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# Antioxidant Activities of Some Selected Wild Edible Plants of Arunachal Pradesh State In India and Effect of Solvent Extraction System

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## ABSTRACT

The aim of present study was to evaluate the antioxidant activities of four different solvent extracts of four wild leafy vegetables e.g. *Solanum nigrum Phytolacca esculenta, Piper pedicellatum and Pouzolzia hirta* and fruits of *Melodinus khasianus* collected from Arunachal Pradesh state in India. The extracts of the plants were examined for their antioxidant activities by using free radical 1,1-diphenyl-2-picryl hydrazyl (DPPH) scavenging method, ABTS radical scavenging ability, reducing power capacity, estimation of total phenolic content, flavonoid content and flavonol content.. The result showed that the total phenolics, flavonoids and flavonols of the different extracts of the investigated samples ranged from 7.49 ±3.55 - 305.38 ±2.78 mg gallic acid equivalents **(**GAE)/g dry extract, 4.13±0.02 - 171.01±0.99 mg rutin equivalent /g dry extract and 18.90±0.15 to 326.21±1.84 mg quercetin equivalent /g dry extract respectively. Furthermore the plant extracts exhibited good free radical scavenging capacity. The solvent used to extract the active constituents from plants were benzene, chloroform, acetone and methanol. The different levels of antioxidant activities were found in the solvent systems used. The results indicate that these wild edible vegetables could be utilized as natural antioxidant.

**Keywords:** Antioxidant activity, Different solvent extracts, Arunachal Pradesh, Wild edible plants

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## INTRODUCTION

Oxidation is a chemical reaction involving the loss of electrons which can produce free radicals. The uncontrolled production of reactive oxygen species affect living cells and these are responsible for many chronic diseases in human being such as atherosclerosis, parkinson's disease, arthritis, alzheimer's disease, stroke, chronic inflammatory diseases, cancers, and other degenerative diseases[1].

Antioxidants are synthetic or natural substances that may inhibit the oxidation of other molecules. As antioxidants have been reported to prevent oxidative damage caused by free radical, it can interfere with the oxidation process by reacting with free radicals, chelating catalytic metals and also by acting as oxygen scavengers [2].

At present, the synthetic antioxidants like butylated hydroxyl anisole (BHA), commonly used to reduce the free radical damage to the human body are supposed to be responsible for liver damage and carcinogenesis. So, it is very much essential to find out and develop potent natural antioxidants which can inhibit the formation of free radicals and will be associated with health maintenance and prevention of chronic diseases of human being.

Plant materials are rich sources of active constituents of varied chemical characteristics. Studies on herbal plants, vegetables, and fruits have indicated the presence of active components *viz.* phenolic compounds, flavones, isoflavones, flavonoids, anthocyanin, coumarin, lignans, catechins and isocatechins and they have been reported to have multiple biological effects, including antioxidant activity [3]. So the consumption of herbaceous plants has been widely promoted to terminate the action of free radicals and thereby protecting the body from various diseases.

The antioxidant activities of plants are strongly dependent on the polarity of the solvents and plant parts used for the complete extraction of active components [4-5]. Solvents, such as methanol, ethanol, acetone, chloroform, ethyl acetate and water have been widely used for the extraction of antioxidant compounds from various plants and plant based foods and medicines.

Therefore, the objective of present study was to investigate the effect of different extracting solvents with different polarity on the antioxidant activities of four wild leafy vegetables e.g. *Solanum nigrum Phytolacca esculenta, Piper pedicellatum and Pouzolzia hirta* and fruits of *Melodinus khasianus*. Thus the results from this preliminary study will enable us to develop and isolate potent antioxidants from plant.

## MATERIALS AND METHOD

### Plant materials

The five plant materials e.g the fruits of *Melodinus khasianus* and the leaves of *Solanum nigrum Phytolacca esculenta, Piper pedicellatum and Pouzolzia hirta* were collected from different market of Arunachal Pradesh state, India on June 2014 and authenticated in our office. The voucher specimens were preserved in the Plant Chemistry department of our office under registry no BSITS 70, BSITS 71, BSITS 72, BSITS 74 and BSITS 75 respectively. The plant parts were shed-dried, pulverized and stored in an airtight container for further extraction.

