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Investigation of phytochemicals from some Sudanese Medicinal Plants and their Antimicrobial potency

دراسة مكونات بعض النباتات الطبية السودانية ومقدرتها التضادية للمايكروبات

A Dissertation Submitted in Partial Fulfillment of the Requirements of the Master Degree in Chemistry

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April 2017

الآية

قال تعالي:

(وَقُلِ اعْمَلُوا فَسَيَرَى اللَّهُ عَمَلَكُمْ وَرَسُولُهُ وَالْمُؤْمِنُونَ ۖ وَسَتُرَدُّونَ إِلَىٰ عَالِمِ الْغَيْبِ وَالشَّهَادَةِ فَيُنَبِّئُكُم بِمَا كُنتُمْ تَعْمَلُونَ)

سورة التوبة(105)

Dedication

To the spirit of my precious mother

And My Father

TO my Brothers and Sisters

They who without this work nothing

Acknowledgment

Thank to Allah the most gracious. The compassionate for giving me strength and health to complete this work .

Iam deeply grateful to mysupervisor prof Mohamed AbdlEkareem for his fruitful guidance. I also special thanks you for all staff of Medical Biochemistry Research Department and micro and photochemistry department in National Centre for Research. Also I want thanks for Bash pharm Co.Ltd for support and motivation .

Finally appreciation to Sudan university of science and Technology .and my colleagues and the best friends whose iam proud to know them, and learning, sharing knowledge and experiences with them wish you all a bright future and happy life.

Abstract

The present study was designed to investigate the antioxidant and antimicrobial activities of three Sudanese plants ;

(***Rheumpalmatum*** ***Rutagraveolens,*** ***Moringaperegrine***) Phyto chemical study was conducted to detect thebioactive compounds. The crude ethanolic extract was partitioned successively by chloroform , ethyl acetate and n-butanol. Different fractoins were assessed for antimicrobial potency against standard bacterial strains: Gram positive (*Bacillus subtilis* (NCTC8236), *Staphylococcus aureus* (ATCC25923); Gram negative (*Escherichia coli* -ATCC25922, *Pseudomonas aeruginosa* -ATCC27853) and the fungal species : *Candida albicans* (ATCC7596). The cup-plate agar diffusion method was used. The antioxidant activities were conducted via DPPH radical scavenging assay.

The results of phytochemical screening showed that all extracts contain flavonoids, saponins, triterpenes, steroids and tannins. This study demonstrated antioxidant and antimicrobial properties for the studied species, and showed interesting correlation between phytochemical constituents and biological activities

مستخلص البحث

اجريت اختبارات فعالية مضادات الاكسده وفعالية مضادات البكتريا لثلاثة نباتات (الراوند ،السذاب و البان) المستخدمه في الطب الشعبى في السودان .تم اجراء المسح الكيميائي للمكونات الطبيعيه في هذه النباتات المسوؤله عن الفعاليه البيولوجيه حيث تمت عملية التجزئه للمستخلص الكحولى باستخدام مذيبات : كلوروفورم ، ايثايل اسيتات والبيوتانول علي التوالي , جميع المستخلصات اختبرت فعالتها البيولوجيه علي عدد من البكتريا الموجبة القياسيه (البكتريا العصويه الرقيقه والبكتريا الكرويه العنقوديه الذهبيه) و البكتريا السالبه القياسيه (الإشريكية القولونية وبكتريا الزائفة الزنجارية)وكذلك طفيل المُبيَضّة البيضاء واجريت هذه الاختبارات بطريقه الانتشار. كذلك اختبرت فعالية جميع المستخلصات كمضدات اكسده وذلك باستخدام طريقه قبض الجذر الحر لمركب ثنائي فينيل بيكريل هيدرازيل .

نتائج المسح الكيميائي للمستخلصات اظهرت انها تحتوي على الصابونينات ، التانينات ، القلويدات، الفلافونويدات والاسترويدات وقد اوضحت هذه الدراسه العلاقه بين الفعاليه البيولوجيه والمكونات الفعالة لهذة النبات .

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Chapter one

INTRODUCTION

1.1-Medicinal plants

Medicinal plants have traditionally occupied an important position in the socio-cultural, spiritual and medicinal arena of rural and tribal lives in Sudan. Through its long history, the Sudan has witnessed the fusion of many cultures, Pharonic, Christian and Islamic along with thelocal indigenous cultures. With this unique history and vast variety of climate and flora, traditional medicine together with use of medicinal plants became an important part of the cultural heritage of the Sudan (El kalifa et al., 1999) Sudan is the largest country in Africa with a diverse flora. Most of the Sudanese people in rural areas rely on traditional medicine for the treatment of many infectious diseases. Sudanese traditional medicine is characterized by a unique combination of knowledge and practices of Arabic, Islamic and African culture (El Hamidi 1970, El Kamali and El Khalifa 1997). Medicinal plants have been used for centuries as remedies for human diseases because they contain components of therapeutic value. Recently, the acceptance of traditional medicine as an alternative form of health care and the development of microbial resistance to the available antibiotics has led authors to investigate the antimicrobial activity of medicinal plants (Bisignano et al. 1996; Lis-Balchin and Deans 1996; Maoz and Neeman 1998; Hammer et al. 1999). Moreover, the increasing use of plant extracts in the food, cosmetic and pharmaceutical industries suggests that, in order to obtain active compounds, a systematic study of medicinal plants is very important**.**

