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Qualitative and quantitative analysis of phytochemicals of *Taraxacum officinale*

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The present study investigates the qualitative and quantitative analysis of the major bioactive constituents of medicinally important plant *Taraxacum officinale* in its aqueous and methanol extract of root, stem and flower. Saponins, flavonoids, alkaloids, phenols were highly concentrated in the stem, root and flower, with the higher concentration of flavonoids in the flower extracts. Phenols and steroids were also found present in the investigated plant parts. The percentage value of plant extracts in water and methanol are, stem (water extract 21%, methanol 18%), root (water extract 22%, methanol 17.8%), flower (water extract 19%, methanol 16%). The significance of the plant in traditional medicine and the importance of the distribution of these chemical constituents are discussed with respect to the role of the plant in ethnomedicine in Kashmir region of India.

Key words: Phytochemical constituents, *Taraxacum officinale,* qualitative and quantitative analysis.

INTRODUCTION

Medicinal plants are of great importance to the health of individuals and communities in general. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds. Many of the indigenous medicinal plants are used as spices and food plants. They also sometimes added to foods meant for pregnant women and nursing mothers for medicinal purposes as reported by (Okwu, 1999, 2001; Hill 1952).

In addition, the use of herbal medicine for the treatment of diseases and infections is as old as mankind. The World Health Organization supports the use traditional medicine provided they are proven to be efficacious and safe (WHO 1985). In developing countries, a huge number of people lives in extreme poverty and some are suffering and dying for want of safe water and medicine, they have no alternative for primary health care (Grieve, 1931). There is therefore the need to look inwards to search for herbal medicinal plants with the aim of validating the ethno-medicinal use and subsequently the isolation and characterization of compounds which will be added to the potential list of drugs.

Dandelion (*Taraxacum spp*) is used in many traditional and modern herbal medical systems, as particularly has been documented in Asia, Europe, and North America. The root is primarily considered a gastrointestinal remedy supporting digestion and liver function, while the leaf is used as a diuretic and bitter digestive stimulant. Preclinical research on dandelion has revealed numerous properties, including its actions as an inflammation modulator, diuretic, digestive stimulant, insulin stimulant, demulcent, prebiotic, immunomodulator, antiangiogenic, and antineoplastic, although not all studies agree.

These traditional sources consistently referred to the roots as helpful for the liver, while the leaves and flowers were regarded as useful diuretics and bitter digestive stimulants (Grieve, 1931) throughout its enormous growing range, all parts of the dandelion were eaten as food. *Taraxacum officinale*, known as dandelion, has been used in folk medicine in the treatment of hepatic disorders, inflammation and several women's diseases such as breast and uterus cancers. In Traditional Chinese medicine, it is also acclaimed as a nontoxic herb with exceptional values for its choleretic, diuretic, anti-rheumatic and anti-inflammatory properties. Several flavonoids including caffeic acid, chlorogenic acid, luteolin, and luteolin 7-glucoside have been isolated from the dandelion (Williams et al., 1996).

Taraxacum officinale leaves are rich in fiber, potassium, iron, calcium, magnesium, phosphorus,

vitamins A and C, the B vitamins thiamine and riboflavin, and protein as studied (Jackson, 1982; Schmidt, 1979) Sesquiterpene lactones impart a bitter taste to the plant, which is especially notable in the leaf but also in the root particularly when spring-harvested (Kuusi, 1985).

These compounds also likely explain the increase in bile production seen in animal studies with dandelion (Faber, 1958), with the studies themselves lending support to the traditional use of dandelion as a bitter digestive stimulant.

Studies on the effects of various dandelion extracts and compounds on the immune system are contradictory, some showing inhibition and some stimulation of tumor necrosis factor (Koo et al., 2004). This may suggest that dandelion extract has various effects on different lymphocyte populations or body tissues, or it may indicate that dandelion can modulate immune reactions.

