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Biodiversity and Healing: Exploring the medicinal potential of wild edible plants abundant in antioxidants

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Abstract

Wild edible plants (WEPs) are rich in antioxidants with a history of traditional medicinal use. This study aimed to find the most efficient polyphenol extraction solvent from WEPs, including *Lasia spinosa, Eriosema chinense, Nasturtium indicum, Begonia hatacoa,* and *Embelia floribunda*, for pharmaceutical and drug industry applications. Polyphenols, crucial antioxidants, were meticulously analyzed via RP-HPLC. Total phenolic, flavonoid, and flavonol levels were measured with four solvents. 80% aqueous (aq.) ethanol proved the most effective, surpassing solvents of varying polarities. The plants exhibited high total phenolics and flavonols, notably gallic acid (30.78±1.67 µg/mg dry extract) and syringic acid (32.03±1.89 µg/mg dry extract) in 80% Aq. ethanol extract of E. floribunda. Correlation analyses revealed strong connections between parameters, with 80% Aq. ethanol and acetone showing the highest correlation values (r and R2), indicating their exceptional polyphenol extraction and antioxidant potential. The principal component analysis emphasized the pharmaceutical potential of WEPs, particularly *E. floribunda*'s 80% Aq. ethanol extract due to its phenolic and polyphenolic content. In conclusion, 80% of ethanol extracts of these plants outperform synthetic derivatives in antioxidant activity, making them promising for pharmaceutical and drug product development with enhanced natural antioxidant properties.

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INTRODUCTION

Oxidation is a chemical process where electrons are lost, leading to the creation of unstable molecules known as free radicals. These free radicals have been associated with the development of various chronic diseases in humans, including atherosclerosis, Parkinson's disease, arthritis, Alzheimer's disease, stroke, chronic inflammatory disorders, malignancies, and other degenerative conditions [1][2].

In the pharmaceutical context, combating the detrimental effects of free radicals is crucial. To

address this challenge, pharmaceutical researchers explore antioxidants with the ability to neutralize free radicals and mitigate the cellular damage they can induce. Antioxidants play a vital role in pharmaceutical applications aimed at preserving cellular health and preventing oxidative stress-related ailments.

Plant materials encompass a diverse array of active compounds with a broad spectrum of chemical characteristics. These compounds, found in herbal plants, vegetables, and fruits, include phenolic compounds, flavones, isoflavones, flavonoids, anthocyanins, coumarins, lignans, catechins, and isocatechins. One of their remarkable attributes is their substantial antioxidant activity [3].

Antioxidants originating from plant materials assume a pivotal role in shielding the body against the detrimental effects of free radicals. These antioxidants act as protective shields, neutralizing unstable molecules and thereby preventing harm to the body. This protective mechanism contributes significantly to safeguarding against a wide range of illnesses and health conditions.

The choice of solvents and plant parts during the extraction process plays a crucial role in determining the antioxidant potential of plants. Various solvents, including methanol, ethanol, acetone, chloroform, ethyl acetate, and water, have been used to extract antioxidant compounds from various sources, such as plants, plant-based foods, and medicinal substances. The selection of the right solvent system is essential for extracting target molecules effectively while preserving their chemical properties. Polar solvents are generally more effective for extracting polyphenols compared to nonpolar solvents. As a result, a combination of water and organic solvents like methanol, ethanol, acetone, and chloroform is commonly employed for extracting plant materials. Solvents can be used alone or in combination to enhance polyphenol production. For instance, a mixture of water and aqueous methanol or ethanol can be used to increase polyphenol yields. It's important to note that no universally recommended solvent maximizes the production of plant phenolics, especially when dealing with wild edible plants. The quality of plant extracts and the complex chemistry of edible plants vary widely, making the selection of an appropriate solvent crucial for optimizing the extraction process [4][5][6].

Antioxidants are categorized into synthetic and natural types. Synthetic antioxidants, such as BHT, BHA, propyl gallate, and TBHQ, have been linked to health concerns like cancer and liver damage. To address these issues, the food and pharmaceutical industries are increasingly turning to natural antioxidants, which offer safety, nutrition, and medicinal benefits.

Natural antioxidants are derived from plant sources rich in compounds like phenolics, flavonoids, vitamin C, carotenoids, tannins, and proanthocyanins. These phytochemicals protect the body against free radicals. Wild edible plants are particularly promising due to their untapped nutritional and bioactive components that combat oxidative stress. They contain phenols, flavonoids, vitamin C, and carotenoids with antioxidant properties.

WEPs, abundant in antioxidants, are of significant interest to the pharmaceutical industry. They provide a diverse range of compounds with potential medicinal and therapeutic applications, drawing from traditional knowledge and promoting sustainability. This makes them a valuable resource for drug development and supplements in the pharmaceutical sector.

Rural communities in Arunachal Pradesh, India, have harnessed various WEPs like *Lasia spinosa*, *Eriosema chinense*, *Nasturtium indicum*, *Begonia hatacoa*, and *Embelia floribunda* for multiple purposes, including food, traditional medicine, and livestock fodder. The ethnomedicinal wisdom surrounding these plants has substantial potential to benefit the pharmaceutical industry.

L. Espinosa, a member of the Araceae family, has a range of traditional uses. Its fresh leaves are used as a culinary vegetable, often cooked or fried. In some culinary traditions, the tender stem is cooked with dry fish (known as "Khumi").

In addition to its culinary applications, this plant has been used as a remedy for colic, rheumatism, and intestinal ailments. The corm of *L. spinosa* is employed to alleviate throat problems. Notably, the leaves and corms are administered to address issues like piles in the Khagrachari region [7]. In regions like Rajshahi and Natore districts of Bangladesh, the plant's tubers are used to treat

conditions such as rheumatoid arthritis, constipation, and blood purification [8].

Research has also highlighted the therapeutic properties of various parts of the plant. The rhizome, for instance, is recognized for its antioxidant capabilities and displays antimicrobial attributes along with cytotoxic activity [9]. The crude extract from the plant's roots has shown antinociceptive, anti-inflammatory, and antidiarrheal effects. In some communities, a porridge or pudding made from the young tender leaves, known as "jurang," is commonly used as a remedy for intestinal worm infections, particularly in Nagaland, India [10].

E. chinense, a member of the Fabaceae family, is recognized for its diverse applications in traditional practices. The fruits and seeds of this plant have been traditionally employed due to their tonic, diuretic, and astringent properties. Notably, in the regions of North East India, tribal communities have utilized the roots of *E. Chinese* as a remedy for the treatment of diarrhoea.

N. indicum a member of the Brassicaceae family, holds significance for its various uses. The leaves of this plant are commonly consumed as a vegetable. Additionally, the herb has been traditionally employed as an herbal remedy, particularly for addressing urinary tract infections and respiratory tract infections [11].

B. hatacoa, part of the Begoniaceae family, plays a meaningful role in cultural traditions due to its established applications. Its leaves are esteemed as a nutritional resource and are embraced as a vegetable in local cuisine, underscoring its significance in culinary heritage. Beyond its culinary contributions, this plant has garnered attention in traditional medicine for its potential health benefits. It has historical use as a remedy for conditions like colic, rheumatism, and intestinal disorders, reflecting its role in supporting various aspects of well-being.

E. floribunda, a member of the Primulaceae family, holds cultural importance for its multifaceted uses. The leaves of this plant are incorporated into diets as a vegetable, contributing to local culinary traditions. Additionally, the leaves are utilized in traditional medicine for their potential therapeutic properties. A decoction of the leaves is specifically employed as a remedy for addressing intestinal worm infestations, reflecting its role in traditional healthcare practices.

