Sudan University of Science and Technology

College of Graduate Studies

A Comparative Study of Antioxidants Activities and Heavy Metals Content of some Medicinal Plants in Sudan

دراسة مقارنة نشاط مضادات الأكسدة و محتوى المعادن الثقيلة لبعض النباتات الطبية في السودان

A Thesis Submitted in Fulfillment of the Requirements for the degree of Doctor of Philosophy in Chemistry

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Dedication

For the spirit of my mother and father

To my brother and sisters

To daughter and sons

Published papers

- **Antioxidant Activity of*Psidium guajava, Schoenanthus cymbopogone, Solenostemma argel , Hyphaene thebaica and Petroselinum crispum***

- **Antimicrobial Activity of*Psidium guajava, Schoenanthus cymbopogone, Solenostemma argel , Hyphaene thebaica and Petroselinum crispum***

بسم الله الرحمن الرحيم

قال تعالى:

اقرأ باسم ربك الذى خلق(١) خلق الانسان من علق (٢) اقرأ وربك

الاكرم (٣) الذى علم بالقلم (٤) علم الانسان ما لم يعلم (٥)

صدق الله العظيم

الايات من(١ إلى ٥) من سورة العلق

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Abstract

Samples of Five different plants(*Psidium guajava (*Aljawafa*),Schoenanthus cymbopogone (*Almharib*) , Solenostemma argel*(Alhargil*), Hyphaene thebaica (*Aldoum*) and Petroselinum crispum (*Albagdunas) used in many parts of Sudan as herbal medicine. The plant samples were collected randomly from different locations of sudan.The aim of study is to evaluate the phytochemicals components of plants and their potentiality as a source of biologically activecompounds , in addition determination of heavy metals content. Four different solvents of varying polarities were used for preparation the crude extract from the plant samples.The dried plants materials were subjected to successive extraction with petroleum ether, then ethyl acetate , acetoneand finally with ethanol .The results clearly indicated variation of the total yields , the*P.guajava* extract gave the highest yield (7.001%) followed by *S. argel*(6.551%),*P. Crispum (*4.760%),*S. Cymbopogone (*4.633%) and*H. thebaica* (2.538%).Antioxidant activityand free radical moietiesanalysedby two reagents 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) and 2.2. azinobisethylbenzoline-4-sulfonic acid (ATBS) using spectrophotometricmeasurement. In the DPPH assaypetroleum ether extract revealed a non-significant free radical scavenging activity, with scavenging activity ranging between 0.16% for *P.crispum* , 29% for *P.guajava* and 30% for *S.cymbopogon* extracts while *S.argel* and *H.thebaica* extracts showed no antioxidants effect,*P. guajava* sample exhibited high activity with scavenging rate (90%, 89.2% and 89%) with ethanol, acetone and ethylacetateextracts respectively.ABTS radical exhibited good antioxidant activities compared with DPPH free radicals.The highest scavenging ability was exhibited by ethanol extract from*P.guajava*(94%), while the lowest was determined in the acetone extract of *P.crispum* (69%).Analysis for heavy metals ,results showed the range (0.037 -0.166 ppm)for Zn, (0.024 -0.2 ppm)for Mn, (103.15 -198.3 ppm)for Fe, (0.001 -0.004 ppm)for Cd , (0.012 -0.099 ppm)for

Co,and (37 -79 ppm)forNi , using atomic absorption spectroscopyand showed that the levelswere in the permissible limits according to World Health Organization (WHO), lead less than detection limit in all samples.Percentages of plants components revealed the amount of moisture(94.1 -94.9), protein(1.4 -10.2), fibre(8.7 -26.5) and ash(4.9 -7.1). The quantitative spectrophotometric analysis indicated that all plant extracts contained amount of plant samples polyphenols (0.103,0.155,0.270,0.314and0.374 mg/ml) for*P.Crispum, S. cymbopogone, S. argel ,P. Guajava and H. thebaica*,flavonoids results ranging between(0.21 -0.55mg/ml) to *S. argel*and*S. cymbopogone* respectively, amount of alkaloids ranging (0.025mg/ml for*P. Guajava* to 0.101mg/ml for *S.argel*)and tannins showed (0.23mg/ml for*S.argel* to 0.56mg/ml for*P.Crispum).*Gaschromatography (GC) was used to determineSaturated and unsaturated fatty acids in all plant extracts. Results showed various kinds of fatty acids ,Cis-10-Heptadecenoic acid methyl ester ranging between(41.99 -70.41%) which was the dominant fatty acid followed by Cis-11-Eicosenoic acid methyl ester(3.25 -11.82%) and pentadecenoic acid methyl ester(4.39 -15.84%) they are prevailing fatty acids. Reliable methods were adopted for testing the bioactivity of the components towards four bacterial species (*Bacillus subtilis Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus),* the obtained results of solvents extract withbacterial strainshowed B.s(12.5 -20mm),E.c (11 -23mm),P.a (11 -23.5 mm) and S.a (13 -21 mm) and two fungal species *A. Niger(*0 -23 mm) *and C. Albicans*(0 -21mm). All plant extracts were non-toxic towards the normal cell line (IC50 > 100 ppm) as compared with triton-x100 as positive control. In conclusion, the obtained results of this study justify the use of these plants in traditional medicine and indicate a promising potential for the development of medicinal agents from them.

المستخلص

أجريت الدراسة على خمسة عينات من نباتات مختلفة تستخدم فى بعض مناطق السودان كعلاجات عشبية تتمثل فى اوراق الجوافة ،المحريب ،الحرجل ،ثمار الدوم و البقدونس وتم جمع العينات عشوائيا من مناطق مختلفة فى السودان. تهدف الدراسة لتقييم المكونات الكيميائية النباتية ودورها كمصدر للمكونات النشطه بيولوجيا بالاضافة لتقدير بعض العناصر الانتقاليه و تم استخلاص المكونات الكيميائية بواسطة مذيبات عضوية تختلف من حيث القطبية (الا يثر البترولى ،اسيتات الايثيل ،الاستون و الايثانول) وخضعت عينات النباتات الجافة الى استخلاص متعاقب بواسطة الايثر البترولى ، الايثيل اسيتات،الاستون واخيرا الايثانول واشارت نتائج الاستخلاص الى اختلاف فى نسبة الحصيلة المئوية. مستخلص اوراق الجوافة اعطى اعلى نسبة ٧,٠٠١٪ تتبعها الحرجل ٦,٥٥٪, البقدونس ٤,٧٦٪ المحريب ٤,٦٤٪ ثم الدوم ٢,٥٢٪ .تم تقييم مضادات الاكسدة باستخدام نوعين من الكواشف ضد انصاف الجزور الحرة ١,١ثنائى فينايل -٢-بيكريل هيدرازيل و٢،٢ ثنائى ازينوبنزول ايثيل-٤- حمض السلفونيك . و تم تحليلها وتميزها باستخدام المطياف الضوئى واظهرت نتائج الكشف ضد(ABTS) مضادات تاكسد عالية مقارنة مع (DPPH). كاشف الDPPH معمستخلص الايثر البترولى اظهر نتائج ضعيفة تجاه انصاف الجذور الحرة فكانت النسبة تتراوح بين ١٦٪ بقدونس, ٢٩٪اوراق الجوافة ٣٠٪ محريب بينما مستخلصات الحرجل والدوم لم تظهر اى نشاط كمضادات اكسدة بينما اعلى نشاط كان لمستخلص اوراق الجوافة بنسب تتراوح بين ٩٠٪،٨٩,٢٪ و ٨٩٪ مع الايثانول ، الاستون و اثيل اسيتات على التوالى. اما كاشف ATBS اظهر نتائج عالية، مستخلص الايثانول لاوراق الجوافة كمضادات اكسدة كانت بنسب عالية ٩٤٪ بينما اقل نشاط كان مع مستخلص الاستون للبقدونس بنسبة ٦٩٪ نتائج الايثر البترولى اقل من ٥٠ ٪ .واشار التحليل بواسطة الامتصاص الذرى لتقدير بعض العناصر الثقيلة نسب تتراوح بين (٣٧, - ١٦٦,جزء من المليون) للزنك ،|(٠٢,- ٢, جزء من المليون)للمنجنيز ، (١٠٣- ١٩٨جزء من المليون) للحديد ، (٠٠١,- ٠٠٤, جزء من المليون) للكادميوم ،(٠,٠١٢- ٠,٠٩٩ جزء من المليون) للكوبلت و(٣٧- ٧٩جزء من المليون) للنيكل واشارت ان النسب فى المستوى المسموح به للاستهلاك على حسب تقدير منظمة الصحة العالمية ويلاحظ وجود الرصاص بنسب منخفضة جدا او تكاد تكون معدومة .كما اوضح التحليل الطيفى الكمى ان جميع المستخلصات النباتية تحتوى على القلويدات وتتراوح كميتها بين٠,٠٢٥ملجم/مل لاوراق الجوافة و٠,١٠١ملجم/مل للحرجل اما الفلافونيدات اظهرت نتائج تتراوح بين (٠,٢١ الى٠,٥٥)ملجم/مل ،الفينولات كانت نتائجها كالاتى (٠,١٠٣، ٠,١٥٥

٠,٣١٤،٠,٢٧٠و٠,٣٧٤) ملجم/مل لكل من البقدونس ،المحريب، الحرجل ،اوراق الجوافة وثمار الدوم على التوالى اما التانينات بين (٠,٢٣للحرجل الى ٠,٥٦)ملجم/مل للبقدونس و كما تم قياس النسب المئوية للمكونات النباتية فكانت نسبةالرطوبة عالية٩٤٪ ،البروتينيتراوح بين (١,٤- ١٠,٢٪) ، الالياف(٨,٧- ٢٦,٥٪) والرماد(٤,٩- ٧,١٪) حسب الطريقه المعتمده .كشف التحليل بواسطة كروماتوغرافيا الغاز وجود مجموعة من الاحماض الدهنية والاسترات الاليفاتية والعطرية و الاميدات فى المستخلصات التى تم تحليلها . واظهرت النباتات بصوره عامة نشاط حيوى و مثبطات ضد الانواع البكتيرية الاربعه (العصويه الرقيقة ، الاشريكية القولونية ،الزائفة الزنجارية والمكورة العنقودية الذهبية)بصورة افضل من الانواع الفطرية (الرشاشية السوداء والمبيضة البيضاء).و اشارات نتائج المستخلصات ضد انواع البكتريا الاتى نسبة التثبيط (١٢,٥- ٢٠م م)ضد العصويه الرقيقة ،(١١- ٢٣م م) ضد الاشريكية القولونية ،(١١- ٢٣,٥م م)ضد الزائفة الزنجارية و(١٣- ٢١م م) ضد المكورة العنقودية الذهبية بالنسبة للفطريات كانت نسب التثبيط (٠- ٢٣م م) ضد الرشاشية السوداء و(٠- ٢١م م) ضد المبيضة البيضاء. جميع النباتات المستخلصة ليس لها سميه تجاه الخلايا الطبيعية (التركيز الموافق للتثبيط النصفى > ١٠٠ جزء من المليون) مقارنة مع تريتون ١٠٠× موجب التحكم. تعتبر النباتات التى اجريت عليها الدراسة من النباتات الطبية بما تحتوية من مركبات فعالة كمركبات مضاده للاكسدة ومضادة للبكتريا يمكن تقديرها طبيا.

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IUCN	International Union of Conservation of Nature
WHO	World Health Organization
UNADIS	United Nation Acquired Immune Deficiency
ROS	Reactive Oxygen Species
RDA	Recommended Dialy Allowance
USDA	United State Department of Agricullure
LD	Lethal Dose
SRL	Sisco Research Lab
DPPH	Diphenyl Picryl Haydrazyl
ABTS	Azino bis ethyl Benzo Thiazoline Sulfonic acid
SAS	Statistical Analysis System
A.O.A.C	Ascocession of Official Analsis Chemists
MTT	Dimethyl Thiazol -2-yl diphenyl Tetrazolium
DMSO	Dimethyl Sulfo Oxide
EPA	Enviromental Protection Egency
MPL	Maximum PermissibleLimit
DNA	Deoxyribo Nucleic Acid
QUE/g	Querctien Equivialent per gram
LDLs	Low Density Lipoproteins
ECG	Epicatechin Gallate
EGCG	Epicatechin Gallo catGallate
MDIZ	Mean Diameter of groth

CHAPTER ONE
INTRODUCTION and LITERATURE REVIEW

1.1Background

Medicinal plants provide a subsidy from nature that can be of great value to rural people and may be of value to global society as a source of new drugs. More than 50% of all the drugs in clinical use currently are of natural product origin (Amit& Shailendra 2005). The cultivation of plants as sources of drugs is the best and the most promising way to satisfy markets expanding demand for these raw materials. This is certainly the only applicable way in which we can stop their decline and secure their long-term survival. In addition, there will be an accelerated poverty reduction through creation of rural income (Mukonyi, et al 2001).

A number of significant global diseases including cancer, malaria, tuberculosis and certain viral, fungal and bacterial infections are showing patterns of resistance to known therapeutic agents. The plant kingdom represents an extra ordinary reservoir of novel molecules. Of the estimated 250,000 – 500,000 plant species around the globe, only small percentage has been investigated phytochemical and the fractions subjected to biological or pharmacological screening (Hostettman, et al 1995).

1.2 Natural products

1.2.1 Development

The World Health Organization estimates that approximately 80 percent of the world's population relies primarily on traditional medicines as sources for their primary health care (Farnsworthet al,1985). Over 100 chemical substances that are considered to be important drugs are either currently in use or have been widely used in one or more countries in the world have been derived from less than 100 different plants. Approximately 75 % of these substances were discovered as a direct result of chemical studies focused on the isolation of

active substances from plants used in traditional medicine (Cragg& Newman 2001).39% of the 520 new drugs approved during the period 1983 through 1994 were either natural products or derivatives of natural products (Harvey, A. 2001). Over 60 percent of antibacterial and antineoplastics were again either natural products themselves or based on structures of natural products(Grabley, & Sattler 2003).

Natural products today are most likely going to continue to exist and grow to become even more valuable as sources of new drug leads (Sandsborg&Rolfsen 1999). This is because the degree of chemical diversity found in the natural products is broader than from any other source, and the degree of novelty of molecular structure found in natural products is greater than that determined from any other source (Choudhary& Atta-Ur-Rhman 2005).

Also it was mentioned that, the revival of interest in the use of medicinal plant products for the treatment of various ailments is mainly due to increase awareness of the limited horizon of synthetic pharmaceutical products to control major diseases, high cost of currently available synthetic medicines, reported cases of adverse side-effects of modern medicines and perceived gentleness of natural medicines (Colegate& Molyneux 1993).

1.2.2 Origin

A wide range of medicinal plants part is used as raw drugs and they posses varied medicinal properties. The activity may reside in a variety of different components, including aldehyde and phenolic compounds, therefore, natural products research remain one of the main means of discovering bioactive compounds. Until recently, most natural products chemists have been more concerned with the isolation and structural elucidation of secondary metabolites than their biological activity. They realize that the, detection, isolation and structure determination of metabolite are only the first step towards answering much broader questions (Dash, et al. 2011).

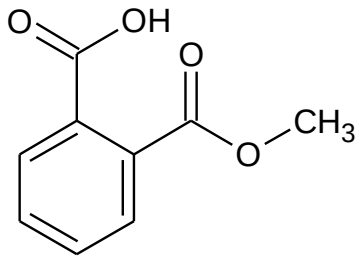
The development of drug resistance in human pathogen against commonly used antibiotics necessitated a search for new antimicrobial substances from other sources including plants. Screening of medicinal plants for antimicrobial activities is important for potential new compounds for therapeutic use (Madhuri& Pandey 2009).Phytochemicals such as vitamins(A, C, E and K), carotenoids, terpenoids, flavonoids, polyphenols, alkaloids, tannins, saponins, pigments, enzymes and minerals proved to have antimicrobial and antioxidant activity (Nascimento, et al 2000).

The specific function of many phytochemicals is still unclear; however, a considerable number of studies have shown that they are involved in the interaction of plants/pests/diseases. Antimicrobial screening of plant extracts and phytochemicals then represents a starting point for antimicrobial drug discovery. Phytochemical studies have attracted the attention of plant scientists due to the development of new and sophisticated techniques. These techniques played a significant role in the search for additional resources of raw material for pharmaceutical industry (Shakeri,et al 2012).Medicinal plants possess immunomodulatory and antioxidant properties, leading to antibacterial activities. They are known to have versatile immunomodulatory activity by stimulating both non-specific and specific immunity. The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficiency. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant (Farnsworth, 1984).

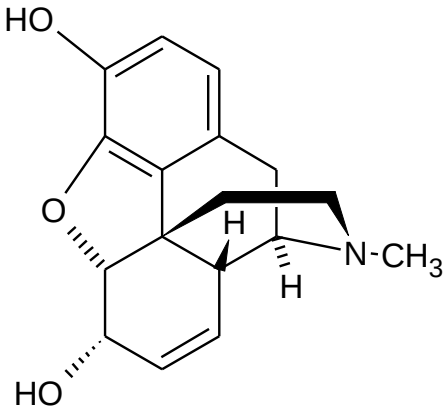
1.3 Pharmaceuticals (non-toxic drug)

Plant-derived substances have traditionally played important roles in the treatment of human diseases.Today, about 80 % of the world population residing in third world countries still rely almost entirely on plant products

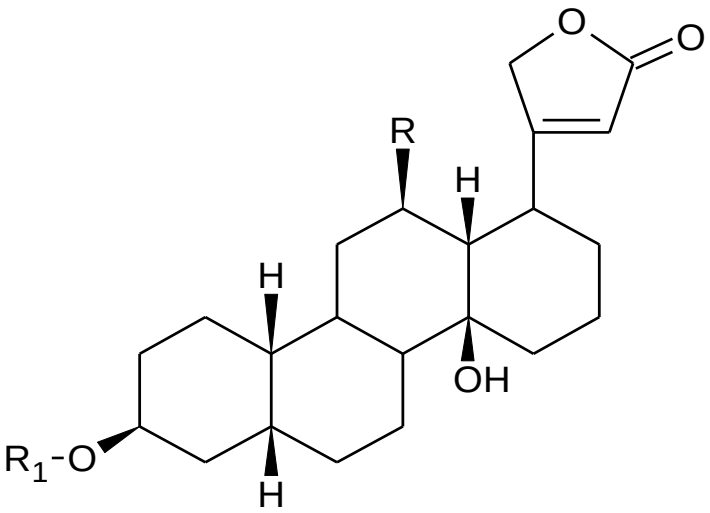
for their primary health care. The remaining 20% of individuals living in the world use, in more than 25% of cases, pharmaceuticals which have been directly derived from plant products(Cox, 1994), (O′Keefe, 2001). These range from common remedies such as aspirin (originally isolated from the Rosacea; *Filipendula ulmaria*), to prescription drugs such as the analgesic morphine and the cardiacglycoside digitoxin and digoxin (isolated from the Papaveracea; *Papaver somniferum*, and the Apocynacea, *Digitalis purpula*, respectively).



Aspirin



Morphine



Digitoxin (R = H, R_1 = sugar residue)Digoxin (R = OH, R = sugar)

Figure(1.1): The chemical structure of aspirin, morphine, **digitoxin and digoxin** (O′Keefe, 2001)

Higher plants also produce a variety of different types of compounds, including biologically active proteins. Some of these types of compounds are even shared with other organisms, and they include such chemical families as lectins, defensins, cyclotides, and ribosome–inactivating proteins (Cox Gad, 2005) Ribosome – inactivating proteins are group of proteins exhibiting a wide spectrum of biological activities, including a ribonucleolytic activity for which the group is named. These compounds can be obtained from *Panax ginseng* and other plants have been reported to demonstrate antifungal and antiviral activities (Manns, et al 2000). Table(1. 1)shows a number of well-known drugs that are directly developed from plant species (Amit, et al2005).

Table(1.1): Non cytotoxic drugs developed from plant sources

Drug	Medical use	Plant source
Aspirin	Analgesic,anti-nflammatory	***Filipendula ulmaria***
Atropine	Pupil dilator	***Atropa pelladona***
Benzoin	Oral disinfectant	***Styrax tonkinensis***
Caffeine	stimulant	***Camellia sinensis***
Codeine	Analgesic, antitussive	***Papaver somniferum***
Digoxin	For arterial fibrillation	***Digitalis purpura***
Eugenol	For toothache	***Syzigium aromaticum***
Hygoscyamine	Anticholinergic	***Hyoscyamus niger***
Morphine	Analgesic	***Papaver somniferum***
Papaverine	Antispasmodic	***Papaver somniferum***

Pilocarpine	For glaucoma	***Pilocarpus japorandi***
Quinine	For malaria prophylaxis	***Cinchona pubescens***
Resepine	Antihypertensive	***Rauwolfia serpentia***
Scopolamine	For motion sickness	***Datura stramonium***
Toxiferine	Relaxant in surgery	***Strychnos guianensis***
Xanthotoxin	For vitiligo	***Ammi majus***

1.4 Plants provide therapeutic benefits

The medical systems of the world have always valued the use of medicinal plants (Heinrich *et al.*, 2004). The plant kingdom has provided therapeutic benefits to humans dating back as far as the earliest recorded knowledge obtained from countless prehistoric references from ancient Egypt, Assyria, China and India. Early explorers of the New World documented the first findings of medicinal plants for many parts of the world (Heinrich *et al.*, 2004).

Throughout human evolution, religious practices and rituals have influenced the treatment of disease and the use of plants . The unwritten purposes for which medicinal plant substances were used by the ancients are analogous to the purposes for which they are utilized nowadays; we have managed to decipher the secret *modus operandi* of Nature. Today, knowledge regarding the biosynthesis and biological effects of secondary plant metabolites has broadened extensively (Raskin *et al*.,2002).

Bioscientific studies of the virtues and chemical properties of indigenous remedies used by native people throughout the world have resulted in the

production of many therapeutic agents used in modern, conventional medicine (Heinrich *et al.*, 2004). Today, opium from *Papaver somniferum* is used as an analgesic, aspirin compounds from *Salix nigra* are used for their analgesic, antipyretic and anti-inflammatory properties, digoxin from *Digitalis purpurea* is used for heart failure treatment, vincristine and vinblastine from Madagascan Periwinkle (*Catharanthus roseus*) are used as antineoplastic agents, taxol from Yew (*Taxus baccata*) is used for the treatment of ovarian and breast cancers, quinine from *Cinchona* species is used in the treatment of malaria, and caffeine from *Coffea arabica* is used as a stimulant (Raskin *et al*., 2002).

1.5The rationale for the use of botanically-derived compounds

A plant may be considered as a complex biosynthetic laboratory, synthesizing many diverse bioactive chemical compounds exerting curative effects. The plant's defense system against predation, herbivores, microbial and viral infection is provided by such bioactive compounds. Only one third of all diseases can be treated efficiently and so after more than 100 years of pharmaceutical research, innovative and effective lead compounds are still needed (Mulzer and Bohlmann, 2000). The idea that complex biologically active compounds in plants have been selected and perfected by evolution for longer than drug companies must be realized (Raskin *et al.*, 2002).

Complex diseases are often treated with a single drug. The problem of resistance to anticancer, antimalarial and antimicrobial drugs coupled with the multifaceted nature of complex diseases brings to light the consideration that a combination of therapeutic agents should be used for the treatment of certain diseases. The traditional medicinal system uses the approach of combining botanical and non-botanical remedies for the treatment of complex diseases and in this way provides a more holistic approach to disease treatment and

prevention. Plants produce an array of compounds to prevent the development of resistance and thus to protect themselves against pathogens. This suggests that the treatment of certain diseases with botanically-derived agents are less likely to lead to resistance problems as the biological effects exerted by plant products may be the result of the multiple and synergistic effects caused by the interactions between many different biochemical compounds (Raskin*et al.*, 2002).

The increasing cost of energy and chemical raw materials, pharmaceutically related environmental concerns as well as the fact that plants are the most abundant and renewable resource makes them more compatible as a source of drug for the future. Furthermore, advances in metabolic engineering, biochemical genomics, chemical separation, molecular characterization and pharmaceutical engineering, have better equipped us to exploit the therapeutics of plants. Numerous bioactive compounds derived from plants are used as prototypes, where their structures are elucidated and can be used as templates for structural modification for the development of better and newer drugs (structure optimization programs) (Heinrich *et al.*, 2004).

1.6 African traditional medicine

African traditional medicine is the oldest and perhaps the most diverse of all medicine systems. Africa is considered to be the cradle or Mankind with a rich biological and cultural diversity marked regional difference in healing practices. Unfortunately, the systems of medicines are poorly recorded and remain so to date. Yet the documentation of medicinal uses of African plants is becoming increasingly urgent because of the rapid loss of the natural habitats of some of these plants because of anthropogenic activities. The African continent is reported to have one of the highest rates of deforestation in the world. The paradox is that it is also a continent with a high rate of endemism with the Republic of Madagascar topping the list at 82%. (Heinrich *et al*., 2004).

1.7 Sudan folklore-medicine

Traditional medical practices play an important role in Sudan, and 90% of population particularly those who are living in frontiers and rural areas depend mainly on herbal medicine for the treatment of various types of diseases (Koko *et al*., 2000). Sudan folklore-medicine represents a unique blend of indigenous cultures with Egyptian, Indian. Arabian, East and West African cultures. This in view of a number of factors, such as draught, desertification, expansion of agricultural schemes and the introduction of health services to primitive areas, which initiated astonishingly rapid changes, leading to the least use of native medicines, which would eventually disappear. As in many developing countries, herbal drugs are of major importance in Sudanese folk medicine (Ali *et al*., 2002).

1.8 Economics of herbal medicine trade

The potential for developing antimicrobials into medicines appears rewarding, from both the perspective of drug development and the perspective of phytomedicine. The immediate source of financial benefit from plant based antimicrobial is from herbal products market. This market offers many opportunities for those cultivating new crops, as many of the plant that are wild grafted today must be cultivated to march the demands of this market.

Medicinal plants provide a subsidy from nature that can be of great value to rural people and may be of value to global society as a source of new drugs. More than 50% of all the drugs in clinical use currently have a natural product origin (Amit&Shailendra 2005). According to International Union of Conservation of Nature (IUCN) and world health organization (WHO) the cultivation of plants as sources of drugs is the best and the most promising way to satisfy market's expanding demand for these raw materials, for wild plant species that are endangered through over exploitation, this is certainly the only applicable way in which we can stop their decline and secure their

long-term survival. In addition, there will be an accelerated poverty reduction through creation of rural income (Mukonyi, et al. 2001).

A number of significant global diseases including cancer, malaria, tuberculosis and certain viral, fungal and bacterial infections are showing patterns of resistance to known therapeutic agents. The plant kingdom represents an extra ordinary reservoir of novel molecules. Of the estimated 250,000 – 500,000 plant species around the globe. Only small percentage has been investigated phytochemically and the fractions subjected to biological or pharmacological screening (Hostettman, et al 1995). A survey conducted by WHO showed that 60% - 80% of the world's population depended on herbal medicine for their health needs, the majority of this population comes from the developing countries and over 75% of the people infected by AIDS live in Africa(UNAIDS &WHO 1998). The vast variety of medicinal plants systems are in African countries. Ethnomedicinal studies are therefore the way forward for the continent. Plants which are observed to be efficacious and frequently prescribed by traditional healers may contain compounds that are potential drug candidates and could rightly be recommended for further examinations(Igoji, et al. (2005).

1.9 Antioxidants

An antioxidant is a substance that delays oxidation by inhibiting initial free radical formation or by preventing them from producing more free radicals which can perpetuate the reaction .Antioxidant scan bind metals, scavenge species that initiate orperpetuate oxidation, quench high energy oxygen species preventing formation of peroxides, or decompose lipid peroxides. They can improve both color and flavor stability in meats .Some vitamins such as ascorbic acid and vitamin E exhibit antioxidative activity. Many herbs and spices (rosemary, oregano,grape seed, sage, and thyme) contain antioxidant components (Ahnetal., 2007; Rojas and Brewer, 2007). Antioxidants are found

in synthetic and natural form.Synthetic antioxidants are widely used as food additives to prevent acidification, owing to their high performance,low cost and wide availability.Therefore, many synthetic antioxidants such as butylated hydroxyl anisole (BHA), tertiary butyl hydroquinone (TBHQ), 2,4,5 tri hydroxyl butyrophenone (THBP), di-tertbutyl-4-hydroxymethyl phenol (IONOX-100), Propyl gallate (PG), octylgallate (OG), nordihydroguaiareticacid (NDGA) and 4-hexylresorcinol (4HR) are used in edible vegetable oil and cosmetics(Guan,et al., 2005; Guo,et al , 2006). Preservatives such as 2-naphthol (2NL), 4-phenylphenol (OPP) and 2.4-dichlorophenoxyacetic acid (2,4-DA), are commonly used in vegetable and fruit to keep them fresh. Propyl gallate and butylated hydroxyl anisole, as synthetic phenolic antioxidants, display high chemical activity for suppressing chain initiation, or breaking chain propagation of the peroxidation of unsaturated fatty acids. Although they are powerful in protecting product quality in food distribution, excess antioxidants added to food might produce toxicities, and thus endanger the health of consumers (Williams 1993, 1994).

1.9.1Antioxidant from natural products

The medicinal plant products, which are derived from plant parts such as stem bark, leaves, fruits and seeds have been part of phyto-medicine that produce a definite physiological action on the human body. The most important of these natural bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds (Hill, 1998). A number of reactive molecules are generated through various biological redox reactions such as superoxide radical (O^{-2}), hydroxyl radical ($O\dot{H}^{-}$), hydrogen peroxide (H_2O_2) and nitric oxide (NO_2) which can directly react with biological macromolecules such as proteins, lipids and DNA of healthy human cells and cause cell membrane disintegration, DNA mutation and protein damage (Hill,1998). Deregulation of these reactive oxygen species (ROS) can further create cancer, atherosclerosis, cardiovascular disease, liver injury, ageing and inflammatory

disease. Antioxidants act as oxygen scavengers by interrupting the oxidation process by reacting with free radicals and chelating catalytic metals. Some synthetic antioxidants were developed in the past few decades but they are suspected of having some adverse effects. Therefore, in search of suitable alternative natural antioxidants has received much attention to identify and develop more potent antioxidants of natural origin to replace synthetic ones. Different kinds of plant material have already been reported as natural antioxidant (Packer and Colman1999).

