academicJournals

Vol. 9(10), pp. 412-420, October 2015
DOI: 10.5897/AJPS2015.1345
Article Number: 5FAC45455775
ISSN 1996-0824

http://www.academicjournals.org/AJPS

African Journal of Plant Science

Full Length Research Paper

Antimicrobial and antioxidant activities of extracts from medicinal plant ginger (*Zingiber officinale*) and identification of components by gas chromatography

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Received 20 August, 2015; Accepted 29 September, 2015

The present study was conducted to investigate antioxidant and antimicrobial activities of *Zingiber officinale*, and detected possible chemicals existence in the plant. Phytochemical screening was carried out using the standard test methods of different chemical groups. Investigating the antioxidant activity, one complementary test method namely 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay was carried out. Disc diffusion method, with minor modifications was used for the evaluation of *in vitro* antimicrobial activity. The extracts were a rich source of phytochemicals. In DPPH free radical scavenging test, the ethanol, acetone and cyclohexane extracts show the highest free radical scavenging activities, although the extracts revealed good antimicrobial activities. Finally, the bioactive compounds of *Zingiber officinale* rhizomes have been evaluated by using GC-spectroscopy.

Key words: *Zingiber officinale*, GC spectroscopy antimicrobial, antioxidant, agar-well diffusion, phytochemical screening.

INTRODUCTION

Ginger is widely used as spices in food and pharmaceutical chemistry. Ginger (*Zingiber officinale*) botanical family is Zingiberaceae and it is a flavoring agent, and herbal medicine used when fresh and dried. Ginger rhizomes are the plant part used for culinary and medicinal aims (Afzal et al., 2001). The root of ginger has been used as a spice for over 2000 years and its cultivated in many tropical and subtropical countries, including India, Nigeria, Fiji, Taiwan, Jamaica, China, Australia, and some area of Kurdistan. Ginger is one of the common additives in some foods and beverages and is valued for its aromatic volatile constituents as well as for its spicy and pungent constituents (Bartley et al., 1994). Traditional medicine in Japan, China and India uses the rhizomes of ginger as a constituent of the herbal treatment for digestive disorders (indigestion, nausea, constipation and flatulence), headaches, rheumatism, colds and cough (Mustafa,1990). A large portion of

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traditional Chinese herbal remedies contain ginger. In the Ayurvedic medical practice in India, ginger is the herbal treatment for colds and other viral infections, poor appetite, digestive problems, arthritis and headache (Ghayur et al., 2005). In Kurdistan, most ginger is marketed as whole fresh rhizomes and used as salad components as a flavored taste and extracted essential oils used as cold treatment among rural peoples.

The antimicrobial properties of ginger have been known and valued for centuries (Gupta et al., 2003). The original discovery of ginger's inhibitory effects on prostaglandin biosynthesis in the early 1970s has been repeatedly confirmed (Raju et al., 2013). This discovery identified ginger as the herbal medicinal product that shares pharmacological properties with non-steroidal anti-inflammatory drugs. Ginger is a strong antioxidant substance and may either mitigate or prevent generation of free radicals. It is considered as a safe herbal medicine with only few and insignificant side effects.

The oxidation importance inside our body and in foodstuffs has been widely recognized. Oxidative metabolism is the main source of the survival of cells. Side effects of this depend on the production of free radicals and other reactive oxygen species that cause oxidative changes. Defense mechanisms against the effects of excessive oxidations are provided by the action of various antioxidants and the need to measure antioxidant activity is well documented (Ebana et al., 1993).

The purpose of the present study was to determine the chemical composition of dry ginger as well as its antioxidant and antimicrobial activities of extracted components. An attempt was also made to investigate association between the antioxidant and antimicrobial activities of extracted components of dry ginger extracted in different solvents.

MATERIALS AND METHODS

Collection and identification of plant material

Rhizome of ginger (*Z. officinale* Rosco) was purchased from a local market of Rania, Kurditsan region-Iraq and the species was taxonomically confirmed by a taxonomist at the Biology Department of Raparin University.

