

Abiotic stress-driven enhancement of metabolites and bioactivity in *Plectranthus amboinicus* (Lour.) Spreng

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Abstract

This study examines the effects of abiotic stress conditions, including heat, drought, salinity, and chilling, on the accumulation of secondary metabolites, as well as the antioxidant and antimicrobial potential of *Plectranthus amboinicus*. The findings indicate that drought stress significantly increased the levels of phenolic compounds and tannins, whereas heat stress enhanced the accumulation of flavonoids and alkaloids. Salinity and low-temperature conditions also promoted the synthesis of phenolics and flavonoids. The antioxidant activity, assessed using the DPPH assay, demonstrated strong free radical scavenging ability, with IC_{50} values of approximately 160 $\mu\text{g/ml}$. Furthermore, antimicrobial analysis showed effective inhibition of the bacterial strain *Bacillus subtilis*, with activity comparable to that of a standard antibiotic. Overall, the results suggest that exposure to abiotic stress enhances both the phytochemical profile and therapeutic potential of *Plectranthus amboinicus*, supporting its potential application in nutraceutical and pharmaceutical fields.

Keywords: *Plectranthus amboinicus*, abiotic stress, drought, salinity, antioxidant, antimicrobial activity, secondary metabolites

Introduction

Plectranthus amboinicus commonly referred to as Indian borage, Mexican mint, or Cuban oregano, is a perennial herb belonging to the Lamiaceae family, valued for its aromatic leaves and traditional medicinal uses (Chinnahong *et al.*, 2025) [26]. The conventional medical system has seen a global upsurge in attention in recent years. Phytochemical research is being driven by the screening of medicinal herbs, which has emerged as a viable source of bioactive chemicals with therapeutic promise. Effective herbal medications can be discovered through ethnomedicobotanical research on tribal communities (Santos Filipe *et al.*, 2025).

It is rich in secondary metabolites including phenols, alkaloids, flavonoids, and tannins, which contribute to its therapeutic potential in treating respiratory disorders, digestive issues, and inflammation (Arumugam *et al.*, 2016)

[1]. Abiotic stress factors namely drought and heat are particularly effective in stimulating such responses and have been shown to elevate antioxidant enzyme activity and metabolite accumulation (Mishra *et al.*, 2023) [3]. Free radicals generated by oxidative stress can damage cellular components, but antioxidant compounds in medicinal plants scavenge these radicals, offering protective health benefits (Lobo *et al.*, 2010) [4], moreover, due to the safety concerns associated with synthetic antioxidants, there is growing interest in natural plant-based alternatives. (Manassis *et al.*, 2020) [5].

This study combines antioxidant, antimicrobial, and effect of abiotic stress conditions on secondary metabolite enhancement to provide a comprehensive evaluation of the *Plectranthus amboinicus*'s medicinal potential.



Fig 1: *Plectranthus amboinicus*

Materials and Methods

a. Sample collection and extraction

Plectranthus amboinicus was collected from Department of Botany, University of Rajasthan and self-grown.

Different stress conditions were given to plants. (Liu *et al.*, 2024) [7].

Few plants were given heat stress (high temperature exposure), some were given drought stress (with holding water), and Salinity stress was induced in few plants (100mM NaCl solution treatment) and Chilling stress (low temperature exposure) in few of them for a period of 2 weeks.

Afterwards, all plant were collected and thoroughly washed under running tap water to remove soil particles. The different plant parts (leaf and stem samples) were separated and allowed to dry at room temperature. Once dried, the plant materials were ground into a fine powder and used for further analysis, with results compared to those obtained from non-stressed (control) samples (Savithamma *et al.*, 2011) [27].

b. Preparation of Plant Extracts for Analysis

5 grams from each dried sample was weighed and were Soxhlet extracted for 24 hours in methanol. Obtained extracts were filtered and dried in Petri dish. Each extract was resuspended in 10ml of methanol at the time of analysis.

Estimation of Secondary Metabolites

1. Estimation of total phenol: The total phenolic content of stem and leaf extracts of *Plectranthus amboinicus* was estimated following the procedure outline by Shamsul method (Shamsul *et al.*, 2019) [8].