Therefore, this study aims to assess the effectiveness of various solvents in extracting polyphenols and the consequent antioxidant activities from wild edible plants (WEPs) like *Lasia spinosa*, *Eriosema chinense*, *Nasturtium indicum*, *Begonia hatacoa*, and *Embelia floribunda*. These plants are traditionally consumed by ethnic communities in Arunachal Pradesh, India. The study may provide baseline data on the application of these wild edible the plants in food and pharmaceuticals industry.

MATERIALS AND METHODS

Plant materials

The plant materials under investigation, specifically *Lasia spinosa*, *Eriosema chinense*, *Nasturtium indicum*, *Begonia hatacoa*, and *Embelia floribunda*, were sourced from various markets within the state of Arunachal Pradesh, India. The identification and authentication of these plant materials were conducted at our institution. Subsequently, the collected plant materials were meticulously stored in our laboratory, each assigned a distinct registry number, namely, BSITS 87, BSITS 88, BSITS 90, BSITS 91, and BSITS 92, respectively. To facilitate further extraction procedures, these plant components underwent a meticulous process involving shade-drying, crushing, and subsequent storage within a hermetically sealed container.

Preparation of plant extracts

For each powdered plant sample (100g), a dual extraction process was employed using 80% aqueous ethanol at ambient temperature. The extractions were repeated, with each instance involving agitation for a duration of 18 to 24 hours. The resulting concentrates from both the initial and subsequent extractions were combined and subjected to concentration within a rotary evaporator operating under reduced pressure. This procedure yielded concentrated extracts characterized by their viscous nature, subsequently subjected to freeze-drying to remove moisture. Similarly, extracts utilizing acetone, chloroform, and benzene were also prepared using this method. All dried extracts obtained from each solvent were carefully preserved at a temperature of -20°C. The weight of the plant material that had

been air-dried was utilized to determine the percentage yield.

Total phenolic content (TPC)

The quantification of total phenolic content within the crude extracts was conducted employing the Folin-Ciocalteu method [12]. In individual test tubes, a volume of 100 µl from each analyzed extract was taken.

To this extract, 1.0 ml of Folin-Ciocalteu reagent and 0.8 ml of a solution containing sodium carbonate (7.5%) were added.

Following a 30-minute incubation period, the reaction mixture's absorbance at 765 nm was measured using a UV-visible spectrophotometer (Shimadzu UV 1800).

The assessment of total phenolic content within the dry plant material was subsequently computed as gallic acid equivalents (GAE) expressed in milligrams per 100 grams (mg GAE/100g).

This calculation was based on the equation y = 0.0013x + 0.0498, where y represented the absorbance, and x symbolized the Gallic acid equivalent content (mg/100g).

Total flavonoid content (TFC)

The method Ordonez was used to determine the total flavonoids in the investigated plants [13]. In a test tube, 0.5 ml $AlCl_3$ ethanol solution (2%) was added to 0.5 ml of extracts. The absorbance of the mixture was measured at 420 nm after one hour at room temperature (UV-visible spectrophotometer Shimadzu UV 1800). The presence of flavonoids was indicated by a yellow colour. The following equation based on the calibration curve was used to compute total flavonoid concentrations as rutin (mg/100g): y = 0.0182x - 0.0222, R^2 = 0.9962, where y was the absorbance and x was the Rutin equivalent (mg/100g).

Total flavonols content (TFLC)

For the quantification of total flavonols within plant extracts, the methodology developed by Kumaran and Karunakaran was employed [14]. In a test tube, a mixture of 2.0 ml of 2% $AlCl_3$ ethanol solution and 3.0 ml of sodium acetate solution (50 g/L) was introduced to 2.0 ml of the plant extracts. Following an incubation period of 2.5 hours at a temperature of 20°C, the absorption at 440 nm was measured using a Shimadzu UV 1800 UV-visible spectrophotometer. The determination of total flavonol content, presented as quercetin equivalent (mg/100g), was executed utilizing the subsequent equation derived from the calibration curve: y = 0.0049x + 0.0047. The correlation coefficient (R^2) for this equation was found to be 0.9935. In this context, 'y' signifies the absorbance, while 'x' corresponds to the quercetin equivalent content (mg/100g).

Reducing power (RP)

The determination of the reducing power of the plant extracts was carried out using the method devised by Oyaizu [15]. In this procedure, 2.5 ml of a phosphate buffer (0.2 M, pH 6.6) was combined with an equal volume of 1% potassium ferricyanide within a test tube. To this mixture, 100 µl of the plant extracts was introduced. The reaction mixture was subjected to incubation at 50°C for 20 minutes. Following this incubation, the mixture underwent centrifugation at 3000 rpm for 10 minutes, after the addition of 2.5 ml aliquots of 10% trichloroacetic acid. The upper layer of the solution (2.5 ml) was then blended with an equal volume of distilled water, and a freshly prepared solution of ferric chloride (0.5 ml, 0.1%) was incorporated. The absorbance of the resulting reaction mixture was measured at a wavelength of 700 nm. The computation of the reducing power, expressed as ascorbic acid equivalent (AAE), was achieved using the subsequent equation derived from the calibration curve: y = 0.0023x - 0.0063. The coefficient of determination (R^2) for this equation was determined to be 0.9955. Within this context, 'x' signifies the ascorbic acid equivalent content (mg/100g), while 'y' denotes the absorbance.

DPPH free radical scavenging activity

The free radical scavenging activity of the plant extracts was assessed using the stable radical DPPH (1,1-diphenyl-2-picrylhydrazyl) as a measure [16]. In individual test tubes, a volume of 100 µl from each analyzed extract was introduced, followed by the addition of 3.9 ml of freshly prepared DPPH solution (25 mg/L) in methanol. The mixture was stirred and left for 30 minutes. Subsequently, the absorbance was gauged at 517 nm using a UV-visible spectrophotometer (Shimadzu UV 1800). The efficacy of scavenging the DPPH radical was computed utilizing the subsequent equation:

Scavenging ability of DPPH (%) = {(Ac – At)/Ac} x 100

Here, 'Ac' signifies the absorbance of the control reaction, while 'At' denotes the absorbance in the presence of the extracted sample.

To express the antioxidant activity of the extract, the term IC_{50} was employed. This parameter represents the quantity of dry extract in milligrams that is required to inhibit 50% of DPPH radical production. The IC_{50} value is calculated through empirical testing and analysis.

ABTS radical scavenging activity

The method outlined by Re et al. was employed to determine the radical scavenging activity of the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation (ABTS.+) [17]. To initiate this process, ABTS was dissolved to achieve a concentration of 7 mM in water. For the generation of ABTS radicals, 2.45 mM of potassium persulfate was introduced, resulting in a final concentration. This radical generation phase spanned a duration of 12 to 16 hours, conducted in darkness at room temperature.

Following this, the absorbance at 734 nm was calibrated to 0.70 ± 0.02 by appropriately diluting the solution with ethanol. To investigate the scavenging activity, 1 ml of the diluted ABTS.+ solution was mixed with 100 µl of the plant extract. After a 15 mins interval, the absorbance at 734 nm was measured.

The computation of the percentage of inhibition was achieved through the utilization of the ensuing equation:

ABTS scavenged (%) = (Ac - At) / Ac x 100

Here, 'Ac' signifies the absorbance of the control, and 'At' represents the absorbance of the test extracts.

Metal chelating activity (MC)

The methodology outlined by Lin and co-workers was adopted to assess metal chelating activity [18]. This procedure involved evaluating the portion of the ferrozine–Fe^{+2} complex formation that was hindered. The estimation of chelating power was accomplished using the subsequent formula:

Chelating power (%) = {(Ac – At)/Ac} × 100

In this equation, 'Ac' signifies the absorbance observed in the control reaction, while 'At' indicates the absorbance associated with the plant extract samples.

