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Comparative Antioxidant Power Determination of *Taraxacum officinale* by FRAP and DTPH Method

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Abstract

Antioxidant activity has been assessed by *in vitro* method for phytochemical fraction of plant; viz. water extracts of root, stem and flower of *Taraxacum officinale* plant. This investigation was under taken to evaluate water extracts of *taraxacum officinale* plant for possible antioxidants potential. Antiradical activity of all extracts was measured by 1, 1, diphenyl-2, picrylhydrazyl (DPPH) assay and was compared to ascorbic acid and Ferric reducing power (FRAP) of the extract. In the present study two *in vitro* models were used for evaluation of antioxidant activity. The first one method was for direct measurement of reducing power and the other one for radical scavenging activity. The present study revealed the *Taraxacum officinale* has significant radical scavenging activity.

Keywords: Antioxidant; Reducing power; Anti-radical; DPPH; *in vitro*; *Taraxacum officinale*

Introduction

The antioxidants are a variety of Vitamins, minerals and enzymes that help to protect the body from the formation and disposal of free radicals. Some people have the idea that an antioxidant is a specific nutrient, yet it actually refers to any nutritional compound that has these qualities. They are useful in the fight against ageing and degenerative diseases, it must be kept in mind that they have a wide sphere of influence on the body, and they can positively influence your general wellbeing.

The big source of antioxidants is the green belt in the form of plants, which make life possible on this planet. The use of herbal medicine for the treatment of diseases and infections are as old as mankind. The World Health Organization supports the use of 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.

Dandelion is considered to be an excellent general tonic and a "natural" diuretic. Dandelion tea has shown to be very helpful as a liver detoxicant. It also improves functions of gallbladder, pancreas, spleen and intestines. Dandelion can reduce inflammations in cases of hepatitis and cirrhosis, help gallstone dissipation and improve kidney functions.

An antitumor action of the aqueous extract of *Taraxacum officinale* has been reported in the scientific literature [1]. Dandelion's active ingredients are found in both the roots and leaves. The leaves contain bitter *sesquiterpene lactones* such as taraxinic acid and *triterpenoids* such as cycloartenol. The roots contain these compounds as well as phenolic acids and inulin [2,3]. Potassium is present in the leaves at 297 mg per 100 grams of leaves [3]. The leaves also contain substantial amounts of Vitamin A (14,000 units per 100 grams of leaves, compared with 11,000 units per 100 grams of carrots) [3]. The sesquiterpene lactones found in both leaves and roots have demonstrated diuretic effects [4]. They also stimulate bile flow from the liver. A Chinese case series reported that an herbal combination including dandelion was helpful in treating 96 adults with chronic hepatitis B infection [5]. In Chinese, Arabian and Native American traditional medicine it is used to treat a variety of diseases including cancer [6,7].

Evidence suggests dandelion may influence nitric oxide production [8]. Nitric oxide is important for immune regulation and defense; however, this molecule can be inhibited by cadmium. Classically listed as a cholagogue, dandelion root is approved by the German Commission E for the treatment of disturbances in bile flow, stimulation of diuresis, loss of appetite, and dyspepsia [9].

Dandelion root contains an abundance of sesquiterpene lactones, also known as bitter elements principally taraxacin and taraxacerin [10]. Other related compounds include beta-amyrin, taraxasterol, and taraxerol, as well as free sterols (sitosterin, stigmasterin, and phytosterin). Other constituents include polysaccharides (primarily fructosans and inulin), smaller amounts of pectin, resin, and mucilage, and various flavonoids. Three flavonoid glycosides – luteolin 7-glucoside and two luteolin 7-diglucosides – have been isolated from the flowers and leaves. Hydroxycinnamic acids, chicoric acid, monocaffeyltartaric acid, and chlorogenic acid are found throughout the plant, and the coumarins, cichoriin, and aesculin have been identified in the leaf extracts [11]. Dandelion leaves are a rich source of a variety of vitamins and minerals, including beta carotene, non-provitamin A carotenoids, choline, iron, silicon, magnesium, sodium, potassium, zinc, manganese, copper, and phosphorous.

