Identification of Dandelion *Taraxacum officinale* Leaves Components and Study Its Extracts Effect on Different Microorganisms

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Abstract

Natural plant Dandelion *Taraxacum officinale* have been used as a phytomedicine, in this study, the chemical components of the Dandelion *Taraxacum officinale* leaves in watery and alcoholic extracts were identified. The results showed that watery extract was alkaline (presence of alkaloids) while the alcoholic extract was acidic in these contained: glycosides, alkaloids, phenolic compounds, tannins, flavonoids and proteins, while the saponins and resins were not found.

The result also showed that high concentrations of the following trace elements were found in the leaves (K ,Ca , Na ,Fe) with 185.1, 22, 19.5, 11.2 ppm, respectively and low concentrations of (Zn, Cd, Cu) with 6.3, 1.3, 0.2 ppm, respectively. The effect of these extracts on the different microorganisms were studied. It has been found that the concentration 0.5 mg/ml was effective on the inhibition of the growth of the intended bacteria (for both extracts) especially Gram positive, *Staphylococcus aureus* and the alcoholic extracts with concentration 0.5 mg/ml was more effective on the Gram negative, *E.coli*, than the watery extract, while less than 0.1 mg/ml failed to inhibit any microorganisms. The High Performance Liquid Chromatography (HPLC) was used to identify some flavonoids as compared to standard one; the analysis showed that both kaempferol and morin were absent.

Keyword: Dandelion *Taraxacum officinale*, Plant extraction, Trace elements, HPLC.

Introduction

Herbs have been used for a large range of purposes .They are found to be potent sources of natural components .Some have been used for hundreds of years, and their clinical and pharmacological effects have been extensively studied from various viewpoints [1].This study focused on a used in folk medicine, *Taraxacum officinale*, commonly called Dandelion, which is a herbaceous perennial plant of the family *Asteraceae* (Compositae). It can be found growing intemperate regions of the world, in lawns, on roadsides, on disturbed banks and shores of water ways and other areas with moist soils. Dandelion is considered a weedy species [2]. It has many english common names including: Blowball, Lion's-tooth, Cankerwort, Swine's snout, …etc [3], Arabic names :Hindiba, Khas berri [4], The genus name *Taraxacum* ,might be from the Arabic word "*Tharakhchakon*" [2], or from the Greek word "*Tarraxos*" .The common name "Dandelion" comes from the French phrase "Dent de lion" which means "Lion's tooth", in references to the jagged shaped foliage [5]. Dandelion serves mainly as a diureticum, and at the same time as a cleanser of the blood and liver. An active substance of dandelion reduces serum cholesterol and triglycerides because it intensifies bile secretion [1,6]. Dandelion improves the function of liver, pancreas and stomach. It is used to treat anemia, cirrhosis of the liver, hepatitis and rheumatism [1,7] anti-inflammatory, anti-oxidative, anti-carcinogenic, analgesic, anti-hyperglycemic, anti-coagulatory and prebiotic effects [8-13].

The leaves can be eaten cooked or raw in various forms, for assembling salads or soup, which also recommended as a natural source of vitamin C in the early spring. Dandelion water extract has anti-tumor activity attributed to polysaccharide [1].The most important biologically active components are sesquiterpenic lactones, biotin, inositol and vitamins B, D, E and phosphorous (P). The leaves possesses a higher content of β-Carotene than carrot and more Fe and Ca level than spinach, along with macro- and micro- elements [1,14].

Flavonoids and coumaric acid derivatives were identified from dandelion flower [7].Its

roots have been used to make a coffee like drink and the plant had used by Native Americans as a food and medicine [15].

However, its use has mainly been based on empirical finding .This contribution provides a comprehensive review of the pharmacologically relevant compounds of *Taraxacum officinale* characterized so far and of the studies supporting its use as a medicinal plant.

Accordingly, the leaves of *Taraxacum officinale* has drawn the attention of researches and consumers and an understanding of the chemical and pharmacological properties should be of importance from scientific points of view, therefore, this study is aimed at evaluating the ash contents, pH, leaves components, trace elements, effect of its extracts on different microorganisms and enzyme,checking of some the presence of flavonoids in these extracts.

