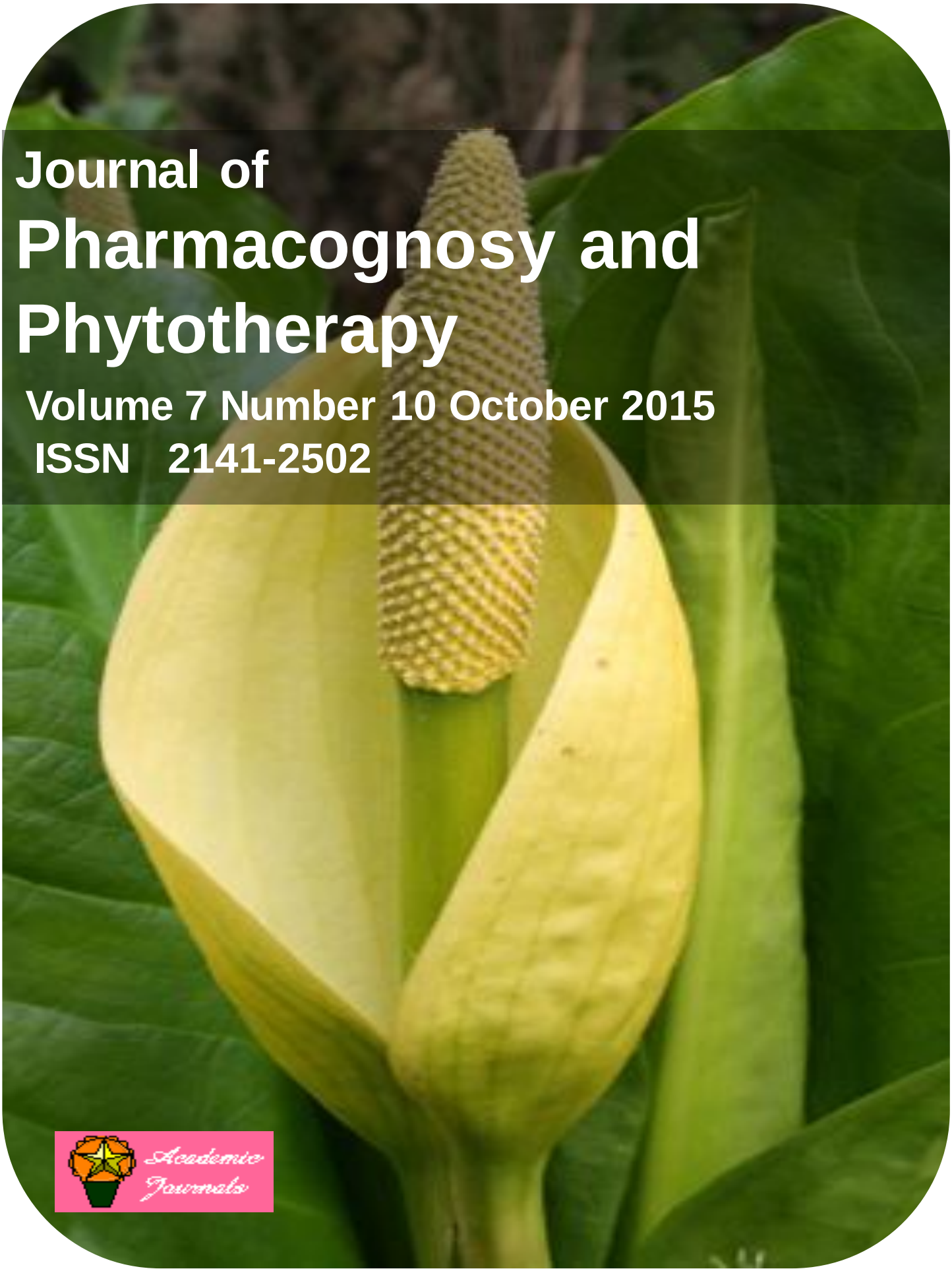

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Full Length Research Paper

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

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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

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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

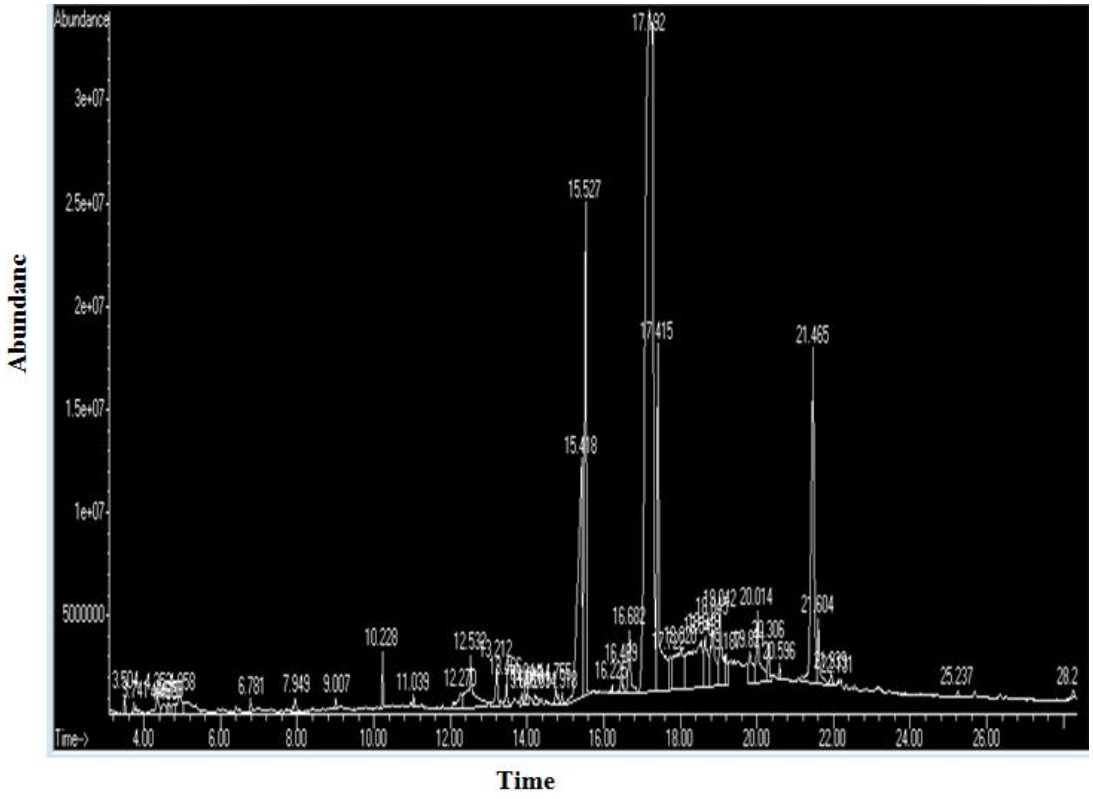


Figure 1. GC-MS chromatogram of methanolic leaves extract of *U. dioica* .

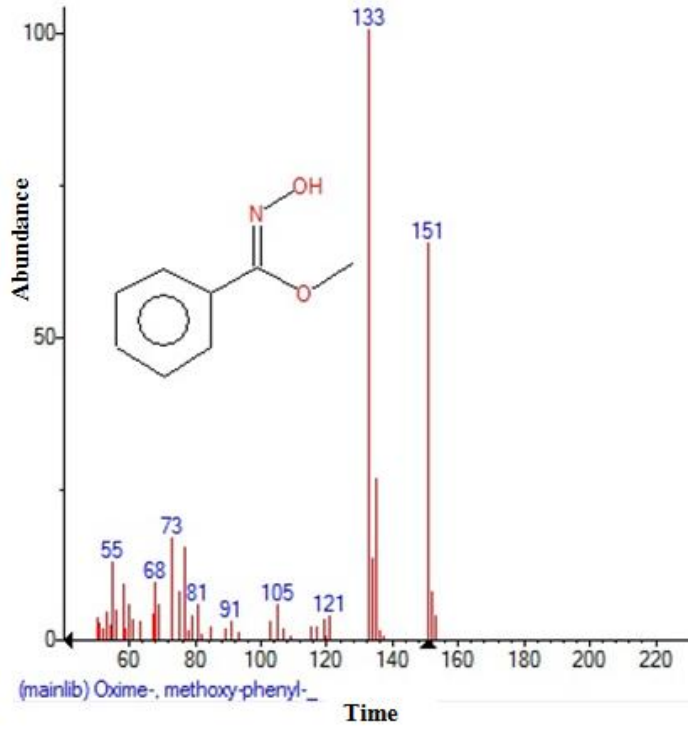


Figure 2. Mass spectrum of Oxime- methoxy-phenyl with retention time (RT) = 3.504.

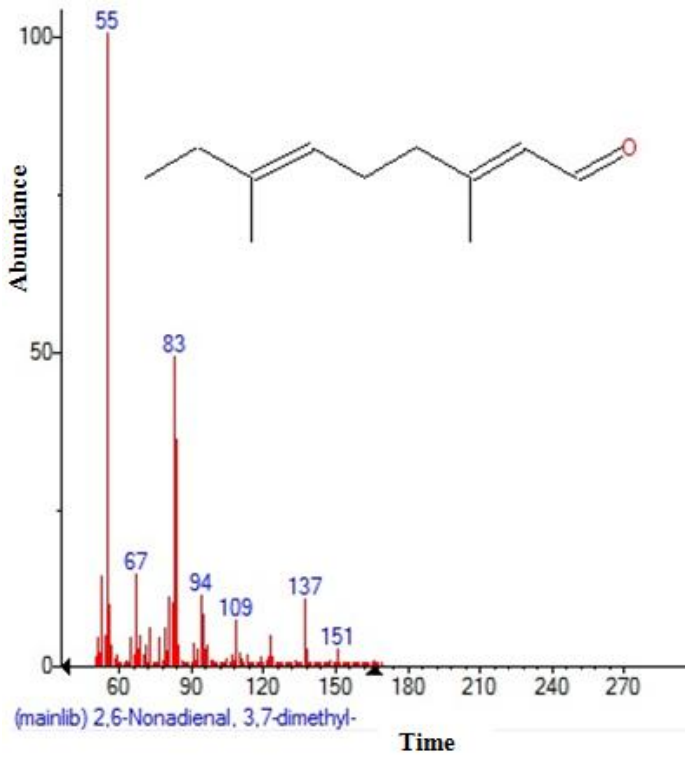


Figure 3. Mass spectrum of 2,6,-Nonadienal,3,7-dimethyl with retention time (RT)= 3.739.

