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GC-MS Charaterization of Phytochemicals and Anti Microbial Properties of *Chromolaena odorata* Leaf Harvested from South Eastern Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

The analysis of the leaf extract of *Chromolaena odorata* was carried out to characterize its components, GC-MS analysis was carried out in our laboratory. The spectrum obtained showed 15 peaks which translates to 15 compounds, with their molecular weight, formula and structures. Initial phytochemical screening showed the presence of alkaloid, saponins, tannins, flavonoids and triterpenoids, cardic glycosides and phenols while antraquinone glycosides was absent. Antimicrobial analysis revealed that the extract showed marked activities against Streptococcus sp, Staphylococcus sp, Pseudomonas aeruginosa but was resistant to Serratia marcescens.

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Keywords: Chromolaena odorata; gas chromatography; phytochemicals; antimicrobial activity.

1. INTRODUCTION

"*Chromolaena odorata* is popular weed that grows in the tropics and commonly called awolowo plant in the Eastern part of Nigeria, several parts of this herb has been used to treat wounds, burns, and skin infections" [1-4]. "It also possesses anticancer, antidiabetic, anti-hepatotoxic, anti-inflammatory, antimicrobial, and antioxidant properties. Its phytochemical components are alkaloids, flavonoids, flavanone, essential oils, phenolics, saponins, tannins, and terpenoids. Some important constituents of this plant includes Eupolin and quercetin is used in traditional medicine for the treatment of inflammation skin infections and wounds .It is used also for the treatment of malaria, abdominal and cervical pain, urinary tract infections, ulcers, diarrhoea, coughs, colds and skin Certain phytochemicals such as (terpenoids, steroids, flavonoids, alkaloids, saponins, tannins, phlobatannin, and phenols and are associated with the plant which are of immense pharmacological importance" [5-9]

"The plant shows antimicrobial activities against Shigella flexneri, Shigella sonnei Neurospora crassa" [10]. "*Chromolaena odorata* has numerous therapeutic potentials, that explains why it is used in Traditional medicine as anti-diarrheal, astringent, antispasmodic, antihypertensive, anti-inflammatory, diuretic tonic, antipyretic and heart tonic agent" [11,12]. "The extract of the plant reduces the bleeding and clotting time, because it contains many antioxidant compounds that enhance wound healing property" [13,14].

"The plant spreads rapidly in lands used for forestry, pasture and plantation crops. It includes 1,200 species of small herbs" [15]. "Essential oils from the plant has antifungal, antimicrobial activities and antiradical potential as it can serve as insecticide" [16,17].

2. MATERIALS AND METHODS

2.1 Sample Collection

Fresh leaves of *Chromolaena ordorata* was obtained from its natural habitat in Amakohia Owerri Imo state. The plant was identified by Dr F. Ibeawuchi of Crop Science Department Federal University of Technology Owerri. The samples were washed, air dried, and pulverized into uniform fine powder using an electric blender and stored in a new air tight clean sample container.

2.2 Phytochemical Screening

2.2.1 Frothing test for saponins

This test is based on the ability of the saponins to produce froth in aqueous solution.5g of the plant extract was weighed into a test tube and 50ml of water was added and extracted after two hours. The water extract was shaken vigorously in a conical flask. The production of a stable froth indicates the presence of saponins in the sample.

2.2.2 Test for flavonoids

5g of the sample was soaked with 20 ml of water and left to stand for 2 hours, it was then filtered and to the filtrate drops of ammonia and 3ml of concentrated H_2SO_4 was added. A yellow precipitate which disappears on storage indicates the presence of flavonoids.

2.2.3 Test for alkaloids

5g of the sample was extracted using 20% acetic acid in ethanol.5ml of the extract was treated with Wagner's reagent (iodine crystals and KI). A yellowish brown precipitate indicates the presence of alkaloids.

2.2.4 Test for tannins

5g of the powdered leave sample was weighed into a beaker and 50ml of water was added and allowed to soak properly for two hours and filtered. The extract was treated with drops of ferric chloride. A blue-black precipitate indicates the presence of tannins.

2.2.5 Terpenoids (Salkowski Test)

To 2 ml of ethanol extract was added 0.5ml of chloroform. Add 1 ml of concentrated H_2SO_4 a reddish brown coloration in the interface of the two layers indicate the presence of terpenoids.

2.2.6 Cardiac glycosides test

5 ml of the water extract was measured into a 50 ml beaker and equal volumes of Fehling

solutions A and B were added this mixture was heated using a heating mantle until it boils reddish brown precipitates was indicative of the presence of cardiac glycoside.

2.2.7 Anthraquinones (Borntrager's Test)

2 ml of ethanol extract measured into a dry beaker and 1.0 ml of chloroform was added and shaken for 5 min. The extract was then shaken with equal volume of 10 % ammonia solution. A pink violet or red color in the ammoniacal layer (lower layer) indicate the presence of anthraquinone.

2.2.8 Test for phenol

2 ml of extract and 2 ml of iron chloride was mixed in a beaker, a deep green or bluish green colouration indicates presence of phenols.