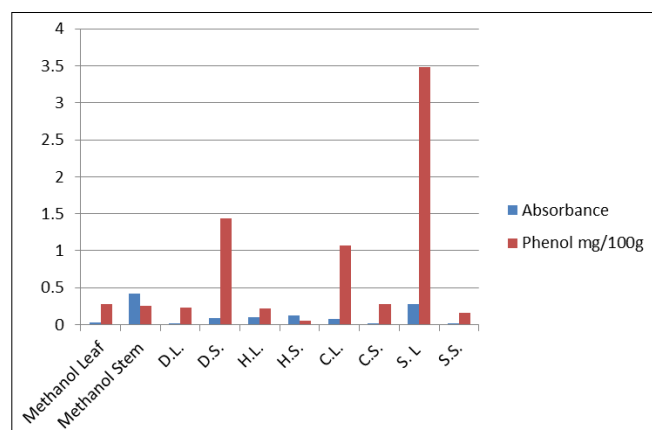


Fig 2: Quantitative estimation of Phenol content in various samples of *Plectranthus amboinicus*.

(Abbre. - MS- methanol stem, ML- methanol leaf, DS-drought stem, DL-drought leaf, HS- heat stem, HL-heat leaf, CS-chilled stem, CL-chilled leaf, SS-salt stem, SL-salt leaf Phenolic compounds were significantly influenced by stress. The highest phenolic content was recorded per 100gm fresh weight under salinity stress in the leaf (3.4842 mg), while the lowest was observed under heat stress in the stem (0.048 mg) in the stressed samples. Generally, leaves accumulated more phenolics than stems across stress treatments. Under chilling stress, the stem recorded moderate levels (0.2742 mg), while the leaf contained 1.0677 mg of phenol. In salinity stress, phenol levels were elevated, with the leaf (3.4842 mg) being markedly higher than the stem (0.1568 mg).

2. Estimated of total flavonoid content: The total flavonoid content of *in vivo* (stem and leaf) sample extracts of *P. amboinicus* plant species was quantified following the aluminum method described by Nguyen *et al.*, 2010[17].

Flavonoids were estimated using the aluminum chloride assay, with quercetin as the standard.

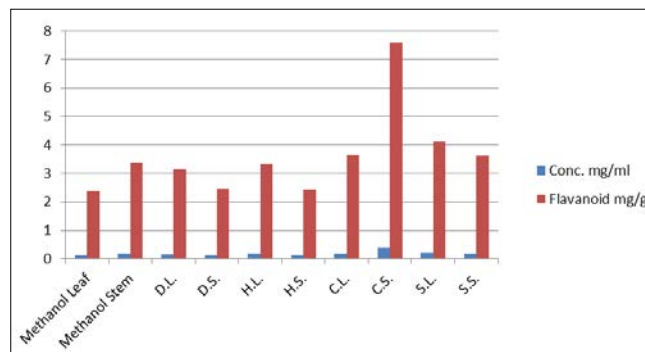


Fig 3: Quantitative estimation of Flavonoid content in various samples of *Plectranthus amboinicus*

Flavonoid concentration showed marked variation among treatments. The chilling stress stem (7.584 mg/g) exhibited the maximum flavonoid content, whereas the lowest content was observed in the methanol leaf extract (2.376 mg/g). Stress conditions induced moderate increases in flavonoid levels, with heat stress leaf (3.322 mg/g) slightly surpassing the stem (2.428 mg/g). Chilling stress showed elevated flavonoids in both stem (7.584 mg/g) and leaf (3.656 mg/g). Under salinity stress, flavonoid levels were higher in the leaf (4.136 mg/g) than the stem (3.612 mg/g).

3. Estimation of Tannins

Estimation of Tannins was carried out by Rhodanine assay that was followed according the method of Scalbert *et al.*, 1992[10]. Tannins were analyzed using the rhodanine assay with gallic acid.

