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

ABSTACTthe analysis of the leaf extract of Chromolaena odorata wsa carried out to characterize its components, 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) ,the spectrum obtained showed 15 peaks which was translates to 15 compounds with their molecular weight, formular and structures. Initial phytochemical screening shows the presence of alkaloids, 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 serratiamarcessen

Key words chromolaena odorata, gas chromatography, phytochemicals ,

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. 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, phenols and are associated with the plant[1-5]

The plant shows antimicrobial activities against Shigella flexneri ,Shigella sonnei Neurospora crassa[6]. Chromolaena odorata has numerous therapeutic potentials,that explains why it is used in Traditional medicine as anti-diarrheal, astringent, antispasmodic, antihypertensive, antiinflammatory, diuretic tonic, antipyretic and heart tonic agent. [7-8].The extract of the plan 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 has antifungal and antimicrobial activities and antiradical potential it can serve as insecticide [12-13].

MATERIALs AND METHOD

SAMPLE COLLECTION

Fresh leaves of *C-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 .

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 50cm^3 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.

Test for flavonoids

5g of the sample was soaked with 20 cm^3 of water and left to stand for 2 hours, it was then filtered and to the filtrate drops of ammonia and 3cm^3 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.5cm^3 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 50cm^3 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.

Terpenoids (Salkowski Test)

To 2 cm^3 of ethanol extractwas added 0.5 cm^3 of chloroform. Add 1cm^3 of concentrated H_2SO_4 a reddish brown coloration in the interface of the two layers indicate the presence ofterpenoids .

Cardiac Glycosides Test

5cm^3 of the water extract was measured into a 50cm^3 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 cm^3 of ethanol extract measured into a dry beaker and 1.0 cm^3 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

2cm^3 of extract and 2 cm^{3}of 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 100cm^{3}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[14]

Determination of tannins

17g of the sample was measured into a beaker and 150 cm^3 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 cm^3 beaker and 200 cm^3 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^oC the mixture was extracted and the residue was extracted with 200cm^3 of 20% ethanol. The combined extract was reduced to 40cm^3 over water bath at 90^oC .the concentrated extract was transferred into a 250cm^3 separation funnel and 250 cm^3 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$cm^3$of n-butanol was added. The mixture was washed twice with 10 cm^3 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

Determination Alkaloid

17 g of the sample was weighed into a 250 cm^3 beaker and 200 cm^3 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 whatman filter paper, the precipitate was dried andweighed[15]

ANTI MICROBIAL ANALYSIS

Sample preparation for microbial analysis

250cm^3 of ethanol was introduced into 100g of the powdered sample in a 500 cm^3beaker. This was allowed to stand for 48 hours filtered and the filtrate concentrated and used for analysis

Collection of Bacterial and Fungal isolates

The isolates wares obtained from clinical and environmental sources using streaking and pour plate methods. Isolates obtained include staphylococcus aureus, Streptococcus sp, Pseudomonas aeruginosa , Serratia marcescens and Penicillium spThe 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 properly identified.

Gram Staining

Heat fixed smears of each of the bacterial isolates was made unto clean grease- free slides The smears were stained with crystal violet for 1 minuteThey were washed in waterThe smears were covered with lugols iodine and allowed for a minute They were washed in waterThe smears were decolorized with acetone until no more colour appeared to ooze outThey were counter stained with safranin for 1 minute They were washed with water The slides were blot- dried with filter paper and allow to dryThey were examined microscopically using X 100 objectives of the microscope

Biochemical test for identification of bacterial isolates

Catalase test

Few colonies of the organisms were emulsified in drop of distilled water on clean slides and placed in Petri- dishes 2 drops of H_2O_2 [hydrogen peroxide] was added and the dishes were coveredThe plates were observed for gas bubbles which indicates a positive result

Coagulase test

A drop of saline was placed on a clean slide One or two colonies of the organism was emulsified unto the drop of saline A straight wire loop dipped into a human plasma was used to mix the bacterial suspension Clumping of the mixture was observed for as it indicates a positive result.

Indole test

The organism was grown overnight in peptone water A few drops of kovacs reagent was added to the overnight peptone water culture.A red ring was observed for above peptone water as this indicates a positive result.

Citrate Utilization Test

A light suspension of the organism was made in saline.It was stab inoculated into Simmons citrate agar.A growth of blue colour in Simmons agar indicates a positive result.

