL, VLDL and cholesterol levels at the finish of the experimental time. HDL level was higher ($p<0.05$) in group (A) followed by C, B, control and lower level in D. This study showed that baobab cream cheese with and without supplemented by BB536 is successful in reducing plasma TC, VLDL and cholesterol levels of rats. The effect of *B. longum* BB536 increased in the presence of a prebiotic. These findings are in agreement with prior studies reportingthat the consumption of fermented milk including *bifidobacteria* (10^9cfu/g) by hypercholesterolaemic human subjects resulted in a reduction of the TC level from 300 to 150 mg/100 ml (Homma, 1988). Consumption of yoghurt containing BB536 resulted in a noteworthy decline in TC and LDL-C levels (Al-Sheraji *et al*., 2012), while consumption of a probiotic yoghurt enriched with *B. longum* 913, *L. acidophilus* 145 and 1% oligofructose caused an increase of plasma HDL-C (Kiessling *et al*., 2002).In addition also baobab cream cheese contain high level of phenol, antioxidant activity and vitamin c those components were affected on Triglyceride TC, HDL, VLDL and cholesterol levels. These results were in agreement with previous result reporting that quercetin dihydrate and gallate supplements both significantly lowered the plasma lipid and hepatic cholesterol levels compared to those of the control (Bok *et al*., 2002),also The present result agree with previous reported by Chatterjea and Shinde (2002) which examined a reduction in serum cholesterol in experimental animals administered with vitamin C, and of recent, the ability of the vitamin to inhibit the oxidation of HDL evenin humans (Hillstrom *et al*., 2003).

4.5.6 Different microbial groups in colon of rats

Chose microbial groups in colon of rats fed with different baobab cream cheese are detailed in Tables 3media. Based on feeding trials, changes in colonic microbial groups of the rats are ensured. Different microbial groups in colon of rats as presented in Table (33).

Total aerobes in the large bowel changed little due to the ingestion of baobab cream cheese compared with the control.

Total aerobes of colon significant different increased in all groups of rats received the different baobab cream cheese as compared to the control. Group (D) showed the highest increase in total aerobes in colon (8.56log CFU/ g) However, (B) group recorded deceased of 7.07log CFU/ g that might be due to activity of the viable supplements. In general, total anaerobes were also higher for rats fed with baobab cream cheese compared with the control, but deceased in D group. This result may be affected by *bifidobacteriumBB536* and fiber contain in baobab pulp. Although prebiotics offer one rational approach to the probiotic concept, the health consequences have not yet been defined. In theory, a number of potential benefits may arise (Loo *et al*., 1999). However, it may be that enhanced resistance to pathogens offers the most feasibility. The bifidobacteruim and lactic acid bacteria of the gastrointestinal tract are thought to play a significant role in improved colonization resistance.

Feeding with baobab cream cheese and cream cheese spread encouraged the highest lactobacillus increase in colon compared with control group. Bifidobacteria displayed the highest increased among all microbiota communities in colon as fine. However, the increases were significant ($P < 0.05$) in all groups with baobab cream cheese (Table 33) was recorded increased in group B (7.77) followed C (6.43) and A (6.22 CFU/g).Increased bifidobacterial numbers in the gut may be one factor that contributes towards improved competitive exclusion of pathogens. Moreover, many lactobacilli

and bifidobacteria are able to produce natural antibiotics, which can have a broad spectrum of activity against various intestinal pathogens (Gibson and Wang, 1994; Shiba *et al*., 2003).Lactobacillusand Bifidobacterium are non-pathogenic bacteria of the bowel tract. These micro-organisms may increase resistance to disease by reducing the growth of pathogenic and putrefactive bacteria by producing inhibitorysubstances, competing directly for substrates and mucosal attachment sites (Coconier *et al*., 1998; Finlay and Falkow, 1989; Jacobsen *et al*., 1999).

The harmful pathogens of gut microbiota contain species of Coliform Salmonella and Staphylococcus in Table (30) all microbial groups were showed significant different ($p<0.05$) decreased in all groups treated compared with control. The *coliform* was not found in colon of treated C and D but decreased in other groups also salmonella was not found in colon of A and, C this result may be affected by high doses of bifidobacterium and level of baobab pulp in C on the other wise in D may be affected by potassium sorbet while in D may be affected by antioxidant activity in baobab pulp .While Staphylococcus was decreased in treated D followed by B, C and A. These results showed that baobab cream cheese may be different effected on the growth of the harmful pathogens which decreased in the colon compared with other. This affected may be due to the high fiber and antioxidant contains in baobab fruit.

Table (32): Biochemistry parameters of rats orally treated of different cream cheese for a period of 30 days

Parameter	Treatments				
	Control	A	B	C	D
Glucose(mg/dl)	108.00±0.58^{ab}	97.33± 2.52^{ab}	101.33± 0.97^{ab}	93.67± 1.16^{b}	111.33±2.31^{a}
T. protein(g/dl)	6.27 ± 0.15^{b}	6.47± 0.12^{ab}	6.70 ± 0.27^{a}	6.40 ± 0.10^{ab}	6.50± 0.00^{ab}
Albumin. (g/dl)	2.50 ±0.36^{a}	2.73 ±0.12^{a}	2.63 ±0.12^{a}	2.63± 0.06^{a}	2.67± 0.31^{a}
Globulin.(g/dl)	3.77 ± 0.23^{a}	3.73± 0.12^{a}	4.07 ± 0.15^{a}	3.77 ±0.06^{a}	3.83±0 .31^{a}
Na(mmol /L)	136.58±1.23^{bc}	139.83±0.91^{a}	135.77± 0.03^{c}	139.15±0.04^{ab}	133.81±1.81^{c}
K/mmol/l	3.05±0.03^{ab}	2.96± 0.13^{b}	3.20± 0.13^{ab}	3.35± 0.27^{a}	3.07± 0.01^{ab}
phos/mmol/l	4.87± 0.01^{c}	5.38± 0.05^{a}	4.01± 0.05^{e}	5.28± 0.00^{b}	4.75± 0.02^{d}
Ca/mmol/l	2.13± 0.03^{b}	3.03± 0.00^{a}	2.61± 0.24^{ab}	2.55± 0.41^{ab}	2.63± 0.21^{ab}
blood urea/mg/dl	40.67± .58^{b}	50.00± 3.46^{a}	42.67 ± 3.51^{b}	46.67±1.53^{ab}	42.00^{b}±1.00
creat/mg/dl	0.80±0.10^{a}	0.63± 0.15^{a}	0.80± 0.30^{a}	0.83± 0.21^{a}	0.60± 0.10^{a}

uric acid/mg/dl	4.30± 0.44[a]	4.77± 0.21[a]	4.47± 0.16 [a]	4.30±0.27 [a]	4.37± 0.21[a]
D.Billi/mg/dl	1.13± 0.49[a]	0.57± 0.21[a]	0.87± 0.21[a]	1.33±0.12 [a]	0.87±0.31[a]
AST/U/L	171.67±5.13[a]	160.00± 12.73[a]	154.33±16.86[a]	164.67±12.50[a]	181.33± 3.21[a]
ALT/U/L	86.33± 8.33[a]	98.7± 20.6 [a]	95.67± 6.66[a]	89.67± 11.59[a]	100.33±10.97[a]
ALP/U/L	97.67± 6.11[c]	122.00± 0.65[a]	107.33 ± 0.21[bc]	116.33± 3.06[ab]	124.33±5.13[a]
HDL/mg/dl	34.27± 1.29[b]	46.17 ± 3.19[a]	38.88± 0.53[b]	37.50± 0.44[b]	27.47± 2.28[c]
Cholesterol /mg/dl	95.67 ±3.51[b]	90.67± 2.08[b]	80.67± 2.08[c]	89.33± 1.53[bc]	114.00± 6.00[a]
Triglyceride/mg/dl	132.00±0 .65[a]	117.33± 8.14[ab]	101.33 ± 3.21[b]	110.33± 1.[ab]	128.70± 21.2[ab]
VLDL	26.40± 0.53[a]	23.47± 1.63[ab]	20.27± 0.64[b]	22.07± 0.31[ab]	25.73 ± 4.24[ab]

Values are means ±SD (n=3); means with different superscripts in the same row are significantly different ($p \leq 0.05$).All groups feed on normal diets feed and orall of 2.5 ml of different diets treatment

Control group: orally fed sterile waterA: orally fed baobab cream cheese without added *Bifidobacterium longum 536*.

B: orally fed baobab cream cheese supplemented with 2% *BB536*

.C: orally feed duple dose of baobab cream cheese supplemented with 2% *BB536*. D: orally feed cream cheese spread.

Table (33): Effect oral feeding of baobab cream cheese on intestinal flora of rats

Bacterial groups	Treatments				
	control	A	C	B	D
Total anaerobe	6.97±0.60^{b}	7.39±0.60^{b}	7.40 ±0.03^{b}	7.07±0.04^{b}	8.56±0.49^{a}
Total aerobe	6.12± 0.565^{b}	6.48±0.35^{ab}	7.45±0.44^{a}	7.50 ±0.40^{a}	6.42± 0.34^{ab}
Lactobacillus	5.59 ± 0.43^{b}	7.13 ±0.04^{a}	6.57± 0.43^{ab}	7.59 ± 0.47^{a}	7.38 ±0.57^{a}
Bifidobacteria	5.28 ±0.99^{b}	6.22±0.07^{ab}	6.43± 0.95^{ab}	7.77 ±0.04^{a}	6.20±0.11^{ab}
Coliform	4.21±0.04^{b}	3.89±0.01^{c}	0.0±0.00^{d}	4.48 ±0.05^{a}	0.00±0.00^{d}
Salmonella	4.86±0.05^{a}	0.00±0.00^{b}	0.00±0.0^{d}	3.48±0.15^{c}	3.89±0.04^{b}
Staphylococcus	4.19± 0.14^{a}	3.29± 0.17^{c}	3.54 0.27^{bc}	3.82±0.03^{ab}	4.00±0.01^{a}

Values are means ±SD (n=3); means with different superscripts in the same row are significantly different ($p \leq 0.05$).