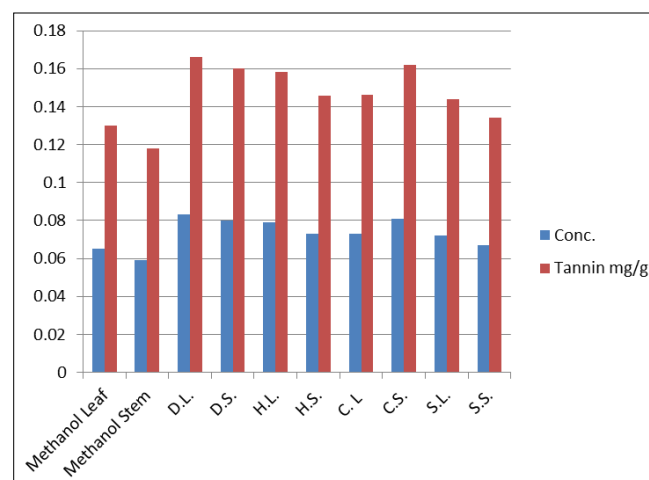


Fig 4: Quantitative estimation of Tannin content in various samples of *Plectranthus amboinicus*

The tannin concentration varied among treatments and between stem and leaf samples. In general, tannin levels were higher in leaves than stems except chilling stress. Under heat stress, leaf tannin (0.1582 mg/g) was slightly higher compared to stem (0.1456 mg/g). Chilling stress resulted in elevated tannin accumulation, with leaf (0.162 mg/g) higher than stem (0.081 mg/g). Similarly, salinity stress induced notable tannin production, with the leaf (0.146 mg/g) showing slightly higher levels than the stem (0.134 mg/g). Under drought stress, tannin was also higher in the leaf (0.165 mg/g) than in the stem (0.160 mg/g). Overall, leaf samples consistently accumulated more tannins across all stress conditions.

4. Estimation of Alkaloid

The alkaloid content was estimated following the method described by sreevidya *et al.*, 2003^[11]. The method is based on the precipitation by Dragendorff's reagent (DR), resulting in the formation of a yellow bismuth complex in nitric acid medium in the presence of thiourea. The yellow complex formed follows Lambert-Beer's Law, and its concentration can be determined spectrophotometrically.

Alkaloids were measured using Dragendorff's reagent and bismuth nitrate standards.

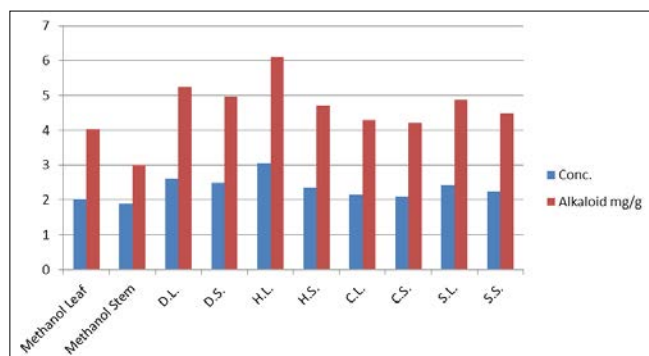


Fig 5: Quantitative estimation of alkaloid content in various samples of *Plectranthus amboinicus*

Alkaloid concentration was strongly affected by stress treatments. The highest level was observed in the heat-stressed leaf (6.1 mg/g), whereas the methanol stem extract showed the lowest value (3.008 mg/g). Under chilling stress, the stem contained 4.216 mg/g while the leaf showed 4.296 mg/g. Salinity stress enhanced alkaloid accumulation, with the leaf (4.868 mg/g) slightly exceeding the stem (4.478 mg/g). Drought stress also induced alkaloid production, with the leaf (4.868 mg/g) consistently higher than the stem (4.478 mg/g).

Antioxidant Activity Estimation

1. Materials and Methods

Plant extract preparation: Leaves of *Plectranthus amboinicus* were shade dried, powdered, and Soxhlet extracted in 80% methanol. The extract was incubated at 37

°C for 24 h, then filtered, dried, and subsequently reconstituted in 10 mL of 80% methanol. Ascorbic acid (1 mg/ml in 80% methanol) as standard was used as positive control, three different concentrations of the plant leaf sample *via* serial dilutions (100–300 µg/ml) were prepared. DPPH solution (0.004% w/v) was prepared by dissolving 0.004 g of DPPH in methanol.

For the assay of 3 ml of DPPH solution was added to 1 ml of the test extract, standard, or blank. The mixture was incubated in the dark at room temperature for 30 minutes, after which absorbance was recorded at 517 nm. The percentage inhibition was calculated, IC₅₀ values were obtained from inhibition curves, and the Antioxidant Activity Index (AAI) was calculated as IC₅₀ of ascorbic acid (µg/mL) divide by IC₅₀ of the sample (µg/mL) (Leaves *et al.*, 2009).

