**Sudan University of Science and Technology**

**College of Graduate Studies**

# Constituents of Maharaib Herb(*Cympobogon Proximus*) and Its Efficiency in Removing Heavy Metals from Aqueous Media

# مكونات عشبة المحريب وفعاليتها في إزالة العناصر الثقيلة من الأوساط المائية

**A thesis Submitted in Partial Fulfillment for the Requirement of Master Degree in Chemistry**

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**صدق الله العظيم**

التوبة: ١٠٥

# DEDICATION

**To my parents and husband**

**Brothers and sisters**

**Close relatives**

**With love and respect**

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First and foremost, praises and thanks to the Allah, the Almighty, for His showers of blessings throughout my research work to complete the research successfully.

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# Abstract

*Cymbopogon proximus* is considered one of medicinal plant which is widely used especially in Sudan. The present study was conducted to evaluate the phytochemical composition of the leaves extracts (Distilled Water and Chloroform) of *C.proximus*. In Preliminary phytochemical analysis showed that the aqueous extraction possess higher amount of phytochemical constituents compared to chloroform, the results showed that the saponins ,tannins and terpenes were found in a large quantities comparing to others. The amount of essential oil obtained by hydro distillation was 4.46%w/w. the chemical composition of essential oil of *C.proximus* was analyzed by GC/MS system. A total of 13 constituents were identified; Piperitone (43.2%), elemol ( 15.45%), 4-carene (7.55%), β-eudesmol (7.49%), limonene (4.92%) and ɣ-eudesmol (4.38%) , where other minor components were less than 3 to 0.1%. The monoterpenes constitute (4-carene, 2-carene, limonene, p-menth-2-en-1-ol, Cis-p-mentha-2,8dien-1-ol, piperitone, 1,8-cineole) represented about (68.63%) of the oil and The oxygenated sesquiterpenes constitute (Elemol, ß-eudesomal, ɣ-eudesomal, Cuparene) represented about (29.74%). In another investigation the plant was used according to their folkloric use to remove heavy metals from water. The heavy metals in this study were analyzed using Atomic Absorption Spectrophotometer (AAS) and the results showed that *C.proximus* has a good capability to remove the lead and zinc from aqueous medium, the lead concentration was decreased from 10.0ppm to 5.77 ,4.30 ,and 3.41 ppm at 10,20,30 minutes respectively and the zinc concentration was decreased from 10.0ppm to 7.52, 4.10 and 2.7 ppm at 10,20,30 minutes respectively when treated with 2.0(g) of plants powder, due to specific chemical structure of flavonoids which can chelate metal ions and form complexes.

# مستخلص البحث


يعتبر المحريب أحد النباتات الطبية التي تستخدم على نطاق واسع خاصة في السودان. اجريت هذه الدراسه لتحديد التركيب الكيميائي النباتي لمستخلصات الأوراق (الماء المقطر والكلوروفورم) للمحريب. وكشف التحليل الكيميائي النباتي الأولي للمستخلص المائي احتوائه على كميه عاليه من المكونات مقارنه بمستخلص الكلوروفورم واظهرت النتائج وجود الصابونين والمواد القابضه والتربينات بكميات كبيرة مقارنة بالمركبات الاخرى , ووجد ان كمية الزيت العطري المتحصل عليه بالتقطير المائي (4.46٪ وزن / وزن) ، المكونات الكيميائيه لزيت المحريب العطري تم تحليلها بواسطه جهاز كروماتوغرافيا الغاز البايبريتون (43.2%) ,2- كارين(9.07%)، اليمول(15.45%)، بيتا-ايدوسومال (7.49%) والليمونين (4.92%)، وجاما-ايدوسومال (4.38%) حيث تمثل المكونات الثانوية الأخرى أقل من 3 إلى 0.10٪ ,التربينات الاحاديه (4-كارين ,2- كارين ,ليمونين ,-p-menth-2-en-1-ol, Cis-p-mentha-2,8dien ,بايبريتون ,1,8-سينيول) تمثل حوالي (68.63%)،التربينات نصف الاحاديه (اليمول، جاما-ايدوسومال, بيتا-ايدوسومال, كوبارين) تمثل حوالي (29.74%). وفي دراسه تشخيصيه اخرى يستخدم هذا النبات وفقا لاستعماله الشعبي التقليدي لأزاله العناصر الثقيله من الاوساط المائيه ،العناصر الثقيله في هذه الدراسه تم تقديرها بجهاز الامتصاص الذري (AAS) النتائج لهذه الدراسه اظهرت ان المحريب له فعاليه جيده في ازاله الرصاص والزنك من المحاليل المائيه حيث نجد ان تركيز الرصاص تقلص من 10.0 ppm الى5.77 ,4.30 , 3.41 ppm عند20,10, 30 دقيقه على التوالي ونجد ان تركيز الزنك قد تقلص من 10.0 ppm الى 7.52, 4.10 ,2.77 ppm عند20,10, 30 دقيقه على التوالي وهذا بعد اضافه 2جرام من بدره المحريب وهذا نسبه لوجود الفلافونويد الذي يتميز بتركيبه خاصه تمكنه من تكوين معقدات مع ايونات المعادن .

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## Introduction & Literature Review

# CHAPTER ONE

## INTRODUCTION & LITREATURE REVIEW

### 1. General introduction

Natural products have been playing a significant role in human disease therapy and prevention. More than 60% and 75% of the chemotherapeutic drugs for cancer and infectious disease respectively are of natural origin, because since the beginning of human civilization, medicinal plants have been used by mankind for its therapeutic value. Almost 60% of the world population and about 80% of the population in developing countries rely on traditional medicine, mostly plant drugs, for their primary health care needs. And hence natural products play an important role in drug development programs in the pharmaceutical industry (Khalid et al., 1986).

The medicinal value of plants lies in some chemical substances due to secondary metabolites where most of them are bioactive constituents such as alkaloids, terpenoids, volatile oil, flavonoids and phenols that produce a definite physiological action in human body (Elujoba et al., 2005).

*Cymbopogon proximus* (locally named, Maharabe) from Family: Poaceae (Graminae), is a great interest due to its commercially valuable essential oils and widely used in traditional medicine, and thus the potentiality of *Cymbopogon proximus* essential oil which could be the alternative approach for the treatment of chronic diseases such as chronic Kidney disease and failure (Khalid et al., 2012). The Essential oil of *C. proximus* has a strong aromatic odor and has great medicinal value such that traditionally it is widely used as antispasmodic, a protection against fever, anti-intestinal ailment problems, anti-malarial, and anthelminthic (especially against Guinea worms) (Elujoba et al., 2005).

Here in Sudan the *Cymbopogon proximus* is used traditionally as a diuretic to inhibit kidney stone formation, an anti-infectious agent in urinary tract infections, an antibacterial and anti-fungal *Cymbopogon proximus* is used in the treatment of colds, epilepsy, abdominal cramps and pains, as well as in culinary and perfume products. It is therefore very important for this study to be carried out scientifically to prove the activities of this plant and to validate its traditionally claimed therapeutic properties (Hostettmann et al., 2000).

Some heavy metals have bio-importance as trace elements but the toxic effects of many of them in human are of great concern, although several adverse health effects of heavy metals have been known for a long time, exposure to heavy metals continues, and is even increasing in some parts of the world, particularly in less developed countries. The main threats to human health from heavy metals are associated with exposure to lead and zinc. The use of certain plant and plant extracts is reported to possess an ability to serve in water purification ( Eltahir and Ereish,2010).

main objectives of this study are to test certain Sudanese plant namely c.proximus for their ability to purify water from lead and zinc. Analysis of the heavy metals lead and zinc were performed before and after treatment of water with c.proximus.

Heavy metals are causing serious health hazard due to its toxicity and persistence in soil and aqueous medium too. Heavy metals contamination in drinking water has become a major cause of concern for the environmentalists. lead and zinc is widely distributed in aqueous medium in the Gangetic plain causing skin pigmentation and liver disorders. Several methods of removal of heavy metals from aqueous medium e.g. precipitation, adsorption, ion exchange are in practice medicinal plants can be utilized for the removal of heavy metals from aqueous medium. Medicinal plants e.g. Cymbopogon proximus. (Hostettmann et al., 2000).

## 1.2 LITREATURE REVIEW

### 1.2.1 Traditional medicine in Sudan

Sudan has been home to indigenous civilization, such as Meroe, and road for others, namely pharaonic, Christian and Islamic civilizations. The country has been heavily influenced by fusion of different cultures. The immigrant Arab culture and the neighboring cultures (mainly Egyptian and West African cultures) have strongly influenced Sudanese culture. However, there is a wide range of practices, which fall under the umbrella of traditional medicine.

Medicinal plants represent an important component of traditional medicine in Sudan and the flora of Sudan is relatively rich in medicinal plants corresponding to the wide range of ecological habitats and vegetation zones. These are coupled with ample inherited information in the field of medicinal plants and herbal traditional users which originally were unique blends of indigenous cultures of various nations (Khalid et al., 1986).

