ORIGINAL ARTICLE Open Access

ISSN 2278-1404

International Journal of Fundamental & Applied Sciences

# Scrutinizing the antioxidant potential of *Prunella vulgaris* L. : A medicinal plant from central Himalayan region

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Manuscript received 3rd Aug, 2014, revised 23rd Dec, 2014 & accepted 1st Jan, 2015

***Abstract***

**Background & Aim:** The present study was carried out to evaluate the *in vitro* antioxidant activities of methanolic extract of *Prunella vulgaris*, a valuable medicinal plant from Central Himalayans. **Methods:** This was achieved by screening of the plant extracts at varying concentrations (1-70µg/ml), using 2,2-diphenyl-1-picryl- hydrazyl (DPPH) radical scavenging activity, reducing power assay superoxide radical scavenging activity, metal chelating activity, and analysis of total antioxidant activity by ABTS method. **Results:** Total phenol and flavonoid contents (17.200±0.306 mg gallic acid equivalent (GAE)/g dry weight and 3.920±0.042 mg quercetin equivalents (QE)/g dry weight) were found respectively. Scavenging effect of methanolic extracts of *P. vulgaris* was four times greater than that of the synthetic antioxidant ascorbic acid. **Conclusion:** Results also suggests a close relationship between total phenolic content and antioxidant activity, reducing power and radical scavenging effect on DPPH radicals, which proves *P. vulgaris* is a potential source of useful natural antioxidants.

***Keywords****: Prunella vulgaris;* antioxidants; Phenol; Flavonoid; DPPH; ABTS; metal chelating



## *1. Introduction*

Free radicals are essential part of life that modulates diverse physiological functions[1,2]. Their excessive generation may disrupt the body's antioxidant system that sometimes may lead to "oxidative stress", such situation contributes to a variety of diseases. Although the development of some synthetic antioxidants in the past few years has flourished, however they are not widely used as therapeutic agents due to their possible toxicity. As a result, the development of natural antioxidant has drawn the attention of scientific community. Ethno-botany has emerged as an important branch of study, which focuses on the utility of different plants and their properties as food and medicine. *P. vulgaris* L. (Labiateae), is a perennial herb belonging to the mint family also known as self-heal[3], is very popular in European, Asian and Chinese medicine. Dried fruit spikes with flowers are used for various pharmaceutical purposes, besides leaves and stems are used in olive green dye. Leaves are also used as raw or cooked in salads and soups[4]. Fresh leaves and stem of this herb are rich in protein, plant fat, carbohydrate, carotene, vitamin B and nicotinic acid[3]. The whole plant is considered as alterative, anti-HIV1[5], antibacterial, antipyretic, antiseptic, antispasmodic, astringent, carminative, diuretic, febrifuge, hypotensive, stomachic, styptic, tonic, vermifuge and vulnerary[3]**.** It was used to heal wounds, ulcers and sores[6], as a tea in treatment of fever, diarrhoea, sore mouth and internal bleeding[7]. It is used as antibiotic and hypotensive drug[8]. *P. vulgaris* is rich in phenolic acids[9] such as rosmarinic acid (RA). RA exhibits a wide spectrum of biological activities[10], including lipoperoxidation suppression[11], scavenging superoxide radicals[12] and antioxidant[9] and anti-inflammatory effects[2]. The Chinese Pharmacopoeia 2010 considers rosmarinic acid to be the only criterion for the quality control of Prunellae Spica. In addition, triterpenes are the dominant compounds in *P. vulgaris*[13].All the triterpenes, ursolic acid and oleanolic acid are most prevalent in *P. vulgaris* and exhibit many bioactivities including their role as antioxidants. Therefore, this study is aimed to scrutinize the correlation between phytochemicals and antioxidant activity of *P.*

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*vulgaris* extract.