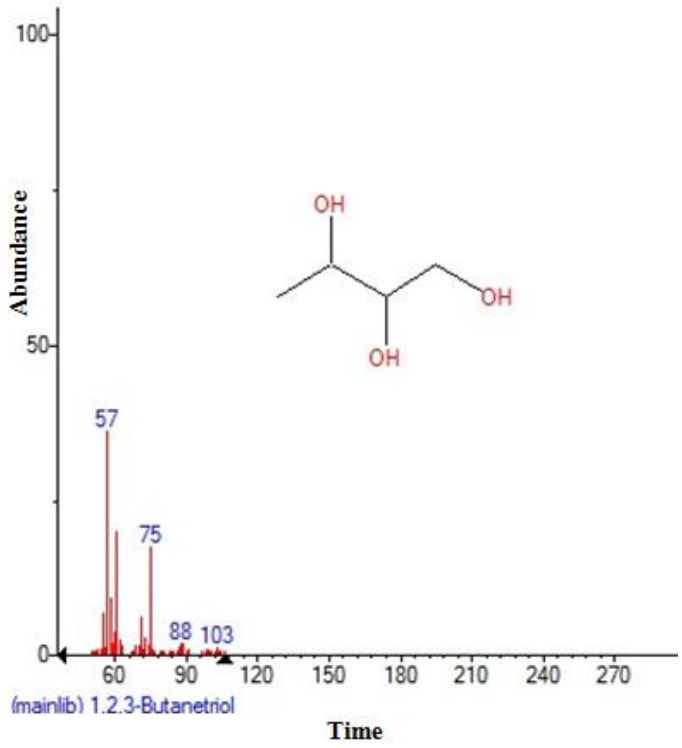


Figure 4. Mass spectrum of 1, 2, 3-Butanetriol with retention time (RT)= 4.380.

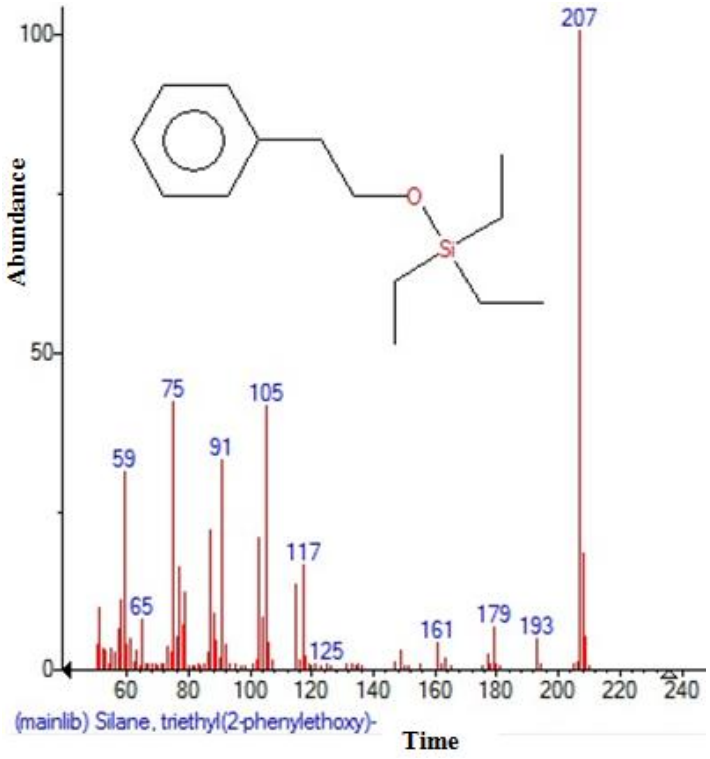


Figure 5. Mass spectrum of Silane, triethyl(2-phenylethoxy) with retention time (RT)= 4.975.

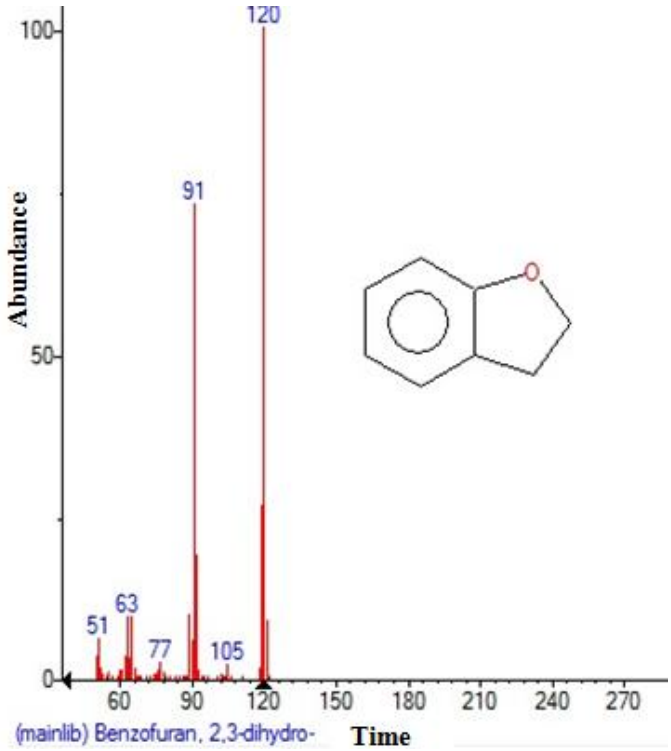


Figure 6. Mass spectrum of Benzofuran, 2,3,-dihydro with retention time (RT)= 6.777.

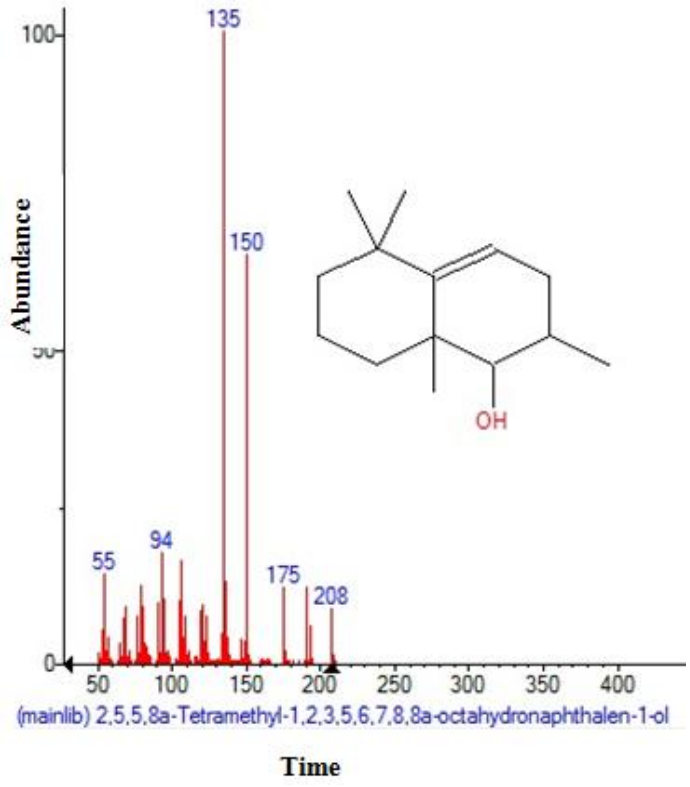


Figure 7. Mass spectrum of 2,5,5,8a-Tetramethyl-1,2,3,5,6,7,8, 8a-octahydronaphthalen-1-ol with retention time (RT)= 7.939.

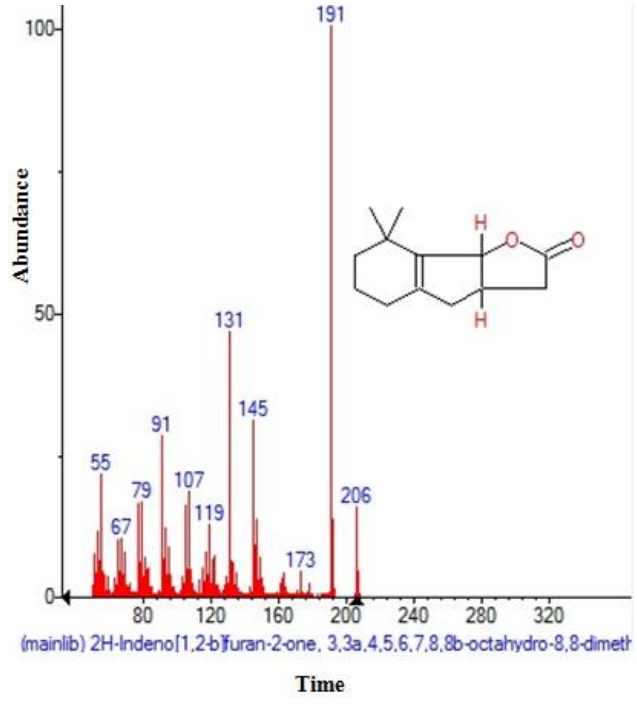


Figure 8. Mass spectrum of 2H-Indeno[1,2-b]furan-2-one, 3,3a, 4,5,6,7,8, 8b-octahydro-8,8-dimet with retention time (RT)= 8.992.

