Sudan University of Science and Technology

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Physicochemical Characterization of *Ziziphus Spnia-christi Fruit Pulp* (Nabag)

التوصيف الفيزيوكيميائي للب ثمرة النبق

A Thesis Submitted In Partial Fulfillment of the Requirements for the Degree of Master in Chemistry

By

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Dedication

To my

Parents, Brothers and Sisters

For my elegant Madam

With you life is possible

Acknowledgments

My thank and praise to Al mighty Allah for guiding me to the right path of life. Without his grace this project could not become a reality.

I am feeling oblige to take this opportunity to sincerely thank my supervisor Dr. Omer A. Gibla. Moreover I am highly obliged in taking the opportunity to sincerely thank all the staff members of chemistry department for their generous attitude and friendly behavior.

At last but not the least I am thankful to all my teachers and friends who have been always helping and encouraging me though the years. When I go to their classes, I leave energized and excited.

Abstract

The aim of this study was to measure the nutritional value of *Ziziphus spinachristi* (L) (Nabag) fruits pulp as one of the forest foods that are frequently consumed by children and adults in Southern Darfur. The samples were collected from Nyala city market. AAS and ICP analysis were carried to measure the availability of some minerals as essential constituents of food. HPLC analysis was used to determine sugars and protein content. Total carbohydrates, ash, moisture, fibre, fats and protein were determined by other methods. The results showed minerals mean concentrations as Ca(57.22 mg/100g), Mg(72.11 mg/100g), Na(9.12 mg/100g), V(0.14 mg/100g), Fe(2.81 mg/100g), Cu(0.34 mg/100g), Ni(0.25 mg/100g), Al(3.42 mg/100g), As(0.37 mg/100g)and Pb(0.31 mg/100g).

Sugars content were fructose (175.58 g/kg), glucose (158.34 g/kg) and sucrose (132.02g/kg). The results of manual methods showed ash content as (3.34 %), moisture (8.16 %), protein (5.38 %), fibre (4.77 %), fat (1.09 %), and total carbohydrates (77.26 %). The total results showed that Nabag fruits pulp was very rich with sugars and nutritionally essential minerals.

المستخلص:

الهدف من هذه الدراسة هو قياس القيمة الغذائية للب ثمار النبق كواحد من الاطعمة الغابية التي يستهلكها الاطفال والكبار احيانا.استخدم كل من جهاز مطيافية الامتصاص الذري (AAS) و(ICP) لقياس وجود بعض المعادن الاساسية كمكونات غذائية.استخدمت جهاز (HPLC) لتحديد محتوي السكر والبروتين.

تم تحديد محتوي كاربوهيدرات الكلية، الرماد، الرطوبة، الالياف، الدهون و البروتين بطرق اخري.

اظهرت النتائج متوسط تراكيز المعادن كالاتي (Ca(57.22 mg/100g), Mg(72.11 mg/100g), Na(9.12 mg/100g), V(0.14 mg/100g), Fe(2.81 mg/100g), Cu(0.34 mg/100g), Ni(0.25 mg/100g), Al(3.42 mg/100g), As(0.37 mg/100g)and Pb(0.31 mg/100g)).

وجد ان محتوي السكريات هو الفركتوز(175.58 g/kg)، الجلكوز(158.34 g/kg) و السكروز(132.02g/kg).

نتائج الطرق اليدوية اظهرت محتوي رماد(% 3.34)، رطوبة(% 8.16)، الياف(% 4.77)، دهون(1.09 %)، بروتين(% 5.38) وكاربوهيدرات كلية(% 77.26).

اظهرت النتائج الكلية ان لب ثمرة النبق غني جدا بالسكريات و المعادن ذات الاهمية الغذائية Ca(57.22 mg/100g), Mg(72.11 mg/100g), Na(9.12 mg/100g), V(0.14 mg/100g), Fe(2.81 mg/100g), Cu(0.34 mg/100g), Ni(0.25 mg/100g), Al(3.42 mg/100g), As(0.37 mg/100g)and Pb(0.31 mg/100g).

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Chapter One

Introduction

1. Introduction

1.1. The study area:

Darfur is located in the western part of Sudan in an area between the 10th and 16th degree latitude and the 22nd and 30th degree longitude, covering 510,888 square kilometers. It is surrounded by four countries Libya, Chad, Central African Republic and South Sudan. This location has given the region strategic and economic significance.

Although western Sudan is sub-Sahara, there are three climates, semi-desert in the north semi-mountainous Jebal Marra and savannah in the south and west. (FAO 1994)

There is more than 160 tribes, most of them speak local languages in addition to Arabic and each tribe has it is their own culture, norms, traditions and features. These elements have played an integral part in Darfur's old and recent history.

Nyala means in Daju the place of chatting or a theatre. Nyala city 673 meters above sea level, it is 1,200 km far from Khartoum.

Nyala's infrastructure is among the best in Darfur, with a number of cultural and business opportunities exist. Indeed, Nyala is located in arid to semi-arid region with a typical Mountainous climate, characterized by irregular rainfall events and a harsh dry summer period. Annual precipitation is around 186 mm and annual mean temperature is 19.4°C with a minimum temperature of 11°C in January and a maximum of 40.9°C in April. (hi.globldatalab.org.Retrieved 2018-09-13).

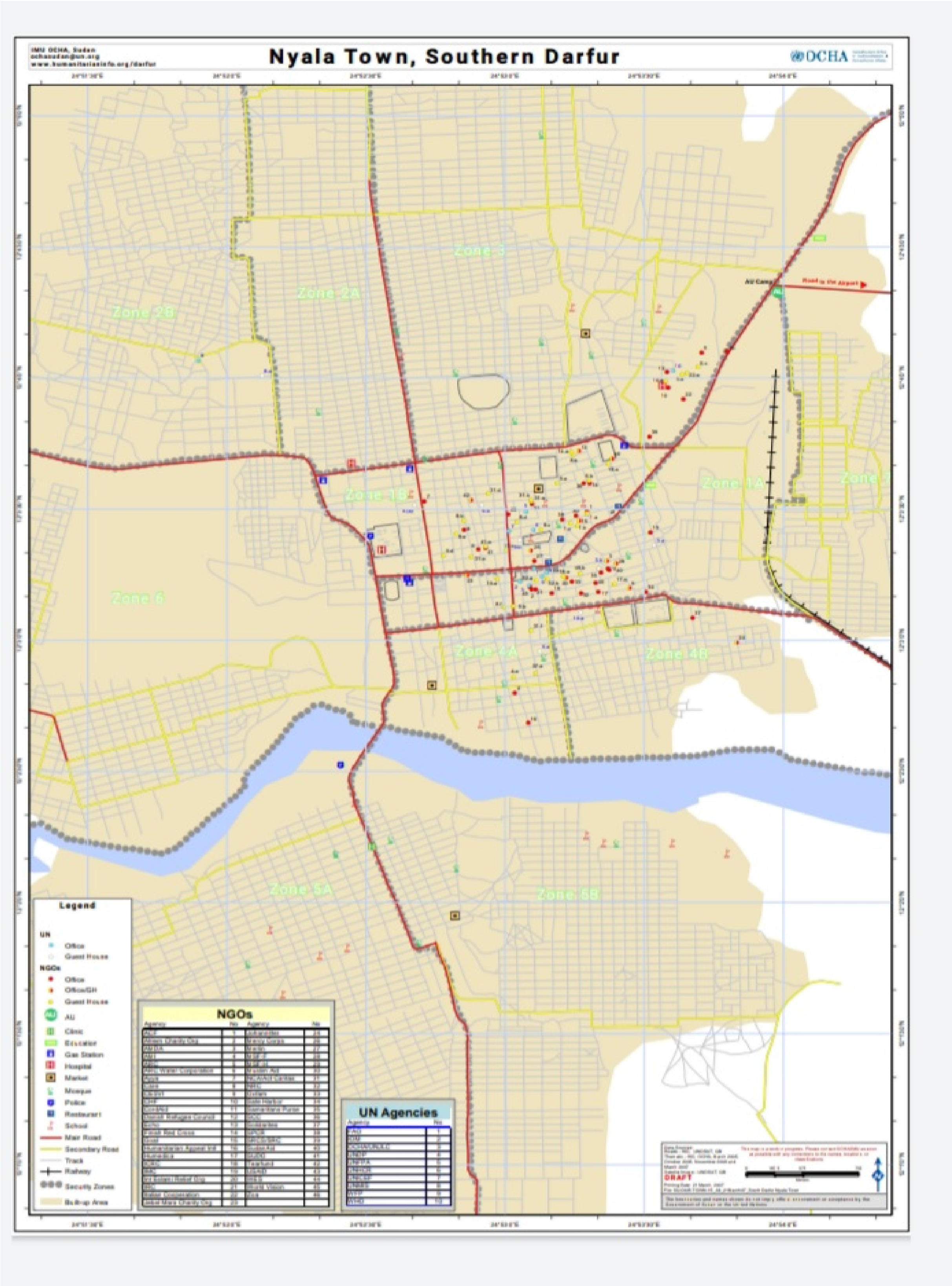

Fig.1. Map of Southern Darfur

1.2. Common and popular forest foods

Over the last few years, there has been an increasing global trend toward the use of natural antioxidants present in fruits and green leafy vegetables because of the fact that consumers are more concerned regarding the safety of using synthetic compounds in convenient food products (Swenson, 2002).

The major factors that encourage the use of vegetable sources as antioxidants and antimicrobials are the low cost, plants that contain variable chemical families and amounts of antioxidants with high bioavailability for the human body, and have less adverse effects in the human body compared with synthetic ones(Swenson,2002).

