Wild Edible Plants of Meghalaya State in India: Assessment of Nutritional and Toxicological Potential

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ABSTRACT

Wild edible plants (WEPs) have long been recommended as a functional food and played a vital role in meeting food and nutritional needs, as well as improving the health of underprivileged communities in many rural areas around the world. This study aims to investigate the dietary value, micronutrients, and toxicological status of five WEPs such as *Herpetospermum pedunculosum*, *Coix lacryma-jobi*, *Sonchus asper*, *Plukenetia corniculata*, and *Streptolirion volubile,* consumed by indigenous people in Meghalaya, India. All of the plants included significant amounts of protein (5.42-22.16%), carbohydrate (4.47-44.22%), minerals, and vitamins, all of which were measured according to WHO guidelines. Principal component analysis (PCA) was applied to the collective proximate composition, vitamins, and minerals content to discriminate among the plants. The amounts of anti-nutrients and heavy metals in all plants are below lethal limits. Water extracts of all WEPs have been tested for haemolytic toxicity, cytotoxicity, and genotoxicity, ensuring that they are safe for human consumption. Therefore, the focus of this research is on the use of WEPs as a source of dietary supplements, which could lead to the commercialization of the product and, as a result, assess consumer perceptions of wild edible plants in India.

Key Words: *Wild edible plants, Nutritional quality, Minerals content, Antinutrients, Vitamins, Toxicity*

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INTRODUCTION

Meghalaya is a state in north-eastern India where a variety of wild plants are being studied for their medicinal properties. The availability of protein, starch, and vitamins in wild edible plants reduces the risk of many infections such as cancer, heart attack, diabetes, and so on. Because pesticides and manure deposits are not present, wild vegetables have also evolved into a business crop with expanding market potential [1]. However, they lack adequate logical information about the nutraceutical scenario of such wild vegetables, and individuals lack sufficient knowledge of their beneficial and hazardous features. It has been previously studied that, compared to regular vegetables, wild plants have a greater nutraceutical value [2]. To find viable sources of discretionary sustenance in this situation, a detailed examination of wild edible plants is required. Though wild edible plants are delicious and healthy, overconsumption of them may be harmful to human health due to some antinutrients found in the plants. Protein processing, growth, and iron and zinc absorption are all harmed by anti-nutritional substances like saponin, phytic acid, oxalic acid, tannin, and cyanogen glycoside [3-5]. Phytic acid reduces mineral bioavailability [6], and through hydrogen bonding and hydrophobic processes, tannins bind to proteins, reducing their quality [7]. Plants have been utilized as food and medicine since ancient times, and it is now known that green plants in general, like regular hazardous substances, are a key source of antimutagens [8]. Therefore, it is vital to determine whether wild edibles can have harmful effects on living organisms before employing them.

In this regard, the objective of the present study was to investigate the nutraceutical potential, antinutritional characteristics, and toxicity of five wild edible plants such as *Herpetospermum pedunculosum Coix lacryma-jobi*, *Sonchus asper*, *Plukenetia corniculata*, and *Streptolirion*

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volubile allegedly used by the tribal people of Meghalaya state in India as food.

MATERIALS AND METHODS

Collection of plant materials

The five plant materials e.g *Coix lacryma-jobi* L. (Poaceae), *Herpetospermum pedunculosum* (Ser.) C.B.Clarke (Cucurbitaceae), *Plukenetia corniculata* Sm. (Euphorbiaceae), *Sonchus asper* (L.) Hill (Asteraceae) and *Streptolirion volubile* Edge. (Commelinaceae) were collected from several markets in the state of Meghalaya, India, and identifications were authenticated. The voucher specimens were kept at our Department under the registry numbers BSITS 111, BSITS 110, BSITS 115, BSITS 112, and BSITS 116. The edible parts were shed-dried, crushed, and preserved for analysis in an airtight container.

Nutritional composition

The nutritional composition of the edible plants was assessed using the Association of Official Analytical Chemists' (AOAC) standard food analysis methodologies [9].

The ash content of the wild edibles was determined by burning them for 5-6 hours at 500°C in a muffle furnace, and the moisture content was quantified by heating them in an air oven at 100–110°C. Petroleum ether (60-80°C) was used to extract crude fat in a Soxhlet system. The crude fibre content was estimated by treating fat and moisture-free plant materials with 1.25 % dilute acid and then 1.25 % alkali, followed by washing with water and ignition of the remainder. According to the AOAC recommendations, the crude protein concentration was obtained using the micro Kjeldahl method [8]. Carbohydrate content in the wild plants was estimated following the method given by Hedge and Hofreiter in 1962 [10]. Each plant sample's energy content was calculated by multiplying the protein, fat, and carbohydrate amount by 4.00, 9.00, and 4.00, respectively, and following the addition of the values [9].

Estimation of minerals

Plant material was burned at 600°C in a muffle furnace until ash formed and ash was then dissolved in 100 mL of 5% hydrochloric acid (HCl) to provide a solution appropriate for minerals analysis by atomic absorption spectroscopy (AAS) (AA 800, Perkin-Elmer Germany). Each mineral's standard solution was prepared to draw calibration curves for each element, and minerals were estimated [11].

HPLC analysis for water-soluble vitamins

Freeze-dried plant materials (1 gm) were extracted with 10ml HPLC grade water, 1 ml 0.1M NaOH, 10 ml phosphate buffer (1M, pH 5.5) and the mixture was kept in the dark for 24 hours. The solution was filtered and the filtrate was then transferred to a volumetric flask and volume made up to 25ml with HPLC grade water. The mixture of standard vitamin (C, B1, B5, B6, B2, B3, and B9) solutions was also prepared using the technique described by Seal *et al*., 2018 [12]. The standard solutions were kept refrigerated at 4°C in amber glass bottles.

