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Wild Edible Plants: Antioxidant Activities in Different Solvents and Quantification of Phenolic Compounds by HPLC

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Wild edible plants (WEPs) are rich in antioxidants and diverse sections of the plant had been notably utilized as conventional and folklore medicine to cure various human ailments. This study was designed to evaluate the most effective solvent for extracting polyphenols from medicinally important WEPs which includes *Coix lacryma-jobi*, *Herpetospermum pedunculosum*, *Plukenetia corniculata*, *Sonchus asper* and *Streptolirion volubile*, which allows to offer medical guide for conventional use of the plant. Individual phenolic component concentrations were determined using RP-HPLC and total phenolic, total flavonoid and total flavonol were quantified using four different solvents. Aqueous ethanol (80%) is most effective solvent for polyphenol extraction amongst solvents of diverse polarities. The studied plants were found to be rich in total phenolics, flavonols, especially gallic acid (1.12 ± 0.33 µg/mg dry extract) and ferulic acid (4.03 ± 0.53 µg/mg dry extract) in the 80% aq. ethanol extract of *H. pedunculosum*. The correlation analyses of each solvent found out sturdy to susceptible connections amongst all examined parameters, with the best values (r and R^2) in 80% aq. ethanol and chloroform, indicating that those solvents have an excessive ability for polyphenol extraction and antioxidant activity. The Principal component analysis revealed that based on the phenolics and polyphenolics content, the 80% aq. ethanol extract of *H. pedunculosum* was shown to be more potent than the other plants under investigation. Present findings display that aqueous ethanol extracts of the studied plants have better antioxidant activities than synthetic derivatives, indicating their prospective usefulness and ability to replace synthetic derivatives in consumable and medical products.

Keywords: HPLC, Ethanol, Phenolic compounds, *C. lacryma-jobi*, *H. pedunculosum*, *P. corniculata*, *S. asper*, *S. volubile*.

INTRODUCTION

Free radicals are formed as a result of oxidation, a chemical reaction in which electrons are lost. It cause many chronic diseases in humans, including atherosclerosis, Alzheimer's disease, arthritis, Parkinson's disease, stroke, chronic inflammatory disorders, malignancies and other degenerative diseases. Antioxidants are compounds that help prevent or reduce cell damage caused by free radicals, which are unstable molecules produced by the body in response to environmental and other stresses [1,2]. Antioxidants can come from both natural and synthetic sources. Certain plant based diets are known to be high in antioxidants.

Active compounds with a wide range of chemical characteristics abound in plant materials. Active components found in herbal plants, vegetables and fruits include isoflavones, flavones, lignans, phenolic compounds, flavonoids, anthocyanin, coumarin, catechins and isocatechins, which have been shown to have a variety of biological effects, including antioxidant activity [3]. Antioxidants formed from plant materials prevent free radicals from causing harm to the body, protecting it from a number of illnesses.

The antioxidant activities of plants are influenced by the polarity of the solvents and plant sections used for complete extraction of active components. Solvents like methanol, ethanol, acetone, chloroform, ethyl acetate and water have been used to extract antioxidant compounds from various plants and plant based foods and medications. A suitable solvent system provides for the most effective extraction of target molecules while preserving their chemical properties. It's also been noted that polar rather than nonpolar solvents produce the best polyphenol extraction. As a result, plant materials are commonly extracted using water and organic solvents (methanol, ethanol, acetone and chloroform) [4]. Furthermore, these solvents can be employed

alone or in combination, for example, water and an aqueous mixture of methanol and ethanol can be used to increase polyphenol production [5]. The results show that polyphenol yield is influenced by the type and polarity of extraction solvents, as well as the physical characteristics of plant materials [6]. To yet, no specific or acceptable solvent has been indicated for maximizing plant phenolic production, especially in wild edible plants. Because of the wide range of quality of plant extracts and the chemistry of edible plants, choosing the right solvent is crucial for optimizing the extraction process. The goal of this study was to determine the efficacy of different solvents for polyphenol extraction and subsequent antioxidant activities of wild edible plants such as *Coix lacryma-jobi*, *Herpetospermum pedunculosum*, *Plukenetia corniculata*, *Sonchus asper* and *Streptolirion volubile*, which are consumed by ethnic people in Arunachal Pradesh, India.

EXPERIMENTAL

The investigated wild edible plants (WEPs) namely *Coix lacryma-jobi* L. (Poaceae), *Herpetospermum pedunculosum* (Ser.) C.B.Clarke (Cucurbitaceae), *Plukenetia corniculata* Sm. (Euphorbiaceae), *Sonchus asper* (L.) Hill (Asteraceae) and *Streptolirion volubile* Edgew. (Commelinaceae) were obtained from several markets of Arunachal Pradesh state, India and authenticated. The collected plant materials were preserved under registry numbers BSITS 111, BSITS 110, BSITS 115, BSITS 112, BSITS 116, respectively in Botanical Survey of India. For further extraction, the plant components were shed-dried, crushed and stored in an air-tight container.

Preparation of plant extracts: Each powdered plant (100 g) was extracted twice with 80% aq. ethanol at room temperature, each time with agitation for 18-24 h. The first and succeeding extractions' concentrates were mixed and concentrated in a rotary evaporator at reduced pressure to produce viscous extracts, which were then dried with a freeze drier. Acetone, chloroform and benzene extracts were also prepared in this method. The dried extracts from each solvent were stored at –20 ºC. The weight of air dried plant material was used to compute the% yield.

