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GC-MS Profiling and Antioxidants Activity of *Zingiber officinale*, *Citrus limon* and Apple Cider Vinegar Extracts

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Abstract

The objective of this study was to evaluate the bioactive compounds and antioxidants activity Zingiber officinale, Citrus limon and apple cider vinegar extracts. The antioxidant activity was determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH). Methanol extracts of Zingiber officinale, Citrus limon and apple cider vinegar extracts and evaluated for its bioactive compound and antioxidant activity. Gas Chromatography-Mass analysis of Zingiber officinale, Citrus limon and apple cider vinegar extracts revealed the presence of various groups of bioactive compounds for example, Hypocholesterolemia, antimicrobial, anticancer, antioxidant, antidiabetic, antimicrobial, anti-inflammatory, anti-plasmodial, Antitumor, Antiviral, anti-obesity. Ginger extract possessed the highest antioxidant activity (DPPH) 90.59 %. Zingiber officinale, Citrus limon and apple cider vinegar extracts is considered an excellent source of natural bioactive molecules that could be an interesting material for medicinal.

Keywords: *Zingiber officinale*, *Citrus limon*, apple cider vinegar, Antioxidant.

Introduction

Medicinal plants have many bioactive compounds components and are considered plants that have many health benefits and used to treat many diseases and play important role in healing. Medical plants were plants used for purpose of maintaining health [1].as they play useful role in health care. Medical plants which have in part or all of their parts, therapeutic properties for various diseases or reduce their symptoms, or have physiological impact on body of human or animal, and impact performance of organs in human or animal body, if its effect is stimulating or inhibiting, and it also impact on living organisms that parasitize human or animal body either outside or inside inhibiting, killing or expelling [2][3].. Lemon fruits have contains many chemicals that play important role in protecting body from many diseases. Lemon peels also consist of various plant chemicals as glycosides, volatile oils and sitosterol, and there are many important activities found in lemon as ascorbic acid, flavones and phenolic compounds, that exceptional in other [4].. As for ginger, which is type of medical plant and contains effective chemical compounds, including volatile oils at rate of 3-2.5, ginger has several properties. Including anti-tumor, many recent studies have confirmed that ginger contains compounds that inhibit various types of cancer [5].. The apple plant is one of plants that have many benefits that affect the health of body and is important source of flavonoids and other nutrients in human diet [6]. The aim of this study was to determine the chemical content and antioxidant activity *Zingiber officinale* rhizome, *Citrus limon* fruits and apple cider vinegar extract.

Materials and methods

Samples collection

Samples of *Zingiber officinale* rhizome, *Citrus limon* fruits and apple cider vinegar were collected from the local market obtained from Nasiriyah city, Thi -Qar, Iraq, October 2024. The samples were identified at the Lab of Plant Taxonomy, Iraq Natural History Research Center and. The ginger sample was then pulverized using a mechanical grinder and then stored at 4 °C until use.

Preparation of crud alcoholic extracts of medicinal plants

Extracts are prepared by mixing 1 ml to lemon juice to 9 ml methanol, and ginger extract is prepared by mixing 1 gram from mold ginger to 9 ml methanol, and apple cider vinegar extract is prepared by taking 1 ml of apple cider vinegar and mixing it with 9 ml methanol, then put these solutions for hour in magnetic rotor device Hotplate Stirrer. After that, take clear solution using pipettes and put it in tubes, then it is placed in centrifuge for 15 minutes at speed of 3 thousand revolutions per minute. After that, take clear solution and send it to GC-Ms device [7].

Antioxidant extraction

Antioxidant extracts are made using acetone solvent, where take 1 ml of lemon juice with 10 ml of acetone, 1 gram of ground ginger with 10 ml of acetone, 1 ml of apple cider vinegar with 10 ml of acetone at concentration of 50% in 30 ml test tube. Then tubes are placed in shaking incubator for hour, then placed in refrigerator for 24 hours. Then take filtrate and place it in centrifuge with speed 4500 rpm in 5 min. Then filtrate is placed in sealed containers and stored until use.