Lipid peroxidation assay (LP)

The assessment of anti-lipid peroxidation was conducted through Amabye's method with specific adaptations [19]. In this process, all reagents, excluding the extract, were utilized in a negative control setup. The following formula was employed to compute the inhibition of peroxidation:

Lipid peroxidation inhibition (%) = {(Ac – At)/Ac} × 100

Here, 'Ac' represents the absorbance observed in the control reaction, while 'At' corresponds to the absorbance associated with the extracts being examined.

Phenolic acids and flavonoids quantification by HPLC

HPLC equipment

HPLC analyses were conducted using a Dionex Ultimate 3000 liquid chromatograph equipped with a diode array detector (DAD) featuring a 5 cm flow cell. The data processing was managed by a Chromeleon system manager. For the separation of samples, a reversed-phase Acclaim C18 column (with a molecular size of 5 microns and dimensions of 250 mm x 4.6 mm) was employed. A volume of 20 µl from the test sample was introduced into the HPLC column for analysis.

Standard solutions

For the preparation of the stock solution at a concentration of 1 mg/ml, 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) was dissolved in methanol. The preparation of working solutions involved dilution of the standard solution with the mobile phase solvent system.

Before introducing the standard and working solutions into the HPLC apparatus, a filtration step was undertaken using a 0.45 µm PVDF-syringe

filter. This process ensured the removal of particulate matter and other impurities, contributing to the accuracy and precision of the HPLC analyses.

In summary, the standard solutions were meticulously prepared and subsequently diluted to prepare working solutions for HPLC analysis, and both types of solutions underwent filtration to ensure the purity and quality of the samples.

Estimation of phenolic acids and flavonoids by HPLC

HPLC analysis was harnessed for the quantification of phenolic acids and flavonoids within an 80 percent aqueous ethanol extract of the investigated plants, following the methodology outlined by Datta et al. [20]. The analysis was carried out utilizing a Dionex Ultimate 3000 liquid chromatograph furnished with a diode array detector (DAD) incorporating a 5 cm flow cell. Data processing was facilitated by a Chromeleon system manager. A reversed-phase Acclaim C18 column with a particle size of 5 microns and dimensions of 250 x 4.6 mm was employed for sample separation. The HPLC column received an injection of 20 µl of the sample.

By the stipulations of the US Pharmacopeia (USP) and the International Council for Harmonisation (ICH), the method underwent validation. The mobile phase constituted methanol (Solvent A) and a 0.5 percent aqueous acetic acid solution (Solvent B). The column temperature was maintained at a constant 25°C, with each injection involving a volume of 20 µl. A gradient elution was achieved by altering the ratio of solvent A to solvent B. The total analysis time per sample spanned 105 minutes. HPLC chromatograms were detected using a photodiode array UV detector at three distinct wavelengths (272 nm, 280 nm, and 310 nm).

The retention time for each chemical compound was determined by introducing standards under analogous conditions. The quantification of phenolic acids and flavonoids within the extracts was carried out using the integrated peak area. The concentrations were deduced through reference to a calibration curve that correlated peak area with the corresponding standard sample concentration.

The data were meticulously presented in triplicate, accompanied by a convergence limit to enhance the robustness of the analysis.

Statistical analysis

The data analysis was conducted using triplicate samples, and the resultant outcomes were presented as the mean along with the standard error of the mean (SEM). To assess variations among the groups and to distinguish plants exhibiting comparable characteristics concerning their total phenolic content (TPC), total flavonoid content (TFC), total flavonol content (TFLC), radical scavenging activities, phenolic acids, and flavonoid content, a one-way analysis of variance (ANOVA) was carried out. Subsequently, a Tukey test (with a significance level of $p \leq 0.05$) was performed to pinpoint significant differences.

In order to explore relationships and connections among various parameters, correlation analyses were executed. Both the correlation coefficient (r) and the coefficient of determination (R^2) were used to gauge the strength and extent of these relationships. Additionally, a Principal Component Analysis (PCA) was conducted to provide a visual representation of the data's patterns and variances.

For these statistical analyses, the SPSS software (version 11.0 for Windows) was employed, facilitating the rigorous examination and interpretation of the obtained results.

RESULTS AND DISCUSSION

Extractive value

The data presented in **Table 1** and **Table 2** illustrates the extractive values of various wild plants when subjected to extraction using four distinct solvents such as benzene, chloroform, acetone, and 80 percent aqueous ethanol. A comparative analysis of these solvents reveals that 80 % aqueous ethanol stands out as the most effective solvent for achieving maximum extract yield from all the investigated plants. Notably, when 80 % aqueous ethanol is employed to extract the leaves of *N. indicum*, it results in the highest yield (16.00±1.05 g/100g), surpassing the yields obtained from alternative extraction solvents. This pattern is consistent with the trends observed in the extraction of other components of these plants. The observed variations in extractive values among the plant materials can be attributed to the diverse chemical constituents present and the polarity of the selected extraction solvent, providing insights into the disparities noted [21].

Table 1: Antioxidant activities of wild edible plants in different solvent extraction systems

Antioxidant parameters	Solvent	*L. spinosa*	*E. chinense*	*N. indicum*	*B. hatacoa*	*E. floribunda*
Extractive value (g/100g)	Benzene	1.00±0.01^{c}	3.50±0.03^{a}	1.00±0.02^{c}	0.95±0.03^{d}	1.45±0.11^{b}
	Chloroform	1.50±0.06^{c}	3.50±0.01^{a}	1.50±0.03^{c}	0.10±0.02^{d}	1.80±0.23^{b}
	Acetone	1.00±0.004^{b}	3.00±0.01^{a}	1.00±0.03^{b}	0.25±0.03^{c}	1.00±0.08 ^{b}
	80 % Aq. ethanol	4.00±0.02^{c}	4.00±0.03^{c}	16.00±1.05^{a}	2.95±0.34^{d}	5.55±0.22^{b}
Total phenolic content (GAE, mg/100g DPM)	Benzene	20.19±0.65^{d}	56.28±1.48^{a}	20.00±3.60^{d}	46.29±2.66^{b}	24.61±1.21^{c}
	Chloroform	65.77±4.60^{d}	163.38±3.20^{a}	24.55±0.66^{e}	76.41±6.50^{c}	147.31±2.66^{b}
	Acetone	90.79±6.78^{d}	311.09±1.54^{a}	30.96±4.60^{e}	128.08±1.15^{c}	234.87±8.37^{b}
	80 % Aq. ethanol	204.87±3.5^{d}	776.15±8.67^{b}	162.74±5.68^{e}	226.41±7.91^{c}	850.77±9.24^{a}
Total flavonoids content (RE, mg/100g DPM)	Benzene	23.29±0.45^{d}	66.69 ±2.04^{b}	23.68±0.34^{d}	63.49±0.68^{c}	69.52±1.11^{a}
	Chloroform	30.48±0.96^{d}	100.30±6.75^{b}	27.88±0.59^{e}	81.79±1.80^{c}	114.58±1.27^{a}
	Acetone	25.76±1.58^{e}	205.80±2.60^{a}	39.77±0.57^{d}	124.91±0.60^{c}	145.69±2.89^{b}
	80 % Aq. ethanol	74.43±1.68^{e}	504.24±3.30^{a}	116.05±1.07^{d}	178.17±1.89^{c}	276.61±7.48 ^{b}
Total flavonol content (QE, mg/100g DPM)	Benzene	32.47±5.23^{d}	46.17±5.23^{b}	21.24±1.06^{e}	51.51±6.46^{a}	41.12±1.75^{c}
	Chloroform	41.31±3.98^{d}	62.75±3.98^{c}	32.98±2.38^{e}	127.14±2.64^{a}	91.79±2.87^{b}
	Acetone	43.35±2.97^{c}	185.77±2.97^{a}	38.26±3.28^{d}	156.58±3.98^{b}	155.05±2.33^{b}
	80 % Aq. ethanol	65.80±5.17^{e}	313.68±5.17^{a}	101.86±1.72^{d}	186.36±2.54^{c}	210.85±8.34^{b}
DPPH (% of inhibition)	Benzene	1.50±0.07^{c}	31.14±0.11^{a}	1.63±0.16^{c}	1.85±0.13^{c}	3.54±0.32^{b}
	Chloroform	4.03±0.03^{c}	55.14±0.23^{a}	2.82±0.31^{d}	2.47±0.12^{d}	6.38±0.44^{b}
	Acetone	4.03±0.02^{d}	55.43±0.68^{a}	3.29±0.33^{e}	5.05±0.14^{c}	8.01±0.57^{b}
	80 % Aq. ethanol	18.30±0.82^{d}	88.73±1.31^{a}	16.97±1.89 ^{e}	27.66±2.14^{c}	80.53±4.27^{b}

