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Original Article

Ethnomedicinal Plants of North-Eastern India: Unveiling Phenolic Composition and Antioxidant Activity for Skin Health

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ABSTRACT

Background: Skin diseases are a major health concern due to their link with oxidative stress, inflammation, tissue damage and impaired skin function. Antioxidants play a crucial role in addressing these issues and offer potential therapeutic benefits. Medicinal plants rich in antioxidants can combat pathogen resistance to pharmaceutical treatments. The primary objective of this study was to explore and analyze the antioxidant activity and composition of both free and bound phenolic compounds in *Nyctanthes arbor-tristis, Hibiscus rosa-sinensis, Lawsonia inermis, Mimosa pudica, Cassia alata* and *Cassia occidentalis*, which are commonly used by the indigenous population of North-Eastern India to treat skin conditions. **Materials and Methods:** The total phenolic content and total flavonoid content were determined using spectrophotometric methods. The assessment of radical scavenging activity was carried out utilizing ABTS and DPPH assays. RP-HPLC was employed to detect and quantify the specific concentrations of individual phenolic components. **Results:** The 80% aqueous ethanol extract of *Nyctanthes arbor-tristis* exhibited high levels of myricetin (22.18±0.33 µg/mg dry extract) and quercetin (18.35±0.53 µg/mg dry extract). Correlation analysis demonstrated strong relationships between total phenolic content, flavonoid content and radical scavenging activities. Furthermore, Principal Component Analysis revealed that *Nyctanthes arbor-tristis* exhibited superior antioxidant potency compared to the other plants studied. **Conclusion:** These findings offer valuable insights into the phenolic composition, antioxidant activity and medicinal potential of these plants. They contribute to a medical guide for their ethnomedicinal use and establish a foundation for conventional medical practices. Understanding their phenolic composition and antioxidant properties could harness their potential in treating skin diseases and addressing oxidative stress, inflammation, tissue damage and impaired skin function.

Keywords: Ethnomedicinal plants, Skin diseases, Antioxidant properties, Phenolics by HPLC.

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INTRODUCTION

Ethnomedicinal plants have been revered for their therapeutic properties and have been widely utilized in traditional medicine to treat various ailments, including skin diseases. These plants possess bioactive compounds that demonstrate antimicrobial, anti-inflammatory and antioxidant properties, which contribute to their effectiveness in managing skin disorders. Embracing the use of ethnomedicinal plants for treating skin diseases not only showcases the wealth of traditional knowledge but also provides a sustainable and natural approach to caring for dermatological conditions.[1]

In the realm of skin diseases, antioxidants play a vital role and hold significant therapeutic potential. These conditions frequently involve heightened oxidative stress and inflammation, which can result in tissue damage and impaired skin functionality. Antioxidants act as powerful agents against oxidative stress by neutralizing detrimental free radicals and alleviating inflammation within the skin. By doing so, they can help mitigate the onset and progression of various skin diseases, including acne, psoriasis, eczema and signs of ageing. The incorporation of antioxidants into skincare routines and diets has the potential to enhance skin health and aid in the management of skin conditions.[2]

As secondary metabolites, phenolic compounds bestow plants with their unique color, taste and aroma. These compounds are extensively acknowledged for their potential antioxidant

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characteristics, playing a vital role in counteracting harmful free radicals and safeguarding the human body against damage induced by oxidative stress.[3] Moreover, phenolic compounds have been linked to various health advantages, including the prevention of chronic ailments like cancer, cardiovascular diseases and neurodegenerative disorders.[4]

For centuries, *Aloe vera*, a succulent plant, has held a prominent place in traditional medicine, particularly in the treatment of diverse skin conditions. It is well-known for its remarkable antioxidant, anti-inflammatory and wound-healing attributes. The gel derived from *Aloe vera* leaves is rich in bioactive compounds, such as polysaccharides, vitamins, minerals and phenolic compounds, all of which contribute to its therapeutic effects on the skin.[5]

Centella asiatica, commonly known as Gotu Kola or Indian pennywort, is an herbaceous plant that has been extensively utilized in traditional medicine systems like Ayurveda and traditional Chinese medicine. This plant exhibits notable antioxidant, anti-inflammatory and wound-healing properties, which make it highly advantageous for addressing diverse skin conditions.[6]

In recent years, there has been an increasing interest in studying the phenolic composition, antioxidant activity and medicinal potential of wild edible plants. These plants hold significant value in traditional medicine, as they provide a wide range of bioactive compounds that can be utilized for therapeutic purposes.

By analyzing the phenolic content using RP-HPLC and quantifying the total phenolic and flavonoid contents using spectrophotometric methods, we aim to provide insights into the bioactive profile of these plants. Additionally, the assessment of antioxidant activity using ABTS and DPPH assays will help evaluate their potential as natural antioxidants.

Exploring the phenolic composition, antioxidant activity and medicinal potential of these ethnomedicinal plants holds the potential to integrate them into mainstream healthcare systems. This research can establish a solid foundation for their incorporation in the prevention and treatment of various ailments, offering new possibilities for traditional medicine in modern healthcare practises. Additionally, this research opens avenues for the development of innovative phytopharmaceuticals and functional food products enriched with these bioactive compounds. Such advancements have the potential to bring about a new era of health-promoting interventions and products with enhanced therapeutic benefits.

This study aims to explore the phenolic composition and antioxidant activity of six ethnomedicinal plants of significant medicinal value. These plants, namely *Nyctanthes arbor-tristis, Hibiscus rosa-sinensis, Lawsonia inermis, Mimosa pudica, Cassia alata* and *Cassia occidentalis*, are traditionally used by the ethnic population of North-eastern India for treating various skin conditions.

Nyctanthes arbor-tristis L., belonging to the family Nyctaginaceae, has been traditionally used by the indigenous population of North-eastern India for treating ringworms. The fresh leaves of this plant are boiled in mustard oil and applied externally to the affected area.[7]

Hibiscus rosa-sinensis L., from the family Malvaceae, is known for its traditional use in treating dandruff. The method involves mixing 10-15 leaves with coconut oil and cooking them on a low flame until the leaves are completely cooked. The oil is then drained and ready for use.[8]

Lawsonia inermis L., a plant from the family Lythraceae, has ethnomedicinal importance in preventing dandruff formation. The leaves are soaked in water overnight and then transformed into a paste. This paste is applied to the hair and scalp, serving as a remedy for dandruff. It is also applied topically for skin conditions. The local application of the leaf paste has shown efficacy in treating scabies.[9]

Mimosa pudica L., from the family Mimosaceae, is used in traditional medicine for its beneficial effects on skin cuts and wounds. The leaves of this plant are ground into a paste and applied locally to the infected regions of the skin.[10]

Cassia alata L., from the family Fabaceae, is employed for its therapeutic properties against ringworm. The leaf paste is applied to the affected area until the condition is cured, or leaf juice is directly applied to the affected region.[11]

Cassia occidentalis L., from the family Caesalpinaceae, is known for its traditional use in treating skin diseases. The leaves and roots of this plant are utilized for their medicinal properties.[12]

MATERIALS AND METHODS

Plant materials

The investigated plant materials, namely *Nyctanthes arbor-tristis* L. (Oleaceae), *Hibiscus rosa-sinensis* L. (Malvaceae), *Lawsonia inermis* L. (Lythraceae), *Mimosa pudica* L. (Leguminosae), *Cassia alata* (L.) Roxb (Leguminosae) and *Cassia occidentalis* (L.) Link (Leguminosae), were obtained from several markets in Assam, India and the identifications were authenticated in our office. The collected plant materials were kept in our laboratory under registry numbers PS 01, PS 02, PS 03, PS 05, PS 08 and PS 10, respectively. Plant materials were shed-dried, crushed and stored in an airtight container for subsequent extraction.

Preparation of plant extracts

The procedure involved the extraction of 100 g of powdered plant material using 80% aqueous ethanol. Each extraction was carried out twice at room temperature, with agitation for duration of 18-24 hr. The resulting extracts from the initial and

subsequent extractions were combined and concentrated using a rotary evaporator under reduced pressure, resulting in the formation of thick extracts. These extracts were then subjected to freeze-drying for dehydration. All dried extracts obtained from each solvent were kept at minus (-) 20°C. The percentage (%) yield was estimated using the weight of the air-dried plant material.

Total Phenolic Content (TPC)

To determine the total phenolic content in the crude extracts, the Folin-Ciocalteu method developed by Singleton and Rossi in 1965 was employed.[13] In this method, 100 µL of each examined extract was mixed with 1.0 mL of Folin-Ciocalteu reagent and 0.8 mL of 7.5% sodium carbonate in test tubes. The reaction mixture was allowed to stand for 30 min, after which the absorbance at 765 nm was measured using a Shimadzu UV 1800 UV-visible spectrophotometer. Subsequently, the total phenolic content within the plant material was determined in milligrams per 100 g (mg GAE/100 g), employing the equation y=0.0013x+0.0498, where y denoted the absorbance and x represented the gallic acid equivalent measured in mg/100 g. The coefficient of determination (R^2) for the equation was 0.999.