Materials and Methods

Protocol of Method

[A] Collection and treatment of samples:

The leaves of *Taraxacum officinale* were collected from the north of Baghdad, Iraq.The leaves were transported to the laboratory, washed with water, cleaned with filter paper or soft clothes to remove all traces of dust and insects, then dried in shad 25-30ºC for one week, with continuous overturn to prevent mould. weighed, ground in a mortar and pestle, placed in airtight bottles and stored in dissicator to by used for extraction.

[B] Preparation of extracts:

a) Watery extract:

Air dried leaves 50 g were suspended in 1 liter of distilled water and left for 24 hrs at 35ºC with continuous stirring in shaking incubator. Then the mixture was filtered by filter paper. The filtrate was centrifuged for 10 min. at 2500 rpm, and the extract was evaporated to dryness at 40ºC in the incubator. The pH of the extract was determined using pH-meter (Orion,SA720).

b) Alcoholic extract:

Prepared as in water extract described above, but with using ethanol 70% (v/v) instead of water to give alcoholic extract powder [16-19]. The pH of this extract was also determined.

[C] Determination of Ash content:

Dried leaves 2 g were taken and heated at 900ºC for 20 min. in muffle furnace until the material converted to white powder, after its cooling the percentage of ash content was determined [20].

[D]Chemical detection of the plant components:

The chemical components of the prepared watery and alcoholic extract were detected as shown in Table (1). They included: glycosides, alkaloids, saponins, phenolic compounds, tannins, resins, flavonoids [16-18] and proteins [21].

[E] Determination of trace element levels:

Dried leaves 3 g were taken and mixed with 8 ml of concentrated nitric acid and 2 ml of 60% prechloric acid in a conical flask, the mixture was kept for 24hrs covered with watch glass. After that it was left for 6hrs at sand bath at 80ºC, until the digestive material converted to white powder. Deionized water 8 ml were added to this powder, and the trace elements were determined [20] by (Shimadzu AA-670, Flame Atomic Absorption Spectrophotometer).

[F] Inhibition of hyaluronidase:

Hyaluronidase inhibition activity was determined turbidimetrically by the method of Kass *et al* [22] by using 0.01 mg/ml enzyme mixed with 250 µg/ml from the extracts with inhibition time 45 min. and the percentage of inhibition %I was calculated according to this equation [23]:

$$\%\,\text{Inhibition} = \frac{\text{Activity of control} - \text{Activity in the presence of Extract}}{\text{Activity of control}} \times 100$$

[G]The biological activity:

The biological activity against various bacterial species was determined. As gram negative bacteria, *Proteus mirabilis* and *E.cole* were used, while *Staphylococcus aureus* was used as gram positive bacteria. These isolates were obtained from department of Biology/College of Science /Al-mustansiriyah University .The concentrations used for each extracts were 0.1, 0.5 and 1 mg/ml [18, 19].

[H] HPLC Analysis:

Analysis of flavonoids, Kaempferol and Morin, were carried out in the chemical research center, ministry of science and technology, Baghdad, Iraq, by using (Shimadzu, LC2010A, Japan) HPLC. Standard solution were prepared (standard Kaempferol and Morin were gift supplied from Dr.Mohammed Mustafa Radi).

A luna 5U C-18Column(250mm x 4.6 x 5µm)Was used and coupled with 20µl of sample at 40ºC with a linear gradient mobile phase containing solvent A (water), solvent B (acetonitrile) and solvent C (5% formic acid in water, v/v) with flow rate set at 0.2ml/min.

The linear gradient program started with 88% A:10% B:2% C (v/v) and finished at 73% A:25% B:2% C (v/v) with retention time 10min. The chromatograms were recorded at 280nm. Standard and leaves extracts samples were made at 25ppm then analyzed directly by HPLC [24].

Results and Discussion

Our results showd that pH value for watery and alcoholic extracts are pH =8.15 and 4.81, respectively (This may reflect the presence of alkaloids in the watery extracts). The Ash content for the *Taraxacum officinale* leaves is (12.8%). The qualitative chemical analysis of the watery and alcoholic extracts are represented on Table 1,which show that leaves contents are (glycosides, alkaloids, phenolic compounds, tannins, flavonoids and proteins) in watery extract and the absence of alkaloids in the alcoholic extract. Other studie show that the major phenolic fraction in dandelion is anthocyanins, a type of flavonoids [1,14], phenolic acid [25] and poly phenolic compounds [26], these phenolic compound are an ecologically significant class of secondary metabolites expressed in all higher plants with high chemopreventive potential [27]. The saponins and resins are not found in both extracts.

