

Exploration of Nutritional and Bioactive Potential from Aromatic Herbal Plants of Gilgit-Baltistan: A Pathway to Functional Food Development

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This study explored the phenolic, flavonoid, tannin, ascorbic acid and mineral contents, as well as the antioxidant and antimicrobial activities of methanolic and hot water extracts of six aromatic herbal plants viz. *Hippophae rhamnoides* L., *Thymus serpyllum*, *Taraxacum officinale*, *Mentha arvensis* L., *Rosa rugosa* and *Prunus persica*, from Gilgit-Baltistan, Pakistan. Phytochemical screening revealed the presence of bioactive compounds in the studied plants, including proteins, phenols, tannins, flavonoids, saponins, oils and resins. The methanolic extracts showed higher values for all parameters compared to hot water extracts. Furthermore, *T. serpyllum* had the highest total phenolic content (18.769 mg GAE/g), flavonoid content (2.445 mg QE/g), antioxidant activity (91.72%) and significant antibacterial effects, surpassing the green tea sample (12.901 mg GAE/g). In contrast, the *H. rhamnoides* L., leaves exhibited the highest tannin content (195.744 mg GAE/g) and significant levels of Ca (19.64 mg/kg), Zn (10.43 mg/kg), Mg (12.73 mg/kg) and P (27.71 mg/kg), whereas *T. officinale* leaves had the highest vitamin C content (278.59 mg/100 g). Overall, the nutritional and bioactive properties of the studied plants were comparable to the green tea sample, highlighting their potential as nutraceuticals and their suitability for functional tea preparation and traditional therapeutic applications.

Keywords: Aromatic herbal plants, Bioactive compounds, Functional tea, Gilgit-Baltistan, Phytochemicals, Human health.

INTRODUCTION

Natural products derived from plants, animals and micro-organisms play a crucial role in discovering new bioactive compounds and enhancing food quality. Among these, plants are particularly valued for their renewability and substantial contributions to human well-being. Medicinal plants, used for centuries to treat various ailments, have inspired many modern pharmaceuticals [1]. Herbal medicines, comprising complex mixtures of plant-derived ingredients, are widely used globally to address diverse health concerns [2]. According to the WHO, herbal drugs consist of active plant ingredients combined with solvents, diluents or preservatives [3].

Aromatic plants, containing compounds extractable from flowers, seeds, leaves, bark, wood, fruits and roots [4], are prominent in traditional medicine, food preservation and flavour enhancement [5]. As consumer demand for organic products rises and concerns about synthetic additives grow, aromatic plants have gained popularity as functional ingredi-

ents across pharmaceutical, food and feed industries [6]. These plants contain polyphenolic compounds with antimicrobial, antioxidant and anti-inflammatory properties, making them eco-friendly, safe and ideal for health products [7]. Industries seek safe, effective and affordable bioactive compounds and plant-derived components show considerable promise in meeting these needs.

Aromatic herbs are primarily used for the essential oil extraction, with applications in cosmetics, spices, flavouring, fragrances, pesticides, repellents and herbal beverages [8]. Extensive research on their antioxidant and antimicrobial properties highlights their potential to prevent and reduce risks of diseases such as inflammation, cardiovascular disorders and cancer [9]. These plants also exhibit secondary metabolites like terpenoids, steroids and flavonoids, which further enhance their nutraceutical potential [10].

In recent years, neurodegenerative diseases have become a rising concern. Several studies suggest that various teas may provide protective effects against neurodegenerative and cardio-

vascular diseases, largely due to beneficial polyphenols [11,12]. New flavoured green tea varieties, such as lemon, ginger-mint and jasmine green tea, have been developed. Therefore, leveraging native plant materials could expand flavoured tea options, enhancing both health benefits and sensory appeal [13].

Gilgit-Baltistan, renowned for its biodiversity, hosts approximately 5,000 medicinal plant species. Among these, sea-buckthorn (*Hippophae rhamnoides* L.), thyme (*Thymus linearis*), duck rose, dandelion (*Taraxacum* spp.) and peach are abundant yet under utilised despite their nutritional and medicinal potential. Sea-buckthorn, known as the miracle plant, thrives in Gilgit-Baltistan, covering 5,700 hectares with over 12 million plants. Effective management could increase berry production, potentially generating over 300 million PKR annually [14]. Despite its therapeutic and nutritional benefits, the leaves and stems remain under utilised, leading to waste [11,15]. Likewise, *Thymus serpyllum* is traditionally used for respiratory and digestive ailments and shows potential in food and pharmaceutical applications [16,17]. Dandelion (*Taraxacum mongolicum*) possesses strong nutritional and medicinal properties, with roots supporting digestion and liver function, while leaves serve as diuretics and digestive stimulants [18]. Mint (*Mentha arvensis* L.), widely cultivated for its medicinal benefits, treats digestive issues and skin allergies [19]. The Rosa genus, with over 300 species, is known for its ornamental value and notable antioxidant and antimicrobial properties [20] and peach (*Prunus persica* L. Batsch.) exhibits high antioxidant activity [21].

