Proximate Composition, Phenolic Contents and in vitro Antioxidant Properties of *Pimpinella stewartii* (A Wild Medicinal Food)

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Abstract Present study was intended to examine proximate nutritional and minerals composition, phenolic contents and in vitro antioxidant properties of *Pimpinella stewartii*, a commonly utilized species as medicinal and flavoring agent by the inhabitants of Pakistani Himalayas. Ethnomedicinal data were collected by semi structured interviews and questionnaires. Standard analytical methods were applied to determine nutrients, minerals and phenolic contents, whereas free radical scavenging activities and total antioxidant properties were estimated by different assays. Results indicated that *Pimpinella stewartii* is an excellent source of carbohydrates, proteins and dietary fibers. Likewise, this specie also contains significant levels of K (6332 ± 56.1), Ca (3141 ± 47.0), Fe (1512 ± 18.7) and Mg (478.6 ±11.4) mg/kg. Comparatively, water extracts showed higher concentrations of phenolic contents than acetone extracts, showing significant difference \((p<0.05)\). Flavonoid content was maximum in water extract at 98.67 ± 0.14 mg Rt Eq/100 g FW, followed by flavonols, total phenolics and ascorbic acid. Phosphomolibdenium complex assay (PMA) exhibited highly significant total antioxidant capacity at 86.26 ± 0.53 µM AAE/100g FW for acetone extract, followed by % DPPH radical scavenging activity (62.39 ± 0.40), whereas in water extracts measured levels were highest for ferrous ion chelating activity and ferric ion reducing antioxidant power (FRAP). *Pimpinella stewartii* was found rich source of nutrients and phenolic contents, and demonstrated significant in vitro antioxidant properties. Furthermore, in depth investigation of phytochemical profiling, in vivo antioxidant properties and biological activities could be useful to promote consumer health and prevention of degenerative diseases.

Keywords: nutrients, phenolics, antioxidant properties, Pimpinell stewartii, Himalayas, Pakistan


1. Introduction

The tradition of eating wild plants has not been completely disappeared due to their significant contribution in food security and human health since time immemorial [1,2]. Wild edible plants are significant for the livelihoods of rural communities as being an essential part of their survival strategy in many developing countries. Apart from their traditional use of food, wild edible plants are also used as medicine, fodder, and for rituals functions and as genetic resources for new crop species development. Wild food plants serves as substitute to staple food during periods of food shortage and are the valuable supplements for a nutritional balanced diet [3]. Consumers now demand healthy, tasty and uncontaminated natural functional foods. In this context wild edible plants are used because of their assumed health benefits and thus can be called as medicinal foods [4].

Reactive oxygen species (ROS) including superoxide anion, hydroxyl radical species, singlet oxygen, hydrogen peroxide and nitrogen species are generated due to oxidative stress [5]. ROS counter bio-molecules and caused cellular damage, which leads to chronic diseases such as cancers, cardio vascular disorders, arteriosclerosis, malaria, diabetes, stroke, rheumatoid arthritis, ageing processes as well as neurodegenerative diseases that may be lead to death [6,7]. Human body has an antioxidant defense system, which maintains a balance between the production and inactivation of ROS. However, pathological conditions and inadequate endogenous antioxidant defense result overproduction of ROS, which leads to lipid per-oxidation, damage to DNA and causes oxidative stress [8]. Growing knowledge about the health-promoting impact of antioxidants in everyday foods is combined with the assumption, that a number of common synthetic preservatives may have hazardous effects on health [9], this fact has led to increase investigations in the field of natural antioxidants [10].
Epidemiological studies showed contrary association between the consumption of fruits and vegetables, and the risk of degenerative and chronic diseases. Health benefits of fruits and vegetables are mainly attributed to natural antioxidant compounds including polyphenols, flavonoids, carotenoids, anthocyanin, flavonols, vitamin C and E present in them [11], owning the ability of body protection against degenerative diseases [12]. Information about such food is a part of traditional knowledge, transmitted from one generation to next through the contribution of household individuals [13]. Globally, the traditions of using wild edible plants as food and medicine are at the risk of disappearing. Therefore it is important to collect data about popular uses of such plants species before vanishing of this knowledge, and to find innovative ways of infusing them for the future generations [14].

