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Original research paper

NUTRITIONAL AND TOXICOLOGICAL EVALUATION OF WILD EDIBLE PLANTS FROM NORTH-EAST INDIA: IMPACT OF VARIOUS COOKING METHODS

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Abstract: This study aimed to explore the nutritional value, anti-nutritional factors, mineral content, and *in vitro* toxicity of ten wild edible plants from the North-Eastern region of India, including *Meynia laxiflora*, *Castanopsis indica*, *Docynia indica*, *Flemingia vestita*, *Bauhinia purpurea*, *Dillenia pentagyna*, *Diplazium esculentum*, *Elaeagnus latifolia*, *Elaeagnus pyriformis*, and *Fagopyrum cymosum*. The impacts of cooking techniques including boiling and microwaving were also investigated in the study.

Nutritional analysis assessed fat, protein, fibre, carbohydrate, and mineral content in both raw and cooked samples. The anti-nutritional properties were evaluated by measuring the content of cyanogenic glycosides, oxalates, tannins, saponins, and phytates. *In vitro* toxicity was assessed through haemolytic assays on rat erythrocytes, cytotoxicity was measured using the MTT assay, and genotoxicity was evaluated using the comet assay.

The findings revealed that both cooking methods reduced ash, fat, mineral content, anti-nutritional factors, and plant toxicity. At the same time, increasing the relative concentration of fibre and carbohydrates due to water loss or the breakdown of other components, the absolute content of these nutrients remained unchanged. Microwave cooking significantly enhanced the measured protein content ($p<0.05$), with increases ranging from 9.58% to 33.95%. This effect is likely due to structural modifications in the proteins caused by microwave treatment, which enhance their availability or digestibility rather than increasing the actual protein content, whereas boiling caused a reduction in protein levels, ranging from 9.66-23.25%. Additionally, microwave cooking resulted in lower mineral losses than boiling and was more effective in reducing anti-nutritional factors and toxicity ($p<0.05$). As a result, microwaving is recommended to improve nutritional quality, reduce fat content, and decrease anti-nutritional components and toxicity. Toxicity studies at the cellular and genomic levels indicated that these plants are safe for consumption.

Key words: *wild edible plants, thermal treatments, nutritional composition, minerals content, anti-nutrients, toxicity*

INTRODUCTION

Wild edible plants are commonly utilized as food sources in developing countries. Because of rapid population growth, scarce arable land, and the high cost of staple foods, many people depend on wild edible plants and other natural resources to fulfil their nutritional requirements. In rural areas, a diverse array of wild vegetables is often gathered without cultivation, motivated by cultural traditions, taste preferences, or food scarcity. Biochemical techniques have been deve-

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loped to cultivate desirable plant species on a large scale in gardens and fields to address caloric needs. However, research suggests that cultivated plants that require substantial chemical inputs, such as fertilizers, plant growth regulators, and herbicides, often lose their natural flavour, appearance, and nutritional value (Sekeroglu, Ozkutlu, Deveci, Dede & Yilmaz, 2016).

Recently, there has been an increasing interest in assessing wild edible plants which are vital components of the human diet, providing essential minerals, vitamins, hormone precursors, protein, and energy. These plants are crucial in lowering the risk of diseases such as cancer, coronary heart disease, and diabetes because they are rich in protein, carbohydrates, and various macronutrients. Wild vegetables are also becoming commercial crops with growing market potential, as they typically lack pesticide or fertilizer residues (Weng, Huang & Yang, 2001). The literature suggests that the nutritional and therapeutic benefits of unconventional plant foods can be comparable to or even surpass that of common vegetables (Aberoumand, 2011).

In addition to their nutritional benefits, wild edible plants offer valuable products such as medicines, fibre, fodder, and dyes. While traditionally consumed by tribal and rural communities for both food and medicinal purposes, there is a lack of scientific data on the proximate and chemical composition of these wild vegetables.

Many people are not fully aware of their beneficial and potentially toxic properties. Analysing wild edible plants are crucial to identify alternative food sources. Although these plants are nutritious and flavourful, excessive consumption can be harmful due to the presence of anti-nutritional compounds. Anti-nutritional factors such as phytic acid, tannins, saponins, oxalic acid, and cyanogenic glycosides can negatively impact health by inhibiting protein digestion, growth, and the absorption of essential minerals like iron and zinc (Liener & Kakade, 1980; Larsson, Rossander-Hulten, Sandstrom & Sandberg, 1996). Phytic acid, for instance, reduces mineral bioavailability (Reddy, Sathe & Pierson, 1988), while tannins decrease nutritional quality by binding to proteins through hydrogen bonding and hydrophobic interactions (Hahn, Rooney & Earp, 1984).

Plants have served as sources of both food and medicine since ancient times. Green plants are recognized as primary sources of both antimutagens and natural toxins (Plewa & Wagner, 1993). Therefore, it is crucial to evaluate whether wild plants might have harmful effects on living beings before they are consumed. Although the intake of fresh vegetables is highly recommended, wild edible plants are seldom eaten raw and are usually cooked before consumption. Understanding how domestic cooking methods affect the proximate composition and mineral content of wild edibles is essential. It is also important to identify cooking methods that enhance the nutritional value and health-related properties while reducing anti-nutritional factors and toxicity.

This study aims to evaluate the nutritional value, anti-nutritional factors, and toxicity of ten wild edible plants viz. *Meynia laxiflora* Robyns (Family: Rubiaceae), *Castanopsis indica* Roxb (ex Lindl.) A.DC (Family: Fagaceae), *Docynia indica* (Wall.) Decne. (Family: Rosaceae), *Flemingia vestita* Benth. ex Baker f. (Family: Fabaceae), *Bauhinia purpurea* L. (Family: Fabaceae), *Dillenia pentagyna* Roxb. (Family: Dilleniaceae), *Diplazium esculentum* (Retz.) Sw. (Family: Aspleniaceae), *Elaeagnus latifolia* L. (Family: Elaeagnaceae), *Elaeagnus pyriformis* Hook. f., (Family: Elaeagnaceae) and *Fagopyrum cymosum* (Trev.) Meisn (Family: Polygonaceae) sourced from the North-Eastern region of India. In Northeast India, these plants are locally named as Soh-mon, Chakkum-khokrok, Soh-phoh, Soh-phlang, Muyung-laphang, Dieng Soh karbam, Jhur-tyrkhang, Soh-shang, Heiyai and Jarain respectively. These plants are widely consumed by tribal communities in the area. The study also investigates the effects of common domestic cooking methods, namely boiling and microwaving, on the overall nutritional quality and toxicity of these wild edibles. The findings will help identify the cooking methods that best preserve nutrients and phytochemicals while reducing anti-nutritional components and toxicity.

MATERIALS AND METHODS

Plant samples

The edible portions of fresh plants viz. *Meynia laxiflora*, *Castanopsis indica*, *Docynia indica*, *Flemingia vestita*, *Bauhinia purpurea*, *Dillenia pentagyna*, *Diplazium esculentum*, *Elaeagnus latifolia*, *Elaeagnus pyriformis*, and *Fagopyrum cymosum*, were obtained from North-East region in India. The plant identifications were confirmed by the Botanical Survey of India, Howrah.

Voucher specimens were stored in the Plant Chemistry Department of our office and catalogued under registry numbers BSITS 11, BSITS 12, BSITS 13, BSITS 14, BSITS 15, BSITS 16, BSITS 17, BSITS 18, BSITS 19, and BSITS 20, respectively. The edible parts of wild plants were then dried at room temperature, finely ground into powder, and kept in sealed containers. Nutritional composition, mineral content, anti-nutritional factors, and toxicity analysis were then performed in our laboratory.

Cooking by boiling

Five grams of each plant were boiled in distilled water at 100 °C at a ratio of 1:10 (w/v) on a hot plate for 1 h until they softened, following the method described by Hefnawy (2011). The plant samples were then drained using a sieve and dried in an air oven at 50 °C for 2 h. After drying, the samples were stored for further analysis.

Microwave cooking

Five grams of each plant were added to a glass beaker containing distilled water at a 1:10 (w/v) ratio and microwaved for 15 min until softened. After cooking, the plants were drained, dried in an air oven at 50 °C for 2 h, and then stored for further analysis (Hefnawy, 2011).

Nutritional parameters

The nutritional composition of the samples under investigation was assessed using standard food analysis methods specified by the Association of Official Analytical Chemists (AOAC, 2000). The ash content was measured by heating the plant samples in a muffle furnace at 500 °C for approximately 5-6 hours (AOAC 923.03). Moisture content was assessed by drying the samples in an air oven at 100-110 °C (AOAC 925.09). Crude protein was determined using the micro Kjeldahl method (AOAC 984.13). Total carbohydrate content was measured using the method described by Hedge & Hofreiter (1962), often cited as (AOAC 978.10).

Crude fat was extracted from the moisture-free samples using petroleum ether (60-80 °C) in a Soxhlet apparatus for 6-8 hours (AOAC 920.39). Crude fibre content was determined by treating the fat and moisture-free materials with 1.25% dilute acid and 1.25% alkali, followed by washing with water and igniting the residue (AOAC 962.09). The energy content of each plant sample was calculated by multiplying the values obtained for protein, fat, and available carbohydrates by 4.00, 9.00, and 4.00, respectively, and then summing these values (AOAC, 2000).