Table 1: Antioxidant activity reported in leaf sample of *Plectranthus amboinicus*

S.No.	Conc. (µg/ml)	Standard	Sample
		Ascorbic Acid	Leaf
1.	100	95.83	80.90
2.	200	93.86	91.51
3.	300	92.65	94.77
IC50 Value			160.62
Activity Index			0.41

Antimicrobial Assay

1. Materials and Methods

The test was carried out using the agar well diffusion method on Mueller Hinton agar (MHA) medium and the antimicrobial activity was assessed against *Bacillus subtilis*. Microbial cultures were swabbed uniformly on agar plates. Wells (6 mm) were made and filled with 50–100 µL of plant leaf sample extracts. Controls used were Standard antibiotic (Azithromycin) and solvent control for the comparative antimicrobial activity analysis (Chavez-Esquivel *et al.*, 2021)^[31].

Methanol and Ethyl acetate extracts prepared from *Plectranthus amboinicus* leaves were screened for antimicrobial efficacy against *Bacillus subtilis*. Subsequently, the plates were incubated at 37 °C for 18–24 h and the Zone of inhibition were recorded to evaluate antimicrobial activity (Abrah *et al.*, 2023)^[32].

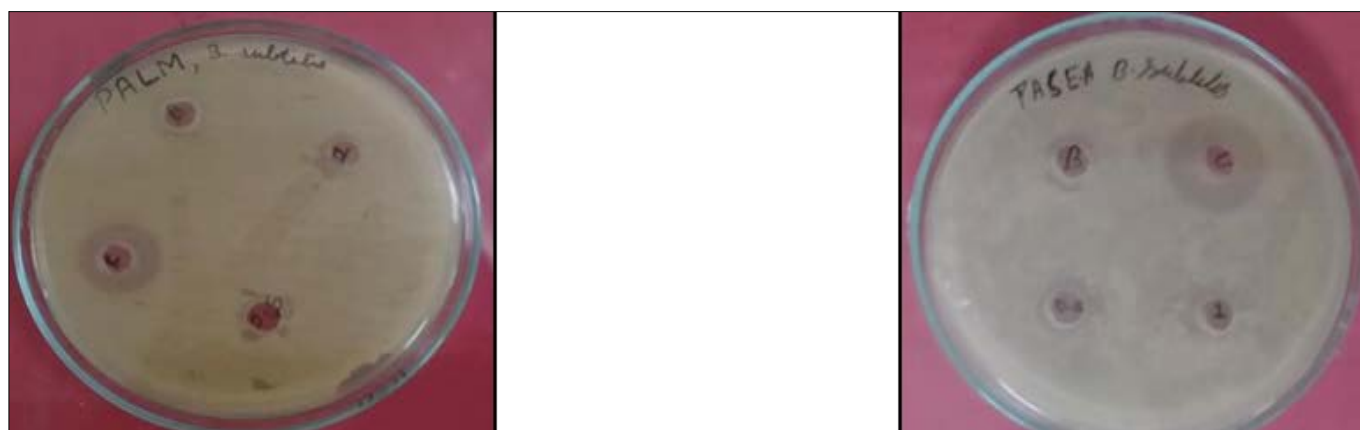


Fig 6: Antimicrobial activity assessment of *Plectranthus amboinicus* leaf Methanol (PALM) extract and *Plectranthus amboinicus* leaf Ethyl acetate extract against *Bacillus subtilis*.

Table 2: Antimicrobial activity reported at different concentrations of various sample extracts.

Sample	Concentration of sample	Inhibition zone of standard	Inhibition zone of sample
PALM	0.5	18	10
PALM	1.0	20	14
PALE.A	0.5	16	12
PALE.A	1.0	21	15

Results

1. Phytochemical Analysis of *Plectranthus amboinicus* under Abiotic Stress

Quantitative estimation of major phytochemicals, including phenols, flavonoids, tannins and alkaloids, was performed in methanolic extracts of leaf and stem samples exposed to various abiotic stress conditions such as drought, heat, chilling and salinity. The results revealed noticeable variation in phytochemical content across different treatments and tissues.