1.9.2 Antioxidant compounds

Antioxidant compounds are chemicals that prevent the oxidation of other chemicals. They protect the key cell components by neutralizing the damaging effects of free radicals, which are natural by- products of cell metabolism.Antioxidants, including phenolic compounds (e.g., flavonoids, phenolic acids and tannins), have diverse biological effects, such as anti-inflammatory, anti-carcinogenic and anti-atherosclerotic effects, as a result of their antioxidant activity (Shenoy and Shirwaikar, 2002).A factor that provides a distinct challenge in the assay of antioxidant capacity is that within biological systems, there are at least four general sources of antioxidants: (i) enzymes, for example, superoxide dismutase, glutathione peroxidase, and catalase; (ii) large molecules (albumin, ceruloplasmin, ferritin, other proteins); (iii) small molecules [ascorbic acid, glutathione, uric acid, tocopherol, carotenoids, (poly)phenols]; and (iv) some hormones (estrogen, angiotensin, melatonin, etc.). On the other hand, there are multiple free radical and oxidant sources [e.g., $O_2^{\bullet-}$, $1O_2$, HO•, NO•, ONOO-, HOCl, RO (O)•, LO(O)•], and both oxidants and antioxidants have different chemical and physical characteristics. Individual antioxidants may, in some cases, act by multiple mechanisms in a single system or by a different single mechanism depending on the reaction system. Furthermore, antioxidants may respond in a different

manner to different radical or oxidant sources. For example, carotenoids are not particularly good quenchers of peroxyl radicals relative to phenolics and other antioxidants but are exceptional in quenching singlet oxygen, at which most other phenolics and antioxidants are relatively ineffective. However, singlet oxygen is not a radical and does not react via radical mechanisms but reacts mostly by the addition to double bonds, forming endoperoxides that can be reduced to alkoxyl radicals that initiate radical chain reactions. Because multiple reaction characteristics and mechanisms as well as different phase localizations are usually involved, no single assay will accurately reflect all of the radical sources or all antioxidants in a mixed or complex system (Manach *et al.,* 2004).Oxidative cell damages arising from free radicals are in the basis of many diseases. Thus, the intake of natural antioxidants is very important for humans. Medicinal and edible plants are rich source of such kind of compounds. Relatively new direction which is developed very fast is investigations concerning the antioxidant capacity of medicinal plants (Acuna *et al.,* 2002; Manach *et al.,* 2004; Bajpai *et al.,* 2005). Survey studies in this direction have been made with Flora of India, China, South Africa and other countries (Atawodi, 2005; Qiong *et al.*, 2004; Gacche and Dhole, 2006). From Vietnamese Flora many plant species have been investigated in the search for novel antioxidants, but there is still a demand to find more information concerning potential of plant species(Marinova *et al.* 2005)

1.9.4 Natural antioxidant

Endogenous antioxidant systems, because, they are under constant oxidative stress from free radicals,reactive oxygen species and prooxidants. Biological tissues from which meat is derived have developed antioxidant systems to protect them. Processing can initiate oxidation by damaging tissue integrity and functionality.Cooking denatures the protein structure of these antioxidant enzyme systems (Decker and Mei, 1996; Chenet al., 1999).

1.9.5 Free radicals

A free radical is a molecule with one or more unpaired electrons in its outer orbital. Many of these molecular species are oxygen (and sometimes nitrogen) centered. These highly unstable molecules tend to react rapidly with adjacent molecules, donating, abstracting, or even sharing their outer orbital electron(s). This reaction not only changes the adjacent, target molecule, sometimes in profound ways, but often passes the unpaired electron along to the target, generating a second free radical or other reactive oxygen species (ROS), which can then go on to react with a new target. In fact, much of the high reactivity of ROS is due to the generation of such molecular chain reactions; effectively amplifying their deleterious effects many folds (Gutteridge and Halliwell, 2000). The later include various diseases like cancer, ischemia, atherosclerosis, diabetes, Alzheimer's disease etc.Many antioxidant defense systems in the body such as superoxide dismutase (in mitochondria and cytosol), catalase (in peroxisomes), glutathione peroxidase, α-tocopherol (in membranes and lipoproteins) etc. limit the levels and the damage caused by free radicals (Anderson, 1996). When this multiple defense system fails due to increased production of reactive oxygen species or decreased level of cellular antioxidants, the net result is (i) DNA damage leading to mutations and cancer; (ii) oxidative inactivation and accumulation of metabolic enzymes and an increase in the level of oxidative modified proteins resulting in aging; (iii) induction and initiation of lipid peroxidation and oxidative modification of low density lipoproteins leading to deleterious cardiovascular effects.

Due to the above-presented pathological implications of ROS, it is important to find an antioxidant, which may scavenge multiple ROS so that it can be used in multiple disease states and also to maintain a healthy status. The need to identify antioxidants, which can scavenge several free radicals and prevent multiple diseases, can be achieved by simple *in vitro* test systems. Further the

in vitro results can be confirmed with *ex vivo* and *in vivo* systems(Gutteridge and Halliwell, 2000).

1.10 Heavy metals

Heavy metals as micronutrients are important for the proper functioning of vital organs in the body. For example, iron is a component of hemoglobin and other compounds used in respiration. Heavy metals are widespread in soil as a result of geo-climatic conditions and environmental pollution. Therefore, their assimilation and accumulation in plants is obvious. Together with other pollutants, heavy metals are discharged into the environment through industrial activity, automobile exhaust, heavy-duty electric power generators, municipal wastes, refuse burning and pesticides used in agriculture (Flora ,et al.2008). Human beings, animals and plants take up these metals from the environment through air and water. Heavy metals have the tendency to accumulate in both plants and human organs.

The term heavy metal refers to any metallic chemical element that has a relatively high density and is toxic or poisonous at low concentrations. Examples of heavy metals mercury (Hg), cadmium (Cd), arsenic (As), chromium (Cr)and lead (Pb) (Saper et a,2004).

Heavy metals are natural components of the Earth's crust. They cannot be degraded or destroyed. To a small extent they enter our bodies via food, drinking water and air. As trace elements, some heavy metals (e.g. copper, selenium, zinc) are essential to maintain the metabolism of the human body. However, at higher concentrations they can lead to poisoning. Heavy metal poisoning could result, for instance, from drinking-water contaminated (e.g. lead pipes), high ambient air concentrations near emission sources, or intake via the food chain.Heavy metals are dangerous because they tend to bioaccumulate. Bioaccumulation means an increase in the concentration of a chemical in a biological organism over time, compared to the chemical's

concentration in the environment. Compounds accumulate in living things any time they are taken up and stored faster than they are broken down (metabolized) or excreted. Heavy metals can enter a water supply by industrial and consumer waste, or even from acidic rain breaking down soils and releasing heavy metals into streams, lakes, rivers, and groundwater (Harris, et al 2011).The toxicity of heavy metal to aquatic organisms has been the focus of interest to scientist for numerous years. Important toxic metals include Cd, Hg, Pb, Cu, Cr, Ni, Mn, Ca, Au, Ag, Li, Ce, Ga and Fe.The heavy metals find their means to water bodies through wastewater from diverse industries (Meena et al., 2008).Some plants even have the ability to accumulate metals like Hg and Cr that have no known biological function to them. Reports have also shown that the levels of minerals in the plants depend on the soil properties and the geographical locations where the plant grows.(Meena et al., 2008).

Trace element ingestion, even in very small quantity, can affect not only the physiological function and health of living organisms but also demographic, distribution and reproduction, as well as survival of organism. Toxic elements like Pb , Cd, and Hg are widely distributed and mobilized in environment, and human exposure to these non-essential elements has been consequently elevated (Hsu et al., 2006). At higher level of exposure of human beings, there is damage to almost all organs and organ system, most not ably central nervous system, kidneys and circulatory system , leading to death at excessive levels. At very low level, biochemical, psychological and neuron, which are the heavy oral-processes, are influenced mostly (Flora ,et al2008). Pb persists to be a significant public health problem in various countries in Asia, not only between human but also among a variety of species of other organisms (Hsu et al., 2006). It is necessary to know about the level of diverse heavy metal in medicinal plants used in herbal medicine. Some of the heavy metals may be

lethal and these are frequently present in environmental matrices. They are establishing in soil, water and plant and readily mobilize by human activities as waste. The presence of heavy metals (Pb and Cd) was reported in various herbal products and even standard extracts in Iran, India, Pakistan, and China (Asghari,2008). The presence of metal residues in the herbal plants is prevalent since they are easily contaminated during growth, development, and processing. After collection and transformation into drug form, heavy metals confined in plants finally enter the human body and may disturb normal functions of different organ systems. WHO has emphasized the need for quality assurance of herbal products, including testing heavy metals (WHO, 1990).

1.10.1 Essential elements and health

Essential elements are chemical nutrients needed by the body in large or tiny amounts and are vital for growth and development for example Zn, Mn, Cr and Fe. Essential elements are usually associated with chemical reactions in the body; hence they are essential for the control and maintenance of bodily functions (WHO, 1996). However, through these chemical reactions the body grows, cells are replaced, energy is produced for essential life processes, reproductive functions are developed and used, muscles are made to contract and nerve impulses are transmitted.

They are in fact the inorganic counterparts of essential biological nutrients namely vitamins and proteins (WHO, 1996).

The need for the presence of essential trace elements in the body in proper amounts cannot be overemphasized since vitamins can neither function nor be assimilated without the help of essential elements. Essential elements activate enzymes and vitamins hence lack of them can present serious health challenges including clinical and pathological disorders in animals, plant sand man. Some essential elements make vitamins work more effectively for

instance, vitamin A works best with Se and Zn, while B-vitamins are potentiated by Se, Zn, Co, Cu, Fe and Mn. For vitamin C the essential elements found to promote its effectiveness are Co, Cu and Se, while vitamin E is activated by Fe , Mn , Se and Zn (Mindell and Mundis, 2004).

Elements such as Zn and Cu are known to be components of enzymes which play key roles in the growth of both plants and animals. Certain essential elements are important co-factors of certain hormones that control chemical reactions in human body, dictating when to stop or slow down for example; somebody suffering from diabetes or thyroid could be due to hormone deficiency.

Hormones need minerals to function properly, therefore lack of minerals especially essential trace elements lead to immobilization of hormones resulting in inefficient control. Essential elements cannot be manufactured by the body, hence must be acquired through diet. Human being obtain essential elements from plants consumed or animals which have themselves eaten plants, and since the main source of essential elements is the soil, their uptake by plants is an essential part of the process (Underwood, 1977).

The uptake of essential elements from soil is determined by many factors including their amounts in soil, genetic makeup of plant species, physical condition of soil and pH of soil (Underwood, 1977).

Certain plant species have the ability to accumulate uniquely high concentrations of a particular essential element. Many interactions between elements in the soil have been shown to be of profound nutritional importance. Most of the essential trace elements have unfilled d-orbital and readily form coordination complexes with electron rich elements like nitrogen, oxygen and sulphur thus competing with each other for such ligands .Interactions can result in competitive inhibition or in stimulation of uptake, for example; high concentration of Cd and P in the soil can prevent uptake of Zn by plants (Underwood, 1977). Even when trace element intake can be estimated, the

amount available to the body may be significantly less because the absorption and metabolism of individual trace element is strongly influenced by mineral interactions. Over abundance of Zn or Fe in the body can interfere with absorption of the other minerals (Solomon, 1986). Copper has tendency to accumulate in the blood and deplete the brain Zn supplies, high dietary levels of Zn induce signs of Cu deficiency anaemia and decreased activity of the enzyme cytochrome oxidase, whereas presence of Fe reduces the absorption of Mn. There is unequivocal evidence that physiological levels of Cu interact positively with Fe to facilitate the absorption and utilization by extra hepatic tissues (O'Dell, 1989). Essential mineral interaction must be based on the physico-chemical properties of their ions. Ions whose valence shell electronic structures are similar would be antagonistic to each other; therefore minerals such as Mn, Fe, Zn and Cu compete with each other because they have similar molecular weights, sizes and charges (O'Dell, 1989).

1.10.1.1 Iron and human health

Iron is a widely distributed mineral, vital for plant and animal life. The common oxidation states of Fe are Fe^{+2} and Fe^{+3}. Human body contains 4.0 to 5.0 g of Fe (Underwood, 1977). Most of the body's Fe exists in complex forms bound to protein, either as porphyrin or as non-haeme protein-bound compounds such as ferretin and transferrin. Iron is also present in nature as haem iron in meat and non-haem iron found in cereals and vegetables. Iron is necessary for production of haemoglobin, myoglobin and certain enzymes. Haemoglobin iron occupies 60 to 70% of body iron inhuman (Underwood, 1977). Highest concentrations of Fe are in the liver, spleen, kidney and heart. Pregnancy increases the requirement of Fe by approximately 3.5 mg per day because of extra demand by the foetus (Williams and Caliendo, 1988). Children and adolescents need Fe not only to maintain their haemoglobin concentration but also to increase Fe storage during their period of growth. Iron is necessary for proper metabolization of B-vitamins. Vitamin C

improves the absorption of Fe from the food thus the presence or absence of vitamin C can influence the availability of Fe greatly. Infants may be prone to Fe deficiency because the Fe content of cow's milk is low and the amount of mineral passed on by the mother during development up until birth is usually not sufficient to meet the child's need beyond the age of 6 years (Underwood, 1977). The main vital function of Fe is the transfer of oxygen to various sites in the body. Iron boosts immune system of the body because it is responsible for the formation of white blood cells. Therefore, when the body Fe levels is low or deficient, the bone marrow cannot supply the necessary white blood cells because Fe containing enzymes are unable to function. Studies in humans have confirmed that, anaemic individuals have greater chances of developing infectious diseases particularly those associated with respiratory system (Mindell and Mundis, 2004). Iron helps in eliminating old blood cells and building of new ones. Iron is also involved in the synthesis of the protein collagen and contributes to drug detoxification pathways in the liver.The absence of Fe in the body can impair vital functions like oxygen carrying capacity of red blood cells, haemoglobin formation and functions of blood and enzymatic activity of respiratory function, especially the cytochrome involved in cellular respiration. Only about 8% of total Fe intake is absorbed and actually enters the blood stream (Mindell and Mundis, 2004). Iron rich food sources are pork, beef, liver, red meat, oysters, nuts, legumes and green leafy vegetables (USDA, 2007). The recommended daily allowance of Fe is 10.0 to 15.0 mg for adults and 30 mg for pregnant women. Iron deficiency is common among small children, women at child bearing age and persons with chronic blood loss. Symptoms of iron deficiency are fatigue, giddiness, blurred vision, pallor of the skin, anorexia and headaches. A curious symptom sometimes seen in Fe deficiency individuals is an appetite for ice, clay, paste or other non -nutritional substances. However anaemia and malaria are the main deficiency diseases. Causes of Fe deficiency include inadequate intake from ignorance of

what foods to choose, lack of food and high consumption of Fe poor foods (USDA, 2007).Toxicity of Fe occurs when ingested Fe exceeds 3.0 g and symptoms include gastro-intestinal bleeding and restlessness (Williams and Caliendo, 1988).

1.10.1.2 Zinc and human health

Zinc is essential for higher plants and normal levels in plants range from 25 to150 ppm (Underwood, 1971). Zinc is a versatile trace element required as a co-factor by more than 200 enzymes. Zinc mass transport vehicle in the blood is the protein albumin. Human body contains 1.4 to 2.3 g of Zn (Williams and Caliendo, 1988). The highest concentration of Zn occurs in the eyes, male sex organs, liver and kidneys. Zinc is present also in the plasma, erythrocytes, leucokocytes and platelets. Absorption of Zn depends on age, amount digested and kind of food eaten (WHO, 1998). Zinc acts as a traffic director, overseeing the efficient flow of body processes, maintenance of enzyme systems and cells. It is essential for protein, DNA and RNA synthesis. Zinc is also essential for collagen formation and governs the contractibility of muscles.

Zinc also plays a major role in the synthesis and degradation of carbohydrates, lipids, proteins and nucleic acids. It also plays an important role in polynucleotide transcription and translation; hence it is essential in all forms of life. Zinc plays fundamental role in the structure and function of numerous proteins including metallo -enzymes and hormone receptors (Kinghorn et al., 2011).Zinc reinforces and rejuvenates immune system, facilitates digestion and aids in the transportation of vitamin A from the liver. Therefore some of the symptoms of vitamin A deficiency may be due to reduced body levels of Zn. Zinc rich foods can enhance immunity functioning, by increasing many aspects of immunity including production of antibodies and T-cells as well as other white blood cell activity .Italian studies have discovered that low dosages of Zn results in an amazing 80% re-growth of thymus glands, increase

in active hormones and T-cells that fight infections (Bakhru, 2006). Zinc helps in the formation of insulin that is responsible for maintaining blood glucose level. Zinc reduces the incidence of opportunistic infections for example pneumonia in HIV and AIDS patients by up to 45% (Kabi, 2004). It also exerts a normalizing effect on prostate glands and important in development of all reproductive organs. Zinc is a constituent of antioxidant superoxide dismutase that prevents damaging effects of oxygen free radicals on cells. Therefore, dietary Zn is important in preventing most of the chronic diseases like cancer, heart disease and bronchitis. Zinc important for blood stability since it maintains the proper concentration of vitamin E in the blood and in maintenance of body's alkaline balance. Zinc is the key mineral required for foetus growth in the womb. Zinc interacts with platelets in blood clotting and also affects thyroid hormone function. Zinc is essential to wound healing and taste perception. Zinc deficiency can result to poor learning ability in children as a result of impaired neuropsychological function. The recommended daily allowance(RDA) value for adults, range from 12 to 15 mg of Zn but slightly higher for nursing mothers (Williams and Caliendo, 1988). Dietary reference values for Zn vary according to the dietary pattern of the country, bioavailability of dietary Zn, age, sex and physiological status. Intakes of Zn tend to be low in relation to the recommended value and there are few foods that are really good sources of Zn; therefore it may take special care to ensure an adequate Zn intake (Walsh, 1994).

Zinc deficiency cause poor wound healing, arteriosclerosis, infertility (men), prostate hypertrophy and difficulties with parturition (women). Zinc deficiency diseases include diabetes, anaemia, kidney disease, prostate cancer and cardio vascular disease . Low levels of Zn in the body can contribute to pre-menstrual tension. Zinc deficiency symptoms include; white spots on fingernails, slow hair growth, reduced sense of smell, spotty skin and love for salt in food. During processing of wheat flour, up to 80% of Zn is lost (Walsh

et al., 1994). Soil exhaustion and careless cooking also contribute to low levels of Zn. Best natural Zn sources include liver, sea food, wheat germ, brewer's yeast, pumpkin seeds, and leafy vegetables. Zinc toxicity occurs at levels exceeding 1,000 mg and effects include vomiting, nausea and impaired immune function (Mindell and Mundis, 2004).

1.10.1.3 Manganese and human health

It is a widely distributed mineral, essential for both plants and animals. Manganese is essential for normal growth, skeletal formation and for normal reproductive functions in mammals and poultry. Normal levels in plants range from 20 to 50 ppm (Underwood, 1977). The biologically active form of Mn is Mn^{2+}

Many enzymes are activated by Mn and human body content range from 12 to 20 mg (Underwood, 1977). Manganese content in human blood, range from 8 to 20 μg per dm^{-3} (Williams and Caliendo, 1988).Manganese plays the role of activating enzymes necessary for the body's proper use of biotin, vitamin B^1 and vitamin C. It is also responsible for synthesis of glycoprotein in body cells that act as protein coating against viruses (Bakhru, 2006). Manganese is also needed for normal bone structure and formation of thyroxin which is a principal hormone of the thyroid gland (Mindell and Mundis, 2004). Manganese is crucial for synthesis of interferon which is directly associated with body's defence mechanism. Manganese aids in maintenance of blood sugar level, hence may help to prevent/cure diabetes. Previous studies indicate that diabetics invariably have low Mn levels in blood; therefore lack of Mn in regular diets could be linked to prevalence of diabetes in man. It is needed for utilization of fats, lipid metabolism and building of nucleic acid (carrier of genetic information). Manganese can block the replication of HIV inside cells; hence can prevent it from causing AIDS(Williams and Caliendo, 1988)

The recommended adult daily allowances of Mn range from 2 to 5 mg (Mindell and Mundis, 2006). About 3.7 mg of Mn are ingested daily from a

well balanced diet (William and Caliendo, 1988). Manganese deficiency diseases include diabetes, anaemia and cardiovascular disease. Manganese toxicity occurs at levels exceeding 20 mg and symptoms include anorexia, impulsiveness, spastic gait and Parkinson disease (WHO, 1998). Good sources of Mn include cereals, green leafy vegetables and peas (Mindell and Mundis, 2004)

1.10.1.4 Lead

Lead is a highly toxic metal whose widespread use has caused extensive environmental contamination and health problems in many parts of the world. Lead is a bright silvery metal, slightly bluish in a dry atmosphere. It begins to tarnish on contact with air, thereby forming a complex mixture of compounds, depending on the given conditions. The sources of lead exposure include mainly industrial processes, food and smoking, drinking water and domestic sources. The sources of lead were gasoline and house paint, which has been extended to lead bullets, plumbing pipes, pewter pitchers, storage batteries, toys and faucets (Thürmer*et al.*, 2002). In the US, more than 100 to 200,000 tons of lead per year is being released from vehicle exhausts. Some is taken up by plants, fixation to soil and flow into water bodies, hence human exposure of lead in the general population is either due to food or drinking water (Goyer, 1990). Lead is an extremely toxic heavy metal that disturbs various plant physiological processes and unlike other metals, such as zinc, copper and manganese, it does not play any biological functions. A plant with high lead concentration fastens the production of reactive oxygen species (ROS), causing lipid membrane damage that ultimately leads to damage of chlorophyll and photosynthetic processes and suppresses the overall growth of the plant (Han *et al.*, 2009). Some research revealed that lead is capable of inhibiting the growth of tea plant by reducing biomass and debases the tea quality by changing the quality of its components (Yongsheng*et al.*, 2011). Even at low concentrations, lead treatment was found to cause huge instability

in ion uptake by plants, which in turn leads to significant metabolic changes in photosynthetic capacity and ultimately in a strong inhibition of plant growth (Mostafa *et al.*, 2012).

1.10.1.5 Cadmium

Cadmium is the seventh most toxic heavy metal as per ATSDR ranking. It is a by-product of zinc production which humans or animals may get exposed to at work or in the environment. Once this metal gets absorbed by humans, it will accumulate inside the body throughout life. This metal was first used in World War I as a substitute for tin and in paint industries as a pigment. In today's scenario, it is also being used in rechargeable batteries, for special alloys production and also present in tobacco smoke. About three-fourths of cadmium is used in alkaline batteries as an electrode component, the remaining part is used in coatings, pigments and platings and as a plastic stabilizer. Humans may get exposed to this metal primarily by inhalation and ingestion and can suffer from acute and chronic intoxications. Cadmium distributed in the environment will remain in soils and sediments for several decades. Plants gradually take up these metals which get accumulated in them and concentrate along the food chain, reaching ultimately the human body. In the US, more than 500,000 workers get exposed to toxic cadmium each year as per. The Agency for Toxic Substances and Disease Registry (Bernard, 2008; Mutlu *et al.*, 2012). Researchers have shown that in China the total area polluted by cadmium is more than 11,000 hectares and its annual amount of industrial waste of cadmium discharged into the environment is assessed to be more than 680 tons. In Japan and China, environmental cadmium exposure is comparatively higher than in any other country (Han *et al.*, 2009). Cadmium is predominantly found in fruits and vegetables due to its high rate of soil-to-plant transfer (Satarug *et al.*, 2011). Cadmium is a highly toxic nonessential heavy metal that is well recognized for its adverse influence on the enzymatic

systems of cells, oxidative stress and for inducing nutritional deficiency in plants (Irfan *et al.*, 2013).

1.10.1.6Cobalt

Cobalt is an essential element for humans and animals , a physiological function of this element in higher plants has not been identified. Where as normal Cobalt concentrations in plants are cited to be as low as 0.1–10 mg kg-1dry weight, its beneficial role as a trace element has been described (Palit S, et al1994). Cobalt is an essential element for the synthesis of vitamin B12 which is required for human and animal nutrition. Unlike other heavy metals, cobalt is safe for human consumption and up to 8 mg can be consumed on a daily basis without health hazard (Young, 1983).Cobalt at lower concentration help in better nodulation and consequently a better growth and yield, but at higher concentration cobalt reduced the bacterial population in the rhizosphere and as a result nodulation was hampered which led to a lower growth and yield of crop.In other words, cobalt addition increased the nodules formation of root and atmospheric nitrogen fixation by microorganisms which increase the nitrogen content in leguminous plants. This was confirmed by(Young, 1983). Moreover, cobalt application increases the formation of loghaemoglobin required for nitrogen fixation, there by improves the nodules activity .Cobalt, a transition element, is an essential component of several enzymes and co-enzymes. It has a role inaffecting growth and metabolism of plants in different stages, depending on the concentration and status of cobalt in rhizosphere and soil (Palit and Sharma, 1994). The beneficial effects of Cobalt include retardation of senescence of leaf, increase in drought resistance in seeds, regulation of alkaloid accumulation in medicinal plants, and inhibition of ethylene biosynthesis (Palit and Sharma, 1994).

1.11Primary and secondary metabolites

The biosynthesis and breakdown of proteins, fats, nucleic acids and carbohydrates, which are essential to all living organisms, is known as primary metabolism with the compounds involved in the pathways known as "*primary metabolites"*. The mechanism by which an organism biosynthesizes compounds called "*secondary metabolites"* (natural products) is often found to be unique to an organism or is an expression of the individually of a species and is referred to as "*secondary metabolism"*(Maplestone, et al 1992).The biosynthesis of secondary metabolites is derived from the fundamental processes of photosynthesis, glycolysis and Krebs cycle to afford biosynthetic intermediates which, ultimately, results in the formation of secondary metabolites also known as natural products. Plants have been well documented for their medicinal uses for thousands of years. They have evolved and adapted over millions of years to withstand bacteria, insects, fungi and weather to produce unique, structurally diverse secondary metabolites. Their ethno pharmacological properties have been used as a primary source of medicines for early drug discovery (McRae, et al 2007). The knowledge associated with traditional medicine (complementary or alternative herbal products) has promoted further investigations ofmedicinal plants as potential medicines and has led to theisolation of many natural products that have become well known pharmaceuticals. The use of natural products as medicines has been described throughout history in the form of traditional medicines, remedies, potions and oils with many of these bioactive natural products still being unidentified. The dominant source of knowledge of natural product uses from medicinal plants is a result of man experimenting by trial and error for hundreds of centuries through palatability trials or untimely deaths, searching for available foods for the treatment of diseases (King horn, et al 2011).

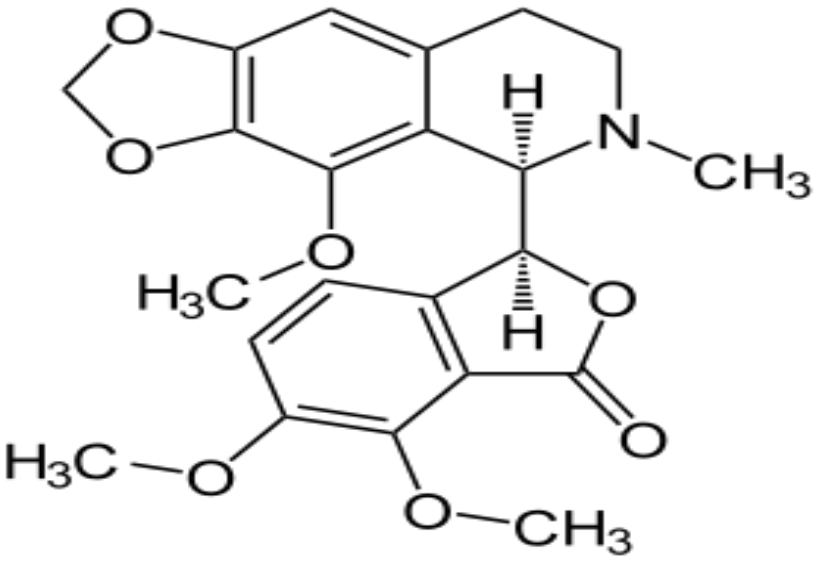
1.11.1 Secondary metabolites

1.11.1.1 Alkaloids

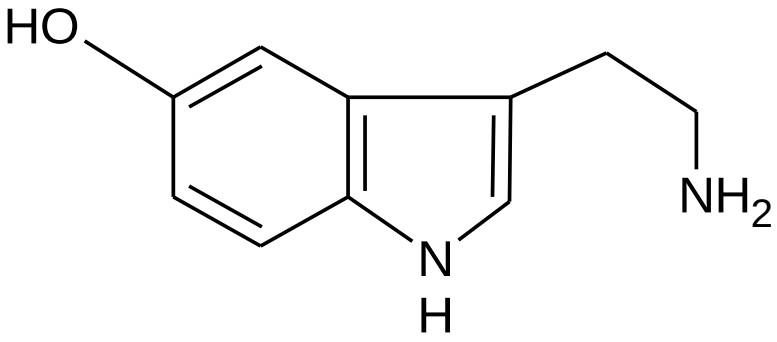
Alkaloids are the natural compounds found in all vegetables that include nitrogen and are considered to be disintegrated of substances that comprise proteins. They are exuded in particular cells or tubes and can be of great use in safeguard against normal enemies as they have a bitter flavor. Generally, alkaloids are amalgams that do not have any scent and boast of a distinctive outcome on animal's body mechanism or function. Owing to these properties, alkaloids have significant therapeutic value and form the ingredients of many important medicines. The alkaloids also comprise strong vegetable toxics and sedatives. Substances like caffeine and theobromine that are closely associated with natural purine (substance that can be converted to uric acid in the body) are normally categorized as alkaloids (Pettite, et al 1986).