Africa has faced with the serious problem of not being able to feed its population or supply them with fuel wood (FAO, 2001). Frequent crop failure in arid and semi-arid areas often results in the poor nutrition of local people (Maxwell.1991, Waterlow, 1998). For these reasons it is important to find other resources for getting enough food for the growing population.

Sudan country includes different ecological zones from the desert in the north to the tropical rainforest in the south. The savanna areas are vast, and occupy at least 37% of the entire land in Sudan (Crail, 1991). They are habitats for numerous plant species (Elamin, 1990).

Trees and shrubs play a significant role in maintaining the natural ecosystem and in preventing and combating desertification in the Sahel. They provide a multitude of useful products for the people (Von Maydell, 1990). With a new understanding of the value of indigenous fruit trees in providing food security and meeting nutritional needs, these trees receive increasing attention, especially in semi-arid zone.

Non-wood forest products have been defined as "all goods of biological origin other than wood in all of its forms, as well as services derived from forest or any land under similar use." In many parts of the world, these products still play an

important biological and social role in local food systems. They can contribute substantially to nutrition, either as part of the family diet or as a means to achieve household food security. They can also improve health through the prevention and treatment of diseases.
Poor households residing in and around forest areas particularly landless people, women and children depend to a greater or lesser extent on the exploitation of common property forest resources in their everyday life or in periods of crisis (FAO, 1995). Although one should not expect forest foods to ensure food self-sufficiency of the local population, they can nevertheless constitute an important element of sustainable diets. Such resources which are often seen as relics of the past should actually be considered as underexploited opportunities for the future (FAO 1995). In Africa, local communities depend on forests and plants for their daily needs including goods and services. These needs are principally food, medicine, wood and fodder. In Sudan, about Non- wood forest products that can be harvested in their natural environments could be produced from forest plantations or from trees outside forest zones, there are 830 types of wood plants such as trees, shrubs and creeping plants. Amongst these types, there are 154 types classified as non-wood products. about 90 of these are considered to be food stuffs, beverages and fodder whereas 67 other types are considered as medicinal herbs and chemical products together with 18 types as fiber sources and 19 types as raw materials for rural and handicraft industries(Badi, 1993). There are 16 types that have their place in the Sudanese folklore especially the traditional medicine (Badi, 1993). Despite this wide diversity, a very limited number of these products are considered to be of commercial value and are used or marketed in their raw material form (FNC, 2001). According to Suliman and Eldoma (1994) gathering of NWFP in Sudan started to gain considerable importance for food supply, especially during the dry season; develop good market values and are used more frequently in many industries. The contribution of NWFPs in livelihood

support has been underlined. They are particularly important to rural communities in terms of food and nutritional requirements, medicines, fodder for livestock and related domestic requirements. The demand for a number of NWFPs, especially those for food and medicines, is increasing in urban centers and cities as members from respective communities migrate and desire to use these commodities (FAO, 1995b). Gradually, influence spills over to other communities in the cities, thereby increasing the markets. An analysis done some time back showed that NWFPs are important to three main groups (FAO, 1995b); rural populations who have traditionally used these items for livelihood, social and cultural purposes; urban consumers and traders or product processors whose numbers in the NWFPs sector increase as urban markets for these products grow. Usually it provide essential food and nutrition, medicine, fodder and other related domestic requirements to rural populations as well as urban consumers (Wilson,1990).They are particularly important in relieving hunger periods in the agricultural cycle, can provide employment during slack periods and act as a buffer against risk and house-hold emergencies (Wilson, 1990; Campbell, 1991; FAO, 1995). The complete lack of reliable information on Sudanese NWFP in general and fruits in particular constitute the main research problem.

Most people in Sudan consume forest foods in one form or another. Innumerable cultivated trees produce food: fruit and nut trees coconut *palms*, *date*, *Nabag*, *Lalob*, *Cawal*, local *rice*, *Gudeim*, *guddain*, *Doum* and so on. Many household throughout the world use these as supplementing to their diets with fruits, nuts, edible leaves and other foodstuffs.

1.2.1. Physicochemical properties of forest fruits

The physical properties of forest fruits were studied for three sizes of fruits: small, medium and large. Results of physical properties (Tables 1.1, 1.2, 1.3 and 1.4) were in agreement with results reported by Vogt, (1995) and El-Amin, (1990). However red colour of *Vangueria madagascariensis (kirkir)* was reported by Abdel Muti (2002). The weight of *Grewia tenax* (*Gudeim*) fruit was obtained from 100 seeds, and which weighed 14.0 g. 52.20 % of this weight is edible part and peels together, consequently the seeds weight 47.80 %.

Physico-chemical composition of forest fruits were showed in Table (.1.4), moisture content of the forest fruits was in the range from 5.47 % *Hyphaene thebaica* to 60.52% (*karmadoda*), the first value was higher than the findings (FA0, 2006) for African *Hyphaene thebaica* of 4.00 %. While, the second value was not coping with the result 70.1 % (Kuria, 2005). The differences in moisture content are influenced by cultivation and post-harvest conditions (Bates, 2001). The protein content obtained ranged from 3.80 to 7.71% for *Hyphaene thebaica* and *Vangueria madagascariensis*, respectively. The value obtained for *Grewia tenax* was similar to the value of African *Hyphaene thebaica* (3.80 %). (Layman, 2003) mentioned that the minimum recommendation target of dietary protein of approximately 70 g/day for adults. Moreover, the plants are inexpensive source of protein; therefore *Vangueria madagascariensis* could be exploited for protein supplementation. The changes in protein contents however, indicate variations in metabolic activity during the different development stages (Wills, 1981).

Vangueria madagascariensis gave the highest fat content (2.35 %), and *Hyphaene thebaica* fruit gave the lowest value 0.95 %. (Abdel Muti, 2002) reported 1.20 % for *Vangueria madagascariensis* and (Nwosu, 2008) reported the same value for *Hyphaene thebaica*. Values of fibre content varied from 7.30 *Grewia tenax* to 18.89 % *Vangueria madagascariensis* but most values were in the range of 16.03 to 18.89 %. These results were between the ranges of 2.30 – 45.30 % obtained by (Saka, 1994) for some fruits of wild trees from Malawi.

The result of ash contents of the forest fruits ranged between 2.48 % for *karmadoda* to 7.17 % for *Hyphaene thebaica*. The last result is exactly coincides to 7.17 % and higher than 2.03 % (Abdel Rahman, 2007) for two Sudanese varieties of mango called Baladi and Abu-Samaka. This increase in ash content gives a sign to increase of minerals content of *Hyphaene thebaica* fruit. Carbohydrates content of fruit ranged from 67.42 to 84.49 % for *Vangueria madagascariensis* and *Grewia tenax*, respectively. This level is superior than the range of 3.00 - 25.00 % mentioned by Bates (2001) for some common fruits. The forest fruits recorded high percentages of crude protein, fat, crude fibre and carbohydrates. Consequently, this designate that the fruits constitute significant source of energy. The energy values obtained were 309.90, 327.28, 338.49 and 371.98 Kcal for *Hyphaene thebaica*, *Vangueria madagascariensis*, *karmadoda* and *Grewia tenax*, respectively.
The highest total sugars (83.04 %) were recorded for *Grewia tenax* and the lowest (29.59 %) for *Vangueria madagascariensis*, whereas the maximum level of reducing sugars was 77.05 % (*Grewia tenax*) and lowest was 4.67 % (*Hyphaene thebaica*). The values of total sugars in this study were higher than 15.21, 12.30 and 9.82 % for apple, pineapple and orange, respectively obtained (Fasoyiro, 2005). These differences could be attributed to the difference between species and varieties. *Grewia tenax* have a very high level of sugars in the form of D-glucose and D-fructose which are readily available nutritionally (Abdel Muti, 1991).
Ascorbic acid content of the investigated plants varied between 31.74 – 389.82 mg/100g. (Rathore, 2009) gave a vitamin C content of 30.00 mg /100g for orange. Moreover, the values are within the range of Baobab (Tabaldi) of 150 – 499 mg/100g (Manfredini, 2002).
Hyphaene thebaica fruit contains low level of ß-carotene ;(27.49 IU) vitamin (A/100 g); while *karmadoda* and *Grewia tenax* contains high level of (178.45 IU)

vitamin (A/100 g). These results are within the range from 0 to 4162.5 IU vitamin A/100g for pumpkins (Cucurbitaceae) (Adebooye, 2007). The studies of ß-carotene during storage and processing of vegetables showed no definite trend of nutrient retention, but fluctuate among samples. The decrease in ß-carotene during thermal processing was negligible particularly at the temperature and time of preparation of sample (Howard, 1999). According to (Karel, 2003) the D value which is the time needed to reduce the concentration of vitamin A by 90 % at 121 °C was reported 12.4 days. The pectin content of forest fruits were 0.27 % (*Vangueria madagascariensis*) - 1.02 % (*karmadoda* and *Grewia tenax*). The value of *Hyphaene thebaica* fruit is within the range from 0.28 to 0.48% stated (Zyren, 2006) for 10 kinds of fruits. Whereas, the higher values were cope with level (Ibrahim, 2000) to Hallway date cultivar.

