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Original Article

Phytochemical Insights into Antioxidant and Antimicrobial Potentials Across Five Indian Medicinal Plants

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ABSTRACT

Background: Traditional medicine systems like Ayurveda use plant extracts; Indian medicinal plants like *Alternanthera sessilis, Cassia auriculata, Murraya koenigii, Celastrus paniculatus,* and *Moringa concanensis* are rich in bioactive compounds such as phenolics, flavonoids, and tannins, known for antioxidant and antimicrobial effects, prompting scientific validation of their traditional use and understanding their pharmacological properties. **Objectives:** This study aims to investigate the antibacterial and antioxidant qualities of hydroalcoholic extracts from the aforementioned Indian medicinal plants, employing Ultrasound Assisted Extraction (UAE) for enhanced extraction efficiency. The research seeks to establish connections between the phytochemical compositions and bioactivities of the extracts through comprehensive analyses. **Materials and Methods:** The study involves the characterization of physical properties, phytochemical screening, and quantitative assessment of total phenolic, flavonoid, and tannin content in the hydroalcoholic extracts. Antioxidant activities are evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assays, as well as the Ferric Reducing Antioxidant Power (FRAP) assay. The antimicrobial potential against various pathogens is assessed using the Minimum Inhibitory Concentration (MIC) calculation method. **Results:** The study reveals a diverse array of bioactive compounds present in the hydroalcoholic extracts, including phenolics, flavonoids, and tannins. *Cassia auriculata* and *Celastrus paniculatus* extracts exhibit notable antioxidant activity, supported by high levels of total phenolic and tannin content. *Cassia auriculata* demonstrates consistent strong scavenging action in both DPPH and ABTS assays. *Alternanthera sessilis, Cassia auriculata,* and *Celastrus paniculatus* show significant efficacy against *Pseudomonas aeruginosa* during antimicrobial testing. **Conclusion:** This investigation establishes correlations between the phytochemical compositions and the antimicrobial/antioxidant activities of hydroalcoholic extracts from Indian medicinal plants. The findings contribute to a deeper understanding of the medicinal properties of these plants and underscore the potential of *Cassia auriculata* and *Celastrus paniculatus* extracts as natural antioxidants. Such insights hold promise for future advancements in medicinal research and the development of pharmaceuticals derived from natural sources.

Keywords: *Cassia auriculata*, DPPH, ABTS, Antimicrobial, Antioxidant, MIC.

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INTRODUCTION

The traditional Indian medicinal herbs *Alternanthera sessilis* R.Br., *Cassia auriculata* Linn., *Murraya koenigii* (L.) Spreng, *Celastrus paniculatus* Willd *and Moringa concanensis* Nimmo have medicinal significance due to their therapeutic potential in traditional medicine.[1] These herbs have been studied for their antimicrobial,[2] antioxidant,[3] hypoglycemic,[4] immunostimulating,[5] and hypolipidemic properties.[6] Which aligns with the growing interest in exploring their therapeutic potential. Additionally, the Indian Pharmacopoeia has provided standards for herbal drugs, reflecting the increasing recognition of the importance of these medicinal herbs.[7] *Alternanthera sessilis*, a member of the Amaranthaceae family, holds a significant place in Traditional Siddha Indian Medicine, emphasizing the concept of ‘Food as Medicine’. The plant is widely consumed in Southern India particularly Tamil Nadu, is valued for its medicinal properties. Siddhas recognised its benefits, promoting its use for a variety of health purposes, including its role as an antioxidant, relief for abdominal issues, liver diseases and wounds.[8] Phytochemical analysis reveals the presence of diverse compounds, contributing to its therapeutic actions. *Cassia auriculata* Linn. commonly known as Tanners Senna, is renowned in Ayurvedic medicine. Found in dry regions of India, its various parts are employed for treating conditions like diabetes, rheumatism and more. The

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plant's chemical composition includes flavonoids, anthracene derivatives and fatty acids, aligning with its traditional uses and contributing to its medicinal properties.[9] *Murraya Koenigii*, the curry-leaf tree, holds a crucial place in traditional medicine across South Asia. Known for its diverse applications, it serves as a stimulant, anti-dysenteric and aids in managing diabetes. The plant's leaves and roots possess bitter, cooling and analgesic properties, enriched with vitamins and minerals. Chemical analysis highlights the presence of triterpenoids, alkaloids and various compounds, underscoring its medicinal significance.[10] *Celastrus paniculatus* Willd. is a woody climbing shrub with a rich history in Ayurveda. Recognized for its multifaceted properties, it is used for conditions ranging from skin diseases to cardiac debility. Chemical examination reveals the presence of fatty acids, sterols and sesquiterpene polyalcohols, providing a scientific basis for its traditional use.[11] *Moringa concanensis* Nimmo, locally known as Kattumurungai, is a plant acknowledged by tribal communities in Tamil Nadu. Traditional medicinal practices attribute therapeutic properties to its chemical constituents, including ascorbic acid, fatty acids, flavonoids and glycosides. This plant showcases a convergence of traditional knowledge and modern scientific understanding, making it a subject of research interest.[12]

