Research Article

Qualitative and Quantitative Analysis of content in *Ginger* and its Antimicrobial, *Anttioxidant* Activities

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Abstract

This study investigates phytochemical constituents, oil content, antimicrobial and antioxidant properties for *Ginger*. Results showed the presence of Alkaloids, Tannins, Carbohydrates, Phenols, Cardiac Glycosides, Quinones, Anthraquinone, Terpenoids, Coumarins and Steroids. Quantitative analysis for bioactive constituents in oil using GC-MS. Constituents are: Cyclohexene,3-(1,5-diethyl-4-hexenyl)-6-methylene(16.98%),1,3-Cyclohexadine,5-(1,5-dimethyl-4-hexenyl)-2-(14.94%), alpha.- franesene(10.01%), beta.-Bisabolene (7.60%), beta.-Copaene (5.06%), Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-(4.98%), Eucalyptol(3.74%), Camphene(2.65%), Cyclopetene,3-isopropenyl-5,5-dimethyl-Eucalyptol(2.27%). In the disc diffustion bioassay, *Ginger* oil showed good activity against *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aureus*. Testing of antioxidant activity using 1,1-diphenyl-2-picrylhidrazil (DPPH) as free radicals, *Ginger* oil showed very high DPPH radical scavenging activity.

Keywords: *Ginger*, antioxidant, antimicrobial, Phytochemical

Introduction:

Medicinal plants, since times immemorial, have been used in virtually all cultures as a source of medicine. The wide spread use of herbal remedies and health care preparations, as those described in ancient texts such as the Vedas and the Bible, and obtained from commonly used traditional herbs and medicinal plants, has been traced to the occurrence of natural products with medicinal properties [1]. Essential oils are the collection of hydrophobic secondary

metabolites that can be extracted from plants and are used in perfumes, flavorings and alternative medicine techniques such as aromatherapy. While a single compound may have a distinct smell, most essential oils are actually hundreds of compounds that, when combined, create the smell associated with that particular plant[2].*Ginger* (Zingiber officinale) is a herbaceous perennial plant of the family *Zingiberaceae*, used as a spice, food, flavouring agent, and medicine. Its generic name, Zingiber is derived from the Greek gingivitis, *Ginger* was one of the first oriental spices known in Europe. Throughout the early centuries, *Ginger* was thought to have medicinal powers. It was often used by pregnant women for morning sickness. The spice has a slightly biting taste and is used, usually dried and ground, to flavour breads, sauces, curry dishes, confections, pickles, and *Ginger* ale. Its fresh rhizome is used in cooking. It is most alkaline-promoting foods. It has a pH of 5.6 to 5.9, similar to that of figs, fennel, leeks, parsnips and romaine lettuce. The alkalinity of foods depends on many variables, including growing conditions and processing[3]. There are many studies which prove the hypotensive effect of ginger when it was given at 0.3-3 mg/kg. It helps to reduce a trial blood pressure by blocking calcium channels or by acting on muscarinic receptor[4]. *Ginger* also gave an excellent result in treating nausea. A study showed that acetone and methanol extract of *Ginger* strongly inhibits gastric ulceration. *Ginge*r oil is produced from fresh or dried rhizomes. The phytochemicals of *Ginge*r consist of volatile oils (bisabolene, cineol, phellandrene, citral, borneol, citronellol, geranial, linalool, limonene, zingiberol, zingiberene, camphene), oleoresin (gingerol, shogaol), Phenol (gingeol, zingerone), Proteolytic enzyme (zingibain), Vitamin B6, Vitamin C, Calcium, Magnesium, Phosphorus, Potassium, Linoleicacid. The pungency of *Ginger* is due to gingerol which is the alcohol group of the oleoresin [5]. The composition of fresh ginger oil shows that it contains more of oxygenated compounds (29%) compared to dryginger oil (14%). The higher content of Geranial (monoterpenoid) and other oxygenated compounds makes fresh *Ginge*r oil more potent than dry *Ginge*r oil. The content of hydrocarbon compounds are more in dry ginger oil compared to fresh ginger oil.