GC-MS analysis

GC-MS analysis of lemon, ginger and apple cider vinegar extracts was performed by Shimadzu PLUS2010QP MS-C system equipped with capillary column (30m × 0.25mm ID × 0.25µm thick) depended to method by [8]. less divided injections performed in 2-minute purge time. Helium was carrier gas at flow rate of 1 ml/min. The column temperature was 50°C which maintained for 3hours.. 1 min, and it was programmed at 5°C to 80°C, and from 10°C to 340°C. The inlet temperature 250°C., solvent 4 min, and solvent delay 4min. The peak determined depended on matching computer-generated 340 C detector temperature mass spectra with NIST 08 NlST and 08 NIST libraries and direct comparison with data.

DPPH radical scavenging activity test

The determination of antioxidant activity through 2,2-diphenyl-1- picrylhydrazyl (DPPH) scavenging system was carried out according to the method of. [9].Stock solution was prepared by dissolving 40 mg DPPH in 100 ml methanol and kept at -20°C until used. About 350 mL stock solution was mixed with 350 ml methanol to obtain the absorbance of 0.70±0.01 unit at 517 nm wavelength by using spectrophotometer (Epoch, Biotek, USA). About 300

µL samples with 2.700 ml methanolic DPPH solution prepared were kept overnight for scavenging reaction in the dark. Percentage of DPPH scavenging activity was determined as follow:

% Antioxidant Activity = $DPPH_{blank}$- $DPPH_{sample}$ / $DPPH_{blank}$ *100

Results and Discussions:

Gas Chromatography-Mass analysis of ginger extract

Gas Chromatography-Mass analysis of ginger extract revealed the presence of various groups of bioactive compounds (Table 1) Hexanal, Octanal, Decanal, Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-, Beta -Bisabolene, 1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, 1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, Neral, Trisilane, 2-Butanone, 4-(4-hydroxy-3-methoxyphenyl)-, (2E,6E)-3,7,11-Trimethyldodeca-2,6,10-trien-1-yl stearate, 9,10-ethanoanthracene, tetradecahydro-, Phenol, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, (R)-, Tetradecanoic acid, 5,9-Undecadien-2-one, 6,10-dimethyl-, (Z)-, 7-(2-Hydroxypropan-2-yl)-1,4a-dimethyldecahydronaphthalen-1-ol, Cyclopropanemethanol, .alpha.,2-dimethyl-2-(4-methyl-3-pentenyl)-, [1.alpha.(R*),2.alpha.]-, Spiro[4.5]decan-7-one, 1,8-dimethyl-8,9-epoxy-4-isopropyl-, n-Hexadecanoic acid, 1,6,10,14,18,22-Tetracosahexaen-3-ol, 2,6,10,15,19,23-hexamethyl-, (all-E)-(.+/-.)-m 9,12-Octadecadienoic acid, methyl ester, (E,E)-, 3-Penten-2-one, 4-(2,2,6-trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)-, (E)-, 9,12-Octadecadienoic acid (Z,Z)-, Octadecanoic acid, (E)-1-(4-Hydroxy-3-methoxyphenyl) dec-3-en-5-one, 3-Decanone, 1-(4-hydroxy-3-methoxyphenyl)-, 1-(4-Hydroxy-3-methoxyphenyl)decane-3,5-dione, Gingerol, (E)-1-(4-Hydroxy-3-methoxyphenyl)dodec-3-en-5-one, Phenol, 2-methoxy-4-(methoxymethyl)-, (3R,5S)-1-(4-Hydroxy-3-methoxyphenyl) decane-3,5-diyl diacetate, 1-(3,4-Dimethoxyphenyl)decane-3,5-diyl diacetate, N-(2-Methoxy-5-methylphenyl)-2-phenylcyclopropanecarboxamide, 5-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)dodecan-3-one, (E)-1-(4-Hydroxy-3-methoxyphenyl) tetradec-3-en-5-one, 3,7-Dimethyloct-6-en-1-yl tetradecanoate, 1-(4-Hydroxy-3-methoxyphenyl)tetradecane-3,5-dione, Adipic acid, di(.beta.-citronellyl) ester, (9Z,12Z)-(E)-3,7-Dimethylocta-2,6-dien-1-yl octadeca-9,12-dienoate. In the current study, some of the identified compounds have been reported to have several biological activities. The identified compounds from ginger extract have been reported to have therapeutic properties by several researchers. For example, Hypocholesterolemia, antimicrobial, anticancer, antioxidant, antidiabetic, antimicrobial, anti-inflammatory, anti-plasmodial, Antitumor, Antiviral, anti-obesity [10].