The chromatographic analysis was conducted according to Seal *et al*., 2018 [12]'s procedure. The mobile phase was made up of acetonitrile (Solvent A) and aqueous trifluoroacetic acid (TFA, 0.01 % v/v) (Solvent B), and the column was maintained at 22^0C with a 20 µl injection volume. The amount of solvents A to B was varied in gradient elution. According to the absorption maxima of the studied plants, the detection of vitamins was done using a photodiode array (PDA) detector configured to different wavelengths (210, 245, 275, and 290 nm). Spiking with standards under the same conditions was used to determine the retention time of each component.

Anti-nutritional composition

Munro and Bassir [13] established a method for determining the oxylate content in edible plants. 50 ml of 0.3M HCl were applied to 1 gm of powdered samples. The solution was agitated intermittently for 1 hour using a magnetic stirrer, then filtered and diluted to 100ml. After that, 25 ml of the filtrate was collected and titrated against 0.1 N $KMnO_4$ solution for 30 seconds until a light pink colour developed. The method of Reddy and Love [14] was used to determine phytate. One gm of powdered plants was steeped in 100 ml of 2% HCl for 5 hours and then filtered. 5 ml of 0.3 % ammonium thiocyanate solution was added to 25 ml of filtrate. After that, the mixture was titrated with Iron (III) chloride solution until a brownish-yellow tint was achieved and lasted for 5 minutes. The method of Hudson and El-Difrawi (1979) [15] was used to determine saponin. Tannins were measured using Price *et al*. 1978's modified vanillin-HCl technique [16], with tannic acid serving as the reference standard. The alkaline titration method was used to determine the cyanogenic glycoside content of the sample, with the end-point being persistent turbidity against a black backdrop [9].

Toxicity studies

Plant extracts

Aqueous extracts were prepared by soaking 5 gm powdered plant materials in 50ml distilled water, for 24 hours at room temperature and filtered. The filtrate was concentrated using a rotary evaporator under reduced pressure. Preservation of the dry extracts was done at -20°C until needed.

Haemolytic toxicity

The aqueous extracts of five wild edible plants were tested for haemolytic toxicity using Malagoli's approach [17]. Healthy rats' blood was drawn, and a 10% erythrocyte solution was prepared in sterile Phosphate buffered saline (PBS, pH 7.4). Plant extracts in various concentrations (100-1000 µg/ml) were added to a 10% erythrocyte solution, which was then incubated for one hour at 37°C. The combination was centrifuged, the supernatant was collected, and a UV-VIS spectrophotometer (Model Shimadzu, UV 1800) was used to measure the absorbance of the released haemoglobin at 540 nm. The negative control was PBS, and the positive control was hydrogen peroxide (50-200 µM). The cell viability of each sample was calculated by dividing the absorbance of the sample by the negative control absorbance multiplied by a factor of a hundred.

Cytotoxicity

Aqueous extracts of five wild edible plants were investigated on isolated goat liver cells using 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) according to Mosmann's technique [18].

Fresh goat livers were used for the isolation of hepatocytes followed by perfusion with collagenase in PBS (pH 7.4). Aqueous extracts (100µl) at various concentrations (100-1000 µg/ml) were incubated with separated hepatocytes for 2 hours at 37°C in a CO_2 incubator before centrifugation. The supernatant was discarded and MTT (5 mg/ml, in PBS, pH 4.5) was added to achieve a final concentration of 0.5 mg/ml. The mixture was again incubated at 37°C for 1 hour until purple intracellular formazan crystals could be seen under the microscope. 100µl DMSO was added to the mixture for the dissolution of formazan crystals and incubated for 30 min to 1 hour. In a UV-VIS spectrophotometer, the absorbance of the purple solution was measured at 570 nm, and the hepatotoxicity of plant extracts was analysed.

Genotoxicity

Singh *et al*. [19] used single-cell gel electrophoresis comet analysis to assess the genotoxic potential of the plant extracts. a 100 µl solution of extracts of plants of various concentrations (100-1000 µg/ml) was mixed with 100 µl of heparinised whole blood and then added to and incubated at 37^0C for two hours. The incubated mixture (100 µl) was embedded in 0.5 % low melting point agarose (LMPA), then spread on a slide coated with a 1 % normal melting point agarose film (NMPA) and solidified at 4°C. For 1 hour, slides were immersed in cold lysis buffer (pH 10) and then placed in a horizontal gel electrophoresis chamber filled with alkaline electrophoresis buffer for 20 minutes (pH 13.0). The slides were neutralised and stained with ethidium bromide solution (20mg/ml) after 30 minutes of electrophoresis at 25V/300mA. A fluorescence microscope was used to observe the coloured nuclei. The Olive Tail Moment (OTM) of individually tagged nuclei was calculated using comet assay software. Because the plant extract had a higher percentage of tail DNA, it was more genotoxic and had more DNA damage.

Statistical analysis

The data was analysed using triplicate samples, and the results were provided as mean standard error mean (SEM). One-way analysis of variance (ANOVA) was performed to investigate the differences and identify plants with similar nutritional composition, minerals, and vitamin content, followed by the Tukey test ($p< 0.05$) and Principal Component Analysis (PCA). The data was analysed using Minitab version 18.0 (State College, PA, USA) from Minitab Inc.

