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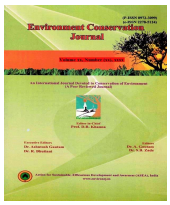

Standardization of roots of *Taraxacum officinale*

Somesh Thapliyal ✉
Department of Pharmaceutical Sciences, Hemvati Nandan Bahuguna Garhwal University,Srinagar Garhwal, Uttarakhand, India.
Mohd. Imtiyaz Ahmad
Azad Institute of Pharmacy and Research Lucknow, Uttar Pradesh, India.

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ABSTRACT

Taraxacum officinale **(Dandelion) belongs to the Asteraceae family; an edible herb commonly found in subtropical and temperate regions worldwide. Traditionally dandelion is used in diarrhea, gout, jaundice, diabetes, pneumonia, urinary problems and to purify the blood. The phytochemical study of dandelion discovered the occurrence of β- sitosterol, stigmasterol, taraxsterol, lactucopicrin, lactucin, cichorin, taraxacoside, taraxacerin, campesterol, homotaraxasterol, etc. Anti-inflammatory, immunostimulating, antimicrobial, antioxidant and antidiabetic activities of dandelion were studied. This plant has great therapeutic value and in order to overcome the problem of adulteration, the present study was aimed to standardize and preserve the quality parameters of the plant. Roots of *Taraxacum officinale* were subjected to measurements including macroscopy, microscopy, foreign organic content, ash value, extractive value, phytochemical screening, fluorescence analysis and chromatographic analysis. HPTLC analysis confirmed the presence of various phytocomponents. The macroscopic, microscopic and physico-chemical criteria presented here can help to identify the drug and to prepare the monograph.**

Introduction

Medicinal plants have historically been used to treat various ailments (Barnes *et al.*, 2007). Medicinal plants are found all over the world, but tropical countries have the greatest abundance (Duke and Martinez, 1994). Now a day's in medical practice there is a growing awareness and acceptance of the role of herbal drugs. Over eighty percentages of the global residents in underdeveloped nations depend on herbal treatments to lead a healthy life (Bodeker, 2005). The increasing demand of medicinal plants has resulted in a range of adulteration of herbal products, leading to consumer disappointment and, in some cases, serious consequences (Tewari, 1991). The main disadvantage of herbal medicines is lack of standardization techniques. Because of lack of standardization there is a chance of adulteration of plant material. To overcome the problem of adulteration plant must be properly standardized (Bauer, 1998).

The plant *Taraxacum officinale* belongs to the Compositae or Asteraceae family. It is an edible herb commonly known as dandelion. The plant is commonly found in open meadows and grassland up to 3500 m of the temperate and subtropical constituency of Asian and European, countries (Stewart-Wade *et al.*, 2002). It is the most recognizable weed in the world. Plant leaves are adopted as a salad; traditionally used as a laxative, stomachic, and hepatic stimulant (Dearing *et al.*, 2001). It has been used to treat diarrhea, gout, liver and spleen problems, diabetes, pneumonia, urinary problems, blood purifier, and diuretic (Schütz *et al.*, 2006). Liver protecting impact of root extract of dandelion investigated (Mahesh *et al.*, 2010). Mosquito repellant action of the milky latex of *Taraxacum officinale* was also reported (Stuart, 1979). Biologically this plant has been assessed for antiviral activity (Rehman *et al.*, 2016), anti-inflammatory (Koh *et al.*, 2010), immunostimulatory (Yoon, 2008), antidiabetic (Akhtar *et al.*, 1985), antimicrobial and antioxidant (Ghaima *et al.*, 2013) activities. Various parts of

Corresponding author E-mail: somesh.thapliyal@gmail.com
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dandelion are used to prepare foodstuff in addition to being employed in therapeutic applications. The underground parts of plant after roasting are used as a coffee substitute. Moreover, plant extracts are used as flavoring agent for a variety of foods, drinks, soft drinks, and frozen dairy items (Hfaiedh *et al.*, 2016).

Phytochemically major active constituents present in the root of *Taraxacum officinale* are β- sitosterol, stigmasterol, and taraxsterol. Taraxinic acid or lactucopicrin, lactucin, cichorin, taraxacoside, taraxacerine, taraxasterol , campesterol, homotaraxasterol, luteolin-7-glucoside, caffeic acid, ferulic acid, quercetin-7-glucoside, α-amyrin, β-amyrin, lupeol, taraxol, taraxaserol, arnidiol, faradiol, neoxanthin, flavoxanthin, chrysanthe-maxanthin, β-D-glucopyransoides, lutein-5-6-epoxide, taraxacoside, asparagine, apigenin-7-glucoside, chlorogenic acid are isolated from different parts of *Taraxacum officinale* (Singh *et al*., 2008**).**

This plant has great therapeutic value; hence the current study was done to standardize the root of selected plant to establish the quality parameters so that plant can be differentiate from other similar plant species. Roots of *Taraxacum officinale* were subjected to macroscopy, microscopy, foreign organic content, ash value, extractive value, phytochemical screening, fluorescence analysis, and chromatographic analysis.

