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Research Article

## SCREENING OF MEDICINAL PLANTS FOR IRON CHELATING AND ANTIOXIDANT ACTIVITY

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### ABSTRACT

A study was carried out to determine the iron chelating and antioxidant activity of methanolic extracts of five medicinal plants from Uttarakhand, India. The antioxidant property was studied using ABTS radical scavenging, phosphomolybdenum and iron chelating potential assays. A strong correlation was found between antioxidant potential with that of phosphomolybdenum potential in the methanolic plant leaves extracts. A satisfactory correlation was calculated between total phenolic content and antioxidant potential but no satisfactory relationship was found with iron chelating potential indicating that polyphenols are not main chelating particles. The values of ABTS and iron chelating assay indicate that all the five plants were good sources of antioxidants.

**Keywords:** ABTS, antioxidant, iron chelation, medicinal plant, methanolic extract.

### INTRODUCTION

Recent years, research to determine the active compounds from various traditionally used natural sources including medicinal plants has received attention (Schaich *et al.*, 2015). Free radical is an atom, molecule, or ion that has unpaired valence electrons or an open electron shell or unpaired electron spinning on the peripheral layer around the nucleus. The family of free radicals generated from the oxygen is called reactive oxygen species (ROS) which cause damage to other molecules by extracting electrons from them in order to attain stability (Zheng and Huang, 2001). ROS are various forms of activated oxygen, which

include free radicals such as superoxide anion radicals ( $O_2^-$ ), hydroxyl radicals ( $OH^\cdot$ ), non-free radicals ( $H_2O_2$ ) and singlet oxygen (Halliwell, 1995). The ROS and Reactive nitrogen species (RNS) are produced through normal cellular metabolism and concentrate in high concentration which becomes toxic. Mammalian cells hold intracellular defenses such as superoxide dismutase, catalase or glutathione peroxidase, consecutively to protect the cells from excessive levels of free radicals. Exogenous addition of compounds such as vitamins (A, E), minerals (Se, Zn), or proteins (transferrin, ceruloplasmin, albumin) can

provide additional protection (Di Carlo *et al.*, 1999). The oxidative stress caused from imbalance between the generation and the neutralization of ROS by antioxidant is responsible for many human diseases such as aging, cancer, and neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, and Huntington's disease (Finkel and Holbrook, 2000; Engelhart *et al.*, 2002; Ebrahimzadeh and Bahramian, 2009). Antioxidants have been used in the food industry to extend the shelf life of foods, particularly those rich in polyunsaturated fats which are readily oxidized by molecular oxygen and are a major cause of quality deterioration, nutritional losses, off-flavor development and discoloration. Artificial antioxidants, such as propyl gallate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertiary butylhydroquinone have been widely used in industry to control lipid oxidation in foods. However, these synthetic antioxidants have been barred due to their potential health risks and toxicity (Kahl and Kappus, 1993; Kalt *et al.*, 1999). The interest for searching antioxidants from natural sources has received much consideration and efforts have been put into recognizing compounds and source of plants that can act as antioxidants. These natural antioxidants or other compounds that can neutralize free radicals may be of crucial importance in the prevention of vascular diseases, some forms of cancer (Pulido *et al.*, 2000; Sekine *et al.*, 2002) and oxidative stress responsible for DNA, protein and membrane damage. External antioxidants, like antioxidants extracted from plants, can be administered in order to combat those radicals (Miller, 1996; Tachakittirungrod *et al.*, 2007; Patel *et al.*, 2010).

In this study, we investigated phenolic, flavonoid content and antioxidant activity of *Thymus serpyllum* (TS), *Barleria prionitis* (BP), *Spilanthes acmella* (SA), *Clerodendrum indicum* (CI) and *Pogostemon cablin* (PC) that

were commonly found in the area of FRI, Uttarakhand.