**Chemicals**

1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), butylated hydroxytoluene (BHT), ascorbic acid, quercetin were purchased from Sigma Chemical Co. (St. Louis, MO, USA)., Folin-Ciocalteus's phenol reagent, gallic acid, potassium ferricyanide, potassium per sulphate, Aluminium chloride, $FeCl_3$ and sodium carbonate were from Merck Chemical Supplies (Damstadt, Germany). All the chemicals used including the solvents, were of analytical grade.

**Extraction of plant material (Benzene, chloroform, acetone and methanol )**

One gram of each plant material were extracted with 20 ml each of benzene, chloroform, acetone and methanol with agitation for 18 -24 h at ambient temperature. The extracts were filtered and diluted to 50 ml and aliquot were analyzed for their total phenolic, flavonoid and flavonol content, reducing power and their free radical scavenging capacity.

**Estimation of total phenolic content**

The amount of total phenolic content of crude extracts were determined according to Folin-Ciocalteu procedure [6]. 20 - 100 µl of the tested extracts were introduced into test tubes. 1.0 ml of Folin-Ciocalteu reagent and 0.8 ml of sodium carbonate (7.5%) were added. The tubes were mixed and allowed to stand for 30 min. Absorption at 765 nm was measured (UV-visible spectrophotometer Shimadzu UV 1800). The total phenolic content was expressed as gallic acid equivalents (GAE) in milligram per gram (mg/g) of extract using the following equation based on the calibration curve $y = 0.0013x + 0.0498$, $R^2 = 0.999$ where y was the absorbance and x was the Gallic acid equivalent (mg/g).

**Estimation of total flavonoids**

Total flavonoids were estimated using the method of Ordonez et al., 2006 [7]. To 0.5 ml of plant extract, 0.5 ml of 2% $AlCl_3$ ethanol solution was added. After one hour, at room temperature, the absorbance was measured at 420 nm (UV-visible spectrophotometer Shimadzu UV 1800 ). A yellow color indicated the presence of flavonoids. Total flavonoid contents were calculated as rutin

(mg/g) using the following equation based on the calibration curve: y = 0.0182x - 0.0222, $R^2$ = 0.9962, where y was the absorbance and x was the Rutin equivalent (mg/g).

**Estimation of total flavonols**

Total flavonols in the plant extracts were estimated using the method of Kumaran and Karunakaran, 2006 [8]. To 2.0 ml of extract, 2.0 ml of 2% $AlCl_3$ ethanol and 3.0 ml (50 g/L) sodium acetate solutions were added. The absorption at 440 nm (UV-visible spectrophotometer Shimadzu UV 1800) was read after 2.5 h at 20°C. Total flavonol contents were calculated as quercetin (mg/g) using the following equation based on the calibration curve: y = 0.0049 xs + 0.0047, $R^2$ = 0.9935, where y was the absorbance and x was the quercetin equivalent (mg/g).

**Measurement of reducing power**

The reducing power of the extracts were determined according to the method of Oyaizu, 1986 [9]. Extracts (100 µl) of plant extracts were mixed with phosphate buffer (2.5 ml, 0.2 M, *p*H 6.6) and 1% potassium ferricyanide (2.5 ml). The mixture was incubated at 50°C for 20 min. Aliquots of 10% trichloroacetic acid (2.5 ml) were added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared ferric chloride solution (0.5 ml, 0.1%). The absorbance was measured at 700 nm. The reducing power of the plant extracts were given in ascorbic acid equivalent (AAE) in milligram per gram (mg/g) of dry material using the following equation based on the calibration curve : y = 0.0023x - 0.0063, $R^2$ = 0.9955 where y was the absorbance and x was the ascorbic acid equivalent (mg/g).

**Determination of DPPH free radical scavenging activity**

The free radical scavenging activity of the plant samples and butylated hydroxyl toluene (BHT) as positive control was determined using the stable radical DPPH (1,1-diphenyl-2-picrylhydrazyl) [10]. Aliquots (20 - 100 µl) of the tested sample were placed in test tubes and 3.9 ml of freshly prepared DPPH solution (25 mg $L^{-1}$) in methanol was added in each test tube and mixed. 30 min later, the absorbance was measured at 517 nm (UV-visible spectrophotometer, Shimadzu UV 1800). The capability to scavenge the DPPH radical was calculated, using the following equation:

$$\text{DPPH scavenged (\%)} = \{(Ac - At)/Ac\} \times 100$$

Where Ac is the absorbance of the control reaction and At is the absorbance in presence of the sample of the extracts. The antioxidant activity of the extract was expressed as $IC_{50}$. The $IC_{50}$ value was defined as the concentration in mg of dry material per ml (mg / ml) that inhibits the formation of DPPH radicals by 50%. Each value was determined from regression equation.

