academicJournals

Vol. 7(10), pp. 238-252, October 2015
DOI: 10.5897/JPP2015.0361
Article Number: 8FCE0C855983
ISSN 2141-2502

http://www.academicjournals.org/JPP

Journal of Pharmacognosy and Phytotherapy

Full Length Research Paper

Phytochemical analysis of *Urtica dioica* leaves by fourier-transform infrared spectroscopy and gas chromatography-mass spectrometry

Huda Jasim Al-Tameme, Mohammed Yahya Hadi and Imad Hadi Hameed*

Department of Biology, Babylon University, Hilla City, Iraq.

Received 17 August, 2015; Accepted 21 September, 2015

Phytochemicals are chemical compounds formed during the plants normal metabolic processes. These chemicals are often referred to as secondary metabolites. The objective of this research was to determine the chemical composition of leaves extract from methanol. The phytochemical compound screened by gas chromatography-mass spectrometry (GC-MS) method. Fifteen bioactive phytochemical compounds were identified in the methanolic extract of *Urtica dioica*. The identification of phytochemical compounds is based on the peak area, retention time molecular weight, molecular formula, mass spectrometry (MS) fragment-ions and pharmacological actions. GC-MS analysis of *U. dioica* revealed the existence of the Oxime- methoxy-phenyl, 2, 6,-Nonadienal, 3, 7-dimethyl, 1, 2, 3-Butanetriol, Silane, triethyl(2-phenylethoxy), Benzofuran, 2,3,-dihydro, 2,5,5,8a-Tetramethyl-1,2,3,5,6,7,8, 8a-octahydronaphthalen-1-ol, 2H-Indeno[1,2-b]furan-2-one, 3,3a, 4,5,6,7,8, 8b-octahydro-8,8-dimet, 1-Dodecanamine, N, N-dimethyl, 2(3H)-Naphthalenone, 4, 4a,5,6,7,8-hexahydro-1-methoxy, D-Fructose, diethyl mercaptal, pentaacetate, [1,1-Bicyclopropyl-2-octanoic acid 2hexyl-methyl ester, Estra-1,3,5(10)-trien-17B-ol, Cyclopropaneoctanoic acid, 2-[2-pentylcycloproyl)methyl]-methyl, 1-Hydroxy-2-(2,3,4,6-tetra-O-acetyl-beta-d-glucopyranosyl)-9H-xanthe and Ethyl iso- allochlate. The FTIR analysis of *U. dioica* leaves proved the presence of aromatic rings, alkenes, aliphatic fluoro, alcohols, ethers, carboxlic acids, esters, nitro compounds, hydrogen bonded alcohols and phenols. It contain chemical constitutions which may be useful for various herbal formulation as anti-inflammatory, analgesic, antipyretic, cardiac tonic and antiasthamatic.

Key words: GC-MS analysis, fourier-transform infrared, phytochemicals, *Urtica dioica*.

INTRODUCTION

Phytochemicals are defined as bioactive non-nutrient plant compounds in fruits, vegetables, grains, and other plant foods that have been linked to reducing the risk of major chronic diseases (Hai, 2004; Magee and Rowland, 2004; Altameme et al., 2015; Hameed et al., 2015a). General description of *Urtica dioica* erect perennial, 50 to 300 cm tall with 4-sided stems, armed with stinging hairs, opposite leaves, 7 to 15 cm long, the stalks from about 1/10 as long to nearly 1/2 as long as the blades, depending on variety. The stipules prominent, mostly 10

*Corresponding author. E-mail: imad_dna@yahoo.com.

to 15 mm long. Fruits are achenes, lens-shaped, flattened, about 1.5 mm long, enclosed by the 2 inner sepals. *U. dioica* has many hollow stinging hairs called trichomes on its leaves and stems, which act like hypodermic needles that inject histamine and other chemicals that produce the stinging sensation when contacted by humans and other animals (Kavalali, 2003; Petlevski et al., 2003; Gulcin, 2004).

The other compounds isolated are derivatives of the terpenoids previously isolated from the roots and flowers of *U. dioica* (Gozum et al., 2003; Luo, 2009), and they include stigmasterol derivative, sitosterol derivative and ethyl cholestanol (Belyakova et al., 2002; Benkeblia, 2004; Golalipour et al., 2009).

This study aims to analyze the chemical compounds of *U. dioica* leaves by fourier-transform infrared (FT-IR) spectroscopy and gas chromatography-mass spectrometry (GC-MS).

MATERIALS AND METHODS

Collection and preparation of plant material

The leaves were dried at room temperature for seven days and when properly dried then powdered using clean pestle and mortar, and the powdered plant was size reduced with a sieve (Hameed et al., 2015). The fine powder was then packed in airtight container to avoid the effect of humidity and then stored at room temperature (Hussein et al., 2015).

Preparation of sample

About 9 g of the plant sample powdered were soaked in 100 ml methanol individually. It was left for 72 h so that alkaloids, flavonoids and other constituents if present will get dissolved. The methanol extract was filtered using Whatman's No.1 filter paper and the residue was removed (Jasim et al., 2015).

Gas chromatography-mass spectrum analysis

The GC-MS analysis of the plant extract was made in a (Agilent 7890 A) instrument under computer control at 70 eV. About 1 µL of the methanol extract was injected into the GC-MS using a micro syringe and the scanning was done for 45 min. As the compounds were separated, they eluted from the column and entered a detector which was capable of creating an electronic signal whenever a compound was detected (Mohammed and Imad, 2013; Kareem et al., 2015; Imad et al., 2014). The greater the concentration in the sample, bigger was the signal obtained which was then processed by a computer. The time from when the injection was made (Initial time) to when elution occurred is referred to as the retention time (RT). While the instrument was run, the computer generated a graph from the signal called chromatogram. Each of the peaks in the chromatogram represented the signal created when a compound eluted from the gas chromatography column into the detector. The X-axis showed the RT and the Y-axis measured the intensity of the signal to quantify the component in the sample injected. As individual compounds eluted from the gas chromatographic column, they entered the electron ionization (mass spectroscopy) detector, where they were bombarded with a stream of electrons causing them to break apart into fragments. The fragments obtained were actually charged ions with a certain mass .The mass/charge (M/Z) ratio obtained was calibrated from the graph obtained, which was called the Mass spectrum graph which is the fingerprint of a molecule (Imad et al., 2014).

Before analyzing the extract using GC-MS, the temperature of the oven, the flow rate of the gas used and the electron gun were programmed initially. The temperature of the oven was maintained at 100°C. Helium gas was used as a carrier as well as an eluent. The flow rate of helium was set to 1 ml per min. The electron gun of mass detector liberated electrons having energy of about 70eV. The column employed here for the separation of components was Elite 1 (100% dimethyl poly siloxane) (Imad et al., 2014). The identity of the components in the extracts was assigned by the comparison of their retention indices and mass spectra fragmentation patterns with those stored on the computer library and also with published literatures.