Human beings have used plants for the treatment of diverse ailments for thousands of years (Sofowara, 1982; Hill, 1989). According to the World Health Organization, most populations still rely on traditional medicines for their psychological and physical health requirements (Rabe and Van Stoden, 2000),

since they cannot afford the products of Western pharmaceutical industries (Salie*et al.*, 1996), together with their side effects and lack of healthcare facilities (Griggs *et al*., 2001). Rural areas of many developing countries still rely on traditional medicine for their primary health care needs and have found a place in day-to-day life. These medicines are relatively safer and cheaper than synthetic or modern medicine (Iwu*et al.*, 1999; Idu*et al*., 2007; Mann *et al*., 2008; Ammara*et al.*, 2009). People living in rural areas from their personal experience know that these traditional remedies are valuable source of natural products to maintain human health, but they may not understand the science behind these medicines, but knew that some medicinal plants are highly effective only when used at therapeutic doses (Maheshwari*et al.*, 1986; Van Wyk*et al.*, 2000).

Herbal medicines are in great demand in both developed and developing countries as a source of primary health care owing to their attributes having wide biological and medicinal activities, high safety margins and lesser costs. Herbal molecules are safe and would overcome the resistance produced by the pathogens as they exist in a combined form or in a pooled form of more than one molecule in the protoplasm of the plant cell (Lai and Roy, 2004; Tapsell*et al.*, 2006). Even with the advent of modern or allopathic medicine, Balick and Cox (1996) have noted that a number of important modern drugs have been derived from plants used by indigenous people.

Traditional use of medicine is recognized as a way to learn about potential future medicines. Researchers have identified number of compounds used in mainstream medicine which were derived from "ethnomedical" plant sources (Fabricant and Farnsworth, 2001). Plants are used medicinally in different countries and are a source of many potent and powerful drugs (Srivastava, *et al*., 1996; Mahesh and Sathish, 2008).

1.2-Natural antibiotic properties of plant secondary metabolites

The plant chemicals are classified as primary or secondary metabolites. Primary metabolites are widely distributed in nature, occurring in one form or another in virtually all organisms. In higher plants such compounds are often concentrated in seeds and vegetative storage organs and are needed for physiological development because of their role in basic cell metabolism. Primary metabolites obtained from higher plants for commercial use are high volume-low value bulk chemicals (e.g. vegetable oils, fatty acids, carbohydrates etc.)

Plants generally produce many secondary metabolites which are biosynthetically derived from primary metabolites and constitute an important source of microbicides, pesticides and many pharmaceutical drugs . From a long period of time medicinal plants or their secondary metabolites have been directly or indirectly playing an important role in the human society to combat diseases (Wink *et al.*, 2005).

Secondary metabolites (compounds) have no apparent function in a plant's primary metabolism, but often have an ecological role, as pollinator attractants, represent chemical adaptations to environmental stresses or serve as chemical defense against micro-organisms, insects and higher predators and even other plants (allelochemics). Secondary metabolites are frequently accumulated by plants in smaller quantities than the primary metabolites (Karuppusamy**,** 2009; Sathishkumar and Paulsamy, 2009).

In contrast to primary metabolites, they are synthesized in specialized cell types and at distinct developmental stages, making their extraction and purification difficult. As a result, secondary metabolites that are used commercially as biologically active compounds, are generally high value-low volume products than the primary metabolites (e.g. steroids, quinines, alkaloids, terpenoids and flavonoids), which are used in drug manufacture by the pharmaceutical industries. These are generally obtained from plant materials by steam

distillation or by extraction with organic or aqueous solvents and the molecular weight are generally less than 2000.

Some biologically active plant compounds have found application as drug entities or as model compounds for drug synthesis and semi-synthesis. A survey of current pharmaceutical use revealed that, of the total prescription drugs dispensed, 25% are plant derived (Farnsworth and Morris, 1976; Ogundipe*et al.*, 1998). Plant compounds are highly varied in structure; many are aromatic substances, most of which are phenols or their oxygen-substituted derivatives. However, there is an increased attention on extracts and biologically active compounds isolated from plant species used in herbal medicine, due to the side effects and the resistance that pathogenic micro-organisms build against the antibiotics (Essawi and Srour, 1999). New compounds inhibiting microorganisms such as benzoin and emetine have been isolated from plants (Cox, 1994). Of the various pharmaceuticals used in modern medicine, aspirin, atropine, ephedrine, digoxin, morphine, quinine, reserpine and tubocurarine serve as examples of drugs discovered through observations of indigenous medical practices (Gilani and Rahman, 2005). Eloff (1999) stated that the antimicrobial compounds from plants may inhibit bacteria by a different mechanism than the presently used antibiotics and may have clinical value in the treatment of resistant microbial strains.

Plant constituents may be isolated and used directly as therapeutic agents or as starting materials for drug synthesis or they may serve as models for pharmacologically active compounds in drug synthesis. The general research methods includes proper selection of medicinal plants, preparation of crude extracts, biological screening, detailed chemo pharmacological investigations, toxicological and clinical studies, standardization and use of active moiety as the lead molecule for drug design (Wink *et al.*, 2005).