Pimpinella genus belongs to family Umbellifereae is represented by 150 species, which are distributed in Africa, Europe, Asia and South America. In Pakistan Pimpinella stewartii (Dunn) E. Nasir, synonyms Eriocyca stewartii, or Pituranhos stewartii is mainly distributed in Murree, Abbottabad and Haripur hills from 700 to 2500 meters elevation. It is an annual herb up to one meter in height, having whitish flowers and long ovoid fruits. It grows along the margins of cultivated fields and on dry or rocky places along with grasses (Figure 1). The roots of P. stewartii are fragrant, and fruits are carminative, used cure indigestion, gastric and griping pain in stomach [15,16,17,18]. To our knowledge phenolic contents and antioxidant properties of P. stewartii has never been reported before. In this context present study was aimed to assess proximate nutritional, mineral and phenolic composition, and in-vitro antioxidants properties of P. stewartii.

Figure 1. Pimpinella stewartii (Dunn) E. Nasir

2. Materials and Methods

2.1. Ethnobotanical Data Collection

Data on ethnomedicinal value were collected through interviews, questionnaires, and focus group conversation with local informants having traditional knowledge of wild edible plants [1], from 25 different localities of Abbottabad, Haripur and Murree Hills. Cultural value, including cultural importance index (CI) and mean cultural importance index (mCI) were also calculated as reported previously [1].

\[
CI = \frac{1}{N} \sum_{i=1}^{N} URi
\]

Where, N is the number of informants and UR is the use report in each use category.

2.2. Sampling

Freshly collected specimen were identified by Prof. Dr. Mir Ajab Khan, Department of Plant Science, Quaid-i-Azam University-Islamabad. In brief, about 1-2 kg fresh sample was collected and washed vigilantly with tap water followed by distilled water. Afterwards, sample was dried.
at 55°C in electric oven until constant weight is obtained [19], then ground with a porcelain pestle and mortar. Fine powder was sieved through muslin cloth, stored in polythene bottles and was kept in desiccators for analysis.

2.3. Standards and Reagents

Ascorbic acid; ethylenedi-aminetetraacetic acid (EDTA); aluminum chloride, ferric chloride, ferrous chloride, ferrous sulphate, Folin-Ciocalteu; 2,2-diphenyl-1-picrylhydrazyl (DPPH); sodium acetate, rutin, gallic acid, meta-phosphoric acid, 1,10-phenanthroline, ferrozine, dichloroindophenol, trichloroacetic acid (TCA) were purchased from Sigma Co. (St. Louis, MO, USA). Standards solutions of different metals were purchased from Merck Co. (Darmstadt, Germany). Sulphuric acid; sodium carbonate, sodium hydroxide, sodium nitrite, disodium hydrogen phosphate and hydrogen peroxide were obtained from Wako Co. (Osaka, Japan). Potassium ferricyanide, sodium dihydrogen phosphate, Nitric acid, perchloric acid, from Wako Co. (Osaka, Japan). Potassium ferricyanide, hydrogen phosphate and hydrogen peroxide were obtained from Wako Co. (Osaka, Japan). Potassium ferricyanide, sodium dihydrogen phosphate, Nitric acid, perchloric acid, hydrochloric acid, potassium sulphate, sodium sulphate, copper sulphate, ammonium molybdate, selenium dioxide, boric acid, sodium carbonate, acetone, petroleum ether, methyl red/bromo-cresol green indicators were used of analytical grade and purchased from Merck Co. (Darmstadt, Germany).

2.4. Determination of Nutrients

Proximate composition of crude proteins, fats, carbohydrates, crude fibers, ash, moisture, dry matter and calorific values was determined by the methods as explained by AOAC [20] and final values were represented as means of triplicate ± SD.

2.5. Quantification of Minerals

To estimated mineral contents (~1.0 g) of powder sample was digested in a mixture of nitric, sulphuric and perchloric acids (1:2:1) at 80-85°C until a clear solution was obtained [21]. A blank was also prepared likewise and trace metals including Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Na, Pb, Sr and Zn were quantified using atomic absorption spectrophotometer (AAS). Final concentrations in (mg/kg) were represented as means of three replicates ± SD.

2.6. Estimation of Phenolic and Ascorbic acid Contents

2.6.1. Extraction

A two step hydrophilic (aqueous) and hydrophobic (acetone) extraction procedure was adopted as reported before [22]. Briefly, 1,000g of sample was blended with 10 mL distilled water, then centrifuged at 6000rpm for 15 min, and supernatant was collected in a clean test tube. This procedure was repeated thrice and supernatants were pooled into a flask. The solid residue was then re-extracted 3 times in acetone (1:10 w/v) and supernatants were collected in another flask.