Control group: orally fed sterile water.A: orally fed baobab cream cheese without added *Bifidobacterium longum 536*. B: orally fed baobab cream cheese supplemented with 2% *BB536*.C: orally feed duple dose of baobab cream cheese supplemented with 2% *BB536*. D:orally feed cream cheese spread.

CHAPTER FIVE
CONCLOUSIONS AND RECOMMENDATIONS

5.1 Conclusion:

Baobab fruits from different locations of Sudan have high variation in morphological characteristics, minerals, pectin, sugars contents and very rich in vitamin C.

Sudanese baobab fruit pulp showed characteristic qualitative profile of flavonoids and phenolic compounds, high antioxidant capacity, is rich in phenolics, flavonoids, and a good source of natural antioxidant.

Baobab fruit pulp from Damazin has high contents of phenolics, flavonoids and antioxidant activity compared with that for from other locations.

Baobab pulp showed variaty of carbohydrates(Maltotriose ,Sucrose, Maltose, Arabinose , Glucose ,and Xylose) , organic acids(Citric acid), vitamin (Ascorbic acid), amino acids(Glycine ,Aspartic acid , Glutamic acid and protein amino acid (GABA), phenolic compounds (Protocathecuic acid, Caffeoyl-α-$_D$-glucose, Feruloylcatalpol , Caffeoylajugol, and Methylgallic acid)Alkaloids(3-Methylxanthine) , (1-Methylnicotinamide(1),(β-Sitosterol)and Fatty acids.

Baobab fruit pulp contains several beneficial phytochemicals and could have potential positive effect on general human's health.

The inference from experiment of bioassay was that the baobab pulp extract had inhibitory effect on alpha glycosidase and NO activity.

Baobab fruits pulp cream cheese could be produced from milk without rennet and whey separation with high yield.

Baobab cream cheese supplemented with BB536 on rats based on weight gains, blood hematology and biochemistry analysis and it has promoted improved intestinal environment, and as such could be considered as functional food.

5.2 Recommendations:

1/ Incorporation of baobab fruit pulp into industrials dairy products.

2/Further investigations for:

- Quantitative phytochemical profile of Sudanese baobab fruit pulp.

- Qualitative and quantitative of bioactive components in baobab (seed, epicarp and red fiber).

- Using baobab fruit pulp for treatment of cancer, anemia, cholesterol and type*2diabetes (*T2D).

REFERENCES

Abas, F.; Lajis, N.H; Israf, D.A; Khozirah, S. and UmiKalsom, Y. **(**2006). Antioxidant and nitric oxide inhibition activities of selected Malay traditional vegetables. Food Chemistry; 95:566–573.

Abd El-Salam, M. H; Hippen, A. R; Salem, M. M; Assem F. M. and Aassar, M. El. (2012). Survival of probiotic Lactobacillus casei and Enterococcus fecium in Domiati cheese of high conjugated linoleic acid content. Emir. J. Food Agric. 24 (2):98-104.

Abdo, K.M; Cunningham, M.L; Snell, M.L; Herbert, R.A; Travlos, G.S; Eldrige,S.R. and Bucher, J.R. (2001). 14 Week toxicity and cell proliferation of methyl eugenol administered by gavage to F344 rats and B6C3F1 mice. Food and Chemical Toxicology 39: 303 – 316.

Adams, M.R. (1999). Safety of industrial lactic acid bacteria. J. Biotechnology. 68: 171–178.

Afolabi, O.R. and Popoola, T.O.S. (2005). The effects of baobab pulp powder on the micro flora involved in Tempe fermentation. European Food Research and Technology.220 (2):187–190.

Agbessi Dos-Sintos, D. and Tom, I. (1996).Manual dentitionAfricaine; elements de base appliqué. Dahar: acct, IPDetEdition karthala (cited in Agroforesty today, Vol. 8-12).

Ajayi, I.A; Dawodi, F.A. and Oderinde, R.A. (2003). Fatty acid composition and metal content of Adansonia digitata seeds and seed oil. La Rivista Italiana delle Sostanze Grasse, 80: 41-43.

Akalin, A.S; Gonc, S; Unal, G; and Fenderya, S. (2007). Effects of Fructooligosaccharide and whey protein Concentrate on the Viability

of Starter Culture in Reduced-Fat Probiotic Yogurt during Storage. Journal of Food Science; 72: M222-M227.

Alander, M; Satokari, R; Korpela, R; Saxelin, M; Vilpponen- Salmela, T;Mattila-Sandholm,T. and von Wright, A. (1999). Persistence of colonization of human colonic mucosa by a probiotic strain, Lactobacillus rhamnosus GG, after oral consumption. ApplyEnvironment Microbiol. 65: 351–354.

Alkalin, A.S; Fendery, S.A.K; and bulut, N. (2004).Viability and activity of bifidobacteria in Yoghurt containing fructose oligo saccharine during refrigerated storage. International Journal Food Science. Tecknol, 39:613-621.

Al-Qarawi, A.A; Al-Damegh, M. A; and El-Mougy, S. A. (2003). Hepatoprotective Influence of Adansonia digitata Pulp, Journal of Herbs, Spices and Medicinal Plants, 10:3, 1-6.

Al-Sheraji, S. H;Ismail, A; Manap, M. Y;Mustafa, S; Yusof, R. M. and Hassan, F. A. (2012).Hypocholesterolaemic effect of yoghurt containing Bifidobacterium pseudocatenulatum G4 or Bifidobacterium longum BB536. Food chemistry, 135(2): 356-361.

Antolovich, M; Prenzler, P; Robards, K. and Ryan, D. (2000).Sample preparation in the determination of phenolic compounds in fruits.Analyst, 125(5): 989-1009.

Anuradha, S. and Rajeshwari, K. (2005). Probiotics in Health and Disease . Journal, Indian Academy of Clinical Medicine; 6: 67-72.

AOAC. (1990). Official Methods for Analysis (15th ed). Association of Official Analytical Chemists. Washington, D.C., USA.

AOAC. (1984). Association of Official Analytical Chemists. Official Methods of Analysis. 14th ed., Published by the AOAC Inc., North Nineteenth Street, Suite 210 Arlington. Virginia 22209. USA.

AOAC. (2003). Association of Official Analytical Chemists.Official Methods of Analysis, 17th ed. Arlington, Virginia, USA.

Arai, S. (1996). Studies on functional foods in Japan–State of the art.Biosci.Biotech. Biochem.60:9-15.

Arnold, T.H., Well, M.J. and Wehmeyer, A.S. (1985). Khoisan Food Plants Taxa with Potential for Economic Exploitation. In: Wickens, G.E., J.R.Goodin and D.V. Field (Eds.), Plants for Arid Lands.Allen and Unwin, London, 69-86.

Arroyo, P. L.; Hatch-Pigott, V.; Mower, H. F and Cooney, R. V. (1992). Mutagenicity of nitric oxide and its inhibition by antioxidants.Mutat. Res. 281, 193-202.

Azuma, N., Yamauchi, K. and Mitsuoka, T.(1984). Bifidus Growth promoting activity of a Glycomacropeptide Derived from Human K-Casein. Agricultural and Biological Chemistry; 48: 2159-2162.

Babu, K ;Tiwari A.K.,Srinivas P.V.,Ali A.Z., Raju B.and Rao, J.M.(2004). Yeast and mammalian α-glucosidase inhibitory constituents from Himalayan rhubarb *Rheum emodi* Wall.Ex Meisson. Bioorg. Med. Chem. Lett. ;14:3841–3845.

Bagchi, D; Bagchi, M; Stohs, S. J; Das, D. K., Ray, S. D; Kuszynski, C. A; Joshi, S. S. and Pruess, H. G. (2000). Free radicals and grape seed proanthocyanidin extract: importance in human health and disease prevention. Toxicology, 148: 187–197.

Baiano, A; Terracone, C; Viggiani, I; Del Nobile, M.A. (2013). Effects of cultivars and location on quality, phenolic content and antioxidant activity of extra-virgin olive oils.Journal of the American Oil Chemists' Society, 90:103–111.

Banasaz, M;Norin, E; Holma, R; and Midtvedt T. (2002).Increased Enterocyte Production in Gnotobiotic Rats Mono-associated with Lactobacillus rhamnosus GG. Appl. Environ. Microb., 68:3031-3034.

Baobab Fruit Company, (2002). Nella Tradizione Africana Baobab. From: www.baobabfruitco.com

Barrangou ,R; Lahtinen, S.J; Ibrahim ,F. and Ouwehand, AC. (2011). Genus *Lactobacillus*. In: Lactic Acid Bacteria: Microbiological and Functional Aspects. London: CRC Press. 77-91.

Barrow, G.I. and Feltham, R.K.A. (1993). Cowan and Steel's Manual for the Identification of Medical Bacteria. (Third edition), Cambridge University Press.

Barwick, M. (2004).Tropical and Subtropical Trees: A worldwide Encyclopaedic Guide, Thames and Hudson, London, UK.

Bas, E; Recio, M. C; Abdallah, M; Manez, S; Giner, R. M., CerdaNicolas, M. and Rios, J.L.(2007).Inhibition of the pro-inflammatory mediators'production and anti-inflammatory effect of the iridoidscrovalentinoside. J.Ethnopharmacol.,110: 419-427.

Baum, D. A. (1995) .The comparative pollination and floral biology of Baobabs (Adansonia- Bombacaceae).Ann. Missouri Bot. Gard., 82**:** 322-348.

Baydar, N. G; Özkan, G. and Yaşar, S. (2007). Evaluation of the antiradical and antioxidant potential of grape extracts. Food control, 18(9): 1131-1136.

Becker, B. (1983). The contribution of wild plants to human nutrition in the Ferlo (Northern Senegal).Agroforestry Systems, 1**:** 257-267.

Becker, E. M., Nissen, L. R. and Skibsted K. (2004). Antioxidant evaluation protocols: Food quality or health effects. European Food Research Technology, 219: 561-571.

Besco, E. Bracioli, E. Vertuani, S.Ziosi, P. Brazzo, F. Bruni, R. Sacchetti , G. and Manfredini ,S. (2007). The use of photochemiluminescence for the measurement of the integral antioxidant capacity of baobab products. Food Chemistry. 102:1352-1356.