2.2.9 Determination of flavonoid

"17 g of sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The solution obtained was filtered with Whitman filter paper No 45. The filtrate was later transferred into a crucible and was evaporated to dryness over a water bath and weighed" [18].

2.2.10 Determination of tannins

17 g of the sample was measured into a beaker and 150 ml of water was added. The sample was stirred and allowed to stand for 4 hours before filtration with Whatman filter paper. Few drops of conc HCl was added to the clear solution to acidify it, this is followed by the addition of ethyl acetate. The solution was properly mixed and separated with a separating funnel. This was repeated twice the aqueous solution obtained while the ethyl acetate solution discarded. The aqueous solution was heated to dryness and tannin was obtained and weighed.

2.2.11 Determination of saponins

"17 g of the sample was weighed into a 250 ml beaker and 200 ml of 20% ethanol was added and stirred using glass rod. The mixture was heated over water bath for 4 hrs with continuous stirring while the temperature was maintained at 55oC the mixture was extracted and the residue was extracted with 200 ml of 20% ethanol. The combined extract was reduced to 40 ml over water bath at 90oC.The concentrated extract was transferred into a 250 ml separation funnel and 250 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the diethyl ether was discarded. The process was repeated thrice .60 ml of n-butanol was added. The mixture was washed twice with 10 ml of 5 % sodium chloride. The remaining solution was heated over water bath and the residue dried to constant weight. The saponin content was calculated in percentages" [19].

2.2.12 Determination alkaloid

"17 g of the sample was weighed into a 250 ml beaker and 200 ml of 29 % acetic acid in ethanol was added and covered to stand for 6hrs. This was filtered and the extract was concentrated using a water bath to one quarter of the original volume. The Alkaloid was precipitated out using concentrated ammonium hydroxide which was added drop by drop until precipitation was complete. The solution was allowed to settle and the precipitation was collected by filtration using Whatmann filter paper, the precipitate was dried andweighed" [19].

2.3 Anti Microbial Analysis

2.3.1 Sample preparation for microbial analysis

250 ml of ethanol was introduced into 100 g of the powdered sample in a 500 ml beaker. This was allowed to stand for 48 hours filtered and the filtrate concentrated and used for analysis

2.3.2 Isolation of bacterial and fungal isolates

The isolates were obtained from clinical and environmental sources using streaking and pour plate methods. Isolates obtained were biochemically characterized and includes; Staphylococcus aureus, Streptococcus sp, Pseudomonas aeruginosa, Serratia marcescens and Penicillium sp. The organisms were subcultured unto plates of nutrient agar, macconkey and potato dextrose agar to obtain pure cultures of the organisms. Smears of the bacterial isolates were made unto clean grease-free slides, air- dried and heat fixed.Gram staining was done for each of the bacterial isolates Catalase test, coagulase test, indole test, and citrate utilization test were carried out to further identify the bacterial isolates using the standard methods Organisms were identified.

2.3.3 Gram staining

Heat fixed smears of each of the bacterial isolates was made unto clean grease- free slides and were stained with crystal violet for 1 minute. Washed in water and covered with lugols iodine and allowed for a minute The smears were decolorized with acetone until no more colour appeared to ooze out They were counter stained with safranin for 1 minute .The slides were blot-dried with filter paper and allow to dry

2.3.4 Inoculum preparation

Serial dilutions of the test organisms were made to get a concentration that corresponds to 0.5 ml McFarland's turbidity standard. The diluted bacterial suspension was compared visually against the 0.5 ml McFarland's turbidity standard by placing against a white background This procedure was repeated for each of the bacterial isolates.

2.3.5 Preparation of bacterial and fungal suspensions

"2mls of normal saline was aseptically poured into sterile 5ml test tube. The test tube. The test tubes were labeled with the names of each isolate; bacteria and fungi. Two gram positive bacteria*, Staphylococcus aureus and Streptococcus pneumonia,* one gram negative bacteria (*Klebsiella specie* , two fungi specie *Aspergillus niger* and *Mucors specie.* These five test tubes were set up on test tube rack. With the help of a sterile wire loop, each fungal and bacteria were transferred to each tube bearing each isolates name according to the previous labeling of the test tubes. The test tubes were swirled after each inoculation until the isolate suspension becomes turbid. The color of each tube was marched with that of a 5% Marc Farlard standard. Bacterial suspension had four test tubes while the fungal suspensions had two test tubes" [20].

2.3.6 Dilution of extract

Varying concentration of each of the extracts was obtained using doubling dilution The dilution was achieved using four test tubes for each extract The four tubes were labeled as follows tube 1 [Neat], tube 2 [1/10], tube 3[1/20], tube 4 [1/40] 1 ml of ethanol was added to each tube from test tube 2- tube 4 Another 1ml of extract was added to tube 2- a homogenate of the mixture was achieved by gentle and careful shaking of the tube 1ml of the homogenate was taken using a sterile pipette and transferred to tube 3. The content of tube 3 was also mixed properly and then 1 ml of the homogenate was aspirated using a sterile pipette and transferred to tube 4. The content of tube 4 was carefully mixed and then 1 ml of the homogenate was aspirated and discarded.

