Natural bioactive compounds of Asparagus: A study of antioxidant and antibacterial activity

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Abstract. Ornamental plants, beyond their aesthetic appeal, possess notable therapeutic and medicinal properties. Asparagus setaceus, commonly cultivated in tropical and subtropical regions, is known for its bioactive compounds, including phenols, tannins, saponins, and flavonoids. This study aimed to investigate the phytochemical composition, antioxidant activity, antimicrobial efficacy, and bioactive compound profiling of A. setaceus leaf extract. Soxhlet extraction with methanol was used in the analysis; the total polyphenol and flavonoid content were estimated to be 121 mg GAE/g and 0.16 mg QE/g, respectively. Dose-dependent antioxidant activity was obtained in the DPPH radical scavenging assay. Through agar diffusion, an inhibition zone of 20 mm as compared to standard antibiotic control was shown, revealing significant inhibition of the Escherichia coli bacterium in antimicrobial activity assayed. GC-MS analysis revealed the presence of 168 bioactive compounds, among which dimethyl ether and hexadecanoic acid were the most abundant, and therefore, have potential therapeutic use, especially in antimicrobial and antioxidant activities. The present findings support the pharmacological importance of A. setaceus as a source of bioactive constituents, which merits further research for therapeutic purposes.

1. Introduction

Ornamental plants are grown for their aesthetic qualities in gardens and landscape design projects, as houseplants, specimen displays, and cut flowers. Flower crops and ornamentals are cultivated for their nutritional and therapeutic qualities in addition to their aesthetic value [1]. Utilizing garden settings as therapeutic entities to improve the healing process that takes place in healthcare settings has attracted attention. Therapeutic gardens can keep people healthy or aid in their recovery from disease by reducing the stress reaction [2].

Asparagus setaceus is globally popular as an ornamental plant usually cultivated in tropical and subtropical regions. *A. setaceus* is commonly named as asparagus fern, lace fern, asparagus grass, climbing asparagus or ferny asparagus [3]. *A. Kunth* is a perennial herb that is classified as a sub shrub in the Asparagaceae family. *A. setaceus* is scrambling evergreen perennial with tough green meters in length. The plant consists of bell-shaped flowers

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(greenish-white and 0.4cm long) followed by small berries which change colour from green to black upon maturity. The leaves have a soft green fern-like foliage which are 7mm long with leaf- like cladodes features. It has historically been applied to skin diseases, such as cough, lung infections, andrology, diuretics, tonics, demulcents, and diuretics, as well as having antiepileptic potential. They belong to the liliaceaea family [4].

There are over 300 species of asparagus in the genus asparagus worldwide. The primary source of the crude medication shatawar, which is used to increase milk supply and enhance appetite in nursing women. *A. setaceus* is described to offer abundant health benefits, as it richly contains vitamin, mineral content, antioxidants, and essential nutrients in its composition [5]. This herb used in traditional Chinese medicine is an effective remedy against disorders of the urinary system; it is attributed to health concerns associated with several illnesses. The plant might also have diuretic properties, which can possibly prevent UTIs and other urinary issues. It is also pretty common for its capacity to purify the air when grown indoors [6]. Ethanolic extract of *A. setaceus* was shown to possess various phenolic compounds, flavonoids, tannins, and saponins. It had a CL50 of 90.22 μg/mL and showed the inhibition of standard Gram-positive and Gram-negative bacteria but failed against multidrug-resistant strains [7]. Agar diffusion and broth dilution methods were applied to study various extracts of *A. racemosus* like ethanol, hexane, and chloroform against various microorganisms. Ethanol extract was found active against all the tested organisms and had highly potent activity against *Staphylococcus aureus, Escherichia coli*, and *Candida albicans*. The lowest minimal inhibitory concentration and minimum bactericidal concentration was noted for *S. aureus, E. coli*, and *C. albicans*. Chloroform extract was found to be moderately active, while hexane extract had the lowest antimicrobial activity [8].

Though the plant is rich in various bioactive compounds, characterization of this plant is limited, and therefore, the possible use remains underexplored. With an attempt to elucidate the bioactive constituents of *A. setaceus*, with possible health benefits, this work focused on antioxidant and antimicrobial activity of leaf extract.