Determination of mineral composition

The plant material was placed in a pre-cleaned, consistently weighed silica crucible and heated in a muffle furnace at 400 °C until smoke production ceased. After that, the crucible was placed in a desiccator to cool to room temperature. The carbon-free ash was then moistened with concentrated sulfuric acid and heated on a heating mantle until sulfuric acid fumes were no longer emitted. After that, the crucible with the sulfated ash was heated to 600 °C in a muffle furnace for 2-3 h, or until the content's weight didn't change.

Atomic absorption spectroscopy (AAS) was used to analyse the mineral elements in a solution made from one gram of sulphated ash and 100 ml of 5% hydrochloric acid (HCl) (AA 800, Perkin-Elmer, Germany).

Standard solutions for each element were prepared, and calibration curves were developed. The mineral content was then measured using atomic absorption spectrophotometry (Gawalko, Nowicki, Babb & Tkachuk, 1997).

Determination of anti-nutritional parameters

The oxalate content in the edible plants was measured following the procedure described by Munro and Bassir (1980). For each ground plant sample, one gram was extracted in triplicate by heating at 50 °C and stirring with a magnetic stirrer for 1 h using 0.3M HCl. The extracts were combined, diluted to 100 ml with water, and then used to estimate the total oxalate content.

The phytate content was measured following the method of Reddy and Love (1999). One gram of the ground plant material was soaked in 100 ml of 2% HCl for 5 h and then filtered. To 25 ml of the filtrate, 5 ml of 0.3% ammonium thiocyanate solution was added, and the mixture was titrated with Iron (III) chloride solution until a brownish-yellow colour that persisted for 5 min was observed.

The saponin content was determined by applying the technique outlined by Hudson and El-Difrawi (1979). Tannic acid was used as the reference standard for the modified vanillin-HCl method of Price, Scoyoc and Butler's (1978) analysis of tannin concentration.

The alkaline titration method was used to measure the cyanogenic glycoside concentration; a

persistent turbidity against a black background was established as the endpoint (AOAC 2000).

Toxicity study

Preparation of plant extracts

Five grams of shade-dried powdered plant material were soaked in 50 ml of distilled water at room temperature for 24 h and then filtered. The remaining plant material was soaked again in the same solvent for another 24 hours and filtered. The filtrates from both extractions were combined and concentrated with a rotary evaporator under reduced pressure to produce viscous extracts, which were subsequently dried using a freeze-dryer. The resulting dried extracts were stored at -20 °C until required.

Five milligrams of each crude extract were dissolved in 10 ml of phosphate-buffered saline (PBS, pH 7.4) to reach a concentration of 500 µg/ml. The resulting solutions were filtered through 0.22 µm syringe filters to remove any particulate matter and were then stored at -20°C until needed.

Haemolytic toxicity

Haemolytic toxicity studies of aqueous extracts of ten wild edible plants were carried out using Malagoli's method (Malagoli, 2007).

A 10% erythrocyte suspension was prepared in sterile phosphate-buffered saline (PBS, pH 7.4) for the haemolytic studies. Plant extracts were combined with the 10% rat erythrocyte suspension at doses of 100, 300, and 500 µg/ml.

After centrifuging the mixture for 1h at 37 °C, the supernatant was extracted. Utilising a Shimadzu UV 1800 model UV-VIS spectrophotometer, the absorbance of the liberated haemoglobin was measured at 540 nm. There were two controls: the only ingredient in the negative control was sterile phosphate-buffered saline, and the only ingredient in the positive control was hydrogen peroxide (50–200 µM). Three duplicate assays were used to calculate the average values. Each sample's cell viability was calculated by multiplying the absorbance by 100 and dividing it by the absorbance of the negative control.

Cytotoxicity

Ten wild edible plant aqueous extracts were assessed using the 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) test on isolated goat liver cells, following the guidelines provided by Mosmann (1983).

Collagenase was added to fresh goat livers that had been perfused in PBS (pH 7.4). The liver was then finely chopped and the cells were separated using a 40µl hole size cell strainer (Genetix cell strainer, S. Korea). After that, the cells were centrifuged at 800 g to remove any fine debris and cleaned with HBSS. The trypan blue exclusion test yielded a cell viability range of 85 to 95%. Phase-contrast microscopy was utilised to assess the hepatocytes' purity. 10% FBS and 0.5 ml of RPMI were added to an Eppendorf tube holding the separated cells. The freshly extracted hepatocytes were treated with several doses (100, 300, and 500 µg/ml) of edible plant aqueous extracts (100 µl), and the mixture was incubated for two hours at 37 ^{o}C in a CO_2 incubator.

Both the medium control (blank medium) and the cell control (cells not treated with extract) were obtained and cultured under identical experimental conditions. After centrifuging each tube, the supernatant was disposed of. To get a final concentration of 0.5 mg/ml, Thiazolyl Blue Tetrazolium Bromide, MTT (5 mg/ml, in PBS, pH 4.5) (Sigma, USA) were added to the tube. It was then incubated for 1 h at 37 °C until intracellular purple formazan crystals were visible under a microscope.

Following an hour, the MTT-containing culture media was carefully removed by centrifugation, and 100 µl of DMSO was added. The mixture was then incubated for 30 min to 1 hour to dissolve the formazan crystals. The UV absorbance of the resulting purple solution was spectrophotometrically measured at 570 nm in a UV-VIS spectrophotometer (Model Shimadzu, UV 1800) and the percentage of cell viability was calculated to determine the hepatotoxicity of plant extracts.

Genotoxicity

The comet assay for single-cell gel electrophoresis, as reported by Singh, McCoy, Tice, and Schneider (1988), was utilised to assess the genotoxic potential of the extracts. A healthy rat's tail vein was used to draw one millilitre of blood, and 100 µl of heparinised whole blood was combined with 100 µl of plant extracts at varying concentrations (100, 300, and 500 µg/ml) and incubated for two hours at 37 °C in a CO2 incubator. In the experiment, both positive and negative controls were present.

Sample preparation involved embedding 100 µl of cell suspensions in 100 µl of 0.5% low melting point agarose (LMPA), which were subsequently spread over a slide that had been previously

coated with a 1% normal melting point agarose film (NMPA).

For every sample, two slides were produced, and the agarose cell suspensions were left to harden at 4 °C. Following solidification, the slides spent an hour in a cold lysis buffer that contained the following ingredients: 2.5 M NaCl, 100 mM EDTA, 10 mM Tris buffer, 10% DMSO, 0.8% Triton X-100, pH 10.

To allow the DNA to unwind, the slides were taken out of the lysis buffer and put in a horizontal gel electrophoresis chamber that was filled with alkaline electrophoresis buffer (1 mM EDTA, 0.3 N NaOH, pH 13.0) for 20 minutes. At 25V/300mA, electrophoresis was run for 30 minutes.

The slides were electrophoretically sepa-rated, neutralized three times, and then stained with a 20 mg/ml ethidium bromide solution. The dyed nuclei were captured on camera and seen under a fluorescence microscope.

The Olive Tail Moment (OTM) of individual stained nuclei was calculated using comet assay software. A higher percentage of tail DNA indicated a higher level of DNA damage and a higher level of genotoxicity of the plant extract.

Statistical analysis

Samples were analysed in triplicate for every analysis. The statistical software for the social sciences (SPSS version 7.5) was used to do a univariate analysis of variance (ANOVA) and then a Tukey's test ($p \leq 0.05$) on the experimental results.

RESULTS AND DISCUSSION

Nutritional composition

Raw plant materials

The proximate compositions of raw and cooked plants are presented in Table 1. The highest ash content was found in the raw leaves of *D. esculentum* (12.20±0.24%) and the lowest in the uncooked fruits of *D. indica* (0.70±0.04%). The inorganic material of the plant found in ash includes oxides and salts that have cations like sodium, potassium, calcium, magnesium, iron, and manganese and anions such phosphates, sulphates, chlorides, and other halides (Gopalan, Rama Sastri & Balasubramanian, 2004). The amount of minerals in the food is indicated by the ash content.

The ash content of these wild vegetables corroborates results reported for some commonly used edibles in Bangladesh, Arunachal Pradesh, and Meghalaya, India (Satter et al., 2016; Seal, Chaudhuri & Pillai, 2013; Seal, Pillai & Chaudhuri, 2017), and is higher than that of the commonly consumed leafy vegetables by the Bodo tribe in Assam, India (Narzary, Swargiary & Basumatary, 2015).

This variation could be attributed to ecological factors or the age of the plant samples being studied. The highest fat content (2.45±0.78%) was found in the fruits of *M. laxiflora*, while the lowest was observed in *D. esculentum* (0.18±0.06%). The fat levels in these plants are consistent with findings from other studies on wild edible plants in Bangladesh and India (Satter et al., 2016; Seal et al., 2013, 2017).

Essential fatty acids, such as linoleic and linolenic acid, are found in fats and must be consumed through diet and these acids are essential for controlling blood coagulation, inflammation, and brain growth.

