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RESEARCH ARTICLE

A comparative analysis of phytochemical constituents and bioactivity of two wild medicinal herbs of the Amaranthaceae family

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Abstract

Alternanthera sessilis and *Alternanthera paronychioides* are 2 common herbaceous plants in the North Eastern region of India. The 2 species are known traditionally for edibility and medicinal use. The objectives are to qualitatively screen phytochemicals and test for antioxidant activity and antimicrobial activity between the 2 species– *Alternanthera sessilis* and *Alternanthera paronychioides.* The samples for analysis were collected from aerial leaves and air-dried for phytochemical screening and biological activity tests. Antioxidant assays used are DPPH (2,2-diphenyl-1-1picrylhydrazyl), H_2O_2, and FRAP (ferric reducing antioxidant power) assay. For antimicrobial tests, 2 different strains of microbes were used to test the bioactivity of the plants. Phytochemical screening showed the presence of several antioxidant potential groups of phytochemicals like phenols, flavonoids, etc. Phytochemical constituents of *Alternanthera sessilis* showed more antioxidant potential than *Alternanthera paronychioides* in DPPH and H_2O_2 assays but *Alternanthera paronychioides* showed more potential than *Alternanthera sessilis* in FRAP assay. The phytochemicals also showed positive tests for antimicrobial activity against 2 bacterial strains, i.e., *Escherichia coli* (MTCC 443) and *Staphylococcus aureus* (MTCC 737). This study reveals the presence of phytonutrients which suggest the antioxidant and antibacterial activities exhibited by the 2 species of the family. Further exploration of phytochemicals in different aspects could lead to better-performing drugs for various ailments.

Keywords

Alternanthera; antioxidant; antimicrobial; antibacterial; phytochemical

Introduction

Herbaceous plants have been sources of nutrition since ancient times. The uses of such plants for medicinal purposes are known from traditional knowledge from the native people of the particular region (1). Herbs like *Centella asiatica*, *Tinospora cordifolia*, *Amaranthus spinosus*, *Alternanthera sessilis*, *Ocimum sanctum,* etc. are known traditionally to be medicine for curing a variety of ailments (2). Majorities of such chronic diseases are due to inflammation.

Phytonutrients are biological compounds or bio constituents that impart various health benefits to the animal body. Such phytonutrients include alkaloids, carbohydrates, tannins, amino acids, terpenoids, flavonoids, etc. that help reduce inflammation (3). These compounds are

known to possess healing properties for most human ailments. The results were positive for diuretic, antidiabetic, anticancer, antimicrobial, antioxidants, etc. (4, 5). The majority of recent studies are focused on screening processes to procure plant-based drugs for diseases. The antioxidant potential provided by polyphenols is known to affect the mechanism of allergies, free radicals, hepatotoxins, and aggregations of platelets (6, 7).

Extensive research for a better understanding of nutrients from plant and their beneficial direction about how to prepare such food perfectly is still a challenge. The 2 species of Amaranthaceae, *Alternanthera sessilis* and *Alternanthera paronychioides* were consumed by local communities of Assam, North East region of India (8, 9). Therefore, based on some prior knowledge of traditional communities on the herb uses, comparative phytochemical and bioactive analyses of *Alternanthera sessilis* and *Alternanthera paronychioides* were performed in this study. These 2 plants have not been studied side by side as per the literature search.

Materials and Methods

Collection and preparation of plant material

2 plant species (Fig. 1a and 1b) were gathered from the campus of Gauhati University and the surrounding area of Jalukbari, Guwahati, Assam state, region of India. The samples were collected fresh and shade-dried for a few days. The leaves were later ground into a semi-fine powder. The plant species were identified based on the Gauhati University Botany Herbarium (GUBH), Department of Botany collections. *A. sesslis* (L.) R. Br. ex DC was identified with the help of voucher specimen accession number 20475 and *A. paronychioides* A. St. -Hil. with voucher specimen accession number 20476.