Table (1.1) the physical properties of forest fruits

Parameter	*Doum*	*Kirkir*	*Karmadoda*	*Gudeim*
Figure	Not symmetric	Bally	Bally	Not symmetric
Skin colour	Brown to red	Bright yellow-brown	Crimson	Orange
Edible part colour	Bright yellow	Bright yellow-yellow	Red – brown	Bright orange
Taste	Sweetly	Acidic	Acidic sweet	Very sweet
Seeds count	Single	2 – 5	Numerous	2 - 4

The data appear to designate that all samples are important sources of nurture crude protein, crude fibre and ash (mineral); in addition, to carbohydrates and energy values. *Karmadoda* is excellent source of vitamin C; *Vangueria* *madagascariensis* and *karmadoda* are bestsource of ß-carotene. These forest fruits require more investigation in the area of nutritive value, as the determination of abundant elements and amino acids profile.

Table (1.2) Physical properties of different sizes of *Hyphaene thebaica* fruit

Parameter	Fruit size			Lsd0.05	SE
	Small	Medium	Large		
Length (cm)	5.01±0.03c	5.73±0.01b	8.29±0.05a	0.06318	0.01826
Width (cm)	4.62±0.08c	5.67±0.03b	6.43±0.06a	0.1264	0.03651
Thickness (cm)	5.12±0.07c	5.35±0.02b	6.15±0.06a	0.1094	0.03162
Weight (g)	100.22±0.2c	113.31±2.07b	137.22±0.5a	2.474	0.7151
Edible part (%)	30.87±0.38b	32.53±0.89a	22.37±0.12c	1.127	0.3256
Seeds (%)	63.12±0.60a	56.82±0.16c	61.65±0.60b	0.9929	0.2869
Peels (%)	6.27±0.11c	10.97±0.10b	15.65±0.68a	0.8041	0.2324

* Means±SD bearing different superscript letters within rows are significantly different (P≤0.05)

Table (1. 3) Physical properties of different sizes of *Vangueria madagascariensis* fruit

Parameter	Fruit size			Lsd0.05	SE
	Small	Medium	Large		
Length (cm)	1.05±0.17c	1.58±0.02b	2.03±0.05a	0.2095	0.06055
Width (cm)	0.92±0.02c	2.08±0.03b	2.22±0.07a	0.08935	0.02582
Thickness (cm)	0.90±0.10c	2.03±0.03b	2.23±0.03a	0.1264	0.03651
Weight (g)	4.09±0.01c	6.78±0.03b	9.46±0.02a	0.0006318	0.0001826
Edible part (%)	13.99±0.04c	30.83±0.07b	35.59±0.50a	0.5825	0.1683
Seeds (%)	64.87±0.76a	49.80±0.05c	53.07±1.00b	1.453	0.4199
Peels (%)	20.83±0.30a	19.49±0.13b	11.43±0.42c	0.6093	0.1761
Count of seeds	2.00±0.00c	4.00±0.00b	5.00±0.00a	0.0006318	0.0001826

Table (1.4) physicochemical composition of forest fruits (on dry weight basis)

Component	*Doum*	*Kirkir*	*Gudeim*
protein	3.80	7.71	5.35
Fat	0.95	2.35	0.38
Total sugars (%)	50.00	29.59	79.86
Carbohydrates	69.72	67.42	84.49
Reducing sugars (%)	4.67	17.25	77.05
Energy value (Kcal)	309.90	327.28	338.49

1.2.2. Some examples of southern Darfur forest foods:

1.2.2.1. *Grewia tenax* (Gudeim)

A number of species of genus *Grewia* tenax have been used as medicinal agents to treat several diseases. The large flowering plant genus *Grewia* belongs to family *Tiliaceae* and reported for its medical importance. *Grewia* is a genus of 150 species in the tropical and subtropical areas of Africa, Asia and Australia (Van Wyk1997) Eleven species of *Grewia* were identified in the Sudan, particularly on sandy or clay soils in the savanna zone of central Sudan, Darfur, Kordofan and Red Sea

Hills (Elamin H.M. 1990). These species were: *Grewia tenax*, *G.villosa*, *G.flavescens*, *G.mollis*,*G.bicolor*, *G.occidentalis*, *G.ferrugineaG.asiatical*, *G.erythraea*, *G.tembensis*and *G.stolzii*. *Grewia*"Gudeim plant" is classified locally in Sudan into two distincttypes: 1- Sharwi type, which is preferred by consumers because of its high quality (shiny red color, sweet scent, stores well). 2- Early maturing type which is produced in Southern Kordofan and is considered of inferior quality (Elamin, 1990).

Fruit may be eaten fresh or dried for later consumption, but after chewing is not actively sought after. In Sudan, a drink is prepared by soaking the fruits "Gudeim drink",*Grewai*s pp. Flour may be mixed with custard to form Nesha drink which is given to pregnant and lactating women to improve their health and milk production. It may be used as fermented drink in Sudan and Southern Africa (FAO/WHO 1988). The dead leaves are eaten, but only while they remain on the plant. There is a high and increasing demand on the fruits of this species due to the general belief that it cures malaria and iron deficiency anemia. The fruit is an important economic commodity, both locally, where it is used as food and folk medicine, and internationally where it has great export value for use in food and pharmaceutical industries (Nuha and Fatima, 2018).

1.2.2.2. *Balamities aegyptiaca (Heglig*)

Balamities aegyptiaca tree is a plant with multiple benefits and has played a major role both ancient and modern. The fruits, leaves, stem and roots are used to make laundry soap. The fruit is a natural laxative for the stomach and treats indigestion, neurological diseases, and some diseases that affect the reproductive system (syphilis) infertility, the manufacture of sex hormones, cough and chest diseases and (epilepsy) yellow fever. Alcoholic drinks are also made from the fruits which may be source of alcohol, such as ethanol (Kuhnlein 1989; Kuhnlein and Turner 1995, 1997).

Fruit seeds contain a high amount of protein and oil, which is used in the industry of cosmetics, food and oil and in the treatment of rheumatism, yellow in camels, influenza and headache. The inner core is used in the treatment of hemorrhoids and outer peel of the fruit is used in the treatment of the fever in western Sudan (P.kari 1987; Andre, 2006).

Fruit contain sugars that used locally in manufacture and considered a meal with special nutritional value to strengthen the breastfeeding women to production milk; it was found that the fruits of the *Balamities aegyptiaca* have an effect on treatments related to women and obstetrics. The twigs used as smoke to treat rheumatism , the bark is used in the treatment of malaria, jaundice and dental , and it has been found in experiments that it reduces the level of Uric acid in the blood, by removing the high urinal (P.kari 1987; Andre, 2006).

Balamities aegyptiaca as medicinal plants differ from others in that they contain active ingredients, which are volatile oil, fixed oils, soaps, carbohydrates, fats, gums and steroids (Carrier Linguistic Committee 1973).

Fruits contain oil, protein, sugar vitamin, mineral salts, soap and diosgenin. It is also an important source of steroids and high foaming glucomides when dissolved in water and is similar to soap and is used for various hygiene purposes and washing cotton and silk clothes.

It's an important source of many steroid drugs that are used as a feedstock in the manufacture of sex hormones. An example of these medical drugs is corticosteroids. It's also source of some contraceptives and sex hormones such as progesterone and others (Carrier Linguistic Committee 1973).

1.2.2.3. *Cawal*

Cawal has many names in different regions of Sudan and is mainly used as powder in the salt of asida in western Sudan. It is used in the manufacture of papers, fodder, food and pharmaceutical industries and sometimes seeds used as an alternative to coffee. It also used as seed to treat jaundice and is used as tea to

treat headaches, stomach pain, and known diseases. Internationally, the Chinese use it as remedy for red eyes and headaches, and it's considered an alternative to meat. *Cawal* is a perennial plant belonging to the legume family and classified as a shrub, herbaceous plant and noxious herb. In western Sudan called *Cawal* and in its east called sorib, it's found abundantly in America, Asia and Africa. leaves are compound consisting of three pairs of leaves , oval in shape it's flowers are yellow to orange in color and it's stems can be up to 2 meters long. The pair of leaves are smaller than the pair of upper leaves and its leaves are sensitive to light.
the fermentation vessel (Zeer) is prepared as follows:
the inside of the fermentation pot is wiped with a type of clay of the same type of clay in which the fermentation pot was made, then it was wiped from inside with a layer of sticky substance usually *okra* and sometimes a wild plant called abadeib is used so that the *Cawal* does not mix with the soil. The fermenter buried in a cool area down to the neck of the pot.

Cawal leaves are cleaned from the stemsn, seeds, flowers and foreign materials and the leaves are pounded, taking into account not to lose juice. Then the dough is placed in the fermentation vessel and cover with sorghum leaves. Then well-washed stones are placed on top of the corn leaves to press the dough down, then the mouth of the pot covered with a metal tray or metal plate, and the mouth of the container and the metal cover are closed with clay to prevent insects from entering the fermentation pot. every three days , the ingredients are mixed inside the bowl well by hand and cover with fresh fine corn leaves and after 14 days .the *Cawal* (black substance with strong smell) is formed and the dough is formed in the form of the small balls and placed in the sun for a period of 3-4 days until it dries and almost black *Cawal* contains a high percentage of proteins that are free of cholesterol and beneficial bacteria and is cooked alone or considered as one of

most important spices in the west and is added as a powder to other foods such as *okra* , *luba*, yoghurt and so on.
the powder is added to any salt according to the mood, and the *Cawal* has a strong pungent odor.
Cawal have strong benefits for humans body such as balancing the natural microbes in the gastrointestinal tract and the respiratory system, lowering the level of cholesterol and limiting high cholesterol by breaking down fats in the viscera which prevents the body from reabsorbing ,reducing high blood pressure, improving mineral absorption especially calcium and overcoming osteoporosis.