Antioxidants are molecules that play a crucial role in the body by neutralising harmful compounds known as free radicals, which can cause oxidative damage to cells and tissues. These molecules occur naturally in the body and are essential for maintaining the integrity of cell membranes and protecting against oxidative stress. Antioxidants can be enzymatic, such as superoxide dismutase, glutathione peroxidase and catalase, or non-enzymatic, including vitamins like vitamin E and vitamin C, as well as compounds like melatonin. They function by scavenging free radicals and preventing oxidative damage, thereby contributing to overall health and well-being.[13] The antioxidant properties of medicinal herbs have been associated with the prevention and treatment of various diseases, including cancer, liver disorders and inflammatory conditions.[14-16] Medicinal plants and their derivatives have been found to reduce nephrotoxicity and side effects of anticancer drugs through their antioxidant and anti-inflammatory properties.[11,12] Additionally, antioxidants are essential in neutralising reactive oxygen species and preventing diseases such as cancer, diabetes and cardiovascular diseases.[17] *In vivo* studies for antioxidants face challenges related to the extreme reactivity and metabolic conversion of antioxidants, making them unsuitable as test analytes except at high levels of oxidative stress.[18] Additionally, the poor systemic bioavailability of antioxidants poses a significant challenge in conducting *in vivo* studies, leading to mixed results. The *in vitro* methods for determining antioxidant activity are valuable for assessing the potential health benefits of compounds. *In vitro* methods for determining antioxidant activity include assays based on Single Electron Transfer (SET) reactions, such as the Ferric Reducing Antioxidant Power (FRAP) and ABTS bleaching assay and Hydrogen Atom Transfer (HAT) assays, which measure the activity of antioxidants to scavenge peroxyl radicals.[19] Additionally, the DPPH assay, 2,2'-azino-bis-(3-ethylbenzo thiazolin-6-sulfonic acid) diammonium Salt (ABTS) method and Ferric Reducing Antioxidant Power (FRAP) method are commonly used for evaluating antioxidative activities *in vitro*.[20] Antimicrobial activity in herbal drugs is of significant importance due to their potential as alternative sources of antimicrobial, antiviral and antifungal agents. Herbal materials have been found to exhibit radical scavenger ability, efficacy in eliminating bacteria and viruses and scavenging free radicals, highlighting their potential in combating microbial infections.[20] The antimicrobial activity of plant extracts and phytochemicals has been evaluated against antibiotic-resistant microorganisms, emphasizing the relevance of herbal drugs in addressing antibiotic resistance.[21] Additionally, the search for novel antimicrobial compounds from aromatic and herbal plants is essential in the fight against multidrug-resistant pathogens.[22] The Minimum Inhibitory Concentration (MIC), which is a measure of the effectiveness of an antimicrobial agent, is the lowest concentration that stops the visible growth of a microorganism after it has been incubated overnight.[23] It is a crucial parameter in determining the effectiveness of antimicrobial agents against specific pathogens. The MIC is determined through various methods such as the broth microdilution assay and agar dilution method, providing valuable information for guiding treatment decisions and assessing the susceptibility of microorganisms to antimicrobial agents.[24,25]

The objective of this work is to thoroughly investigate the antibacterial and antioxidant properties of five extracts derived from medicinal plants, while establishing a correlation with their varied phytochemical compositions. The study uncovers a range of substances, including as alkaloids, carbohydrates and phenolic compounds, which serve as the foundation for further investigations. The quantitative estimations establish strong correlations between particular phytochemicals and their antioxidant capacities. This study effectively reveals the connections between the compositions of phytochemicals and their biological activities, providing valuable insights for the development of natural medicine and pharmaceuticals.

MATERIALS AND METHODS

Materials

The study involved meticulous collection and authentication of plant materials, where fresh aerial parts of *Cassia auriculata* Linn.(CAE) was gathered from Nagamangala taluk, Mandya district, Karnataka. Additional plant specimens, including *Alternanthera sessilis* (ASE), *Murraya koenigii* (MKE), *Celastrus paniculatus* Willd (CPE) and *Moringa concanensis* Nimmo (MCE), were collected from the botanical garden premises of

the Foundation for Revitalisation of Local Health Tradition (FRLHT), Bangalore. Dr. Ganesh Babu, an Assistant Professor at TDU, FRLHT, Bangalore, validated their authenticity, providing an authentication certificate.

For the preparation of plant extracts, leaves were carefully obtained from the authenticated aerial parts, cleaned and dried before being ground into a fine powder. Ultrasound-assisted extraction, utilising a hydroalcoholic mixture, was performed to extract bioactive compounds. The extracts underwent filtration, Rota vapor dehydration and desiccation for further processing. Quantitative estimations of total phenolic, flavonoid and tannin content were conducted using specialised reagents purchased from Sigma Aldrich such as Folin-Ciocalteau, aluminum chloride, gallic acid standards and catechin standards. Measurements were carried out using a UV-visible spectrophotometer. Phytochemical screening involved qualitative tests for various chemical constituents using Harborne's method.

Antioxidant activity assessments included DPPH, ABTS and FRAP assays, with specific reagents and standards purchased from Sigma Aldrich, HiMedia and the results were measured using a UV-visible spectrophotometer. Antibacterial susceptibility tests for Minimum Inhibitory Concentration (MIC) utilized HiMedia branded Muller Hinton Agar and broth, bacterial cultures, resazurin and a fluorescence spectrophotometer, performed in Tarsons 96-well culture plates under sterile conditions to ensure reliable results.

Methods

Preparation of Plant Extracts

Leaf samples were obtained from the collected aerial portion after authentication. Subsequently, they were cleaned extensively under running tap water to eliminate any earthy, dusty, or foreign substances. The samples were then dried in the shade for duration of 25 days. After the leaves had dried, they were ground into a fine powder using a kitchen grinder. To safeguard the powder from moisture attack, they were stored in a desiccator. Following this, the material underwent an extraction procedure.

The process of extracting all five plant leaf samples using ultrasound-assisted extraction involved combining 50 g of powder samples of the drugs with a hydroalcoholic mixture (20:80, water:ethanol ratio) in a 1:10 ratio of drug to solvent. The mixture was sonicated for four periods of 15 min each at room temperature (27-30°C). This temperature was maintained continuously over the process of sonication to prevent overheating and ensure that the sample was close to room temperature. Solid detritus was eliminated from the extracts by filtration them through (ash-less) filter paper (Whatman number 4) following sonication. The resulting extracts underwent Rota vapor dehydration in order to eliminate any residual solvent. In the end, the extracts were desiccated by transferring them to tared petri dishes and placing them in an electric water immersion set to 40°C.