Total phenolic content (TPC) estimation: Total phenolic content in crude extracts was determined using the Folin-Ciocalteu method [7]. In test tubes, 100 µL of each of the examined extracts were taken. It was mixed with 1.0 mL Folin-Ciocalteu reagent and 0.8 mL sodium carbonate (7.5%). After allowing the reaction mixture to stand for 30 min, the absorbance at 765 nm was measured (UV-visible spectrophotometer Shimadzu UV 1800). The total phenolic content of dry plant material was calculated as gallic acid equivalents (GAE) (mg GAE/100 g) using the equation: $y = 0.0013x + 0.0498$, $R^2 = 0.999$, where y was the absorbance and x is the Gallic acid equivalent (mg/100 g).

Total flavonoids content (TFC) estimation: The method of Ordonez *et al.* [8] was used to determine total flavonoids in the investigated plants. In a test tube, 0.5 mL $AlCl_3$ ethanol solution (2%) was added to 0.5 mL of extracts. The absorbance of mixture was measured at 420 nm after 1 h 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/100 g): $y = 0.0182x - 0.0222$, $R^2 = 0.9962$, where y is the absorbance and x is the rutin equivalent (mg/100 g).

Total flavonols content (TFLC) estimation: Kumaran & Karunakaran's method [9] was applied to quantify the total flavonols in plant extracts. In a test tube, 2.0 mL 2% $AlCl_3$ ethanol and 3.0 mL (50 g/L) sodium acetate solutions were added to 2.0 mL extracts. After 2.5 h at 20 ºC, the absorption at 440 nm was measured with a Shimadzu UV 1800 UV-visible spectrophotometer. The following equation, based on the calibration curve, was used to compute total flavonol content as quercetin (mg/100 g): $y = 0.0049x + 0.0047$, $R^2 = 0.9935$, where y is the absorbance and x is the quercetin equivalent (mg/100 g).

Reducing power analysis: The reducing power of the plant extracts was determined using Oyaizu's method [10]. In a test tube, 2.5 mL phosphate buffer (0.2 M, pH 6.6) and equal volume of 1% potassium ferricyanide were added to 100 µL plant extracts. At 50 ºC, the reaction mixture was incubated for 20 min. The mixture was then centrifuged at 3000 rpm for 10 min after aliquots of 10% trichloroacetic acid (2.5 mL) were added. The solution's upper layer (2.5 mL) was mixed with equal volume of distilled water and freshly made $FeCl_3$ solution (0.5 mL, 0.1%) was added to it. At a wavelength of 700 nm, the absorbance of reaction mixture was determined. The following equation was used to calculate reducing power in ascorbic acid equivalent (AAE) as $y = 0.0023x - 0.0063$, $R^2 = 0.9955$ where x is the ascorbic acid equivalent (mg/100 g) and y is the absorbance.

DPPH free radical scavenging activity: The stable radical DPPH (1,1-diphenyl-2-picrylhydrazyl) was used to test the free radical scavenging activity of the plant extracts [11]. In each test tube, 100 µL of the examined extracts were taken, then 3.9 mL of freshly prepared DPPH solution (25 mg/L) in methanol was added and stirred. The absorbance was measured at 517 nm after 30 min (UV-visible spectrophotometer Shimadzu UV 1800). Using the following equation, the ability to scavenge the DPPH radical is calculated as

$$\text{Scavenging ability of DPPH (\%)} = \frac{A_c - A_t}{A_c} \times 100$$

where A_c represents the absorbance of control reaction and A_t denotes the absorbance in presence of the extracts sample. Antioxidant activity of the extracts was expressed as IC_{50} which was calculated as the quantity in mg dry extract that prevents 50% of DPPH radical production.

ABTS radical scavenging activity: The radical scavenging activity of 2,2′-azino-*bis*(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation ($ABTS^{\bullet+}$) was determined using the method described by Re *et al.* [12]. The absorbance at 734 nm was adjusted to 0.70 ± 0.02 by diluting the solution with ethanol. To examine the scavenging activity, 1 mL of diluted $ABTS^{\bullet+}$ solution was added to 100 µL of plant extract and the absorbance at 734 nm was measured after 15 min. The following equation was used to compute the percentage of inhibition:

$$\text{ABTS scavenged (\%)} = \frac{A_c - A_t}{A_c} \times 100$$

here, A_c and A_t are the absorbencies of the control and test extracts, respectively.

Metal chelating activity: Lin *et al.* [13] procedure was followed for determining the metal chelating activity. The fraction of ferrozine–Fe^{+2} complex forms that were inhibited was estimated using the formula below:

$$\text{Chelating power } (\%) = \frac{A_c - A_t}{A_c} \times 100$$

where A_c denotes the absorbance of the control reaction and A_t represents the absorbance of the extracts

Lipid peroxidation assay: Anti-lipid peroxidation was measured using Amabye's technique with certain modifications [14]. All of the reagents were kept aside from the extract in a negative control. The folowing formula was used to calculate peroxidation inhibition:

$$\text{Lipid peroxidation inhibition } (\%) = \frac{A_c - A_t}{A_c} \times 100$$

where A_c denotes the absorbance of the control reaction and A_t signifies the extracts' absorbance.

Phenolic acids and flavonoids quantification by HPLC

HPLC equipment: HPLC experiments were carried out on a Dionex Ultimate 3000 liquid chromatograph with a diode array detector (DAD) and a 5 cm flow cell, as well as a Chromeleon system manager as a data processor. A reversed-phase Acclaim C18 column (5 micron molecular size, 250 4.6 mm) was used to separate the samples. Sample test (20 µL) was introduced into the HPLC column.