Table (1): List of compounds from methanol ginger extract observed in GC-MS with their retention time and biological activity

No.	Compound name	R.T.	Area	Biological activity
1	Hexanal	4.346	2448475	Antimicrobial
2	Octanal	9.342	2999036	Antioxidant Antibacterial
3	Decanal	12.877	6890298	Antimicrobial
4	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	16.711	2471974	Antioxidant, Antitumor

5	Beta -Bisabolene	16.868	4549843	Antibacterial Anti-inflammatory Anti-oxidant
6	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-	17.669	2303084	Antimicrobial
7	Neral	18.297	3167734	Antioxidant
8	Trisilane	18.431	1494100	**Antimicrobial**
9	2-Butanone, 4-(4-hydroxy-3-methoxyphenyl)-	18.714	4590641	anti-inflammatory, antidiabetic, antilipolytic
10	(2E,6E)-3,7,11-Trimethyldodeca-2,6,10-trien-1-yl stearate	19.23	5148966	Antioxidant Antimicrobial
11	9,10-ethanoanthracene, tetradecahydro-	19.71	2256596	Antimicrobial
12	Phenol, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, (R)-	19.80	1286183	Antioxidant, **antibacterial**, antiplasmodial, anticancer
13	Tetradecanoic acid	19.87	2695132	Antioxidant Antimicrobial Anticancer
14	5,9-Undecadien-2-one, 6,10-dimethyl-, (Z)-	20.43	7380722	Antioxidant Anti-inflammatory
15	7-(2-Hydroxypropan-2-yl)-1,4a-dimethyldecahydronaphthalen-1-ol	20.55	2518770	Anti-inflammatory
16	Cyclopropanemethanol, .alpha.,2-dimethyl-2-(4-methyl-3-pentenyl)-, [1.alpha.(R*),2.alpha.]-	21.29	1969120	Antidepressant
17	Spiro[4.5]decan-7-one, 1,8-dimethyl-8,9-epoxy-4-isopropyl-	21.4	1264327	**Antimicrobial**
18	n-Hexadecanoic acid	22.02	8939143	Antimicrobial, antioxidant, antiatherosclerotic
19	1,6,10,14,18,22-Tetracosahexaen-3-ol, 2,6,10,15,19,23-hexamethyl-, (all-E)-(.+/-.)-	22.61	2542234	Antioxidant Antimicrobial Anticancer
20	9,12-Octadecadienoic acid, methyl ester, (E,E)-	23.16	2649964	Antimicrobial
21	3-Penten-2-one, 4-(2,2,6-trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)-, (E)-	23.37	1959297	Antimicrobial
22	9,12-Octadecadienoic acid (Z,Z)-	23.63	5049826	Antimicrobial
23	Octadecanoic acid	23.82	6629137	Antiviral, antibacterial, antioxidant
24	(E)-1-(4-Hydroxy-3-methoxyphenyl)dec-3-en-5-one	24.37	1681853	Antimicrobial
25	3-Decanone, 1-(4-hydroxy-3-methoxyphenyl)-	24.44	4428913	Antimicrobial
26	1-(4-Hydroxy-3-methoxyphenyl)decane-3,5-dione	25.28	1430497	Antioxidant Antimicrobial Anticancer
27	Gingerol	25.35	1413493	Anticancer, Anti-inflammation Anti-oxidation.
28	(E)-1-(4-Hydroxy-3-methoxyphenyl)dodec-3-en-5-one	26.06	1927805	Anti-inflammatory, Antioxidant, Anticancer
29	Phenol, 2-methoxy-4-(methoxymethyl)-	26.47	2170995	Anti-inflammatory, Antioxidant
30	(3R,5S)-1-(4-Hydroxy-3-methoxyphenyl) decane-3,5-diyl diacetate	26.79	5957889	can reduce blood lipids and antithrombotic. Antioxidant, Anticancer

