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Mohamed Ahmad Malin
Department of Applied Industrial Chemistry, International university of Africa, 2469-Khartoum, Sudan

Dr. Mahmoud Mohamed Ali
Department of Applied Industrial Chemistry, International university of Africa, 2469-Khartoum, Sudan

Ashraf Mahmoud Ramadhani
Department of Applied Industrial Chemistry, International university of Africa, 2469-Khartoum, Sudan

Correspondence
Mohamed Ahmad Malin
Department of Applied Industrial Chemistry, International university of Africa, 2469-Khartoum, Sudan

GC-MS analysis and antimicrobial activities of *Cymbopogon proximus* essential oil and phytochemical screening of its crude extracts

Mohamed Ahmad Malin, Dr. Mahmoud Mohamed Ali and Ashraf Mahmoud Ramadhani

Abstract
Use of plant based drugs and chemicals for curing various ailments and personal adornment is as old as human civilization. Plants and plant-based medicaments are the basis of many of the modern pharmaceuticals we use today *for our various* ailments. *Cymbopogon proximus* is considered one of medicinal plant which is widely used especially in Sudan. In the present study, an attempt was made to determine the phytochemical composition of the leaves extracts (Distilled Water, Chloroform and Hexane) of *C. proximus*, Its Vitro effects against *Aspergillus niger*, *Candida albicans*, *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* showed high activity on both Gram-positive, Gram-negative bacteria and the two tested fungus however no activity observed against Pseudomonas aeruginosa. In Preliminary phytochemical analysis revealed the presence of Saponins, tannins and terpenes in large quantities compared to others and for GC-MS analysis, it was founded that the major compounds was Piperitone (50.30%), 2-Carene, β-Elemol, β-Eudesmol, Eudesm-7(11)-en-4-ol and D-limonene, where other minor components range less than 3 to 0.02% were also considered in its activities such as thymol and Camphene The amount of essential oil obtained by hydro distillation was 4.46% w/w and the minimum inhibition concentration (MIC) ranged between 0.25 and 0.125 µl/ml. From our data, we conclude that the leaf of *Cymbopogon proximus* extract can be explored further as an option for efficacious and safe drug as bactericidal and fungicidal agents.

Keywords: *Cymbopogon proximus*, essential oil, phytochemical screening, antimicrobial activity

1. 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 (Shrestha and Dhillions, 2003) [21]. And hence natural products play an important role in drug development programs in the pharmaceutical industry (Baker *et al.*, 1995) [4].

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. (Edeoga, *et al.*, 2005 and Abubakar, *et al.*, 2008) [6, 1].

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 . 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) as pointed out by Yentéma *et al*., (2007) [24] and Marwat *et al*., (2009) [15]. 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. According to different researches done on *C. proximus* it was founded to be effective renal antispasmodic and diuretic agent (El-Askary *et al*., 2003; El-hardallou, 2011; Sabry *et al*.,

2014) [7, 8, 20] possessing a sedative, digestive and anti-parasitic properties (Sousa *et al.*, 2005) [22]. Norbert *et al.* (2014) [17] demonstrated that *C. proximus* is an antifungal and anti-inflammatory agent used for the prevention and treatment of acute inflammatory skin conditions. Furthermore, *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.

2. Materials and methods

2.1 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.

2.2 Chemicals and reagents

N-hexane, Chloroform, Distilled Water, Methanol, Anhydrous Calcium Chloride, dilute hydrochloric acid, Wagner's reagent (Iodine in Potassium Iodide), Lead acetate, Sodium hydroxide, concentrated H_2SO_4, Fehling's solution, Ferric chloride $FeCl_3$, Nutrient agar and Sabouraud-dextrose were all analytical grade.

2.3 Standard Microorganisms

Six strains of common bacteria and fungi were used in this study was obtained from the Microbiology laboratory, Faculty of Pure and Applied Science, International University of Africa as shown in table below:

Table 1: Shows standard Micro-organism and its species

Species / Microorganisms	Source
Aspergillus niger	*ATCC 1015 (*Fungi)
Candida albicans	*ATCC* 7596 (Fungi)
Escherichia coli	*ATCC 25922(*Gram -ve bacteria)
Klebsiella pneumonia	*ATCC* 53657(Gram -ve bacteria**)**
Pseudomonas aeruginosa	*ATCC* 27853 (Gram -ve bacteria)
Staphylococcus aureus	*ATCC* 25923(Gram +ve Bacteria)

ATCC = American Type Culture Collection.

2.4 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 polythene bag sealed ready for extraction.