RESULTS AND DISCUSSION

GC-MS analysis of compounds was carried out in methanolic leaves extract of *U. dioica*, as shown in Table 1. The GC-MS chromatogram of the 15 peaks of the compounds detected was shown in Figure 1. Chromatogram GC-MS analysis of the methanol extract of *U. dioica* showed the presence of fifteen major peaks and the components corresponding to the peaks were determined as follows. The first set up peak was determined to be Oxime- methoxy-phenyl (Figure 2). The second peak indicated to be 2, 6,-Nonadienal, 3, 7-dimethyl (Figure 3). The next peaks considered to be 1, 2, 3-Butanetriol, Silane, triethyl(2-phenylethoxy), Benzofuran, 2,3,-dihydro, 2,5,5,8a-Tetramethyl-1,2,3,5,6,7,8, 8a-octahydronaphthalen-1-ol, 2H-Indeno[1,2-b]furan-2-one, 3,3a, 4,5,6,7,8, 8b-octahydro-8,8-dimet, 1-Dodecanamine, N, N-dimethyl, 2(3H)-Naphthalenone, 4, 4a,5,6,7,8-hexahydro-1-methoxy, D-Fructose, diethyl mercaptal, pentaacetate, [1,1-Bicyclopropyl-2-octanoic acid 2hexyl-methyl ester, Estra-1,3,5(10)-trien-17B-ol, Cyclopropaneoctanoic acid, 2-[2-pentylcycloproyl)methyl]-methyl, 1-Hydroxy-2-(2,3,4,6-tetra-O-acetyl-beta-d-glucopyranosyl)-9H-xanthe and Ethyl iso- allochlate. (Figure 4-16).

The FTIR analysis of *U. dioica* leaves proved the presence of aromatic rings, alkenes, aliphatic fluoro, alcohols, ethers, carboxlic acids, esters, nitro compounds, hydrogen bonded alcohols and phenols which shows major peaks at 891.11, 958.69, 1010.70, 1091.71, 1242.16, 1319.31, 2686.84 and 3363.86 (Table 2; Figure 17). Polar extract of the *U. dioica* contains lignans +)-neoolivil, (-)-secoisolariciresinol, Dehydrodiconiferyl alcohol, isolariciresinol, pinoresinol, and 3,4divanillyltetrahydrofuran, and has antiinflammatory effects and stimulates the proliferation of human lymphocytes (Obertreis et al.,1996; Harput et al.,2005; Kanter et al., 2005; Hameed et al., 2015c). Traditionally, it has been used for uterine hemorrhage, cutaneous eruption, infantile and psychogenic eczema, epistaxis, and melena and specifically for nervous eczema (Bandow et al., 2003; Burt, 2004; Banso and

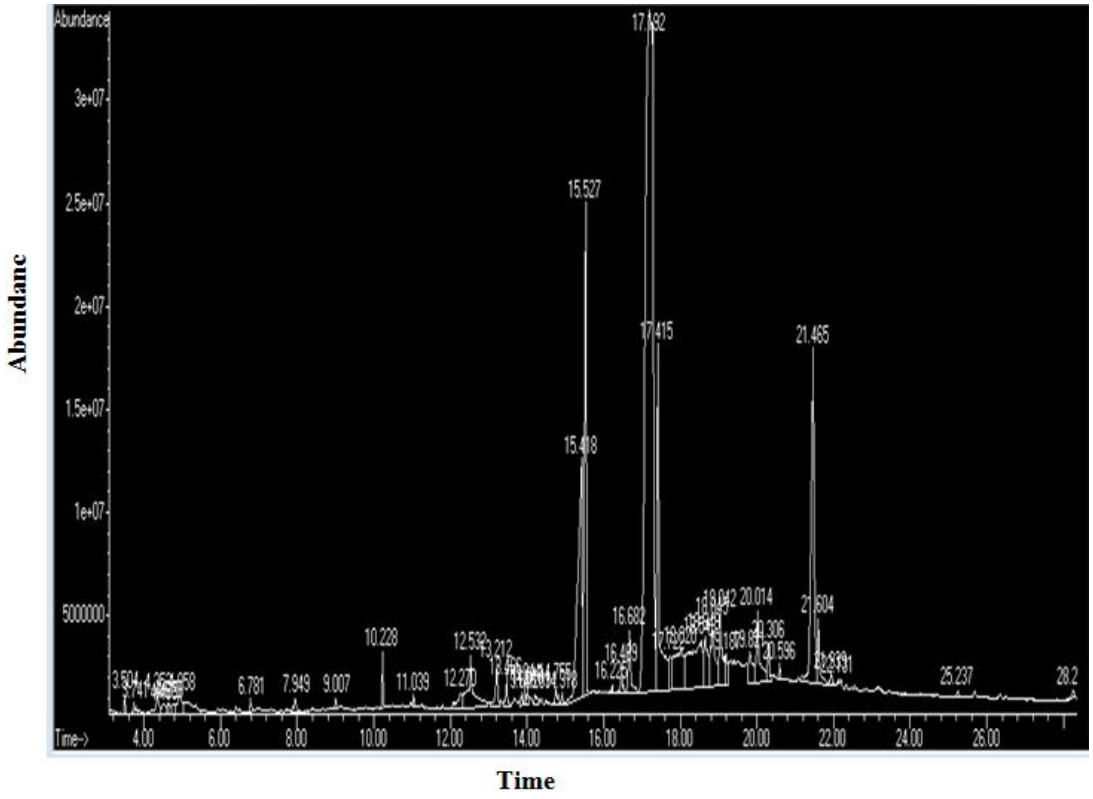


Figure 1. GC-MS chromatogram of methanolic leaves extract of *U. dioica* .

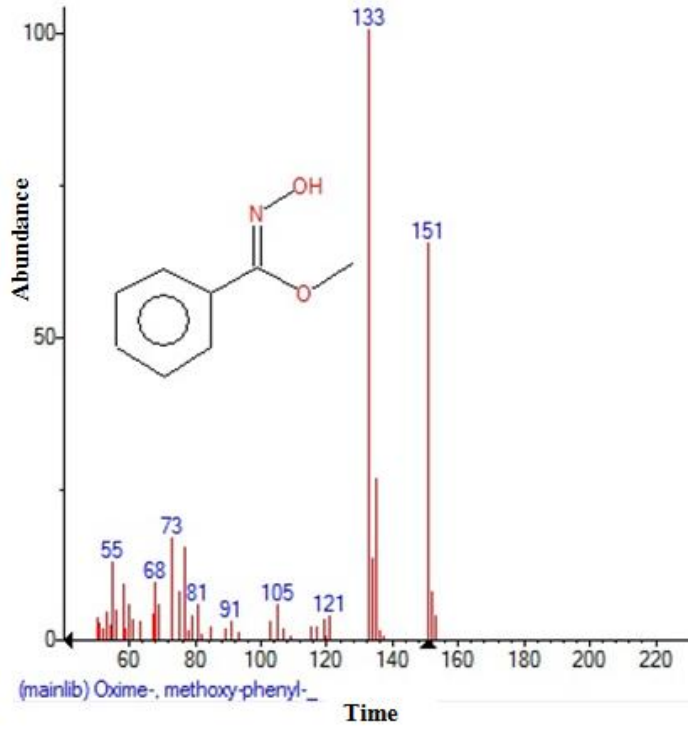


Figure 2. Mass spectrum of Oxime- methoxy-phenyl with retention time (RT) = 3.504.