Standard solutions: To prepare a stock solution (1 mg/mL), the standard phenolics 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. Diluting the standard solution with the mobile phase solvent system yielded the working solutions. Before introducing the standard and working solutions into the HPLC apparatus, they were filtered using a 0.45 µm PVDF-syringe filter.

Estimation of phenolic acids and flavonoids by HPLC: HPLC analysis was used to quantify the phenolic acids and flavonoids in an 80% aq. ethanol extract of the examined plants, following the method as described by Datta *et al.* [15]. This study used a Dionex Ultimate 3000 liquid chromatograph with a diode array detector (DAD) and a 5 cm flow cell, as well as a Chromeleon system manager as a data processor. A reversed phase Acclaim C18 column was used to separate the samples (5 µ particle size, 250 × 4.6 mm). The HPLC column was filled with 20 µL of sample. According to USP and ICH requirements, the approach was validated. The mobile phase consists of methanol (solvent A) and 0.5% aq. acetic acid solution (solvent B) and the column was held at a constant temperature of 25 ºC with a 20 µL injection volume. The ratio of solvent A to solvent B was varied to achieve a gradient elution. Per sample, the total analysis time was 105 min. A photo diode array UV detector was used to detect HPLC chromatograms at three different wavelengths (272, 280 and 310 nm). The retention period of each chemical was determined by spiking with standards under the same conditions. The integrated peak area was used to quantify phenolic acids and flavonoids in the extracts and the contents were determined using a calibration curve that plotted peak area against concentration of the corresponding standard sample. The data were presented in triplicate with a convergence limit.

Statistical analysis: The data was analyzed using triplicate samples and the results were provided as mean standard error mean (SEM). To evaluate the differences and identify the plants with similar characteristics in relation to their TPC, TFC, TFLC, radical scavenging activities, phenolic acids and flavonoid content, one-way analysis of variance (ANOVA) followed by Tukey test ($p \leq 0.05$), correlation analyses ($p < 0.05$) among different parameters were also performed using both correlation coefficient (r) and coefficient of determination (R^2) and Principal Component Analysis (PCA) were used. SPSS software (version 11.0 for Windows) was used to conduct statistical analysis.

RESULTS AND DISCUSSION

Extractive value: Table-1 shows the extractive value of the wild plants tested with four different solvents. In comparison to the other solvents utilized for extraction, such as benzene, chloroform and acetone, the results demonstrate that 80% aq. ethanol is the best suited solvent for obtaining the maximal extract from all the plants under consideration. When the leaves of *S. asper* are extracted with 80% aq. ethanol, the highest yield (10.00 ± 0.04 g/100 g) is obtained, whereas the lowest yield is obtained with other extracting solvents. Similarly, extracts of other plant components followed a similar pattern to *S. asper* extracts. The different nature of chemical components present, as well as the polarity of the solvent used for extraction, could explain the discrepancies in the extractive value of the plant materials [16].

Total phenol, flavonoid and flavonol content: The amount of total phenolics in benzene, chloroform, acetone and 80% aq. ethanol extracts of five wild plants varied greatly, ranging from 9.36 ± 1.48 mg GAE/100 g dry plant material (DPM) to 522.05 ± 3.05 mg GAE/100 g DPM (Table-1).

The presence of a high level of phenolic content was detected in the 80% aq. ethanol extract of *H. pedunculosum* (522.05 ± 3.50 mg GAE/100 g DPM) followed by the same solvent extract of *C. lachryma-jobi* (142.05 ± 8.67 mg GAE/100 g DPM), whereas the benzene extract of *C. lachryma-jobi* contains the least amount (9.36 ± 1.48 mg GAE/100 g DPM). A significant amount of phenolic compounds was also quantified in the acetone extract of *H. pedunculosum* and 80% aq. ethanol extract of *P. corniculata* and *S. volubile.*

In terms of rutin equivalent, the flavonoid concentration of the extracts ranged from 12.07 ± 2.04 to 116.56 ± 1.16 mg/100 g DPM (Table-1). The 80% aq. ethanol extract of *S. asper* contained the most flavonoid (116.56 ± 1.16 mg/100 g DPM) and a substantial amount of flavonoid was also identified in *H. pedunculosum*. The flavonoids in the chloroform and acetone

extracts of the other four plants under examination are similarly quite high.

The flavonol concentration of various extracts of plant materials is measured in quercetin equivalents (Table-1). The 80% aq. ethanol extract of *S. asper* (102.60 ± 2.73 mg/100 g DPM) contained the most flavonol, followed by the ethanol and acetone extracts of *H. pedunculosum*. The flavonol content of the 80% aq. ethanol extracts of *S. volubile* and *C. lachrymal-jobi* is likewise quite high.

The results clearly demonstrate that phenolics have a significant influence in the composition of these plants. Plants' radical scavenging action is mediated by other phenolic compounds containing hydroxyls, such as flavonoids and flavonols. In our research, the optimal solvent for isolating phenolic compounds, flavonoids and flavonols from plant sources was found to be 80% aq. ethanol. The presence of phenolic compounds in *H. pedunculosum*, *P. corniculata* and *C. lachryma-jobi* could explain their strong radical scavenging ability.