Preparation of extract

Maceration of dried leaves with 4 different solvents such as methanol, aqueous, chloroform, and hexane for 24 h at 37 °C was done to prepare the plant extract. According to the polarity level, solvents were favored from aqueous to methanol to chloroform to hexane. The filtrate was gathered through a rotary evaporator (Rotavapour® R II, BUCHI, Switzerland) that was heated at 37 °C. The process of evaporation was proceeded by the collection of the crude extract.

Qualitative test

12 distinct phytochemicals, including alkaloids, phenols, tannins, saponins, flavonoids, steroids, cardiac glycosides, glycosides, terpenoids, reducing sugar, carbohydrates, and coumarins (10).

Test for Alkaloids (Wagner's Reagent):

Wagner's reagent was added to 1 or 2 mL of extract along the test tube's side. A reddish-brown precipitate was produced.

Test for Phenols (Ferric Chloride Test):

1 or 2 drops of 5% $FeCl_3$ were added to a 1 mL of the extract and the creation of a strong colour of blue indicated the presence of phenols.

Test for Tannins (Lead Acetate):

1 mL of plant extract was mixed with 1 mL of lead acetate. White precipitate appears.

Test for Saponin (Froth Test):

2 mL of distilled water was added to 1 mL of extract, which was then forcefully shaken. The presence of saponins is indicated by the formation of foam layers.

Test for Flavonoids:

1 mL of extract and 1 mL of 20%NaOH were combined. The yellow colour appears.

Test for Steroids:

1 mL of the extract was added along with 2 mL of chloroform and 2 mL of concentrated H_2SO_4. The creation of a red colour confirms the presence of steroids.

Test for Cardiac Glycosides:

0.5 mL of 10% $FeCl_3$ and 1 mL each of glacial acetic acid and H_2SO_4 were added to 1 mL of extract.

Test for Glycosides:

2 mL of chloroform, 2 mL of glacial acetic acid, and a few drops of conc. H_2SO_4 was added and mixed with 1 mL of the extract. Glycosides are present when a violet-to-yellow coloration forms.

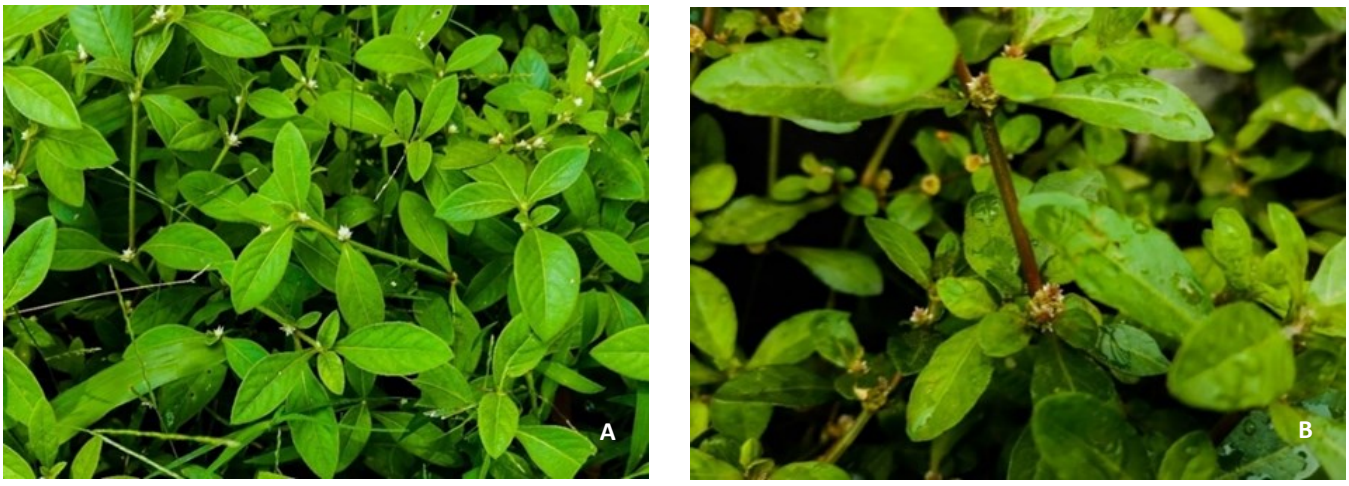

Fig. 1. Showing the natural habitat of the two plants; A: Habit of *Alternanthera paronychioides* A. St.-Hil, B: Habit of *Alternanthera sessilis* (L.) R. Br. ex DC.