1.2.2.4. *Hyphaene thebaica (Doum)*

the fruit has a quite spongy (porous) wall that is very rich in carbohydrates and is a good source of iron and niacin (FAO, 1988). Fruit pulp(pore) of *Hyphaene thebaica*, that covering of the fruit is edible(eaten) and can either be pounded to form a powder or cut off in slices, the powder is often dried then added to food as flavoring agent; also fruit pulp is chewed to control hypertension. It tastes like gingerbread and when soaked in water until the pulp becomes soft and syrupy is much enjoyed by children (FAO, 1988). Generally, polysaccharides are the main cause of precipitation of fruit drinks. Cellulose ranks second in importance in the formation of precipitated layer in fruit beverages due to their high molecular weights. Starch probably ranks third in the formation of precipitate. In addition, the hardness of water, the method of filtering of the extract, type of the fruit and the viscosity of drink probably affect formation of precipitates (A.Azim, 1981).

1.3. *Ziziphus spinachristi* (L) tree (*Nabag*)

1.3.1. Plant classification

The genus has been divided into two subgenera, which are increasingly treated as separate genera:

Subgenus Rhamnus: flowers with four petals, buds with bud scales, leaves opposite or alternate, branches with spines. Species include

-Rhamnusalaternus-Italian buckthorn

-Rhamnusalnifolia- alder leaf buckthorn, alder-leaved buckthorn

-Rhamnuspumila_dwarf buckthorn and so on.

Subgenus frangula: flowers with five petals, buds without bud scales, leaves always alternate, branches without spines. Species include:

-Rhamnusbetulifolia-birch leaf buckthorn

-Rhamnus California-California buckthorn, coffee berry

-Rhamnuscaroliniana -Carolina buckthorn, Indian chery

-Rhamnusfrenula (frangulaalnus)-alder buckthorn, glossy buckthorn, breaking buckthorn and back dogwood as so on.

1.3.2. Plant description

Ziziphus spinachristi (L) Willd is a spiny shrub or small tree (Fig.1.2) that strongly resists heat and drought (National Academy of Sciences 1980). Normally the species grows into a tree form ,but it often acquires a bush form due to intensive grazing during the later part of the dry seasons (Obeid and Mahmoud, 1971) and heavy destructive cutting for fencing material and fuel (Miehe, 1986).

It develops very deep taproot and has an extraordinary regenerative power. It is evergreen but can drop some of its leaves during very dry seasons (Maydell1986). The tree can reach a size of 5–10 m and a trunk diameter (DBH) of up to 45 cm. The bark is whitish brown or pale grey and is deeply fissured.

The crown is rounded or umbelliform with dense branches that spread widely and have a tendency to weep at the ends. Spines are light brown in color and paired (Fig. 3) with one of each pair being up to 8 mm long, straight and directed forward while the other is shorter and slightly curved (El amin, 1990).

Leaves are simple, alternate, narrowly ovatelanceolate (Fig.1. 3), varying from 1 to 9 cm in length and 1–3.5 cm in width, are glabrous above, minutely and densely pubescent beneath, have three basal, conspicuous veins running up to the apex and around 0.5–1 cm long petioles (Maydell, 1986; Arbonnier, 2004).

The flowers have a sweet scent and are found in dense clusters in the axils of the leaves. They are small, greenish yellow, sub-sessile with 5 min sepals 2 mm long and five petals 1.5 mm long (El Amin 1990). Stamens are five opposite the petals and inserted at the base of flat lobed disc (Fig.1. 3). The ovary is 2-locular; styles short and divided above into two lobes (Miller and Morris, 1988).

In Sudan the flowering time is August until December. Fruit is a globose drupe about 1–1.5 cm in diameter, red-brown, with a hard stone (Fig.1.3) surrounded by a sweet edible pulp. Fruiting time extends from October until April.

spinachristi (L) (Nabag)

Fig.1. 2.Goats feeding on fallen leaves and fruits of Willd *Ziziphus spinachristi (L) (Nabag)*

Fig.1.3.Leaves, spines, fruits and seeds of Wild *Ziziphus spinachristi (L)* (*Nabag*)

1.3.3. Ecological and Geographical distribution

The genus *Ziziphus spinachristi* is known to be drought tolerant and very resistant to heat (Paroda, 1989). It can be found in desert areas with very low rainfall (Jawand , 1978). An indication of the importance of the root is the high root-to-shoot ratio measured on *Z. Mauritania Lam.* and the characteristic deep rooting nature of both *Z. nummularia Burm. F* (Wealth of India, 2004) and *Z. Mauritania* (Depommier, 1988). The large carbohydrate reserves in the roots contribute to the strong regeneration potential of *Ziziphus plants. Z. Mauritania* is reported as having a great capacity of recovering from injury of any kind, including fire (Grice, 1996).

Some species like *Z. Mauritania and Z. jujube Mill. (Ziziphus zizyphus .L.) H. Karst.* nom. Are found on almost every continent (Kirkbride, 2006), whereas other species like *Z. spina-christi, Z. nummularia and Z. mucronata* are restricted to specific areas. *Z. spina-christi* is found over the whole Sahelian area from Senegal to Sudan and across a large area in North Africa, Middle East, east Afghanistan and North West India (Maydell, 1986; Arbonnier, 2004). The species is native to Sudan (El Amin, 1990; Vogt, 1995; Dafni, 2005), where it grows extensively in the north and centre of the country and along the Nile banks in Khartoum (Vogt, 1995).

Ziziphus spina-christi can generally be found at altitudes up to 600 m. However, in the United Arab Emirates it has been reported to grow at elevations of around 1,500 m (Jongbloed, 2003). The species can tolerate high temperatures. It grows in desert areas with an annual rainfall of 50– 300 mm (Maydell, 1986), but is often also found in wadis where underground water is available. In Egypt apart from spontaneous growth, the tree is cultivated in the Nile valley in villages and parks (Boulos, 2000). The shrub thrives on a wide range of soil types, but prefers light silty ones (Vogt, 1995).

1.3.4. Chemical Constituents and structures of Nabag fruit

Nabag berries and leaves are considered to be a rich source of bioactive substances like isoflavones and flavonoids, which have various beneficial effects on health, such as anti-atherogenic, antioxidant, anticancer, and antibacterial effects (Suomela, 2006).

Oxidation is a natural consequence of metabolism in biological organisms. The result is the formation of detrimental reactive oxygen species (ROS) and reactive nitrogen species (RNS), such as superoxide, hydrogen peroxide, singlet oxygen and nitric oxide radicals. Normally, the antioxidant system in the human body can scavenge these radicals, thereby maintaining the balance between oxidation and antioxidation. However, when the body cannot eliminate excessive ROS using intracellular antioxidant enzyme system and extracellular antioxidant compounds, oxidative stress will occur, leading to chronic and degenerative diseases, such as osteoarthritis, atherosclerosis, cancer and other degenerative diseases related to aging (Saikia, 2013).

The antioxidative properties are attributed to hydrophilic and lipophilic compounds including ascorbic acid, flavonoids, proanthocyanidins, and carotenoids (Michel, 2012).

In particular, the leaves of sea buckthorn have been reported to contain higher levels of phenolic compounds and antioxidant activities than the berries, and also as having a higher content of nutrients and bioactive compounds such as minerals, vitamins, fatty acids, carotenoids, and phenolic compounds. Antioxidants protect the body from the detrimental effects of free radicals generated as byproducts of normal metabolism and play important roles in preventing pathogenic processes related to cancer and cardiovascular disease, and they can also enhance immune function. In addition to antioxidative roles, phenolic compounds from SB leaves had been reported to have antimicrobial activity against several pathogenic microorganisms. Further, sea buckthorn leaf extracts are reported to have marked antibacterial, antitumoral, anti-inflammatory, and antioxidative activities (Geetha, 2002).

1.3.1. Sugars

Sugar is an important component of *Ziziphus* berries, as it plays a significant role in determining the sweetness of its juice. Typically, only glucose and fructose are reported to be the sugars found in buckthorn of all the three major subspecies (*H. rhamnoides ssp. sinensis*, *ssp. rhamnoides*, and *ssp. mongolica*) (Yang, 2009). One compound detected but often reported as "unknown" was subsequently identified as ethyl β-D-glucopyranoside (Tiitinen, 2006), the presents the biochemical constituents observed in berries from the four Romanian sea buckthorn cultivars studied. Fructose and glucose were the major sugars detected. While the concentrations of fructose in Colosal, Carmen, and Golden Abundant were similar (0.18% ±**v** 0.12%, 0.18% ± 0.25%, and 0.19% ± 0.12%, respectively), it was significantly higher ($p < 0.05$) in the SF6 variety (1.10% ± 0.13%)

The glucose content was seen to be higher than that of fructose, and in all cases, it was almost double, but with a similar pattern in each of the cultivars.

The glucose concentrations determined were similar to those reported by Yang et al. (Yang, 2009) in all samples of analyzed, but much lower than the sugar content of *Nabag*. The sum of fructose and glucose in the samples analyzed by (Yang, 2011) varied widely from (0.6% - 24.2%) in berries of *ssp. sinensis*, while *ssp. Mongolica* had a similar level of glucose but less fructose than had *ssp. sinensis.* A glucose/fructose ratio between 1:1 and 1:10 has been reported for buckthorn (Ficzek, 2019), and in this study, the glucose/fructose ratio was at the lower end of those values ranging from 1:1 in the Colosal variety to 1:2.4 in SF6. Sucrose was only detected, and at a very low level, in the Carmen variety, but it was previously reported at trace levels in most of the varieties studied by (Yang, 2009).