Quantitative estimation of total phenolic, flavonoid and tannins content

Estimation of total phenolic content

This study aimed to analyse phenolic and polyphenol molecules, which are secondary metabolites found in plant extracts, in order to understand their antioxidant effects. The quantification of total phenolic content was conducted using spectrophotometry with the use of Folin-Ciocalteau reagents. The methodology, in accordance with the procedure described by Singleton and Rossi (1965),[26] consisted of combining two milliliters of pre-diluted Folin-Ciocalteau reagent with a sample of known concentration. Following a 5 min interval of incubation, a 1.6 mL solution of sodium carbonate (7.5% w/v) was introduced to commence the reaction. The resultant solution was subsequently incubated for 60 min in the absence of light at ambient temperature. Afterwards, the absorbance was quantified at a wavelength of 765 nm using a UV-visible spectrophotometer. The reference blank, which did not contain the sample but had 400 μL of methanol, was utilized to compensate for the absorbance originating from the background. In order to create a calibration curve, gallic acid standards with different concentrations (ranging from 10 to 100 μg/mL) were employed. The total phenolic content values were quantified in milligrams of Gallic Acid Equivalent (mg GAE) per gram of dry extract. In order to achieve accuracy, the measurements were carried out three times, resulting in strong and dependable results for the quantification of phenolic compounds and evaluation of the antioxidant capabilities in the analysed plant extracts.

Estimation of total flavonoid content

The analysis of flavonoids, polyphenolic compounds characterised by benzo-γ-pyrone structures, was conducted to determine the total flavonoid content in plant extracts. Colorimetry employing an aluminum chloride reagent was employed for this purpose. The fundamental principle of this method involves the formation of a stable yellow compound through the complexation of aluminum chloride with keto clusters on a C-4 atom and hydroxy clusters on C-3 or C-5 atoms, particularly from the group of flavones and flavonols. Following the methodology outlined by Sakanaka *et al.* (2005),[27] 250 μL of the Sample or catechin standard solution was mixed with 1.25 mL of distilled water in a test tube. Subsequently, 75 μL of a 5% sodium nitrite solution was added and after six min, 150 μL of a 10% aluminum chloride solution was introduced. The mixture was left to stand for an additional 5 min before the addition of 0.5 mL of 1 M sodium hydroxide. To achieve a final volume of 2.5 mL, distilled water was added and the solution was thoroughly mixed. The absorbance of the resulting solution was promptly recorded at 510 nm using a UV-visible spectrophotometer. Calibration curves were constructed using

catechin standards of various concentrations (10-100 μg/mL) and the total flavonoid content was expressed as milligrams Catechin Equivalents (mg CAE) per gram of dry extract. To ensure accuracy, all measurements were performed in triplicate, providing reliable data for the quantification of flavonoids in the examined plant extracts.

Estimation of total tannin content

The Vanillin test was used to quantify condensed tannins in this investigation. The Vanillin-HCl technique exploits the interaction between condensed tannins and vanillin in the presence of mineral acid, leading to the production of a red hue. This assay was especially tailored for the analysis of flavon-3-ols-dihydrochalcones and proanthocyanidins. Following the protocol described by Burns (1971),[28] 0.250 mL samples of the desired concentration in methanol were combined with a reagent combination (2.250 mL) consisting of 4% vanillin in methanol and 8% concentrated HCl in methanol, in a 4:1 ratio. The reaction mixture was vigorously mixed using a vortex mixer and then left undisturbed for duration of 20 min to enhance the progress of the reaction. Following the incubation period, the resulting color was measured at a wavelength of 500 nm using a UV-visible spectrophotometer. The calibration curves were created by utilising catechin standards with different concentrations ranging from 20 to 400 μg/mL. The total tannin content was then quantified as milligrams of Catechin Equivalents (CAE) per gram of dry extract. In order to guarantee precision, all measurements were carried out three times, yielding dependable and uniform results for the quantification of condensed tannins in the analysed plant extracts.

Phytochemical screening

A preliminary phytochemical screening procedure was conducted on hydroalcoholic extracts of five different plant species. This involved general qualitative chemical tests to identify the chemical composition of active constituents in the extracts. The standard method outlined by Harborne (1998) was utilized to detect alkaloids, glycosides, carbohydrates, fixed oils, fats, tannins, gum and mucilage, flavonoids, saponins, terpenoids, lignin, phytosterols.[29]

Antioxidant activity

DPPH Assay

The leaf extracts was tested using a 1,1-diphenyl2-picryl hydroxyl (DPPH) technique. A total of 24 milligrams of DPPH were dissolved in 100 mL of methanol to make the stock solution. Filtration of DPPH stock solution using methanol yielded a usable mixture with an absorbance of around 0.973 at 517 nm. In a test tube, 3 mL DPPH workable solutions were combined with 100 μL of leaf extract (concentration ranging from 25 μg to 800 μg/ml. Three millilitres of solution containing DPPH in 100 μL of methanol is often given as a standard. After that, the tubes were kept in complete darkness for 30 min. The absorbance was therefore determined at 517 nm. The following formula was used to compute the percentage of antioxidants.[30]

$$\% \text{ of antioxidant activity} = [(Ac - As) \div Ac] \times 100$$

Where, Ac-Control reaction absorbance;

As-Testing specimen absorbance.