RESULTS AND DISCUSSION

Proximate composition of wild edible plants

The edible sections of five fresh plant materials taken from various locations in Meghalaya, India were studied for proximate composition, and the results are presented in **Table 1**. The moisture substance was found to be most abundant (85.63±0.17%) in the leaves of *S. volubile* and least abundant (69.68±0.26%) in *C. lachrymal-jobi*. The amount of water present in the food is indicated by the content of moisture in it and controls its true quality before ingestion [20]. Of plants grown in India, the moisture content found in the studied plants was close to some ordinary vegetables like spinach (92.1%), cabbage (91.9%), and broad beans (82.4%) [21]. The vegetable residue was found to be highest (23.56±0.17%) in *P. corniculata*, and the substantial amount was analysed in different plants, implying that the vegetables were mineral-rich and might give a substantial amount of mineral components in our diet [22]. For the body to retain fat-soluble vitamins like carotene and vitamin A fat in the diet is important [23]. A significant amount of fat was found in *P. corniculata* (3.00±0.20%) as well as a large proportion in the other plants studied.

Vegetables are high in fibre, which helps to reduce the risks of a variety of diseases and is essential for the absorption and urge waste ejection [24-26]. The amount of crude fibre in the studied wild vegetables ranged from 1.01±0.013 % to 13.03±0.05 % **(Table 1)**, with the lowest value in *S. volubile* and the highest in *H. pedunculosum*, and was similar to commercial fruits and vegetables [21].

Table 1. Proximate composition, minerals, vitamin and antinutritional content in wild edible plants

Sl No	Parameters/ Plants		*H. pedunculosum*	*C. lachryma-jobi*	*S. asper*	*P. corniculata*	*S. volubile*
1	Proximate composition (%) Energy (kcal/100gm)	Ash	7.68±0.29^{d}	2.80±0.08^{e}	10.43±0.22^{c}	23.56±0.17^{a}	18.65±0.27^{b}
		Moisture	79.76±0.29^{c}	69.68±0.26^{e}	73.55±0.26^{d}	82.39±0.27^{b}	85.63±0.17^{a}
		Fat	1.05±0.02^{c}	2.53±0.05^{b}	2.39±0.04^{b}	3.00±0.20^{a}	1.02±0.03^{c}
		Crude fibre	13.03±0.05^{a}	10.38±0.14^{b}	10.41±0.21^{b}	1.39±0.14^{c}	1.01±0.013^{d}
		Protein	11.72±0.03^{d}	5.42±0.04^{e}	17.93±0.04^{c}	19.41±0.15^{b}	22.16±0.07^{a}
		Carbohydrate	6.54±0.18^{c}	44.22±0.06^{a}	4.47±0.11^{d}	8.67±0.20^{b}	6.58±0.17^{c}
		Energy	82.91±0.12^{e}	221.00±0.59^{a}	111.68±0.55^{d}	139.34±0.15^{b}	124.17±0.66^{c}
2	Minerals (mg/100gm dry plant material)	Na	17.24±1.38^{c}	4.50±0.27^{e}	60.49±2.53^{a}	11.97±0.29^{d}	21.07±0.58^{b}
		K	496.86±8.02^{d}	149.66±1.14^{e}	597.01±8.16^{b}	560.66±3.33^{c}	1172.66±2.67^{a}
		Ca	636.32±6.25^{e}	720.04±9.57^{d}	914.11±3.28^{b}	753.87±0.72^{c}	1153.73±1.99^{a}
		Cu	BDL	BDL	0.51±0.02^{a}	0.066±0.002^{c}	0.086±0.003^{b}
		Mg	151.00±0.41^{a}	58.00±0.55^{b}	151.07±0.49^{a}	13.91±0.33^{d}	32.69±0.27^{c}
		Zn	10.29±0.04^{b}	7.55±0.03^{c}	12.68±0.09^{a}	1.09±0.033^{d}	1.92±0.033^{d}
		Fe	7.68±0.11^{b}	2.74±0.06^{e}	7.43±0.12^{c}	8.72±0.27^{a}	5.61±0.033^{d}
		Mn	BDL	BDL	8.05±0.05^{a}	1.45±0.015^{c}	1.75±0.02^{b}
3	Heavy metals (mg/100gm dry plant material)	Pb	0.006±0.0001^{d}	0.02±0.005^{a}	0.016±0.004^{b}	0.012±0.001^{c}	0.011±0.005^{c}
		Cr	0.09±0.004^{d}	0.07±0.005^{e}	0.15±0.005^{a}	0.11±0.014^{c}	0.14±0.015^{b}
		Cd	Not detected	Not detected	Not detected	Not detected	Not detected
		Hg	Not detected	Not detected	Not detected	Not detected	Not detected
4	Water soluble vitamin (mg/100gm dry plant material)	C	0.32±0.02^{c}	0.034±0.02^{e}	0.87±0.03^{b}	0.042±0.003^{d}	2.24±0.03^{a}
		B1	0.18± 0.003^{c}	0.25± 0.023^{b}	0.25± 0.02^{b}	0.92± 0.02^{a}	0.02± 0.002^{d}
		B2	0.30± 0.02^{b}	0.18± 0.001^{c}	0.08± 0.002^{e}	0.16± 0.003^{d}	0.76± 0.03^{a}
		B3	1.29± 0.02^{a}	ND	ND	0.87± 0.03^{b}	ND
		B5	0.20±0.016^{b}	0.18±0.003^{c}	0.18±0.013^{c}	0.11±0.003^{d}	8.86±0.04^{a}
		B6	0.15±0.016^{d}	0.14±0.016^{e}	0.34±0.016^{b}	0.21±0.02^{c}	0.35±0.03^{a}
		B9	0.36±0.02^{d}	0.14±0.02^{e}	0.75±0.02^{c}	1.12±0.01^{a}	0.82±0.026^{b}
5	Antinutrient (%)	Oxalate	0.18±0.006^{b}	0.08±0.006^{d}	0.13±0.002^{c}	0.20±0.005^{a}	0.18±0.003^{b}
		Phytate	0.43±0.07^{a}	0.31±0.05^{b}	0.29±0.04^{c}	0.31±0.04^{b}	0.44±0.06^{a}
		Saponin	0.14±0.002^{a}	0.021±0.002^{e}	0.12±0.002^{b}	0.058±0.005^{d}	0.074±0.008^{c}
		Tannin	0.29±0.08^{c}	0.25±0.02^{d}	0.24±0.03^{e}	1.52±0.03^{a}	0.42±0.07^{b}
		Cyanogenic glycoside	0.0013±0.0005^{d}	0.0013±0.0006^{d}	0.0065±0.0001^{b}	0.0097±0.0005^{a}	0.0019±0.0002^{c}