Each value in the table was obtained by calculating the average of three experiments and data are presented as Mean ± Standard error of the mean (SEM). Statistical analysis were carried out by Tukeys test at a 95% confidence level and statistical significance was accepted at the $p < 0.05$ level. The superscript letters a,b,c,d and e denote the significant differences within the same parameters of individual solvent extract among the plants

Total phenol, flavonoid and flavonol content

The quantities of total phenolics found in the extracts obtained through benzene, chloroform, acetone, and 80% aqueous ethanol from five wild plant species exhibited substantial variation, spanning from 20.00±3.60 mg GAE/100g dry plant material (DPM) to 850.77±9.24 mg GAE/100g DPM (**Table 1** and **Table 2**).

Notably, the 80% aqueous ethanol extract of *E. floribunda* demonstrated a notably elevated phenolic content (850.77±9.24 mg GAE/100g DPM), closely followed by the same solvent's extract of *E. chinense* (850.77±9.24 mg GAE/100g DPM). In contrast, the benzene extract of *N. indicum* exhibited the lowest phenolic content (20.00±3.60 mg GAE/100g DPM). Substantial quantities of phenolic compounds were also quantified in the acetone extract of *E. floribunda* and the 80% aqueous ethanol extracts of *B. hatacoa* and *L. spinosa*, measuring 234.87±8.37, 226.41±7.91, and 204.87±3.5 mg GAE/100g DPM, respectively.

The extracts displayed a wide range of flavonoid concentrations, expressed in terms of rutin equivalent, spanning from 23.29±0.45 to 504.24±3.30 mg/100g DPM as shown in Table 1. Among the extracts, the 80% aqueous ethanol extract of *E. chinense* exhibited the highest flavonoid content (504.24±3.30 mg/100g DPM). Remarkable flavonoid levels were also detected in

E. floribunda, *B. hatacoa*, and *N. indicum*, with concentrations of 276.61±7.48, 178.17±1.89, and 116.05±1.07 mg/100g DPM, respectively. Furthermore, the chloroform and acetone extracts of the remaining four plants examined also displayed notably elevated levels of flavonoids.

The quantification of flavonol concentrations across different plant extracts is reported in terms of quercetin equivalents, as detailed in **Table 1** and Table 2. The 80% aqueous ethanol extract of *E. chinense* exhibited the highest flavonol content (313.68±5.17 mg/100g DPM), followed by the ethanol extract of *E. floribunda* (276.61±7.48 mg/100g DPM). Additionally, noteworthy flavonol amounts were observed in the acetone extracts of *E. floribunda* and *E. chinense*, measuring 155.05±2.33 and 185.77±2.97 mg/100g DPM, respectively. The chloroform extract of *B. hatacoa* and the ethanol extract of *E. floribunda* also displayed considerable flavonol content.

Due to their potent redox properties, phenolic compounds stand out as the predominant plant constituents exhibiting remarkable antioxidant capabilities. These compounds, recognized as phenolic antioxidants, have the ability to bind with and neutralize free radicals [22]. Among these phenolic antioxidants, flavonoids and flavonols are prevalent natural components frequently encountered in plant species. Through scavenging or chelation mechanisms, flavonoids and flavonols have demonstrated their efficacy in quenching oxidative stress and displaying antioxidant prowess [23]. The outcomes of our investigation unequivocally underscore the substantial impact of phenolic compounds on the constitution of these plants. The radical-scavenging attributes of these plants are substantially mediated by other phenolic compounds featuring hydroxyl groups, such as flavonoids and flavonols. In our study, the most effective solvent for extracting phenolic compounds, flavonoids, and flavonols from plant sources was determined to be 80% aqueous ethanol. The presence of phenolic compounds within *E. chinense*, *E. floribunda*, and *B. hatacoa* potentially elucidates their formidable capacity for scavenging radicals.

Reducing power

As delineated in **Table 1** and **Table 2**, the reduction potential of the five wild edible plant species is gauged in terms of milligrams of ascorbic acid equivalents (AAE) per 100 grams of dry plant material (DPM). Remarkably, the 80% aqueous ethanol extract derived from *E. chinense* (232.93±1.34 mg AAE/100g DPM), a source rich in flavonoids and flavonols, emerges as the frontrunner in terms of its robust reducing ability. Conversely, the benzene extract obtained from *N. indicum* displays the lowest activity (9.22±0.45 mg AAE/100g DPM) when measured in terms of ascorbic acid equivalent.

In the assay conducted, the extracts' antioxidants facilitate the conversion of the Fe+3/ferricyanide complex to its ferrous state, thus highlighting the inherent reduction capabilities. This reducing capacity exhibited by the extracts assumes significance as it could potentially serve as an indicator of anticipated antioxidant effects. This phenomenon is driven by the extracts' capacity to disrupt the propagation of free radical chains through the donation of hydrogen atoms, thereby curbing oxidative processes [24]. This intricate process underscores the significant role that these plant extracts could play in mitigating oxidative stress and conferring potential health benefits.

DPPH radical scavenging activity

The assessment of anti-radical abilities in five wild food plants was conducted using the DPPH radical scavenging test. The DPPH stable free radical method for assessing the antioxidant activity of a chemical or plant extract is a simple, rapid, and sensitive method [25]. This test is a widely used method for evaluating the antioxidant activity of chemical compounds or plant extracts. The basic principle involves measuring the ability of a sample to neutralize the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical. The DPPH molecule is purple and absorbs light at 517 nm. As antioxidants interact with DPPH, they donate hydrogen atoms or electrons, leading to the conversion of the purple DPPH radical into a colorless compound, and consequently, a decrease in absorbance at 517 nm.

The effectiveness of an antioxidant is often indicated by the extent of suppression of the DPPH radical, and a higher value suggests stronger antioxidant activity. The findings from the study illustrate the results of this assessment for different plant components. Among the tested plant extracts, the 80% aqueous ethanol extract of *E. chinense* demonstrated the highest radical

scavenging activity with an inhibition rate of 88.73% ± 1.31%. On the other hand, the benzene extract of *L. spinosa* showed the lowest activity, inhibiting the DPPH radical by only 1.50% ± 0.07%. This suggests that the components of *L. spinosa* were not very effective at neutralizing the DPPH radicals.

Notable anti-radical effects in the chloroform and acetone extracts of *E. chinense*, as well as the 80% aqueous ethanol extract of *E. floribunda* were also detected. These extracts exhibited considerable inhibition of the DPPH radicals, indicating their potential as antioxidants.

The observed differences in antioxidant activity among these plant extracts can be attributed to the presence of certain chemical constituents in the plants. Specifically, the plants with higher radical scavenging activity are likely to contain compounds with hydroxyl (OH) groups. These hydroxyl groups are capable of acting as effective radical scavengers. When these compounds interact with DPPH radicals, they donate hydrogen atoms or electrons, leading to the formation of a stable and colorless compound, which in turn results in reduced absorbance at 517 nm.