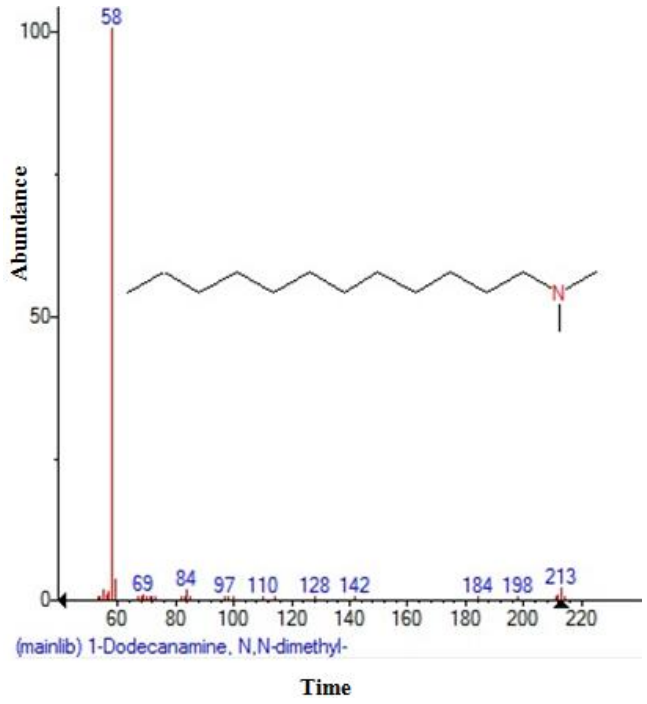


Figure 9. Mass spectrum of 1-Dodecanamine, N, N-dimethyl with retention time (RT)= 10.228.

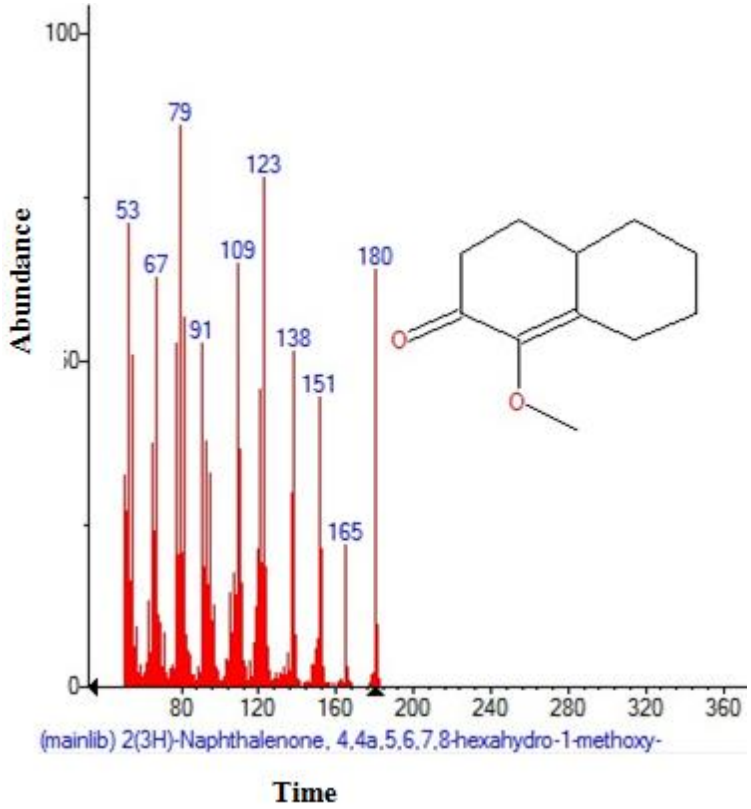


Figure 10. Mass spectrum of 2(3H)-Naphthalenone, 4, 4a,5,6,7,8-hexahydro-1-methoxy with retention time (RT)= 11.029.

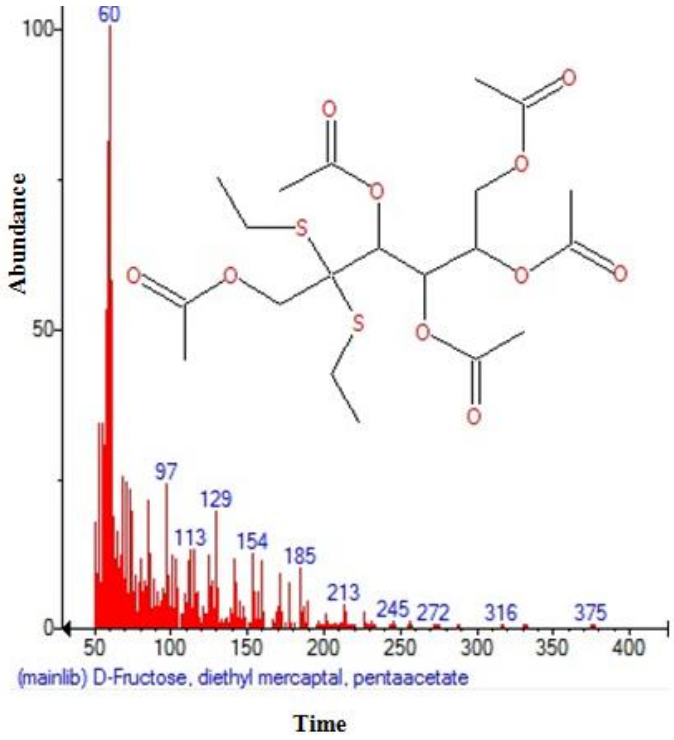


Figure 11. Mass spectrum of D-Fructose, diethyl mercaptal, pentaacetate with retention time (RT)= 13.243.

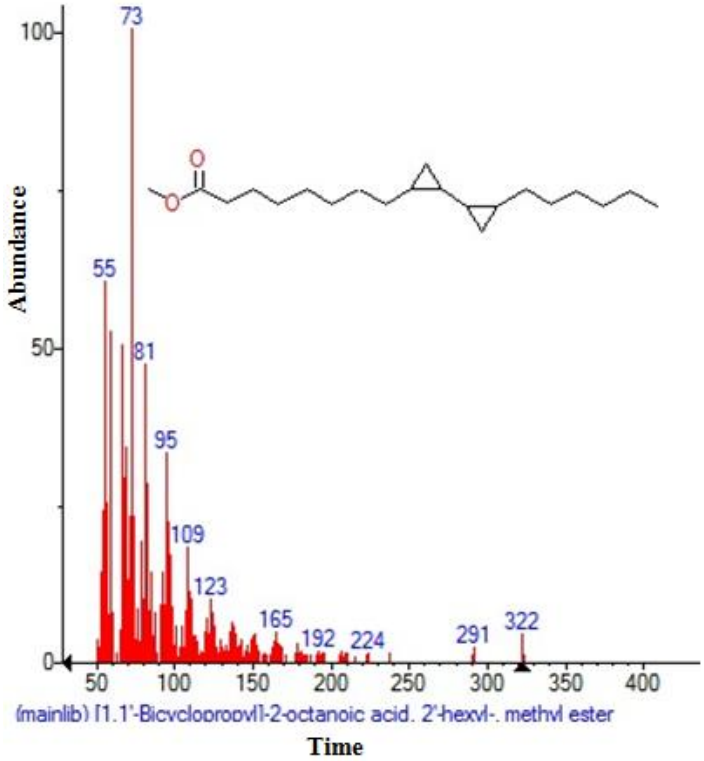


Figure 12. Mass spectrum of [1,1-Bicyclopropyl-2-octanoic acid 2hexyl-methyl ester with retention time (RT)= 13.501.

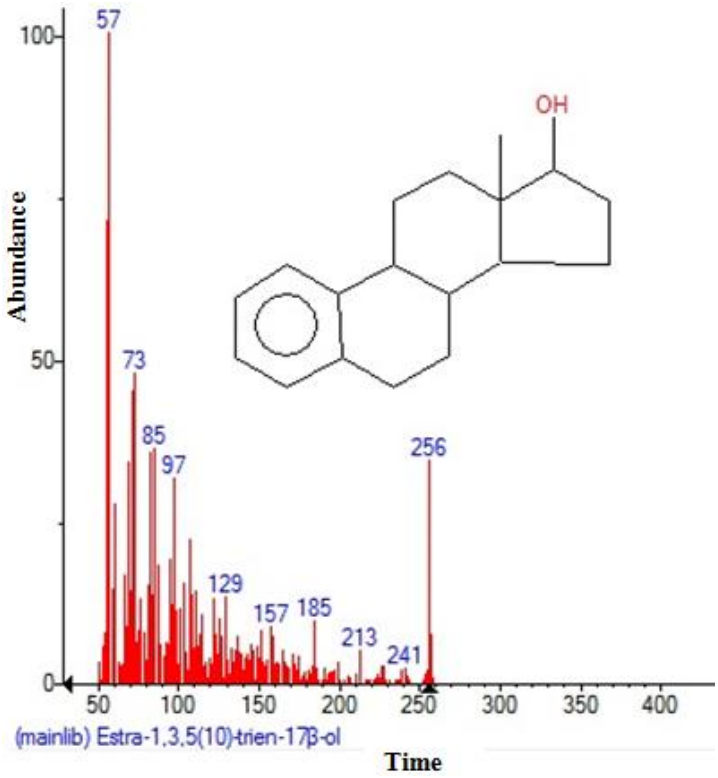


Figure 13. Mass spectrum of Estra-1,3,5(10)-trien-17B-ol with retention time (RT)= 15.561.