2.3.7 Evaluation of antibacterial activity using well in agar diffusion method

Standardized concentration of the test bacteria [S. aureus, streptococcus sp, p. auroginosa and Serratia marcescens] and fungi [penicillium sp] were spread on the streaked unto the surface of freshly prepared Mueller-Hinton Agar plates and potato dextrose agar plates with a sterile wire loop. These were allowed for 30 minutes to diffuse and a no 4 cork borer was used to bore hole of 8mm diameter on each of the agar plates containing the five isolates. A volume of 0.1 ml [100ų] of each of the three extracts was used to fill the agar wells made in the Mueller – Hinton agar plates and potato dextrose plates. The Mueller –Hinton plates were allowed to stand for I hour to allow the extract diffuse into the agar and were incubated at 37o C for 24 hours while the potato dextrose plates were incubated at room temperature for 5 days. After incubation, the zones of inhibition around the extract was measured using a ruler. Zones greater than 8mm were regarded as sensitive while zones less than 8 mm were regarded as resistance.

2.4 GC-MS Experimental Procedures

2.4.1 Preparation of samples for GC-MS analysis

30g of the sample was repeatedly extracted with 400 ml of ethanol using soxhlet extractor; another 30 g of each sample was soaked in 200 ml ethanol for 48 hours and extracted, this extract was concentrated using rotary evaporator and regarded as cold extract. The extracts from the soxhlet extracts and that obtained from cold extracts were combined and they were re-extracted using chloroform to obtain chloroform soluble extract which was used for analysis. The extract obtained here was used only for Gc-Ms analysis

2.4.2 GC-MS experimental determination

"GC-MS analysis was carried out with SHIMAZU Japan Gas Chromatography 5890-11 with a fused GC column OV 101 coated with polymethyl silicon (0.25 mm x 50 m) and the conditions are

as follows: Temperature programming from 80-200oC held at 80oC for 1 minute, the rate is 5oC/min and at 200oC for 20minutes. FID Temperature of 300oC, injection temperature of 250oC, carrier gas is Nitrogen at a flow rate of 1cm3/min and split ratio of 1:75. GC-MS Gas chromatography, Mass spectrum analysis were conducted using GC-MS QP 2010 Plus Shimazu Japan with injector Temperature at 230oC and carrier gas pressure of 100kpa. The column length was 30m with a diameter of 0.25mm and the flow rate of 50m/min. The eluents were automatically passed into the Mass Spectrometer with a detector voltage set at 1.5kv and sampling rate of 0.2 seconds. The Mass Spectrometer was also equipped with a computer fed Mass Spectra data bank, HERMCEZ 233M-Z centrifuge Germany was used. Reagents and solvents such as Ethanol, Chloroform, Diethyl ether, hexane all of analytics grade was obtained from Merck Germany" [20].

3. RESULT AND DISCUSSION

3.1 Results of Phytochemical Screening

Results for phytochemical screening as presented in Table 1 below, shows the presence of alkaloids, saponins, tannins, flavonoids and triterpenoids, cardic glycosides and phenols while antraquinone glycosides was absent.

The phytochemical quantification result in Table 2, the leaf extract contains 1.98g given 0.11% of tannins. "Tannins have astringent properties, hastening the healing of wounds and inflamed mucous membrane" [21]. The presence of Tannins in the sample supports the use in treating wounds, varicose ulcers, hemorrhoids, frost bites and burns in herbal medicine.

Tannins are polyphenol compounds that are well known with its protein inhibition property. Tannins interfere with the process of protein synthesis by binding to the proline rich protein. Besides, high concentration of tannins also shows antimicrobial and antifungal activities by coagulating the protoplasm of microorganisms. The presence of tannins in this study give credence to the antimicrobial effects of *Chromolaena odorata* on some known human pathogens such as Staphyloccocus aureus, Escherichia coli and Candida albicans.

The leaf of *Chromolaena Odorata contains* 0.25g alkaloid given 0.014% as shown in Table 2. "Alkaloid rank among the most efficient therapeutically significant plant substance. Pure isolated alkaloids and their synthetic derivatives are used by Etinomedicinal practitioners for their analgesic, antispasmodic and bactericidal effects" [22]. "They exhibit marked physiological activity when administered to animals; the high alkaloid content of this sample may be the reason for the use in the treatment of wounds, rheumatism and skin infections. Most samples containing alkaloid are used in Nigeria for the treatment of malaria and fever" [23].