Oxidase Test

A loop full of oxidase reagent was added to a filter paper in a petri-dish.
With the aid of a platinum loop, a colony of the organism was smeared unto the welted filter paper.A purple colour was observed for as it indicates a positive result.Fungal Identification using Potassium Hydroxide Preparation A drop of potassium hydroxide was placed on a slide The fungal isolated was placed unto the dropIt was teased using two dissecting needlesA cover slip was placed over itThe preparation was allowed to stand at room temperatureIt was then observed microscopically.

Evaluation of Antimicrobial Activity

A Dilution of the organism

Serial dilution of the organisms were made to get a concentration that corresponds to 0.5cm^3 McFarland's turbidity standard.0.5cm^3 McFarland's turbidity standard was prepared by mixing 0.05 cm^3 of 1.175 % barium chloride dihydrate [$BaCl_2.2H_2O$] with 9.95 $cm^3$of 1% sulphuric acid [H_2 SO_4]0.9cm^3 of sterile nutrient broth was added to four clean test tubes set up on a rack 0.l$cm^3$of the broth cultures of one of the organisms was added to the first tube containing 0.9cm^3 of nutrient brothIt was well mixed and 0.1 cm^3 of the mixture was pipette and transferred to the second tube Content of the tube 2 was well mixed and 0.1 cm^3 of the mixture was pipette out and transferred to tube 3Content of tube 3 was then mixed properly while 0.1 cm^3 of the mixture was pipette out and discardedThe diluted bacterial suspension was compared visually against the 0.5 cm^3McFarland's turbidity standard by placing against a white background This procedure was repeated for each of the bacterial isolates.

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] 1cm^3 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 cm^3 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 cm^3 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 streaked on 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 4mm diameter on each of the agar plates containing the five isolates.A volume of 0.1cm^3 [10ul] 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 cm^3 of ethanol using soxhlet extractor; another 30 g of each sample wassoaked in 200 cm^3ethanol for 48 hours and extracted. 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

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 20 minutes. 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.

RESULT DISCUSSION

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 Results of phytochemical screening of leave extract of *c odorata*

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

Key; ++ present, - - absent

Results of phytochemical quantification 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[16]. The presence of Tannins in the sample supports the use intreating wounds, varicose ulcers, hemorrhoids,frostbitesand burnsin herbalmedicine.

Tannins are polyphenol compounds that 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 significantplant substance. Pure isolated alkaloids and their synthetic derivatives are used by Etinomedicinal practitioners for their analgesic,antispasmodic and bactericidal effects [17]. 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.[18]

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.[19,20] .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 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 [21,22]. 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[23]. 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. [24-26]. 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
31mm	16mm	20mm	R	Neat
20mm	12mm	15mm	R	1/10
8mm	10mm	8mm	R	1/20
3mm[R]	8mm	4mm[R]	R	1/40

The extract showed marked activities against Streptococcus sp, Staphylococcus sp, Pseudomonas aeruginosa but was resistant to serratiamarcessen 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]. Pseudomonas aeruginosa is a gram negative gamma-proteobacteria which belong to the family Pseudomonaceae. It causes bacteremia, pneumonia, folliculitis, swimmer ear which is an ear infection accompanied with swelling, ear pus. Itching, discharge and difficulty in hearing, eye inflammation with associated pains, pus, swelling redness and impaired vision. 29