Similar to other developing countries, traditional medical practices play an important role in Sudan. Herbal drugs are of major importance in Sudanese folk medicine (Elujoba et al., 2005)

In the past people depended exclusively on traditional medicine. They also used some wild plants for cosmetics and perfume by extracting the oils with primitive methods. In recent years, however, medicinal plants have represented a primary health source for the pharmaceutical industry. Large quantities are used for the preparation of infusions and decoctions both in the countries where traditional medicine is still of great therapeutic, social and economic importance, and in the production of important pharmaceutical products.

The government is now giving greater attention to the cultivation of crops in demand on the world market. On the other hand, the government has imported some improved hybrids and varieties which have adapted to the Sudan environment eg. Datura inermis , Cymbopogon proximus and Hyocyamus niger.

If these plants are cultivated on a large scale, they could represent an important source of hard currency, besides satisfying the market needs.

## 1.2.2 Herbal Medicine

Herbs are used in many domains, including medicine, nutrition, flavorings, beverages, dyeing, repellents, fragrances and cosmetics.

Traditional herbal medicine as a major African socio-cultural heritage, obviously in existence for several hundreds of years, was once believed to be primitive and wrongly challenged with animosity, especially by foreign religions. However, today traditional medicine has been brought into focus for meeting the goals of a wider coverage of primary health care delivery, not only in Africa but also to various extents in all countries of the world.

Herbal medicine is still the mainstay of about 75-80% of the world population, mainly in the developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and lesser side effects. However, the last few years have seen a major increase in their use in the developed world (Elujoba et al., 2005).

The world Health Organization (WHO) has for several decades, supported, promoted and assisted the development of traditional medicine in the bid to move the African health agenda forward, particularly for the less-developed countries. The WHO has recently defined traditional medicine including herbal drugs as comprising therapeutic practices that have been in existence, often for hundreds of years, before the development and spread of modern medicine and are still in use today. Plants have a long history of use in the African continent for the treatment of different diseases and complaints. In certain African countries, up to 90% of the population still relies exclusively on plants as a source of medicines.

Despite the great advances observed in modern synthesis-based pharmacy, medicinal plants still make an important contribution to health care. There has been a growing interest in the alternative therapies in recent years, especially those from plants (Elujoba et al., 2005).

WHO estimates that about 65-80% of the world's population living in developing countries depends essentially on medicinal plants (herbs) for primary health care.

Many species have been recognized to have medicinal properties and beneficial impact on health, e.g. antioxidant activity, digestive stimulation action, anti-inflammatory, antimicrobial, hypolipidemic, antimutagenic effects and anti-carcinogenic potential (Hostettmann et al., 2000).

### 1.2.3 Cymbopogon proximus:

Description**:**

The *Cymbopogon proximus* is a herbal plant. Common name is camel's hay and is known locally as (Maharaib). It is a perennial herb, erect, tufted 9 cm long, culms slender, glabrous and 3 – 4 nodes. Leaf simple, alternate, linear 5-7 cm long, 1cm wide, sheathed apex spiny entire, and inflorescence spikelet's highly branched 5 cm log (Eltahir and Ereish,2010).

The plant is widely distributed in Africa (northwest tropical, northeast tropical and east tropical), temperate Asia (western Asia and Arabia) and tropical Asia (Indian and Indo-China). In addition, Cymbopogon proximus is found in northern and Central Sudan (Clayton et al., 2005).

### 1.2.4 Classification of Cymbopogon proximus:

Kingdom: Plantae .

Phylum: Magnoliophyta.

Class: Liliopsida.

Order: Poales.

Family: Poaceae.

Genus: Cymbopogon .

Species: Cymbopogon proximus.

## 1.2.5 Traditional uses:

*Cymbopogon proximus* (Family Poaceae) is a traditional medicinal Sudanese plant commonly known as "Mahareb", which is used in folk medicine. It is extensively used as folk medicine to promote diuresis, to alleviate colic pain and as antipyretic plant against fever (Khalid et al., 2012).

In the Sudanese folk medicine, it is famous as an effective diuretic and renal antispasmodic.

A decoction of the entire dried herb has been used for centuries by certain tribes in South sudan as a diuretic, colic pain killer, aid for removal of small stones from the urinary tract, and antipyretic. The plant has been found to possess antispasmodic, antioxidant. Antibacterial affects. Hypotensive, antiemetic, anticonvulsant properties. Hypoglycemic properties. Fungicidal properties. Ovicidal and larvicidal properties (Bassole et al., 2003).

It was also used to treat constipation, intestinal complaints, carminative, stomachic and as an appetizer, Treatment of drinking water with herbal plants as traditional medicine is worldwide.

In particular, the *Cymbopogon proximus* (CP) is widely used in Sudan in a purpose of folk medicine. The addition of *Cymbopogon proximus* (Maharaib) at a rate of 5g CP/1.5 liter water ($3.3L^{-1}$) to ground water removes $NO^{-3}$ in 48 h and at the same time increases total dissolved solids (TDS) and fluoride (F- ) levels and consequently increases the risk of dental fluorosis incidence and other fluoride adverse effects (Abdellah et al.,2012).

## 1.2.6 Medical uses:

According to phytochemical tests of Cymbopogon proximus the presence of saponins, Flavonoids, glycosides ,alkaloids, and tannins may be a rationale for the use of the plant in medicine preparations.

Flavonoids are known to protect against allergies, diabetes, inflammations, malaria, platelet aggregations and microbial infection.

Phytochemical content of Cymbopogon proximus and chemical composition of its oil indicate an herb with good potentials in medicinal applications and pest control.

The Cymbopogon proximus treated lower levels of serum calcium, serum blood urea nitrogen (BUN) and kidney calcium. The Cymbopogon proximus has a significant protective effect against ethylene glycol-induced nephrolithiasis in rats (Warrag et al.,2014).

Terpenes are naturally occurring substances produced by a wide variety of plants and animals. A broad range of the biological properties of terpenoids is described, including cancer chemopreventive effects, antimicrobial, antifungal, antiviral, antihyperglycemic, anti-inflammatory, and antiparasitic activities. Terpenes are also presented as skin penetration enhancers and agents involved in the prevention and therapy of several inflammatory diseases. Moreover, a potential mechanism of their action against pathogens and their influence on skin permeability are discussed. The major conclusion is that larger-scale use of terpenoids in modern medicine should be taken into consideration.

Tannins are poly phenolic compounds that are broadly categorized into two major groups:

hydrolyzable tannins, consisting of a central core of carbohydrate to which phenolic carboxylic acids are bound by ester linkage. The other type is condensed tannins, or proanthocyanidins, consisting of oligomers of two or more flavan-3-ols, such as catechin, epicatechin, or the corresponding gallocatechin. Tannins have a very high affinity for proteins and form protein-tannin complexes. The ingestion of a plant containing condensed tannins decreases nutrient utilization, protein being affected to a great extent, and decreases feed intake. On the other hand, hydrolyzable tannins are potentially toxic to animals. Consumption of feeds containing high levels of hydrolyzable tannins cause liver and kidney toxicity and lead to death of animals. Oak and yellow wood poisonings are attributed to hydrolyzable tannins (Makkar et al., 2007).

Saponins are steroid or triterpenoid glycosides, common in a large number of plants and plant products that are important in human and animal nutrition. Several biological effects have been ascribed to saponins. Extensive research has been carried out into the membrane permeabilising, immunostimulant, hypocholesterolaemic and anticarcinogenic properties of saponins and they have also been found to significantly affect growth, feed intake and reproduction in animals. These structurally diverse compounds have also been observed to kill protozoans and molluscs, to be antioxidants, to impair the digestion of protein and the uptake of vitamins and minerals in the gut, to cause hypoglycaemia, and to act as antifungal and antiviral agents. These compounds can thus affect animals in a host of different ways both positive and negative (Francis et al., 2002).

Flavonoids are plant pigments that are synthesized from phenylalanine, generally display marvelous colors known from flower petals, mostly emit brilliant fluorescence when they are excited by UV light, and are ubiquitous to green plant cells. The flavonoids are used by botanists for taxonomical classification. They regulate plant growth by inhibition of the exocytosis of the auxin indolyl acetic acid, as well as by induction of gene expression, and they influence other biological cells in numerous ways.

Flavonoids kill many bacterial strains, inhibit important viral enzymes, such as reverse transcriptase and protease, and destroy some pathogenic protozoans. Yet, their toxicity to animal cells is low.

Flavonoids are major functional components of many herbal and insect preparations for medical use, which have been used since ancient times. The daily intake of flavonoids with normal food, especially fruit and vegetables is 1–2 g. Modern authorized physicians are increasing their use of pure flavonoids to treat many important common diseases, due to their proven ability to inhibit specific enzymes, to simulate some hormones and neurotransmitters, and to scavenge free radicals.

Alkaloids many substances which interfere with the inflammatory response have been isolated from plants. Some alkaloids of vegetal origin which in the period of 1907 to 2000 were evaluated regarding a possible anti inflammatory activity. The alkaloids were classified in sub groups in accordance with their chemical structures and the pharmacological data were obtained from different experimental models. Of the 171 evaluated alkaloids, 137 presented anti-inflammatory activity, and among those, the isoquinoline type was the most studied. The carrageenin-induced paw edema was the most used model for evaluating the anti-inflammatory activity (Makkar et al., 2007).