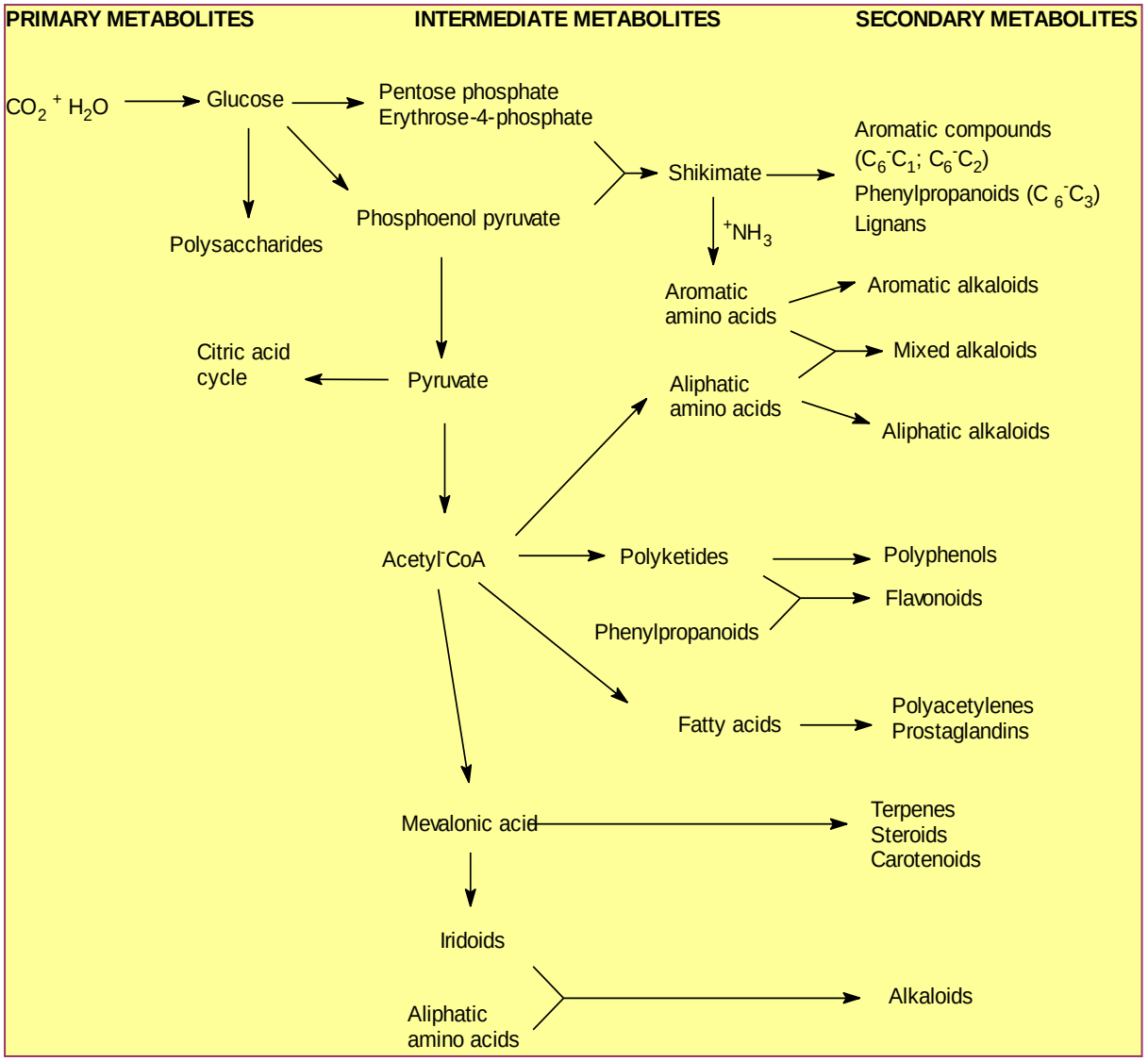

Figure 1.1: biosynthesis of secondary metabolites

1.3-Alkaloids

Alkaloids are naturally occurring chemical compounds containing basic nitrogen atoms and are produced by a large variety of organisms including bacteria, fungi, plants, and animals. Many alkaloids are toxic and often have a pharmacological effect, which makes them to be used as medications and recreational drugs. Some alkaloids have a bitter taste (Manske, 1965; Guillermo and Victor**,** 1999).

1.4-Flavonoids

Flavonoids are derived from 2-phenylchromen-4-one (2-phenyl-1-4-benzopyrone) and are commonly known for their antioxidant activities. Flavonoids, which are widely distributed in plants, fulfill many functions including producing yellow, red or blue pigmentation in flowers and protection from attacks by microbes and insects. Compared to other active plant compounds, they are low in toxicity. Flavonoids are referred to as nature's biological response modifiers because of their inherent ability to modify the body's reaction to allergens, viruses and carcinogens. They show anti-allergic, anti-inflammatory, antimicrobial and anticancer activity (Rauha*et al.*, 2000; Cushnie and Lamb, 2005; Filippos*et al.*, 2007; Spencer and Jeremy, 2008).