2.5 Crude Extracts preparation

The n-hexane, 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 sample were LH-Leave n-Hexane, LC-Leaves Chloroform and LD-Leave Distilled water extract ready for phytochemical screening.

2.6 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.

$$\text{Weight of oil} = \text{density} \times \text{volume of oil} \quad (1)$$

$$\text{Volatile oil \%} = \frac{\text{weight of oil}}{\text{Sample weight}} \times 100(\text{w/w}) \quad (2)$$

2.7 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.

2.8 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.

2.8.1 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.

2.8.2 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.

2.8.3 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.

2.8.4 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.

2.8.5 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.

2.8.6 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.

2.8.7 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 or blue colored precipitate indicates the presences of tannins.

2.9 Antibacterial assay of *C. proximus* Essential Oil
Agar well diffusion method reported by Robert *et al.,* (2003) [19] were used for testing antibacterial activity of *Cymbopogon proximus* essential oil. Sterile nutrient agar plates were prepared. A sterile cork borer No. 4 was used for preparation of cups on the nutrient agar plates. Different concentrations of *Cymbopogon proximus* essential oil (100, 50, 25, 12.5 and 6.25µg/ml) and its pure Essential oil were pipetted into cups using micro titer-pipette and allowed to diffuse at room temperature for one hours. Marked at the bottom of each plate the specific concentration placed. The viable organisms tested were grown in broth overnight to contain 10^8 CFU/mL (colony-forming units per milliliter). Loop-full of diluted culture was spotted on the surface of sub-culture plate and was incubated at 37 ºC for 24 hours. The broth media containing the test bacteria were prepared using Norma Saline and were applied on the agar surface before incubation for 24 hrs at 37 °C. Methanol was used as control in separate cup.
After incubation the resulted diameters of growth inhibition zones were measured, averaged and the mean values were tabulated followed by tabulation of minimal inhibition concentration (MIC) results which were reported in details as the MIC in µg/ml.

2.10 Anti-fungal assay of *C. proximus* Essential Oil
The same methods used for the bacteria were adopted for Fungi. Using Sabouraud- dextrose agar instead of nutrient agar. The inoculated media were incubated at 25ºC overnight for the Aspergillus niger and Candida albicans.

3. Results
3.1 Result of Weight Extracts of *C. proximus*
Table (2) below shows extraction and percentage yields by weight of 25g of the *C. proximus* (leaves) using 125ml of 3solvents (n-hexane, chloroform and distilled water) respectively in order of polarity.

Table 2: Weight Extracts of *C. proximus*

Plant extract	Part used	Solvents	Yield (g)	Yield (%)
Cymbopogon Proximus Locally known [Maharaib]	Leaves	n-Hexane	0.7g	2.8%
		Chloroform	0.82g	3.28%
		Aqueous	1.92g	7.68%

In hexane extraction, leaves showed smallest values of crude extract comparable with chloroform and Aqueous extraction. Where aqueous extract showed highest %yield compared with chloroform and n-hexane.
These different results may be due to the difference of nature and polarity of each solvent. It's therefore; the distilled water represents as best solvents to extract *C. proximus*.

3.2 Results of Weight Extracts 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. Hence there is correlation with the results of (Kamal and Maged, 2008) [13] who reported that **"**the percentage of volatile oil yield from *C. proximus* prepared by hydro-distillation method was 5.44% w/w".

3.3 Phytochemical Screening Results
Table (3) below showed a phytochemical screening of *Cymbopogon proximus* extracts by using solvents, n-hexane, chloroform and Water.

Table 3: Phytochemical screening of the plant using different solvents

Plant Extract	Solvents	Alkaloid	Flavonoid	Glycoside	Phenol	Saponins	Tannin	Terpene
Cymbopogon Proximus	n-hexane	+	+	+	-	+	+	+
	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 solvents, 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 its traditionally claimed therapeutic properties. This study is in agreement with the results of (Dalziel, J.M. 1965) [10] 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) [23]. According to flavonoids are known to protect against allergies, diabetes, inflammations, malaria, platelet aggregations and microbial infection (Okwu, and Omodiromiro, 2005) [6]. 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) [23]. 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 GC-MS results

In the GC-MS analysis 55 phytochemical compounds were identified in the Essential oil of *C. proximus* leaves. Table 4 indicated 20 selected compounds in which the identification of phytochemicals was based on the molecular weight, molecular formulae and peak area. The identified compounds were fifty five, out of which six were found in abundance and classified as major compounds of the extract which were Piperitone (50.30%), 2-Carene (9.88%), β-Elemol (8.47%), β-Eudesmol (3.53%), Eudesm-7(11)-en-4-ol (3.21%) and D-limonene (3.08%) as major components, where other forty nine were classified as minor components which range in 3-0.02% in which some were significant components in *Cymbopogon proximus* essential oil such as thymol (0.11%) and Camphene (0.02%). 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.