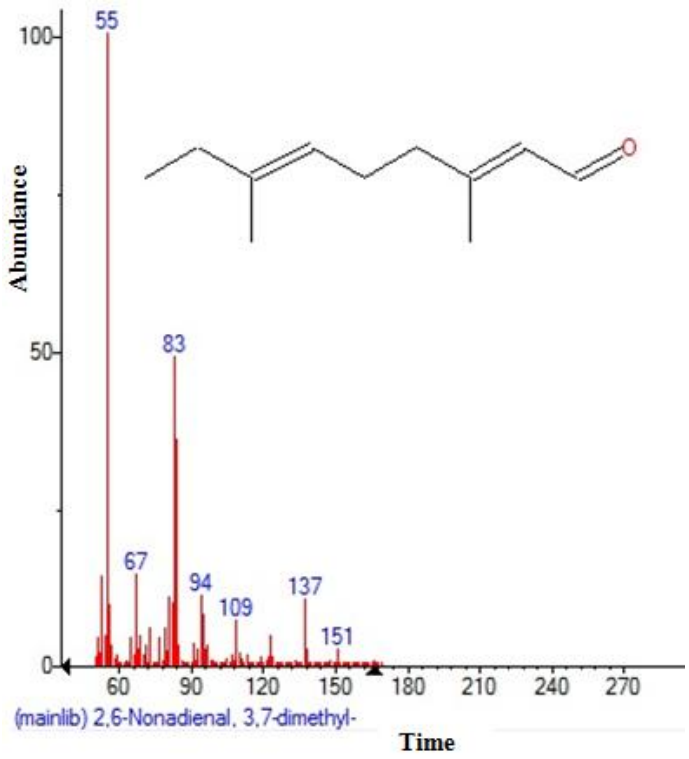


Figure 3. Mass spectrum of 2,6,-Nonadienal,3,7-dimethyl with retention time (RT)= 3.739.

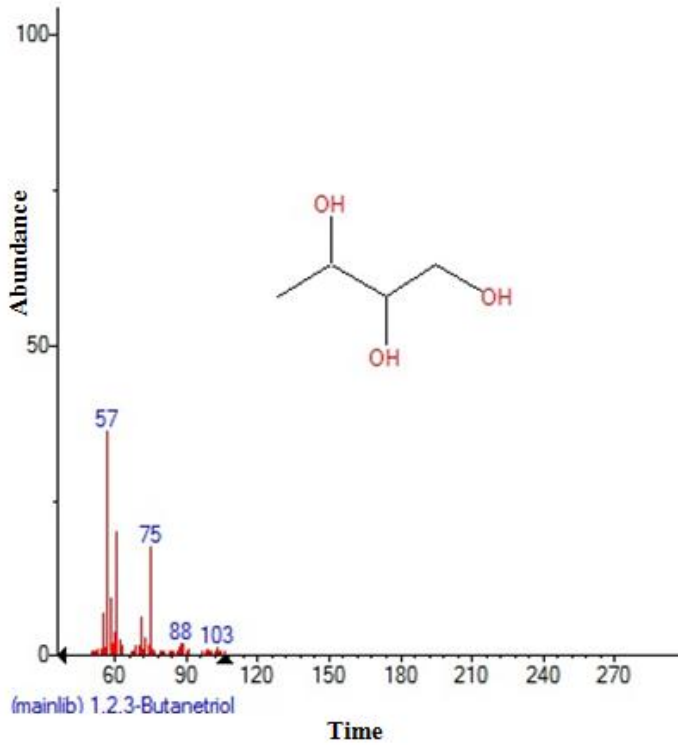


Figure 4. Mass spectrum of 1, 2, 3-Butanetriol with retention time (RT)= 4.380.

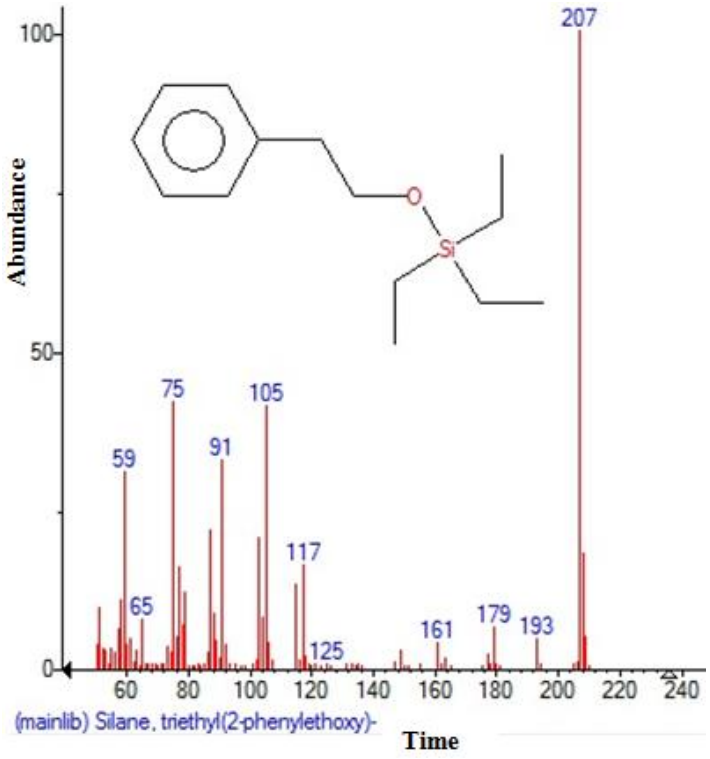


Figure 5. Mass spectrum of Silane, triethyl(2-phenylethoxy) with retention time (RT)= 4.975.

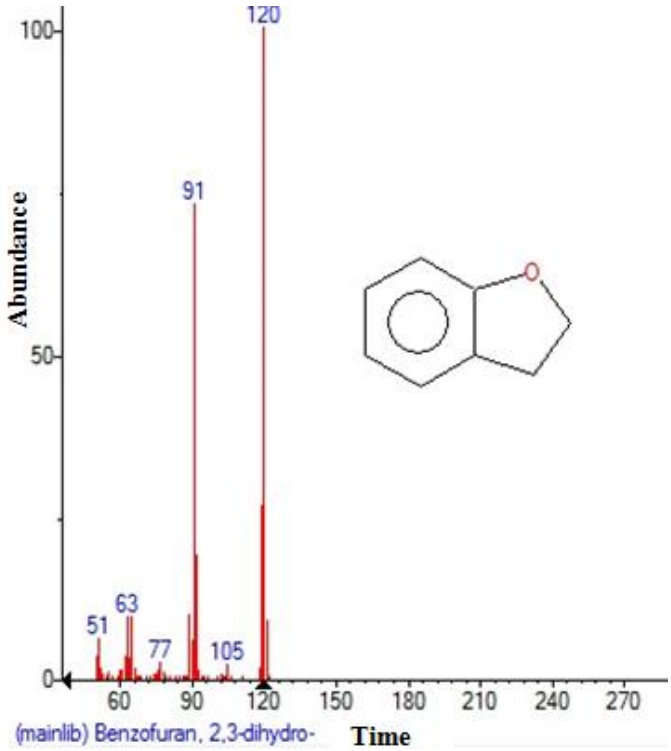


Figure 6. Mass spectrum of Benzofuran, 2,3,-dihydro with retention time (RT)= 6.777.