Bialonska, D; Kasimsetty, S. G; Schrader, K. K; and Ferreira, D. (2009).The effect of pomegranate (Punica granatum L.) byproducts and ellagitannins on the growth of human gut bacteria.Journal of agricultural and food chemistry, 57(18): 8344-8349

Blanchette, L; Roy, D; Belanger, G. and Gauthier, S.F. (1996). Production of cottage cheese using dressing fermented by bifidobacteria. J Dairy Sci 79:8–15.

Bok, S. H; Park, S. Y; Park, Y. B; Lee, M. K; Jeon, S. M., Jeong, T. S., and Choi, M. S. (2002). Quercetin dihydrate and gallate supplements lower plasma and hepatic lipids and change activities of hepatic antioxidant enzymes in high cholesterol-fed rats. International journal for vitamin and nutrition research, *72*(3): 161-169

Bouhallab, S; Favrot, C. and Maubois, J.L. (1993). Growth-promoting activity of tryptic digest of caseinomacropeptide for Lactococcus lactis subsp lactis. Lait; 73: 73-77.

Bourlioux, P; Koletzko, B; Guarner, F. and Braesco, V. (2003). The intestine and its microflora are partners for the protection of the host: report on the Danone Symposium "The Intelligent Intestine". American Journal for clinical Nutrition; 78:675-83.

Bravo, L. (1998). Polyphenols: chemistry, dietary sources, metabolism and nutritional significance. Nutrition Reviews, 56:317–333, ISSN , 0229-6643.

Bredt, D.S and Snyder, S.H. (1990). Isolation of nitric oxide synthetase, a calmodulin – requiring enzyme. Proc. Natl. Acad. Sci. USA 87:682–685.

Bruno, R. S; Leonard, S. W. and Atkinson J.(2006). Faster plasma vitamin E disappearance in smokers is normalized by vitamin C supplementation. Free Radical Biology and Medicine, 40: 689-697.

Cai, Y.Z., Luo, Q., Sun, M. and Corke, H. (2004). Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Scince .74: 2157-2184.

Carr, W. R. (1955). Ascorbic Acid Content of Baobab Fruit. Nature, 176 (4496): 1273. Government Analyst's Laboratory, Salisbury.

Carr**,** A. C**.** and Frei, B. (1999) . Toward a new recommended dietary allowance for vitamin C based on antioxidant and health effects in humans. American Journal of Clinical Nutrition,69**:** 1086-1107.

Ceriello, A. and Motz, E. (2004). Is oxidative stress the pathogenic mechanism underlying insulin resistance, diabetes, and cardiovascular disease? The common soil hypothesis revisited. Thrombosis, and Vascular Biology 24: 816-823

Chadare, F. J., Linnemann, A. R. (2009). Baobab Food Products: A review on their composition and nutritionalvalue. Critical Reviews in Food Science and Nutrition 49: 254-274.

Chalasani, N; Aljadhey, H; Kesterson. J;, Murray, M.D. and Hall, S.D. (2004). Patients with elevated liver enzymes are not at higher risk for statin hepatotoxicity. Gastroenterology, 126: 1287-1292.

Champagne, C.P; Gardner, N.J. and Roy, D. (2005). Challenges in the addition of probiotics cultures to foods. Critical Review in Food Science and Nutrition; 45: 61-84.

Chatterjea, M. N; and Shinde, R. (2002). Serum enzymes in heart diseases. Textbook of Medical Biochemistry (5th ed.) Jappe brothers, New Delhi, Med. Publ. Ltd, 555-557.

Chen, R. M; Wu, J. J; Lee, S. C; Huang,H. and Wu, H. M. (1999). Increase of Intestinal Bifidobacterium and Suppression of Coliform Bacteria with ShortTerm Yogurt Ingestion. Journal of Dairy Science; 82: 2308-2314.

Chouraqui, J.P; Van Egroo, L.D.and Fichot, M.C. (2004).Acidified Milk Formula Supplemented with Bifidobacterium lactis: Impact on Infant Diarrhea in Residential Care Settings. Journal of Pediatric Gastroenterology and Nutrition; 38: 288-292.

Christen, G. L.; Davidson, P. M.; McAllister, J.S. and Roth, L.A. (1992). Coliform and other indicator bacteria. In Marshall RT (ed.): standards methods for examination of dairy products: 16- 23.

Cisse, M; Sakho, M;Dornier, M; Diop, C.M;Reynes, M. and Sock, O. (2009). Caractérisation du fruit du baobab et étude de sa transformation en nectar, Fruits, 64: 19-34.

Coconier, M.H; Lievin, V;Hemery, E; Servin, A.L. (1998). Antagonistic activity against Helicobacter infection in vitro and in vivo by the human Lactobacillus acidophilus strain LB. Appl. Environ.Microbiol.64: 4573–4580.

Coe, S.A; Clegg, M; Armengol, M. and Ryan, L.(2013). The polyphenol-rich baobab fruit (*Adansonia digitata L.*) reduces starch digestion and glycemic response in humans. Nutrition Research.33(11):888–96.

Coinu, R; Carta, S; Urgeghe, P. P;Mulinacci, N;Pinelli, P;Franconi, F. and Romani, A. (2007). Dose-effect study on the antioxidant properties of leaves and outer bracts of extracts obtained from Violetto di Toscana artichoke. Food Chemistry, 101:524–531.

Collins, M.D; and Gibson, G.R. (1999). Probiotics, prebiotics, and synbiotics: approaches for modulating the microbial ecology of the gut. *American Journal of Clinical Nutrition*; 69: 1052S-7S.

Conforti. F, and Menichini, F. (2011). Phenolic compounds from plants as nitric oxide production inhibitors. Curr Med Chem, 18: 1137-45.

Cornwell, T; Cohick, W; and Raskin, I. (2004).Dietary phytoestrogens and health.Phytochemistry, *65*(8): 995-1016.

Coutouly, A;Riaublanc, A;Axelos, M. and Gaucher, I.(2014). Effect of heat treatment, final pH of acidification, and homogenization pressure on the texture properties of cream cheese. Dairy Science and Technology, 94:125-144.

Crittenden, R.G ; Morris, L.F;Harvey, M.L; Tran, L.T; Mitchell, H.L. and Playne, M.J. (2001). Selection of a Bifidobacterium strain to complement resistant starch in a synbiotic yogurt. Journal of Applied Microbiology; 90: 268-278.

Dalzeil, J.M. (1948). The useful plants of west Tropical Africa Grown Agents for Overseas Governments and Administrations, 4 Mill bank, London,SWI

Dave, R. I. and Shah, N. P. (1997). Effect of Cysteine on the viability of yogurt and probiotic bacteria in yogurt made with commercial starter cultures. International Dairy Journal, 7: 537–545.

Dave, R. I; and Shah, N. P. (1998). Ingredient supplementation effects on viability of probiotic bacteria in yogurt. Journal of Dairy Science; 81: 2804–2816.

De Roos, N.M. and Katan, M.B. (2000). Effects of probiotic bacteria on diarrhea, lipid metabolism, and carcinogenesis: a review of papers published between 1988 and 1998. American Journal of Clinical Nutrition; 71:405-11.

De Smedt ,S; Alaerts, KK; ouyate, A.M; Van Damme, P; Potters, G. and Samson, R. (2011). Phenotypic variation of baobab *(Adansoniadigitata L.)* fruit traits in Mali.AgroforestSyst 82:87-97.

De Vrese, A., Stegelmann, A., Richter, B., Fenselau, S., Laue, C., and Schrezenmeir, J. (2001). Probiotics-compensation for lactase insufficiency. American Journal of Clinical Nutrition; 73: 421–429.

Deguchi, Y; Morishita, T; and Mutai, M. (1985).Comparative studies on synthesis of water-soluble vitamins among human species of bifidobacteria. Agricultural and Biological Chemistry; 49: 13-19.

Desai, A.R; Powell, I.B. and Shah, N.P. (2004). Survival and Activity of probiotic Lactobacilli in skim milk containing prebiotics. Journal of Food Science; 69: S57- S60.

Desjardins, M; Roy.D. and Goulet, J.(1990). Growth of bifidobacteria and their enzyme profiles. Journal of Dairy Science;73: 299–307.

Desmazeaud, M. (1983) .Nutrition of Lactic Acid Bacteria: State of the Art. Le lait. 63:267-316.

Di Carlo, G.; Mascolo, N.; Izzo, A.A. and Capasso, F. (1999). Flavonoids: old and new aspects of a class of natural therapeutic drugs. Life Sciences, Vol.65, No.4, (June 1999), pp. 337-353, ISSN 0024-3205.

Diop, A.G., Sakho, M; Dornier; M., Cisse, M. and Reynes, M. (2005). Le baobab africain (Adansonia digitata L.): principales caractéristques et utilisations. Fruits, 61:55-69.

Donnet-Hughes, A; Rochat, F; Serrant, P;Aeschlimann, J.M. and Schiffrin, J.E. (1999). Modulation of nonspecific mechanisms of defense by lactic acid bacteria: effective dose. J. Dairy Sci. 82: 863–869.

El Amin, H. M. (1990).Trees and. Shrubs of the Sudan. Ithaca Press, UK, ISBN 0863721168. FAO 1988: Traditional Food Plants. FAO food and nutrition paper 42, 63-67.

Eurostat .(2007). Metadata categories. Website, 17 October. Luxembourg European Europen Communities.http://ec.europa.eu/eurostat/ramon.

Fagbohun, A. A; Ikokoh, P. P; Afolayan, M. O; Olajide, O. O; Fatokun, O. A ; and Akanji, F. T. (2012). Chemical Composition and Anti-Oxidant Capacity of the Fruit Pulp of *Adansonia digitata L*., 8(3): 165-172.

Fandi, K.G; Ghazali, H.M; Yazid, A.M. and Raha, A.R. (2001). Puri®cation and N-terminal amino acid sequence of fructose-6-phosphate phosphoketolase from Bifidobacterium longum BB536. Letters in Applied Microbiology; 32: 235-239.