2. Materials and methods

2.1. Sample collection and preparation of extract

A. setaceus was procured from Talegaon, Pune, India. Leaf samples were washed, shade-dried, ground into fine powder, and subsequently stored in an airtight container. Secondary metabolites were extracted using Soxhlet extractor (Kasiramar & Gopalasatheeskumar, 2019) with, methanol as the solvent and made concentrated using rotary vacuum evaporator (Figure 1). The concentrated leaf extract was stored at a temperature of 4°C.

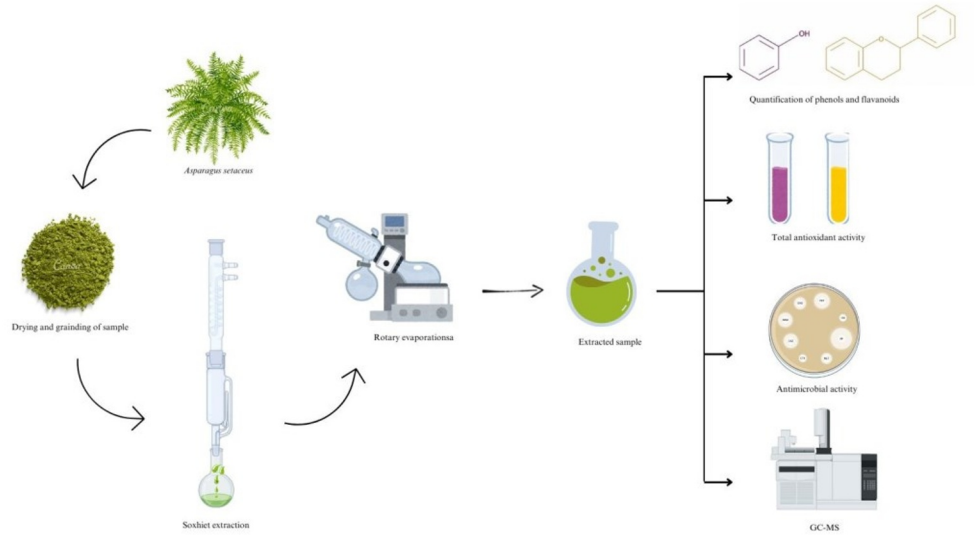

Fig. 1. Process of secondary metabolite extraction from *A. setaceus* leaves, including powdering, Soxhlet extraction with methanol, concentration using a rotary vacuum evaporator. The extract was utilized for the determination of bioactive compounds, as well as for evaluating antioxidant and antimicrobial activities.

2.2. Quantification of the total polyphenol content in extract

The Folin-Ciocalteu reagent technique was used to calculate the extract's total polyphenol content (TPC) [9]. The solution was prepared from 1 ml of the stock plant extract solution with 5 ml of 7% sodium carbonate, followed by the addition of 10% Folin-Ciocalteu reagent. The mixtures were then allowed to incubate at room temperature in the dark for a duration of 90 minutes. Absorbance measured at the wavelength of 760 nm. The TPC in the extract was reported as micrograms of Gallic acid equivalents (mg GAE/g) per gram, or as milligrams of phenolic content based on dry weight. All samples underwent analysis in triplicate, and the results were subsequently averaged.

2.3. Quantification of the total flavonoid content in extract

The aluminum chloride technique was used to calculate the extract's total flavonoid content (TFC). The solution was prepared by mixing 1 ml of plant extract solution diluted with 4 ml of distilled water, 0.3ml of 5% of NaNo3, 0.3 ml of 10% Al (NO3) and 2ml of freshly prepared NaOH [10]. After 15 minutes of room temperature incubation, the absorbance of the solution was measured at 415 nm using a UV-visible spectrophotometer. Per milligram of the extract, the TFC was measured in micrograms of quercetin equivalent (mg QE/g). Measurements were done in triplicate and the results were averaged.

2.4. DPPH radical Scavenging assay

According to [11] the plant samples extracted with methanol used for analysing the radical scavenging activity with different concentration ranges (100,200,300 and 400 µg/ml) in 0.1 mM of DPPH reagent with quercetin as the standard. Kept for 20 min dark incubation at room temperature. The UV-Visible Spectrophotometer was used to measure the absorbance

at 517 nm in order to quantify the decolorization of DPPH. The percentage of inhibition of the extract was calculated using the equation:

$$\text{Scavenging rate} = [1-(A1-A2)/A0\} \text{ X } 100\%$$

Where, the absorbance of the control (only DPPH, without extract) was denoted by A0, absorbance of the extract with DPPH using A1, and the absorbance of the extract without DPPH with A2.