## *2. Material and Methods*

### 2.1 Chemicals and reagents

2,2-diphenyl-1-picryl-hydrazyl (DPPH), quercetin, sodium nitrite ($NaNO_2$), ascorbic acid, Ferric chloride ($FeCl_3$), gallic acid, Potassium di-hydrogen phosphate ($KH_2PO_4$), di-potassium hydrogen phosphate ($K_2HPO_4$), sodium carbonate ($Na_2CO_3$), aluminium chloride ($AlCl_3$), sodium hydroxide (NaOH), sodium carbonate, potassium acetate, Potassium persulphate, TPTZ (2,4,6- tripyridyl 1,3,5 triazine), Sodium acetate ($CH_3COONa$), Ferrozine, Ferrous chloride, Ethylene diamine tetra acetic acid (EDTA), $Na_2EDTA$, Riboflavin and Nitro-blue tetrazolium (NBT) were obtained from Himedia Laboratories Pvt. Ltd, Mumbai, India. Folin-Ciocalteu's reagent, Molisch's reagent, conc. $H_2SO_4$, Fehling's reagent, conc. HCl, $NH_4OH$, Meyer's reagent (potassiomercuric iodide solution), Keller-Kiliani reagent, 2,2 [azinobis (3ethyl benothiazoline-6sulphonic acid) diammonium salt] (ABTS), chloroform, ethanol and. methanol were obtained from Merck, Mumbai, India. All chemicals used were of analytical grade.

### 2.2 Plant material and extraction

*P. vulgaris* was collected from Central Himalayan region (Nainital), altitude ranging from 1500-1900m. *Prunella* sample (deposition specimen no. is 115359) were identified by Botanical Survey of India (BSI) Dehradun, Uttarakhand.

### 2.3 Phytochemical Studies

#### 2.3.1 Extract Preparation

#### 2.3.1.2 Preparations of dried extract of *P. vulgaris*

Whole plant of *P. vulgaris* was washed with clean sterile water and oven-dried for 72 hours at 50°C. 1gm of dry plant material was blended into fine powder and soaked in 10ml of 80% methanol solvent (the ratio of plant material to solvent was 1:10 w/v) for 48 hours in shaking conditions (100 rpm at 35ºC). The resultant suspension was centrifuged at 10,000 rpm for 10 min. Supernatant was used and stored at 4ºC for further studies.

#### 2.3.2 Determination of Plant Extract Yield

The percentage yield of methanol extract (ME) from each sample was calculated using the following formula:

Percentage of extraction (%)

$$= \frac{\text{Weight of the extract (g)}}{\text{Weight of the plant material (g)}} \times 100$$

#### 2.3.3 Preliminary Phytochemical Screening

The various phytochemical constituents present in the 80% methanolic extract (ME) were analyzed by previous methods[14]. Followed protocols are as described below.

#### 2.3.3.1 Carbohydrates

1 ml of 80% methanolic extract were taken, 1 ml of Molisch's reagent and 1ml of conc. $H_2SO_4$ was added to it. Formation of reddish ring indicates the presence of carbohydrates[15].

#### 2.3.3.2 Reducing Sugars

1 ml of 80% methanolic extract were taken and 2ml of Fehling's reagent was added to it. It was boiled for 5 minutes. Appearance of brick red precipitate confirms the presence of reducing sugars[15].

#### 2.3.3.3 Tannins

2 ml of 80% methanolic extract were taken and 1ml of 1M $FeCl_3$ was added to it. Blue-black or greenish black precipitate confirms the presence of tannins[15].

#### 2.3.3.4 Saponins – Frothing Test

0.5ml of 80% methanolic extract were added to 5ml of millipore water and shakind was done for 30 seconds. The presence of persistent frothing confirms the presence of saponins[15].

#### 2.3.3.5 Flavonoids – Shinoda'sTest

1ml of 80% methanolic extract were taken. Few magnesium ribbons and 36% conc. HCl (11.65 N) was added to it. Appearance of red or pink colour indicates the presence of flavonoids[15].