One special feature of sea buckthorn berry is the high oil content in the soft parts, in addition to the oil found in the seeds (Yan, 2002),the oil content of the whole berries can vary considerably with the variety and other factors (Yang, 2001) In our samples, the differences in total fat content are statistically insignificant between Golden Abundant (4.86), SF6 (4.61%), and Colosal (4.21%), but a significantly higher concentration of (5.71%) was found in the Carmen variety. Previous studies reflect the importance of the specific variety of *Hippophae*spp. In relation to the fat content of fresh berries; the oil level ranges reported range from (1.4% - 13.7%) in *ssp. Turkestanica* from the Western Pamirs (Yang, 2001).

1.3.2. Flavonoids

Flavonoids are natural polyphenol compounds widely distributed in *Nabag*. They are mostly found in the fruits and leaves of the plant. According to their parent nuclear, flavonoids are divided into flavonoids, flavanols and flavanones in Nabag. Isorhamnetin, quercetin and kaempferol(Lv, 2016) are the main flavonoids found in *Nabag*.

The flavonoids of *Nabag* are often used as inhibitors of ascorbic acid oxidation, especially in treating cardiovascular diseases (Wang, 2003). Xing (Xing, 2018) found that the total flavonoids in the leaves, pulp, pericarp and seeds of *Nabag* were (2.24%, 0.95%, 0.51% and 0.31%) respectively, indicating that the leaves have the highest total flavonoid content.

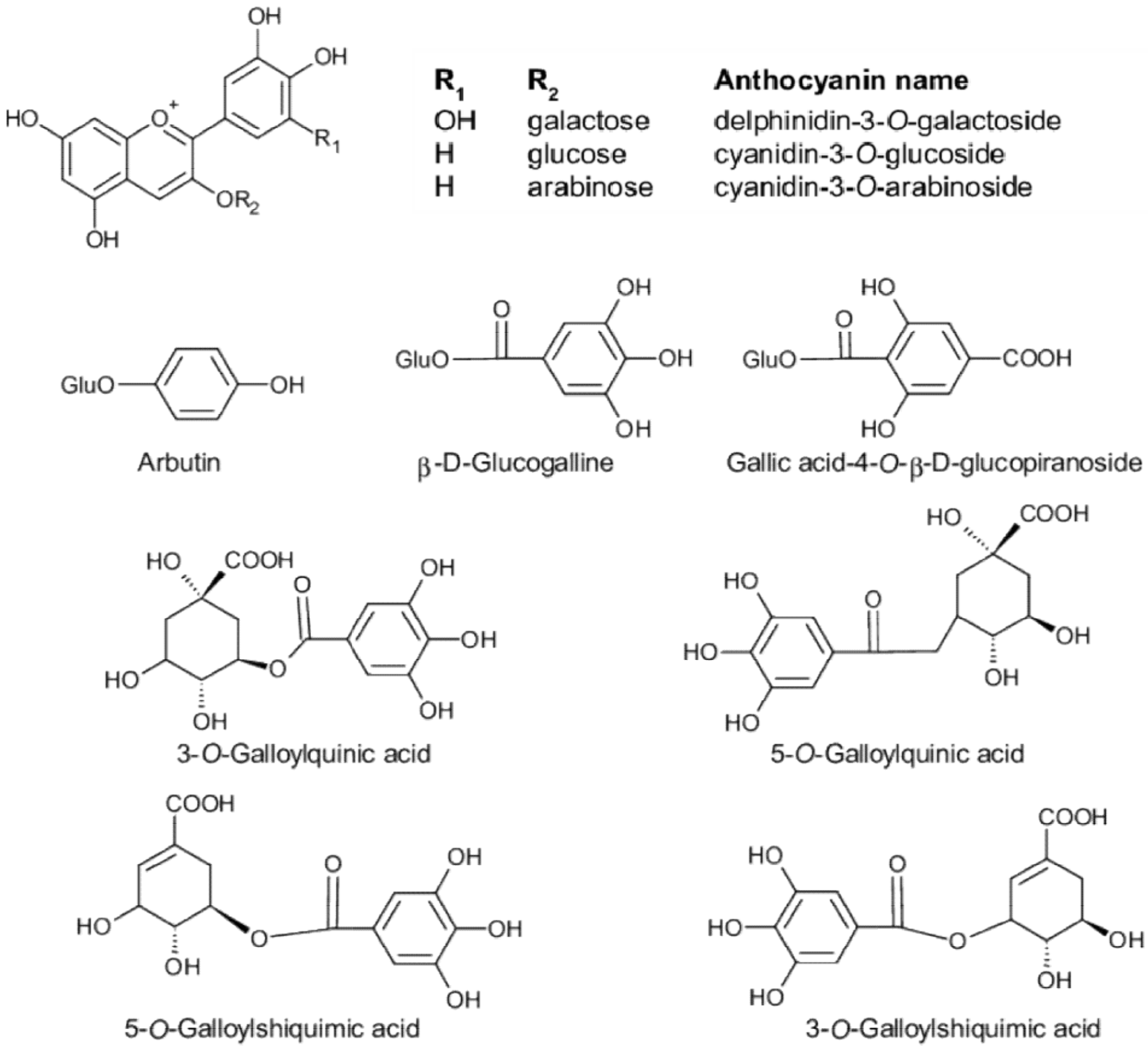

Fig.1.4.Chemical structures of flavonoids from *ziziphus spniachristi* (Mingyue, 2020)

1.3.3. Phenolic Acids

Phenolic acids are the organic acids containing an aromatic ring; these compounds are abundant in *Nabag* in the form of free radicals or bound as esters and glycosides (Kumar, 2011). Phenolics are the major compounds of *Nabag* plants with antioxidant activity (Zhang, 2004.). They are divided into hydroxybenzoic acid, hydroxycinnamic acid, and their derivatives, according to the number and position of the hydroxyl and methoxy groups in their aromatic ring. The total phenolic acid content in the seeds of *Nabag* is higher than that in the fruits and seed coat. In a study, the total phenolic acid content in the seeds was released as a soluble ester (57.3%) of the total phenolic acid; free phenolic acid and phenolic acid released by the glycosidic bond accounted for (8.4% and 34.3%)of the total phenolic acid, respectively (Liu, 2014). Phenolic acids in *Nabag* mainly include gallic acid, syringic acid, protocatechuic acid, salicylic acid, vanillic acid, gentisic acid, caffeic acid, sinapic acid, ferullic acid, cinnamic acid, 1-feruloyl-*β*-D-glucopyranoside and chlorogenic acid (Singh, 2017). Among them, gallic acid is the predominant phenolic acid in both the fruits and leaves of *Nabag*.

The phenolic compounds quantified are considered to be the major determinants for the antioxidant capacity of plants. Therefore, due to their considerable content of phenolics and carotenoids buckthorn plants studied are a promising source of natural antioxidant compounds.

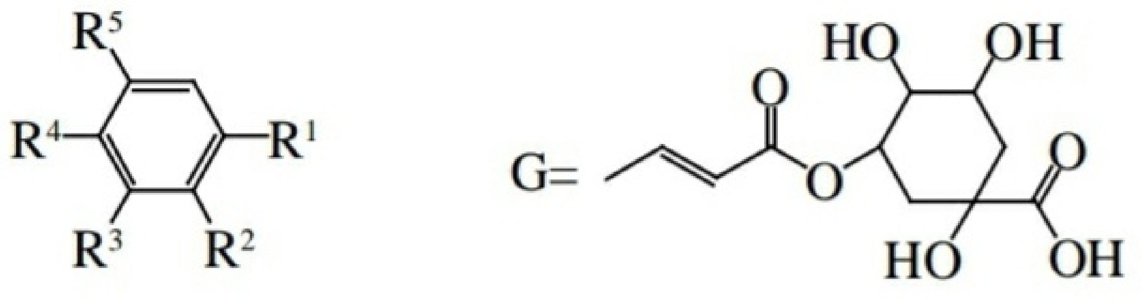

Fig.1.5.Chemical structures of phenolic acids from *ziziphus spniachristi* (Mingyue, 2020)

1.3.4. Antioxidants

Antioxidants are effective compounds that can delay, interrupt or inhibit the process of oxidative reactions by neutralizing free radicals via donation of hydrogen atom or electron, quenching singlet and triplet oxygen and chelating metals and thus play a proactive role towards improving shelf-life of food products as well as reducing the incidence of different ailments such as cancer, aging and inflammation (Lobo, 2010; Khadri, 2010; Dalleau, 2013). Currently, owing to the perceived toxic effects of synthetic antioxidants and multiple microbial drug resistance, the use of plants derived natural antioxidants and bioactives, is therefore focus of current scientific research (Alnajar, 2012).Plants based phenolic antioxidants, due to their unique structural features and multiple biological actions, play a promising role in the treatment of certain cancer and infectious diseases (Bhalodia&Shukla, 2011; Prasad, 2012; Miyasaki, 2013).

Dietotherapy with antioxidant foods is a very convenient and effective method for the supplementation of endogenous antioxidants to alleviate damage due to free radicals (Tan, 2010).

Phenolic compounds are secondary metabolites with antioxidant activity. Their biological activity depends on their structures, combination with other compounds, solubility, absorption and metabolism. Plasma iron and copper are catalysts of many free radical synthetic reactions; especially, iron ions catalyze lipid peroxidation and free radical synthesis. Polyphenols can bond with free metal ions. For example, the catechol structure of the quercetin molecule can bind with Fe^{2+} or Cu^{2+} to form inactive metal complexes, which can reduce oxidation reactions and the damage caused by free radicals to the body

The antioxidant mechanisms of polyphenols from *Nabag* species (Fig.1.6) can be summarized as follows.