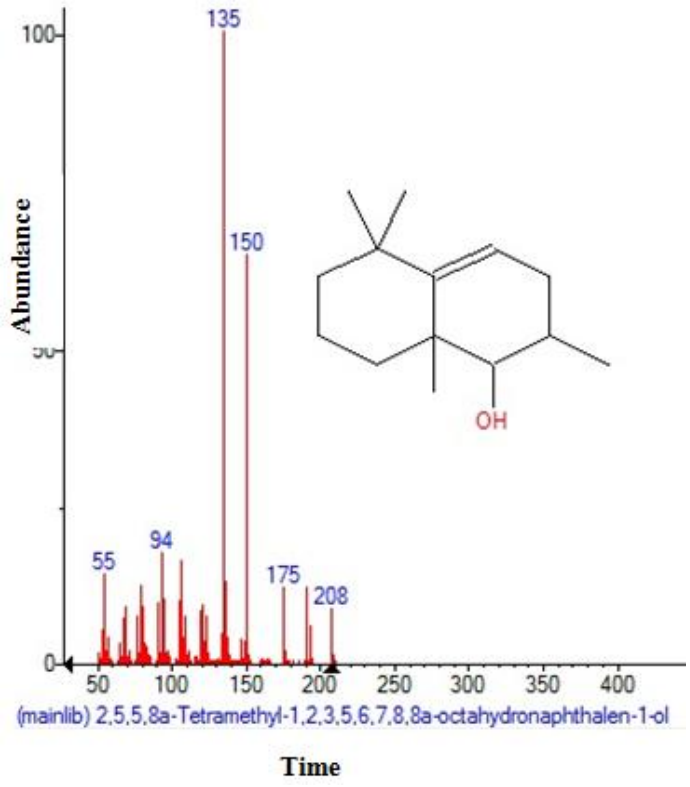


Figure 7. Mass spectrum of 2,5,5,8a-Tetramethyl-1,2,3,5,6,7,8, 8a-octahydronaphthalen-1-ol with retention time (RT)= 7.939.

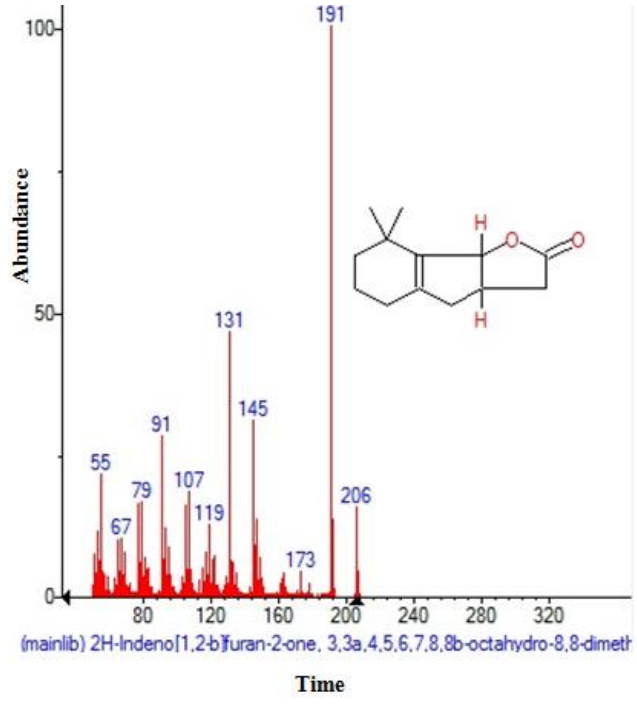


Figure 8. Mass spectrum of 2H-Indeno[1,2-b]furan-2-one, 3,3a, 4,5,6,7,8, 8b-octahydro-8,8-dimet with retention time (RT)= 8.992.

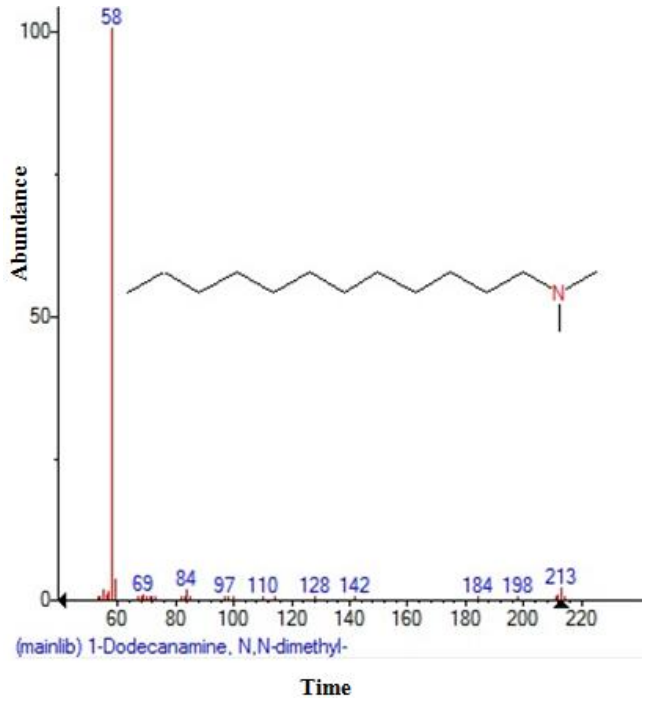


Figure 9. Mass spectrum of 1-Dodecanamine, N, N-dimethyl with retention time (RT)= 10.228.

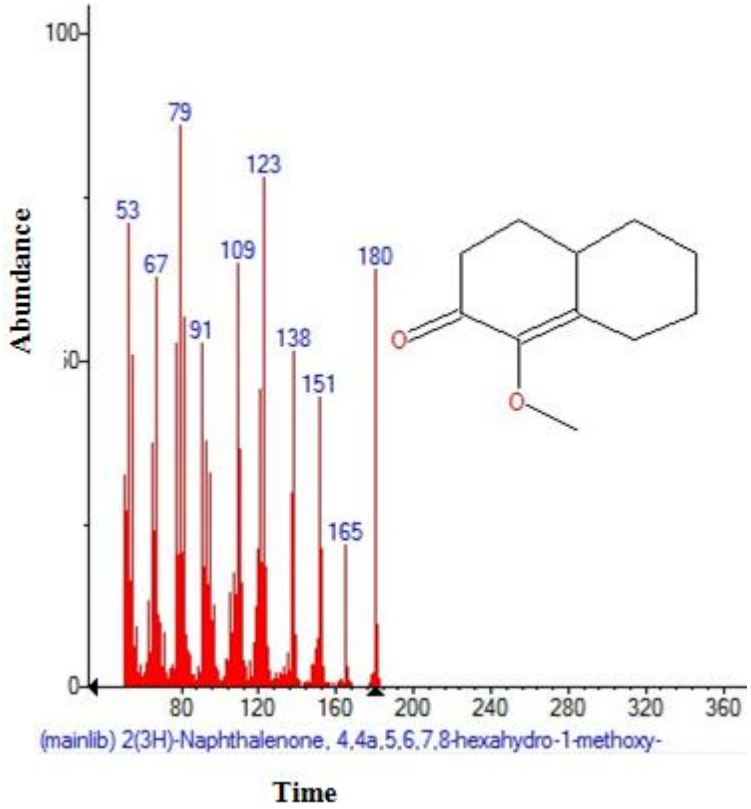


Figure 10. Mass spectrum of 2(3H)-Naphthalenone, 4, 4a,5,6,7,8-hexahydro-1-methoxy with retention time (RT)= 11.029.

