Exploring the nutraceutical potential and safety assessment of wild edible plants in Sikkim, India: A comprehensive analysis

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Abstract

The consumption of wild edible plants has been instrumental in addressing food and nutritional needs and improving the health of marginalized communities in rural areas worldwide. This study aimed to assess the nutritive value, mineral content, vitamin content, and toxicological status of ten wild edible plants, namely *Aralia armata*, *Macropanax dispermus*, *Fagopyrum dibotrys*, *Heracleum wallichii*, *Tupistra clarkei*, and *Rumex nepalensis*. These plants are commonly consumed by tribal communities in Sikkim, India. Samples of these plants were collected from various regions in Sikkim, and their nutraceutical potential was evaluated using established food analysis methods recommended by the Association of Official Analytical Chemists. The analysis revealed high protein contents in several species, notably in *A. armata* (19.30±1.01%). Carbohydrate content ranged from 6.42±0.10% (*R. nepalensis*) to 11.16±0.48% (*T. clarkei*). Moreover, all plants exhibited significant mineral content, including sodium, potassium, calcium, iron, zinc, and magnesium. Additionally, water-soluble vitamins such as vitamin C (11.51±0.24 - 87.56±0.41 mg/100g of dry plant material) as well as B1, B2, B3, B5, B6, and B9 were present in appreciable amounts. The levels of antinutrients, such as oxalate, phytate, and saponin, as well as heavy metals (lead, chromium, cadmium, and mercury), were found to be below the established toxic thresholds in all plants. Thus, they pose no immediate health risks associated with these compounds. Furthermore, assessments of haemolytic toxicity, cytotoxicity, and genotoxicity of water extracts from the wild edible plants indicated their safety for human consumption. Therefore, this study highlights the potential utilization of wild edible plants as a source of dietary supplements, potentially leading to their commercialization. Understanding consumer perceptions towards wild edible plants in India is crucial in this regard.

Introduction

Sikkim, located in north-eastern India, boasts a rich abundance of wild plants that have attracted considerable attention for their potential benefits. Consequently, there has been a surge in interest regarding the investigation of various wild edible plants. These plants hold immense significance in human diets as they provide essential nutrients, enhance vitality, and have long been recommended for the general population to mitigate the risk of chronic diseases like cardiovascular disease, cancer, and type 2 diabetes. Additionally, the absence of pesticides or fertilizers in wild vegetables has facilitated their emergence as a commercially viable crop with substantial market potential [1]. Apart from their nutritional value, edible wild plants offer various useful substances including medicinal compounds, fiber, grains, and natural colorants. Several widely circulated publications have suggested that the nutraceutical potential of wild plants may be equivalent to, or even surpass, that of commercially cultivated vegetables [2].

However, despite the deliciousness and health benefits associated with wild edible plants, excessive consumption can be detrimental to human health due to the presence of antinutritional compounds. Antinutrients such as phytic acid, tannins, saponins, oxalic acid, and cyanogen glycosides have negative impacts on health by interfering with protein digestion, development, and the absorption of vital minerals such as iron and zinc [3–4]. Phytic acid, for instance, reduces the bioavailability of minerals [5], while tannins bind to proteins through hydrogen bonding and hydrophobic interactions, thereby diminishing their nutritional quality [6]. Nevertheless, it is worth noting that plants, including wild edible ones, have been used as food and medicine since ancient times, and it is now understood that green plants, in general, serve as important sources of antimutagens, similar to conventional pharmacological agents [7]. Therefore, it is crucial to assess the potential harmful effects of wild edible plants on living organisms before considering their consumption.

In view of the aforementioned concerns, the primary objective of this study was to determine the nutrient composition, antinutritional properties, and toxicity of six specific wild edible plants such as the leaves of *Aralia armata*, *Macropanax dispermus*, *Fagopyrum dibotrys*, *Heracleum wallichii*, *Tupistra clarkei*, and *Rumex nepalensis*, that are reportedly consumed by tribal communities in the state of Sikkim, India. By conducting this research, the aim was to provide a comprehensive understanding of the safety and nutritional value of these plants, thereby enabling informed decision-making regarding their consumption.

Materials and methods

Collection of plant materials

Six plant materials, namely *Aralia armata* Wall. ex G.Don) Seem.(Araliaceae), *Macropanax dispermus* (Blume) Kuntze (Araliaceae), *Fagopyrum dibotrys* (D.Don) H.Hara (Polygonaceae), *Heracleum wallichii* DC (Apiaceae), *Tupistra clarkei* Hook.f.(Asparagaceae), and *Rumex nepalensis Spreng. (Polygonaceae)*, were collected from various locations in Sikkim, India. The identification of these plant materials was authenticated in our office. Voucher specimens of each plant were preserved at the Plant Chemistry department under the respective registry numbers: BSITS 148, BSITS 149, BSITS 150, BSITS 151, BSITS 152, and BSITS 153.

The edible parts of these plants were shed-dried and ground into a fine powder. Subsequently, the powdered samples were stored in an impenetrable compartment. The proximate composition, mineral content, vitamin content, and toxicity of the plant materials were then evaluated. By evaluating these parameters, valuable information about the proximate composition, mineral content, vitamin content, and toxicity of the plant materials was obtained.

Estimation of nutritional composition

In our laboratory, the nutritional composition of the ground plant samples was analyzed following the standardized food analysis methods outlined in the Association of Official Analytical Chemists [8].

The ash content was determined by subjecting the plant samples to a temperature of 500°C in a muffle furnace for approximately 5–6 h. Moisture content was measured by heating fresh plant samples in an air oven at 100–110°C. For the extraction of crude fat, a moisture-free sample was treated with petroleum ether (60–80°C) in a Soxhlet apparatus for about 6–8 h.

To estimate the crude fiber content, the fat and moisture-free materials were initially treated with a 1.25% dilute acid, followed by treatment with a 1.25% alkali. The residue was then washed with water and subjected to ignition. The determination of crude protein was carried out using the micro-Kjeldahl method, as specified in the AOAC procedures [8].

The total carbohydrate content was estimated according to the method described by Hedge and Hofreiter in 1962 [9]. The energy content of each plant sample was calculated by multiplying the values obtained for protein, fat, and available carbohydrate by 4.00, 9.00, and 4.00, respectively, and summing up the results [8].

Estimation of minerals

To determine the mineral elements in the plant materials, a silica crucible was pre-cleaned and weighed before adding 5g of the sample. The crucible was heated in a muffle furnace at 400°C until no smoke was evolved. After cooling in a desiccator, the carbon-free ash was moistened with concentrated sulphuric acid and heated on a heating mantle until sulphuric acid fumes ceased. The crucible with the sulphated ash was further heated in a muffle

furnace at 600°C until a constant weight was achieved. The obtained sulphated ash (1g) was dissolved in 100 ml of 5% hydrochloric acid (HCl) to create a solution for mineral element determination using atomic absorption spectroscopy (AAS) with the AA 800 instrument from Perkin-Elmer Germany. Standard solutions of each element were prepared, and calibration curves were constructed. Minerals such as Na, K, Ca, Mn, Fe, Mg, Zn, Cu, as well as heavy metals including Pb, Cr, Cd, and Hg, were determined by atomic absorption spectrophotometry based on the calibration curves [10].

Estimation of water soluble vitamins by HPLC

Preparation of mixture standard vitamin solutions

The stock standard solutions of vitamin C, B1, B3, B5, and B6 were prepared by dissolving 25 mg of each standard in 1 ml 0.1M hydrochloric acid in 25 ml standard volumetric flask. For preparation of standard stock solutions of vitamin B9 and B2, 25 mg of the each standard were dissolved in 1 ml 0.1M sodium hydroxide in a 25 ml standard volumetric flask [11]. The standard solution was stored in amber-glass bottles in the refrigerator at 4^{o}C. The working standards were prepared by diluting with phosphate buffer (1M, pH 5.5).

Preparation of sample solution

Plant materials were washed with distilled water and then cut into very small pieces, frozen in liquid nitrogen, and kept at -20^{o}C until analysis. Each freeze-dried sample (1g) was soaked in 10 ml of water and extracted with 1 ml 0.1M NaOH and 10 ml phosphate buffer (1M, pH 5.5) were added to it and kept in the dark for 24h. The solution was first filtered through a Whatman No. 1 filter paper and the resulting filtrate was taken in a 25 ml volumetric flask. The solution was topped up to the mark with HPLC grade water. The sample solution was filtered through a 0.45 mm membrane filter before injection into the HPLC system. The stock solutions of the sample were kept in a refrigerator for further use [11].

Chromatographic analysis of water soluble vitamins

The chromatographic analysis was carried out following the method described by Seal et al. [11]. The mobile phase contains acetonitrile (Solvent A) and aqueous trifluoro acetic acid (TFA, 0.01% v/v) (Solvent B). The column was thermostatically controlled at 22^{o}C and the injection volume was kept at 20 ml. A gradient elution was performed by varying the proportion of solvent A to solvent B. Total analysis time per sample was 35 min. HPLC Chromatograms of all vitamins were detected using a photo diode array UV detector at four different wavelengths (210, 245, 275 and 290 nm) according to the absorption maxima of analysed compounds. Each compound was identified by its retention time and by spiking with standards under the same conditions. The quantification of vitamins in the extracts was carried out by the measurement of the integrated peak area and the contents were calculated using the calibration curve by plotting peak area against the concentration of the respective standard sample. The data were reported as means ± standard error of means of three independent analyses.

Estimation of anti-nutritional composition

Oxalate contents of edible plants were determined utilizing the strategy depicted by Munro and Bassir [12]. Powdered plants (1g) were extracted thrice by warming (50^{o}C) and stirring with a magnetic stirrer for 1h with 0.3M HCl. The combined extracts were diluted to 100 ml with water and used for total oxalate estimation. Phytate was determined using the method Reddy and Love [13]. Powdered plants (1g) were soaked in 100 ml of 2% HCl for 5h and filtered. To 25 ml of the filtered, 5 ml 0.3% ammonium thiocyanate solution was added. The mixture was then

titrated with Iron (III) chloride solution until a brownish-yellow color that persisted for 5min was obtained. Saponin was determined using the method of Hudson and El-Difrawi [14]. Tannins were assayed in accordance with the modified vanillin-HCl method of Price et al. [15] and tannic acid was used as the reference standard. Cyanogenic glycoside contents of sample were determined by alkaline titration method, where the end-point was noted as a permanent turbidity against a black background [8].

Toxicity studies of wild edible plants

Preparation of plant extracts

To prepare the extracts from the powdered plant materials, 5g of the powder was macerated in 50 ml of distilled water at room temperature for 24 h. The mixture was then separated using cotton wool to remove any solid particles. The defatted plant materials underwent another round of maceration in the same solvent for an additional 24 h. The extracts obtained from the initial and subsequent extractions were combined and concentrated using a rotary evaporator under reduced pressure, resulting in viscous extracts. These extracts were further dried using a freeze dryer and stored at -20°C for future use.

For the experimental setup, 10 mg of each crude extract was dissolved in 10 ml of phosphate buffered saline (PBS) with a pH of 7.4. To ensure the removal of any particulate matter, the sample solutions were filtered through syringe-adapted filters with a pore size of 0.22 µm. The filtered solutions were then stored at -20°C until they were ready for use.

Haemolytic toxicity study

In the evaluation of haemolytic toxicity, Malagoli's methodology [16] was employed using aqueous extracts of six wild edible plants. Blood samples were collected from healthy rats and mixed with EDTA. The mixture was then centrifuged at 5,000 rpm for five minutes to obtain the red blood cell (erythrocyte) component. A 10% erythrocyte suspension was prepared using sterile phosphate buffered saline (PBS) with a pH of 7.4, which served as the medium for haemolytic studies.

