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Determination Of Bioactive Molecules And Antioxidant Activity In Stinging Nettle (Urtica Dioica)

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***Abstract*—Medicinal plants are considered an important source for the production of new drugs due to their richness in bioactive compounds. Urtica dioica is one of those medicinal plants that are widely distributed throughout the world, but unfortunately, this plant is under-evaluated especially in the Middle East region. In this study, three different bioactive molecules were identified in the hexane extract of Urtica by GC-MS and IR spectroscopy analysis. Ethanolic and aqueous extracts of Urtica dioica showed a high antioxidant activity, as revealed by diphenyl picryl hydrazyl (DPPH) and H_2O_2 tests, thus raising the therapeutic importance of these bioactive compounds. Moreover, the antioxidant activity was found to be fairly correlated with the extracts concentration.**

Keywords—medicinal plant, Urtica dioica, bioactive compounds, GC-MS, antioxidant activity, DPPH, H_2O_2.

I. Introduction

Plants are a natural source of biologically active compounds such as carbohydrates, proteins, oils, minerals, vitamins, alkaloids, quinones, terpenoids, and polyphenols...(1). These compounds are grouped into two classes: primary metabolites which are essential for the growth and the development of the plant and secondary metabolites which play a key role in the adaptation of the plant to its environment (2). In addition to their importance to the plant, these compounds are currently used for commercial purposes in medicine, cosmetics applications or as food products (3).Stinging nettle (*Urtica dioica*) is a herbaceous and perennial plant, distributed throughout the world (4). It has a great medicinal potential with a rich history of use in folk medicine to treat arthritis and rheumatism since it provides temporary relief of pain (5). Several compounds occurring in the nettle were previously shown to exhibit antioxidant, antimicrobial, antiulcer and analgesic activities (6).

Overproduction of free radicals leads to an oxidative stress which is a deleterious process that can cause the damage of cellular structures, including lipids, proteins and DNA (7). Plants are a potential source of natural antioxidants which are used by them to neutralize reactive oxygen species and thus to survive (8). The importance of the antioxidant properties of plant constituents in the maintenance of health and protection against coronary heart disease and cancer has increased the interest of scientists, food manufacturers and consumers as the trend for the future is moving toward functional foods with specific health effects (9). Moreover, these compounds (natural antioxidants) possess biological effects against tumors, heart disease, AIDS and other diseases because of their scavenging activity (7). Therefore, it is interesting to evaluate the antioxidant activity of the stinging nettle.

Gas chromatography-mass spectrometry (GC-MS) and infrared spectroscopy (IR) analysis were performed in this study to identify some bioactive compounds found in the hexane extract of *Urtica dioica*. Moreover, the antioxidant activity of ethanolic and aqueous extracts was assessed by DPPH and H_2O_2 assays.

II. Materials and Methods

A. Collection of plant material and sample preparation:

Fresh *Urtica Dioica* was collected from south Lebanon and the whole plant leaves and stems were washed thoroughly under running tap water first then

shade dried. The dried plants were then pulverized to powder using a mechanical grinder. The plant powder was extracted using Ultrasound Assisted Extraction (UAE) technique using 5 different solvents separately: Hexane, dichloromethane, acetone, ethanol and distilled water for 1 hour. The extracts were then filtered using Whatman No. 1 filter paper. The obtained crude extracts were concentrated by rotary evaporator at 60°c for the aqueous extract and 40°c for the others. The extracts were then stored in sterile bottles at 5°C for further use.

B. Purification and identification

- Bioassay guided isolation of active compound(s)

The hexane extract of *U. dioica* was fractionated using silica gel 60 (0.063 to 0.200 mm) column chromatography (CC). The column was eluted with a solvent gradient of hexane-ethyl acetate (EtOAc) in 10:0 and 0:10 ratios to give 11 fractions as follows:

10% EtOAc – hexane (3 fractions)
20% EtOAc – hexane (2 fractions)
30% EtOAc – hexane (2 fractions)
50% EtOAc – hexane (3 fractions)
100% EtOAc (1fraction)

Eleven fractions were collected, analyzed by a thin layer chromatography (TLC) (analytical plate) on silica gel 60 PF254 (Merck) aluminum sheets and pooled together due to similarity in TLC profile to give over three fractions: FI, FII and FIII. Each fraction was then purified by a preparative plate of thin layer chromatography (TLC): FI gave 6 sub-fractions, FII gave 4 and FIII gave 3 subfractions. These subfractions were submitted to GC- MS and IR analysis.