Table 1. phytochemical screening of leaf extract of *C- odorata*

Phytochemical Constituents	Inference
Alkaloid	++
Saponins	++
Cardic glycoside	++
Anthraquinone glycoside	--
Tannins	++
Flavonoids	++
Terpenoids	+
Phenols	+

Key; ++ present, - - absent
Results of phytochemical determination of c-odorata

Table 2. Results of determination of phytochemical in the leaves extracts of *C odorata*

Phytochemical components	Mass (g)	Percentage yield %
Tannins	1.98	0.11
Saponins	2.48	0.14
Alkaloid	0.25	0.014
Flavonoid	0.47	0.027

"Alkaloids are vast and vary a lot in their activity when ingested by man and livestock. Some alkaloids are useful and important in medicine and constitute most of the valuable drugs currently used by humans. They are reported to have marked physiological effect on animals" [24,25]. Saponins was found to be available at 2.48g in the leaf of *Chromolaena Odorata* constituting the highest value of 0.14% as shown in Table 2. "The saponin content fortifies the use of the extract from this plants in the treatment of wounds. Some of the general characteristic of saponins includes; formation of forms in aqueous solutions, hemolytic activity and cholesterol binding properties" [26,27]. Saponin has the natural tendency to ward off microbes and this makes them good for treating fungal and yeast infections. These compounds serve as natural antibiotic, helping the body to fight infections and microbial invasion. Saponins mostly are soap forming compounds that also have antimicrobial property. The flavonoid content of *C odorata* leaf was found to be 0.47g given 0.027 % as shown in Table 2 result. "Flavonoids are distributed group of polycyclic compounds characterized by a common Benzo pyrone ring structure that has been reported to act as antioxidants in many biological systems. The family encompasses flavonoids, flavones, chalcones, catechins, anthocyanidins and isoflavonoids" [28]. "In addition to their free radical scavenging activities, Flavonoids have multiple biological activities including – vasodilatory, anti-carcinogenic, anti-allergic, antiviral, estrogenic effects as well as being inhibitors of phospholpase H_2, cycloxygenase, glutathione reductase and xanthine oxidase" [29-31]. They support lactogenecity. Flavonoids in intestinal tracts lower the risk of heart diseases. As anti-oxidant, favonoids provide anti-inflammatory actions Antibacterial activity has been displayed by a number of flavonoids. Flavonoids also possess anti-inflammatory and analgesic effect as well as anti-cancer properties [32].

3.2 Antimicrobial Analysis

"The extract showed marked activities against Streptococcus sp, Staphylococcus sp, Pseudomonas aeruginosa but was resistant to Serratia marcessen Most of these pathogens have been implicated to be the main causes of some human ailments. Staphylococcus aureus is a gram positive coccus that causes skin infection such as; pimples, impetigo, boils, cellulitis, folliculitis, carbuncles, scalded skin syndrome, abscesses, pneumonia, toxic shock syndrome, bacteremia and sepsis" [31]. "It has been reported that extracts from this plant has activity against gram positive bacteria Staphylococcus aureus and gram negative bacterial" [32].

Table 3. Antimicrobial activity of *c-odorata* extract against the bacterial isolate

Streptococcus Sp	Staphylococcus aureus	Pseudomonas aeruginosa	Serratia marcescens	Dilutions	Penicillium sp
31mm	16mm	20mm	R	Neat	R
20mm	12mm	15mm	R	1/10	R
8mm	10mm	8mm	R	1/20	R
3mm [R]	8mm	4mm [R]	R	1/40	R

3.3 GC/MS Results

3.3.1 The GC/MS results obtained are enlisted below

Table 4. Compounds obtained from GC-MS spectrum of the leaf extracts of *c-odorata*

Peak	Chemical name	Molecular formula	Molecular weight
1	1,3-diethyl-5-methyl- Benzene	$C_{11}H_{16}$	148
2	1,2,3,4-tetrahydro- Naphthalene	$C_{10}H_{12}$	122
3	α-Terpineol	$C_{10}H_{18}O$	154
4	cyclopentylmethyl – Cyclohexane	$C_{12}H_{12}$	166
5	Tridecane	$C_{13}H_{28}$	184
6	1-methyl- Naphthalene	$C_{11}H_{10}$,	142
7	Decahydro-1,1,4a,5,6-pentamethylnaphthalene.	$C_{15}H_{28}$	206
8	(3aR,4R,7R,8aS)-4,9,9-Trimethyl-1-methylene-4,5,6,7,8,8a-hexahydro-1H-3a,7-	$C_{15}H_{22}$	202

Peak	Chemical name	Molecular formula	Molecular weight
	methanoazulene		
9	1,6-dimethyl- Naphthalene	$C_{12}H_{12}$	156
10	Pentadecane	$C_{15}H_{32}$	212
11	Propylidene-bicyclo [4.1.0] heptanes	$C_{10}H_{16}$	136
12	8-Isopropyl-1,5-dimethyltricyclo [4.4.0.02,7] dec-4-en-3-one	$C_{15}H_{22}O$	218
13	5-Hydroxy-4',7-dimethoxyflavanone	$C_{17}H_{16}O_5$	300
14	5,6,7,4'-Tetramethoxyflavanone	$C_{19}H_{20}O_6$	344