Preparation of plants for extraction

The collected rhizome was separated from undesirable materials. They were dried in open air under shade for three weeks. The shade plants were grinded to coarse powder with the help of a suitable grinder (pistol and mortar). The powder was stored in an airtight container and kept in a cool, dark and dry place until extraction process started with selected solvent.

Solvent extraction

For acetone, ethanol, and cyclohexane extractions, about 1 kg of air dried, powdered sample were immersed in 150 ml of 80% acetone, 95% ethanol and 99% cyclohexane separately in a clean and sterilized glass containers separately. The glass containers with its contents were sealed and kept for maceration for 3 days accompanying occasional shaking and stirring. At the end of third day, the whole mixtures were filtered carefully using Whatman filter paper NO.1. The resultant filtrates were then allowed to evaporate in water bath maintained about 37°C to dryness and thus a greenish black semisolid masses of each extracts were obtained (yield 30, 38 and 43 g consequently). Those gummy semisolid masses were designated as crude extracts of each solvent.

GC-spectroscopy

Preparation of extract

Two microliter (2 µl) of the ethanol, cyclohexane and acetone extracts of *Z. officinale* was employed for GC/MS analysis (Shahidi et al., 1992).

Instruments and chromatographic conditions

GC-Spectroscopy was carried out on a GC clarus 500 Perkin Elmer system comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer (GC-S) instrument employing the following conditions: column Elite-1 fused silica capillary column (30 × 0.25 mm ID ×1EM df, composed of 100% Dimethyl polysiloxane), operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1.2 ml/min and an injection volume of 0.5 EI was employed (split ratio of 10:1) injector temperature 250°C; ion-source temperature 280°C. The oven temperature was programmed from 40°C (isothermal for 2 min), with an increase of 5°C/min, to 200°C/min, then 5°C/min to 250°C/min, ending with a 10 min isothermal at 250°C. Mass spectra were taken at 70 eV, a scan interval of 0.5 s and fragments from 40 to 550 Da.

Identification of components

Interpretation on mass spectrum of GC-Spectroscopy was done using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The retention time of the unknown component was compared with the retention time of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

Phytochemical screening of extract

The method described with slight modification was used for screening of alkaloid, steroids, phlobotannins, flavonoids, glycosides, saponins, tannin and terpenoids.

Alkaloids test

One gram (1 g) of the ginger and 5 ml of honey was stirred with 5 ml of 1% aqueous hydrochloric acid on a steam bath. One milliliter (1 ml) of the filtrate was treated with few drops of Draggendoff's reagent. Blue black turbidity serves as preliminary evidence of alkaloids.

Saponins test

One gram (1 g) of the extracts and 5 ml of honey was shaken with distilled water in a test tube. Frothing which persists on warning was taken as preliminary evidence of the presence of saponins.

Tannins

One gram (1 g) of extracts and 5 ml of honey was stirred with 100ml distilled water and filtered. Ferric chloride reagent was added to the filtrate. A blue-black or blue green precipitate determines the presence of Tannins (Ahmed et al., 2013).

Phlobotannins test

Disposition of red precipitate when an aqueous extract of the test samples was boiled with 1% hydrochloric acid determines the presence of phlobotannins (Merlin et al., 2009).

Flavonoids test

One milliliter (1 ml) of diluted ammonia solution was added to aqueous filtrate of the test samples followed by the addition of concentrated H_2SO_4. A yellow coloration observation determines the presence of flavonoids.

Cardiac glycosides (keller-killiani test)

One gram (1 g) of the extracts and 5 ml of honey was dissolved in 2 ml glacial acetic acid containing a drop of ferric chloride solution. This was underplayed with 1 ml concentrated H_2SO_4. A brown ring of the interface indicates a deoxy-sugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a green ring may form just gradually spread throughout this layer (Sadhu et al., 2007).

Steriods

Two milliliter (2 ml) of acetic anhydride was added to 0.5 g of extract and 2 ml of sulphuric acid was added by the sides of the test tube and observed the colour change from violet or blue-green.