Additionally, zingiberol is also another predominant to 3% of volatile oils; these are bisabolene, zingiberene and zingiberol[6]. The applicability of soxhlet extraction for extraction of ginger oil by using acetone, methanol, n-hexane, water, and ethanol as solvents ~~have~~ was studied. The experimental data was presented in terms of the effect of different methods, effect of solvent, effect of time and effect of temperature on extraction of *Ginge*r oil[7]. It has also been reported that ginger (rich in sesquiterpenes) essential oils posse-ssed a wide spectrum of antimicrobial activity However, antimi- crobialactivity (bioactivity)of essential oils was dependent not only on the major components but also on the chemical structures of these components[8]. The pungent principles of ginger rhizomes, the phenolic compounds zingerone, gingerols and shogaols, are the main factors determining the antioxidant activity of the rhizome. These natural antioxidants not only inhibit lipid oxidation in food but also prevent free-radical-induced diseases caused by oxidative tissue degeneration. Oxidative stress is a major factor in the pathogenesis of diabetic complications. Mechanisms to counteract oxidative stress in tissues of living organisms are associated with the modulation of antioxidant enzymes. Several spices, among others *Ginge*r, have been proven to possess antidiabetic potential. Antioxidant activity of *Ginge*r *invivo* also influences the xenobiotic metabolism by inducing drug metabolizing enzymes such as glutathione-S-transferase, quinine reductase and glutathione peroxidase[9]. This study was aimed to determine the phytochemical constituents presence in the rhizome extract of *Ginger,* determine the yield percentage of flavonoids, Alkaloids and Saponins in the rhizome extract, analyzed oil of *Ginger* by GC-MS and study antimicrobial antioxidant activities of oil.

Materials and methods:

Plant material:

*Ginge*r from Omdurman market – Sudan. The plants seeds were blended to powder using an electric blender and stored in air-tight bottles.

Bacterial microorganisms:

- *Bacillus subtilis* NCTC 8236 (Gram + ve bacteria)
- *Staphylococcus aureus* ATCC 25923(Gram +ve Bacteria)

- *Escherichia coli* ATCC 25922(Gram -ve bacteria)
- *Pseudomonas aeruginosa* ATCC27853 (Gram -ve bacteria)

Fungal microorganisms:

A. - *Candida albicans* ATCC7596

Preparation of extracts:

The *Ginger* plant was washed with distilled water to remove pollutant particles, then was air dried at ambient temperature for six weeks.The dried materials were pounded using asterile laboratory mortal and grander blended using asterile electric blender to obtain a homogenous sample. 50 g of each powdered dried Rizome were extracted for 72 hours using 95% ethanol in a 200 ml beaker and later transferred to an air-tight bottle to avoid escape of the solvent. The sample was filtered using muslin cloth and later filter paper. The organic solvent filtrates were concentrated in vacuum using a rotary evaporator to obtain crude extracts. Extract obtained was kept in an air-tight bottle until it was used for phytochemical screening test.

Phytochemical screening:

The phytochemical screening of secondary metabolites of the plant extracts were evaluated according to the method of harborne(1973). The phytochemical screening was done to confirm the presence or absence of certain phytochemical components that are responsible for the anti-microbial activities of plant extracts such as triterpenes, carbohydrates, flavonoids, glycosides, saponins, steroids, tannins, and alkaloids[8].

Test for Flavonoids:

To 2ml of plant extract, 1ml of 2N sodium hydroxide was added. Presence of yellow color indicated the presence of flavonoids.

Test for Alkaloids:

To 2ml of plant extract, 2ml of concentrated hydrochloric acid was added. Then few drops of Mayer's reagent were added. Presence of green color or white precipitate indicated the presence of alkaloids.