To study the haemolytic effects, various concentrations (100, 200, 300, 500, and 1000 µg/ml) of the plant extracts were added to the 10% erythrocyte suspension. The mixture was incubated at a temperature of 37°C for 1 h. After incubation, the cells were centrifuged, and the supernatant was used to measure the absorbance of the liberated haemoglobin at 540 nm using a UV-VIS spectrophotometer (Model Shimadzu, UV 1800).

Two controls were prepared for comparison. The negative control received sterile phosphate buffer saline, while hydrogen peroxide (ranging from 50 to 200 µM) served as the positive control. Triplicate assays were performed, and the average value was calculated.

To assess cell viability, the absorbance of each sample was divided by the absorbance of the negative control and multiplied by one hundred. This calculation provided the cell viability percentage for each sample.

Cytotoxicity study

On isolated goat liver cells, aqueous extracts of ten wild edible plants were examined using 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay according to Mosmann's protocol [17].

Fresh goat livers were procured from local market and were perfused with collagenase in PBS (pH 7.4). The liver was then minced into minute pieces, and cells were separated using cell strainer having 40µl pore size (Genetix cell

strainer, S.Korea). The cells were then washed with HBSS and centrifuged to eliminate fine debris. The cell feasibility extends somewhere in the range of 85–95%, as controlled by the trypan blue rejection test. The immaculateness of hepatocytes was examined by phase-contrast microscopy. In an eppendorf tube containing 0.5 ml RPMI and 10% FBS, the separated cells were placed. Aqueous extracts (100µl) of edible plants were applied to freshly isolated hepatocytes at various concentrations (100, 200, 300, 500 and 1000 µg/ml) and incubated for 2h at 37^{o}C in a CO_2 incubator. Cell control (cells without extract treatment) and medium control (blank media) were likewise obtained and cultured under the identical experimental conditions. All the tubes were then centrifuged and supernatant was discarded. Thiazolyl Blue Tetrazolium Bromide, MTT (5 mg/ml, in PBS, pH 4.5) (Sigma, USA) were added into tube to achieve a final concentration of 0.5 mg/ml and incubated for 1h at 37°C until intracellular purple formazan crystals are visible under microscope. After 1h, the culture medium with MTT was carefully removed by centrifugation, and 100µl DMSO was added to it and incubated for 30 min to 1h to dissolve formazan crystals. The UV absorbance of the purple solution was spectrophotometrically measured at 570 nm in a UV-VIS spectrophotometer (Model Shimadzu, UV 1800), and the hepatotoxicity of plant extracts was assessed.

Genotoxicity study

The genotoxic potential of the plant extracts was evaluated by single-cell gel electrophoresis comet examine as depicted by Singh et al. [18]. One (1) ml blood was drawn from a healthy rat's tail vein, and 100 µl of heparinised whole blood was mixed with 100 µl plant extracts of various strengths (100, 200, 300, 500 and 1000 µg/ml) and kept at 37°C in a CO_2 incubator for two hours. 100 µl cell suspension were embedded in 100 µl of 0.5% low melting point agarose (LMPA), which was then distributed on a slide coated with a 1% normal melting point agarose film (NMPA). Two slides were prepared for each sample in which agarose cell suspensions were allowed to solidify at 4°C. Slides were submerged in cold lysis buffer (2.5M NaCl, 100mM EDTA, 10mM Tris buffer, 10% DMSO, Triton X-100 0.8 percent, pH 10) for 1h after solidification. For the unwinding of DNA, the slides were taken from the lysis buffer and placed in a horizontal gel electrophoresis chamber filled with alkaline electrophoresis buffer (1 mM EDTA, 0.3N NaOH, pH 13.0) for 20 min. Electrophoresis was performed for 30 min at 25V/300mA and electrophoresis slides were neutralized (three times) and stained with ethidium bromide solution (20mg/ml). The stained nuclei were visualized under fluorescent microscopy and photographed. The Olive Tail Moment (OTM) of individual stained nuclei was calculated using comet assay software. A higher percentage of tail DNA suggested that the plant extract had more DNA damage and was more genotoxic.

Ethical clearance for performing experiments on rats to get erythrocytes, was obtained from Institutional Animal Ethics Committee (Approval No.-04/P/S/IAEC/2017), Serampore College, West Bengal, India conforming to the CPCSEA guidelines. Goat liver was procured fresh from local abattoir and brought on ice within 30 min of death.

Statistical analysis

The analysis was conducted using triplicate samples, and the results were reported as mean ± standard error mean (SEM). To evaluate the differences and identify plants with similar characteristics regarding their nutritional composition, mineral content, and vitamin content, one-way analysis of variance (ANOVA) followed by Tukey's test ($p \leq 0.05$) was employed. Additionally, Principal Component Analysis (PCA) was conducted. Data analysis was performed using Minitab version 18.0 (Minitab Inc, State College, PA, USA).

Results and discussion

Proximate composition of wild edible plants

The proximate composition of the edible parts of six fresh plant materials collected from various locations in Sikkim was investigated, and the results are presented in Table 1. Among the plant materials, the leaves of *F. dibotrys* exhibited the highest moisture content (84.22 ± 2.07%), while the leaves of *M. dispermus* had the lowest moisture content (75.16 ± 1.34%).

Table 1
Nutritional, antinutritional and vitamin content in wild edible plants

Sl No	Parameters/ Plants		*A. armata*	*M. dispermus*	*F. dibotrys*	*H. wallichii*	*T. clarkei*	*R. nepalensis*
1	Proximate composition (%) Energy (kcal/100gm)	Ash	8.61 ± 0.07^{b}	8.81 ± 0.55^{b}	14.86 ± 1.33^{a}	7.67 ± 0.51^{c}	7.03 ± 0.32^{c}	14.18 ± 1.67^{a}
		Moisture	81.22 ± 1.45^{b}	75.16 ± 1.34^{d}	84.22 ± 2.07^{a}	79.32 ± 1.28^{c}	82.45 ± 2.54^{b}	79.36 ± 2.31^{c}
		Fat	2.04 ± 0.13^{b}	0.24 ± 0.05^{d}	1.90 ± 0.34^{c}	2.68 ± 0.54^{b}	1.37 ± 0.46^{c}	6.34 ± 0.72^{a}
		Crude fibre	6.00 ± 1.14^{b}	6.70 ± 1.73^{a}	5.92 ± 0.44^{b}	4.96 ± 0.84^{c}	5.01 ± 0.63^{c}	6.35 ± 0.65^{a}
		Protein	19.30 ± 1.01^{a}	7.74 ± 1.02^{d}	9.47 ± 1.02^{c}	7.67 ± 1.02^{d}	7.19 ± 1.04^{e}	11.14 ± 1.04^{b}
		Carbohydrate	9.49 ± 0.11^{b}	7.45 ± 0.19 ^{c}	6.77 ± 0.31^{d}	7.86 ± 0.27^{c}	11.16 ± 0.48^{a}	6.42 ± 0.10^{d}
		Energy	133.54 ± 4.39 ^{a}	62.94 ± 1.65 ^{e}	82.11 ± 1.66 ^{d}	86.27 ± 1.61^{c}	85.76 ± 2.69 ^{c}	127.33 ± 2.86 ^{b}
2	Minerals (mg/100gm dry plant material)	Na	1.03 ± 0.06^{c}	2.10 ± 0.05 ^{a}	1.02 ± 0.02^{c}	1.13 ± 0.05^{b}	1.22 ± 0.03^{b}	2.02 ± 0.03^{a}
		K	20.13 ± 0.08^{e}	20.43 ± 0.12^{e}	79.40 ± 0.25^{b}	34.20 ± 0.12^{d}	48.16 ± 0.12^{c}	95.4 ± 0.20^{a}
		Ca	23.73 ± 0.08^{c}	20.84 ± 0.04^{d}	18.4 ± 0.05^{e}	63.43 ± 0.15^{b}	80.76 ± 0.38^{a}	17.18 ± 0.02^{e}
		Cu	0.28 ± 0.03^{d}	0.45 ± 0.08^{c}	0.78 ± 0.006 ^{a}	0.43 ± 0.04^{c}	BDL	0.69 ± 0.05^{b}
		Mg	23.39 ± 1.41^{c}	28.40 ± 1.56 ^{b}	39.28 ± 1.19 ^{a}	22.81 ± 1.24^{c}	38.56 ± 2.78^{a}	27.67 ± 1.18 ^{b}
		Zn	12.19 ± 0.43^{b}	8.56 ± 0.06 ^{d}	15.43 ± 0.72^{a}	8.32 ± 0.09 ^{d}	2.97 ± 0.13^{e}	9.25 ± 0.02 ^{c}
		Fe	6.81 ± 0.28 ^{e}	11.32 ± 0.14^{c}	7.57 ± 0.49 ^{d}	11.42 ± 0.12^{c}	16.45 ± 0.16^{b}	21.44 ± 0.37 ^{a}
		Mn	1.72 ± 0.06 ^{f}	6.32 ± 0.22 ^{a}	2.18 ± 0.34 ^{e}	5.24 ± 0.17^{b}	3.18 ± 0.18^{d}	4.56 ± 0.19^{c}
3	Heavy metals (mg/100gm dry plant material)	Pb	0.02 ± 0.004 ^{a}	0.02 ± 0.008 ^{b}	0.016 ± 0.004 ^{c}	0.015 ± 0.004 ^{c}	0.011 ± 0.01 ^{d}	0.015 ± 0.001^{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 and f denotes the significant differences within same parameters of individual plant.

Sl No	Parameters/ Plants		*A. armata*	*M. dispermus*	*F. dibotrys*	*H. wallichii*	*T. clarkei*	*R. nepalensis*
		Cr	0.04 ± 0.001^{e}	0.07 ± 0.002^{d}	0.18 ± 0.05 ^{a}	0.13 ± 0.011^{b}	0.09 ± 0.002 ^{c}	0.081 ± 0.002 ^{c}
		Cd	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected
		Hg	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected
4	Water soluble vitamin (mg/100gm dry plant material)	C	13.64 ± 0.33 ^{e}	22.04 ± 0.17 ^{d}	87.56 ± 0.41^{a}	11.51 ± 0.24 ^{f}	78.71 ± 0.22 ^{b}	54.81 ± 0.19 ^{c}
		B1	ND	0.02 ± 0.03 ^{d}	0.35 ± 0.03 ^{b}	0.23 ± 0.03 ^{c}	0.85 ± 0.11^{a}	0.37 ± 0.03^{b}
		B2	0.04 ± 0.002 ^{d}	0.30 ± 0.02^{b}	0.28 ± 0.02^{b}	0.82 ± 0.03 ^{a}	0.30 ± 0.01^{b}	0.06 ± 0.001^{c}
		B3	ND	0.82 ± 0.03^{a}	0.19 ± 0.01^{b}	ND	0.03 ± 0.006^{c}	0.18 ± 0.005^{b}
		B5	ND	0.52 ± 0.03 ^{b}	0.34 ± 0.04 ^{c}	0.29 ± 0.03 ^{d}	1.40 ± 0.13^{a}	0.33 ± 0.14 ^{c}
		B6	1.66 ± 0.11 ^{a}	0.21 ± 0.04^{d}	0.22 ± 0.04 ^{d}	0.33 ± 0.05 ^{b}	0.33 ± 0.03 ^{b}	0.26 ± 0.20 ^{c}
		B9	0.87 ± 0.04 ^{c}	0.63 ± 0.014 ^{d}	2.41 ± 0.02 ^{b}	3.78 ± 0.02 ^{a}	0.58 ± 0.014 ^{d}	2.80 ± 0.06 ^{b}
5	Antinutrient (%)	Oxalate	0.15 ± 0.02 ^{b}	0.15 ± 0.03 ^{b}	0.16 ± 0.09 ^{a}	0.13 ± 0.05 ^{c}	0.13 ± 0.09 ^{c}	0.13 ± 0.04 ^{c}
		Phytate	0.29 ± 0.04^{c}	0.23 ± 0.07^{d}	0.38 ± 0.09 ^{b}	0.18 ± 0.08 ^{e}	0.15 ± 0.07 ^{f}	0.44 ± 0.08 ^{a}
		Saponin	3.86 ± 0.03^{b}	0.09 ± 0.003^{d}	4.92 ± 0.02^{a}	2.54 ± 0.02^{c}	3.88 ± 0.01^{b}	0.05 ± 0.004^{e}
		Tannin	0.68 ± 0.06^{a}	0.09 ± 0.02 ^{d}	0.34 ± 0.017^{b}	0.04 ± 0.003^{e}	0.26 ± 0.007 ^{c}	0.29 ± 0.003^{c}
		Cyanogenic glycoside	0.15 ± 0.06^{a}	0.13 ± 0.03^{b}	0.15 ± 0.06^{a}	0.09 ± 0.007^{c}	0.13 ± 0.07^{b}	0.14 ± 0.004^{b}

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 and f denotes the significant differences within same parameters of individual plant.