2.5. Antimicrobial Assay

Antimicrobial activity of the plant extract was determined using agar well diffusion method in Muller Hinton Agar. *Escherichia coli* was selected as the test microorganism which was inoculated in nutrient broth and incubated overnight at 37°C. Bacterial lawns were prepared on agar plates and wells of ~10 mm was made using a cork borer. Antibacterial activity of the plant extract was evaluated on agar plates with streptomycin (10 mg) used as a control and incubated for 24 hours at 37 °C. After incubation, clear zone was observed on the plates which corresponds to the antimicrobial activity of extract.

2.6. Gas Chromatography- mass spectrometry analysis

The bioactive compounds of the leaf extract of *Asparagus setaceus* were identified using gas chromatography-mass spectrometry (GC-MS) analysis. The samples were analysed using the GC-MS (detector CH-GCMSMS-02). A capillary column of HP 5mts (30 m × 0.25 mm × 0.25 mm) was used, with a constant flow of helium at 1.5 ml/min and a source temperature of 280 °C. The temperature of the oven was initially set at 80 °C for 2 minutes, then ramped three times: first at 15 °C/min to 200 °C, then at 4 °C/min to 240 °C, and lastly at 15 °C/min to 280 °C, which was held for 5 minutes. The total run time was 34 minutes and the results were analysed using NIST MS Search V.2.3-2017.

3. Results and Discussion

3.1. Phytochemical composition of the extract

Total phenol and flavonoid content of the extract was determined using Folin-Ciocalteu and aluminium chloride method, respectively. The results were described in milligrams of GAE/ g of the extract for phenol content whereas for flavonoid it was milligrams of QE/ g of the extract. Total phenol content was observed to be 121 mg GAE/g whereas 4.14 mg QE/g of flavonoid was found in the leave extract. The results indicate the potential richness of flavonoids and phenol in *A. setaceus*, attributing to its biological and pharmacological significance. Asparagus contains many bioactive compounds comprising of phenols, carotenoids, sterols, and fructo-oligosaccharides [12]. Phenols and flavonoids have been reported in ethanolic extract of *A. setaceus* were high levels of condensed tannins, anthocyanins, flavonols, aurones, anthocyanidins, xanthones, and chalcones were observed [7].

3.2. Radical scavenging activity

The antioxidant activity of the extract was evaluated using the widely employed DPPH radical scavenging assay. The scavenging effect of the leaf extract on DPPH radicals was

measured as % inhibition and compared to the standard antioxidant, quercetin. The extract demonstrated dose-dependent DPPH scavenging activity, comparable to the standard antioxidant with activity of 12.76% of scavenging radicals. Similar results have been reported from another species i.e., *A. officinalis* (activity of 16.07 %) [13].

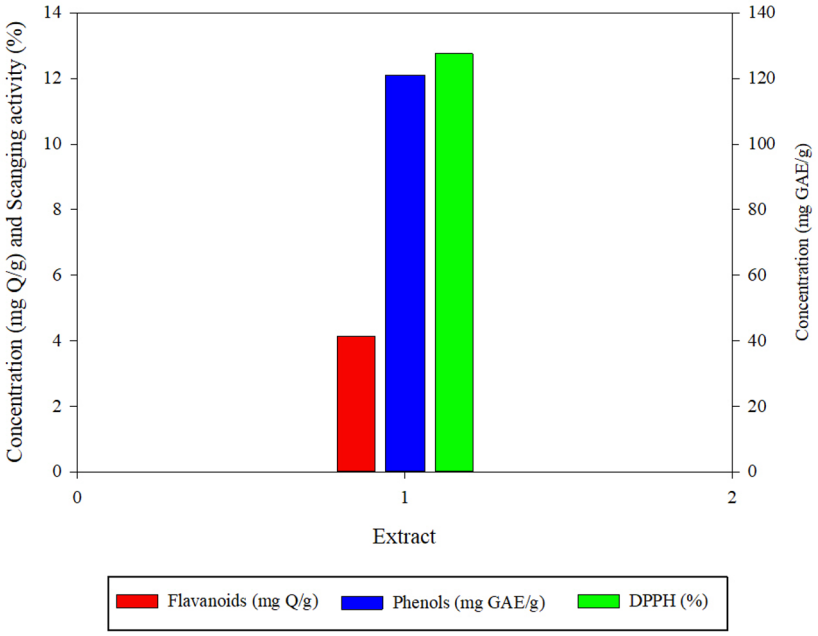

Fig. 2. Presence of bioactive compounds and antioxidant activity in leave extract of *A. setaceus*.