Test for Saponins:

To 2ml of plant extract, 2ml of distilled water was added and shaken in a graduated cylinder for 15minutes lengthwise. Formation of 1cm layer of foam indicated the presence of saponins.

Test for Tannins:

To 1ml of plant extract, 2ml of 5% ferric chloride was added. Formation of

dark blue or greenish black indicated the presence of tannins.

Test for carbohydrates:

Fehlings test, Equal volume of Fehling A (copper sulphate in distilled water) and Fehling solution B (Potassium tartarate and sodium hydroxide in distilled water) reagents were mixed with few drops of crude extract and boiled, a brick red precipitate of cuprous oxide forms, indicate the presence of reducing sugar.

Test for Phenols:

To 1ml of the extract, 2ml of distilled water followed by few drops of 10% ferric chloride Wasadded. Formation of blue or green color indicated the presence of phenols.

Test for Glycosides:

To 2ml of plant extract, 3ml of choloroform and 10% ammonia solution was added. Formation of pink color indicated the presence of glycosides.

Test for Cardiac Glycosides:

To 0.5ml of extract, 2ml of glacial acetic acid and few drops of 5% ferric chloride were added. This was under layered with 1 ml of concentrated sulphuric acid. Formation of brown ring at the interface indicated the presence of cardiac glycosides.

Test for Quinones:

To 1ml of extract, 1ml of concentrated sulphuric acid was added. Formation of red color indicated the presence of quinones.

Anthraquinone:

To 1ml of plant extract few drops of 10% ammonia solution was added and the appearance of pink color precipitate indicated the presence of anthraquinones.

Test for Terpenoids:

To 0.5ml of extract, 2ml of chloroform was added and concentrated sulphuric acid was added carefully. Formation of red brown color at the interface indicated the presence ofterpenoids.

Test for Coumarins:

To 1 ml of extract, 1ml of 10% NaOH was added. Formation of yellow color indicated the presence of coumarins.

Steroids and Phytosteroids:

To 1ml of plant extract equal volume of chloroform was added and subjected with few drops of concentrated sulphuric acid where the appearance of brown ring indicated the presence of steroids and appearance of bluish brown ring indicated the presence of phytosteroids.

Oil Extraction:

The oil of the Rizome was extracted with the steam distillation method according to the methods of analysis used for chemical control of food of the National Institute of Health, 1996. A sample of 500 g of mature Rizome was extracted using hexane for 6 hours. The solvent was removed with a rotary evaporator and the oil was kept in the fridge at 4°C for further manipulation. The qualitative and quantitative analysis of the sample was carried out using GM/MS technique model (GC/MS-QP2010-Ultra) from japans 'Simadzu Company, with capillary column (Rtx-5ms-30m×0.25 mm×0.25µm). The sample was injected by using split mode, helium as the carrier gas passed with flow rate 1.69ml/min, the temperature program was started from 50^{0}C with rate 7^{0}C/min reaching 280^{0}C as final temperature degree, the injection port temperature was 300^{0}C, the ion source temperature was 200^{0}C and the interface temperature was 250^{0}C. The sample was analyzed using scan mode in the range of m/z 40-500 charges to ratio. Identification of components for the sample was achieved by comparing their retention times and mass fragmentation patterns with those available in the library, the National Institute of Standards and Technology (NIST). Results were recorded.