Table 4: chemical composition of *C. proximus* essential oil as identified by GC-MS

Selective GC peak	Retention Time RT[1](min)	Chemical compound	Peak area (%)
2	5.135	Camphene	0.02
6	6.109	2-carene	9.88
10	6.667	D-limonene	3.08
17	8.646	p-2-Menthen-1-ol	3.05
20	9.637	p-Mentha-1,5-dien-8-ol	1.11
22	10.046	p-Cymen-8-ol	0.63
23	10.148	α-terpineol	2.38
25	10.510	2-pentylcyclopentanone	0.66
27	11.619	p-Menth-1-en-3-one (Piperitone)	50.30
28	12.282	Thymol	0.11
31	13.977	Geranyl acetate	0.63
34	14.864	Caryophyllene	1.22
43	16.192	Dihydro-beta-agarofuran	0.35
44	16.503	Cuparene	0.51
49	17.263	β-Elemol	8.47
50	17.950	Caryophyllene oxide	1.03
51	18.759	Guaiol	2.14
52	19.111	β-Eudesmol	3.53
53	19.150	α-Eudesmol	2.63
54	19.271	Eudesm-7(11)-en-4-ol	3.21

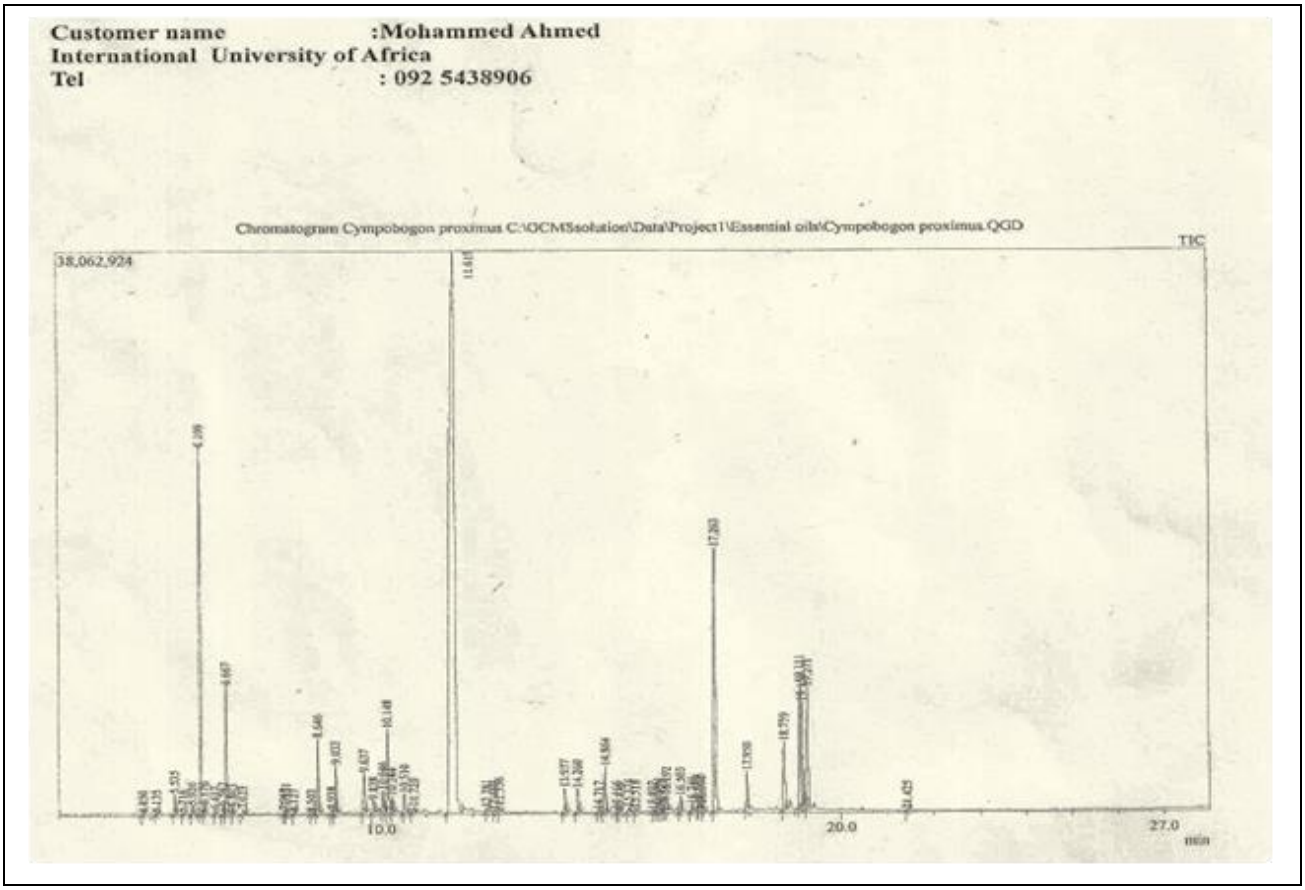

Fig 1: GC-MS -Chromatogram of *Cymbopogon proximus* essential oil

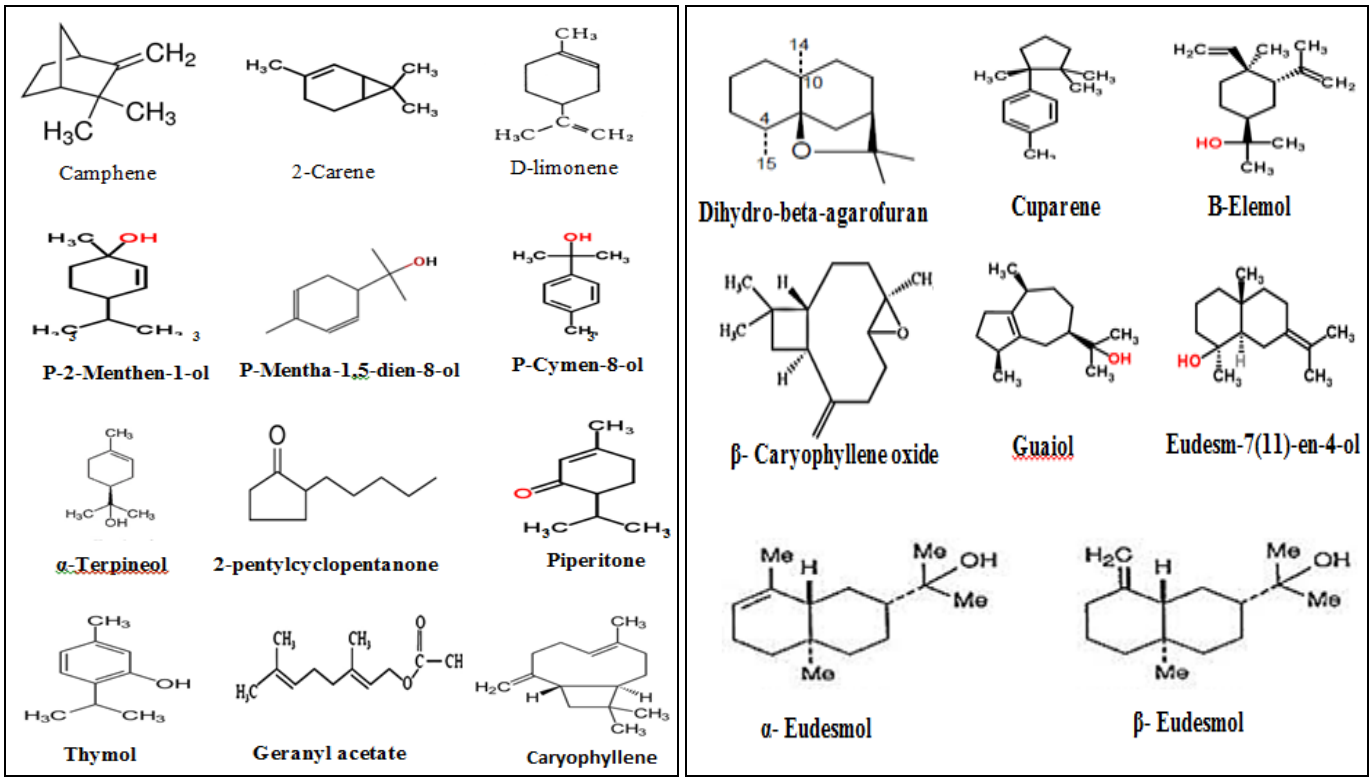

Fig 2: Structures of the compounds that were obtained by GC-MS analysis.