Total Flavonoids Content (TFC)

The total flavonoid content in the investigated plants was determined using the method described by Ordonez *et al.*, in 2006.[14] For this analysis, 0.5 mL of a 2% $AlCl_3$ ethanol solution was added to 0.5 mL of the extracts in a test tube. After allowing the mixture to stand at room temperature for one hour, the absorbance was measured at 420 nm using a Shimadzu UV 1800 UV-visible spectrophotometer. The detection of flavonoids was established through the appearance of a yellow colour within the solution. For quantification of total flavonoid levels in terms of rutin equivalents (mg/100 g), the subsequent equation was employed, derived from the calibration curve, y=0.0182x-0.0222, where an R^2 value of 0.9962 was attained. Within this formula, y signifies the absorbance and x signifies the quantity of Rutin equivalent measured in mg/100 g.

Total Flavonols Content (TFLC)

The quantification of total flavonols in the plant extracts was carried out using the method established by Kumaran and Karunakaran in 2006.[15] In this approach, a test tube was used to mix 2.0 mL of a 2% $AlCl_3$ ethanol solution and 3.0 mL of a sodium acetate solution (50 g/L) with 2.0 mL of the extracts. The solution was left to stand at a controlled temperature of 20°C for duration of 2.5 hr. Subsequently, the absorbance of the solution was measured at 440 nm using a Shimadzu UV 1800 UV-visible spectrophotometer. The total flavonol content in terms of quercetin equivalents (mg/100 g) was calculated using the following equation derived from the calibration curve: y=0.0049x+0.0047. The coefficient of determination (R^2) for the equation was determined to be 0.9935. In the equation, y represents the absorbance and x denotes the quercetin equivalent in mg/100 g.

Reducing Power (RP)

To evaluate the reducing power of the plant extracts, Oyaizu's method was employed.[16] In this method, 100 µL of the plant extracts were mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and an equal volume of 1% potassium ferricyanide. The reaction mixture was incubated at 50°C for 20 min. Afterward, 2.5 mL aliquots of 10% trichloroacetic acid were added and the mixture was centrifuged at 3000 rpm for 10 min.

The upper layer of the solution (2.5 mL) was mixed with an equal volume of distilled water and then 0.5 mL of a freshly prepared 0.1% ferric chloride solution was added. The absorbance of the resulting reaction mixture was measured at a wavelength of 700 nm using a spectrophotometer.

Based on the calibration curve, the following equation was used to determine the reducing power in terms of Ascorbic Acid Equivalents (AAE), y=0.0023x-0.0063. For this equation, the coefficient of determination (R^2) was found to be 0.9955. In the equation, y stands for the absorbance at 700 nm and x represents for the ascorbic acid equivalent in mg/100 g.

DPPH free radical scavenging activity

To evaluate the free radical scavenging activity of the plant extracts, the stable radical DPPH (1,1-diphenyl-2-picrylhydrazyl) was employed, following the method described by Blois in 1958.[17] The method entails mixing 100 µL of the tested extracts with 3.9 mL of freshly prepared DPPH solution (25 mg/L) in methanol, followed by stirring the mixture. After a 30-min incubation period, the absorbance was measured at 517 nm using a Shimadzu UV 1800 UV-visible spectrophotometer.

The ability of the extracts to scavenge the DPPH radical was calculated using the following equation:

$$\text{Scavenging ability of DPPH (\%)} = \{(Ac-At)/Ac\} \times 100$$

Here, Ac represents the absorbance of the control reaction (without extracts) and at represents the absorbance in the presence of the extract sample.

The antioxidant potential of the extracts was quantified as IC_{50}, representing the amount in milligrams of dried extract necessary to inhibit 50% of DPPH radical generation. The IC_{50} parameter was ascertained by analyzing the concentration of the extract at which a 50% reduction in absorbance was observed.

ABTS radical scavenging activity

The method described by Re *et al.*, in 1999 was employed to determine the radical scavenging activity of the 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation (ABTS.+).[18]

In this procedure, ABTS was dissolved in water, resulting in a concentration of 7 mM. To initiate the formation of ABTS radicals, a final concentration of 2.45 mM potassium persulfate was introduced. This radical generation process took place in darkness at room temperature over a period of 12-16 hr. To achieve the desired consistency, the absorbance of the solution was calibrated to 0.70±0.02 at 734 nm by appropriately diluting it with ethanol.

For the assessment of scavenging activity, a combination of 1 mL of the diluted ABTS+ solution and 100 µL of the plant extract was prepared. Subsequently, the absorbance at 734 nm was determined after a 15 min interval using a spectrophotometer.

The percentage of inhibition, signifying the extent of ABTS scavenged, was calculated through the utilization of the subsequent equation:

$$\text{ABTS scavenged (\%)} = (Ac - At)/Ac \times 100$$

In the equation, Ac represents the absorbance of the control reaction (without extracts) and At represents the absorbance in the presence of the test extracts.

By comparing the absorbencies of the control and test extracts, the percentage of inhibition was determined, indicating the scavenging activity of the plant extract against the ABTS radical cation.

Quantification of phenolic acids and flavonoids using HPLC

HPLC equipment

HPLC analyses were conducted using a Dionex Ultimate 3000 liquid chromatograph equipped with a Diode Array Detector (DAD) and a 5 cm flow cell. The Chromeleon system manager was employed as the data processing software. Separation of the samples was achieved using a reversed-phase Acclaim C18 column with a molecular size of 5 microns and dimensions of 250×4.6 mm. A volume of 20 µL of the test sample was introduced into the HPLC column.

Standard Solutions

For the preparation of a stock solution at a concentration of 1 mg/mL, the standard phenolic acids (gallic acid, protocatechuic acid, gentisic acid, chlorogenic acid, p-hydroxy benzoic acid, vanillic acid, caffeic acid, syringic acid, p-coumaric acid, ferulic acid, sinapic acid, salicylic acid and ellagic acid) and flavonoids (catechin, rutin, myricetin, quercetin, naringin, naringenin, apigenin and kaempferol) were meticulously dissolved in methanol. Subsequently, by blending the standard solution with the mobile phase solvent system, the working solutions were prepared. Prior to their introduction into the HPLC apparatus, both the standard and working solutions underwent filtration using a 0.45 µM PVDF-syring.

Estimation of phenolic acids and flavonoids by HPLC

An 80% aqueous ethanol extract of the plant material was subjected to High-Performance Liquid Chromatography (HPLC) analysis to quantify the levels of phenolic acids and flavonoids. The analysis method employed was based on the procedure outlined by Datta *et al.*[19] The equipment used included a Dionex Ultimate 3000 liquid chromatograph equipped with a Diode Array Detector (DAD) featuring a 5 cm flow cell. Data processing was managed through the Chromeleon system manager.

For separation, a reversed-phase Acclaim C18 column with dimensions 250×4.6 mm and particle size of 5 microns was utilized. The HPLC column was loaded with a 20 µL sample volume. The methodology adhered to the validation criteria set by the United States Pharmacopeia (USP) and the International Conference on Harmonization (ICH).

The mobile phase consisted of two components: methanol (Solvent A) and a 0.5% aqueous acetic acid solution (Solvent B). The column temperature was maintained at 25°C throughout the analysis and each injection involved an injection volume of 20 µL. To achieve a gradient elution, the ratio of Solvent A to Solvent B was adjusted. The entire analytical process for each sample had a duration of 105 min.

Detection of HPLC chromatograms occurred using a photo diode array UV detector, employing three distinct wavelengths: 272 nm, 280 nm and 310 nm. The retention times of individual chemical compounds were established by introducing standard solutions under equivalent conditions. Quantification of phenolic acids and flavonoids within the extracts was carried out by measuring the integrated peak areas. The concentrations were determined through the application of a calibration curve, which correlated the peak area to the concentration of the corresponding standard reference.

The analytical data were presented in triplicate, indicating that each analysis was repeated three times to ensure consistency. A convergence limit was applied to ensure the accuracy and reliability of the measurements.

Statistical analysis

The dataset was subjected to analysis using triplicate samples and the results were presented as the mean accompanied by the Standard Error of the Mean (SEM). To assess variations and ascertain similarities among plants concerning Total Phenolic Content (TPC), Total Flavonoid Content (TFC), Total Flavonol Content (TFLC), radical scavenging activities, as well as phenolic acid and flavonoid contents, a series of statistical methods were employed. Initially, a one-way Analysis of Variance (ANOVA) was conducted, followed by the Tukey test (with a significance level set at $p \leq 0.05$). This enabled the identification of statistically significant differences and the grouping of plants displaying similar attributes. Furthermore, correlation analyses were performed

to explore the relationships between various parameters. The significance level was set at $p<0.05$. Both correlation coefficient (r) and coefficient of determination (R2) were employed to measure the strength and direction of correlations. To comprehensively assess the interplay among variables and to potentially identify patterns, Principal Component Analysis (PCA) was executed. This technique helps reduce the dimensionality of the data while retaining crucial information, allowing for the visualization of trends and similarities within the dataset.

All statistical analyses were conducted using SPSS software, specifically version 11.0 for Windows. This software facilitated the systematic exploration and interpretation of the complex relationships and trends present within the data.

RESULTS AND DISCUSSION

Total phenolic, flavonoid and flavonol content

The study conducted on the 80% aqueous ethanol extracts from six ethnomedicinal plants revealed a substantial variation in the overall phenolic content. The recorded values ranged from 15.88±1.48 mg GAE/100 g of Dry Plant Material (DPM) to 142.77±2.05 mg GAE/100 g DPM, as outlined in Table 1. Among the evaluated extracts, the highest concentration of phenolic content was detected in the 80% aqueous ethanol extract of *N. arbor-tristis*, measuring 142.77±2.05 mg GAE/100 g DPM. A significant quantity of phenolic compounds was also evident in the 80% aqueous ethanol extract of *L. inermis*, with a content of 121.62±2.67 mg GAE/100 g DPM. In contrast, *M. pudica* exhibited the lowest phenolic content, recording a concentration of 15.88±1.48 mg GAE/100 g DPM. Additionally, a substantial presence of phenolic compounds was identified in the 80% aqueous ethanol extract of *C. occidentalis*.