2.6.2. Determination of Phenolic Contents

Total phenolics content (TPC) was determined by using the method described previously [23], with slight modifications. In brief, 1.0 mL aliquots of water or acetone extracts were mixed with 5 mL of 10 fold diluted Folin-ciocalteu reagent and 4 mL of 7.5% sodium carbonate. The mixture was allowed to stand for 90 minutes at room temperature and absorbance was measured at 760 nm. Final values were articulated as mg gallic acid equivalents in 100g on fresh weight basis of the sample (mg GAE/100g, FW) and presented as mean of three replicates ± SD.

Total flavonoids content (TFC) was estimated by modified colorimetric method as reported earlier [23]. Briefly, 5 mL of water or acetone extract was mixed with 0.3 mL of 5% sodium nitrite for 5 minutes in a test tube followed by the addition of 0.3 mL of 10% aluminum chloride. After 6 min, 2 mL of sodium hydroxide was added to stop the reaction and mixture was further diluted with deionized water up to 10 mL. Absorbance was immediately measured at 510 nm and final values were expressed as mg rutin equivalents in 100g of sample on fresh weight basis (mg Rt/100g, FW) and presented as mean of triplicates ± SD.

Total flavonols content (TFC) of the studied sample was determined following the method as reported by [24]. In short, 2.0 mL of aluminum trichloride (2%) and 3 mL sodium acetate (50 g/L) solutions were mixed in 2.0 mL of sample extract. The absorption was measured at 440 nm after 2.5 h at 20°C and final values were expressed as mean of three replicates ± SD in mg rutin equivalents in 100g on fresh weight basis of sample (mg Rt/100g, FW).

2.6.3. Determination of Ascorbic Acid

Ascorbic acid content (AAC) was calculated as described earlier [25]. Briefly, water and acetone extracts were re-extracted with meta-phosphoric acid (1%, 10 mL) for 45 min at room temperature and filtered. The filtrate (1.0 mL) was mixed with 9 mL of 2, 6-dichloroindophenol (0.8 g/1000 mL) and absorbance was measured within 30 minutes at 515 nm. Ascorbic acid content was estimated on the basis of calibration curve of L-ascorbic acid (0.006-0.1 mg/mL; y = 3.006x + 0.007; R² = 0.999). Mean final concentrations were represented as mg ascorbic acid equivalents in 100g of sample on fresh weight basis (mg AA/100g FW) for three replicates ± SD.

2.7. Antioxidant Assays

2.7.1. DPPH Radical Scavenging Activity

DPPH free radical scavenging activity was calculated following the method as explained previously [26]. Briefly 2.0 mL of the extracts or standards were mixed vigorously in 5 mL of DPPH solution (0.1 mM in methanol), and incubated in dark for 30 minutes at room temperature. The decolourization of DPPH was measured against blank at 517 nm. The percentage inhibition was calculated using formula and represented as mean of three replicates ± SD.

\[
\text{Inhibition} = \left( \frac{A_{\text{Blank}} - A_{\text{Sample}}}{A_{\text{Blank}}} \right) \times 100.
\]

2.7.2. Hydroxyl Radical Scavenging Activity

Hydroxyl radical scavenging activity was measured as reported earlier [27]. In brief, 2.0 mL of 0.2 M phosphate
buffer (pH 7.2), 0.04 mL ferrous sulphate (0.02 M), 2 mL of extract and 1 mL of 1, 10-phenanthroline (0.04 M) were mixed in a test tube. The Fenton reaction was initiated by the addition of 0.1 mL of 7 mM H₂O₂. Absorbance was measured at 560 nm after 5 min incubation at room temperature and expressed in (%) relative hydroxyl radical scavenging activity as mean of triplicate ± SD

\[
Scavenging\text{ }Activity(\%) = \frac{(A_{\text{Blank}} - A_{\text{Sample}})}{(A_{\text{Blank}})} \times 100.
\]