A glycoside consists of two components, an aglycone (non-sugar) part and a sugar part. The aglycone portion may be of several different types of secondary metabolites, including coumarin, flavonoids or hydroxyanthracene. The sugar moiety is linked to the aglycone by a direct carbon to carbon bond (C-glycoside), or through oxygen to carbon bond (O-glycoside). Cyanide glycosides, release toxic hydrogen cyanide when cells are damaged and act as a defence mechanism (Filho et al., 2006).

## 1.3 Essential oils

Essential oils are volatile and liquid aroma compounds from natural sources, usually plants.

The odoriferous substances (essential oils) themselves are formed in the chloroplast of the leaf, vesinogenou layer of cell wall or by the hydrolysis of certain glycosides. They may be found in different parts of the plant. Some could be in leaves (oregano), seed (almond), flower (jasmine), peel (bergamot), berries (juniper), rhizome (galangal ginger), root (angelica archangelica), bark (sassafras), wood (agar wood), resin (frankincense), petals (rose). Essential oils from different parts of the same plant may have completely different scents and properties. Geranium for instance, yield oil both from the flowers and the leaves, and the oil from both parts differ in constituents, scents and some other properties. The quantity of essential oil extracted from the plant is determined by many interrelated Factors, climatic, seasonal and geographical conditions, harvest period and extraction techniques (Pannizi et al., 1993).

The yield of oils from the plants can also be affected by the stages of the plant growth. Science regards essential oils in terms of functionality. They are considered" the chemical weapons" of the plant world as their compounds may deter insects, or protect the plant against bacterial or fungal attacks. They also act as "plant pheromones" in an effort to attract and seduce their pollinators. The oxygenated molecules of essential oils, which serves as chemical messengers to the cells bring life to the plants, destroying infestation, aiding growth and stimulating healings. More poetically inclined souls regard them as the essence of the plant's soul, their ethereal nature concentrated as scents, through which plants communicate with their surrounding world. Therapeutic properties of the essential oils have been reported by previous researchers. These properties were established after the oils have been extracted from the plant materials (Pannizi et al., 1993).

### 1.3.1 Extraction of essential oils

Essential oils are valuable plant products, generally of complex composition comprising the volatile principles contained in the plant and the more or less modified during the preparation process. The oil droplets being stored in the oil glands or sacs can be removed by either accelerate diffusion through the cell wall or crush the cell wall. The adopted techniques depend on the part of the plants where the oil is to be extracted, the stability of the oil to heat and susceptibility of the oil constituents to chemical reactions. Common techniques used for the extraction of essential oils are;

#### 1.3.1.1 Hydro distillation

The technique involves distillation of water that is in direct contact with fresh or sometimes dried macerated plant materials. Plant material is grinded and weighed, then transferred into the Clevenger set up. Plant material is heated in two to three times its weight of water with direct steam. The distillation vessel is heated over heating mantle and the water vapor and oil are removed through a water cool condenser.

#### 1.3.1.2 Hydro diffusion

Hydro diffusion is a method of extracting essential oils in which steam at atmospheric pressure (low-pressure steam <0-1 bar) is passed through the plant material from the top of the extraction chamber, thus resulting in the oils that retain the original aroma of the plants

### 1.3.1.3Enfleurage

This process is applicable to flowers such as jasmine or tuberose, that have low content of essential oil and so delicate that heating would destroy the blossoms before releasing the essential oils. Flower petals are placed on trays of odorless vegetable or animal fat which will absorb the flowers essential oil. Every day or every few hours after the vegetable or fat has absorbed as much essential oil as possible; the depleted petals are removed and replaced with fresh ones. This procedure continues until the fat or oil becomes saturated with the essential oil. This is called enfleurage mixture. Addition of alcohol helps to separate the essential oil from the fatty substances. The alcohol then evaporates leaving behind only the essential oil; hence enfleurage method is the best method when the source from the oil is to be extracted from flower or petals.

### 1.3.1.4Cold pressing

Another method of extracting essential oil that has not found high application in scientific research is cold pressing. It is used to obtain citrus fruits oils such as bergamot, grape fruit, lemon, lime, etc. The fruits to be extracted are rolled over a trough with sharp projections that penetrate the peels, this pierce the tiny pouches containing the essential oil. The whole fruit is pressed to squeeze the juice and is separated from the juice by centrifugation.

### 1.3.1.5 Steam distillation

This is the most common method of extracting oils and is the oldest form of essential oils extraction. In this technique, the desired plant (fresh or sometimes dried) is first placed into the vessel.

steam is added and passed through the plant that contains the plants aromatic molecules or oils. Once upon, the plant releases these aromatic molecules and in the state, the fragrant molecules travel within a closed system towards the cooling device. Cold water is used to cool vapors. As they cool, they condense and transform into a liquid state.

### 1.3.1.6 Solvent extraction

This method involves the extraction of the oils from the oil bearing materials with the use of solvent. Solvent used depends on the part of the plant to be used for extraction. For instance, leaves, roots, fruits are extracted with benzene with or without mixture of acetone or petroleum ether, in the cold or at boiling point while flowers are extracted with ethers. The solvent enters the plant to dissolve the oil waxes and color. After the extraction, the solvent is removed by distillation under reduced pressure leaving behind the semisolid concentrate, this concentrate is extracted with absolute ethanol. The second extract is cooled to precipitate the waxes and then filtered. This wax free alcoholic solution is distilled under reduced pressure to remove alcohol and finally the essential oil.

### 1.3.1.7Microwave assisted process (MAP)

The MAP process uses microwave to excite water molecules in plant tissue causing the cells to rupture and release the essential oil trapped in the extra cellular tissue of the plants. this technique has been developed and reported by many authors as a technique for extraction of essential oils in order to obtain a good yield of the essence and to reduce the time of extraction. This technique has also been applied for the extraction of saponins from some medicinal plants (Safir et al., 1998).

### 1.3.1.8 Carbon dioxide extraction

In this technique, plant material is placed in a high pressure vessel and carbon dioxide is passed through the vessel. The carbon dioxide turns into liquid and acts as a solvent to extract the essential oil from the plant material. When the pressure is decreased, the carbon dioxide returns to a gaseous state leaving no residue behind. Qualities of essential oil extracted with any of the techniques described above depend on the chemical composition of the oil.

## 1.3.2 CHEMICAL COMPOSITION OF ESSENTIALOILS

Most constituents of oil belong to the large group of terpenes. terpenes usually refer to hydrocarbon molecules consisting of isoprene (2-methylbuya-1, 3-diene). The isoprene unit, which can build upon it in various ways, is a five-carbon molecule. Two of the molecules of isoprene give mono terpenes, sisquiterpenes contain three molecule of isoprene, four isoprene gives di terpene, five isoprene gives Sesquiterpenes.

The two main purposes of analyzing essential oils are:

(i) To identify and quantify as many constituents as possible.

(ii) To evaluate the quality of the oils and detect any possible adulteration that may affect their usage.

Analysis of essential oils is generally performed using Gas chromatography (qualitative analysis) and Gas chromatography-mass spectroscopy (qualitative analysis).Gas chromatography analysis is a common Confirmation test.

## 1.3.3 Gas Chromatography Analysis (GC)

Gas chromatography analysis is a chemical instrument used for separating chemicals in a complex sample and provides a representative spectral output. The gas chromatography instrument vaporizes the sample and then separates and analyzes the various components. Each component ideally produces a specific spectral peak. The time elapsed between injection and evaluation is called “Retention time”.

The sample is injected to the injection port with a hypodermic needle and syringe, the injection port is maintained at a temperature at which the sample vaporizes immediately. The carrier gas propels the oils down the column and the oil spread evenly along the crosses section of the column, the column allows the various substances to partition themselves. Substances that do not like to stick to the column or packing are impeded but eventually elute from the column. Ideally, the various compounds in the sample separate before eluting from the column end. The detector measure different compounds as they emerge from the column.

### 1.3.4 Gas Chromatography-Mass Spectroscopy Analysis (GC/MS)

Gas chromatography-Mass spectroscopy analysis is a method which combines the features of gas, liquid chromatography and mass spectroscopy to identify different substances within a test sample. The gas chromatography-mass spectroscopy instrument is made of two parts: The gas chromatography (GC) portion separates the chemical mixture into pulses of pure chemicals and mass spectrometer (MS) identifies and quantifies the chemicals. After the sample has passed through the GC, the chemical pulses continue to the MS. The molecules are blasted with electron, which causes them to break into pieces and turns into positively charged particles called ions.

This is important because the particles must be charged to pass through the filter. As the ions continue through, they travel through an electromagnetic field that filters the ions based on mass. The filter continuously scans through the range of masses as the stream of ions come from the ion source. They enter the detector and then the detector counts the number of ions with specific mass. This information is sent to the computer and a mass spectrum is created. The mass spectrum is a graph of the number of ions with different masses that travelled through the filter. The data from the mass spectrometer is sent to a computer and plotted on a graph called the mass spectrum. The importance of analysis is to know the quality of the constituent, so that it can be put into various uses (Kagawa et al., 2003).