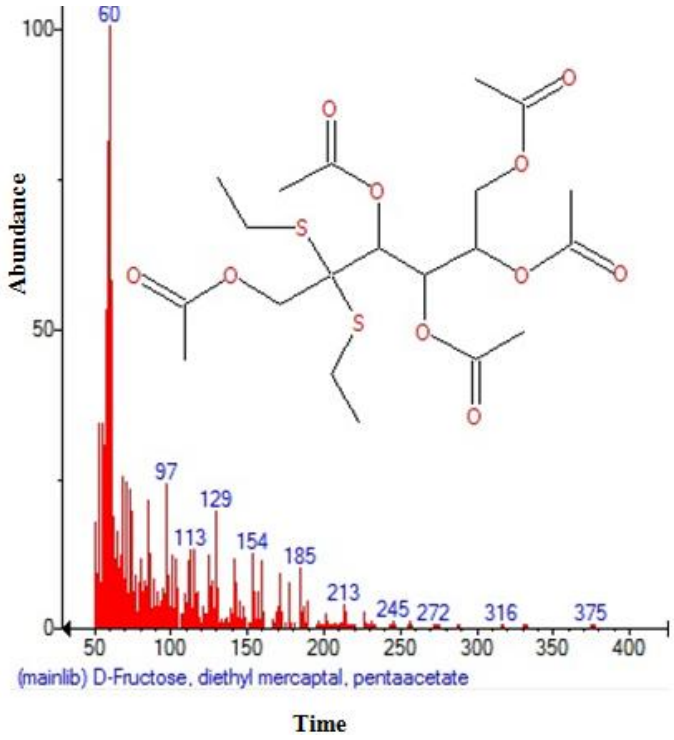


Figure 11. Mass spectrum of D-Fructose, diethyl mercaptal, pentaacetate with retention time (RT)= 13.243.

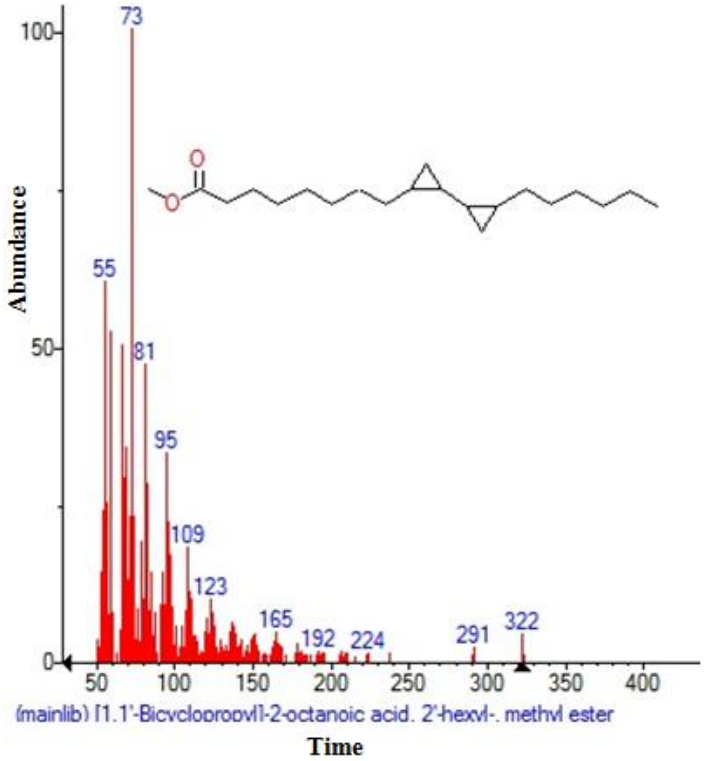


Figure 12. Mass spectrum of [1,1-Bicyclopropyl-2-octanoic acid 2hexyl-methyl ester with retention time (RT)= 13.501.

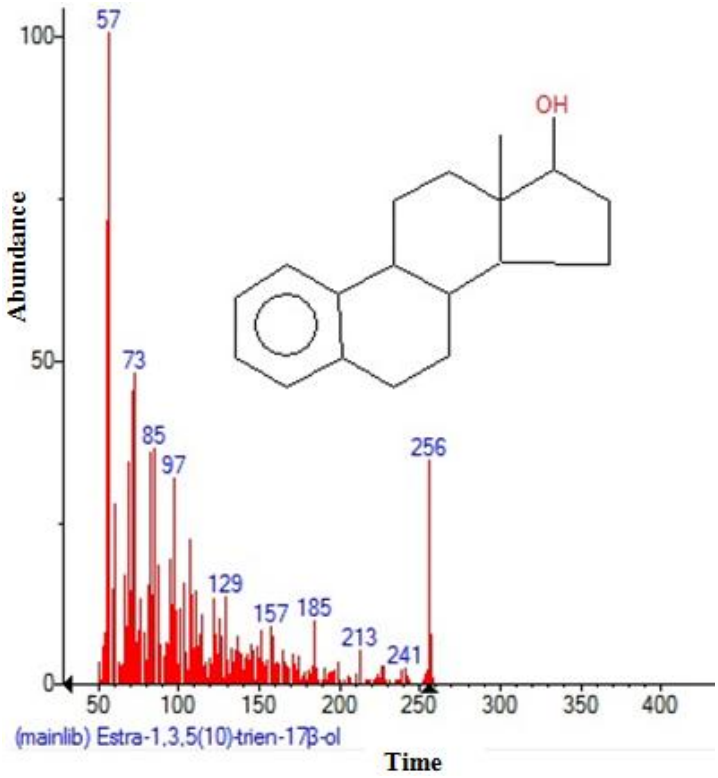


Figure 13. Mass spectrum of Estra-1,3,5(10)-trien-17B-ol with retention time (RT)= 15.561.

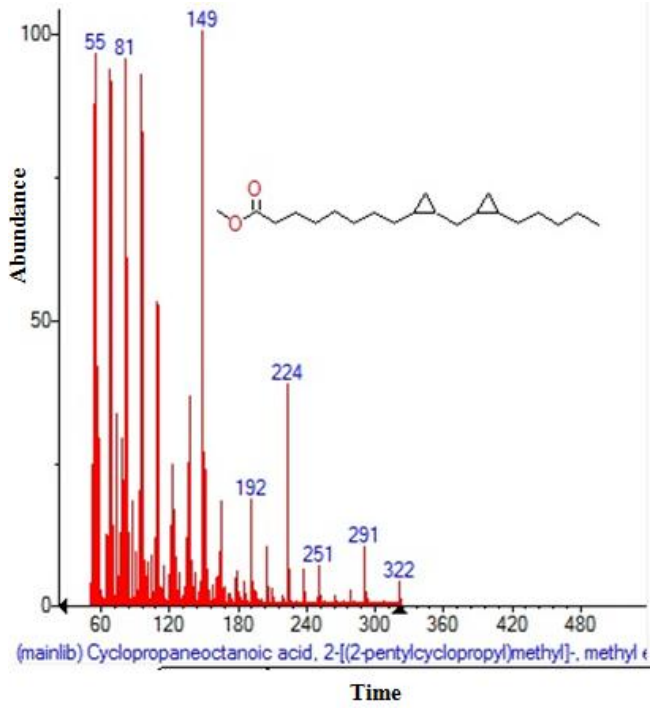


Figure 14. Mass spectrum of cyclopropaneoctanoic acid, 2-[2-pentylcycloproyl)methyl]-methyl with retention time (RT)= 20.327.