FAO .(1988).Traditional Food Plants. FAO food and nutrition paper 42: 63-67.

FAQ/WHO. (2001). Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. Cordoba, Argentina: Food and Agriculture Organization of the United Nations and World Health Organization Expert Consultation Report.

Finlay, B.B. and Falkow, S. (1989).Common themes in microbial pathogenicity.Microbiol. Rev. 53, 210–230.

Fraga C.G. (2010). Plant Phenolics and Human Health: Biochemistry, Nutrition and Pharmacology. John Wiley and Sons Inc., New Jersey.

Fraga, C. G; Shigenaga, A. M. K; Park, J. W;Degan, P. and Ames, B. N. (1990).Oxidative damage to DNA during aging - 8-hydroxy-2'-deoxyguano-sine in rat organ DNA and urine. Proceedings of National Academy of Science, 87: 4533-4537.

Frank J.F; Christen G.L. and Bullerman L.B. (1992). Tests for groups of microorganisms. In Standard Methods for the Examination of Dairy Products, 16th edition. Marshall R.T. (ed.). Washington, DC: American Public Health Association: 271–286.

Fridovich, I. (1986). Biological effects of the superoxide radical. Archives of Biochemistry and Biophysics, 247: 1–11.

Fukushima , Y; Kawata, Y; Hara, H ; Terada, A and Mitsuoka, T. (1998). Effect of a probiotic formula on intestinal immunoglobulin A production in healthy children. Int. J. Food Microbiol., 42: 39–44.

Fuller ,R . and Gibson, G.R. (1997). Modification of the intestinal microflora using probiotics and probiotics .Scand .Jornal of Gastoenterology 222,28-31.

Fuller, R.(1989). Probiotics in man and animals. Journal of Applied Bacteriology, 66: 365-378.

Gao, H; Huang, Y; Gao, B. and Kawabata, J. (2008). Chebulagic acid is a potent a-glucosidase inhibitor. BiosciBiotechnolBiochem 72: 601-603.

Gaydou, E.M; Bianchi,J.P;Ralamanavaro ,A. and Waegell, B. (1982). Hydro-carbons, Sterols and Tocopherols in the seeds of six *Adonsonia* species. Phytochem., 21(8): 1981-1987.

Gebauer, J. and luedeleng, E. (2013).A note on Baoab (Adansonia digitata L.) in Kordofan, Sudan.Genetics Resources Crop Evolution.DOI 10.1007/s100722-013-9964-5.

Gebauer, J; El-Siddig, K. and Ebert, G. (2002). Baobab (Adansonia digitata L.): a Review on a Multipurpose Tree with Promising Future in the Sudan. Gartenbauwissenschaft, 67, 155-160.

Giannini, E.G; Testa, R. and Savarino, V.(2005). Liver enzyme alteration: A guide for clinicians. *Can. Med. Assoc. J., 172*: 367–379.

Gibson, G.R and Roberfroid, M.B. (1999).Colonic microbiota, nutrition and health. Dodrecht: Kluwer Acdemic Publishers.

Gibson, G.R. and Rastall, R.A. (2004).Functional foods. Bioscience explained; 2: 1-7.

Gibson, G.R. and Roberfroid, M. (1995) . Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. Journal of Nutrition; 125: 1401- 1412.

Gibson, G.R., Wang, X., (1994). Inhibitory effects of bifidobacteria on other colonic bacteria. J. Appl. Bacteriol. 77, 412–420.

Glew, R. H; Vanderjagt, D.J; Lockett, C; Grivetti, L.E; Smith, G.C; Pastuszyn, A. and Millson, M.(1997) Amino acid, fatty acid, and mineral composition of 24 indigenous plants of Burkina Faso. Journal of Food Composition and Analysis, 10 (Article No. FC970539)**:** 205-217.

Goktepe, I; Juneja ,V.K. and Ahemedna, M. (2006). Probiotic in food safety and Human Health . Taylor and Francis Group . Boca Raton , New York .

Gomes, A.M.P; Malcata, F.X. (1998). Development of probiotic cheese manufactures from goat milk: response surface analysis via technological manipulation. J Dairy Sci 81:1492–1507.

Gorbach, S. (2002). Probiotics in the Third Millennium. Digest liver dis. 34:S2-S7.

Granato, D; Branco, G. F; Cruz, A. G;Faria, J. A .F. and Shah, N. P. (2010).Probiotic dairy products as functional foods. Comprehensive Reviewsin Food Science and Food Safety 9 455–470.

Gruenwald, J; Galizia ,M.D. (2005). Market Brief in the European Union for selected natural ingredients derived from native species. *AdansoniaDigitata L.*Baobab.*The United Nations Conference on Trade and Development* (UNCTAD). Bio Trade Initiative/BioTrade Facilitation Programme (BTFP)

Guarner, F. and Malagelada. J,R. (2003). Gut flora in health and disease. lancet 361.512-519.2.

Guinee, T.P; Pudja, P.D. and Farkye, N.Y. (1993). Fresh acid-curd cheese varieties, in P.F. Fox (Ed),Cheese: Chemistry, Physics and Microbiology.Chapman & Hall, London, pp. 363-419.

Guler-Akın, M.B. and Akın, M.S. (2007). Effects of cysteine and different incubation temperatures on the microflora, chemical composition and sensory characteristics of bio-yogurt made from goat‘s milk. *Food Chemistry;* 100: 788-793.

Guteridge, J. M. (1989). Iron and oxygen: A biologically damaging mixture. ActaPaediatrica Scandinavia, 36: 78-85.

Halliwell, B. (1997). Antioxidants and human disease: A general introduction. Nutr.ReV., 55, 44S-49S.

Halliwell, B. and Gutteridge, J. M. C.(1989). Free radicals in biology and medicine, 2 Ed., Clarendon, UK, Oxford science publications. pp. 22–85.

Halliwell, B. Antioxidants and human disease: A general introduction. Nutr.ReV. 1997, 55, 44S-49S.

Halliwell, B.(1994). Free radicals and antioxidants free radicals, ntioxidants, and human disease: curiosity, cause, or consequence. Lancet, 344: 721-4.

Halvorsen, R; Berstad, A; Lassen, J; Midtvedt ,T. and Narvhus J. (2000). The Use of Probiotics for Patients in Hospitals: A Benefit and Risk Assessment. Norwegian Scientific Committee for Food Safety. 07/112-FINAL: 1-29. ISBN: 978-82-8082-291-8.

Hamid, A.A; Shah, Z.M; Muse, R. and Mohamed, S. (2002) .Characterisation of antioxidative activities of various extracts of *Centella asiatica* (L) Urban. Food Chem. 77: 465-469.

Harrigan, W.F. and Mc Cane, E. (1976). Laboratory Methods in food and Dairy Microbiology, Acadamic press, London.

Henry, J.B. (1984). Clinical Diagnosis and Management by Laboratory Methods.17th ed. W.B. Saunders, London.

Hillstrom, R. J; Yacapin-Ammons, A. K;Lynch, S. M. (2003). Vitamin C inhibits lipid peroxidation in human HDL. J.Nutr.; 133 (10): 3047 – 3051

Holzapfel, W.H; Geisen, R; Schillinger, U. (1995). Biological preservation of foods with reference to protective cultures, bacteriocins and food-grade enzymes. International Journal of Food Microbiology 24: 343 – 362.

Homma, N. (1988). Bifidobacteria as a resistance factor in human beings. Bifidobacteria and Microflora, 7: 35-43

Hou, J.W; Yu, R.C. and Chou, C.C. (2000). Changes in some components of soymilk during fermentation with bifidobacteria. Food Research International; 33: 393-397.

Houghtby, A.G.; Maturin, L.J. and Koenig, K.E. (1992).Microbiological count methods. In Standard Methods for the Examination of Dairy Products, 16th edition, Ed., R.T. Marshal. Washington, DC: American Public Health Association, pp: 213-246

Hsu, C.Y.; Lin, G.M.; Lin, H.Y.; Chang, S.T.(2018). Characteristics of proanthocyanidins in leaves of Chamaecyparis obtusa var. formosana as strong α-glucosidase inhibitors.J. Sci. Food Agric. 98, 3806–3814.

Hu, M.L. (2011) .Dietary polyphenols as antioxidants and anticancer agents: more questions than answers. Chang Gung Medical Journal, 34: 449-460.

Huis -Veld, J. H. J. Havenaar, R. (1991).Journal of Chemical.Technology and Bio-Teechnology, 51,562-567.

Husebye, E.; Hellstrm ,PM.; Sundler, F.; Chen, J. and Midtvedt,T. (2001). Influence of Microbial Species on Small Intestinal Myoelectric Activity and Transit in Germ-free Rats. Am. j. physiol-gast. l. 280:G368-G380.

Ibrahim, C.; Didier, M.; Max, R.; Pascal, D.; Benjamin, Y.; and Renaud, B.(2013). Biochemical and Nutritional Properties of Baobab Pulp from Endemic Species of Madagascar and the African Mainland.African Journal of Agricultural, 8(47):6046– 6054

Igbabul, B. D.; Amove, J. and Twadue, I. (2014).Effect of fermentation on the proximate composition, antinutritional factors and functional properties of cocoyam (Colocasia esculenta) flour.African Journal of Food Science and Technology, 5(3), 67-74.

IOM/NAS. (1994). "Opportunities in the Nutrition and Food Sciences", ed. P.R. Thomas and R. Earl, p. 109. Institute of Medicine/National Academy of Sciences, National Academy Press, Washington, D.C

Isolauri, E. (2001). Probiotics in human disease. American Journal of Clinical Nutrition; 73: 1142s-1146s.

Isolauri, E.; da Costa Ribeiro, H.; Gibson, G.;Saavedra, J.; Saliminen, S.; Vanderhoof, J. and Varavithya, W. (2002). Functional Foods and robiotics: Working Group Report of the First World Congress of Pediatric Gastroenterology, Hepatology, and Nutrition, Journal of Pediatric Gastroenterology and Nutrition, 35: S106-S109.