In regard to hormone detoxification, a recent study compared the effects of an herbal formula containing dandelion (specifically, *T officinalis*), turmeric (*Curcuma longa*), artichoke (*Cynara scolymus*), rosemary (*Rosmarinus officinalis*), Schisandra (*Schisandra chinensis*), and milk thistle (*Silybum marianum*), a healthy diet, and placebo on hormone levels in 40 premenopausal women (Greenlee, 2007).

MATERIALS AND METHODS

Extraction

The plant (stem, leaves and roots) was thoroughly washed. Every part was cut into pieces, and were dried in an oven at 60°C for 9 hrs and pulverized. 50, 60 and 65g of the powdered material (stem, leaves and roots) were extracted first with 95% (v/v) hexane by Soxhlet apparatus, and then the residues were further extracted with dichloromethane separately. Same procedure was repeated for ethyl-acetate, methanol and water with same type of repeated residues.

All the solvents were used based upon their increasing polarity index. The extracts were evaporated to dryness on a water-bath. The plant extracts were distilled off with distillation apparatus and yielded quantities of (leaf, stem and root) extracts in different solvents were obtained and were further taken to evaluate the phytochemical studies. The percentage yield of plant extracts are shown in Table1.

Phytochemical analysis

Chemical tests for the screening and identification of bioactive chemical constituents in the medicinal plants under study were carried out in extracts using the standard procedures as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973).

Quantitative analysis of phytochemical constituents

Tannins

0.5g of powdered sample of each plant is boiled in 20ml of distilled water in a test tube and filtered 0.1% $FeCl_3$ is added to the filtered samples and observed for brownish green or a blue black colouration which shows the presence of tannins.

Saponins

2g of powdered sample of each plant is boiled together with 20ml of distilled water in a water bath and filtered. 10ml of the filtered sample is mixed with 5ml of distilled water in a test tube and shaken vigorously to obtain a stable persistent froth. The frothing is then mixed with 3 drops of olive oil and for the formation of emulsion which indicates the presence of saponins.

Flavonoids

A few chop of 1% NH_3 solution is added to the aqueous extract of each plant sample in a test tube. A yellow coloration is observed if flavonoids compound are present.

Terpenoids

5ml of aqueous extract of each plant sample is mixed with 2ml of $CHCl_3$ in a test tube 3ml of concentrated H_2SO_4 is carefully added to the mixture to form a layer. An interface with a reddish brown coloration is formed if terpenoids constituent is present.

Glycosides

1ml of concentrated H_2SO_4 is prepared in test tube 5 ml of aqueous extract from each plant sample is mixed with 2ml of glacial CH_3CO_2H containing 1 drop of $FeCl_3$. The above mixture is carefully added to 1ml of concentrated H_2SO_4 so that the concentrated H_2SO_4 is underneath the mixture.

If cardiac glycoside is present in the sample, a brown ring will appear indicating the presence of the cardiac glycoside constituent.

Alkaloids

5g o f the plant sample is prepared in a beaker and 200ml of 10% CH_3CO_2H in C_2H_5OH is added to the plant sample nearly 0.5g.

Table 1. The percentage yield of different extracts of different parts of *Taraxacum officinale.*

Solvent	Stem	Flower	Root
Hexane	12%	14%	11%
Dichloromethane	10%	11%	13%
Ethyl acetate	13%	15%	9%
Methanol	19%	21%	21%
Water	25%	27%	26%

Phenolic compounds

The extract (500 mg) was dissolved in 5 ml of distilled water. To this, few drops of neutral 5% ferric chloride solution were added. A dark green colour indicated the presence of phenolic compounds.

Quantitative determination of phytochemicals

Preparation of fat free sample

2g of the sample were defatted with 100 ml of diethyl ether using a Soxhlet apparatus for 2 h.

Determination of total phenols by spectrophotometric method

The fat free sample was boiled with 50ml of ether for the extraction of the phenolic component for 15 min. 5 ml of the extract was pipetted into a 50ml flask, then 10ml of distilled water was added. 2ml of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol were also added. The samples were made up to mark and left to react for 30 min for colour development. This was measured at 505nm.