The considerable disparity noted in the total phenolic content among these wild plants implies the existence of a wide array of phenolic compounds across distinct plant species. Phenolic compounds are well-known for their antioxidant properties and have been associated with potential health benefits, including the prevention of chronic diseases such as cardiovascular disorders, cancer and neurodegenerative conditions.[20,21] Thus, the variation in phenolic content among these extracts implies that certain wild plants, like *N. arbor-tristis* and *L. inermis*, might possess higher antioxidant and health-promoting potential compared to others, such as *M. pudica*.

These findings highlight the importance of exploring and studying the phenolic content of wild plants, as they can serve as valuable sources of natural antioxidants and bioactive compounds. Further research could focus on identifying the specific phenolic compounds present in these extracts and investigating their individual contributions to the observed antioxidant activity. Moreover, exploring the potential health benefits and therapeutic applications of these wild plant extracts, rich in phenolic content, could provide valuable insights for the development of novel natural remedies or functional foods.[22]

The analysis of the extracts showed a significant variation in flavonoid concentration, measured in rutin equivalent, ranging from 2.64±0.44 to 17.49±1.06 mg/100 g DPM (Table 1).

Among the extracts, the 80% aqueous ethanol extract of *N. arbor-tristis* exhibited the highest flavonoid content, measuring 116.56±1.16 mg/100 g DPM. Conversely, *M. pudica* displayed the lowest amount of flavonoids, with a concentration of 2.64±0.44 mg/100 g DPM. Furthermore, appreciable amounts of flavonoids were detected in the other four plants under examination, namely *L. inermis, C. occidentalis, C. alata* and *H. rosa-sinensis*, indicating a similarly high presence of these compounds.

Table 1: Antioxidant activities of ethnomedicinal plants.

Antioxidant parameters	*N. arbor-tristis*	*H. rosa-sinensis*	*L. inermis*	*M. pudica*	*C. alata*	*C. occidentalis*
Extractive value(g/100 g).	6.5±0.78c	9.0±0.63b	16.1±1.24a	3.5±0.58e	4.5±0.89d	16.2±1.39a
Total phenolic content (GAE, mg/100 g DPM).	142.77±2.55a	28.54±1.23e	121.62±1.18b	15.88±2.90f	54.69±1.46d	74.56±1.62c
Total flavonoids content (RE, mg/100 g DPM.)	17.49±2.35a	5.36±1.39e	15.05±1.94b	2.64±0.75f	9.09±0.34d	13.35±1.08c
Total flavonol content (QE, mg/100 g DPM).	40.88±2.49a	8.43±3.45d	31.96±1.43b	4.73±1.57e	20.98±2.16c	30.77±3.05b
Reducing power (AAE, mg/100 g DPM).	75.50±4.39a	20.72±2.45e	64.56±1.43b	9.21±1.57f	43.39±3.16d	50.50±2.65c
DPPH(% of inhibition).	60.05±3.07 a	22.15±1.12 e	47.88±2.33 c	14.59±2.12 f	40.37±1.17 d	43.62±2.29 b
ABTS(% of inhibition)	55.92±3.42a	23.34±2.14f	43.67±1.35c	25.68±2.59e	40.61±1.28d	46.28±4.06b

Each entry in the table was derived from the calculation of the mean of three separate experiments and the data are expressed as Mean±Standard Error of the Mean (SEM). Statistical analyses were performed using Tukey's test at a 95% confidence level and statistical significance was acknowledged at the $p<0.05$ threshold. The subscript letters a, b, c, d, e and f are used to indicate significant differences within the same parameter among the various plants.

Flavonoids are a diverse class of compounds with well-documented antioxidant and health-promoting properties. They have been associated with various biological activities, including anti-inflammatory, anticancer and cardiovascular benefits. The significant variation in flavonoid concentrations among the examined plant extracts suggests differences in the composition and content of specific flavonoid compounds within each plant species.[23,24]

The high flavonoid content observed in the 80% aqueous ethanol extract of *N. arbor-tristis* (17.49±2.35 mg/100 g DPM) indicates its potential as a valuable source of flavonoids.

Similarly, the appreciable flavonoid amounts (mg/100 g DPM) found in *L. inermis* (15.05±1.94), *C. occidentalis* (13.35±1.08), *C. alata* (9.09±0.34) and *H. rosa-sinensis* (5.36±1.39) highlight the potential health benefits associated with these plant species. The presence of significant flavonoid concentrations in these extracts further supports their potential as natural antioxidants and suggests their possible contribution to the observed biological activities.[24]

Further research can focus on identifying and characterizing the specific flavonoid compounds present in these extracts, as well as evaluating their individual bioactivity and potential synergistic effects. This knowledge can contribute to the development of functional foods, nutraceuticals, or natural remedies targeting various health conditions.

The concentration of flavonols in the 80% aqueous ethanol extracts of the plant materials was quantified using quercetin equivalents, as depicted in Table 1. Among the extracts, *N. arbor-tristis* exhibited a flavonol concentration of 40.88±2.49, followed by *C. occidentalis* with 30.77±3.05, *L. inermis* with 31.96±1.43, *C. alata* with 20.98±2.16, *H. rosa-sinensis* with 8.43±3.45 and *M. pudica* with 4.73±1.57.

Flavonols are a subclass of flavonoids known for their antioxidant and health-promoting properties. Quercetin, a well-studied flavonol, is often used as a reference compound to quantify the flavonol content in plant extracts. The variation in flavonol concentrations among the different plant materials indicates differences in their flavonol profiles and potential health benefits.

Table 2: Phenolic acids and flavonoid content in Ethnomedicinal plants.

	mg/100 gm plant material					
Phenolic acids/ Flavonoids	***N. arbor-tristis***	***H. rosa-sinensis***	***L. inermis***	***M. pudica***	***C. alata***	***C. occidentalis***
Gallic acid	ND	0.24±0.04^{b}	0.45±0.08^{a}	ND	ND	ND
Protocatechuic acid	0.34±0.08^{a}	ND	0.10±0.003^{b}	ND	ND	ND
Gentisic acid	ND	ND	ND	ND	ND	ND
p-Hydroxy benzoic acid	0.093±0.004^{d}	ND	0.10±0.003^{c}	0.012±0.002^{e}	0.76±0.09^{a}	0.35±0.02^{b}
Catechin	ND	ND	40.44±1.06^{a}	ND	9.21±0.86^{b}	ND
Chlorogenic acid	ND	0.91±0.08^{b}	21.24±1.11^{a}	ND	ND	0.37±0.09^{c}
Vanillic acid	2.34±0.77^{a}	ND	2.95±0.92^{a}	ND	ND	ND
Caffeic acid	ND	1.25±0.67^{a}	0.24±0.07^{b}	ND	0.13±0.02^{c}	ND
Syringic acid	0.39±0.05^{bc}	0.43±0.08^{b}	1.39±0.52^{a}	0.31±0.02^{c}	0.16±0.08^{d}	0.025±0.003^{e}
p-Coumaric acid	2.70±0.48^{a}	2.16±0.59^{b}	2.06±0.81^{b}	1.07±0.09^{c}	0.16±0.03^{d}	1.07±0.44^{c}
Ferulic acid	3.42±0.29^{b}	3.87±0.47^{a}	1.6±0.22^{c}	ND	0.33±0.05^{d}	0.34±0.05^{d}
Sinapic acid	0.65±0.05^{c}	0.52±0.02^{d}	1.39±0.19^{b}	ND	0.042±0.002^{e}	3.81±0.72^{a}
Salicylic acid	ND	ND	ND	ND	ND	ND
Naringin	0.48±0.07^{c}	0.28±0.02^{d}	1.43±0.21^{b}	ND	2.78±0.39^{a}	0.19±0.07^{e}
Rutin	2.49±0.09^{b}	0.31±0.02^{d}	1.89±0.34^{c}	ND	ND	6.20±0.88^{a}
Ellagic acid	2.38±0.67^{b}	1.65±0.28^{c}	5.05±0.59^{a}	0.046±0.003^{f}	0.066±0.002^{e}	0.28±0.07^{d}
Myricetin	22.18±1.36^{a}	1.94±0.77^{c}	21.71±1.89^{a}	0.108±0.06^{d}	0.066±0.001^{e}	4.72±0.82^{b}
Quercetin	18.35±1.79^{b}	0.99±0.56^{c}	0.45±0.09^{d}	ND	0.033±0.001^{e}	30.77±2.78^{a}
Naringenin	ND	ND	ND	ND	ND	1.28±0.28^{a}
Apigenin	3.47±0.86^{a}	0.85±0.02^{b}	0.33±0.06^{c}	0.099±0.008^{d}	ND	0.28±0.042^{c}
Kaempferol	2.03±0.29^{a}	ND	0.69±0.07^{b}	0.05±0.003^{d}	ND	0.62±0.06^{c}

ND: Not detected. Each value in the table was derived from averaging three separate experiments and the data are presented as Mean±Standard Error of the Mean (SEM). Statistical analysis was performed using Tukey's test at a 95% confidence level and statistical significance was recognized at the $p<0.05$ threshold. The superscript letters a, b, c, d, e and f are used to indicate significant differences within the same parameter among the various plants.