ABTS Assay

The technique described by Re *et al.*[31] was utilized to determine the ABTS activity of the formulation (VRF). This assay relies on the decolorisation process that takes place when the radical cation ABTS. + is converted to ABTS'(2, 2'-azino-bis(3 -ethylbenzthiazoline-6-sulfonic acid). Simply put, the radical was formed when a 7 mM solution of ABTS in water reacted with 2.45 mM potassium persulphate ($K_2O_8S_2$) in a 1:1 ratio. The combination was incubated in a light-free environment at a temperature of 27ºC for a duration of 16 hr, which was the required time to get a consistent absorbance reading at a wavelength of 734 nm. Following incubation, the radical solution was diluted with water (1 mL of ABTS reagent+27 mL DW) until the absorbance value at 734 nm reached the Desired value

In order to analyse the test samples, a mixture of 980 μL of ABTS. + reagent and 20 μL of the sample (concentration ranging from 25 μg to 800 μg/ml) or standard was prepared. The measurement of absorbance was conducted 6 min after the start, using a wavelength of 734 nm. The optical density (ΔO.D.) was determined by comparing the first reading (at 0 min) with the value taken at the 6th min. For the purpose of this experiment, Quercetin was employed at concentrations ranging from 0.31 μg/mL to 10 μg/mL, the scavenging inhibitory ability of the extract was quantified as a percentage using the ABTS+ assay. This value was then compared to the scavenging capacities of Quercetin.[32]

$$\% \text{ inhibition} = [(Abs_{control} - Abs_{sample})]/(Abs_{control})] \times 100$$

FRAP Assay

The FRAP assay was conducted using a slightly altered version of the Benzie and Strain technique. The stock solutions included of a 300 mM acetate buffer (consisting of 3.1 g CH_3COONa and 16 mL CH_3OOH, with a pH of 3.6), a 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) solution in 40 mM HCl and a 20 mM $FeCl_3 \cdot 6H_2O$ solution. The procedure consisted of the following steps: (i) preparing a fresh FRAP solution by combining 25 mL of acetate buffer, 2.5 mL of TPTZ and 2.5 mL of $FeCl_3 \cdot 6H_2O$, (ii) heating the solution to a temperature of 37ºC, (iii) allowing plant extracts (150 μL) to react with 2850 μL of the FRAP solution for 30 min in the absence of light and (iv) measuring the absorbance of the resulting-colored product (ferrous tripyridyl triazine complex) at a wavelength of 593 nm. The relationship

between the concentration of FeSO4 and the response in the standard curve was a straight line within the range of 200 to 1000 µM. The results are quantified in micromoles of Fe (II) per gram of dry mass.[33]

Antibacterial susceptibility tests

The antibacterial effects of raw extract, were assessed using broth dilution assay (Resazurin) for Minimum Inhibitory Concentration (MIC).[34]

Media: Muller Hinton Agar and broth (MHB).

Test organisms: Four bacteria were selected for the study i.e., *Streptococcus mutans; Staphylococcus aureus; Psedomonas aerugenosa* and *Escherichia coli.*

Inoculum preparation: Inoculum preparation was carried out in specific broth media. The standard colonies of the same morphological type were selected from an agar culture plate further, each colony was scooped with a sterile loop and the grown bacteria were transferred into a tube containing 4-5 mL of MHB broth. The broth culture was incubated at 37°C for 8-14 hr until it achieved the turbidity of the 0.5 McFarland standards. The turbidity of actively growing bacterial culture was adjusted with broth to obtain a final turbidity of 0.5 McFarland standards.

Resazurin method

To the 96 well culture plates equal quantity of microbiological media in specified wells 100 µL was added. Further, plant extracts at 733 µg concentration in 100 µL were added, after adding plant extracts in first well, extracts were serially diluted to the requisite concentrations. To the mixture 20 µL of inoculum of bacterial in each well was inoculated, except positive control, followed by addition of 20 µL resazurin solutions to each well. The resultant mixture was incubated for 1 to 4 hr at 37°C. Fluorescence was recorded using a 560 nm excitation/590 nm emission filter set. The active metabolite of the organisms reduces resazurin into resorufin which is pink and fluorescent in colour which is further reduced to dihydroresorufin, which is indicated by disappearance of pink colour and the end point is taken as MIC.

Statistical analysis

Statistical analyses, including ANOVA and post-hoc Tukey's test, were conducted to assess differences in phenolic, flavonoid, and tannin content among plant extracts. For antioxidant activity assays, ANOVA was used to evaluate variations, followed by post-hoc tests for specific comparisons. Antibacterial susceptibility tests involved statistical analysis to determine minimum inhibitory concentration (MIC) values and compare efficacy among extracts. These analyses ensured rigorous evaluation and interpretation of data, enhancing the reliability of study findings.

RESULTS

Physical properties of extract

Ultrasound Assisted Extraction was used to successfully extract hydroalcoholic extracts from five plant samples after the authentication procedure. The leaves underwent a rigorous process of washing, drying, grinding into a fine powder and extraction using a hydro alcoholic combination. A Rota vapor was then used to filter and concentrate the resultant extracts. Each extract's qualities were recorded, exposing unique elements. The foundation for additional research into the possible bioactive components within each plant sample is laid by these findings, which offer insightful information about the physical properties and yield of the extracted compounds (Table 1).

Phytochemical Screening

Phytochemical composition of hydroalcoholic extracts from five distinct plant species ASE, CAE, MKE, MCE, CPE. Employing Harborne's standard method (1998) for preliminary phytochemical screening, the extracts underwent comprehensive qualitative analyses to detect the presence or absence of various bioactive compounds. The results, detailed in Table 2, provide a clear overview of the phytochemical landscape of each plant extract. Alkaloids were notably present in CPE, ASE, MKE and MCE, with increasing intensities denoted by "+", "++" and "+++" respectively. Carbohydrates were consistently present in all extracts, not in CPE varying in intensity. Fixed oils and fats were detected in ASE, CAE and MCE, while glycosides and saponins were present in multiple extracts, demonstrating the diverse chemical profiles. Tannins and Phenolic compounds exhibited high presence (+++ or ++++ intensity) across all extracts, emphasizing their widespread distribution. Phytosterols and triterpenoids varied among the extracts, indicating diversity in secondary metabolites. Proteins were present in ASE, CAE, MKE

Table 1: Physical properties of the extracts.