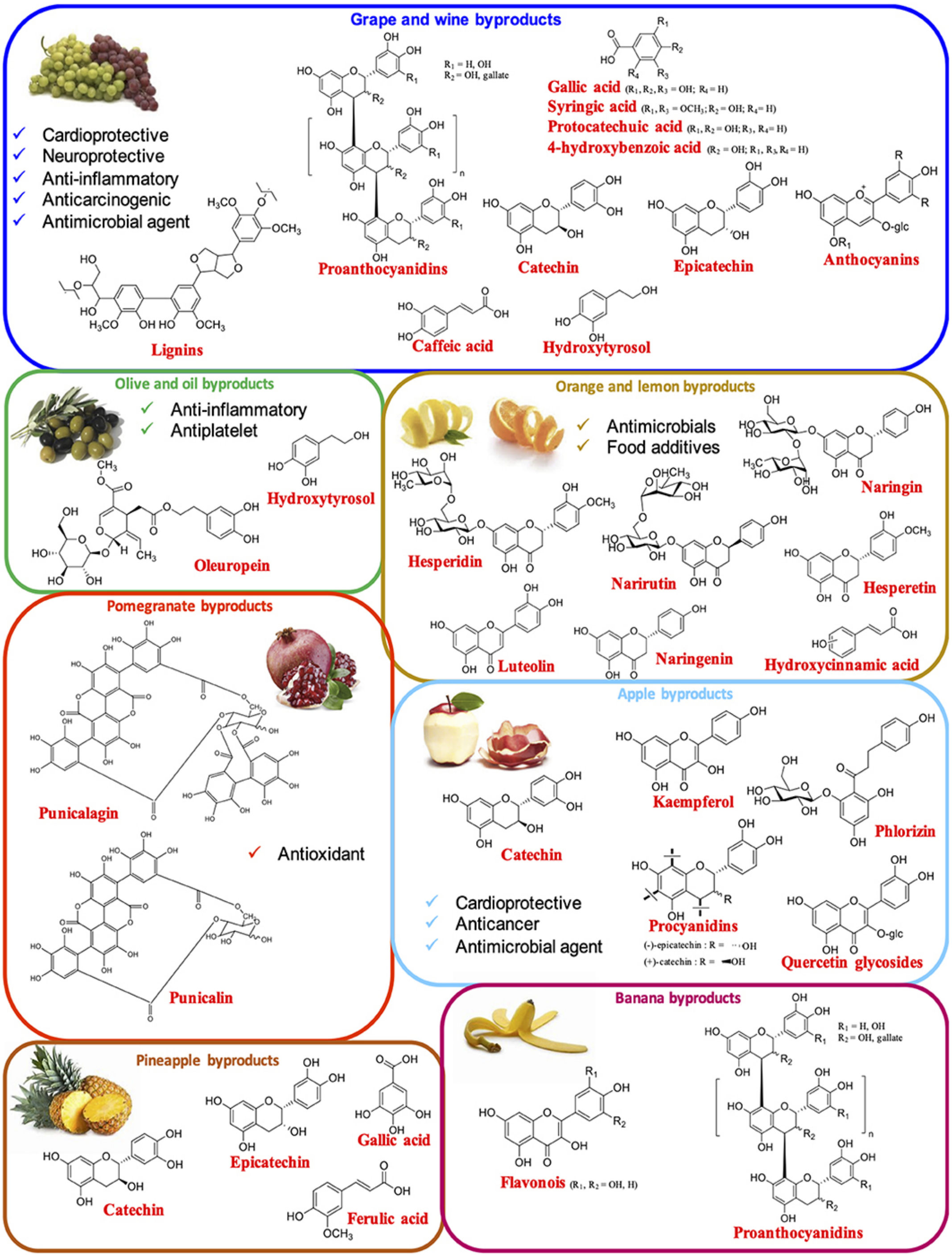

Fig.1.6.the antioxidant mechanism of polyphenols from *ziziphus spniachristi* (NADPH).

1.3.4.1. Regulation of Enzyme Activity

The enzymes related to free radicals are divided into two categories: oxidase and antioxidant enzymes (McAuley, 2002). An antioxidant effect refers to enhancing the activity of antioxidant enzymes and inhibiting the activity of related oxidase. Among antioxidant enzymes, superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT) plays an important role in the induction of reactive oxygen-scavenging enzymes, and glutathione (GSH) synthase can induce the synthesis of endogenous antioxidant enzymes. On the contrary, NADPH oxidase (NOX), xanthine oxidase (XO), lipoxygenase (LOX), monoamine oxidase (MAO) and inducible nitric oxide synthase (iNOS) promote the production of ROS. For example, XO can catalyze the oxidation of xanthine and hypoxanthine, thus producing peroxide free radicals (Lu, 2012). Free radicals can be produced by redox and peroxide of transition metal ions (iron and copper). Therefore, promoting antioxidant enzyme production as well as reducing oxidase and metal ion formation can achieve good antioxidant effect.

1.3.4.2. Enhancing Antioxidant Enzyme Activity

Endogenous antioxidant enzymes include SOD, GPX, CAT, GSHreductase (GR) and glutathione thiotransferase (GST) (Wang, 2017) SOD is a very effective antioxidant enzyme that converts decomposed superoxide anions into H_2O_2 and O_2, thereby producing alcohols and water that are harmless to the body. CAT reduces H_2O_2 to H_2O and O_2, resulting in free radical detoxification. GPX reduces active peroxide to alcohol and water, whereas GSH is oxidized to glutathione disulfide (GSSG). It reclaimed into GSH by GR, which is the main enzyme maintaining the glutathione redox state.

GST is generally regarded as a 2-phase enzyme, which mainly exists in electrophilic compounds for detoxification (Maheshwari, 2011). Several studies have also shown that GST can catalyze the decomposition of the lipid hydrogen peroxide produced by oxidative damage of lipid molecules.

1.3.5. Minerals

Minerals are inorganic elements that cannot be synthesized in the body but obtained from the diet. They are naturally present in soil and water. Some are essential to living organisms while some are very toxic. Plants absorb significant amount of minerals from the environment and usually passed them along the food chain to animals. The deficiency of such nutritionally important minerals usually proves fatal. Minerals are key elements of the body. They are needed in the buildup and function of important biomolecules in the human body. Although, minerals are not a source of energy in the body but they are necessary for the maintenance of normal biochemical processes in the body (Zhao, 2016). Based on the body needs, these essential minerals can be classified as either a macro or micro (trace) minerals.

1.3.6. Applications

the fruits and leaves of *Nabag* are rich in various bioactive components and nutritional ingredients, which are useful in the fields of healthcare, the food industry and the cosmetic industry. In recent years, researchers in the fields of nutrition, food science, medicine, sports science, agriculture and forestry have performed numerous studies on *Hippophae* species, supporting its use as a medicine and food. They believe that the leaves, fruits and seeds of *Hippophae* plants will become ideal high-grade raw materials of nutritious health food with great ecological, social and economic benefits.

1.3.6.1. Medicinal values of *Nabag*

Studies performed worldwide have found that *Hippophae* species contain rich biologically active components that have important medicinal effects on human health; thus, these species are referred to as vitamin storehouses and mystery fruits (Qi, 2001). They have been widely used in the field of medicine, and scientific interest in *Hippophae* species as therapeutic agents is rapidly increasing (Guo.2018.) developed a fermentation method to enhance the active substances with the intestinal autoimmunity function found in *H. rhamnoides*L. Leaves, in which beneficial bacteria is firmly wrapped using a drying technique, thereby enabling it to reach the intestine and exert antioxidant effects (Li, 2009), formulated a hepatoprotective natural product from *H. rhamnoides*L. By inoculating mixed strains of yeasts and lactic acid bacteria to composite culture medium, this was then subjected to a closed culture to obtain a strain solution. The solution was then mixed with *H. rhamnoides*L. And subjected to closed fermentation before elution to obtain the hepatoprotective natural product. The final product contains a large number of active components of detoxification and antioxidant reactions in the liver, and has the following beneficial effects: oxidation resistance, free radical-scavenging, chemical liver injury repair-promoting and liver function-improving effects.

1.3.6.2. Food values of *Nabag*

Fruit of *Hippophae* species is called a third-generation fruit. *Hippophae* plants contain more than 100 types of compounds; this includes vitamin C content of up to 25 mg/g (Liu, 2014). As of 2018, there have been more than 200 kinds of products derived from *Hippophae* species, including nonalcoholic beverages, wines, jams, ice creams, candies and natural additive pigments (Li, 2009).(Zou, 2018)Applied for a patent for the *H. rhamnoides*L. Fruit peel powder, which is rich in antioxidant nutrients, as a nutritional supplement that can significantly enhance the body's free radical-scavenging function and improve antioxidant function in immunocompromised people or people with antioxidant hypofunction. (Yan, 2018), provided a health-promoting edible salt product of *H. rhamnoides*L. That has high nutritional value and exerts excellent antioxidant effects; this product also has a unique taste and is additive-free.