Reducing power: As stated in Table-1, the reduction capabilities of the studied five wild food plants are measured in mg AAE/100 g DPM. The 80% aq. ethanol extract of *H. pedunculosum* (53.74 ± 1.49 mg AAE/100 g DPM), which also contains a high amount of flavonoids and flavonols, has the strongest reducing power. In terms of ascorbic acid equivalent, the benzene extract of *S. asper* had the lowest activity (7.23 ± 0.83 mg AAE/100 g DPM). In this assay, the presence of antioxidants in the extracts reduced the Fe^{3+}/ferricyanide complex to ferrous form. The extracts' reducing capacity may serve as a marker of probable antioxidant effects by breaking the free radical chain by donating hydrogen atoms [17].

DPPH radical scavenging activity: The antiradical abilities of the studied five wild food plants were assessed using the DPPH radical scavenging test. Table-1 illustrates the suppression of the DPPH radical by various plant components at 50%; a higher value implies that the sample has superior antioxidant activity. The radical scavenging activity of the 80%

TABLE-1
ANTIOXIDANT ACTIVITIES OF WILD EDIBLE PLANTS IN DIFFERENT SOLVENT EXTRACTION SYSTEM

Antioxidant parameters	Solvent	*H. pedunculosum*	*C. lachryma-jobi*	*S. asper*	*P. corniculata*	*S. volubile*
Extractive value (g/100 g)	Benzene	0.30 ± 0.01^{e}	3.20 ± 0.03^{a}	0.95 ± 0.02^{c}	2.50 ± 0.02^{b}	0.50 ± 0.03^{d}
	Chloroform	0.60 ± 0.06^{d}	3.05 ± 0.01^{a}	0.80 ± 0.03^{c}	2.50 ± 0.03^{b}	0.50 ± 0.02^{e}
	Acetone	0.65 ± 0.004^{d}	2.25 ± 0.01^{b}	1.00 ± 0.03^{c}	2.50 ± 0.03^{a}	2.50 ± 0.03^{a}
	80% Aq. ethanol	8.40 ± 0.02^{b}	3.05 ± 0.03^{d}	10.00 ± 0.04^{a}	7.50 ± 0.05^{c}	8.50 ± 0.04^{b}
Total phenolic content (GAE, mg/100 g DPM)	Benzene	51.47 ± 0.65^{a}	9.36 ± 1.48^{e}	22.99 ± 1.54^{c}	15.54 ± 3.60^{b}	13.85 ± 2.66^{d}
	Chloroform	81.79 ± 4.60^{a}	27.95 ± 3.20^{d}	26.62 ± 1.17^{e}	39.49 ± 0.66^{b}	36.92 ± 6.50^{c}
	Acetone	106.15 ± 6.78^{a}	69.62 ± 1.54^{b}	38.38 ± 2.70^{e}	50.39 ± 4.60^{d}	55.51 ± 1.15^{c}
	80% Aq. ethanol	522.05 ± 3.5^{a}	142.05 ± 8.67^{b}	109.36 ± 1.37^{e}	140.51 ± 5.68^{c}	118.97 ± 7.91^{d}
Total flavonoids content (RE, mg/100 g DPM)	Benzene	32.11 ± 0.45^{a}	12.07 ± 2.04^{e}	19.67 ± 0.61^{c}	13.56 ± 0.34^{d}	39.50 ± 0.68^{b}
	Chloroform	48.07 ± 0.96^{b}	12.62 ± 6.75^{d}	38.81 ± 0.13^{c}	38.26 ± 0.59^{c}	56.07 ± 1.80^{a}
	Acetone	58.08 ± 1.58^{d}	13.44 ± 2.60^{e}	70.86 ± 0.30^{a}	67.98 ± 0.57^{c}	65.87 ± 0.60^{b}
	80% Aq. ethanol	105.45 ± 1.68^{c}	26.03 ± 3.30^{e}	116.56 ± 1.16^{a}	95.40 ± 1.07^{d}	107.35 ± 1.89^{b}
Total flavonol content (QE, mg/100 g DPM)	Benzene	27.23 ± 5.23^{b}	10.54 ± 5.23^{d}	17.06 ± 1.08^{c}	9.79 ± 1.06^{e}	29.33 ± 6.46^{a}
	Chloroform	34.67 ± 3.98^{b}	30.94 ± 3.98^{c}	34.40 ± 2.59^{b}	34.22 ± 2.38^{b}	39.62 ± 2.64^{a}
	Acetone	91.60 ± 2.97^{a}	45.73 ± 2.97^{c}	51.13 ± 1.86^{b}	45.57 ± 3.28^{c}	42.11 ± 3.98^{d}
	80% Aq. ethanol	100.97 ± 5.17^{b}	61.89 ± 5.17^{d}	102.60 ± 2.73^{a}	54.93 ± 1.72^{e}	82.08 ± 2.54^{c}
DPPH (% of inhibition)	Benzene	7.75 ± 0.07^{a}	2.89 ± 0.11^{b}	2.56 ± 0.22^{d}	2.99 ± 0.16^{b}	2.67 ± 0.13^{c}
	Chloroform	10.57 ± 0.03^{b}	4.36 ± 0.23^{d}	5.12 ± 0.09^{c}	12.65 ± 0.31^{a}	5.37 ± 0.12^{c}
	Acetone	14.12 ± 0.02^{b}	5.33 ± 0.68^{d}	8.81 ± 0.08^{c}	17.52 ± 0.33^{a}	3.18 ± 0.14^{e}
	80% Aq. ethanol	42.89 ± 0.82^{a}	14.13 ± 1.31^{d}	40.79 ± 0.62^{b}	19.82 ± 1.89^{c}	8.78 ± 2.14^{e}
ABTS (% of inhibition)	Benzene	9.23 ± 0.03^{b}	4.45 ± 0.04^{e}	8.05 ± 0.01^{c}	7.64 ± 0.07^{d}	10.63 ± 0.04^{a}
	Chloroform	11.36 ± 0.01^{b}	6.77 ± 0.01^{d}	11.26 ± 0.01^{b}	10.23 ± 0.05^{c}	17.64 ± 0.07^{a}
	Acetone	13.94 ± 0.44^{d}	16.23 ± 0.51^{c}	22.20 ± 0.66^{a}	14.41 ± 0.01^{d}	17.09 ± 0.32^{b}
	80% Aq. ethanol	64.89 ± 1.34^{a}	46.35 ± 1.01^{c}	27.24 ± 1.14^{d}	22.53 ± 1.21^{e}	49.87 ± 1.12^{b}
Reducing power (AAE, mg/100 g DPM)	Benzene	7.90 ± 0.95^{c}	8.86 ± 0.41^{b}	7.23 ± 0.83^{c}	8.97 ± 0.45^{b}	14.09 ± 0.63^{a}
	Chloroform	11.57 ± 1.91^{c}	17.36 ± 0.95^{a}	7.72 ± 0.98^{d}	12.66 ± 1.51^{b}	17.72 ± 1.30^{a}
	Acetone	32.54 ± 2.61^{a}	21.34 ± 1.58^{d}	13.03 ± 2.39^{e}	24.96 ± 6.19^{c}	27.14 ± 0.83^{b}
	80% Aq. ethanol	53.74 ± 1.49^{a}	33.66 ± 1.34^{b}	20.76 ± 1.38^{c}	34.71 ± 1.86^{b}	31.49 ± 3.14^{a}
Metal chelating activity (% of inhibition)	Benzene	14.18 ± 0.11^{a}	11.33 ± 0.19^{b}	5.12 ± 0.08^{e}	8.66 ± 0.07^{c}	7.56 ± 0.07^{d}
	Chloroform	25.13 ± 0.11^{a}	19.88 ± 0.21^{b}	7.64 ± 0.12^{e}	11.87 ± 0.04^{c}	10.48 ± 0.04^{d}
	Acetone	36.24 ± 0.09^{a}	29.57 ± 0.18^{b}	8.26 ± 0.09^{e}	15.32 ± 0.07^{c}	11.22 ± 0.08^{d}
	80% Aq. ethanol	48.51 ± 0.39^{a}	37.35 ± 1.17^{b}	11.55 ± 1.02^{e}	29.77 ± 1.24^{c}	22.55 ± 2.33^{d}
Lipid peroxidation assay (% of inhibition)	Benzene	7.21 ± 0.10^{a}	4.55 ± 0.34^{b}	1.02 ± 0.05^{d}	4.01 ± 0.11^{c}	4.37 ± 0.17^{c}
	Chloroform	14.23 ± 0.08^{a}	9.66 ± 0.55^{b}	3.55 ± 0.12^{e}	8.16 ± 0.04^{c}	6.18 ± 0.04^{d}
	Acetone	18.38 ± 0.12^{a}	11.49 ± 0.11^{b}	7.44 ± 0.09^{e}	9.12 ± 0.37^{d}	10.12 ± 0.22^{c}
	80% Aq. ethanol	26.41 ± 1.52^{a}	19.32 ± 1.46^{b}	9.32 ± 1.09^{e}	11.62 ± 1.39^{d}	14.34 ± 1.42^{c}