N. arbor-tristis demonstrated the highest flavonol concentration among the examined plant materials. This finding suggests that *N. arbor-tristis* may serve as a valuable source of flavonols, including quercetin, which is associated with various biological activities, such as anti-inflammatory and antioxidant effects.

L. inermis, C. occidentalis and *C. alata* also exhibited significant flavonol concentrations, indicating their potential as sources of flavonols with health-promoting properties. These plant materials may contribute to the antioxidant and other beneficial effects attributed to flavonols in various health conditions.

H. rosa-sinensis displayed a relatively lower flavonol concentration compared to other plants in the study. However, it is worth noting that even at a lower concentration, *H. rosa-sinensis* may still provide some amount of flavonols that contribute to its overall antioxidant activity and potential health benefits.

M. pudica had the lowest flavonol concentration among the examined plant materials. While its flavonol content is relatively lower, other phytochemicals present in *M. pudica* may still contribute to its potential health benefits and biological activities.

Overall, the variation in flavonol concentrations among these plant materials underscores the importance of considering the specific plant species and their phytochemical composition when evaluating their potential health effects.[25] Further research can focus on identifying the specific flavonols present in these extracts and elucidating their individual contributions to the observed biological activities.

Phenolic compounds have been found to be abundant in plants and possess remarkable antioxidant activity due to their redox properties. These compounds, known as antioxidants, have the ability to bind to free radicals and counteract their harmful effects.[26] Within the array of natural constituents present in plants, flavonoids and flavonols stand out as frequently encountered compounds. These substances have demonstrated their ability to display antioxidant activity through mechanisms like scavenging or chelation.[27] The obtained results unmistakably underscore the noteworthy contribution of phenolic compounds to the constitution of these plants. Additional phenolic compounds containing hydroxyl groups, notably flavonoids and flavonols, play a pivotal role in facilitating the plants' radical scavenging capabilities. The inclusion of these compounds significantly enhances the overall antioxidant potency of the plants.[26,27]

The strong radical scavenging ability observed in *N. arbor-tristis, H. rosa-sinensis, L. inermis, M. pudica, C. alata* and *C. occidentalis* can be attributed to the presence of phenolic compounds. These plants contain a significant amount of phenolic compounds, which contribute to their potent antioxidant properties and ability to neutralize free radicals. Phenolic compounds have been widely recognized for their ability to scavenge and eliminate harmful radicals, thus protecting cells from oxidative damage.[26,27] Therefore, the abundance of phenolic compounds in these plants likely plays a crucial role in their strong radical scavenging ability.

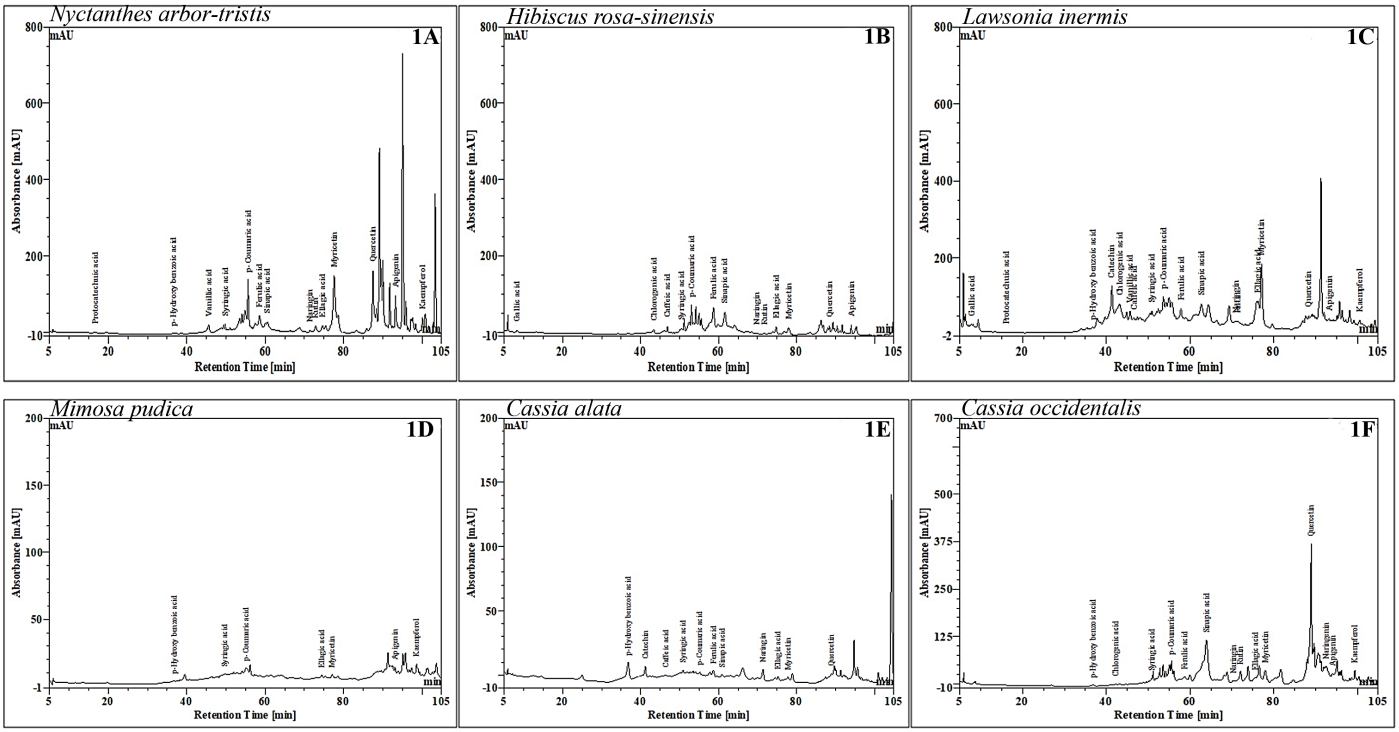

Figure 1: HPLC chromatogram of the 80% aqueous ethanol extract of *Nyctanthes arbor-tristis* (1A), *Hibiscus rosa-sinensis* (1B), *Lawsonia inermis* (1C), *Mimosa pudica* (1D), *Cassia alata* (1E), *Cassia occidentalis* (1F).

Reducing power

The study conducted an analysis of six ethnomedicinal plants, namely *N. arbor-tristis, H. rosa-sinensis, L. inermis, M. pudica, C. alata* and *C. occidentalis*. The researchers evaluated the reduction capabilities of these plants using the metric of mg AAE/100 g DPM. The reduction capabilities were assessed using the 80% aqueous ethanol extracts derived from these plants.

The results indicated that the 80% aqueous ethanol extract of *N. arbor-tristis* demonstrated the highest potential reducing power, with a value of 75.50±4.39 mg AAE/100 g DPM. This suggests that *N. arbor-tristis* possesses a significant capacity for reducing certain compounds. On the other hand, *H. rosa-sinensis* exhibited a relatively lower reducing power of 20.72±2.45 mg AAE/100 g DPM. The reduction capabilities of the other plants were as follows: *L. inermis* (64.56±1.43 mg AAE/100 g DPM), *M. pudica* (9.21±1.57 mg AAE/100 g DPM), *C. alata* (43.39±3.16 mg AAE/100 g DPM) and *C. occidentalis* (50.50±2.65 mg AAE/100 g DPM). These values indicate the extent to which the respective plant extracts can facilitate reduction reactions.

It is important to note that the reducing power of a plant extract is often associated with its antioxidant activity. Antioxidants play a crucial role in neutralizing harmful free radicals and protecting cells from oxidative damage. Therefore, the observed reducing power of these plant extracts suggests their potential to scavenge free radicals and exert antioxidant effects. In this assay, the extracts were evaluated for their reducing capacity as a measure of their antioxidant potential. The presence of antioxidants in the extracts facilitated the reduction of the Fe^{3+}/ferricyanide complex to its ferrous form. The capacity of the extracts to reduce the complex indicates their potential antioxidant effects.

The reducing capacity of the extracts can be considered a marker or indicator of their ability to act as antioxidants. By donating hydrogen atoms, these extracts may break the free radical chain, thus neutralizing and scavenging free radicals. This process is essential for protecting cells from oxidative damage and maintaining their overall health.[28]

DPPH radical scavenging activity

The anti-radical abilities of six ethnomedicinal plants were assessed using the DPPH radical scavenging test, which is a widely used method to evaluate antioxidant activity. This method provides a simple, rapid and sensitive way to measure the antioxidant potential of chemical compounds or plant extracts.[29]

In the DPPH radical scavenging test, the antioxidant effect is determined by the reduction of the purple color of DPPH (2,2-diphenyl-1-picrylhydrazyl) in the presence of test samples. Antioxidant compounds possess the capability to contribute hydrogen atoms or electrons, thereby neutralizing DPPH free radicals and leading to the creation of a colorless and stable molecule known as 2,2-diphenyl-1-hydrazine. The decrease in absorbance observed at 517 nm signifies the scavenging activity of the samples under examination.