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 e,f,g, h and i denotes the significant differences within same parameters of individual plant

The most remarkable measure of carbohydrate was recognized in *C. lachrymal-jobi* (44.22±0.06 %) while the lowest was found in the leaves of *S. asper* (4.47±0.11%). Other edible plants have a lot of carbohydrates, especially when compared to other common eatable plants like apple (13.7%), wood apple (18.1%), potato (20.9%), ripe mango (14.9%), and so on [21]. Consequently, these edible plants are a good carbohydrate source for human beings are these edible plants.

One of the most vital supplements are proteins that should be included in sufficient amounts in the diet for working of antibodies against disease, the repair of body tissue, etc [21]. The highest concentration of protein was found in the leaves of *S. volubile* (22.16±0.07%) and the lowest concentration in the edible sections of *C. lachrymal-jobi* (5.42±0.04%). The other plants studied, *P. corniculata*, *S. asper*, and *H. pedunculosum*, all contained high levels of protein, with 19.41±0.15%, 17.93±0.04%, and 11.72±0.03% respectively.

Mineral composition of wild edible plants

Table 2 shows the minerals including sodium, potassium, calcium, manganese, magnesium, iron, zinc, and copper, contained in the edible sections of all plants studied. High

sodium (Na) convergences were found, ranging from 4.50±0.27mg/100g (*C. lachrymal-jobi*) to 60.49±2.53mg/100g (*S. asper*). The edible parts of *S. volubile* had the highest concentration of potassium (1172.66±2.67mg/100g), whereas the fruits of *C. lachrymal-jobi* had the lowest concentration (149.66±1.14mg/100g). Na is vital for metabolite transport, while K is well-known for its diuretic characteristics. Our bodies' K/Na ratio is important for preventing hypertension, and it should be greater than one since K lowers blood pressure while Na raises it [27] and thus the utilization of these vegetables are useful for human and may almost certainly control the hypertension of our body.

Table 2. Toxicity of wild edible plants

Name of the plant	The concentration of the extract (µg/ml)	RBC cell viability (%)	Hepatocytes cell viability (%)	Olive tail moment (OTM) of stained nuclei
H. pedunculosum	100	95.16±1.01	95.90±1.01	3.11±0.18
	200	91.04±0.82	89.38±1.56	3.43±1.25
	300	90.76±0.54	88.72±1.11	3.59±0.94
	500	88.01±0.38	88.14±1.38	4.74±0.88
	1000	86.32±1.15	85.11±1.01	5.26±0.55
C. lachryma-jobi	100	96.47±1.08	96.81±1.86	1.81±1.22
	200	94.76±1.11	95.17±1.08	1.87±0.68
	300	93.04±0.98	93.02±1.88	1.88±1.08
	500	91.27±0.67	92.81±1.06	1.99±1.15
	1000	90.56±0.85	91.13.±0.35	2.06±0.42
S. asper	100	98.70±2.11	93.76±1.55	2.85±0.87
	200	97.36±2.56	92.44±2.11	3.01±1.04
	300	96.43±1.87	91.10±1.28	3.38±1.19
	500	96.85±1.09	90.93±1.09	3.56±1.05
	1000	88.23±0.32	88.67±0.68	4.25±0.15
P.corniculata	100	96.67±1.77	94.56±1.77	1.98±0.89
	200	94.52±1.05	92.11±1.09	2.19±0.33
	300	93.78±1.11	91.76±0.94	2.76±0.64
	500	91.84±0.78	90.34±1.24	3.24±0.29
	1000	87.06±1.12	86.71±1.84	3.97±0.25
S. volubile	100	97.71±1.34	98.76±2.33	1.82±1.43
	200	95.90±1.08	96.44±1.06	1.91±1.04
	300	94.63±0.68	95.01±1.62	2.18±0.82
	500	92.05±1.26	93.67±1.34	2.67±0.89
	1000	90.65±1.44	91.46±0.88	2.89±0.28
Negative control	0	100.18±2.08	99.72±1.56	1.79±1.81
Positive control (H_2O_2)	50 **µM**	79.18±1.54	76.58±1.88	6.18±1.06
	100 **µM**	66.35±1.06	63.20±1.28	12.46±1.44
	200 **µM**	48.25±1.55	39.25±1.11	21.38±1.48

The values within this table were obtained by computing the mean of three experiments and data are presented as Mean ± Standard error of the mean (SEM)

Ca is required for optimal cardiovascular muscle function as well as the formation of strong bones [28]. The leaves of *S. volubile* (1153.73±1.99 mg/100g) had the highest quantity of Ca, followed by *S. asper* (914.11±3.28 mg/100g), *P. corniculata* (753.87±0.72 mg/100g), and *C. lachrymal-jobi* (720.04±9.57mg/100g). The results reveal that the wild vegetables utilized in this research have a high calcium content and could be an excellent source of calcium in our regular diet.