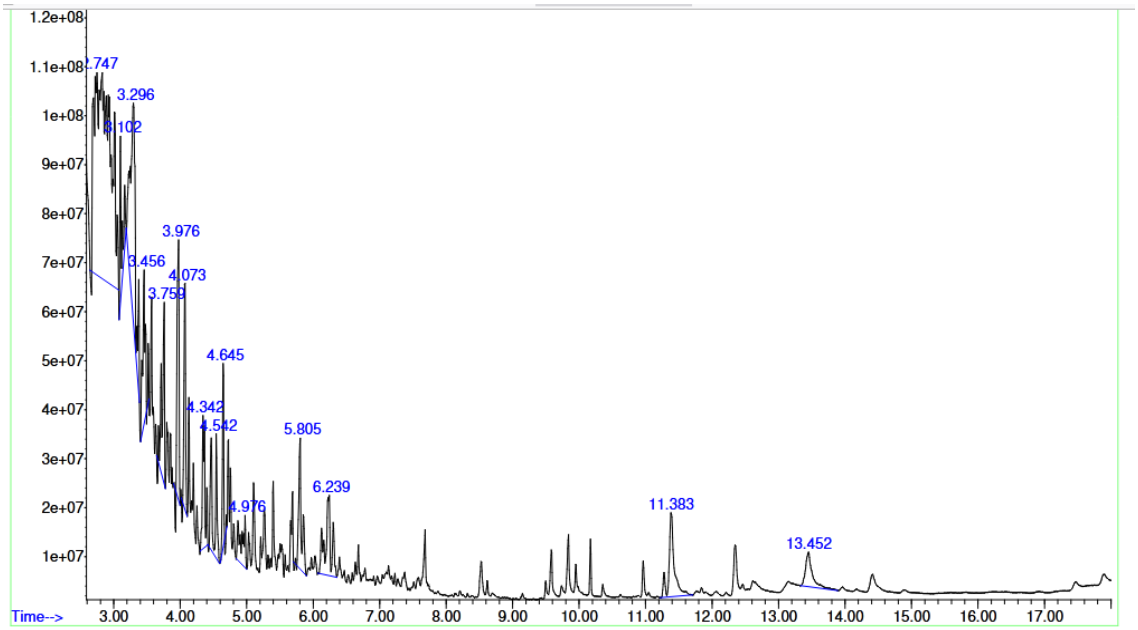

Fig. 1. GC/MS spectrum of the crude leave extracts of C -odorata

3.3.2 Chemical structures from GC-MS analysis of leaf of C-odorata

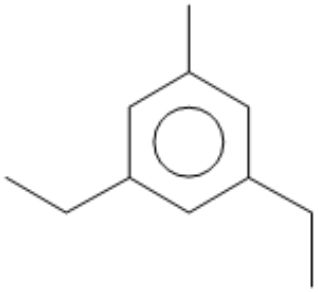

Fig. 2a. 1,3-diethyl-5-methyl- Benzene

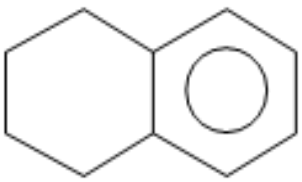

Fig. 2b. 1,2,3,4-tetrahydro- Naphthalene

OH

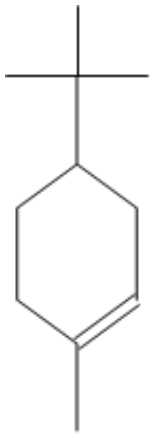

Fig. 2c. α-Terpineol

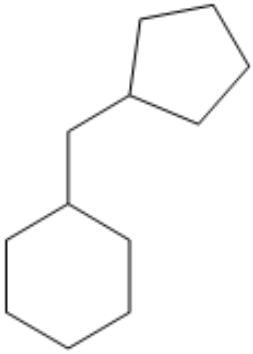

Fig. 2d. cyclopentylmethyl – Cyclohexane

Fig.2e. Tridecane

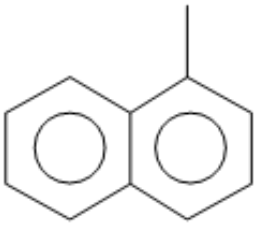

Fig. 2f. 1-methyl- Naphthalene

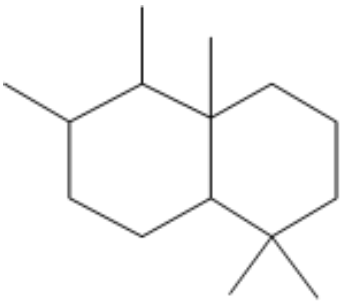

Fig. 2g. Decahydro-1,1,4a,5,6-pentamethylnaphthalene

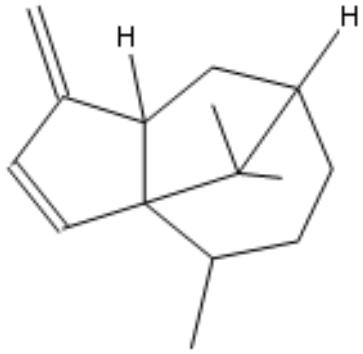

Fig. 2h. (3aR,4R,7R,8aS)-4,9,9-Trimethyl-1-methylene-4,5,6,7,8,8a-hexahydro-1H-3a,7-methanoazulene