Alkaloid determination using Harborne (1973) method

5g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

Flavanoid determination by the method of Bohm and Kocipai- Abyazan (1994)

10g of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper No 42 (125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

Saponin determination

20g of plant sample was dispersed in 200 ml of 20% ethanol. The suspension was heated over a hot water bath for 4 h with continuous stirring at about 55ºC. The mixture was filtered and the residue re-extracted with another 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90ºC. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of normal butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the sample were dried in the oven into a constant weight. The saponin content was calculated in percentage (Nahapetian and Bassiri, 1975).

RESULT AND DISCUSSION

The present study carried out on the *Taraxacum officinale* revealed the presence of medicinal active constituents. The phytochemical active compounds of *Taraxacum officinale* were qualitatively analyzed for stem, roots and flowers separately and the results are presented in Table 2, 3, 4. In these screening process alkaloids, tannins, saponins, flavonoids and terpenoids, glycosides, phenols shows different types of results in different solvents.

The medicinal value of plants lies in some chemical substances that have a definite physiological action on the human body. Different phytochemicals have been found to possess a wide range of activities, which may help in protection against chronic diseases. For example, alkaloids protect against chronic diseases. Saponins protect against hypercholesterolemia and antibiotic properties. Steroids and triterpenoids show the analgesic

Table 2. Phytochemical tests for stem part of plant.

		Hexane	Di-chloro methane	Ethyl acetate	Methanol	Water
1	**Alkaloid Test**					
A	Hager's Test	-ve	+ve	+ve	+ve	+ve
b	Wagner's Test	-ve	-ve	-ve	+ve	-ve
2	**Glycoside Test**	-ve	+ve	+ve	+ve	+ve
3	**Flavanoid Test**	+ve	+ve	+ve	+ve	+ve
4	**Tannins Test**	-ve	+ve	+ve	+ve	+ve
5	**Saponin Test**	+ve	+ve	+ve	+ve	+ve
6	**Terpenoid Test**	-ve	+ve	+ve	+ve	-ve
7	**Phenol Test**	-ve	-ve	+ve	+ve	+ve

Table 3. Phytochemical tests for roots.

		Hexane	Di-chloro methane	Ethyl acetate	Methanol	Water
1	**Alkaloid Test**					
a	Hager's Test	-ve	+ve	+ve	+ve	+ve
B	Wagner's Test	-ve	-ve	-ve	+ve	-ve
2	**Cardiac Glycosides**	+ve	+ve	+ve	+ve	+ve
3	**Flavanoid Test**	-ve	-ve	+ve	+ve	+ve
4	**Tannins Test**	-ve	+ve	+ve	+ve	+ve
5	**Saponin Test**	+ve	+ve	+ve	+ve	+ve
6	**Terpenoid Test**	+ve	+ve	+ve	+ve	+ve
7	**Phenol Test**	-ve	-ve	-ve	+ve	+ve

Table 4. Phytochemical tests for flower.

		Hexane	Di-chloro methane	Ethyl acetate	Methanol	Water
1.	**Alkaloid Test**					
a.	Hager's Test	-ve	+ve	+ve	+ve	+ve
b.	Wagner's Test	-ve	-ve	-ve	+ve	-ve
2	**Cardiac Glycosides**	+ve	+ve	+ve	+ve	-ve
3.	**Flavanoid Test**	-ve	-ve	+ve	+ve	+ve
4.	**Tannins Test**	-ve	+ve	+ve	+ve	+ve
5.	**Saponin Test**	+ve	+ve	+ve	+ve	+ve
6.	**Terpenoid Test**	+ve	+ve	+ve	+ve	+ve
7.	**Phenol Test**	-ve	-ve	+ve	+ve	+ve

properties. The steroids and saponins were responsible for central nervous system activities.