Many plant-derived drugs to treat cancers are alkaloids, others biological activities have been established including antimalarial, antiviral and anticholinesterase. More than 10 000 alkaloids are now known, the first discovered being narcotine, isolated from opium by Derosne in 1803.Alkaloids exist as salts in the cell sap. They may be extracted from the cell with acidified water or alcohol, or alternatively they are soluble in organic solvents (e.g. chloroform) when the plant is rendered alkaline. For insects, it is believed that alkaloids have an ecological role in two species of butterflies since their larvae become distasteful to predators because of alkaloidal accumulations in their tissues (Mann, 1978).

In man, the alkaloid serotonin is a natural nerve transmitter in the central nervous system, responsible in part for control of sleep patterns



Narcotine



Serotonin

Figure(1.2):Chemical structure of narcotine and serotonin (representative alkaloids compounds)(Mann,1978)

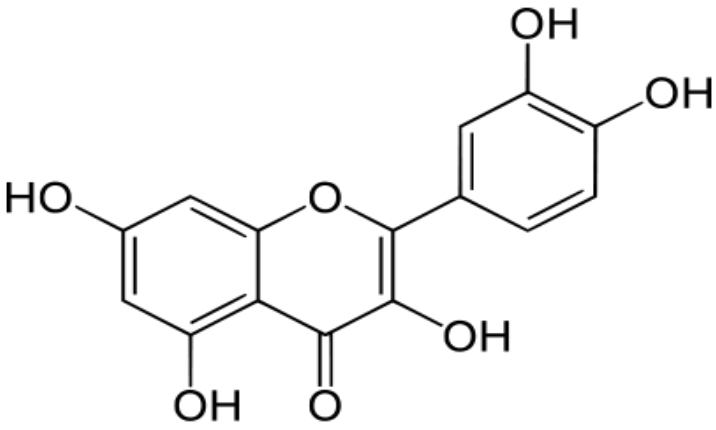
1.11.1.2 Flavonoids

Flavonoids represent one of the largest and most studied classes of phenylpropanoid-derived plant specialized metabolites, with an estimated 10,000 different members. Structurally, they consist of two main groups, the 2-phenylchromans (the flavonoids, including flavanones, flavones, flavonols, flavan-3-ols, and anthocyanidins) and the 3-phenylchromans (the isoflavonoids, including isoflavones, isoflavans, and pterocarpans). Flavonoids act as attractants to pollinators and symbionts, as sunscreens to protect against UV irradiation, as allelochemicals, and as antimicrobial and antiherbivory factors (Jorgensen, 1995).Their importance in plant biology goes beyond their specific functions within the plant. For example, the early advances in floral genetics were primarily the result of the ease of screening for mutations impacting flavonoid-derived flower colors, and the first demonstration of epigenetic gene silencing in plants was likewise associated with flavonoid biosynthesis (Jorgensen, 1995). Flavonoids have been ascribed positive effects on human and animal health and are central to the current interest in "botanicals" for disease therapy and chemoprevention.

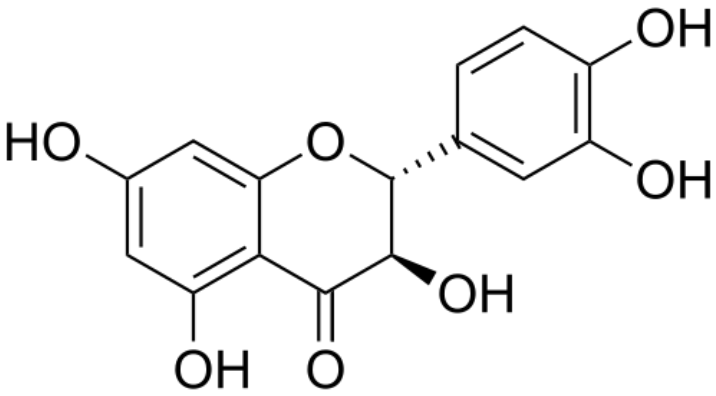
1.11.1.3 Phenolic compounds

Dietary phenolic compounds are the most abundant antioxidants in human diets. With over 8,000 structural variants, they are secondary metabolites of plants and denote many substances with aromatic ring(s) bearing one or more hydroxyl moieties. They are subdivided into groups by the number of phenolic rings and of the structural elements that link these rings. The phenolic acids with the subclasses derived from hydroxybenzoic acids such as gallic acid and from hydroxycinnamic acid, containing caffeic, ferulic, and coumaric acid (Kehrer, 1993).Certain phenolic compounds have been identified in fruits and vegetables that may account for this cancer prevention, e.g. apigenin was found to be cancer chemo preventive agent (Wang, et al2000). It has been shown to be growth inhibitory in many cancer cell lines such as human breast

cancer cells (Hirano, et al1989)., leukemia cells (Lee, et al1995), and solid malignant tumor cells (Fotsis, et al1997).

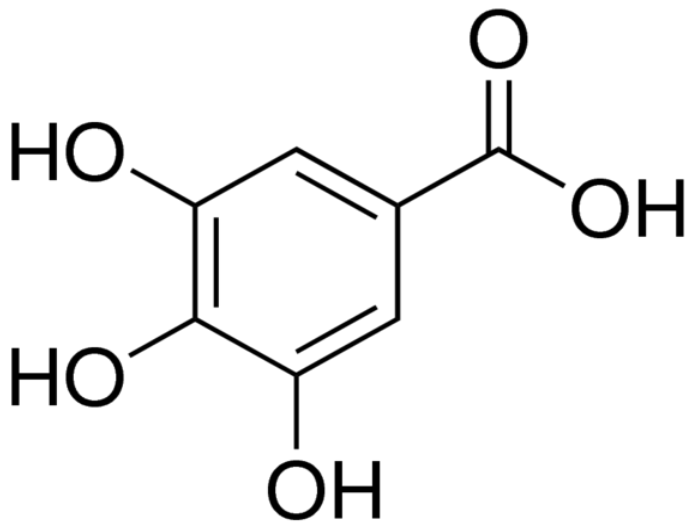


Quercetin

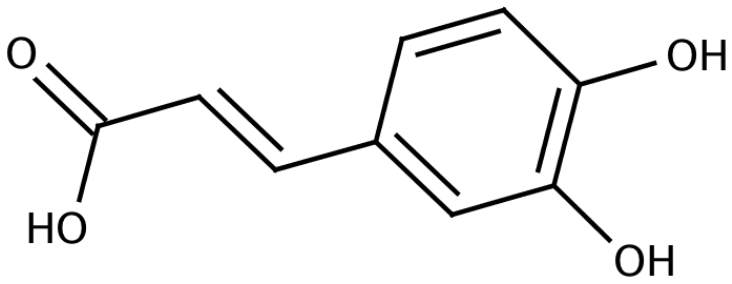


Taxifolin

Figure(1.3): Chemical structure of quercetin andtaxifolin (representative flavonoids compounds) (Shashank, et al 2013).



Gallic acid

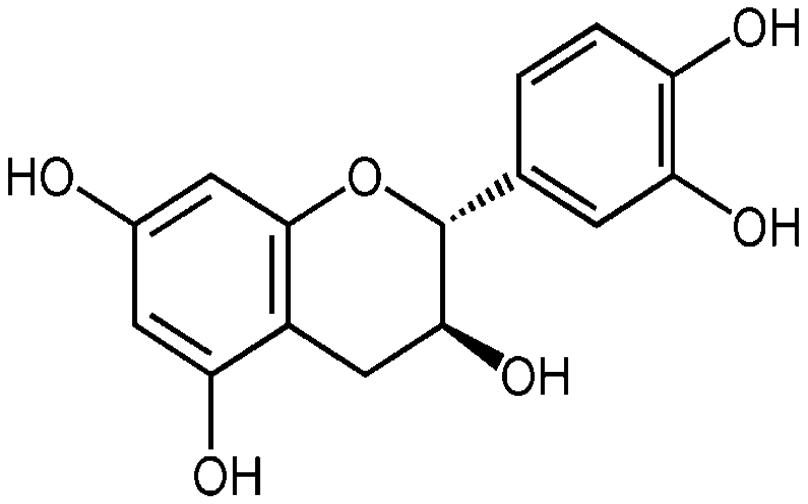


Caffeic acid

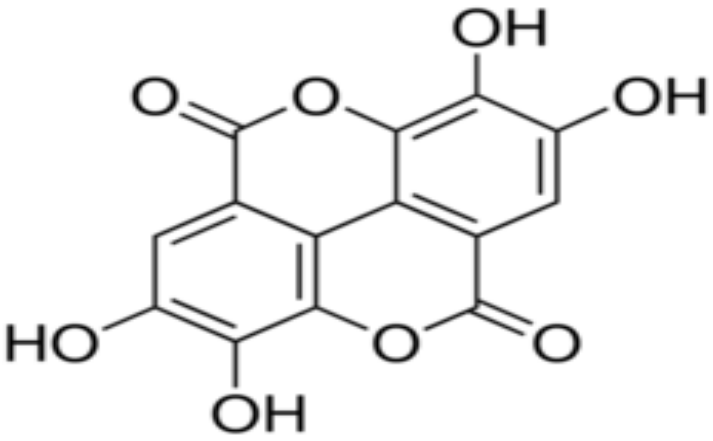
Figure(1.4): Chemical structure of gallic acid and caffeic acid (representative phenolic compounds)(Hagerman, et al 1998)

1.11.1.4 Tannins

Tannins are polyphenolic compounds found in many plants and are by far the most common secondary compound found in *Acacias*. Tannins can be beneficial or detrimental to the ruminant, depending on concentration. From 2-4 % tannins in the diet protects protein from rumen degradation and increases the absorption of essential amino acids whereas 4-10% depresses voluntary feed intake (VFI) (Terrill, et al 1992) and (Barry, 1992). Benefits reported include increases in wool production, milk protein secretion, ovulation rate and the development of more nutritionally based and ecologically sustainable systems for disease control in grazing animals. Tannins may also appear to have some protective affects against the establishment of, and tissue damage caused by, gastrointestinal nematodes (Kahn& Hernandez 2000). Tannins levels in excess of 50 g/kg dry matter, on the other hand, can lead to low palatability, reduce digestibility, lower intake, inhibit digestive enzymes and be toxic to rumen micro-organisms (Vaithiyanathan& Kumar 1993). The availability of sulphur and iron also becomes limiting to animals consuming tannin-rich foliage. Sheep ingesting 0.9g hydrolysable tannins per kg body weight can show signs of toxicity in 15 days. Animals such as mule deer, rats and mice have been shown to secrete proline-rich proteins in saliva and these constitute the first line of defence against ingested tannin. Unfortunately, the proline-rich protein defence is not present in domestic ruminant species to enable domestic livestock to consume plants high in tannins.



Catechin



Ellagic acid

Figure(1.5): Chemical structure of catechin and ellagic acid (representative tannins compounds) (Makkar, et al 2007)

1.12 Approximate analysis

Proximate and nutrient analyses of edible plantand vegetables play a crucial role in assessing their nutritional significance (Pandey , 1999). As various medicinal plant species are also used as food along with their medicinal benefits, evaluating their nutritional significance can help to understand the worth of these plant species (Pandey,1999). For herbal drug's standardization is concerned, WHO also emphasized on the need and importance of determining proximate and micronutrients composition of the herbal plants. Such herbal formulations must pass through standardization processes (Pandey,1999). Medicinal plants play a significant role in providing primary health care services to rural people and are used by about 80% of the marginal communities around the world. Each medicinal plant species has its own nutrient composition besides having pharmacologically important phytochemicals. These nutrients are essential for the physiological functions of human body.Such nutrients and biochemicals like carbohydrates, fatsand proteins play an important role in satisfying human needs for energy and life processes (Mann,et al 2000).

1.13 Fatty acid

Chemically, a fatty acid is an organic acid that has an acid group at one end of its molecule, and a methyl group at the other end. Fatty acids are typically categorized in the omega groups 3, 6 and 9 according to the location of their first double bond (there is also an omega 7 group, but these are less important to human health). (Jones ,et al 2001). Now don't panic if you are not up on your chemistry; this isn't going to be a chemistry lesson. I just wanted you to understand why a fatty acid might be called an omega 3 or omega 6 fatty acid. The term essential fatty acid refers to a fatty acid which the body cannot manufacture, and must obtain from dietary sources. These essential fatty acids were originally designated as Vitamin F, until it was realized that they must be

classified with the fats.(USDA, 2015) There are two fatty acids designated as essential fatty acids: linoleic acid and alpha linolenic acid. This does not mean that the other 15 or so fatty acids found in the omega 3, 6 and 9 groups aren't important, just that a healthy body can manufacture them as long as it gets enough linoleic acid and alpha-linolenic acid.

1.14Cytotoxicity

Bioactive compounds are almost always toxic in high doses. Pharmacology is simply toxicology at a lower dose, and toxicology is simply pharmacology at a higher dose. Thus *in vitro* lethality in simple zoological organism can be used as a convenient monitor for screening and fractionation in the discovery and monitoring of bioactive natural products. Brine shrimp nauplii have been used previously in a number of bioassay systems, but (Patel, et al .2009) have developed a method where by natural products extracts, fractions or pure compound are tested using African green monkey kidney normal cell line (*Vero)* and cervical cancer cell line *(Hella)*

Table(1.2) : Details of cell lines(Patel, et al 2009)

Cell line	**Morphology**	**Origin**	**Species**	**Ploidy**	**characteristic**	**Supplier**
Hella	Epithelial	Cervix	Human	Aneuploid	G6PDtypeA	NCCS, Pune
Vero	Epithelial	Kidney	Monkey	Aneuploid	Viral subtract And assay	NCCS, Pune

NCCS is National Centre for Cell Science

1.15 Biological screening of plants

1.15.1Screening of plants for antimicrobial effects

It was reported by (Bax& Mullan 2000) that global antibacterial resistance is becoming an increasing public health problem. Bacterial resistance to almost all available antibacterials been recorded. According to these authors, the pharmaceutical industry and fledgling biotechenology companies are intensifying efforts to overcome bacterial resistance. On the other hand, some of the drugs currently in use result in adverse side effects (Covington, 1988).. Therefore, the research for new antimicrobial substance exhibiting minimal side effects is warranted. One of the most promising areas in search for new biologically active compounds is the plants used in traditional medicine (Alonso, et al1995). Hence screening of plants for new antimicrobial agents possess an enormous challenge, especially with the emergence of drug resistant strains. Fungal infection is an inflammatory condition in which fungi multiply and invade the skin, the digestive tract, the genitals and other body tissues, particularly, the lungs and liver. Microscopic fungi, which are called dermatophytes, often live exclusively on such dead body tissue as hair, the outer layer of skin and the nails. The fungus grows best in moist, damp, dark places with poor ventilation and on skin that is irritated, weakened or continuously moist. (Rebecca, et al (2005).

Systemic fungal infections occur when spores are touched or inhaled or there is an overgrowth of fungi in or on the body. Such infections are most often a serious problem in those with suppressed immune system. Candidiasis is a rather common fungal infection. When it occurs in the mouth, it is called thrush. Less often it occurs in the mucosa membranes of other parts of the digestive system, or in the vagina, heart valves, urinary tract, eyes or blood. Other systemic fungal infections include aspergillosis, which mostly affects the lung and may also spread to the brain and kidneys; blastomycosis, a lung infection that may spread through the bloodstream; coccidioidomycosis, also

known as San Jaquin or valley fever; mucormycosis, which can develop into a very serious, live-threatening infection; and histoplasmosis (Garrod, et at1981).

1.15.2 General characteristics of tested organisms

1.15.2.1Bacillus subtilis

Bacillus subtilis, known also as the hay bacillus or grass bacillus, is a Gram-positive, catalase-positive bacterium, found in soil and the gastrointestinal tract of ruminantsand humans. A member of the genus*Bacillus*, *B. subtilis* is rod-shaped, and can form a tough, protective endospore, allowing it to tolerate extreme environmental conditions. *B. subtilis* has historically been classified as an obligate aerobe, though evidence exists that it is a facultative aerobe. *B. subtilis* is considered the best studied Gram-positive bacterium and a model organism to study bacterial chromosome replication and cell differentiation. It is one of the bacterial champions in secreted enzyme production and used on an industrial scale by biotechnology companies (Euzéby, *2008).*

1.15.2.2Escherichia coli

Escherichia coli is one of the common microbial flora of gastrointestinal tract of poultry and human being including other animals but may become pathogenic to both although most isolates of *E. coli* are nonpathogenic but they are considered as indicator of fecal contamination in food and about 10 to 15% of intestinal coliforms are opportunistic and pathogenic serotypes and cause a variety of lesions in immune compromised hosts as well as in poultry. Among the diseases, some are often severe and sometimes lethal infections such as meningitis, endocarditis, urinary tract infection, septicemia, epidemic diarrhea of adults and children and yolk sac infection, omphalitis, cellulitis, swollen head syndrome, coligranuloma, and colibacillosis. During the past two decades, severe outbreaks of gastrointestinal illness have occurred by food borne pathogenic *E. coli*, especially 0157:H7 (Akond, et al 2009).

1.15.2.3Pseudomonas aeruginosa

Pseudomonas aeruginosa is a Gram-negative rod, non-capsulated, measuring about 0.6 X 2 µm, occurs in single forms, pairs and occasionally in short chains. It causes urinary tract infection, respiratory infection, dermatitis, soft tissue infections, bacteremia, bone and joint infections, gastrointestinal infections and variety of systemic infection, particular in patients with severe burns. The bacterium is the 4th most commonly isolated nosocomial pathogen account for 10.1% of all nosocomial infections (Nelson &Reginald 2007).

1.15.2.4Staphylococous aureus

Stphylococcusaureus is a Gram-positive spherical organism, non-spore-forming, arranged usually in grape-like irregular cluster, non-motile and non-capsulated. It is found as normal flora in nose and intact skin, but it is an opportunistic pathogen that can cause infection of damaged skin, wound and mucous membrane (Jawets, et al 2001)..

1.15.2.5Aspergillus niger

Aspergillus niger belongs to the family of Ascomycetes, which are filamentous saprophytic fungi found everywhere. *Aspergillus* species are common in the environment and therefore are common contaminants of clinical specimens in the absence of diseases. Serious infection is usually enclosed in deep tissue; definitive diagnosis depends on histopathology showing septate hyphae (Jawets,et al 2001).

1.15.2.6Candida albicans

Candida albicans appears as Gram-positive, small, oval thin walled yeast and sometime shows budding. It is a common human pathogen of candidosis. *C. albicans* can cause mouth infection, (thruch), vaginal infection, bowel infection and rarely septicemia, endocarditis, meningitis and lung abscesses (Jawets, et al 2001).

1.16 Studied plants

1.16.1 *Psidium guajava*

Scientific classification

Kingdom: Plantae

Order : Myrtales

Family: Myrtaceae

Genus*: Psidium*

Species: *P.Guajava*

It is a low evergreen tree or shrub 6 to 25 feet high, with wide-spreading branches and square, downy twigs, is a native of tropical America. It is a common vegetation cover by roads and in waste places in Hawaii. Guava is a tropical and semitropical plant. It is well known in the islands for its edible fruit. It is common in the backyards. The branches are crooked, bringing opposite leaves. The flowers are white, incurved petals, 2or 3 in the leaf axils, they are fragrant, with four to six petals and yellow anthers. The fruit is small, 3 to 6 cm long, pear-shaped, reddish-yellow when ripe.(Dey,1986)

Guava is native to the American tropics. The English name guava probably came from the Haitian name, guajaba. The Spanish explorers took the guava to the Philippines and the Portuguese disseminated it from the Philippines to India. Then it spreaded easily and rapidly throughout the tropics because of the abundance of seeds with long viability and became naturalized to the extent that people indifferent countries considered the guava to be indigenous to their own region. It is now also grown in the subtropics.(Nakason& Paull 1998). Psidium guajava is medium sized tree with thin smooth, patchy, peeling whitish brown bark,(WHO1998)but under high moisture conditions, grows to 6-9 m in height.(Nakason&Paull 1998).

Leaves are simple, alternate, short-petiolate, exstipulate, gland dotted, aromatic, entire, apex ovate.(Pandey,1999).All parts of the young fruit are

astringent. Guava exhibits antibacterial action against intestinal pathogens such as Staphyloccocus. The dried ripe fruits are recommended as a remedy for dysentery, while the leaves and fruits are used as a cure for diarrhoea. Oil contains bisabolene and flavinoides that exhibit anti-inflammatory properties. A decoction of the leaves or bark is taken externally as a lotion for skin complaints, ringworm, wounds, and ulcers. Water from soaking the fruit is good to treat diabetes. The leaves are made into a cataplasm; cooked, they are given to horses with strangle.Some suggested treatments are digestive tract ailments, cold, and high blood pressure: leaf decoction or fruit juice with salt or sugar taken orally. Trauma, pain, headache, and rheumatism: hot leaf decoction compress. Sore throat, hoarse throat: leaf decoction, gargle. Varix, ulcer: leaf decoction, treated with warm water, bath. Hepatitis, gonorrhoea, and diarrhoea: clear fruit juice.(Orwa et al.2009)

1.16.1.1 PharmacologIcal actions of P. guajava leave

1.16.1.2Anticough action

Guava leaf has been used in Bolivia and Egypt for a long time to treat ailments including cough and pulmonary diseases. (Prance & Kallunki 1984).The aqueous extract decreased the frequency of cough induced by capsaicin aerosol within 10 minutes afterintraperitoneal injection of the extract. The LD50of guava leaf extract was more than 5g/kg. These results suggested that guava leaf extract is recommended as a cough remedy (Jaiari ,et al 1999).Meanwhile a recent study conducted on the Egyptian plant showed that the alcoholic extract (in a dose starting from 4µg/ml), the aqueous extract(from 8µg/ml), the ethyl acetate extract (from 6 µg/ml), the essential oil (16 µg/ml) as well as quercetin (30 µg/ml),quercetin has shown many important biological properties which include anti-diarrhoea effect, antioxidant and spasmolytic effects, inhibition on skeletal muscles

contraction, antimicrobial, anti-inflammatory and induced reduction of presynaptic molecular activity (Jaiari ,et al 1999).

1.16.1.3Antioxidant activity

Guava fruit is a suitable source of natural antioxidants. Peel and pulp could also be used to obtain antioxidant dietary fiber (AODF), a new item which combines in a single natural product the properties of dietary fiber and antioxidant compounds.(Jimenez,et al 2001)

Meanwhile the leaf was proven to have strong antioxidant activity, (Hui-Yin, 2007) since it possesses strong DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity, potent inhibitory activity of lipid peroxidation and strong inhibition against oxidative cell death (H_2O_2-induced oxidative cell death) (Masuda,et al 2002) and therefore guava could be used to extend the shelf life of food stuffs, to reduce wastage and nutritional losses by inhibiting and delaying oxidation. As a conclusion, supplementing a balanced diet with guava leaf extracts may provide health-promoting effects (Qian He,2004)

1.16.2 *Schoenanthus cymbopogon*e

Scientific classification

Kingdom: Plantae

Class : Liliopsida

Order : Poales

Family :Poaceae

Genus : *Cymbopogon*

Species : *C.Schoenanthus*

Schoenanthus (L.) Sprengel, pl. Min.Cog. Pugil Prim 2:15 (1815), family Poaceae(Graminae) and to outline the differences between them. These plants were selected for their great importance in Sudanese folkloric medicine Plant anatomy deals with the structure, contents and development of cells and

tissues. It is of primary importance for all aspects of research in plant sciences such as morphogenesis, physiology, ecology, taxonomy, evolution, genetics, reproduction etc (Eltahir& Ahlam 2002).

The systematic anatomy is mainly aimed towards relating structure particularly of vegetative organs, to taxonomic classification of the plants in which the characters are exemplified. Accurate microscopical and macroscopical descriptions of the medicinal plants must be carried out to maintain standards of safety and quality andto authenticate the crude drug materials properly (Cutler, 1978). Most of the drugs that are extracted from leaves, barks, roots and rhizomes may be difficult to identify from their macroscopical appearance only; they must be complemented by microscopical characterization. The microscopical features of the medicinal plants were studied for different purposes. They may be studied to outline the diagnostic features; thushelping to identify them, to classify them using the anatomical characters and to distinguish between similar species to avoid adulteration (Eltahir& Ahlam 2002). Family Poaceae (Graminae) plants are herbaceous or perennial herbs, rarely shrubs or trees; stems erect, ascending or prostrate and creeping, usually branched at the base. Leaves alternate, distichously, simple sometimes crowded at the bases of the stems, consisting of sheath, legule and lamina. Flowers usually hermaphrodite, sometimes unisexual, small and inconspicauos consisting of stamens and pistils. *C.Schoenanthus* common names, Arabic:Ethkher, Tibn Makkah, Hashishat El Gamal, Halfa Bar, Sinbel Al-Arab, Askhabar, Abo Rekba, (United State)

English: Camel grass, Camel hay, Geranium grass,Camel hay grass, Lemon-scented grass, Sweet cane, Sweet rush, Spikenard oil (USDA,2015).

Densely tufted perennial; culms erect, 60-130 cm high, enclosed at the base by tight bundles of old sheaths. Leaf-blades narrowly linear to filiform, up to 30 cm long, 1-3 mm wide, glaucous, asperulous. (Watson , et al 2015).

Cymbopogon schoenanthus was an aromatic herb consumed in salads and used to prepare traditional meat recipes(Khadri et al2008). The plant was used in traditional medicine as antihelminthes , antidiarrhea, antirheumatic, carminative, diaphoretic, stomachic, diuretic, emenagogue, antipyretic, for treatment of jaundice and as tonic. It was also used for anorexia; astringent, sudorific and to cure dromedary wounds. In Morocco and Egypt an infusion of the flowers and the whole plant were used as febrifugal, diuretic, antirheumatismal and antigastralgic (Atyat, 1995). The plant was used in Sudan for the treatment of gout, prostate inflammation, kidney diseases, and for stomach pains (El Ghazali,1997).Parts Used: Aerial parts of the plant are used dried, powdered, or fresh (Rizk A. 1986)

1.16.2.1 Pharmacological effects

1.16.2.1.1 Antioxidant effect

The antioxidant activity of the essential oils from fresh leaves, dried leaves and roots of *Cymbopogon schoenanthus* was assayed using β-carotene–linoleic acid bleaching method. Aqueous extract, of Cymbopogon schoen from three different locations in Southen Tunisia were screened for their antioxidant activity.Antioxidant activity of the essential oils of Cymbopogon schoenanthus was measured by DPPH assay. The results of antioxidant effect ranged from 36.0% to 73.8% (2µl of essential oil per ml of test solution)(Khadri et al2008).

1.16.3 Hargel (*Solenostemma argel*)

Scientific classification

Kingdom : Plantae

Order : Gentianales

Family : Apocynaceae

Genus : *Solenostemma*

Species : *S.Argel*

Hargel (Solenostemma argel) is a plant or plant part valued forits medicinal, aromatic or savory qualities. Herb plants produce and contain a variety of chemical substances (Shayoub, 2003). Herbs had been used all cultures throughouthistory. The primitive man observed and appreciated the great diversity of plants available to him. Theplants provided food, clothing, shelter, and medicine (Shayoub, 2003).Certainly herbs used in some infections, cough, cold, stomach upset, indigestion, catarrh, constipation and so on (WHO, 2002; Mcntyres2003). Herbal medicine has provided the world's population with safe,effective and low cost natural substances (medicine) for centuries (Shayoub, 2003).

The plant hargel is a member ofthe family Apocynaceae that comprises numerous medicinal plants, like Calotropis procera, Marsdenia obyssinicna and Huernia mecrocarpa, known for their cardiac activity. Hargel grows naturally in the northern parts of the Sudan and extends from Berber to Abu-Hamad. Hargel has antimicrobial effect to some bacteria and fungi.(Abd Elhady,et al1994) and has antiviral activity to new castle disease virus.Hargel leaves are used in indigenous medicine for the treatment of some diseases such as the disease of liver and kidney. It is an effective remedy for bronchitis and is used to treat neuralgia. It is used as incense in the treatment of measles and sometimes crushed and used as remedyfor healing wounds. The leaves are infused to treatgastro-intestinal cramps and stomach colic (Abd Elhady,et al 1994).

The plant has a long flowering period from March to June (El-kamali,2001).

Regional(Algeria, Libya and Egypt). No particular threat is reported forAlgeria, but in Egypt the plant isvulnerable because of its intensiveoveruse. The largest population of Solenostemma hargel grows in theupper part of the Wadi Allaqiconservation area and from 1989 on hasbeen protected by Egyptian law.The plant is cultivated on a farm in the downstream part ofWadi Allaqi to increase its population and promote the cultivation ofthis

economically important plant. Hargel is Tropical plant thatspreads across the central Sahara to the Sinai and the southeastern(Arabian) desert.

The plant grows in extremely dry conditions with a yearlyrainfall of around 50-100 mm. It grows on the gravelly soils and on the stony and pebbly soils(El-kamali, 2001).Part used is the leaves and stems which arecollected in the spring andprepared as an infusion, a decoction or a powder. This can be taken bymouth or used externally. Many phytochemical constituents have been detected, Acylated phenolic glycosides,namely argelin and argelosid. (El-kamali, 2001).

1.16.3.1 Pharmacological action

Hargel isused for colds, diabetes, respiratory troubles, rheumatism, stomachpain, urinary infection. The bitter sap from the stem is used for cold(El-kamali, 2001).

1.16.3.2 Chemical composition

Leaf of hargel is characterized by high carbohydrates and low crude fiber, contained protein, crude oil, ash,and moisture content. the leaf contained high potassium calcium ,magnesium and sodium ,but it ischaracterized by low copper, iron,manganeseand lead . Leaf contained phytic acid and tannin content as reported by(Sabah El-Kheir & Murwa, 2010).