#### 2.3.3.6 Steroids – Liebermann-Burchard'sTest

2ml of 80% methanolic extract were taken. 2ml of 100% acetic anhydride and 1ml of 98% conc. $H_2SO_4$ (18.4 M) was added to it. The formation of blue green ring confirms the presence of steroids[16].

#### 2.3.3.7 Alkaloids

5 drops of $NH_4OH$ (14.5 M) was added to 1ml of 80% methanolic extract. This was followed by addition of 20 ml of 100% chloroform. From the two layers separated out, the chloroform layer was extracted using 20 ml dilute 98% $H_2SO_4$ (18.4 M), following the addition of 5 drops of Meyer's reagent (potassiomercuric iodide solution), a creamy/ brownish red/orange red precipitate is indicative of the presence of alkaloids[15].

#### 2.3.3.8 Anthraquinones – Borntrager's Test

2ml of 80% methanolic extract were taken and 2ml of 10% $NH_4OH$ was added to it. Appearance of pink colour confirms the presence of anthraquinones[15].

#### 2.3.3.9 Glycosides – Keller-Kiliani Test

2 ml of 80% methanolic extract were taken and 1ml of

Keller-Kiliani reagent (1ml of 100% glacial acetic acid, 1ml of 10% of $FeCl_3$ and 1ml of conc. $H_2SO_4$) was added to it. A blue green colour indicates the presence of glycosides[16].

**2.3.3.10 Terpenoids – Salkowski Test**

5 ml of 80% methanolic extract were taken and 2ml of chloroform was mixed with it. 3 ml of conc. $H_2SO_4$ was layered over it. A reddish brown colouration at the interface indicates the presence of terpenoids[17].

**2.3.3.11 Coumarins – Fluorescence Test**

1 ml of 80% methanolic extract were mixed with 1 ml of 2.5N NaOH. Development of blue green fluorescence indicates the presence of coumarins[18].

**2.3.3.12 Phenolics**

2ml of 80% methanolic extract, 5% ferric chloride solution was added. Deep blue black colour indicates the presence of phenolics[6].

**2.3.4 Quantitative Phytochemical Assays**

**2.3.4.1 Determination of Total Phenolic Content (TP)**

The total phenolic content of the sample extract was determined by Folin-Ciocalteu's colorimetric method[19] with certain modifications. 5µl of sample extract was taken and make it up to 100µl with double distilled (d.d.) water. 450µl of distilled water and 50µl of Folin-Ciocalteu's reagent were added further and allowed to stand for 5min. This mixture was then neutralized by adding 500µl of 7 % (w/v) sodium carbonate and kept at room temperature in dark for 90 minutes. The resulting blue colored solution was measured spectrophotometrically (UV-VIS) at 765nm. Quantification of total phenolic content was based on standard curve of gallic acid prepared in the two solvents respectively. The results were expressed in mg gallic acid equivalent (GAE) per gram dry weight of the material.

**2.3.4.2 Determination of Total Flavonoid Content (TF)**

Content of flavonoids of the sample extract were determined by $AlCl_3$ colorimetric method[20] with certain modifications. 10µl of sample extract was taken and make it up to 100µl with double distilled (d.d.) water. 200µl distilled water was added further; 100µl of 10 % (W/V) $AlCl_3$ was added followed by the addition of (20µl) 1M potassium acetate and 500µl of distilled water. Then the reaction mixture was incubated at room temperature for 30 minutes. Thereafter the absorbance was recorded at 415nm using UV-VIS spectrophotometer. Quantification of total flavonoid content was done on the basis of standard curve of quercetin prepared in the two solvents and the results were expressed in mg quercetin equivalent (QE) per gram dry weight of the material.