Terpenoids (Salkowski test)

To 0.5 g of the extracts, was added 2 ml of chloroform. Then concentration H_2SO_4 (3 ml) was carefully added to form a layer. A reddish brown coloration of the interface indicates the presence of terpenoid.

Determination of antimicrobial activity

In this test, two strains of Gram-positive (*Staphylococcus aureus* and *Klebsiella*) and two strains of Gram-negative bacteria (*Escherichia coli* and *Streptococcus*), were used to evaluate the Anti-inflammatory potency. These Microorganisms were sub-cultured properly in nutrient broth and nutrient agar. They were collected from the clinical central laboratory of Rania.

Test microorganisms

For the determination of antimicrobial activity, disc diffusion method is widely acceptable. In this method, antibiotics were diffused from a source through the nutrient agar and a concentration gradient was created. Dried, sterilized filter paper discs (6 mm diameter, HI-Media, China) containing the extracts of known amounts (0.1, 0.2 and 0.4 µg/ml per disc) were applied on nutrient agar medium consistently seeded with the test microorganisms (Pisoschi et al., 2009). Then standard antibiotic of ciprofloxacin (10 µg per disc) and blank discs were used as positive and negative control. For the maximum diffusion of the test materials to the surrounding media, these plates were kept at low temperature (4°C) for about 24 h. Then the plates were incubated (at 37°C) for about 24 h to allow optimum growth of the organisms. The test materials with antimicrobial potency inhibited microbial growth in plates and thereby yielded a clear, distinct zone defined as zone of inhibition (ZOI). Thus, the antimicrobial activity of the extracts was determined correctly by measuring the zone of inhibition expressed in millimeter (Zheng et al., 2001).

Determination of DPPH antioxidant activity

The antioxidant activities of plant extract and the standard antioxidant, Vitamin E were assessed on the basis of free radical scavenging effect of the stable DPPH free radical (Valadez-Vega et al., 2013). Stock solutions (10 mg/ml) of the ethanol, acetone and cyclohexane extracts of *Z. officinale* were prepared in respective solvent systems from which serial dilutions were carried out to obtain concentrations of 3, 6, 12, 25, 50 and 100 µg/ml, respectively. In this assay, an equal amount of sample solution was added to an equal amount of 0.1 mM ethanolic DPPH solution, vortexes and allowed to stand at the dark place at 25°C for 30 min for the reaction to occur (Larson, 1988). After 30 min of incubation period, the absorbance was read against a blank at 517 nm with (Jen way, UK) UV/Visible spectrophotometer. The radical scavenging activity was expressed as the percentage of inhibition (I%) and calculated as per the equation:

$$I\ (\%) = (Abs\ blank - Abs\ sample/Abs\ blank) \times 100$$

Where Abs blank is the absorbance of the control reaction (containing all reagents except the extracts) and Abs sample is the absorbance of the defined concentration of extracts with all reagents.

IC_{50} value is the concentration of sample required to scavenge 50% DPPH free radical and was calculated from the plot of inhibition (%) against logarithm of extracts concentration (Gao et al., 2000). All the tests were carried out in triplicate and average of the absorptions was noted. Vitamin E was used as positive control standard for this study (Yu et al., 2005).

RESULTS AND DISCUSSION

The phytochemical screening of *Z. officinale* extracts gave different results as shown in Table 1. Depending on test results, extracts mostly contained alkaloids followed by flavonoids and then saponins.

GS-Spectroscopy

The GC-spectroscopy study of *Z. officinale* has shown

Table 1. Results of phytochemical screening of Zingiber officinale extracts.

Bioactive principles	Ethanol extract of ginger	Cyclohexane extract of ginger	Acetone extract of ginger
Alkaloids	+++	+++	++++
Tannins	++	++	++
Glycosides	++	++	++
Saponins	+++	+++	++
Steriods	+	+	-
Flavonoids	+++	++	+++
Terpenoids	+	+	+
Phlobotannins	+	-	+

Table 2. Extracted compounds of Figure 1, Figure 2 and Figure 3 of ginger extracts.