The present investigation involves the assessment of radical scavenging activities exhibited by the 80% aqueous ethanol extract derived from a diverse range of ethnomedicinal plants. The results showed that *N. arbor-tristis* exhibited the highest radical scavenging activity, with a percentage inhibition of 60.05±3.07. On the other hand, *M. pudica* leaves demonstrated the lowest activity, with a percentage inhibition of 14.59±2.12.

Additionally, other edible plants such as *L. inermis* (47.88±2.33%), *C. occidentalis* (43.62±2.29%) and *C. alata* (40.37±1.17%) also showed significant inhibition of the DPPH radical in the study.

The observed radical scavenging activity of these plant extracts can be attributed to the presence of hydroxyl groups, which have the potential to act as radical scavengers. By donating hydrogen atoms or electrons to the DPPH radical, these hydroxyl groups effectively neutralize and reduce the radical's reactivity, thus exhibiting strong antioxidant properties.

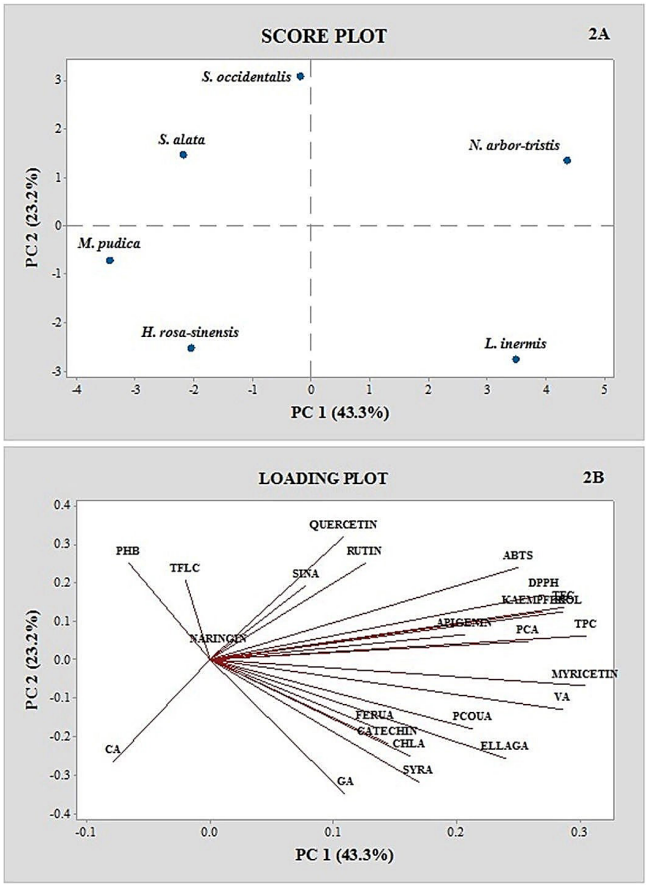

Figure 2: Principal Component Analysis utilizing the variables outlined in Tables 1 and 2. The score plot (2A) and loading plot (2B) display the initial two principal components for the clustering of plant samples. The variables taken into account include 18 parameters (TPC, TFC, TFLC, RP, DPPH, ABTS, MC, LP, Gallic acid, Protocatechuic acid, Syringic acid, p-Coumaric acid, Ferulic acid, Catechin, Rutin, Myricetin, Apigenin, Kaempferol).

The findings highlight the potential of these ethnomedicinal plants as natural sources of antioxidants. Incorporating these plants into the diet or utilizing their extracts in various applications may provide health benefits by combating oxidative stress and protecting against free radical-induced damage.

Further discussions and analyses, including the specific mechanisms of action and the identification of active compounds responsible for the observed antioxidant activity, may be available in the reference provided or other related studies.

ABTS radical scavenging activity

Table 1 presents the results of the ABTS scavenging activity test conducted on diverse extracts of 6 ethnomedicinal plants. The ABTS test measures the ability of the extracts to eliminate the color of ABTS, which is indicative of their antioxidant action. The higher the radical scavenging activity, the greater the inhibition percentage observed.

In the study, the 80% aqueous ethanol extract of *N. arbor-tristis* exhibited the highest radical scavenging activity, with a percentage inhibition of 55.92±3.42. Following *N. arbor-tristis, C. occidentalis* demonstrated significant radical scavenging activity with a percentage inhibition of 46.28±4.06. *L. inermis* also displayed notable scavenging activity, with a percentage inhibition of 43.67±1.35.

The remaining investigated plants, namely *C. alata, M. pudica* and *H. rosa-sinensis*, showed potential ABTS radical scavenging activities. Although specific percentage inhibition values for these plants were not provided in the information provided, their inclusion in the study suggests that they exhibited antioxidant properties to some extent.

These results underscore the antioxidative capacity of the examined indigenous plants, as evaluated using the ABTS scavenging activity assay. The ability of the extracts to scavenge the ABTS radical indicates their capacity to neutralize free radicals and protect against oxidative stress.[30]

Estimation of phenolic acids and flavonoids in plant materials under study by RP-HPLC

Table 2 outlines comprehensive information concerning the levels of phenolic acids and flavonoids present in the 80% aqueous ethanol extracts derived from the plants subject to this study. The analysis was conducted using High-Performance Liquid Chromatography (HPLC) and the quantities are expressed as µg/mg of dry extract. The HPLC chromatogram of the 80% aqueous ethanol extract of all investigated plants has been presented in Figure 1A, 1B, 1C, 1D, 1E and 1F.

The extracts contain a range of phenolic acids, namely gallic acid, protocatechuic acid, gentisic acid, chlorogenic acid, p-hydroxybenzoic acid, vanillic acid, caffeic acid, syringic acid, p-coumaric acid, ferulic acid, sinapic acid, salicylic acid and ellagic acid. These phenolic acids are known for their various bioactive properties and have been extensively studied for their potential health benefits.

The flavonoids detected in the extracts include catechin, rutin, myricetin, quercetin, naringin, naringenin, apigenin and kaempferol. Flavonoids are a class of plant compounds with antioxidant, anti-inflammatory and other beneficial effects on human health.

The HPLC analysis allowed for the successful separation and quantification of these phenolic acids and flavonoids. The sensitivity of the compounds at 280 nm enabled their detection and differentiation in the extracts. The obtained absorption spectra were contrasted with those of standard reference materials in order to detect and verify the presence of these particular compounds.

The information provided in Table 2 gives insight into the phenolic acid and flavonoid profiles of the investigated plants.

Table 3: Correlation between TPC and DPPH, TPC and ABTS, TFC and DPPH, TFC and ABTS, TFLC and DPPH, TFLC and ABTS.

	TPC X DPPH			TPC X ABTS		
Solvent	**r**	**R^2**	**Equation**	**r**	**R^2**	**Equation**
80% aq. ethanol	0.944	0.891	y=0.3143x+15.165	0.897	0.805	y=0.2222x+23.032
	TFC X DPPH			TFC X ABTS		
	r	R^2	Equation	r	R^2	Equation
80% aq. ethanol	0.977	0.955	y=2.8426x+8.2619	0.944	0.893	y=2.0443x+17.788
	TFLC X DPPH			TFLC X ABTS		
	r	R^2	Equation	r	R^2	Equation
80% aq. ethanol	0.985	0.971	y=1.1679x+11.296	0.975	0.951	y=0.8601x+19.506

TPC: Total phenolic content; **TFC:** Total flavonoid content; **TFLC:** Total flavonol content.

The presence and quantities of these compounds contribute to the overall chemical composition of the extracts and may be associated with the observed antioxidant and health-promoting activities of the plants.

Further details on the specific quantities of each phenolic acid and flavonoid in the extracts, as well as their potential biological activities and implications, could be explored in the reference or related studies associated with Table 2.

The presence of gallic acid, either in its free form or as an ester, in plants contributes to their antioxidant activity. The HPLC investigation revealed varying quantities of gallic acid within the analyzed plants. Especially, *L. inermis* exhibited a significant content of gallic acid (0.45±0.08 µg/mg dry extract), whereas *H. rosa-sinensis* showcased the lowest level (0.24±0.04 µg/mg dry extract). The gallic acid levels found in the 80% aqueous ethanol extracts of *L. inermis* and *H. rosa-sinensis* were comparable to those present in commonly consumed vegetables like spinach (1.82 µg/mg), lemon (2.03 µg/mg), onion bulb (1.55 µg/mg), chilli pepper (3.33 µg/mg) and cabbage (0.49 µg/mg).[31]

The presence of gallic acid in these wild food plants, especially in considerable amounts, highlights their potential as natural sources of this antioxidant compound. Gallic acid is known for its ability to scavenge free radicals and protect against oxidative stress, which is associated with various health benefits.

Comparing the levels of gallic acid in the investigated plants with those found in common vegetables provides context for their relative abundance of this compound. The quantities found in *L. inermis* and *H. rosa-sinensis* suggest that these plants can contribute a significant amount of gallic acid to the diet, similar to or in some cases even surpassing the levels found in commonly consumed vegetables.

The information presented in the HPLC study and the comparison with known vegetable sources of gallic acid indicate the potential nutritional value and antioxidant capacity of the investigated plants. Incorporating these plants into the diet may provide a natural source of gallic acid, which can contribute to overall health and well-being.

Protocatechuic acid belongs to the category of phenolic acids and is commonly discovered in wild plant sources. It displays structural resemblances akin to widely acknowledged antioxidant compounds such as gallic acid, caffeic acid, vanillic acid and syringic acid. Among the investigated plants, protocatechuic acid was specifically detected in the 80% aqueous ethanol extract of *N. arbor-tristis*, with a quantity of 0.34±0.08 µg/mg dry extract.