2.7.4. Ferrous Ion Chelating Activity

Ferrous ion chelating activity was measured following the method as described before [29]. Briefly, 2.0 mL of extract and ferrous sulphate (0.125 mM) each was mixed together, following the addition of 2 mL of 0.3125 mM ferrozine to start reaction. The mixture was shaken vigorously, and left at room temperature for 10 min before taking absorbance at 562 nm against blank. EDTA (0.625-5.0 mg) was used as positive control and sample without extract or EDTA served as negative control. Final results expressed as percentage inhibition of ferrozine-Fe (II) complex were calculated using formula as given, and represented as mean of three replicates ± SD.

\[
Chelating\text{ }Activity(\%) = \frac{(A_{\text{Control}} - A_{\text{Sample}})}{(A_{\text{Control}})} \times 100.
\]

2.7.5. Ferric Ion Reducing Antioxidant Power (FRAP)

Total antioxidant activity of the water and acetone extracts was estimated using the method as previously [30]. In brief, 2.0 mL of each, sample extract, phosphate buffer (0.2 M, pH 6.6) and potassium ferricyanide (0.1%) were mixed, followed by incubation at 50°C in water bath for 20 minutes. Then 2 mL of trichloroacetic acid (10%) was added to stop the reaction. About 2 mL supernatant was separated and mixed with 2 mL of distilled water and 2 mL of 0.01% ferric chloride. Mixture was kept at room temperature for 20 min before taking absorbance at 700 nm against blank. Gallic acid was used as positive control. Final values were expressed as micromole gallic acid equivalent per 100g on fresh weight basis of sample (µM AAE/100 g, FW), and presented as mean of three replicates ± SD.

2.7.6. Phosphomolydenium complex assay (PMA)

Total antioxidant capacity (TAC) was calculated using phosphomolydenium complex assay as reported before [31]. In short, 2.0 mL of extract and 6.6 mL of reagent mixture (0.6 mol/L sulphuric acid, 28 mol/L sodium phosphate and 4 mol/L ammonium molybdate) were mixed in flask, then capped and incubate at 95°C for 90 minutes. Absorbance was measured at 695 nm against blank after cooling at room temperature. Final results were expressed as micromole ascorbic acid equivalent per 100g fresh weight of sample (µM AAE/100 g, FW), and presented as mean of triplicate value ± SD.

3. Results and Discussion

3.1. Ethnomedicinal Uses and Culture Values

Inhabitants of the study area used shade dried leaves of *Pimpinella stewartii* to treat gastrointestinal disorders including gas trouble, indigestion and griping pain, which are in agreement as reported earlier [16,17,18,32]. Fresh leaves and aerial parts of this species are also used as flavoring agent in curries and vegetables. Based on use reports mean cultural important index (mCI) of the taxon was 0.793, which revealed that ethno-medicinally *P. stewartii* is a well-known species among the rural communities of the Haripur, Abbottabad and Murree particularly reside in the mountainous areas. Present findings also indicated strong interaction between local communities and natural flora in surrounding.

3.2. Proximate Nutritional Composition

Approximate values of nutrients in *P. stewartii* are mentioned in Table 1, which indicated that percentage carbohydrates content calculated by difference method was highest at 61.09 ± 0.14, followed by % crude proteins (13.35 ± 0.39), and % crude fibers (12.19 ± 0.47) contents on dry weight basis of the sample with significant difference at difference at p<0.05. Measured values for proteins and crude fats were in agreement as reported for *Pimpinella anisum* [33]. Energy value was calculated at 322.4 ± 0.41 Kcal/100 g, while fresh sample contained 80.42% moisture and 19.80% dry matter contents.

<table>
<thead>
<tr>
<th>Table 1. Proximate composition of <em>Pimpinella stewartii</em></th>
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<tbody>
<tr>
<td>Proximate composition</td>
</tr>
<tr>
<td>Crude proteins (%)</td>
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<tr>
<td>Crude fats (%)</td>
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<tr>
<td>Crude fibers (%)</td>
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<tr>
<td>Ash content (%)</td>
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<tr>
<td>Carbohydrates content (%)</td>
</tr>
<tr>
<td>Dry matter content (%)</td>
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<tr>
<td>Moisture content (%)</td>
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</tbody>
</table>

Different letters (a-g) within the columns indicate significant difference at p<0.05. Values are means of three replicates ± SD.