Table (1)
Chemical components analysis (qualitative methods) for watery and alcoholic extracts of Taraxacum officinale leaves.

Components	*Reagents*	*Note*	*Result Watery extract*	*Result Alcoholic extract*
Glycosides	Iodine test Molish test Benedict test	Brown ppt. Violet ring Orange ppt.	Ve+ Ve+ Ve+	Ve- Ve+ +Ve
Alkaloids	Mayer's reagent Wagner reagent Picric acid	No white ppt. Brown ppt. Yellow ppt.	Ve- Ve- Ve-	Ve- Ve- Ve-
Saponins	Fast stirring Mercuric Chloride	No dense foam for long time No White ppt.	Ve - Ve -	Ve- Ve-
Phenolic compounds	Aqueous%1 Ferric chloride	Green ppt.	Ve+	Ve+
Tannins	Aqueous%1 Ferric chloride Lead acetate%1	Green ppt. Preface yellow ppt.	Ve+ Ve+	Ve+ Ve+
Resins	Ethanol + Boiling + Distilled Water	No turbidity	Ve-	Ve-
Flavonoids	aqueous%1 Ferric chloride Ethanol hydroxide alcohol	Green ppt. Yellow ppt.	Ve+ Ve+	Ve+ Ve+
Proteins	Folin-Ciocalteau reagent	Blue color	Ve+	Ve+

The concentration trace elements in *Taraxacum officinale* leaves are represented in Table (2) which shows, high concentrations of (K, Ca, Na, Fe) with (185.1, 22, 19.5, 11.2) ppm, respectively, and low concentrations of (Zn, Cd, Cu) with (6.3, 1.3, 0.2) ppm, respectively, other reports indicated the presence of these metal in this plant by using different method [28,29], while (Cr, pb) were not found. The high concentration of potassium cause the leaf acts as a diuretic; this spares the human body's potassium, which tends to be excreted with diuretic use. The effect is more therapeutic and beneficial overall. The young leaves that come up in the spring may also be add to spring salads for this benefit [14]. Regardless of its intended use, the high mineral content of this herb greatly increases the chance of drug-drug interactions with the conventional medicines that are sensitive to cations [13].

Table (2)
The concentration trace elements content of Taraxacum officinale leaves.

Trace elements	*symbol*	*Concentration (ppm)*
Potassium	**K**	**185.1**
Calcium	**Ca**	**22**
Sodium	**Na**	**19.5**
Iron	**Fe**	**11.2**
Zinc	**Zn**	**6.3**
Cadmium	**Cd**	**1.3**
Cupper	**Cu**	**0.2**
Chrome	**Cr**	**Nil**
Lead	**Pb**	**Nil**

This work shows, the two extracts were examined for their effects on hyaluronidase. The percentage of inhibition for each extract was 1.3% with respect to control assays run simultaneously. Kuppusamy, et al. show that morin and kaempferol, types of flavonoids, having potent inhibitory effect on this enzyme [30] with 56%, 31%, respectively. Absence of these two flavonoids, as shown latter by HPLC, may cause the lower inhibitory effects of these extracts on hyaluronidase.

The effect of these extracts on different microorganisms were studied and compared. However, the results in Table (3), show that the concentrations 0.5 and 1 mg/ml exhibit effective inhibition towards the growth of intended bacteria for both extracts specially on Gram positive, *Staphylocoous aureu*, while less inhibition effects were seen for Gram negative, *Proteus mirabilis* and *E.coli*.

Alcoholic extract with concentrations 0.5 mg/ml was more effective inhibitor for the Gram negative, *E.coli* than that for the watery extract; and the concentration 0.1 mg/ml of this extract failed to inhibit any microorganisms.

Table (3)
The effect of watery and alcoholic extracts of Taraxacum officinale represented by inhibition zone (mm) against different bacteria species.