Material and Methods

Taraxacum officinale root was procured from the Doddabetta area of Ootacamund, Tamilnadu, India and was identified by Dr. S. Rajan, Survey of Medicinal Plants and Collecting Unit, Arts College, Ootacamund. Proper examination of the untreated root sample of *Taraxacum officinale* was performed according to WHO guidelines (1998) under diffused sunlight and an artificial source to observe the color of selected root sample, other organoleptic characters like size, odor, shape and taste were also determined. To evaluate the microscopic features 10 to 12 mm thick sections were prepared using a rotary microtome. Toluidine blue used to stain sections. After staining, sections were examined under a microscope (Sanderson, 2020). The alcohol extract of coarse powder of *Taraxacum officinale* root was prepared. Hot extraction methods and cold extraction method were used for extraction of phytoconstituents; and qualitatively tested by phytochemical screening using the standard procedure (Sati and Kumar, 2015)(Archana *et al.*, 2012). Various physico-chemical parameters such as total ash value, acid-insoluble ash value, and water-soluble ash value, loss on drying, foreign organic matter and fluorescence analysis were carried out according to WHO (1998) guidelines. For chromatographic analysis, 1 g powdered drug was extracted with ethanol to prepare the sample, followed by filtration through Whatmann filter paper. Precoated HPTLC silica gel G254 plates (Merck) were used in the study. A solvent solution of chloroform: methanol (8:2) was used as the mobile phase. Sample was applied to the HPTLC plate as an 8 mm bend with the help of CAMAG Linomat IV applicator. CAMAG Twin Trough Chamber was used to develop the plate. A densitometer was used to scan a plate of *Taraxacum officinale* alcoholic extract at 254 nm (Sherma, 2010).

Results and Discussion

A systematic approach is required in pharmacognostic study to confirm and determine the identity, purity and quality of a raw drug. This comprehensive and rigorous pharmacognostic study will provide useful information for future research. It is important to note that macroscopic plant evaluation is subjective; hence substitutes or adulterants can be very similar to the original material (Rehman *et al*., 2016). Therefore, the macroscopic findings must be validated and authenticated. Morphological evaluation of *Taraxacum officinale* root was performed based on organoleptic parameters such as color, smell, shape, size and taste. It was found that the length of the root was about 30 cm and the thickness was about 15-25 cm. The outer surface of the fresh root was yellowish brown while the inner surface was white and fleshy. Thin and fibrous rootlets were present. Upon drying, the occurrence of longitudinal wrinkles was observed. The taste was bitter. The fracture was short (Fig. 1).

The microscopic evaluation is essential to identify the crude drugs. It is one of the crucial pharmacognostic parameter in preparation of modern monographs (Koh *et al.,* 2010).T.S. and

L.S. of the root sample is studied. Microscopic study showed the occurrence of periderm, secondary phloem, secondary xylem, vessels and xylem parenchyma and seive tube member. The periderm consists four to five layers of cells. The cortex region is wide and homogenous. The cortical cells are parenchymatous in nature. Secondary phloem is cylindrical in shape having sieve element and parenchymatous cells. Secondary xylem has several vessels and fibres. The vessels are variable in diameters. The xylem fibers are thick and lignified (Fig. 2). Analysis of physicochemical parameters of a crude drug is very important as it helps in identification and in setting of proper standards (World Health, 1998).

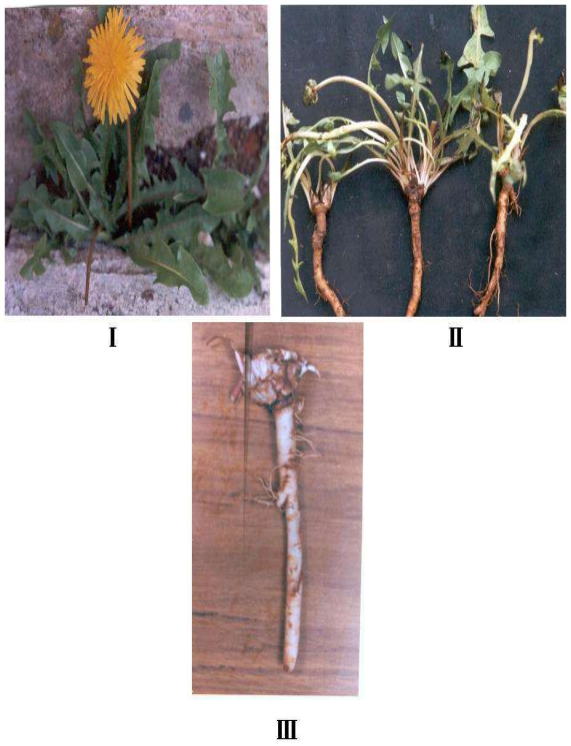

Figure 1: *Taraxacum officinale* (I: Flower, II: Aerial parts and Root, III: Root).