**2.3.5 Determination of Antioxidant Activity**

The antioxidant potential of *P. vulgaris* was evaluated by different methods such as FRAP (ferric reducing antioxidant power), DPPH (1,1-diphenyl 2-picryl-hydrazyl), ABTS 2,2 [azinobis (3ethyl benothiazoline-6sulphonic acid) diammonium salt], MCA (Metal chelating activity) and SSA (Superoxide scavenging activity).

**2.3.5.1 FRAP Antioxidant Assay**

Ferric reducing antioxidant power (FRAP) assay was performed[21] with minor modification. 10µl of sample extract was taken and make it up to 100µl with double distilled (d.d.) water and was mixed with 1.5ml of pre-warmed FRAP reagent (370C) and kept at 370C for 10 minutes. Absorbance was taken at 593 nm. Standard was prepared by using ascorbic acid. For control absorbance of FRAP reagent (Acetate Buffer: TPTZ solution: Ferric chloride ratio is 10:1:1) was taken without adding sample extract. Results were expressed in mg ascorbic acid equivalent (AAE) per gm dry weight of the material.

**2.3.5.2 DPPH Antioxidant Assay**

The DPPH assay was done according to the original method[14] with certain modifications.100µM DPPH (50ml) was added to equal volume of 20% (v/v) ethanol (50ml) to generate DPPH cations (DPPH*). DPPH* (400µl) was taken and different concentrations of sample extract were added in different test tubes and makeup the volume to 100µl with double distilled (d.d.) water further addition of 500µl of double distilled water was done to make the final volume to 1ml . Then it was shaken vigorously and kept in dark for 20 minutes at room temperature. The reduction in absorbance was recorded at 520 nm in UV-VIS spectrophotometer. Ascorbic acid was used as standard and for control; absorbance of DPPH cations was taken without adding sample extract.

% inhibition of free radical DPPH was calculated as:

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

$IC_{50}$ for the assay was calculated graphically using a calibration curve by plotting antioxidant capacity or percentage inhibition versus the corresponding sample concentration.

**2.3.5.3 ABTS Antioxidant Activity Assay**

Total antioxidant activity was measured by ABTS methods[22] with some modification. 1mM ABTS (3.5ml) and 1mM Potassium persulphate (1.2ml) was mixed and volume was made up to 10ml with d.d water and kept in

dark condition for 16 hours at $25^0C \pm 2^0C$ for production of ABTS cations (ABTS*). Absorbance of ABTS solution was recorded at 734nm and diluted with phosphate buffer saline till the absorbance reached in the range of 0.70±0.05. For sample analysis, 1.90 ml of ABTS* solution was added to different concentrations of sample extract and makeup the volume to 100µl with double distilled (d.d.) water, mixed thoroughly and incubated for 6 minutes in dark at 23°C. The absorbance of the resulting solution was measured using UV-VIS spectrophotometer at 734nm. Ascorbic acid was used as standard and for control; absorbance of ABTS cations was taken without adding sample extract.

Total antioxidant activity % was calculated as:

% Antioxidant Activity

$$= \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

$I_{C50}$ for the assay was calculated graphically using a calibration curve by plotting antioxidant capacity or percentage inhibition versus the corresponding sample concentration.

**2.3.5.4 Metal chelating assay (MCA)**

Metal chelating assay was performed[23] with some modification. Different concentrations of sample extract were added in different test tubes and makeup the volume to 100µl with double distilled (d.d.) water. The mixture was diluted to 400µl with double distilled water, 700µl Ferrozine (2.5mM) and 20µl $FeCl_2$ were added in the test tube followed by the addition of 100µl methanol(80%). The resulting reaction mixture was shaken vigorously and incubated in dark for 20 minutes. Absorbance was recorded at 562nm. $Na_2EDTA$ was used as standard. For control absorbance of reagent was taken without adding sample extract.

% Chelating activity was calculated as:

% Chelating activity

$$= \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

$I_{C50}$ for the assay was calculated graphically using a calibration curve by plotting antioxidant capacity or percentage inhibition versus the corresponding sample concentration.