Ethanol extract			Acetone extract		Cyclo-hexane extract	
Peaks	**compounds**	**RT**	**Compounds**	**RT**	**Compounds**	**RT**
1	Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl-6-pentyl)	3.37	Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl-6-pentyl)	3.40	1,3-cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl	3.31
2	4-(3-hydroxy-2-methoxyphenyl) butan-2-one	4.13	n-Hexadecanoic acid	5.47	trans-.alpha.-Bergamotene	3.38
3	n-Hexadecanoic acid	5.49	E-1,9-Tetradecadine	6.30	2- butanone, 4-(4-hydroxy-3-methoxyphenyl)	4.08
4	9-Octadecyne	6.33	2- butanone, 4-(4-hydroxy-3-methoxyphenyl)	7.19	Sesquirosefuran	5.47
5	2-Butanone, 4-(4-hydroxy-methoxyphenyl)	6.84	Gingerol	8.03	E-12-Tetradecenal	6.31
6	Butan-2-one 4-(3-hydroxy-2-methoxyphenyl)	7.12	Dihydrocapsacin	8.82	Capsaicin	7.2
7	Capsaicin	7.98	Furan, 2,5-dibutyl	9.11	6-(3,5-Dimethyl-furan-2-yl)-6-methyl-hept-3-en-2-one	7.61
8	Bis(2-methylphenylthio)-methane	8.03	4-Hexanoyal resorcinol	10.98	Gingerol	7.95
9	6-(3,5-Dimethyl-furan-2-yl)-6-methyl- hept-3-en-2-one	8.87	Campesterol	12.5	2H,6H-Pyrano[3,2-b]xanthen-6-one, 5,9-dihydroxy-8- methoxy-2,2-dimethyl-7-(3-methyl-2-butenyl)-	8.91
10	4-Hexanoyl resorcenol	9.14	Stigmasterol	Squalene	Squalene	9.06
11	Campesterol	12.52			Propan-2-one, 1-(4-isopropoxy-3-methoxyphenyl)-	10.47
12	Stigmasterol	12.84			5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-3-decanone(Gingerol	10.97
13					Campesterol	12.49
14					Stigmasterol	12.82

RT: Retention time.

many phytochemicals (Table 2) with peaks (Figures 1, 2 and 3) which contribute to the medicinal activity of the plant. Analysis of the chemical composition of the extract by GC-spectroscopy helped in the identification of components in ethanol extract. The major compounds identified in ginger ethanol extract were Bicyclo [3.1.1]