				Anti-inflammatory,
31	1-(3,4-Dimethoxyphenyl)decane-3,5-diyl diacetate	26.97	2640396	Antioxidant, anti-inflammatory, anticancer, antibacterial and anti-platelet aggregation
32	N-(2-Methoxy-5-methylphenyl)-2-phenylcyclopropanecarboxamide	27.09	1109795	Antibacterial
33	5-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)dodecan-3-one	27.41	1655427	Antioxidant
34	(E)-1-(4-Hydroxy-3-methoxyphenyl)tetradec-3-en-5-one	27.66	2581985	Antioxidant
35	13-Octadecenal, (Z)-	28.01	1244710	Anti-oxidant, anti-inflammatory and hypocholesterolemic
36	3,7-Dimethyloct-6-en-1-yl tetradecanoate	28.16	2416096	Antimicrobial Antioxidant
37	1-(4-Hydroxy-3-methoxyphenyl)tetradecane-3,5-dione	28.52	1159495	Antimicrobial Antioxidant
38	Adipic acid, di(.beta.-citronellyl) ester	29.39	1564053	Antimicrobial
39	(9Z,12Z)-(E)-3,7-Dimethylocta-2,6-dien-1-yl octadeca-9,12-dienoate	29.64	1645079	Antimicrobial Antioxidant

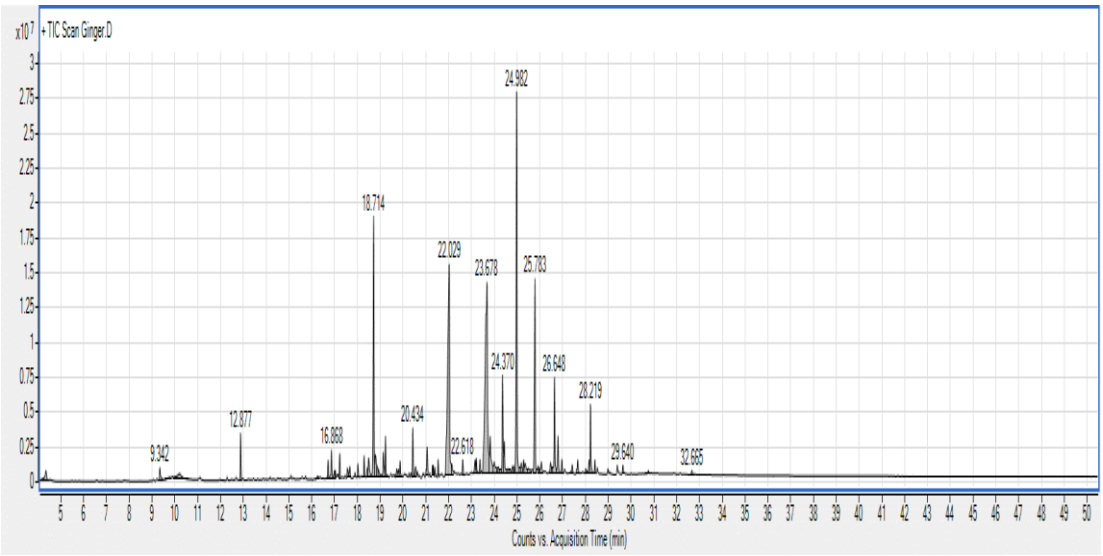

Fig1: Gas chromatogram of methanolic ginger extract

Gas Chromatography-Mass analysis of lemon fruit extract

Gas Chromatography-Mass analysis of ginger extract revealed the presence of various groups of bioactive compounds (Table 2) Furfural, beta.-Myrcene, D-Limonene, .gamma.-Terpinene, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, Terpinen-4-ol, 5-Hydroxymethylfurfural, Neral, Citral, 2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (Z)-, trans-.alpha.-Bergamotene , beta.-Bisabolene, n-Hexadecanoic acid. . The identified compounds from lemon fruit extract have been reported to have therapeutic properties by several

researchers. For example, Antimicrobial, antiviral antioxidant antitumor, antihistaminic, Anti-inflammatory and anticancer [11].

