GC-MSCHARATERIZATIOF OF PHYTOCHEMICALS AND ANTI MICROBIAL PROPERTIES OF CHROMOLAENA ODORATA LEAF HARVESTED FROM SOUTH EASTERN NIGERIA

ABSTACT.The analysis of the leaf extract of Chromolaenaodorata 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 thatthe extract showed marked activities against Streptococcus sp, Staphylococcus sp, Pseudomonas aeruginosa but was resistant to Serratia marcescens

Key words chromolaenaodorata, gas chromatography, phytochemicals ,Antimicrobial activity

INTRODUCTION

"Chromolaenaodoratais 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"[36,37,38,39]. "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 Eupolinand 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 plantwhichare of immense pharmacological importance"[1-5]

"The plant shows antimicrobial activities against Shigella flexneri,Shigella sonnei Neurospora crassa"[6]. "Chromolaenaodorata 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". [7-8]."The extract of the plant reduces the bleeding and clotting time, because it contains many antioxidant compounds that enhance wound healing property". [9-10]

" The plant spreads rapidly in lands used for forestry, pasture and plantation crops . It includes 1,200 species of small herbs"[11]. "Essential oils from the plant hasantifungal , antimicrobial activities and antiradical potential as it can serve as insecticide"[12-13].

MATERIALs AND METHOD

SAMPLE COLLECTION

Fresh leaves of *Chromolaenaordorata* 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 .

PHYTOCHEMICAL SCREENING

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 50mlof 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.

Test for flavonoids

5g of the sample was soaked with 20 mlof 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.

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.

Test for tannins

5g of the powdered leave sample was weighed into a beaker and 50mlof 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.

Terpenoids (Salkowski Test)

To 2 ml of ethanol extractwas 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 ofterpenoids .

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

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 .

Test for phenol

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

Determination of flavonoid

"17g of sample was extracted repeatedly with 100mlof 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"[14]

Determination of tannins

17g of the sample was measured into a beaker and 150ml 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

Determination of saponins

"17g of the sample was weighed into a 250 mlbeaker 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 55^{o}C the mixture was extracted and the residue was extracted with 200ml of 20% ethanol. The combined extract was reduced to 40ml over water bath at 90^{o}C .the concentrated extract was transferred into a 250ml 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 .60mlof 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" [15]

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 for6hrs. This was filtered and the extract was concentrated using a water bath to one quarter of the original volume. The Alkaloid wasprecipitated out using concentrated ammonium hydroxide which was added drop by drop until precipitation was complete. Thesolution was allowed to settle and the precipitation was collected by filtration using Whatmann filter paper, the precipitate was dried andweighed"[15]

ANTI MICROBIAL ANALYSIS

Sample preparation for microbial analysis

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

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 methodsOrganisms were identified.

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 waterand covered with lugols iodine and allowed for a minute The smears were decolorized with acetone until no more colour appeared to ooze outThey were counter stained with safranin for 1 minute .The slides were blot- dried with filter paper and allow to dry

Inoculum preparation

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

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"[16]

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 extractThe four tubes were labeled as follows tube 1 [Neat], tube 2 [1/10], tube 3[1/20], tube 4 [1/40] 1ml of ethanol was added to each tube from test tube 2- tube 4Another 1ml of extract was added to tube 2- a homogenate of the mixture was achieved by gentle and careful shaking of the tube1ml 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 1ml of the homogenate was aspirated and discarded.

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 0.1ml [100uḷ] 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 37^{o} 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.

GC-MSEXPERIMENTALPROCEDURES

PreparationofSamplesforGC-MSAnalysis

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

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 80-200^{o}C held at 80^{o}C for 1 minute, the rate is 5^{o}C/min and at 200^{o}C for 20minutes. FID Temperature of 300^{o}C, injection temperature of 250^{o}C, carrier gas is Nitrogen at a flow rate of 1cm^{3}/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 230^{o}C 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"[16].

RESULT ANDDISCUSSION

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

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

The phytochemical quantification result in table 2, the leaf extract contains 1.98g given 0.11% of tannins. "Tanninshave astringent properties, hastening the healing ofwounds and inflamed mucous membrane"[17]. The presence of Tannins in the sample supports the use intreating wounds, varicose ulcers, hemorrhoids,frostbitesand burnsin herbalmedicine.

Tannins are polyphenol compounds thatare 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 *Chromolaenaodorata* on some known human pathogens such as Staphyloccocus aureus, Escherichia coli and Candida albicans .