3.3. Minerals Contents

Measured levels of selected metals expressed as mg/kg are given in Figure 2. It is evident that among essential elements, potassium metal showed maximum contribution at 6332 ± 56.1 mg/kg, followed by Ca (3141 ± 47.0 mg/kg), Fe (1512 ± 18.7 mg/kg), Mg (478.6 ± 11.4 mg/kg), Na (157.3 ± 0.55 mg/kg), Mn (15.85 ± 0.56 mg/kg) and Zn (14.31 ±0.43 mg/kg) showing significant
difference at \( p < 0.05 \). Among other elements notable level was measured for Co (33.41 ± 0.40 mg/kg), whereas copper metal exhibited lowest concentration at 4.531 ± 0.18 mg/kg. Measured values of Cd, Sr, Cr, Li, and Pb levels were in the range of 4.871 mg/kg to 22.47 mg/kg with no significant difference at \( p < 0.05 \).

Figure 2. Measured levels of metals (mg/kg) in *Pimpinella stewartii*

Different letters (a-f) indicate significant difference at \( p < 0.05 \)

Values are means of three replicates ± SD.

3.4. Total Phenolics and Ascorbic Acid Contents

Results of phenolic and ascorbic acid contents are presented in Table 2. In general, water extracts exhibited elevated levels of phenolics, flavonoids, flavonols and ascorbic acid contents compared to corresponding acetone extracts. Phenolics and flavonoids are powerful free radical scavengers and significantly important natural antioxidants [34]. Among phenolics, total flavonoids content exhibited highest value (98.67 ± 0.41 mg Rt/100g) in the water extract, followed by flavonols and total phenolic contents at 51.15 ± 0.29 mg Rt/100g FW and 29.67 ± 0.52 mg GAE/100 g FW respectively. Estimated level of ascorbic acid was high in water extract (1.633 ± 0.34 mg AA/100g). In the case of acetone extract flavonoids content was 38.19 ± 0.33 mg Rt/100 g FW, followed by flavonols, phenolics and ascorbic acid contents. It has been reported that total phenolics content was ranged 1.071 to 1.441 \( \mu \)g/ml in *Pimpinella tirupatiensis*, 7.275-29.05 mgGAE/g in *Pimpinella brachycarpa* and 0.014-0.220 mg GAE/100g in *Pimpinella barbata* collected from different parts of India and China [35,36,37]. Likewise, 331.1-872.0 \( \mu \)gQE/g of flavonoids contents were reported in *Pimpinella brachycarpa* [37]. However, these results are not comparable to our findings due to difference in plant species, climatic conditions and analytical techniques.

<table>
<thead>
<tr>
<th>Contents</th>
<th>Water extract</th>
<th>Acetone extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total phenolics (mg GAE/100 g, FW)</td>
<td>29.67± 0.52</td>
<td>12.74± 0.54</td>
</tr>
<tr>
<td>Flavonoids (mg Rt/100 g, FW)</td>
<td>98.67± ± 0.41</td>
<td>38.19± 0.33</td>
</tr>
<tr>
<td>Flavonols (mg Rt/100 g, FW)</td>
<td>51.15± 0.29</td>
<td>26.11± 0.62</td>
</tr>
<tr>
<td>Ascorbic acid (mg AA/100 g, FW)</td>
<td>1.633± ± 0.34</td>
<td>1.114± 0.13</td>
</tr>
</tbody>
</table>

Different letters (a-d) within the columns indicate significant difference at \( p < 0.05 \)

Values are means of three replicates ± SD.

3.5. Antioxidant Properties

Antioxidant properties of *P. stewartii* were estimated by different assays such as, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Ferrous ion (Fe\(^{2+}\)), hydrogen peroxide (H\(_2\)O\(_2\)), hydroxyl ion (OH\(^{-}\)) free radicals scavenging activities, phosphomolybdenum complex assay (PMA) and Ferric ion reducing antioxidant power (FRAP) as given in Table 3. Usually, 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay is used to assess preliminary antioxidant activity, which is based on ability of DPPH compound to react proton donors such as phenolics [38]. Present study indicated that
acetone extracts showed better results for DPPH, H₂O₂, OH⁻ and PMA assays, whereas estimated values in Fe²⁺ and FRAP assay were high in water extracts.

Percentage DPPH radical scavenging activity measured for acetone extract of *P. stewartii* was 62.39 ± 0.40, which was slightly higher than water extract showing significant difference (*p*<0.05). Though different workers [35,36,37] have reported DPPH radical scavenging activity in other species of the genus *Pimpinella*, such as *P. barbata*, *P. brachycarpa* and *P. tirupatiensis*. However, present findings are not comparable due to variations in species.