1.5-Saponins

Saponins are the glycosides of 27 carbon atom steroids, or 30 carbon atom triterpenes in plants. They are found in various plant parts; leaves, stems, roots, bulbs, flowers and fruits. They are characterized by their bitter taste and their ability to haemolyze red blood cells. They are used medically as expectorant, emetic and for the treatment of excessive salivation, epilepsy, chlorosis and migraines. They are used in Ayurvedic medicine as a treatment for eczema, psoriasis and for removing freckles. Saponins are believed to be useful in the human diet for controlling cholesterol. Digitalis-type saponins strengthen the heart muscle causing the heart to pump more efficiently (Oakenfull and Sidhu, 1990). Saponins also inhibit cancer tumor growth in animals, particularly, lung and blood cancers, without killing normal cells. Saponins are the plant's immune system acting as an antibiotic to protect the plant against microbes and fungus (Shideler, 1980; Chatterrjee and Chakravorty, 1993).

1.6-Anthraquinones

Anthraquinones are aromatic organic compounds and is a derivative of anthracene. It has the appearance of a yellow or light-gray to gray-green, solid, crystalline powder. It is fairly stable under normal conditions.

Anthraquinonesnaturally occur in some plants, fungi, lichen and insects, wherein they serve as a basic skeleton for their pigments. Anthraquinones are used in the production of dyes and are also used as a laxative (Chatterrjee and Chakravorty, 1993; Samp, 2008).

1.7-Cardiac glycosides

Cardiac glycosides are drugs used in the treatment of congestive heart failure and cardiac arrhythmia. These glycosides are found as secondary metabolites in several plants and in some animals. Some of these compounds are used as arrowhead poisons in hunting (Filippos*et al*., 2007).

1.8-Antimicrobial activity of plants

Medicinal plants have always been considered as a source for healthy life for people. Therapeutical properties of medical plants are very useful in healing various diseases and the advantage of these medicinal plants are natural (Kalemba and Kunicka, 2003). In many parts of the world, medicinal plants have been used for its antibacterial, antifungal and antiviral activities for hundreds of years (Ali *et al.,* 1998; Barbour *et al.,* 2004; Yasunaka*et al.,* 2005). Researchers are increasingly turning their attention to natural products and looking for new leads to develop better drugs against cancer, as well as viral and microbial infections (Ibrahim, 1997; Towers *et al*., 2001; Koshy*et al*., 2009).

Antimicrobial studies have shown that Gram-negative bacteria show a higher resistance to plant extracts than Gram-positive bacteria. This may be due to the variation in the cell wall structures of Gram-positive and Gram-negative bacteria. More specifically, Gram-negative bacteria has an outer membrane that is composed of high density lipopolysaccharides that serves as a barrier to many environmental substances including antibiotics (Paz *et al.*, 1995; Vlietinck*et al.*, 1995; Kudi*et al*., 1999; Palambo and Semple, 2001). Although hundreds of plant species have been tested for antimicrobial properties, the vast majority have not been adequately evaluated (Onwuliri and Dawang**,** 2006; Mahesh and Sathish, 2008).

The antimicrobial compounds found in plants may prevent bacterial infections by different mechanisms than the commercial antibiotics and therefore may have clinical value in treating resistant microorganism strains (Eloff, 1999). The indiscriminate use of antibiotics has resulted in many bacterial pathogens rapidly becoming resistant to a number of originally discovered antimicrobial drugs (Barbour *et al*., 2004). There is, thus, a continuous search for new antibiotics, and medicinal plants may offer a new source of antibacterial agents. This is indeed very important because *Candida albicans, Staphylococcus aureus, Pseudomonas aeruginosa*and *Escherichia coli* are some of the important human pathogens that have developed resistance to antimicrobials (Barbour *et al.,* 2004).

Microorganisms are very diverse and even though their different cells look similar in morphology and produce similar colonies, it becomes necessary to identify the organisms by their biochemical characteristics that help to properly classify the organisms, causing diseases that kill people, animals and plants .

1.8.1-*Staphylococcus aureus*

is a common coloniser of human skin and mucosa. *S. aureus*can cause disease, particularly, if there is an opportunity for the bacteria to enter the body. Prescott *et al*., (2005) states that *S. aureus*is the most important human staphylococcal pathogen and causes boils, abscesses, wound infections, pneumonia, toxic shock syndrome amongst other diseases.*S. aureus*is also a pathogen frequently reported to produce food poisoning, which leads to cramps and severe vomiting. Most strains of this bacterium are sensitive to many antibiotics, and infections can be effectively treated (Abbas *et al.,* 2004).

1.8.2-*Pseudomonas aeruginosa*

is an opportunistic pathogen and exploits some break in the host defenses to initiate an infection. It is a common environmental microorganism present in water and soil and is notorious for its resistance to antibiotics and is, therefore, a particularly dangerous and dreaded pathogen (Prescott *et al*., 2005). The

bacterium is naturally resistant to many antibiotics due to the impermeability characteristics of the outer membrane. Moreover, its tendency to colonize surfaces in a biofilm form makes the cells impervious to therapeutic concentrations of antibiotics (Craig, 1997; Okemo*et al.,* 2001).

1.8.3-*Bacillus subtilis*

is a food-poisoning, Gram-positive, facultative, aerobic, sporulating bacteria normally found in soil. *B. subtilis*is normally considered as being non-pathogenic; but it has been linked to food-borne illnesses, causing diarrhoea, nausea, vomiting, and associated with rice dishes served in oriental restaurants and its infection is self-limiting (Willey *et al*., 2008). *B. subtilis*produces subtilism, which is an extracellular enzyme that catalyzes the breakdown of proteins into polypeptides, resembles trypsin in its action, and has been shown to be a potent occupational allergen (Willey *et al.,* 2008).

