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

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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).

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

**INTRODUCTION**

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, 2,5,6,8a-Tetramethyl-1,2,3,4,5,7,8, 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 mercapta, pentacetate, [1,1-Bicyclopropyl-2-octanoic acid 2hexyl-methyl ester, Estra-1,3,5(10)-trien-17B-oI, Cyclopropaneoctanoic acid, 2-[2-pentylcyclopropyl]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, carboxic 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.

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; Gobes et al., 2004; Gobes 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 whenever a compound was detected (Mohammed and Imad, 2013; Hameed et al., 2005; Kanter et al., 2005; Hameed et al., 2015c).

The GC column employed here for the separation of components was Elite 1 (100% dimethyl poly siloxane) (Imad et al., 2014). 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-Nonadienone, 3, 7-dimethyl (Figure 3). The next peaks considered to be 1, 2, 3-Butanetriol, Silane, triethyl(2-phenylethoxy), Benzo furan, 2,3-dihydro, 2,5,5,8a-Tetramethyl-1,2,3,5,6,7,8, 8a-octahydropentaphenanthro-1-ol, 2H-Indeno[1,2-b]furan-2-one, 3,3a, 4, 5,6,7, 8b-octahydro-8,8-dimethyl, 1-Dodecanamine, N, N-dimethyl, 2(3H)-Naphthalenone, 4, 4a,5,6,7,8-hexahydro-1-methoxy, D-Fructose, diethyl mercaptal, pentacetaete, [1,1-Bicyclopropyl-2-octanoic acid 2hexyl-methyl ester, Estra 1,3,5(10)-trien-17B-ol, Cyclopropanoacetic acid, 2-[2-pentylcyclopropyl]methyl]-methyl, 1-Hydroxy-2-(2,3,4,6-tetra-O-acetyl-beta-d-glucopyranosyl)-9H-xanthe and Ethyl iso-alloclolate. (Figure 4-16).

The FTIR analysis of *U. dioica* leaves proved the presence of aromatic rings, alkenes, aliphatic fluoro, alcohols, ethers, carboxic acids, esters, nitro compounds, hydrogen bonded alcohols and phenols which shows major peaks at 891.11, 589.69, 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 (+)-neooolivil, (-)-secoisolariciresinol, Dehydrodiconiferyl alcohol, isolariciresinol, pinosinol, and 3,4divanillyltetrahydrofuran, and has anti-inflammatory 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-octahyronaphthalen-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-17β-ol with retention time (RT)= 15.561.

Figure 14. Mass spectrum of cyclopropanoctic acid, 2-[2-pentylcyclopropyl]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   | C9H8NO2     | 151              | 151.063329       | ![Chemical structure](image1.png)     | 55,68,73,81,91,105,121,133,151          | Antioxidant and antimicrobial activity    |
| 2.  | 2, 6, -Nonadienal, 3, 7-dimethyl    | 3.739   | C11H18O2    | 166              | 166.13576        | ![Chemical structure](image2.png)     | 55,67,83,94,109,137,151                 | Anti-inflammatory and antioxidant activity|
| 3.  | 1, 2, 3-Butanetriol                 | 4.380   | C9H18O3     | 106              | 106.062994       | ![Chemical structure](image3.png)     | 57,75,88,103                          | Wide range of biological properties including antitumor activity |
| 4.  | Silane, triethyl(2-phenylethoxy)    | 4.975   | C16H26OS    | 236              | 236.159642       | ![Chemical structure](image4.png)     | 59,66,75,91,105,117,125,161,17,9,193   | Biocontrol                               |
| 5.  | Benzofuran, 2,3-dihydro             | 6.777   | C10H9O2     | 120              | 120.057514       | ![Chemical structure](image5.png)     | 51,63,77,91,105,120                    | Antiarrhythmic, spasmodilic, antiviral    |
Table 1. Condt.

|  | Compound Description                                                                 | C, H, O, N | Molecular Weight | Mass Spectrum | Pharmacological Action                                                                 |
|---|--------------------------------------------------------------------------------------|------------|-----------------|---------------|--------------------------------------------------------------------------------------------|
| 6. | 2,5,5,8a-Tetramethyl-1,2,3,5,6,7,8, 8a-octahydronaphthalen-1-ol                        | C_{20}H_{22}O | 208             | 208.182715    | 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-dimethyl              | C_{18}H_{18}O | 206             | 206.13068     | New chemical compound                                                                       |
| 8. | 1-Dodecanamine, N, N-dimethyl                                                        | C_{36}H_{78}N | 213             | 213.24565     | Anti-Staphylococcal Activity                                                               |
| 9. | 2(3H)-Naphthalene-4, 4a,5,6,7,8-hexahydro-1-methoxy                                  | C_{16}H_{16}O_2 | 180             | 180.115029    | Pharmacological action of this product is unknown                                            |
| 10. | D-Fructose, diethyl mercaptal, pentaacetate                                          | C_{20}H_{32}O_10S_2 | 496             | 496.14369     | Antitumor and antibacterial activity                                                        |
| 11. | [1,1-Bicyclopentyl-2-octanoic acid 2hexyl-methyl ester                                | C_{26}H_{50}O_3 | 322             | 322.28718     | Anti-diabetic and anti-inflammatory and 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 1. Condt.

| No. | Name                                             | MW | log P | Formula | Anti-proliferative effect |
|-----|--------------------------------------------------|-----|-------|---------|---------------------------|
| 12. | Estra-1,3,5(10)-trien-17B-ol                     | 256 | 256.18271 | C_{21}H_{24}O | 57,73,85,97,129,157,185,213,24,1256 |
| 13. | Cyclopropaneoctanoic acid, 2-[2-pentylcycloproyl](methyl)-methyl | 322 | 322.28718 | C_{20}H_{34}O | 55,81,149,192,224,251,291,322 |
| 14. | 1-Hydroxy-2-(2,3,4,6-tetra-O-acetyl-beta-d-glucopyranosyl)-9H-xanthe | 718 | 718.174515 | C_{25}H_{44}O_{24} | 55,81,95,147,213,247,280,368,4,52 |
| 15. | Ethyl iso-alloclate                               | 436 | 436.31887 | C_{12}H_{22}O_{2} | 55,69,81,95,253,400 |

**Antimicrobial, Antioxidant, Anti-inflammatory**
Table 2. FT-IR peak values of *Urtica dioica* methanol leaf extract.

| S/N | Peak (Wave number cm⁻¹) | 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.

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Conflicts of interest
The authors have none to declare.

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