In summary, the assessment of anti-radical abilities using the DPPH radical scavenging test provides insight into the antioxidant potential of different plant extracts. The presence of hydroxyl groups in the compounds present in the plants contributes to their radical scavenging activity. The variations in inhibition rates among the tested plant extracts indicate their varying degrees of antioxidant effectiveness. These findings offer valuable information about the potential health benefits of these wild food plants, as antioxidants play a crucial role in neutralizing harmful free radicals and preventing oxidative damage to cells and tissues.

ABTS radical scavenging activity

Table 1 and **Table 2** provides insights into the ABTS scavenging activity across a range of extracts derived from five distinct wild food plants, as assessed using the ABTS test. This test involves observing the reduction of color intensity in ABTS due to the antioxidant properties of the tested samples of particular note, the 80% aqueous ethanol extract obtained from *E. chinense* showcased the most potent radical scavenging activity, boasting an impressive inhibition rate of 90.13±1.01%. This signifies that the components within this extract were highly efficient at neutralizing ABTS radicals, leading to a substantial reduction in color intensity. Following closely, *E. floribunda* exhibited significant antioxidant prowess with an inhibition rate of 79.35±2.86%, indicating its ability to effectively counteract ABTS radicals.

Conversely, the chloroform extract originating from *B. hatacoa* demonstrated the lowest activity, revealing an inhibition rate of merely 1.93±0.07%. This suggests that the components within this extract were less effective at scavenging ABTS radicals, resulting in a minimal reduction in color intensity.

Furthermore, the extracts of *E. chinense* prepared using benzene, chloroform, and acetone displayed robust radical scavenging properties. These extracts exhibited inhibition rates ranging from 60% to 73%. This range indicates that the components within these extracts were capable of neutralizing a significant portion of ABTS radicals, leading to substantial reductions in color intensity.

In summary, the data from the ABTS test sheds light on the diverse levels of radical scavenging activity exhibited by extracts from the five wild food plants. *E. chinense* and *E. floribunda* emerged as particularly strong contenders in terms of antioxidant potential, while the chloroform extract of *B. hatacoa* displayed the lowest activity. Additionally, various extracts of *E. chinense*, especially those obtained using benzene, chloroform, and acetone, demonstrated noteworthy radical scavenging capabilities within the 60% to 73% inhibition range. This implies their significant ability to counteract ABTS radicals and mitigate oxidative stress.

Metal chelating activity

Table 1 and **Table 2** presents the metal chelating activity as a percentage inhibition of metal ions for the various samples studied. Notably, the 80% aqueous ethanol extract derived from *E. chinense* displayed the highest chelating activity at 77.35±1.17%. This extract effectively inhibited the binding of metal ions, indicating its strong potential for chelation. Following closely was *E. floribunda*, with a chelating activity of 68.27±2.24%.

Table 2: Antioxidant activities of wild edible plants in different solvent extraction systems

Antioxidant parameters	Solvent	*L. spinosa*	*E. chinense*	*N. indicum*	*B. hatacoa*	*E. floribunda*
ABTS (% of inhibition)	Benzene	2.10±0.03^{d}	60.66±0.04^{e}	6.84±0.07^{b}	2.11±0.04^{d}	3.60±0.72^{c}
	Chloroform	5.88±0.01^{d}	64.09±0.01^{a}	7.08±0.05^{c}	1.93 ±0.07^{e}	10.62±2.13^{b}
	Acetone	6.65±0.44^{d}	73.62±0.51^{a}	7.36±0.01^{c}	5.04±0.32^{e}	11.29±1.52^{b}
	80 % Aq. ethanol	40.41±1.34^{d}	90.13±1.01^{a}	52.35±1.21^{c}	41.30±1.12^{d}	79.35±2.86^{b}
Reducing power (AAE, mg/100g DPM)	Benzene	15.18±0.95^{d}	48.89±0.41^{b}	9.22±0.45^{e}	36.92±0.63^{c}	70.94±2.48^{a}
	Chloroform	23.51±1.91^{d}	90.74±0.95^{a}	12.64±1.51^{e}	60.72±1.30^{c}	66.59±3.01^{b}
	Acetone	33.30±2.61^{d}	152.14±1.58^{a}	15.18±6.19^{e}	41.20±0.83^{c}	85.43±2.79^{b}
	80 % Aq. ethanol	55.76±1.49^{d}	232.93±1.34^{a}	45.63±1.86^{e}	68.04±3.14^{c}	164.09±5.38^{b}
Metal chelating activity (% of inhibition)	Benzene	4.18±0.11^{b}	21.33±0.19^{a}	2.66±0.07^{c}	2.56±0.07^{c}	2.79±0.55^{c}
	Chloroform	7.13±0.11^{c}	58.88±0.21^{a}	5.87±0.04^{d}	4.48 ±0.04^{e}	11.69±1.82^{b}
	Acetone	9.24±0.09^{d}	69.57±0.18^{a}	8.32±0.07^{e}	10.22±0.08^{c}	18.35±2.03^{b}
	80 % Aq. ethanol	21.51±0.39^{d}	77.35±1.17^{a}	19.77±1.24^{e}	32.55±2.33^{c}	68.27±2.24^{b}
Lipid peroxidation assay (% of inhibition)	Benzene	2.21±0.10^{c}	24.55±0.34^{a}	2.01±0.11^{c}	1.37±0.17^{d}	3.77±0.28^{b}
	Chloroform	6.23±0.08^{c}	39.66±0.55^{a}	4.16±0.04^{d}	3.18 ±0.04^{e}	8.49±0.73^{b}
	Acetone	8.38±0.12^{d}	51.49±0.11^{a}	6.12±0.37^{e}	11.12±0.22^{c}	17.44±1.29^{b}
	80 % Aq. ethanol	36.41±1.52^{c}	79.32±1.46^{a}	17.62±1.39^{e}	24.34±1.42^{d}	69.76±3.54^{b}

Each value in the table was obtained by calculating the average of three experiments and data are presented as Mean ± Standard error of the mean (SEM). Statistical analysis were carried out by Tukeys test at a 95% confidence level and statistical significance was accepted at the $p < 0.05$ level. The superscript letters a,b,c,d and e denote the significant differences within the same parameters of individual solvent extract among the plants

In comparison to the other wild edible plants investigated, the chloroform and acetone extracts of *E. chinense* exhibited promising metal chelating activity. These extracts demonstrated significant capability in preventing metal ions from binding.

Metal ions can play a role in initiating lipid peroxidation, a process that triggers a chain reaction leading to the deterioration of food products [26]. Additionally, metal ion catalysis has been linked to conditions like cancer and arthritis [27]. In contexts of lipid peroxidation and oxidative degradation of proteins, ferrous ions facilitate the conversion of hydrogen peroxide to hydroxyl radicals through processes like the Fenton reaction. Ferrous ions are particularly potent pro-oxidants and are often present in food systems [28]. It's important to note that the chelating capacity of the plant extracts exhibited a trend where decreased polarity correlated with reduced chelating capacity. This pattern aligns with what has been observed for reducing and radical scavenging properties in similar studies. The chelating ability observed in the methanol extracts can be attributed to their high content of phenols and flavonoids. Polyphenols with dihydroxy groups are known to form complexes with metals, limiting the generation of metal-catalyzed free radicals [29].

In summary, **Table 1** and **Table 2** highlights the metal-chelating activity of the studied plant extracts. *E. chinense* and *E. floribunda* extracts showcased notable chelating potential, with the former displaying the highest activity. The ability of these extracts to chelate metal ions is of significance in preventing processes like lipid peroxidation and metal ion catalysis, which can have implications for food preservation and health outcomes. The observed trends in chelating capacity based on polarity and the role of polyphenols provide insights into the mechanisms underlying these antioxidant properties.