- GC-MS Analysis

GC-MS analysis of the hexane extract of *U. dioica* was performed using a Perkin–Elmer GC Clarus 500 system comprising an AOC-20i auto-sampler and a Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with a Elite-5MS (5% diphenyl/95% dimethyl poly siloxane) fused with a capillary column (30 × 0.25 µm ID × 0.25 µm df). For GC-MS detection, an electron ionization system was operated in fast atomic bombardment mode with ionization energy of 70 eV. Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1 ml/min, and an injection volume of 2 µl was employed (a split ratio of 10:1). The injector temperature was maintained at 250 °C, the ion-source temperature was 180 °C, the oven temperature was programmed from 50°C (isothermal for 2 min), with an increase of 10 °C/min to 60°C, then 20°C/min to 80°C, with a 3 min isothermal at 80 °C. then 10°C/min to 90°C, with a 2 min isothermal at 90 °C, then 30°C/min to 120°C, with a 3 min isothermal, then 30°C/min to 150°C, with a 5 min isothermal, then 100°C/min to 250°C, with a 10 min isothermal at 250 °C, then 50°C/min to 300°C, ending with a 5 min isothermal. Mass spectra were taken at 70 eV; a scan interval of 0.5 s for all fragments mass. The solvent delay was 0 to 2 min, and the total GC/MS running time was 70 min.

- Identification of phytocomponents

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

- .Infrared spectroscopy analysis (IR)

FTIR (Fourier transform Infra red) (Model - Varian 3600; Range: 400-4000 cm-1) was obtained for different subfractions. Sample (1-2 mg) was crushed with KBr (3-4 mg) and the pellet was formed with the help of mechanical pressure. This pellet was further observed at different wavelengths in FTIR instrument.

C. Evaluation of antioxidant activity

- Radical scavenging activity of DPPH

1 ml of different concentrations of samples diluted in ethanol was added to1 ml of DPPH (0.15 mM in ethanol), and at the same time, a control comprising 1ml DPPH with 1 ml ethanol was prepared. The mixtures were well mixed by hand and then incubated in the dark at room temperature for 30 min. The absorbance is measured at 517 nm by a UV-Vis spectrophotometer.
The capacity of free radical scavenging DPPH by nettle extracts is calculated according to the following equation:

% scavenging activity = [(Abs control- Abs sample)] / (Abs control)] x 100

- 2- Radical scavenging activity of the hydrogen peroxide (H_2O_2)

A H_2O_2 solution (40 mM) was prepared in PBS (pH 7.4). The absorption was determined spectrophotometrically (Gene Quant1300 UV-Vis) at 230 nm. Various concentrations of plant extracts diluted in distilled water were added to the H_2O_2 solution (0.6 ml, 40 mM) and the absorbance was measured after 10 min. A solution containing a buffer blank where an extract H_2O_2 free was used.

The scavenging percentage was calculated using the following equation:

% scavenging activity = [(Abs control- Abs sample) / Abs control] × 100

III. Results and discussion

A.1. Identification of Compound A

The structure of this compound after the evaluation of its MS and IR spectrum was established as **A** and its name is Ribitol, 1,3:2,4-di-O-benzilidene (fig.4). The IR spectrum showed a -C-C- bond of aromatic ring at 1460 cm^{-1} and -C-H- bond of aromatic ring at 848 cm^{-1} (fig. 1). The analysis of the peak obtained in GC at 12.5 min by MS (fig. 2) gave these results: the ion peak observed at *m / z* 329 is obtained on the basis of the [MH+] ion. The fragment observed at m/z 179, the base peak at m/z 149 and the fragment at m/z 81 correspond to the loss of parts 1, 2 and 3 (fig.3) of this molecule respectively. This compound is known to exhibit an activity against breast and colon cancer and also antimicrobial properties (10).