Sl. No.	Name of the extract	Colour	Percentage yield
1.	*Alternanthera sessilis* extract (ASE)	Dark green	15.64%
2	*Cassia auriculata* extract (CAE)	Reddish brown	35.78%
3	*Celastrus paniculatus* extract (CPE)	Greenish brown	19.88%
4	*Moringa concanensis* extract (MCE)	Yellowish brown	17.04%
5	*Murraya koenigii* extract (MKE)	Greenish brown	19.66%

Table 2: Phytochemical screening.

Sl. No.	Phytochemical	ASE	CAE	MKE	MCE	CPE
1	Alkaloids	++	-	++	++	+++
2	Carbohydrates	++	++	++	++	-
3	Fixed oils and Fats	+	+	-	+	+
4	Glycosides	+	+	-	+	-
	Saponins	+	+	-	+	-
	Cardiac Glycosides	-	-	-	-	-
	Anthracene Glycosides	-	-	-	-	-
5	Tannins and Phenolic	+++	+++	+++	+++	+++
6	Phytosterols and Triterpenoids	++	-	+	+	++
7	Proteins	++	++	++	++	-
8	Gum's and Mucilage's	-	-	-	-	-
9	Carotenoids	+	-	-	-	-

Table 3: Total phenolic content, total flavonoid content and total tannin content of samples (*n*=3)

Sl. No	Sample	TPC (mg GAE/g sample)	TFC (mg CAE/g)	TTC (mg CAE/g)
1	MCE	194.78±7.24[a]	633.18±9.09[b]	126.06±6.94[d]
2	CPE	424.33±7.43[c]	866.52±9.64[e]	167.73±3.21[e]
3	CAE	793.40±7.43[d]	821.06±6.42[d]	63.18±3.21[c]
4	MKE	265.57±9.45[b]	727.12±9.64[c]	47.27±6.42[a]
5	ASE	264.19±9.30[b]	605.91±6.42[a]	51.82±6.42[b]

Values are represented as mean ± standard deviation. Values in the same column with different alphabets indicates the presence of significant differences.

Table 4: FRAP activity, DPPH and ABTS radical scavenging activity of samples IC_{50} values expressed in µg/ml.

Sample	FRAP	DPPH	ABTS
CAE	740.0[e]	71.5[a]	79.37[a]
ASE	141.0[b]	374.7[d]	112.5[b]
MCE	80.9[a]	386.2[e]	272.3[d]
CPE	635.5[d]	178.1[b]	81.46[a]
MKE	319.8[c]	226.1[c]	163.6[c]

Values in the same column with different alphabets indicates the presence of significant differences.

and MCE, while gums and mucilage's were absent in all extracts. Carotenoids were present in ASE, highlighting the variability in pigment composition. These findings underscore the diverse phytochemical composition of the studied plant extracts, offering valuable insights into their potential health-promoting properties. Further investigations into the specific bioactivities associated with these identified compounds are warranted for a comprehensive understanding of the therapeutic potential of these plant species (Table 2).

Quantitative estimation of total phenolic, flavonoid and tannins content

Estimation of total phenolic content

The determination of Total Phenolic Content (TPC) in the hydroalcoholic extracts of ASE, CAE, MKE, MCE and CPE was conducted using gallic acid as a standard.

The TPC values, expressed in mg Gallic Acid Equivalents (GAE) per gram of dry extract, were calculated based on the absorbance at 765 nm. Table 3 presents the TPC results for each extract,

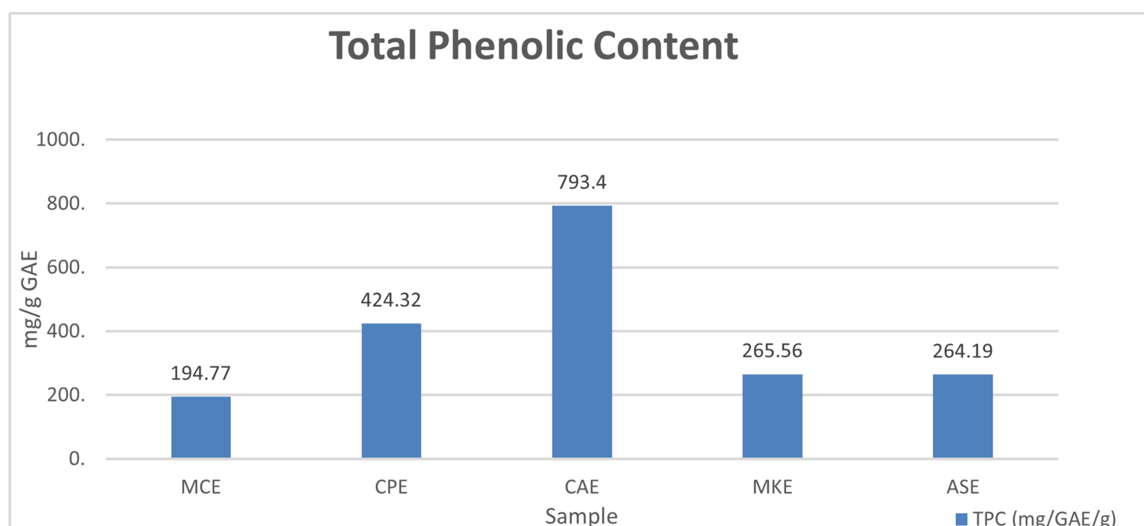

Figure 1: Graphical representation of total phenolic content of different extracts.