Bacterial species	*Alcoholic extract (mg/ml)*			*Watery extract (mg/ml)*		
	0.1	**0.5**	**1**	**0.1**	**0.5**	**1**
Staphylococcus aureus **(gram + ve)**	–	++	++	–	++	++
Proteus mirabilis **(gram - ve)**	–	+	+	–	+	+
E.coli **(gram - ve)**	–	+	+	–	–	+

(-) No inhibition zone
(+) Inhibition zone between (1-4) mm .
(++) Inhibition zone between (4-10) mm .

This inhibitory effects may be duo to the (glycosides and/or phenolic compounds and/or tannins and/or flavonoids and/or alkaloids and/or proteins) presence in the plant extracts. Such compounds had been reported to have an active effect on the bacterial cells membrane, which may caused destroy these microorganisms [7, 18]. The alkaloids interact with the DNA, the tannins inhibit the carrier enzymes and proteins present in the cells membrane, while the phenolic compounds form complex with dissolved protein out of the cells or with cells membrane which made to destroy the bacteria [18, 22].

The HPLC chromatogram in Fig.(1), shows the standard Morin (A), standard Kaempferol (B) and leaves extract sample (C).

Identification of these flavonoids in leaves extract sample was checked according to retention time obtained from standard run at identical conditions.

The major peaks for Morin standard had retention time was 2.35min (Fig.(1, A)), while for that of Kaempferol was 3.3 and 3.56 min. (Fig.(1, B)). As it is clear from the chromatogram. These two flavonoids were absent in the leaves of *Taraxacum officinale*.