GC/MS Results

The Gc/Ms results obtained are enlisted below

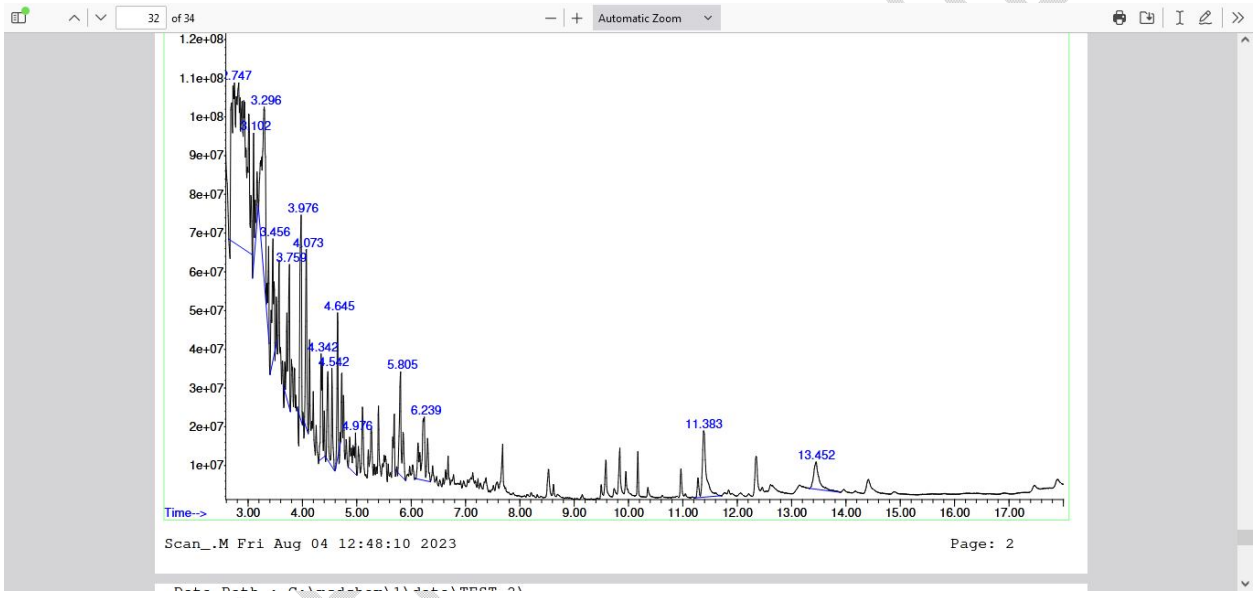


Fig 1 GC/MS spectrum of the crude leave extracts of C -odorata

.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

The interpreted values of the chromatogram are in table 5 and the structures of the compounds obtained are in fig 3. Peak 1 occurred at m/z 148, with molecular formula $C_{11}H_{16}$ and is named as 1,3-diethyl-5-methyl- Benzene, .Peak 2, occurred at m/z 122 with molecular formula $C_{10}H_{12}$ and is named,1,2,3,4-tetrahydro-Naphthalene. Similarly other peaks are interpreted as follows; Peak 3, m/z 154, molecular formula $C_{10}H_{18}O$, name. α-Terpineol Peak 4, molecular formula $C_{12}H_{12}$,m/z 166, name cyclopentylmethyl – Cyclohexane Peak 5 molecular formula $C_{13}H_{28}$ m/z 184, name Tridecane . Peak 6,molecular formula $C_{11}H_{10}$, m/z 142, name 1-methyl- Naphthalene .Peak 7, molecular formula $C_{11}H_{10}$, m/z 142, Name:,1-methyl- Naphthalene. Peak 8 ,molecular formula $C_{15}H_{28}$,m/z 208, Name:Decahydro-1,1,4a,5,6-pentamethylnaphthalene. Peak 9 molecular formula $C_{15}H_{22}$,m/z 202, Name:(3aR,4R,7R,8aS)-4,9,9-Trimethyl-1-methylene-4,5,6,7,8,8a-hexahydro-1H-3a,7-methanoazulene . Peak 10 ,molecular formula $C_{12}H_{12}$, m/z 156 , Name:,1,6-dimethyl- Naphthalene . Peak 11, molecular formula $C_{15}H_{32}$,m/z 212 , Name: Pentadecane Peak 12, molecular formula $C_{10}H_{16}$,m/z 136, Name:7-Propylidene-bicyclo[4.1.0] heptane . Peak 13, molecular formula, $C_{15}H_{22}O$ m/z 218, Name:8-Isopropyl-1,5-dimethyltricyclo[4.4.0.02,7] dec-4-en-3-one . Peak 14, molecular formula $C_{17}H_{16}O_5$, m/z 300 name,5-Hydroxy-4',7-dimethoxyflavanone Peak 15, molecular formula $C_{19}H_{20}O_6$. m/z 344. name, 5,6,7,4'-Tetramethoxyflavanone

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

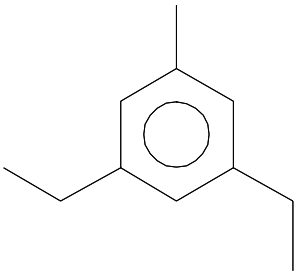


Fig2.a 1,3-diethyl-5-methyl- Benzene

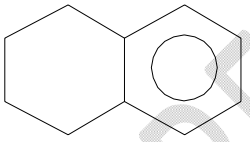


Fig2 .b 1,2,3,4-tetrahydro- Naphthalene

OH

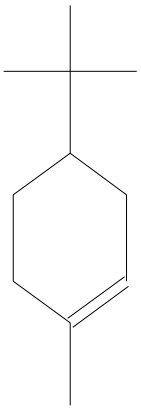


Fig2.c α-Terpineol

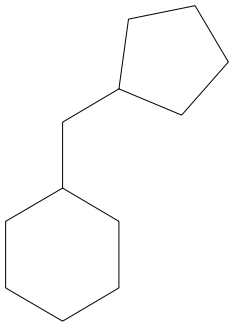
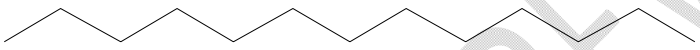