Copper (Cu) is a necessary component for the consolidation of iron into red platelets, which helps to avoid sickness [29]. An adequate measure of Cu was available in the leaves of *S. asper* (0.51±0.02) and the leaves of *S. volubile* (0.086±0.003).

The Zn present in the wild plants under scrutiny was discovered most elevated in the edible parts of *S. asper*

(12.68±0.09 mg/100g) which played an essential role in nucleic acid digestion and its deficiency leads to slow improvement in gonadal capacity [29].

Manganese (Mn) concentrations in the investigated plants ranged from 1.45±0.015 to 8.05±0.05 mg/100g and these plants might play an important role in protein, sugar, fat digestion, and the production of steroid sexual hormones [30].

Fe is necessary for the production of haemoglobin and iron-rich foods should be consumed regularly to avoid iron deficiency weakness [30] and it was found at the highest concentration in the leaves of *P. corniculata* (8.72±0.27 mg/100g), trailed by *H. pedunculosum* (7.68 ±0.11 mg/100gm) and in *S. asper* (7.43 ±0.12 mg/100g) which are very much contrasted with some regular verdant vegetables.

The plants studied had Mg contents ranging from 13.91±0.33 to 151.07±0.49 mg/100g and regular ingestion of these plants helps to maintain a healthy immune system and regulate blood glucose levels [30].

The heavy metal concentration of the wild vegetables under research is shown in **Table 1**. Lead (Pb), one of the heavy metals, is a potential contaminant that quickly accumulates in soils and wastes.

Although Pb is not a necessary component for plants, it is accumulated in numerous areas of the plant and harms the liver, kidneys, vascular system, and immune system. The concentration of Pb was most minimal (0.006±0.001 mg/100g) in *H. pedunculosum* and the most elevated was recognized in *C. lachrymal-jobi* (0.02±0.005 mg/100g). Pb levels in wild vegetables were below the WHO's permissible limit of 0.03 mg/100g in our study. In this study, Pb levels are equivalent to those found in Indian basil (0.009 mg/100g), cabbage (0.013 mg/100g), and water leaf (0.018 mg/100g) [30].

The improvement of insulin sensitivity, sugar influences, protein, and fat digestion is the result of Chromium however, over-exposure to it can injure the kidneys and liver. Cr concentrations were lowest (0.07±0.005 mg/100g) in *C. lachrymal-jobi* and highest (0.15±0.005 mg/100g) in *S. asper*. The Cr level in the veggies was found to be lower than the WHO's allowed guideline of 0.29 mg/100g during the current investigation [30].

Quantifications of water-soluble vitamins by HPLC

Water-soluble vitamins such as ascorbic acid (C), thiamine (B1), riboflavin (B2), niacin (B3), pantothenic acid (B5), pyridoxine (B6), and folic acid (B9) were satisfactorily estimated using the HPLC method. An effectual separation of the all-standard vitamin mixture at 210 nm, is shown in **Figure 1** by a typical HPLC chromatogram. As demonstrated in **Table 1**, the amount of vitamins in all plant materials has been presented in mg /100 gm of dry plant material. To avoid scurvy, Vitamin C, a vital nutrient in fruits and vegetables, is required [31]. The results revealed that the vitamin C amount in our plants varied between 0.034±0.02 to 2.24±0.03 mg/100gm and thus might be responsible to reduce the risk of atherosclerosis [32].

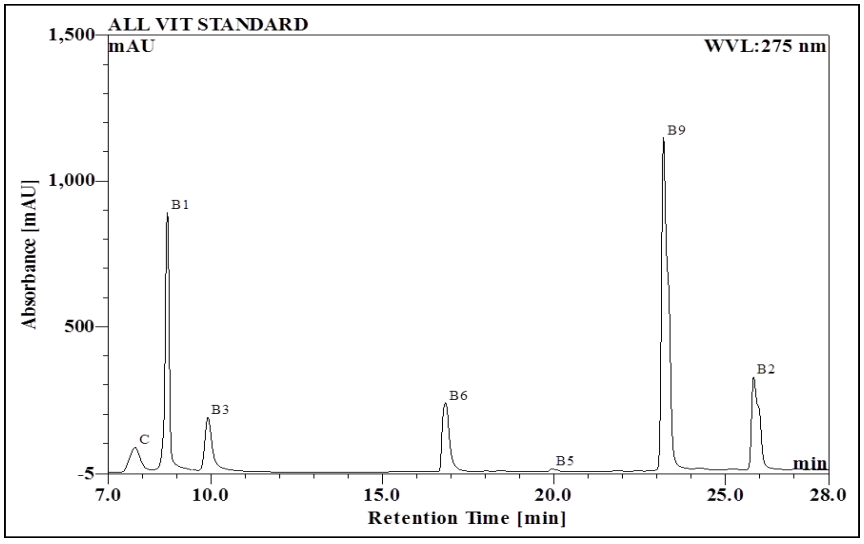

Figure 1. HPLC Chromatogram of the mixture of Standard vitamin

Thiamine (B1) is a vital nutrient and its inadequate levels in food cause body degeneration as well as hypertension and cardiovascular diseases [33]. The greatest concentration of B1 was found in the *P.corniculata*, followed by *S. asper*, *C. lachrymal-jobi*, and *H. pedunculosum*, leaves, these quantities being similar to the thiamine content in some common vegetables and fruits [21].