Lipid peroxidation assay

Table 1 and Table 2 provides the outcomes of a lipid peroxidation assay conducted using various plant extracts, with results expressed as percentage inhibition. Notably, the 80% aqueous

ethanol extract from *E. chinense* exhibited the highest efficacy in the anti-lipid peroxidation assay, displaying an inhibition rate of 79.32±1.46%. Following closely was *E. floribunda* with an inhibition rate of 69.76±3.54%.

Moreover, the acetone extracts from these two plants also demonstrated notable effectiveness in inhibiting lipid peroxidation, surpassing the inhibitory effects of other plant extracts. When compared to the effects of other plant extracts, the 80% aqueous ethanol extract emerged as particularly potent in inhibiting lipid peroxidation.

Lipid peroxidation is recognized as a significant contributor to the degradation of lipid food quality, leading to the development of rancid and stale flavours [30]. Synthetic antioxidants like BHA are incorporated into foods during processing to counteract lipid peroxidation, thereby enhancing food quality and stability. Additionally, lipid peroxidation within cell membranes has been linked to a range of clinical disorders including atherosclerosis, inflammation, and liver injury [31].

Phenolic compounds and other chemical components may employ various molecular mechanisms to curtail lipid peroxidation, such as free radical quenching, electron transfer, radical addition, and radical recombination [32]. The connection between total phenolic content and peroxidation activity is underscored by the observation that the 80% aqueous ethanol extract from all the studied plants, possessing the highest phenolic content, also exhibited the most robust anti-lipid peroxidation effect. This finding aligns with prior research on fruits and vegetables, which has consistently demonstrated a substantial correlation between total phenolic content and peroxidation activity [33][34].

To summarize, the results provided in **Table 1** and **Table 2** elucidate the outcomes of a lipid peroxidation assay using various plant extracts. The 80% aqueous ethanol extract from *E. chinense* showcased the greatest potential in inhibiting lipid peroxidation, a process crucial for preserving lipid-based food quality. This finding resonates with the critical role of phenolic content in the anti-lipid peroxidation effect and underscores the potential of these plant extracts as natural sources of antioxidants to enhance food stability and counteract lipid-related health disorders.

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

Table 3 provides a comprehensive overview of the quantities of phenolic acids, including 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, as well as flavonoids such as catechin, rutin, myricetin, quercetin, naringin, naringenin, apigenin, and kaempferol. These components were present in the 80% aqueous ethanol extracts of the investigated plants. The quantification was achieved using High-Performance Liquid Chromatography (HPLC), and the results are expressed in micrograms (µg) per milligram (mg) of dry extract.

The successful separation and identification of these compounds were enabled by their sensitivities to the 280 nm wavelength, allowing for accurate analysis. The absorption spectra recorded during the analysis were uniform for both the plant extracts and the standard reference materials. This consistency in absorption spectra was crucial in confirming the identity of the compounds under investigation.

Gallic acid, whether in its free form or as an ester, remains within the plant and functions as an antioxidant. The HPLC study conducted unveiled the presence of gallic acid in varying quantities across the investigated plants. Notably, *E. floribunda* exhibited a notably high concentration of gallic acid (30.78±1.67), while *N. indicum* displayed the lowest amount (0.28±0.09). In *L. Spinosa* (2.99±0.48) and *E. chinense* (1.74±0.33), the levels of gallic acid were comparable to those found in common 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) [35]. This data underscores the significant presence of gallic acid in the investigated plants, with *E. floribunda* standing out as having an especially high content. It's worth noting that gallic acid is recognized for its antioxidant properties, which contribute to the plant's potential health benefits.

Protocatechuic acid is a notable type of phenolic acid that is abundantly present in nature. This compound shares structural similarities with other well-recognized antioxidant compounds such as

Table 3: Quantification of phenolics and polyphenolic compounds in the 80% aq. ethanol extract of wild edible plants by HPLC

Phenolic acids/Flavonoids	Phenolics and polyphenolic compounds by HPLC µg/mg plant extract				
	L. spinosa	*E. chinense*	*N. indicum*	*B. hatacoa*	*E. floribunda*
Gallic acid	2.99±0.48	1.74±0.33	0.28±0.09	0.47±0.07	30.78±1.67
Protocatechuic acid	ND	0.01±0.002	0.24±0.07	0.30±0.09	0.46±0.05
Gentisic acid	ND	ND	ND	0.12±0.03	ND
p-Hydroxy benzoic acid	ND	ND	ND	-	ND
Catechin	ND	0.82±0.08	0.86±0.06	-	0.60±0.05
Chlorogenic acid	0.21±0.06	0.07±0.004	1.46±0.72	0.07±0.006	1.63±0.29
Vanillic acid	0.09±0.007	ND	0.09±0.001	ND	2.05±0.34
Caffeic acid	0.76±0.09	0.14±0.07	0.03±0.002	0.01±0.003	1.09±0.49
Syringic acid	0.16±0.06	0.36±0.09	1.76±0.67	0.18±0.06	32.03±1.89
p-Coumaric acid	0.09±0.005	1.85±0.67	0.14±0.03	0.06±0.007	1.94±0.29
Ferulic acid	0.04±0.002	0.86±0.08	1.77±0.52	1.27±0.29	2.37±0.19
Sinapic acid	0.30±0.06	1.16±0.33	0.07±0.002	0.09±0.001	0.09±0.002
Salicylic acid	1.20±0.38	0.06±0.004	ND	ND	ND
Naringin	0.11±0.03	0.02±0.007	0.02±0.006	ND	ND
Rutin	ND	0.15±0.08	0.23±0.02	ND	ND
Ellagic acid	ND	0.29±0.06	0.26±0.07	0.07±0.003	ND
Myricetin	0.03±0.003	0.63±0.08	0.09±0.006	0.11±0.06	0.99±0.07
Quercetin	2.92±0.69	11.10±1.78	1.53±0.34	ND	0.01±0.0002
Naringenin	0.55±0.09	4.50±0.78	0.47±0.08	0.10±0.003	0.35±0.06
Apigenin	1.94±0.78	4.03±0.29	0.47±0.08	0.19±0.04	0.25±0.06
Kaempferol	1.84±0.05	3.69±0.79	1.01±0.38	0.12±0.02	0.39±0.05

Each value in the table was obtained by calculating the average of three experiments and data are presented as Mean ± Standard error of the mean (SEM). Statistical analysis were carried out by Tukeys test at a 95% confidence level and statistical significance was accepted at the $p < 0.05$ level.

gallic acid, caffeic acid, vanillic acid, and syringic acid. In the context of the study, the 80% aqueous ethanol extracts from *E. floribunda*, *B. hatacoa*, and *N. indicum* exhibited significant quantities of protocatechuic acid, measuring approximately 0.46±0.05, 0.30±0.09, and 0.24±0.07 µg/mg respectively.

The substantial presence of protocatechuic acid in these plant extracts is likely a contributing factor to their robust antioxidant attributes. These properties have the potential to play a role in both the prevention and treatment of ailments associated with oxidative stress, including conditions such as neurodegenerative and hepatic diseases [36]. Catechins, a class of flavanols, are found in a variety of plant-derived foods and beverages. Among the plants studied, *N. indicum* exhibited the highest content of catechin at a level of 0.86±0.06, closely followed by *E. chinense* (0.82±0.08), and *E. floribunda* (0.60±0.05). This suggests that *N. indicum* in particular may play a significant role in contributing to its healing and cell-strengthening properties [37]. Chlorogenic acid is a compound found in coffee, coffee beans, and various higher plants. It's an ester of caffeic acid and quinic acid. Studies have shown that it possesses anti-diabetic effects and can contribute to the reduction of blood sugar levels [38]. In our study, substantial quantities of chlorogenic acid were identified, particularly in *E. floribunda* (1.63±0.29) and *N. indicum* (1.46±0.72). The presence of these significant amounts of chlorogenic acid in these plants suggests their potential to contribute to the reduction of blood sugar levels [39].