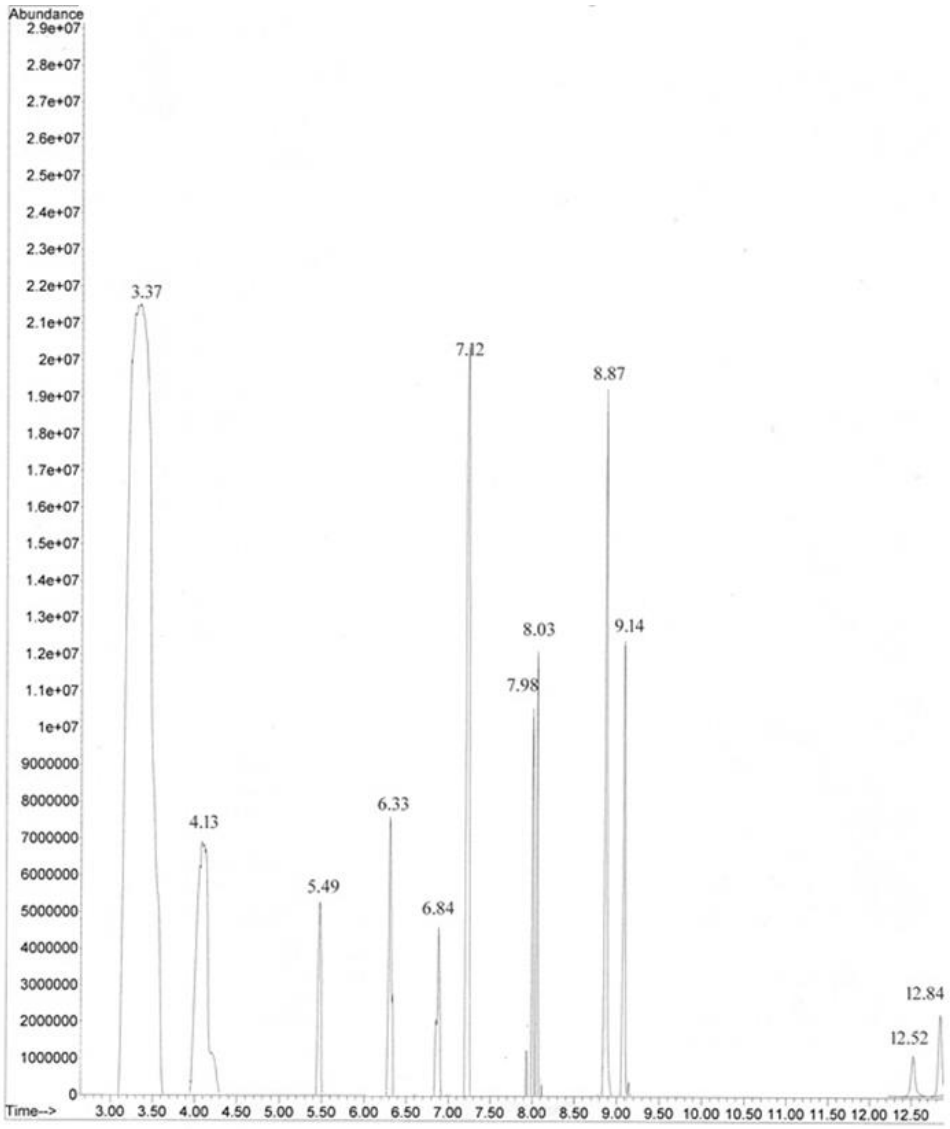

Figure 1. GC-spectroscopy of ethanol extracts.

hept-2-ene, 2, 6-dimethyl-6-(4-methyl-6-pentyl) and 4-(3-hydroxy-2-methoxyphenyl butan-2-one, 9-Octadecyne, 4-Hexanoyl resorcinol. The other compounds were Capsaicin and Campesterol (Figure 1). The acetone extract was subjected to identification of compounds in the extract. The major compounds identified in extract were Bicyclo[3.1.1]hept-2-ene,2,6-dimethyl-6-(4-methyl-6-pentyl) and 4-(3-hydroxy-2-methoxyphenylbutan-2-one, Gingerol, Furan, 2,5-dibutyl,4-Hexanoyal resorcinol (Figure 2). The cyclohexane extract was performed for the identification of the compounds in the extract. The major compounds identified in this extract were 1,3-cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl, trans-.alpha.-Bergamotene, Sesquirosefuran, E-12-Tetradecenal, Gingerol, 2H,6H-Pyrano[3,2-b]xanthen-6-one, 5,9-dihydroxy-8-methoxy-2,2-dimethyl-7-(3-methyl-2-butenyl) (Figure 3).

Determination of antimicrobial activity

The zones of inhibition observed in the disk diffusion bio assay are shown in Table 3. Plants with an average zone of inhibition in diameter of ≥ 2.8 mm was considered as those recording a significant antimicrobial activity. It indicates that *Z. officinale* has strong antimicrobial activity against all selected organisms. The cyclohexane extract of *Z. officinale* failed to show any activity against the selected bacterial isolates and it showed activity against only Klebsiella.

