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Antibacterial and antioxidant activities of ethyl acetate extract of nettle (*Urtica dioica*) and dandelion (*Taraxacum officinale*)

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ABSTRACT

The antibacterial and antioxidant activities of ethyl acetate extract of nettle (*Urtica dioica*) and dandelion (*Taraxacum officinale*) was studied, also phytochemical screening and determination of total phenolic content has been investigated. The results revealed that ethyl acetate extract of nettle was more effective on all bacterial isolates (*Aeromonas hydrophila, Salmonella typhi, Staphylococcus aureus, Bacillus cereus, Escherichia coli*) than dandelion with highest inhibition zone (24 mm) towards *B. cereus, A hydrophila* was more resistant than other bacteria. Also it was found that nettle gave large inhibition zone to *S. typhi* (22mm). Ethyl acetate of nettle had the highest content of phenolic compounds (48.3mg GAE/gdw) while dandelion had only (10.2 mg GAE/gdw) of phenolic content. The phytochemical qualitative screening exhibited flavonoid, glycosides and phenols were present in nettle and dandelion. In nettle, alkaloid, tannins and terpenoids were present, while absent in dandelion, on the other hand, dandelion had the saponins which not found in nettle. Steroids not present in the tow plants. Evaluation of antioxidant activities of ethyl acetate extract of nettle and dandelion by ferric thiocyanate method (FTC) exhibited that nettle caused 76% lipid peroxidation in inhibition of linoleic acid emulsion; this activity was greater than dandelion (44%) and α-tocopherol (65%).

INTRODUCTION

The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body (Hedges and lister, 2007).

Nettle "*Urtica dioica*" has medical properties and its extract have been used for hundreds of years in world traditional medicine for treating diseases such as eczema, digestion, joints pain and anemia (Chrubasik *et al*., 2007). Dandelion "*Taraxacum* spp" used as diuretic and bitter digestive stimulant, preclinical research on has revealed numerous properties, antiangiongenic and prebiotic also the leaves are rich in fibers, potassium, iron, calcium and some vitamins(Clyton, 2000) .The therapeutic effects of many plant spices and herbs suggests the presence of antioxidative and antimicrobial constituents in their tissues. Antioxidants can be used to reverse the harmful and pathological effect of the free radicals, such as flavonoids, phenolic acids and diterpenes (Yanishiera *et al*., 2006). Because of the need for new antimicrobial agents and strategies for their use in the treatment of serious gram- negative and gram- positive infections is evident and is greater than ever because of emergence or multidrug resistance in common pathogens, the rapid emergence or new infections, and the potential for use or multidrug – resistant agents in bioweapons (Spellberg *et al*., 2003).

The aim of this study is evaluation the antibacterial and antioxidant activities of ethyl acetate extract of leaves of nettle and dandelion, preliminary phytochemical screening of the extract was also done along the determination of total phenolic content and study the comparison between the tow plants.

MATERIALS AND METHODS

Plant material

Fresh leaves of Nettle (*Urtica dioica*) and Dandelion (*Taraxacum officinale*) were procured from markets of Arbil city, North of Iraq. Authentication and identification of the plant was carried out by Dr. Ali Al- Mosawy, Department of Biology, College of Science, University of Baghdad.

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Sample preparation

The plants were cleaned and cut into small pieces, and then air dried. The dried samples were then pulverized into fine powder in a grinder, which was then stored at 4 ^{o}C until use.

Preparation of crude ethyl acetate extract

Air dried leaves sample (20 gm) was soaked in 100 ml of 95 % ethyl acetate, and shaken at 150 rpm for 24 hr. at ambient temperature. The extract was filtered through filter paper (Whatman No.1) which was impregnated with same solvent. The ethanol was concentrated to near dryness under reduced pressure below 40 ^{o}C using rotary evaporator. The amount of the concentrate extract was noted down. The extracts were diluted to 20 mg/ml with 10 % dimethyl sulfoxide (DMS) solution and stored in air tight glass bottles in a refrigerator till further use (Mingarro *et al*., 2006).

Microorganisms and media

The bacterial isolates *A. hydrophila* and *S. typhi* isolated from patients with food poisoning (gastrointestinal infections). The bacteria were obtained, as clinical isolates, from Al–Yarmook Teaching Hospital, Baghdad, Iraq.

While, *S. aureus* isolated from the salted white cheese and *B. cereus* isolated from spoiled rice. Bacterial cultures were maintained on nutrient agar (NA) slops. Subcultures were made monthly and stored at 4 °C.

Culture preparation

A loopfull of 24 hr. surface growth on a NA slope of each bacterial isolate was transferred individually to 5ml of Brain heart infusion broth (pH 7.6) and incubated at 37^{o}C for 24 hr. bacterial cells were collected by centrifugation at 3000 rpm for 15 min, washed twice and resuspended in 0.1% pepton water. Turbidity was adjusted to match that of as McFarland standard (10^{8} CFU/ml). Then 1:10 dilution of the cell suspension was performed to give an inoculums concentration of 10^{7}(CFU/ml).