Moisture content is an essential parameter that indicates the amount of water present in a food sample and plays a significant role in determining the quality and freshness of the food before consumption. It also affects the physical and chemical characteristics that influence the long-term preservation and stability of food [19]. The observed moisture content in the studied plants was comparable to the moisture content found in common vegetables grown in India, such as spinach (92.10%), cabbage (91.90%), and broad beans (82.40%) [20].

The findings of this study suggest that the plant materials analyzed possess moisture content within the range commonly observed in vegetables. This indicates that the studied plants are likely to exhibit similar properties related to freshness and preservation as other commonly consumed vegetables. Further analysis of the proximate composition and nutritional content of these plant materials will provide a comprehensive understanding of their potential as food resources.

The ash content of the studied vegetables varied among the different plant materials. *F. dibotrys* exhibited the highest ash content (14.86 ± 1.33%), while the other plants showed substantial amounts within the range of 7.03 ± 0.32% to 14.18 ± 1.67%. The ash content reflects the mineral composition of the plants and indicates their potential to provide significant amounts of mineral components in our diet. The high ash content observed in *F. dibotrys* suggests that this plant is rich in minerals. Including such plants in our diet can contribute to fulfilling our mineral requirements. Minerals play crucial roles from a physicochemical, mechanical, and physiological perspective, supporting various bodily functions and maintaining overall health [21].

The range of ash content found in the studied plants indicates their potential as valuable sources of essential minerals. Incorporating these plants into our regular diet can contribute to meeting our nutritional needs and promoting a balanced intake of minerals. Further exploration of the mineral composition of these plants would provide more insights into their potential health benefits and nutritional value.

Fat plays a crucial role in a balanced diet as it provides essential unsaturated fats that are not produced by the body and must be obtained from food. Among these unsaturated fats, linoleic and linolenic acids are particularly important for functions such as inflammation control, blood coagulation, and mental well-being. Additionally, fat serves as an energy reserve in the body, storing excess calories.

In addition to its energy-providing role, fat is essential for the absorption and utilization of fat-soluble vitamins, including vitamin A and carotene [20]. These vitamins require the presence of fat in the diet for effective absorption and utilization by the body.

The analysis of the studied plant materials revealed varying amounts of fat content. *R. nepalensis* exhibited the highest fat content (6.34 ± 0.72%), followed by *H. wallichii* (2.68 ± 0.54%), *A. armata* (2.04 ± 0.13%), *F. dibotrys* (1.90 ± 0.34%), as well as other plants investigated.

The presence of relatively higher amounts of fat in these plant materials suggests their potential contribution to dietary fat intake. Including these plants in our diet can provide a natural source of fats, including important unsaturated fatty acids. This can support various physiological functions, including the absorption of fat-soluble vitamins and overall maintenance of good health.

It is important to maintain a balanced intake of fats, including both saturated and unsaturated fats, in order to meet the body's nutritional needs. Incorporating a variety of plant-based sources of fats, such as the studied plant materials, can contribute to a well-rounded and nutritious diet. Further exploration of the specific types of fats present in these plants would provide deeper insights into their potential health benefits and dietary value

Vegetables are known to be rich sources of dietary fiber, which plays a crucial role in reducing the risk of various diseases, including obesity, constipation, diabetes, high serum cholesterol levels, heart disease, breast and colon cancer, and hypertension [22]. The World Health Organization (WHO) recommends a daily intake of 22–23 g of fiber per 1000 kcal in the diet, as it is essential for healthy digestion and promotes regular bowel movements [23–24]. The crude fiber content of the wild vegetables studied (Table 1) ranged from 4.96 ± 0.84% to 6.70 ± 1.73%. *H. wallichii*

exhibited the lowest crude fiber content, while *M. dispermus* had the highest. These findings indicate that the studied wild vegetables can contribute to a high-fiber diet.

The crude fiber content observed in the studied vegetables is comparable to that found in commonly consumed fruits and vegetables such as apples (3.20%), broad beans (8.90%), cabbage (2.80%), potatoes (1.70%), and spinach (2.50%) [20]. This similarity suggests that incorporating these wild vegetables into the human diet can help meet the WHO's recommended fiber intake.

Including wild vegetables in the diet provides an opportunity to enhance fiber consumption and reap the associated health benefits. By incorporating these vegetables into our meals, we can contribute to the promotion of healthy digestion, regular bowel movements, and the prevention of various diseases linked to fiber intake. The findings highlight the potential of wild vegetables as a valuable dietary component to meet the WHO's fiber recommendations [24].

Among the studied wild vegetables, the leaves of *T. clarkei* exhibited the highest carbohydrate content (11.16 ± 0.48%), while the leaves of *R. nepalensis* had the lowest (6.42 ± 0.10%). Additionally, the other edible plants analyzed in this study also contained appreciable amounts of carbohydrates. These findings are consistent with the carbohydrate content observed in commonly consumed edible plants such as apples (13.70%), wood apples (18.10%), potatoes (20.90%), and ripe mangoes (14.90%) [20] The significant carbohydrate content observed in the studied wild vegetables indicates their potential as a valuable source of carbohydrates in human diets. Including these vegetables in the diet can contribute to meeting the body's energy requirements and fulfilling the need for dietary carbohydrates. The carbohydrate content found in these wild vegetables is comparable to that of well-known edible plants, suggesting that they can serve as a reliable source of carbohydrates for human consumption.

By incorporating these edible plants into our meals, we can benefit from the carbohydrates they provide, which are essential for energy production and various physiological functions in the body. These findings highlight the nutritional value of wild vegetables as a viable option for obtaining carbohydrates in our diet [20].

Proteins play a vital role in the human diet, as they are essential for various physiological functions within the body. Adequate protein intake is necessary for tissue repair, hormone regulation, and antibody production to support the immune system and combat diseases [20]. In this study, the leaves of *A. armata* exhibited the highest concentration of crude protein (19.30 ± 1.01%), while the edible sections of *T. clarkei* had the lowest concentration (7.19 ± 1.04%).

The other plant materials examined, namely *R. nepalensis*, *F. dibotrys*, and *M. dispermus*, also displayed considerable levels of protein content, with values of 11.14 ± 1.04%, 9.47 ± 1.02%, and 7.74 ± 1.02% respectively. These findings suggest that these wild edible plants can serve as valuable sources of dietary protein.

Including these plant materials in the diet can contribute to meeting the body's protein requirements and ensuring the availability of essential amino acids necessary for various bodily functions. The observed protein content in these wild vegetables is noteworthy and demonstrates their potential as beneficial protein sources in the human diet.

By incorporating these plants into our meals, we can enhance our protein intake, which is essential for maintaining overall health and supporting important physiological processes. The presence of significant protein levels in these wild vegetables further highlights their nutritional value and potential as valuable components of a balanced diet [20].

Mineral composition of wild edible plants

The study examined the mineral composition of various plant species, focusing on minerals such as sodium, potassium, calcium, manganese, magnesium, iron, zinc, and copper found in their edible parts. The results showed that all plants studied contained these minerals, with sodium and potassium being particularly noteworthy due to their significant roles in the human body.

The concentration of sodium in the plants ranged from 1.02 ± 0.02 mg/100g to 2.10 ± 0.05 mg/100g. Notably, the edible parts of *R. nepalensis* had the highest concentration of potassium at 95.4 ± 0.20 mg/100g, while *A. armata* leaves had the lowest concentration at 20.13 ± 0.08 mg/100g.

The K/Na ratio is essential for maintaining optimal health, as it influences blood pressure. It is desirable for this ratio to be greater than one, as potassium helps lower blood pressure while sodium tends to raise it. The study's findings indicate that several of the plants examined have favorable K/Na ratios in their edible parts. The proportions of K/Na were significant in the edible parts of *A. armata* (19.54), *M. dispermus* (9.72), *F. dibotrys* (77.84), *H. wallichii* (30.27), *T. clarkei* (39.47), *R. nepalensis* (47.22). These are compared to some regular fruits and vegetables (Amla 45.00, papaya ripe 11.50, tomato 11.31, *Castanea sativa* 56.67, *Punica granatum* 1400.00) [24]. This information suggests that the consumption of these vegetables could potentially help regulate blood pressure and control hypertension in our bodies. It highlights the importance of including these plant species in our diets to maintain a healthy balance of essential minerals.

In conclusion, the study emphasizes the significance of certain minerals like sodium and potassium found in the edible parts of various plants. The favorable K/Na ratios observed in these plants compared to regular fruits and vegetables underscore their potential role in controlling hypertension. Incorporating these vegetables into our diets may contribute to overall health and well-being by ensuring a proper balance of essential minerals. Further research and awareness of these findings could benefit public health strategies aimed at preventing and managing hypertension.

Calcium (Ca) is an essential micronutrient that plays a vital role in various physiological functions within the human body. It is known to have a significant impact on cardiovascular muscle function, blood clotting, milk coagulation, and regulation of cell permeability [25]. Adequate calcium intake is necessary for maintaining overall health and preventing calcium deficiency-related disorders.

In a study reported by Sundriyal and Sundriyal in 2004 [24], the calcium content of several cultivated vegetables and fruits was found to range between 10–130.0 mg/100g. These findings highlight the variability in calcium levels among different plant-based food sources. However, it is worth noting that the calcium levels in wild vegetables examined in this particular investigation were comparatively higher.

Among the wild vegetables analyzed, *T. clarkei* exhibited the highest calcium content at 80.76 ± 0.38 mg/100g, followed by *H. wallichii* at 63.43 ± 0.15 mg/100g, *A. armata* at 23.73 ± 0.08 mg/100g, and *M. dispermus* at 20.84 ± 0.04 mg/100g. These results indicate that wild vegetables can serve as a valuable source of dietary calcium due to their elevated calcium concentrations.