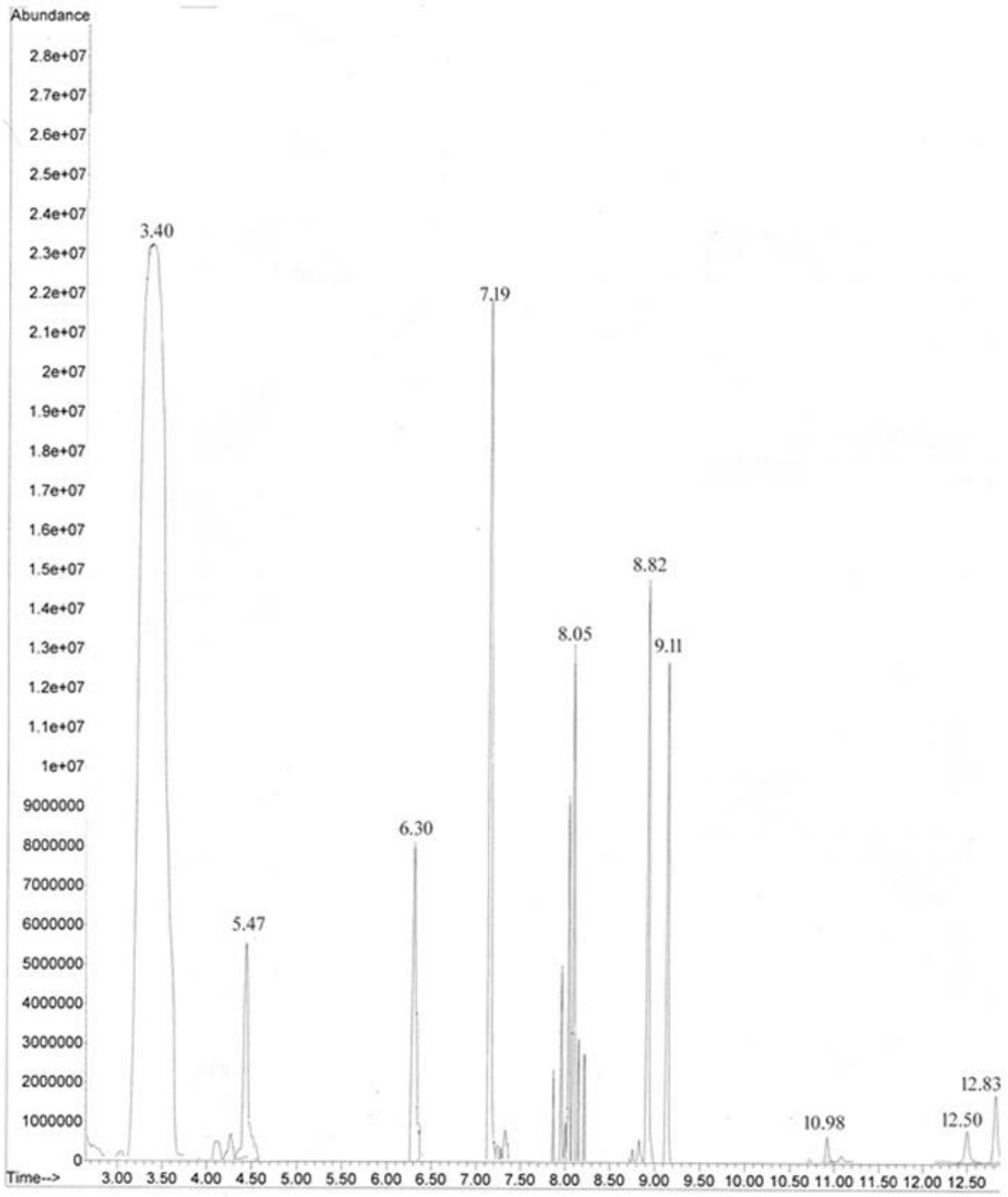

Figure 2. GC-Spectroscopy of acetone extracts.