The physical properties of cymbopogon proximus essential oils are founded (specific gravity at $15^0$ is 0.9149 $\pm$ 0.005, the optical rotation, at $20^0$ is -$59^0$22' ,refractive index ,at $20^0$ is 1.4831 $\pm$ 0.001)(Tajidin NE et al., 2012).

### 1.3.5 MEDICINAL AND PHAMACOLOGICAL USES OF ESSENTIAL OILS

Essential oils are valuable natural products used as raw materials in many fields, including perfumes, cosmetics, aromatherapy, phytotherapy, spices and nutrition, insecticides. Aromatherapy is the therapeutic use of fragrances or at least mere volatiles to cure or mitigate or prevent diseases, infection and indisposition by means of inhalation. Inhalation of essential oils or their individual volatile terpenes has a significant role in controlling the central nervous system. For instance, aroma inhibit of storax pill essential oil and pre inhalation of Aconus gramineus rhizome essential oils are used in Chinese folk medicine in the treatment of epilepsy. The fragrance compounds, cisjasmonate, which characterized the aroma of Jasminum grandiflorum have a tranquilizing effect on the brain upon inhalation. They significantly increased the sleeping time of mice induced by pentobarbital. Cendrol, which is a major component of cardwood essential oil, shows a sedative effect and prolonged pentobarbitalinduced sleeping time on rats upon inhalation. The vapor of lavender essential oil or one of its main component linalool may also be applicable to the treatment of menopausal disorder through inhalation. Lavender essential oil demonstrated an analgesic activity, mainly relevant after inhalation at the doses devoid of sedative side effects. Medical professionals are more interested in the medicinal properties of essential oils.

Many oils show antibacterial, fungicidal, relaxant, stimulating, antidepressant effect and can be very effective therapeutic agent. Essential oils are known for their therapeutic properties hence, used in the treatment of various infections caused by both by pathogenic and non-pathogenic diseases. Pathogenic diseases caused by bacterial, virus, and the fungi can be treated with essential oils. Strong in vitro evidence indicates that essential oil can act as antibacterial agent against a wide spectrum of pathogenic bacteria strains including; Listeria monocytogenes, Linnocua, Salmonella typhimurium, Shigella dysentria, Bacilluscerus , and Staphylococcus aureus.

Thyme and oregano essential oils can inhibit some pathogenic bacteria strains such as E.coli, Salmonella typhimurium, Salmonella entritidis and Salmonella choleraesuis , with the inhibition directly correlated to the phenolic components carvacrol and thymol. Eugenol and carvocrol showed an inhibitory effect against the growth of four strains of Escherichia coli 0157:H7 and Listeria monocytogens. Also, the presence of phenolic hydroxyl group in carvacrol particularly is credited with its activity against pathogens such as Bacillus cereus (Motakii et al., 2002).

Essential oil with high concentration of thymol and carvacrol e.g. oregano, savory and thyme, usually inhibit gram positive more than gram-negative pathogenic bacteria. However the antibacterial activity against gram- negative Haemophilus influenza and Pseudomonas aeruginosa respiratory pathogens, while gram-positive streptococcus pyrogens was the most resistant to the oil. Essential oils show bactericidal activity against oral and dental pathogenic microorganisms and can be incorporated into rinses or mouth washes for pre-procedural mouth control , general improvement of oral health , interdental hygiene and to control oral malodour . Mouth rinses containing essential oils with chlorhexine gluconate are commonly used as preprocedural preparations to prevent possible disease transmission, decreases chances of postoperative infections, decreases oral bacterial load and decrease aerolization of bacteria. Mouth washes containing essential oils could also be used as part of plaque-control routine since they can penetrate the plaque biofilm, kill pathogenic-wall and inhibiting their enzymatic activity. In addition, essential oil in mouth washes prevent bacterial aggregation slows the multiplication and extract bacterial endotoxins . Croton cajucara benth essential oil was found to be toxic to some pathogenic bacteria and fungi associated with oral cavity diseases . Besides their antibacterial and antifungal activities, essential oils have also been reported to posses interesting antiviral activities alternative to synthetic antiviral drugs. They have demonstrated virucidal properties with the advantages of low toxicity , Herpes simplex virus (type III) causes some of the most common viral infections in human and can be fatal. Synthetic antiviral drugs have been used to treat Herpes infection , but not all are efficacious in treatment of genital herpes infections. Incorporation of Artemisia arboreseens essential oils in multi lamella liposomes greatly improved its activity against intra cellular herpes simplex virus type 1 (HSV-1). Due to the presence of Citra and citronellal in Melissa officinalis L. essential oil, it also inhibits the replication of HSV-2 and the ability to replicate of HSV-1 can be suppressed incubation with different essential oils *in vitro.* also demonstrated the effect of five essential oils on Epstein-Barr virus (EBV) (viridae) which caused the infectious mononucleosis associated with Burkitt lymphoma and naso-pharynx carcinoma. The study aimed the effect of these oils on the expression of EBV viral capside antigen (VCA) in the marmouset B95-8 lymphoblastoid cellular line using the indirect immune-flourescence technique. The result showed a cytotoxic effect of tested oils at a dilution factor lower than 1: 500. Moreover, the vapor of cellularviability was not affected. Treatment of B95-8 cells with 1: 1000 dilution of thymus oil increased the fluorescence intensity VCA-positive cells in two separate experiments. In three other tests, only fluorescence intensity was increased by oil from thymus while the percentage of the fluorescent cells did not increase significantly (Tan et al., 2000).

Essential oils can also be used for the treatment of non-pathogenic diseases. For instance, Garliessential oil significantly lowered serum cholesterol and triglycerides while raising the level of high-density lipoproteins in patients with coronary heart diseases. The hypolipidemic action of garlic oil is primarily due to a decrease in hepatic cholestrogenesis (Mathew et al., 1996).

Some essential oils also exert hypotensive activity when applied in vivo and they are used for treating hypertension.

Oral administration of combination of oregano, cinnamon, cumin, and other essential oils decreases systolic blood pressure in rats and intravenous administration of the essential oil from the aerial parts of Menthax villosa induced in a significant and dose-dependent hypotension associated with decrease in heart rate. This activity was attributed to volatile component, piperitenone oxide which represents 55.4% of the oil. The hypotensive effect induced by the oil is probably due to its direct cardiodeplesant action and peripheral vasolidation, which can be attributed to both endothelium dependent and endothelium-independent mechanism. Intravenous administration of essential oil of basil (Ocimum gratissium) induced an immediate and significant hypotension and bradycardia. The hypotensive activity of the essential oil resulted from its vasodilator effect, acting directly upon vascular smooth muscles. This effect was attributed to the suction of eugenol known about 80%, but from a safety point of view, care must be taken in dealing with eugenol due to its suspected carcinogenicity andm hepatotoxicity. Intravenous injection of the monoterpene alcohol terpinen-4-ol decrease main aortic blood pressure in a dose related manner, in a conscious DOCA-salt hypertensive rats. The mechanism of action was related to the induction of vascular smooth muscle relaxation rather than enhanced sympathetic nervous system activity. Terpinene-4-ol is a major constituent of several essential oil, particularly tea tree and sweet marjoram essential oils.

Some essential oils may aggravate diabetes, for instance rosemary essential oil showed hyperglycaemic and insulin release inhibitory effect in diabetic rabbits has emphasized that the lipophilic fraction of aromatic plants are not generally responsible for any anti-diabetic activity showed by these plants, but it was also indicated that an oral administration of a combination of essential oils including cinnamon, cumin, oregano, fennel, myrtle besides others was able to enhance insulin sensitivity in type II diabetes, in addition to lowering circulating glucose in the tolerance testing a rat.

The essential oil of satureja khuzestanica results in significant decreases in fasting blood glucose level in diabetic rats. Essential oils and their individual aroma components showed cancer suppressive inactivity when tested on a number of human cancer cells lines including glioma, tumours, breast cancer, leukaemia and others. Glioma is one of the most malignant human tumours. A significant effect on the treatment of glioma using the sesquiterpene hydrocarbon element which is found in small amounts in many essential oils, it prolonged quality survival time of patients with glioma. Antiangiogenic therapy is one of the most promising approaches to control cancer. Perillyl alcohol (POH) which is the hydroxlated analogue of d-limonene has the ability to interfere with angiogenesis. POH either alone or with PA (perillic acid, the major metabolite of POH in the body), has the potential use as an anticancer drug that stimulates different types of tumour to apoptosis inhibit their proliferation of overcomes their resistance to chemo/radiotherapy.

Treatment of human leukaemia cells with eucalyptus oil showed morphological changes (fragmentation of DNA) indicating an induction of apoptosis (Motakii et al., 2002).

The essential oil of lemon balm (mellisa oficinalis L) was found to be effective against a series of human (A549, MCF-7, Caco-2, HL-60, K562) and a mouse cell line (B16F10) and that of Artemisia annua L. Induced apoptosis of cultured SMMC-7721 hepatocarcinoma cells.