1.8.4-*Escherichia coli*

are usually found in the gastro-intestinal tracts of warm blooded organisms. The most common cause of urinary tract infection in humans is *E. coli,* causing at least five types of gastro-intestinal diseases in humans. Pathogenicity is generally due to the presence of one or more virulence factors, including invasiveness factors, heat-liable and heat-stable enterotoxins, verotoxins and colonization factors. Pathogenic strains are usually identified by detection of specific virulence factors or of a serotype associated with a virulence factor (Willey *et al*., 2008).

E. coli is an emerging cause of food-borne infection which leads to bloody diarrhoea and occasionally to kidney failure. Most cases of the illness have been associated with eating under-cooked, contaminated, ground beef. Person-to-person contact in families and child care centers is also an important mode of transmission if hygiene is inadequate. *E. coli* infection can also occur after drinking raw milk and after swimming or drinking contaminated water (Akinnibosun*et al.,* 2008).

1.8.5-Candida albicans

is the most common organism implicated in fungal infections, which is found in the human digestive tract, mouth, and genital region (Eggiman and Garbino, 2003). Under normal circumstances, levels of *Candida* are controlled by beneficial bacteria. However, if the bacteria-fungus balance is upset, by the use of antibiotics or if the immune system is compromised, an overgrowth of *Candida* can occur, resulting in infection (Braunwald and Kasper, 2001).

1.9-Antioxidant of medicinal plants

In living systems, oxidation is a basic part of the normal metabolic process, in which Reactive oxygen species (hydrogen peroxide and hypochlorous acid) and many free radicals (hydroxyl radical (OH) and superoxide anion) are generated (Finkel and Holbrook, 2000; Halliwell, 2000; Pietta, 2000; Vijayabaskaran*et al.*, 2010). Rapid production of free radicals may cause alteration in the structure and function of cell constituents and membranes and can results in human neurologic and other disorders such as cancer, diabetes, inflammatory disease, asthma, cardiovascular, neurodegenerative diseases, and premature aging (Mclarty, 1997; Young and Wood, 2001; Yang *et al.*, 2001; Sun *et al.*, 2002; Bimal*et al.*, 2011). Therefore, the prevention of the above conditions requires the presence of antioxidants or the free radical scavenging molecules in the body.

There are plenty of antioxidant substances present in plants (fruits, vegetables, medicinal herbs, etc.) and the free radical scavenging molecules present in them are in the form of phenolic compounds (e.g. phenolic acids, flavonoids, quinones, coumarins, lignans, tannins), nitrogen compounds (alkaloids, amines), vitamins, terpenoids (including carotenoids), and some other endogenous metabolites, (Zheng and Wang 2001; Cai*et al.*, 2003; Govindarajan*et al.*, 2005; Naruthapata and Supaporn, 2009).

The most commonly used methods for measuring antioxidant activity are those which involve the generation of free radicals which are then neutralized by

antioxidant compounds. DPPH is a well-known radical and a trap ("scavenger") for other radicals (Husain *et al.*, 1987, Visioli*et al*., 2000; Parr *et al*,. 2004; Solai*et al.*, 2010). Therefore, rate reduction of a chemical reaction upon addition of DPPH is used as an indicator of the radical nature of that reaction. Because of a strong absorption band centered at about 520 nm, the DPPH radical has a deep violet color in solution, and it becomes colorless or pale yellow when neutralized. This property allows visual monitoring of the reaction, and the number of initial radicals can be counted from the change in the optical absorption at 520 nm. DPPH method measures electron donating activity of other compounds in the mixture and hence provides an evaluation of antioxidant activity due to free radical scavenging. Any molecule that can donate an electron or hydrogen will react with DPPH, thus neutralizing its colour from a deep purple to a light yellow by electrons from the oxidant compounds. The concentration of DPPH at the end of a reaction will depend on the concentration and the structure of compound being scavenged (Naik*et al.*, 2005; Balasundram*et al.*, 2006; Masoko, 2007).

1.10- plants under study

i)Rutagraveolens(Rue ,herb-of –grace**).**Family: Rutaceae

Habitat: Rocks, old walls and dry hills, mainly on limestone

Rue is a small evergreen subshrub or semiwoody perennial 2-3 ft (0.6-0.9 m) tall and almost as wide. The stems become woody near the base, but remain herbaceous nearer the tips. The 3-5 in (7.6-12.7 cm) long leaves are dissected pinnately into oblong or spoon shaped segments.

Figure *1.2: Rutagraveolens*

The commonly known phytochemical compounds from *R. graveolens*are acridone alkaloids, coumarins, volatile substances, terpenoids, flavonoids and furoquinolines (kuzovkina et al., 2004). The existence of saponin, tannins and glycosides has also been proven (Hashemi et al., 2011) .

Extracts from *R. graveolens* have been used as an antidote for toxins such as snake and scorpion venoms (Sallal and Alkofahi, 1996). For a long time, R. graveolens has been used as a folklore medicine for treatment of various conditions such as eye problems, rheumatism, dermatitis, pain and many inflammatory diseases (Ratheesh and Helen, 2007)

ii)Rheum palmatum(Rhubarb,Atrosanguineum or Da huang)

This species belongs to the family :polygonaceae. It usually grows in forest edge near the mountain or grassy slopes, wild or cultivated.