Including wild vegetables rich in calcium in our regular diet can contribute to meeting the body's calcium requirements. Calcium plays a crucial role in maintaining strong bones and teeth, muscle function, nerve transmission, and proper blood clotting. By incorporating these calcium-rich wild vegetables into our meals, we can enhance our overall calcium intake and support various physiological processes.

Copper (Cu) is a vital trace element that is considered an essential component for the human body. Unlike some other nutrients, the body cannot synthesize copper, and therefore, it must be obtained through dietary sources. Copper plays a crucial role as a cofactor for various enzymatic reactions and is involved in numerous physiological processes [26].

One of the significant functions of copper is its involvement in the catalyst that enables iron to bind to red blood cells, thereby promoting proper oxygen transportation throughout the body. This interaction between copper and iron is essential for preventing diseases related to iron deficiency, such as anemia.

Our investigation revealed that *F. dibotrys* exhibited the highest copper content at 0.78 ± 0.006 mg/100g, followed by *R. nepalensis* at 0.69 ± 0.05 mg/100g. On the other hand, *A. armata* showed the lowest copper content among the analyzed plants, with a concentration of 0.28 ± 0.03 mg/100g.

These findings highlight the variability in copper levels among different plant species. Incorporating copper-rich plants, such as *F. dibotrys* and *R. nepalensis*, into our diet can contribute to meeting the body's copper requirements. Adequate copper intake is crucial for the proper functioning of enzymes involved in energy production, connective tissue formation, and antioxidant defense mechanism.

Zinc (Zn) is a crucial micronutrient in human nutrition, serving as an essential component of various molecules involved in important physiological processes. It plays a key role in the digestion of nucleic acids and functions as a catalyst for immune reactions and a stabilizer of cell membranes. Inadequate zinc levels can result in growth retardation and impaired gonadal function, leading to delayed development [26]. The investigation revealed that wild plants, including *F. dibotrys*, contained significant amounts of zinc. The edible parts of *F. dibotrys* were found to have the highest zinc content at 15.43 ± 0.72 mg/100g. Furthermore, other plant species examined in the study exhibited zinc levels ranging from 2.97 ± 0.13 to 12.19 ± 0.43 mg/100g, as reported in Table 1.

These findings demonstrate that the zinc levels in the analyzed edible wild plants are comparable to those observed in other wild and leafy vegetables in India [24]. Including these zinc-rich plants in our diet can contribute to meeting the body's zinc requirements. Zinc is essential for numerous physiological processes, including DNA synthesis, immune function, growth, and reproductive health. By consuming wild plants such as *F. dibotrys*, individuals can enhance their zinc intake and support various vital functions within the body. However, it is important to consider a balanced diet that incorporates a variety of zinc sources to ensure optimal nutrient intake.

Manganese (Mn) is an essential trace element found in various plant species. It plays a crucial role in human nutrition, particularly in processes such as protein, sugar, and fat digestion, as well as the production of steroid sexual hormones [27]. Adequate manganese intake is necessary for maintaining optimal health and supporting these physiological functions.

In the investigated plants, manganese concentrations ranged from 1.72 ± 0.06 to 6.32 ± 0.22 mg/100g. This variability in manganese levels highlights the diversity of manganese content among different plant species. Consuming manganese-rich wild edible plants can contribute to meeting the body's manganese requirements and support the essential functions associated with this trace element.

Manganese is involved in various enzymatic reactions, acting as a cofactor for several enzymes that play a role in energy metabolism, antioxidant defense, and the synthesis of essential compounds. Additionally, manganese is important for proper bone development and maintenance, as well as for the functioning of the nervous system.

By incorporating manganese-containing wild edible plants into our diet, we can ensure an adequate intake of this essential trace element. However, it is important to maintain a balanced and varied diet to obtain a range of essential nutrients, including manganese, from different food sources

Iron (Fe) is an essential mineral that plays a critical role in various physiological processes within the human body. It is necessary for the production of hemoglobin, which is responsible for transporting oxygen to cells and tissues. Iron is also involved in the normal functioning of the central nervous system and plays a crucial role in the digestion of carbohydrates, proteins, and fats [27]. Consuming iron-rich foods is vital to prevent iron deficiency anemia and associated weakness.

Iron concentrations of different wild edible plants were analyzed. Among the analyzed plants, the leaves of *R. nepalensis* exhibited the highest iron content at 21.44 ± 0.37 mg/100g. It was followed by *T. clarkei* at 16.45 ± 0.16 mg/100g and *H. wallichii* at 11.42 ± 0.12 mg/100g. These iron levels observed in wild edible plants contrast significantly with those found in common leafy vegetables.

The variations in iron content among different plant species can be attributed to various soil characteristics in their growth zones. The composition and nutrient content of the soil play a significant role in the absorption and accumulation of minerals by plants, including iron. Factors such as soil pH, organic matter content, and mineral composition can influence the availability of iron to plants, ultimately affecting their iron content.

Including iron-rich wild edible plants in our diet can help ensure an adequate intake of this essential mineral. Iron is crucial for maintaining overall health, supporting oxygen transport, and preventing iron deficiency-related conditions.

It is worth noting that the iron content of foods can also be influenced by other factors such as plant variety, cultivation practices, and food processing methods. Therefore, it is important to consider a diverse and balanced diet that includes various sources of iron to meet the body's iron requirements.

Magnesium (Mg) is an essential mineral that plays a crucial role in various physiological functions within the human body. It is particularly important for normal neuronal and muscle function, as well as for maintaining a healthy immune system. Adequate magnesium intake is necessary to support these vital processes [27].

Our investigation revealed a range of magnesium levels among the investigated plants. *H. wallichii* exhibited the lowest magnesium content at 22.81 ± 1.24 mg/100g, while *T. clarkei* had the highest magnesium content at 38.56 ± 2.78 mg/100g.

Regular consumption of these magnesium-rich plants can have several benefits for human health. Magnesium plays a crucial role in regulating blood glucose levels, supporting proper nerve function, and maintaining healthy muscles. Additionally, magnesium is involved in numerous biochemical reactions within the body and is essential for the functioning of enzymes that contribute to energy production and DNA synthesis.

Maintaining adequate magnesium levels is important for overall well-being. However, it's essential to note that magnesium requirements may vary depending on factors such as age, sex, and individual health conditions. Incorporating magnesium-rich plants into the diet can be an effective way to ensure an adequate magnesium intake, but it is also recommended to have a balanced diet that includes a variety of magnesium sources.

Table 1 presents the heavy metal content, including lead (Pb) and chromium (Cr), in the wild vegetables studied. Lead, a non-essential heavy metal, can accumulate in soils and waste and poses a potential contaminant. Although

plants do not require lead for their growth, it can be accumulated in various plant parts. The consumption of vegetables containing lead can have immediate and long-term detrimental effects, negatively impacting the liver, kidneys, vascular system, and immune system [28]. In the investigated wild vegetables, the concentration of lead varied. The lowest lead concentration was found in *T. clarkei* (0.011 ± 0.01 mg/100g), while the highest was detected in the leaves of *A. armata* (0.025 ± 0.004 mg/100g). It is noteworthy that the lead concentrations observed in the wild vegetables studied were below the permissible limit set by the World Health Organization (WHO) of 0.03 mg/100g. Moreover, the lead levels found in this study are comparable to those reported in other Indian food items such as Indian basil (0.01 mg/100g), cabbage (0.01 mg/100g), and water leaf (0.02 mg/100g) [27].

Chromium, another essential mineral, plays a role in improving insulin sensitivity and influencing the digestion of sugar, protein, and fat. However, prolonged exposure to high levels of chromium can lead to liver and kidney damage. Among the wild vegetables studied, the lowest chromium concentration was found in the leaves of *A. armata* (0.04 ± 0.001 mg/100g), while the highest was observed in the leaves of *F. dibotrys* (0.18 ± 0.05 mg/100g). Importantly, the chromium levels in these vegetables were lower than the WHO's guideline limit of 0.29 mg/100g [27].

The findings of this study suggest that the lead and chromium concentrations in the analyzed wild vegetables are within safe limits according to the WHO guidelines. However, it is important to note that long-term exposure to even low levels of heavy metals can have adverse health effects. Thus, it is crucial to practice a varied and balanced diet, incorporating a diverse range of food sources, to minimize the potential risks associated with heavy metal exposure.

Quantifications of water soluble vitamin by HPLC

The estimation of water-soluble vitamins, including ascorbic acid (C), thiamine (B1), riboflavin (B2), niacin (B3), pantothenic acid (B5), pyridoxine (B6), and folic acid (B9), was successfully conducted using the HPLC technique. A representative HPLC chromatogram of the standard vitamin mixture is shown in Fig. 1, where all vitamins were effectively separated and detected at 210 nm. The identification of these constituents was further confirmed through absorption spectra analysis, which showed comparable results between the plant extracts and standard substances. The content of all vitamins in the plant materials is presented as mg/100g dry plant material, as detailed in Table 1.

Among the various nutrients present in fruits and vegetables, vitamin C holds significant importance. It is renowned for its antioxidant properties, which support the body's defense against viral infections, bacterial diseases, and harmful substances. Vitamin C is crucial for preventing scurvy and maintaining healthy skin, gums, and blood vessels. Its deficiency can lead to wounds, lethargy, dry skin, and overall poor health [29]. In our study, the analysis revealed a range of vitamin C content in the six wild edible plants, varying from 11.51 ± 0.24 to 87.56 ± 0.41 mg/100g. The inclusion of vitamin C-rich plants in our diet may contribute to reducing the risk of atherosclerosis and certain types of cancer due to their antioxidant properties [30]. Specifically, the vitamin C content in the investigated wild edible plants, such as *T. clarkei*, *R. nepalensis*, and *M. dispermus*, was determined to be 78.71 ± 0.22, 54.81 ± 0.19, and 22.04 ± 0.17 mg/100g dry plant material, respectively.

The results of this study provide valuable insights into the vitamin C content of wild edible plants and highlight their potential health benefits. By incorporating these plants into our regular diet, we can enhance our antioxidant intake, support our immune system, and promote overall well-being

Thiamine (B1) is an essential nutrient that plays a vital role in maintaining cellular function and supporting the proper functioning of various organs in the body. It is involved in energy production, starch digestion, and the normal functioning of nerve cells. Inadequate levels of thiamine can lead to degeneration, particularly in the nervous and circulatory systems, as well as an increased risk of hypertension and cardiovascular diseases [31].

In a study on wild edible plants, the thiamine levels were measured and found to range from 0.02 ± 0.03 to 0.85 ± 0.11 mg/100g. Among the plants tested, the highest concentration of thiamine was found in the leaves of *T. clarkei*, followed by *R. nepalensis*, *F. dibotrys*, and *H. wallichii*. Interestingly, the thiamine content in these wild edible plants was found to be comparable to the levels found in certain common vegetables and fruits, such as apples (0.02 mg/100g), beans (0.13 mg/100g), cauliflower (0.07 mg/100g), and spinach (0.08 mg/100g) [20].

This finding highlights the potential of these wild edible plants as a source of thiamine in the diet. Including these plants in one's regular diet can contribute to meeting the recommended thiamine intake and ensuring adequate levels of this important nutrient in the body. Thiamine plays a crucial role in various physiological processes, including the metabolism of carbohydrates, the proper functioning of the nervous system, and the maintenance of overall health.

Riboflavin (B2) is a crucial nutrient that plays a vital role in maintaining energy production, promoting proper digestion, and supporting various cell functions. It works synergistically with thiamine to ensure the overall reinforcement of foods and the optimal functioning of the body [32].