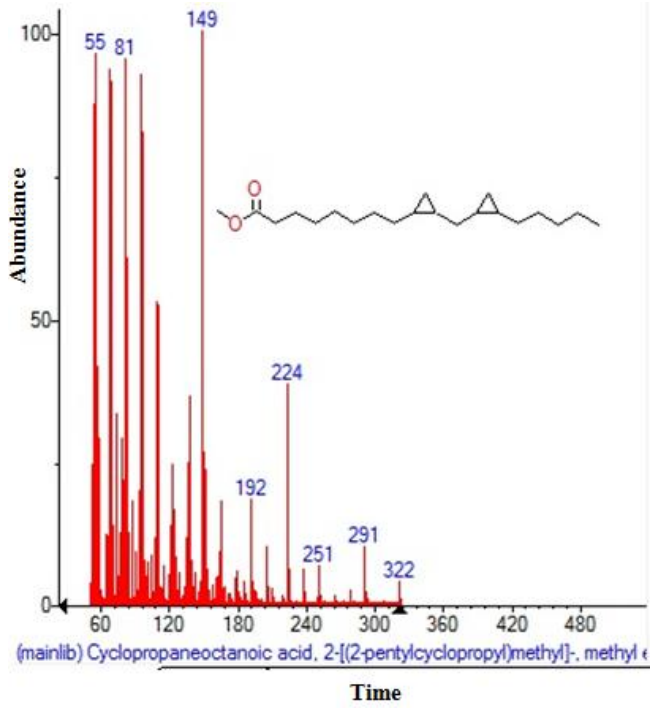


Figure 14. Mass spectrum of cyclopropaneoctanoic acid, 2-[2-pentylcycloproyl)methyl]-methyl with retention time (RT)= 20.327.

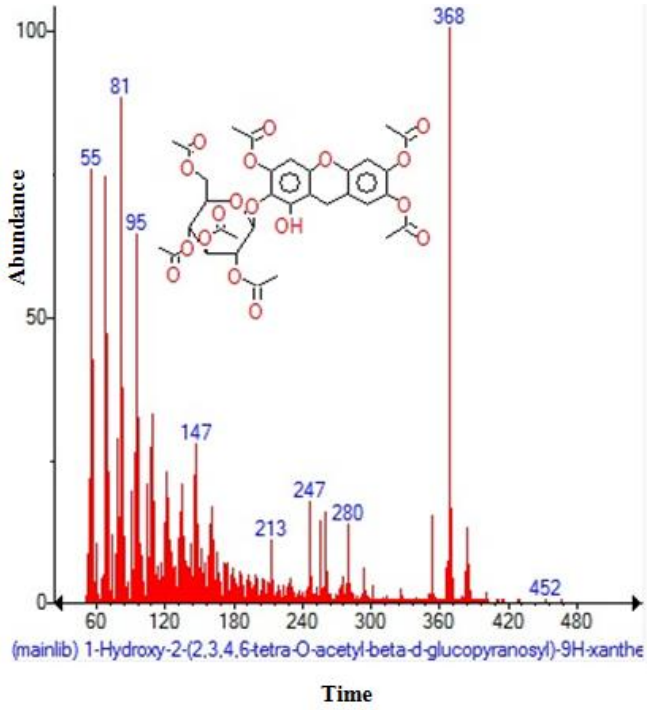


Figure 15. Mass spectrum of 1-Hydroxy-2-(2,3,4,6-tetra-O-acetyl-beta-d-glucopyranosyl)-9H-xanthe with retention time (RT)= 20.585.

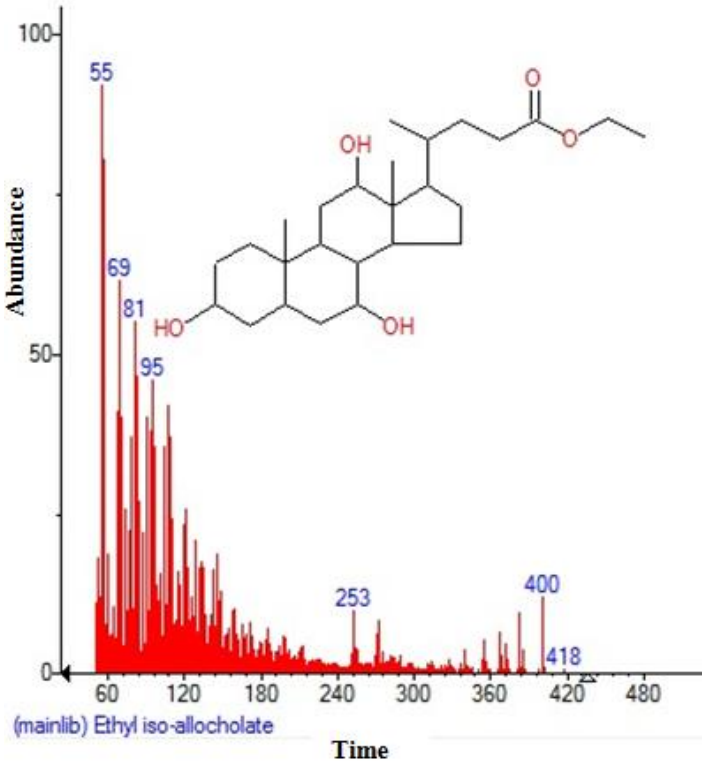


Figure 16. Mass spectrum of Ethyl iso- allochlate with retention time (RT)= 25.277.

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

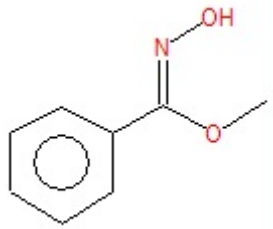
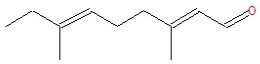
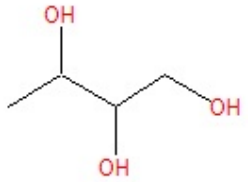
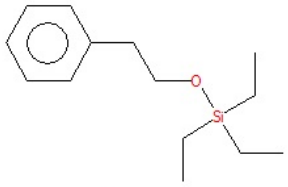
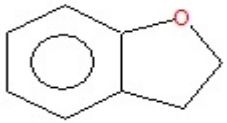
S/N	Phytochemical compound	RT(min)	Formula	Molecular weight	Exact mass	Chemical structure	MS Fragment- ions	Pharmacological actions
1.	Oxime- methoxy-phenyl	3.504	$C_8H_9NO_2$	151	151.063329		55,68,73,81,91,105,121,133,151	Antioxidant and antimicrobial activity
2.	2, 6,-Nonadienal, 3, 7-dimethyl	3.739	$C_{11}H_{18}O$	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.

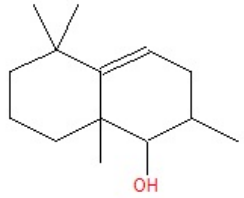
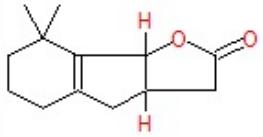
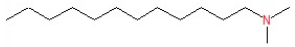
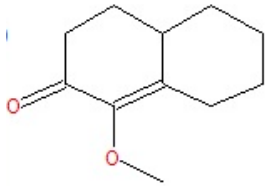
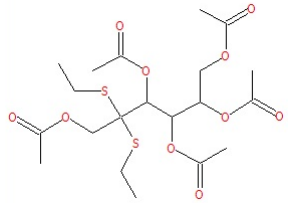
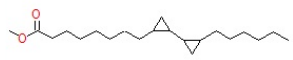
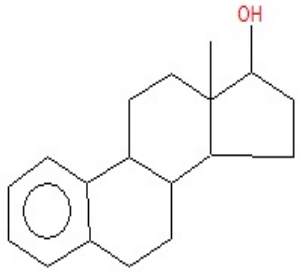
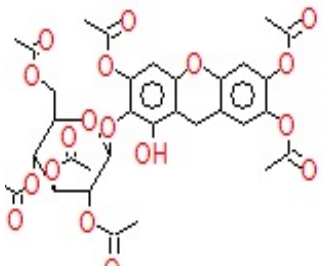
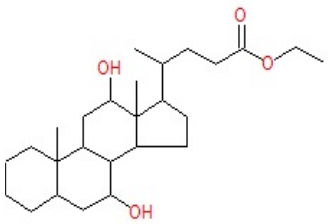
6.	2,5,5,8a-Tetramethyl-1,2,3,5,6,7,8, 8a-octahydronaphthalen-1-ol	7.939	$C_{14}H_{44}O$	208	208.182715		55,94,135,150,175,208	Pharmacological action of this product is unknown
7.	2H-Indeno[1,2-b]furan-2-one, 3,3a, 4,5,6,7,8, 8b-octahydro-8,8-dimet	8.992	$C_{13}H_{18}O_2$	206	206.13068		55,67,79,91,107,119,131,145,173,191,106	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

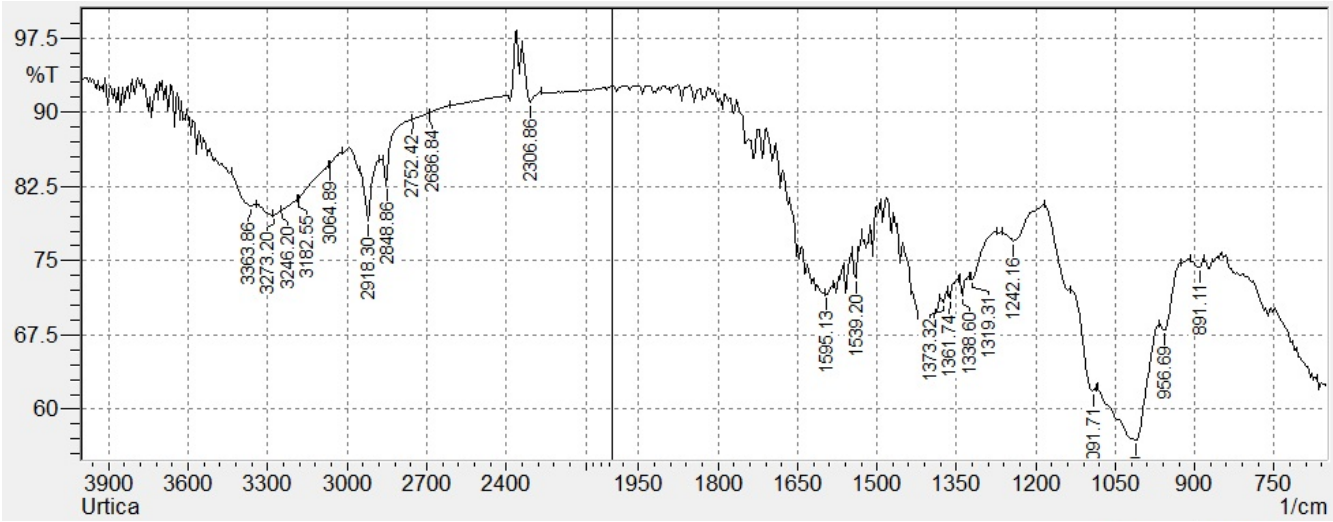


Figure 17. Fourier-transform infrared profile of leaves extract of *Urtica dioica*.