Riboflavin (B2) is an essential component for proper vitality digestion and is the companion to thiamine, which

is used in the reinforcement of foods [34]. Among the investigated plants, the highest concentration of B2 was found in *S. volubile* (0.76± 0.03 mg/100gm), while the lowest concentration was detected in *S. asper* (0.08± 0.002 mg/100gm). These plants have a B2 content that is nearly equal to that of other common foods such as spinach (0.24 mg/100g), almonds (1.10 mg/100g), beet greens (0.41 mg/100g), potato (0.023 mg/100g), green beans (0.122 mg/100g), and other foods [35].

Vitamin B3 is an important essential for fat metabolism and plays a role in DNA synthesis [12] and our investigation, it was detected highest in *H. pedunculosum* (1.29± 0.02 mg/100gm).

a basic component required by the body for the maintenance of fat and cell generation is Vitamin B5 and its deficiency causes muscle spasms [36]. It was distinguished most remarkable in *S. volubile* (8.86±0.04 mg/100gm).

Pyridoxine (B6) is a water-soluble vitamin played a role in homocysteine synthesis widely used to boost the nutritional value of foods [12]. The most elevated B6 was seen in *S. volubile* (0.35±0.03mg/100gm) though the lowest was recognized in *C. lachrymal-jobi* (0.14±0.016 mg/100gm). The B6 levels found in these wild edible plants were comparable to those seen in common vegetables and fruits [26].

Vitamin B9 (folic acid) is needed for a variety of bodily functions and a lack of folate can lead to iron insufficiency [12]. In addition, to avoiding lipid peroxidation, it performed a significant role in cancer prevention [37]. The degree of B9 in wild eatable plants extended from 0.14±0.02 to 1.12±0.01 mg/100gm. The highest measure of B9 was found in *P. corniculata* and the leaves of *S. volubile* contained the second most astounding quantity of B9 (0.82±0.026 mg/100gm).

Anti-nutritional composition

The outcomes of the anti-nutrient analysis of the wild edible plants under scrutiny were introduced in **Table 1**. Oxalate is a supplement antagonist that binds to minerals in the gastrointestinal tract, leading to minerals deficiency in the body [38, 39]. In the current study, oxalate levels were highest in *P. corniculata* (0.20±0.005 %) and lowest in *C. lachrymal-jobi* (0.08±0.006 %). The oxalate levels in the examined plant are identical to some regular foods like spinach (0.658%), almond (0.407%), amla (0.296%), and amaranth (0.772%) [21] and the oxalate levels in the plants studied are not regarded to be harmful.

Phytic acid produces an insoluble complex with minerals like iron, zinc, calcium, and magnesium [40, 41]. Phytic acid levels in wild edible plants vary from 0.29±0.04 % in *S. asper* to 0.44±0.06% in *S. volubile*. The phytate levels found in our study were less than 10-60 mg/g, which had to be accounted for to overcome the minerals' bioavailability difficulties [42, 43].

Saponins are responsible for causing red platelet damage and interfering with thyroid function [44]. The amount of saponin in the plants varies depending on the species. The highest amount of saponin was detected in *H. pedunculosum* (0.14±0.002 %) whereas *C. lachrymal-jobi* had the most minimal focus (0.021±0.002%).

Tannin is a significant anti-nutritional component found in food that prevents the enzymes amylase, lipase, trypsin, and chymotrypsin from performing their functions efficiently. As a result, the nature of protein is degraded, and iron absorption is hampered [40]. The leaves of *P. corniculata* (1.52±0.03%) showed the highest tannin content, while the lowest concentration was found in the leaves of *S. asper* (0.24±0.03%), and these low concentrations may not have any harmful effects on the human being.

Cyanogenic glycosides are found all over the plant kingdom, and their enzymic hydrolysis releases HCN, which can cause cyanide poisoning [45]. This investigation discovered that the cyanide content of the plants studied ranges from 0.0013±0.0005 to 0.0097±0.0005 % and the number of cyanogenic glycosides in these plants was low and safe to devour as sustenance.

Toxicity studies

Plants with powerful nutraceutical characteristics were subjected to haemolytic tests because they may not be consumed if they have a haemolytic effect. Furthermore, this information may reveal some information on the cytotoxicity of the investigated plants.

Table 2 shows the results of toxicity investigations of edible plants at various doses (100-1000 µg/ml), including cell viability and DNA damage, using buffer (negative control) and hydrogen peroxide (positive control).

Haemolytic toxicity

In vitro haemolytic tests using rodent erythrocytes with various concentrations of wild plants under investigation were carried out. Haemolysis produced by red platelet concentrates varies by concentration, even though all extracts had a lower haemolytic impact on mouse red platelets at all concentrations. The haemolytic cell had the maximum viability (90.65 %) in the case of *S.volubile* at the highest concentration of 1000 µg/ml, and the lowest (86.32 %) in the case of aq. extract of *H. pedunculosum* at the same concentration. H_2O_2 (200µM) resulted in 51.75% haemolysis whereas negative control caused 100.18% cell viability of rodent erythrocytes **(Figure 2)**.