Additionally, the presence of fat enhances the body's absorption of lipid-soluble vitamins like vitamin A and β-carotene (Gopalan et al., 2004).

Cooked plant materials

Cooking treatments led to a significant reduction in both ash and fat contents. The loss of ash content in the wild plants due to boiling (8.75-49.88%) was more pronounced ($p<0.05$) than that observed with microwave heating (4.88-21.43%, $p>0.05$), likely because of diffusion into the boiling water (Tsungai, Munyanyi & Mduluza, 2017). Microwave cooking may influence the fats in edible plants, potentially altering their distribution (1.86-28.19%) and promoting lipid breakdown.

This process can involve increased lipase activity, denaturation of the fat fraction, and the breakdown of lipids into glycerol and fatty acids. However, this does not necessarily equate to a reduction in total fat content; rather, it reflects a shift in fat composition and structure. The fat content in the microwaved edible plants also significantly decreased (1.86-28.19%, $p<0.05$), possibly due to increased lipase activity, denaturation of the fat fraction, and the breakdown of lipids into glycerol and fatty acids during microwave cooking (Tsungai et al., 2017).

Table 1.
Nutritional composition of wild edible plants and impact of cooking

Plant	Thermal treatment	Ash (%)	Fat (%)	Fibres (%)	Proteins (%)	Carbohydrates (%)
Meynia laxiflora	Raw	2.50±0.14	2.45±0.78	1.80±0.49	6.62±0.14	8.66±0.53
	Boiling	1.94±0.19 (-22.4)	2.11±0.42 (-13.95)	0.95±0.28 (-47.22)	5.32±1.08 (-19.64)	7.78±0.15 (-10.16)
	Microwave cooking	2.01±0.32 (-19.6)	2.34±0.77 (-4.57)	1.01±0.34 (-43.89)	6.18±0.18 (-6.64)	8.11±0.45 (-6.35)
Castanopsis indica	Raw	1.08±0.27	0.81±0.07	0.60±0.05	4.45±0.05	9.30±0.22
	Boiling	0.92±0.08 (-14.81)	0.76±0.18 (-5.59)	0.34±0.04 (-43.33)	4.02±0.39 (-9.66)	8.76±0.59 (-5.81)
	Microwave cooking	0.98±0.19 (-9.25)	0.79±0.09 (-1.86)	0.47±0.08 (-21.67)	5.25±0.28 (+17.98)	9.86±0.43 (+6.02)
Docynia indica	Raw	0.70±0.04	3.51±0.36	0.40±0.07	3.59±0.22	9.18±2.11
	Boiling	0.47±0.05 (-32.85)	3.18±0.14 (-9.32)	0.21±0.08 (-47.5)	3.02±0.43 (-15.87)	8.02±0.16 (-12.64)
	Microwave cooking	0.55±0.03 (-21.43)	3.09±0.38 (-11.89)	0.31±0.05 (-22.5)	4.11±0.78 (+14.49)	9.34±0.55 (+1.74)
Flemingia vestita	Raw	2.90±0.33	0.81±0.04	1.63±0.45	9.34±0.30	8.53±1.25
	Boiling	1.87±0.25 (-35.51)	0.67±0.14 (-16.77)	0.95±0.08 (-41.72)	8.26±1.14 (-11.56)	7.32±0.65 (-14.19)
	Microwave cooking	2.54±0.33 (-12.41)	0.71±0.66 (-11.80)	1.15±0.35 (-29.45)	10.78±2.56 (+15.42)	7.98±1.02 (-6.45)
Bauhinia purpurea	Raw	8.71±0.20	1.24±0.06	1.63±0.53	7.62±0.41	8.10±2.26
	Boiling	6.67±0.66 (-23.42)	1.13±0.44 (-8.87)	0.78±0.02 (-52.15)	6.29±0.85 (-17.45)	7.79±0.78 (-3.83)
	Microwave cooking	7.23±32 (-16.99)	1.04±0.23 (-16.13)	0.96±0.06 (-41.10)	8.35±0.75 (+9.58)	8.67±0.66 (+7.04)
Dillenia pentagyna	Raw	9.82±0.03	1.26±0.05	5.75±0.33	0.72±0.05	8.24±0.50
	Boiling	8.96±0.16 (-8.75)	1.11±0.44 (-12.14)	3.78±0.46 (-34.26)	0.55±1.55 (-23.26)	7.59±0.45 (-7.89)
	Microwave cooking	9.34.18±0.55 (-4.88)	1.18±0.56 (-6.59)	4.63±0.34 (-19.48)	0.96±1.44 (+33.95)	8.82±1.25 (+7.04)
Diplazium esculentum	Raw	12.20±0.24	0.18±0.06	3.88±0.49	14.39±0.42	6.83±0.26
	Boiling	10.55±0.78 (-13.52)	0.11±0.04 (-38.89)	2.72±0.58 (-29.89)	12.67±1.12 (-11.93)	5.76±0.19 (-15.67)
	Microwave cooking	11.76±0.22 (-3.60)	0.13±0.05 (-27.78)	3.02±0.34 (-22.16)	13.28±1.35 (-7.69)	6.09±0.65 (-10.83)
Elaegnus latifolia	Raw	8.62±0.07	1.51±0.05	0.71±0.16	14.82±1.06	7.43±0.34
	Boiling	6.76±0.19 (-21.57)	1.11±0.59 (-26.65)	0.36±0.05 (-49.29)	12.84±1.19 (-13.38)	6.55±0.66 (-11.84)
	Microwave cooking	7.34±0.34 (-14.85)	1.34±0.23 (-11.45)	0.58±0.08 (-18.31)	13.68±1.77 (-7.71)	7.11±0.35 (-4.30)
Elaegnus pyriformis	Raw	5.40±0.21	1.49±0.11	0.78±0.06	23.19±1.06	6.91±0.36 [c]
	Boiling	4.76±0.29 (-11.85)	1.12±0.44 (-24.83)	0.46±0.02 (-41.03)	20.56±2.17 (-11.33)	5.98±0.29 [b] (-13.46)
	Microwave cooking	4.92±0.42 (-8.88)	1.32±0.64 (-11.40)	0.61±0.08 (-21.79)	21.49±3.76 (-7.32)	6.54±0.46 [a] (-5.35)
Fagopyrum cymosum	Raw	4.25±0.56	1.41±0.41	2.76±0.55 [c]	25.49±2.42	6.60±1.75
	Boiling	2.13±0.78 (-49.88)	0.98±0.09 (-30.33)	1.47±0.18 (-46.74)	21.56±2.33 (-15.44)	7.01±0.56 (+6.21)
	Microwave cooking	3.67±0.58 (-13.64)	1.01±0.12 (-28.19)	1.94±0.11 (-29.71)	23.76±3.04 (-6.81)	7.38±0.32 (+11.82)
Range of Loss/Increase (%)	Boiling	Loss (8.75-49.88)	Loss (5.59-38.88)	Loss (29.89-49.29)	Loss (9.66-23.25)	Loss (3.82-15.66) Increase 6.21
	Microwave cooking	Loss (4.88-21.43)	Loss (1.86-28.19)	Loss (18.30-43.88)	Loss (6.64-7.71) Increase (9.58-33.95)	Increase (1.74-11.81)

Each value in the table represents the average of three experiments, presented as Mean ± Standard Error of the Mean (SEM). Statistical analysis was conducted using Tukey's test at a 95% confidence level, with statistical significance accepted at $p < 0.05$. A negative value within brackets indicates a percentage decrease, while a positive value indicates a percentage increase in the tested parameters

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Boiling also resulted in a more substantial depletion of fat content (5.59-38.88%) compared to microwaving. Microwave cooking may retain fats differently than boiling, as boiling often involves higher temperatures that can lead to fat breakdown or degradation.

However, factors like cooking time and specific conditions (e.g., temperature and moisture levels) are essential to consider when comparing fat retention between these methods, as they also play a significant role in the final fat content.

The microwave method preserved more fat than boiling, likely because the extremely high temperatures in boiling caused more fat to denature than microwave cooking (Tsungai et al., 2017).

Among the wild edibles studied, raw *D. esculentum* had the highest fibre content (3.88±0.49%), while the raw fruits of *D. indica* had the lowest (0.40±0.07%).

Dietary fibres are beneficial for increasing bulk in the diet due to their water-absorbing properties, which help facilitate intestinal transit (Jenkin, Jenkin, Wolever, Rao & Thompson, 1986). The recommended daily intake (RDA) of dietary fibre is 38 g/day for adult males and 25 g/day for adult females (Trumbo, Schlicker, Yates & Poos, 2002).

Fibre has been shown to reduce the risk of coronary heart disease, lower serum cholesterol, decrease hypertension, and help prevent diabetes, as well as breast and colon cancer (Rehman et al., 2014; Vadivel & Janardhanan, 2005). Thus, incorporating these wild vegetables, which have high fibre content, into the diet can help meet the recommended daily fibre intake.

The uncooked wild foods have a protein level ranging from 0.72% to 25.49%. These results were nearly identical to several underutilised green leafy vegetables as mentioned by Gupta, Lakshmi, Manjunath, and Prakash (2005). Foods that provide more than 12% of their calorific value from proteins are considered good sources of proteins (Aberoumand, 2009).