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 95% confidence level and statistical significance were accepted at the $p < 0.05$ level. The superscript letter a, b, c, d and e denotes the significant differences within same parameters of individual solvent extract among the plants.

aq. ethanol extract of *H. pedunculosum* was the highest (42.89 ± 0.82% inhibition), while the benzene extract of *S. asper* had the lowest activity (2.56 ± 0.22% inhibition) in the current study. *S. asper* extracts in 80% aq. ethanol also demonstrated considerable inhibition. The presence of hydroxyl groups, which can act as a radical scavenger, could explain why these plants are so effective at scavenging radicals.

ABTS radical scavenging activity: Table-1 shows ABTS scavenging activity in diverse extracts of the studied five wild food plants using the ABTS test. The 80% aq. ethanol extract of *H. pedunculosum* had the highest radical scavenging activity (64.89 ± 1.34% of inhibition), followed by *S. volubile* (49.87 ± 1.12% of inhibition) and the benzene extract of *C. lachryma-jobi* had the lowest activity (4.45 ± 0.04% of inhibition) in this study. *P. corniculata* and *H. pedunculosum* extracts in acetone and chloroform had strong radical scavenging properties.

Metal chelating activity: Metal chelating activity is given in Table-1 as a percentage inhibition of metal ions. The 80% aq. ethanol extract of *H. pedunculosum* (48.51 ± 0.39%) had the highest chelating activity, followed by *C. lachryma-jobi* (37.35 ± 1.17%). In comparison to the other wild edibles studied, the acetone extract of these two plants showed promising metal chelating activity.

Lipid peroxidation assay: Table-1 shows the results of a lipid peroxidation assay utilizing all of the plant extracts, which were expressed as% inhibition. *A* 80% aq. ethanol extract of *H. pedunculosum*'s had the highest anti-lipid peroxidation assay (26.41 ± 1.52%), followed by *C. lachryma-jobi* (55.37 ± 0.09%). These two plants' acetone extracts were also more effective than other plant extracts at inhibiting lipid peroxidation. When compared to other plant extracts, 80% aq. ethanol extract is more effective at inhibiting lipid peroxidation.