The essential oils of Australian tea tree (Melaleuca alternifolia) and its major monoterpene alcohol, terpinen-4-ol, were able to induce caspasedependent apoptosis in human melanoma M14 WT cells and their drug-resistant counterparts, M14 adriamicin-resistant.

There was evidence to suggest that the effect of the toil of terpinen-4-ol was meditated by their interaction within the plasma membrane and subsequent reorganisation of membrane lipids. Hepatic arterial infusion with Curcuma oil had a similar positive effect in treating primary liver cancer as that of the chemical drugs.

The essential oil of Tetraclinis articulate, (a conifer tree) showed the hallmarks of apoptosis when tested on a number of human cancer cell lines including melanoma, breast and ovarian cancer in addition to peripheral blood lymphocytesal (Cheng et al.,2001).

## 1.4 Heavy metals

As a result of different human activities, the world is facing serious threats of air, land, and water pollutions. Water pollution in particular, has raised severe environmental impacts. In addition to the shortage of resources of water due to drought and misuse, production of large volumes of wastewater has put a lot of pressure on the humankind. There are different types of water contaminants **(infectious agents, inorganic pollutants, organic pollutants, thermal pollutants, radio- active pollutants, oxygen demanding wastes**). Among these pollutants, the inorganic pollutants are extremely harmful due to their high toxicity and non-biodegradability.

### 1.4.1 Sources of inorganic pollutants

Heavy metals, like mercury, lead, tin, cadmium, selenium, and arsenic are introduced to the environment by different human activities and deposit slowly in the surrounding water and soil. In many developing counties, little attention is paid to the environmental issues that the drainage of waste water into lakes and rivers is very common. The uncontrolled activities cause poisoning of fresh water resources which affects the entire eco-system.

Recently, water pollution as results of industrial and economical progression is becoming a significant environmental problem. Metals are widely distributed throughout nature and occur freely in soil and water (Kumiawan et al., 2006).

Heavy metals are defined as metallic elements that have relatively high density compared to water. Heavy metal pollution is identified as one of the consequences brought about by development and economic progress. Heavy metals can cause brain damage and many diseases in human beings. They cannot be degraded easily and their cleanup usually requires their removal. Therefore, the direct release of reused waste water for the irrigation of agriculture and horticultural is viewed as posing potential risk to human health. There are 59 elements classified as heavy metals and out of these five are considered to be highly toxic and hazardous. These are cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb) and zinc (Zn), which are released into the environment by human activities or through natural constituents of the earth's crust. Traditional systems of medicine continue to be widely practiced on many accounts. Centellaasiatica and Orthosiphonstamineus as phytoremediation agents, both species were grown in contaminated soil obtained from industrial land. Plant growth response and their ability to accumulate and translocate zinc, copper and lead were assessed (Gunatilake SK et al., 2015).

### 1.4.2 Hazardous effect of heavy metals

As potent pollutants, heavy metals were intensively investigated from the point of view of persistence and toxicity. The accumulation of heavy metals certainly has adverse effect on aquatic flora and fauna and may constitute a public health problem where contaminated organisms are used for food. They can cause poisoning, initiate cancer, and result in brain damage when found above the tolerance levels. The agencies for the environmental monitoring have set permissible limits for heavy metals levels in drinking water because of their harmful effects.

### 1.4.3 Conventional Methods for Heavy Metal Removal

The conventional technologies include physical, chemical or biological. Each of these methods has its advantages and disadvantages

### 1.4.4 Removal of heavy metals by adsorption

Adsorption is known to be one of the best of the technologies for the decontamination of water because it is an effective, economical and ecofriendly treatment technique. It is a process strong enough to realize water reuse obligation and high runoff standards in the industries. Adsorption is basically a mass transfer process by which the metal ion is transferred from the solution to the surface of sorbent, and becomes bound by physical and/or chemical interaction.

Chelation of heavy metals is considered very important and very useful, especially for plants, which are economics. Different plants have been used to achieve maximum heavy metals removal from metaliferous waste water, in recent years (Gunatilake SK et al., 2015).

Cymbopogon proxmus belong to family Poaceae, locally known as muharaib , is an aromatic densely- tufted grass growing wildly and widely in Upper Egypt and sudan. The herb is highly reputed in folkloric medicine. Cymbopogon essential oils are characterized by monoterpene constituents piperitone is one of the important components of the oil present in several species of C. proxmus with wide industrial uses such as raw material for perfumery, confectionery and vitamin A. In sudan Cymbopogon proxmus used to purify drinking water.

## 1.5 Objectives

This study will achieve the following aims:

To investigate the chemical composition of the essential oil of C.proximus using GC/MS, And phytochemical screening of it is crude extracts.

To study the ability of C.proximus to remove the heavy metals (Zn and Pb) from aqueous medium.

# Chapter Two
# Materials & Methods

CHAPTER TWO

# MATERIALS & METHODS

## 2 Sample collection and identification

The plant *Cymbopogon proximus* (locally named Maharaib), Family: Poaceae (Graminae) was collected from herbalist at administration gardens Khartoum State. The botanical classification of the plant was confirmed at the Medicinal & Aromatic Plants & Traditional Medicine Research Institute, Khartoum, Sudan.

## 2.1 Chemicals and reagents

All of these chemicals were analytical grade.

Distilled Water $H_2O$ ,Chloroform $CHCl_3$, Methanol $CH_3OH$, Anhydrous Calcium Chloride $CaCl_2$, dilute hydrochloric acid HCl, Wagner's reagent (Iodine in Potassium Iodide 2.5gm iodine is dissolve in 12.5gm of potassium iodide $KI_2$), Lead acetate $(CH_3COO)_2Pb$ , Sodium hydroxide NaOH, concentrated $H_2SO_4$, Fehling's solution , lead chloride $PbCl_2$, zinc chloride $ZnCl_2$, Ferric chloride $FeCl_3$.

## 2.2 Preparation of plant Material

The leaves of *Cymbopogon proximus* was dried under the shade for seven days, chopped and pulverized into a fine powder using grinder, stored into a sealed polythene bag ready for extraction.

## 2.3 Crude Extracts preparation

The Chloroform and Distilled water extracts were prepared by extracting 25g of pulverized sample with 125mL of solvent under continuous agitation using shaker apparatus for seven days. The extracts were filtered and solvent were evaporated under room temperature for 3days. The test samples were Leave Chloroform and Leave Distilled water extract ready for phytochemical screening.

## 2.4 Preparation of Volatile Oil by hydro-distillation

50g of the powdered leaves were transferred into 2 liter round-bottomed flask followed by addition of 500 ml of distilled water and distilled for 4hr using a Clevenger apparatus (AOAC, 1990). The floated oil was separated from the water using a micro titer-pipette and dried using anhydrous calcium chloride. The percentage of oil was calculated as follow.

**Weight of oil = density × volume of oil…………………………………….. (1)**

**Volatile oil % = (weight of oil / plant (g)) ×100(w/w)………………………. (2)**

## 2.5 GC-MS Analysis of Essential Oil

The qualitative and quantitative analysis of *C. proximus* essential oil was carried out by using GC/MS technique model (GC/MS-QP2010-Ultra) from Japan's Shimadzu Company, with capillary column (Rtx-5MS- (30m) in length, diameter (0.25mm) and thickness (0.25µl). The sample was injected by using split mode, helium as the mobile phase (carrier gas) passed with flow rate 1.69 ml/min, the temperature program was started from 50 °C reaching 280 °C as final temperature degree with a temperature program rate of 10 ºC/min, starting at three minutes and finishing at twenty eight minutes, the injection port, ion source and interface temperature were (280, 200 and 250) °C respectively.

The sample was analyzed by using scan mode to identify chemical composition of the essential oil in the range of m/z 40-550 charges to ratio and the total run time was 28 minutes. Identification of components for the sample was achieved by comparing their retention times and mass fragmentation patents with those available in the library, the National Institute of Standards and Technology (NIST). The percentage of each compound was based on the peak area divided by the total area of component peaks. The instrument was connected to a computer coupled with special software that was used to analyze the data and the results were recorded.

## 2.6 Phytochemical analysis

Phytochemical examinations were carried out for all the extracts as per the standard methods. Phytochemical screening for major constituents was undertaken using standard qualitative methods as described by (Harborne, 1973). In quantitative test, the extracts were evaluated qualitatively for the presence of alkaloids, flavonoids, glycosides, phenols, saponins, tannins and terpenes.

### 2.6.1 Detection of Alkaloids

The extracts were dissolved individually in 2ml of dilute hydrochloric acid and filtered. Wagner’s Test: 1ml of the Filtrate was treated with few drops Wagner’s reagent (Iodine in Potassium Iodide). Formation of reddish brown precipitate was indicating the presence of alkaloids in the extract.

### 2.6.2 Detection of Flavonoids

**Lead acetate Test:** 1ml of extract was treated with few drops of 10% lead acetate solution. Formation of yellow color precipitate was indicating the presence of flavonoids.