Table (1): Chromatographic condition

Column oven temperature	150.0°C
Injection temperature	300.0°C
Injection mode	Split
Flow control mode	Linear velocity
Pressure	139.3KPa
Total flow	50.0ml/ min
Column flow	1.54ml/sec.
Linear velocity	47.2cm/sec.
Purge flow	3.0ml/min.
Spilt ratio	- 1.0

Antimicrobial test:

Bacterial suspensions:

The method was employed in the standardization of the test organism[10]. One ml aliquots of a 24 hour broth

culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37º C for 24 hours. The bacterial growth was harvested and washed off with 100 ml sterile normal saline, to produce a suspension containing about 108- 109 C.F.U/ ml. The suspension was stored in the refrigerator at 4° C till used. Serial dilutions of the stock suspension were made in sterile normal saline solution and 0.02 ml volumes of the appropriate dilution were transferred by micro pipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drops to dry and then incubated at 37 °C for 24 hours. After incubation, the number of developed colonies in each drop was counted. The average number of colonies per drop (0.02 ml) was multiplied by 50 and by the dilution factor to give the viable count of the stock suspension, expressed as the number of colony forming units per ml suspension. Each time a fresh stock suspension was prepared. All the above experimental conditions were maintained constantly so that suspensions with very close viable counts would be obtained.

Preparation of fungal suspension:

The fungal cultures were maintained on Sabouraud dextrose agar, incubated at 25 °C for 4 days. The fungal growth was harvested and washed with sterile normal saline and finally suspended in 100ml of sterile normal saline, and the suspended was stored in the refrigerator until used.

Testing of antimicrobial susceptibility:

The paper disc diffusion method was used to screen the antimicrobial activity of plant extracts and performed using Mueller Hinton agar (MHA). The experiment was carried out according to the National Committee for Clinical Laboratory Standards Guidelines (NCCLS, 1999). Bacterial suspension was diluted with sterile physiological solution to 10^8cfu/ ml (turbidity = McFarland standard 0.5). One hundred microliters of bacterial suspension were swabbed uniformly on surface of MHA and the inoculum was allowed to dry for 5 minutes. Sterilized filter paper discs (Whatman No.1, 6 mm in diameter) were placed on the surface of the MHA and soaked with 20 µl of a solution of each plant extracts. The inoculated plates were incubated at 37 °C for 24 h in the inverted position. The diameters (mm) of the inhibition zones were measured.

Antioxidant activity of Rhizome oil:

DPPH radical scavenging assay:

Ginger is having powerful antioxidant activity due to its oil, which has protective effect on DNA damage. They have demonstrated this effect in many cell culture [4] with some modification. In 96-wells plate, the test samples were allowed to react with 2.2Di (4-tert-octylphenyl)-1-picrylhydrazyl stable free radical (DPPH) for half an hour at 37°C. The concentration of DPPH was kept as 300 µm. The extract was dissolved in DMSO (500µg/ml. concentration), while DPPH was prepared in ethanol. After incubation, decrease in absorbance was measured at 517 nm using multiplate reader spectrophotometer. Percentage radical scavenging activity by samples was determined in comparison with a DMSO treated control group and propyl gallate (PG). All tests and analysis were run in triplicate.

Results and Discussion:

The *Ginger* oil which are key species in Sudanese have own medicinal property. When volatile oil of these species extract was analyzed by GC-MS result indicated to a lot of Photochemical compounds was existed in *Ginger* oil. Furthermore, the oil have been assessed for Antimicrobial activity via the cup plate agar Diffusion bioassay against five standard human pathogens and Anti-oxidant activity.

Photochemical analysis:

Phytochemical screening of *Ginger* for various compound classes was carried out by using standard procedures as described by Harbome 1998, Sofowora 1993 and Trease and Evans 1989. Phytochemical screening of the rhizome extracts revealed that Flavonoids, Alkaloids, Saponins, Tannins, Carbohydrates, Phenols, Glycosides, Cardiac Glycosides, Quinones, Anthraquinone, Terpenoids, Coumarins and Steroids are present (Table 2).