Izhaki, I.; Tsahar, E.; Paluy, I. and Friedman, J. (2002). Within population variation and interrelationships between morphology, nutritional content, and secondary compounds of Rhamnus alaternus fruits. New Phytologist, 156(2): 217–223.

Jacobsen, C.N.; Nielsen, V.R.; Hayford, A.E.; Moller, P.L.;Michaelsen, K.F.; Paerregaard, A.; Sandstro ¨ m, B.; Tvede, M.; Jakobsen, M. (1999).Screening of probiotic activities of forty-seven strains of Lactobacillus spp. by in vitro techniques and evaluation of the colonization ability of five selected strains in humans. Appl. Environment Microbiol. 65: 4949–4956.

Jain, P.K.; McNaught, C.E.; Anderson, A.D.G.; MacFie, J. and Mitchell, C.J. (2004). Influence of synbiotic containing Lactobacillus acidophilus La5, Bifidobacterium lactis Bb 12, Streptococcus thermophilus, Lactobacillus bulgaricus and oligofructose on gut barrier function and sepsis in critically ill patients: a randomised controlled trial. Clinical Nutrition; 23:467-475.

Janer, C.; Pelaez, C. and Requena, T. (2004). Caseinomacropeptide and whey protein concentrate enhance *Bifidobacterium lactis* growth in milk. Food Chemistry; 86: 263-267.

Jawez, M. and Adel, B. (1990).Medical Microbiolgy, 19th ed. USA.

Johansson, M.L.; Nobaek, S.; Berggren, A.; Nyman, M.; Bjo¨ rck, I.; Ahrne, S.; Jeppsson, B.and Molin, G. (1998) .Survival of *Lactobacillusplantarum* DSM 9843 (299v), and effect on the short-chain fatty acid content of faeces after ingestion of a rose-hip drink with fermented oats.Int. J. Food Microbiol. 42: 29–38.

Jozef, M; Meessen, K. and Harro, P.(2002). “Urea” in Ullmann's Encyclopedia of Industrial hemistry, Wiley-VCH,Weinheim,

Juvan, S.; Bartol, T.and Boh, B. (2005). Data structuring and classification in newly-emerging scientific fields. Online Information Review 29: 483-498. http://dx.doi.org/10.1108/14684520510628882.

Kaboré, D.; Sawadogo-Lingani, H.; Diawara, B.; Compaoré, C.; Dicko, M. and Jakobsen, M. (2011). A review of baobab (Adansonia digitata) products: Effect of processing techniques, medicinal properties and uses. African Journal of Food Science.5(16):.833–844.

Kailasapathy, K. and Chin, J. (2000) .Survival and therapeutic potential of probiotic organisms with reference to Lactobacillus acidophilus and Bifidobacterium spp. Immunology and Cell Biology; 78: 80-88.

Kamatou, G.P.P., Vermaak, I., and Viljoen, A.M. (2011), an updated review of Adansonia digitata: A commercially important African tree. South African Journal of Botany, 77, 908–919.

Katsvanga, C.A.T.; Jim ,L.; Gwenzi, D.; Muhoni, L.; Masuka, P.and Moyo, M. (2007). Characterisation of community identified Uapakakirkiana phenotypes for domestication. *Journal of Sustainable Development in Africa* **9**:356-366

Kaur, C and Kapoor, C. (2001). Antioxidants in fruits and vegetables the millennium's health. International Journal of Food Science and Technology, 703-725.

Kawada, N.; Seki, S. and Kuroki, T. (1998).Effect of antioxidants resveratrol, quercetin and N-acetylcystein, on the functions of cultured rat hepatic stellate cells and kupfercells.Hepatology, 27, 1265-1274.

Kheiri, N. A. (1996). Evaluation of baobab (Gonglase) solution for total home management of diarrhea in Sudan children.Ph .D. Thesis, University of Khartoum. Sudan.

Kiessling, G.; Schneider, J. and Jahreis, G.(2002). Long-term consumption of fermented dairy products over 6 months increases HDL cholesterol. uropean Journal of Clinical Nutrition 56: 843-849.

Kilic ¸ G.B.; Kuleasan, H.;Eralp, I.; Karahan, A.G. (2009). Manufacture of Turkish Beyaz cheese added with probiotic strains. LWT—Food Sci Technol 42:1003–08.

Kim JH, Kang MJ, Choi HN, Jeong SM, Lee YM, Kim JI. (2011) Quercetin attenuates fasting and postprandial hyperglycemia in animal models of diabetes mellitus. Nutr Res Pract;5: 107–11.

Kim, D.O.; and Lee, C. Y. (2002). Extraction and isolation of polyphenolics.In R. E. Wrolstad (Ed.) Current protocols in food analytical chemistry. New York: John Wiley and Son, I1.2.1–I1.2.12.

Kim, D.O.; Jeong, S.W. and Lee C.Y. (2002).Antioxidant capacity of phenolicphytochemicals from various cultivas of plums. Food . Chem 81**:** 321-326.

Kim, D.O.; Jeong, S.W. and Lee, C.Y. (2003).Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. Food Chemistry 81: 321-326.

Kim, H.K.; Cheon, B.S.; Kim, Y.H.; Kim, S.Y. and Kim, H.P. (1999). Effects of naturally occurring flavonoids on nitric oxide production in the macrophage cell line RAW 264.7 and their structure-activity relationships. Biochem. Pharmacol,58: 759-65.

Kim, H.K.; Choi, Y.H.; Verpoorte, R. (2010). NMR-based metabolomic analysis of plants. Nat. Protoc. 5, 536–549.

Kitson, R.E., and Mellon, MG.(1944).Colorimetric determination of phosphorus as molybdivanadophosphoric acid. Ind. Eng. Chem. Anal. Ed.16:379-83.

Klaver, F.A.M.; Kingma, F. and Weerkamp, A.H. (1993).Growth and survival of bifidobacteria in milk. Netherland Milk and Dairy Journal; 47: 151-164.

Kojima, T.; Yaeshima, T.; Ishibashi, N.; Shimamura, S.and Hayasawa, H. (1996).Inhibitory effects of human-drived bifidobacterium on pathogenic *Escherichia coli* serotype O-111. Bioscience Microflora ., 15(1): 17 – 22

Komatsu, W.; Ishihara, K.; Murata, M.; Saito, H. and Shinohara, K. (2003). Docosahexaenoic acid suppresses nitric oxide production and inducible nitric oxide synthase expression in interferon-γ plus lipopolysaccharide-stimulated murine macrophages by inhibiting the oxidative stress. Free Radical Biology and Medicine, 34(8): 1006-1016.

Kris-Etherton, P.M.; Lefevre, M.; Beecher, G.R.; Gross, M.D.; Keen, C.L. and Etherton, T.D. (2004). Bioactive compounds in nutrition and health-research methodologies for establishing biological function: The Antioxidant and Anti-inflammatory Effects of Flavonoids on Atherosclerosis. Annual Review of Nutrition. 24 (1):.511–538.

Kullisaar,T.; Zimer, Mikelsaar, M.; Vihalemn, T.; Annuk, H.;Kairance, C. and Kill,. A. (2002).Two antioxidative Lactobacilli strain as promising probiotics .Int. J. Food Maicrobial., 72:21-224.

Lamien-Meda, A.; Lamien, C. E.; Compaoré, M. M. Y.; Meda, R.T.N.; Kiendrebeogo, M., Zeba, B., Millogo, J.F and Nacoulma, O.G.(2008). Polyphenol content and antioxidant activity of fourteen wild edible fruits from Burkina Faso. Molecules 13: 581–594.

Laranjinha, J.; Alemeida, L. and Madeira, V. (1994).Reactivity of dietary phenolic acids with peroxyl radicals: Antioxidant activity upon low-density lipoprotein peroxidation. Biochem. Pharm., 48,87-494.

Laroia, S., and Martin, J.H. (1991) .Methods for enumerating and propagating bifidobacteria. Cultured dairy products Journal; 26:32–33.

Larsen, C.N.; Nielsen, S.; Kæstel, P.; Brockmann, E.; Bennedsen, M.;Christensen, H.R.; Eskesen,D.C.; Jacobsen,B.L.; and Michaelsen, K.F. (2006).Dose-response study of probiotic bacteria Bifidobacterium animalis subsp. lactis BB-12 and Lactobacillus paracasei subsp. paracasei CRL-341 in healthy young adults. European Journal of Clinical Nutrition; 60:1284-1293.

Lattanzio, V.; Lattanzio, V.M.T. and Cardinali, A. (2006).Role of phenolics in the resistance mechanisms of plants against fungal pathogens and insects. Phytochemistry: Advances in Research, Kerala.

Leahy, S.C.; Higgins, D.G.; Fitzgerald, G.F. and Sinderen, D. van (2005).Getting better with bifidobacteria. Journal of Applied Microbiology; 98: 1303–1315.

Lee, Y.K.; and Salminen, S. (1995). The coming of age of probiotics.Trends Food Sci. Technol., 6: 241–245.

Legua, P.; Melgarejo, P. and Martinez, J.J. (2012). Evaluation of Spanish pomegranate juices: Organic acids, sugars, and anthocyanins. International Journal of Food Properties, 15, 481–494.

Lilly, D. M. and Stillwell, R. H. (1965). Probiotics: growth-promoting factors produced by microorganisms. *Science*, *147*(3659), 747-748.

Liong, M.T.; Shah, N.P.(2006). Effects of a Lactobacillus casei synbiotics on serum lipoprotein, intestinal microflora, and organic acids in rats.Journal of Dairy Science.; 89: 1390 - 1399.

Loo, J.V.; Cummings, J.; Delzenne, N.; Englyst, H.; Frank, A.; Hopkings, M.; Kok, N.; Macfarlane, G.; Newton, D.; Quigley, M.; Roberfroid,M.; van Vliet, T. and van den Heuvel, E.(1999). Functional food properties of non-digestible oligosaccharides report from the ENDO project (DGXII AIRII-CT94-1095). Br. J. Nutr. 81, 121–132.

Lourens-Hattingh, A. and Viljoen, B.C. (2001).Yogurt as probiotic carrier food. International Dairy Journ al; 11: 1-17.