Ash values, extractive values, loss on drying, and fluorescence reaction with various chemical reagents were investigated and the results are present in Table 1. Any organism or part other than plant material are known as foreign organic material; was determined according to WHO guideline, found to be 0.189%. The presence of moisture in the drug sample can lead to microbial contamination due to enzymatic hydrolysis.The loss of water content on drying was determined, The ash value is useful for identifying excess sand and depleted low-grade drugs. and was found to be 7.863%. This test is based on gravimetric analysis.

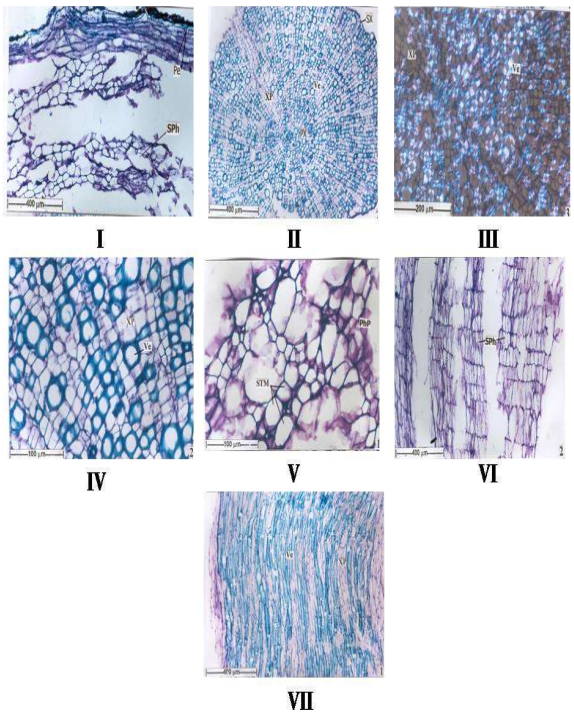

Figure 2: Microscopic Characters of root of Taraxacum officinale (I to VII). (Pe:Periderm, SPh: Secondary Phloem, SX: Secondary Xylem, XP: Xylem Parenchyma, Ve: Vessel, PX: Primary xylem, PhP: Phloem Parenchyma, STM: Sieve Tube Members).

Table 1: Results of physiochemical parameters

Parameters	Percentage
Foreign organic matter	0.189 %
Loss on Drying	7.863 %
Total ash	5.546 %
Sulphated ash	3.571 %
Acid insoluble ash	1.496 %
Water soluble ash	1.581 %
Alcohol soluble extractive value (Hot Extraction Method)	12.57 %
Alcohol soluble extractive value (Cold Extraction Method)	8.943 %

If the value of the ash is not within the limit, then this directly affects the purity of the drug, and indirectly affects the quality of the drug. Inorganic radicals like phosphates, carbonates and silicates of sodium, calcium and magnesium etc. are present in ash. When a powdered drug is ignited at a temperature 400°C, ash is obtained, which is known as total ash. It was determined and was 5.546%. When the residue obtained after boiling the total ash with dilute hydrochloric acid and then ignited at 400°C, it is known as acid-insoluble ash; gives an idea about the concentration of silica, which is mainly present as sand. Hydrochloric acid is used because silica is soluble in hydrochloric acid. The acid-insoluble ash was found to be 1.496%. When powder drugs are first burned to ash, then treated with sulfuric acid and burned to in turn get the ash known as sulphated ash, and it was found to be 3.571%. The weight difference between the total ash and the residue obtained after treating the total ash with water is known as water soluble ash; was determined and found to be 1.581%. The extractive value helps to evaluate solubility of drug constituents that cannot be calculated in any other way. It gives an idea about the solubility pattern of phytoconstituents based on the polarity of solvent. The extractive value can be determined by hot extraction methods and cold extraction methods. Depending on the choice of solvent, different solvents can be used to determine the extraction value. The alcohol soluble extract value of the root of *Taraxacum officinale* was determined by the hot extraction method and the cold extraction method and was found to be 12.57% and 8.943%, respectively. Fluorescence analysis of the drug was observed under daylight and UV light using different solvents such as hydrochloric acid, sulfuric acid, ammonia solution, aqueous sodium hydroxide. The fluorescence behavior tabulated in Table 2.