The presence of protocatechuic acid in these plants could contribute significantly to their strong antioxidant properties. Antioxidants play a crucial role in preventing and treating conditions associated with oxidative stress, such as neurodegenerative and hepatic diseases.

The prevalence of protocatechuic acid within these plants implies their prospective utility as natural reservoirs of this phenolic compound. Such a presence could potentially enhance their collective antioxidant ability. Considering the structural similarities of protocatechuic acid with other well-known antioxidants, it is plausible that this compound contributes to the observed antioxidant qualities of the investigated plants. The antioxidant capacity of protocatechuic acid can help neutralize free radicals and reduce oxidative damage in the body.

The identification of protocatechuic acid in *N. arbor-tristis* emphasizes the potential health benefits associated with the consumption of this plant. Further exploration of the specific effects and mechanisms of protocatechuic acid in oxidative stress-related ailments would provide valuable insights into its potential therapeutic applications.[32]

Catechins, a type of flavanol, are widely present in various plant-based foods and beverages. In the investigated plants, a particularly high amount of catechin (40.44±0.08 µg/mg dry extract) was quantified in *L. inermis*. This finding highlights the potential of *L. inermis* as a rich source of catechins. Additionally, *C. alata* was also found to contain an appreciable amount of catechin (9.21±0.86 µg/mg dry extract), suggesting its potential contribution to the plant's curative and cell-strengthening capabilities.

Catechins are well-known for their antioxidant properties and they have been extensively studied for their potential health benefits. They exhibit strong free radical-scavenging activity, which helps protect cells from oxidative damage. The presence of a substantial amount of catechin in *L. inermis* suggests that this plant may possess significant antioxidant capacity, which could be beneficial for human health.

Furthermore, the detection of catechin in *C. alata* indicates that this plant also contains this important flavonoid. The presence of catechin in *C. alata* suggests its potential role in contributing to the plant's curative and cell-strengthening properties. Catechin's antioxidant activity can help combat oxidative stress and may have protective effects on cellular health.

The high amount of catechin in *L. inermis* and its presence in *C. alata* highlight the nutritional and potential therapeutic value of these plants. Incorporating these plants into the diet may provide a natural source of catechins, which could contribute to overall well-being and potentially offer health benefits.[33]

Chlorogenic acid, an ester formed by caffeic acid and quinic acid, can be found in coffee, coffee beans and a variety of higher plants. This particular compound has demonstrated anti-diabetic properties and is linked to the reduction of blood sugar levels.[34] In our investigation, the highest amount of chlorogenic acid was detected in *L. inermis* (21.24±1.11 µg/mg dry extract), while the

lowest amount was found in *H. rosa-sinensis* (0.91±0.08 µg/mg dry extract).

The detection of significant levels of chlorogenic acid in *L. inermis* suggests that this plant can be considered a rich source of this compound. Chlorogenic acid has been studied for its potential benefits in managing diabetes and reducing blood sugar levels. Its ability to inhibit glucose absorption and promote glucose metabolism contributes to its anti-diabetic effects.[34]

On the other hand, *H. rosa-sinensis* exhibited a lower content of chlorogenic acid compared to *L. inermis*. However, it is important to note that the presence of chlorogenic acid in *H. rosa-sinensis*, albeit in a lesser amount, still contributes to the overall phytochemical profile of this plant.

Consuming vegetables and plants rich in chlorogenic acid has been associated with potential benefits in regulating blood sugar levels. The presence of chlorogenic acid in *L. inermis* and other plants suggests that their consumption may offer advantages in terms of diabetes management and blood sugar control.[35]

Vanillic acid, a flavoring ingredient, was detected in the leaves of *N. arbor-tristis* and *L. inermis*, with quantities of 2.34±0.77 and 2.95±0.92 µg/mg dry extract, respectively. Extensive research has been conducted on vanillic acid due to its potential hepatoprotective attributes, particularly concerning its effects on mitigating liver damage induced by concanavalin A.[36]

The presence of vanillic acid in *N. arbor-tristis* and *L. inermis* suggests that these plants may possess compounds that could potentially contribute to hepatoprotective effects. Hepatoprotection refers to the ability of certain substances to protect the liver from damage and promote its overall health and function.

The identification of vanillic acid in these plants highlights their potential in the field of hepatoprotection. The hepatoprotective properties of vanillic acid have been studied in the context of concanavalin A-induced liver damage, suggesting its potential therapeutic application in liver-related disorders.

The HPLC analysis conducted in this investigation further supports the potential hepatoprotective properties of the investigated plants. HPLC is a powerful analytical technique that allows for the separation, identification and quantification of various compounds in a sample. The use of HPLC in this study provides valuable information on the presence and quantities of vanillic acid in *N. arbor-tristis* and *L. inermis*, linking these plants to potential hepatoprotective effects.

Caffeic acid, a potent antioxidant, has been shown to enhance resistance, regulate blood lipid levels and provide protection against mutagenic agents. As a key hydroxycinnamic acid component, it is prominently identified in wine and frequently detected within fruits, vegetables and herbs, often existing as an ester, such as in the form of chlorogenic acid.

The present investigation unveiled that the leaves of *H. rosa-sinensis* possess a comparatively higher content of caffeic acid (1.25±0.03 µg/mg dry extract). This finding is noteworthy as it demonstrates the presence of this beneficial compound in *H. rosa-sinensis*, which can be considered a valuable source of caffeic acid. The detected amount of caffeic acid in *H. rosa-sinensis* is comparable to its content in other commonly consumed vegetables such as lettuce (1.57 µg/mg), carrot (0.09 µg/mg), cauliflower (0.058 µg/mg) and potato (2.80 µg/mg).

The substantial presence of caffeic acid in *H. rosa-sinensis* indicates its potential role in enhancing the overall antioxidant capacity and potential health benefits linked to the consumption of this plant. Caffeic acid's antioxidant properties are well-documented and its ability to scavenge free radicals and protect against oxidative stress makes it valuable for human health.

By comparing the caffeic acid content in *H. rosa-sinensis* to that in other common vegetables, the study provides insights into the relative abundance of this compound. It highlights the potential nutritional value of *H. rosa-sinensis* and its capacity to contribute to a well-rounded diet rich in beneficial phenolic compounds.[37]

Among the plants under scrutiny, the 80% aqueous ethanol extract from *L. inermis* displayed the most substantial syringic acid content (1.39±0.04 µg/mg). Syringic acid is a recognized compound esteemed for its plausible anti-cancer, anti-proliferative and hepatoprotective attributes.[38]

The significant presence of syringic acid in *L. inermis* suggests that this plant extract may possess bioactive compounds that contribute to its observed health benefits. The anti-cancer and anti-proliferative properties of syringic acid make it a valuable component for potential therapeutic applications against various types of cancer and abnormal cell growth.

Additionally, the hepatoprotective effects of syringic acid indicate its potential in promoting liver health and protecting against liver damage. These properties can be particularly important for individuals with conditions related to liver function and those seeking preventive measures against liver diseases.

It is worth noting that other plants studied, including *N. arbor-tristis* (0.39±0.09 µg/mg), *H. rosa-sinensis* (0.43±0.08 µg/mg) and *M. pudica* (0.39±0.05 µg/mg), also exhibited considerable levels of syringic acid. This suggests that these plants may also possess potential bioactive properties associated with syringic acid.

The presence of syringic acid in multiple plant extracts underscores the significance of this compound in the context of medicinal and functional foods. Its diverse health benefits, particularly its anti-cancer, anti-proliferative and hepatoprotective effects, make it an intriguing subject for further investigation and potential therapeutic applications p-Coumaric acid, a constituent present in a range of foods including barley, peanuts, navy beans,

tomatoes, carrots and more, exhibits antioxidant properties and has been acknowledged for its capacity to impede the creation of carcinogenic nitrosamines within the stomach.[39]

Within the scope of this study, the highest concentration of p-coumaric acid was identified in *N. arbor-tristis* (2.70±0.04 µg/mg), signifying the abundant presence of this antioxidant compound. Following *N. arbor-tristis, H. rosa-sinensis* exhibited a significant amount of p-coumaric acid (2.16±0.067 µg/mg), while *L. inermis* contained a slightly lower level (2.06±0.81µg/mg). Both *M. pudica* and *C. occidentalis* were found to contain similar amounts of p-coumaric acid.

The presence of p-coumaric acid in these investigated plants suggests their potential as dietary sources of this antioxidant compound. The antioxidant properties of p-coumaric acid contribute to its ability to scavenge free radicals and provide protection against oxidative stress, thereby promoting overall health and reducing the risk of certain diseases, including cancer.

Furthermore, the inhibition of carcinogenic nitrosamine formation in the stomach by p-coumaric acid highlights its potential role in cancer prevention. By impeding the generation of these harmful compounds, p-coumaric acid may offer additional protection against the development of gastrointestinal cancers.

The significant levels of p-coumaric acid found in *N. arbor-tristis, H. rosa-sinensis, L. inermis, M. pudica* and *C. occidentalis* further emphasize the presence of this beneficial compound in these plant extracts. These findings support the potential utilization of these plants as dietary sources for obtaining p-coumaric acid and harnessing its antioxidant and cancer-preventive effects.