1.16.4 *Hyphaene thebaica*

Scientific classification

Kingdom: Plantae

Class : liliopsida

Order : Arecaceae

Family : Arecaceae

Genus : *Hyphaene*

Species : *H.Thebaica*

Doum palm fruit (Hyphaene thebaica) is a desert palm tree with edible oval fruit, originally native to the Nile valley. It also grows very well in the northern partof Nigeria. It is a member of the palm family, Arecaceae(Fletcher,1997). It is represented by the genus Hyphaene, the fruit of interest in the current study. Its fibre and leaflets are usedby people along the Nile to weave baskets. Doum palm fruit is also a source of potent antioxidants (Hsu& Coupar 2006).

The fruit has a brown outer fibrous flesh which is normally chewed and spewed out. Doum palm kernel is edible when it is unripe but hard when it is ripe,the root is also medicinal. The foliage is used to make mats, ropes, baskets ,and hats while the stem with the leaves are used for construction purpose (Moussa et al., 1998).

Roots of doum palm are used for treatment of bilharzias while the fruit is often chewed to control hypertension (Orwa et al., 2009).

Doum fruit, (Hyphaene thebaica) is a desert palm growing wild throughout the drierregions in Egypt, (Sub-Saharan) Africa, and west India. It listed as one of the useful plants of the world (Fletcher,1997).

Doum is one of commonly consumed beverages in traditional places in Egypt. The drink has been prepared from the fruits by infusing the dried ground fruit pulp in hot water. This drink is widely consumed as a health tonic as a remedy for hypertension (Hashem,1994).

Doum fruits are relatively rich in protein and in essential minerals. It contains high amounts of essential minerals which in most instances exceed the recommended daily allowance (RDA), and thus may keep the balance and ratios between those in need (Cook,et al.2000). Doum was reported to lower the blood pressure, when its biological activity was evaluated in rat feeding experiments(Sharaf,et al1972)(Betty,et al.2006). It has been reported that Na/K ratios less than one haveagreat importance in the body for the control of

high blood pressure(Aremu,et al.2006). The high fiber content of doum fruit is suggesting as a potential to be used in the formulation of bakery products such as bread, biscuits, cookies, cakes and pan cakes to satisfy consumer needs for increased fiber intake without sacrificing sensoryattributes and enrich their texture, flavor and nutritional value (Dubois,1978)(Fondroy,et al.1989). Besides, it is great contribution to the health and wellbeing of humankind by preventing the gastrointestinal problems such as constipation and therefore, it is regarded as a natural anti colon cancer (Coimbra& Jorge,2011). Doum pulp contains 4.91% proteins, 5.26% fat, 4.50% ash and 85.33% total carbohydrate (Eissa,et al.2008).The current focus is toward natural antioxidant especially plant polyphenolics (Katalinic,et al.2006)-(Eldahshan,et al.2009). Doum is rich in polyphenolic compounds;its extract contains 1.20 μg/g dried plant extract as Gallic and 3.70 μg/g dried plant extract as quercetin

which act to reduce the oxidative stress by scavenging free radicals (Eldahshan,et al.2008)(Kamis,et al.2003)-(Jeong, et al 2009).

1.16.5 Petroselinum crispum

Scientific classification

Kingdom :Plantae

Class : Mangoliopsida

Family : Umbelliferae

Order : Apiales

Genus : *Petroselinum*

Species : *P.Crispum*

Medicinal plants and herbs play an important role in the prevention and treatment of kidney diseases. Parsley (Petroselinum crispum, Family: Umbelliferae/Apiaceae) is used as a culinary, garnishing and medicinal herb in the Mediterranean region of Southern Europe. Parsley extract was reported to produce a diuretic effect and good antioxidant activity (Kreydiyyeh& Usta

2002). Parsley leaves are rich in apigenin and its glucosidal flavonoids that were found to possess anti-inflammatory especially for renal inflammation; antioxidant and anticancer activities(Papay, et al2012).In addition, the aqueous extract of parsley reduced the number of calcium oxalate deposits and therefore parsley can be used for kidney and bladder stones (Saeidi, et al2012). Phytochemically, the leaves and seeds of P.crispum has been shown to contain xanthotoxin, ficusin, bergapten, majudin, heraclin and antimicrobial furocoumarins namely 8 methoxypsoralen, 5-methoxypsoralen, oxypuecedanin, isopimpinellin, 6'-acetylopin and a new monoterpene glycoside(Kreydiyyeh& Usta 2002). Furthermore, the plant is a good source of iron, calcium, phosphorous and antioxidants like luteolin, vitamin C, vitamin A and zinc and these may likely account for its hepatoprotective effect (Popovic,et al2010). However, the nephroprotective and antidyslipidemic effect of P.crispumhas not been scientifically investigated. Genetically modified(GM) administration in rats could be considered one of the best experimental models to study, due its availability, low cost and reproducibility of the renal lesions. So, the present studywas designed to evaluate the efficacy of parsley (P.crispum) extract and decoction on the kidney of gentamicin-induced nephrotoxicity in rats.

1.17 General objective

The objective of this study arises from the fact that our Sudanese flora comprises a very rich and characteristic plants that covers a wide area of Sudanese land. Many of these plants are used as natural medicines by the natives to treat tropical diseases including, malaria, fungal ,bacterial,cough and stomach pain.Thusthe overall aim of the study is to investigate five medicinal plants [*Psidium guajava, Schoenanthus cymbopogone, Solenostemma argel, ,Hyphaene thebaical, and petroselinum crispum*] from,different places of Sudan as a potential source of heavy metals possessing antioxidant activities. The selection

of these plants is based on the known use of these species by various people as herbal remedies.

1.17.1 Aim of the study:

The aim of the study is to evaluate the use and effectiveness of the selected medicinal plants in health care:

1. Evaluating the antioxidant activities of the selected medicinal plants.
2. Qantification of moisture ,crued protein , crued fiber ,ash and ether extract contents
3. Determination of heavy metals levels(Zn, Mn, Fe, Ni, Pb, Cd and Co) in the selected plants.
4. Screening for the major classes of secondary metabolites(alkaloids ,flanonoids ,polyphenol and tannin) contents
5. Evaluation of the microbial activity of the extracted compounds aginstdifferent bacterial and fungal strains.

CHAPTER TWO

2.MATERIALS AND METHODS

2.1 Materials

2.1.1 Plant materials

The samples of the plants *Psidium guajava (*Aljawafa*), ,Schoenanthus cymbopogone(*Almharib*), Solenostemma argel*(Alhargil*) ,Hyphaene thebaica (*Aldoum*) and Petroselinum crispum(*Albagdunas) were collected randomly from different location of Sudan. The plant materials were dried under shade for 7 days except the fruits of *doom palm* which was found dried. They were ground into fine powder in preparation for extraction. The finely powdered materials were weighed separately and stored at room temperature.

2.1.2Chemicals

TheOrganic solvents used in this study petroleium ether, ethyl acetate, acetone and ethanol from Sisco Research Lab (SRL), India. culture media were from Merck, Germany.and the other chemical were used 2,2-diphenyl-1-picrylhydrazyl (DPPH),(2, 2´-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)ABTS,propyl glate,ascorbic acid (ANKARA,TORKIYE),dimethyl sulfuric, peptone water , Mueller hinton agar ,saboraud dextrose agar, normal saline, bromocresol green (TLC) hemicals (india), sodium hydroxide (CDH), phosphate buffer solution, sodium sulfate, citric acid (Fluka ,chemika), quercetin , aluminum chloride, distilled water, deionized water, gali acid, sodium carbonate, ferric chloride, tannic acid, nitric acid (Sigma – Aldrich), aqua regia, sulfuric acid,hydrochloric acid (LOBACHEMIE), glucose solution,anthron reagent, cupric crystal, boric acid, perchloric acid, sodium chloride, hexane, potassium bicarbonate, 3-(4,5-dimethyl thiazol-2-yl)-2,5diphenyl tetrazolium bromide

2.1.3 Equipment

UV/VIS Spectro star (Nano BMG LABTECH) with 1cm matched quartz cell, Atomic absorption spectrophotometer Model 3300 , UV/vis spectrophotometer (Jasco) model V-530 (Germany), Gaschromatograph,Sohxlet,Oven ,crucible, muffle furnace,desiccator and Incupater

2.2 Methods of extraction

Hundred gm of the finely ground material was soaked successive in four solvents vary in polar. Firstly soaked in petroleum ether (a least polar solvent) for 48 hours with frequent swirling to ensure through extraction. The soaked material was filtered and the crude extract were collected in clean containers. The crude extract was dried at room temperature and weighed. The residue after extraction with petroleum ether was air dried under room temperature and soaked in ethyl acetate for 48 hours with occasional swirling to ensure through extraction. The soaked material was filtered and the crude extract collected in clean containers. The crude extract was dried at room temperature and weighed. The residue after extraction with ethyl acetate was air dried and soaked in acetone for 48 hours with occasional swirling. This was followed by filtering, drying and weighing. The residue was air dried and soaked in ethanol (high polar solvent) and the above procedure was repeated. Finally, residue obtained from each extract was air dried under room temperature and its yield percentage was determined, colour and texture were reported.

$$\text{Yield\%} = \frac{\text{Wieght of extract}}{\text{Wieght of samples}} \times 100$$

Results obtained shown in table 3.1 —— 3.5

2.3 Antioxidant activity

2.3.1 DPPH radical scavenging assay:

The DPPH radical scavenging was determined according to the method of Villano*et. al.*(2007). with some modification. In 96-wells plate, the samples were allowed to react with 2.2 Di (4-tert-octylphenyl)-1-picryl-hydrazyl stable free radical (DPPH) for half an hour at 37ºC. The concentration of DPPH was kept at (300µM). The samples were dissolved in DMSO while DPPH was prepared in ethanol. After incubation, decrease in absorbance was measured at 517nm using multiplate reader spectrophotometer. Percentage radical scavenging activity by the samples was determined in comparison with a DMSO treated control group. All tests and analysis were run in triplicate.

The ability of the extract to scavenge DPPH radical was calculated by the following equation:

DPPH radical scavenging activity (%) = 1- [($Abs_{sample} - Abs_{blank}$)]/ ($Abs_{control}$)] x 100

Where;

Abs_{sample} is the absorbance of DPPH radical + sample;

Abs_{blank} is the absorbance of sample + methanol;

$Abs_{control}$ is the absorbance of DPPH radical + methanol

Result obtained shown in table (3.6) (p-74).

2.3.1.1 Ic_{50} Calculations:

The IC_{50} (the concentration of test material, which possess 50% inhibition of free radicals) of all the extracts and their fractions was determined by monitoring the effect of different concentrations ranging from 0.5-0.0035mg/ml. the IC_{50} of the extracts and their fractions were calculated using EZ-Fit Enzyme Kinetic Program (Perrella Scientific Inc, U. S.A).

2.3.2 ABTS radical scavenging activity

The scavenging activity of the studied extracts on ABTS (2, 2´-azinobis (3-ethylbenzothiazoline- 6-sulfonic acid) radical cation was estimated according to the method of (Rao*et al*. 2014). Briefly, ABTS radical cation was freshly prepared by mixing 14 mm ABTS with an equal volume of 4.95 mm potassium persulphate and kept for 16 h in dark atroom temperature. This ABTS radical cation solution was used for the assay after dilution in phosphate buffer saline (PBS) appropriately. To 50 µl of sample or standard, 150 µl of ABTS radical solution was added. After 6 min incubation at room temperature, the absorbance was measured at 734 nm. Methanol was used as blank solution, and ABTS solution without the sample served as control. Scorbic acid was used as reference synthetic antioxidant compound. Reduction of ABTS radical in percent (R %) was calculated as the same as described in DPPH radical assay.The result obtained shown in table 3.7 (p-77)

2.4Sample Preparation and Analysis of heavy metals

The plants dried in an oven at 70^{o}C to a constant weight and then ground into a powder with mortar and pestle (Maimon et. al, 2012).

For total digestion of plant samples, dry ashing-aqua regia method was used. 1g of dried plant samples were weighed and placed in silica crucible in a muffle furnace for 4hours at 500°C until white ash appeared. Sample is removed and cooled in the dessicators. Moisten sample with 1ml of concentrated nitric acid (HNO_3) and evaporated to dryness at 150°C by using hot plate. Evaporated sample was placed in the heated furnace at 400°C for 15 minutes. After that, the digested sample was solubilized with 5 mL of aqua regia of the digestion method required the beaker used to be covered with watch glasses to

prevent evaporation of the sample (Chen & Ma, 1998). The samples of plants were then filtered using Whatman no.1 filter paper and made up to 50 mL with distilled water for further analysis. A blank was prepared with an equal amount of acids. All reagents were of analytical grade and contained very low concentrations of trace metals. Normal precautions for trace metals analysis were observed throughout.

Sample solutions and reagent blanks were analyzed for Fe, Zn,Mn, Ni, Pb, Co, and Cd by using atomic absorption spectrophotometry (AAS) To determine heavy metal concentrations . All analyses were replicated three times. Significant differences between concentrations of heavy metals, following different sampling stations were analyzed (SAS Institute, 1982).

2.5 Proximate analysis

The collected dry plants were subjected to chemical analysis. The following parameters were determined moisture, fibre, ash, crude protein contents and ether extract.

2.5.1 Moisture content

The moisture content of the sample was determined according to a known procedure (A.O.A.C 2000). Two grams of the sample (in triplicate) were weighed in an empty, dry and clean moisture dish with a known weight. The moisture dish containing the sample was then placed in an oven at 105 °C for 18 hours. After that, the moisture dish was removed and placed in a desiccators containing anhydrous sodium sulfate and weighed three times at 10 minutes intervals and the weight was calculated as an average. The moisture content as percentage was calculated according to the equation:

Moisture content (%) $= \frac{W1-W2}{S} X100$

Where:

W_1 = Weight of crucible and sample before drying.

W_2 = Weight of crucible and sample after drying.

S = Original weight of the sample

See table 3.9 (p-86).

2.5.2 Total dietary fiber

Crude fiber was determined using the Pearson method (1970). Three grams of defatted sample was placed in 1- liter conical flask, 200 ml of H_2SO_4 (0.255N) was added, the conical flask was placed on digestion apparatus with pre adjusted hot plate and boiled for 30 min, the flask was rotated periodically to keep solids from adhering to sides; after that the content was filtered through Buchner funnel using filter paper (Whatman No.2) and the conical flask was rinsed with hot water several times and washed through Buchner. The residue transferred to the conical flask and 200 ml of sodium hydroxide (0.313N) was added and boiled for 30 min, the content was filtered as above with filter paper (Whatman No. 2). The residue was washed first with enough 1% HCl until the paper and content acidic, then with hot water to remove acid, then wash with alcohol and Diethyl ether until substantially all the water was removed. The air dried residue was transferred to ashing crucible and dried to constant weight in drying oven, cooled in desiccator and weighted, then ignited at 500 ˚C in muffle furnace, cooled in desiccator and reweighted.

$$\text{Crude fibre \%} = \frac{\text{loss of weight in ignition x 100}}{\text{Weight of sample}}$$

See table 3.9 (p-86).

2.5.3 Ash content

The ash content of the sample was determined according to aknown procedure(A.O.A.C 2000). Two grams of each line sample were weighed. The crucible containing the sample was placed in a muffle furnace at 550 ˚C for 8 hours. Then the crucible was removed and cooled in a desiccator and weighed again (repeated until constant weight). The ash content was calculated as percentage according to the equation:

Ash content (%) $= \frac{W1 - W2}{S} X100$

Where:

W_2= Weight of empty crucible.

W_1= Weight of the crucible containing the ash (after ashing).

S = Original weight of the sample.

See table 3.9 (p-86).

2.5.4 Ether extract

2g of sample was weighed and placed in to soxhlet extraction, 150 ml of petroleum ether was added then open the soxhlet apparatus 6 hours, collected petroleum ether, the flask which contain the ether extract was dried and then cooled in adesicator and weighed

Ether extract% = $\frac{W1 - W2}{S} X100$

Where w_2 is the weight of the flask and extract

W_1 is the weight of the empty flask .

S is the weight of the sample

See table 3.9 (p-86).

2.5.5 Crude protein

Protein content was determined using the standard kjeldahl method A.O.AC (2000). 2 g of dry sample was placed in 100 ml kjeldahl digestion flask, 0.4 g of mixed catalyst (copper sulfate + sodium sulfate) and 3.5 ml of concentrated sulfuric acid were added. The flask was heated in an electrical heater for 2 hours. The sample was cooled and diluted with distilled water and then placed in the distillation apparatus. 20 ml of 40% sodium hydroxide (NaOH)were added and the distillation was applied for 10 minutes. The ammonia evolved was received in 10 ml of 2% boric acid contained in 100 ml conical flask attached to the receiver end. The trapped ammonia was titrated against 0.02 N hydrochloric acid (HCL) using universal indicator (Bromocresol green and methyl red in alcohol). The crude protein content percentage was calculated according to the following equation:

$$\text{Protein content (\%)} = \frac{\text{Volume of HCL X 0.02 X 14X 6.25 X 100}}{\text{Sample weight X 1000}}$$

See table 3.9 (p-86).

2.5.6 Determination of nitrogen free extract:

Nitrogen Free Extract (NFE) was calculated by difference after analysis of all the other items method in the proximate analysis.

NFE =(100-% moisture + % crude protein + % crude fat+ % crude fiber + % ash) .

See table 3.9 (p-86).

2.6 Phytochemical Screening

The extract of each powdered sample was subjected to general phytochemical tests for identifying the major phytoconstituents in each plants. Standard procedures were carried out as described by Sofowara. Alkaloids, flavonoids, polyphenols and tannins were estimated following standard methods (Sofowara, 1993).

2.6.1 Quantitative determination of the chemical constituents

2.6.1.1 Alkaloid determination

Preparation of solutions

The method described by (Shamsa,et al 2008). was used for determining the total alkaloids. Bromocresol green solution (1×10^{-4}) was prepared by heating 69.8 mg of bromocresol green with 3 ml of 2M NaOH and 5ml distilled water until completely dissolved and the solution was diluted to 1000 ml with distilled water. Phosphate buffer solution (pH 4.7) was prepared by adjusting the pH of 2M sodium phosphate (71.6 g Na_2HPO_4 in 1 L distilled water) to 4.7 with 0.2M citric acid (42.02 g citric acid in 1 L distilled water).

Preparation of standard curve

Atropine standard solution was prepared by dissolving 1 mg pure atropine (Sigma Chemical, USA) in 10 ml distilled water. Accurately measure aliquots (0.4, 0.6, 0.8, 1 and 1.2 ml) of atropine standard solution were transferred each to different separatory funnels. Then, 5 ml phosphate buffer (pH 4.7) and 5 ml of bromocresol green solution were added and mixed with 1, 2, 3 and 4 ml of chloroform. The extracts were collected in a 10-ml volumetric flask and then diluted to adjust volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm against blank prepared as above but without atropine.

Procedure

The plant materials (100g) were ground and then extracted with methanol for 24 hours. The extract was filtered and methanol was evaporated on a rotary evaporator under vacuum at a temperature of 45°C to dryness. Part of this residue was dissolved in 2 M HCl and then filtered. One ml of this solution was transferred to a separatory funnel and washed with 10 ml chloroform (3 times). The pH of this solution was adjusted by adding 2 ml of 0.1 M NaOH. Then 5 ml of bromocresol green reagent and 5 ml of

phosphate buffer were added to this solution. The mixture was shaken and the yellow complex formed was extracted with 1, 2, 3, and 4 ml chloroform by vigorous shaking. The extracts were collected in a 10-ml volumetric flask and diluted to volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm using a UV-visible spectrophotometer model 6505 Jenway (Germany). See table 3.15 (P-100)

2.6.1.2 Flavonoid determination

Total flavonoid contents were measured with the aluminum chloride colorimetric assay. Aqueous and ethanolic extracts and different dilution of standard solution of quercetin (100 – 1000 µg/ml) were added to 10 ml volumetric flask containing 4ml of water. To the above mixture, 0.3 ml of 1M $NaNO_2$ was added. After 5 minutes, 0.3 ml of 10% $AlCl_3$ was added. After 6 minutes, 2 ml of 1M NaOH was added and the total volume was made up to 10 ml with distilled water then the solution was mixed well and the absorbance was measured against a freshly prepared reagent blank at 510 nm. Total flavonoid content of the extract was expressed as percentage of quercetin equivalent per 100 g dry weight of sample (Patel, A., Patel, A. and Patel, N. (2010).See table 3.15 (P-100)

2.6.1.3 Polyphenol determination

The total phenolic content was determined using Folin – Ciocalteu reagents with analytical grade gallic acid as the standard. One ml of extract or standard solution (0 – 500 mg/L) was added to deionized water (10 ml) and Folin – Ciocalteu phenol reagents (1 ml). After 5 minutes, 20% sodium carbonate (2 ml) was added to the mixture. After being kept in total darkness for 1 hour, the absorbance was measured.

Amounts of total polyphenol (TP) were calculated using gallic acid calibration curve. The results were expressed as gallic acid equivalents (GAE) g/g of dry plant matter (Patel, and Patel, N. (2010)See table 3.15 (P-100)

2.6.1.4 Total tannins

The tannins content was determined by using $FeCl_3$ and gelatin test (Shivakumar, et al (2012) with some modification. About 1 ml of extract was transferred to vials, 1 ml of 1% $K_3Fe(CN)_6$ and 1 ml of 1% $FeCl_3$ were added, and the volume was made up to 10 ml with distilled water. After 5 min, absorbance was measured at 510 nm against a blank using Shumadzu model 1800 double beam spectrophotometer. A calibration curve was constructed, using tannic acid (100 – 800 mg/l) as standard, and total tannin content of the extracts was expressed as tannic acid equivalents.See table 3.15 (P-100)

2.7 GC/Ms of petroleum ether extract

The petroleum ether extract of all samples were subjected to chemical analysis using GC analysis. It was performed on a Thermo Finnegan Focus GC with a FID detector using a capillary column, CP Sil 8 CB low bleed MS (30 m × 0.25 mm; film thickness: 0.25 _m, Varian). Samples were injected in split mode under the following conditions: injector temperature, 250°C; oven temperature program:

Rate	Temperature (°C)	Hold time (min)
-	35.0	3.00
5.00	240.0	0.00
3.00	280.0	4.00

The carrier gas, helium at 1.2 ml/min.

2.7.1 Preparation of methyl ester

5 mg of the sample was dissolved in 1 ml of toluene in a test tube, 2 ml of 1% sulfuric acid in methanol was added and the mixture was left overnight. 5 ml of sodium chloride solution (5%) was added and the required esters were extracted with hexane using Pasteur pipettes to separate the layers. The

hexane layer was washed with 4 ml of water containing 2% potassium bicarbonate and dried over anhydrous sodium sulfate. The solution was filtered to remove the drying agent, and the solvent was removed by evaporating at room temperature.

2.8Biological Activity

2.8.1 Determination of antimicrobial activity

2.8.1.1Culture media

Nutrient broth

This medium contains peptone, yeast extract and sodium chloride. It was prepared according to Barrow and Feltham (Barrow, et al (1993) by dissolving 13 grams of the medium in one liter of distilled water. The pH of the medium was adjusted to 7.4 and the medium was then distributed into screw capped bottles, 5ml of each was taken and sterilized by autoclaving at 121˚C for 15 minutes.

Nutrient agar

The medium contained lab-lemco powder (1.0 g), yeast extract (2.0 g), peptone (5.0 g) and agar No.3 (15.0 g). 28 grams of dehydrated medium were dissolved in one liter of distilled water and the pH was adjusted to 7.4. The dissolved medium was sterilized by autoclaving at 121˚C for 15 minutes.

Mueller Hinton agar

Mueller Hinton dehydrated media (38 g) was dissolved in liter of purified water and heated with frequent agitation. Media was sterilized at 121˚C for 15 minutes and cooled to 45 - 50˚C and dispensed into sterile Petri dishes.

Saboraud dextrose agar

The medium contained mycological peptone (10.0 g), dextrose (D-glucose) (40.0 g) and agar (15.0 g). 65 grams of dehydrated medium were suspended

in a liter of distilled water, dissolved by heating and the pH was adjusted to 5.6. The medium was sterilized by autoclaving at 121˚C for 15 minutes.

2.8.1.2 Preparation of reference strains of bacteria

One ml aliquots of 24 hours' broth culture of tested organisms were aseptically added to nutrient agar slopes and incubated (Griffin and George Ltd, England) at 37˚ C for 24 hours. The bacterial growth was harvested and washed off by addition of sterile normal saline. The harvested bacteria were suspended in a suitable volume of normal saline to produce a suspension containing about 10^{8}- 10^{9} colony forming units per ml (cfu/ml). The suspension was stored in the refrigerator at 4˚C till used. The average number of viable organisms per ml of the stock suspension was determined by means of the surface viable counting technique (Miles& Misra 1938)

2.8.1.3 Preparation of reference strains of fungi

The fungal culture was grown on saboraud dextrose agar, incubated at 25˚C for 4 days. The suspended fungal growth was harvested in 100 ml of sterile normal saline and the suspension was stored in refrigerator till used.

2.8.1.4 Testing for antibacterial activity

The antibacterial activity test was performed using the disc diffusion assay (Shankar, et al (2010). Sterile filter paper discs (Whatman No. 1, 6 mm in diameter) were impregnated with one of each extract (20mg/ml) and left to dry to remove residual solvent, which might interfere with the determination. Twenty ml aliquots of the molten Mueller Hinton agar were distributed into sterile Petri-dishes.

About 0.1 ml of the standardized bacterial stock suspension 10^{8} –10^{9} C.F.U/ ml were streaked on Mueller Hinton agar medium plates using sterile cotton swab. The sterilized filter paper disc were soaked in the prepared extracts, and then were placed on surface of the test bacteria plates. Reference drugs

and 10% Dimethyl sulfoxide (DMSO) were used as the positive and negative controls, respectively.The plates were incubated at room temperature for 24 hours (Onkar, et al (1995). After the incubation period, the inhibition zone around the discs were measured.Mean and standard error values were tabulated, each test was carried out in triplicates.See tables 3.17 – 3.20 |(P -118 -121)

2.8.1.5 Testing for antifungal activity

The same method as for bacteria was adopted. Instead of nutrient agar, Saporaud dextrose agar was used. The molten medium was incubated with the specific organism and the medium was incubated at 25˚C for one day.See tables 3.17 – 3.20 |(P -118 -121)

2.9 Cytotoxicity screening

2.9.1 Principle

This colorimetric assay is based on the capacity of mitochondria succinate dehydrogenase enzymes in living cells to reduce the yellow water soluble substrate 3- (4,5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) into an insoluble, blue colored formazan product which is measured spectrophotometrically. Since reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells (Khalighi-Sigaroodi,et al 2012)

2.9.2 Preparation of extract solutions

Using a sensitive balance 5mg of each extract (direct methanolic extract) were weighted and put in eppendorf tubes. 50µl of DMSO were added to the extract and the volume was completed to 1ml with distilled water obtaining a concentration of 5mg/ml. The mixture was vortexed and stirred by magnetic stirrer to obtain a homogenous solution.

Cell line and culturing medium

Vero (Normal, African green monkey kidney) cells were cultured in a culturing flask containing a complete medium consisting of 10% fetal

bovine serum and 90% minimal essential medium (MEM) and then incubated at 37˚C. The cells were cultured twice a week.

Cell counting

Cell counts were done using the improved Neubauer champer. The cover slip and chamber were cleaned with detergent, rinsed thoroughly with distilled water and swapped with 70% ethanol, then dried. An aliquot of cell suspension was mixed with equal volume of trypan blue (0.4%) in a small tube. The chamber was charged with cell suspension. After cells, had settled, the chamber was placed under light microscope. Using 40 X objective, cells in the 4 large corner squares (each containing 16 small squares) were counted. The following formula was used for calculating cells:

(Cells/ml) N = Number of cells counted X Dilution Factor X 10^4/ 4

Procedure

The monolayer cell culture formed in the culturing flasks was trypsinized and the cells were put in centrifuging tube and centrifuged for 5 minutes separating the cells from the supernatant that flicked out. One ml complete medium was added to the cells and all the cell suspension was contained in a basin. In a 96- well microtitre plate, serial dilutions of each extracts were prepared. Three duplicated concentrations for each extract i.e. 6 wells for each of 8 extracts. All wells in rows A, B and C were used in addition to first 4 wells from each rows D, E and F. The first 2 wells of row G were used for the negative control and the first 2 wells of row H were used for the positive control Triton X. 20 µl complete medium pipetted in all wells in rows B, C and mentioned wells of rows E and F. Then 20 µl from each extract were pipette in rows A and B and first 4 wells of rows E and F. 20 µl

taken from row B were pipette and mixed well in row C from which 20 µl were taken and flicked out. The same was done from E to F. After that 80 µl complete medium were added to all used wells. Then adjusting the cell account to 3000 cell/well, 100 µl of cell suspension were added completing all wells to the volume 200 µl. Now, we have duplicated three concentrations 500, 250, 125 µg/ml for each extract. Then the plate was covered and incubated at 37°C for 96 hours.On the fourth day, the supernatant was removed from each well without detaching the cells. MTT suspension stock (5 mg/ml) prepared earlier in 100 ml phosphate buffer solution (PBS) was diluted (1: 3.5) in a culture medium. To each well of the 96- well plate, 50 µl of diluted MTT were added. The plate was incubated for further 4 hours at 37°C. MTT was removed carefully without detaching cells, and of DMSO (100 µl) was added to each well. The plate was agitated at room temperature for 10 minutes then read at 540 nm using microplate reader. The percentage growth inhibition was calculated using the formula below:

% cell inhibition = 100 - [(Ac – At)/Ac] x 100

Where At = Absorbance value of test compound

Ac = Absorbance value of control

Results obtained shown in table 3.16 (P-115)

Chapter three

Results and discussion

P.guajava, S.cymbopogon, S.argel, H.thebaica and P.crispum aremedicinal plants used in traditional medicine. They belong to different families, and are geographically distributed at different areas in Sudan. This study was carried out to evaluate chemical components and biological activities beside determination of heavy metals. The collected samples were subjected sequential extraction using petroleum ether, ethyl acetate, acetone and ethanol, the extracted components were used quantitatively and qualitatively to determined heavy metals and phytochemical constituents, also antimicrobial and cytotoxicity and antioxidant were done.