Table (2): List of compounds from methanol lemon extract observed in GC-MS with their retention time and biological activity

No.	Compound name	R.T.	Area	Biological activity
1	Furfural	5.446	2824480	Antimicrobial, antiviral antioxidant antitumor, antihistaminic
2	.beta.-Myrcene	7.371	9101831	Anticoagulant, anti-inflammatory, anti-microbial
3	D-Limonene	9.696	4.2208	Antibacterial and antifungal
4	.gamma.-Terpinene	10.301	53253915	Antioxidant and antibacterial
5	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	12.029	21291302	Antimicrobial, antioxidant
6	Terpinen-4-ol	12.477	3378068	Antimicrobial
7	5-Hydroxymethylfurfural	13.364	11775582	Antioxidant and protection against acute hypobaric hypoxia
8	Neral	13.435	3964731	Anti-inflammatory antioxidant
9	Citral	13.867	3498053	Anti-inflammatory, anti-microbial antioxidant, anticancer
10	2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (Z)-	15.14	2427579	Anti-microbial
11	trans-.alpha.-Bergamotene	16.122	2726745	Attract ectoparasites
12	beta.-Bisabolene	17.033	3989550	anticancer,, anti-tumor and synergistic bactericidal
13	n-Hexadecanoic acid	21.895	2638716	Antioxidants, hypocholesterolemic

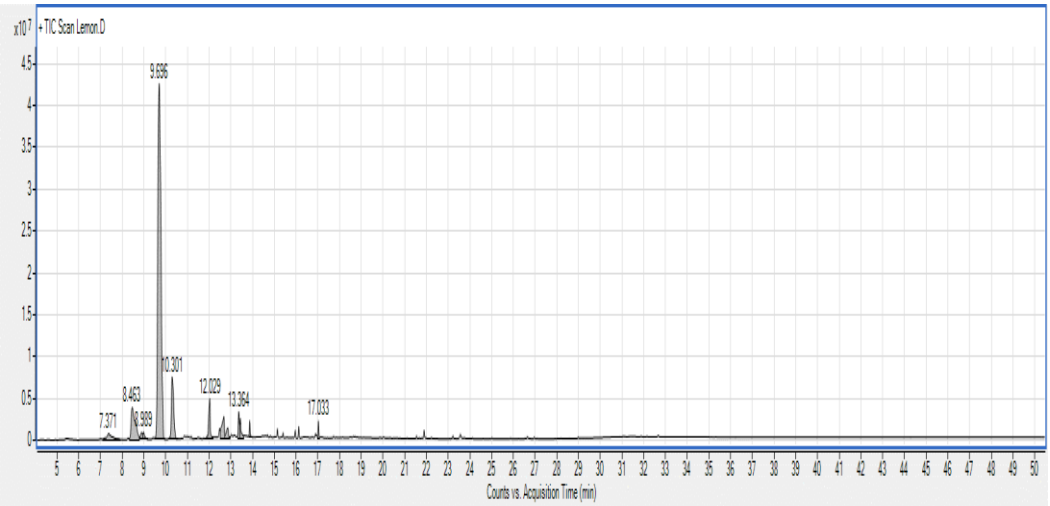

Fig2: Gas chromatogram of methanolic lemon extract

Gas Chromatography-Mass analysis of apple cider extract

Gas Chromatography-Mass analysis of apple cider extract discovered the presence of several groups of bioactive compounds (Table 2), Ethylene glycol monoisobutyl ether, 2-(3,4-Dimethyl-.alpha.-thiosemicarbazonobenzyl) benzoic acid, N-t-Butyl-N'-2-[2-thiophosphatoethyl] aminoethylurea, Methanamine, N-methoxy-, Erythritol, Undecane, Dimethylamine, N-(neopentyloxy)-, Acetamide, N-cyclopentyl-, Benzoic acid, 1,4:3,6-Dianhydro-.alpha.-d-glucopyranose, Naphthalene, 1,2-dihydro-1,1,6-trimethyl-, 2-Amino-4,6-diphenyl-6H-1,3,5-thiadiazine, .alpha.-Methyl-D-mannopyranoside, Pentadecanoic acid, 14-methyl-, methyl ester, N-[3-t-Butylimino-1,2-dimethylpropyl]aziridine, n-, Hexadecanoic acid, 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-, 2(1H)-Naphthalenone, octahydro-4a-methyl-7-(1-methylethyl)-, (4a.alpha.,7.beta.,8a.beta.)-, Benzoic acid, undecyl ester, Benzoic acid, tetradecyl ester. The identified compounds from apple cider extract have been reported to have therapeutic properties by several researchers. For example, antioxidant, anti-ulcer hypocholesterolemia, antimicrobial, anti-inflammatory, anticancer, antidiabetic, antitumor, antiviral, anti-obesity [12].