## MATERIALS AND METHODS

### Plant materials

Five plants *Thymus serpyllum* (Lamiaceae), *Barleria prionitis* (Acanthaceae), *Spilanthes acmella* (Compositae), *Clerodendrum indicum* (Verbena), and *Pogostemon cablin* (Lamiaceae) were collected from local area of Forest Research Institute, Dehradun in the month of January 2013. The plant samples were washed with tap water to remove debris, soil particles and damaged portions were detached. The leaves of the samples were undressed from the plants and dried in a convection oven at 45°C for 48 h until there was no change in weight. The dried leaves were stored in sealed vacuum desiccator with silica gel as a desiccant (Martyn *et al.*, 2002).

### Extraction of plant materials

The dried leaves were ground to get into powdered using a domestic blender and 0.5 g of powder was extracted using 50 of methanol in a reflux setup for about 12 h. The obtained methanol extract was filtered through Whatman No. 1 filter paper and the liquid extracts were concentrated under vacuum to yield dry extracts. The obtained concentrated extracts were used for analysis without further treatment.

### Total phenol content

The total phenolic contents of different medicinal plants were quantified using the Folin-Ciocalteu method (Singleton *et al.*, 1965) with few modifications. In brief, 0.125 ml of a properly diluted sample (0.1-1mg/ml) was added to 0.5 ml of distilled water and was mixed with 0.125 ml of the Folin-Ciocalteu phenol reagent. The reaction mixture was incubated for 6 min at room temperature, then 1.25 ml of 7% Na<sub>2</sub>CO<sub>3</sub> and 3 ml of distilled water were added to the solution. The reaction mixtures were

incubated for 90 min at room temperature, and then absorbance at 765 nm was measured using a Perkin Elmer UV-Vis Lambda 25 model spectrophotometer with matched cuvettes. A calibration curve was prepared using the standard solution of gallic acid (0.2-1 mg/ml,  $r^2 = 0.9994$ ). The results were expressed as milligrams of gallic acid equivalents (GAE) per gram of dry powder. All determinations were performed in triplicate (n=3).

#### **Flavonoid content**

The amount of flavonoids was determined using spectrophotometric method (Quettier-Deleu *et al.*, 2000) with slight modifications. Plant extract (1ml of 1mg/ml) in methanol were separately mixed with 0.5 ml methanol, 1 ml 2% aluminum chloride, 0.1 ml 1 M potassium acetate and 2.8 ml distilled water. The samples in triplicate were incubated for 1 h at room temperature and the absorbance was read at  $\lambda$  max = 415nm and the mean value was obtained. The same procedure was repeated for the rutin and the calibration line was constructed. Concentration of flavonoids in extracts was expressed in terms of rutin equivalent (mg of Ru/g of extract).

#### **Iron chelation activity**

The chelating effect on ferrous ions was determined according to the method of Dinis *et al.* (1994) with few changes. The reaction solution was prepared by mixing 0.25ml of extract at different concentration with 0.06 ml of 2 mM FeCl<sub>2</sub>. This was followed by the addition of 0.2 ml of 2 mM ferrozine and finally the volume increased by adding 3 ml of solvent, shaken and left to react at room temperature for 10 min before determining the absorbance at 562 nm. All samples are analyzed in triplicates and mean value was represented. The chelating effect (%) was calculated from the formula:

Metal chelating effect (% inhibition) =  $[(A_0 - A_1) / A_0 \times 100]$ ; where  $A_0$  = absorbance of

control/blank,  $A_1$  = absorbance of the extract/ standard.

#### **Molybdenum reduction potential**

The phosphomolybdenum reduction capacity of extracts was evaluated using method of Prieto *et al.* (1999). The 0.4 ml of extract at concentration of 1 mg/ml was mixed with 4 ml of reagent. The reagent was prepared using 0.6 M sulphuric acid, 28 mM sodium phosphate, 4 mM Ammonium molybdate. The tubes was covered and incubated in water bath at 90°C for 45 min then bring down the temperature to RT and absorbance was measured using double beam spectrophotometer at 695nm. The blank solution was prepared by adding 4 ml reagent solution and an appropriate volume of the same solvent was used for the extract. The mean of 3 readings was used and expressed as mg of ascorbic acid equivalents (AAE)/g extract.