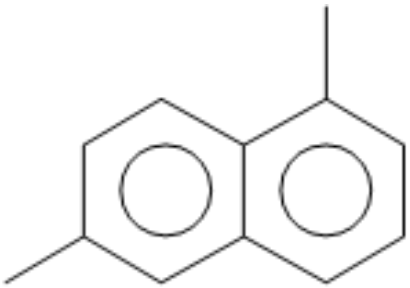

Fig. 2i. 1,6-dimethyl- Naphthalene

Fig. 2j. pentadecane

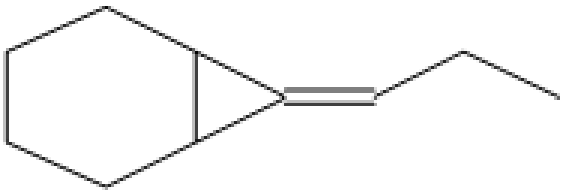

Fig. 2k. Propylidene-bicyclo[4.1.0]heptan

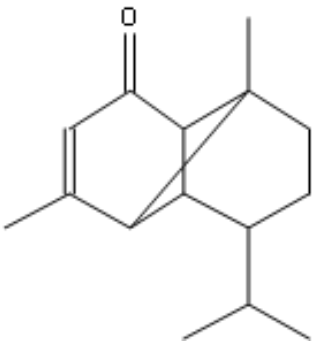

Fig. 2l. 8-Isopropyl-1,5-dimethyltricyclo [4.4.0.02,7] dec-4-en-3-one

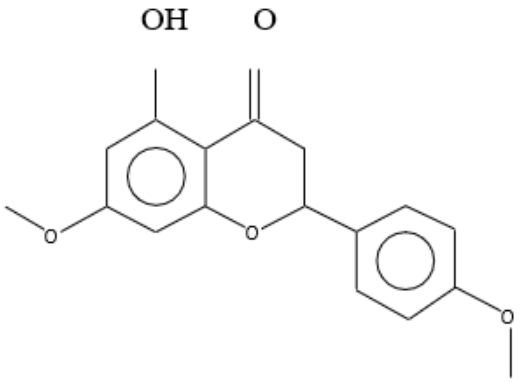

Fig. 2m. 5-Hydroxy-4',7-dimethoxyflavanone

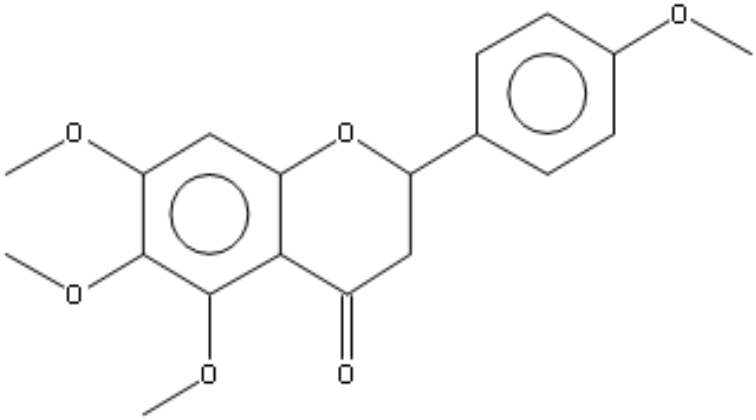

Fig. 2n. 5,6,7,4'-Tetramethoxyflavanone

Some of the important compounds like α-Terpineol is of great interest as it has wide range of biological application as an antioxidant, anti -cancer, anticonvulsant compound .It is also used to enhance skin penetration and also has insecticidal effect .Tridecane is used with other alkanes such as undecane pentadecane or dodecane to form a light weight smooth glide base for skin care formulations 1,3-diethyl-5-methyl- Benzene is used as reactive agent for pesticides synthesis and for denitrification reaction. The compound 1,2,3,4-tetrahydro-Naphthalene is used as a high boiling point solvent and can be used for producing lubricants and alpha tetralone Methoxy flavones are found naturally in plants and foods .Reports have shown that flavones play important roles in biotic and abiotic interaction and can serve as nutraceutical in human and animal foods [33,34].Flavones have antioxidant activities and thus can fight free radicals that cause ailment in animals Thy have anti-cancer and anti-inflammatory properties, they can fight chronic inflammation and pains. The presence of those compounds contributed to a wider range of medicinal effects such as cellular metabolism regulating activity [35], the anticancer activity of the plant has been studied, while the vascular relaxation and cardioprotective activity has been obtained [36], Mehoxyflavones are known to possess activities against *E. coli*, K. pnuemoniae and has vasorelaxation properties [37]. They are known for anticancer and cardioprotective activities [38]. Propylidene-bicyclo [4.1.0] heptanes inhibit protein synthesis in bacteria including staphylococci [39].