In a study investigating wild edible plants, the concentrations of B2 were assessed. Among the plants examined, the highest levels of B2 were found in the edible sections of *H. wallichii*, with a concentration of 0.82 ± 0.03 mg/100g. On the other hand, *A. armata* displayed the lowest concentration of B2 at 0.04 ± 0.002 mg/100g. Notably, the B2 content in these wild edible plants was found to be comparable to the levels found in common fruits and vegetables, such as almonds (1.10 mg/100g), spinach (0.24 mg/100g), beet greens (0.41 mg/100g), green beans (0.12 mg/100g), and potatoes (0.03 mg/100g) [33].

These findings highlight the potential of these wild edible plants as a valuable source of B2 in the diet. Including these plants in one's regular diet can contribute to meeting the recommended B2 intake and ensuring sufficient levels of this important nutrient in the body. B2 plays a crucial role in various physiological processes, including energy metabolism, antioxidant activity, and the maintenance of healthy skin, eyes, and nervous system.

Vitamin B3, also known as niacin, plays a critical role in various physiological processes, including fat metabolism, cholesterol regulation, and blood sugar control. It is involved in DNA synthesis, calcium digestion, intracellular respiration, as well as the synthesis of unsaturated fats and steroids [11]. During our investigation, the highest concentration of vitamin B3 was detected in *M. dispermus*, with a value of 0.82 ± 0.03 mg/100g. Additionally, appreciable amounts of vitamin B3 were observed in *F. dibotrys* and *R. nepalensis*.

The presence of significant levels of vitamin B3 in these wild edible plants highlights their potential as a natural source of this essential nutrient. Including these plants in one's diet can contribute to meeting the recommended intake of vitamin B3 and supporting various physiological functions. Vitamin B3 plays a crucial role in energy production, maintaining healthy cholesterol levels, and regulating blood sugar levels. These findings highlight the potential of these wild edible plants as a valuable source of vitamin B3, supporting various physiological functions and offering nutritional benefits.

Vitamin B5, also known as pantothenic acid, is an essential nutrient that plays a fundamental role in cell formation and optimal fat metabolism. Insufficient intake of vitamin B5 can lead to symptoms such as irritability, fatigue, apathy, numbness, tingling sensations, and muscle spasms in individuals [34]. During our analysis, we observed the highest concentration of vitamin B5 in the leaves of *T. clarkei* at 1.40 ± 0.13 mg/100g. This was followed by *M.*

dispermus (0.52 ± 0.03 mg/100g) and *F. dibotrys* (0.34 ± 0.04 mg/100g). Additionally, other plants investigated in our study also exhibited a generally good amount of vitamin B5.

The identification of significant levels of vitamin B5 in these wild edible plants highlights their potential as a valuable source of this essential nutrient. Including these plants in one's diet can help fulfill the recommended intake of vitamin B5 and support optimal cellular function and fat metabolism. Vitamin B5 is involved in various physiological processes, including energy production, hormone synthesis, and the maintenance of healthy skin and hair.

Moreover, the symptoms associated with vitamin B5 deficiency emphasize the importance of ensuring an adequate intake of this nutrient. By incorporating these wild edible plants into dietary practices, individuals can enhance their vitamin B5 intake and potentially alleviate or prevent the symptoms associated with its insufficiency.

Pyridoxine (B6) is an essential water-soluble vitamin that plays a critical role in various bodily functions, including red blood cell metabolism, nervous system function, immune system support, and numerous other important processes. It is also involved in the synthesis and breakdown of homocysteine, an amino acid [11]. This nutrient is naturally present in most food items, and due to its stability, it is often used for fortifying food products [11]. In our investigation, we evaluated the levels of B6 in all the wild edible plants under examination.

Among the plants studied, the highest concentration of B6 was observed in *A. armata*, with a value of 1.66 ± 0.11 mg/100g. On the other hand, *M. dispermus* displayed the lowest concentration of B6 at 0.21 ± 0.04 mg/100g. It is worth noting that the amounts of B6 obtained from these wild edible plants were comparable to those found in common vegetables and fruits such as bananas (0.37 mg/100g), avocados (0.29 mg/100g), spinach (0.24 mg/100g), broccoli (0.13 mg/100g), cauliflower (0.12 mg/100g), and cucumber (0.20 mg/100g) [24]. Therefore, the regular consumption of these plants would provide sufficient B6 to support healthy bodily functions.

These findings emphasize the potential of these wild edible plants as a valuable source of B6 in the diet. Incorporating these plants into one's regular meals can contribute to meeting the recommended B6 intake and supporting optimal bodily functions. B6 is involved in various processes, including protein metabolism, neurotransmitter synthesis, and immune system regulation.

Additionally, the presence of significant levels of B6 in these plants suggests that they can serve as a natural and accessible source of this essential nutrient. Incorporating these plants into local dietary practices and promoting their cultivation and consumption can have significant nutritional benefits, particularly in regions where access to commercial sources of B6 may be limited.

Vitamin B9, also known as folic acid, is a water-soluble B vitamin that can be obtained from a variety of natural sources. It plays a crucial role in several important bodily functions, including DNA synthesis and repair, cell division, and cell growth. Insufficient folate levels in adults can lead to iron deficiency, while in children, it can result in slower growth and development [11, 35–36]. Additionally, folic acid plays a significant role in cancer prevention by counteracting the detrimental effects of reactive oxygen species (ROS) and inhibiting lipid peroxidation [37].

During our investigation, the levels of B9 were analyzed in six different wild edible plants, ranging from 0.58 ± 0.014 to 3.78 ± 0.02 mg/100g. The highest concentration of B9 was found in *H. wallichii*, indicating its potential as a rich source of this essential nutrient. The leaves of *R. nepalensis* also exhibited a considerable amount of B9, with a concentration of 2.80 ± 0.06 mg/100g.

The wide range of B9 levels in these wild edible plants underscores their significance as a potential source of folic acid. Including these plants in one's diet can contribute to meeting the recommended intake of B9 and supporting essential bodily functions. Folate is particularly important for processes involving cell division and growth, making it crucial for normal development and overall health.

Moreover, the role of folic acid in cancer prevention further emphasizes the importance of maintaining adequate levels of B9. By incorporating these wild edible plants into dietary practices, individuals can potentially benefit from the protective effects of folic acid against oxidative stress and lipid peroxidation, reducing the risk of certain cancers

Anti-nutritional composition

The results of the anti-nutrient analysis conducted on the edible plants under investigation are presented in Table 1. Among the anti-nutrients, oxalate is a compound found in certain plants as soluble and insoluble salts of oxalic acid. It has the ability to bind with nutrients in the gastrointestinal tract, preventing their absorption by the body [38]. Consumption of wild edible plants with high levels of oxalic acid may potentially lead to nutrient deficiencies. In our study, the levels of oxalate were found to be highest in the leaves of *F. dibotrys* (0.16 ± 0.09%) and lowest in the leaves of three other plants, namely *H. wallichii*, *T. clarkei*, and *R. nepalensis*. It is worth noting that the oxalate levels in the examined plants are comparable to those found in common fruits and vegetables such as spinach (0.66%), almonds (0.41%), amla (0.30%), and amaranth (0.77%) [20].

Although oxalate has been known to inhibit the absorption of renal calcium, particularly at concentrations around 45 g/100g [39], it is important to note that the oxalate levels observed in the plants studied are not at the threshold considered harmful. While high oxalate consumption can potentially contribute to the formation of kidney stones in susceptible individuals, the levels found in the investigated plants are within acceptable limits and are not expected to pose significant health risks.

Considering the relatively moderate levels of oxalate in the edible plants under scrutiny, their consumption as part of a balanced diet is unlikely to result in adverse effects. It is important to maintain a diverse and varied diet to ensure an adequate intake of essential nutrients while minimizing the risk of excessive exposure to any specific anti-nutrients. Furthermore, proper cooking and processing techniques can help reduce the oxalate content in foods, further mitigating any potential concerns.

Phytic acid and phytates are naturally occurring compounds predominantly found in vegetables, although they can also be found in low concentrations in certain plants. These compounds have a tendency to bind to minerals such as iron, zinc, calcium, and magnesium, forming insoluble complexes. Additionally, they can interact with proteins and carbohydrates, potentially impacting their digestion and utilization in the body [40]. In our study, the levels of phytic acid in the wild edible plants varied, ranging from 0.15 ± 0.07% in *T. clarkei* to 0.44 ± 0.08% in *R. nepalensis*. These concentrations are comparable to those found in commonly consumed foods such as rice (0.15%) and beans (0.25%) [20]. The phytic acid levels observed in our study also fall within the range of 0.37–0.90 mg/g reported in various fruits, including guava, mango, orange, and pineapple [41]. It is important to note that the phytate levels found in our study were below the range of 10–60 mg/g, which is considered significant in terms of mineral bioavailability challenges [42]. While phytic acid and phytates can potentially inhibit the absorption of certain minerals, their levels in the investigated plants do not appear to pose significant concerns in this regard.

To overcome any potential mineral bioavailability difficulties associated with phytates, it is recommended to adopt certain food processing techniques. Methods such as soaking, fermenting, and germination can help reduce the

phytate content in foods, thereby enhancing mineral absorption. By employing these strategies, the nutritional value of the consumed plants can be optimized, ensuring an adequate intake of essential minerals.

Saponins are a naturally occurring mixture of compounds that can be found in various plant species. They exhibit detergent-like properties when added to fluids, leading to a foaming effect. However, the use of the term "synthetic" in relation to saponins is incorrect, as they are naturally produced by plants.

The foaming effect of saponins can interfere with the normal functioning of the epithelial cells lining the stomach, which may contribute to stomach-related disorders. Additionally, saponins have been associated with adverse effects such as red blood cell damage, interference with certain chemicals, and disruption of thyroid function [43]. It is important to note that these effects are typically observed when saponins are consumed in high amounts or in concentrated forms, rather than in the normal dietary context.

The concentration of saponins can vary among different plant species. For example, *F. dibotrys* was found to have the highest amount of saponins, with a concentration of 4.92 ± 0.02%. On the other hand, the leaves of *R. nepalensis* had the lowest concentration, measuring only 0.05 ± 0.004%. These variations in saponin levels reflect the natural diversity among plants and their biochemical composition.

In conclusion, saponins are natural compounds found in plants, and they possess detergent-like properties when added to fluids. While they can cause stomach-related disorders and interfere with certain physiological functions in high concentrations, it is important to consider the context in which saponins are consumed. The concentration of saponins varies among plant species, with some plants containing higher levels than others. Further research is necessary to fully understand the potential benefits and risks associated with saponins and their impact on human health.

Tannins are a prevalent group of anti-nutritional compounds that can be found in many vegetables. It is believed that these compounds, due to their polyphenolic properties, can interact with proteins and other natural substances such as alkaloids and amino acids, potentially causing adverse effects or complex formations.

Tannins, commonly present in various foods, have been found to inhibit the proper functioning of enzymes such as amylase, lipase, trypsin, and chymotrypsin. This interference can lead to the degradation of protein quality and hinder the absorption of iron [40]. It's important to note that the effects of tannins on human health depend on the concentration consumed and the overall dietary context.

The concentration of tannins can vary among different plant species. For instance, the leaves of *A. armata* were found to have the highest concentration of tannins, measuring 0.68 ± 0.06%. On the other hand, the leaves of *H. wallichii* had the lowest concentration, measuring 0.04 ± 0.003%. It is worth mentioning that these lower concentrations may not pose significant harm to humans [40].