Ferulic acid, a significant phenolic compound detected within the plants investigated in our study, has gained substantial recognition due to its versatile physiological roles. These encompass antimicrobial, anti-inflammatory, antidiabetic and anti-cancer attributes.[40] Additionally, it has been associated with cholesterol-lowering effects and improvements in sperm viability.

Among the plants analyzed, *H. rosa-sinensis* exhibited the highest concentration of ferulic acid, indicating its potential as a rich source of this beneficial compound. This was followed by *N. arbor-tristis*, which displayed a significant level of ferulic acid (3.42±0.29 µg/mg dry extract). *L. inermis* also contained a notable amount of ferulic acid (1.60±0.22 µg/mg dry extract).

The antimicrobial properties of ferulic acid contribute to its ability to combat various microorganisms, potentially aiding in the prevention and treatment of microbial infections. Its anti-inflammatory effects make it valuable in mitigating inflammation-related conditions. Additionally, the antidiabetic properties of ferulic acid are associated with its ability to regulate blood sugar levels and improve insulin sensitivity.

Furthermore, the anti-cancer properties of ferulic acid have been attributed to its ability to inhibit cancer cell growth and induce apoptosis (programmed cell death) in certain cancer types. Its cholesterol-lowering effects contribute to cardiovascular health by reducing serum cholesterol levels and potentially lowering the risk of cardiovascular diseases.

Moreover, the improvement of sperm viability by ferulic acid highlights its potential role in male fertility and reproductive health.

The wide range of physiological functions associated with ferulic acid underscores its significance as a bioactive compound present in these investigated plants. The varying concentrations of ferulic acid observed in *H. rosa-sinensis, N. arbor-tristis* and *L. inermis* further highlight the diversity in phenolic composition across different plant species.

Sinapic acid is a naturally emerging molecule within plants, showcasing a variety of potential advantages such as antioxidant, antimicrobial, anti-inflammatory, anti-cancer and anti-anxiety properties. In the investigated plants, *C. occidentalis, L. inermis, N. arbor-tristis* and *H. rosa-sinensis*, sinapic acid was found in significant concentrations (3.81±0.72 µg/mg dry extract, 1.39±0.19 µg/mg dry extract, 0.65±0.05 µg/mg dry extract and 0.52±0.02 µg/mg dry extract, respectively). The consumption of ethnomedicinal plants containing sinapic acid may contribute to potential benefits for human health.[41]

Rutin, classified as a polyphenolic compound, manifests diverse biological impacts, including antidiabetic and anticancer activities. Its potential utility as a medicinal agent is promising.[42,43] The significant presence of rutin in *C. occidentalis* (6.20±0.88 µg/mg dry extract), *N. arbor-tristis* (2.49±0.09 µg/mg dry extract) and *L. inermis* (1.89±0.34 µg/mg dry extract) indicates that these plants could be considered as potential therapeutic agents. The substantial presence of rutin in *C. occidentalis, N. arbor-tristis* and *L. inermis* indicates the potential for these plants to be further investigated for their therapeutic capabilities.

Ellagic acid, classified as a phenolic antioxidant, is distributed among a variety of fruits and vegetables. Remarkably, within the plants under scrutiny, *L. inermis* emerged as having the most abundant ellagic acid content (5.05±0.59 µg/mg dry extract). *N. arbor-tristis* and *H. rosa-sinensis* also contained significant amounts of ellagic acid, with values of 2.38±0.67µg/mg dry extract and 1.65±0.28 µg/mg dry extract, respectively. These findings suggest that both plants may possess potential medicinal properties, including anti-cancer and anti-heart disease effects.[41] However, it's important to note that while the presence of ellagic acid suggests the potential for these plants to possess medicinal properties, further research is required to fully understand the extent of their effectiveness and to explore their specific mechanisms of action. Additionally, individual variations in absorption and metabolism should be taken into consideration.

Myricetin, extracted from plants, is renowned for its nutraceutical and diverse medicinal qualities. As determined through HPLC analysis, the myricetin content within the studied plants displayed a spectrum spanning from 0.066±0.001 to 22.18±1.36 µg/mg dry extract. Notably, a high amount of myricetin was detected in the leaves of *N. arbor-tristis* (22.18±1.36 µg/mg dry extract), while significant concentrations were found in 80% aq. ethanol extracts of *L. inermis* (21.71±1.89 µg/mg dry extract). Consequently, these plants have the potential to provide a wide array of effects, encompassing robust antioxidant, anticancer, antidiabetic and anti-inflammatory attributes. Furthermore, they might hold promise in the realm of illness prevention, including conditions like Parkinson's and Alzheimer's disease.[44]

Quercetin is extensively recognized for its diverse range of health advantages, including its anti-cancer, anti-histamine and anti-inflammatory properties, all stemming from its potent antioxidant capacities.[45] It is naturally present in several plant-based foods, with citrus fruits, apples, onions, parsley, sage, tea and red wine serving as potent sources of quercetin. These foods are widely consumed and offer a natural dietary means of incorporating quercetin into one's daily intake. The presence of high amounts of quercetin in *C. occidentalis* (30.77±2.78 µg/mg dry extract) and *N. arbor-tristis* (18.35±1.79 µg/mg dry extract) highlights the potential nutraceutical properties of these plants.

C. alata and *L. inermis* were found to contain a significant amount of naringin, with concentrations of 2.78±0.39 µg/mg dry extract and 1.43±0.21 µg/mg dry extract, respectively. Supplementing with naringin has been the focus of numerous studies, revealing its potential advantages in the management of conditions such as obesity, diabetes, hypertension and metabolic syndrome.[46]

Naringenin, a flavonoid known for its antioxidant, anti-fibrogenic, anti-inflammatory and anti-cancer properties, has been found to offer protective effects on the liver against various substances. In the present study, the content of naringenin was specifically analyzed and detected in *C. alata*, with a concentration of 1.28±0.28 µg/mg dry extract. This finding suggests that the plants investigated in this research may possess this beneficial flavonoid and potentially contribute to safeguarding against liver disorders.[47]

Apigenin, a flavonoid present in various fruits and vegetables such as parsley, chamomile, celery and kumquats, has gained recognition as a potential cancer chemopreventive agent.

The anticarcinogenic effects of apigenin have been substantiated through numerous studies, showcasing its ability to modulate cellular responses to oxidative stress and DNA damage, suppress inflammation and angiogenesis, decelerate cell proliferation and stimulate autophagy mechanisms.[48]

In the investigated plants, the highest level of apigenin was detected in *N. arbor-tristis*, with a concentration of 3.47±0.86 µg/mg dry extract. *H. rosa-sinensis* also contained a notable amount of apigenin, measuring 0.85±0.02 µg/mg dry extract. These findings suggest that these plants may possess potential anticancer properties attributed to their apigenin content.

The presence of significant levels of apigenin in *N. arbor-tristis* and *H. rosa-sinensis* underscores the potential of these plants as sources of this beneficial compound with potential anticancer properties.

Kaempferol, a flavonoid found in diverse plant sources, has demonstrated potential health advantages, encompassing anti-atherogenic and anti-cancer attributes. Its capacity to hinder the oxidation of low-density lipoproteins implies its potential to mitigate the risk of atherosclerosis, a condition marked by arterial plaque accumulation.

In a study by Calderon-Montao *et al.*, it was found that consuming foods high in kaempferol is associated with a lower risk of stomach cancer.[49] This suggests that kaempferol may possess anti-cancer properties, particularly in relation to stomach cancer.

N. arbor-tristis was found to contain a significant amount of kaempferol, measuring 2.03±0.29 µg/mg dry extract. *L. inermis* also exhibited a notable concentration of kaempferol, with 0.69±0.07 µg/mg dry extract. These findings indicate that consuming these plants may provide protection and potential health benefits attributed to their kaempferol content.

Overall, kaempferol has been implicated in preventing the oxidation of low-density lipoproteins and reducing the risk of atherosclerosis. Furthermore, its association with a lower risk of stomach cancer suggests its potential as an anti-cancer agent. The presence of significant amounts of kaempferol in *N. arbor-tristis* and *L. inermis* highlights the potential benefits of consuming these plants for health protection.

Phenolic acids, a group of phytochemicals found in various plant sources, have been studied for their potential role in skin health and the management of skin diseases. These bioactive compounds exhibit a range of properties that can benefit the skin.

Phenolic acids possess antioxidant activity, which helps protect the skin from oxidative stress and damage caused by free radicals. This is particularly relevant in skin diseases associated with increased oxidative stress, such as photoaging and inflammatory skin conditions.[50] By neutralizing free radicals, phenolic acids contribute to the prevention of cellular damage and maintain skin integrity.

Additionally, phenolic acids have anti-inflammatory effects, which can help alleviate symptoms associated with inflammatory skin diseases like acne, psoriasis and atopic dermatitis. They modulate various inflammatory pathways, reducing redness, swelling and other inflammatory responses in the skin.[51]

Phenolic acids also demonstrate antimicrobial properties, which can be beneficial in the management of skin infections related to certain skin diseases. They help inhibit the growth of bacteria and fungi that contribute to the development or exacerbation of skin infections.[52]

Furthermore, phenolic acids have been reported to influence the process of wound healing. They promote collagen synthesis, which is essential for tissue repair and the formation of new skin. Phenolic acids also exhibit potential antiproliferative effects on abnormal skin cells, suggesting their potential in the treatment of certain skin conditions, including skin cancer.[53]

By incorporating phenolic acid-rich foods or using skincare products containing these compounds, it is possible to harness their potential benefits for skin health and the management of skin diseases. However, further research is still needed to fully understand the mechanisms and specific applications of phenolic acids in dermatology.[54]

Flavonoids, a class of phenolic compounds abundantly present in various plants, have shown promising potential in the context of skin diseases. These bioactive compounds have been found to exhibit a range of beneficial effects on the skin.