3.1Yield and physical characteristics of the components

The percentage yields of the extracted component from each sample using four different solvents (petroleum ether, ethyl acetate, acetone and ethanol) of varying polarities were calculated. The colour and texture of each extract was recorded. The obtained results are presented in Tables (3.1, 3.2, 3.3, 3.4 and 3.5). A clear variation was noticed among the different extracts in their yield percentage as well as the texture and colour. This disparity in the result could be due to the phytochemical constituents of the plants. The ethanol extract of studied plants gave the highest yield in form of gummy or waxy extracts, while petroleum ether extracts recorded the lowest yield. Both ethyl acetate and acetone extracts of all plants gave relatively moderate yield. P.guajava gave the highest yield with all solvents while H.thebaica was the lowest one.Harbone and Mabry (Harborne, (1992). reported that the less polar solvents (such as chloroform) are particular useful for the extraction of less polar flavonoid aglycones such as flavanone,

dihydroflavonles, flavones and flavonols, which are highly methylated while the more polar plant metabolites are generally isolated with alcohol and water. It seems most of secondary metabolites have solubility in methanol and chloroform.However, the extract yields of the plant materials are strongly dependent on the nature of extracting solvent and method. The variations in the extract yields from plants using different solvents might be explained by the polarity of extracted components and solvents applied (Hsu , (2006).

Table (3.1): percentageYields% and physical characteristics of the extracted components from P.guajava

Solvent	Wieght(g/100g)	Yield	Colour	Texture
P. ether	0.01261	1.261	Dark green	Sticky
E. acetate	0.01345	1.345	Dark green	Pawder
Acetone	0.01744	1.744	Dark green	Sticky
Ethanol	0.02651	2.651	Dark green	Waxy
Total yield		**7.001%**		

Table (3.2): percentageYields% and physical characteristics of the extracted components from S.cymbopogon

Solvent	Wieght(g/100g)	Yield (%)	Colour	Texture
P.ether	0.00563	0.563	Brown	Waxy
E. acetate	0.00865	0.865	Brown	Waxy
Acetone	0.01324	1.324	Light brown	Waxy
Ethanol	0.01881	1.881	Dark brown	Waxy
Total yield		**4.633%**		

Table (3.3): percentageYields% and physical characteristics of the extracted components from S.argel

Solvent	Wieght(g/100g)	Yield (%)	Colour	Texture
P. ether	0.00761	0.761	Dark green	Waxy
E. acetate	0.00945	0.945	Green	Waxy
Acetone	0.02344	2.344	Dark green	Sticky
Ethanol	0.0251	2.501	Brown	Waxy
Total yield		**6.551%**		

Table (3.4): percentageYields% and physical characteristics of the extracted components from H.thebaica

Solvent	Wieght(g/100g)	Yield (%)	Colour	Texture
P, ether	0.00254	0.254	Yellow	Waxy
E. acetate	0.00721	0.721	Light brown	Waxy
Acetone	0.00331	0.331	Light brown	Sticky
Ethanol	0.01232	1.232	Brown	Waxy
Total yield		**2.538%**		

Table (3.5): percentageYields% and physical characteristics of the extracted components from P.crispum

Solvent	Wieght(g/100g)	Yield (%)	Colour	Texture
P. ether	0.00501	0.501	Dark green	Waxy
E. acetate	0.00766	0.766	Dark green	Waxy
Acetone	0.00943	0.943	Dark green	Waxy
Ethanol	0.02550	2.550	Dark green	Waxy
Total yield		**4.760%**		

Tables above gives brief descriptions of the physical appearances and percentage yields of petroleum ether ,ethyl acetate ,acetone and ethanol extracts . The results clearly indicated variation in the percentage yields. The obtained totals yields of p.ether ,eth. Acetate, acetone and ethanol extracts were (3.34 ,4.64 ,6.69 and 10.82 %) respectively.The results showed that ethanol extract of P.guajava 2.651% contained agreater proportion by mass of the total components while petroleum ether extract of H.thebaica appeared to have produced the lowest yield (0.254%).

3.2 antioxidant activity

The antioxidant capacities of plant extracts largely depend on the composition of the extracts and conditions of the test system. The antioxidant capacities are influence by many factors, which cannot be fully described with one single method. Therefore, it is necessary to perform more than one type of antioxidant capacity measurement to take into account the various mechanisms of antioxidant action (Wang,*et al* 2006). In this experiment, different extracts of the studied plants were evaluated for antioxidant capacities using DPPH and ABTS assays.See table 3.6 & 3.7 P:74 ,77

3.2.1 Scavenging of DPPH radical

The antioxidant activity of different plant from*P.guajava, S.cymbopogon, S.argel, H.thebaica and P.crispum* were determined using a solution of DPPH reagent. DPPH is a molecule containing a stable free radical. The presence of antioxidant substances could be revealed by the decrease of the intensity of thetypical purple colour of the free DPPH radical (Abdualmjid and Sergi 2013). It is a direct and dependable method for determining the radical scavenging action. DPPH is very stable free radical. Unlike in vitro generated free radicals such as the hydroxyl radical and superoxide anion, DPPH has the advantage of being unaffected by certain side reactions, such as metal ion chelation and enzyme inhibition. A freshly prepared DPPH

solution exhibits a deep purple colour with an absorption maximum at 517 nm.This purple colour generally fades when antioxidant molecule quench DPPH free radicals (i.e. by providing atoms or by electron donation, conceivably via a free-radical attack on the DPPH molecule) and convert them into a colourless /bleached product (i.e. 2,2-diphenyl-1-hydrazine, or a substituted analogous hydrazine), resulting absorbance at 517 nm band (Gülçin, et al 2009). Results of antioxidant activity of samples extract were presented in Table (3.6).

Table (3.6): antioxidant activity of petroleum ether, acetone, ethyl acetate and ethanol extracts of*P.guajava, S.cymbopogone, S.argel, H. thebaica* and *P.crispumusing*2, 2-diphenyl-1-pycrilhydrazil hydrate reagent

No.A	**Sample**	**%RSA ±SD (DPPH)**	IC_{50}**±SD mg/ml (DPPH)**
1	P.ether extract of *P.guajava*	29 ± 0.0.05	-
2	P.ether extract of *S.cymbopogone*	30 ± 0.04	-
3	P.ether extract of *S.argel*	Inactive	-
4	P.ether extract *H. thebaica*	Inactive	-
5	P.ether extract of *P.crispum*	16 ± 0.07	
6	E.acetate extract *P.guajava*	89 ± 0.01	0.038±0.03
7	E.acetate extract S.*cymbopogone*	43± 0.07	-
8	E.acetate extract *S.argel*	35± 0.08	-
9	E.acetate extract *H.thebaica*	23 ± 0.09	-
10	E.acetate extract. *P.crispum*	27± 0.08	-

11	Acetone extract of *P.guajava*	89.2 ± 0.02	0.029±0.01
12	acetone extract *S.cymbopogo*	21 ± 0.15	
13	acetone extract *S.argel*	32 ± 0.05	-
14	acetone extract of *H. thebaica*	47 ± 0.3	-
15	acetone extract *P.crispum*	30± 0.6	-
16	ethanol extract *P.guajava*	90 ± 0.02	0.477±0.02
17	ethanol extract *S.cymbopogo*	45 ± 0.13	-
18	ethanol extract *S.argel*	18 ± 0.07	-
19	ethanol extract *H.thebaica*	22 ± 0.06	-
20	ethanol extract *P.crispum*	07 ± 0.07	-
Standard	Propyl Gallate	93± 0.01	0.077µg/ml± 0.01

The obtained results were compared with Propyl gallate as a good antioxidant agent. For successive extraction, petroleum ether extract revealed a non-significant free radical scavenging activity with scavenging activity ranging between 0.16% for *P.crispum* , 29% for *P.guajava and* 30% for *S.cymbopogon* extracts while *S.argel* and *H.thebaica* extracts showed no antioxidants effect. Concerning the antioxidant activity of ethyl acetate only *P. guajava* sample that exhibited high activity with scavenging rate 89%, the otherness samples their activity scavenging rates 23%, 27%, 35% and 43% for *H.thebaica, P.crispum, S.argel* and *S.cymbopogon* respectively. All acetone extracts showed no antioxidant effect except *P.guajava* that exhibited high activity with scavenging rate 89.2%, but their activities were relatively high than those of petroleum ether. All ethanol extract showed lowest effective free radical scavenging in the DPPH assay, while

P.guajava extract exhibited a remarkable antioxidant effect which showed scavenging rate of 90%.

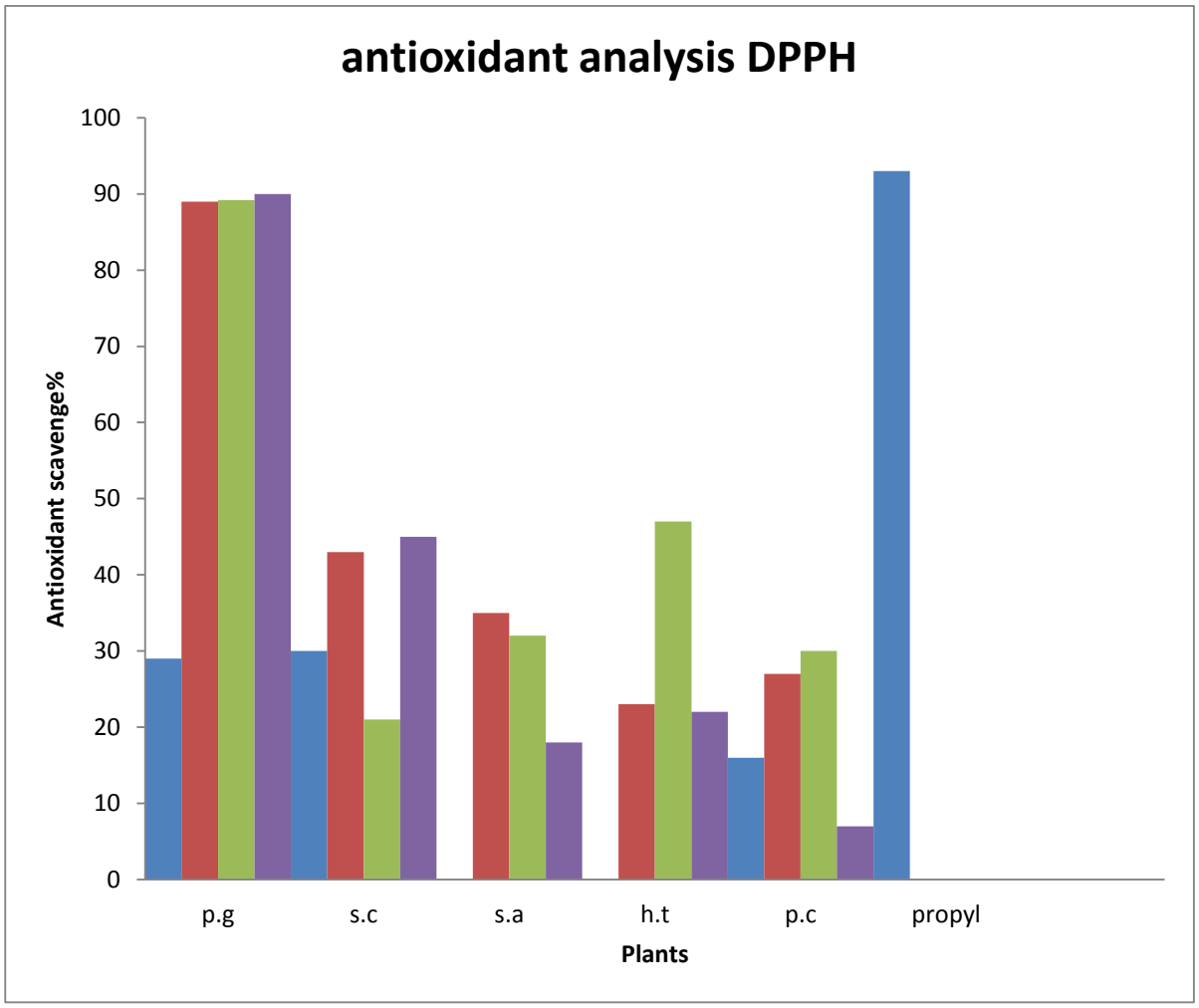


Figure 3.1 antioxidant activity of petroleum ether, ethyl acetate, acetone and ethanol extracts of *P.guajava*, *S.cymbopogone*, *S.argel*, *H. thebaica* and *P.crispumusing*2, 2-diphenyl-1-pycrilhydrazil hydrate reagent

3.2.2Scavenging of ABTS radical

The ABTS method is widely employed for measuring the relative radical scavenging activity of hydrogen donating and chain breaking antioxidants in many plant extracts. It is recommended to be used for plant extracts because the long wavelength absorption maximum at 734 nm eliminates colour interference in plant extracts (Awika*et al*., 2003). The capability of the plant samples extracts to scavenge ABTS radicals are shown in Table (3.7).

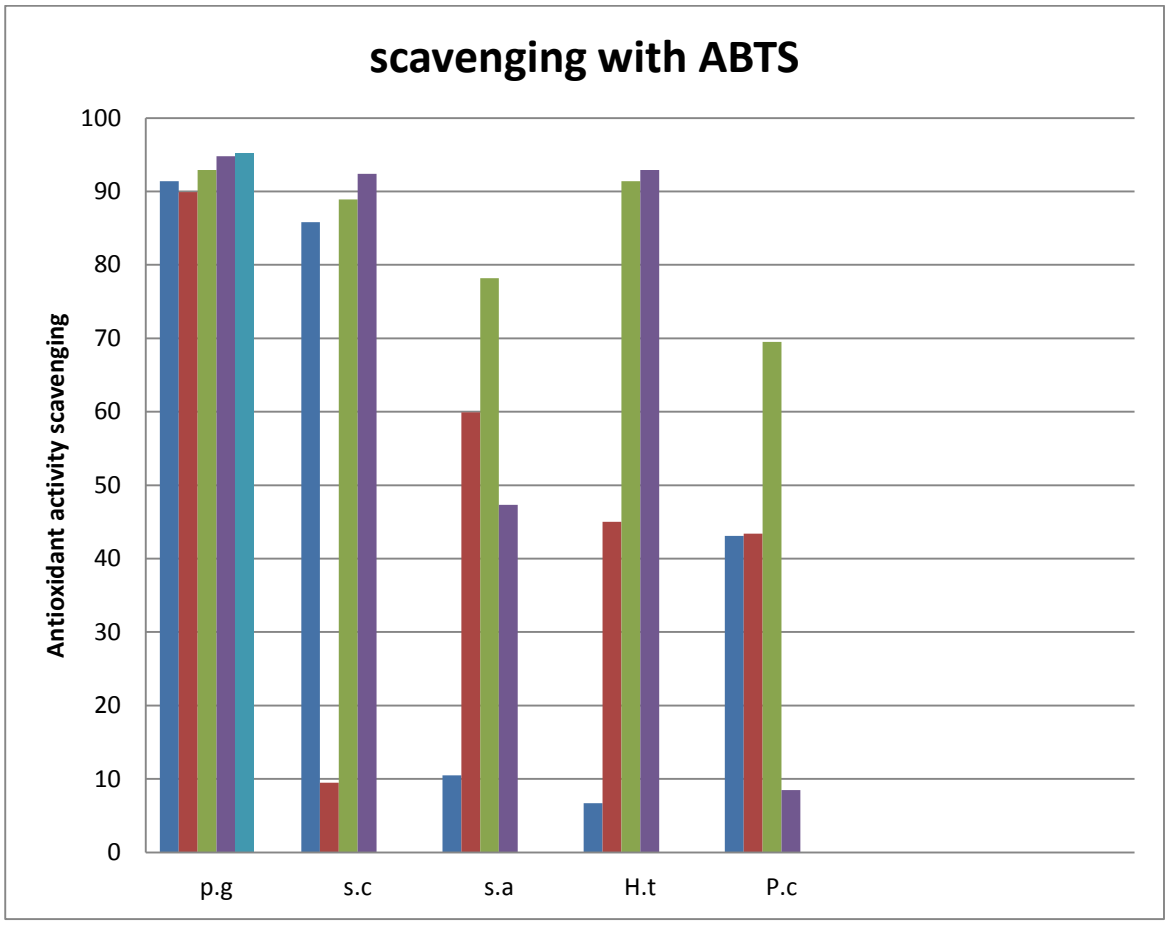
Table (3.7): antioxidant activity of petroleum ether,ethyl acetate,acetone, and ethanol extracts of*P.guajava, S.cymbopogone, S.argel, H. thebaica* and *P.crispumusing*2, 2´-azinobis (3-ethylbenzothiazoline- 6-sulfonic acid reagent

No.	**Sample**	**%RSA ±SD (ABTS)**	IC_{50}**±SD mg/ml (ABTS)**
1	P.etherextract of *P.guajava*	91.4±0.02	0.02±0.02
2	P.ether extract of *S.cymbopogon*	85.8±0.04	0.09±0.03
3	P.ether extract of S.*argel*	-10.5±0.03	-
4	P.ether extract of . H.*thebaica*	6.7±0.01	-
5	P.ether extract of *P.crispum*	43.1±0.1	-
6	E.acetate extract of *P.guajava*	89.9±0.02	0.15±0.01
7	E.acetate extract S.*cymbopogone*	90.5±0.003	0.09±0.002
8	E.acetate extract of *S.argel*	59.9±0.08	0.11±0.004
9	E.acetate extract of *H.thebaica*	45.5±0.02	
10	E.acetate extract. of P. *P.crispu*	34.4±0.05	

11	Acetone extract of *P.guajav*	92.9±0.02	0.04±0.01
12	acetone extr ofS.*cymbopogone*	88.9±0.02	0.09±0.02
13	acetone extract of *S. S.argel*	78.3±0.3	0.08±0.01
14	acetone extract of *H. thebaic*	91.4±0.01	0.12±0.003
15	acetone extract of *P.crispum*	69.5±0.1	0.240±0.02
16	ethanol extract of *P.guajava*	94.8±0.01	0. 4±0.008
17	ethanol extract *S.cymbopogone*	92.4±0.01	0.09±0.02
18	ethanol extract of *S.argel*	47.3±0.08	
19	ethanol extract of *H.thebaic*	92.9±0.003	0.06±0.001
20	ethanol extract of *P.crispum*	88.5±0.02	0.17±0.004
Standard	Ascorbic acid	95.2±0.01	0.0616 µg/ml ±0.01

The ability of the tested samples to scavenge ABTS radicals were compared to ascorbic acid (vitamine c) standard. Most crude extracts exhibited good antioxidant activities (69% – 94%) compared to ascorbic acid (95.2%). The highest scavenging ability was exhibited by ethanol extract from *P.guajava* (94%), while the lowest was determined in the acetone extract of *P.crispum* (69%). At petrleum ether extract two samples (*P.guajava , S. cymbopogon*) showed high antioxidant effect rates 91.4% ,85% respectively while S.argel inactive and *P.crispum*, *H.thebaica* no antioxidant effect.The antioxidant activity of ethyl acetate three samples (*P.guajava,S. cymbopogon and S.argel*) that exhibited highest activity with scavenging rates 89.9%,90.5% and 59.9% respectively except *H.thebaica and P.crispum* no antioxidant

effect.All acetone extract showed a high effective free radical scavenging in the ABTS assay, it exhibited a remarkable antioxidant effect specially *P.guajava* extract which showed scavenging rate of 92.9%.Then Concerning the antioxidant activity of ethanol extract all samples showed ahigh effective free radical scavenging except *S.argel* which showed scavenging rate of 47%. However, the extensive investigations on antiradical and antioxidant activities of small phenolics including flavonoids and phenolic acids have been reported (Heim et al., 2002). Extract from the plant samples howed to possess potential antioxidant activity which related to the phenolic constituents (Sayed et al., 2011). Previous studies (Huang, et al 1998) indicated that the active ingredients in Poaceae family plants are mixtures of homologous long chain phenolic compounds. They are natural products isolated mainly from the Poaceae family; possess a wide range of biological properties and antioxidant activity (Evans& Schmidt 1980).ethanol extract of*P.guajava* showed a potential antioxidant activity. This activity might be due to the presence of phenolic groups, terpenoids and alkaloids (Jones, et al 1995) .It has been proven that numerous natural compounds show different antioxidant activity that depends on their origin, chemical structure, bioavailability, or polarity of solvents ,*etc*, and that some of these compounds work together in protecting against various degenerative disorders including cancer, stroke, cardiovascular, Alzheimer's and Parkinson's diseases (Halliwel., 2000).Flavonoids and phenolic compounds are the main antioxidative compounds of fruits and vegetables (Amarowicz & Karamac2003). In this study, the phytochemical screening of plant studies showed the presence of large amounts of phenolic compounds and flavonoids.The high antioxidant activity of in most of the studied plant extracts could be attributed to these compounds.



Figure(3.2): antioxidant activity of petroleum ether,ethyl acetate,acetone, and ethanol extracts ofP.guajava, *S.cymbopogone, S.argel, H. thebaica* and P.crispumusing 2, 2´-azinobis (3-ethylbenzothiazoline- 6-sulfonic acidreagent

3.3 Heavy metals

Concentration of essential and non-essential heavy metals in medicinal plants beyond permissible limit is a matter of great concern to public safety all over the world .The contributions of medicinal plants in the traditional system of medicine for curing diseases has been documented. Nowadays increased scientific interest and consumer demand have promoted the development of herbal products as dietary supplements. In view of renewed interest, oriental herbal medicines have a prominent role to play in the pharmaceutical and health markets of the 21stcentury (Kleinschmidt & Johnson, 1977). It has been reported that whatever is taken as food could cause metabolic disturbance subject to the allowed upper and lower limits of trace metals (Prasad, 1976). Both the deficiency and excess of essential micronutrients and trace of toxic metals may cause serious effects on human health (Underwood, 1997 & Reilly, 1980). WHO recommends that medicinal plants which form the raw materials for the finished products may be checked for the presence of heavy metals, further it regulates maximum permissible limits of toxic metals like arsenic, cadmium and lead, which amount to 1.0, 0.3 and 10 ppm, respectively (WHO, 1989, 1998). Medicinal herbs are easily contaminated during growth, development and processing. After collection and transformation into dosage form the heavy metals confined in plants finally enter the human body and may disturb the normal functions of central nervous system, liver, lungs, heart, kidney and brain, leading to hypertension, abdominal pain, skin eruptions, intestinal ulcerand different types of cancers.The concentrationof Zn,Mn , Fe, Ni,Pb, Cd and Co in selected medicinal plants are appended (Table-3.8).

Table(3.8):concentration Heavy metals (ppm) of *P.guajava, S. cymbopogon, S.argel , H.thebaica, P.crispum*

Samples / element	**P.g**	**S.C**	**S.a**	**H.t**	**P.c**
Zn	0.076	0.059	0.166	0.037	0.115
Mn	0.200	0.088	0.187	0.026	0.024
Fe	122.0	103.15	198.3	122.4	131.4
Ni	79.00	37.00	38.20	63.40	60.99
Pb	<LTD	<LTD	<LTD	<LTD	<LTD
Cd	0.003	0.004	0.001	0.001	0.002
Co	0.036	0.094	0.037	0.012	0.031

LTD mean less than detection limit, LTD for Pb=0.8

3.3.1 Manganese

As evident from Table (3.8), maximum concentration of Mn was found in *P.guajava* 0.2 ppm, followed by *S.argel* 0.187 ppm, *S. cymbopogon* 0.088 ppm, *H.thebaica* 0.026 ppm and *P.crispum* 0.024 ppm. Manganese concentration is low in all plants, however it is within normal background level for the element in plants under the critical concentration of 300-500 ppm DW. Mn deficiency in plants causes chlorosis. The estimated safe and adequate daily dietary intake in adults is 11 mg/day (Pendias,1992). Deficiency of Mn in human causes myocardial infarction and other cardiovascular diseases, also disorder of bony cartilaginous growth in infants & children and may lead to immuno-deficiency disorder and rheumatic arthritis in adults (Smith, 1990).

3.3.2 Zinc

As evident from Table-3.8, high concentration of Zn was found in *S. argel* 0.166 ppm followed by *P.crispum0*.115 ppm, *P.guajava* 0.076 ppm,

S.cymbopogon 0.059 ppm and *H.thebica* 0.037 ppm. Zinc is an essential trace element for plant growth and also plays an important role in various cell processes including normal growth, brain development, behavioural response, bone formation and wound healing. Zinc deficient diabetics fail to improve their power of perception and also causes loss of sense of touch and smell (Hunt, 1994). The dietary limit of Zn is 100 ppm (Jones, 1987).

3.3.3 Iron

Iron is an essential element for human beings and animals and is an essential component of hemoglobin. It facilitates the oxidation of carbohydrates, protein and fat to control body weight, which is very

important factor in diabetes. Results in table 3.8 reveal that maximum concentration of Fe was found in *S.argel* 198.3 ppm and 131.4, 122.4, 122, 103.15 for *P.crispum, H,thebica, P.guajava, S.cymbopogon* respectively .The results suggest that high amount of Fe in plants may also be due to the foliar absorption from the surroundings air. The dietary limit of Fe in the food is 10-60 mg/day (Kaplan et al., 1993). Low Fe content causes gastrointestinal infection, nose bleeding and myocardial infarction. (Hunt, 1994).

3.3.4 Nickel

In case of Ni the concentration in different plants was in the order of *P.guajava* 79.00 ppm, *H. thebica* 63.40 ppm, *P.crispum* 60.99 ppm, *S.argel* 38.20 ppm, *S.cymbopogon* 37.80 ppm, . The higher concentration of Ni in plants may be due to anthropogenic activities. The most common ailment arising from Ni is an allergic dermatitis known as nickel itch, which usually occurs when skin is moist, further more Ni has been identified as a suspected carcinogen and adversely affects lungs and nasal cavities. Although Ni is required in minute quantity for body as it is mostly present in the pancreas and hence plays an important role in the production of insulin. Its deficiency results in the disorder of liver (Pendias , 1992). EPA has recommended daily intake of Ni should be less than 1 mg beyond which is toxic (McGrath &.Smith, 1990).

3.3.5 Cobalt:

It is evident from the data (Table 3.8) that cobalt concentration was found in the range of 0.012- 0.094 mg/kg. That high concentration of cobalt 0.094 mg/kg was found in *S.cymbopogon* followed by *S.argel* 0.037 mg/kg, *P.guajava* 0.036mg/kg and *P.crispum* 0.031 mg/kg while lowest concentration 0.012 mg/kg was found in *H.thebica*. Maximum permissible limit (MPL) for Cobalt (Co) recommended by World Health

Organization (WHO) is 0.2- 0.3 mg/kg (WHO, 1998) while the daily dietary intake (DDI) is 0.04 mg. Although cobalt is toxic at elevated concentration, however the body needs only in trace amount. High intake of cobalt causes vomiting, nausea,vision and heart problems and also damage of thyroid (Smith ,1990).

3.3.6 Lead and cadmium

Lead and Cadmium are non-essential trace elements having functions neither in humans body nor in plants. They induce various toxic effects in humans at low doses. WHO (1998) prescribed limit for Pb contents in herbal medicine is 10 ppm while the dietary intake limit for Pb is 3 mg/week. The typical symptoms of lead poisoning are colic, anemia, headache, convulsions and chronic nephritis of the kidneys, brain damage and central nervous system disorders. Cadmium accumulates in human body and damages mainly the kidneys and liver. The lowest level of Cd which can cause yield reduction is 5- 30 ppm, while the maximum acceptable concentration for food stuff is around 1 ppm (Neil, 1993). High concentration of Cd was found in *S.cymbopogon* 0.004 ppm followed by *P.guajava* 0.003 ppm, *P.crispum* 0.002 ppm *S. argel* and *H.thebica* were detected the sam concentration 0.001ppm. Surprisingly no Pb in plant samples (Below detection limit).

3.4 Proximate analysis

Table 3.9. The proximateconstituentsof the plant samples of P.guajava , S.cymbopogon, S.argel, H.thebaica and P.crispum

Sample	DM	CP	CF	EE	Ash	NFE
P.guajava	9.42^{bc}	10.15^{a}	13.11^{c}	1.21^{a}	4.92^{d}	64.87^{c}
S.cymbopogon	9.49^{a}	3.17^{d}	15.29^{b}	0.80^{c}	5.15^{c}	70.49^{b}
S.argel	9.43^{bc}	5.24^{c}	9.18^{d}	1.07^{b}	6.92^{b}	71.98^{a}
H.thebaica	9.46^{ab}	1.35^{e}	26.49^{a}	0.33^{d}	7.10^{a}	59.38^{d}
P.crispum	9.41^{c}	7.57^{b}	8.65^{e}	0.23^{e}	7.10^{a}	70.66^{b}
±SEM	0.16	0.03	0.05	0.01	0.02	0.18

DM:moisture, CP: crued protein , CF: crued fiber , ash , EE ether extract and NFE nitrogen free extract

3.4.1 Moisture content

As seen from Table 3.9 The rang of moisture between (9.41 -9.49) % .The highest level of moisture (9.49%) detected in the S.cymbopogonand H.thebaicashowed nerly equal amount content (9.46%) the S.argel and P.guajava had equal amount of moisture (9.43%). While the lowest amount of moisture was detected in the P.crispumcontained (9.41%)It was clear that the amount of water in all samples plant was equal approximately.

