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Phytochemical analysis and antioxidant activities of *Artemisia stelleriana* Besser leaf extracts

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Abstract

The present study aims to report the proximate and mineral composition, phenolic contents, and antioxidant potential of *Artemisia stelleriana* leaves. The leaf extracts were prepared using various solvents like distilled water, methanol, ethanol and acetone and analyzed for their phenolic and flavonoid contents and antioxidant activity. The methanolic extracts showed the highest total phenolic and flavonoid contents (10.09 ± 0.24 mg GAE/g and 225.04 ± 0.38 mg QE/g respectively). The methanolic extracts showed significantly higher 1,1-Diphenyl-2-picrylhydrazyl radical scavenging assay (DPPH-RSA), Reducing power assay and total antioxidant capacity compared to distilled water, ethanol and acetone extracts. Gas Chromatography-Mass Spectroscopy revealed that the methanolic extracts of leaves to be a good source of bioactive compounds like 2,4-di-tert-butylphenol (2,4-DTBP), neophytadiene, octacosane and eucalyptol.

Keywords

Artemisia stelleriana, Total phenolic content, Antioxidant activity, DPPH

Introduction

Medicinal plants have been helping humankind for thousands of years to fight ailments and act as dietary supplements. In recent years many medicinal plants have been studied to examine their antioxidant activity, as these natural antioxidants have been associated with a lower risk of various diseases (1). Polyphenols are a complex group of secondary metabolites that can be categorized into flavonoids and phenolic acids. They are involved in protecting the cell constituents against oxidative stress and damage through their free radical scavenging activity (2). Some phenolic compounds act as antibiotics and antidiarrheal, antiulcer and anti-inflammatory agents and are capable of preventing genotoxicity by reducing exposure to oxidative and carcinogenic factors (3).

The genus *Artemisia*, belong to Asteraceae family, has been explored, since time immemorial for its medicinal properties and has shown promising benefits in medical sciences. These species are known to produce aromatic oils, and several other products are used for pharmaceuticals, flavoring agents, hallucinogens. Phytochemicals obtained from these species have been used for the treatment of various ailments like *A. campestris* has been used for correcting hyperglycemia and preventing diabetic complications (4); *A. absinthium* possessed acaricidal properties (5) and *A. annua* has been used for the treatment of chloroquine-resistant and cerebral malaria (6) and possess antioxidant, antibacterial, anticoccidial and antiviral properties (7, 8).

A. stelleriana Besser commonly known as 'dusty miller' or 'beach wormwood' is a native species of Asia and cultivated as an ornamental in Europe and Asia. It a perennial shrub with aromatic leaves. The leaves of *A. stelleriana* have been traditionally used in the treatment of peptic ulcers, hair loss and are used to stimulate mental faculties and carminative (9, 10). It has also been used in China as a hair tonic, stomachic, antifatulent and to treat sores and skin disorders (11, 12). Previous studies have revealed the essential oils from these plants to be a rich source of monoterpenoids along with sesquiterpenoids (13, 14) and α -linoleic acid and linoleic acid (15). However, there has been no systemic report regarding the phytochemical composition and antioxidant potential of these plants. The objective of this study was to estimate the proximate and mineral composition, phytochemical composition and antioxidant activity and chemical constituents using GC/MS analysis of *A. stelleriana* leaves extract.

Materials and Methods

Materials

Leaves of *A. stelleriana* was collected from the Ethnomedicinal Garden of FRLHT, Bengaluru, and grown in the polyhouse (12°56'04.9"N 77°36'24.5"E). Folin-Ciocalteu reagent (FC), 1,1-Diphenyl-2-picrylhydrazyl (DPPH), Gallic acid, Sodium nitrate, Aluminium chloride, Ferric chloride, Sodium carbonate, Sodium hydroxide, Quercetin, Sodium phosphate, Ammonium molybdate, Potassium ferrocyanide, Ascorbic acid Methanol, Ethanol, Acetone were purchased from Sigma Aldrich (St. Louis, MO, USA).

Sample preparation

Ten grams (10 g) of the fine powder of dried leaves of *A. stelleriana* was extracted using 100 ml of deionized water using a water bath at 100 °C for 20 min. 100 ml each of methanol, ethanol and acetone was utilized for extraction of a 10 g sample at 40 °C for 24 h. The cooled samples were centrifugated at 6000 rpm for 10 min. The supernatant obtained was concentrated using a rotary shake evaporator and the diluted extracts were utilized for various analytical methods.

Proximate Analysis

Dried and powdered leaf samples were analyzed for moisture, ash, protein, carbohydrate and fiber content was determined as previously described (16). Total chlorophyll content was obtained as described by Arnon (17).