Cyanogenic glycosides are secondary metabolites that are widely present in various plant species. These compounds have the ability to release hydrogen cyanide (HCN) through enzymatic hydrolysis, which can potentially lead to cyanide poisoning. Therefore, it is crucial to understand and manage the presence of cyanogenic glycosides in foods for the sake of dietary benefits and safety [44].

Research has indicated that the cyanide content in plants investigated ranged from 0.09 ± 0.007% to 0.15 ± 0.06%. It is important to note that the levels of cyanogenic glycosides in these plants were relatively low. These low concentrations suggest that the consumption of these plants as food is considered safe [44]. While cyanogenic

glycosides can potentially release cyanide, it is essential to understand that the toxicity risk is largely associated with the amount of cyanide released, rather than the presence of cyanogenic glycosides themselves. Proper processing and cooking techniques can effectively reduce the cyanide content in foods, ensuring their safety for consumption.

In conclusion, cyanogenic glycosides are widely distributed across the plant kingdom and can release cyanide through enzymatic hydrolysis. However, the levels of cyanogenic glycosides found in plants are generally low, indicating that these plants can be safely consumed as food. It is important to employ appropriate processing methods to mitigate the risk of cyanide poisoning and enhance the dietary benefits and safety of cyanogen-containing foods.

Toxicity studies

Table 2 presents the findings of toxicity studies conducted on edible plants, assessing cell viability and DNA damage levels. The experiments involved the use of buffer (as a negative control) and hydrogen peroxide (as a positive control) to evaluate the effects. The haemolytic tests were conducted on plants known for their potent nutraceutical properties, aiming to determine if they possess any haemolytic effects. Additionally, these tests may provide insights into the cytotoxicity of the plants under investigation.

Table 2
Toxicity of wild edible plants

Name of the plant	Concentration of the extract (µg/ml)	RBC cell viability (%)	Hepatocytes cell viability (%)	Olive tail moment (OTM) of stained nuclei
A. armata	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	85.01 ± 0.38	88.14 ± 1.38	3.74 ± 0.88
	1000	83.28 ± 1.25	85.35 ± 1.78	3.98 ± 1.11
M. dispermus	100	96.47 ± 1.08	86.81 ± 1.86	3.26 ± 1.22
	200	92.76 ± 1.11	86.17 ± 1.08	3.41 ± 0.68
	300	91.04 ± 0.98	86.02 ± 1.88	3.65 ± 1.08
	500	87.27 ± 0.67	85.81 ± 1.06	3.82 ± 1.15
	1000	85.51 ± 1.87	83.22 ± 1.12	4.21 ± 1.05
F. dibotrys	100	98.70 ± 2.11	83.76 ± 1.55	2.85 ± 0.87
	200	97.36 ± 2.56	83.44 ± 2.11	3.01 ± 1.04
	300	96.43 ± 1.87	83.10 ± 1.28	3.38 ± 1.19
	500	96.85 ± 1.09	82.93 ± 1.09	3.56 ± 1.05
	1000	93.08 ± 1.04	81.26 ± 1.07	3.89 ± 1.11
H. wallichii	100	96.67 ± 1.77	84.56 ± 1.77	2.98 ± 0.89
	200	94.52 ± 1.05	82.11 ± 1.09	3.19 ± 0.33
	300	93.78 ± 1.11	81.76 ± 0.94	3.76 ± 0.64
	500	91.84 ± 0.78	81.34 ± 1.24	3.94 ± 0.29
	1000	89.76 ± 1.55	80.02 ± 1.55	4.34 ± 0.58
T. clarkei	100	89.71 ± 1.34	83.76 ± 2.33	3.02 ± 1.43
	200	88.90 ± 1.08	83.44 ± 1.06	3.31 ± 1.04
	300	87.63 ± 0.68	83.01 ± 1.62	3.58 ± 0.82
	500	87.05 ± 1.26	82.67 ± 1.34	3.97 ± 0.89
	1000	84.71 ± 1.89	79.31 ± 1.68	4.36 ± 1.11
R. nepalensis	100	92.38 ± 2.65	86.33 ± 3.98	2.37 ± 0.78
	200	90.54 ± 1.97	84.15 ± 4.67	2.86 ± 0.84

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)

RBC : Red blood cell

Name of the plant	Concentration of the extract (µg/ml)	RBC cell viability (%)	Hepatocytes cell viability (%)	Olive tail moment (OTM) of stained nuclei
	300	88.11 ± 3.65	83.68 ± 5.11	3.26 ± 0.35
	500	86.32 ± 2.68	81.45 ± 2.65	3.96 ± 0.62
	1000	85.11 ± 2.79	80.56 ± 3.44	4.11 ± 0.28
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
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)				
RBC : Red blood cell				

Haemolytic toxicity

In order to assess the potential haemolytic effects of the investigated wild plants, in vitro haemolytic tests were conducted using rodent erythrocytes. Different concentrations (100, 200, 300, 500, and 1000 µg/ml) of the plants were tested. Hydrogen peroxide (H_2O_2) at a concentration of 200 µM resulted in 51.75% haemolysis, while the buffer (negative control) showed 100.18% cell viability in rodent erythrocytes (Fig. 2).

The haemolytic effects observed in the rodent erythrocytes varied depending on the concentration of the plant extracts. However, it was noted that all concentrations of the extracts demonstrated a reduced haemolytic impact on the rodent erythrocytes. The viability of the haemolytic cells was most significantly affected (93.08%) at the highest concentration of 1000 µg/ml in the case of *F. dibotrys*, while the lowest impact was observed in the aqueous concentrate of *A. armata* (83.28%) at the same concentration (Fig. 2).

These findings indicate that the haemolytic effects of the plant extracts were concentration-dependent, with higher concentrations generally resulting in greater haemolysis. However, it is important to note that even at the highest tested concentrations, the haemolytic impact on rodent erythrocytes was relatively low for all investigated plants. This suggests that the tested wild plants may have a minimal haemolytic effect and could potentially be safe for consumption.

Cytotoxicity

To evaluate the impact on hepatocyte cell viability, hepatocytes were isolated from fresh goat liver and exposed to various concentrations (100, 200, 300, 500, and 1000 µg/ml) of aqueous concentrates derived from edible plants. Figure 3 presents the results, showcasing the effects of the plant concentrates on hepatocyte cell viability.

Among the tested plants, *A. armata* exhibited the highest suitability for hepatocyte cells at a concentration of 1000 µg/ml, with a viability rate of 85.35%. Conversely, *T. clarkei* showed the lowest viability rate (79.31%) at the same concentration (Fig. 3).

Comparing the viability rates of red blood cells (RBCs) and hepatocyte cells across all concentrations (100–1000 µg/ml) of the plant extracts, they were found to be similar to the negative control. This indicates that the plant extracts did not significantly affect the viability of RBCs and hepatocyte cells in comparison to the negative control. However, when exposed to the positive control, H_2O_2 at a concentration of 200 µM, both RBCs and hepatocyte cells exhibited viability rates of less than 50%.

These findings suggest that the aqueous concentrates of the edible plants tested in this study had a relatively high suitability for hepatocyte cells, with minimal adverse effects on their viability. The viability rates of both RBCs and hepatocyte cells in the presence of the plant extracts were comparable to the negative control, indicating a lack of significant cytotoxicity.

It is important to note that the viability of hepatocyte cells is crucial as they are primary functional cells in the liver responsible for various metabolic processes. The findings suggest that the tested plant extracts may have a favorable impact on hepatocyte cell viability, which could be indicative of their potential beneficial effects on liver health.

Further research is needed to elucidate the underlying mechanisms and evaluate the overall safety and potential benefits of these edible plants on liver function and health.

Genotoxicity

To evaluate the genotoxicity of plant extracts, rat lymphocytes were incubated with various concentrations (100–1000 µg/ml) of the plant concentrates in a low-melting-point agarose suspension. The lysed cells were subjected to alkaline conditions, followed by electrophoresis and visual inspection under a fluorescence microscope. The Olive Tail Moment (OTM) of stained nuclei was calculated using comet assay software, providing a measure of DNA damage. Negative controls (whole blood and RPMI-1640) and positive controls (whole blood, 50, 100, and 200 µM H_2O_2, and RPMI-1640) were included in the study [45].

The comet assay results, represented in Table 2 and Figs. 4 and 5A to 5F, revealed that the aqueous concentrate of *T. clarkei* at a concentration of 1000 µg/ml had the highest OTM (4.36) of tail DNA (Fig. 5E), while *F. dibotrys* at the same concentration exhibited the lowest OTM (3.89) (Fig. 5C). The negative control showed an OTM of 1.79 of tail DNA (Fig. 5G), while the positive control (a mixture of whole blood, RPMI 1640, and 200 µM H_2O_2) indicated an OTM of 21.38 of tail DNA (Fig. 5H).

Mutagenicity and genotoxicity can arise from the formation of free radicals during normal metabolism. The positive controls in this experiment exhibited significant genetic alterations and comet formation, indicating genotoxic effects, while the plant extracts showed no significant genotoxic impact at concentrations ranging from 100 to 1000 µg/ml (Fig. 5A-5F). Hydrogen peroxide, as an oxidative stress inducer, caused DNA damage (OTM 6.18–21.38 of tail DNA) as detected by the comet assay.

The results of this study indicated that the degree of DNA damage caused by the plant extracts at various concentrations was comparable to the negative control. Regular consumption of antioxidants, such as vitamins E and C, lycopene, and β-carotene, derived from dietary sources, has been shown to lower levels of free-radical damage and provide protection against degenerative conditions, including cancer, by reducing DNA damage [46].

Plants contain a wide range of pharmacologically active phytochemicals, some of which have been found to be beneficial in treating various human ailments. However, certain phytochemicals, such as saponins, tannins, and

cyanogenic glycosides, have been associated with harmful effects and can contribute to mutagenicity and genotoxicity upon exposure [47].

In conclusion, the genotoxicity assessment of plant extracts using the comet assay demonstrated that the tested plant extracts did not exhibit significant genotoxic effects at concentrations ranging from 100 to 1000 µg/ml. Further research is required to fully understand the potential genotoxicity and safety profiles of these plant extracts and their phytochemical constituents.

Principal component analysis

To improve the discrimination among the samples, a principal component analysis (PCA) was conducted on the combined data of proximate composition, Vitamin C, and mineral content. The PCA score plots (Fig. 6A) depict the distribution of all plant samples based on these variables, while the corresponding loading plots (Fig. 6B) illustrate the associations between the variables and the principal components.

The PCA yielded four principal components (PC) with eigenvalues greater than 1. However, for simplicity, only the first two PCs were retained for further analysis. These two PCs accounted for 65.1% of the total variance explained by the pooled proximate, vitamin C, and mineral values. PC1 contributed 34.5% of the variance, approximately 1.13 times more than PC2, which accounted for 30.6%.

In the loading plot (Fig. 6B), PC1 exhibited negative associations with protein, carbohydrate, and calcium, while demonstrating positive associations with fat, sodium, potassium, iron, zinc, and vitamin C variables. On the other hand, PC2 showed negative correlations with protein and zinc, but displayed positive correlations with the remaining variables.

Upon examining the score plot (Fig. 6A), it becomes evident that the leaves of *A. armata*, *R. nepalensis*, and *F. dibotrys* were distinctly separated and located far from all other samples on both the left and right sides. This separation can be attributed to their high contents of carbohydrate, protein, minerals, and vitamin C.