Values are presented as mean ± standard error mean of three replicates. The total phenolic content, flavonoid content, flavonol content, reducing power and $IC_{50}$ value of each plant material was calculated by using Linear Regression analysis.

**Determination of scavenging activity of ABTS radical cation**

The 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation ($ABTS^{\cdot+}$)-scavenging activity was measured according to the method described by Re *et al*.[11]. ABTS was dissolved in water to a 7 mM concentration. The ABTS radicals were produced by adding 2.45 mM potassium persulphate(final concentration). The completion of radical generation was obtained in the dark at room temperature for 12–16 h. This solution was then diluted with ethanol to adjust its absorbance at 734 nm to 0.70 ± 0.02. To determine the scavenging activity, 1 ml of diluted $ABTS^{\cdot+}$ solution was added to 10 µl of plant extract (or water for the control), and the absorbance at 734 nm was measured 6 min after the initial mixing, using ethanol as the blank. The percentage of inhibition was calculated by the equation:

$$\text{ABTS scavenged}(\%) = (A_{cont} - A_{test}) / A_{cont} \times 100$$

Where $A_c$ and $A_s$ are the absorbencies of the control and of the test sample, respectively. From a plot of concentration against % inhibition, a linear regression analysis was performed to determine the $IC_{50}$ value of the sample.

## RESULTS AND DISCUSSION

**Extractive value**

The extractive value of the tested wild edible plants with four different solvents are depicted in Table 1. The result showed that, methanol is the most suitable solvent to obtain the maximum extract from all the plants under investigation in comparison to the other solvents like benzene, chloroform and acetone used for extraction. The fruits of *M. khasianus* give maximum yield (5.425±0.02 g/100g) when it is extracted with methanol and the least amount is observed with benzene. Likewise, the leaf extract of other plant materials also followed the same order of *M. khasianus* extracts. The differences in the extractive value of the plant materials may be due to the varying nature of the chemical components present and the polarities of the solvent used for extraction [12].

**Table 1. Extractive value of wild edible plants collected from Arunachal Pradesh using different solvents**

| Sl No | Name of the plant | Parts used | Extractive value (g / 100g dry material) | | | |
|---|---|---|---|---|---|---|
| | | | **Benzene** | **Chloroform** | **Acetone** | **Methanol** |
| 1 | *M. khasianus* | Fruits | 2.95±0.01 | 3.92±0.06 | 3.85±0.04 | 5.425±0.02 |
| 2 | *P. esculenta* | leaves | 0.60±0.04 | 0.90±0.04 | 0.60±0.02 | 3.0±0.07 |
| 3 | *S. nigrum* | leaves | 0.65±0.03 | 0.60±0.01 | 0.50±0.01 | 2.7 ±0.03 |
| 4 | *P. pedicellatum* | leaves | 0.52±0.02 | 1.60±0.03 | 1.30±0.03 | 6.1±0.04 |
| 5 | *P. hirta* | leaves | 0.57±0.02 | 0.92±0.03 | 1.02±0.03 | 5.30±0.05 |

Each value in the table was obtained by calculating the average of three experiments and data are presented as Mean ± SEM

**Total phenol, flavonoid and flavonol content of the extracts**

Phenols are known to be the effective antioxidants in plants due to their hydroxyl groups. Typical phenols with antioxidant activity have been characterized as phenolic acids and flavonoids. Phenolic acids are repeatedly implicated as natural antioxidants in fruits, vegetables and other plants.

It has been established that phenolic compounds are the major plant compounds with antioxidant activity and this activity is due to their redox properties. Phenolic compounds are a class of antioxidant agents which can adsorb and neutralize the free radicals [13].

The screening of the benzene, chloroform, acetone and methanol extracts of five wild plants revealed that there was a wide variation in the amount of total phenolics ranging from 7.49 ±3.55 to 305.38 ±2.78 mg GAE/g dry extract (Table 2).