Flavonoids possess antioxidant, anti-inflammatory, antimicrobial and wound-healing properties, which can contribute to the prevention and management of various skin diseases.[55] Their antioxidant activity helps neutralize harmful free radicals, reducing oxidative stress and protecting skin cells from damage. This is particularly relevant in skin diseases associated with increased oxidative stress, such as aging and inflammatory skin conditions.[56]

The anti-inflammatory properties of flavonoids can help alleviate symptoms associated with skin diseases characterized by inflammation, including acne, eczema and psoriasis. Flavonoids modulate inflammatory pathways, reducing redness, swelling and discomfort in the skin.[57] Additionally, their antimicrobial activity helps inhibit the growth of bacteria and fungi that can worsen or trigger skin infections associated with certain skin diseases.[52]

Moreover, flavonoids promote wound healing by enhancing collagen synthesis and supporting tissue regeneration. They play a role in angiogenesis, the formation of new blood vessels, which is essential for proper wound healing and the repair of skin lesions.[58]

By incorporating flavonoid-rich foods, supplements, or topical applications, it is possible to harness the potential benefits of flavonoids for skin health and the management of skin diseases. However, further research is still needed to fully understand the mechanisms and specific applications of flavonoids in dermatology.[59]

Correlation between total phenolic content and antioxidant assays

The Pearson correlation coefficients for DPPH and the antioxidant parameters TPC, TFC, ABTS, RP and TFLC were determined to be 0.944, 0.977, 0.969, 0.990 and 0.985, respectively. These results demonstrate a strong positive linear relationship between DPPH and the assessed antioxidant parameters and have been presented in Table 3.

Based on these correlation coefficients, it can be concluded that a significant portion of the antioxidant potential exhibited by the tested plants, at least 90%, is attributed to their high phenolic content. This finding is consistent with previous studies conducted by several researchers, which have also reported a positive correlation between TPC and antioxidant capacities.[60-64]

The antioxidant activity assay methods employed in this study revealed that the tested plants exhibited good antioxidant capacities. These investigations indicate a promising role for these ethnomedicinal plants as valuable natural reservoirs of antioxidants. As a result, their potential extends to being utilized for the preparation of crude extracts, which could hold significance in the treatment of skin ailments. Moreover, there lies a prospective avenue for isolating and refining antioxidant compounds sourced from these plants, thereby establishing a correlation with the management of skin diseases.

By harnessing the potency of these extracts abundant in antioxidants, a multitude of sectors such as pharmaceuticals, nutraceuticals and functional foods can pave the way for products imbued with heightened antioxidant attributes. This endeavour aligns with the potential to create interventions relevant to skin diseases. The refinement and exploration of isolated antioxidant compounds from these extracts could yield opportunities for innovative applications, potentially offering novel strategies in addressing skin-related ailments.

To embark on a more comprehensive exploration, it becomes crucial to examine the distinct traits and possible utilities of the antioxidant elements inherent in these botanical specimens. Subsequent investigations could concentrate on pinpointing and elucidating the nature of these elements, while also scrutinizing their absorption within the body and plausible contributions to well-being. In the context of skin conditions, directing attention towards these endeavors could potentially unravel insights into the suitability of these antioxidant compounds for addressing dermatological concerns and promoting skin health.

Principal component analysis

In order to better differentiate and compare the samples, a Principal Component Analysis (PCA) was conducted using a combination of variables including TPC, TFC, ABTS, RP, TFLC, phenolic acids and various polyphenolic compounds such as Gallic Acid (GA), protocatechuic acid (PCA), p-Hydroxybenzoic

acid (PHB), catechin, Chlorogenic acid (CHLA), Vanillic Acid (VA), Caffeic Acid (CA), Syringic Acid (SYRA), p-Coumaric Acid (PCOUA), Ferulic Acid (FA), Sinapic Acid (SINA), naringin, rutin, Ellagic Acid (ELLAGA), myricetin, quercetin, apigenin and kaempferol (Figure 2). The PCA score plots (Figure 2A) and loading plots (Figure 2B) were generated to visualize the results.

The PCA results revealed that the first two principal components (PC1 and PC2) accounted for 66.5% of the total variance. PC1 (43.3% variance) contributed significantly more to the overall variation compared to PC2 (23.2% variance). For simplification, only the initial two Principal Components (PCs) were taken into account for the analysis.

In the loading plot (Figure 2B), PC1 showed positive associations with TPC, TFC, ABTS, RP and various phenolic acids and polyphenolic compounds. On the other hand, PC2 exhibited a negative correlation with certain phenolic compounds while positively correlating with TPC, TFC, DPPH, ABTS, PHB, SINA, TFLC, rutin, quercetin, apigenin and kaempferol.

The PCA score plot (Figure 2A) demonstrated a clear separation of *N. arbor-tristis* and *L. inermis* from the other samples. These two samples appeared on the right side and were distinct due to their high contents of TPC, TFC, TFLC, DPPH, ABTS and RP. Moreover, based on the phenolic and polyphenolic content, *N. arbor-tristis* exhibited higher potency compared to the other five plants under investigation.

The results of the PCA analysis provide valuable insights into the relationships between the different variables and samples. It helps in identifying the key factors contributing to the variance and allows for a better understanding of the overall antioxidant potential of the tested plants. By utilizing PCA, researchers can effectively compare and select plants with higher phenolic and polyphenolic content for potential applications in the development of antioxidant-rich extracts and isolation of specific antioxidant molecules.

CONCLUSION

This study systematically evaluated the antioxidant potential and phenolic composition of six traditionally used medicinal plants from North-Eastern India viz. *Nyctanthes arbor-tristis*, *Hibiscus rosa-sinensis*, *Lawsonia inermis, Mimosa pudica, Cassia alata*, and *Cassia occidentalis*, with a focus on their relevance in the treatment of skin diseases. Spectrophotometric and chromatographic analyses revealed that *Nyctanthes arbor-tristis* possessed the highest levels of phenolic antioxidants, particularly myricetin and quercetin, which are known for their potent free radical scavenging properties. Strong correlations between total phenolic and flavonoid content with DPPH and ABTS radical scavenging activities affirm the central role of these bioactive compounds in the observed antioxidant effects. Principal Component Analysis further confirmed the superior antioxidant profile of *Nyctanthes arbor-tristis* among the studied species.

The results substantiate the traditional ethnomedicinal use of these plants in treating skin conditions associated with oxidative stress, inflammation, and tissue degeneration. By elucidating the phenolic profiles and antioxidant capacities, this study provides a scientific foundation for their potential therapeutic application in modern dermatological formulations. These findings also support further pharmacological investigations and the development of plant-based alternatives for managing oxidative skin disorders, thereby bridging traditional knowledge with evidence-based medicine.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

ABTS: 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); **CA:** Caffeic acid; **CHLA:** Chlorogenic acid; **DPM:** Dry plant material; **DPPH:** 1,1-Diphenyl-2-picrylhydrazyl; **ELLGA:** Ellagic acid; **FA:** Ferulic acid; **GAE:** Gallic acid equivalent; **GA:** Gallic acid; **HPLC:** High-performance liquid chromatography; LP: Lipid peroxidation; **M:** Molar; **MC:** Metal chelating; **mg:** Milligram; **PCA:** Principal component analysis; **PCAT:** Protocatechuic acid; **PCOUA:** p-Coumaric acid; **PHB:** p-Hydroxybenzoic acid; **RE:** Rutin; **RP:** Reducing power; **SEM:** Standard error of Mean; **SINA:** Sinapic acid; **SYRA:** Syringic acid; **TFA:** Trifluoroacetic acid; **TFC:** Total flavonoids; **TFLC:** Total flavonol content; **TPC:** Total phenolic content; **VA:** Vanillic acid; **µG:** Microgram; **µM:** Micromolar.

SUMMARY

The primary objective of this study was to delve into the antioxidative traits and phenolic composition of six indigenous medicinal plants such as *Nyctanthes arbor-tristis, Hibiscus rosa-sinensis, Lawsonia inermis, Mimosa pudica, Cassia alata* and *Cassia occidentalis*, widely employed in North-Eastern India for

addressing skin-related issues. Our research endeavors divulged substantial antioxidative capabilities and phenolic constitution within these plants. Notably, the *Nyctanthes arbor-tristis* variety exhibited elevated levels of total phenolic content and flavonols, underscoring its potent potential as an antioxidant reservoir. The correlation analysis and Principal Component Analysis lent further validation to the antioxidant attributes of the phenolic compounds housed within these botanicals.

These revelations not only contribute to augmenting our comprehension of the curative possibilities these plants offer but also lay a foundation for their seamless integration into conventional medical approaches geared towards mitigating skin ailments rooted in oxidative stress, inflammation, tissue impairment and compromised skin functionality. The study notably underscores the essence of scrutinizing natural founts of antioxidants for devising efficacious treatments, while concurrently spotlighting the value of traditional medicinal wisdom in tackling present-day skin health concerns

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