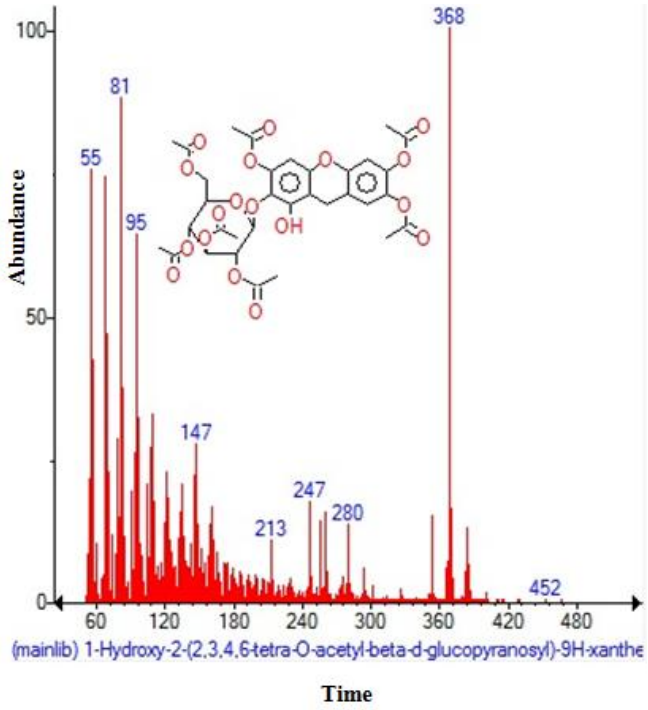


Figure 15. Mass spectrum of 1-Hydroxy-2-(2,3,4,6-tetra-O-acetyl-beta-d-glucopyranosyl)-9H-xanthe with retention time (RT)= 20.585.

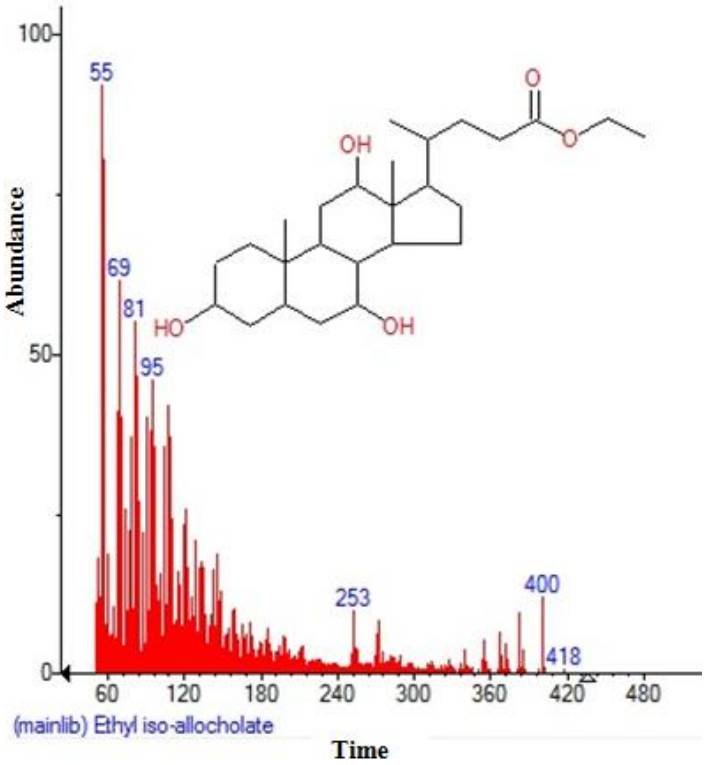


Figure 16. Mass spectrum of Ethyl iso- allochlate with retention time (RT)= 25.277.

Table 1. Major phytochemical compounds identified in methanolic leaves extract of *Urtica dioica*.

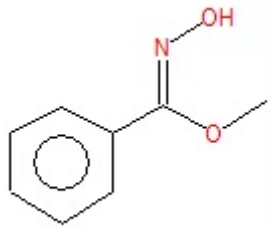
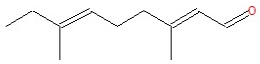
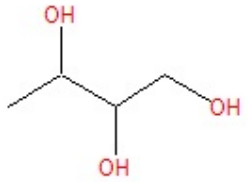
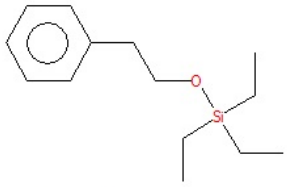
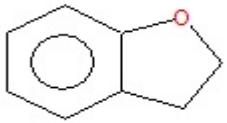
S/N	Phytochemical compound	RT(min)	Formula	Molecular weight	Exact mass	Chemical structure	MS Fragment- ions	Pharmacological actions
1.	Oxime- methoxy-phenyl	3.504	$C_8H_9NO_2$	151	151.063329		55,68,73,81,91,105,121,133,151	Antioxidant and antimicrobial activity
2.	2, 6,-Nonadienal, 3, 7-dimethyl	3.739	$C_{11}H_{18}O$	166	166.13576		55,67,83,94,109,137,151	Anti-inflammatory and antioxidant activity
3.	1, 2, 3-Butanetriol	4.380	$C_4H_{10}O_3$	106	106.062994		57,75,88,103	Wide range of biological properties including antitumor activity
4.	Silane, triethyl(2-phenylethoxy)	4.975	$C_{14}H_{24}OSi$	236	236.159642		59,65,75,91,105,117,125,161,179,193	Biocontrol
5.	Benzofuran, 2,3,-dihydro	6.777	C_8H_8O	120	120.057514		51,63,77,91,105,120	Antiarrhytmic, spasmolitic, antiviral

Table 1. Condt.