According to the Food and Nutrition Board (2001), food plants that provide more than 12% of their calorific value of protein are a good source of protein. In that context, *P. oleracia* L. leaves and stem (23.47%) are a good source of protein.

As a result, consuming these plants can significantly contribute to rural people's access to affordable and readily available proteins.

The digestible carbohydrate content in the raw plants varied from 6.6% to 9.3%. This range is comparable to the carbohydrate contents reported for certain green leafy vegetables from the Sonitpur district of Assam, India, which ranged from 5% to 11% (Saha, Biswal, and Deka, 2015). Similarly, the carbohydrate content in wild edibles consumed by the Bodo tribe of Assam, India, was reported to be between 4% and 12% (Narzary et al., 2015), which aligns closely with the values observed in this study.

The crude fibre content in edible plants decreased significantly ($p < 0.05$) with both cooking treatments. This reduction may be attributed to the formation of protein-fibre complexes (Bressani, 1993) resulting from the chemical changes induced by soaking and cooking. The results of the present study showed that the decrease in fibre content was less after microwaving (18.30-43.88%) compared to boiling (29.89-49.29%).

The current study demonstrated a significant increase in protein content (9.58-33.95%, $p < 0.05$) in wild edible plants following microwave treatment, whereas there was an insignificant decrease (9.66-23.25%, $p > 0.05$) in protein content in the boiled samples. The reduction in protein content in the boiled samples may be due to the solubilisation and leaching of nitrogenous substances during boiling. The increase in crude protein with microwaving could be attributed to the enhanced availability of protein resulting from enzymatic hydrolysis of insoluble proteins. This trend aligns with Bliss's (1975) findings, which suggested that increased protein content could be due to enzymatic hydrolysis releasing free amino acids (Tsungai et al., 2017).

All cooking methods resulted in a significant increase ($p < 0.05$) in carbohydrate content. Microwaved edibles showed a higher increase in carbohydrates (1.74-11.81%), with boiling yielding a 6.21% increase in the leaves of *F. cymosum*. This increase in carbohydrate content with cooking may be attributed to the destruction of plant cell walls, which enhances the solubility of carbohydrates in water. The smaller increase observed in boiled samples could be due to carbohydrates leaching into the boiling water before analysis (Tsungai et al., 2017).

Table 2.
Mineral composition of wild edible plants and cooking's impact

Plant	Thermal treatment	Minerals (mg/100 g of edible portion)							
		Na	**K**	**Ca**	**Cu**	**Mg**	**Zn**	**Fe**	**Mn**
Meynia laxiflora	Raw	24.00±1.26	403.67±11.44	75.00±2.83	0.028±0.003	12.05±1.02	2.05±0.09	1.46±0.25	0.103±0.06
	Boiling	21.45±3.09 (-10.62)	390.56±9.46 (-3.24)	71.24±2.03 (-5.01)	0.021±0.002 [c] (-25.00)	9.56±0.18 (-20.67)	1.94±0.72 [c] (-5.42)	0.98±0.06 (-32.99)	0.07±0.009 [c] (-32.03)
	Microwave cooking	23.26±2.18 (-3.08)	399.87±2.34 (-0.94)	73.86±4.12 (-1.52)	0.025±0.004 [b] (-10.71)	11.23±0.34 (-6.81)	1.99±0.68 [b] (-2.98)	1.16±0.35 (-20.69)	0.09±0.005 [b] (-12.62)
Castanopsis indica	Raw	20.00±3.52	164.33 ±1.53	115.00±4.66	0.039±0.002	14.95±1.02	4.95±0.32	1.65±0.13	1.25±0.34
	Boiled	17.67±2.08 (-11.65)	157.35±2.39 (-4.24)	109.56±2.44 (-4.73)	0.028±0.003 [b] (-27.58)	10.67±2.11 (-28.63)	2.67±0.23 (-46.08)	0.89±0.05 (-46.12)	0.98±0.09 (-17.88)
	Microwave cooking	19.01±3.03 (-4.95)	161.27±3.08 (-1.86)	113.67±5.89 (-1.15)	0.035±0.002 [a] (-9.48)	13.45±1.48 (-10.04)	3.44±0.06 (-30.53)	1.19±0.27 (-27.97)	1.08±0.34 (-13.6)
Docynia indica	Raw	26.33±3.15	295.33±5.27	113.67±2.33	0.162±0.02 [2]	12.66±1.09	2.66±0.09	8.83±0.34	2.95±0.12
	Boiling	24.67±2.85 (-18.12)	259.07±4.88 (-12.28)	109.54±2.28 (-3.63)	0.116±0.03 [b] (-8.33)	9.67±0.29 (-23.61)	1.34±0.02 (-49.60)	6.53±0.29 (-26.03)	1.11±0.36 (-62.41)
	Microwave cooking	25.51±0.09 (-3.12)	270.66±2.56 (-8.35)	111.78±5.02 (-1.65)	0.153±0.04 [a] (-5.36)	10.34±1.06 (-18.32)	2.09±0.05 (-21.39)	7.67±0.47 (-13.11)	2.27±0.53 (-23.14)
Flemingia vestita	Raw	22.67±1.33	305.00±6.27	58.67 ±2.33	0.149±0.04	16.57±1.29	6.57±0.29	10.64±0.39	3.05 ±0.72
	Boiling	18.56±2.11 (-18.11)	254.37±3.82 [c] (-16.6)	43.67±2.99 (-25.56)	0.097±0.03 [b] (-38.86)	11.35±2.08 (-31.51)	4.61±0.28 (-29.85)	7.92±0.28 (-25.54)	1.87±0.41 (-38.68)
	Microwave cooking	21.45±3.08 (-5.37)	287.78±4.95 [b] (-5.64)	51.78±3.65 (-11.73)	0.121±0.06 [b] (-23.74)	14.53±1.35 (-12.32)	4.99±0.35 (-24.08)	8.65±0.66 (-18.67)	2.11±0.36 (-30.81)
Bauhinia purpurea	Raw	23.67±4.52	485.67±4.03	117.33±3.66	0.294±0.02	13.29± 1.32	4.21±0.17	3.36±0.26	0.70±0.026
	Boiling	19.55±2.19 (-17.39)	437.89±4.11 (-9.84)	97.32±2.24 (-17.05)	0.178±0.03 [c] (-39.38)	8.55±1.06 (-35.66)	2.87±0.36 (-31.83)	1.56±0.81 (-53.61)	0.49±0.04 (-30.33)
	Microwave cooking	21.45±2.44 (-9.37)	462.49±5.33 (-4.77)	105.33±4.08 (-10.23)	0.238±0.04 [b] (-20.32)	11.78±2.08 (-11.36)	3.96±0.23 (-5.94)	2.81±0.45 (-16.43)	0.56±0.08 (-20.37)
Dillenia pentagyna	Raw	16.30 ±1.45	663.00±2.66	266.67±4.66	0.76±0.04	13.75±2.22	3.35 ±0.12	3.24±0.05	2.07±0.32
	Boiling	14.27±3.17 (-12.45)	592.25±3.21 (-10.67)	178.89±2.39 (-32.92)	0.62±0.02 (-18.64)	10.56±1.06 (-23.20)	1.89±0.29 [c] (-43.64)	2.33±0.51 (-28.16)	1.18±0.44 (-43.08)
	Microwave cooking	15.22±2.54 (-6.63)	601.26±4.89 (-9.31)	204.33±3.44 (-23.37)	0.71±0.07 (-6.82)	11.34±1.44 (-17.53)	2.11±0.13 [b] (-37.08)	2.91±0.73 (-10.27)	1.89±0.73 (-8.84)
Diplazium esculentum	Raw	23.67 ±1.08	874.67±5.34	174.67±4.66	0.53±0.04	17.22±2.27	3.35±0.27	5.14±0.81	1.03±0.51
	Boiling	19.78±2.72 (-16.42)	789.54±2.74 (-9.73)	111.53±2.04 (-36.15)	0.37±0.02 (-29.57)	14.46±2.09 (-16.01)	1.69±0.26 [c] (-49.55)	3.86±0.23 (-24.95)	0.68±0.09 (-34.00)
	Microwave cooking	22.41±2.55 (-5.31)	801.24±4.55 (-8.39)	154.64±4.28 (-11.46)	0.16±0.01 (-21.95)	15.78±1.12 (-8.35)	2.65±0.64 [b] (-20.89)	4.79±0.27 [a] (-6.87)	0.96±0.07 (-6.82)
Elaegnus latifolia	Raw	19.30 ±3.45	271.67±3.83	117.33±3.65	0.13±0.04	1.25±0.28	4.97±0.38	3.45±0.23	0.42±0.02
	Boiling	17.46±2.75 (-9.53)	205.28±2.98 (-24.43)	98.62±2.66 (-15.95)	0.08±0.05 [b] (-38.62)	0.97±0.23 (-22.45)	2.45±0.18 (-50.70)	2.61±0.19 (-24.32)	0.23±0.07 (-45.58)
	Microwave cooking	18.28±3.78 (-5.28)	259.05±3.56 (-4.64)	103.55±3.80 (-11.75)	0.11±0.06 [b] (-15.60)	12.28±0.08 (-10.46)	3.56±0.29 (-28.37)	2.98±0.32 (-13.58)	0.35±0.08 (-17.19)
Elaegnus pyriformis	Raw	19.00±2.95	288.83±5.33	125.33±3.66	0.25±0.03	12.66±3.02	1.52±0.12	3.18±0.15	0.72±0.08
	Boiling	16.97±3.18 (-10.68)	211.56±7.55 (-26.62)	96.87±2.38 (-22.71)	0.11±0.02 (-56.00)	9.87±0.63 (-22.06)	0.87±0.32 (-8.33)	1.98±0.31 (-37.79)	0.39±0.03 (-45.75)
	Microwave cooking	18.05±2.55 (-5.00)	267.93±4.38 (-7.07)	111.35±2.52 (-11.16)	0.19±0.03 (-24.00)	10.87±2.12 (-14.17)	1.34±0.54 (-12.03)	2.67±0.24 (-16.12)	0.56±0.05 (-22.11)
Fagopyrum cymosum	Raw	23.00±4.85	613.67±7.66	303.33±11.40	0.19±0.03	10.93±2.26	4.21±0.32	10.45±0.14	1.69±0.32
	Boiling	20.57±2.12 (-10.56)	567.89±4.24 (-7.45)	217.88±6.11(-28.17)	0.09±0.003 (-53.53)	7.99±2.51 (-26.90)	2.87±0.43 (-31.77)	7.79±0.32 (-25.47)	1.09±0.001 (-35.73)
	Microwave cooking	21.22±3.18 (-7.73)	601.25±3.98 (-2.02)	287.45±3.45(-5.24)	0.11±0.001 (-43.20)	8.83±0.08 (-19.22)	3.88±0.67 (-7.76)	8.25±0.74 (-21.07)	1.56±0.009 (-8.01)
Range of loss (%)	Boiling	6.31 - 18.11	3.24-26.62	3.63-36.14	18.63-56.00	16.01-35.66	5.42-50.70	24.31-53.61	21.6-62.41
	Microwave cooking	3.08-9.36	0.94-9.31	1.15-23.37	5.36-43.20	6.81-19.22	2.98-37.07	6.87-27.97	6.82-30.81