Test for Terpenoids:

1 mL of extract was added together with 2 mL of chloroform and 2 mL of concentrated H_2SO_4. The coloration of reddish brown indicated the presence of terpenoids.

Test for Reducing Sugars:

2 mL of 1:1 Fehling solution A & B were added to 1 mL of extract, which was then maintained in a water bath. Red precipitate formation denotes the presence of sugar.

Test for Carbohydrates:

0.5 mL of Benedict's reagent and 0.5 mL of extract were mixed into the test tube and heated in boiling water for 2 min. The presence of carbohydrates is shown by colored precipitate.

Test for Coumarins:

1 mL of extract and 1 mL of concentrated H_2SO_4 were combined. The presence of coumarins is indicated by a yellow colour appearance.

Quantitative test

Total phenols and total flavonoids were done by following the Folin Ciocalteu (11) and Aluminium chloride tests respectively (12).

Total phenolic content determination

5 mL of 7.5% Na_2CO_3 and 0.1 mL of extract were combined with 1.5 mL of the Folin Ciocalteu reagent. With distilled water, the mixture was thinned to a volume of 10 mL and incubated in the dark at room temperature for 90 min. The mixture's absorbance was measured using the Systronics 119 UV-VIS spectrophotometer at 765 nm. The gallic acid in methanol served as the standard curve for quantification and the values of the results were expressed as mg of gallic acid equivalent per g of extract using the formula in eqn 1:

$$T = C \times \frac{V}{M} \qquad (1)$$

Total flavonoid content determination

The sample had a concentration of 1 mg/mL in methanol. The standard calibration curve was developed using quercetin. Different quercetin in methanol concentrations (20, 40, 60, 80, and 100 µg/mL) were used. 1 mL of methanol and 0.1 mL of 10% aluminum chloride were added to 0.1 mL of each solution. Approximately 0.1 mL of sodium acetate was added to this combination and the final volume was increased to 3 mL using distilled water. At room temperature, the mixture was vortexed briefly (Fig. 3). At 415 nm, the extract's absorbance was measured using the Systronics 119 UV-Vis spectrophotometer. With the aid of the following formula, the values of the results were converted into units of mg/mL of quercetin equivalent or mg QE/g extract in eqn 2:

$$T = C \times \frac{V}{M} \qquad (2)$$

Determination of Antioxidant

DPPH (2,2-diphenyl-1-1picrylhydrazyl) Assay

With a few modifications of Brand and Williams's work of DPPH assay (13, 14) *in vitro,* radical scavenging activity was tested. The extract or standard solution was measured out at 750 µL and a matching volume of DPPH solution made in methanol was added (0.1 mM). The mixture was incubated at room temperature for 30 min. An estimation was made by measuring DPPH decolorization at 517 nm using a spectrophotometer. Ascorbic acid was used as a reference and the test was performed in triplicate. To get the IC_{50} value, the absorbance values of the tested substances and the control were compared. The ratio between the difference in absorbance between the control and the sample, multiplied by 100, was used to calculate the inhibition percentage of free radicals, which indicated the DPPH scavenging activity.

H_2O_2 Assay

A hydrogen peroxide buffer solution of 40 mM and pH 7.4 was prepared (15). 0.6 mL of buffer solution was added to the ascorbic acid solution of concentrations 15, 50, and 200 µL/mL. For 10 min, absorbance was measured at 230 nm. Hydrogen peroxide in phosphate solution was used as a control. The ratio between the difference absorbance of the control and absorbance of the sample and absorbance of the control multiplies by 100.