2. Total phenolic content

The total phenolic content varied among the different samples. Methanolic extract of stem and leaf exhibited phenolic values of 0.2532 mg/100 g and 0.2736 mg/100 g, respectively. Among the stress-treated samples, drought-stressed leaf and stem showed phenolic contents of 0.228 mg/100g and 1.44 mg/100g, respectively. Heat-stressed leaf and stem exhibited values of 0.2232 mg/100g and 0.048 mg/100g. Chilling-stressed leaf and stem samples recorded phenolic contents of 1.0677 mg/100g and 0.2742 mg/100g, respectively. The highest phenolic content was observed in salinity-stressed leaf (3.4842 mg/100g) followed by salinity-stressed stem (0.1568 mg/100g).

3. Total Flavonoid Content

Flavonoid content also varied significantly among the samples. Methanolic stem extract showed a flavonoid content of 3.394 mg/g, while methanolic leaf extract showed 2.376 mg/g. Drought-stressed leaf and stem samples recorded values of 3.148 mg/g and 2.464 mg/g, respectively. Heat-stressed leaf showed 3.322 mg/g, whereas heat-stressed stem recorded 2.428 mg/g. Chilling-stressed leaf showed 3.656 mg/g, and chilling-stressed stem exhibited the highest flavonoid content of 7.584 mg/g. Salinity-stressed leaf and stem samples showed flavonoid contents of 4.136 mg/g and 3.612 mg/g, respectively.

4. Total Tannin Content

The tannin content across the samples ranged from 0.118 mg/g to 0.166 mg/g. Methanolic extracts stem and leaf showed values of 0.118 mg/g and 0.130 mg/g, respectively. Drought-stressed leaf and stem recorded values of 0.166 mg/g and 0.160 mg/g. Heat-stressed leaf showed 0.1582 mg/g, while heat-stressed stem showed 0.1456 mg/g. Chilling-stressed leaf and stem samples recorded tannin contents of 0.146 mg/g and 0.162 mg/g, respectively. Salinity-stressed leaf and stem showed values of 0.144 mg/g and 0.134 mg/g.

5. Alkaloid Content

Alkaloid estimation revealed variation among different treatments. Methanolic stem extract showed an alkaloid content of 3.008 mg/g, while methanolic leaf extract showed 4.022 mg/g. Drought-stressed leaf and stem samples

recorded alkaloid contents of 5.238 mg/g and 4.974 mg/g, respectively. Heat-stressed leaf showed the highest alkaloid content of 6.1 mg/g, whereas heat-stressed stem recorded 4.706 mg/g. Chilling-stressed leaf and stem showed alkaloid contents of 4.296 mg/g and 4.216 mg/g, respectively. Salinity-stressed leaf and stem recorded values of 4.868 mg/g and 4.478 mg/g.

6. Antioxidant Activity (DPPH Assay)

The antioxidant activity of methanolic leaf extract was assessed using the DPPH radical scavenging assay. The results indicated a concentration dependent increase in scavenging activity. At 100 µg/ml, the percentage inhibition of the sample was 80.90%. At 200 µg/ml, inhibition increased to 91.51%, and at 300 µg/ml, maximum inhibition of 94.77% was observed. The IC₅₀ value of the sample was calculated as 160.62 µg/ml, and the activity index was 0.41. Ascorbic acid used as standard showed inhibition values of 95.83%, 93.86% and 92.65% at corresponding concentrations.

7. Antimicrobial Activity

The antimicrobial activity of different sample extracts was evaluated using the zone of inhibition method at two concentrations. The PALM extract produced inhibition zones of 10 mm at 0.5 concentration and 14 mm at 1.0 concentration, whereas the standard showed larger zones of 18 mm and 20 mm, respectively. Similarly PALE.A extract exhibited inhibition zones of 12 mm at 0.5 concentration and 15 mm at 1.0 concentration, while the standard recorded zones of 16 mm and 21 mm at the corresponding concentrations.

Discussion

Total phenolic content (TPC) in *Plectranthus amboinicus* varied significantly across control and stress-treated samples, indicating abiotic stress-induced modulation of phenylpropanoid metabolism. The elevated flavonoid levels observed under salinity and drought stress further corroborate earlier findings that osmotic and ionic stresses stimulate secondary metabolite production to mitigate reactive oxygen species and maintain cellular homeostasis (Gill & Tuteja, 2010^[17]). Overall, the enhanced flavonoid accumulation under stress conditions highlights the adaptive biochemical response of *Plectranthus amboinicus* and supports its potential as a rich source of antioxidant compounds, particularly when subjected to controlled abiotic stress.