The data are presented as mean ± standard error of the mean (SEM), with each value in the table derived by averaging three experiments. Tukey's test was used to conduct the statistical analysis at a 95% confidence level, and statistical significance has been accepted at the $p < 0.05$ level. The test parameters' % rise is indicated by a positive value inside a bracket, while their percentage decline is indicated by a negative value

The mineral composition of the wild edible plants

The mineral content of both raw and cooked plants is shown in Table 2. The sodium (Na) concentration in the plants studied ranged from 16.30 ± 1.85 to 24.66 ± 1.26 mg/100 g of the edible portion in uncooked plants. For comparison, sodium levels in some cultivated vegetables and fruits vary from 3.0 to 124.9 mg/100 g (Gopalan et al., 2004). The potassium (K) content in the raw plants ranged from 164.33 ± 1.53 to 874.67 ± 5.34 mg/100 g. Both tissue excitability and preserving the body's ionic balance depend on Na and K. Potassium is significant due to its diuretic properties, whereas Na is crucial for the transportation of metabolites.

The K/Na ratio in every food significantly influences the development of arteriosclerosis and hypertension. Blood pressure is increased by Na and decreased by K (Saupi, Zakaria & Bujang, 2009). The quantities of calcium (Ca) in the raw plants ranged from 58.67±2.33 to 303.33±11.40 mg/100 g, while other cultivated vegetables, such as spinach, cabbage, and lettuce, have values between 39 and 73 mg/100 g (Gopalan et al., 2004). The study's findings were remarkably similar to the wild greens eaten in Bangladesh (Satter et al., 2016). The outcome suggests that these wild vegetables might be a valuable supply of Ca for our diet. Additionally, blood coagulation and the healthy operation of the heart muscles depend on it (Sundriyal & Sundriyal, 2004).

The iron (Fe) content of these plants ranged from 1.46 to 10.64 mg/100 g, which compares favourably with the values reported for widely consumed leafy vegetables in Kano, Nigeria (Mohammed & Sharif, 2011) and for wild leafy vegetables in Meghalaya, India (Seal et al., 2013), which ranged from 21.30 mg/100 g to 33.40 mg/100 g. In addition to catalyzing numerous enzymes, including cytochrome oxidase, Fe is necessary for oxygen to bind to haemoglobin (Geissler & Powers, 2005). The results infer that including the plants from our study in diet could help lower anaemia.

Magnesium (Mg) plays a critical role in preventing muscle degeneration, growth retardation, cardiomyopathy, immunologic dysfunction, impaired spermatogenesis, and bleeding disorders (Chaturvedi, Shrivastava & Upreti, 2004). The highest Mg content was found in uncooked *F. vestita* (16.57 ± 1.29 mg/100 g), while the lowest was observed in *E. latifolia* (1.25 ± 0.28 mg/100 g).

Manganese (Mn) serves as a cofactor for enzymes such as arginase and glycosyl transferase, and it also activates other enzymes like phosphoenolpyruvate carboxykinase and glutamine synthetase. Additionally, Mn is crucial for the formation of haemoglobin (Indrayan, Sharma, Durgapal, Kumar & Kumar, 2005). The concentration of Mn in the uncooked plants ranged from 0.10 to 2.95 mg/100 g. The stabilisation of macromolecular production and structure is aided by zinc (Zn). Zinc has a well-established function in the synthesis of DNA and RNA, and zinc is required for the activity of both DNA and RNA polymerases. The content of zinc varied from 1.52 to 6.57 mg/100g. These values are comparable to those found in certain green and wild vegetables in Bangladesh (Satter et al., 2016), Nigeria (Mohammed & Sharif, 2011), and India (Saikia & Deka, 2013). Copper (Cu) plays a crucial role in the formation of copper protein. The three main metalloenzymes that contain copper are tyrosine oxidase, lysyl oxidase, and cytochrome C oxidase. The uncooked plants from this study had Cu contents ranging from 0.028 to 0.762 mg/100g. Therefore, each of the studied plants significantly contributes to meeting the diet's mineral requirements.

Boiling treatments of the edible plants led to varying losses of minerals: Na (6.31 - 18.11%), K (3.24 - 26.62%), Ca (3.63 - 36.14%), Cu (18.63 - 56.00%), Mg (16.01 - 35.66%), Zn (5.42 - 50.70%), Fe (24.31 - 53.61%), and Mn (21.60 - 62.41%). In contrast, the decreases in mineral content for microwaved plants were comparatively minor ($p < 0.05$). During cooking, minerals leached into the water at different rates. Microwave cooking resulted in better mineral retention across all minerals compared to boiling.

The composition of anti-nutrients in wild edible plants

The anti-nutrient composition of both raw and cooked edible plants is presented in Table 3. The results indicate a significant reduction in anti-nutrient levels in the wild edible plants following both boiling and microwave treatments.

Oxalate is an anti-nutrient that occurs naturally in certain plants as oxalic acid and as soluble and insoluble salts. In the gastrointestinal tract, it binds to nutrients, making them inaccessible to the body (Ilelaboye, Amoo, & Pikuda, 2013).

Consuming edible plants with high levels of oxalic acid can potentially lead to nutritional deficiencies. The concentration of oxalate in the raw edible plants ranges from 0.228% in *D. pentagyna* to 0.74% in *E. pyriformis*.

Boiling treatment reduced the oxalate content in the edible plants by 17.78-25.95%, while microwave cooking resulted in more significant decreases of 7.75-32.20% ($p < 0.05$). The greatest loss of oxalate (32.30%) was observed in microwaved *B. purpurea*, followed by *E. pyriformis* at 30.05%. Both boiling and microwaving affected the oxalate levels in edible plants mainly by causing the loss of soluble oxalate in the cooking water, thereby enhancing the nutritional quality of the plants.

Phytic acid, also known as myoinositol hexakisdihydrogen phosphate, is primarily found in legumes and, to a lesser extent, in vegetables. It has a strong affinity for minerals such as iron, zinc, calcium, and magnesium, forming insoluble complexes that can reduce the bioavailability of these nutrients. Additionally, phytic acid can bind with proteins and starch, further impacting nutrient absorption (Yasir & Ahmad, 2018). The phytic acid content in wild edible plants varied, ranging from 0.17±0.02% in *C. indica* to 0.48±0.09% in *F. cymosum*. The impact of various cooking methods on the phytic acid content of these edible plants is presented in Table 3. Boiling treatments resulted in a reduction of phytate content by 20.52% to 35.46%, while microwave treatments led to a more substantial decrease, ranging from 9.63% to 41.68% ($p < 0.05$).

The observed reduction in phytic acid content in edible plants during boiling could be partially attributed to the leaching of phytic acid into the cooking water. Since phytic acid is relatively heat-labile, the more pronounced decrease seen with microwave cooking could be due to its sensitivity to heat (Shruti, Singh, Sharma, Kumar & Yadav, 2018).