1.3.6.3. Cosmetic values of *Nabag*

Cosmetics made from *Hippophae* plant extracts have been produced abroad; these products have natural skin-nourishing and hair-protecting properties. Cosmetic products made of *Hippophae* plants include shampoo; skin cream and bath soak (Xiong, 2004). Smida (2019) evaluated the antioxidant activities of *Nabag*-based mouthwash; the free radical-scavenging activity of sea buckthorn was assessed by DPPH and superoxide anion scavenging (NBT) assays to elucidate the role of buckthorn in reducing oxidative stress and damage linked to periodontitis. The results suggested that the superoxide radical-scavenging activities of the buckthorn pulp oil-based mouthwash increased markedly with increasing concentrations, suggesting that the mouthwash is a potent scavenger of superoxide radicals.

Furthermore, numerous beauty care products derived from *H. rhamnoides*L. Such as hair lotions, moisturizing creams, facial creams and facial masks, are popular in Russia (Wu, 1997).

2.4. Objectives of the study:

*To study the physicochemical properties of *Ziziphus spina-christi* fruit pulp (*Nabag).*

* To determine the minerals content of *Ziziphus spina-christi* fruit pulp (*Nabag)* by (AAS) and (ICP) instrumentation.

*To measure the sugars content *of Ziziphus spina-christi* fruit pulp (*Nabag)* by HPLC analysis.

*To evaluate the suitability of *Ziziphus spina-christi* fruit pulp (*Nabag)* as source of energy and essential minerals.

Chapter Two

Materials and methods

2. Materials and methods

2.1. Materials

Fresh Nabag fruit samples were brought from Nyala market in January 2021. The fruit pulp powder of each sample was dried and used for analysis.

2.2. Chemicals

Sugars standards and reagents obtained from Sigma-Aldrich Co. and Fluka (Saint Louis, MO, USA). (Darmstadt, Germany).

-Hydrochloric acid (37%), code: 12726309

-Sodium Sulphate (Anhydrous (96%)), code NO.: 7757-82-6

- Copper Sulphate (96%), Code: 28332500

-Sulphuric acid (purity 98 %), code: 12458.

-Sodium hydroxide (molar mass: 39.9971g/ ml), color: yellow, code: 4100000

-Nitric acid (with purity range (69-72%), product code: 16560).

-Boric acid (99.9%), Appearance: white powder, Shandong Above Energy Technology co.ltd is located in Jinan city

-Potassium hydroxide (95.0%), white deliquescent, central drug house (P) LTD, Code. No: 2815200000.

-Acetonitrile (99.9%), Size: 2.5 L n Packaging: Glass bottle, Description: Acetonitrile gradient grade for liquid chromatography LiChrosolv Reag. Ph.Eur, Supplier No: 1.00030.2500., code: 41120000

- Perchloric acid: assay (70-72%), description: clear colorless liquid.

2.3. Instruments

***Atomic absorption spectrophotometer**

Model 210 VGP Cat# 28750-15 Cole-Partmer, India. The conditions were adjusted according to the Atomic Absorption Spectrophotometer (WHO, 1998).

*** Inductively coupled plasma-optical emission spectrophotometer (ICP-OES)** ICP-9000 multitype emission spectrophotometer

***HPLC**

A Shimadzu Liquid Chromatography model SLC-10 Avp with a refractive index detector (HPLC/IR) , stainless steel column, length 250 mm, diameter 4.6 mm (Alltech, Nicholasville, KY, USA), and particle size 5 µm. temperature inside the column was maintained at 30 °C by means of a CTO-10 AS thermostatic heater. Column pressure 6.3 MPa. The mobile was phase acetonitrile/water (75:25 v/v) at a flow rate of 1.3 mL/min.

2.4. Methods of analysis

2.4. 1. Determination of minerals content (AAS)

Mineral content of Nabag fruit pulp samples were determined according to the method of A.O.A.C (2000).

Exactly 1g of sample was taken in digestion tubes 10ml of conc. HNO_3 was added and the mixture was kept overnight 0 .4ml of $HCLO_4$was added to that mixture and kept in fumes block for digestion. The temperature was increased gradually starting from 50˚C up to 300˚C. The digestion was completed at 80min as indicated by the appearance of white fumes. The mixture was left to cool and quantitatively transferred to a 100ml volumetric flask. The volume was then completed to the mark with distilled water. A.A.S was used for determine of Mg, Ca, Na, Fe, V, Cu, Ni, Al, As and Pb and used specific lamp for each element.

2.4. 2.Determination of minerals content (ICP-OES)

0.5 gram from each samples were digested with 10ml of conc. HNO3 using hot plate. After digestion, the samples were evaporated to dryness and the residues were dissolved in conc. HNO_3 and H_2O_2, diluted to 10 mL with conc. HNO_3. Finally, the proper concentrations of dissolution samples were determined by Shimadzu's ICP-900multiple ICP emission spectrometry (ICP-OES).

2.5. Determination of moisture content

Moisture content was determined according to the Association of official's analytical chemists AOAC (1990).

2 grams of each sample was accurately weighed in dry, clean, pre-weighed crucible and left overnight at 105°C in a hat air oven. Each crucible was then cooled in desiccator and reweighed. The process of drying, cooling and weighing was repeated until constant weight was measured.

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

Where: MC: moisture content, W1: weight of empty crucible, W2: weight of crucible with the sample and W3: weight after drying.

2.6. Determination of ash content

Ash content of the sample was determined according to the method of AOAC (1990).2 grams from each samples were placed in a clean, dry and pre-weighed crucible; each crucible was heated in a muffle furnace at 550°C for 3hours until light gray ash was obtained. Each crucible was cooled in a desiccator and weighed. The ignition, cooling and weighing were repeated until constant weights were obtained. Ash content was calculated using equation:

$$AC\% = \frac{W2-W1}{W3} \times 100$$

Where, Ac: ash content.

W1: weight of empty crucible.

W2: weight of crucible with ash.

W3: weight of sample.

2.7. Determination of protein content

Protein content was determined by using the micro-Kjeldahl method according to AOAC (1990).

0.2 g of sample was weighed and placed in small digestion flask (50 ml). About 0.4 g catalyst mixture (anhydrous sodium sulphate and copper sulphate), 3.5 ml of conc. H_2SO_4 was added. The contents were heated on an electrical heater for 2 hours until the color changed to blue-green.

After cooled the sample was transferred to the distillation unit and 20 ml of NaOH was added. The ammonia was received in 100 ml conical flask containing10ml of boric acid plus 3-4 drops of methyl red indicator. The distillation was continued until the volume reached 50 ml.

The content of the flask were titrated against 0.02 N HCL. The titration reading was recorded. The protein content was calculated using the equation;

$$PC\% = \frac{(T - B) \times N \times 14 \times 100 \times 6.25}{W_s \times 1000}$$

Where, PC = protein content

T = Titration reading

B = Blank titration reading

N = normality of HCL

W_s = sample weight

1000 = to convert to mg

2.8. Determination of fat content

Fat was determined according to the method of AOAC (1990) using soxhlet apparatus.

An empty clean and dry boiling flask was weighed. 2 g of sample was weighed and placed in a clean extraction thimble and covered with cotton wool. The thimble was placed in an extractor. Extraction was carried out for 8 hours with petroleum ether. The heat was regulated to obtain at least 15 siphoning per hour. The residual ether was dried by evaporation. The flask was placed in an oven at 105°C until dried completely and then cooled in a desiccator and weighed. The fat content was calculated using the equation:

$$FC (\%) = \frac{W2 - W1}{Ws} \times 100$$

Where, FC= Fat content

W1= Weight of extraction flask

W2= Weight of extraction flask with fat

Ws= Weight of sample

2.9. Determination of fiber content

Fiber content was determined according to AOAC (1990). 2g of defatted sample were treated successively with boiling solution of H_2SO_4 and KOH (0.26 N and 0.23 N, respectively). The residue was then separated by filtration, washed and transferred into a crucible then placed into an oven adjusted to 105°C for 18 – 24 hours. The crucible then with the sample was weighed and ached in a muffle furnace at 500°C and weighed. The content fiber was calculated using equation:

$$FC\ (\%) = \frac{W1 - W2}{Ws} \times 100$$

Where, FC = content fiber

W1 = Weight of crucible with sample before ashing

W2 = Weight of crucible with sample after ashing

Ws = weight of sample

2.10. Determination of total sugars content in *ziziphus spniachristi* fruits pulp

25 g was transferred into a beaker and mixed with 150 mL distilled water. The resultant mixture was heated at 80 °C in a water bath for 30 min, and then filtered into a 250-mL volumetric flask. Subsequently, 3 mL of the mixture

containing 1 M Zinc acetate and 0.25 M Potassium ferrocyanide was added to the volumetric flask, and then the flask was brought up to the volume using distilled water. Finally, 10 mL of the resultant mixture was titrated using Fehling's method to measure the total soluble sugar content (Association of Official Analytical Chemists, 2010). Glucose was used as the external standard, and the total sugar content was expressed as gram glucose equivalents per 100 g of fresh fruit weight g/100 g.

2.11. Determination of Sugars content (HPLC)

2.5 g Samples of Nabag fruit pulp, previously ground using a mortar and a pestle, were homogenized with ultrapure water for 1 h on a multiposition magnetic stirrer (RT series, 10 Carl Roth, Germany). The mixture was transferred to a 50 mL graduated flask containing 12.5 mL methanol of HPLC purity and filled up to the volume with ultrapure water. The solution was filtered through a 0.45 µm Millipore syringe filter, collected in vials, and stored at 4 °C until further analysis. Chromatographic separation of carbohydrates was performed on an Altima Amino 100 stainless steel column, with a length of 250 mm, diameter of 4.6 mm and particle size of 5 µm. The temperature inside the column was maintained at 30 °C by means of a CTO-10 AS thermostatic heater. Column pressure was 6.3 MPa. The mobile phase was acetonitrile/water (75:25 *v/v*) at a flow rate of 1.3 mL/min and it was filtered through a membrane filter (0.45 µm) before chromatographic analysis. The injection volume was 10 µL. A calibration curve was made for each sugar using standard solutions of different concentrations (0.5–80 mg/mL). The linear regression factor of the calibration curves was higher than 0.9982 for all sugars.
Sugars were quantified by comparison of the peak area obtained with those of standard sugars. The results for each sugar were expressed as g/k g.