### 2.6.3 Detection of Glycosides

Two and half milliliter (2½ ml) of 50% $H_2SO_4$ was added to 5cm3 of the extracts in a test tube. The mixture was heated in boiling water for 15 minutes. It was then cooled and neutralized with 10% NaOH, 5ml of Fehling’s solution was added and the mixture was boiled. A brick red precipitate was observed which indicated the presence of glycosides.

### 2.6.4 Detection of phenols

**Ferric Chloride Test:** Extracts were treated with 3-4 drops of 10% ferric chloride solution. Formation of bluish black color was indicating the presence of phenols.

## 2.6.5 Detection of Saponins

**Froth Test:** 0.5ml Extracts were diluted with 5ml of distilled water. The solution was shaken vigorously and observed for the stable persistent froth. Formation of 1 cm layer of foam was indicating the presence of saponins.

## 2.6.6 Detection of Terpenoids (Salkowski test)

0.5ml of each of the extract was added 2ml of chloroform and then 3ml of the concentrated $H_2SO_4$ was carefully added to from a layer. A reddish brown colour of the interface was indicating the presence of terpenoids / steroids.

## 2.6.7 Detection of Tannins

Five per cent Ferric Chloride solution (5% $FeCl_3$) were added drop by drop to 2-3ml of the extract. A dark green colored precipitate indicates the presences of tannins.

## 2.7 Preparation of heavy metals solution

The concentrations of metals (zinc and lead) were prepared in the linear range with some modification. The concentration was prepared as 5ppm and 10ppm. For specific metal analysis, standard solutions of known concentrations were used and the effect of the addition samples of extracts metal adsorptions was tested. The samples of extracts were prepared by taking approximately 2g of the sample (C.p) and mixing it with about 50 mL of prepared solution. All experiments were conducted at room temperature and after being allowed to stand for 10, 20 and 30 minutes the samples were analyzed using Atomic Absorption Spectrophotometer (AAS) (Model 210 VGP). The concentrations of the metals zinc, and lead adsorbed by sample were determined after and before adding the plant powder.

# Chapter Three
# Results & Discussion

# CHAPTER THREE
# RESULTS & DISCUSSION

## 3. Extracts Weight of C. proximus Essential Oil

For 50g of the pulverized C. proximus, it was found that only 2.23g equivalent to 4.46% w/w of the constituent was oil. The color of the oil obtained from the *C.* proximus was yellow.

## 3.1 Essential Oil Yield:

The yield of oil was calculated by the following equations:

The yield of oil (v/w %) = (volume of Oil (ml)) / (Plant (g)) × 100

The yield (v/w) % = (2.437 / 50) × 100

The yield (v/w) % = 4.874 %

The yield of oil (w/w %) = density × volume of oil (ml) / (plant (g)) × 100

The yield (w/w) % = ((0.9149 × 2.437) / 50) × 100

The yield (w/w) % = 4.46 %

Hence there is correlation with the results of (Kamal and Maged, 2008) who reported that "the percentage of volatile oil yield from *C. proximus* prepared by hydro-distillation method was 5.44% w/w".

## 3.2 Extracts Weight of C. proximus

Table (1) below shows extraction and percentage yields by weight of 25g of the *C.proximus* (leaves) using 125ml of 2 solvents (chloroform and distilled water) respectively in order of polarity.

**Table (3.2.1):** Extracts Weight of *C. proximus*

| Plant Extract | Part Used | Solvents | Yield (g) | Yield (%) |
|---|---|---|---|---|
| cymbopogon proximus locally known (Muharaib) | **Leaves** | Chloroform | **0.96** | **3.84** |
| | | Water | **1.86** | **7.44** |

When chloroform was used for extraction, leaves showed small values of crude extraction compared with aqueous extraction. Where aqueous extract showed high % yield compared with chloroform.

These different results may be due to the difference of nature and polarity of each solvent. Therefore; the distilled water consider as a best solvent to extract *C. proximus.*

## 3.3 Phytochemical Screening

Table (**3.3.1**) below showed a phytochemical screening of *Cymbopogon proximus* extracts by using Chloroform and Water as solvents.

| ***Plant Extract*** | **Solvents** | **Alkaloid** | **Flavonoid** | **Terpene** | **Glycoside** | **Saponins** | **Tannin** | **Phenol** |
|---|---|---|---|---|---|---|---|---|
| Cymbopogon proximus | Chloroform | + | + | ++ | + | ++ | + | + |
| | Water | ++ | ++ | +++ | ++ | +++ | +++ | ++ |

+ = present in trace amount, ++ = present in moderate amount, +++ = present in large amount

The obtained results showed that Aqueous extract possess higher amount of phytochemical constituents compared to other solvent, where the extract showed the presence of saponins, tannins and terpenes as larger quantity compared to alkaloids, flavonoids glycosides and phenols. The presence of seven phytochemical constituents in *Cymbopogon proximus* indicates its richness in chemical constituents and hence indicates its Herbal potentials in medicinal applications and partially confirmed it is traditionally claimed therapeutic properties. This study is in agreement with the results of (Dalziel, J.M. 1965) who reported **“**the high presence of saponins, tannins and terpenes may be a rationale for the use of the plant in medicine preparations**”**. This is because saponins are active agents with soap like properties**”**. Tannin when present helps in healing of wounds and also has antimicrobials properties (Trease and Evans, 1996) According to flavonoids are known to protect against allergies, diabetes, inflammations, malaria, platelet aggregations and microbial infection (Okwu, and Omodiromiro,2005). Accordingly there may be scientific basis for use of *C. proximus* as traditional medicinal aliment in Sudan, Egypt, Ghana and Nigeria. Glycoside was found in moderate amount, which is reportedly used for treatment of heart diseases (Trease and Evans, 1996) Reported that, the main constituents of Cymbopogon Proximus are saponins, tannins and flavonoids. The results obtained were in agreement with the above authors’ means there is correlation between the results obtained and the results reported.

## 3.4 Chemical Compounds by GC-MS

Table (**3.4.1**) list the main components of the EO of C. proximus revealed by the (GC/MS) technique.

| **Compound Name** | **Area,%** |
|---|---|
| **p-cymene** | **0.86** |
| **4-carene** | **7.55** |
| **2-carene** | **9.07** |
| **o-cymene** | **0.76** |
| **limonene** | **4.92** |
| **p-menth-2-en-1-ol** | **1.81** |
| **Cis-p-mentha-2,8dien-1-ol** | **1.47** |
| **piperitone** | **43.2** |
| **Elemol** | **15.45** |
| **ß-eudesomal** | **7.49** |
| **ɣ-eudesomal** | **4.38** |
| **Cuparene** | **2.42** |
| **1,8-cineole** | **0.61** |

The *Cymbopogon proximus* essential oil was analyzed using GC-MS technique. Identification of compounds from essential oil was based on comparison of their mass spectra with the, computer library of NIST or Wiley Registry of Mass Spectral Data. GC-MS of the hydro distilled essential oil of *C. proximus* leaves detection and identification of thirteen (13) compounds (Table 3.4.1 ).

This analysis of *C.proximus* essential oil revealed some of the chemical components and diverse range of bioactive molecules with high therapeutic value making *C.proximus* being a rich source of different types of medicines and plays an important role in drug development programs in the pharmaceutical industry and perfume industry.

The monoterpenes constitute (**4-carene, 2-carene, limonene, p-menth-2-en-1-ol, Cis-p-mentha-2,8dien-1-ol, piperitone, 1,8-cineole**) represented about (68.63%) of the oil and The oxygenated sesquiterpenes constitute (**Elemol, ß-eudesomal, ɣ-eudesomal, Cuparene**) represented about (29.74%).

Piperitone (43.2%), elemol ( 15.45%), 4-carene (7.55%), β-eudesmol (7.49%), limonene (4.92%) and ɣ-eudesmol (4.38%), were the main components identified in the essential oil of *C. proximus* leaves (table3.4.1). 2-carene which was detected in *C. proximus* leaves essential oil in considerable amount (9.07%).

This study shows an agreement with the results of Gasal, *et al*., (2016), Kamal and Maged (2008), El-kamali, *et al.*, (2010) and Banthorpe, *et al.*, (1976) whom reported, the major component of *C. proximus* was piperitone, elemol, ɣ-eudesmol, and β-eudesmol ( Meanwhile Al-Taweel, *et al.*, (2013).

The present study is contrary to the results of (Menut *et al*., 2000) who concluded, analysis of the volatile oil sample from Burkina-Faso showed that piperitone is the major component (55.5%) while Sudanese sample was free from that compound. But our experimental results in this study were proved the presence of piperitone as a major component (43.2%) in Sudanese *C. proximus* as showed GC-MS results above.