Mineral analysis

Weighed 0.5 g of dried plant sample and a mixture containing 2 ml 65% HNO₃ (wt/vol) and 0.5ml 30% H₂O₂ (wt/vol) was added and left to stay overnight. The samples were digested by heating the sample at 220 °C for 5 min. Thereafter, samples were allowed to cool down and diluted with 25 ml of distilled water. The samples were filtered and used for the determination of mineral content. The minerals were determined using Flame Atomic Absorption Spectrometer-128 (Systronics, India) (18).

Analytical methods

The Total Phenolic Content (TPC) of deionized water, methanol, ethanol, and acetone leaf extracts was determined using Folin-Ciocalteu reagent as described (19). The results were expressed as mg of Gallic acid equivalent per gram of dry plant matter (mg GAE/ g). Total flavonoid content (TFC) was determined by Aluminium chloride colorimetric assay (20) and was expressed as mg of quercetin equivalent per gram of dry plant matter (mg QE/ g). The antioxidant capacity of the extracts was determined by 1,1-Diphenyl-2-picrylhydrazyl radical scavenging assay (DPPH-RSA), Reducing power assay and phosphomolybdenum method (21).

Gas Chromatography/ Mass spectroscopy Analysis (GC/MS)

Gas Chromatography/ Mass spectroscopy Analysis was performed using a Shimadzu GC-17A equipped with GCMSSQP5050A mass detector and an HP-5 MS capillary column (30 m × 0.25 mm, film thickness 0.25 μm). Detection of the phytochemicals was done using NIST 2011 library.

Statistical analysis

The analysis was performed in triplicates and the results are expressed as Mean ± Standard deviation. The data obtained were analyzed statistically using one-way ANOVA SPSS- 21.0 statistical software followed by Tukey's test and p ≤ 0.05) is considered as statistically significant.

Results and Discussion

Proximate Analysis

The results obtained from the proximate analysis are

Table 1. Proximate composition of *A. stelleriana* leaves

Component	
Moisture (%)	21.95 ± 0.45
Total ash (%)	29.74 ± 0.29
Carbohydrate (%)	17.96 ± 0.89
Crude protein (%)	11.19 ± 0.55
Total Chlorophyll content (mg/g)	0.56 ± 0.006

Data are Mean ± Standard deviation (n=3)

shown in Table 1. The moisture content and total ash were found 21.95 ± 0.45 mg/g (fresh weight) and 29.741 ± 0.2 mg/g (dry weight) respectively. Excess moisture leads to an increased enzymatic activity which results in the breakdown of important constituents of plants as this moisture promotes the growth of fungi and yeast. Ash content is considered to be an indication of mineral content (22). The carbohydrate and crude protein were found 17.96 ± 0.89% and 11.19 ± 0.55% respectively which is more than that found in the leaf sample of *Talinum triangulare* (23). High protein and carbohydrate content was also reported in fresh leaves of *A. argyi* (24). The presence of carbohydrate and protein content improves the nutritional aspects of the substance (25). The total chlorophyll content of the leaf sample was estimated 0.56 ± 0.00mg/g (Table 1). The results indicated that carbohydrates constituted the major

constituent in the leaves of *A. stellariana* and can naturally serve as a source of energy in dietary supplements.

Mineral analysis

Mineral content in the leaves is shown in Table 2. Ca was present in the highest concentration followed by Mg, Fe, Zn,

Table 2. Mineral composition in *A. stellariana* leaves

Type of element	Element	Concentration (ppm)
Macro-elements	Ca	8.886
	Mg	1.764
	Fe	0.339
	Zn	0.126
Micro-elements	Cu	0.121
	Mn	0.114
	Pb	0.072
	Cd	0.000

Cu, Mn, Pb and Cd. Similar observations were reported in *A. argyi* leaves (24). Calcium is the basic component of our skeletal system, as it provides rigidity and strength to our bones. Calcium regulates heartbeat, nerve transmission, muscle contraction as well as blood coagulation. Mg can activate as many as 300 enzyme systems in the body and is involved in the conversion of glucose to glucose-6-phosphate (26). Fe has a major role to play in the synthesis of DNA, activating various enzymes involved in brain neurotransmitters. Zn is mandatory for carbohydrate metabolism and the synthesis of DNA. Cu acts as a cofactor for many of the enzymes and is necessary for the oxidation of vitamin C which along with Fe which helps in the production of hemoglobin. Mn regulates neurotransmitter activity related to nerve-muscle disorders. Lead and cadmium are considered to be toxic metals (27).