Chapter Three

Results and Discussion

3. Results and discussion

Table (.3.1.) AAS and ICP analysis of *ziziphus spniachristi* (L) fruits pulp

Mineral	Concentration(mg/100g)						
	AAS				ICP		
	S1	S2	S3	mean	S4	S5	mean
Mg	73.06	69.40	62.88	68.45	78.52	76.67	77.60
Ca	60.55	54.49	45.50	53.51	62.77	62.78	62.78
Na	11.93	8.28	6.60	8.94	10.35	8.51	9.43
Fe	2.96	2.48	2.69	2.71	2.72	3.19	2.96
V	0.12	0.15	0.16	0.14	0.12	0.13	0.125
Cu	0.33	0.27	0.37	0.32	0.36	0.39	0.38
Ni	0.24	0.25	0.24	0.24	0.25	0.26	0.255
Al	3.88	3.55	3.01	3.48	2.53	4.12	3.33
As	0.38	0.42	0.37	0.39	0.401	0.298	0.35
Pb	0.28	0.34	0.34	0.32	0.34	0.27	0.31

Table.3.1 results by A.A.S analysis showed high availability of Mg in *Nabag* fruits pulp samples with concentrations (68.45 mg/100g). Ca was the second element of high availability with concentrations as (53.51 mg/100g). Na concentrations (8.94 mg/100g).Iron showed almost similar concentrations in the three samples with mean values (2.71 mg/100g). V, Cu and Ni have relatively low concentrations with mean values (0.14 mg/100g), (0.32 mg/100g) and (0.24 mg/100g) respectively.

Aluminum has mean value as (3.48 mg/100g) and this may be due to the considerable natural availability of this element in the earth crust. The toxic minerals As and Pb showed low availability with concentrations with maen value (0.39 mg/100g) for As and (0.32 mg/100g).

The ICP of Nabag fruits pulp results showed that fruit pulp was very rich with essential nutrients Mg, Ca, Na, Fe, Ni, Cu and V (Table.3.1). Aluminum concentrations were different to some extent. Arsenic and Lead showed relatively low concentrations.

Keta (2017) reported that the Na concentration as (7.67 ± 0.138mg/100g), K as (306.67±11.55mg/100g), calcium value obtained were 0.033±0.003mg/100g, Mg as (0.16±0.005mg/100g) and P as (1.582±3.34mg/100g) in dry weight.

Barde (2012) reported that the Ca (143.47-143.30mg), Mg (40.39-48.50mg), P (13.20mg and 13.20mg), Fe (5.50-3.98mg), Cu (0.25-0.43mg), Mn (3.09- 1.02mg), S (70.50- 50.47mg) and Zn (1.28- 1.42mg).

Maina (2016) reported that The result for the various mineral elements indicated in mg/kg Na(181.35), Mg (162.35), K(384.43), Zn(3.57), Mn(1.38),Fe(1.29), P(371.43) and Ca(194.35).

Table (.3.2.) Chemical compositions of *ziziphus spnia-christi* fruits pulp

Test	Sample1	Sample 2	Sample3	Mean
Moisture content%	8.26	8.03	8.19	8.16
Ash content%	3.31	3.24	3.46	3.34
Fibre content%	3.58	5.45	5.28	4.77
Fat content%	1	1.36	0.90	1.09
Protein content%	5.53	5.99	4.63	5.38
Fructose content%	32.67	31.66	30.67	31.67
Glucose content%	31.17	28.17	28.28	29.21
Disaccharides content%	68.03	68.65	67.60	68.07
Monosaccharide content%	62.63	63.38	62.36	62.79
Carbohydrate content%	78.32	75.93	77.54	77.26

Table (.3.2) results showed the chemical composition of Nabag fruits pulp samples and their values, Ash content (3.34 %), moisture content (8.16 %), fibre content (4.77 %), protein content (5.38%), fat content (1.09 %), monosaccharide content (62.79 %), disaccharides content (68.07 %), and carbohydrate content as (77.26%).

Li (2007) reported moisture contents ranging from 17.38% to 22.52%, carbohydrate contents ranging from (80.86% - 85.63%), protein contents ranging from (4.75% - 6.86%), lipid contents ranging from (0.37% - 1.02%), ash contents ranging from (2.26% - 3.01%), insoluble fiber contents ranging from (5.24% - 7.18%).

Table (3.3) Determination of Sugars in *ziziphus spnia-christi* fruit pulp (HPLC)

Sucrose	Fructose	Glucose
132.02g/kg	175.58 g/kg	158.34 g/kg

HPLC analysis showed that, Nabag fruits pulp rich of protein and sugars content included fructose (175.58 g/kg), glucose (158.34 g/kg) and sucrose (132.02g/kg). The fructose revealed high concentration in Nabag fruit pulp.

Qiong (2020) reported that the Sucrose is the prominent sugar (40.3%) contained in the fruit followed by mannose (21%), galactose (18.8%), fructose (10.4%), glucose (8.7%), maltose (0.6%), and L-rhamnose (0.2%). Maud Muchuweti (2005) reported that the sugars identified in Z. mauritiana fruit were fructose, glucose and galactose.

Conclusion:

* Nabag fruits pulp was found to be rich with many essential minerals especially Mg, Ca, Na, Fe, V, Cu and Al.

* Carbohydrates content of Nabag fruits pulp was significantly high.

* Nabag fruits pulp was found to be rich with fructose, glucose and sucrose.

* The availability of carbohydrates may indicates that, Nabag fruits pulp possess high potential energy source for our day activities.

* We can conclusively say that Nabag fruits pulp is good for human consumption especially as it is cheap and available in rural areas.

* Nabag fruits pulp content of protein, fibre and fats were found to be relatively high.

Recommendations:

- Further studies may be require including, wide sampling to represent most of Nabag tree growing areas throughout the States of Sudan.
- More care should be given to Nabag plant propagation.
- Quality control research and scientific methods of cultivation may be required.
- More chemical and nutritional values of Nabag fruit may need to be characterized, since the fruit is one of the essential forest food sources in the rural areas and poor communities.
- Problems arising from mineral nutrient deficiencies can be remedied substantially by adequate intake of Nabag, Since it is grown in the wild, it

is cheap and can therefore serve as an affordable source of mineral nutrition.

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Appendixes

Appendix (I) HPLC analysis of sugars content in Nabag fruit pulp

وزارة الزراعة
مركز البحوث الزراعية
المركز الاقليمي للاغذية والاعلاف

شهادة تحليل تخصصية

التاريخ: 2021/2/24

اسم العميل : Yahya Mohammed Abdullah

Sucrose	Fructose	Glucose	نوع العينة	رقم العينة
132.02 g/kg	175.58 g/kg	158.34 g/kg	Buckthorn Fruit	٤٢٨٨

ملخص النتائج : النتيجة تمثل العينة الواردة للمعمل فقط

مدير المركز الاقليمي
"د/ اشرف هاشم جمعه"

٩ش الجامعة - الجيزة , ت: 5731989 - 5732280 - فاكس : 5732280 ص.ب 588 الأورمان e-mail : clff@intouch.com

Appendix (II) ICP analysis of minerals content in Nabag fruit pulp

ICP ANALYSIS 01/10/2020

Research

Sample	Na mg/kg	Mg mg/kg	Ca mg/kg	V mg/kg	Fe mg/kg	Ni mg/kg	Cu mg/kg	As mg/kg	Al mg/kg	Pb mg/kg
DIW	0.038	0.036	0.009	0.112	0.111	0.124	0.025	0.251	0.066	0.234
CRM 5.0 ppm	4.513	5.519	4.229	5.19	4.966	4.961	4.91	6.044	4.167	5.159
CRM 10 PPM	8.407	10.655	8.145	10.077	9.636	9.525	9.481	11.815	8.039	9.913
BLANK	0.248	0.191	0.28	0.115	0.196	0.142	0.036	0.269	0.162	0.253
1	103.491	785.189	627.651	1.215	27.186	2.484	3.56	4.011	25.252	3.421
2	85.135	766.71	627.779	1.273	31.885	2.581	3.93	4.081	42.362	3.444
A1	83.905	828.588	489.36	1.15	42.428	1.946	4.289	2.98	41.175	2.74
A2	> 15712	16.431	55.386	1.093	51.44	1.985	0.45	25.091	74.023	2.907
A3	> 15699	7.364	28.85	1.302	20.874	1.486	0.367	13.41	26.383	2.621
A4	14.146	1.087	1.865	0.099	0.284	0.123	0.035	0.44	0.231	0.289
H1	214.082	975.508	625.521	2.313	259.562	5.309	5.562	6.407	447.481	4.872
H2	> 15241	14.791	53.327	1.275	44.687	1.645	0.646	24.368	63.921	2.751
H3	> 15924	6.815	25.428	1.148	19.958	1.611	0.604	12.298	23.547	2.917
H4	10.215	0.09	0.218	0.115	0.229	0.132	0.028	0.345	0.213	0.283