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.

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Full Length Research Paper

Hepatoprotective effects of the decoction and macerated leaves of *Rhamnus alaternus* L. on rats exposed to carbon tetrachloride

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The plant of *Ramnus alaternus* L. (Rhamnaceae) has been object of many therapeutic indications for traditional medicine. Many studies have showed a hepatoprotective activity of *R. alaternus* L. The objective of this study was to compare the hepatoprotective activities of decoction and macerated *R. Alaternus* L. leaves extracts on rats initially exposed to carbon tetrachloride CCl_4 (1 ml/kg). The macerated leaves (250 mg/kg body weight) showed a highly significant hepatoprotective activity ($p < 0.01$) expressed by a significant decrease in enzymatic biochemical markers such as total bilirubin, alkaline phosphatase (ALP) and transaminases (GOT and GPT). Mean serum biochemical marker levels were 4.5 ± 0.9 mg/dL, 143 ± 3.7, 43.5 ± 9.2 and 32.2 ± 5.1 U/L, respectively. These results illustrate the dominance of the hepatoprotective pharmacological activity of macerated *R. alaternus* L. leaves.

Key words: Rhamnaceae, traditional medicine, hepatoprotective activity, biochemical markers, alcaline phosphatase.

INTRODUCTION

Liver diseases have a multifactorial etiology including infectious factor. High mortality rate has been correlated with two types of liver diseases namely, jaundice and hepatitis. (Pang et al., 1992). Hepatitis, has been associated with the drinking of contaminated water and poor hygiene (WHO, 2005). Jaundice which is common in children has been characterized by yellow skin because of an excess of bilirubin (Sourabie et al., 2012). Hepatoprotective effects of drugs and plant extracts have been studied by using carbon tetrachloride (CCl_4) induced hepatotoxicity in animal models (Suja et al., 2002). Studies have shown toxicity of CCl_4 which leads to free radicals in tissues such as liver, kidney, heart, lung, testis, brain and blood (Kumar et al., 2005; Khan and Ahmed, 2009). Research in herbal medicine has been an alternative therapy for liver disease (Pramyothin et al., 2005). Plant drugs have been known for their role in the management of hepatic diseases.

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Figure 1. The shrub of *Rhamnus alaternus* L.
Source: Rameau et al. (2008).

Medicinal plants have been an important source of bioactive molecules, used in the food industry, cosmetics and pharmacy.

These molecules include coumarins, alkaloids, phenolic acids, tannins, flavonoids and terpenes (Bahorun et al., 1996). Polyphenols have therapeutic virtues, mainly against cancer and cardiovascular disease. These molecules are involved in the protection of plants against microbial attacks (Bruneton, 1999). Different medicinal plants species such as *Tinospora bakis*, *Cochlospermum tinctoria*, *Nauclea latifolia* and *Argemone mexicana*, have been used in traditional medicine for the therapy of jaundice (Sourabie et al., 2012). Through a rich literature on the phototherapy, it has been found that *Rhamnus alaternus* L. known by populations of different areas of Algeria for its therapeutic activities against jaundice (Figure 1). Important works on the chemistry of *R. alaternus* L. have been carried out and significant pharmacological properties have been reported by studies in Algeria and Tunisia (Ben Ammar et al., 2008; Chemli et al., 2006). Therapeutic properties of this species have been demonstrated *in vitro* because of their active compounds (Ben Ammar et al., 2008). The present study aimed to compare the hepatoprotective effects of the decoction and macerated leaves of *R. alaternus* L. on rats exposed to CCl_4.

MATERIALS AND METHODS

Description of plant

Leaves of *R. alaternus* have been collected during the month of October, 2013 in rural areas of Mohammedia, located in North-West of Algeria (Figure 2). *R. alatenus* L. is a sun-loving plant, growing in Mediterranean areas. It is a shrub of 5 m high with non spiny leaves and fruits (Chemli et al., 2006; Rameau et al., 2008). The leaves are oval or lance-shaped with slightly toothed edges, buckthorn, short-stalked, thick and leathery. The fruits appear in late fall. There are small red and black berries, grouped in compact clusters. Identification of the plant was made by M. Terras, researcher and team member of Laboratory of water sources and environment Biology Department at the University of Saida (Algeria). Dried leaves have been transformed into powder using an electric mill (Moulinex model D5001). Powders have been used to prepare aqueous decoction and macerated extract of the leaves.

Preparation of extract

To prepare an aqueous decoction, two hundred fifty grams (250 g) of powder leaves of *R. alaternus* have been used in a flask containing 1000 ml of distilled water. Decoction has been maintained under continuous reflux for 2 h at 80°C (Belhattab et al.,

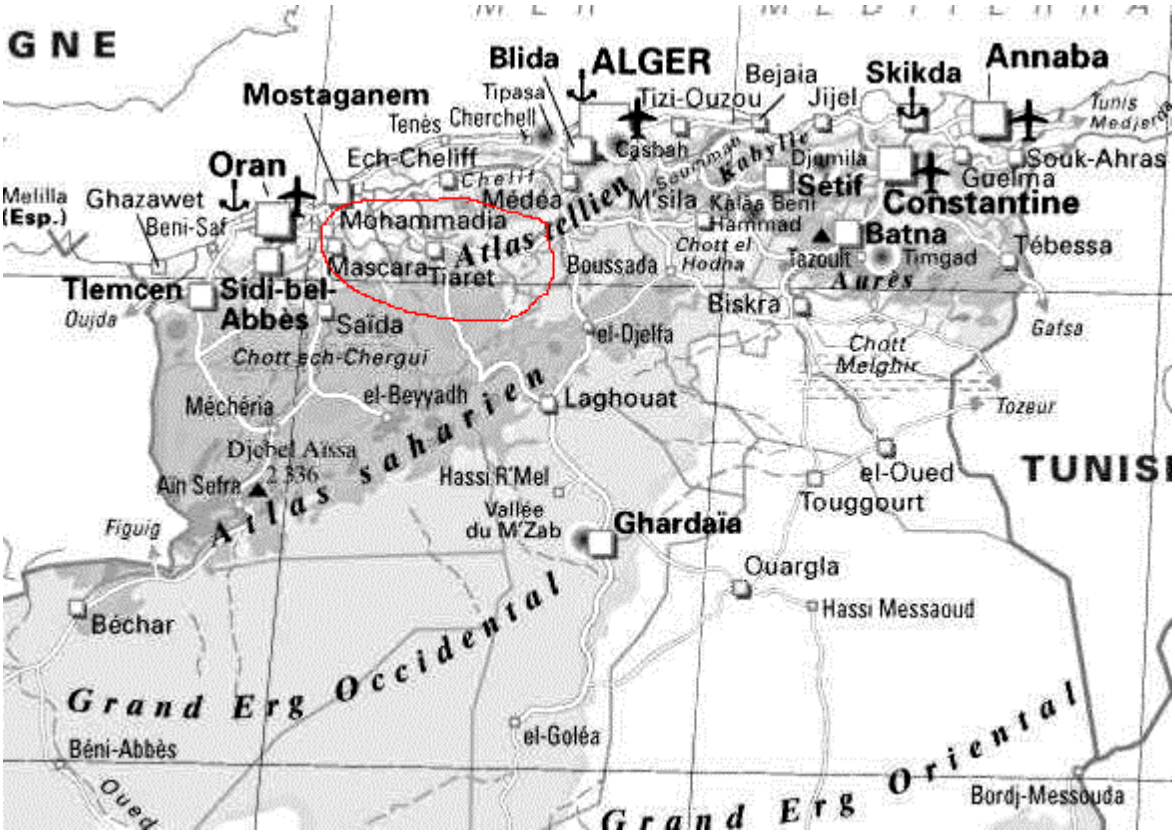


Figure 2. Geographical map of Mohammedia, located in North- West of Algeria. (Diagram tourism development of the department of Mascara, 2015).

2004). Decoction has been filtered through a funnel containing cotton wool and then centrifuged at 2500 rpm for 5 min. Aqueous macerated extract of the leaves was obtained in the same conditions as the decoction with the only difference that it has made macerated extract (cold extraction) without any heating process of the test portion (250 g).