4. CONCLUSION

Antimicrobial analysis revealed that the extract showed marked activities against *Streptococcus sp, Staphylococcus sp, Pseudomonas aeruginosa but was resistant to Serratia marcescens*. The compound is effective against skin infections such as abrasions due to its ability to inhibit protein synthesis in bacteria It can be used against uncomplicated skin infections while in complicated cases it can be combined with other antimicrobials

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Hanuman, Donga Durga Veera, KV Venkata Rao, Gandham Nagarjuna, Kamishetty Mounika, Bhaskar Debbarma, Vemula Sravathi, Vankudothu Venkatesh, Pechetty Sravani, Mullu Atchuta Rao, and Amgoth Vamsi Kiran. Antioxidant effect of lemongrass (cymbopogon citratus) extract on paraquat-induced oxidative stress in sheep red blood cells using in-vitro phytochemical analysis. Journal of Advances in Biology & Biotechnology. 2024;27(5):730-38. Available:https://doi.org/10.9734/jabb/2024/v27i5835.
2. Adeniyi, Sunday Adegoke, Gabrial Ademola Olatunji, Olubunmi Stephen Oguntoye, and Ezekiel Olatoye Solanke. Phytochemical properties and in vitro antimicrobial activity of methanolic leaf extract of durio zibethinus murr. on selected clinical isolates. Asian Journal of Biology. 2024;20(4):22-29. Available:https://doi.org/10.9734/ajob/2024/v20i4399.
3. Jamilah J, Sharifa A, Sharifah NR. GC-MS analysis of various extracts from leaf of Plantago major used as traditional medicine. World Appl Sci J. 2012;17:67-70.
4. Annalakshmi R, Mahalakshmi S, Charles A, Sahayam CS. GC–MS and HPTLC analysis of leaf extract of Madhuca longifolia (Koenig) Linn. Drug Invention Today. 2013;5(2):76-80.
5. Anushika S, Wannee J, Sirinthipaporn A, Jiraungkoorskul W. Wound healing property review of siam weed, *Chromolaena odorata*. Phcog Rev. 2017;11:35-8.
6. Paterson ID, Zachariades C. ISSRs indicate that *Chromolaena odorata* invading southern Africa originates in Jamaica or Cuba. Biol Control. 2013;66: 132–9.
7. Shao X, Li Q, Lin L, He T. On the origin and genetic variability of the two invasive biotypes of *Chromolaena odorata*. Bio Invas. 2018;20(8):2033–46.
8. Bédi G, Tonzibo ZF, Chopard C, Mahy JP, N'Guessan TY. Etude des effects anti douleurs des huiles essentielles de *Chromolaena odorata* et de Mikania Cordata, par action sur la lipoxygenase. Phys Chem News. 2004;15:124–7.
9. Omokhua AG, McGaw LJ, Finnie JF, Van Staden J. *Chromolaena odorata* (L.) R.M. King & H. rob. (Asteraceae) in sub Saharan Africa: a synthesis and review of its medicinal potential. J Ethnopharmacol. 2016;83:112–22.
10. Foluke O, Cajethan E, Taofikat K, Daniel A, Grace M. Phytochemical Screening and Antimicrobial Activity of *Chromolaena odorata* Leaf Extract against Selected Microorganisms Journal of Advances in Medical and Pharmaceutical Sciences 13(4): 1-9,
11. Suksamrarn A, Chotipong A, Suavansri T, Boongird S, Timsuksai P, Vimuttipong S, Chuaynugul A. Antimycobacterial activity and cytotoxicity of flavonoids from the flowers of *Chromolaena odorata*. Arch Pharm Res. 2004;5:507-511.
12. Vaisakh MN, Pandey A. The invasive weed with healing properties: A review on *Chromolaena odorata*. Int. J. Pharm Sci. Res. 2012;3:80-83
13. Akomas SC, Ijioma SN. Bleeding and clotting time effect of ethanolic extracts of *Chrolmolaena* odorata versus Ocimum gratissimum treated albino rats. Compr J Med Sci 2014;2:9-13
14. Phan TT, Allen J, Hughes MA, Cherry G, Wojnarowska F. Upregulation of adhesion complex proteins and fibronectin by human keratinocytes treated with an aqueous extract from the leaves of *Chromolaena odorata* (Eupolin). Eur J Dermatol 2000;10:522-7.
15. Debashisha P, Santoshkumar D, Gourikumar D. Qualitative phytochemical analysis and investigation of anthelmintic and wound healing potentials of various extracts of *chromolaena odorata* linn. collected from the locality of mohuda village, berhampur (south orissa). Int J Pharm Biol Sci. 2010;1:56-67.
16. Félicien A, Alitonou GA, Djenontin TS, Tchobo F, Yèhouénou B, Chantal M,

Sohounhloué D. Chemical composition and biological activities of the essential oil extracted from the fresh leaves of *Chromolaena odorata* (L. Robinson) growing in Benin. ISCA J Biol Sci. 2012;1(3):7-13