This study shows an agreement with the results of Gasal, *et al*., (2016) [11], Kamal and Maged (2008) [13], El-kamali, *et al*., (2010) [9] and Banthorpe, *et al*., (1976) [5] whom reported, the major component of *C. proximus* was piperitone, α- elemol, β-elemol, α-eudesmol, and β-eudesmol. Meanwhile Al-Taweel, *et al*., (2013) [3] confirmed the presence of additional compounds which are α-Terpineol, β-Caryophyllene and Caryophyllene oxide in *C. proximus*.

The present study is contrary to the results of (Menut *et al*., 2000) [16] 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 (50.30%) in Sudanese *C. proximus* as showed GC-MS results above.

3.5 Antimicrobial Activity Assay

In vitro antimicrobial activity of the essential oil of *C. proximus* showed antimicrobial activity against the tested fungi and bacteria using the agar well-diffusion method. Antimicrobial activity was recorded as the clear zone of inhibition surrounding the agar well. The mean of the zones of inhibition are shown in Table below for essential oil before dilution.

Table 5: Shows Antimicrobial activity of *C. proximus* essential oil and inhibition zones (mm) with concentration of 100 µl/ml against six standard microorganisms.

Test Microorganisms	Inhibition zones (mm)	Activity
Aspergillus niger	58	+++
Candida albicans	60	+++
Escherichia coli	78	+++
Klebsiella pneumonia	75	+++
Pseudomonas aeruginosa	0	-
Staphylococcus aureus	63	+++

(+++) = high activity, (–) = no antimicrobial activity and (0) = no inhibition was detected

Inhibitory effect was detected on five pathogens, including Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (*E. coli* and *Klebsiella*) where as essential oil showed high antifungal activity against the two tested fungi (*Aspergillus Niger* and *Candida albicans*). No antimicrobial activity was observed against *Pseudomonas aeruginosa.* This was followed by study of minimum inhibition concentration of essential Oil of *C. proximus* to test specimens that was positively inhibited by 100 µl/ml as tabulated bellow.

Table 6: Shows the mean of Minimum Inhibition Concentration (MIC) against five standard microorganisms

Microorganisms	Concentrations %	Inhibition zones (mm)
Aspergillus niger	50	36
	25	18
	12.5	0
	6.25	0
Candida albicans	50	39
	25	24
	12.5	15
	6.25	0
Escherichia coli	50	42
	25	26
	12.5	15
	6.25	0
Klebsiella pneumoniae	50	34
	25	20
	12.5	16
	6.25	16
Staphylococcus aureus	50	29
	25	19
	12.5	12
	6.25	0
(0) = no inhibition was detected		

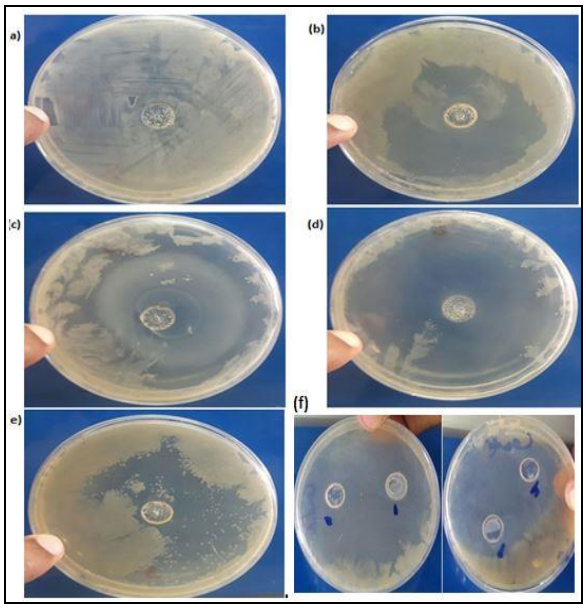

Fig 3: Zone of Inhibition of: (a) *Pseudomonas aeruginosa,* (b) *Candida albicans,* (c) *Staphylococcus aureus,* (d) *Escherichia coli,* (e) *Klebsiella pneumonia* and (f) E. coli

4. Conclusion

The essential oil and an aqueous extract of *C. proximus* showed high antimicrobial activities against gram positive and gram negative bacteria similar to fungus with negative result on pseudomonas. The presence of phytochemicals may be responsible for its therapeutic effects; this was further verified through GC-MS results showing presence of vital compounds. The plant could be a source of new antibiotic compounds which could be more effective against multidrug resistant strains of micro-organisms. More tests on experimental animals must be done using *Cymbopogon proximus* as herbal medicine for the treatment of human diseases, infections

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