Preparation of animals

Forty (40) male adult Wistar rats, 2 months old and weighing about (180 to 200 g) from the central animal house of the Department of Biology of Saida University, were used in this study. They were kept under standard environmental conditions at 25°C with 12:12 h light-dark cycle in ventilated plastic cages. The rats were fed with standard feed livestock and water ad libitum. The animals were divided into 4 groups (10 rats per group) as follow:

Group 1: served as normal controls (NC) and rats received a tap water daily for 7 days orally.
Group 2: served as toxic control and the rats were injected intraperitoneal with carbon tetrachloride (CCl_4) (1 ml/kg body weight) daily for the same period (7 days).
Group 3: (CCl_4 + decoction of *R. alaternus* leaves): the rats were injected intraperitoneal with CCl_4 (1 ml/kg body weight) and treated orally with the decoction of *R. alaternus* leaves (250 mg/kg body weight) daily for the same period.
Group 4: (CCl_4 + macerated *R. alaternus* leaves): the rats were injected intraperitoneal with CCl_4 (1 ml/kg body weight) and treated orally with the macerated *R. alaternus* leaves (250 mg/kg body weight) daily for the same period (Sourabie et al., 2012).

Biochemical study

At the end of the experiments, all animals were sacrificed and the blood from each animal was taken into haemolysis tubes (5 mL). The blood samples were centrifuged at 2500 rpm for 10 to 15 min and the sera isolated were used for estimation of the serum biochemical markers of liver; as serum glutamic oxaloacetic transaminase (GOT), serum glutamic pyruvic transaminase (GPT), alkaline phosphatase (ALP) and total bilirubin.

Histopathology

The animals were dissected to isolate liver tissues. After draining the blood, liver samples were excised, washed with normal saline and processed separately for histological observations. Initially, the materials were fixed in 10% buffered neutral formalin for 48 h and then with bovine solution for 6 h. Paraffin sections were taken at 5 mm thickness, processed in alcohol-xylene series and were stained

Table 1. Effects of decoction and macerated *Rhamnus alatenus* leaves on serum hepatic parameters in groups of control rats and exposed to CCl_4.

Groups of rats (n = 40)	Total bilirubin (mg/dL)	ALP (U/L)	SGOT (U/L)	SGPT (U/L)
Group 1 (NC)				
Mean ± SD	1.49 ± 0.03	130.25 ± 4.4	26.5 ± 4	26 ± 4.6
Min-Max	1.41 - 1.56	120 - 139	17 - 36	19 - 39
Mean CI (95%)	1.47 - 1.50	128.09 - 132.4	24.53 - 28.46	17 - 28.2
Group 2 (CCl_4)				
Mean ± SD	**11.0 ± 0.94	**185.5 ± 5.95	**88.25 ± 6.96	**69.25 ± 2.92
Min-Max	9.88 - 13.74	169 - 197	69 - 99	66 - 78
Mean CI (95%)	10.53 - 11.46	182.58 - 188.41	84.83 - 91.66	67.81 - 70.68
Group 3 (CCl_4 + D)				
Mean ± SD	10.00 ± 1.07	161.5 ± 8.7	49 ± 1.08	61.5 ± 3.77
Min - Max	7.13 - 12.04	145 - 186	46 - 51	55 - 69
Mean CI (95%)	9.47 - 10.52	157.23 - 165.76	48 - 57	59.65 - 63.34
Group 4 (CCl_4 + M)				
Mean ± SD	4.51 ± 0.45	135.25 ± 0.85	30.75 ± 3.54	34.5 ± 2.21
Min - Max	3.33 - 5.33	133 - 137	22 - 37	29 - 39
Mean CI (95%)	4.28 ± 4.73	134.83 - 135.66	29.01 - 32.48	33.47 - 35.58

NC: Normal control; D: Decoction, M: Maceration; SD: Standard deviation; Min: Minimum; Max: Maximum; CI: Confidence interval; ALP: Alkaline phosphatase; GOT: Glutamic oxaloacetic transaminase; GPT: Glutamic pyruvic transaminase; P**: Highly significant (< 0.001).

with alum haematoxylin and eosin (Galighter et Koyloff, 1971). The sections were examined microscopically for histopathological changes.

Statistical analysis

Data are expressed as mean ± SD, with a value of $p < 0.05$ considered statistically significant. Statistical evaluation was performed by one way analysis of variance (ANOVA) followed by the Tukey's t-test for multiple comparisons. All analysis was made with the statistical software Sigmaplot (version 11.0).

RESULTS

As shown in Table 1 and Figure 3, body weight of rats exposed to CCl_4 (Group 2) was highly decreased significantly ($p^{**} < 0.01$) compared with normal control rats (Group 1). The median body weight in group of rats administered CCl_4 was lower (174.57 ± 5.2 g) compared to normal control rats (Group1) which showed a higher mean body weight (224.28 ± 6.6 g). Whereas the body weight of rats (Groups 3 and 4) has not showed a significant difference. Their mean body weights were 179.85 ± 1.68 and 196.28 ± 1.63 g, respectively.

The administration of carbon tetrachloride CCl_4 (1 ml/kg body weight) developed a significant increase in serum enzymatic markers ($p^{**} < 0.01$), especially serum transaminase (GOT and GPT) and alkaline phosphatase (ALP). Similarly, it was observed concomitantly, a significant increase in serum total bilirubin ($p^{**} < 0.01$) (Table 1). This phenomenon was particularly observed in rats of Group 2 which were administrated CCl_4, in comparison with the normal control rats of Group 1 as shown in Figures 4, 5, 6, 7.

The aqueous extracts (decoction and macerated) administered at the same dose (250 mg/kg) developed a significant decrease in serum enzymes (GOT and GPT) ($p^{**} < 0.01$) and a decrease in the serum total bilirubin concentration as compared to animals which were administered CCl_4 (Group 2) (Figures 4, 5, 6, 7). The effect of different treatments (decoction and macerated) on serum enzyme markers and serum total bilirubin is reported in Figures 4, 5, 6, 7. From the perspective of pharmacological activity, the decrease of biological parameters observed in the test Groups 3 and 4 is a clear sign and an illustration of the ability of these extracts to reduce the hepatotoxicity induced by the administration of toxic organochlorine (CCl_4, 1 ml/ kg B.W). Histopathologies of various groups are shown in Figure 8. In Group 1 (normal control rats), liver tissue section

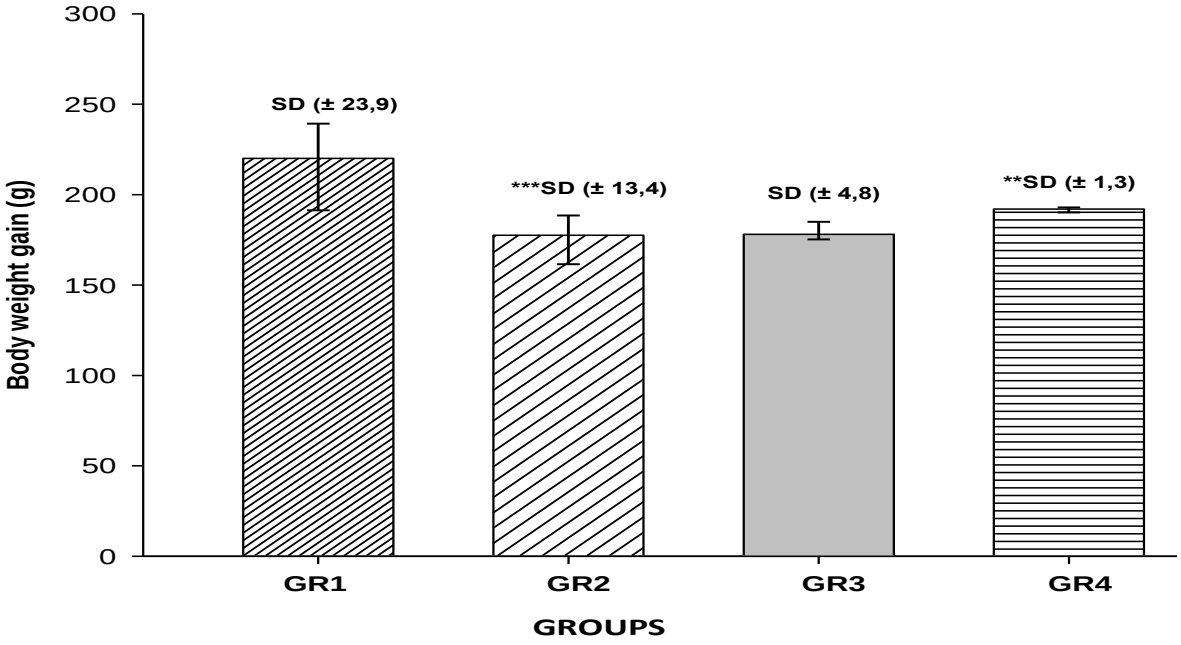


Figure 3. Variation of body weight in groups of animals. GR1 (NC): Group 1 of normal control rats; GR2 ($CC1_4$): Group 2 of treated rats with CCl_4; GR3 (CCl_4-D): Group 3 of treated rats with CCl_4 and decoction *Rhamnus alaternus* leaves; GR4 (CCl_4-M): Group 4 of treated rats with CCl_4 and macerated *Rhamnus alaternus* leaves; SD: standard deviation; ** ($p < 0.01$): statistically significant difference between the groups of animals.