3.4.2 Crude protein

Table 3.9 shows the percentage crude protein in each plants. It was observed that som of the studied plants contained considerable amount of protein (7.57 ,10%) for P.crispumand P.guajava respectively. H.thebaica, S.cymbopogonand S.argel were significantly different

amount in their crude protein content. The highest level of crude protein (10%) was detected in the P.guajava while the lowest amount of crude protein (1.35%) was detected in theH.thabica. S.argel and P.crispum showed moderate amount of crude protein content. The obtained results showed that the crude protein content of the studied plant was sufficiently high to warrant consideration of their use as protein supplement to low quality diets. The study observed that the sampl plant which contain high amount of fiber contain low amount of protein.

3.4.3 Fibre content

Table 3.9 shows the fibre contents of the studied plants. H.thebaicashowed highest amount of fibre contents (26.49%) while P.crispumhad lowest amount of fibre content (8.7%), and the other samplles showed moderate amount of fiber.The fiber contents increase with advantage foliage maturity as a result of lignification. Fiber and particularly lignin content are key factors influencing the digestibility of dry mater and consequently the availability of energy in forage (Alam*et al*., 2007). However, any assessment of the extent to which fiber content impedes energy availability in tanniniferous forages is complicated by the ability of tannins to interfere with current fibre assays

3.4.4 Ash content

The amount of ash in each plants is illustrated in Table 3.9H.thebaicaand P.crispumcontained equal amount of ash (7.10%). These values were higher than others 6.9% value reported for S.argel and 5.15% value reported forS.cymbopogonwhile the *P.guajava* had low amount of ash (4.92%). The amount of ash in samples plants indicated that the plants were rich in mineral contents

3.4.5 Ether extract content (E.E)

The amount of (E.E) ranged from (0.23 -1.21%) showed in Table (3.9). *P.guajava*showed highest amount of (E.E) contents (1.21%) while P.crispumhad lowest amount of(E.E) content (0.23%)and the other samplles showed various amount of (E.E)0.33 ,0.8 and 1.7 for H.thebaica, S.cymbopogon and S.argel respectively.The higher value of E.E in som of the tested samples is an indecation of higher energy level.(Odedire & Babayemi 2008)

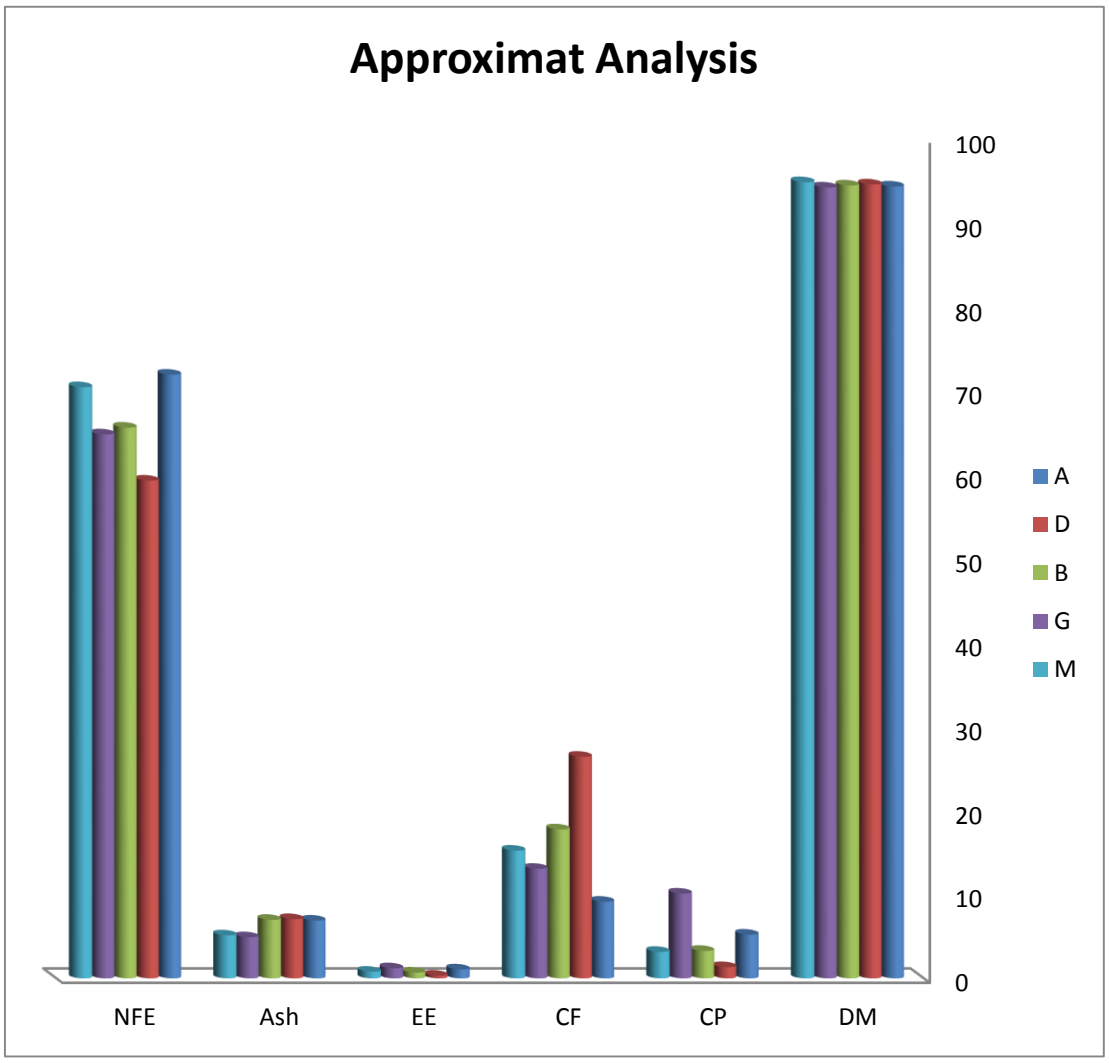
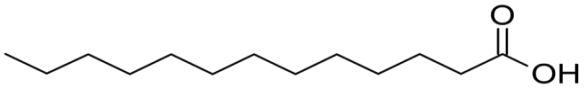
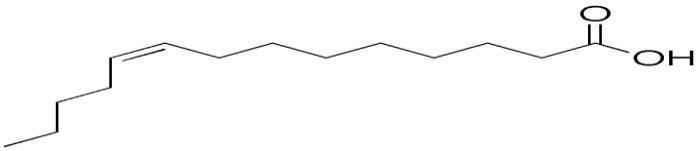
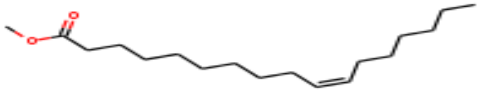
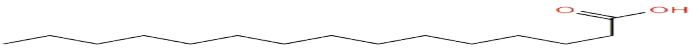
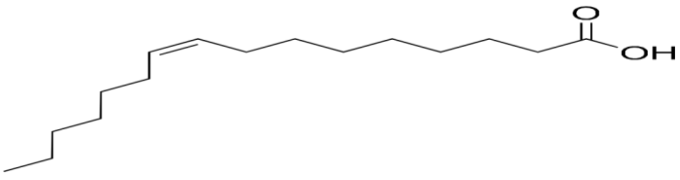


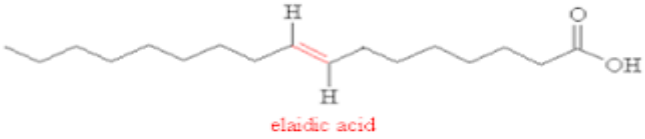
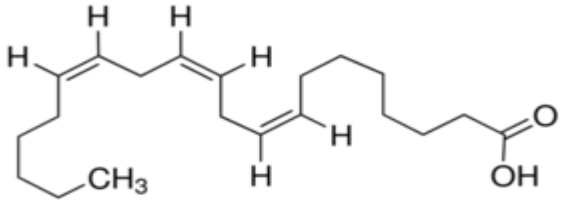
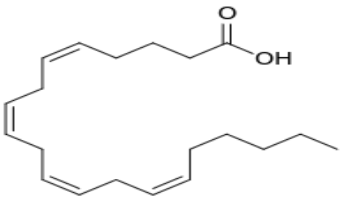
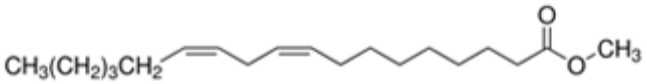
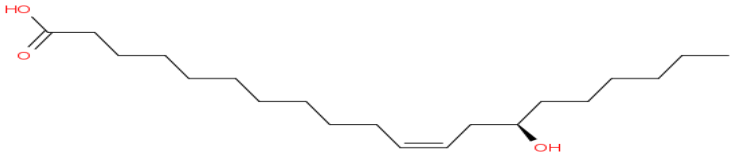
Figure 3.3Approximat analysis from moisture, crude protein , crude fiber , ash , ether extract and nitrogen free extract of (G)P.guajava,(M) S.cymbopogon, (A) S.argel, (D) H.thebaica and (B) P.crispum

3.5Fatty acids composition

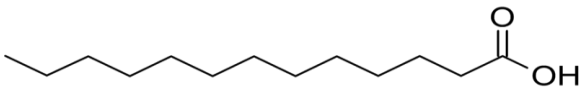
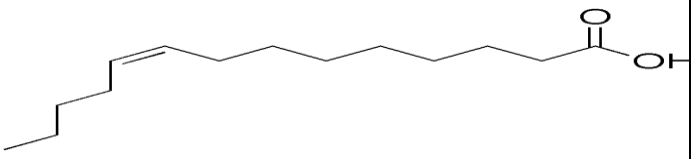
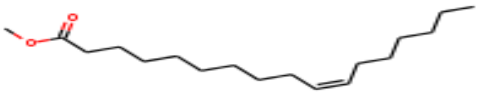
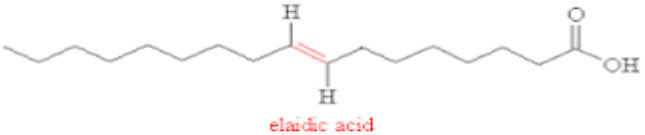
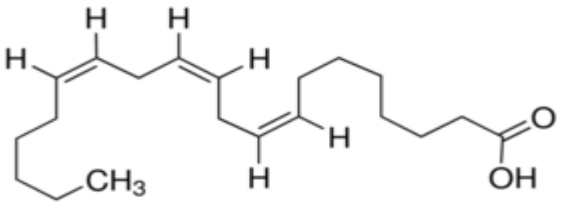
The fatty acid composition of analyzed plant samples is shown in Tables (3.10, 3.11, 3.12, 3.13, 3.14) The analysis revealed the presence of both saturated and unsaturated fatty acids in all sample.

Table (3.10): Fatty acids composition of *Psidium guajava* leaves extract

Fatty acid	%	RT (min)	Chemical structure
Tri decanoic acid methyl ester (Tridecylic acid)	0.78	27.37	
Myristoleic acid methyl ester (9-tetradecenoic acid)	0.29	29.71	
Cis-10-Pentadecenoic acid methyl ester	0.56	31.41	
Pentadecenoic acid methyl ester (pentadecanoic acid)	7.74	32.12	
Palmitoleic acid methyl ester (9-*cis*-Hexadecenoic acid)	2.33	33.01	

Cis-10-heptadecenoicM.E	44.84	35.49	
Elaidic acid methyl ester ((E)-9-Octadecenoic acid)	5.36	38.51	
cis- 8 11 14-eicosatrienoic acid methyl ester (homo-γ-linolenic acid)	3.95	40.85	
Arachidonic acid methyl ester ((5Z,8Z,11Z,14Z)-5,8,11,14-Eicosatetraenoic acid)	0.62	42.42	
Lamda-Linolenic acid M.E	3.22	37.26	
Cis- 11-Eicosenoic acid methyl ester (Arachidic acid)	11.04	41.48	

Table(3.11): Fatty acids composition of Schoenanthus cymbopogone extract

Fatty acid	%	RT (min)	Chemical structure
Tri decanoic acid methyl ester (Tridecylic acid)	1.24	27.36	
Myristoleic acid methyl ester (9-tetradecenoic acid)	0.1678	29.23	
pentadecenoic acid methyl ester (pentadecanoic acid)	4.39	32.14	
Cis-10-heptadecenoic acid M.E	41.989	35.490	
Cis-10-Pentadecenoic acid methyl ester	1.22	31.41	
Elaidic acid methyl ester ((E)-9-Octadecenoic acid)	1.25	38.48	
cis- 8 11 14-eicosatrienoic acid methyl ester (homo-γ-	4.04	40.87	

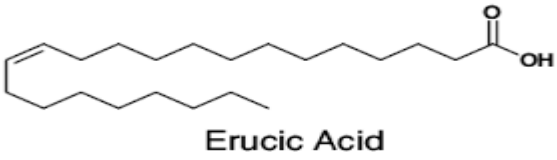
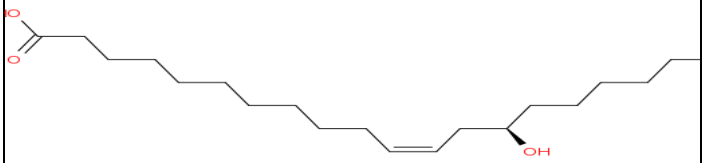
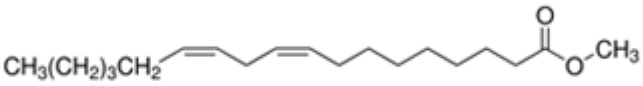
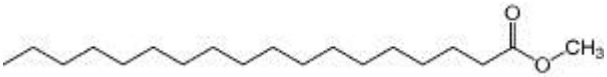
linolenic acid)			
Erucic acid methyl ester (Cis-13-Docosenoic acid)	2.96	44.70	 Erucic Acid
Cis- 11-Eicosenoic acid methyl ester (Arachidic acid)	2.16	41.783	
Lamda-Linolenic acid M.E	2.898	37.28	
Stearic acid M.E	0.441	39.24	

Table (3.12): Fatty acids composition of Solenotemma argle leaves extract

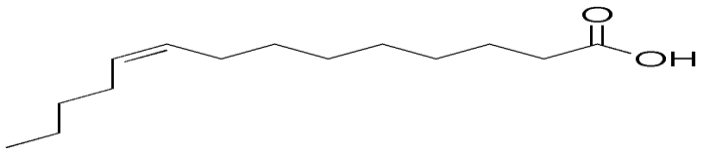
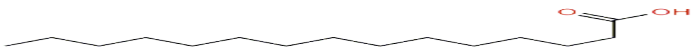
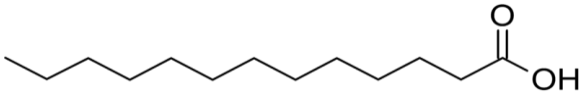
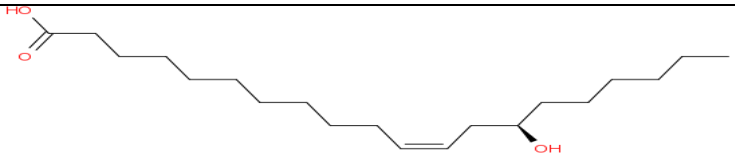
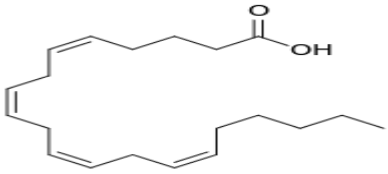
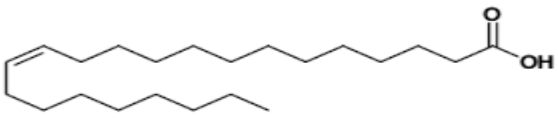
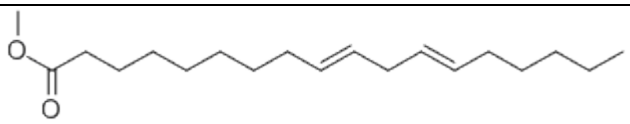
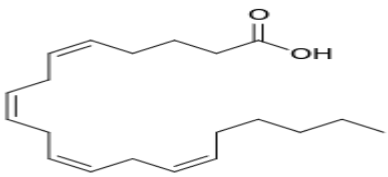
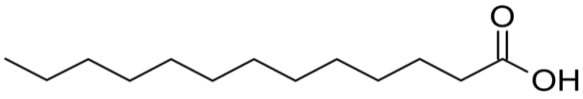
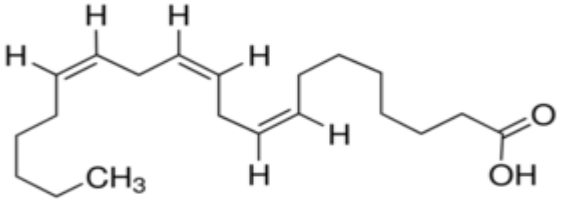
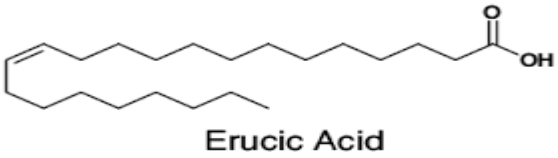
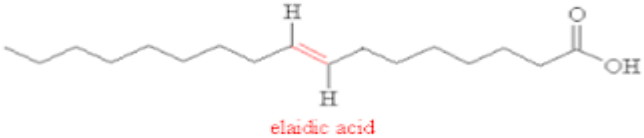
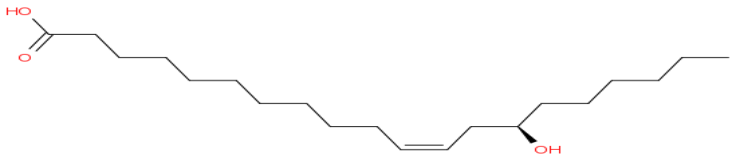
Fatty acid	%	RT (min)	Chemical structure
Myristoleic acid methyl ester (9-tetradecenoic acid)	0.38	29.23	
Pentadecenoic acid methyl ester (pentadecanoic acid)	4.72	32.09	
Tridecanoic methyl ester (Tridecylic acid)	0.86	27.40	
Cis- 11- Eicosenoic acid methyl ester (Arachidic acid)	7.85	41.43	
Arachidonic acid methyl ester (5,8,11,14-all-*cis*-Eicosatetraenoic acid)	1.06	42.39	
Erucic acid methyl ester (Cis-13-Docosenoic acid)	0.72	44.67	 Erucic Acid
Linolelaidic acid M.E	6.85	37.75	
Cis-10-heptadecenoic acid M.E	33.164	35.314	
Cis-5,8,11,14,17-Eicosenoic acid M.E	3.86	40.8	

Table (3.13): Fatty acids composition of Hyphaene thebaica fruits extract

Fatty acid	%	RT (min	Chemical structure
Pentadecenoic acid methyl ester (pentadecanoic acid)	15.84	32.07	
Tridecanoic methyl ester (Tridecylic acid)	1.84	27.89	
cis- 8 11 14-eicosatrienoic acid methyl ester (homo-γ-linolenic acid)	1.25	40.91	
Erucic acid methyl ester (Cis-13-Docosenoic acid)	0.35	45.19	 Erucic Acid
Elaidic acid methyl ester ((E)-9-Octadecenoic acid)	3,77	38.59	 elaidic acid

Cis- 11-Eicosenoic acid methyl ester	3.25	41.53	

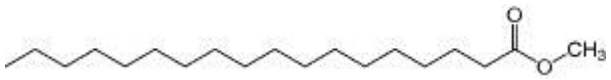
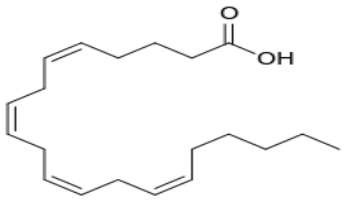
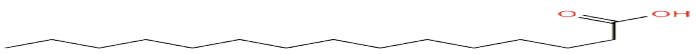
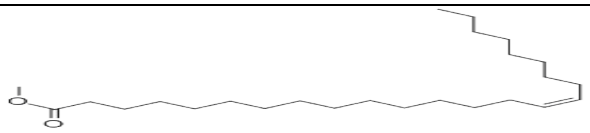
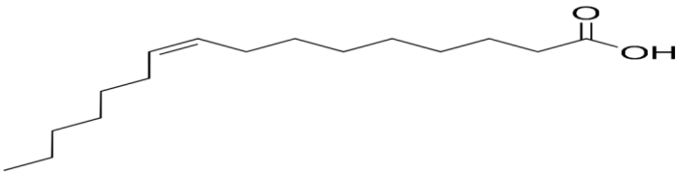
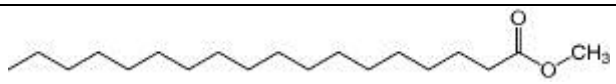
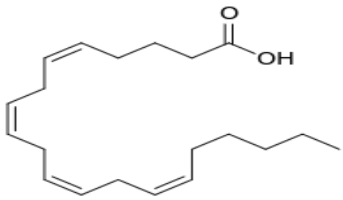
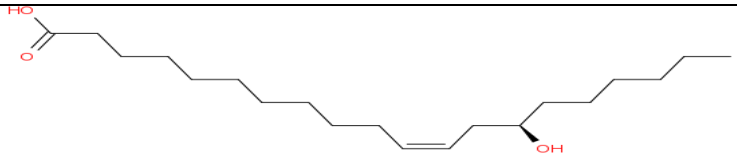
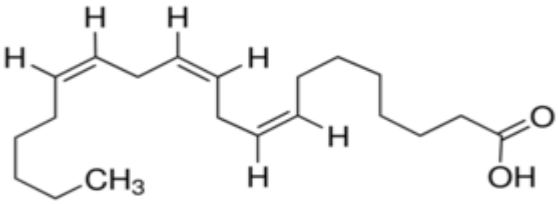
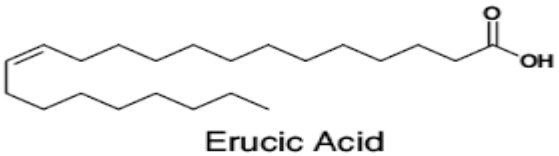
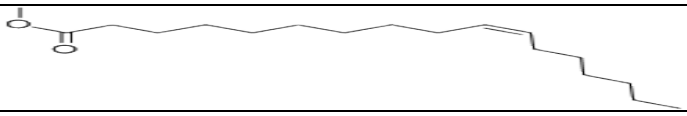
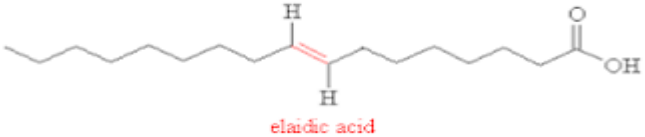
Stearic acid M.E	0.443	39.31	
Cis-10-heptadecenoic acid M.E	2.94	36.54	
Arachidonic acid methyl ester	1.07	42.43	

Table (3.14): Fatty acids composition of Petroselinum crispum leaves extract

Fatty acid	%	RT (min)	Chemical structure
Pentadecenoic acid methyl ester (pentadecanoic acid)	6.97	32.07	
Nervonic acid methyl ester (selacholeic acid)	1.44	52.04	
Palmitoleic acid methyl ester (9-*cis*-Hexadecenoic acid)	0.33	33.37	
Stearic acid M.E	0.654	39.30	
Arachidonic acid methyl ester ((5*Z*,8*Z*,11*Z*,14*Z*)-5,8,11,14-Eicosatetraenoic acid)	1.66	42.37	
Cis- 11- Eicosenoic acid methyl ester (Arachidic acid)	7.88	41.46	
cis- 8 11 14- eicosatrienoic acid methyl ester (homo-γ-linolenic acid)	1.41	40.86	
Erucic acid methyl ester (Cis-13-Docosenoic acid)	2.56	44.73	 Erucic Acid
Cis-10-heptadecenoic acid M.E	44.65	35.30	
Elaidic acid methyl ester ((E)-9-Octadecenoic acid)	0.86	38.54	 elaidic acid

As shown in Table (3.10) the *Psidium guajava* leaves extract contained eleven different fatty acids.Cis-10-Heptadecenoic acid methyl ester was found in high value (44.84%), followed by Cis-11-Eicosenoicacid methyl ester(11.04%),pentadecenoic acid methyl ester(7.73%),Elaidic acid methyl ester ((E)-9-Octadecenoic acid) (5.36%), palmitoleic acid methyl ester (Hexadecanoic acid) (2.33%) andthe other detected fatty acids were in the range of 0.29% for Myristoleic acid methyl ester to (0.77%) forTri decanoic acid methyl ester.In *S.cymbopogone* extract, also Cis-10-Heptadecenoic acid methyl ester obtain high amount(41.99%),followed by Cis-11-Eicosenoicacid methyl ester(11.82%),pentadecenoic acid methyl ester(4.39%), Erucic acid methyl ester (Cis-13-Docosenoic acid) (2.95%) and lamda-linolenic acid methyl ester(2.89%) just found inS.cymbopogone extract only. Elaidic acid methyl ester ((E)-9-Octadecenoic acid),Tri decanoic acid methyl esterand Cis-10-pentadecenoic acid methyl ester showed (1.25%),(1.24%) ,(1.22) respectively,the other detected fatty acids were in range of (0.17%) for Myristoleic acid methyl ester to (0.44%) for stearic acid methyl ester.*In S.argel*extract Table (3.12)contained nine different fatty acids,Cis-10-Heptadecenoic acid methyl ester showed high value (63.16%) , the other fatty acids Cis-11-Eicosenoicacid methyl ester(7.86%),,linoleladic acid methyl ester(6.85%) this is only one fatty acid were recorded,not present in other samples,while the lowest values represented in Myristoleic acid methyl ester(0.378%), Erucic acid methyl ester(0.718%), Tri decanoic acid methyl ester (0.863%) andArachidonic acid (1.06%).Table (3.13) of *Hyphaene thebaica* extract revealed the presence of nine fatty acids,Cis-10-Heptadecenoic acid methyl ester(69.24%), pentadecenoic acid methyl ester(15.84%),Elaidic acid methyl ester (3.77%),Cis-11-Eicosenoicacid methyl ester (3.25%) , Tri decanoic acid methyl ester(1.84%), Cis-5,8,11,14,17-Eicosapentaenoic acid (1.25%),Arachidonic acid(1.07%), stearic acid methyl ester

(0.44%)andErucic acid methyl ester(0.35%).Table (3.14) showed the *P. crispum* extract, was explain various amount of ten fatty acids, the majority of amount in lowest values ranged between (0.33%) for palmitoleic acid methyl esterto (7.88%) for Cis-11,14- Eicosanoic acid methyl ester except Cis-10-Heptadecenoic acid methyl ester (70.41%) which was the dominant fatty acid. The.Nervonic acid methyl ester (1.44%) was not present in extract ofother samples. On the other hand, Palmitoleic acid methyl ester was found in leaves extract of P.*guajava* and P. crispum not detected in other plantsamples extract.

However, a lot of research studies have documented the significant roles of essential fatty acids in many biochemical pathwaysresulting in cardio protective effect because of theirconsiderable antiatherogenic, antithrombotic,anti-inflammatory, antiarrhytmic, hypolipidemic effect, because of the potential of reducing the risk ofserious diseases, especially cardiovascular diseases, cancer, osteoporosis, diabetes and other healthpromotion activities following from their complex influence on concentrations of lipoproteins, fluidityof biological membranes, function of membraned enzymes and receptors, modulation of eicosanoidsproduction, blood pressure regulation, and finally, on the metabolism of minerals (Hatano, et al1989and Matsuo, et al 1978).Biological activities of an individual fatty acid might be derived from the character andthree-dimensional configuration of molecules and their subsequent enzymatic transformation in a widescale of compounds named eicosanoids. Eicosanoids derived from n-6 and n-3 fatty acids have antagonistic effects. Eicosanoids from the first group promote an inflammation; the latter are much lessinflammatory or even anti-inflammatory (Mišurcová, et al (**2011).**

3.6Quantitative analysis

Ethanolic extract of different parts of P.guajava ,S.cymbopogone ,S.argel ,H.thebaica and P.crispum were subjected to quantitatively analysis to determine the concentrations of alkaloids, flavonoids, phenolic compounds and tannins in mg/ml.The results are summarized in Table (3.15).These compounds are often used to treat a broad spectrum of health disorders and not a single condition.It is likely that the activity of an extract can be due to synergistic interactions of several secondary metabolites present, which cannot be detected when single compounds are evaluated alone (Aleinein,et al **2014,** Eid, et al**2013&**Hamoud, **2015**).

Table(3.15): Quantitative analysis of total alkaloids, flavonoids, phenolic compounds and tannins in mg/ml of methanolic extracts of P.guajava S.cymbopogone ,S.arelg ,H.thebaica and P.crispum

Sample	**Total alkaloids**	**Total flavonoids**	**Total phenolics**	**Total tannin**
P.g	0.2	580	490	434
S.c	0.6	730	430	229
S.a	0.1	277	375	190
H.t	0.5	476	135	284
P.C	0.7	380	225	350

P.g :P.guajava, S.c:S.cymbopogone, S.a:S.arelg, H.t:H.thebaica and P.C: P.crispum

3.6.1 Total alkaloids

The concentration of alkaloid compounds in each sample was calculated from the equation of the chart ($y = 0.109 x+0.007$, $R^2 = 0.997$) as shown in Figure(3.4)

Alkaloid was present in all studied samples . The range of alkaloids vary between (0.025-0.101) mg/ml for*P.guajava* and*S.arelg* respectively. Alkaloids play some metabolic role and control development in living systems (Edeoga, & Eriata 2001). They are also involved in protective function in animals and are used as medicine especially the steroidal alkaloids (Stevens, et al 1997). Alkaloids are often unevenly distributed in plant

families. Common alkaloid containingplants can be found in theLeguminosae, the Liliaceae, the Solanaceae and the Amaryllidaceae. They have diverse pharmacological effects and have a long history in medication.As well as the large number of useful pharmacological properties of thealkaloids utilized by man, alkaloids have been shown to be important resistance factorsagainst herbivorous pests (Irchhaiya, et al (2015).