Despite Gilgit-Baltistan's rich medicinal plant resources, much of their nutritional and bioactive potential remains under explored. Detailed investigation into their secondary metabolites could unlock new nutraceutical applications and provide economic benefits to local communities. This study aims to explore the nutritional and bioactive properties of selected medicinal plants from Gilgit-Baltistan, focusing on their potential for functional herbal tea production.

EXPERIMENTAL

Collection and processing of plant materials: Samples of sea-buckthorn (*H. rhamnoides* L.), thyme, duck rose, dandelion (*Taraxacum* spp.), mint (*M. arvensis*) and peach (*P. persica* L.) were collected from various locations in the Baltistan region, Pakistan. The plants were taxonomically identified and authenticated by Department of Plant Science at Karakoram International University, Gilgit, Pakistan. The collected plant specimens were washed with water to remove dust and soil particles. They were then shade-dried for 10 days to prevent chemical transformation and degradation by UV light. Once dried, the materials were ground into a fine powder.

Preparation of plant extracts: The powdered plant materials were soaked in 80% methanol for 72 h and then filtered through Whatman filter paper. The solvent was evaporated using a rotary evaporator to obtain concentrated extracts. The plant material powder was soaked in hot boiling water on water bath for 30 min and then the extract was filtered by Whatman filter paper.

Qualitative phytochemical analysis: Flavonoids were identified using the sulfuric acid and lead acetate test [22].

The presence of saponins and tannins was assessed by the methods outlined in Hussain *et al.* [22]. The ferric chloride assay was employed to detect phenols, while oil and resin presence was confirmed following Santhi & Sengottuvel's method [23]. Biuret, Millon's and ninhydrin tests were used for the identification of amino acids and proteins.

Determination of mineral content: Mineral contents, including Na, K, Ca, Mg, Fe, Mn, Cu and Zn, were determined after digestion with HNO₃/HClO₄ using atomic absorption spectrophotometry, following the method by Gokoglu *et al.* [24]. Phosphorus was measured using the ammonium molybdate method [25].

Determination of total phenolic content: Total phenolic compounds in both extracts were quantified by the Folin-Ciocalteu method, with gallic acid as the standard. The results were expressed as mg/g of gallic acid equivalents (GAE) [26]. After sample and standard preparation, 0.5 mL of each sample was mixed with 2.5 mL of 10-fold diluted Folin-Ciocalteu reagent and 2 mL of 7.5% sodium carbonate. The mixture was left to react for 30 min at room temperature before measuring the absorbance at 760 nm. All determinations were performed in triplicate.

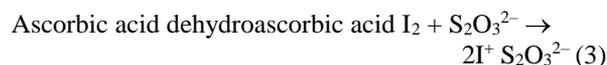
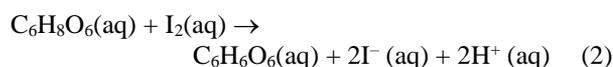
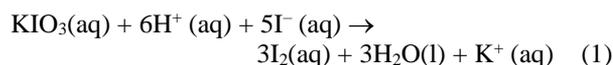
Determination of total flavonoid content: The total flavonoid content was measured using the aluminum chloride colorimetric assay. Quercetin was used as the standard, with results expressed as quercetin equivalents (mg QE/g). Absorbance was recorded at 510 nm using a spectrophotometer and each measurement was performed in triplicate.

Determination of total tannin content: Tannin content was determined using the Folin-Ciocalteu method, with results expressed as mg GAE/g of dry extract. After the addition of Folin-Ciocalteu reagent, sodium carbonate and distilled water, the mixture's absorbance was measured at 725 nm on a UV-visible spectrophotometer after 30 min [26].