Table (2): phytochemical screening of *Ginger*

Phytochemical Compounds	Plant extract *Ginger*
Flavonoids	-
Alkaloids	+
Saponins	-
Tannins	+
Carbohydrates	+
Phenols	+
Glycosides	-
Cardiac Glycosides	+
Quinones	+
Anthraquinone	+
Terpenoids	+
Coumarins	+
Steroids	+

+ (contain this phytochemical compound)

GC-MS analysis of *Ginger*oil:

The oil of *Ginger* was analyzed by GC-MS. Identification of the constituents was accomplished by consulting the MS (NIST); the observed fragmentation pattern was also interpreted .74 components The typical total ion chromatogram (TIC) is depicted in (Fig1). Different components were quantified and identified by their retention time and mass spectra and a tabulation of constituents is presented in table (3).

Table (3): constituents *Ginger*roots oil

ID#	Name	Ret.Time	Area	Area%
	Camphene	4.413	11439735	2.65
	Cyclopentene, 3-isopropenyl-5,5-dimethyl-	5.837	9810498	2.27
	Eucalyptol	5.883	16158329	3.74
	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	14.528	21502456	4.98
	1,3-Cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, [S-(R*,S*)]-	14.790	64547675	14.94
	.beta.-copaene	14.843	21847402	5.06
	.alpha.-Farnesene	14.948	43243848	10.01
	.beta.-Bisabolene	14.985	32820913	7.60
	Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R*,S*)]-	15.269	73334748	16.98

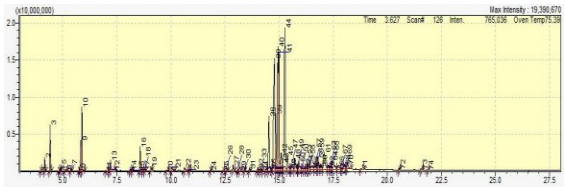


Fig.1: Total ion cromatograms of *Ginger* oil

The following major constituents were detected by GC-MS are:

Cyclohexene,3-(1,5-dimethyl-4-hexenyl)-6methylene-,[S-(R*,S*)(16.98%):

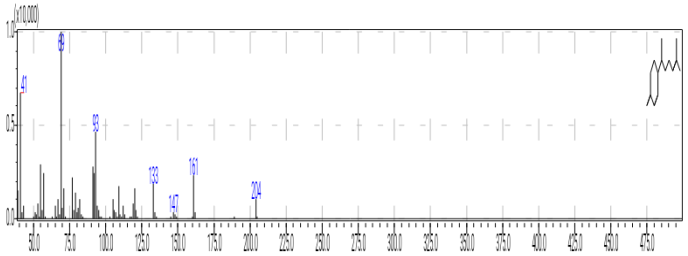


Fig.2:MassspectrumofCyclohexene,3-(1,5-dimethyl-4-hexeneyl)-6methylene-, [S-(R*,S*)]-

The El mass ofCyclohexene. In Fig. 2the peak at m/z 2o4, which appeared at R.T. 16.98 in total ion chromatogram, corresponds to $M^+[C_{15}H_{24}]^+$ The peak at m/z 189

corresponds toloss of a methyl function.

1,3Cyclohexadine,5-(1,5-dimethyl-4-hexenyl)-2-methyl-,[S-(R*,S*)]-(14.94%):

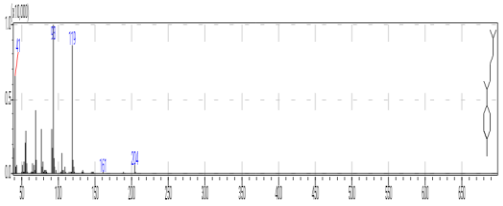


Fig.3:mass spectrum of 1,3 cyclohexadine,5-(1,5-dimethyl-4-hexenyl)-, [S-(R*,S*)]-

The El mass of Cyclohexadine. In Fig. 3 the peak at m/z 119, which appeared at R.T. 16.98 in total ion chromatogram, corresponds to $M^{+}[C_{15}H_{24}]^{+}$ The peak at m/z 105corresponds to loss of a methoxy function.

alpha.-Farnesene (10.01%):