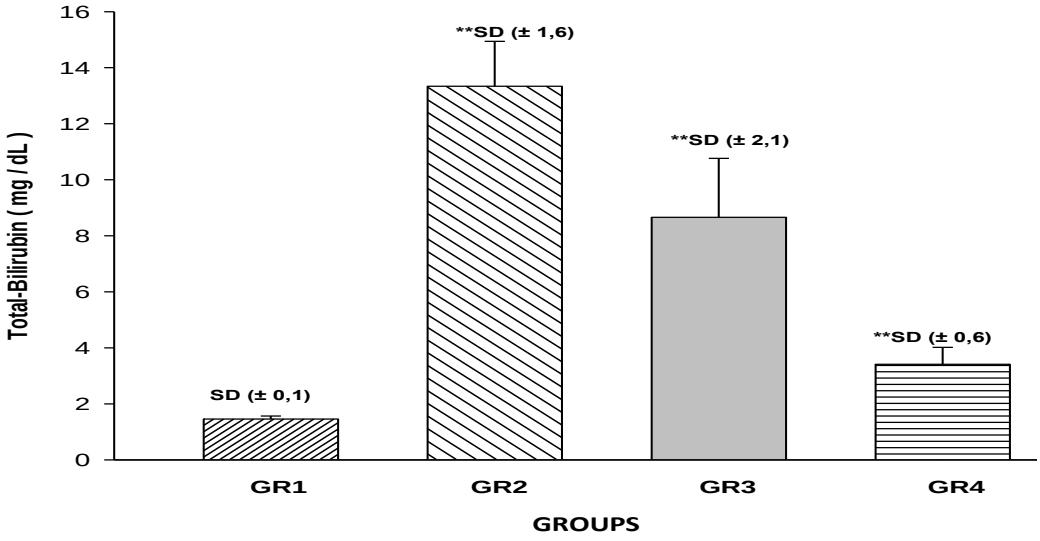


Figure 4. Effects of decoction and macerated *Rhamnus alatenus* leaves on serum total-bilirubine in groups of animals exposed to CCl_4.

(Figure 8A) shows a normal architecture of liver cells. Hepatocyte, hepatic sinusoids, portal tract, shows normal size and Shape. The nuclei are round and are uniform with little variation in size.

In Group 2 (Rats treated with CCl_4), liver tissue section (Figure 8B) shows extreme degeneration of hepatic

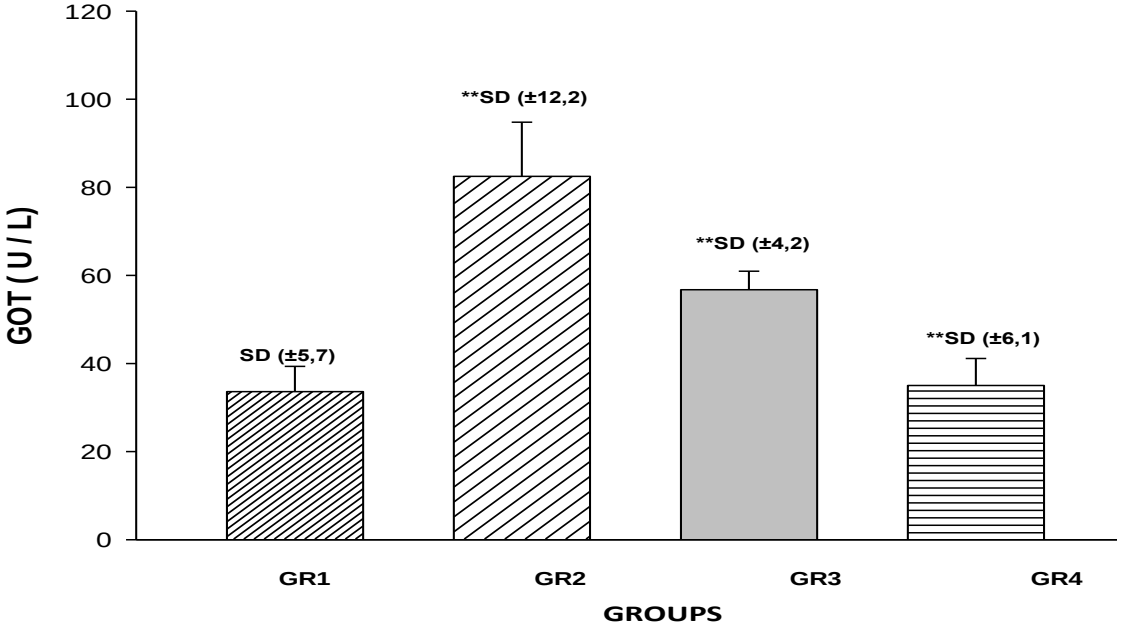


Figure 5. Effects of decoction and macerated *Rhamnus alatenus* leaves on serum glutamic oxaloacetic transaminase (GOT) in groups of animals exposed to CCl_4.

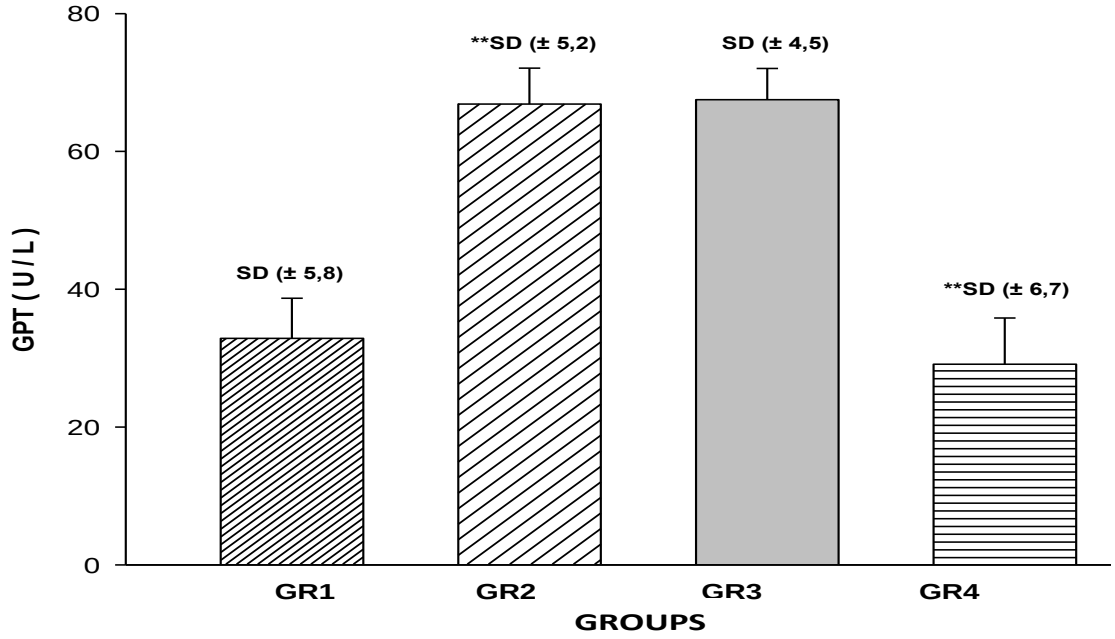


Figure 6. Effects of decoction and macerated *Rhamnus alatenus* leaves on serum glutamic pyruvic transaminase (GPT) in groups of animals exposed to CCl_4.

architecture by necrosis, foci of haemorrhage, fatty changes and vein crowding. Hepatocyte are arranged like disks and shows a typical proliferation. In Group 3 (Rats treated with CCl_4 and decoction of *R. alaternus* leaves),

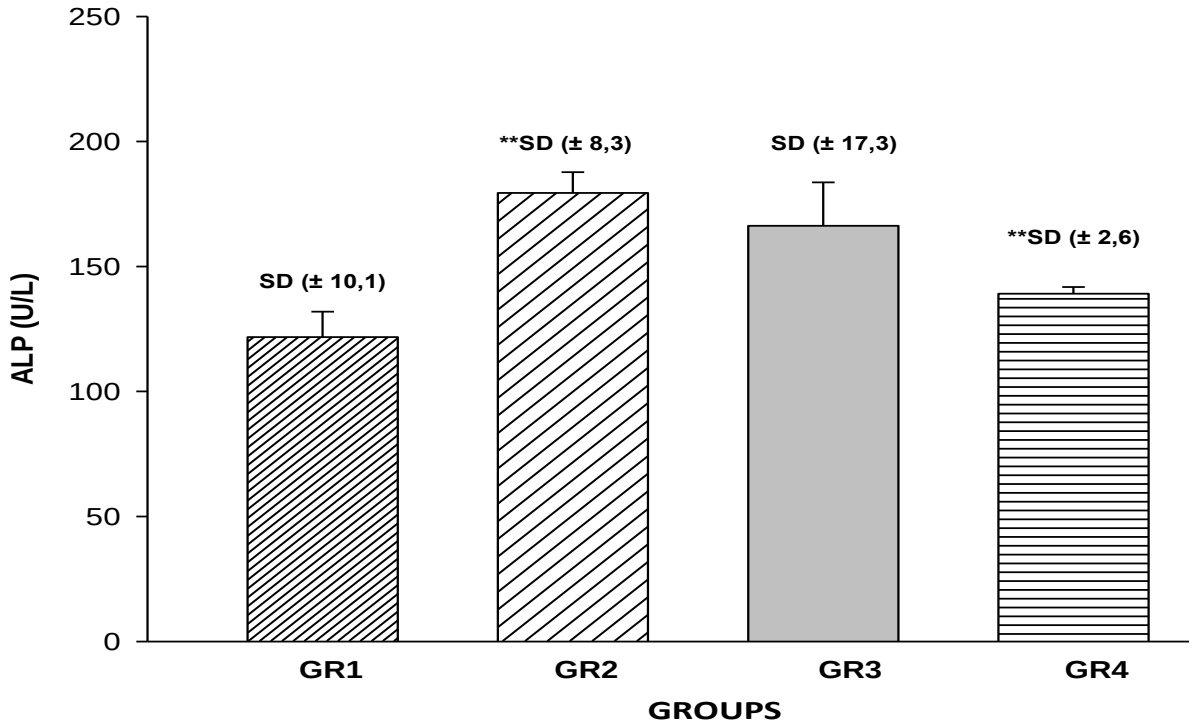


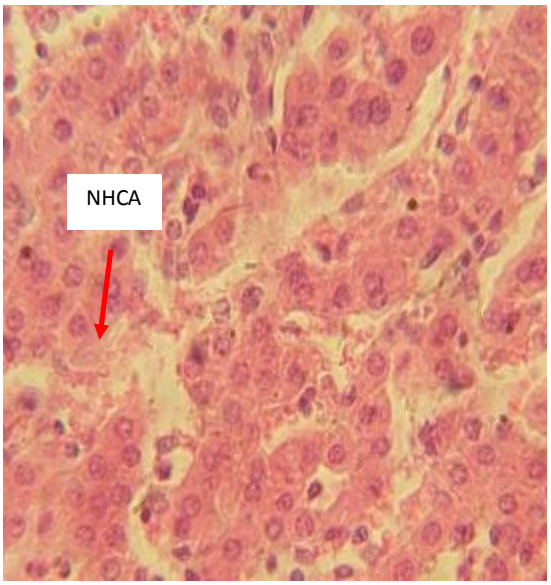
Figure 7. Effects of decoction and macerated *R. alatenus* leaves on serum alkaline phosphatase (ALP) in groups of animals exposed to CCl_4.