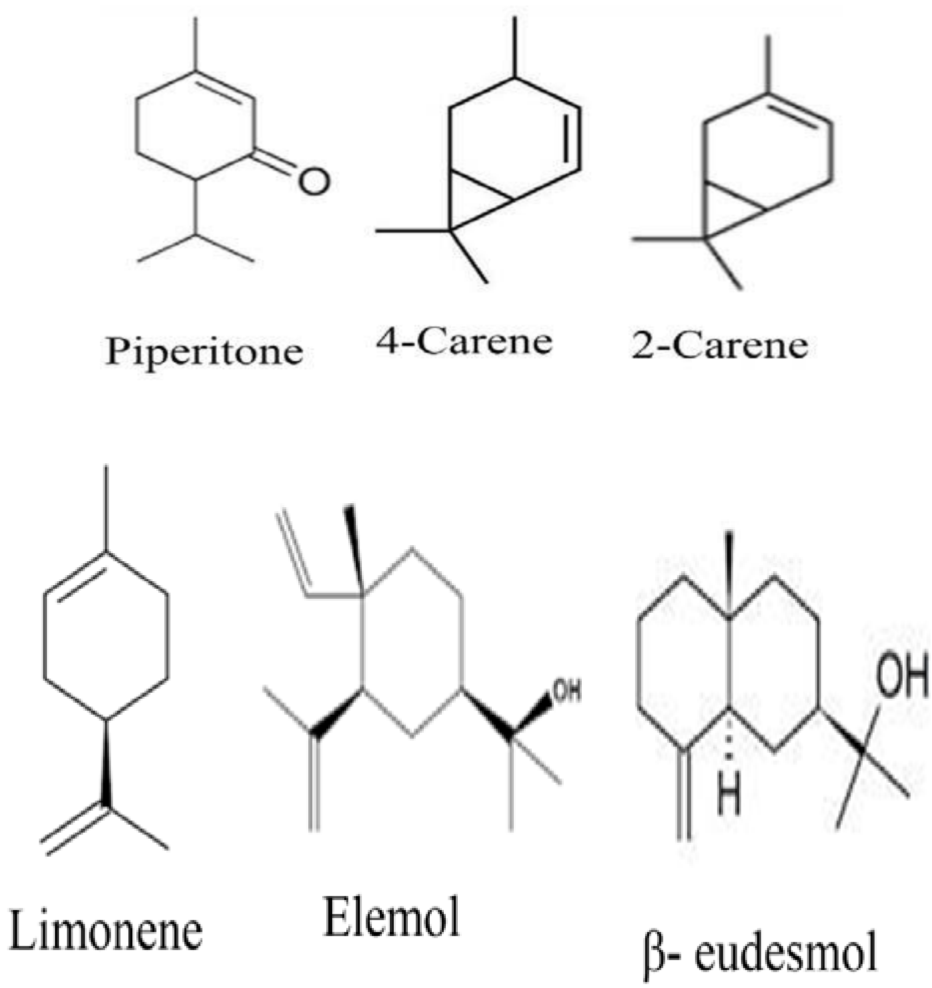


**Fig (3.4):**C. proximus essential oils main constituents

## 3.5 Removal of heavy metals by *C.Proximus*

**Table (3.5.1):** Zn concentration at different times with *C.proximus* in nutrient medium

| **Initial concentration of Zn in ppm** | **Weight of *C.proximus* (g)** | **Residual concentration ppm at different times** | | |
|---|---|---|---|---|
| | | **10 min** | **20 min** | **30 min** |
| **50 ml 5.00 ppm** | **2.00** | **2.34** | **2.17** | **1.70** |

**Table (3.5.2):** Zn concentration at different times with C.proximus in nutrient medium

| **Initial concentration of Zn in ppm** | **Weight of C.proximus (g)** | **Residual concentration ppm at different times** | | |
|---|---|---|---|---|
| | | **10 min** | **20 min** | **30 min** |
| **50 ml 10.00 ppm** | **2.00** | **7.52** | **4.10** | **2.77** |

**Table (3.5.3):** Pb concentration at different times with C.proximus in nutrient medium

| **Initial concentration of Pb in ppm** | **Weight of C.proximus (g)** | **Residual concentration ppm at different times** | | |
|---|---|---|---|---|
| | | **10 min** | **20 min** | **30 min** |
| **50 ml 5.00 ppm** | **2.00** | **2.70** | **2.42** | **2.42** |

**Table (3.5.4):** Pb concentration at different times with *C.proximus* in nutrient medium

| **Initial concentration of Pb in ppm** | **Weight of *C.proximus* (g)** | **Residual concentration ppm at different times** | | |
|---|---|---|---|---|
| | | **10 min** | **20 min** | **30 min** |
| **50 ml 10.00 ppm** | **2.00** | **5.77** | **4.30** | **3.41** |