#### **ABTS radical cation decolorization assay**

ABTS<sup>+</sup> radical stock solution was prepared by reacting 7mM ABTS (2,2'-azino-bis- (3- ethylbenzothiazoline -6-sulfonic acid) and 2.45 mM potassium persulphate (K<sub>2</sub>S<sub>2</sub>O<sub>8</sub>) in a ratio of 1:0.5 results incomplete oxidation and allocate the mixture to stand in the dark at room temperature for 12–16 h before use (Roberta *et al.*, 1994). The stock solution was diluted with solvent and PBS (pH 7.4) to give absorbance of 0.7 ( $\pm 0.02$ ) at 734 nm at RT. The radical discoloration assay was performed in a volume of 1ml diluted ABTS<sup>+</sup> solution and 20  $\mu$ l of extract or standard compound (final concentration 10-50  $\mu$ g/ml) and absorbance at 734 nm was recorded at RT accurately 1 min after initial mixing up to 20 min. Respective solvent blank was run and determinations were carried at least five times of standard and samples. The IC<sub>50</sub> values calculated:

% Inhibition =  $[(A_0 - A_1) / A_0 \times 100]$ , where  $A_0$  = e absorbance of the

control/blank, A1= absorbance of extract/standard.

## RESULTS AND DISCUSSION

The total phenolic and flavonoid content obtained are shown in Table 1. The phenolic compounds exhibit scavenging efficiency of free radicals and reactive oxygen species was due to their phenolic hydroxyl groups that have the ability to scavenge radicals and widely distributed in plant kingdom (Elliot, 1999). The study of phenolic compounds in food has been exhaustive after the investigation on red wine explained that moderate consumption of red wine which contains polyphenols help in the prevention of cardiovascular diseases (Marinova *et al.*, 2005). Phenolic compounds also can do other biochemical activities such as antimutagenic, anticarcinogenic and the ability to modify expression of genes (Nakamura *et al.*, 2003).

### Flavonoid content

The amount of flavonoids in the five medicinal plants ranged from 19-42 mg/g of plant in terms of rutin equivalent.

**Table 1. Phenolics and flavonoids in medicinal plants.**

Medicinal plant	Total Phenolic content * (mg/g)	Total flavonoids content (mg/g)
TS	91.29±2.58	35±1.98
BP	53.49±3.85	19±2.5
SA	31.8±2.81	26±1.99
CI	47.24±1.98	30±0.98
PC	108.39±2.11	42±1.11

\* (*Thymus serpyllum* (TS), *Barleria prionitis* (BP), *Spilanthes acmella* (SA), *Clerodendrum indicum* (CI), *Pogostemon cablin* (PC))

The pharmacological effect of flavonoids is associated with their antioxidant activities (Shi *et al.*, 2006). The management of hypertension, obesity and diabetes could be

done by five methanolic extracts which have high concentration of flavonoids.

### Iron chelation activity

The chelating activity of medicinal plant extract is shown in Table 2. Metal ion chelating property is a sign of antioxidant activity; it reduces the concentration of the catalyzing transition metal in LPO (Duh *et al.*, 1999). All the five extracts and standard compound interfered with the formation of ferrous and ferrozine complex, signifying the chelating activity. These methanolic extract may be explored for clinical relevance in the treatment of iron-overload disorders such as thalassemia and results in the production of iron chelators due to its elevated chelating ability *in vitro* at low doses. Iron chelators from the plant methanolic extract will decrease iron availability in the blood circulation of thalassemia patients.