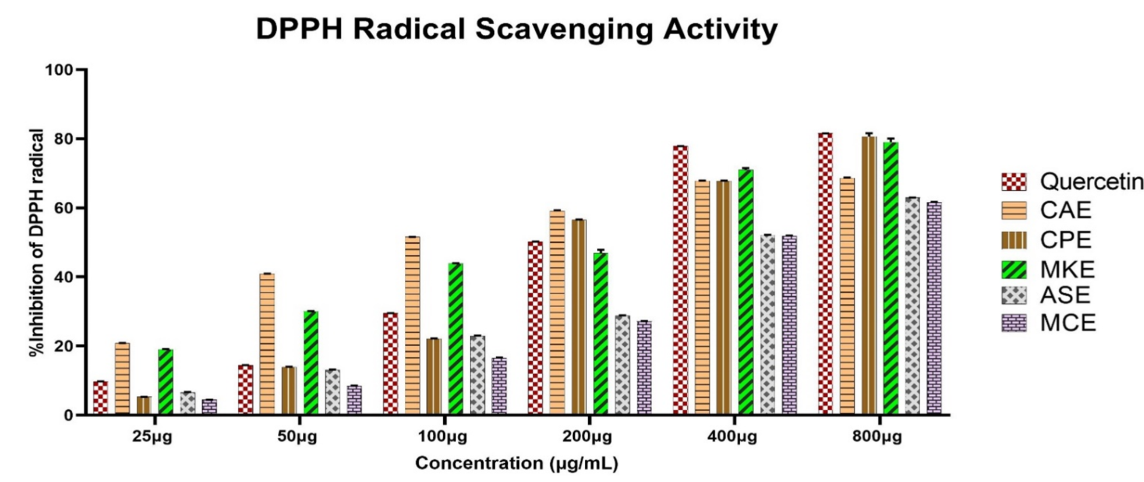

Figure 2: Comparison of DPPH radical scavenging activity of hydroalcoholic extracts from five distinct plant.

indicating the mean values of three replicates along with standard deviations. The TPC values were found to be 194.78±7.24, 424.33±7.43, 793.40±7.43, 265.57±9.45 and 264.19±9.30 mg GAE/g of dry extract for MCE, CPE, CAE, MKE and ASE, respectively.

These findings, represented in Figure 1, demonstrate the varying phenolic content among the studied plant extracts. The results highlight *Cassia auriculata* (CAE) as having the highest TPC, followed by *Celastrus paniculatus* (CPE), *Murraya koenigii* (MKE), *Alternanthera sessilis* (ASE) and *Moringa concanensis* (MCE). This quantitative analysis provides valuable insights into the antioxidant potential of the plant extracts, paving the way for further exploration of their health-promoting properties.

Estimation of total flavonoid content

The determination of Total Flavonoid Content (TFC) in the hydroalcoholic extracts of ASE, CAE, MKE, MCE and CPE was conducted using catechin as standard.

The TFC values, expressed in mg Catechin Equivalents (CAE) per gram of dry extract, were calculated based on the absorbance at 510 nm. Table 3 presents the TFC results for each extract, indicating the mean values of three replicates along with standard deviations. The TFC values were found to be 633.18±9.09, 866.52±9.64, 821.06±6.42, 727.12±9.64 and 605.91±6.42 mg CAE/g of dry extract for MCE, CPE, CAE, MKE and ASE, respectively.

Figure 4 visually represents the varying flavonoid content among the studied plant extracts. The results highlight CAE, MKE, ASE and MCE. This quantitative analysis provides valuable insights into the flavonoid composition of the plant extracts,

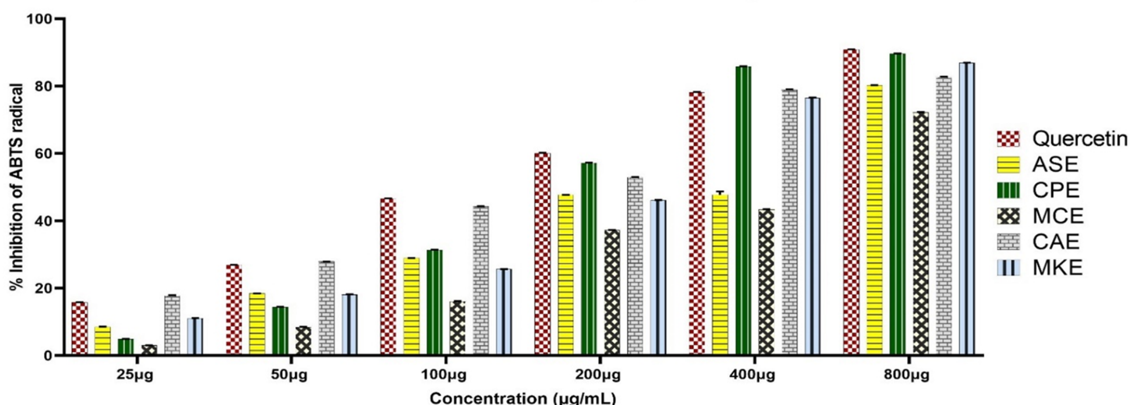

Figure 3: Comparison of ABTS radical scavenging activity of hydroalcoholic extracts from five distinct plant.

allowing for a comprehensive understanding of their potential health-promoting properties and antioxidant capacity.

Estimation of tannin content

The determination of Total Tannin Content (TTC) in the hydroalcoholic extracts of five plants was conducted using catechin as a standard. The TTC values, expressed in mg catechin equivalents (CAE) per gram of dry extract, were calculated based on the absorbance at 500 nm.

Table 3 presents the TTC results for each extract, indicating the mean values of three replicates along with standard deviations. The TTC values were found to be 126.06±6.94, 167.73±3.21, 63.18±3.21, 47.27±6.42, 51.82±6.42 mg CAE/g of dry extract for MCE, CPE, CAE, MKE and ASE, respectively.

These results highlight the varying tannin content among the studied plant extracts, with CPE exhibiting the highest TTC, followed by MCE, CAE, ASE and MKE. This quantitative analysis provides valuable insights into the tannin composition of the plant extracts, contributing to the overall understanding of their potential health-promoting properties and antioxidant capacity.

Antioxidant activity

DPPH Assay

The data presented in Table 4 regarding the DPPH radical scavenging activity of hydroalcoholic extracts from five distinct plant species is adapted from a study on antioxidant activity using the DPPH assay. Additionally, Figure 2 illustrates a comparison of the DPPH radical scavenging activity of these plant extracts.