Determination of ascorbic acid content: Ascorbic acid content was determined by an iodometric method [27]. Plant samples were mixed with potassium iodide and sulphuric acid before titration against sodium thiosulfate. A blank titration with distilled water was also performed and the ascorbic acid content was calculated based on the following reactions:



Equations for the reaction:



Antioxidant activity: Antioxidant properties of the plants extract were assayed by using DPPH free radical scavenging method [28] with a little modification. Briefly, the extracts were dissolved in DMSO and added 12 mg/mL standard and shaken well to fully dissolve the solution. Then DPPH-ethanol solution was made, 19.50 mg of DPPH was dissolved in 165 mL ethanol in a flask and covered it with an aluminum foil and shaken well. Three samples for each extract were prepared. Three were blank without samples with only DMSO

antioxidant mechanisms are primarily attributed to their hydrogen-donating and metal ion-chelating properties, which play a crucial role in scavenging free radicals and mitigating oxidative damage [35]. The elevated TPC in *T. serpyllum* suggests its potential as a significant source of natural antioxidants, offering promising benefits such as cancer prevention and antimicrobial activity. Conversely, *T. officinale* displayed the lowest phenolic content, which may correlate with its reduced antioxidant activity. Despite this, the high TPC observed in *T. serpyllum* and *R. rugosa* compared to the green tea sample suggests that these plants could provide a comparable or even superior source of phenolic compounds. Such findings are promising for the development of functional foods or supplements with enhanced antioxidant properties.

Total flavonoid contents: The total flavonoid content (TFC) was significantly higher in the methanolic extracts, ranging from 0.566 to 2.445 mg QE/g, compared to the water extracts, which ranged from 0.157 to 0.265 mg QE/g (Fig. 2). The higher TFC in methanolic extracts compared to water extracts is due to methanol's moderate polarity, which allows it to dissolve a wider range of flavonoids, including both polar and slightly non-polar types [36]. Water, being highly polar, is less effective for extracting partially non-polar flavonoids, resulting in lower TFC. Moreover, flavonoids are more stable in methanol, as they are susceptible to hydrolysis and degradation in aqueous solutions, especially under heat [37]. *T. serpyllum* had the highest flavonoid content (2.445 mg QE/g) and *R. rugosa* had the lowest (0.685 mg QE/g). Remarkably, *T. serpyllum* also exhibited the highest TFC in water extracts, while *R. rugosa* again had the lowest. *T. serpyllum* analysis align with previous research that highlights the significance of flavonoid-rich plants in promoting overall health and combating oxidative stress [37]. The higher flavonoid content in *T. serpyllum* suggests that it may offer enhanced health benefits compared to other plants studied.

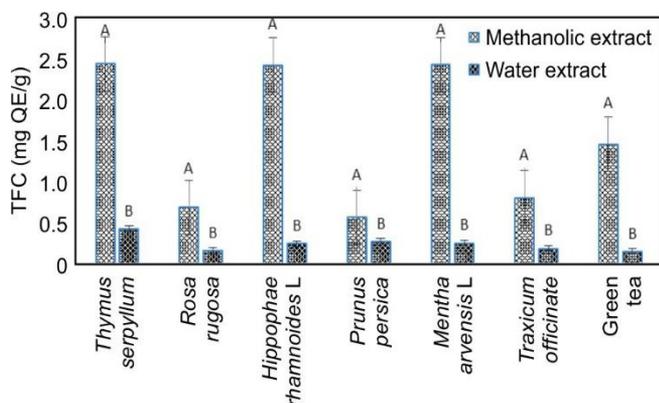


Fig. 2. Total flavonoid content in different aromatic herbal plants extract (mg QE/g) fresh weight; Note: Values having same superscripts do not differ considerably significantly in methanolic and hot water extract at $p \geq 0.05$

Total tannin contents: The current study assessed the tannin content in various herbal plant extracts, revealing a significant range across different plants and solvents. In methanolic extracts, tannin content ranged from 23.585 to 195.744 mg GAE/g, while in water extracts, it varied from 12.533 to 98.04 mg GAE/g (Fig. 3). *H. rhamnoides* L., leaves demonstrated