Fig 2.dcyclopentylmethyl – Cyclohexane

2.5 Tridecane



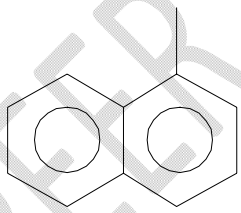


Fig2.e 1-methyl- Naphthalene

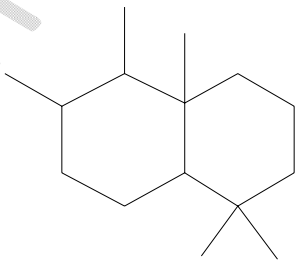


Fig2.f ;Decahydro-1,1,4a,5,6-pentamethylnaphthalene

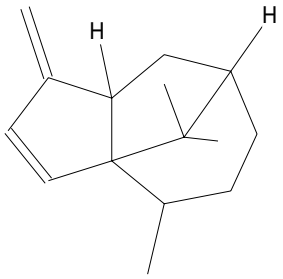


Fig2.g(3aR,4R,7R,8aS)-4,9,9-Trimethyl-1-methylene-4,5,6,7,8,8a-hexahydro-1H-3a,7-methanoazulene

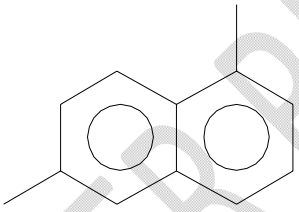


Fig2.h1,6-dimethyl- Naphthalene

Fig2.i pentadecane

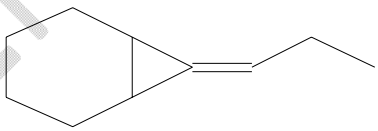


Fig2.jPropylidene-bicyclo[4.1.0]heptan

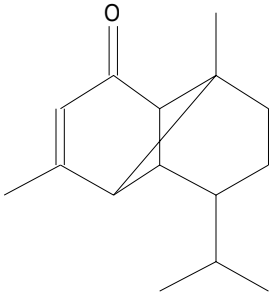


Fig2.k8-Isopropyl-1,5-dimethyltricyclo[4.4.0.02,7]dec-4-en-3-one

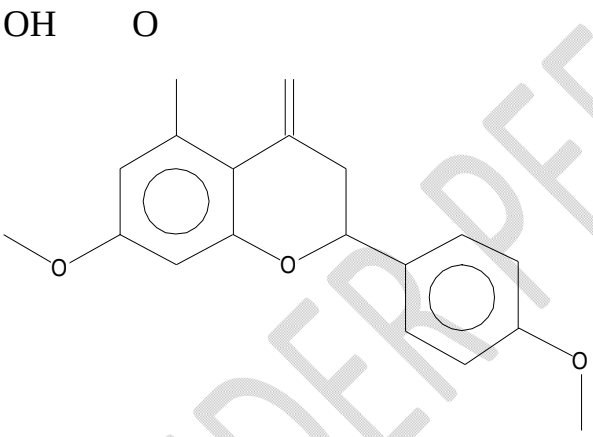


Fig2.l; 5-Hydroxy-4',7-dimethoxyflavanone

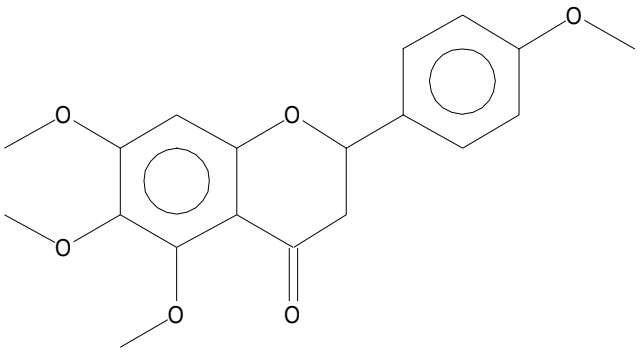


Fig2.m; 5,6,7,4'-Tetramethoxyflavanone

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[30-31].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[32] ,the anticancer activity of the plant has been studied, while the vascular relaxation and cardioprotective activity has been obtained [33], Mehoxyflavones are known to possess activities against E. coli, , K. pnuemoniae and has vasorelaxation properties[34]. They are known for anticancer and cardioprotective activities[35].Propylidene-bicyclo[4.1.0]heptanes inhibit protein synthesis in bacteria including staphylococci. 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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