DPPH- radical scavenging activity

Cancer, malignant tumor or neoplasm is a broad term for a large group of diseases that can affect any part of the body. 8.2 million people worldwide died from cancer in 2012 and 60% of world's total new annual cases occur in Africa, Asia and Central and South America (American Society, 2009). So, cancer and reactive species are causes of many lots of health complication and the pace is increasing in a surprisingly higher level. Studies for new source of antioxidant compounds are the major concern of the time. As certain groups of plant secondary metabolites like tannin, reducing sugar, alkaloid, flavonoid, gum, saponin and steroidal compounds are responsible for some specific pharmacological actions, the cyclohexane, ethanol and acetone extracts of *Z. officinale* were tested to determine whether these definite groups were present in the extract (Khamis et al., 1997). The study demonstrated the presence of alkaloids, tannins, gums, flavonoids and saponins as the major secondary metabolites (Newman et al., 2002). There has been a great deal of interest of late in the role of complementary and alternative drugs for the treatment of various acute and chronic diseases. Among the several

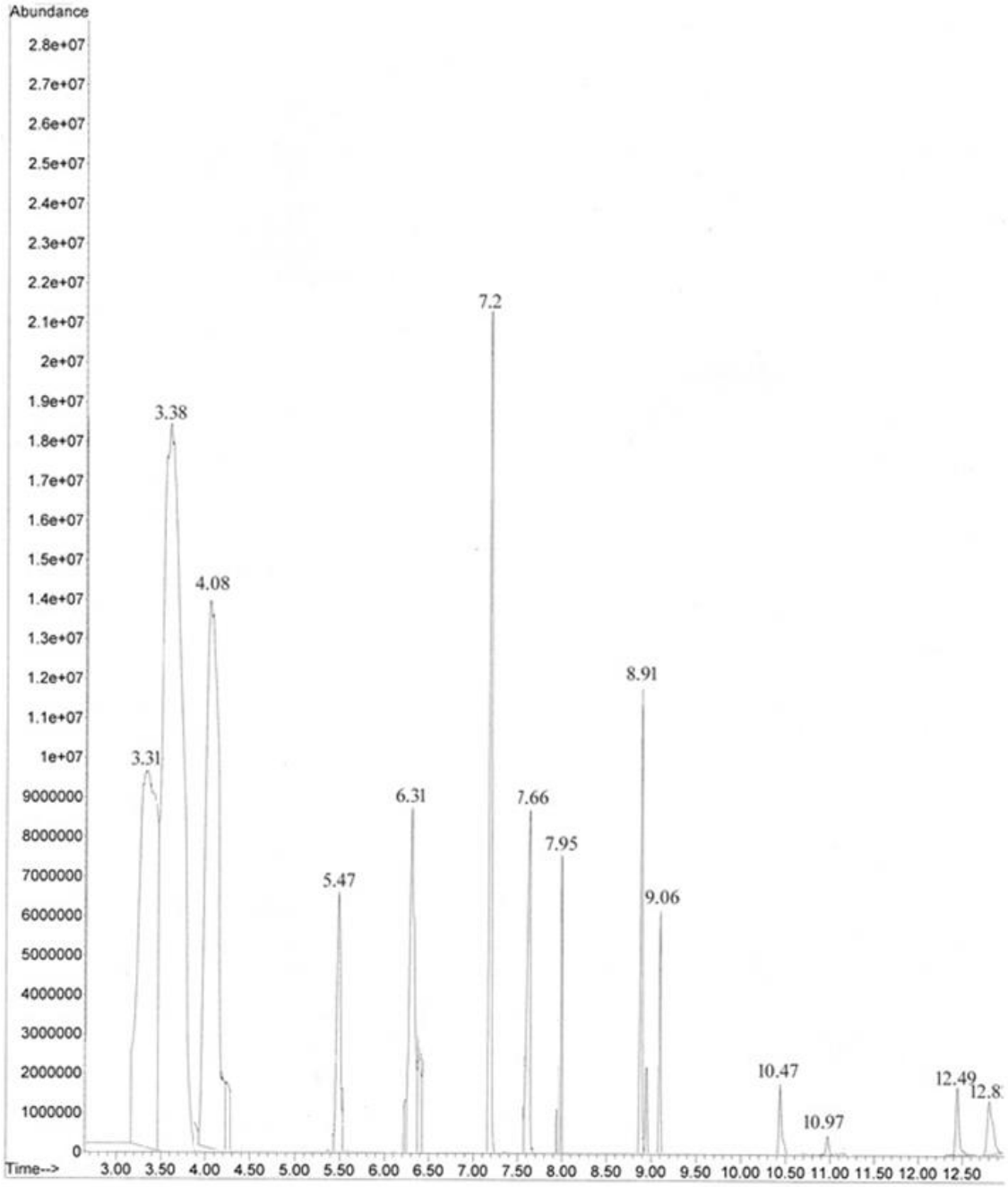

Figure 3. GC- spectroscopy of cyclohexane extracts.