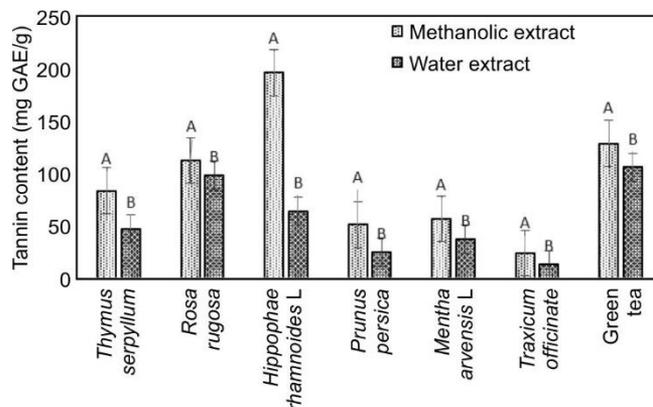


Fig. 3. Total tannin content in different aromatic herbal plants extract (mg GAE/g fresh weight); Note: Values having same superscripts do not differ considerably significantly in methanolic and hot water extract at $p \geq 0.05$

the highest tannin content in methanolic extract (195.744 mg GAE/g), while *R. rugosa* showed the highest tannin content in water extracts (98.04 mg GAE/g). Conversely, *T. officinale* exhibited the lowest tannin content in both aqueous (12.533 mg GAE/g) and methanolic (23.585 mg GAE/g) extracts. Significantly, *H. rhamnoides* L. had a higher tannin content (195.744 mg GAE/g) compared to the green tea reference (128.15 mg GAE/g). High tannin concentration in *H. rhamnoides* could be linked to its effectiveness in traditional medicine and its potential for developing therapeutic products.

Ascorbic acid contents: The ascorbic acid (vitamin C) content across the studied plants demonstrated considerable variability, ranging from 91.15 to 278.59 mg/100 mL in methanolic extract, which were generally higher than those in hot water extracts, which ranged from 91.53 to 185.23 mg/100 mL (Fig. 4). This suggests that methanol is more effective at extracting and preserving ascorbic acid due to its solvent properties and ability to stabilize thermolabile compounds [38]. Ascorbic acid is heat-sensitive and degrades at high temperatures, so the heating involved in hot water extractions can reduce its concentration due to thermal degradation and oxidation, while methanol extraction at lower temperatures preserves ascorbic acid more effectively [39]. Also, methanol stabilizes ascorbic acid better than water by reducing oxidation, leading to higher retention of this thermolabile compound [40]. Consequently, methanol is considered a superior solvent for retaining higher levels of ascorbic acid in plant extracts. In this study, *T. officinale* leaves exhibited the highest vitamin C content in methanolic extracts (278.59 mg/100 mL), while *M. arvensis* L. showed the highest ascorbic acid content in water extracts (191.53 mg/100 mL). Conversely, *T. officinale* had the lowest ascorbic acid content in hot water extracts (91.53 mg/100 mL). Remarkably, the ascorbic acid content in all studied plants was lower compared to the Green Tea reference sample (348.24 mg/100 mL). These results are consistent with previous studies that highlight the significant vitamin C content of *T. officinale* [41] and *M. arvensis* L. [42]. In contrast, the ascorbic acid content in *T. officinale* was found lower in hot water extracts, indicating that the extraction method can influence the vitamin C yield. The results suggest that some plants such as *T. officinale* and *M. arvensis* L. exhibit considerable ascorbic acid content, green tea remains the

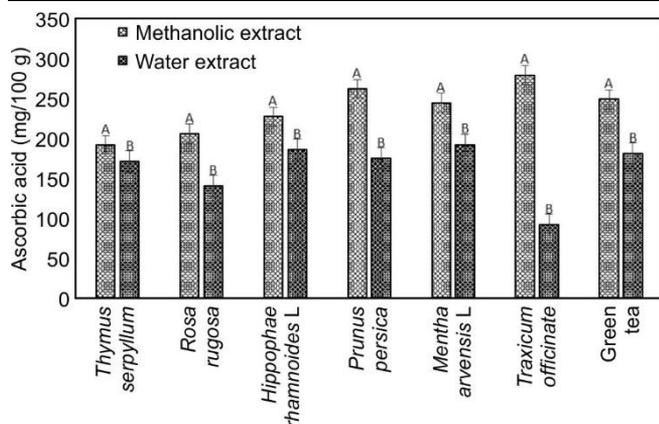


Fig. 4. Ascorbic acid content in different aromatic herbal plants extracts (mg/100 g); Note: Values having same superscripts do not differ considerably significantly in methanolic and hot water extract at $p \geq 0.05$