Overall, the PCA analysis revealed that the combined proximate composition, Vitamin C, and mineral data effectively differentiate the plant samples, with the first two principal components explaining a substantial portion of the overall variance. The findings suggest that these variables play a significant role in distinguishing the samples under investigation

Conclusion

The research conducted on wild edible plants collected from Sikkim highlighted their high nutritive value, making them a potential source of essential nutrients for maintaining human health. These plants exhibited remarkable levels of protein, fat, sugar, and fiber, surpassing those found in ordinary vegetables. Furthermore, they were found to be rich in various minerals such as sodium, potassium, calcium, iron, copper, magnesium, and zinc.

Importantly, the study addressed concerns regarding the presence of toxic metals in the plants. While hazardous metals like cadmium and mercury were not detected, the presence of lead and chromium was noted within permissible limits, as recommended by the WHO. Consequently, the levels of these heavy metals are unlikely to pose a threat to human health.

The wild edible plants also demonstrated the presence of water-soluble B and C vitamins in varying amounts, as determined through RP-HPLC analysis. These findings can contribute to estimating the dietary intake of vitamin C

and B vitamins in the general population.

Additionally, the study evaluated the anti-nutritional factors in these plants, including oxalate, phytate, saponin, cyanogenic glycoside, and tannin. However, the levels of these compounds were found to be lower than the established toxic thresholds, indicating that these plants can be safely consumed without restrictions.

Moreover, assessments of haemolytic toxicity, cytotoxicity, and genotoxicity of water extracts from these edible plants revealed no harmful effects at the cellular and genomic levels, further confirming their safety for consumption.

Based on these findings, it is suggested that these wild edible plants possess excellent nutritive properties and can contribute to mitigating the risk of nutritional deficiencies and related diseases. Furthermore, promoting the cultivation of these wild species on a large scale can bring economic benefits to underprivileged farmers while ensuring a sustainable supply of nutrient-rich food for the indigenous population.

In conclusion, the study underscores the significance of wild edible plants as a valuable source of nutrients and advocates for their cultivation and utilization to improve human health and well-being.

Abbreviations

MTT: 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide ; **HPLC**: High-performance liquid chromatography; **Na**: Sodium; **K**: Potassium; **Ca**: Calcium; **Mn**: Manganese; **Cu**: Copper; **Mg**: Magnesium; **Fe**: Iron; **Zn**: Zinc; **Pb**: Lead; **Cr**: Chromium; **Cd**: Cadmium; **Hg**: Mercury; **%**: Percentage; **nm**: Nanometer; **mg**: Milligram; **µg**: Microgram ; **SEM** : Standard error of Mean; **µM** : Micromolar; **AAS**: Atomic absorption spectroscopy; **M**: Molar; **TFA**: Trifluoro acetic acid; **EDTA**: Ethylene diamine tetra acetic acid; **RBC** : Red blood cells; **PBS**: Phosphate buffer saline; **HBSS** : Hank's Balanced Salt Solutions; **RPMI**:*Roswell Park Memorial Institute;* ***FBS***: Fetal bovine serum; **DMSO**: Dimethyl sulfoxide; **LMPA**:Low melting point agarose; **NMPA**: Normal melting point agarose; **OTM**: Olive tail moment ; **AOAC** : Association of Official Analytical Chemists ; **h** : hour; **^{o}C** : Degree centigrade; HCl : Hydrochloric acid; **%** : Percentage; **PCA** : principal component analysis; **PC1** : First principal component; **PC2** : Second principal component; **g** : Gram

Declarations

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Author contributions

Tapan Seal conceptualized and designed the research, supervised the study, interpreted the data and drafted the manuscript.

Kausik Chaudhuri and Basundhara Pillai analysed the samples

Norbu Sherpa collected the plant materials

Rajib Gogoi identified the investigated plants, reviewed the final manuscript draft.

All authors read and approved the final manuscript.

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Availability of data and materials

The raw data and materials for this study will be made available by the authors on request.

Competing interests

The authors have no competing interest regarding this study.

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Figures

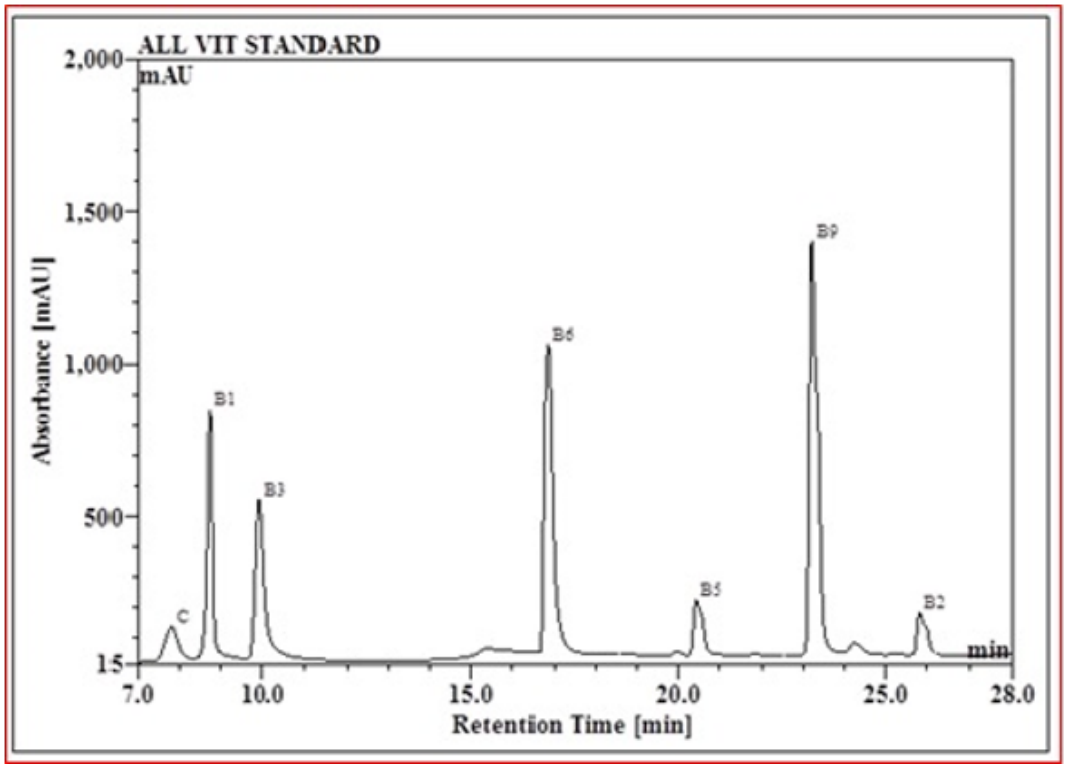


Figure 1

HPLC Chromatogram of mixture of Standard vitamin

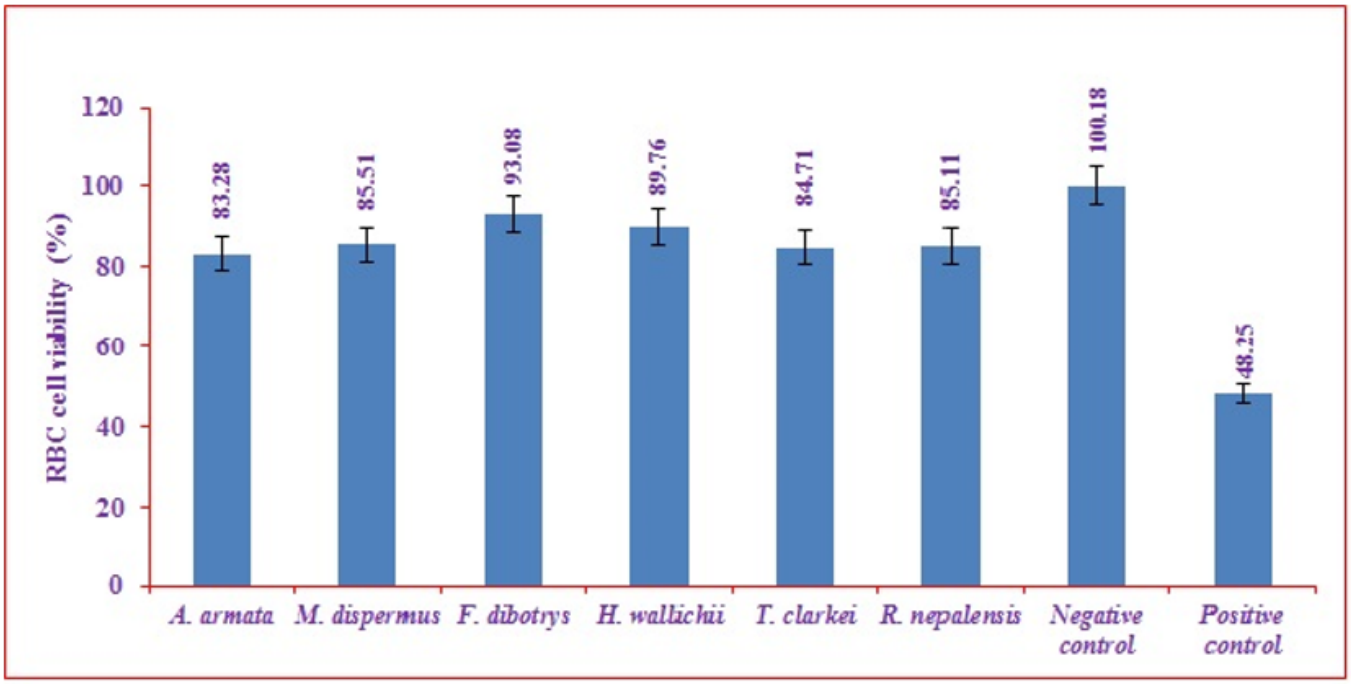


Figure 2

Haemolytic toxicity of Plant extracts (1000 µg/ml), negative control and positive control (H_2O_2 ; 200µM)

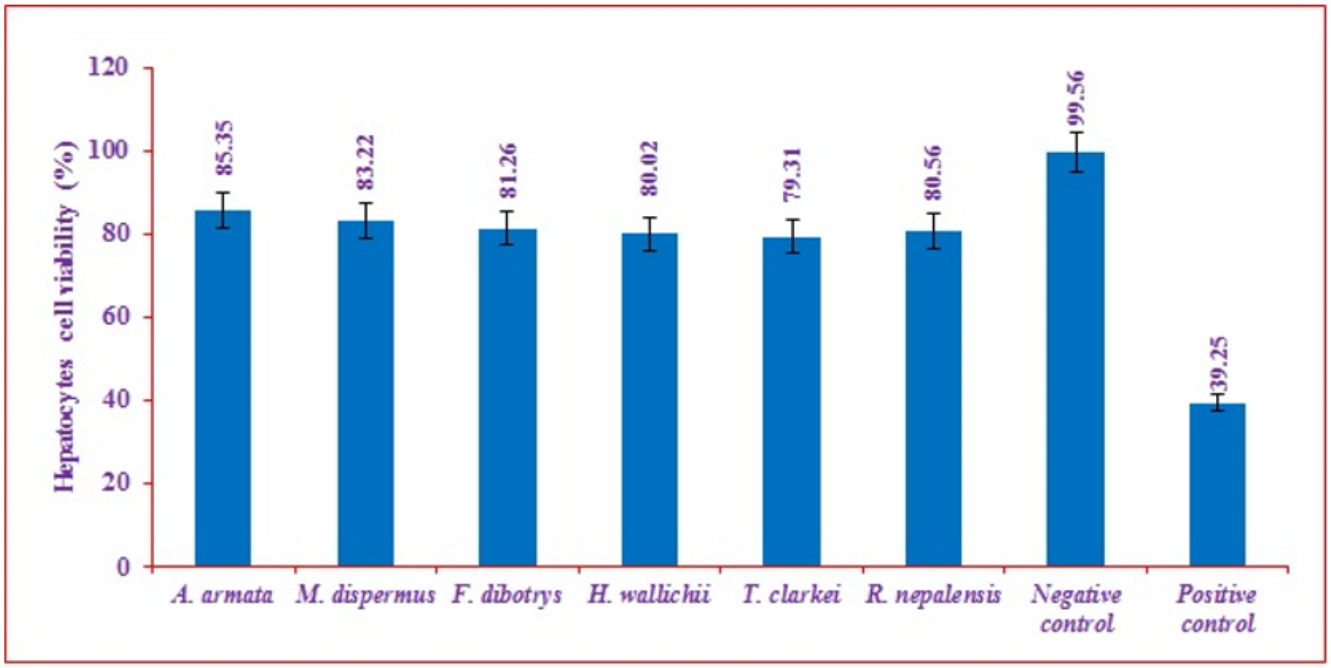


Figure 3

Hepatotoxicity of Plant extracts (1000 µg/ml), negative control and positive control (H_2O_2 ; 200µM)