classes of phytochemicals, interest has focused on the antioxidant property of the polyphenols that are found in various botanical agents. Plant vegetables and spices used in folk and traditional medicine have gained wide acceptance as one of the main sources of prophylactic and chemo preventive drug discoveries and development.

In our study to show the antioxidant activity of extracted ginger, the standard (vitamin E) showed IC_{50} 31.62 µg/ml after 30 min. In free radical scavenging assay, IC_{50} values of ethanol, acetone and cyclohexane extracts were 83, 81 and 25.11 µg/ml, respectively in comparison to the standard V. E IC_{50} 31.62 µg/ml. From this study, it was evident that, the ethanol and acetone extracts of *Z. officinale* showed lowest antioxidant activities, while the cyclohexane extract showed the highest and significant

Table 3. Antimicrobial activity extracts from *Zingiber officinale.*

Bactria species		Inhibition zone diameter in mm of Zingiber officinale extracts												Ciprofloxacin
Gram negative bacteria	Gram positive bacteria	Cyclohexane extract (µg/ml)				Ethanol extract (µg/ml)				Acetone extract (µg/ml)				(µg/ml)
		0.4	0.2	0.1	0	0.4	0.2	0.1	0	0.4	0.2	0.1	0	
	Staphylococcus aureus sensitivity ZOI (mm)	0	0	0	0	2.1	1.1	0.5	0	2.3	1.1	0.5	0	0.5
Escherichia														
coli sensitivity ZOI (mm)		0	0	0	0	2.2	2.0	0.3	0	2.1	1.2	0.2	0	1.3
Streptococcus		0	0	0	0	2.5	1.6	0.7	0	3.1	1.1	0.1	0	0.8
	Klebsiella	0.07	0.03	0.025	0	2.0	1.9	1.0	0	2.8	2.6	2.0	0	1.2

ZOI: Zone of inhibition.

Table 4. Radical scavenging activities of *Zingiber officinale* extracts determined by the reduction of DPPH free radical.

Concentration ug/ml	*Zingiber officinale*		Standard vitamin E	
	% inhibition	IC_{50} µg/ml	% inhibition	IC_{50} µg/ml
Cyclohexane extracts				
100	44.93		12.67	
75	40.56		10.15	
50	32.93		9.92	
25	29.12	25.11	8.17	
12	22.8		8.1	
6	13.2		6.8	
3	5.1		4.2	
Ethanol extract				
100	28.17			
75	6.50			
50	4.41			
25	3.78			
12	2.26	83		31.62
6	2.03			
3	1.99			
Acetone extract				
100	57.42			
75	34.71			
50	33.17			
25	31.21	81		
12	27.13			
6	18.60			
3	12.19			

antioxidant activity (Table 4).

Conclusion

Out of the three different solvent extracts of *Z. officinale* for the evaluation of antioxidant activities, ethanol and acetone extracts exhibited the greater antioxidant capacity. The antioxidant activities of medicinal plants may be due to the presence of flavonoids compounds containing the hydroxyl groups that confers the hydrogen

donating ability; the antimicrobial activity of selected plant materials against the above mentioned bacterial pathogens in the laboratory. Again Ethanol and Acetone extracts of *Z. officinale* showed significant inhibitions against all selected organisms, while cyclohexane extract showed insignificant inhibition against selected species. Thus, these products may be useful as potential sources for the future drug development.

Conflict of interest

The authors have not declared any conflict of interest.

ACKNOWLEDGEMENTS

The authors are thankful to Dr. Frooq Emam of Salahaddin University for the corrections made in this paper.

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