FRAP (Ferric reducing antioxidant power) Assay

FRAP assay to determine plant extract's ability to donate hydrogen and convert ferric ions to ferrous ions or its antioxidant potential (16). This reduction creates a blue-colored compound that aids in explaining the absorption at 593 nm. The FRAP assay used the following reagents: 2.5 mL of 1% ferric cyanide, 2.5 mL of 10% trichloroacetic acid, and 0.1% $FeCl_3$ (v/v) added to each 0.5 mL well and thoroughly mixed. The absorbance after 30 min helps to account for the antioxidant potential. Fresh preparations of the solutions and reagent were made on the day of the experiment. The analysis was conducted in duplicate and a standard curve was created using ascorbic acid at various concentrations. The more antioxidant potential is indicated by a more concentrated colour appearance.

Determination of Antimicrobial activity

The Agar Cup Diffusion Method was utilized for the antimicrobial experiment and a 6 mm cup with 100 µL of crude extract dissolved in DMSO was used (16). First, the bacterial strains ((*Escherichia coli* (MTCC 443) and *Staphylococcus aureus* (MTCC 737)) and fungal strain ((*Candida albicans* (MTCC 227)) grown on slants were incubated for 24 h at 35 °C. Nutrient agar and potato dextrose agar were the media utilized for antibacterial and antifungal activities respectively. Replicates were assessed and the results were matched with the control. Tetracycline and fluconazole were employed as standards to calculate the inhibition zone by measuring the diameter of inhibition and comparing it to the zone of inhibition they caused.

Results

Qualitative Analyses

A. paronychioides demonstrated the presence of alkaloids, phenols, flavonoids, tannins, terpenoids, carbohydrates, saponins, and steroids in methanolic extract (Table 1). Alkaloids, phenols, saponins, glycosides, terpenoids, and coumarins in aqueous solution. Alkaloids, terpenoids, and reducing sugars are found in chloroform extract. Additionally, it only revealed the presence of alkaloids in the hexane extract. Methanolic extract from the other plant, *A. sessilis*, showed that it included alkaloids, phenols, tannins, terpenoids, flavonoids, and carbohydrates (Table 2). Saponins, glycosides, and the rest are prevalent in aqueous extract as they are in methanolic extract. Alkaloids, phenols, and reducing sugars were found in the extract of chloroform. Finally, only alkaloids and terpenoids are found in hexane extract. The same extract was subjected to subsequent tests, including quantitative, antioxidant assay, and antimicrobial test. When compared to gallic acid, the phenolic content of the methanolic extract of *A. paronychioides* and *A. sessilis* was determined to be 0.0209 mg g^{-1} and 0.0139 mg g^{-1} respectively. According to the linear equation Y= 0.0034x + 0.0038 of the linear regression curve, the flavonoid content of leaves from *A. paronychioides* and *A. sessilis*is 0.0021 mg/mL and 0.0077 mg/mL respectively **(**Fig. 2 and 3).

DPPH Assay

This technique involved testing the concentration of ascorbic acid against methanol extracts of *A. paronychioides* and *A. sessilis*. The findings are reported as a % inhibition of the standard extract and 2 species' scavenging activities. When it comes to scavenging, *A. sessilis* outperforms *A. paronychioides*. The IC_{50} value was dosage dependent, with *A. paronychioides* having the greatest value at 20.329 µL/mL and *A. sessilis* having the lowest value at 6.2725 µL/mL. The IC_{50} value for ascorbic acid was 5.83624 µL/mL. The IC_{50} number represents the concentration required to neutralize 50% of radicals. Therefore, the antioxidant activity increases as the IC_{50} value decreases (Fig. 4).

Table 1. Qualitative analysis of *Alternanthera paronychioides*

Test	Methanol	Aqueous	Chloroform	Hexane
Alkaloid	+	+	+	+
Phenols	+	+	+	-
Tannins	+	+	-	-
Saponins	-	+	-	-
Flavonoids	+	+	-	-
Steroids	-	-	-	-
Cardiac glycosides	-	-	-	-
Glycosides	-	+	-	-
Terpenoids	+	+	-	+
Reducing Sugars	-	-	+	-
Carbohydrate	+	-	-	-
Coumarins	-	-	-	-