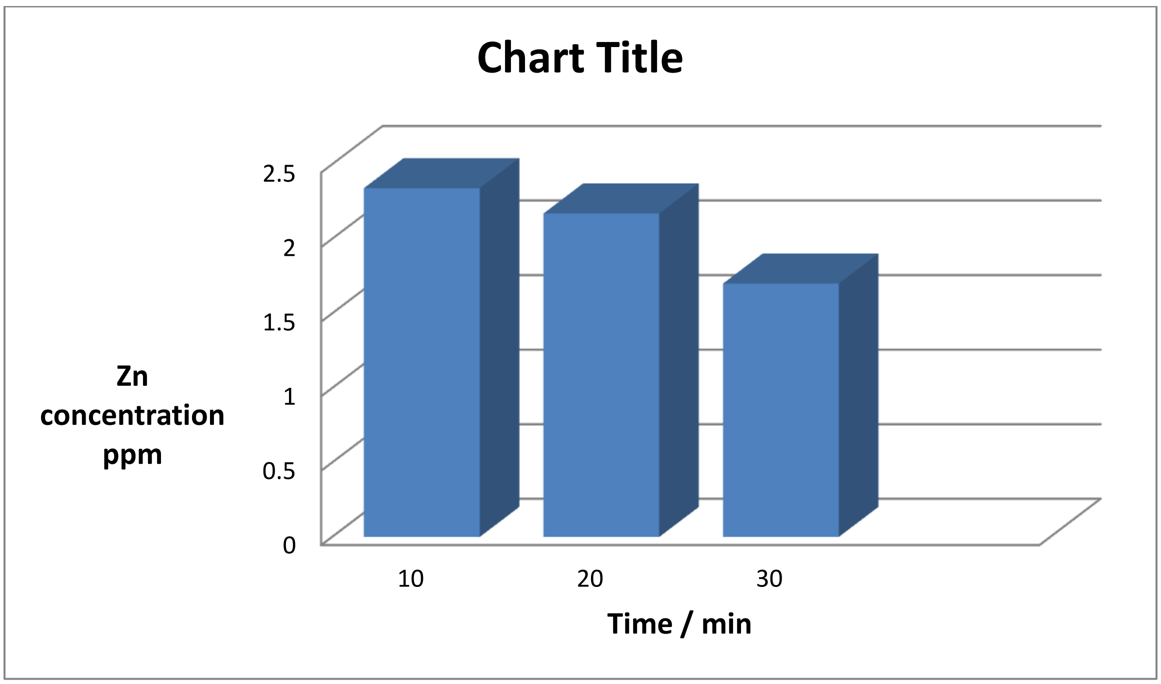


**Fig (3.5.1): Adsorption of Zinc (5.00ppm) using *Cymbopogon proximus***

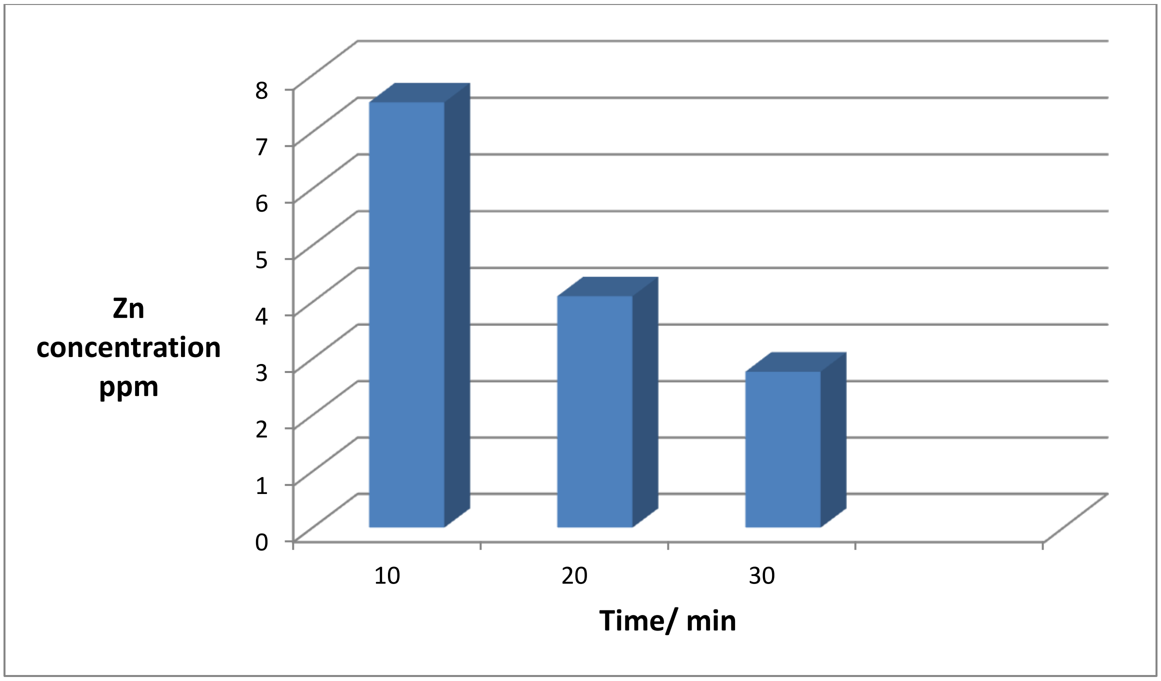


**Fig (3.5.2): Adsorption of Zinc (10.00ppm) using Cymbopogon proximus**

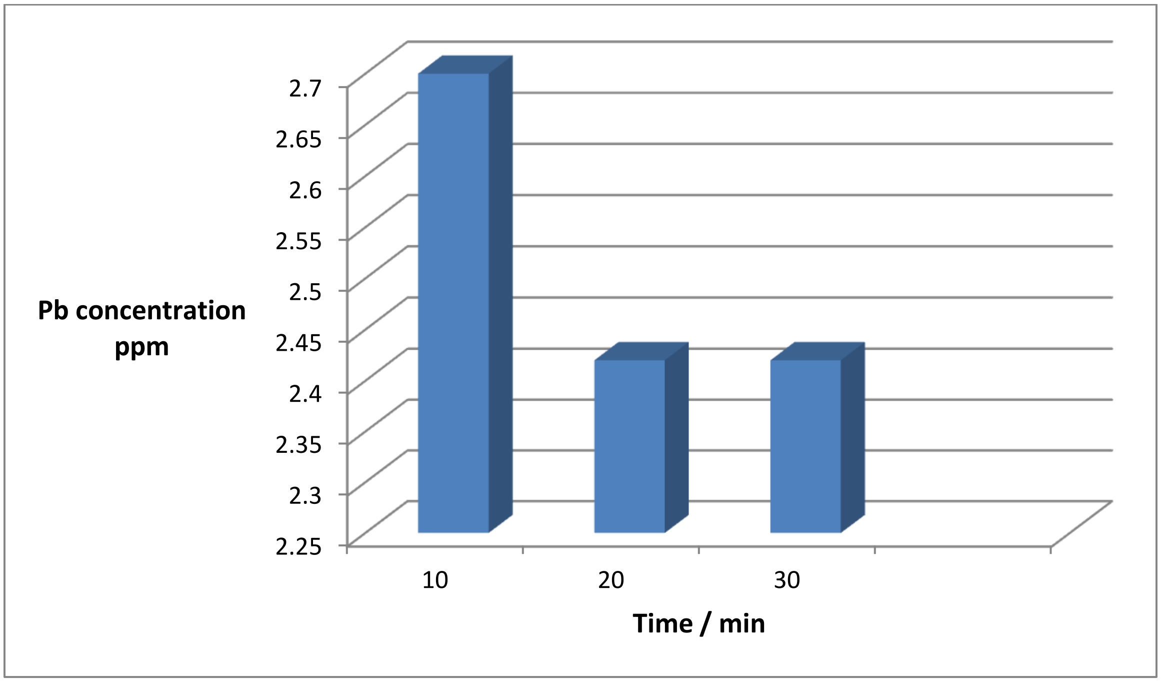


**Fig (3.5.3): Adsorption of Lead (5.00ppm) using *Cymbopogon proximus***

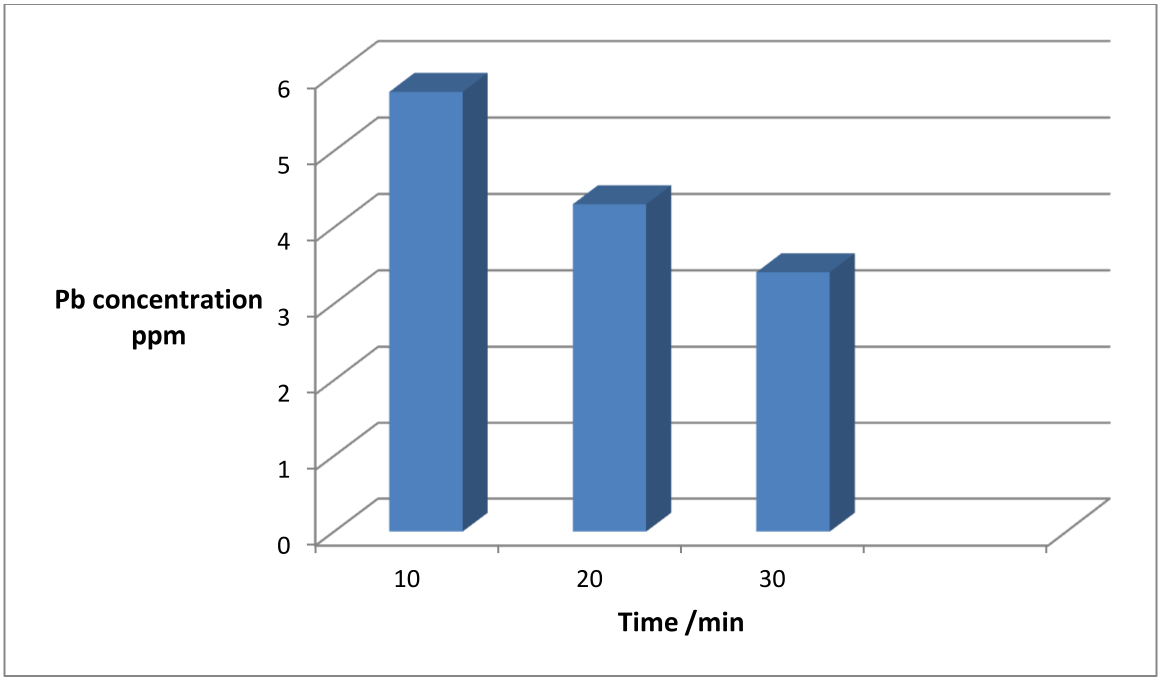


**Fig (3.5.4): Adsorption of Lead (10.00ppm) using *Cymbopogon proximus***

*Cympopogon proximus* commonly grown in this area of Sudan has been tested for adsorption capacity of Zn(II) and Pb(II). *Cympopogon proximus* has been put in water with and without nutrient medium. 50 ml 5 ppm Zn(II) concentration decreased to 2.34 ppm with 2.0 g *Cympopogon proximus* in nutrient medium at 10 minutes and 5 ppm Zn(II) concentration decreased to 2.17 ppm at 20 minutes and became 1.70 ppm at 30minutes.(Table 3.5.1). 50 ml 5 ppm Pb(II) concentration decreased to 2.7 ppm and 2.42 ppm and 2.42 ppm with 2.0 g *Cympopogon proximus* at 10,20,30 minutes respectively (Table 3.5.2).

The results are more satisfactory when 10 ppm Zn(II) solution treated with almost 2.0 g of *Cympopogon proximus* in nutrient medium. The residual Zinc is 7.52 ,4.10 and 2.77 at 10,20,30 minutes respectively (Table 3.5.3) 50 ml .10 ppm Pb(II) concentration decreased to5.77 ppm and 4.30 ppm and 3.41 ppm when treated with 2.0 g *Cympopogon proximus* at 10,20,30 minutes respectively (Table 3.5.4).

Whole set of experiments have been carried out with the dried and powdered plants in nutrient medium. *Cympopogon proximus* was found to be a good accumulator of Lead and Zinc. The mechanism of biosorption may be through intracellular uptake are important for detoxification of Pb(II) and Zn(II) by the application of medicinal planta. The phytochemical compounds (**Flavonoids**) of these plants are reactive towards dissolved metals. Thus the investigation presents the applicability of *Cymbopogon proximus* in order to remove covalent Lead and Zinc from aqueous medium.

# Conclusion

*Cympobopogon proximus* have been used as traditional medicine in many countries, *C.proximus it* most widely used in traditional and in conventional medicine due to the pharmacological potential of their phytochemicals. The majority of these species contain a voluminous amount of essential oils which have shown several biological activities such as insecticidal, anti-protozoan, anticancer, anti-HIV, anti-inflammatory and anti-diabetes effects.

the major component of *C. proximus* oil was piperitone, elemol, eudesmol, β-eudesmol. confirmed the presence of additional compounds which are 4-carene, 2-carene, limonene, p-cymene in *C. proximus.*

Heavy metals can cause many dangerous diseases in human beings so that we must remove it using chemicals and other methods. However, these methods were not very effective and highly cost, but plants and biological methods is best choice in this field. We use the plant according to their folkloric use in water purification. The results of this study showed that the tested plant have capability to remove such metals and the study had confirmed the traditional use of these plants in water purification. *C.proximus* contained flavonoid ,tannin due to the chemical structure, flavonoids can chelate metal ions and form complexes so the efficacy of this plant may be to the presence of these compounds.

It can reasonably conclude that, *cymbopogon proximus* had capability to remove such metals also this finding confirm the traditional use of this plant in water purification. Therefore, a wide investigation of this plant for their removal potent of heavy metals and the identification of the phytochemicals (Flavonoids) of this plant would be an interesting line of inqurity.

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