The leaf of *ChromolaenaOdorata contains* 0.25g alkaloid given 0.014% as shown in table 2. "Alkaloid rank among the most efficient therapeutically significantplant substance. Pure isolated alkaloids and their synthetic derivatives are used by Etinomedicinal practitioners for their analgesic,antispasmodic and bactericidal effects"[18]."They exhibit marked physiological activity when administered toanimals; the high alkaloid content of this sample may be the reason for the use in the treatment of wounds, rheumatism andskin infections. Most samples containing alkaloid are used in Nigeria for the treatment of malaria and fever".[19]

"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".[20,21] .Saponins was found to be available at 2.48g in the leaf of *ChromolaenaOdorata*constituting the highest value of 0.14% as shown in **table 2** . "The saponin content fortifies the use of theextract from this plants in the treatment of wounds. Some of the general characteristic of saponins includes; formation of forms inaqueous solutions, hemolytic activity and cholesterol binding properties"[22,23]. Saponin has the naturaltendency to ward off microbes and this makes them good for treating fungal and yeast infections. These compounds serveasnatural antibiotic,helpingthebodytofightinfectionsandmicrobial 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 compoundscharacterized by a common Benzo pyrone ring structure that has been reported to act as antioxidants in many biological systems. Thefamily encompasses flavonoids, flavones, chalcones, catechins, anthocyanidins and isoflavonoids"[24]."In additionto 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 andxanthine oxidase". [25-27]. They support lactogenecity. Flavonoids in intestinal tracts lower the risk ofheartdiseases.Asanti-oxidant,favonoidsprovideanti-inflammatoryactions Antibacterial activity has been displayed by a number of flavonoids. Flavonoids also possess anti-inflammatory and analgesic effect as well as anti-cancer properties

Antimicrobial analysis

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

Streptococcus Sp	Staphylococcus aureus	Pseudomonas aeruginosa	Serratia marcescens	Dilutions	Penicilliu m 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

"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"[27]."It has been reported that extracts from this plant has activity against gram positive bacteria Staphylococcus aureus and gram negative bacterial"[28].

GC/MS Results

The Gc/Ms results obtained are enlisted below

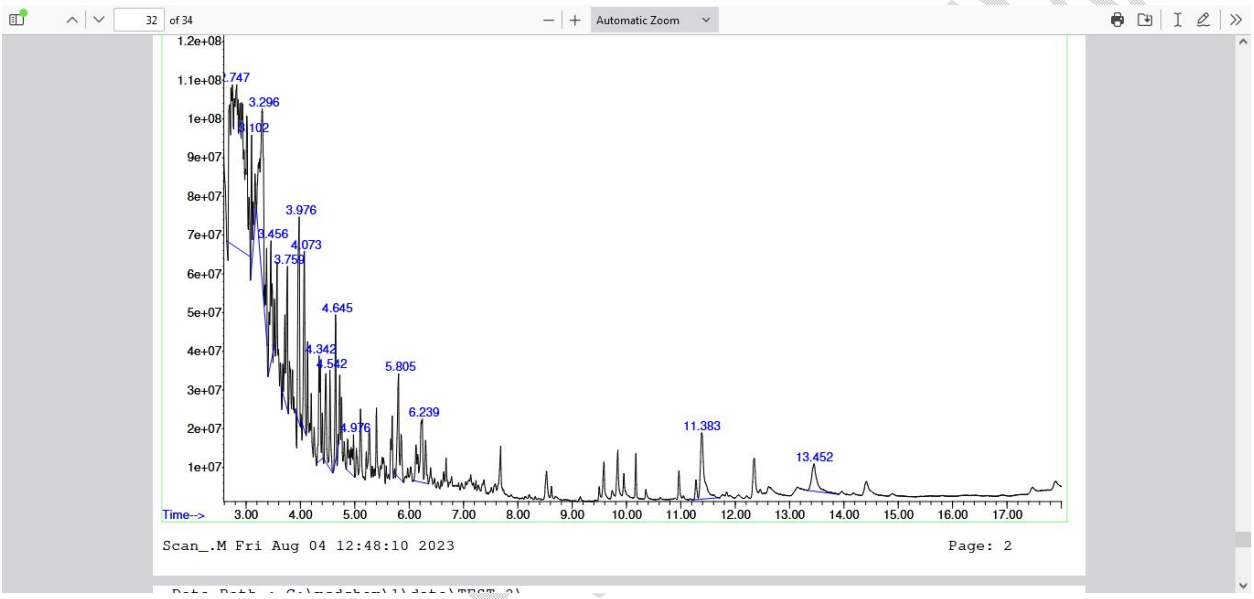

Fig 1 GC/MS spectrum of the crude leave extracts of C -odorata

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-methanoazulene	$C_{15}H_{22}$	202
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