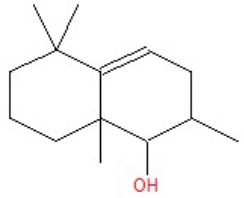
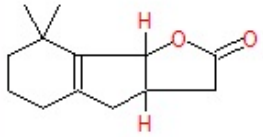
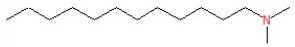
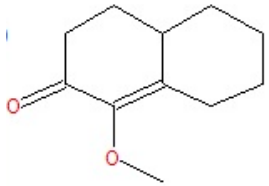
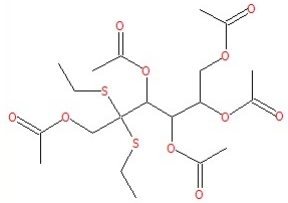
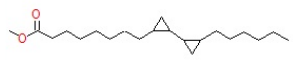
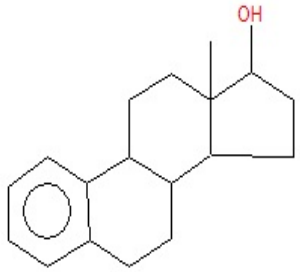
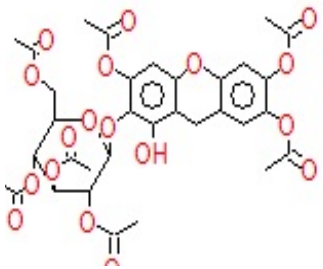
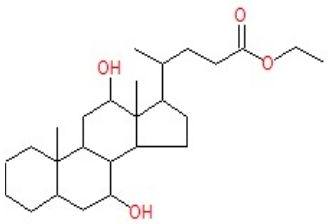
6.	2,5,5,8a-Tetramethyl-1,2,3,5,6,7,8, 8a-octahydronaphthalen-1-ol	7.939	$C_{14}H_{44}O$	208	208.182715		55,94,135,150,175,208	Pharmacological action of this product is unknown
7.	2H-Indeno[1,2-b]furan-2-one, 3,3a, 4,5,6,7,8, 8b-octahydro-8,8-dimet	8.992	$C_{13}H_{18}O_2$	206	206.13068		55,67,79,91,107,119,131,145,173,191,106	New chemical compound
8.	1-Dodecanamine, N, N-dimethyl	10.228	$C_{14}H_{31}N$	213	213.24565		58,69,84,97,110,128,142,184,198,213	Anti-Staphylococcal Activity
9.	2(3H)-Naphthalenone, 4, 4a,5,6,7,8-hexahydro-1-methoxy	11.029	$C_{11}H_{16}O_2$	180	180.115029		53,67,79,91,109,123,138,151,165,180	Pharmacological action of this product is unknown
10.	D-Fructose, diethyl mercaptal, pentaacetate	13.243	$_{20}H_{32}O_{10}S_2$	496	496.14369		60,97,113,129,154,185,213,245,272,316,375	Antitumor and antibacterial activity
11.	[1,1-Bicyclopropyl-2-octanoic acid 2hexyl-methyl ester	13.501	$C_{21}H_{38}O_2$	322	322.28718		55,73,81,95,109,123,165,192,224,291,322	Anti-diabetic and anti-inflammatory and anti-inflammatory,

Table 1. Condt.

12.	Estra-1,3,5(10)-trien-17B-ol	15.561	$C_{18}H_{24}O$	256	256.18271		57,73,85,97,129,157,185,213,241,256	anti-proliferative effect
13.	Cyclopropaneoctanoic acid, 2-[2-pentylcycloproyl)methyl]-methyl	20.327	$C_{18}H_{20}O_3$	322	322.28718		55,81,149,192,224,251,291,322	New chemical compound
14.	1-Hydroxy-2-(2,3,4,6-tetra-O-acetyl-beta-d-glucopyranosyl)-9H-xanthe	20.585	$C_{33}H_{34}O_{18}$	718	718.174515		55,81,95,147,213,247,280,368,452	New chemical compound
15.	Ethyl iso- allochlate	25.277	$C_{26}H_{44}O_5$	436	436.31887		55,69,81,95,253,400	Antimicrobial. Antioxidant. Anti-inflammatory

Adeyemo, 2006). Among those identified, phytocompounds have the property of antioxidant and antimicrobial activities (Silva et al., 2004; Sein et al., 2008). Plant based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects. Continued

Table 2. FT-IR peak values of *Urtica dioica* methanol leaf extract.

S/N	Peak (Wave number cm-ˡ)	Intensity	Bond	Functional group assignment	Group frequency
1.	891.11	74.304	C-H	Aromatic rings	690-900
2.	958.69	68.024	C-H	Alkenes	675-995
3.	1010.70	56.914	C-F stretch	Aliphatic fluoro compounds	1000-10150
4.	1091.71	61.891	C-F stretch	Aliphatic fluoro compounds	1000-10150
5.	1242.16	76.996	C-O	Alcohols, Ethers, Carboxlic acids, Esters	1050-1300
6.	1319.31	73.166	NO2	Nitro Compounds	1300-1370
7.	1338.60	71.524	NO2	Nitro Compounds	1300-1370
8.	1361.74	71.150	NO2	Nitro Compounds	1300-1370
9.	1373.32	70.723	C-H	Alkenes	1340-1470
10.	1539.20	73.241	NO2	Nitro Compounds	1500-1570
11.	1595.13	71.600	C-C	Aromatic rings	1500-1600
12.	2306.86	90.993	-	Unknown	-
13.	2686.84	89.928	O-H	Hydrogen bonded Carboxylic acids	2500-2700
14.	2752.42	89.287	-	Unknown	-
15.	2848.86	82.640	-	Unknown	-
16.	2918.30	79.097	C-H	Alkanes	2850-2970
17.	3064.89	84.666	H-O	H-bonded H-X group	2500-3500
18.	3182.55	81.242	H-O	H-bonded H-X group	2500-3500
19.	3246.20	80.081	O-H	Hydrogen bonded Alcohols, Phenols	3200-3600
20.	3273.20	79.592	O-H	Hydrogen bonded Alcohols, Phenols	3200-3600
21.	3363.86	80.541	O-H	Hydrogen bonded Alcohols, Phenols	3200-3600

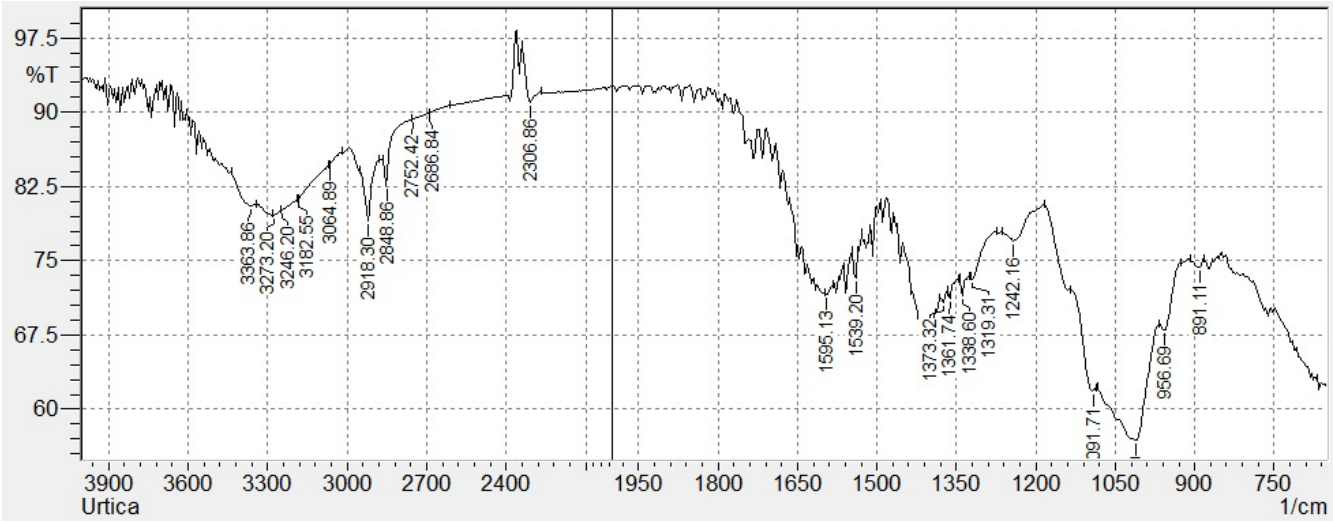


Figure 17. Fourier-transform infrared profile of leaves extract of *Urtica dioica*.

further exploration of plant derived antimicrobials is needed today.