**Table 2. Total phenolic content in wild edible plants collected from Arunachal Pradesh using different solvents**

| Sl No | Name of the plant | Parts used | Total phenolic content (GAE mg / g dry extract) | | | |
|---|---|---|---|---|---|---|
| | | | **Benzene** | **Chloroform** | **Acetone** | **Methanol** |
| 1 | *M. khasianus* | Fruits | 9.90 ±0.99 | 13.32 ±0.28 | 8.92 ±0.44 | 49.81 ±0.47 |
| 2 | *P. esculenta* | leaves | 25.21 ±2.82 | 32.47 ±2.46 | 19.87 ±1.85 | 68.71 ±1.85 |
| 3 | *S. nigrum* | leaves | 7.49 ±3.55 | 56.19 ±1.06 | 25.12 ±2.56 | 72.55 ±1.55 |
| 4 | *P. pedicellatum* | leaves | 53.23 ±3.23 | 194.71 ±6.93 | 106.11 ±3.55 | 305.38 ±2.78 |
| 5 | *P. hirta* | leaves | 11.81 ±2.94 | 26.74 ±2.49 | 26.01 ±2.72 | 218.43 ±2.09 |

Each value in the table was obtained by calculating the average of three experiments and data are presented as Mean ± SEM

The highest amount of phenolic content was found in the methanol extract of *P. pedicellatum* (305.38 ±2.78 mg GAE/g dry extract) followed by the same solvent extract of *P. hirta* (218.43 ±2.09 mg GAE/g dry extract) **.** While lower amount was observed in the benzene extract of *Solanum nigrum* (7.49 ±3.55 mg GAE/g). The chloroform and methanol extracts of *S. nigrum, P. pedicellatum* and *P. hirta* were found to contain a very good amount of phenolic compounds. Numerous studies have conclusively shown that consumption of foods high in phenolic content reduce the risk of heart disease [14].

Flavonoids and flavonols were regarded as one of the most widespread groups of natural constituents found in the plants. It has been recognized that both flavonoid and flavonol show antioxidant activity through scavenging or chelating process [15].

The flavonoid contents of the extracts in terms of rutin equivalent were between 5.21±0.06 to 330.08±3.34 mg/g dry material (Table 3).

**Table 3. Total flavonoid content in wild edible plants collected from Arunachal Pradesh using different solvents**

| Sl No | Name of the plant | Parts used | Total flavonoid content(Rutin equivalent mg / g dry extract) | | | |
|---|---|---|---|---|---|---|
| | | | Benzene | Chloroform | Acetone | Methanol |
| 1 | *M. khasianus* | Fruits | 5.30±0.04 | 4.13±0.02 | 4.65±0.01 | 5.45±0.02 |
| 2 | *P. esculenta* | leaves | 54.22±0.40 | 111.59±0.08 | 130.92±0.20 | 49.41±0.02 |
| 3 | *S. nigrum* | leaves | 40.61±0.07 | 113.82±0.26 | 104.26±0.87 | 50.91±0.07 |
| 4 | *P. pedicellatum* | leaves | 70.43±0.15 | 171.01±0.99 | 92.53±0.62 | 150.69±0.45 |
| 5 | *P. hirta* | leaves | 44.08±0.21 | 71.65±0.34 | 72.17±0.82 | 123.85±0.62 |

Each value in the table was obtained by calculating the average of three experiments and data are presented as Mean ± SEM

The highest amount of flavonoid (171.01±0.99 mg/g dry extract) was found in the chloroform extract of *P. pedicellatum* and the benzene and acetone extract of this plant also contain a very good amount of flavonoids. The benzene, chloroform, acetone and methanol extract of other four plants under investigation were also found to contain a very good amount of flavonoids.

The flavonol contents in the different extracts of plant materials are evaluated in terms of quercetin equivalent (Table 4).

**Table 4. Total flavonol content in in wild edible plants collected from Arunachal Pradesh using different solvents**

| Sl No | Name of the plant | Parts used | Total flavonol content (Quercetin equivalent mg / g dry extract) | | | |
|---|---|---|---|---|---|---|
| | | | Benzene | Chloroform | Acetone | Methanol |
| 1 | *M. khasianus* | Fruits | 19.42±0.19 | 26.20±0.15 | 18.90±0.15 | 23.38±0.16 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 2 | *P. esculenta* | leaves | 33.98±0.28 | 49.86±1.05 | 100.31±1.02 | 52.71±0.24 |
| 3 | *S. nigrum* | leaves | 44.97±0.45 | 129.50±0.98 | 125.81±2.12 | 84.77±0.72 |
| 4 | *P. pedicellatum* | leaves | 80.30±0.85 | 326.21±1.84 | 184.14±3.26 | 298.03±2.78 |
| 5 | *P. hirta* | leaves | 90.77±1.20 | 129.60±1.02 | 107.49±1.41 | 291.68±5.55 |

Each value in the table was obtained by calculating the average of three experiments and data are presented as Mean ± SEM

The highest amount of flavonol is observed in the chloroform extract of *P. pedicellatum* (326.21±1.84 mg/g dry extract) followed by the methanol extract of this plant. Very good amounts of flavonol are also found to contain in the chloroform, acetone and methanol extract of *P. esculenta*, *S. nigrum* and *P. hirta*.