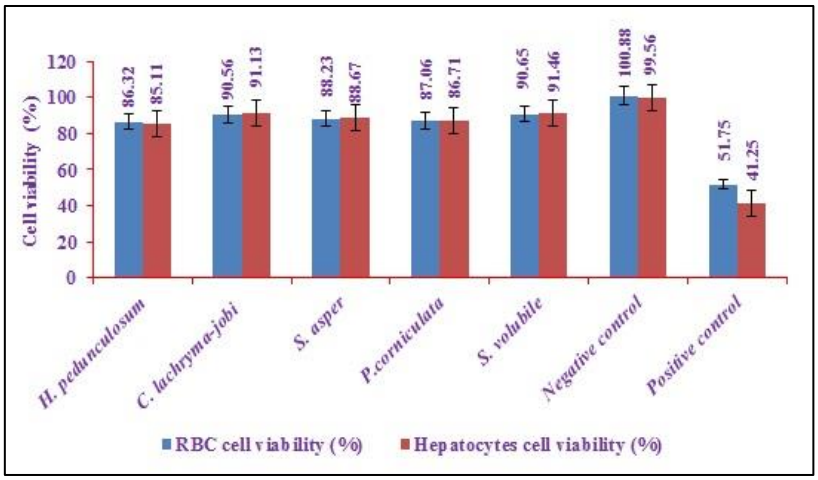

Figure 2. Haemolytic toxicity and cytotoxicity of plant extracts (1000 µg/ml), negative control and positive control (H_2O_2 ; 200µM)

Cytotoxicity

Fresh goat liver hepatocytes were extracted, and the effects of extracts at various doses on hepatocyte cell reasonability were investigated. *S. volubile* had the highest hepatocyte cell viability (91.46 %) at a concentration of 1000µg/ml, whereas *H. pedunculosum* had the lowest (85.11 %) at the same concentration **(Figure 2)**. The vitality rate of RBC and hepatocyte cells was very similar to the negative control for all plant extracts at all fixations (100-1000 µg/ml), however, the viability rate of both RBC and hepatocyte cells employing H_2O_2 (Positive control) at a concentration of 200 µM was less than 50%.

Genotoxicity

The detection of genotoxic characteristics of plant extracts can be done using DNA damage measurement as a sensitive marker with a high predictive value. A higher rate of tail DNA indicated that the plant extract was more prone to DNA damage and genotoxicity. The comet assay is a low-cost, precise, and rapid method of determining DNA strand fragmentation [46].

The results of comet assessments of plant extracts, as well as negative and positive controls **(Table 2 and Figure 3)**, revealed that the aq. concentrate of *H. pedunculosum* at a concentration of 1000 µg/ml had the highest OTM (5.26) of tail DNA, while *C. lachrymal-jobi* at the same concentration had the lowest OTM (2.06). Negative control yielded OTM 1.79 of tail DNA, but positive control produced OTM 21.38 of tail DNA. The formation of free radicals during normal digestion causes mutagenicity and genotoxicity. The positive controls in this experiment showed highly significant aberrant nucleoid genetic alterations and comet formation (OTM 6.18-21.38 of tail DNA), whereas the plant extracts had no significant genotoxic effect at concentrations ranging from 100 to 1000µg/ml. The degree of DNA damage induced by the plant extract at various concentrations was especially close to the negative control, according to the findings. Plants contain a wide range of pharmacologically intriguing chemicals, including vitamin E and C, lycopene, and ß-carotene, which have been proven to have a protective impact in human trials by lowering DNA damage [47]. Phytochemicals, such as saponin, tannin, and cyanogenic glycosides can operate as master oxidants and are responsible for mutagenicity and genotoxicity [48].

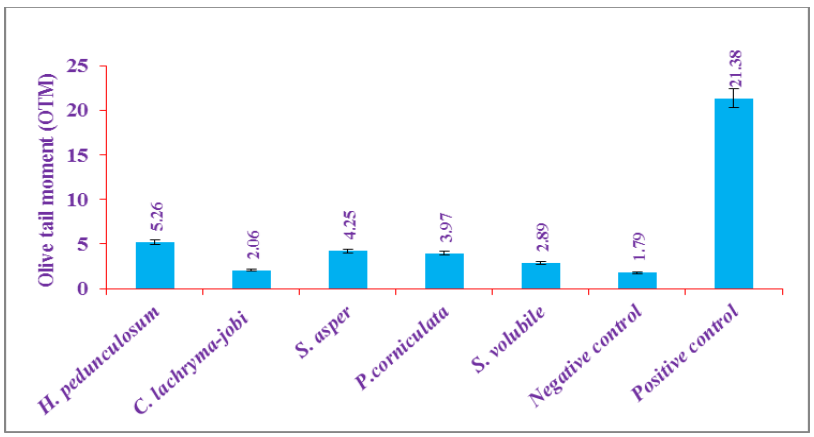

Figure 3. Genotoxicity of plant extracts (1000 µg/ml), negative control and positive control (H_2O_2 ; 200µM)