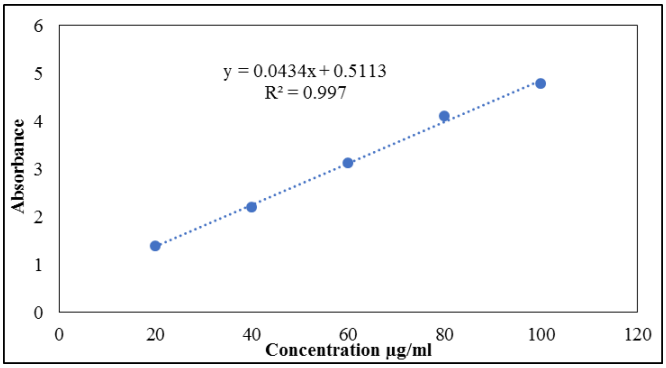

Fig. 2. Gallic acid standard curve for determination of total phenolic

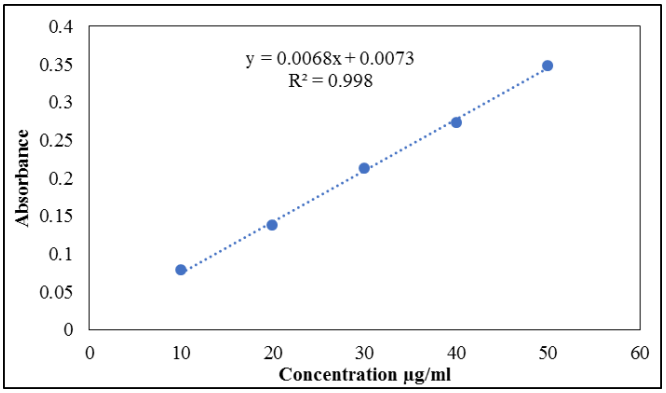

Fig. 3. Quercetin standard graph for flavonoid content estimation.

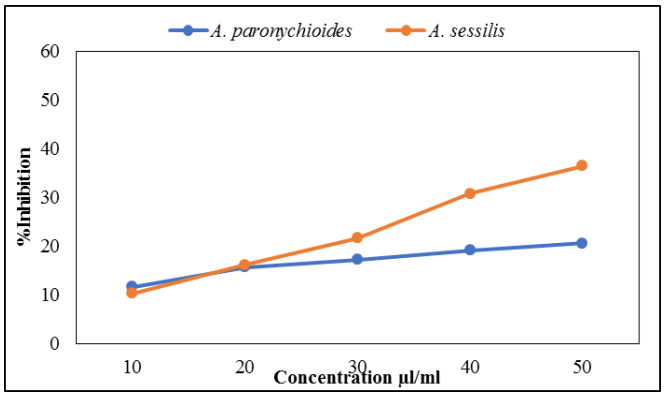

Fig. 4. DPPH antioxidant activity of *A. paronychioides* and *A. sessilis.*

Table 2. Qualitative analysis of *Alternanthera sessilis*

Test	Methanol	Aqueous	Chloroform	Hexane
Alkaloid	+	+	+	+
Phenols	+	+	-	-
Tannins	+	-	-	-
Saponins	+	+	-	-
Flavonoids	+	-	-	-
Steroids	+	-	-	-
Cardiac glycosides	-	-	-	-
Glycosides	-	+	-	-
Terpenoids	+	+	+	-
Reducing Sugars	-	-	+	-
Carbohydrate	+	-	-	-
Coumarins	-	+	-	-

In vitro H_2O_2 assay

The experiment's estimates were based on how well peroxide scavenged free radicals in samples at progressively higher concentrations of methanol. The strongest antioxidant activity was demonstrated by *A. sessilis*, with an IC_{50} value of 46.38 µL/mL, and *A. paronychioides*, with an IC_{50} value of 59.593 µL/mL. Ascorbic acid's capacity to scavenge free radicals had an IC_{50} value of 8.185 µL/mL (Fig. 5).