3.3. Antimicrobial activity

Antimicrobial activity was assessed using the zone of inhibition method on Mueller-Hinton Agar (MHA) plates. Significant zone of inhibition was observed for extract against *E. coli* (20 mm) which were close to that of control (25 mm). Similarly, antibacterial activity of *A. setaceus* has been reported against gram-positive and gram-negative bacteria [7].

3.4. GC-MS analysis

GC-MS analysis of the plant samples showed total compound number of 168. Compounds with area % more than 10 lakh was taken into consideration in the study where we found different unique compounds in *A. setaceus*. Dimethyl ether and hexadeconic acid showed the highest area% among the other compounds showing its uniqueness in the given plant sample. The unique compounds were identified with a cut off value of 10.0 L (which reveals the highest abundance) and biological properties were assigned for such compounds from NCBI. The assigned therapeutic activity includes antimicrobial, antioxidant, neuroprotective, antifungal, etc. thus confirming the expected activity of the plant extract.

Table 1. Details of bioactive compounds (based on relative distribution) identified using GC-MS in leave extract of *A. setaceus*.

RT Compound	*Asparagus* Compound Name	Formula	Property
41672802	Dimethyl ether	C_2H_6O	Ethers
41672802	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	Anti-inflammatory
41672802	Vitamin E	$C_{29}H_{50}O_2$	Antioxidant property
41672802	Cyclodecasiloxane, eicosamethyl-	$C_{20}H_{60}O_{10}Si_{10}$	Antimicrobial
41672802	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$	Anticancer and antimicrobial
41672802	Cyclodecasiloxane, eicosamethyl-	$C_{20}H_{60}O_{10}Si_{10}$	Antimicrobial
41672802	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$	Anticancer and antimicrobial
41672802	Tetracosamethyl-cyclododecasiloxane	$C_{24}H_{72}O_{12}Si_{12}$	Antidiabetic and antioxidant
41672802	Cyclooctasiloxane, hexadecamethyl-	$C_{16}H_{48}O_8Si_8$	Antimicrobial
41672802	L-Lactic acid	$C_3H_6O_3$	Antioxidant
41672802	Tetracosamethyl-cyclododecasiloxane	$C_{24}H_{72}O_{12}Si_{12}$	Antimicrobial
41672802	Cycloheptasiloxane, tetradecamethyl-	$C_{14}H_{42}O_7Si_7$	Anticancer and antimicrobial
41672802	Octacosanol	$C_{28}H_{58}O$	Antiaggregatory properties
41672802	Neophytadiene	$C_{20}H_{38}$	Antimicrobial, anti-inflammatory
41672802	trans-Sinapyl alcohol	$C_{11}H_{14}O_4$	Anti-inflammatory
4834767.5	Tetrahydroionone	$C_{13}H_{24}O$	Antifungal agent
4817604.8	.delta.-Tocopherol	$C_{27}H_{46}O_2$	Antioxidant
4749973.5	Cyclohexasiloxane, dodecamethyl-	$C_{12}H_{36}O_6Si_6$	Antimicrobial
4195476.8	Butyl 2-(2-(2-butoxyethoxy)ethoxy)acetate	$C_{14}H_{28}O_5$	Antidiabetic

4. Conclusion

The findings of this study highlight the rich phytochemical profile and biological activities of *A. setaceus* leaf extract, underscoring its antioxidant and antimicrobial potential. GC-MS analysis revealed unique bioactive compounds with therapeutic properties, supporting its traditional medicinal uses. Although the plant demonstrates significant pharmacological potential, further research is warranted to elucidate its mechanisms of actionand evaluate its efficacy in clinical applications. This study contributes to the understanding of *A. setaceus* as a promising candidate for natural therapeutic development.

Declarations

Ethics approval and consent to participate Not applicable

Availability of data and materials

All data generated or analysed during this study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare no competing interests.

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Authors' contributions

Jibu Thomas- Conceptualization, Project administration, Methodology, Review and editing.

Aswathy Sasi- Investigation, Data curation, and writing original draft.

Pema Lhamo- Formal analysis, Software, and Writing original draft.

Shiny Evangeline- Writing original draft.

Vinuba N- Software, and Writing original draft.

Swetha S- Investigation, and Data curation.

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