**2.3.5.5 Superoxide scavenging assay** (SSA)

Superoxide scavenging assay was performed by modified method[24]. Different concentrations of sample extract were diluted with phosphate buffer saline to make up the volume to 100µl, to this 100µl of each riboflavin, EDTA, and absolute methanol were added by keeping the reaction mixture in dark. This step was followed by vigorous shaking and addition of NBT (50µl) and 500µl of phosphate buffer saline. The reaction mixture was allowed to stand for 10min. in fluorescent light. Absorbance was recorded at 590nm. Ascorbic acid was used as standard and phosphate buffer saline as blank. For control absorbance of reagent was taken without adding sample extract.

% scavenging /Inhibition were calculated as:

% scavenging /Inhibition

$$= \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

$IC_{50}$ for the assay was calculated graphically using a calibration curve by plotting antioxidant capacity or percentage inhibition versus the corresponding sample concentration.

**2.3.6 Statistical analysis**

All determinations of antioxidant capacity by DPPH, ABTS, FRAP, metal chelating, superoxide scavenging assay and phytochemicals such as total phenol and flavonoid content were conducted in triplicates.The value for each sample was calculated as the mean± standard error. Analysis of variance and significant difference among the means were tested by one way ANOVA[25] and Correlation coefficients of determination using SPSS (version 19 for window).

## *3. Results and Discussion*

### 3.1 Preliminary Phytochemical Screening

The preliminary phytochemical analysis conducted on *P. vulgaris* extract revealed the presence of various bioactive components like carbohydrates, reducing sugers, tannins, saponins, flavonoids, steroids, alkaloids,

**Table I: Phytochemical activity of *P. vulgaris* extract**

| Tests | ME of *P. vulgaris* |
|---|---|
| Carbohydrates | + |
| Reducing sugars | - |
| Tannins | + |
| Saponins | + |
| Flavonoid | + |
| Steroids | + |
| Alkaloids | + |
| Anthraquinone | + |
| Glycosides | + |
| Tepenoids | + |
| Coumarins | + |
| Phenolic | + |

+=presence, - = absence

anthroquinone, glycosides, terpenoids, coumarins and phenolic (**Table I**).

### 3.2 Plant Yield

The wild whole plant yield of 80% methanolic extract was found to be 8.958±0.324 % w/w (mean ± standard error).

### 3.3 Determination of Total Phenolic Content

The total phenolic content in Methanolic extract (ME) was 17.200±0.306 mg gallic acid equivalent (GAE)/g dry weight. High phenolic contents in methanolic extracts show that 80% methanol is a suitable solvent for the preparation of extracts.

### 3.4 Determination of Total flavonoids contents

The total flavonoid content of ME was 3.920±0.042 mg quercetin equivalents (QE)/g dry weight. Higher level of flavonoids in ME can be attributed to the fact that methanol is less polar than water and thus has the potential to release the bound flavonoids and polyphenols from the cell wall of the plant[26].

### 3.5 FRAP Antioxidant Assay

Ferric reducing power potential of methanolic extracts was 31.209±0.150 mg ascorbic acid equivalent (AAE)/g dry weight. Higher reducing potential of the plant may be attributed to high phenolic content of *P.vulgaris.* The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. However, the activity of antioxidants may be due to various mechanisms such as prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging[27].

### 3.6 DPPH scavenging activity

DPPH antioxidant assay is the most commonly used assay to evaluate the antioxidant activity. It is based on the ability of DPPH to decolorize from violet to yellow in presence of antioxidants thus leads to decrease in absorbance at 520nm. The DPPH free radical scavenging activity is due to the neutralization of DPPH free radical by extract either by transfer of hydrogen or of an electron[28].The concentration of the extract or standard at which about 50% of DPPH radicals are inhibited is known as $IC_{50}$ value. The screening results of the DPPH activity along with standard ascorbic acid are optimized and scavenging effect is expressed as $IC_{50}$. $IC_{50}$ of ME was 30.958 µg/ml, this was comparable to ascorbic acid which was 19.7037 µg/ml (**Table II**).