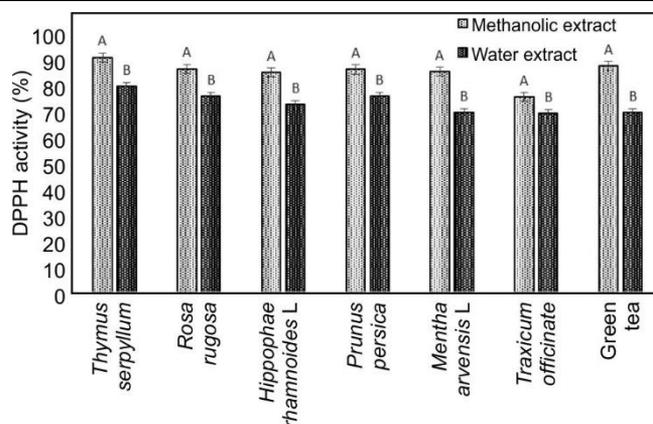


Fig. 5. Antioxidant activities using DPPH (RSA) in different aromatic herbal plant extracts; Note: Values having same superscripts do not differ considerably significantly in methanolic and hot water extract at $p \geq 0.05$

highest in vitamin C among the samples studied. This information could be valuable for selecting plants with high ascorbic acid content for nutritional and therapeutic applications.

Mineral contents: Important minerals such as iron (Fe), calcium (Ca), zinc (Zn), magnesium (Mg) and phosphorus (P) contents were determined in the six plants by using atomic absorption spectrophotometer (Table-2). The Fe contents in these plants were found in the ranged from 4.43 to 10.83 mg/Kg, Ca ranged from 7.88 to 19.64 mg/Kg Mg ranged from 4.79 to 10.43 to mg/Kg and P ranged from 18.72 to 27.71 mg/Kg. *T. officinale* contained higher amount of Fe 11.62 mg/Kg whereas Ca, Zn, Mg and P were highest in *H. rhamnoides L.* On the other hand, the lowest value of Fe found in (PP), Ca in (RR), Zn in (MA), Mg in (TO) and P in (RR). The minerals content in all six plants are comparable with green tea sample. It is suggested that these plants could offer beneficial mineral content in dietary applications also.

Antioxidant capacity: The antioxidant capacity of the medicinal plants was evaluated using the DPPH radical scavenging assay, revealing a range of inhibition percentages from 73.75% to 91.72% in methanolic extracts and from 73.75% to 88.41% in hot water extracts (Fig. 5). These results indicate that methanolic extracts generally exhibit slightly higher antioxidant activity than hot water extracts, likely due to the high polar protic nature of methanol, which enhances the extraction and stabilization of the antioxidant phtocompounds present in the plant material. The *T. serpyllum* leaves showed the highest DPPH value in both water (88.41% and methanolic extract (91.72 %), whereas *T. officinale* showed

the lowest DPPH value in methanolic and water extract. The percentage of DPPH inhibition of investigated plants was greater than the green tea sample. The high antioxidant activity observed in *T. serpyllum* can be attributed to its rich phenolic and flavonoid content, which are known to exhibit potent antioxidant properties [22]. The strong DPPH radical scavenging activity of plant supports its traditional use in medicine and its potential as a source of natural antioxidants. In contrast, *T. officinale* demonstrated the lowest antioxidant activity among the tested plants, which is attributed to its lower phenolic and flavonoid contents, as previous studies have linked these compounds to antioxidant efficacy [43]. Interestingly, the antioxidant activity of all investigated plants was greater than that of the green tea sample.

Antimicrobial activity: In this study, the antibacterial activity of extracts from six plants was assessed using the well diffusion method across various agar media such as nutrient agar (NA), tryptic soy agar (TSA), Sabouraud dextrose agar (SBA), Simmon's citrate medium (SIM) and Mueller-Hinton agar (MHA). Each plant was tested with both aqueous and methanolic extracts and antibiotic erythromycin was used as reference. Table-3 showed that *T. serpyllum* and *R. rugosa* exhibited positive antibacterial effects in TSA, SIM and NA, with no activity in MHA and SBA. *H. rhamnoides L.* demonstrated antibacterial activity in NA and SIM but was ineffective in TSA, MHA and SBA. *P. persica* had activity only in NA, with no significant effects in the other media, whereas *M. arvensis L.* was effective in NA and SIM but not in TSA, MHA and SBA. *T. officinale* exhibited positive effects in NA