Antibacterial activity test of extracts (*in vitro*) using agar diffusion assay method

0.2 ml volume of the standard inoculums (10^{7} CFU/ml) of the test bacterial isolate was spread on Mueller Hinton Agar (MHA) with a sterile glass rod spreader and allowed to dry. Then 6 mm. diameter wells were bored using cork borer in the MHA. Plant extracts (1,5 and 10 mg/ml) were introduced into each well and allowed to stand for 1 hr. at room temperature to diffuse the plant extracts into medium before incubation at 37 ^{o}C for 24 hr. The inhibition zone diameter (IZD) was measured by transparent ruler to nearest mm.

Cephalothin (30 µg/ml) (Oxoid) was used as positive control inhibition zone with diameter less than 12 mm. were considered as having no antibacterial activity , diameter between 12 and 16 mm. were considered moderately active , and these with > 16 mm. were considered highly active (Indu *et al*., 2006).

Determination of total phenolic content

Total phenolic contents of all dry plants were determined using Folin- Ciocalteu assay as described by Attanassova *et al.* (2011). An alquot (1 ml) of extracts or a standard solution of gallic acid (20, 40, 60, 80 and 100 mg / L) was added to a 25 ml volumetric flask, containing 9 ml of distilled deionised water (dd H2O). A reagent blank using (dd H2O) was also prepared. One ml of Folin- Ciocalteu phenol reagent was added to the mixture and shaken. After 5 min., 10 ml of 7% Na2CO3 solution was added to the mixture. The solution was diluted to a 25 ml with dd H2O and mixed. After incubation for 90 min. at room temperature, the absorbance against the prepared reagent blank was determined at 750 nm using spectrophotometer. The data for the total phenolic content of the plants were expressed as milligram of gallic acid equivalents (GAE) per gram dry mass (mg GAE / g dw). All samples analyzed in duplicated.

Phytochemical screening

The ethyl acetate extract of nettle and dandelion were evauated for qualitative determination of major phytoconstituents , alkaloids, saponins, tannins, flavonoids, steroids, terpenoids, glycosides, and phenols (Boxi *et al*., 2012).

Antioxidant activity of extracts

Antioxidant activity of ethylacetate extracts and standard was determined by FTC (Ferric Thiocyanate) method (Gülcin *et al*., 2006). For preparation of stock solutions, 10 mg of each extract was dissolved in 10 ml of distilled water. Then, the solution which contained 50 µg/ml of stock nettle and dandelion solutions or α- tocopherol as standard sample (50 µg /ml) in 2.5 ml of potassium phosphate butter (0.04 M, pH 7.0), was added to 2.5 ml of linoleic acid emulsion in potassium phosphate butter (0.04 M, pH 7.0). The mixed solution (5 ml) was incubated at 37 ^{o}C in flask. The peroxide level was determined by reading the absorbance at 500 nm in a spectrophotometer, after reaction with $FeCl_2$ and thiocyanate at intervals during incubation. During the linoleic acid oxidation, peroxides are formed, which oxidize Fe^{+2} to Fe^{+3}, The latter Ions form a complex with thiocyanate and this complex has a maximum absorbance at 500 nm. Five ml linoleic acid emulsion: contained 17.5 µg Tween – 20, 15.5 µl Linoleic acid and 0.04 M potassium Phosphate buffer (pH 7.0). On the other hand, the 5 ml control was composed of 2.5 ml linoliec acid emulsion and 2.5 ml 0.04 M potussium phosphate buffer (pH 7.0). This step was repeated every 5 hr. until the control reached its maximum absorbance value. Therefore, high absorbance indicates a high linoleic acid emulsion oxidation. Solution without added extracts was used as blank samples. All data on total antioxidant activity are the average of duplicate analyses. The percentage inhibition of lipid peroxidation (%) in linoleic acid emulsion was estimated by the following formula:

$$\% \text{ Inhibition} = 100 - ((A1 - A0) \times 100),$$

Where A0 is the absorbance of the control and A1 is the absorbance of the sample or standard compound (α- tocopherol) .

RESULTS AND DISCUSSION

Antibacterial activity of ethyl acetate extract of nettle and dandelion against some of food borne bacteria at 10 mg/ml showed in (table 1.).

Table. 1: Antibacterial activity of ethyl acetate extracts on some of food borne pathogenic bacteria.

s. no.	Pathogenic bacteria	Diameter of inhibition zone(mm)		
		Nettle (10mg\ml)	Dandelion (10mg\ml)	Cephalothin (30µg\ml)
1	*A.hydrophila*	14	N.I.	20
2	*S.typhi*	22	14	18
3	*S.aureus*	20	16	24
4	*B.cereus*	24	18	22
5	*E.coli*	10	16	20