17. Wang O. Biological activities of the volatile oil from *Chromolaena odorata* on fungi and insects and its chemical constituent. Nat Prod Res Dev. 2013;15(3):183-187.
18. Boham BA, Kocipai AC. Flavonoids and condensed tannins from leaves of Hawaina Vaccinium and V. Calycinium .Pacific Sci. 1994;48;458-463 15.
19. Obadoni B O, Ochuko P O. Phytochemical studies and comparative efficacy of the crude extract of some homeostatic plants in Edo state and Delta state of Nigeria. Global journal of Pure and Applied Sciences. 2001;8:203-208
20. Iwu IC, Alisa CO, Anukam B, Ali B,Ezekoye MO, Igbomezie MC, Anozie RC,Okoro MU, Obiagwu I. Chemical and Medicinal Properties of Spondias *mombim* (linn) Leaf Harvested from the South Eastern Nigeria Global Journal of Pure and Applied Chemistry Research. 2022;10(1):1-22.
21. Okwu DE, Okwu ME. Chemical composition of Spondia Mombium Linn plant parts. J. Sustain. Agric Environs. 2004;6(2);30-37
22. Iwu IC, Ogukwe C, Akah M, Chijioke-Okere M, Onu U, Iwu JO. Phytochemical and Antimicrobial Properties of the Root and leaf Extract of Carica papaya. International Journal of Innovative Research and Development. 2016;5(8):173-179
23. Adesuga SA, Cooker. HAB. Plants used in traditional Medicine against Malaria. Nig Journal of Pharm. 2001;32;5060.
24. Iwu IC, Ogukwe C, Akah M, Chijioke-Okere M, Onu U, Iwu JO. Phytochemical and Antimicrobial Properties of the Root and leaf Extract of Carica papaya. International Journal of Innovative Research and Development. 2016;5(8):173-179.
25. Iwu IC, Onu UL, Ukaoma AA, Oze RN. Phytochemical, Antimicrobial and GC/MS Analysis of the Root of Stachytarpheta Cayennensis (L. Vahl) Grown in Eastern Nigeria International Research Journal of Natural Sciences. 2018;6(2):1-14.
26. Okwu D E. Phytochemicals, Vitamins and Mineral Contents of Two Nigerian Medicinal Plants. International. Int. J.Mol. Med. Adv. Sci. 2005;1: 375-381.
27. Sodip OA, Akiniyi JA. Studies on certain characteristics of Extracts from bark of Pansinysstalia Macrucerus (K. Schum) pierre Exbeille. Global. J. Pure and Applied Sci. 2000;6:83 -87.
28. Okwu D E, Aluwuo C J. Studies on the phytochemical composition and fermentation of the seed of African oil bean tree Pentaclethra Macrophylla Benth. Int. journal of chemical societies. 2008;6:773-788. 30 .
29. Saleh W, Miller NJ, Paganga G, Tijburg GP. Bolwel E, Rice E, Evans C. Polyphenolic Flavonoids as scavangers of aqeous phase radicals as chain breaking anti 0xidants. Arch –Bio Chem Biorh. 1995;2:339-346.
30. Del-Rio A, Obdullio G, Casfillo J. Marin FG, Ortuno A. Uses and properties of Citrus Flavonoids. J. Agric Food Chem. 1997;45:4505-4515.
31. Okwu DE, Orji B. Phytochemical and Nutritional composition of selected Tropical edible grains of Northern Nigeria Food. 2007;4(2)347-350.
32. Iwu I C, Onu U, Ukaoma A A, et al. Characterization of the Volatile Components of the Leaf of Starchytarpheta cayennesis (Rich) Vahl. International Journal of Herbs, Spices and Medicinal Plants. 2019;4:041-049. 23.
33. Ikeyi A P, Ogbonna A O, Ibekwe R O, et al. antimicrobial activity of xylopia aethiopica (uda) on escherichia coli and staphylococcus aureus isolates from gastroenteritic patients. International journal of life science Biotechnology and Pharma Research. 2013;2:3.
34. Hsiao PC, Lee WJ, Yang SF, Tan P, Chen HY, Lee LM, Chang JL, Lai GM, Chow JM, Chien MH. Nobiletin suppresses the proliferation and induces apoptosis involving MAPKs and caspase-8/9/-3 signals in human acute myeloid leukemia cells. Tumor Biol. 2014;35:11903-11911
35. Okabe Y, Shimada T, Horikawa T, Kinoshita K, Koyama K, Ichinose K, Aburada M, Takahashi K. Suppression of adipocyte hypertrophy by polymethoxyflavonoids isolated from Kaempferia parviflora. Phytomedicine. 2014;21(6):800–806.

36. Potikanond S, Sookkhee S, Na Takuathung M, Mungkornasawakul P, Wikan N, Smith DR, Nimlamool W. Kaempferia parviflora extract exhibits anti-cancer activity against HeLa cervical cancer cells. Frontiers in Pharmacology. 2017;8:630.
37. Wattanapitayakul SK, Chularojmontri L, Herunsalee A, Charuchongkolwongse S, Chansuvanich N. Vasorelaxation and antispasmodic effects of Kaempferia parviflora ethanolic extract in isolated rat organ studies. Fitoterapia. 2008;79(3): 214–216 Available:https://doi.org/10.1016/j.fitote.2007.11.017.
38. Chen D, Li H, Li W, Feng S, Deng D. Kaempferia parviflora and Its methoxyflavones: Chemistry and biological activities. Evidence-Based Complementary and Alternative Medicine. 2018:4057456
39. Azuma T, Tanaka Y, Kikuzaki H. Phenolic glycosides from Kaempferia parviflora. Phytochemistry. 2008;69(15):2743–2748. Available:https://doi.org/10.1016/j.phytochem.2008.09.001.

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