TABLE-2
VALUES OF MINERALS CONTENT (mg/Kg) IN DIFFERENT AROMATIC HERBAL PLANTS

Species	Fe	Ca	Zn	Mg	P
<i>Thymus serpyllum</i>	10.83 ± 0.03	12.94 ± 0.06	10.42 ± 0.04	12.067 ± 0.06	23.417 ± 0.08
<i>Rosa rugosa</i>	9.35 ± 0.04	7.88 ± 0.05	9.76 ± 0.05	10.3 ± 0.05	19.87 ± 0.07
<i>Hippophae rhamnoides L.</i>	9.35 ± 0.05	19.64 ± 0.06	10.43 ± 0.03	12.73 ± 0.06	27.71 ± 0.09
<i>Prunus persica</i>	9.26 ± 0.03	16.29 ± 0.05	5.42 ± 0.02	12.56 ± 0.06	23.69 ± 0.08
<i>Mentha arvensis L.</i>	9.92 ± 0.05	17.97 ± 0.06	4.79 ± 0.02	12.42 ± 0.06	23.69 ± 0.08
<i>Traxicum officinale</i>	11.62 ± 0.06	8.89 ± 0.05	10.13 ± 0.04	8.37 ± 0.04	26.63 ± 0.09
Green tea	4.43 ± 0.01	18.43 ± 0.07	6.09 ± 0.03	10.13 ± 0.03	18.72 ± 0.06

Values are mean ± SE.

TABLE-3
ANTIMICROBIAL ACTIVITIES DATA IN AROMATIC HERBAL PLANTS

Species		NA		TSA		MHA		SBA		SIM	
		MOH	H ₂ O								
<i>Thymus serpyllum</i>	A	+	+	+	+	-	-	-	-	+	+
<i>Rosa rugosa</i>	B	+	+	+	+	-	-	-	-	+	+
<i>Hippophae rhamnoides</i> L.	C	+	+	-	-	-	-	-	-	+	+
<i>Prunus persica</i>	D	+	+	-	-	-	-	-	-	-	-
<i>Mentha arvensis</i> L.	E	+	+	-	-	-	-	-	-	+	+
<i>Traxicum officinate</i>	F	+	+	+	+	-	-	-	-	-	-
Green tea	G	+	+	-	-	-	-	-	-	+	+
Reference	R	+	+	+	+	+	+	+	+	+	+

Note: Nutrient Agar (NA), Tryptic Soy Agar (TSA), Sabouraud Dextrose Agar (SBA), Simmon's Citrate medium (SIM) and Mueller-Hinton Agar (MHA).

and TSA but not in MHA, SIM and SBA. Green tea showed activity in NA and SIM but lacked effectiveness in TSA, MHA and SBA, while the reference antibiotic, erythromycin, displayed positive antibacterial effects across all agar media, serving as a benchmark for comparison. The antibacterial activity results suggest that while none of the plant extracts exhibited broad-spectrum activity across all tested media, certain extracts demonstrated effectiveness against environmental non-pathogenic bacteria in specific media. *T. serpyllum* and *R. rugosa* showed the most consistent activity, indicating their potential as sources of antimicrobial agents whereas, *T. officinale* and *P. persica* showed more limited activity.

Conclusion

In conclusion, the present results showed that flavonoid, steroids, saponins, tannins and phenols were detected in hot water and methanolic extracts of *T. serpyllum*, *R. rugosa*, *H. rhamnoides* L., *P. persica*, *M. arvensis* L. and *T. officinale* from Gilgit-Baltistan region of Pakistan. Furthermore, the selected plant has significance amount of phenolic content, flavonoid, tannin and vitamin C and health benefit minerals in addition to have antioxidant and antibacterial activities. The vitamin C content in these plants is comparable to pepper and citrus fruits which are considered as good sources of vitamin C. In addition to nutritional and medicinal values these plants also have exhibited aromatic and natural fragrance. Moreover, the phytochemicals and mineral contents of the studied plants are comparable to green tea of Lepton Company. The antioxidant activity suggests that these plants could serve as natural sources of antioxidant compounds for use as food additives. As rich sources of bioactive compounds and minerals, they may also be suitable for preparing aromatic herbal tea. Further fractionation of methanolic and hot water extracts, isolation of phenolic compounds and evaluation of their biological activities *in vitro* and *in vivo* are required to better understand their mechanisms of action.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

DECLARATION OF AI-ASSISTED TECHNOLOGIES

During the preparation of this manuscript, the authors used an AI-assisted tool(s) to improve the language. The authors reviewed and edited the content and take full responsibility for the published work.

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