The plant has stout rhizomes. Stems are erect, about 2m high, hollow, smooth, and hairless. Large basal leaves are with stout fleshy long handle, which is in similar length with the leaf. Inflorescence is large panicles, with terminal flowers. Pedicels are slender and with joints in the middle to lower part. Flowers are purplish red or mixed with red purple. Dark brown achene has 3 ridges, wings along the ridges, hollow top, and heart-shaped base. It blossoms from June to July and fruits from July to August

Figure 1.3: Rheum palmatum

Rheum palmatum has anthraquinone glycosides and dianthrone glycosides, which are the main cause why it is used as a laxative. Compared to its aglycone, these components have a stronger purgative effect. Anthraquinone glycosides include: chrysophanol-1-monoglucoside or chrysophaein, emodin-6-monoglucoside, aloe-emodin-8-monoglucoside, physcionmonoglucoside, hrein-8-monoglucoside.Rheum palmatum L. also contains emodindiglucoside, aloe-emodindiglucoside, and chrysophanoldiglucoside. Dianthrone glycosides include sennoside A、B、C、D、E、F. Free anthraquinones include chrysophanol, emodin, physcion, aloe-emodin, rhein. In addition, rhubarb also contains fatty acid, calcium oxalate, glucose, fructose and tannins (5% - 10%), gallotannin, catechin, procyanidin, pectin and phenolic carboxylic acids(Ball,1992).-**Folkloric uses include:**

1- Rhubarb can increase peristalsis, inhibit intestinal absorption of water and promote defecation
2- Rhubarb has anti-inflammatory effects on a variety of Gram-positive and-negative bacteria. The most sensitive ones are staphylococcus and streptococcus, which are followed by diphtheria, typhoid and paratyphoid bacillus, pneumococcus, Shigella
3- Rhubarb also inhibits influenza virus

4- Rhubarb cause constipation after the diarrhea due to the tannin contained

5- Rhubarb has cholagogue and stomachic effects

6- Rhubarb stops bleeding, protect liver, lower blood pressure reduce serum cholestero

iii)**Moringaperegrina(**Ben tree, wispy-needled yasartree,wild drum-stick tree)

This species belong to the family Moringaceae. It is a deciduous tree, 3-10 m high, green, glaucous with erect trunk, and white bark. Leaves are 30 cm long, the axes persistent, imparipinnate with early deciduos leaflets. Each leaf is formed of 3 pairs of long, slender junciform pinnae looking like opposite virgate branches. Leaflets are remote, small, oblong. Flowers appear before leaves in May. The pendulous pods ripen in October. The pod is pendulous and contains angled, nut-like white seeds (behen nuts) which are of bitter sweet taste and rich in oil (ben oil). Flowering and fruiting: February-Apri (Ball, J. 1992).

Figure *1.4: Moringa peregrine*

Moringa peregrine has a wide geographic range, growing from the Dead Sea area sporadically along the Red Sea to northern Somalia and around the Arabian Peninsula to the mouth of the Arabian (Persian) Gulf, Red sea coast Sinai Mountains (Täckholm, 1974; Boulos, 1999).

*Moringaperegrina*contains a high level of oleic (70.5%), followed by gadoleic (1.5%), while the dominant saturated acids are palmitic (8.9%) and stearic (3.82%). Tocopherols were also detected. B-sitosterol was found as the most predominant component of the sterolic fraction of the oil. Campesterol, stigmasterol, brassicasterol and cholesterol were also found (Batanouny,1999). *Moringaperegrina*is highly nutritious, which contains more vitamin A than carrots, more calcium than milk, more iron than spinach, more vitamin C than oranges and more potassium than bananas and more protein than milk and eggs (Sreelatha,2011). The plant is said to treat headaches, fevers constipation, burns, abdominal pains, back and muscle pains and labor pains (Boulos, 2000). treated many diseases such as inflammation, cardiovascular and liver diseases, immune boosting agent, blood sugar and cholesterol regulator (Rao and Misra, 1998).

Chapter two

Material and Methods

2.1. Materials

2.1.1 Plant material

The stems of *Moringaperegrina* were collected from Khartoum State . Fruits of *Rutagraveolens* and stems of *Rheum palmatum*were purchased from the local market-Khartoum.The plant was authenticated by the Department of Phytochemistry and taxonomy, National Research Center, Khartoum.

2.2. Methods

2.2.1 Preperations of reagents for phytochemical screening.

i)Flavonoid and phenolic test reagents

- Alumnium chloride solution

1 g of aluminum chloride was dissolved in 100 ml methanol

- Potassium hydroxide solution

1 g of potassium hydroxide was dissolved in 100 ml distilled water.

-Ferric chloride solution

1 g of ferric chloride was dissolved in 100 ml methanol.

ii)Alkaloid test reagents

Maeyer reagent

- Mercuric chloride solution: 1.36 g in 60 ml. distilled water.

- Potassium iodide solution : 5 g in 10 ml. distilled water

The two solutions were combined and then diluted with distilled water up to 100 ml.

-Wagner reagent

1.27 g iodine and 2 g of potassium iodide in 100 ml distilled water.

2.2.2 Preparation of plant extracts for phytochemicalscreening

100 g of powdered air- dried plant material were extracted with 80% aqueous methanol (soxhelt) until exhaustion. This prepared extract(PE) was used for phytochemical screening.