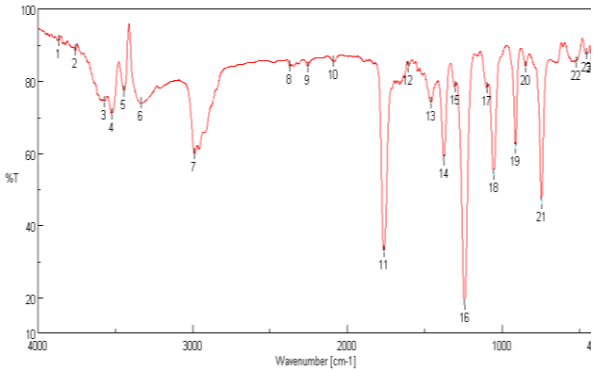

Figure 1: IR spectrum of compound A

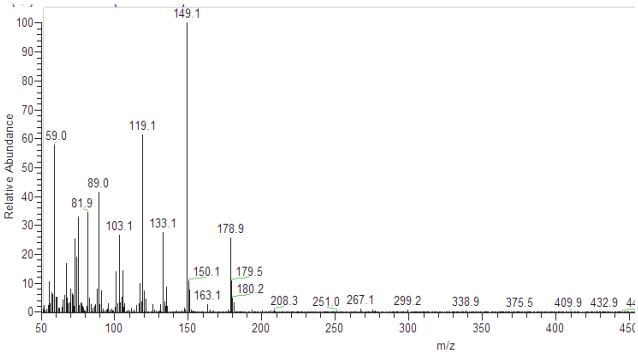

Figure 2: MS spectrum of compound A

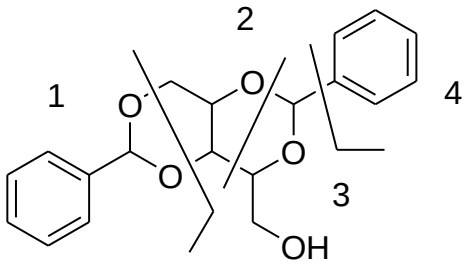

Figure 3: Fragmentation of compound A by MS

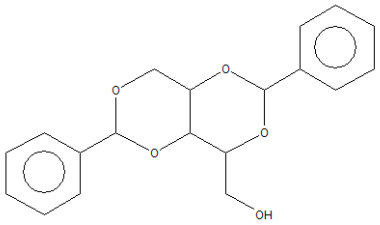

Figure 4: Chemical structure of compound A

A.2. Identification of compound B

The analysis of the fourth fraction showed the presence of an alkaloid containing a thiazole skeleton. The IR absorptions displayed an amine group (3306 cm^{-1}) , a bond for hydroxyl group of an acid (2990 cm^{-1}), a nitrosyl group –NO- at (1513 cm^{-1}), -C-C- bond of aromatic ring (1461 cm^{-1}), amine group of aromatic ring at (1242 cm^{-1}), amine group of aliphatic compound at (1054 cm^{-1}) and a -C-H- bond of an aromatic at 848 cm^{-1} (fig. 5). For the peak corresponding to a retention time of 12.16 min, the superposition of the spectrum obtained in the MS spectrum with the NIST library confirms the presence of Acetic acid, 2-[5-(4-nitrobenzylidene)-4-oxo-2-thioxo-3-thiazolidinyl] (fig.6). Indeed, the fragment observed at m / z 207 corresponds to the loss of the part 1 of the compound. The fragment observed at m / z 179 is assigned to the loss of the part 2 and that observed at m / z 89 corresponds to the loss of the part 3 from this molecule (fig.7). This compound is

known to possess anti-diabetic, anti-carcinogenic, antimicrobial and anti-inflammatory activities (11).