Solvent Extraction

The solubility of the chemical constituents and the polarity of the extracting solvents can influence the extraction of phenolic compounds from the extracts (28-30). To extract the phenolic compounds four solvents were tested. The content of phenolic compounds extracted for all the solvents is shown in Table 3. The TPC measured by methanol has an extraction rate (10.09 ± 0.24 mg GAE/g) which is having the highest significance ($p \leq 0.05$) and is approximately

Table 3. Quantitative determination of phenolic compounds in *A. stellariana* leaf extracts

Extracts	Phenol (mg GAE/g)	Flavonoid (mg QE/g)
Distilled water	2.36 ± 0.03^c	56.78 ± 0.39^c
Methanol	10.09 ± 0.24^a	225.04 ± 0.38^a
Acetone	3.38 ± 0.20^b	153.83 ± 1.37^b
Ethanol	2.60 ± 0.09^c	98.43 ± 0.11^c

Data are Mean \pm Standard deviation ($n=3$); Different uppercase letters in each column indicate a significant difference ($p < 0.05$)

three-fold higher than that of acetone (3.38 ± 0.20 mg GAE/g) and fivefold higher than ethanol (2.60 ± 0.09 mg GAE/g). Water (2.36 ± 0.03 mg GAE/g) gave the lowest TPC in the extracts. The methanol extracts (225.04 ± 0.38 mg QE/g) also showed a significantly higher extraction rate for TFC, followed by acetone (153.83 ± 1.37 mg QE/g), ethanol (98.43 ± 0.11 mg QE/g) and water (56.78 ± 0.39 mg QE/g).

Similar results were observed in the *Artemisia annua* (31), where methanol extracts showed high content of TPC and TFC. The results are also following the previous studies (28-30), suggesting that the extraction of phenolic compounds is much effective in inorganic solvents which have less polarity when compared to solvents with a high polarity like water (32).

Antioxidant capacity

The antioxidant potential of plant extract in different solvent systems was determined using DPPH-RSA, Reducing power assay and Phosphomolybdenum assays (Table 4). Methanolic extracts ($60.6 \pm 0.60\%$) were found to have the

Table 4. Antioxidant activity of *A. stellariana* leaf extracts

Extracts	DPPH assay (%)	Phosphomolybdenum assay (mg/ml)	Reducing Power (mg/ml)
Distilled water	10.56 ± 0.02^c	0.35 ± 0.06^c	0.76 ± 0.08^c
Methanol	60.60 ± 0.60^a	1.23 ± 0.05^a	2.31 ± 0.25^a
Ethanol	20.33 ± 0.31^b	0.46 ± 0.02^c	0.97 ± 0.14^b
Acetone	15.97 ± 0.24^{bc}	0.73 ± 0.03^b	0.98 ± 0.08^b

Data are Mean \pm Standard deviation ($n=3$); Different uppercase letters in each column indicate a significant difference ($p < 0.05$)

highest antioxidant activity when compared to other solvents. The DPPH-RSA activity decreased in the order methanol > ethanol > acetone > water. The result was in agreement with the previous reports on *Artemisia rutifolia*, where the methanolic extracts showed the highest inhibition of DPPH-RSA compared to other solvents (33). In *Cistus ladaniferus* and *Halleria lucida* leaves, a similar tendency was observed (34, 35). Fe reducing power assay measures the reduction of ferric ion ligand complex to ferrous complex in acidic medium by antioxidants. High absorbance indicates high reducing power. The methanolic extracts (2.31 ± 0.25 mg/ml) of the leaves were found to have significantly ($p \leq 0.05$) higher reducing power, compared to other solvents, which was almost two-fold higher than acetone (0.98 ± 0.08 mg/ml), ethanol (0.97 ± 0.14 mg/ml) and water (0.76 ± 0.08 mg/ml). Reductones are usually associated with reducing power and the donation of a hydrogen atom can break radical chains (36), suggesting antioxidant properties being related to the reducing power. Hence, the higher antioxidant activity of the methanolic extract is related to higher reducing power. During phosphomolybdenum assay (Total antioxidant capacity) reduction of Mo (IV) to Mo (V) by the plant, extract takes place which is followed by the formation Mo (V) compounds under acidic pH (37). The antioxidant capacity decreased in the order methanol > acetone > ethanol > distilled water.