2.2.3.1- Test for unsaturated sterols and for triterpenes

10 ml of the (PE) was evaporated to dryness on a water bath, and the cooled residue was stirred with petroleum ether to remove most of the coloring materials. The residue was then extracted with 10 ml chloroform. The chlorform solution was dehydrated over sodium sulphite anhydrous. 5 ml portion of the solution was mixed with 0.5 ml of acetic anhydride, followed by two drops of concentrated sulphuric acid. Two separate layers (green, red) were observed.

2.2.3.2- Test for flavonoids

20 ml of the (PE) was evaporated to dryness on water bath. The cooled residue was defatted with petroleum ether and then dissolved in 30 ml of 30% aqueous methanol and filtered. The filtrate was used for the following tests:

- To 3 ml. of filtrate a fragment of magnesium ribbon was added, shaken and then few drops of concentrated hydrochloric acid were added. Red colour was observed.
- To 3 ml. of the filtrate few drops of aluminium chloride solution were added. A dark yellow colour was formed.
- To 3 ml. of the filtrate few drops of potassium hydroxide solution were added. A dark yellow colour was observed.

2.2.3.3- Test for alkaloids

10 ml of the (PE) were evaporated to dryness on water bath and 5 ml of 0.2N hydrochloric acid were added and the solution was heated with stirring for minutes, then cooled and divided into two portions:

To one portion a few drops of Maeyer reagent were added. A white precipitated appeared, to the other portion few drops of Wagner reagent were added. A brown precipitate appeared.

2.2.3.4- Test for tannins

10 ml of (PE) was evaporated to dryness and the residue was extracted with n-hexane and then filtrated. The insoluble residue was stirred with n-hexane and 10 ml of hot saline (0.9% w/v of sodium chloride and freshly prepared distilled water) were added. The mixture was cooled , filtrated and the

volume adjusted to 10 ml. with more saline solution. 5 ml of this solution was treated with few drops of ferric chloride solution. A dark blue colour was observed.

2.2.3.5- Test for Saponins

1 g of dried powdered plant material was placed in a clean test tube. 10 ml of distilled water were added and the tube was stoppered and vigorously shaken for about 30 seconds, and allowed to stand. Honey comb was formed.

2. 3- Biological activity

2.3.1- Preparations of crude ethanolic,chloroform,ethyl acetate and n-butanol fractions for biological study

- Ethanol extract: of *Combretumaculeatum* was prepared by macerating 100g of the air dried powdered leaves in successive portions of methanol (100%) till exhaustion. The methanolic extract was evaporated under reduced pressure to obtain a semi – solid residue.

- Chloroform fraction was prepared by suspending the semisolid residue obtained from the ethanolic extract in the least amount of distilled water, then shaking with successive portions of ethyl acetate till exhaustion. The ethyl acetate extract was evaporated under reduced pressure to obtain a residue. In the same manner the n-butanol and ethyl acetate fractions were obtained.

2.4DPPH radical scavenging assay:

The DPPH radical scavenging was determined according to the method of Shimada et. al.(1992). with some modification. In 96-wells plate, the test samples were allowed to react with 2.2Di (4-tert-octylphenyl)-1-picryl-hydrazyl stable free radical (DPPH) for half an hour at 37ºC. The concentration of DPPH was kept as (300μM). The test 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 samples was determined in comparison with a DMSO treated control group. All tests and analysis were run in triplicate.

Chapter three :Results and Discussion

3.1-Phytochemical screening

Rheum palmatum ,*Moringaperegrina* and *Rutagraveolens* were screened for major secondary metabolites and results are displayed in Table 1 .

Table 1 : Phytochemical screening of target species

Test	*Rheum palmatum*	*Rutagraveolens*	*Moringaperegrina*
Saponins	-	-	+++
cumarins	+	-	+
Tannins	++	++	+++
Alkaloids	+++	+	-
flavonoid	+++	+++	+++
steroid	+	+	+++
Tritrpens	++	+	+++

3.2 antimicrobial activity

The target species were also evaluated for their antimicrobial activity against five standard human pathogens(Table 5). The results were interpreted in commonly used terms (<9mm:inative; 9-12mm: partially active;13-18mm: active;>18mm:very active).Tables (6) and (7) represent the antimicrobial activity of standard antibacterial and antifungal chemotherapeutic agents against standard bacteria and fungi respectively.The inhibition zones for *Rheum palmatum* are displayed in table (2).

Table 2: Inhibition zones of *Rheum palmatum* fractions

Extracts	EC	PS	Sa	BS	Ca
Ethanol Crude	15	8	11	19	19
Chloroform	11	18	20	18	18
Ethyl acetate	16	14	13	--	12
n-butanol	--	14	16	--	--

All extracts ,except the chloroform fraction gave excellent activity against the bacterial strains -*Bacillus subtilis* and *Staphylococcus aureus*. The ethyl acetate fraction showed excellent activity against *Pseudomonas aeruginosa*.The chloroform fraction showed moderate activity against all test organisms ,except *Escherichia coli*.Also the ethanolic extract was inactive against *Escherichia coli*.All fractions failed to show antifungal activity except the ethanolic fraction.