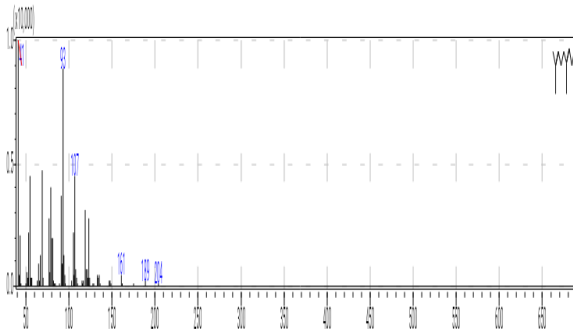


Fig.4:mass spectrum of alph.-farnesene

The El mass of alpha.- franesene. In Fig.4 the peak at m/z 204, which appeared at R.T. 14.948 in total ion chromatogram, corresponds to $M^{+}[C_{15}H_{24}]^{+}$ The peak at m/z 189 corresponds to loss of a methyl function.

beta.- Bisabolene (7.60%):

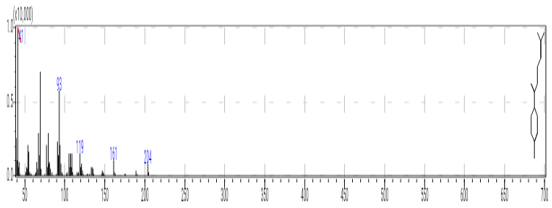


Fig.5:mass spectrum of beta.-Bisabolene

The El mass of beta.-Bisabolene. In Fig.5 the peak at m/z 204, which appeared at R.T. 14.985 in total ion chromatogram, corresponds to $M^{+}[C_{15}H_{24}]^{+}$ The peak at m/z 189corresponds to loss of a methyl function.

beta.- copaene(5.06%):

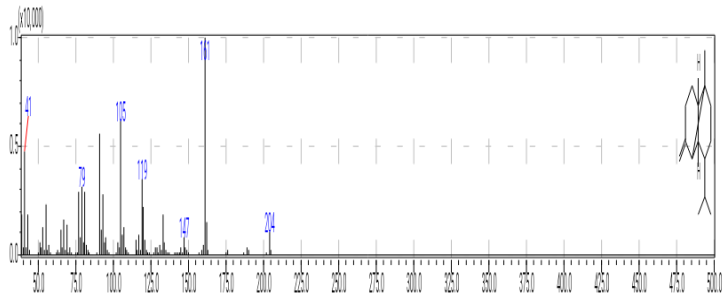


Fig. 6:mass pectrum of beta.- copaene

The El mass of beta.- Copaene. In Fig.6 the peak at m/z 204, which appeared at R.T. 14.843 in total ion chromatogram, corresponds to $M^{+}[C_{15}H_{24}]^{+}$ The peak at m/z 189corresponds to loss of a methyl function.

Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-(4.98%):

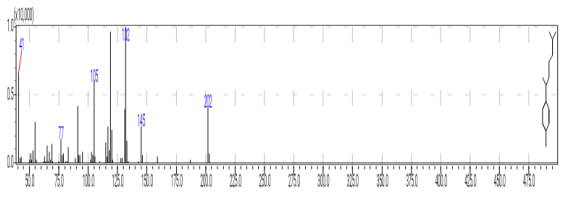


Fig.7: mass pectrum of Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl

The El mass of Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl.In Fig.7the peak at m/z 202, which appeared at R.T. 14.528 in total ion chromatogram, correspond to $M^+[_{C15H22}]^+$ The peak at m/z 159corresponds to loss of a alkyl function.

Eucalyptol (3.74%):

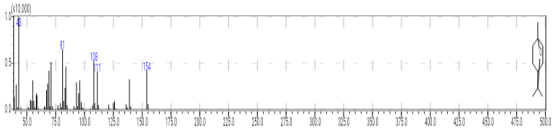


Fig.8: mass pectrum of Eucalyptol

The El mass of Eucalyptol . In Fig.8 the peak at m/z 154, which appeared at R.T. 14.5.883. in total ion chromatogram, corresponds to $M^+[_{C10H18O}]^+$ The peak at m/z 139corresponds to loss of a methyl function.