### 3.7 ABTS assay

In order to calculate the total antioxidant activity of the methanolic plant extract ABTS assay was performed. The $IC_{50}$ value for standard ascorbic acid and methanolic extract was 30.558µg/ml and 52.651µg/ml respectively. This is the method for the screening of antioxidant activity where decolorization assay applicable to both lipophilic and hydrophilic antioxidants. The pre-formed radical monocation of 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS*+) is generated by oxidation of ABTS with potassium persulfate and is reduced in the presence of such hydrogen-donating antioxidants. The influences of both the concentration of antioxidant and duration of reaction on the inhibition of the radical cation absorption are taken into account when determining the antioxidant activity[29] (**Table III**).

### 3.8 Metal chelating assay

Ferrozine can make complexes with ferrous ions. From the result it was evident that methanolic plant extracts possessed $Fe^{2+}$ chelating activity and might play a protective role against oxidative damage induced by metal catalyzed decomposition reactions[30,31]. The metal chelating activity of positive control EDTA was found to be 6.3913µg/ml and that for methanolic extract was 11.839 µg/ml (**Table IV**).

### 3.9 Superoxide scavenging activity

The superoxide scavenging activity of the standard (ascorbic acid) and the methanolic plant extract was 3.776 and 7.279µg/ml respectively. The enhanced

**Table II: % inhibition of DPPH radical scavenging activity and $IC_{50}$ values of ME & standard**

| Standard ( ascorbic acid) Concentration (µg/ml) | % inhibition | IC 50 (µg/ml) | ME Concentration (µg/ml) | ME % inhibition | IC 50 (µg/ml) |
|---|---|---|---|---|---|
| 1 | 5.290 | | 10 | 67.059 | |
| 2 | 7.350 | | 20 | 67.941 | |
| 3 | 9.117 | | 30 | 68.529 | |
| 5 | 12.647 | 19.703 | 40 | 76.471 | 30.958 |
| 10 | 17.647 | | 50 | 77.941 | |
| 15 | 25.000 | | 60 | 79.412 | |
| 20 | 32.352 | | 70 | 80.882 | |

**Table III: % inhibition of ABTS activity and $IC_{50}$ values of ME & standard**

| Standard ( ascorbic acid) Concentration (µg/ml) | % inhibition | IC 50 (µg/ml) | ME Concentration (µg/ml) | ME % inhibition | IC 50 (µg/ml) |
|---|---|---|---|---|---|
| 01 | 10.241 | | 01 | 1.807 | |
| 05 | 14.217 | | 10 | 10.843 | |
| 10 | 19.880 | | 20 | 15.301 | |
| 20 | 30.120 | 30.558 | 30 | 27.711 | 52.651 |
| 30 | 39.157 | | 40 | 36.145 | |
| 40 | 48.795 | | 50 | 47.229 | |
| 50 | 58.434 | | 60 | 63.855 | |
| 60 | 65.663 | | 70 | 80.482 | |

**Table IV: % inhibition of metal chelating capacity and $IC_{50}$ values of ME & standard**

| Standard ($Na_2EDTA$) Concentration (µg/ml) | % inhibition | IC 50 (µg/ml) | ME Concentration (µg/ml) | ME % inhibition | IC 50 (µg/ml) |
|---|---|---|---|---|---|
| 1 | 28.633 | | 1 | 4.167 | |
| 3 | 32.950 | | 3 | 16.833 | |
| 5 | 36.978 | | 5 | 21.667 | |
| 10 | 46.043 | 6.391 | 7 | 31.722 | 11.839 |
| 15 | 54.676 | | 9 | 40.472 | |
| 20 | 62.446 | | 11 | 49.222 | |
| 25 | 70.360 | | 13 | 57.972 | |
| 30 | 78.417 | | 15 | 66.722 | |