In microwave cooking, phytic acid degradation may be enhanced by mechanisms unique to this method, such as rapid dielectric heating, which causes water molecules to vibrate and generate localized heat within the plant matrix. This process can lead to quicker and more uniform heat distribution, potentially breaking down heat-sensitive compounds like phytic acid more efficiently, even over shorter cooking durations. Additionally, microwave-induced thermal and non-thermal effects may contribute to the breakdown of phytic acid by disrupting the cell wall matrix, allowing for better penetration and heat diffusion to the target molecules.

Saponins are a group of chemical compounds present in various plant species, known for their soap-like foaming properties when mixed with liquids. These compounds can disrupt epithelial function and potentially cause digestive issues. Saponins can also damage red blood cells, inhibit enzymes, and interfere with thyroid function (Fan, Guo, Song & Li, 2013). The saponin content in plants varies by species, with the highest concentration found in *M. laxiflora* (0.214±0.03%) and the lowest in the leaves of *D. esculentum* (0.016±0.003%). Boiling treatments led to a reduction of 20.66% to 56.78% in saponin content in edible plants while microwaving resulted in a further decrease, ranging from 7.19% to 39.76%. Boiling treatments resulted in a reduction of 20.66% to 56.78% in the saponin content of edible plants. This reduction can be attributed to multiple mechanisms: thermal degradation, where high temperatures break down the saponin molecules; and leaching, where water-soluble saponins dissolve into the cooking water due to their hydrophilic nature. Additionally, prolonged boiling can disrupt plant cell walls, enhancing the release and loss of saponins into the surrounding water.

Microwaving, on the other hand, caused a further decrease in saponin content, ranging from 7.19% to 39.76%. This reduction occurs due to localized heating effects caused by microwave energy, which generates heat directly within the plant tissues, leading to the structural breakdown of saponins. Furthermore, microwaving may create localized pressure changes and rupture cellular compartments, facilitating the release of saponins. The energy generated can also induce chemical changes, such as partial hydrolysis or degradation of the saponin molecules, leading to further reductions in their content.

These combined effects highlight how different cooking methods influence the bioactive compound content in plants through unique physical and chemical mechanisms. Tannin is an important antinutritional factor that exists in most of the legumes. It is characterized by its bitter polyphenolic compounds that bind or form precipitate with proteins and various other organic com-pounds such as alkaloids and amino acids. Tannins are usually present in food products and inhibit the enzymatic activity of

amylase, lipase, trypsin and chymotrypsin. They decrease the quality of proteins and interfere with iron absorption (Yasir & Ahmad, 2018). The highest concentration of tannin was detected in the nuts of *C. indica* (1.276±0.41%) and the lowest amount was observed in *D. esculentum* (0.096±0.007%).

Cooking treatments led to the greatest reduction in tannin content (29.45%) in microwaved *E. pyriformis*, while the least reduction (21.44%) was observed in boiled *D. pentagyna*. The loss of tannins is likely due to their water-soluble nature, causing them to leach into the cooking medium.

The reduction in tannin content during cooking is primarily due to their water-soluble nature, which facilitates their leaching into the cooking medium. When plant tissues are exposed to heat, the cell walls and membranes begin to break down, increasing permeability and re-leasing intracellular compounds like tannins into the surrounding water.

Heat also disrupts the hydrogen bonding and other interactions that stabilize tannin molecules within the plant matrix, making them more mobile and accessible for diffusion. Furthermore, the elevated temperature of the cooking medium enhances the solubility of tannins, accelerating their migration into the water. Agitation and convection currents during boiling further promote the transfer of tannins from plant tissues into the liquid. Over prolonged cooking times, this process continues, resulting in a significant loss of tannins from the food matrix. These mechanisms collectively explain how cooking, particularly boiling, reduces tannin content in edible plants (Ilelaboye et al., 2013).

Cyanogenic glycosides are secondary metabolites that are found in various plant tissues and produce HCN upon hydrolysis. They are widely distributed in the plant kingdom. The ability of cyanogenic glycosides to release HCN is due to their enzymic hydrolysis which may cause cyanide poisoning. Therefore, the removal of cyanogenic glycosides is necessary to improve the nutritional value and safety of cyanogen-containing foods (Harenčár, Ražná & Nôžková, 2021). This study revealed that the cyanide contents of the raw vegetables range between 0.0033% in *E. pyriformis*, *F. cymosum* and 0.0073% in *B. purpurea***.** Boiling resulted in a reduction of cyanide content ranging from 34.44% to 50.95%, while microwave cooking led to a significant decrease in cyanide levels, varying from 16.07% to 37.24%, in the wild vegetables.

Toxicity of the wild edible plants

Table 4 presents the results of toxicity on both raw and cooked edible plants, including cell viability and the percentage of DNA damage, with buffer serving as the negative control and hydrogen peroxide as the positive control. The result showed the increases of RBC and heaptocytes cell viability and the reduction in the percentage of tail DNA were assessed at three different concentrations of the aqueous extracts from wild edible plants after boiling and microwave treatment. Haemolytic assays were conducted because, despite their potent nutraceutical properties, plants with haemolytic effects may be unsuitable for consumption. Additionally, these assays can provide insights into the mechanisms of cytotoxicity.

Haemolytic toxicity

In vitro haemolytic activities were assessed on human erythrocytes using extracts from edible parts of wild plants at various concentrations (100, 300, and 500 µg/ml). The positive control, H_2O_2 (200 µM), induced 51.75% haemolysis, while the buffer control resulted in 0% hemolysis.

The haemolytic effect of the extracts was concentration-dependent, but all extracts exhibited a lower haemolytic effect on human red blood cells at all concentrations. At the highest concentration (500 µg/ml), the viability of red blood cells was highest with raw *F. vestita* (92.68±1.28%) and lowest with the aqueous extract of raw *C. indica* (88.30±1.04%). At the lowest concentration (100 µg/ml), the highest RBC cell viability was observed with uncooked *P. lineata* (98.19±1.77%), while the lowest was found with uncooked *E. latifolia* (92.88±1.28%).

The investigation revealed that both cooking methods increased the viability of haemolytic cells, thereby reducing the toxicity of wild edible plants across all concentrations. Boiling resulted in the highest RBC cell viability (97.64±1.14%) for *M. laxiflora* at the highest dose, while the lowest viability (91.23±1.57%) was observed in *D. pentagyna* at the same concentration. Microwave cooking demonstrated RBC cell viability ranging from 93.44% to 98.34% at the highest concentration (500 µg/ml) across the samples of wild edible plants.