The inhibition zones for *Rutagraveolens* are displayed in Table (3). Both of the chloroform and ethanol fractions gave excellent antifungal acivity against the test fungi. Furthermore, the chloroform fraction gave excellent activity against the bacterial strains :*Pseudomonas aeruginosa* and *Staphylococcus aureus*.The ethyl acetate fraction exhibited moderate activity against *Pseudomonas aeruginosa* and good activity against *Escherichia coli*.On the other hand, the n-butanol fraction showed good activity against *Staphylococcus aureus* and moderate activity against *Pseudomonas aeruginosa*.

Table 3: Inhibition zones of *Rutagraveolens* fractions

Extracts	EC	**PS**	**Sa**	**BS**	Ca
Ethanol	10	--	23	20	--
chloroform	--	--	19	18	--
Ethyl acetate	17	18	23	20	16
n-butanol	17	--	25	25	24

The zones of inhibition for different fractions of *Moringaperegrina* are displayed in Table (4).

Extract	Ec	Ps	Sa	Bs	Ca
Ethanol	--	14	25	22	17
chloroform	--	15	14	15	--
Ethyl acetate	17	20	22	27	--
n-butanol	14	14	18	20	--

Table 4: Inhibition zones of *Moringaperegrina* fractions

The ethanolic extract showed excellent antibacterial activity against *Staphylococcusaureus* and *Bacillus subtillus*. The same trend was detected for the n-butanol and ethyl acetate fractions. chloroform fraction showed significant activity against these bacteria. Furthemore , both of the ethyl acetate n-butanol fractions displayed significant activity against *Escherichia coli*. The ethyl actate was also very active against *Pseudomonas aeruginosa*. While the ethanol and chloroform fractions did not exhibit any antifungal activity, the n-butanol fraction showed strong activity against the fungus *Candida albicans*. The ethyl acetate showed good activity against the same bacterial strain.

Table 5: Test organisms

Ser. No	Micro organism	Type	Source
1	*Bacillus subtillus*	G+ve	ATCC 2836
2	*Staphylococcus aureus*	G+ve	ATCC 29213
3	*Pseudomonas aeroginosa*	G-ve	NCTC 27853
4	*Escherichia coli*	G-ve	ATCC 25922
5	*Aspergillus Niger*	fungi	ATCC 9736
6	*Candida albicans*	fungi	ATCC 7596

* NCTC. National collection of type culture, Colindale. England

* ATCC. American type culture collection, Maryland, USA

Table (6) : Antibacterial activity of standard chemotherapeutic agents

Drug	Conc. mg/ml	Bs.	Sa.	Ec.	Ps.
Ampicillin	40	15	30	-	-
	20	14	25	-	-
	10	11	15	-	-
Gentamycin	40	25	19	22	21
	20	22	18	18	15
	10	17	14	15	12

Table (7) : Antifungal activity of standard chemotherapeutic agent

Drug	Conc. mg/ml	An.	Ca.
Clotrimazole	30	22	38
	15	17	31
	7.5	16	29

- Sa.*: Staphylococcus aureus*
- Ec.*: Escherichia coli*
- Pa.: *Pseudomonas aeruginosa*
- Ca.: *Candida albicans*
- *Bs.: Bacillus subtilis*
- M.D.I.Z: Mean diameter or growth inhibition zone (mm)..

3.3 Antioxidant activity

Rheum palmatum .Rutagraveolens.Moringa peregrine were evaluated for Antioxidant activity against stable (DPPH) radical.

The DPPH radical scavenging was determined according to the method of Shimada et. al.(1992). with some modification. In 96-wells plate, the test samples were allowed to react with 2.2Di (4-tert-octylphenyl)-1-picryl-hydrazyl stable free radical (DPPH) for half an hour at 37ºC. The concentration of DPPH was kept as (300µM). The test 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 samples was determined in comparison with a DMSO treated control group. All tests and analysis were run in triplicate.

Table 8 Antioxidant activity of target species

No.	Sample Code	%RSA ±SD (DPPH)
1	Crude ethanol (1)	57± 0.14
2	Fraction (1) chloroform	32± 0.02
3	Fraction (1) E.A	67± 0.04
4	Fraction (1) n. B	82± 0.06
5	Crude ethanol (2)	62± 0.01
6	Fraction (2) chloroform	19± 0.01
7	Fraction (2) E.A	89± 0.01
8	Fraction (2) n. B	85± 0.02
9	Crude ethanol (3)	86± 0.02
10	Fraction (3) chloroform	77± 0.05
11	Fraction (3) E.A	80± 0.03
12	Fraction (3) n. B	94± 0.01
Stander	Propyl Gallate	89± 0.01

1: Rheum palmatum

2:Rutagraveolens

3: Moringa peregrine

The n-butanolfraction of rheum palmatum showed signification Antioxidant activity , while both n-butanol and ethyl acetate fractions of rutagraveolensgpave excellent radical scavenhimg properties.

All fraction of Moringa peregrine – specially – the n-butanol fraction exhibited significant antioxidant activity .

Conclusion

Phytochemical study was piloted to detect the bioactive compounds which might have been responsible for the biological. The crude ethanolic extract was suspended in water and extracted successively by: chloroform, ethyl acetate and n-butanol antimicrobial and anti oxidant activities and significant results were obtained.

Recommendation

The bioactive constituents of the target species may be isolated and identified and then evaluated for the antimicrobial and antioxidant potential.

The crude extracts and isolates could be evaluation for other biological activities like antimalarial. Anti-inflammatory, antileshmenial ..etc

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