Phytochemical screening of the various extracts of *Taraxacum officinale* leaves were used to study the presence of contained alkaloids, flavonoids, steroids, saponins, tannins and triterpenoid and also have various medicinal values such as anti-inflammatory, anti-diabetic and analgesic activities and for central nervous system activity.

The importance of alkaloids, saponins and tannins in various antibiotics used in treating common pathogenic strains has recently been reported by (Kubmarawa, 2007; Mensah, 2008) reports alkaloids in 12 leafy vegetables studied. (Ayitey and Addae, 1977) and earlier recorded that bitter leaf contains an alkaloid which is capable of reducing headaches associated with hypertension.

The alkaloid content of the stem in water and methanol extract was found to be (1.1±0.03 and 0.8±0.04) respectively, which is more than the alkaloid content of *C. asiatica* having 0.31±0.06 and *I. cylindrica* 0.45±0.18, but the roots of *Taraxacum officinale* in water and methanolic extract possesses 2.28±0.01 and 2.20±0.02 alkaloid content which is more than *E. officinalis 0.24*±0.03 and *I.cylindrica 0.21*±0.07, also the flowers of the *Taraxacum officinale* are having less alkaloid content in their water and methanolic extract 0.5±0.03 and 0.4±0.01 than A*. indica 0.52*±0.12 H*. rosa - sinensis* 0.51±0.16 (Krishnaiah et al., 2009).

The flavonoid content of stem in water and methanol extract of the plant was found to be 1.0±0.02 and 0.9±0.09 respectively, which is more than found in species like *A. indica* 0.62±0.10 and 0.52±0.20 in *C.*

asiatica, and the flavonoid content in roots were found to be 0.13±0.20 and 0.17±0.20 which is less than found in *E.officinalis and H.rosa-sinensis* having *0.55*±0.13, 0.40±0.15 respectively. The flavonoid content of flower in water and methanol extract of *taraxacum officinale* was found 1.2±0.21, 1.1±0.25, compared with *M. oleifera* and *I. cylindrica* having 0.51±0.18 and 0.32±0.16 much less than the concerned plant. (Krishnaiah et al., 2009).

The saponin content of the plant in its stem portion was found to be 2.95±0.1in water extract and 2.5±0.01 in methanol extract, which is higher as compared to *A.indica* 2.1±0.13 and *C.asiatica* 2.2±0.11. The water extract of the root of the *taraxacum officinale* was found to contain 2.8±0.29 g of saponin and the methanol extract was found to contain 2.671 g of saponin and is much higher than the *E.officinalis* 1.1±0.05 g and *I.cylindrica* 1.4 ± 0.02. The water extract of the flower contained 2.4±0.29 and the methanol extract was found to contain 2.5±0.27, which is also more than *M.oleifera* 2.3±0.04 and *H.rosa-sinensis* 2.0±0.08. (Krishnaiah et al., 2009)

The phenolic content in various parts of plant was studied by spectroscopic method. The phenolic content of the stem in water extract was found to be 0.07±0.01 g and the methanolic extract was found to contain 0.008±0.03, the aqueous extract contained more phenol than *A.indica* 0.024±0.13, but less than *C.asiatica* 0.719±0.23, but the methanolic extract is too less from these two species. The aqueous extract of the root of *taraxacum officinale* contained 0.011±0.25 and the methanolic extract was found to contain 0.012±0.10 of phenol, which is less than *H.rosa-sinensis* 0.680±0.11 and *M.oleifera* 0.08±0.17. The aqueous extract of the flower of the *taraxacum officinale* was found to contain 0.007±0.0003, and the methanolic extract 0.008±0.0001 amount of phenol, which is much less than *E.officinalis* 0.037±0.19 and *I.cylindrica* 0.05±0.25. (Krishnaiah et al., 2009).

Conclusion

The plant screened for phytochemical constituents seemed to have the potential to act as a source of useful drugs and also to improve the health status of the consumers as a result of the presence of various compounds that are vital for good health.

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