Since the 80% aq. ethanol extract of all the plants studied had the highest phenolic content, it also had the strongest anti-lipid peroxidation action. This is in line with the findings of several previous fruit and vegetable studies [18,19], which reveal a substantial connection between total phenolic content and peroxidation activity.

Estimation of phenolic acids and flavonoids by RP-HPLC: Table-2 shows the amounts of phenolic acids such as 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 as well as flavonoids like catechin, myricetin, rutin, quercetin, naringin, naringenin, apigenin and kaempferol, present in the 80% aq. ethanol extracts of the investigated plants. The analysis was done using HPLC and the quantities are represented as µg/mg dry extract. All of the phenolics studied had sensitivities at 280 nm, which allowed them to be successfully separated. The recorded absorption spectra, which were equivalent for both plant extracts and standard material, were also used to identify the ingredients under research.

Gallic acid, whether free or associated as an ester, stays in the plant and acts as an antioxidant. According to the HPLC study, gallic acid was discovered in the investigated plant at various quantities. Gallic acid was detected in substantial amounts in *P. corniculata* (1.30 ± 0.33) and in the least amount in *C. lachryma-jobi* (0.55 ± 0.04). Gallic acid levels in the 80% aq. ethanol extracts of *P. mannii* and *M. cheesmanii* were found to be comparable to those found in common vegetables such as spinach (1.82 µg/mg), lemon (2.03 µg/mg), onion bulb (1.55 µg/mg), chilli pepper (3.33 µg/mg), cabbage (0.49 µg/mg) [20]. Protocatechuic acid is a type of phenolic acid that is found in abundance in nature. It shares structural similarities

TABLE-2
QUANTIFICATION OF PHENOLICS AND FLAVONOIDS IN THE 80% AQUEOUS ETHANOL EXTRACT OF WILD EDIBLE PLANTS BY HPLC

Phenolic acids/ Flavonoids	Phenolics and polyphenolic compound by HPLC (µg/mg plant extract)				
	H. pedunculosum	*C. lachryma-jobi*	*S. asper*	*P. corniculata*	*S. volubile*
Gallic acid	1.12 ± 0.33^{b}	0.55 ± 0.04^{e}	1.07 ± 0.33^{c}	1.30 ± 0.33^{a}	0.58 ± 0.33^{d}
Protocatechuic acid	0.19 ± 0.024^{c}	0.33 ± 0.06^{b}	0.53 ± 0.026^{a}	0.12 ± 0.33^{d}	0.53 ± 0.14^{a}
Gentisic acid	ND	ND	ND	ND	ND
p-Hydroxy benzoic acid	ND	ND	ND	ND	0.03 ± 0.001
Catechin	0.51 ± 0.08^{a}	0.43 ± 0.07^{b}	0.29 ± 0.09^{d}	0.41 ± 0.08^{b}	0.38 ± 0.07^{c}
Chlorogenic acid	ND	0.04 ± 0.0033^{a}	ND	0.45	0.04 ± 0.004^{a}
Vanillic acid	0.28 ± 0.02^{a}	0.03 ± 0.0013^{b}	0.02 ± 0.001^{b}	ND	ND
Caffeic acid	ND	ND	ND	0.70 ± 0.03^{a}	ND
Syringic acid	0.27 ± 0.09^{b}	0.33 ± 0.04^{a}	0.03 ± 0.0013^{e}	0.09 ± 0.02^{d}	0.13 ± 0.033^{c}
p-Coumaric acid	0.28 ± 0.04^{a}	0.19 ± 0.067^{b}	0.01 ± 0.23^{e}	0.09 ± 0.16^{c}	0.03 ± 0.33^{d}
Ferulic acid	4.03 ± 0.53^{a}	1.04 ± 0.06^{b}	0.34 ± 0.09^{d}	0.48 ± 0.097^{c}	0.02 ± 0.006^{e}
Sinapic acid	0.05 ± 0.001^{a}	0.02 ± 0.0066^{bc}	0.01 ± 0.0012^{c}	0.02 ± 0.0014^{bc}	0.03 ± 0.003^{b}
Salicylic acid	0.08 ± 0.001^{b}	ND	ND	ND	0.23 ± 0.033^{a}
Naringin	1.55 ± 0.43^{a}	ND	0.18 ± 0.027^{b}	0.02	ND
Rutin	0.03 ± 0.001^{b}	ND	ND	0.18 ± 0.016^{a}	0.03 ± 0.002^{b}
Ellagic acid	0.12 ± 0.003^{a}	0.09 ± 0.002^{b}	0.03 ± 0.001^{c}	0.02 ± 0.09^{d}	0.03 ± 0.33^{c}
Myricetin	0.19 ± 0.005^{a}	0.15 ± 0.037^{b}	0.03 ± 0.001^{e}	0.11 ± 0.06^{c}	0.09 ± 0.00^{d}
Quercetin	0.05 ± 0.003^{a}	ND	ND	ND	ND
Naringenin	0.01 ± 0.001^{c}	ND	0.13 ± 0.07^{a}	0.13 ± 0.033^{a}	0.12 ± 0.08^{b}
Apigenin	0.003 ± 0.00033^{e}	0.89 ± 0.017^{a}	0.04 ± 0.0033^{d}	0.07 ± 0.004^{c}	0.35 ± 0.07^{b}
Kaempferol	0.09 ± 0.007^{e}	0.30 ± 0.02^{c}	0.13 ± 0.153^{d}	0.51 ± 0.02^{a}	0.34 ± 1.67^{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 95% confidence level and statistical significance were accepted at the $p < 0.05$ level. The superscript letter a, b, c, d and e denotes the significant differences within same parameters among the plants

with well-known antioxidant chemicals such as gallic acid, caffeic acid, vanillic acid and syringic acid. The 80% aq. ethanol extract of *S. asper* and *S. volubile* contained the same amount protocatechuic acid (0.53 µg/mg), followed by in *C. lachrymal-jobi*.