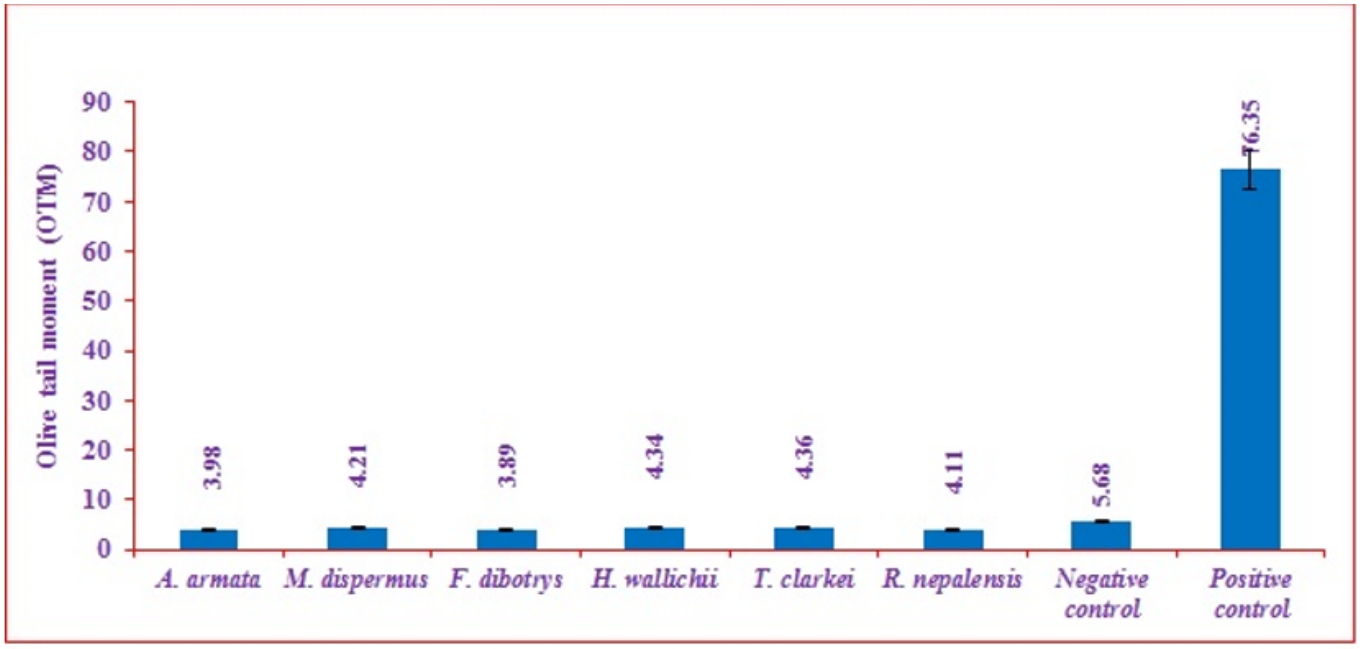


Figure 4

Genotoxicity of Plant extracts (1000 µg/ml), negative control and positive control (H_2O_2 ; 200µM)

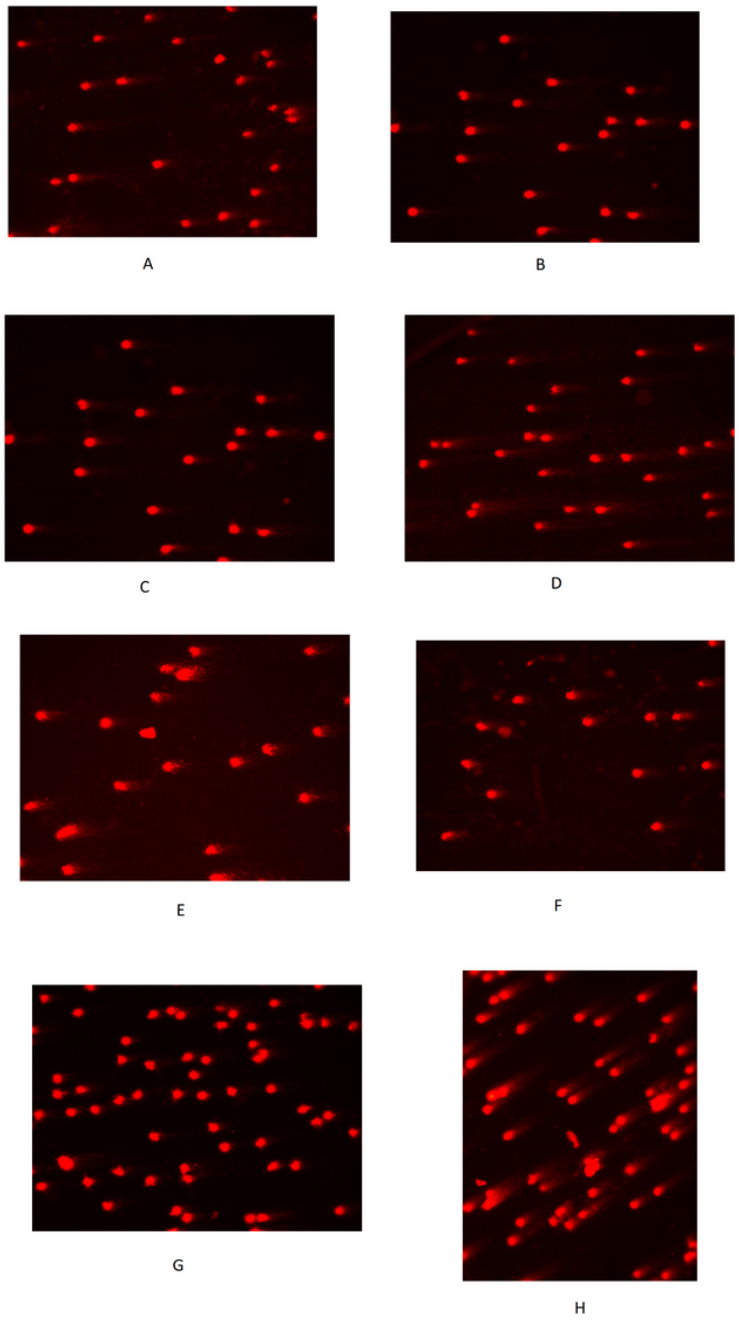


Figure 5

A. Evaluation of genotoxic effect induced by the aqueous extract of A. armata

B. Evaluation of genotoxic effect induced by the aqueous extract of M. dispermus

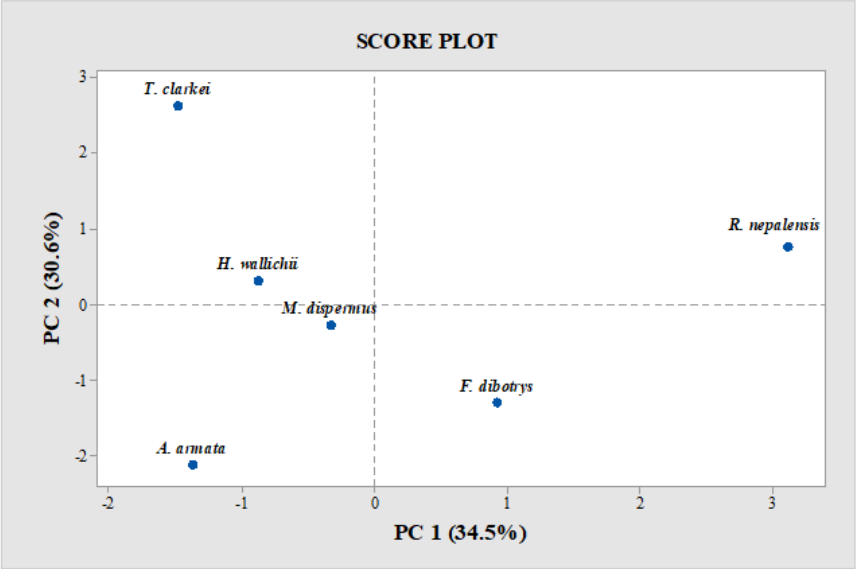
C. Evaluation of genotoxic effect induced by the aqueous extract of F. dibotrys

D. Evaluation of genotoxic effect induced by the aqueous extract of H. wallichii

E. Evaluation of genotoxic effect induced by the aqueous extract of T. clarkeiF. Evaluation of genotoxic effect induced by the aqueous extract of R. nepalensis

G. Evaluation of genotoxic effect induced by negative control

H. Evaluation of genotoxic effect induced by positive control



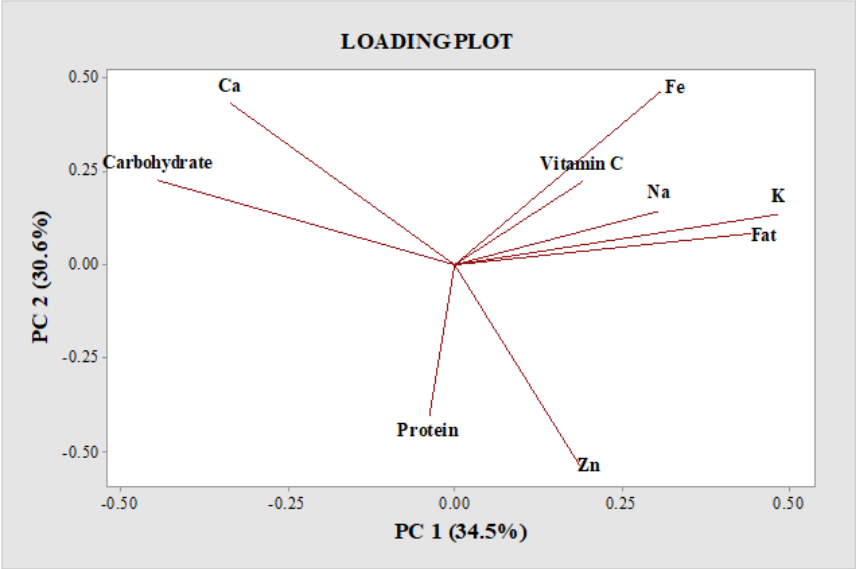


Figure 6

Principal component analysis using variables listed in Tables 1 . Score plot (6A) and loading plot (6B) of first two principal components for clustering of plant samples. Variables: 14 (Ash, fat, protein, carbohydrate, Na, K, Ca, Fe, Mg, Zn, vitamin C)