The present research work was carried out about the antioxidant potential determination of the dandelion. As per the research methodology, the plant is heavily commenced with phytochemical, so could be associated with high potential of antioxidants, therefore

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the determination of antioxidant potential of the plant became a compulsory issue, so was carried out the concerned research work.

Materials and Methods

Plant materials

The plant material was collected from Kupwara region of Kashmir and was authenticated from FRI Dehradun. The collection process was preferably done in the dry condition. Plant was weighed before and after the removal of unwanted material kept under shade at room temperature for the removal of extra moisture. The plant samples were air dried and grounded into uniform powder with a grinder. All the plants parts i.e. stem, flowers and roots were collected separately and were subjected to different operations individually.

Experimental

Extraction

The extraction procedure was carried out with water. The extraction was done by Soxhlet extraction method. A thimble was used in order to get the purest form of extract. 90 g of the root material was used for extraction, 80 g of flower and 105 g of stem plant material was used for extraction purpose. The percentage yields of various extracts are mentioned in table 1.

Antioxidant activity determination: The antioxidant property of plant extracts were determined by two given below mentioned methods.

The antioxidant activities as measured by FRAP method (Ferric reducing ability of plasma or plant) according Benzie and Strain, (1996-19990).

DPPH free radical scavenging assay was measured using DPPH free radical test, employing method of Wong et al. (2005).

Frap- working solution: 25 ml acetate buffer, 2.5 ml TPTZ solution (2, 4, 6- Tripyridyl-S- triazine) and 2.5 ml $FeCl_3.6H_2O$ solution was freshly prepared. Aqueous solution of known $FeCl_4.7H_2O$ was used for calibration.

DPPH method: DPPH Scavenging activity was measured by the spectrophotometric method. A stock solution of DPPH (1.5 mg/ml in methanol) was prepared such that 75 µl of it in 3 ml of methanol. Decrease in the absorbance in presence of sample extract at different concentration (10-100 µg/ml) was noted after 15 min. IC_{50} was calculated from % inhibition.

Protocol for DPPH Free radical scavenging activity

Preparation of stock solution of the sample: 10 mg of extract was dissolved in 10 ml of methanol to get 1000 µg/ml solution.

Dilution of test solution: 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 µg/ml solution of test were prepared from stock solution.

Preparation of DPPH solution: 15 mg for DPPH was dissolved in 10 ml of methanol. The resulting solution was covered with aluminum foil to protect from light.

Estimation of DPPH scavenging activity: 75 µl of DPPH solution was taken and the final volume was adjusted to 3 ml with methanol, absorbance was taken immediately at 517 nm for control reading. 75 µl of DPPH and 100 µl of the test sample of different concentration were put in a series of test-tubes and final volume was adjusted to 3 ml with methanol. Absorbance at zero time was taken in UV-Visible at 517 nm for each concentration. Final decrease in absorbance of DPPH with sample of different concentration was measured after 15 minute at 517 nm.

Percentage inhibitions of DPPH radical by test compound were determined by the following formula.

% Reduction=Control absorbance – Test absorbance/ Control absorbance X 100.

Calculation of IC_{50} value using graphical method.

Observation

Percentage yield of various plants extracts (Table 1)

Antioxidant Property

Preparation of standard solution:- 0.01 ml of $FeSO_4$, solution was mixed with 1.5 ml of FRAP reagent and volume was made up to 5 ml with distilled water, rest of dilutions were prepared by varying the volume of ferrous sulphate solution with distilled water. Monitor up to 5 mM/cm path length 37°C. Absorbance was recorded (Tables 2-5 and Figures 1-4).

Absorbance was recorded at 593 nm/cm and from the standard graph curve the value of (ε) comes out to be

$\varepsilon = 1.5 \times 10^{-4}\ Lmol^{-1}cm^{-1}$.

Antioxidant activity testing by DPPH method

DPPH Free Radical Scavenging Activity of Ascorbic Acid (Table 6).

S/No.	Solvent	Percentage Yield of Plant Extracts		
		Roots	Stems	Flowers
1.	Water	35%	57%	43%

Table 1: Percentage yield of various plant extracts.

Concentration	Absorbance (µM)
10	0.038
20	0.066
30	0.096
40	0.121
50	0.152
60	0.184
70	0.216
80	0.252
90	0.277
100	0.304

Table 2: Preparation of standard solution.