This aligns with previous research highlighting the association between the consumption of vegetables rich in chlorogenic acid and the potential benefits of managing blood sugar levels.

Vanillic acid, a flavouring compound, was discovered in significant quantities in *E. floribunda*

(2.05±0.34) compared to the other plants examined. Notably, vanillic acid has demonstrated hepatoprotective properties in scenarios such as concanavalin A-induced liver damage [40].

The HPLC analysis conducted suggests a link between the investigated plant, *E. floribunda*, and its potential hepatoprotective properties. The substantial presence of vanillic acid within this plant hints at its possible role in offering protection to the liver, as evidenced by prior research on its hepatoprotective effects.

Caffeic acid, a potent antioxidant, exhibits the capacity to enhance immunity, regulate blood lipid levels, and provide protection against mutagenic agents. This hydroxycinnamic acid stands as a prominent constituent within the wine and is frequently present as an ester, similar to chlorogenic acid. It finds its primary occurrence in fruits, vegetables, and herbs.

Recent research has indicated that *E. floribunda* leaves contain a substantial concentration of caffeic acid (1.09±0.49 µg/mg). This concentration bears similarity to levels found in other foods such as lettuce (1.57 µg/mg), carrot (0.09 µg/mg), cauliflower (0.058 µg/mg), and potato (2.80 µg/mg) [41]. Furthermore, the 80% aqueous ethanol extract of *E. floribunda* notably boasts a high content of syringic acid (32.03±1.89 µg/mg). Renowned for its potential anti-cancer, anti-proliferative, and hepatoprotective effects, syringic acid proves to be a significant bioactive compound [42]. Similarly, other plant species like *N. indicum* (1.76±0.67) and *E. chinense* (0.36±0.09) have also exhibited elevated levels of syringic acid.

These findings underscore the potential health benefits associated with the consumption of Eupatorium floribunda and other plants rich in both caffeic and syringic acids. These bioactive compounds contribute to the plants' valuable properties and hold promising implications for human health.

p-Coumaric acid, recognized for its antioxidant properties, is present in a variety of foods like barley, peanuts, navy beans, tomatoes, and carrots. It's notable for its ability to hinder the formation of carcinogenic nitrosamines in the stomach [43]. Remarkably, the highest content of p-coumaric acid was discovered in *E. floribunda* (1.94±0.29 µg/mg), closely trailed by *E. chinense* (1.85±0.67 µg/mg).

Ferulic acid, a prominent phenolic compound identified in our study, demonstrated concentrations spanning from 0.04±0.002 to 2.37±0.19 µg/mg in the plant. This compound is renowned for its diverse physiological functions, encompassing antimicrobial, anti-inflammatory, antidiabetic, and anti-cancer attributes [44]. Moreover, it contributes to the reduction of serum cholesterol levels and the enhancement of sperm viability. In terms of ferulic acid concentration, *E. floribunda* displayed the highest levels, followed sequentially by *N. indicum* and *B. hatacoa*.

Sinapic acid emerges as a multifaceted natural molecule, imbued with antioxidant, antimicrobial, anti-inflammatory, anticancer, and anti-anxiety properties. Within the ambit of our investigation, every plant assessed exhibited a noteworthy content of sinapic acid, encompassing concentrations ranging from 0.09±0.001 to 1.16±0.33 µg/mg. This observation underscores the potential human benefits accrued from the consumption of wild edible plants rich in sinapic acid [45].

Rutin, a flavonol compound, unfolds with a spectrum of biological effects, including antidiabetic [46] and anticancer activities [47], thus positioning it as a valuable medicinal entity. Evidently, *E. chinense* (0.15±0.08) and *N. indicum* (0.23±0.02) both contain substantial rutin levels, suggesting their potential utility as therapeutic agents.

Ellagic acid, categorized as a phenolic antioxidant, finds its presence across an extensive array of fruits and vegetables. *E. chinense* stands out with the highest ellagic acid content (0.29±0.06 µg/mg dry extract). Notably, *N. indicum* and *B. hatacoa* also exhibit noteworthy concentrations of ellagic acid, suggesting potential medicinal attributes encompassing anti-cancer and anti-heart disease properties, among others [45]. Myricetin, a flavonoid sourced from plants, is renowned for its nutraceutical qualities and plays a pivotal role in numerous culinary creations and beverages. As per high-performance liquid chromatography (HPLC) analysis, myricetin content spans from 0.03±0.003 to 0.99±0.07 µg/mg dry extract in the investigated plants. *E. floribunda* and *E. chinense*, both characterized by substantial myricetin

concentrations within their 80% aqueous ethanol extracts, potentially offer an array of effects encompassing robust antioxidant, anticancer, antidiabetic, and anti-inflammatory attributes. Moreover, these plants might hold promise in mitigating illnesses such as Parkinson's and Alzheimer's [48].

Quercetin is widely acknowledged for its antioxidant prowess, which underpins its documented anti-cancer, anti-histamine, and anti-inflammatory attributes [49]. Remarkable sources of quercetin encompass citrus fruits, apples, onions, parsley, sage, tea, and red wine. Notably, among the plants examined, *E. chinense* was the sole one boasting a significant quercetin content (11.10±1.78) as indicated by HPLC analyses.

In the realm of flavonoids, naringin, a flavanone 7-O-glycoside found in grapes and citrus fruits, has been a subject of interest. Research has suggested that naringin supplementation could potentially offer benefits for obesity, diabetes, hypertension, and metabolic syndrome [50]. Our study revealed that *L. spinosa* contains a noteworthy amount of naringin (0.11±0.03 µg/mg dry extract).

Naringenin, another flavonoid, emerges with an impressive array of effects, encompassing antioxidant, anti-fibrogenic, anti-inflammatory, and anti-cancer properties. Furthermore, it exerts protective effects on the liver against various substances. HPLC analysis unveiled *E. chinense* as the higher in naringenin content (4.50±0.78 µg/mg dry extract). Moreover, *L. spinosa* (0.55±0.09 µg/mg dry extract) and *N. indicum* (0.47±0.08 µg/mg dry extract) also exhibited substantial naringenin concentrations. These observations suggest that these plants, hosting this flavonoid, could play a role in safeguarding against liver disorders [51].

Apigenin, a flavonoid distributed among various fruits and vegetables like parsley, chamomile, celery, and kumquats, has garnered a reputation as a potential cancer chemopreventive agent in recent years. Through its influence on cellular responses to oxidative stress and DNA damage, inhibition of inflammation and angiogenesis, regulation of cell proliferation, and promotion of self-digestion mechanisms, apigenin's anticarcinogenic effects have been validated across numerous studies [52]. Among the plants examined, *E. chinense* held the highest concentration of apigenin (4.03±0.29 µg/mg dry extract), followed by *L. Spinosa* (1.94±0.78 µg/mg dry extract).