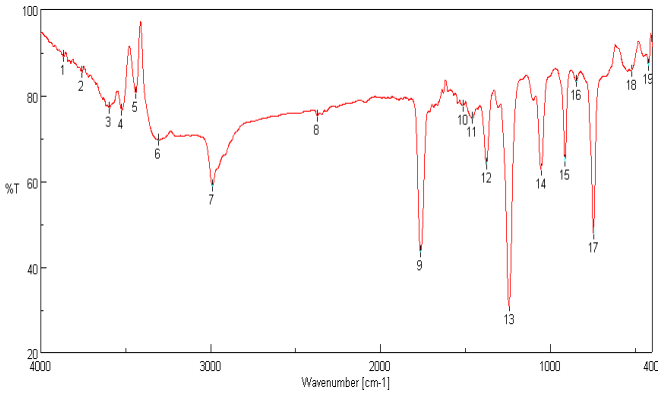

Figure 5: IR spectrum of compound B

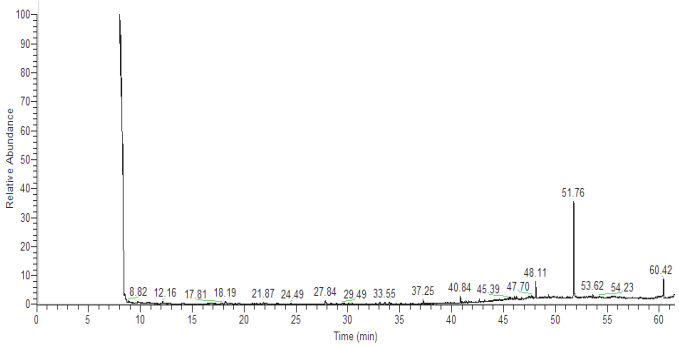

Figure 6: MS spectrum of compound B

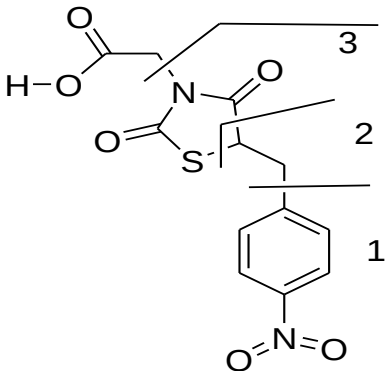

Figure 7: Fragmentation of compound B by MS

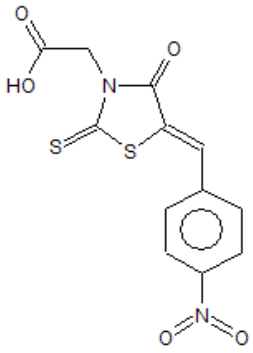

Figure 8: Chemical structure of compound B

A.3. Identification of compound C

The investigation of IR spectrum indicated the presence of an aliphatic compound containing a pantethine skeleton. This compound showed IR bonds (fig.9) for a hydroxyl group at 3441 cm^{-1}, -C-H- bonds of an alkane at 2990 and 1461 cm^{-1}, a carbonyl group at 1764 cm^{-1} and an amine group of an aliphatic compound at 1054 cm^{-1}. The analysis of peak obtained in GC at 18.13 min by MS confirms the presence of 2-myristynoyl panthetine (fig.11). In fact, the observed fragment at m/z 341 is attributed to the loss of the part of the compound C. The peak observed at m/z 191 corresponds to the loss of part 2 and the base peak observed at m/z 57 corresponds to the loss of part 3 of the molecule (fig.10). Compound C is known to have a benefic effect against vascular diseases, prevention of diabetes and peroxidation of lipids (12, 13).

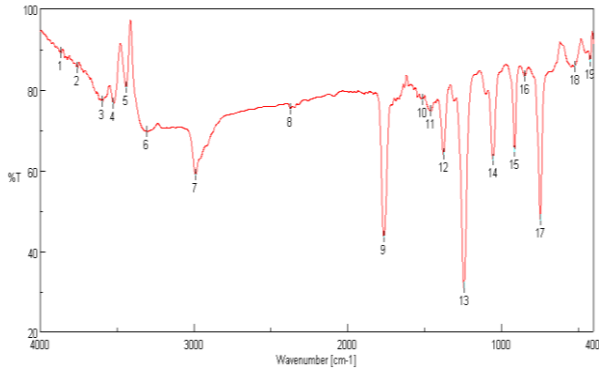

Figure 9: IR spectrum of compound C

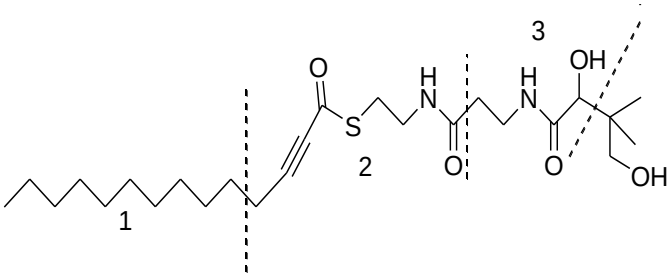

Figure 10: Fragmentation of Compound C by MS

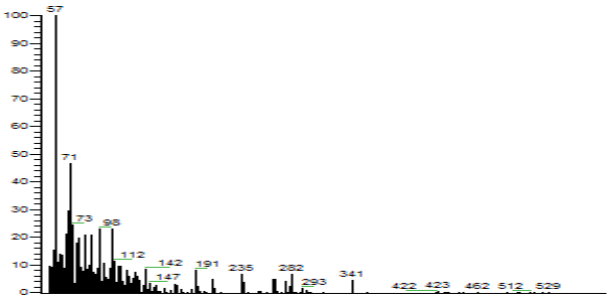

Figure 11: MS spectrum of compound C

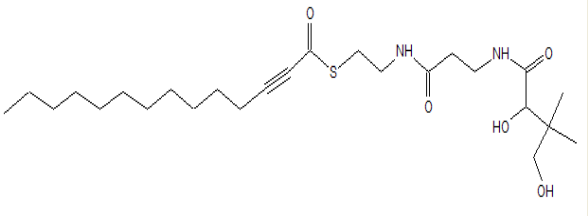

Figure 12: Chemical structure of compound C

B. Antioxidant activity

- DPPH Radical Scavenging Assay

The, α-diphenyl-β-picrylhydrazyl (DPPH) radical scavenging assay is a simple, fast and sensitive method to study the antioxidant activity. In fact, the DPPH owns a free radical which binds to a free electron when an antioxidant compound is present and then the absorbance on the spectrophotometer decreases (14).