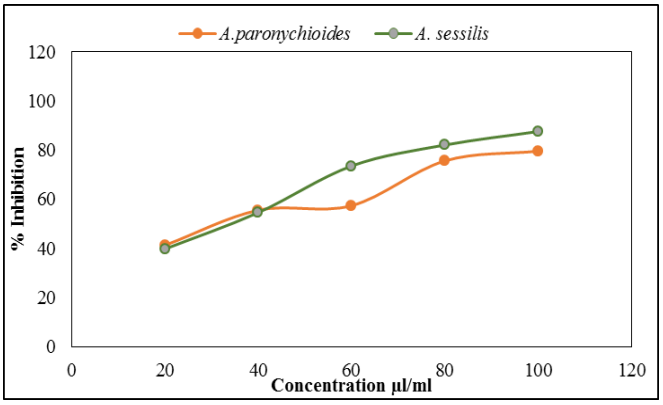

Fig. 5. H_2O_2 scavenging activity of *A. sessilis* and *A. paronychioides.*

FRAP Assay

To assess the oxidizing activity of a component included in the plant extract prepared in methanol, the analysis was conducted using the method for reducing the power of ferrous to ferric ions. The methanolic extract's estimated reducing power was evaluated using ascorbic acid as the reference. *A. paronychioides* and *A. sessilis* both demonstrated 12.23 ± 3.82 µL/mL and 18.98 ± 0.24 µL/mL reductions in the radical respectively (Fig. 6).

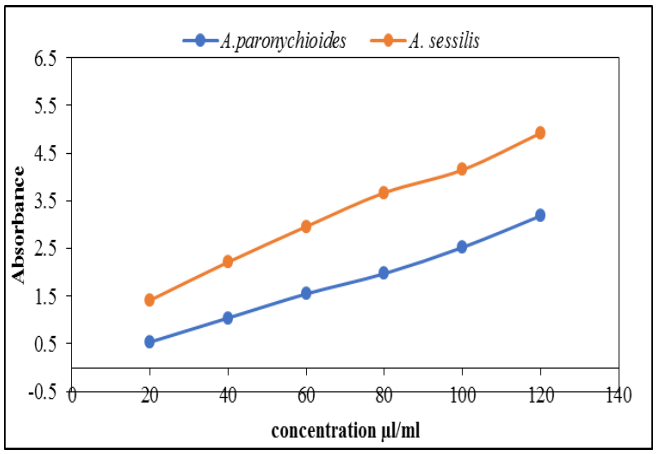

Fig. 6. Reducing power assay of plant extract.

Antimicrobial activity

A. sessilis and *A. paronychioides* methanolic extracts with a 1 mg/mL concentration were used to investigate the antimicrobial activity. 2 bacterial strains and 1 fungal strain were used in the tests. *Candida albicans* MTCC (Microbial type culture collection) 227 was used as a test subject for antifungal activity. Antibacterial activity was also tested against the gram-positive bacteria *Staphylococcus aureus* MTCC 737 and the gram-negative bacteria *Escherichia coli* MTCC 443. The outcomes demonstrated that both species' methanolic extracts had antibacterial properties. In the instance of *A. sessilis*, the extract inhibited growth at MICs of 10 mg/mL in gram-positive bacteria and 15.66 mg/mL in gram-negative bacteria (MTCC 443, MTCC 737). *A. paronychioides*, however, displayed inhibition at 14.66 mg/mL (MTCC A443) and 14 mg/mL (MTCC 737). The test revealed no evidence of antifungal action (Fig.7a and 7b) (Table 3).

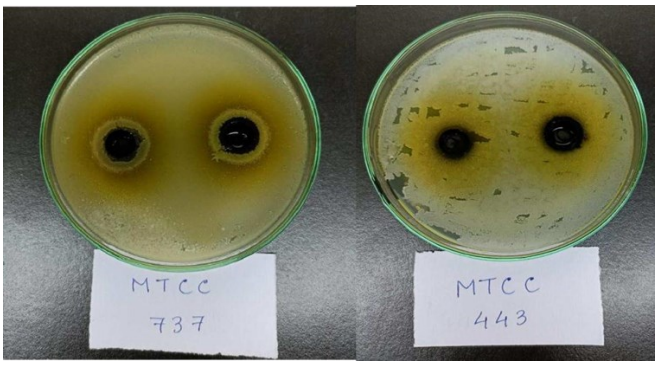

Fig. 7. A: Shows the inhibition of *S. aureus* by AS extract on the left well and AP on the right well; B: Shows the inhibition of *E. coli* growth by AS extract on the left well and AP extract on the right well.