liver tissue section (Figure 8C) shows mild degree of liver necrosis. Hepatocytes are compact. Hepatic sinusoids appear normal. The Hepatocytes are well arranged like clusters. In Group 4 (rats treated with CCl_4 and the macerated *R. alaternus* leaves), liver tissue section (Figure 8D) shows that hepatocytes were regenerative and showed no visible changes and prominent nuclei, reduced score of necrosis and no fatty changes. Thus, confirming the safety of the extract.

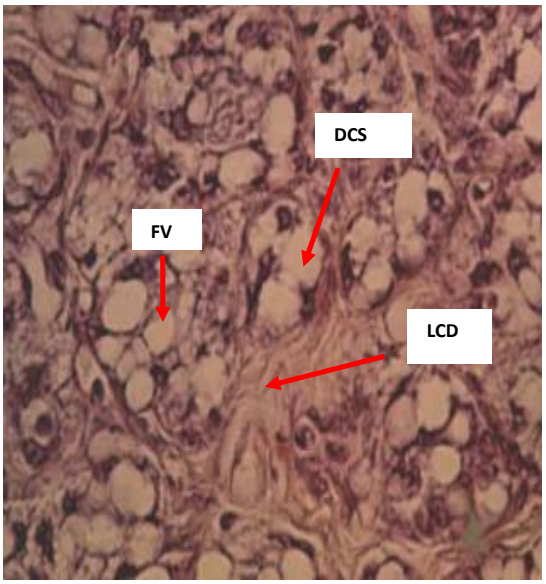
DISCUSSION

CCl_4 has been used in this present study for its liver toxicity and tropism for hepatocytes. Cell mechanism of CCl_4, has been previously described in different studies (Sarada et al., 2012; Letteron et al., 1990), showing alteration and necrosis of liver cells. Hepatic metabolism of CCl_4 has been initiated by transforming it into its primary metabolites (trichloromethyl and trichlorométhyl peroxyde) through the hepatic cytochrome P450 oxidase and main enzymatic system involved into redox reactions of xenobiotics in the liver. Trichloromethyl peroxide is highly reactive free radicals that will initiate a lipid peroxidation (Sourabie et al., 2012). Free radicals have caused an important flow of GOT and GPT from hepatocyte membranes into blood medium (Sourabie et al., 2012). Results of this present study showed that a dose of CCl_4 (250 ml/kg) have caused severe hepatocellular injury as indicated by the massive elevations of GPT, GOT, ALP and total bilirubin levels in rats intoxicated with CCl_4 (Group 2) when compared with normal control animals (Group 1).

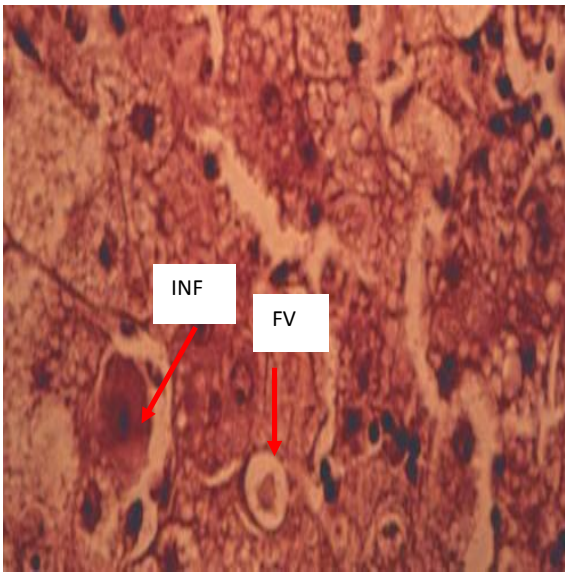
Treated rats (Groups 3, 4) have preserved the integrity of liver cells and significantly decreased elevated enzymatic parameters (GOT, GPT and ALP) and total bilirubin levels. Dose (250 mg/kg) of aqueous extract *R. alaternus* leaves has been used in the present study based on some published studies demonstrating the hepatoprotective activity of this dose (Gopal et al., 2008). In this present study, decoction and specially macerated leaves treated groups (Groups 3 and 4) have exhibited lower levels of GPT, GOT, ALP and bilirubin as compared to CCl_4 treated group (Group 1). Reduction of these parameters is an indication of the stabilization of cell membranes as well as repair of hepatic tissue damage caused by CCl_4 (Lin et al., 2008). The stabilization of serum bilirubin, GPT, GOT, and ALP levels by decoction and macerated plant leaves is a clear indication of the improvement of the functional status of



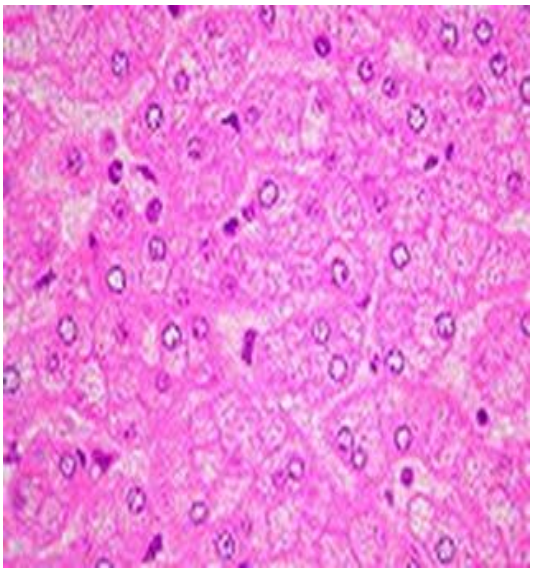
(A): Photographic image of liver tissue shows a normal hepatic cellular arrangements (NHCA) in Group 1 of normal control rats. (Hematoxyline-Eosine × 40).



(B) : In Group 2 (CCl4). Showing liver cell degeneration (LCD), with fatty vacuols (FV) and degenerative cellular swelling (DCS) (H-E × 40).



(C) : In Group 3 (CCl4 + decoction leaves). Less cellular necrosis associated with inflammation (INF) and low presence of fatty vacuoles (FV) (H-E × 40).



(D) : In Group 4 (CCl4 + macerated leaves). Absence of fatty vacuoles and inflammation and normal cell arrangements (H-E × 40).

Figure 8. Histopathologies of various groups.

the liver cells. This indicates the anti-lipid peroxidation of aqueous extract of *R. alaternus* leaves which acted against the damaging effects of free radicals produced by CCl_4.

Histopathological examination clearly reveals that the hepatic cells, central vein and portal triad are almost

normal in decoction and macerated *R. alaternus* leaves treated group (250 mg/kg) in contrast to group which received CCl_4. Thus, decoction and macerated *R. alaternus* leaves can be considered to be an effective hepatoprotective agent as it ameliorated almost to normalcy the damage caused by CCl_4 to hepatic function. In literature, it has been suggested that administration of extract of *R. alaternus* increased enzymatic parameters (GOT, GPT and ALP) and total bilirubin level and it may be due to the presence of active constituents such as flavonoids and alkaloids (Ben Ammar et al., 2009). Studies have shown that phenolic compounds had an antioxidant activity as scavenging free radicals (Seyoum et al., 2006).

Phenolic compounds can also act as antioxidants by chelating metal ions, preventing radical formation and improving the antioxidant endogenous system (Al-Azzawie et al., 2006). These compounds are known to act as antioxidants not only because of their ability to donate hydrogen or electrons but also because they are stable radical intermediates. Probably the most important natural phenolics are flavonoids because of their broad spectrum of chemical and biological activities, including antioxidant and free radical scavenging properties (Kahkonen et al., 1999). In fact, flavonoids have been reported as antioxidants, scavengers of a wide range of reactive oxygen species and inhibitors of lipid peroxidation (Williams et al., 2004).

CONCLUSION

Both decoction and macerated *R. alaternus* L. leaves have exhibited a potent hepato-ameliorating and antioxidant effects in rats exposed to CCl_4. But hepato-ameliorating and antioxidant effects of macerated *R. alaternus* L. leaves were found to be better than those of decoction plant leaves. In two cases, the hepatoprorective qualities of aqueous extract of *R. alaternus* L. leaves need to be addressed by isolating and characterizing the active principle(s) responsible for hepatoprotective activity.

ABBREVIATIONS

(NC): Normal control; **(GOT):** Glutamic oxaloacetic transaminase; **(GPT):** Glutamic pyruvic transaminase; (**ALP):** Alkaline phosphatase; **(SD):** Standard deviation; **(ANOVA):** analysis of variance.

Authors' contributions

All authors read and approved the final manuscript.

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Conflicts of interest

The authors declare that they have no competing interests.

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