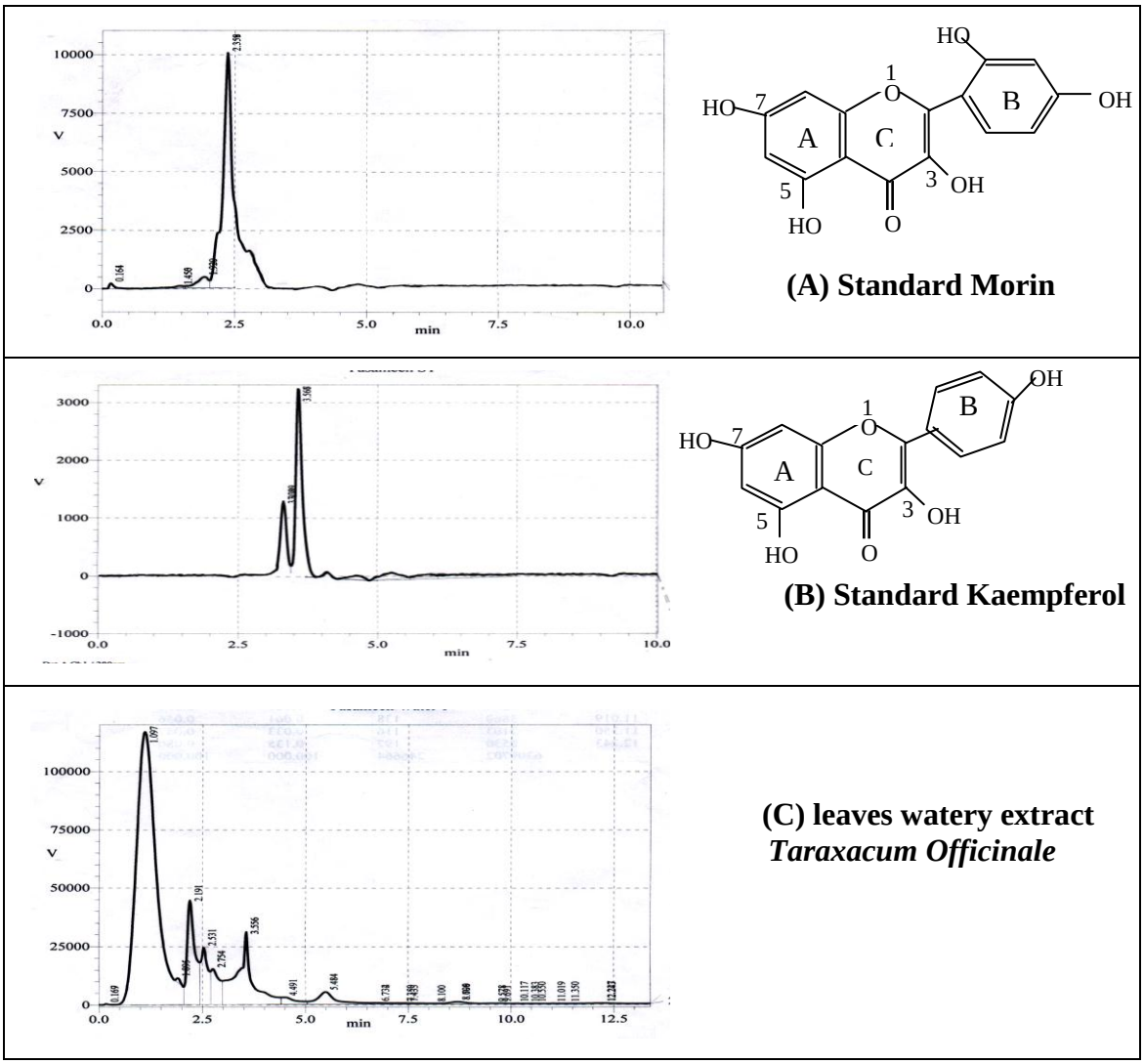

Fig.(1) HPLC chromatogram of (A) standard Morin, (B) standard Kaempferol, (C) leaves watery extract for Taraxacum officinale leaves.
Using luna 5U C-18Column(250mm x 4.6 x 5µm) the mobile phase at 40 ºC containing solvent A (water) ,solvent B (acetonitrile) and solvent C (5% formic acid in water ,v/v) with flow rate set at 0.2ml/min. The absorbance at (280nm).

Conclusion

The present study confirm that plant leaves extracts (watery and alcoholic) for Dendelion, *Taraxacum officinal*e leaves posses *in vitro* antibacterial activity because of its content, glycosides, alkaloids, phenolic compounds, tannins, flavonoids, proteins and trace elements, however, if plant leaves extracts are to be used for food preservation or medical purposes, issues of safety and toxicity will need to be addressed, and this results will serve as a pilot experiment for further research and improvement strategies of this important plant .

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الخلاصة

شملت الدراسة معرفة المكونات الكيميائية الفعّالة الموجودة في أوراق نبات الهندباء Dandelion *Taraxacum officinale*، حيث أظهرت الدراسة أن محلول المستخلص المائي ذا طبيعة قاعدية لاحتوائه على القلويدات بينما كان محلول المستخلص الكحولي ذا طبيعة حامضية، وتحتوي هذه المستخلصات على مجموعة من المركبات الكلايكوسيدية، القلويدات، المركبات الفينولية، العفصيات، الفلافونويدات والبروتينات في حين لا تحتوي على الصابونيات والراتنجات.

كما اثبت التحليل الدقيق للعناصر المعدنية احتواء الاوراق على تراكيز عالية من Fe، Na، Ca، K وهي ١٨٥.١، ٢٢، ١١.٢,١٩.٥ ppm، على التوالي وكميات اقل من Cu , Cd ,Zn وهي٦.٣ ، ٠.٢,١.٢ ppm، على التوالي. كما تم دراسة تأثير المستخلصات المائية والكحولية على أنواع مختلفة من الأحياء الدقيقة حيث لوحظ أن للتركيز ٠,٥ ملغم امل تأثيرا فعّالا تجاه تثبيط نمو البكتريا المستخدمة ولكلا المستخلصين خاصة تجاه البكتريا الموجبة الغرام *Staphylococcus aureus*، في حين كان للتركيز ٠,٥

ملغم \امل للمستخلص الكحولي فعالية تثبيطية اكبر تجاه البكتريا السالبة الغرام *E.coli* من المستخلص المائي، كما لوحظ أن التركيز الأقل من ٠,١ ملغم \امل ليس لها تأثيرا تثبيطيا ولكلا المستخلصين على البكتريا المدروسة.

كما تم استخدام كروماتوغرافيا السائل عالي الأداء HPLC لتشخيص المركبات الفلافونويدية ومقارنتها مع المركبات القياسية، إذ اثبت التحليل عدم احتواء أوراق النبات على مركبي الكمبفيرول و المورين.