Lucey**,** J.A. (2003). Acid and acid/heat coagulated cheese,in ncyclopedia of dairy sciences, Vol. 1 (ed. H.Roginski, J.W. Fuquay, and P.F. Fox), Academic Press, London, pp. 350-356.

Malviya, R.; Srivastava, P.; Bansal, M.and Sharma, P. K. (2010). Mango Peel Pectin as Superdisintegrating Agents. Journal of Scientific and Industrial Research, 69, 688–690.

Manach C., Mazur, A. and Scalbert, A. (2005). Polyphenols and prevention of cardiovascular diseases. Current Opinion in Lipidology 16, 77- 84.

Manach, C.; Scalbert, A.; Morand, C. and Remesy, C. and Jime' nez, L. (2004). Polyphenols: Food sources and bioavailability.American Journal of ClinicalNutrition. 79: 727 - 747.

Manfredini, S.; Vertuani, S.; Braccioli, E. and Buzzoni, V. (2002).Antioxidant capacity of *Adansonia Digitata* fruit pulp and leaves. Acta Phytotherapeutica, 2:2-7.

Mansour, H.A.; Newairy, A.S.; Yousef, M.I. and Sheweita, S.A. (2002). Biochemical study on the effects of some Egyptian herbs in alloxan-induced diabetic rats.Toxicology, 170: 221-228.

Marks, J. (2004). Successful probiotic: Bifidobacteria. Handbook of functional dairy products By Colette Shortt, John OBrien.CRC press LLC. P 33-69.

Marshall, V. and Law, B. (1984).The Physiology and Growth of Dairy lactic-Acid Bacteria. In: Davies FL, Law BA, editors. dvances in the Microbiology and Biochemistry of Chees and Fermented Milk. London: Elsevier Applied Science Publishers Ltd. 67-98.

Marteau, P.; Gerhardt, M.F.; Myara, A.; Bouvier, E.; Trivin, F. and ambaud, J.C. (1995). Metabolism of bile salts by alimentary bacteria during transit in the human small intestine. Microb. Ecol. Health Dis. 8: 151–157.

Martınez-Villaluenga, C. and Gomez, R., (2007).Characterization of bifidobacteria as starters in fermented milk containing raffinose family of oligosaccharides from lupin as erbiootic.International Dairy Journal. 17: 116- 122.

Martınez-Villaluenga,C.; Frıas, J.; Gomez, R. and Vidal-Valverde C., (2006). Influence of addition of raffinose family oligosaccharides on probiotics survival in fermented milk during refrigerated storage. International Dairy Journal; 16: 768– 774.

McIntosh, G.H. (1996). Probiotics and colon cancer prevention. Pacific Journal of Clinical Nutrition; 5:48-52.

Meng, Q. H.; Lewis, P.; Wahala, K.; Adlercreutz, H. and Tikkanen,M. J. (1999).Incorporation of esterified soybean isoflavones with

antioxidant activity into low-density lipoprotein. Biochem.Biophys.Acta, 1438, 369-376.

Mercenier, A.S.and Pavan, B. (2002). Probiotics as biotherapeutic agents : present knowledge and future prospects. Carr.Pharm-Design .,8:99-110.

Metchnikoff, E. (1907). The prolongation of life.Optimistic studies. London: Butterworth-Heinemann.

Meyer, A.(1998). The 1998 top 100® RandD survey. Food Processing 58(8): 32-40.

Min, G. and Chun-zhao, L. (2005).Comparison of techniques for the extraction of flavonoids from cultured cells of Saussurea medusa Maxim. World Journal of Microbiology and Biotechnology *21*: 1461-1463.

Minellia, E.B.; Benini, A.; Marzotto, M.; Sbarbati, A.; Ruzzenente, O.; Ferrario, R.; Hendriks, H. and Dellagli, F. (2004). Assessment of novel probiotic Lactobacillus casei strains for the production of functional dairy foods. International Dairy Journal; 14; 723–736.

Mishra, C. and Lambert, J. (1996).Production of anti-microbial substance by probiotics. Asia Pacific Journal Clinical Nutrition; 5: 20-24.

Mistry, S.; Dutt, K.R.; Jena, J.; Mistry, S.; Dutt, K.R. and Jena, J. (2013) Protective effect of *Sidacordata* leaf extract against CCl4 induced acute liver toxicity in rats. Asian Pac. J. Trop. Med. 6, 280–284.

Mital, B.K. and Garg, S.K. (1995).Anticarcinogenic, hypocholesterolemic, and antagonistic activities of Lactobacillus acidophilus. Critical Review in Microbiology; 21:175-214.Mitsouka, T. (1996).Intestinal

flora and human health. Asia pacific Journal of Clinical Nutrition; 5: 2-9.

Modler, H.W. (1994). Bifidogenic factors- sources, metabolism and applications. International Dairy Journal; 4: 383-407.

Modler, H.W.; Mckellar, R.C. and Yaguchi, M. (1990).Bifidobacteria and bifidogenic factors. Canadian Institute of Food Science; 23: 29-41.

Mohan, R.; Koebnick, C.; Schildt, J.; Schmidt, S.; Mueller, M.; Possner, M.; Radke, M. and Blaut, M. (2006). Effects of Bifidobacterium lactis BB12 supplementation on intestinal microbiota of preterm infants: a double-blind placebo controlled randomized study. Journal of Clinical Microbiology; 44: 4025– 4031.

Mohsen ,M .S and Ammar S M A. (2008).Total phenolic contents and antioxidant activity of corn tassel extracts. Food Chemistry. 112: 595-598.

Moncada, S.; Palmer, R. M. J. and Higgs, E. A. (1991). Nitric oxide: Physiology, pathophysiology, and pharmacology. Pharm. ReV., 43, 109-142.

Monokrousos, N.; Papatheodorou, E. M.; Diamantopoulos, J. D. and Stamou,G. P. (2004). Temporal and spatial variability of soil chemical and biological variables in a Mediterranean shrubland.Forest ecology and management, 202(1), 83–91.

Montoro, P.; Braca, A.; Pizza, C. and De Tommasi, N. (2005). Structure–antioxidant activity relationships of flavonoids isolated from different plant species. Food Chemistry,(2) : 349-355, ISSN 0308-8146.

Morishita, T.; Tamura, N.; Makino, T. and Kudo, S.(1999). Production of menaquinones by lactic acid bacteria. Journal of Dairy Science; 82: 1897-1907.

Munthali, C., Chirwa, P. and Akinnifesi, F. (2012).Phenotypic variation in fruit and seed morphology of *Adansonia digitata L.* (baobab) in five selected wild populations in Malawi.*Agroforest* System, 85:279-290.

Murai, A.; Iwamura, K.; Takada, M.; Ogawa, K. and Usui, T. and Okumura, J. (2002). Control of postprandial hyperglycaemia by galactosylmaltobionolactone and its novel anti-amylase effect in mice. Life Science 71: 1405-1415.

Murray, S.S.; Schoeninger, M.J.; Bunn, H.T.; Pickering, T.R. and Marlett, J.A. (2001). Nutritional Composition of Some Wild Plant Foods and Honey Used by Hadza Foragers of Tanzania. Journal of Food Composition and Analysis, 14: 3-13.

Naidu, A.S.; Bidlac, W.R and Clemens, R.A. (1999).Probiotic spectra of lactic acid bacteria. Critical Reviews in Food Science and Nutrition, 38: 13-126.

Namba, K.; Yaeshima, T.; Ishibashi, N.; Hayasawa, H. and Yamazaki, S. (2003). Inhibitory of *Bifidobacterium longum* on inter hemorrhagic *Escherichia coli* O157: H7. Bioscience Micro flora. 22: 85 – 91.

Narvhus, J. A.; Østeraas, K.; Mutukumira, T. and Abrahamsen, R. K. (1998).Production of fermented milk using a malty compound-producing strain of Lactococcus lactis subsp. lactis biovar.diacetylactis, isolated from Zimbabwean naturally fermented milk. International journal of food microbiology, 41(1), 73-80.

Nasreldin, A.; Gurashi and Maha, A.Y.(2014). Morphological Variation in Fruit Shapes of *Adansonia digitata L*. From Blue Nile and North KordofanStates, Sudan. Journal of Forest products and industries, 3: 106-111.

Ndabikunze .B. K.; Masambu .B. N.; Tiisekwa B. P. M. and Issa-Zacharia,A. (2011), The production of jam from indigenous fruits using Baobab (Adansonia digitata L.) powder as a substitute for commercial pectin ,African Journal of Food Science Vol. 5(3) : 168-175.

Nicol, B. M. (1957). Ascorbic Acid Content of Baobab Fruit. Nature, 180 (4580): 287.

Nnam, N. M.and Obiakor, P.N. (2003). Effect of fermentation on the nutrient and antinutrient composition of baobab (*Adansonia digitata*) seeds and rice (Oryza Sativa) grains. Ecology of Food and Nutrition 42: 265-277.

Nopchinda, S.; Varavithya, W.; Phuapradit, P.; Sangchai, R.; Suthutvoravut, U.; Chantraruksa, V.; and Haschke, F.(2002). Effect of Bifidobacterium Bb12 with or without Streptococcus thermophilus supplemented formula on nutritional status. Journal of the Medical Association of Thailand; 85:1225-1231.

Nour, A. A.; Magboul, B.I.and Kheiri, N.H., (1980).Chemical composition of baobab fruit (*Adansonia digitata*). Tropical Science, 22 (4): 383-388.

Nowacka, N.; Nowak, R.; Drozd, M.; Olech, M.; Los, R. and Malm A. (2014).Analysis of phenolic constituents, antiradical and antimicrobial activity of edible mushrooms growing wild in Poland.LWT - Food Scince and Technology, 59: 689-694.

O'Sullivan, M.G. (1996). Metabolism of bifidogenic factors by gut flora—An overview. IDF Bull. International Dairy Federation, Brussels, Belgium. 313: 23.

Okoho, P.N.(1984). An assessment of the protein, minerals and vitamin losses in sun dried Nigerians vegetables. Nutrition Reports International Zaria, Nigeria.