The graph in figure 13 shows that the aqueous and ethanolic extracts of stinging nettle have a high antioxidant activity at different concentrations. The DPPH test demonstrated that at 0.5 mg/l of extracts, the activity of scavenger increases to 44%.

- Hydrogen peroxide scavenging assay

The H_2O_2 test is another test applied to confirm the antioxidant power of nettle. The hydrogen peroxide is considered as an important reactive oxygen species due to its high ability to penetrate the biological membranes where it plays a significant role but it can be toxic if it is converted to hydroxyl group in the cell. Scavenging of H_2O_2 by the aqueous and ethanolic extracts can be referred to their richness in phenolic compounds, the later have the ability to donate electrons to the H_2O_2 and then reducing it into H_2O (14).

Our results showed that the extracts of the plants are able to scavenge the H_2O_2 in a manner dependent on the concentration. The percentage of scavenging activity of 0.5 mg / ml of aqueous and ethanolic extracts was 51 and 55 respectively (Fig. 14). These results have shown that nettle has a high antioxidant activity particularly in the aqueous extract and, therefore, it can be considered as a good source of natural products that can be used in the treatment of various diseases associated with oxidative stress.

The superior antioxidant activity in the ethanol extract is brought to the presence of higher levels of polyphenols compared to the aqueous extract (15).

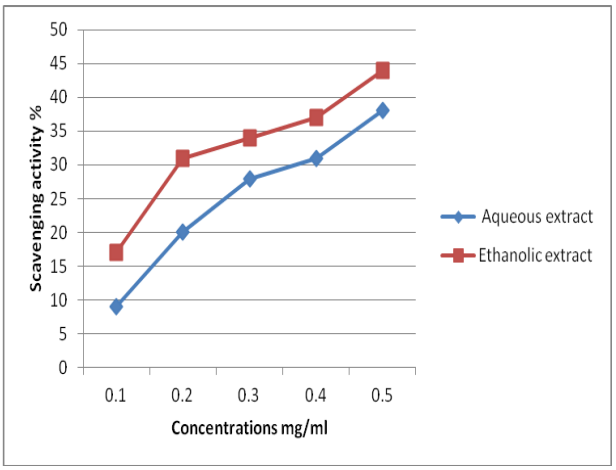

Figure 13: Positive correlation between the concentration of ethanoic and aqueous extracts of stinging nettle and DPPH test.

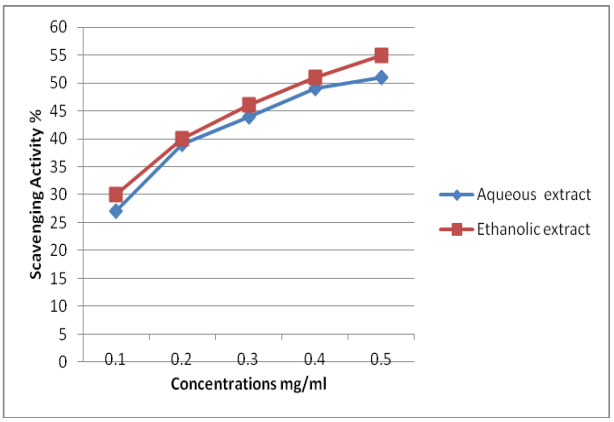

Figure 14: Positive correlation between the concentration of the ethanolic and aqueous extracts of stinging nettle and the H_2O_2 test.

IV. Conclusion

Three bioactive compounds were isolated and identified from hexane extract of the stinging nettle: ribitol, 1,3:2,4-di-O-benzilidene; acetic acid, 2-[5-(4-nitrobenzylidene)-4-oxo-2-thioxo-3-thiazolidinyl] and 2-myristynoyl panthetine. These compounds possess anti-carcinogenic, anti-inflammatory, anti-diabetic properties. Furthermore, the results demonstrated that the Lebanese nettle has a significant antioxidant activity dependent on the nature and the concentration of the extract. This study which reveals the presence of bioactive compounds in *Urtica dioica* suggests that the contribution of these compounds to the pharmacological activity should be investigated in further studies.

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