N.I. =No inhibition

The results revealed that ethyl acetate extract of nettle was more effective on all bacterial isolates than dandelion, with highest inhibition zone (24 mm) towards *B. cereus*. *A hydrophila* was more resistant than other bacteria. It hase been suggested that high resistant to plant extracts in gram negative bacteria is due to the outer membrane of their cell wall, acting as barrier to many substances including antibiotics (Marino *et al*., 2011). Also it was found that nettle gave large inhibition zone to *S. typhi* (22mm), while dandelion exhibited poor antibacterial activity against *E.coli* and their was no. inhibition against *A. hydrophila*. The results of Chahardehi *et al.,* (2012) revealed that ethyl acetate, hexane and chloroform extracts showed higher antimicrobial activity than the other crude extracts, where the ethyl acetate extract showed highest inhibition against *B. cereus*, methicillin resistant *Staphyllococus aureus* and *Vibrio parahaemolyticus*. Terpens and phenols of *U. dioica* are one of the major groups associated with the inhibition of microbial infections and cancer (Dar *et al*., 2012). *U. dioca* is a rich source of phytochemicals such as phenolic compounds and minerals which can be used as a potential source of useful drugs(Ahmed *et al*., 2012). The total phenolic contents of leaves of nettle and dandelion expressed as milligrams of gallic acid equivalent (GAE) per mg dry weight were summarized in table 2.

Table. 2: Total phenolic content of leaves of nettle and dandelion.

Herb	Latin name	Total phenolic content (mg GAE /gdw)
Nettle	*U. dioica*	48.3
Dandelion	*T. officinale*	10.2

GAE = gallic acid equivalent.

Ethyl acetate of nettle had the highest content of phenolic compounds (48.3mg GAE/gdw) while dandelion revealed only (10.2 mg GAE/gdw) of phenolic content. Our results disagree with the results of Chahardehi *et al.* (2004) who found that the total phenolic content of ethyl acetate of *U. dioica* was (18.7 mg GAE/gdw), while the chloroform extract had the highest content (36.4 mg GAE /gdw), also it was found that total phenolic content of dandelion was close to our results (15.5 mg GAE/gdw)(Sengul *et al.* 2004). The result presented in table 3. revealed that flavonoid, glycosides and phenols were present in nettle and dandelion. In nettle, alkaloid, tannins and terpenoids were present, while abscent in dandelion, on the other hand, dandelion had the saponins which not found in nettle. Steroids not present in the tow plants.

Table. 3: Phytochemical analysis (Qualitative) of athyl acetate extract of nettle and dandelion.

Class of compound	*U.dioica*	*T.officinale*
Alkaloids	+	-
Saponins	-	+
Tanins	+	-
Flavonoids	+	+
Steroids	-	-
Terpenoids	+	-
Glycosides	+	+
Phenols	+	+

+ = present, - = absent.

Some studies obtained that *U. dioica* ethyla cetate extract contain flavonoids and alkaloids, phenols, saponins and tannins and this plant was a rich source of flavonoids and alkaloids (Ahmed *et al*., 2012). *T. officnale* had a highly concentrated of some phytoconstituents in the stem, root and flower such as saponins, flavonoids, alkaloids and phenols (Mir *et al*., 2013). Evaluation of antioxidant activity of Nettle and dandelion by using ferric thiocyanate method (FTC) (Fig.1).

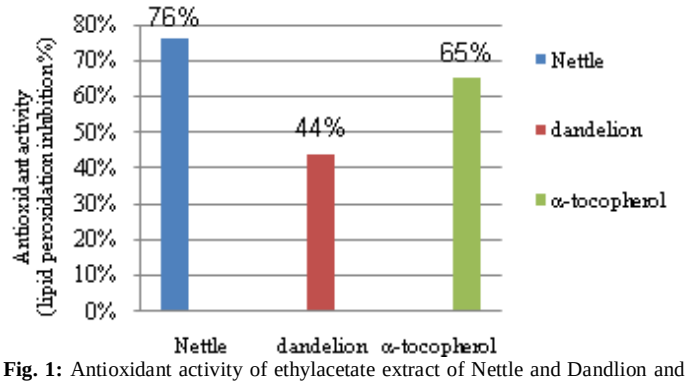

Fig. 1: Antioxidant activity of ethylacetate extract of Nettle and Dandlion and α-Tocopherol as standard (after 40 hr . of incubation using FTC method).

Nettle caused 76% lipid peroxidation in inhibition of linoleic acid emulsion, this activity was greater than dandelion(44%) and α-tocopherol (65%). Some studies found significant antioxidant activity of the methanol extract of *U.dioica* comparable to standard antioxidant compounds like α-tocopherol, ascorbic acid and butylated hydroxyl anisole. This activity may be due to phenols and phenolics, Phenolic compounds have antioxidant properties due to their ability of scavenging free radicals and active oxygen species such as singlet oxygen, free radicals and hydroxyl radicals (Hall, and Cuppett, 1997) . Other researchers mentioned that the total antioxidant activity of water extract of nettle, by using ferric thiocyanate method, exhibited effective antioxidant activity at all doses (50 – 250 µg) (Gulcin *et al*., 2004).The low antioxidant activity of dandelion may be due to the presence of active scavenging compounds in other parts of plants such as flowers and roots more than leaves as in lutiolin and lutiolin-7-o-glycoside (Hu and Kitts, 2004).

CONCLUSIONS

Our results revealed that ethyl acetate extract of nettle had obvious antibacterial and antioxidant activities than dandelion, and this may be due to high phenolic content and presence of active compounds such as alkaloids, tannins and terpenoids in nettle.

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