Table (3): List of compounds from methanol apple cider extract observed in GC-MS with their retention time and biological activity

No.	Compound name	Area	R.T.	Biological activity
1	Ethylene glycol monoisobutyl ether	1740218	5.077	Antimicrobial
2	2-(3,4-Dimethyl-.alpha.-thiosemicarbazonobenzyl) benzoic acid	346142	5.556	Antitumor, antidiabetic anti-fungal
3	N-t-Butyl-N'-2-[2-thiophosphatoethyl]aminoethylurea	1681841	6.271	Antiviral, antimalarial, cytotoxic, anti-inflammatory, antifungal
4	Hexitol, 1,5-anhydro-2,6-dideoxy-	365566	8.093	Anti-viral
5	Methanamine, N-methoxy-	2788712	9.452	Antimicrobial, antioxidant
6	Erythritol	19392631	10.34	Antimicrobial
7	Undecane	780378	11.078	Antimicrobial, antioxidant
8	Dimethylamine, N-(neopentyloxy)-	317776	11.534	Antimicrobial, anti-obesity, anti-inflammatory, antioxidant
9	Acetamide, N-cyclopentyl-	484211	12.037	Anti-inflammatory, antimalarial, anticancer, antifungal
10	Benzoic acid	8487824	12.587	Antimicrobial
11	1,4:3,6-Dianhydro-.alpha.-d-glucopyranose	1056806	12.995	Antifungal antioxidant
12	Naphthalene, 1,2-dihydro-1,1,6-trimethyl-	437187	15.053	Antimicrobial
13	2-Amino-4,6-diphenyl-6H-1,3,5-thiadiazine	337177	15.587	Antioxidant antibacterial
14	.alpha.-Methyl-D-mannopyranoside	330090	16.86	Antibacterial antifungal

15	Pentadecanoic acid, 14-methyl-, methyl ester	1041954	21.542	Antimicrobial
16	N-[3-t-Butylimino-1,2-dimethylpropyl]aziridine	365065	21.762	Anti-inflammatory, antioxidant, antimicrobial
17	n-Hexadecanoic acid	1121539	21.887	Hypocholesterolemic antioxidants, nematicide, pesticide
18	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	1927425	23.223	Hypocholesterolmic anti-ulcer antioxidant, antibacterial, anti-inflammatory
19	2(1H)-Naphthalenone, octahydro-4a-methyl-7-(1-methylethyl)-, (4a.alpha.,7.beta.,8a.beta.)-	280665	23.765	Antioxidant
20	Benzoic acid, undecyl ester	252891	24.126	antibacterial, anti-inflammatory
21	Benzoic acid, tetradecyl ester	449712	25.022	Antioxidant, antibacterial, cytotoxic anti-inflammatory