Oktay, M., Gülçin, İ., and Küfrevioğlu, Ö. İ. (2003). Determination of in vitro antioxidant activity of fennel (Foeniculum vulgare) seed extracts. LWT-Food Science and Technology, 36(2), 263-271.

Osman, M.A. (2004). Chemical and Nutrient Analysis of Baobab *(Adansonia digitata)* Fruit and Seed Protein Solubility. Plant Foods for Human Nutrition, 59, 29-33.

Ouwenhand, A.C. and Salminen, S.J.(1999). The health effects of cultured milk products with viable and non viable bacteria. International Dairy Journal; 8:749- 758.

Parkouda, C., Diawara, B., Ganou, L., and Lamien, N. (2007).Potentialités nutritionnelles des produits de 16 espèces fruitières locales au Burkina Faso. Science et technique. *Sciences appliquées et Technologies*, *1*, 35–47.

Patrut ,A. von Reden, K. Lowy ,D.A., Alberts ,A.H., Pohlman ,J.W., Wittmann ,R. Gerlach, D. Xu., Mitchell, C.S. (2007).Radiocarbon dating of a very large African baobab.Tree Physiology 27(11):1569–74.

PAZ, E., MONTERO, M., ANGULO, O. and GARCIA, H.S., (1998). Preparation of a low fat fresh type of cheese. Milchwissenschaft 53 , 81- 83.

Perdigon, G.; Locascio, M.; Medici, M.; Holgado, A.P.R. and Oliver, G. (2003). Interaction of bifidobacteria with the gut and their influence in the immune function. Biocell; 27: 1-9.

Pessi, T.; Sutas, T.; Sutas .; Hurme, M and Isolauri ,E .(2000). Interleukin-10 generation in atiopic children following oral Lactobacillus rahammosus GG. Clinical and Experimental Allergy; 30: 1804-1808.

Phadungath, C.(2005).Cream cheese products: A review. Songklanakarin. Journal of Science and Technology. 27: 191-199.

Playne, M. J. (2002). The health benefits of probiotics. *Food Australia*; 54: 71-74.

Poch, M. and Bezkorovainy, A. (1988).Growth enhancing supplements for various species of the genus *Bifidobacterium*. Journal of Dairy Science; 71: 3214-3221.

Pramai, P.; Abdul Hamid, N.A.; Mediani, A.; Maulidiani, M.; Abas, F. and Jiamyangyuen, S. (2018). Metabolite profiling, antioxidant and α glucosidase inhibitory activity of germinated rice: nuclear magnetic resonance based metabolomics study. J Food Drug Anal; 26: 47-57.

Prentice, A.;Laskey, M.A.;Shaw, J.; Hudson, G.J.; Day, K.C.; Jarjou, L.M.A.; Dibba, B. and Paul, A.A. (1993). The calcium and phosphorus intakes of rural Gambian women during pregnancy and lactation. British Journal of Nutrition, 69**:** 885-896.

Radostits, O.M.; Gay, C.; Hinchcliff, K.W. and Constable, P.D., (2007). A Textbook of the Diseases of Cattle, Sheep, Goats, Pigs and Horses, 10th edition, (Saunders, Edinburgh, London. 673–753.

Ramassamy ,C. (2006) . Emerging role of polyphenolic compounds in the treatment of neurodegenerative diseases: A review of their intracellular targets. European Journal of Pharmacology 545, 51-64

Ranganna, S. (1979). Manual of Analysis of Fruits and Vegetables Products.Tata Mc-Graw Hill Publishing Company Limited, New Delhi, India.

Ranganna, S. (2001**).** Handbook of Analysis and Quality Control for Fruits and Vegetables Products.2nd ed., Tata. Mc-Grow Hill Published CompanyLimited, New Delhi, India.

Ribeiro, E.P.; Simoes, L.G.and Jurkiewicz, C.H. (2009). Desenvolvimento de queijo Minas frescal adi- ˜cionado de *Lactobacillus acidophilus* produzido a partir de retentados de ultrafiltrac ¸ao. Ci ˜ en ˆTecnol Alim 29:19–23.

Richards, L.A. (1968). Diagnosis and improvement of saline and alkaline soil.1stEd. IBH Publishing Co., New Delhi, India.Agri. Handbook No. 60.

Rinkinen, M.; Jalava, K.; Westermarck, E.;Salminen, S. and Ouwehand, A.C. (2003). Interaction between probiotic lactic acid bacteria and canine enteric pathogens: A risk factor for intestina Enterococcus faecium colonization? Veterinary Microbiology; 92: 111- 119.

Roberfroid, M.B. (1998). Prebiotics and synbiotics: concepts and nutritional properties, British journal of Nutrition; 80: S197-S202.

Roginsky, V. and Lissi, E.A. (2005). Review of methods to determine chain-breaking antioxidant activity in food. Food Chemistry, 92, 235-254.

Rojo, L.E.; Ribnicky, D.; Logendra, S.; Poulev, A.; Rojas-Silva, P. and Kuhn, P. (2012). In vitro and in vivo anti-diabetic effects of anthocyanins

from Maqui Berry (*Aristotelia chilensis*). Food Chemistry;131: 387–96

Rolfe, R.D. (2000). The role of probiotic cultures in the control of gastrointestinal health. Journal of Nutrition; 130: 396S-402S.

Rosander, A.; Connolly, E.;Roos, S. and Connolly.; Roos. (2008). Removal of antibiotic resistance gene-carrying plasmids from Lactobacillus reuteri ATCC 55730 and characterization of the resulting daughter strain, L. reuteri DSM 17938". Appl Environ Microbiol. 74 (19): 6032–40.

Roupas, P. and Williams, P.G. (2007). Regulatory aspects of bioactive dairy ingredients. Bull Intl Dairy Fed 413:16–26.

Ryan ,D. and Robards .K. (1998) . Critical Review: Phenolic compounds in olives. The Analyst 123, 31-44

Ryan ,D. Antolovich M.; Prenzler P.; Robards K.and Lavee S. (2002a). otransformations of phenolic compounds in Olea europaea L. Scientia Horticulturae 92, 147-176 .

Saarela, M.; Morgensen, G.; Fonden, R.; Matto, J. and Mattila-Sandholum, T. (2000). Probiotic bacteria: safety, functional and technological properties. Journal of Biotechnology; 84:197- 215.

Salih, N.K.E. and Yahia, E.M. (2015). Nutritional value and antioxidant properties of four wild fruits commonly consumed in Sudan.International Food Research Journal, *22*: 2389-2395.

Salminen, S., and Gueimonde, M. (2004). Human Studies on Probiotics: What is Scientifically Proven. Journal of Food Science; 69: M137-M140.

Salminen, S.; Bouley, C.,.; Boutron-Ruault, M.C.;Cummings, J.H.;Franck, A.; Gibson, G.R.; Isolauri, E.; Moreau, M.C.and Roberfroid, M., and

Rowland, I. (1998a).Functional food science and gastrointestinal physiology and function. British Journal of Nutrition; 80: S147–71.

Salminen, S.; Isolauri, E. and Salminen, E. (1996). Clinical uses of probiotics for stabilizing the gut mucosal barrier: Successful strains and future challenges. Antonie von Leeuwenhoek; 70: 347-358.

Salminen, S.; Ouwehand, A.; Benno, Y. and Lee, Y.K. (1999). Probiotics: how should they be defined? Trends Food Sci. Technol. 10: 107 – 110.

Salminen, S.; Wright, A. v.; Morelli, L.;Marteau, P.; Brassart, D.; Vos, W. d.; Fonden, R.; Saxelin, M.;Collins, K.; Mogensen, G.; Birkeland, S. E.; Mattila,S.T.; von Wright, A.and Vos, W.M.(1998) .Demonstration of safety of probiotics - a review. International Journal of Food Microbiology ., 44, 93-106.

Salovaara ,H.(1999). Functional Foods : a global prespective. Cereal Food World, 44:98

Sanchez, C., Beauregard, J.L., Chassagne, M.H.,Bimbenet, J.J., and Hardy, J.(1996). Effects of processing on rheology and structure of double cream cheese. Food Res. Intern., 28(6): 547-552.

Sanders, M.E., (2000). Symposium: Probiotic Bacteria: Implications for Human Health - Considerations for use of probiotic bacteria to modulate human health. American Society for Nutritional Sciences; 130: 384S-390S.

Santos A, San Mauro M, Diaz DM.(2006). Prebiotics and their long-term influences on the microbial populations of the mouse bowel.Food Microbiology. 23: 498 - 503.

Sauberlich, H. E. (1994). Pharmacology on Vitamin C. Ann Rev Nutr, 14:371-391.

Saxelin, M., Grenovb, B., Svenssonc, U., Fondénc, R., Renierod, R. and MattilaSandholm, T. (1999).The technology of probiotics. Trends in Food Science and Technology; 10: 387-392.

Scheuring, J. F., Sidibe, M. and Frigg, M. (1999) .Malian agronomic research identifies local baobab tree as source of vitaminA and vitamin c. Sight and Life Newsletter, 1: 21-24.

Schutz, K., Kammerer, D., Carle, R., Schieber, A.(2004). Identification and quantification of caffeoylquinic acids and flavonoids from artichoke (CynarascolymusL.) heads, juice, and pomace by HPLC-DAD-ESI/MSn. J. Agric. Food Chem. 52,4090–4096.

Semjonoves, P., Marauska, M., Linde, R., Grube, M., Zikmanis, P. and Bekers, M. (2004).Development of Bifidobacterium lactis Bb12 on β-(2,6)- linked FructanContaining Substrate. Engineering in Life Sciences; 4: 433-437.

Sena, L. P., Vanderjagt, D.J., Rivera, C., Tsin, A.T.C., Muhamadu, I., Mahamadou, O., Millson, M., Pastuszyn, A., Glew,R.H., (1998). Analysis of nutritional components of eight famine foods of the Republic of Niger. Plant Foods for HumanNutrition, 52: 7-30.

Shah, N.P. (1997). Bifidobacteria: Characteristics and potential for application in fermented milk products. Milchwissenschaft; 52: 16-18.