S. No.	Concentration (µL)	Absorbance	Antioxidant Power (µM/L)
1	10	0.106	7.06
2	20	0.241	16.00
3	30	0.334	22.2
4	40	0.467	31.1
5	50	0.514	34.2
6	60	0.589	39.2
7	70	0.691	46.0
8	80	0.724	48.2
9	90	0.750	50.0
10	100	0.765	51.0

Table 3: Data sheet for the Antioxidant properties of the flower extract.

S. No.	Concentration (µL)	Absorbance	Antioxidant Power (µM/L)
1	10	0.104	6.9
2	20	0.202	13.6
3	30	0.269	17.9
4	40	0.390	26.0
5	50	0.512	34.1
6	60	0.555	37.0
7	70	0.568	37.8
8	80	0.600	40.0
9	90	0.653	43.5
10	100	0.690	46.0

Table 4: Data sheet for the Antioxidant properties of the whole plant extract.

S. No.	Concentration (µL)	Absorbance	Antioxidant Power (µM/L)
1	10	0.094	6.2
2	20	0.267	17.0
3	30	0.345	23.0
4	40	0.428	28.5
5	50	0.457	30.4
6	60	0.474	31.6
7	70	0.478	31.8
8	80	0.489	32.6
9	90	0.500	33.3
10	100	0.510	34.0

Table 5: Data sheet for the Antioxidant properties of the root extract.

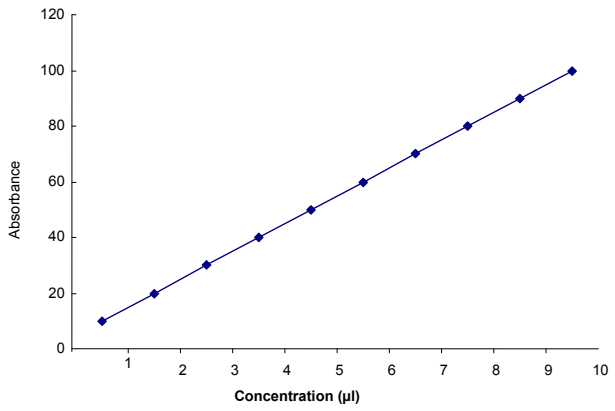

Figure 1: Standard curve for FRAP reagent solution.

Absorbance of the sample at 517 nm

Absorbance of Control = 0.490

Antioxidant Power of *Taraxacum officinale* (Stem) (Table 7)

Absorbance of the sample at 517 nm

Absorbance of Control = 0.490

Antioxidant Activity of Root Extract of *T. officinale* (Table 8)

Absorbance of the sample at 517 nm Absorbance of Control = 0.490

Antioxidant Activity of Flower Extract of *T. officinale* (Table 9)

Absorbance of the sample at 517 nm Absorbance of Control = 0.490

Discussion

Reactive oxygen species (ROS) are involved in the pathogenesis

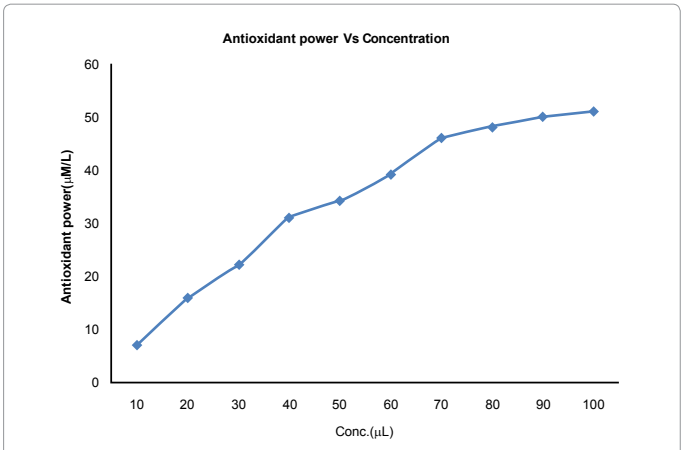

Figure 2: Line Graph showing the variation of antioxidant potential of the flower extract with concentration.