Catechins are a type of flavanol that can be obtained in a range of plant-based foods and beverages. *H. pedunculosum* (0.51 ± 0.08) had the highest level of catechin, followed by *C. lachryma-jobi* (0.43 ± 0.07), indicating that this plant may contribute to its curative and cell strengthening capabilities [21]. In present investigation, chlorogenic acid was detected in *C. lachryma-jobi* and in *S. volubile* in same quantity. As a result, consumption these chlorogenic acid-rich vegetables has been related to reduce the blood sugar levels [22].

Vanillic acid is a flavouring ingredient that was found in high concentration in *H. pedunculosum* (0.28 ± 0.02) among the plants studied. Vanillic acid has been shown to have hepatoprotective properties in concanavalin A-induced liver damage [23]. HPLC analysis revealed that the investigated plant is linked to the hepatoprotective movement.

Caffeic acid is mostly found in fruits, vegetables and herbs as an ester (as in chlorogenic acid). The current study indicated that the leaves of *P. corniculata* contain a generally great amount of caffeic acid (0.70 ± 0.03), which is almost comparable to the equivalent in lettuce (1.57 µg/mg), carrot (009 µg/mg), cauliflower (0.058 µg/mg) and potato (2.80 µg/mg) [24]. The 80% aq. ethanol extract of *C. lachryma-jobi* had the greatest content of syringic acid (0.33 ± 0.04 µg/mg), which is well-known for its anticancer, antiproliferative and hepatoprotective effects [25]. Other plants studied, such as *H. pedunculosum* (0.27 ± 0.09) and *S. volubile* (0.13 ± 0.033), were shown to have high levels of syringic acid.

The maximum concentration of *p*-coumaric acid was found in *H. pedunculosum* (0.28 ± 0.04 µg/mg), followed by in *C. lachryma-jobi* (0.19 ± 0.067 µg/mg). Ferulic acid, one of the primary phenolics detected in the plant in present study at concentrations ranging from 0.02 ± 0.006 to 4.03 ± 0.53 µg/mg dry extract, is well-known for its physiological functions, which include antimicrobial, antimicrobial, anti-inflammatory, antidiabetic and anticancer properties [26]. Hence, the *H. pedunculosum* had the highest concentration of ferulic acid, followed by *C. lachryma-jobi* and *P. corniculata*.

All the studied plants under investigation contained a significant level of sinapic acid. The presence of adequate rutin in *P. corniculata* (0.18 ± 0.016) and *H. pedunculosum* suggests that they could be used as therapeutic agents. Ellagic acid is a type of phenol antioxidant present in a wide range of fruits and vegetables. *H. pedunculosum* has the greatest quantities of ellagic acid (0.12 ± 003 µg/mg dry extract). *C. lachryma-jobi* and *S. asper* contain a considerable amount of ellagic acid, suggesting that both plants may have anticancer, anti-heart disease and other medicinal properties [27].

Myricetin is a flavonoid obtained from plants which is widely known for its nutraceutical properties. According to HPLC analysis, myricetin content in the investigated plants ranged from 0.03 ± 0.001 to 0.19 ± 0.005 µg/mg dry extract. Myricetin was found in significant concentrations in 80% aq. ethanol extracts of *C. lachryma-jobi* and *P. corniculata*.

According to HPLC analysis, only *H. pedunculosum* had a significant quantity of quercetin. Similarly, HPLC analysis also revealed that naringenin content was found to be maximum both in *S. asper* and *P. corniculata*. Present research also found that *H. pedunculosum* contain significant quantity of naringin (1.55 ± 0.43 µg/mg dry extract).

The maximum level of apigenin was found in *C. lachryma-jobi* (0.89 ± 0.017 µg/mg dry extract), followed by in *S. volubile* (0.35 ± 0.07 µg/mg dry extract). Similarly, the significant presence of kaempferol (0.51 ± 0.02 µg/mg dry extract) in *P. corniculata* shows that consuming this plant could provide protection.

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 also 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-3).