Shah, N.P. (2000). Probiotic bacteria: selective enumeration and survival in Dairy food. Journal of Dairy Science; 83: 894-907.

Shah, N.P. (2001). Functional foods from probiotics and prebiotics, Food Technology; 55: 46-53.

Shahat, A. A. (2006). Procyanidins from Adansonia digitata.*Pharmaceutical Biology*.*44*, 445–450.

Shanta-Retenly, V., (2005). Living Bacteria- the Body‘s Natural defense. For The Record; 17: P46.

Sharma, J.N., Al-Omran, A. and Parvathy, S.S.(2007). Role of nitric oxide in inflammatory diseases,Inflammopharmacology, 15 (6) ,252–259.

Sharp, M.D; McMahon, D.J. and Broadbent, J.R. (2008). Comparative evaluation of yogurt and lowfat Cheddar cheese as delivery media for probiotic *Lactobacillus casei*. Journal of Food Science 73:M375–M377.

Sherlock, S.; Dooley, J. (2008).*Diseases of the Liver and Biliary System*; John Wiley & Sons: New, York, NY, USA,

Sheu, F, Lai, H.H.and Yen GC.(2001).Suppression effect of soy isoflavones on nitric oxide production in RAW 264.7 macrophages. J Agric Food Chem.; 49: 1767-72.

Shiba, T., Aiba, Y., Ishikawa, H., Ushiyama, A., Takagi, A., Mine, T.,Koga, Y.,(2003). The suppressive effect of bifidobacteria on Bacteroides vulgatus, a putative pathogenic microbe in inflammatory bowel disease .Microbiol.Immunol. 47, 371–378.

Shihata, A. and Shah, N.P. (2000). Proteolytic profiles of yogurt and probiotic bacteria. International dairy Journal; 10: 401-408.

Sidibe M, Williams JT.(2002). Baobab, Adansonia digitata L. Southampton, UK: International Centre for Underutilized Crops. 96p.

Sidibé, M., Scheuring, J.F., Koné, M., Hofman P., Frigg M., (1998) More on baobab's homegrown Vitamin C. Agroforestry Today,10(4):10.

Sidibe, M., Scheuring, J.F., Tembely, D., Sidibé, M.M., Hofman, P., Frigg, M. (1996).Baobab – Homegrown Vitamin C for Africa. Agroforestry Today, 8 (2), 13-15.

Silva, E.L.; Lobo, J.F.; Vinther, J.M.; Borges, R.M.; Staerk, D.(2016). High-resolution α-glucosidase inhibition profiling combined with HPLC-HRMS-SPE-NMR for identification of antidiabetic compounds in Eremanthus crotonoides (Asteraceae). Molecules, 21, 782.

Simon, J. A. and Hudes, E. S.(2000). Serum ascorbic acid and gallbladder disease prevalence among US adults: the Third National Health and Nutrition Examination Survey (NHANES III). *Archives of International Medicine*, **160**: 931-936.

Stanton C, Gardiner G, Lynch PB, Collins JK, Fitzgerald G, Ross RP. (1998). Probiotic cheese.Intl Dairy J 8:491–6.

Stanton, C., Desmond, C., Coakley, M., Collins, J.K., Fitzgerald, G., Ross, R.P. (2003).Challenges Facing Development of Probiotic-Containing Functional Foods. In: Farnworth E, editor. Handbook of Fermented Functional Foods. Boca Raton: CRC Press., 27-58.

Sybesma, W., Burgess, C., Starrenburg, M., Sinderen, D.V., and Hugenholtz, J. (2004).Multivitamin production in Lactococcus lactis using metabolic engineering. Metabolic Engineering; 6: 109-115.

Taamalli, A., Go ´mez-Caravaca, A.M., Zarrouk, M., Segura-Carretero, A., and Ferna ´ndez-Gutierrez, A. (2010). Determination of polar and minor polar compounds and other chemical parameters for the discrimination of six different varieties of Tunisian extra-virgin olive

oil cultivated in their traditional growing area.*European Food Research and Technology*, *231*, 965–975.

Tembo, David T., Holmes, Melvin J., and Marshall, Lisa J. (2017). Effect of thermal treatment and storage on bioactive compounds, organic acids and antioxidant activity of baobab fruit (Adansonia digitata) pulp from Malawi.*Journal of Food Composition and Analysis* http://dx.doi.org/10.1016/j.jfca..01.002

Tuberoso, I.G., A. Rosa, E. Bifulco, M. P. Melis, A. Atzeri, F. M. Pirisi, M. A. Dessi.(2010). Chemical composition and antioxidant activities of *Myrtus communis* L. berries extracts. Food Chemestery; 123: 1242-1251.

Tundis R., Loizzo M.R., Statti G.A., Menichini F.(2007). Inhibitory effects on the digestive enzyme α-amylase of three *Salsola* species (Chenopodiaceae) *in vitro*. Pharmazie. ;62:473–475.

Udenigwe, C.C., Lu ,Y.L, Han, C.H., Hou, W.C, Aluko, R.E. (2009).Flaxseed protein-derived peptide fractions: Antioxidant properties and inhibition of lipopolysaccharide-induced nitric oxide production in murine macrophages. Food Chemistry.; 116: 277-84.

Ustunol, Z., and Gandhi, H., (2001). Growth and viability of Commercial Bifidobacterium spp. In Honey-Sweetened Skim milk. Journal of Food Protection; 64: 1775-1779.

Van boekel, M. A. J. S., (1993). The transfer of milk components to cheese: Scientific considerations. Proceedings, IDF Seminar on Cheese Yield and Factors Affecting its Control. Cork. Pp 19-27.

Varnam, A.H. and Sutherland, J.P., (1994). Milk and Milk products: Technology, Chemistry and Microbiology. volume 1. In: Food Products Series.London: Chapman and Hall. 307-312.

Vendemiale, G.; Grattagliano, I.; Altomare, E.An .(1999).update on therole of free radicals and antioxidant defense in human disease.Inter. J. Clin. Lab. Res. 29, 49-55.

Vermaak, I., Kamatou, G.P.P., Komane-Mofokeng, B., Viljoen, A.M. and Beckett, K. 2011. African seed oils of commercial importance - Cosmetic applications. South African Journal of Botany. 77(4),pp.920–933.

Vertuani, S., Braccioli, E., Buzzoni, V., Manfredini, S. (2002). Antioxidant capacity of Adansonia digitata fruit pulp and leaves. Acta Phytotherapeutica, 2 (V), 2-7.

Vinderola G, Prosello W, Molinari F, Ghiberto D, Reinheimer J. (2009). Growth of *Lactobacillus paracasei* A13 in Argentinian probiotic cheese and its impact on the characteristics of the product. Intl J Food Microbiol 135(2):171–4.

Vinderola, C. G., Bailo, N., Reinheimer, J. A.(2000). Survival of probiotic microflora in Argentinian yoghurts during refrigerated storage. Food Research International V. 33, n. 2, p. 97-102.

Von May Dell, H. J. (1990). Trees and Shrubs of the Sahel: Their Characteristics and Uses. Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ) GmbH, Germany, ISBN 3823611984.

Von Wright, A., Axelsson L .(2000). Lactic Acid Bacteria: An Introduction. In: Lahtinne S, Salminen, S, Von Wright A, Ouwehand A, editor. Lactic Acid Bacteria: microbiological and Functional Aspects. London: RC Press. pp., 1-16.

Waltham , M.S. (1998). Roadmaps to Market: Commercializing Functional Foods and Nutraceuticals, Decision Resources, Inc., p. 5.

Warminska-Radyko, I., Laniewska-Moroz L. and Babuchowski, A. (2002).Possibilities for stimulation of Bifidobacterium growth by propionibacteria. Lait; 82; 113-121.

Wickens GE, Lowe P. (2008). The Baobabs: Pachycauls of Africa, Madagascar andAustralia: The Pachycauls of Africa, Madagascar and Australia: Springer.

Wickens, G. E. (1982) .The baobab - Africa's upside-down tree. Reprint from Kew Bulletin, 37(2).

Wickens, G.E., 1980. The use of the Baobab (A digitat L) in Africa Pagexx-zz in:LE Houerou, H.N (Ed). Browse in Africa the current state of knowledge.ILCA, Addis Ababa, Ethopia.

Wiehle, M., Prinz, K., Kehlenbeck, K., Goenster, S., Mohamed, S.A., Finkeldey, R., Buerkert, A., & Gebauer, J. (2014). The African baobab (Adansonia digitata L.)—genetic resources in neglected populations of the Nuba Mountains, Sudan. American Journal of Botany, 101, 1489–1507

Willet, W. C. (2001). Eat, drink, and be healthy. The Harvard Medical School guide to healthy eating. New Work: Simon and Schuster.

William, C.F. and Dennis, C.W. (1998). Food Microbiology, 4th ed., New Dalhi, India:56-57.

Yazzie, D., VanderJagt, D.J., Pastuszyn, A., Okolo, A., Glew, H. (1994). The Amino Acid and Mineral Content of Baobab (Adansonia digitata L.)Leaves. Journal of Food Composition and Analysis, 7, 189-193.

Yiming Li, Tran VH, Duke CC, Roufogalis BD. (2012).Preventive and Protective Properties of Zingiber officinale (Ginger) in diabetes mellitus, diabetic complications, and associated lipid and other

metabolic disorders: A Brief Review. Evid-based Complemen Altern Med.

Zhao, Q.Z., Wang, J.S., Zhao, M.M., Jiang, Y.M. and Chun, C. (2006).Effect of Casein Hydrolysates on Yogurt Fermentation and Texture Properties during Storage.Food Technology and Biotechnology; 44: 429–434.

Zheng J., He J., Ji B., Li Y., Zhang X. (2007).Antihyperglycemic effects of *Platycodongrandiflorum* (Jacq.) A. DC extract on streptozotocin-induced diabetic mice. Plant Foods Hum.Nutr. ;62:7–11.

Zin, Z.M., Hamid, A.A., Osman, A. and Saari, N.(2006) Antioxidative activities of chromatographic fractions obtained from root, fruit and leaf of Mengkudu (*Morinda citrifolia* L.). *Food Chemistry*; *94*: 169-178.