Table 3.
Antinutritional properties of wild edible plants and impact of cooking

Plant	Thermal treatment	Oxalates (%)	Phytates (%)	Saponins (%)	Tannins (%)	Cyanogenic glycosides (%)
	Raw	0.328±0.55	0.20±0.07	0.214±0.03	0.234±0.04	0.0059±0.0001
Meynia laxiflora	Boiling	0.267±0.41 (-18.50)	0.146±0.09 (-26.77)	0.139±0.06 (-35.14)	0.151±0.09 (-35.51)	0.0030±0.0005 (-49.38)
	Microwave cooking	0.293±0.64 (-10.43)	0.168±0.07 (-15.88)	0.185±0.03 (-13.44)	0.184±0.04 (21.34)	0.0041±0.0007 (-29.81)
	Raw	0.569±0.48	0.17±0.02	0.038±0.008	1.276±0.41	0.0059±0.0007
Castanopsis indica	Boiling	0.456±0.26 (-19.80)	0.134±0.07 (-20.88)	0.016±0.002 (-56.78)	0.89±0.07 (-30.45)	0.0032±0.0005 (-45.99)
	Microwave cooking	0.485±0.07 (-14.78)	0.123±0.06 (-27.64)	0.023±0.016 (-39.75)	1.014±0.37 (-20.55)	0.0037±0.0003 (-36.59)
	Raw	0.276±0.39	0.19±0.05	0.117±0.08	0.188±0.03	0.005±0.0001
Docynia indica	Boiling	0.206±0.42 (-25.18)	0.149±0.04 (-21.50)	0.091±0.004 (-21.92)	0.139±0.07 (-25.90)	0.0033±0.0008 (-34.44)
	Microwave cooking	0.240±0.61 (-12.91)	0.170±0.05 (-10.61)	0.071±0.006 (-39.08)	0.137±0.9 (-26.67)	0.0040±0.0005 (-19.65)
	Raw	0.319±0.25	0.30±0.04	0.034±0.006	0.421±0.06	0.0057±0.0002
Flemingia vestita	Boiling	0.258±0.38 (-19.05)	0.23±0.08 (-23.43)	0.024±0.005 (-30.47)	0.329±0.08 (-21.73)	0.0029±0.0005(-49.98)
	Microwave cooking	0.286±0.41 (-10.26)	0.22±0.07 (-25.88)	0.031±0.003 (-7.56)	0.321±0.09 (-23.71)	0.0046±0.0002 (-19.65)
	Raw	0.317±0.54	0.27±0.06	0.047±0.009	0.654±0.08	0.0073±0.0005
Bauhinia purpurea	Boiling	0.259±0.69 (-18.40)	0.188±0.05 (-30.47)	0.023±0.006 (-51.74)	0.470±0.09 (-28.18)	0.0039±0.0003 (-46.13)
	Microwave cooking	0.215±0.32 (-32.30)	0.237±0.06 (-12.17)	0.040±0.008 (-15.57)	0.615±0.07 (-6.05)	0.0060±0.0002 (-18.28)
	Raw	0.228±0.04	0.23±0.08	0.016±0.007	0.613±0.06	0.0054±0.0002
Dillenia pentagyna	Boiling	0.169±0.09 (-25.95)	0.148±0.09 (-35.46)	0.011±0.008 (-30.47)	0.481±0.02 (-21.44)	0.0026±0.0007 (-50.95)
	Microwave cooking	0.21±0.06 [b] (-7.75)	0.183±0.07 (-20.22)	0.015±0.007 (-7.19)	0.564±0.04 (-7.95)	0.0034±0.0003 (-37.24)
	Raw	0.265±0.08	0.16±0.09	0.016±0.003	0.096±0.007	0.0056±0.0004
Diplazium esculentum	Boiled	0.218±0.06 (-17.78)	0.127±0.05 (-20.52)	0.008±0.008 (-51.17)	0.066±0.002 (-31.24)	0.0032±0.0006 (-43.15)
	Microwave cooking	0.191±0.05 (-27.85)	0.145±0.06 (-9.63)	0.013±0.0004 (-17.19)	0.075±0.006 (-21.34)	0.0047±0.0005 (-16.07)
	Raw	0.677±0.12	0.31±0.07	0.06±0.003	0.294±0.02	0.0033±0.0004
Elaegnus latifolia	Boiling	0.521±0.09 (-23.03)	0.217±0.03 (-29.99)	0.032±0.007 (-47.13)	0.202±0.04 (-31.24)	0.0019±0.0006 (-41.53)
	Microwave cooking	0.595±0.21 (-12.10)	0.271±0.08 (-12.65)	0.043±0.002 (-28.44)	0.242±0.06 (-17.94)	0.0028±0.0004 (-16.61)
	Raw	0.74±0.09	0.31±0.05	0.102±0.004	0.863±0.06	0.0033±0.0006
Elaegnus pyriformis	Boiling	0.59±0.05 (-20.28)	0.21±0.06 (-33.22)	0.081±0.001 (-20.66)	0.503±0.07 (-41.67)	0.0017±0.0005(-47.59)
	Microwave cooking	0.518±0.04 (-30.05)	0.18±0.07 (-41.68)	0.085±0.002 (-16.38)	0.608±0.09 (-29.45)	0.0024±0.0003 (-28.74)
	Raw	0.43±0.04	0.48±0.09	0.045±0.005	0.415±0.06	0.0055±0.0007
Fagopyrum cymosum	Boiling	0.347±0.08 (-19.23)	0.342±0.08 (-28.85)	0.031±0.006 (-30.47)	0.316±0.03 (-24.01)	0.0027±0.0005 (-50.62)
	Microwave cooking	0.309±0.07 (-28.17)	0.394±0.07 (-17.96)	0.038±0.008 (-15.66)	0.347±0.04 (-16.52)	0.0041±0.0003(-25.10)
Range of loss (%)	Boiling	17.78-25.95	20.52-35.46	20.66-56.78	21.44-41.67	34.44-50.95
	Microwave cooking	7.75-32.20	9.63-41.68	7.19-39.76	6.05-29.45	16.07-37.24

Each value in the table was obtained by calculating the average of three experiments and data are presented as mean ± standard error of the mean (SEM). Statistical analysis were carried out by Tukey's test at 95% confidence level and statistical significance were accepted at the $p < 0.05$ level. The negative value within bracket indicates percentage decrease and positive value within bracket indicates the percentage increase of the test parameters

Cytotoxicity

Hepatocytes isolated from fresh goat liver were exposed to various concentrations (100, 300, and 500 µg/ml) of aqueous extracts from edible plants to assess their effects on cell viability. At the highest concentration (500 µg/ml), the viability of hepatocytes was highest with uncooked *F. vestita* (95.16±1.11%) and lowest with the aqueous extract of raw *M. laxiflora* (89.25±1.78%).

At the lowest concentration (100 µg/ml), uncooked *F. vestita* exhibited the highest hepatocyte cell viability (96.05±1.77%), while the lowest viability (92.08±3.01%) was observed with uncooked *M. laxiflora*.

The investigation demonstrated that both cooking methods increased the viability of heaptocyte cells, thereby reducing the toxicity of wild edible plants at all concentrations. The boiling method resulted in the highest hepatocyte cell viability (95.81±1.67%) for *F. vestita* at the highest dose, while the lowest viability (90.69±1.72%) was observed in *E. latifolia* at the same concentration.

The microwave cooking method showed heaptocyte cell viability ranging from 91.44±1.02% to 96.09±1.65% at the highest concentration (500 µg/ml) across the wild edible plants.

Genotoxicity

Genotoxicity studies involved incubating rat lymphocytes in a low-melting-point agarose suspension with plant extracts at various concentrations (100-500 µg/ml). The cells were lysed under neutral or alkaline conditions (pH > 13), and the lysed cells were analysed using electrophoresis.

The resulting slides were examined imme-diately under a fluorescence microscope to evaluate DNA damage, with the Olive Tail Moment (OTM) of individual stained nuclei calculated using comet assay software.

Negative controls included whole blood and RPMI-1640, while positive controls consisted of whole blood treated with 50, 100, and 200 µM H2O2 and RPMI-1640. A higher percentage of tail DNA indicated greater DNA damage and increased genotoxicity of the plant extract. The comet assay is a cost-effective, simple, and rapid method for detecting DNA strand breaks, providing sensitivity at the individual cell level with minimal sample requirements (Behravan et al., 2011).

The comet assay results showed that uncooked *F. cymosum* had the lowest OTM (3.92±0.81) at the tested concentration, whereas the aqueous extract of raw *M. laxiflora* at 500 µg/ml had the highest OTM (7.11±0.16). For reference, the negative control (whole blood with RPMI 1640) had an OTM of 1.79±1.81, while the positive control (a mixture of whole blood, RPMI 1640, and 200 µM H2O2) had an OTM of 76.35±1.48.

Free radicals, generated during cellular metabolism, contribute to mutagenicity and genotoxicity. The comet assay revealed that hydrogen peroxide caused dose-dependent DNA damage (OTM 25.18–76.35) due to oxidative stress.

The study found that the DNA damage caused by the uncooked plant extracts was comparable to that observed with the negative control. Natural antioxidants, particularly those from dietary sources, are known to combat free-radical damage. Recent human studies suggest that antioxidant supplements, including vitamins E and C, lycopene, and ß-carotene, can reduce free-radical damage and offer protection against degenerative diseases such as cancer by mitigating DNA damage (Kumari & Deshwal, 2011).

The study revealed that microwave cooking led to DNA damage in lymphocytes, with observed values ranging from 3.34 to 6.08 OTM at the highest concentration of edible plants (500 µg/ml). In contrast, boiling resulted in slightly higher DNA damage, with values ranging from 3.05 to 6.79 OTM at the same concentration.

While plants are known for their wide array of pharmacologically active phytochemicals beneficial for treating various human diseases, some compounds such as saponins, tannins, and cyanogenic glycosides, can be harmful. These phytochemicals may act as prooxidants, potentially contributing to their mutagenic and genotoxic effects (Mohamed et al., 2016).

The investigation concluded that both cooking methods effectively reduced the percentage of tail DNA, thereby decreasing the toxicity of wild edible plants across all tested concentrations.