Table 4: Correlation between TPC & DPPH, TPC & ABTS, TFC & DPPH, TFC &ABTS, TFLC & DPPH, TFLC & ABTS using different solvent extracts of wild edible plants

	TPC X DPPH			TPC X ABTS		
Solvent	r	R^2	Equation	r	R^2	Equation
Benzene	0.759	0.576	y = 0.5893x - 11.794	0.736	0.541	y = 1.1229x - 22.525
Chloroform	0.693	0.480	y = 0.2731x - 11.914	0.691	0.478	y = 0.3085x - 11.541
Acetone	0.802	0.644	y = 0.1606x - 10.401	0.785	0.616	y = 0.206x - 11.997
80 % aq. ethanol	0.984	0.969	y = 0.1023x + 0.9743	0.933	0.871	y = 0.0627x + 32.876
	TFC X DPPH			TFC X ABTS		
	r	R^2	Equation	r	R^2	Equation
Benzene	0.453	0.205	y = 0.2481x - 4.3104	0.382	0.146	y = 0.4121x - 5.2671
Chloroform	0.446	0.199	y = 0.2564x - 4.0422	0.438	0.192	y = 0.2851x - 2.3213
Acetone	0.767	0.589	y = 0.2302x - 9.7916	0.742	0.551	y = 0.2918x - 10.838
80 % aq. ethanol	0.908	0.824	y = 0.1868x + 3.5001	0.902	0.813	y = 0.1199x + 33.15
	RP X DPPH			RP X ABTS		
	r	R^2	Equation	r	R^2	Equation
Benzene	0.338	0.114	y = 0.1745x + 1.6098	0.257	0.0668	y = 0.2622x + 5.5617
Chloroform	0.713	0.508	y = 0.5085x - 11.688	0.691	0.477	y = 0.5581x - 10.455
Acetone	0.917	0.841	y = 0.377x - 9.5131	0.907	0.823	y = 0.4892x - 11.226
80 % aq. ethanol	0.975	0.951	y = 0.4195x - 1.0898	0.949	0.902	y = 0.2639x + 30.814

Kaempferol, another flavonoid, has exhibited the capacity to avert the oxidation of low-density lipoproteins, suggesting potential anti-atherogenic properties. Research by Calderon-Montao *et al*. has proposed that a diet rich in kaempferol correlates with a reduced risk of stomach cancer [53]. Notably, *E. chinense* contains a substantial quantity of kaempferol (3.69±0.79 µg/mg dry extract), implying that incorporating this plant into one's diet could potentially offer protective benefits.

Correlation analysis

The correlation coefficient (r) and coefficient of determination (R^2) between antioxidant activities (DPPH and ABTS) and polyphenols (TPC, TFC, and TFLC) from four distinct solvent extracts of wild edible plants were investigated using simple linear regression. When comparing solvents, 80 % aq. ethanol showed the strongest connection (r and R^2) between polyphenols (TPC, TFC, and TFLC) and antioxidant activity (DPPH and ABTS), followed by acetone, whereas chloroform and benzene extracts had a smaller association (**Table 4**).

The extraction of specific groups of antioxidant chemicals and their subsequent antioxidant activity can be affected by changes in solvent polarity [54]. Table 4 shows that antioxidant activity tests (DPPH and ABTS) were substantially linked with TPC, TFC, and TFLC of 80 % aq. ethanol extracts, followed by acetone and chloroform extracts. Higher polyphenols (TPC, TFC, and TFLC) were produced by these solvents, which are the key contributors to overall antioxidant activity. These findings are consistent with prior research, that found a strong link between polyphenols and antioxidant activity [55][56]. Acetone and chloroform, on the other hand, had a weak to poor connection, which is consistent with their polyphenolic yields.

Principal component analysis

Principal component analysis (PCA) was used on the combined TPC, TFC, ABTS, RP, MC, LP, TFLC, rutin, myricetin, apigenin, kaempferol, gallic acid, protocatechuic acid, catechin, syringic acid, p-coumaric acid, ferulic acid profile to better distinguish between the plants under consideration (**Figure 1**).

All of the plant samples' PCA score plots and their corresponding loading plots are shown in **Figure 1**. Even though the PCA results provided three or four principal components (PC) with eigenvalues greater than one, only the first two PCs were retained to simplify the analysis of the results. Based on all variables, the first two PCs explained 76.0 % of the total variance (Figure 1), with PC1 (51.0 %) explaining 2.04 times as much as PC2 (25.0 %).

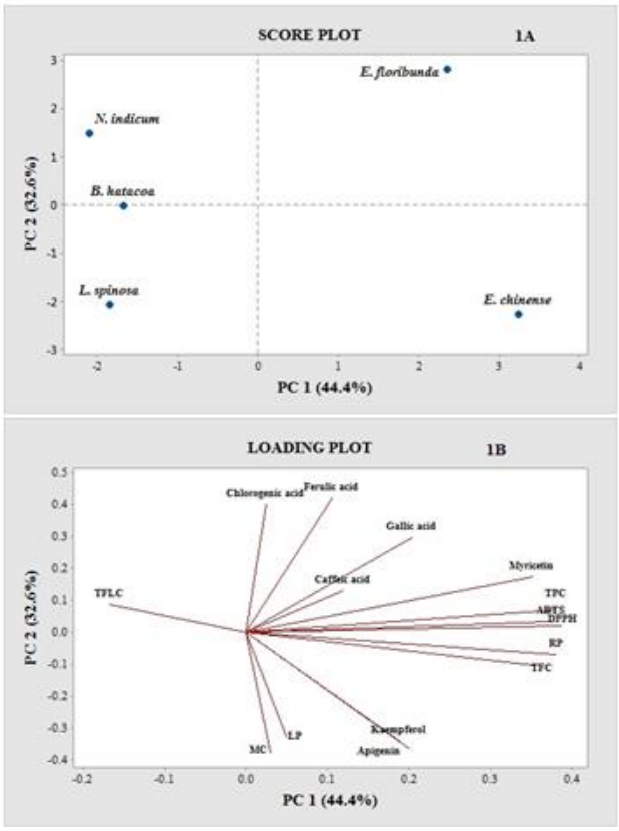

Figure 1: Principal component analysis using variables listed in Tables 1 and Table 2.

Score plot (1A) and loading plot (1B) of first two principal components for clustering of plant samples. Variables: 18 (TPC, TFC,TFLC, RP, DPPH, ABTS, MC, LP, Gallic acid, Protocatechuic acid, Syringic acid, p-Coumaric acid, Ferulic acid, Catechin, Rutin, Myricetin, Apigenin, Kaempferol)

PC1 was found to be positively linked with the variables TPC, ABTS, RP, MC, LP, TFLC, myricetin, apigenin, gallic acid, catechin, syringic acid, p-coumaric acid, and ferulic acid in figure 1B. TPC, TFC, TFLC, DPPH, gallic acid, and ferulic acid were all negatively connected with PC2, while the remainder of the variables were positively correlated. Because of the high concentrations of TPC, TFC, TFLC, DPPH, ABTS, RP, and polyphenolic compounds, *E. chinense* and *E. floribun*da were separated and were distant from all other samples

on the right side. *E. chinense* was shown to be more powerful than *E. floribunda*, *L. spinosa, B. hatacoa*, and *N. indicum* in terms of phenolics and polyphenolics concentration.

CONCLUSION

The 80% aqueous ethanol extract of E. chinense has been identified as having the highest concentration of phenolic compounds, which contribute to its remarkable radical scavenging activity, as per research findings. In addition to E. chinense, flavonoids and flavonols were found to exhibit substantial radical scavenging activity in the benzene, chloroform, and acetone extracts of various plant species when assessed using both the ABTS and DPPH methods. However, it's important to note that despite the positive antioxidant properties of these plant extracts, they are not as potent as synthetic antioxidants like BHT and trolox, which are commercially available. The key difference is that synthetic antioxidants like BHT and trolox are more effective at neutralizing free radicals, but they are also associated with potential toxicity concerns. This is where the significance of these wild edible plants lies in the pharmaceutical industry. Plant extracts are generally considered to be safer and more natural sources of antioxidants when compared to synthetic alternatives. Given the limited use of synthetic antioxidants due to concerns about their potential adverse effects on health, these wild edible plants represent a promising avenue for the development of antioxidant supplements and additives. They can potentially play a crucial role in mitigating oxidative stress-related disorders, which are associated with various health issues, including chronic diseases. In summary, the importance of these plant extracts in the pharmaceutical industry lies in their potential to provide a safer and more natural alternative to synthetic antioxidants. This can lead to the development of antioxidant supplements and additives that have a positive impact on health without the potential toxicity concerns associated with synthetic antioxidants. Further research and development in this area could pave the way for the integration of these plant extracts into pharmaceutical and health-related products.

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Conflict of Interest

The authors declare no conflict of interest, financial or otherwise.

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