This study reveals that among the tested plant extracts, CAE and CPE demonstrated the most potent DPPH radical scavenging activity, with IC_{50} values of 71.5 µg/mL and 178.1 µg/mL, respectively. These IC_{50} values were lower than that of the standard Quercetin, indicating their effectiveness in neutralizing DPPH radicals.

The order of IC_{50} values suggests that CAE and CPE possess stronger DPPH radical scavenging capabilities compared to MKE, ASE and MCE. This variability in antioxidant potential may be attributed to the diverse phytochemical compositions of these plant extracts, as highlighted in earlier sections. The observed DPPH scavenging activity underscores the potential of CAE and CPE as rich sources of natural antioxidants. Further exploration into the specific bioactive compounds responsible for this activity could provide insights into their therapeutic applications. The comparative analysis with Quercetin emphasizes the significance of these plant extracts in the context of natural antioxidant research.

ABTS Radical Scavenging Activity

The ABTS radical scavenging activity of hydroalcoholic extracts from five distinct plant species was evaluated and the results are shown in the Table 4 and Figure 3.

These results reveal that among the tested plant extracts, CAE and CPE displayed the most potent ABTS free radical scavenging activity, with IC_{50} values of 79.37 µg/mL and 81.46 µg/mL, respectively. Notably, the IC_{50} value for standard Quercetin was 1.782 µg/mL, providing a reference point for the efficacy of the plant extracts.

The order of IC_{50} values suggests that CAE and CPE possess superior ABTS radical scavenging capabilities compared to ASE, MKE and MCE. This variation in antioxidant potential may be attributed to the unique phytochemical compositions of these plant extracts. These findings highlight the promising ABTS scavenging activity of CAE and CPE, positioning them as potential sources of natural antioxidants. Further investigation into the specific bioactive compounds responsible for this activity could enhance our understanding of their therapeutic applications. The comparative analysis with Quercetin underscores the significance of these plant extracts in the domain of natural antioxidant research.

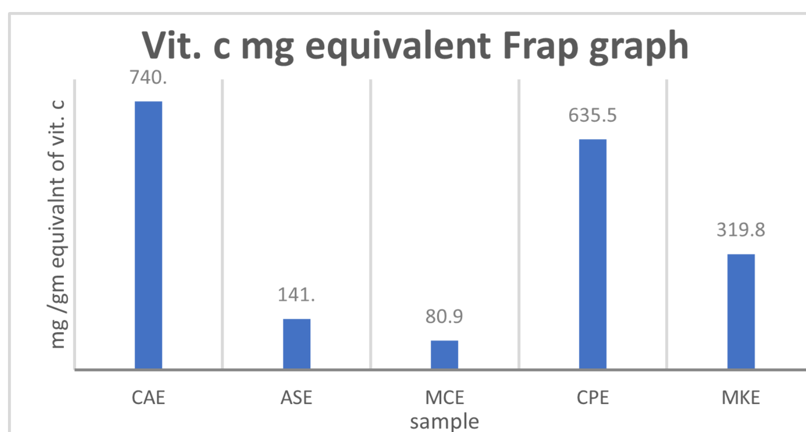

Figure 4: Comparison of Vitamin C Equivalent of hydroalcoholic extracts from five distinct plant.

Table 5: Determination of MIC of 5 hydroalcoholic extracts.

Samples	*E. coli* (µg/100 mL)	*P. auregenosa* (µg/100 mL)	*S. mutans* (µg/100 mL)	*S. aureus* (µg/100 mL)
MKE	350	350	175	700
MCE	350	350	350	700
ASE	262.5	87.5	175	350
CAE	ND	87.5	ND	ND
CPE	ND	87.5	ND	ND

Ferric Reducing Antioxidant Power (FRAP) assay

The Ferric Reducing Antioxidant Power (FRAP) assay was employed to assess the antioxidant potential of hydroalcoholic extracts from five different plant species. The results of FRAP values are shown in the Table 4 and Figure 4 illustrate the comparison of Vitamin C Equivalent of hydroalcoholic extracts from five distinct plant.

Cassia auriculata (CAE) emerged as the most potent antioxidant among the tested extracts, exhibiting a remarkable FRAP activity with a mean value of 740.0±18.6 mg Vitamin C equivalent per gram of dry extract. Following closely, *Celastrus paniculatus* (CPE) displayed substantial antioxidant potential, registering a FRAP value of 635.5±19.8 mg Vitamin C equivalent per gram of dry extract.

Murraya koenigii (MKE) demonstrated moderate antioxidant activity with a FRAP value of 319.8±15.5 mg Vitamin C equivalent per gram of dry extract. In contrast, *Alternanthera sessilis* (ASE) exhibited a lower FRAP value of 141.0±16.1 mg Vitamin C equivalent per gram of dry extract. *Moringa concanensis* (MCE) showed the least antioxidant potency among the studied extracts, with a FRAP value of 80.9±19.1 mg Vitamin C equivalent per gram of dry extract.

These findings highlight the diverse antioxidant capacities of the examined plant extracts, with CAE and CPE standing out as promising sources of natural antioxidants. The variation in FRAP values suggests differences in the phytochemical composition of these plants, emphasizing the need for further exploration into the specific bioactive compounds responsible for their antioxidant activities.

Antimicrobial Activity

The Minimum Inhibitory Concentration (MIC) determination was conducted for all five plant extracts against selected microorganisms and the results are presented in the Table 5.