Camphene(2.65%):

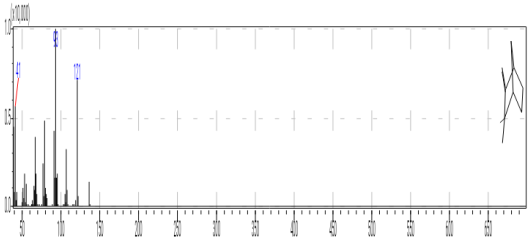


Fig.9:mass pectrum of Camphene

The El mass of camphene. In Fig. 9 the peak at m/z 136, which appeared at R.T. 4.413 in total ion chromatogram, corresponds to $M^+[_{C10H16}]^+$ The peak at m/z 121 corresponds to loss of a methyl function.

Cyclopentene, 3-isopropenyl-5,5-dimethyl-(2.27%):

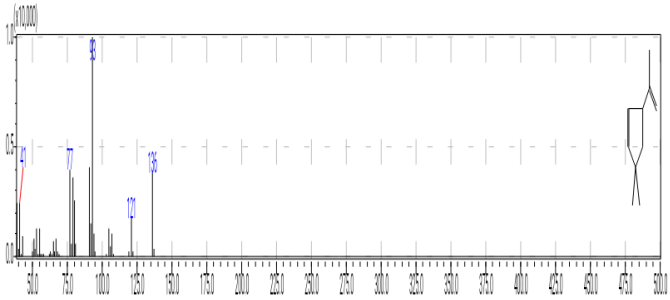


Fig.10: mass pectrum of Cyclopentene, 3-isopropenyl-5,5-dimethyl-

The El mass of in Cyclopentene, 3-isopropenyl-5,5-dimethyl- n Fig. 3.11 The peak at m/z 136, which appeared at R.T. 5.837 in total ion chromatogram, corresponds to $M^+[_{C10H16}]^+$ The peak at m/z 121corresponds to loss of a methyl function.

Antimicrobial activity:

The target oils were evaluated for antimicrobial activity via the cup plate agar diffusion assay. The average of the diameters of the growth inhibition zones are shown in Tabls (4).The results were interpreted in terms of the commonly used terms (<9mm: inactive; 9-12 mm: partially active; 13-18mm: active; >18mm: very active). In the disc diffusion bioassay, *Giger* oil showed good activity against *Bacillus subtilis*, *Staphylococcus aureu* and *Pseudomonas aeruginosa*and Escherichia and good anticandidal potency.

Table (4): The antimicrobial activity of *Ginger*

Microorganism	Species	Type	Inhibition zone diameter (mm) Con (100mg/ml)
Bacteria	*Bacillus subtilis*	+ ve	16
	Staphylococcus aureus	+ ve	17
	Escherichia coli	+ ve	15
	Pseudomonas aeruginosa	+ ve	15
Fungi	*Candida albicans*	+ ve	15

Antioxidant activity:

The target oils were tested for antioxidant screening for their free radical scavenging properties using 2.2Di (4-tert-octylphenyl)-1-picryl-hydrazyl (DPPH assay), while propyl galate was used as standard antioxidant. Based on findings of Dudonne, et al. (2009) [28], DPPH inhibition was categorized as very low (1-10%), low/week (10%-30%), moderate (30-60%), high (61-69%) and very high (70-100%). The Radicals scavenging assay are shown in Table (5). The oils of Ginger showed very high DPPH radical scavenging activity.

Table 5: the radical scavenging assay (DPPH) of *Ginger*:

Sample	RSA%
Ginger	85 ± 0.01
propyl gallate (standard)	92 ± 0.01

RSA* = Radicals scavenging assay

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