The results strongly suggest that phenolics are important components of these plants. The other phenolic compounds such as flavonoids, flavonols, which contain hydroxyls are responsible for the radical scavenging effect in the plants. According to our study, methanol was the most suitable solvent to isolate the phenolic compounds and benzene, chloroform and acetone are the best solvent to isolate the flavonoids and flavonols from the plant materials. The high content of the phenolic compounds in *P. pedicellatum, P. hirta, P. esculenta* and *P. hirta* can explain their high radical scavenging activity.

**Reducing power assay**

The reducing capacity may be a significant index of antioxidant activity. The reducing powers of the five leafy vegetables are evaluated as mg AAE/g dry material as shown in Table 5.

**Table 5. Reducing power (ascorbic acid equivalent) of in wild edible plants collected from Arunachal Pradesh using different solvents**

| Sl No | Name of the plant | Parts used | Reducing power (Ascorbic acid equivalent mg / g dry extract) | | | |
|---|---|---|---|---|---|---|
| | | | Benzene | Chloroform | Acetone | Methanol |
| 1 | *M. khasianus* | Fruits | 11.53±0.76 | 19.09±0.24 | 14.95±0.49 | 16.08±0.30 |
| 2 | *P. esculenta* | leaves | 15.64±0.60 | 35.38±1.39 | 27.71±1.81 | 18.58±0.20 |
| 3 | *S. nigrum* | leaves | 17.22±0.96 | 34.35±2.17 | 48.47±3.76 | 20.38±0.71 |
| 4 | *P. pedicellatum* | leaves | 19.25±1.19 | 67.97±3.92 | 110.25±2.94 | 587.43±5.93 |
| 5 | *P. hirta* | leaves | 34.59±1.09 | 45.00±1.17 | 48.74±2.82 | 518.86±11.84 |

Each value in the table was obtained by calculating the average of three experiments and data are presented as Mean ± SEM

The highest reducing power was exhibited by the methanol extract of *P. pedicellatum* (587.43±5.93 mg/g AAE) which also contain a very good amount of flavonoids and flavonols. The benzene extract of *M. khasianus* showed lowest activity in terms of ascorbic acid equivalent (11.53±0.76 mg/g AAE). In this assay, the presence of antioxidants in the extracts

reduced $Fe^{+3}$/ferricyanide complex to the ferrous form. This reducing capacity of the extracts may serve as an indicator of potential antioxidant activities through the action of breaking the free radical chain by donating hydrogen atom [16].

**DPPH radical scavenging activity**

The evaluation of anti-radical properties of five wild edible leaves was performed by DPPH radical scavenging assay. The 50% inhibition of DPPH radical ($IC_{50}$) by the different plant materials was determined (Table 6), a lower value would reflect greater antioxidant activity of the sample. DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound or plant extracts [17]. The antioxidant effect is proportional to the disappearance of the purple colour of DPPH in test samples. Thus antioxidant molecules can quench DPPH free radicals by providing hydrogen atom or by electron donation and a colorless stable molecule 2, 2-diphenyl-1-hydrazine is formed and as a result of which the absorbance (at 517 nm) of the solution is decreased.

**Table 6. Free radical scavenging ability of the wild edible plants collected from Arunachal Pradesh by the use of a stable DPPH radical (antioxidant activity expressed as $IC_{50}$)**

| Sl No | Name of the plant | Parts used | Free radical scavenging ability $IC_{50}$ mg / g dry extract | | | |
|---|---|---|---|---|---|---|
| | | | Benzene | Chloroform | Acetone | Methanol |
| 1 | *M. khasianus* | Fruits | 3.65±0.81 | 2.66±0.03 | 3.52±0.19 | 0.51 ±0.004 |
| 2 | *P. esculenta* | leaves | 0.84±0.16 | 0.85±0.03 | 1.04±0.13 | 1.37±0.02 |
| 3 | *S. nigrum* | leaves | 1.42±0.11 | 0.76±0.13 | 0.37±0.02 | 1.03±0.001 |
| 4 | *P. pedicellatum* | leaves | 0.20±0.01 | 0.15±0.004 | 0.21±0.002 | 0.041±0.001 |
| 5 | *P. hirta* | leaves | 0.42±0.05 | 0.40 ±0.02 | 0.48±0.02 | 0.047±0.003 |