Principal component analysis

Principal component analysis (PCA) was done individually on the combined proximate composition, vitamins, and mineral data displayed in **Table 1** to better discriminate between the samples. All of the plant samples' PCA score plots (based on all proximate, vitamins, and mineral variables) are shown in **Figure 4**, and their associated loading plots are shown in **Figure 5**. Although the PCA results provided four principal components (PC) with eigenvalues greater than one, only the first two PCs were selected to simplify the analysis of the results. Based on the averaged proximate, vitamin, and mineral values, the top two PCs defined 72.7% **(Figures 4 and 5)** of the entire variation, with PC1 (49.6%) explaining 2.14 times as much as PC2 (23.1 %). PC1 was found to be negatively associated with fat, fibre, carbohydrate, energy, zinc, and magnesium while being positively associated with other components as displayed in **Figure 5**. PC2 has a negative relationship with fat, carbohydrate, energy, vitamin B2, and ash, but a positive relationship with the others. Because of the high contents of carbohydrate, protein, minerals, and water-soluble vitamins C, B2, B6, and B9, the edible sections of *S. volubile*, *C. lachrymal-jobi*, and *P. corniculata* were readily distinguishable and were away from all other samples on the right side as shown in **Figure 4**.

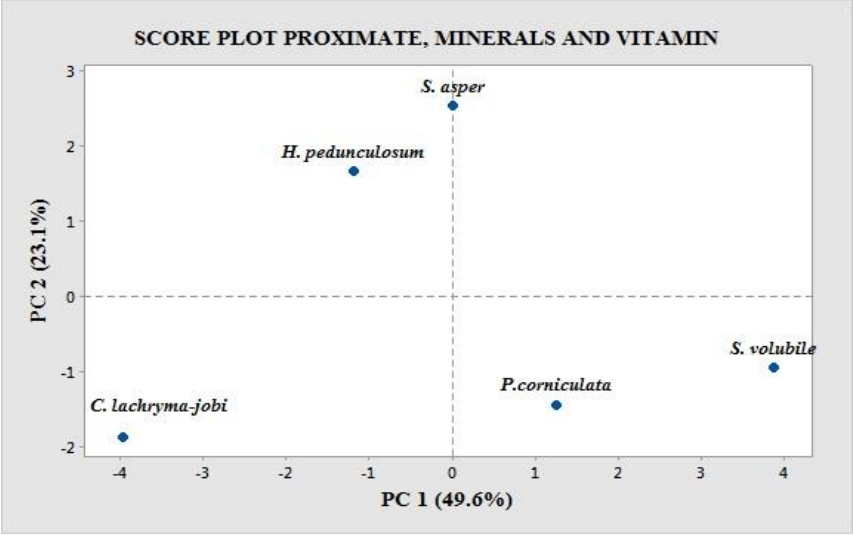

Figure 4. Principal component analysis (Score plot) using 17 variables listed in **Tables 1**

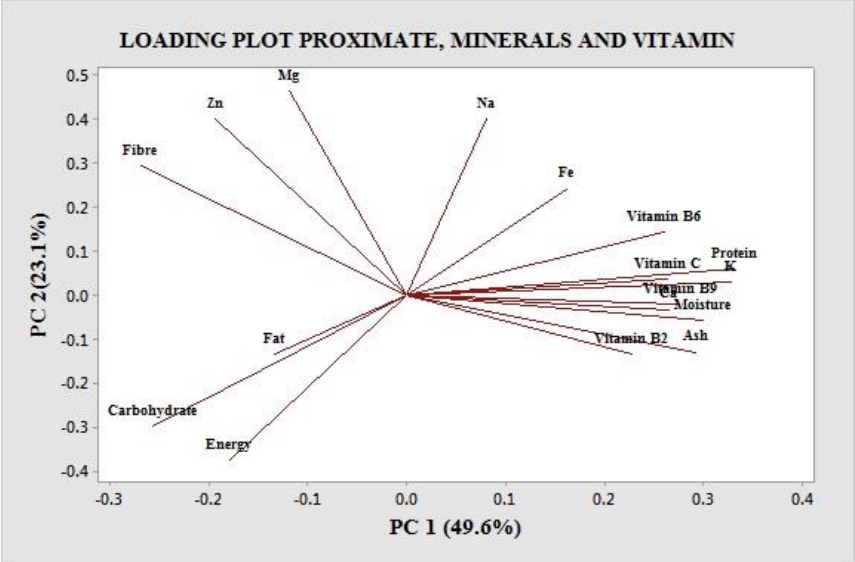

Figure 5. Principal component analysis (Loading plot) using 17 variables listed in **Tables 1**

CONCLUSION

To maintain normal human function these wild edible plants are used for their high fat, carbohydrate, fibre, and minerals protein while providing vital nutrients. Although the hazardous metals Cd and Hg were not found in plant components, Pb and Cr were found in the plant and are unlikely to damage humans. The plants contained a few

water-soluble B and C vitamins in varying amounts, according to RP-HPLC studies. Each of these plants possesses anti-nutrients that are below the recognised lethal level, according to the anti-nutritional study. They are non-harmful at the cell and genome levels, and they are edible, according to the toxicity study. As a result, we believe that these plants could be used to improve human health and provide enough protection against diseases induced by malnutrition. Therefore, the large-scale spread of these wild palatable plants should be encouraged, with economic advantages to poor farmers.

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Conflict of interest: None

Financial support: None

Ethics statement: Serampore College's Institutional Animal Ethics Committee (Approval No.-04/P/S/IAEC/2017) gave ethical authorization for experiments on rats to acquire erythrocytes under CPCSEA guidelines. Fresh goat liver was acquired from a local butcher and placed on ice within 30 minutes of morbidity.

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