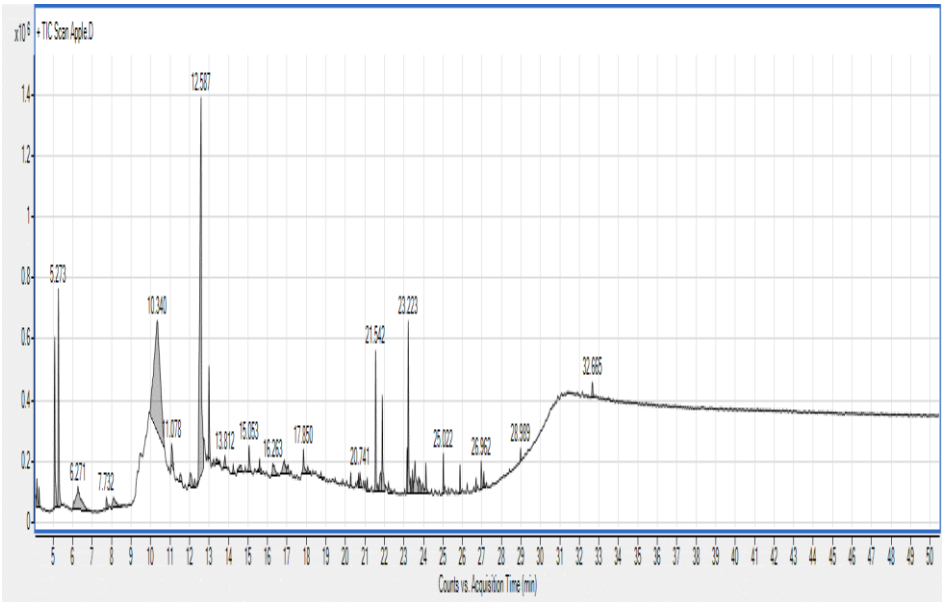

Fig3: Gas chromatogram of methanolic apple cider extract

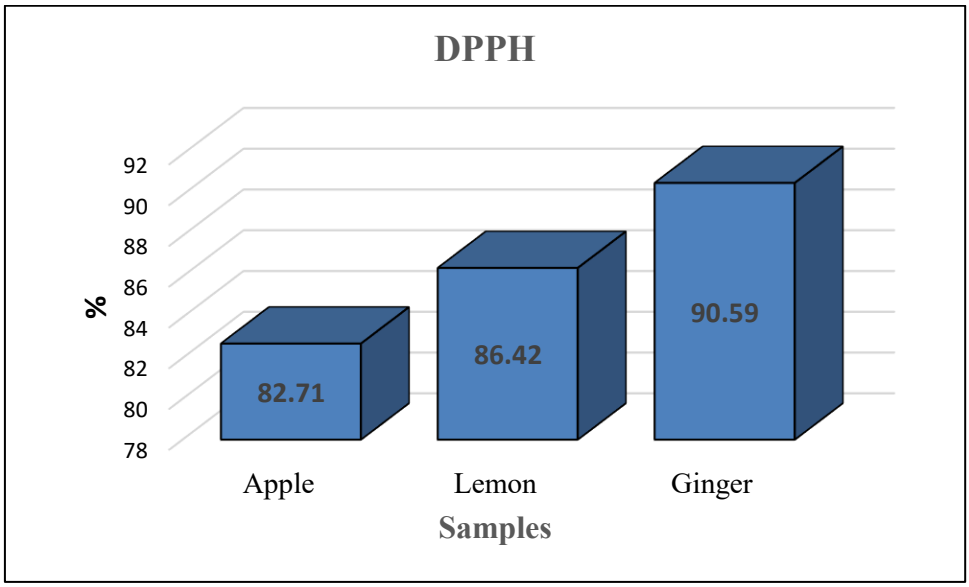

Fig 4: Antioxidants activity of the extracts of ginger, lemon, and apple extract

DPPH radical scavenging activity

Antioxidant effectiveness of plants used in study Fig (4), the results of the statistical analysis showed significant differences between the extracts. as the highest rate of free radical scavenging was recorded for ginger at 90.59%, which was significantly superior to lemon extract, apple cider vinegar, and thyme, which recorded (82.42, 86.71) respectively. The reason for superiority of ginger extract in scavenging free radicals may be due to its containing many effective antioxidants such as (Octanal, Decanal, 1,3-cyclohexadiene, 5-(1,5-dimethi-4hexenyi)-2-methyi- As for lemon, its effectiveness may be due to (4H-pyran-4-one,2,3-dihydro-3,5-dinydroy-6-methyl-5-Hydoxymethyl furfural) we also note presence of some compounds in apple cider vinegar such as (N-t-Butyi-N-2-[2-thi0phosphatoethyl]amino ethyiurea Naphthalene,1,2-dihydro-1'1'6-trimethyl Pentadecanoic acid,14-methl ester Bis(2-ethylhexyl)phthalate) which led to effectiveness of the compound [7] .

Conclusion

Therapeutic properties of ginger, lemon and apple extract may be due to their containing many active medical compounds that have proven their effectiveness as antimicrobial, anti-inflammatory, antiviral, antioxidant, anticancer, antidiabetic, hypocholestrolemic , anti-ulcer and antiparasitic agents such as (Decanal, Octanal, Benzoic acid, Beta-mycene, n-Hexadecanio acid, Erythritol, Beta-bisabolene) through their diagnosis by GC-MS technology. From this study, it could be concluded that ginger, lemon and apple extract have several bioactive compounds and strong antioxidant.

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