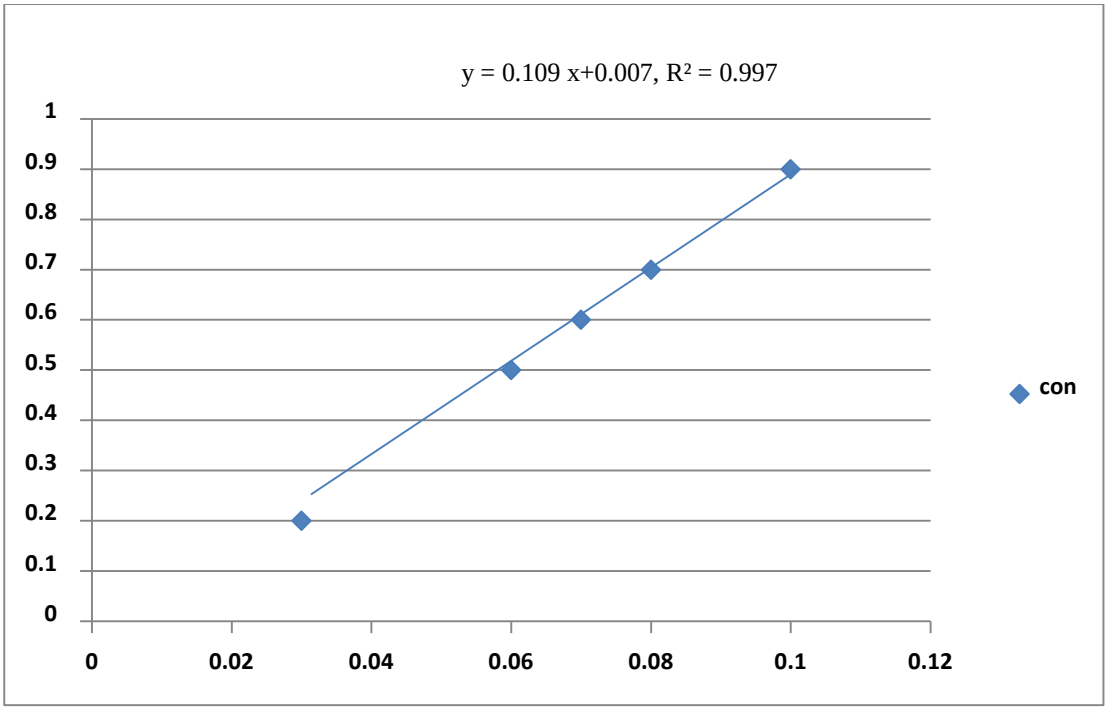


Figure (3.4): Standard curve of Atropine using bromocresol green reagent

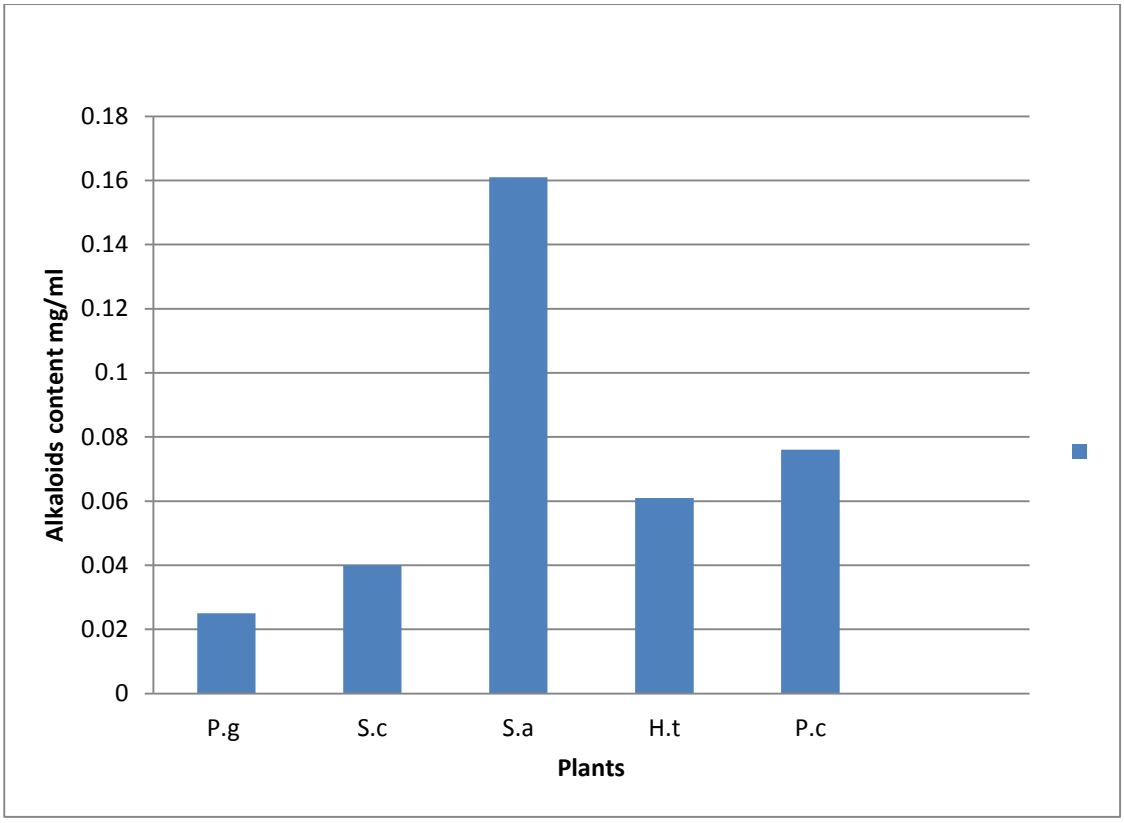


Figure (3.5): Alkaloids content in P.guajava, S.cymbopogone, S.arelg, H.thebaica and P.crispum

3.6.2 Total flavonoids:

The equation of the quercetin calibration curve (Y=0.0007x + 0.0537)was used for determining the concentration of the flavonoids in each extract. Results were expressed as quercetin equivalent per g of dry extract (mg QUE/g). Figure (3. 6) illustrates the amount of flavonoids in each of the five plants sample. All the studied samples showed moderate concentratio of flavonoids.*S.cymbopogone*had high amount of flavonoids (730 mg QUE/g*)* followed by*P.guajava*(580mg QUE/g) andP.crispum (622mg QUE/gwhileS.arelg possess the lowest concentration (277mg QUE/g).

Flavonoides are the largest group of polyphenolic compounds and widely distributed throughout the species of plants sample (Hsu , et al 2006). They are involved in protection against herbivores and micororganisms, both as constitutive agents and as phytoalexins, i.e. produced in response to wounding (Harborne& Williams 2000) and may act as pro-oxidants under certain conditions and have anti-inflammatory and antioxidant properties.(Rietjens, et al2002).In plants, flavonoids are involved in such diverse processes as UV protection, pigmentation, stimulation of nitrogen-fixing nodules and disease resistance.The main subclasses of flavonoids are the flavones, flavonols, flavan-3-ols, isoflavones, flavanones and anthocyanidins (Ju-Sung &Myong 2011).There is information on the levels of flavonols found in commonly consumed fruits, vegetables and beverages. (Harborne & Williams 2000). However, sizable differences are found in the amounts present in seemingly similar produce, possibly due to seasonal changes and varietal differences. (Rietjens, et al2002). Flavonoids also have the ability to inhibit enzymes, but may cause additional mutagenic and toxic effects on DNA via alkylation.Flavonoids and phenolics acids are the most important groups of secondary metabolites and bioactive compounds in plants (Koes, et al 1994).

They are also a kind of natural product and antioxidant substance capable of scavenging free superoxide radicals, anti-aging and reducing the risk of cancer. Flavonoids have been found *in vitro* to be effective antimicrobial substances against a wide array of microorganisms. Their activity is probably due to their ability to complex with extracellular and soluble proteins, and to complex with bacterial cell walls. They can also disrupt microbial membranes (Hertog, et al 1992). In the pathophysiology of diarrhea and cholera toxin, prostaglandins are implicated. Extracts of plants that contain flavonoids are known to modify the production of cyclooxygenase 1 and 2 and lipooxygenase (Crozier, et al1997). Thereby inhibiting prostaglandins production. Some plants show antidairrhoea properties by their antimicrobial activities (Kim, et al 2003 and Stern, et al 1996).or by blocking the eicosanoids (prostaglandins and congeners) (Ladeji, et al 1997). Flavonoids, tannins or steroids are singly or in combination are responsible for the observed effects. Flavonoids are able to inhibit aldose reductase enzyme (that converts sugars to sugar alcohols) and is implicated with diabetic complications, such as neuropathy, heart disease and retinopathy (Dicarlo, et al 1993).

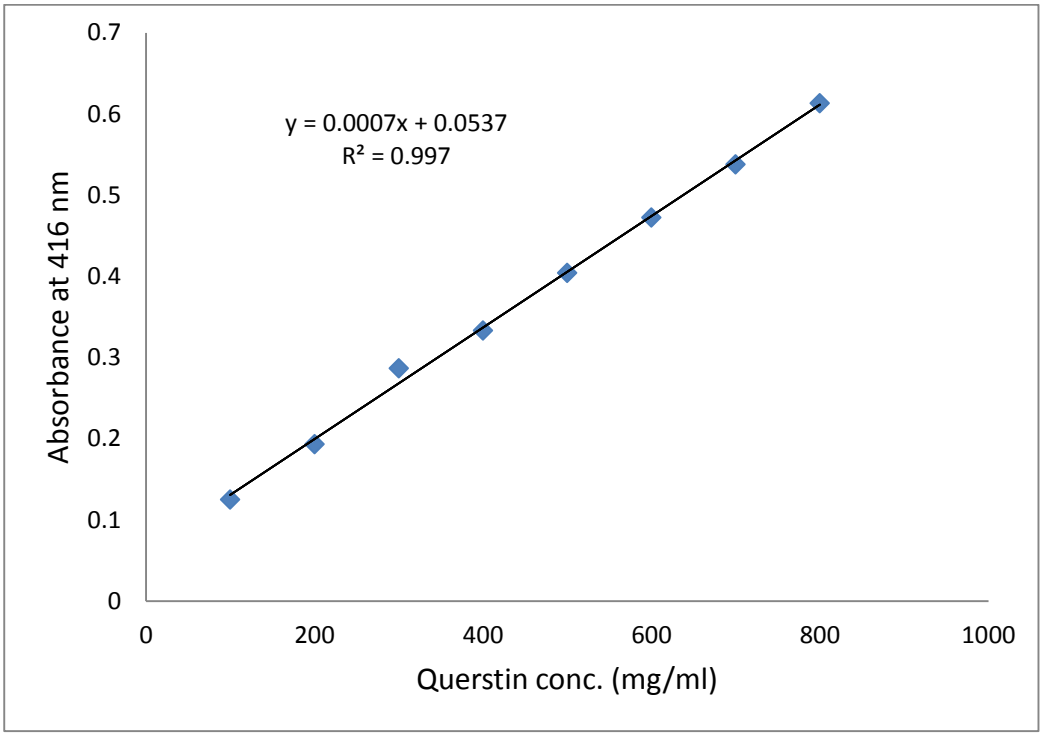


Figure (3.6): Standard curve of quercetin using aluminum chloridereagent

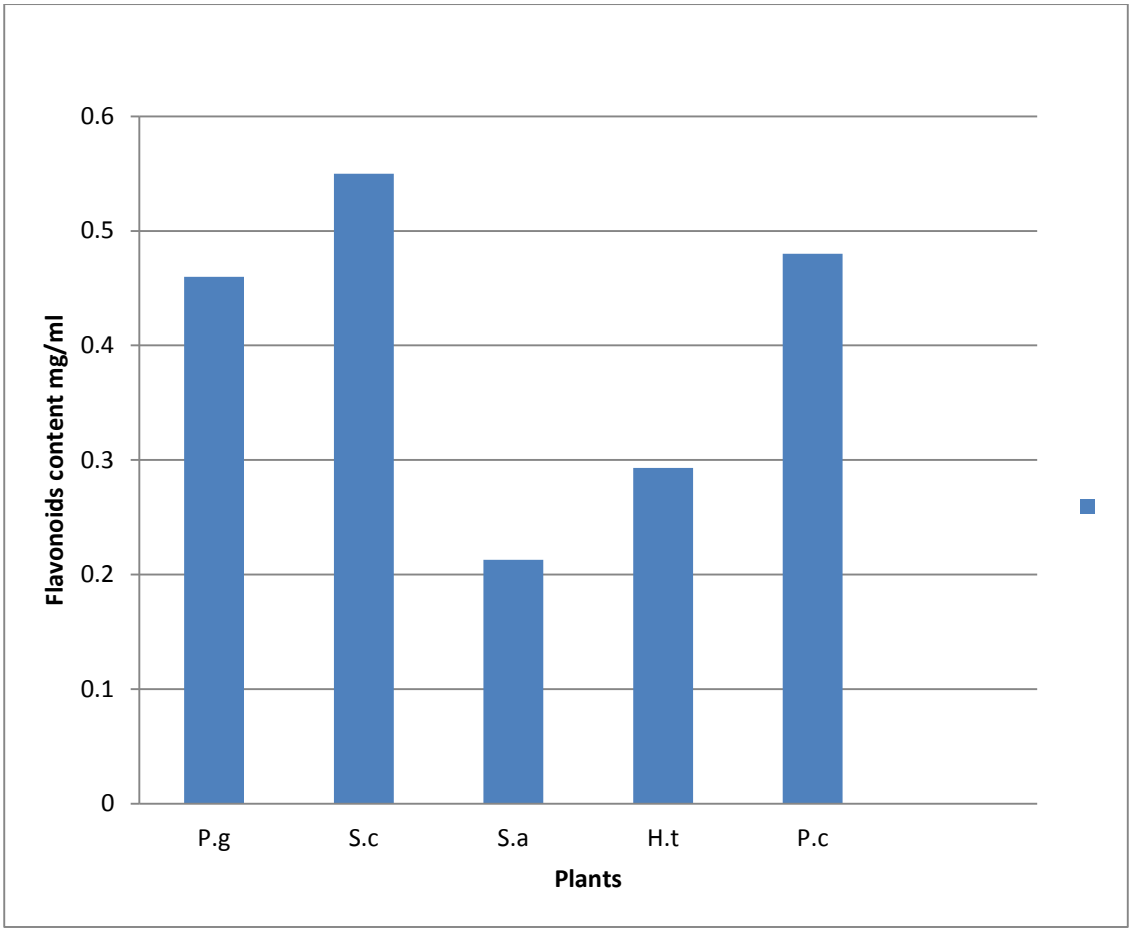


Figure (3.7): Flavonoids content in P.guajava, S.cymbopogone, S.arelg, H.thebaica and P.crispum

3.6.3 Total phenolic compounds:

The methanol extract of the different parts of the plants samples was assayed with the sensitive Folin- Ciocalteu reagent; results were expressed as mg gallic acid equivalents per ml. A constructed curve of gallic acid with chart equation,$Y = 0.0008x + 0.0397$, $R^2 = 0.995$, was used for calculating the concentrations of phenolic compounds in each extract are presented in Figure (3.8). All the studied samples were rich in phenolic compounds. The amount of phenolic compounds in all plants samples was in range of (135 – 418 mg/ml). *P.guajava* contained high amount of phenol content compared with the others kinds of studied plants. The lowest level of phenol content was detected in*P.crispum*.Phenolic compounds are commonly found in plants, and they have been reported to have multiple biological effects, including antioxidant activity. Numerous studies have revealed that some phenolic molecules have antiapoptosis, anti-aging, anticarcinogen, anti-inflammation, anti artherosclerosis, cardiovascular protection, improvement of the endothelial function, as well as inhibition of angiogenesis and cell proliferation activity. Most of these biological actions have been attributed to their intrinsic reducing capabilities (Christopher, et al 1996).Phenolic compounds have also been shown to possess significant pharmacological properties including anti-infl ammatory, antibacterial, antifungal, antiulcer, vascular protective, and anticancer (Ghasemzadeh 2011, Correia, 2001, Han, et al 2007 and Di Carlo, et al (1999).According to several epidemiological studies, polyphenols from grapes andwines have signifcant health benefits, since they possess cancerchemopreventive, cardioprotective, and neuroprotective activities. Among these phenoliccompounds, *trans*-resveratrol, which belongs to the stilbene family, is a major active ingredient and can prevent or slow the progression of major diseases, as well as extend the lifespan of various organisms from yeast to vertebrates (Fresco, et al (2006).

Phenolic compounds like stilbenes have a wide range of pharmacological andbiological actions in three major domains: cancer, cardiovascular disease and neurodegeneration. The response in humans, however, depends on their absorption and in vivo metabolism.Several stilbenes have been isolated from grape cell suspension cultures: resveratrol, piceid, resveratroloside and astringin, all in the two isomeric forms *cis*- and *trans*.Three new resveratrol diglucosides, *cis*- and *trans*-resveratrol 3,5-O-β-diglucoside and *trans*-resveratrol 3,4'-O-β-diglucoside have been isolated together with a new resveratrol triglucoside, *trans*-resveratrol 3,5,4'-O-β-triglucoside (Kandaswami, et al 2005)

In *in vitro* studies with total phenolic compounds from red wine, Frankel *et al*. (Loa, et al (2009). recorded that red wine diluted 1000 fold,containing 10 μmol/l phenolics, inhibited human Low-Density Lipoproteins(LDLs) oxidation significantly more than αtocopherol.Polyphenols such as resveratrol can also act by another mechanism (i.e. their complexation with metal ions, for example iron or copper), which are involved in the generation of free radicals and lipidperoxidation.Moreover, α-tocopherol (the principal form of vitamin E), which functions as a major antioxidant in human LDLs, can be recycled from its free radical form (α-tocopheryl) by a phenolic compound.

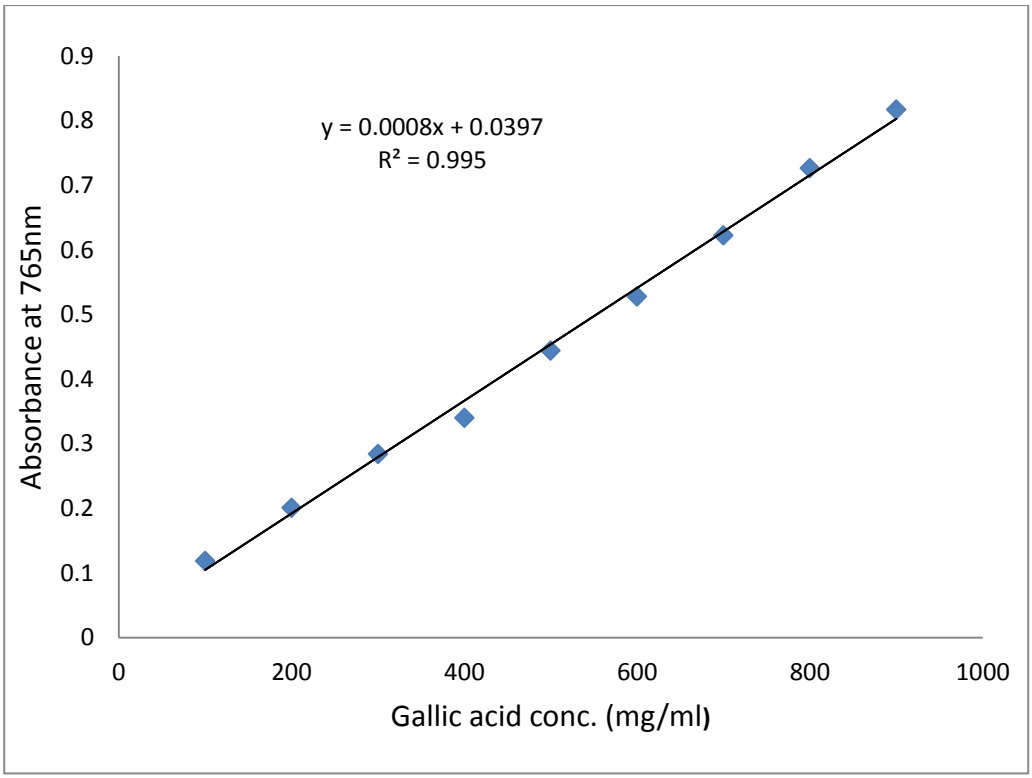


Figure (3.8): Standard curve of gallic acid using Folin–Ciocalteu metho

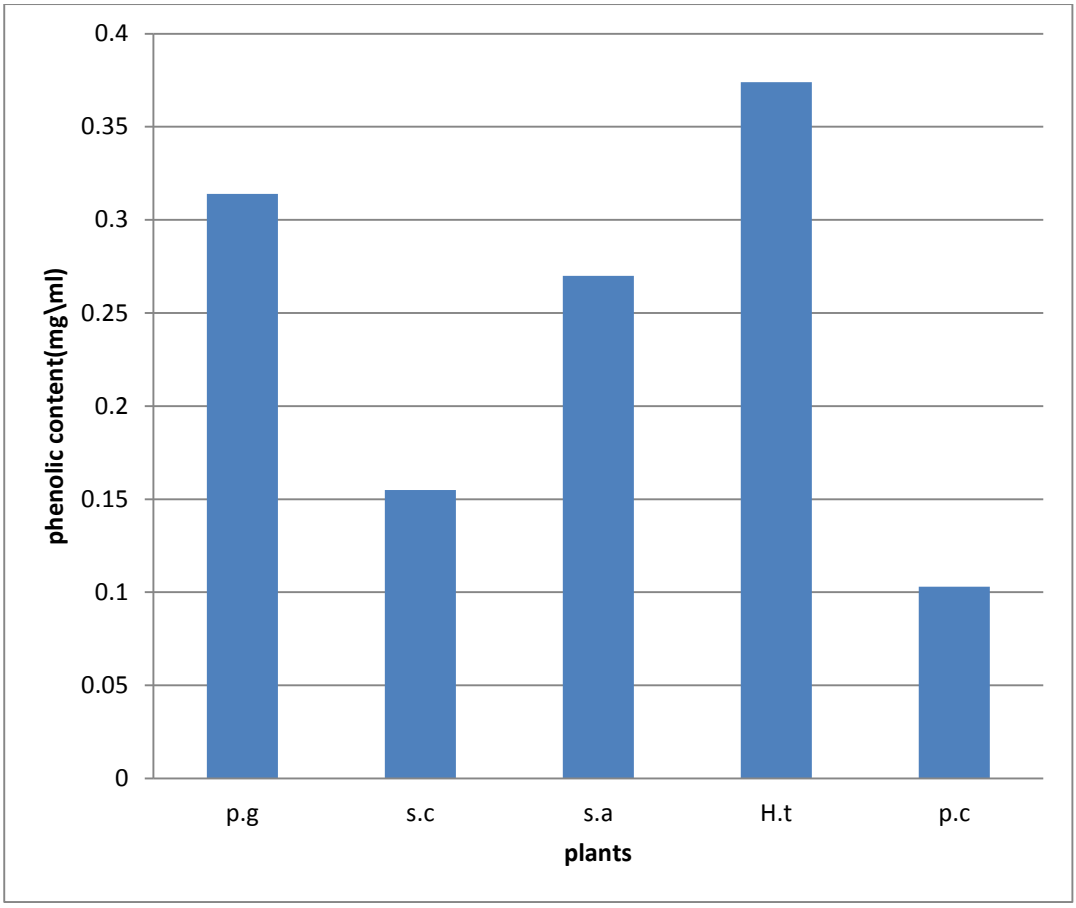


Figure (3.9): phenolic content in P.guajava, S.cymbopogone, S.arelg, H.thebaica and P.crispum

3.6.4 Tannins content:

Tannic acid at different concentrations was used for construction of the standard curve of tannin . The standard curve was used for calculating the amount of tannins in each extract. As shown from Figure (3.10), all studied samples had low concentrations of tannin content. The detected amount of tannin was in the range between 190 to 434 mg/ml. The highest level of tannins was determined in the *P.crispum*, while the lowest level was detected in the *S.argel*. The tannin compounds are widely distributed in many species of plants, where they play a role in protection from predation, and perhaps also as pesticides, and in plant growth regulation (Baur& Sinclair2006).

However, the presence of tannins at high levels can significantly restrict intake and the utilization of nutrients, particularly nitrogen (Larronde , et al 2005). On the other hand, low levels of tannins in foliage can increase the utilization of crude protein by protecting it from digestion in the rumen there by increasing the flow of essential amino acids to the small intestine for absorption (Frankel .et al (1995).Tannins were once classified into two groups: pyrogallol type tannins and catechol type (or catechin type) tannins, according to the polyphenol groups in their molecules. Then, the developments in tannin chemistry led to the renaming of these two groups to hydrolyzable tannins and condensed tannins (Katie, et al (2006), Reed, et al 1990) (Waghorn, G. Caffetannins, labiataetannins and phlorotannins were also referred to tannins (Okuda, et al. (1985**).**

The isolation of bio-active stilbenoids, among which was a monomer piceatannol is regarded as responsible for the tannin activity of the bark of spruce tree (Okuda, (2005**).** and various resveratrol oligomers, and phlorotannins from brown algae, exemplified by monomeric eckol (Okuda, 1999**).** expanded the field of tannins and related polyphenols to these groups

of compounds in the last few decades. At the same time, various biological and pharmacological activities related to the health effects of tannins with a variety of chemical structures, including those of small molecular size, have been found (Grassmann, et al. (1958). As As for the molecular size, (-)-epigallo catechin gallate (EGCG) and (-)-epicatechin gallate (ECG), the main "tannin" in green tea, are examples exhibiting the properties of tannins despite their rather small molecules. They exhibit binding activities towards proteins and other substances and appreciable antioxidant activities, besides their antitumor effects(Nakayama, et al1989**).**Among the biological and pharmacological activities of these tannins are host-mediated antitumor activities, and antimicrobial activities exemplified by those against Helicobacter pylori, antibiotic-resistant bacteria and Leishmania donovani (Yoshida, et al1989**).**Occurrence of galloylated polyhydroxy flavan oligomers(condensed tannins) is limited to the plants of Dicotyledoneae where gallic acid can be biosynthesized. The biological activities of these tannins are generally significantly higher than those of non-galloylated polyhydroxy flavans. (Okuda, (1997).Most of the condensed tannins contained in traditional medicinal plants as their main components are galloylated at 0-3 to a variable extent. Highly Galloylated Polyhydoxyflavan Oligomers was reported in Saxifragastolonifer*a***.** The herb Saxifraga stoloniferais a folk medicine used in Japan for treating earache, painful hemorrhoids, wounds and swelling.

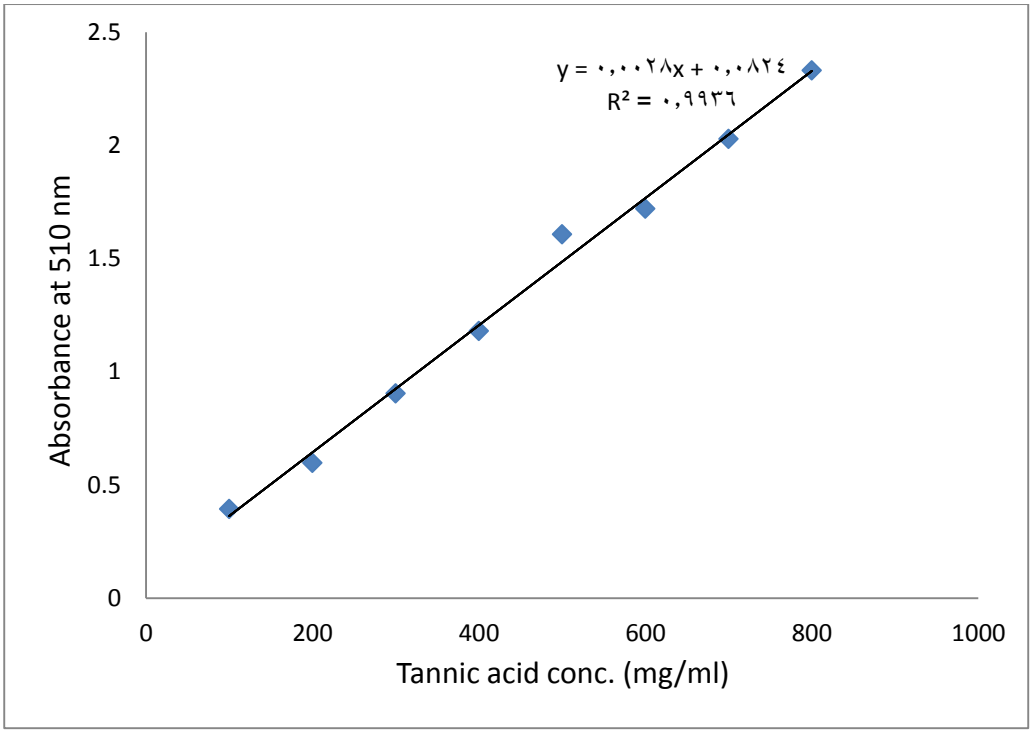


Figure (3.10): Standard curve of tannic acid (mg/ml)

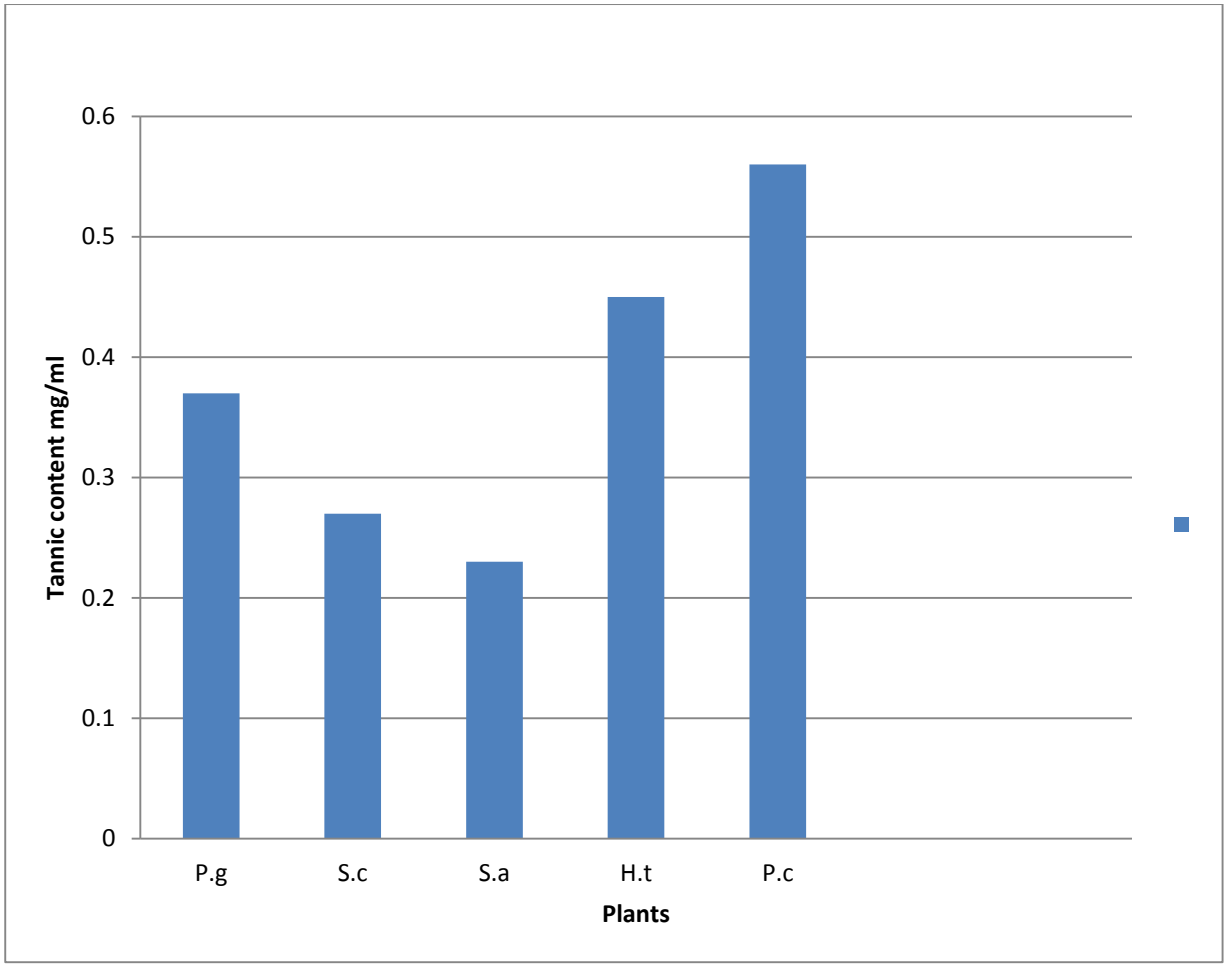


Figure (3.11):Tannic content in P.guajava, S.cymbopogone, S.arelg, H.thebaica and P.crispum

3.7 Cytotoxicity screening

The 3-(4,5- dimethylthiazol-2-yl)-2,5- diphenyl tetrazolium bromide (MTT) colorimetric assay was used to screen the cytotoxic activity of plant extracts. The cytotoxicity of ethanolic extracts from *P.guajava ,S.cymbopogone,S.argel, H.thebacia and P.crispum* was determined on African green monkey kidney ephitelial (Vero) cell lines. Triton-x100 was used as positive control. The obtained results are summarized in Table (3.16).