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

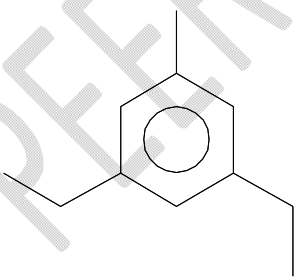

Fig2.a 1,3-diethyl-5-methyl- Benzene

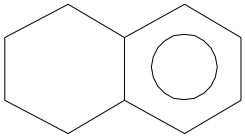

Fig2 .b 1,2,3,4-tetrahydro- Naphthalene

OH

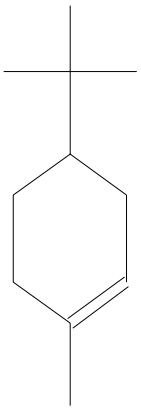

Fig2.c α-Terpineol

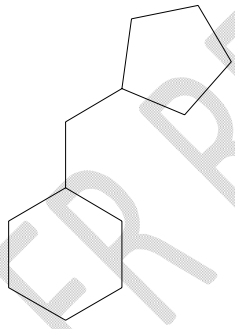

Fig 2.dcyclopentylmethyl – Cyclohexane

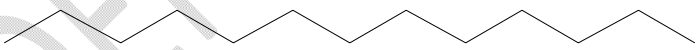

Fig2e Tridecane

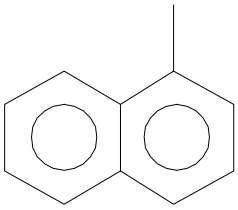

Fig2.f 1-methyl- Naphthalene

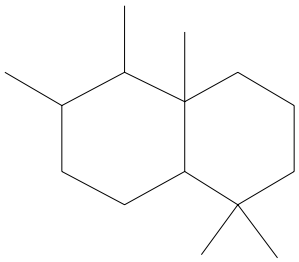

Fig2g ;Decahydro-1,1,4a,5,6-pentamethylnaphthalene

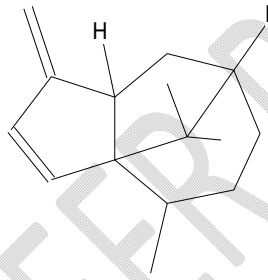

Fig2.h(3aR,4R,7R,8aS)-4,9,9-Trimethyl-1-methylene-4,5,6,7,8,8a-hexahydro-1H-3a,7-methanoazulene

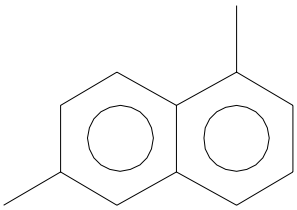

Fig2.i1,6-dimethyl- Naphthalene

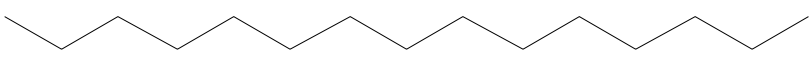

Fig2.j pentadecane

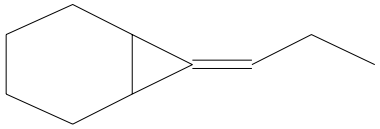

Fig2.kPropylidene-bicyclo[4.1.0]heptan

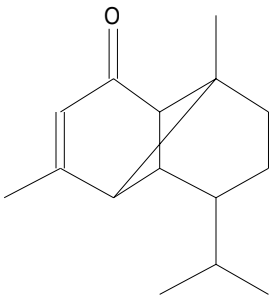

Fig2.l8-Isopropyl-1,5-dimethyltricyclo[4.4.0.02,7]dec-4-en-3-one

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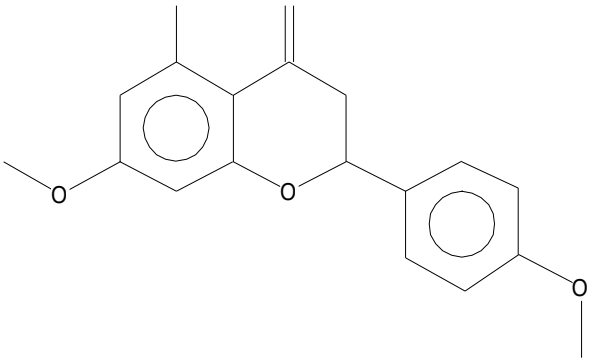

Fig2.m5-Hydroxy-4',7-dimethoxyflavanone

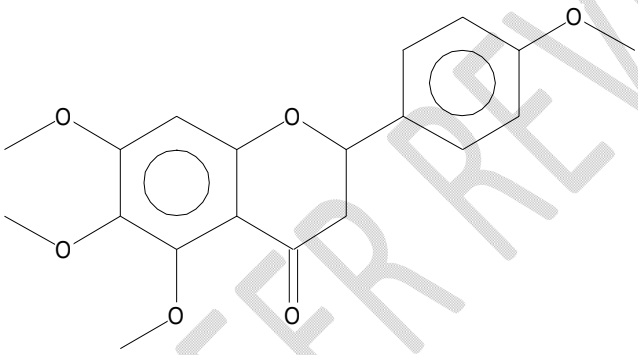

Fig2.n 5,6,7,4'-Tetramethoxyflavanone

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[29-30].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 metabolismregulating activity[31],the anticancer activity of the plant has been studied, while the vascular relaxation and cardioprotective activity has been obtained [32], Mehoxyflavones are known to possess activities against E. coli, , K. pnuemoniae and has vasorelaxation properties[33]. They are known for anticancer and cardioprotective activities[34].Propylidene-bicyclo[4.1.0]heptanes inhibit protein synthesis in bacteria including staphylococci.

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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Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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