The results indicate the lowest MIC value for *P. aeruginosa* was observed with CAE, CPE and ASE at a concentration of 87.5µg/100 mL. However, ASE, CAE and CPE did not show detectable activity against *E. coli, S. aureus* and *S. mutans*. Specifically, ASE exhibited activity against all test organisms, with the order of activity as follows: 87.5 µg/100 mL *P. aeruginosa*<175 µg/100 mL *S. mutans* <262.5 µg/100 mL *E. coli*<350 µg/100 mL *S. aureus*. These results underscore the varied antimicrobial potential of the plant extracts against specific pathogens. Further exploration of the bioactive compounds responsible for this activity is warranted.

DISCUSSION

The comprehensive investigation into the antimicrobial and antioxidant activities of *Alternanthera sessilis* R.Br. (ASE), *Cassia auriculata* Linn. (CAE), *Murraya Koenigii* L. Spreng. (MKE), *Celastrus paniculatus* Willd. (CPE) and *Moringa concanensis* Nimmo. (MCE) yielded insightful correlations with their phytochemical compositions,[35] aligning with the study's primary objectives.

Phytochemical screening unveiled a diverse array of compounds within the extracts, ranging from alkaloids, carbohydrates, fixed oils and fats, glycosides, saponins, to tannins and phenolic compounds, phytosterols and triterpenoids, proteins, gums and mucilage's and carotenoids. This chemical diversity formed the basis for subsequent analyses, contributing to a nuanced understanding of the extracts' potential bioactivities.[36-39] The quantitative estimation of Total Phenolic Content (TPC), Total Flavonoid Content (TFC) and Total Tannin Content (TTC) demonstrated significant correlations between specific phytochemicals and antioxidant capacities.[40,41] CAE and CPE, characterized by elevated TPC and TTC, emerged as robust antioxidants. These findings align with the study's objectives to assess antioxidant properties and quantify antioxidant potential. Antioxidant activities, assessed through DPPH and ABTS radical scavenging assays, further emphasized the link between phytochemicals and antioxidant capacities. CAE consistently demonstrated high scavenging activity, substantiating the quantified antioxidant potential using the Ferric Reducing Antioxidant Power (FRAP) assay.

In antimicrobial evaluation, specificity in response was observed, particularly against *Pseudomonas aeruginosa*. The Minimum Inhibitory Concentration (MIC) results, in line with the study's objectives, showcased the antimicrobial potential of CAE, CPE and ASE. However, the absence of detectable activity against certain pathogens highlights the need for further exploration into the specific bioactive compounds responsible for these observed effects.[42,43] The correlations observed between the phytochemical compositions and bioactivities of the extracts underscore the multifaceted nature of these natural compounds.[44] The intricate interplay between different phytochemicals contributes to the overall antioxidant and antimicrobial capacities, providing a solid foundation for understanding the medicinal potential of these plant extracts. This study successfully achieved its objectives by unraveling the correlations between phytochemical compositions and the antimicrobial and antioxidant capacities of the selected plant extracts. The findings not only contribute to the current understanding of these medicinal plant extracts but also pave the way for future research in natural medicine and pharmaceutical development.

CONCLUSION

This study examined the antimicrobial and antioxidant properties of five medicinal plant extracts: *Alternanthera sessilis* (ASE), *Cassia auriculata* Linn. (CAE), *Murraya Koenigii* (MKE), *Celastrus paniculatus* Willd (CPE) and *Moringa concanensis* Nimmo. The research objectives were carefully satisfied, resulting in relevant phytochemical composition-bioactivity correlations. The phytochemical screening showed alkaloids, carbohydrates, fixed oils and fats, glycosides, saponins, tannins and phenolic compounds, phytosterols and triterpenoids, proteins, gums and mucilage's and carotenoids in the extracts. CAE and CPE were shown to have strong antioxidant potential after quantitative assessments of TPC, TFC and TTC. This shows a good link between phytochemicals and antioxidant capacity. CAE's antioxidant activity was confirmed by DPPH and ABTS radical scavenging tests and the FRAP assay. These findings show that these plant extracts neutralise free radicals, suggesting oxidative stress treatment. CAE, CPE and ASE were found to be selective against Pseudomonas aeruginosa by determining its Minimum Inhibitory Concentration (MIC). The extracts' antibacterial activities suggest they might treat certain microbial strains. The findings improve our understanding of these plant extracts' therapeutic potential. The complex relationships between phytochemical compositions and bioactivities demonstrate the complexity of these natural substances. The knowledge acquired from this study lays the groundwork for future research on these important medicinal plants to produce new medicines and natural cures.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

ASE: *Alternanthera sessilis*; **MKE:** *Murraya koenigii*; **CPE:** *Celastrus paniculatus* Willd; **MCE:** *Moringa concanensis* Nimmo; **UV:** Ultraviolet; **FRAP:** Ferric Reducing Antioxidant Power; **ABTS:** 2,2'-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid); **DPPH:** 2,2-Diphenyl-1-picrylhydrazyl; **HCl:** Hydrochloric Acid; **DW:** Distilled Water; **CH_3COONa:** Sodium acetate; **CH_3OOH:** Acetic acid; **TPTZ:** 2,4,6-Tripyridyl-s-triazine; **$FeCl_3·6H_2O$:**

Ferric chloride hexahydrate; **MIC:** Minimum Inhibitory Concentration; **µL:** Microlitre; **mL:** Millilitre.

SUMMARY

The article explores the antioxidant and antimicrobial properties of hydroalcoholic extracts from five medicinal plants: *Alternanthera sessilis*, *Cassia auriculata*, *Murraya koenigii*, *Moringa concanensis*, and *Celastrus paniculatus*. Through phytochemical screening and quantitative assessments of total phenolic, flavonoid, and tannin contents, the study establishes correlations between phytochemical compositions and bioactivities. *Cassia auriculata* and *Celastrus paniculatus* emerge as potent antioxidants, supported by DPPH, ABTS radical scavenging, and FRAP assays. Additionally, the extracts show selective antimicrobial activity against *Pseudomonas aeruginosa*. The findings underline the therapeutic potential of these plant extracts and provide insights for future research in natural medicine and pharmaceutical development.

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