Table (3.16): Cytotoxicity of plantextracts on normal cell lines (Vero cell line) as measured by the MTT assay:

NO	**Name of Extracts**	**Concentration (µg/ml)**			**IC_{50} (µg/ml)**	**IC_{50}**
		Inhibition (%) ± SD				
		500	**250**	**125**		
1	**S.C**	31.98 ± 0.05	19.00 ± 0.02	6.93 ± 0.04	1365.36	>100
2	**P.g**	49.17 ± 0.03	33.51 ± 0.01	9.55 ± 0.05	485.85	> 100
3	**S.a**	33.02 ± 0.02	26.56 ± 0.09	6.79 ± 0.03	971.00	> 100
4	**P.c**	45.81 ± 0.05	15.13 ± 0.07	2.69 ± 0.04	587.38	> 100
5	**H.t**	42.59 ± 0.06	41.95 ± 0.08	34.74 ± 0.02	1082.43	> 100
6	***Control**	96.28 ± 0.01				< 30

Key:P.g: *P.guajava* ,**S.C:** *S.cymbopogone*,**S.a:***S.argel*,**H.t:** *H.thebacia and* **P.c:***P.crispum*

IC_{50}< 30 µg/ml: high toxic. Control = Triton-x 100 was used as the control positive at 0.2 µg/ml. The maximum concentration used was 500 µg/ml. When this concentration produced less than 50% inhibition, the IC_{50} cannot be calculated.

At 500 µg/ml level of concentration, the maximum percentage rates of inhibition were 31.98± 0.05, 33.02± 0.02 ,42.59±0.06 , 45.81± 0.05 ,49.17± 0.03 for ethanolic extract from S.cymbopogone,S. argel H.thebacia,P. crispum andP .guajava respectively. The maximum percentage rate of inhibition shown by the control at concentration of 0.2 µg/ml was 96.28 ± 0.02%. As compared with control, all the studied plant extracts were exhibited cytotoxicity less than 50%.Extract ofS. cymbopogone showed high inhibitory percentage towards the tested cell at all level of concentrations, while leaves extract of P.guajava exhibited high cytotoxicity towards the tested cell only at high concentration of the crude extract.

At low level of concentration (125 µg/ml), the percentage rate of inhibition was in the range of 2.69 ±0.04 - 34.74 ± 0.02 The calculated IC_{50} of all tested crude extracts were > 100 ppm which confirmed the non-toxicity of the studied plant part extracts.

In general,all samples extracts were weakly inhibited the growth of Vero cells at both lower and higher concentrations except crude extracts o*f* P.guajava leaves andP.crispum which showed mild cytotoxicity at higher concentrations. the ethanolic extracts from Fourty-eight medicinal plants were screened for their toxicity by Ramadhani *et al*. (Ramadhani,2015). The obtained results indicated that fourty-five (93.75%) out of 48 crude extracts assessed using LLC-MK2 cells were non-cytotoxic while three extracts (6.25%) were cytotoxic. However, several medicinal plants have previously been reported to be toxic; such as *Symphytum officinale* L. used for wound healing which contains hepatotoxic pyrrolizidine alkaloids and *Valerian*

officinalis used as asedative for treatment of insomnia and anxiety which causes hepatitis (Stern, 1996).

3.8 Biological activities

3.8.1 Antibacterial and antifungal activities

All the crude extracts, (petroleum ether, ethyl acetate,aceton and ethanol) obtained from plant sample of P.gujava, S.cymbopogone, S.argel,H.thebaicaand P.crispum were screened for their antimicrobial activity against four standard bacteria strains and two fungi to determine their activity. The pathogens used were *Bacillus subtilis*, *Echerichia coli*,*Pseudmonas aeroginosa*, *Staphylococus aureus* ,*Aspergillus niger and Candida albicans*. The antibacterial and antifungal assay for crudes was conducted by using the disc diffusion method. The tests were carried out at a concentration of 20 mg/ml. The zones of inhibition of bacterial and fungal growth were measured after 24 hours and the measurements were done (in mm) from the end of the growth of one side of the disc to the beginning of growth of the other side including the diameter of the disc.

On the basis of the results obtained with standard chemotherapeutic agents against the same standard tested organisms, plant extracts resulting in more than 18 mm growth inhibition zones are considered to possess relatively high antibacterial activity, and those resulting in 14-18 mm inhibition are of intermediate, and those resulting in zones below 14 mm are inactive (Cruickshank, et al1975).

Susceptibility of standard bacteria to different plants extracts were arranged according to their activity. The results clearly indicated variation in theactivity against different bacterial and fungal strains ranging between relatively high to resistant.

The result of petroleum ether extract obtained inTable(3.17);

Table (3.17): Anti-microbial activity of petroleum ether extracts fromP.gujava, S.cymbopogone, S.argel ,H.thebaicaand P.crispumfour bacterial and two fungal strains

Sample	*M.D.I.Z Inhibition zone (mm)					
	Bacterial strains				Fungal strains	
	B. s.	*E. c.*	P. a	S. a	A. n.	C.a.
P.guajava	18.5±0.7	20.5±0.7	18.5± 0.7	21±1.4	22±	16.5±
S.cymbopogone	13±0	18.5± 0.7	13.5±0.7	16.5±0.7	17±0	0
S.argel	20±0	22±0	18.5±0.7	20± 0	23±0	21±1.4
H.thebaica	17±0	11± 0	20± 0	17± 0	20±0	15±0
P.crispum	15.5±0.7	13.5± 0.7	13.5±0.7	16± 1.4	17.5±0.7	0

*M.D.I.ZMean diameter of growth inhibition zone in mm

Key*:* B.s.: *Bacillus subtilis*, E.c.: *Echerichia coli*, P.a.: *Pseudmonas aeroginosa*, S.a.: *Staphylococus aureu*s, A.n.: *Aspergillus niger*, C.a: *Candida albicans*.. Interpretation of results: MDIZ (mm): <9 mm Inactive; 9-15 mm partially active; 15-25mm Active; >25 mm: Very active (Parveen *et al*., 2010).

The activity ranged between high and intermediate activity to resistant against all strains, P.guajava and S. argel are showed high activity or resistant in all strains but*H .thebaica* and *P. crispum* are showed relatively low activity against *E. coli*While*S.cymbopogon* and *P. crispum* are showed no activity or resistant to*C. albicans*.Ethyl acetate extracts showed moderate activity or resistant for almost bacterial and fungal strains with some exceptions, that is showed at Table3.18

Table (3.18): Anti-microbial activity of ethyl acetate extracts fromfromP.gujava, S.cymbopogone, S.argel ,H.thebaicaand P.crispum four bacterial and two fungal strains.

Sample	*M.D.I.Z Inhibition zone (mm)					
	Bacterial strains				Fungal strains	
	B. s.	*E. c.*	P. a	S. a	A. n.	C.a.
P.gujava	17±1.4	16±0	13±0	18.5±0.7	16±0	12±0
S.cymbopogone	14±0	13±0	15±0	15±	0	0
S.argel	12.5±0.7	13±0	15±1.4	14.5±0.7	22±0	10±0.7
H.thebaica	20±0	21±0	18±0	13±0	0	0
P.crispum	13.5±0.7	12.5±0.7	14±1.4	14.5±0.7	17±0	13.5±0.7

*M.D.I.ZMean diameter of growth inhibition zone in mm

Key*:* B.s.: Bacillus subtilis, E.c.: Eche±richia coli, P.a.: Pseudmonas aeroginosa, S.a.: Staphylococus aureus, A.n.: Aspergillus niger, C.a: Candida albicans.Interpretation of results: MDIZ (mm): <9 mm Inactive; 9-15 mm partially active; 15-25mm Active; >25 mm: Very active (Parveen *et al*., 2010).

S.argel showed relatively high activity to the A.niger fungal strains while *S.cymbopogon* and *H.thebaica* showed no activity towards the two fungal.*H.thebaica*showed high activity or resistant to the three bacterial strains except on the test against Staphylococus aureus which give inhibition zone of 13 mm.*P.gujava* showed high activity to the all bacterial strains except on the test against*Pseudmonas aeroginosa* and

S.argel showed low activity against *candida albicans.* Acetone extract results show in Table (3.19)

Table (3.19): Anti-microbial activity of acetone extracts *fromP.gujava, S.cymbopogone, S.argel ,H.thebaicaand P.crispum* four bacterial and two fungal strains.

Sample	*M.D.I.Z Inhibition zone (mm)					
	Bacterial strains				Fungal strains	
	B. s.	*E. c.*	P. a	S. a	A. n.	C.a.
P.gujava	15.5±0.7	20±**0**	16±**0**	19±**1.4**	15±**0**	14±**0**
S.cymbopogone	15±0	19±**1.4**	14.5±**0.7**	17±**1.4**	12±**0**	9±**0**
S.argel	17.5±**0.7**	14±**0**	16±**1.4**	13.5±**0.7**	17±**0**	18.5±**0.7**
H.thebaica	17.5±**0.7**	20±**0**	14.5±**0.7**	18.5±**0.7**	12±**0**	14±**1.4**
P.crispum	12.5±**0.7**	14.5±**0.7**	16±**0**	14±**1.4**	14±**1.4**	12.5±**0.7**

.*M.D.I.ZMean diameter of growth inhibition zone in mm

Key*:* B.s.: Bacillus subtilis, E.c.: Eche±richia coli, P.a.: Pseudmonas aeroginosa, S.a.: Staphylococus aureus, A.n.: Aspergillus niger, C.a: Candida albicans.Interpretation of results: MDIZ (mm): <9 mm Inactive; 9-15 mm partially active; 15-25mm Active; >25 mm: Very active (Parveen *et al*., 2010)

The result of acetone extract indicated that tested of all samples showed high activity or resistant for Escherichia coli bacterial strains while almost results showed moderate activity or resistant for others bacterial and fungal

strains except S.argel which showed low activity againstStaphylococus aureus, allsamples showed low activity against candida albicans fungal while S.argel showed high activity or resistant

Table 3.20 explain the result of ethanol extract for anti-microbial activity:

Table (3.20): Anti-microbial activity of ethanol extracts fromP.gujava, S.cymbopogone, S.argel ,H.thebaicaand P.crispum four bacterial and two fungal strains

Sample	*M.D.I.Z Inhibition zone (mm)					
	Bacterial strains				Fungal strains	
	B. s.	*E. c.*	P. a	S. a	A.n	C.a.
P.gujava	15.5±0.7	17±0	16.5±0.7	17±0	13±0	13.5±0.7
S.cymbopogone	18.5±0.7	20.5±0.7	19±1.4	18±0	23±0	12±0
S.argel	20±0.7	18.5±0.7	23.5±0.7	18±0	18±0	13.5±0.7
H.thebaica	16±1.4	12.5±0.7	11±0	14±1.4	17±0	13±0
P.crispum	15±0	23±0	12.5±0.7	15±0	15±0	7±0

*M.D.I.ZMean diameter of growth inhibition zone in mm

Key*:* B.s.: Bacillus subtilis, E.c.: Echerichia coli, P.a.: Pseudmonas aeroginosa, S.a.: Staphylococus aureus, A.n.: Aspergillus niger, C.a: Candida albicans.Interpretation of results: MDIZ (mm): <9 mm Inactive; 9-15 mm partially active; 15-25mm Active; >25 mm: Very active (Parveen *et al*., 2010)

In ethanol extracts the mean zones of inhibition were found to be in the range of (18- 23.5 mm) for S. argel fowled by S.cymbopogone. except the P.crispumshowed low activity or resistant for C.albicans only while S.argel gave the highest inhibition zone against the all bacterial and fungal strain.

Generally ethanolic extract showed greater antimicrobial activity as compared with other extract may due to the polarity.Therefore the observed antibacterial potency of ethanol extract can be attributed to two reasons: firstly, to the nature of biologically active components (alkaloids, flavonoids, sterols, tannins, phenols etc.) which might be enhanced in the presence of ethanol. It has been documented that alkaloids, flavonoids, tannins and phenols are plants metabolites, well known for their antimicrobial activity (Tschesche, R. 1971). Secondly, the stronger extraction capacity of ethanol could have produced a large number of active constituents responsible for antibacterial activity, which have been found to be present in large quantity in the ethanol extract as per our study. This finding was in agreement with the finding of Agunu (2011) who reports that theethanol extract of S.cymbopogone gave higher antibacterial activity and contain high level of flavonoids. Flavonoids have been found invitro to be effective antimicrobial substances against a wide array of microorganisms. Their activity is probably due to their ability to complex with extracellular and soluble proteins, and to complex with bacterial cell walls (Stern, et al1996).

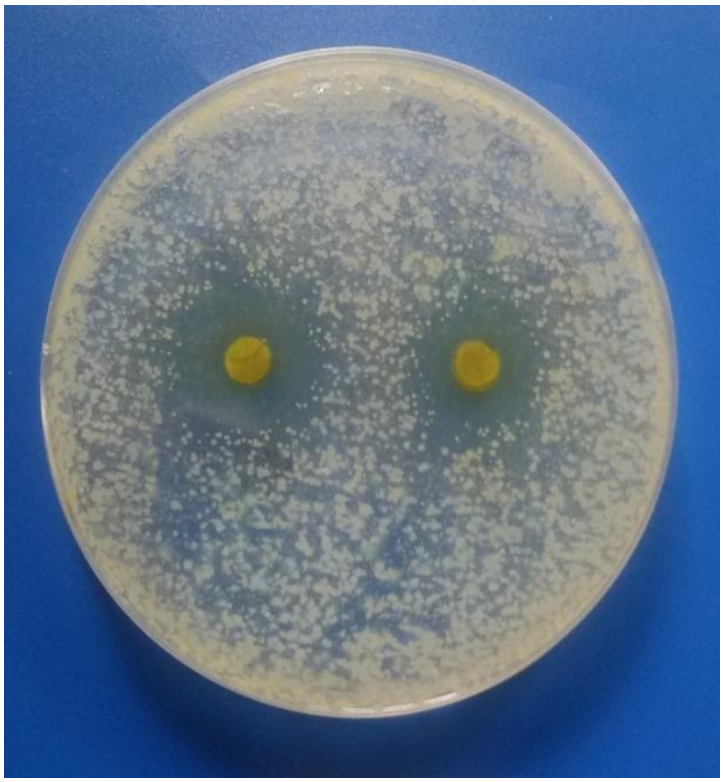


Plate (3.1): Anti-bacterial activity ofpetroleum ether extract of the S.argelagainst *Pseudomonas aeruginosa*

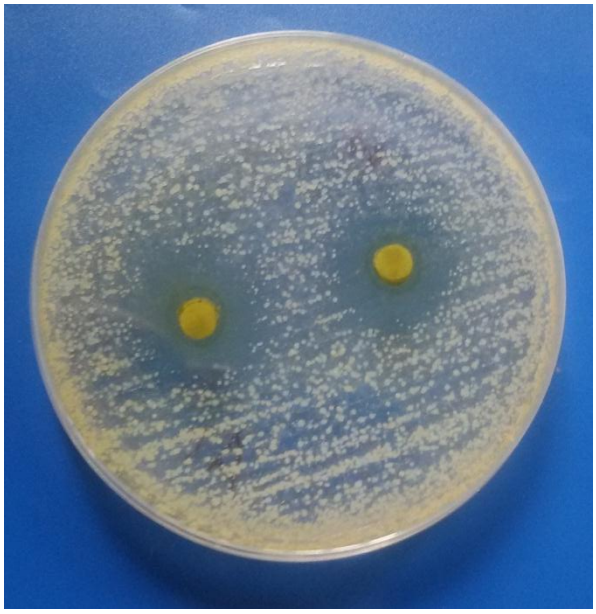


Plate (3.2): Anti-bacterial activity ofpetroleum ether extract of the S.argelagainst Bacillus *s*ubtilis

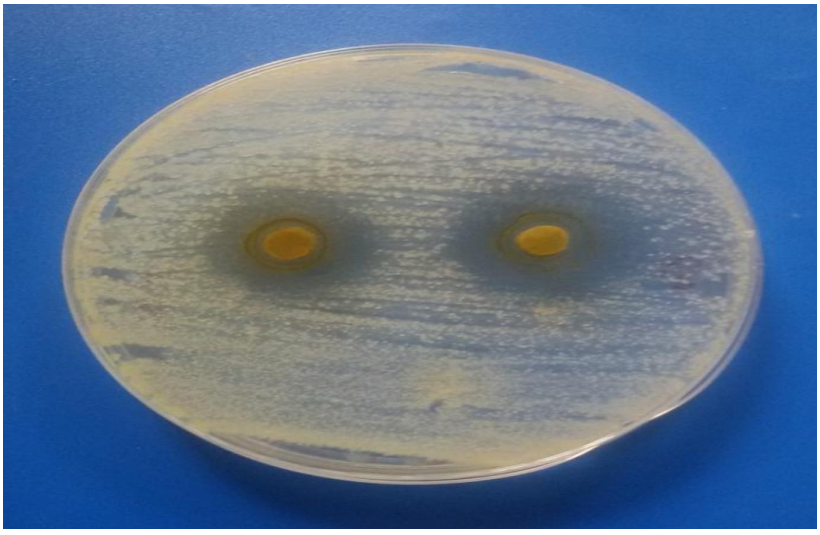


Plate (3.3): Anti-bacterial activity ofethanol Extract ofP.gujavaagainst*Staphylococccus aureus*

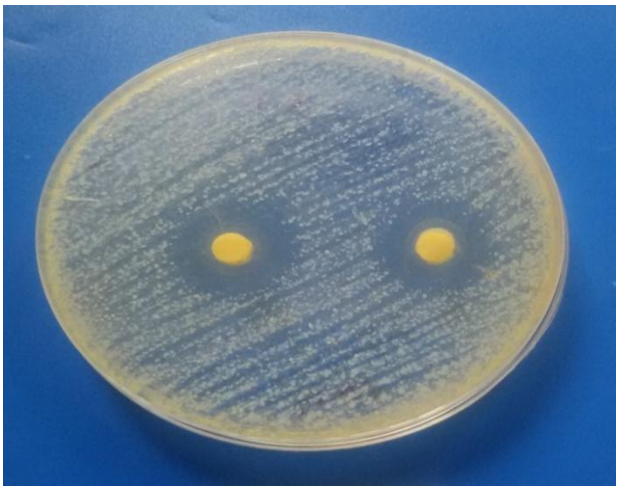


Plate (3.4): Anti-bacterial activity ofethanol extract of S.cymbopogoneagainst Pseudomonas aeruginosa

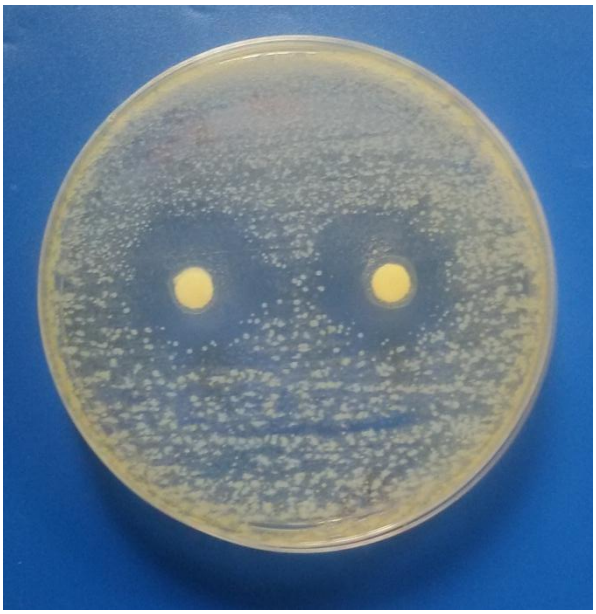


Plate (3.5): Anti-bacterial activity ofethyl acetate extract ofH.thebaicaagainst *Escherichia coli*

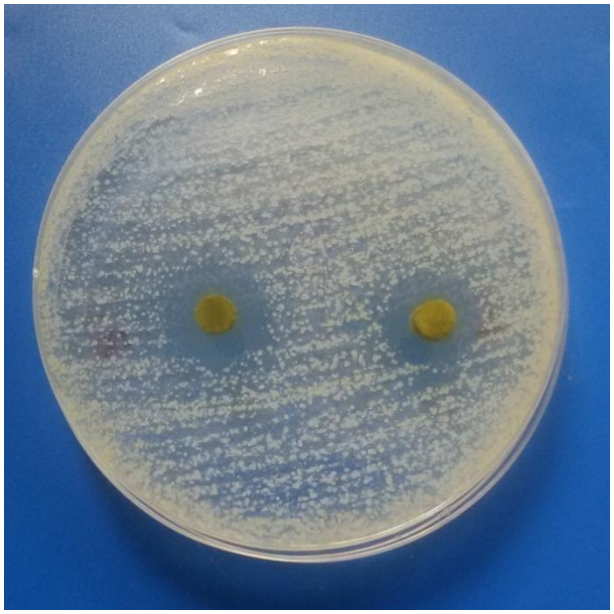


Plate (3.6): Anti-bacterial activity ofpetroleum ether extract ofP.crispumagainst *Bacillus subtilis*

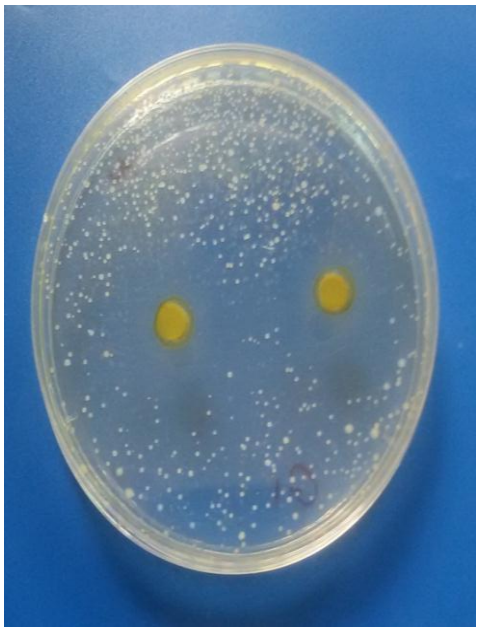


Plate (3.7): Anti-fungal activity ofpetroleum ether extractofP.gujavaagainst *Aspergillus niger*

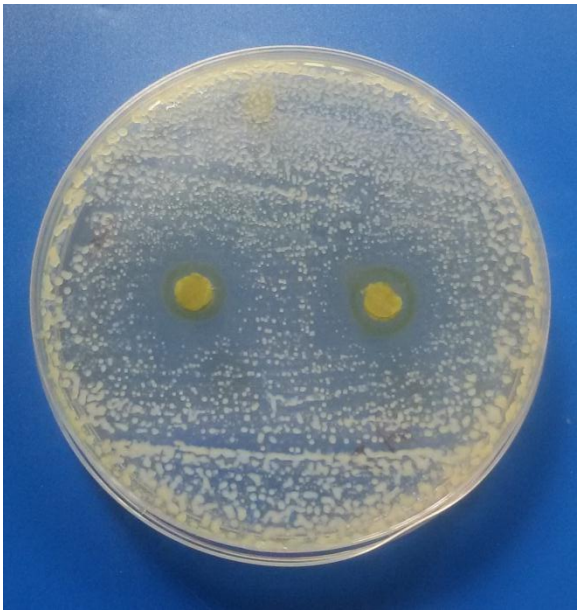


Plate (3.8): Anti-fungal activity ofacetone extract ofS.argelagainst *Candida albicans*

ACONCLUSION AND RECOMMENDATIONS

Conclusion

Plants *Psidium guajava, Schoenanthus cymbopogone, Solenostemma argel , Hyphaene thebaica and Petroselinum crispum* are common plants widely used in many parts of Sudan for medicinal purposesor herbal medicineto treat various diseases. The collected plants were dried under shade, grind then subjected to sequential extraction using petroleum ether, ethyl acetate, acetone and ethanol as solvents.

Phytochemicals that are present in plants have been shown to possess a range of bioactivity, including antioxidant, anti-bacterial properties. Theyare extensively researched by scientists for their health-promoting potential. Through the use of well-established scientific methods and research materials, the present study aimed to investigating the phytochemical components of this plant and evaluating the antioxidant and antimicrobial activities of these components. General standard methods were adopted for extracting and analyzing the target compounds.The highest percentage yield of the extracted components was detected in ethanol extract of P.guajava. The antioxidant activities were evaluated based on the ability of the plant extracts to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2´-Azinobis 3-ethylbenzothiazoline-6-sulfonic acid(ABTS) free radical.Most crude extracts with ABTS reagent exhibited good antioxidant activities (69% – 94%) for percentage70% compared to DPPH reagent (15%). The highest scavenging ability was exhibited by ethanol extract that may be due to polarity of ethanol. P.guajava extract exhibited a remarkable antioxidant effect compared with other plant samples which showed scavenging rate of 94%.The concentration of heavy metals determined in samples plants are well below the critical limit and in permissible limit recommended by World Health Organization (WHO).Proxmiate analysis including moisture,

crude protein, crued fiber,ash and ether extract determined by the Association of official analytical chemists method.The obtained results revealed that all parts of the plant were rich in the secondary metabolites compounds and showed little variation in their chemical profiles.Saturated and unsaturated fatty acids were found in the extract ofplant. Cis-10-Heptadecenoic acid methyl ester, Cis-11-Eicosenoic acid methyl ester and pentadecenoic acid methyl ester were the prevailing fatty acids in the extracts.Cytotoxicity test demonstrated that the studied plants were non-toxic plants towards the normal cell lines.Antimicrobial activities were assayed by the disc diffusion method,The ethanol extracts of all plant exhibited appreciable activity against all the bacterial and fungal species investigated ,on the other hand petroleum ether extract of *S.argel* showed high activity or resistant in all strain.The ability of these extracts of *S.argel* to inhibit the growth of bacteria and fungi is an indication of its broad spectrum antimicrobial potential which may be employed in the management of microbial infections.

These findings may help to discover new chemical classes that could serve as lead for the development of new pharmaceuticals addressing the major therapeutic needs. However, investigations into the pharmacological importance of *Psidium guajava, Schoenanthus cymbopogone and Solenostemma argel* their diversity and detailed phytochemistry may add new knowledge to the information in the traditional medical systems.

Recommendation

1- Comprehensive evaluation of antioxidant activity using more than one method is shown importance in assessing the scavenging power of the extracted components.

2- Using one assay for testing the biological activity of the extracted components seems to be rather unrealistic, so other confirmatory methods are needed.

3-In vivo studies are needed on these active extracts to understand the exact mechanism by which these components exert their antimicrobial and antioxidant properties.

4- Cytotoxicity test should be done on both normal and cancer cell lines.

5- Modern spectroscopic techniques are needed for further characterization, identification and elucidation of the isolated components.

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