**Table V: % inhibition of Superoxide scavenging activity and $IC_{50}$ values of ME & standard**

| Standard ( ascorbic acid) Concentration (µg/ml) | % inhibition | IC 50 (µg/ml) | ME Concentration (µg/ml) | ME % inhibition | IC 50 (µg/ml) |
|---|---|---|---|---|---|
| 1 | 42.073 | | 1 | 8.537 | |
| 3 | 45.122 | | 3 | 14.024 | |
| 5 | 48.171 | | 5 | 26.829 | |
| 10 | 53.659 | 3.776 | 7 | 34.756 | 7.279 |
| 15 | 59.146 | | 9 | 43.902 | |
| 20 | 63.415 | | 11 | 53.049 | |
| 25 | 69.512 | | 13 | 62.195 | |
| 30 | 75.610 | | 15 | 71.341 | |

scavenging activity may be due to the high phenolic and flavonoids found in *P. vulgaris*.

*In-vitro* superoxide radical scavenging activity is measured by riboflavin/light/NBT (Nitro blue tetrazolium) reduction. The method is based on generation of super oxide radical by auto oxidation of riboflavin in presence of light. Super oxide is biologically important as it can form singlet oxygen and hydroxyl radical. Overproduction of superoxide anion radical contributes to redox imbalance and associated with harmful physiological consequences. The super oxide radical reduces NBT to a blue colored formazan that can be measured at 570 nm[24] (**Table V**).

### 3.10 Linear correlation between different parameters of *P. vulgaris*

Linear correlation between the phytochemical constituents and total antioxidant activity was established in order to determine how the antioxidant activity and total phenols or flavonoids level are related to *P. vulgaris*. Metal chelating activity of the methanolic

**Table VI: correlation between the phytochemical constituents and total antioxidant activity**

| | TF | TP | FRAP | SSA | MCA | DPPH | ABTS |
|---|---|---|---|---|---|---|---|
| **TF** | 1 | | | | | | |
| **TP** | -0.052 | 1 | | | | | |
| **FRAP** | 0.863 | -0.55 | 1 | | | | |
| **SSA** | 0.995 | -0.155 | 0.91 | 1 | | | |
| **MCA** | 0.806 | 0.548 | 0.397 | 0.741 | 1 | | |
| **DPPH** | 0.945 | -0.377 | 0.981 | 0.974 | 0.568 | 1 | |
| **ABTS** | -0.951 | 0.358 | -0.977 | -0.978 | -0.585 | -1.000* | 1 |

* Correlation is significant at the 0.05 level (2-tailed)

extract was positively correlated with both phenol and flavonoid content. Our experimentation on the correlation between the total phenol and reducing power led to conclude that they were negatively correlated. The total flavonoid content was positively correlated with other antioxidant activities like DPPH, superoxide scavenging activity but negatively correlated with ABTS total antioxidant activity. ABTS total antioxidant activity was positively correlated with total phenolic content (**Table VI**).

## *Conclusion*

To conclude, this is the first report to concur the quantitative correlations between the polyphenols and the DPPH, ABTS scavenging activity, reducing power, superoxide scavenging activity of *P. vulgaris* and a close linear correlation among each other were established. This study substantiates utilization of this plant as an antioxidant in future. On the other hand, further studies should be continued to obtain appropriate information about the role of *P. vulgaris* in some other dose dependent processes. However, further studies are needed to isolate the active principles, elucidate their structures, and determine their pharmacological activities.

## *Acknowledgement*

The authors are obliged to Uttarakhand State Biotechnology Department (USBD), Govt. Of Uttarakhand for their financial help and support in the form of project funding (Project File No. 482/Tapan K.N/KU/NRIB/R&D Project-1/2011).

## *Conflict of interest*

The author's declares none.

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