The extraction of specific groups of antioxidant chemicals and their subsequent antioxidant activity can be affected by changes in solvent polarity [28]. Table-3 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 previous studies [29,30] that found a strong link between polyphenols and antioxidant activity. 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 (Fig. 1A and 1B). All of the plant samples' PCA score plots are given in Fig. 1A and their corresponding loading plots are shown in Fig. 1B. Despite the fact that 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 results analysis. Based on all variables, the first two PCs explained 76.0% of the total variance (Fig. 1A and 1B), with PC1 (51.0%) explaining 2.04 times as much as PC2 (25.0%). 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 Fig. 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, *H. pedunculosum* and *C. lachrymal-jobi* were clearly

TABLE-3
CORRELATION BETWEEN TPC & DPPH, TPC & ABTS, TFC & DPPH, TFC & ABTS, TFLC & DPPH, TFLC & ABTS USING DIFFERENT SOLVENT EXTRACTS OF WILD EDIBLE PLANTS

Solvent	r	R^2	Equation	r	R^2	Equation
	TPC × DPPH			TPC × ABTS		
Benzene	0.466	0.218	y = 0.0868x + 3.0056	0.4	0.160	y = 0.0547x + 6.7623
Chloroform	0.888	0.790	y = 0.10092x + 2.7408	0.721	0.520	y = 0.1284x + 8.7873
Acetone	0.624	0.390	y = 0.0991x + 1.4504	0.674	0.455	y = -0.0852x + 22.231
80% aq. ethanol	0.925	0.856	y = 0.068x + 7.2244	0.813	0.662	y = 0.0773x + 24.612
	TFC × DPPH			TFC × ABTS		
Benzene	0.446	0.199	y = 0.1555x + 2.2687	0.525	0.276	y = 0.1345x + 5.6623
Chloroform	0.578	0.335	y = 0.1089x + 2.809	0.568	0.323	y = 0.1583x + 9.8323
Acetone	0.482	0.233	y = 0.0915x + 3.2685	0.618	0.382	y = 0.1039x + 11.037
80% aq. ethanol	0.818	0.670	y = 0.5935x -29.852	0.856	0.733	y = 0.4226x + 14.163
	TFLC × DPPH			TFLC × ABTS		
Benzene	0.136	0.0186	y = -0.0467x + 5.8503	0.702	0.494	y = 0.3888x + 1.4953
Chloroform	0.357	0.128	y = 0.2934x - 2.9871	0.454	0.207	y = 0.5778x - 4.4377
Acetone	0.583	0.34	y = 0.1445x + 1.2139	0.747	0.559	y = 0.1715x + 8.702
80% aq. ethanol	0.808	0.654	y = 0.4288x - 9.2358	0.925	0.856	y = 0.3451x + 29.801

TPC = Total phenolic content; TFC = Total flavonoid content; TFLC = Total flavonol content

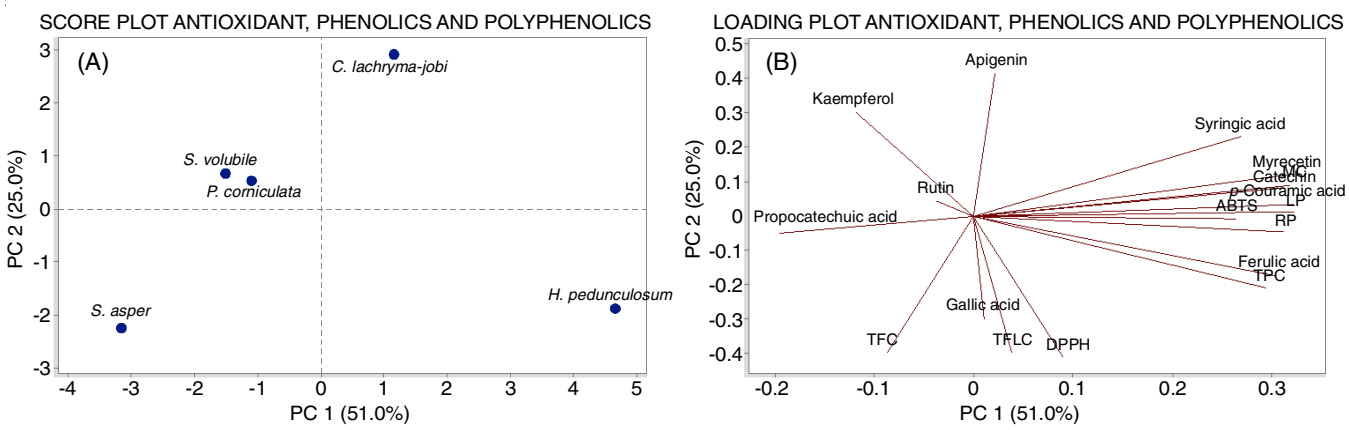

Fig. 1. Principal component analysis using variables listed in Tables 1 and 2. Score plot (1A) and loading plot (1B) of first two principal components for clustering of plant samples. Variables: 18 (TPC, TFC,TFLC, RP, DPPH, ABTS, MC, LP, gallic acid, protocatechuic acid, syringic acid, *p*-coumaric acid, ferulic acid, catechin, rutin, myricetin, apigenin, kaempferol)

separated and distant from all other samples on the right side. *H. pedunculosum* was shown to be more powerful than *C. lachrymal-jobi*, *S. asper*, *S. volubile* and *P.corniculata* in terms of phenolics and polyphenolics concentration.

Conclusion

The 80% aq. ethanol extract of *H. pedunculosum*, which contains the most phenolic compounds, has the strongest radical scavenging activity, according to the findings. Flavonoids and flavonols were found to have high radical scavenging activity in both the ABTS and DPPH methods in benzene, chloroform and acetone extracts of all plants studied. The identified plant extracts' radical scavenging properties are nevertheless less effective than commercially available synthetics like BHT and Trolox. Because plant extracts are relatively harmless and synthetic antioxidants have been limited due to their toxicity, the wild edible plants (*Coix lacryma-jobi*, *Herpetospermum pedunculosum*, *Plukenetia corniculata*, *Sonchus asper* and *Streptolirion volubile*) could be used as antioxidant additions and supplements for disorders linked to the oxidative stress.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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