Table 4.
Toxicity studies of wild edible plants and effect of cooking

Plant	Concentration of the extract (µg/ml)	RBC cell viability (%)			Hepatocytes cell viability (%)			Genotoxicity OTM		
		Raw	Boiled	Microwave cooked	Raw	Boiled	Microwave cooked	Raw	Boiled	Microwave cooked
Meynia laxiflora	100	95.47±3.11	96.28±1.02	98.94±1.68	92.08±3.01	92.87±2.11	96.25±1.88	4.12±0.11	4.56±0.28	4.88±0.99
	300	92.23±1.22	98.07±1.78	97.39±4.01	90.45±1.56	92.29±3.26	96.18±1.06	5.45±0.78	5.67±0.34	4.08±0.53
	500	89.31±1.35	97.64±1.14	96.22±1.09	89.25±1.78	92.02±3.28	95.28±2.15	7.11±0.16	6.29±0.28	6.08±0.18
Castanopsis indica	100	97.47±1.08	95.25±1.19	96.32±2.45	93.83±1.86	94.28±1.98	96.19±2.78	3.36±0.98	2.59±0.76	2.72±0.42
	300	96.85±1.11	88.49±1.82	93.55±2.02	92.60±1.08	93.55±2.54	95.38±1.95	3.82±0.15	3.91±0.61	2.68±0.34
	500	90.42±1.87	96.56±1.34	95.34±3.32	91.22±1.12	93.28±1.05	94.19±0.94	4.11±0.55	3.06±0.69	3.62±0.49
Docynia indica	100	96.70±2.11	99.25±2.38	97.15±1.77	95.19±1.55	96.14±1.29	97.65±1.55	3.65±0.28	3.09±0.33	2.85±0.88
	300	94.36±2.56	98.41±1.77	95.43±1.92	94.23±2.11	95.55±1.88	96.28±1.92	3.92±0.35	3.56±0.38	3.11±0.10
	500	88.30±1.04	95.24±1.68	94.31±1.35	93.02±1.07	94.10±1.34	95.48±1.67	4.08±0.77	4.02±0.61	3.78±0.19
Flemingia vestita	100	98.19±1.77	99.10±1.89	99.78±1.28	96.05±1.77	98.29±1.28	99.06±1.02	4.01±0.52	3.76±0.68	3.18±0.44
	300	97.68±2.05	98.09±1.62	99.12±1.37	94.34±1.09	95.27±1.34	96.38±1.19	4.55±0.11	4.11±0.46	3.98±0.17
	500	92.68±1.28	93.68±1.41	95.33±1.08	95.16±1.11	95.81±1.67	96.09±1.65	4.96±0.28	4.38±0.39	4.02±0.26
Bauhinia purpurea	100	96.90±2.34	97.12±1.55	98.33±2.76	95.12±2.33	96.05±2.11	97.19±1.90	4.16±0.66	3.85±0.18	3.48±0.88
	300	94.19±1.68	95.66±2.01	98.42±1.64	94.19±1.06	95.28±1.33	96.34±2.11	4.98±0.41	4.59±0.55	4.08±0.16
	500	92.27±1.89	94.34±2.18	97.22±2.11	93.76±1.68	94.88±1.88	95.10±2.08	6.18±0.35	5.96±0.68	5.48±0.35
Dillenia pentagyna	100	93.20±2.08	94.11±1.88	97.34±1.29	94.33±1.96	95.01±2.18	96.11±1.35	4.38±0.77	3.96±0.59	3.37±0.66
	300	92.13±2.11	95.44±1.02	98.68±1.89	93.25±1.55	94.28±1.76	95.44±1.48	5.14±0.18	4.98±0.88	4.76±0.28
	500	89.36±1.95	91.23±1.57	97.36±1.76	92.44±1.09	93.08±1.59	95.66±1.69	6.27±0.55	5.95±0.31	5.78±0.19
Diplazium esculentum	100	95.10±2.10	96.12±1.55	98.34±1.76	94.48±1.55	95.28±2.11	96.11±2.88	3.97±0.56	3.63±0.17	3.11±0.24
	300	95.42±1.98	97.33±1.12	98.11±1.23	93.14±1.08	94.36±2.09	95.29±1.25	4.29±0.48	4.07±0.08	3.77±0.16
	500	91.63±1.04	93.78±1.68	95.12±1.11	92.46±1.01	93.65±2.03	95.01±1.92	5.18±0.33	5.01±0.26	3.98±0.32
Elaegnus latifolia	100	92.88±1.28	93.12±1.78	96.56±1.77	92.95±1.99	93.55±1.28	94.68±1.88	4.34±0.33	3.93±0.56	3.55±0.33
	300	95.19±2.09	96.25±0.89	98.21±1.13	91.25±1.02	92.19±1.01	93.27±1.65	5.26±0.47	5.01±0.24	4.78±0.18
	500	92.08±1.58	94.48±1.36	98.34±1.08	90.68±1.15	90.69±1.72	91.44±1.02	6.54±0.38	6.02±0.19	5.79±0.25
Elaegnus pyriformis	100	95.32±3.01	97.26±2.06	96.13±2.48	95.66±1.55	96.38±1.08	97.55±1.11	4.92±0.11	4.77±0.28	4.38±0.99
	300	92.10±1.44	94.75±2.09	96.28±1.17	94.21±1.64	95.11±0.98	96.28±1.73	5.65±0.78	5.19±0.34	4.96±0.53
	500	90.85±1.87	95.35±2.78	94.11±1.02	94.05±1.83	95.18±1.46	96.22±1.52	7.01±0.16	6.79±0.28	6.18±0.18
Fagopyrum cymosum	100	97.27±2.88	99.89±1.83	98.23±1.84	95.24±1.68	96.38±1.08	97.55±2.01	3.28±0.74	2.79±0.38	2.08±0.56
	300	95.13±1.69	98.24±1.34	97.65±2.88	94.21±1.36	95.19±1.72	96.28±1.84	3.61±0.48	3.11±0.32	2.98±0.76
	500	91.14±1.11	94.35±1.98	93.44±1.95	93.38±1.78	94.21±1.81	95.55±1.37	3.92±0.81	3.05±0.56	3.34±0.49
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			25.18±1.06	
	100 µM		66.35±1.06			63.20±1.28			55.46±1.44	
	200 µM		48.25±1.55			39.25±1.11			76.35±1.48	

Data are presented as mean±standard error of the mean (SEM n=3)

CONCLUSIONS

The study demonstrated that various wild edible plant species are rich sources of essential nutrients, including minerals, lipids, proteins, and carbohydrates, as well as anti-nutritional factors like phytates, oxalates, tannins, cyanides, and saponins. However, the nutritional composition of these plants is significantly influenced by cooking methods such as boiling and microwaving.

Boiling led to a reduction in protein content, likely due to protein denaturation and leaching into the cooking water. In contrast, microwaving resulted in a higher concentration of proteins, possibly due to reduced cooking times and minimal leaching. Mineral losses were more pronounced during boiling, likely because of solubility and diffusion into the cooking water, while microwaving caused only slight reductions, preserving mineral content more effectively.

Both boiling and microwaving were effective in reducing anti-nutritional factors to levels considered safe for human consumption. This reduction is attributed to the thermal breakdown of anti-nutrients and their leaching into the cooking medium. Microwaving, however, was particularly efficient in lowering toxicity levels, likely due to localized and rapid heating that disrupts toxic compounds without excessive nutrient loss.

In conclusion, microwave cooking emerges as a preferred method for preparing wild edible plants. It not only minimizes cooking time but also better preserves nutrients and reduces toxicity, enhancing the overall quality and safety of food for improved human health.

AUTHOR CONTRIBUTIONS

Conceptualization, T.S.; Methodology, T.S.; Investigation, K.C., B.P.; Formal analysis, validation, writing-original draft preparation, T.S.; Writing-review and editing, T.S.; Supervision, T.S.

DATA AVAILABILITY STATEMENT

Data contained within the article.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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PREGLED NUTRITIVNIH I TOKSIKOLOŠKIH SVOJSTAVA DIVLJIH JESTIVIH BILJAKA IZ SEVEROISTOČNE INDIJE: UTICAJ RAZLIČITIH POSTUPAKA KUVANJA

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Sažetak: Cilj ove studije je ispitivanje nutritivne vrednosti, antinutritivnih faktora, mineralnog sastava i *in vitro* toksičnosti 10 divljih jestivih biljaka iz severoistočnog regiona Indije, uključujući *Meynia laxiflora, Castanopsis indica, Docynia indica, Flemingia vestita, Bauhinia purpurea, Dillenia pentagyna, Diplazium esculentum, Elaeagnus latifolia, Elaeagnus pyriformis* i *Fagopyrum cymosum*. U studiji su takođe ispitivani uticaji tehnika kuvanja, uključujući kuvanje i mikrotalasno kuvanje.
Nutritivna analiza je obuhvatila određivanje sadržaja masti, proteina, vlakana, ugljenih hidrata i mineralnih materija u sirovim i kuvanim uzorcima. Antinutritivna svojstva su procenjena određivanjem sadržaja cijanogenih glikozida, oksalata, tanina, saponina i fitata. *In vitro* toksičnost je procenjena hemolitičkim testovima na eritrocitima pacova, citotoksičnost je merena MTT testom, a genotoksičnost je procenjena komet testom.
Rezultati su pokazali da su obe metode kuvanja smanjile sadržaj pepela, masti, mineralnih materija, antinutritivnih faktora i toksičnost biljaka. Istovremeno, povećavajući relativnu koncentraciju vlakana i ugljenih hidrata zbog gubitka vode ili razgradnje drugih komponenti, apsolutni sadržaj ovih hranljivih materija ostao je nepromenjen. Mikrotalasno kuvanje značajno je povećalo mereni sadržaj proteina ($p<0.05$), sa povećanjima u rasponu od 9.58% do 33.95%. Ovaj efekat je verovatno posledica strukturnih modifikacija proteina uzrokovanih mikrotalasnim tretmanom, koje povećavaju njihovu dostupnost ili probavljivost, a ne stvarni sadržaj proteina, dok je kuvanje uzrokovalo smanjenje nivoa proteina, u rasponu od 9.66-23.25%. Pored toga, mikrotalasno kuvanje je rezultiralo manjim gubicima minerala nego kuvanje i bilo je efikasnije u smanjenju antinutritivnih faktora i toksičnosti ($p<0.05$). Kao rezultat toga, preporučuje se mikrotalasno kuvanje za poboljšanje nutritivnog kvaliteta, smanjenje sadržaja masti i smanjenje antinutritivnih komponenti i toksičnosti. Studije toksičnosti na ćelijskom i genomskom nivou pokazale su da su ove biljke bezbedne za konzumaciju.

Ključne reči: *divlje jestive biljke, termički tretmani, nutritivni sastav, sadržaj mineralnih materija, antinutrijenti, toksičnost.*

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