Table 2: Observation under fluorescence analysis

Treatment	Visible	Short UV light 254 nm	Long UV 366nm
Powder as such	Light- brown	Dark- brown	Dark- brown
Powder +distilled water	Light- brown	Dark- brown	Dark- brown
Powder +5% aqueous NAOH	Yellowish- brown	Dark -brown	Dark- brown
Powder + ammonia solution	Light- green	Light- green	Black
Powder +conc. H_2SO_4	Light -brown	Light- brown	Black
Powder + 50 % HCL	Light- brown	Dark- brown	Dark- brown

Preliminary phytochemical screening was carried out to determine the presence of chemical constituent's category. The results of phytochemical screening are shown in Table 3. Preliminary phytochemical screening of root of *Taraxacum officinale* gave the colors with modifications according to colors of extract indicating the presence of the various active phytoconstituents metabolites as follows: carbohydrate (Molish's violet ring at the junction of two liquids, Fehling's yellow to red, Bendict's greenish yellow to red), protein (Biuret violet, Xanthoprotein yellow precipitates), amino acids (Ninhydrin purple colour) glycosides (Legal pinkish red), steroids and sterol (Liebermann-Burchard purple / violet , Salkowski red color ring at junction of two layer) tannins (lead acetate white precipitates, Ferric chloride bluish black) and saponin (Foam test persistent of white foam at upper layer). Root extract of *Taraxacum officinale* revealed the presence of cardiac glycosides, terpenoids (antiviral activity), phenols (Immuno modulatory and antihyperglycemic) (Kenny *et al.*, 2015) , tannins (wound healing activity) (Ajaz *et al.*, 2019), flavonoids (antioxidant and anti-inflammatory activity) (Hagymási *et al.*, 2000), steroids and sterols (Antihyperglycemic and Anti inflammatory activity) (Petlevski *et al.*, 2003)(Jones and Persaud, 1998) carbohydrates, proteins and amino acids with absence of alkaloids and fixed oil. Also similar finding reported in the other species of the same family (Jaramillo-Jaramillo *et al.*, 2016). HPTLC is one of the most demanding technical methods for the quantitative and qualitative analysis of medicinal plants (Marston, 2007). According to HPTLC results ethanolic extract of selected sample under 254 nm UV light, showed the presence of seven spots suggesting presence of various compounds in the extract. The Rf value of various phytocomponents

present in alcoholic extracts was found to be 0.07, 0.10, 0.24, 0.34, 0.55, 0.62 and 0.83. HPTLC analysis showed the presence of seven components. It could not be found the name of component there is further need to isolate each component.

Table 3: Result of Phytochemical Screening

Tests	Ethanol extract
Alkaloids	
a) Dragondorff's Test	-
b) Wagner's Test	-
c) Mayer's Test	-
d) Hager's Test	-
Carbohydrates	
a) Molisch Test	+
b) Fehling's Test	+
c) Benedict's Test	+
Proteins	
a) Biuret Test	+
b) Xanthoprotein Test	+
Amino Acid	
a) Ninhydrin Test	+
Glycoside	
a) Legal Test	-
b) BaljetTest	-
Steroids and Sterols	
a) Libermann Burchard Test	+
b) Salkowsky Test	+
Flavonoids	
a) Extract + Tin + HCI	+
Tannins and Phenol	+
Triterpenoids	+
Saponin Test	
a) Foam Test	+
Fixed oils	
a)spot Test	-

+ Represent positive result while – represent negative result

Conclusion

For thousands of years, medicinal plant used as a home remedy to treat a variety of diseases in both developed and developing countries. In recent years, consciousness about the utilization of medicinal plants increased, and pharmaceutical companies are trying to formulate dosage forms based on herbs due to less side effects of medicinal plants compared to synthetic drugs. The overexploitation of crude drugs is increasing due to increasing demand, and instead of genuine drug, lower quality plant materials or substitute or adulterated drugs are being used. Therefore it is necessary to ascertain the quality parameters of herbal drugs. The current study emphasized the standardization of the root portion of *Taraxacum officinale*. The root part was chosen because it has been found in the literature that the root of *Taraxacum officinale* has been reported for various pharmacological activities. In order to overcome the problem of root adulteration with other similar species of genus *Taraxacum*, a standard for identifying the drug needs to be established. Despite the modern method of analysis, identification and standardization by pharmacognostical studies is still more consistent, precise and economical means. The macroscopic, microscopic and physico-chemical criteria presented here could be helpful to enhancement information about the identification, authentication and standardization. Terpenoids, phenols, tannins, flavonoids, steroids, carbohydrate, protein, and amino acid confirmed in selected drug sample. In the HPTLC analysis, the presence of various phytocomponents was confirmed. The information established can also be helpful to arrange monograph of plant.

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Conflict of interest

The authors declare that they have no conflict of interest.

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