### Table 3. Antioxidant properties of *Pimpinella stewartii*

<table>
<thead>
<tr>
<th>Antioxidant assay</th>
<th>Water extract</th>
<th>Acetone extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPPH (%)</td>
<td>61.08 ± 0.47</td>
<td>62.39 ± 0.40</td>
</tr>
<tr>
<td>Fe²⁺ (%)</td>
<td>61.16 ± 0.39</td>
<td>50.76 ± 0.36</td>
</tr>
<tr>
<td>H₂O₂ (%)</td>
<td>25.18 ± 0.75</td>
<td>52.59 ± 0.49</td>
</tr>
<tr>
<td>OH⁻ (%)</td>
<td>0.09 ± 0.07</td>
<td>11.70 ± 0.56</td>
</tr>
<tr>
<td>PMA (µM AAE/100 g, FW)</td>
<td>56.55 ± 0.66</td>
<td>86.26 ± 0.53</td>
</tr>
<tr>
<td>FRAP (µM GAE/100 g, FW)</td>
<td>23.28 ± 0.62</td>
<td>14.24 ± 0.46</td>
</tr>
</tbody>
</table>

Different letters (a-f) within the columns indicate significant difference at *p*≤0.05. Values are means of three replicates ± SD.

Hydroxyl ions (OH⁻) are extremely reactive and most injurious species in free radical patholgy, which cause damage to sugars, amino acids, lipids and nucleotides molecules within the living cells [39]. Results of OH⁻ radical scavenging activity presented in Table 3, revealed that that acetone extract exhibited high OH⁻ radical scavenging activity at 11.70 ± 0.56% compared to water extract. Hydrogen peroxide (H₂O₂) radicals have ability to across the cell membranes rapidly, result in the formation of hydroxyl radicals and inactivation of a few enzymes within the cells. We observed that, acetone extract showed high % H₂O₂ radical scavenging activity (52.59 ± 0.49) than corresponding water extract with significant difference (*p*<0.05). However, measured values for ferrous ions (Fe²⁺) chelating activity in water extract were significantly (*p*<0.05) higher at 61.16 ± 0.39 % compared to acetone extract. In detail literature survey indicated that DPPH, OH⁻, H₂O₂ and Fe²⁺ radical scavenging activities have never been reported for *P. stewartii*.

Ferric ion reducing antioxidant power (FRAP) assay is the only test used for the direct estimation of the reducing ability of antioxidants that react with ferric tripyridyltriazine (Fe³⁺ - TPTZ) complex and produce a colored ferrous tripyridyltriazine (Fe²⁺-TPTZ) [40]. In the same way, phosphomolybdenum complex assay (PMA) is generally used for the detection of ascorbic acid, phenolics, tocopherols and carotenoids. However, PM assay is also used for the determination of total antioxidant capacity (TAC) in food samples [41]. Measured values for FRAP essay were expressed as µM Gallic acid equivalent/100g, and that of phosphomolybdenum complex assay as µM ascorbic acid equivalent/100g on fresh weight of sample. In FRAP assay water extract showed higher concentration (23.28 ± 0.62 µM GAE/100g, FW) compared to the acetone extract with significant difference at *p*≤0.05 (Table 3). According to [37,42] *Pimpinella tragojoides* and *Pimpinella barbata* showed 18.98-153.02 mmol FeSO₄/100g and 1.180-2.371 mmol Fe²⁺/L ferric ion reducing activity respectively, but these values are unmatched to our findings. Nevertheless, in PMA complex assay measured value for acetone extract (86.26 ± 0.53 µM AAE/100g, FW) was more than water extract with significant difference (*p*<0.05).

### 4. Conclusion

*Pimpinella stewartii* is well known plant species among the rural communities of Himalayas in Pakistan because of its use in traditional medicines as carminative agent and as flavoring agent. Our findings revealed that, *Pimpinella stewartii* is an excellent source of nutrients, essential minerals and phenolics contents. Water and acetone extracts of *Pimpinella stewartii* exhibited significant free radical scavenging capacity and antioxidant properties. Furthermore, in depth phytochemical profiling, *in vivo* antioxidant properties and biological activities of this species could be useful to improve consumer health and for pharmaceutical and nutraceutical industries.

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