DISCUSSION

Based on the consumption by the locals, 2 Amaranthaceae edible plants, *Alternanthera sessilis* (AS) and *Alternanthera paronychioides* (AP) of Assam, were obtained for phytochemical investigation (17). A member of this family is known to have therapeutic effects for constipation, pneumonia, leprosy, and diuretics (18). The 2 sample species revealed the presence of crucial phytochemicals such as phenols, flavonoids, tannins, carboxylic acid, reducing sugar, etc. In the initial exploratory test, however, it was discovered that these species lacked cardiac glycosides, anthraquinones, quinones, and free amino acids. The alkaloids, phenolic compounds, flavonoids, and terpenoids can be a useful aid for identifying *Alternanthera* species (19). On the other hand, phenols have

Table 3. Antioxidant assay IC_{50} values of three different radicals

Sample	DPPH method IC_{50} (µL/mL)	H_2O_2 method IC_{50} (µL/mL)	Reducing power Assay IC_{50} (µL/mL)
Ascorbic acid	5.836 ± 0.93	8.185 ± 0.854	2.084 ± 0.86
A. paronychioides	20.329 ± 0.87	59.59 ± 5.630	12.23 ± 3.82
A. sessilis	6.2725 ± 0.93	46.38 ± 2.191	18.98 ± 0.24

Values in given in mean ± standard deviation

a wide variety of phytochemicals that exhibit powerful apoptosis and carcinoma termination by interrupting the cell cycle as well as chemoprotective properties like anti-inflammatory, antioxidant, and antimutagenic properties (20). Antioxidant activity was assessed using the % of phytochemicals in the methanolic extract of the plant specimens that could scavenge free radicals such as DPPH, H_2O_2, and FRAP. In a DPPH assay, *A. paronychioides* seemed to have an IC_{50} value of 20.329 µL/mL and *A. sessilis* seemed to have an IC_{50} value of 6.272 µL/mL. Likewise, *A. paronychioides* had an IC_{50} value of 59.59 µL/mL, and *A. sessilis* also had an IC_{50} value of 46.38 µL/mL in the H_2O_2 assay. *A. sessilis* demonstrated greater antioxidant activity, measuring 18.98 ± 0.24, compared to *A. paronychioides* values, which were 12.23 ± 3.82 according to the results of the FRAP antioxidant assay. Free radicals are expected to form as a result of several metabolic processes and these species play a significant role in multiple metabolic pathways.

The methanolic extract's antimicrobial properties against gram-negative *E. coli* revealed the lowest inhibitory concentrations at 15.66 mg/mL, 14.66 mg/mL, and against gram-positive bacteria strains of *Staphylococcus aureus* at 10 mg/mL and 14 mg/mL respectively. Commonly employed in culinary preparations, edible herbs like *Thymus vulgaris* and *Hibiscus sabdariffa* are tested for their ability to resist bacteria like *Bacillus cereus, Staphylococcus aureus, Escherichia coli*, *Salmonella enteritidis* and fungi like *Candida albicans* (21).

Conclusion

At present the hunt for rich sources of nutritive food, especially plant-based, is at its peak to balance out the unhealthy effects of processed food. Another reason for such a food search is that balanced nutrition is composed of food that can be consumed daily. In the current scenario, many diseases are caused and occur due to excessive consumption of chemically enhanced food or processed food that impacts the normal flow of nutrients inside the human body. The presence of secondary metabolites supports the importance of nutrients and their medicinal value such as antibacterial etc. Additionally, appropriate alternatives for their utilization as edible herbs are a good way to learn more about these plants.

We also can infer from the study that investigating their phytochemical substances in light of their traditional knowledge of gastronomic and medical concerns may lead to the development of novel drugs for the treatment of several ailments.

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Authors' contributions

Jinti M Das and Namita Nath authors designed the main framework and finalized approval of the article, Kumanand Tayung and Anindita Sharma helped to analyze the antimicrobial experiments.

Compliance with ethical standards

Conflict of interest: Authors declare no conflict of interest.

Ethical issues: None

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