Conclusion

U. dioica is native plant of Iraq. It contains chemical constitutions which may be useful for various herbal formulation as anti-inflammatory, analgesic, antipyretic, cardiac tonic and antiasthamatic properties.

ACKNOWLGEMENT

The authors wish to express their deepest gratitude to Prof. Dr. Adul-Kareem for his valuable contributions and support throughout this study. They would also like to express their gratitude to Dr. Ali for his valuable suggestions

and comments.

Conflicts of interest

The authors have none to declare.

REFERENCES

Altameme HJ, Hameed IH, Kareem MA (2015). Analysis of alkaloid phytochemical compounds in the ethanolic extract of *Datura stramonium* and evaluation of antimicrobial activity. Afr. J. Biotechnol.14(19):1668-1674.

Bandow JE, Brotz H, Leichert L (2003). Proteomic approach to understanding antibiotic action. Antimicrob. Agents Chemother. 47:948-955.

Banso A, Adeyemo S (2006). Phytochemical screening and antimicrobial assessment of Abutilon mauritianum, Bacopamonnifera and Daturastr-amonium. Biochemistry 18(1):39-44.

Belyakova VA, Vainshtein KV, Markova Y, Demchenko T, Chibilyaev TH (2002). Extraction of nettle leaves using synthetic esters of fatty acids. Pharm. Chem. J. 39(11):598-602.

Benkeblia N (2004). Antimicrobial activity of essential oil extracts of various onions (*Allium cepa*) and garlic (*Allium sativum*). Lebensm-Wiss u-Technol. 37:263-268.

Burt S (2004). Essentials oils: their antibacterial properties and potential applications in food: a review. Int. J. Food Microbiol. 94:254-259.

Golalipour MJ, Ghafari S, Farsi MM (2009). Effect of *Urtica dioica* extract on quantitative morphometric alterations of liver paranchymal cells in STZ diabetic rats. Int. J. Morphol. 27(4):1339-1344.

Gozum S, Tezel A, Koc M (2003). Complementary alternative treatments used by patients with cancer in eastern Turkey. Cancer Nurs. 26:230–236

Gulcin I (2004). Antioxidant, antimicrobial, antiulcer and analgesic activities of nettle (*Urtica dioica* L.). J. Ethnopharmacol. 90:205-215.

Hai LR (2004). Potential Synergy of Phytochemicals in Cancer Prevention: Mechanism of Action. J. Nutr. 134:3479-3485.

Hameed IH, Hussein HJ, Kareem MA, Hamad NS (2015a). Identification of five newly described bioactive chemical compounds in methanolic extract of *Mentha viridis* by using gas chromatography-mass spectrometry (GC-MS). J. Pharmacogn. Phytother. 7(7):107-125.

Hameed IH, Ibraheam IA, Kadhim HJ (2015b). Gas chromatography mass spectrum and fourier-transform infrared spectroscopy analysis of methanolic extract of *Rosmarinus oficinalis* leaves. J. Pharmacogn. Phytother. 7 (6):90-106.

Hameed IH, Jasim H, Kareem MA, Hussein AO (2015c). Alkaloid constitution of Nerium oleander using gas chromatography-mass spectroscopy (GC-MS). J. Med. Plants Res. 9(9):326-334.

Harput S, Saracoglu I, Ogihara Y (2005). Stimulation of Lymphocyte Proliferation and Inhibition of Nitric Oxide Production by Aqueous *Urtica dioica* Extract U. Phytother. Res.19:346–348.

Hussein AO, Hameed IH, Jasim H, Kareem MA (2015). Determination of alkaloid compounds of *Ricinus communis* by using gas chromatography-mass spectroscopy (GC-MS). J. Med. Plants Res. 9(10):349-359.

Imad H, Mohammed A, Aamera J (2014a). Genetic variation and DNA markers in forensic analysis. Afr. J. Biotechnol. 13(31):3122-3136.

Imad H, Mohammed A, Cheah Y, Aamera J (2014b) Genetic variation of twenty autosomal STR loci and evaluate the importance of these loci for forensic genetic purposes. Afr. J. Biotechnol. 13:1-9.

Imad H, Muhanned A, Aamera J, Cheah Y (2014c). Analysis of eleven Y-chromosomal STR markers in middle and south of Iraq. Afr. J. Biotechnol. 13(38):3860-3871.

Jasim H, Hussein AO, Hameed IH, Kareem MA (2015). Characterization of alkaloid constitution and evaluation of antimicrobial activity of *Solanum nigrum* using gas chromatography mass spectrometry (GC-MS). J. Pharmacogn. Phytother. 7(4):56-72.

Kanter M, Coskun O, Budancamanak M (2005). Hepatoprotective effects of Nigella sativa L. and *Urtica dioica* L. on lipid peroxidation, antioxidant enzyme systems and liver enzymes in carbon tetrachloride-treated rats. World J. Gastroenterol. 42:6684–6688.

Kareem MA, Hussein AO, Hameed IH (2015). Y-chromosome short tandem repeat, typing technology, locus information and allele frequency in different population: A review. Afr. J. Biotechnol. 14(27):2175-2178.

Kavalali G (2003). Hypoglycemic activity of *Urtica pilulifera* in streptozotocin-diabetic rats. J. Ethnopharmacol. 84(2-3):241-5.

Luo JR (2009). Paleophytochemical components from Miocene-Fossil wood of *Pinus griffithii*. J. Chin. Chem. Soc. 56(3):600-605.

Magee PJ, Rowland IR (2004). Phyto-oestrogens, their mechanism of action: current evidence for a role in breast and prostate cancer. Brit. J. Nutr. 91:513-531.

Mohammed A, Imad H (2013). Autosomal STR: From locus information to next generation sequencing technology. Res. J. Biotechnol. 8(10):92-105.

Obertreis B, Ruttkowski T, Teucher T, Behnke B (1996). Ex-vivo in-vitro inhibition of lipopolysaccharide stimulated tumor necrosis factor-alpha and interleukin-1 beta secretion in human whole blood by extractum *Urticae dioicae* foliorum. Arzneimittelforschung 46(9):936.

Petlevski R, Hadzija M, Slijepcević M, Juretić D, Petrik J (2003). Glutathione S-transferases and malondialdehyde in the liver of NOD mice on short-term treatment with plant mixture extract. Phytother. Res. 17(4):311-4.

Sein TT, Spurio R, Cecchini C, Cresci A (2008). Screening for microbial strains degrading glass fiber acrylic composite filters. Int. Biodeterior. Biodegrad. 63:901–905.

Silva BM, Andrade PB, Valentão P, Ferreres F, Seabra RM, Ferreira MA (2004). Quince (*Cydonia oblonga* Miller) fruit (pulp, peel, and seed) and jam: antioxidant activity. J. Agric. Food Chem. 52:4405–4712.