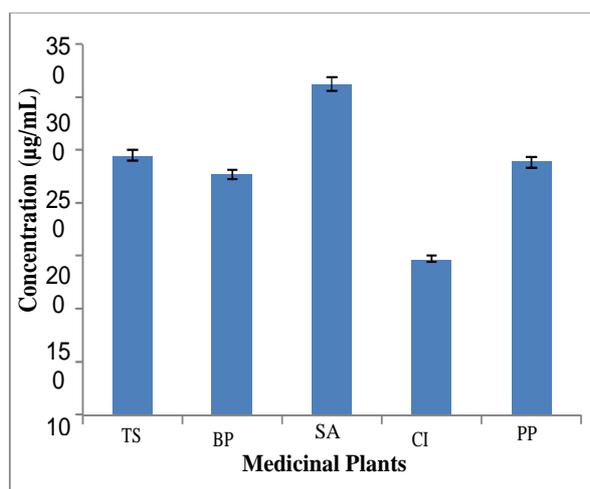
### Molybdenum reduction potential

The results are presented in table 3. Among the examined species, the highest total antioxidant capacity was found in PP with a value of 449 AAE/gm of plant leaf extract.

### ABTS Assay

Fig. 2 shows the per cent inhibition of ABTS radical scavenging activity at different concentrations (50-250 µg/ml). The IC<sub>50</sub> values obtained in this study are 245.0, 227.27, 312.04, 147.05 and 238.77 µg/ml of TS, BP, SA, CI, and PP respectively (Fig.3). The highest ABTS scavenging activity of *Clerodendrum indicum* was attributed due to the presence of higher levels of total phenolic compounds. The scavenging effect of the samples on the ABTS radical was found in order of Ascorbic acid>CI>SA>BP>PP>TS. Among the all tested plant samples, methanolic extract of CI exhibited the most effective radical scavenging activity (IC<sub>50</sub> = 147.05 µg/ml) which was also significantly stronger (p<0.05) than other two samples. This can be attributed mainly to higher

phenolic amount of plant especially phenolic acids, flavonoids than of other plant samples. ABTS is regularly used to determine the antioxidant capacities of foods. This method was not only a rapid and reliable test of total antioxidant capacity but also advantageous assay applicable to both hydrophilic and lipophilic antioxidants/systems (Sasidharan and Menon, 2011).



**Fig. 2. ABTS free radical scavenging effects in medicinal plants.**

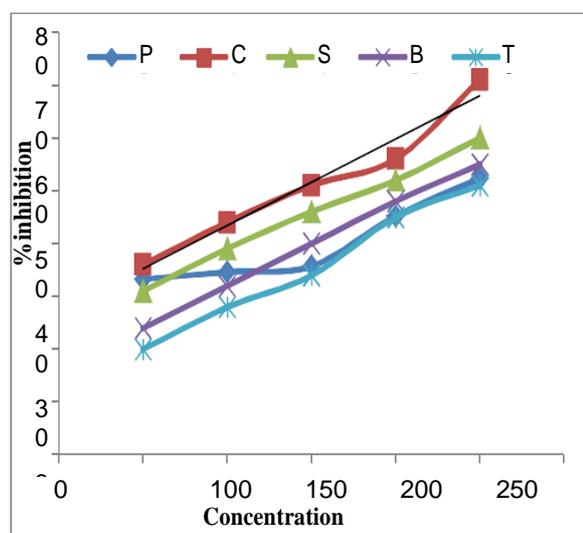
**Table 2. Iron (II) ions chelating activities of methanolic extracts were chelated by 50%**

Extract	IC <sub>50</sub> (µg/ml)*
TS	337± 5.6 µg/ml
BP	392±12.5 µg/ml
SA	426.8±10.1 µg/ml
CI	388.3±4.4 µg/ml
PC	334±4.8 µg/ml
EDTA	13±0.9 µg/ml

\*IC<sub>50</sub>: concentration at which iron (II) ions

**Table 3. Antioxidant activity by molybdenum assay.**

Extract	Antioxidant activity (AAE mg/g)
TS	401.2±3.88
BP	388.6±2.15
SA	332.8±1.71
CI	239.4±1.1
PC	449±2.11



**Fig. 3. ABTS free radical scavenging IC<sub>50</sub> values.**

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#### CONFLICT OF INTERESTS

We declare that we have no conflict of interest.

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