Each value in the table was obtained by calculating the average of three experiments and data are presented as Mean ± SEM

Hence the more potent antioxidant, more decrease in absorbance is seen and consequently the $IC_{50}$ value will be minimum. In the present study the highest radical scavenging activity was shown by the methanol extract of *P. pedicellatum* ($IC_{50}$ = 0.041±0.001 mg dry extract), whereas the benzene extract of *M. khasianus* showed lowest activity ($IC_{50}$ = 3.65±0.81 mg dry material). A strong inhibition was also observed for the methanol extract of *P. hirta* ($IC_{50}$ = 0.047±0.003 mg dry extract) and chloroform extract of *P. pedicellatum* (0.15±0.004 mg dry material). The high radical scavenging property of these plants may be due to the presene of hydroxyl groups that can provide the necessary component as a radical scavenger.

**ABTS radical scavenging activity**

ABTS scavenging activities in various extracts of five leafy vegetables using ABTS assay was shown in Table 7. The antioxidant effect is proportional to the disappearance of the colour of

ABTS in test samples. Concentration of sample that could scavenge 50 % free radical ($IC_{50}$) was used to determine antioxidant capacity of sample compared to standard. Sample that had $IC_{50}$ < 50 ppm, it was very strong antioxidant, 50-100 ppm strong antioxidant, 101-150 ppm medium antioxidant, while weak antioxidant with $IC_{50}$ > 150 ppm [10] . In the present study the highest radical scavenging activity was shown by the methanol extract of *P. pedicellatum* ($IC_{50}$ = 0.15±0.001 mg dry extract), whereas the benzene extract of *M. khasianus* showed lowest activity ($IC_{50}$ =0.54±0.08 mg dry extract). Strong inhibition was also observed for the chloroform and methanol extract of *P. esculenta* and *P. hirta*.

**Table 7. Free radical scavenging ability of the wild edible plants collected from Arunachal Pradesh by the use of a stable ABTS radical cation (antioxidant activity expressed as $IC_{50}$)**

| Sl No | Name of the plant | Parts used | Free radical scavenging ability $IC_{50}$ mg / g dry extract) | | | |
|---|---|---|---|---|---|---|
| | | | Benzene | Chloroform | Acetone | Methanol |
| 1 | *M. khasianus* | Fruits | 0.54±0.08 | 0.47±0.01 | 0.44±0.01 | 0.25 ±0.04 |
| 2 | *P. esculenta* | leaves | 0.48±0.03 | 0.19±0.01 | 0.23±0.01 | 0.18±0.01 |
| 3 | *S. nigrum* | leaves | 0.23±0.01 | 0.21±0.01 | 0.21±0.04 | 0.19±0.001 |
| 4 | *P. pedicellatum* | leaves | 0.29±0.01 | 0.22±0.01 | 0.28±0.03 | 0.15±0.001 |
| 5 | *P. hirta* | leaves | 0.49±0.01 | 0.18 ±0.007 | 0.20±0.03 | 0.16±0.02 |

Each value in the table was obtained by calculating the average of three experiments and data are presented as Mean ± SEM

The benzene, chloroform, acetone and methanol extracts of all of the leafy vegetables under investigation exhibited different extent of antioxidant activity and thus provide a valuable source of nutraceutical supplements.

## CONCLUSION

The result of present study showed that the methanol extract of *P. pedicellatum* , which contain highest amount of phenolic compounds exhibited the greatest radical scavenging activity. The benzene, chloroform, acetone and methanol extract of all leafy plants under investigation contain a very good amount of flavonoids and flavonols also showed strong radical scavenging activity in both ABTS and DPPH method. The radical scavenging activities of the selected plants extracts are still less affective than the commercial available synthetic like BHT and trolox. As the plant extracts are quite safe and the use of synthetic antioxidant has been limited because of their toxicity, therefore, these wild edible plants could be exploited as antioxidant additives and supplements for the diseases associated with oxidative stress.

In addition, naturally antioxidants have the capacity to improve food quality and stability and also act as nutraceuticals to terminate free radical chain reaction in biological systems, and thus may provide additional health benefits to consumers.

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