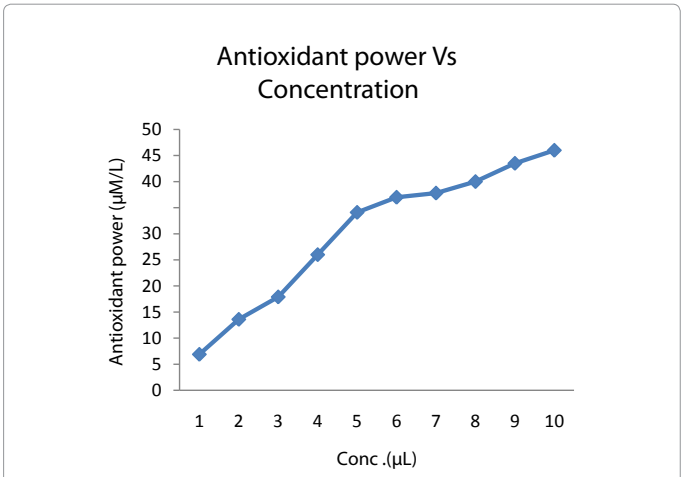

Figure 3: Line Graph showing the variation of antioxidant potential of the whole plant extract with concentration.

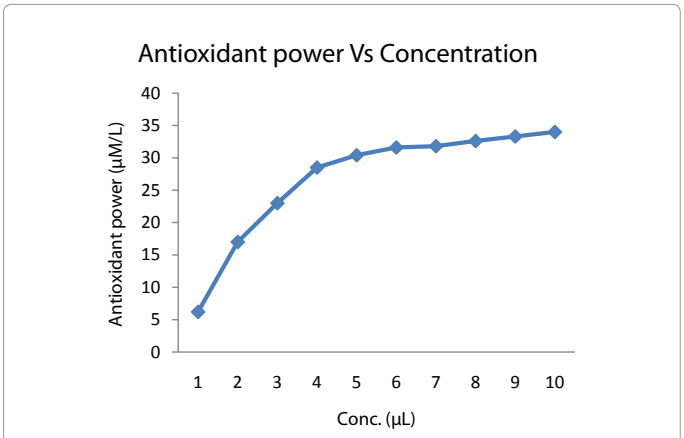

Figure 4: Line Graph showing the variation of antioxidant potential of the root extract with concentration.

S.No	Concentration In (3.3 µg/ml)	Absorbance	% Reduction	IC_{50} Value (µg/ml)
1.	10	0.292	40.63	27
2.	20	0.269	45.90	
3.	30	0.244	50.60	
4.	40	0.226	54.45	
5.	50	0.195	60.20	
6.	60	0.177	63.33	
7.	70	0.162	67.21	
8.	80	0.141	71.45	
9.	90	0.122	75.30	
10.	100	0.088	82.16	

Table 6: DPPH Free Radical Scavenging Activity of Ascorbic Acid.

S.No	Concentration In (3.3 µg/ml)	Absorbance	% Reduction	IC_{50} Value (µg/ml)
1.	10	0.272	44.48	37
2.	20	0.260	46.93	
3.	30	0.258	47.34	
4.	40	0.248	49.38	
5.	50	0.210	57.14	
6.	60	0.175	64.28	
7.	70	0.160	67.34	
8.	80	0.140	71.42	
9.	90	0.131	73.26	
10.	100	0.121	75.30	

Table 7: Antioxidant Power of *Taraxacum officinale* (Stem).

S.No	Concentration In (3.3 µg/ml)	Absorbance	% Reduction	IC_{50} Value (µg/ml)
1.	10	0.277	43.46	55
2.	20	0.270	44.89	
3.	30	0.265	45.91	
4.	40	0.254	48.16	
5.	50	0.249	49.18	
6.	60	0.241	50.81	
7.	70	0.217	55.71	
8.	80	0.190	61.22	
9.	90	0.177	63.87	
10.	100	0.164	66.53	

Table 8: Antioxidant Activity of Root Extract of *T. officinale* Absorbance of the sample at 517nm Absorbance of Control = 0.490.