GC-MS analysis

GC-MS analysis of *A. stellariana* methanolic leaf extract revealed the presence of different bioactive compounds (Fig. 1). The spectral footprint of the bioactive compounds identified using the NIST library and the molecular weight are shown in Table 5. Based on abundance the major components present in the methanolic extract were 2,4-di-tert-butylphenol (2,4-DTBP), neophytadiene, octacosane and eucalyptol. *Artemisia* species like *A. dracunculus*, *A. abrotanum*, *A. absinthium*, *A. vulgaris* etc. have been reported for

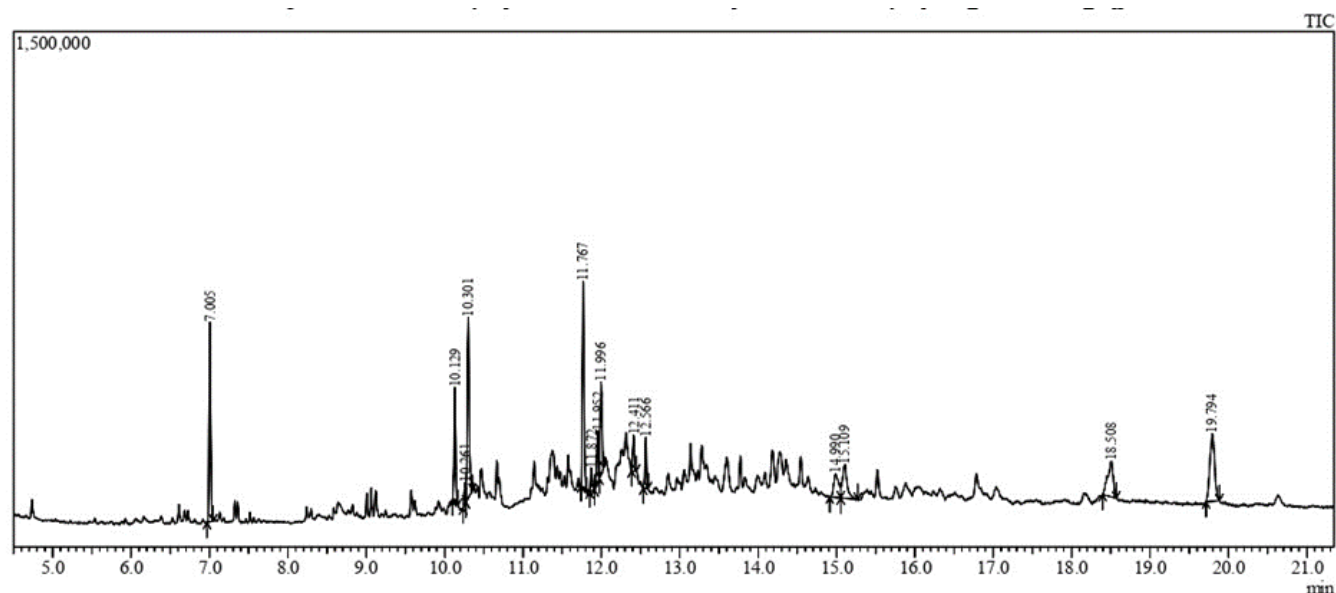


Fig. 1. GC-MS chromatogram of methanolic extracts of *Artemisia stellariana* leaves.

Table 5. Chemical composition of active compounds in methanolic extracts of *A. stellariana* leaves using GC-MS analysis

N	RT (min)	Compounds	% of compounds
1	7.005	Eucalyptol	12.48
2	10.129	2,5-cyclohexadiene-1,4-dione	7.74
3	10.261	Germacrene D	1.68
4	10.301	2,4-Di-tert-butylphenol	14.06
5	11.767	Neophytadiene	14.23
6	11.872	3,7,11-Trimethyl-2,4-dodecadiene	1.75
7	11.952	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	3.68
8	11.996	4-Tetradecanol	6.54
9	12.411	1-Heptadecene	2.48
10	12.566	Cyclooctasiloxane, hexadecamethyl	3.25
11	14.99	beta. Carotene	4.83
12	15.109	Cyclohexane, 1,3,5-triphenyl-	6.29
13	18.508	Squalene	7.97
14	19.794	Octacosane	13.05

the presence of various bioactive compounds (38-40). Various reports have shown 2,4-DTBP possesses antimicrobial, antioxidant, anti-inflammatory, cytotoxic, insecticidal and nematocidal properties (41). Similarly, eucalyptol, a terpenoid compound that has been reported to have antioxidant and anti-inflammatory properties against various ailments like colon damage, pancreatitis, cardiovascular diseases etc (42). However, octacosane has been reported for mosquitoicidal activity (43), which could be potentially used as an insecticidal.

Conclusion

Leaves of *A. stellariana* thus have high phenolic content, flavonoid content and exhibits antioxidant potential. From this phytochemical analysis, it can be concluded that this herbaceous plant has the potential to act as a source of many important secondary metabolites reserves which can be of utmost benefit to humankind. This study conducted justifies the presence of eucalyptol which is a promising compound that has shown anti-inflammatory and antioxi-

ant effects in various diseases including cardiovascular and neurodegenerative diseases. Its potential for herbal drug development can be exploited in future studies.

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Authors contributions

JKS designed and coordinated the experimental work, MM and AD performed the experiments. JKS, MM and AD analyzed the data, read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None.

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