Tannins are significant polyphenolic compounds that contribute to antioxidant, antimicrobial, and stress related protective functions in plants. In the present study, the tannin content of *Plectranthus amboinicus* ranged from 0.118 mg/g to 0.166 mg/g across all samples, suggesting a marked influence of different abiotic stress conditions on tannin biosynthesis. This baseline level is consistent with previous reports suggesting that tannins are present in moderate amounts in *P. amboinicus* under normal growth conditions (Kumar *et al.*, 2014^[18]). Overall, the findings demonstrate that abiotic stresses, especially drought and temperature extremes, significantly enhance tannin accumulation in *Plectranthus amboinicus*. This stress-induced increase in tannin content underscores the plant's adaptive metabolic plasticity and supports its medicinal relevance, as tannins are closely associated with antioxidant

and therapeutic properties. Heat stress resulted in the highest alkaloid content in leaves (6.1 mg g^{-1}), indicating a strong induction of alkaloid biosynthesis under elevated temperature conditions. Heat stress can alter enzymatic activities and activate stress-responsive signaling pathways, leading to enhanced synthesis of nitrogen-containing secondary metabolites such as alkaloids (Isah, 2019^[19]).

Only leaf samples were used for antioxidant and antimicrobial studies. The methanolic leaf extract exhibited a clear concentration-dependent DPPH radical scavenging activity, indicating the presence of effective hydrogen- or electron-donating antioxidant compounds. At $100 \text{ }\mu\text{g/ml}$, ascorbic acid shows higher antioxidant activity than the leaf extract. At $200 \text{ }\mu\text{g/ml}$, the activity of the leaf extract increases and becomes comparable to the standard. At $300 \text{ }\mu\text{g/ml}$, the leaf extract shows slightly higher activity than ascorbic acid. Overall, antioxidant activity increases with concentration for the leaf sample. The progressive increase in inhibition from 80.90% at $100 \text{ }\mu\text{g/mL}$ to a maximum of 94.77% at $300 \text{ }\mu\text{g/mL}$ suggests strong free radical neutralization potential, which is a characteristic feature of phenolic- and flavonoid-rich plant extracts. (Phongpaichit *et al.*, 2007^[20]). The results clearly demonstrate a concentration-dependent increase in antimicrobial efficacy, a trend widely reported for plant-derived extracts (Parekh & Chanda, 2007^[21]). The PALE.A extract showed comparatively higher antimicrobial potential, with inhibition zones of 12 mm at 0.5 concentration and 15 mm at 1.0 concentration. Although the activity of PALE.A extract was still lower than the standard antibiotic (16 mm and 21 mm), its performance indicates promising antimicrobial efficacy. Such plant-based extracts could serve as alternative or complementary therapeutic agents, particularly in the context of rising antibiotic resistance. Although less potent than ascorbic acid overall, the leaf extract shows significant free radical scavenging activity, especially at higher concentrations.

Conclusion

Different stresses activate distinct metabolic pathways: heat favors flavonoid and alkaloid biosynthesis, drought enhances phenolics and tannins, while salinity and chilling stress promote phenolic and flavonoid accumulation. Antioxidant and antimicrobial assays confirm the plant's therapeutic value. Phenylalanine elicitor further amplified metabolite accumulation, highlighting its role as a metabolic enhancer. The results support findings from previous studies (Grace *et al.* 2012; Rivero-Monejo *et al.*, 2021; El-Serafy *et al.*, 2022)^[13] where elicitation and stress were shown to boost bioactive compound levels in *P. amboinicus*. The antioxidant assay further validated that these compounds can enhance free radical scavenging, making the plant a valuable source of natural antioxidants.

The combined study demonstrates that *Plectranthus amboinicus* responds to abiotic stress (heat, salinity, drought and chilling) and elicitor treatment by significantly enhancing secondary metabolite biosynthesis. These phytochemicals contribute to its strong antioxidant and antimicrobial activities. The results establish stress-elicitation as a practical approach to improve the medicinal and nutraceutical value of *Plectranthus amboinicus*, reinforcing its role as a promising source of bioactive compounds.

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