S.No	Concentration In (3.3 µg/ml)	Absorbance	% Reduction	IC_{50} Value (µg/ml)
1.	10	0.370	24.48	47
2.	20	0.353	27.95	
3.	30	0.321	34.48	
4.	40	0.286	41.63	
5.	50	0.255	48.97	
6.	60	0.216	55.91	
7.	70	0.192	60.81	
8.	80	0.165	66.32	
9.	90	0.128	73.87	
10.	100	0.102	79.18	

Table 9: Antioxidant Activity of Flower Extract of *T. officinale* Absorbance of the sample at 517 nm Absorbance of Control = 0.490.

of various diseases. Uncontrolled oxidation is caused by free radicals. Natural antioxidants that are present in herbs and spices are responsible for inhibiting or preventing the deleterious consequences of oxidative stress. Spices and herbs contain free radical scavengers like polyphenols, Flavonoid and Phenolic compounds. The effect of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen donating ability. The DPPH assay is technically simple, but some disadvantages limit its applications. Besides the mechanistic difference that normally occurs between antioxidants and peroxyl radicals, DPPH is long-lived nitrogen radical, which bears no similarity to the highly reactive and transient peroxyl radicals involved in lipid peroxidation. Many antioxidants that react quickly with peroxyl radicals may react slowly or may even be inert to DPPH [12].

Phenolic compounds are known as powerful chain breaking antioxidants. Phenols are very important plant constituents because of their scavenging ability due to their hydroxyl groups. The Phenolic compounds may contribute directly to antioxidative action (Table 10, Figure 5).

Ferric reducing antioxidant power (FRAP) measures the ability of antioxidants to reduce ferric 2, 4, 6- Tripyridyl-s-triazine complex to intensively blue colored ferrous complex in acidic medium. Hence any compound which is having redox potential lower than that of redox pair Fe (III)/Fe (II) can theoretically reduce Fe (III) to Fe (II) [12].

Conclusion

Taraxacum officinale showed strong antioxidant activity by inhibiting DPPH, and reducing power activities when compared with standard L-ascorbic acid. In addition, all the extracts of plant was found to contain a noticeable amount of total phenols, which play major role in controlling oxidation. The results of this study show that the extracts of *Taraxacum officinale* can be used as easily accessible source of natural antioxidant. However, the chemical constituents present in the extract, which are responsible for this activity, like flavonoid,

S. No	Concentration	% Inhibition of Various Plant Parts			
		Ascorbic acid	Plant Stem	Plant Roots	Plant Flowers
1	10	40.63	44.48	43.46	24.48
2	20	45.9	46.93	44.89	27.95
3	30	50.6	47.34	45.91	34.48
4	40	54.45	49.38	48.16	41.63
5	50	60.2	57.14	49.18	48.97
6	60	63.33	64.28	50.81	55.91
7	70	67.21	67.34	55.71	60.81
8	80	71.45	71.42	61.22	66.32
9	90	75.3	73.26	63.87	73.87
10	100	82.16	75.3	66.53	79.18

Table 10: showing % Inhibition of various plant extracts & Ascorbic Acid.

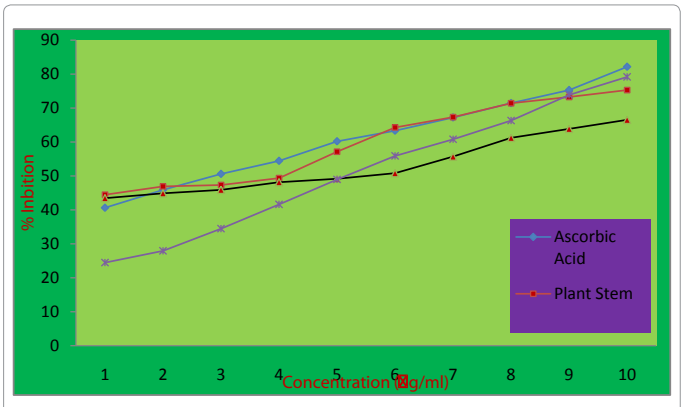

Figure 5: Graph Showing the Comparative Study of Antioxidant Potential of Ascorbic Acid and Various plant extracts.

alkaloids, steroids, terpenoids, tannins, reducing sugars and proteins present in the extract may be responsible for such activity. Some of these constituents have already been isolated from this plant. Hence, the observed antioxidant activity may be due to the presence of any of these constituents.

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