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RESEARCH ARTICLE

Phytochemical screening, nutritional, antinutritional, antioxidant, GCMS and mineral analysis of *Zanthoxylum rhetsa* (Roxb.) DC: Insights from tribal consumption in Mizoram, Northeast India

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Abstract

The plant *Zanthoxylum rhetsa* Roxb, a member of the Rutaceae family, holds dual significance in certain communities, particularly in Mizoram, Northeast India, where it is valued for its medicinal properties and consumed as a vegetable. This study comprehensively analyzes the phytochemical, nutritional, antinutritional and antioxidant properties and the mineral content of *Z. rhetsa*, a plant traditionally consumed by tribal communities in this region. Minerals such as aluminum, copper, iron, manganese, nickel and zinc were measured using Atomic Absorption Spectroscopy (AAS). Gas Chromatography-Mass Spectrometry (GC-MS) analysis was conducted to determine the chemical composition of the samples. Phytochemical screening revealed the existence of several bioactive components, such as tannins and flavonoids, while nutritional studies validated the plant's abundance of key nutrients. Among the minerals, manganese was found in the highest concentration (481.356 mg/100 g of DW), while nickel was present in the lowest concentration (1.614 mg/100 g of DW). The total calorific content was ascertained to be 51.344 Kcal/100g, adequate for dietary requirements. Additionally, the total phenolic content measured was 8.28 mg/g in gallic acid equivalent and the total flavonoid content was 43.04 mg/g in quercetin equivalents. The existence of antinutritional compounds in differing amounts necessitates meticulous evaluation, particularly with the intake of fruits that may possess elevated levels of these substances. This comprehensive assessment highlights the nutritional significance of *Z. rhetsa* in local diets, emphasizing its dual role as a source of beneficial nutrients and potential antinutritional factors.

Keywords

Antinutritional; *Zanthoxylum rhetsa*; methanol extract; minerals; nutritional; GCMS

Introduction

Wild plants are among the most affordable sources of diverse nutrients, including minerals, vitamins and essential oils. They provide essential fatty acids and enhance the taste and colour of diets. Food and Agriculture Organisation (FAO) defines wild edible plants (WEPs) as "plants that naturally grow in self-sustaining populations within natural or semi-natural ecosystems, existing without direct human intervention." (1) (2). Wild edible

fruits are well recognized for their high content of essential minerals, fiber and vitamins, which are vital for human health. Many of these wild fruits have nutritional values comparable to commercially cultivated fruits (3). However, several fruits contain antinutritional components, such as tannins, phytic acid, oxalates, saponins and alkaloids, which can hinder nutrient bioavailability (4).

One of the Rutaceae family's largest and most distributed genera is *Zanthoxylum*, native to warm temperate and subtropical regions (5). The medium-sized deciduous tree, colloquially known as "prickly ash" or "satinwood," often thrives in shaded, damp regions of tropical India at elevations reaching 1,800 meters. It has distinctive light corky bark (6). The plant contains various chemicals associated with multiple therapeutic effects, including terpenoids, xanthyletin, sesamin, alkaloids, flavonoids and sabinene (7). It has traditionally been used to treat various diseases, including diabetes, pain, seizures, bacterial infections, tumors, spasms, diuretic issues and inflammation (8).

Research suggests that *Zanthoxylum* is abundant in crucial nutrients vital for human nutrition and health, promoting bone strength, muscle and nerve function and fluid balance (9). Deficiencies in these vital minerals can compromise immune function by disrupting inflammatory regulation over time, resulting in numerous health complications and growth concerns (10). Bhowmik *et al*., 2012 emphasized that wild edible plants are a crucial source of nutrients and minerals necessary for human health, including protein, vitamins, iron and calcium. (11). Despite their value, wild food plants are often overlooked compared to domesticated food counterparts. Indigenous communities, however, possess valuable knowledge of gathering and preparing food from these wild plant resources, as they are abundant in natural environments (12). Although molecular oxygen is essential for life, it can be harmful as it can be transformed into reactive oxygen species (ROS) by physiological processes. Reactive oxygen species, which include singlet oxygen, hydroxyl radicals, superoxide radicals, and hydrogen peroxide (H_2O_2), have been linked to the onset and progression of various diseases such as atherosclerosis, cancer and cardiovascular conditions (13). Maintaining a healthy biological system requires a favorable balance between oxidation and antioxidation. Natural antioxidants have received significant attention, mainly due to apprehensions regarding the potential adverse effects of synthetic antioxidants on the human body (14). This study seeks to enhance our comprehension of the specific nutritional value, antioxidant characteristics, mineral composition, antinutritional elements and other chemical constituents of wild edible plants frequently utilized as vegetables.

Materials and Methods

Collection of samples

The plant specimens were collected from the tropical wet evergreen forest in Kolasib District, 77.8 kilometres from Aizawl, Mizoram (24°13’36.3792” N, 92°40’39.900” E). This district spans an area of 1,383 km^2 (535 square meters). The collection of wild edible plants was guided by the traditional knowledge of local people and based on their seasonal availability.

Plant material and extraction

The plant specimens were thoroughly washed with distilled water and then shade-dried. The dried samples were ground into a fine powder using a Bajaj Glory (410167) mixer grinder, followed by sieving to ensure uniform particle size. Next, 100 grams of the homogenized powder were extracted using a Soxhlet apparatus with methanol as the solvent. The resulting crude extracts were concentrated with a rotary evaporator at 55-60°C and preserved for further analysis.

For essential oil extraction, the plant material was subjected to hydrodistillation using a Clevenger-type apparatus for 6 hours. The extracted essential oils were dried with anhydrous sodium sulfate and stored at -20°C in a sealed, light-protected bottle until they were ready for chemical and biological analysis.

Qualitative phytochemical screening

The methanol extract of *Z. rhetsa* was examined according to the method outlined by Evans *et al*., 2009 (15) to detect the presence of alkaloids, flavonoids, reducing sugars, carbohydrates, glycosides, phytosterols, saponins, proteins, amino acids and tannins.

Proximate analysis of nutrients

The samples' total moisture, fat and ash content were assessed according to the technique established by the Association of Official Analytical Chemists (AOAC) (16). Protein and carbohydrate content were estimated using the method developed by Artinigam and Ayyagari (17).

Determination of minerals

The inorganic contents were analyzed using the acid digestion method by Toth *et al*., 1948 (18). Plant samples were cleaned, dried and heated at 600°C before being ground into a fine powder. This powdered sample was then digested by mixing it with concentrated nitric acid, allowing it to react for an hour and heating it until fully dissolved. After cooling, the solution was diluted with distilled water, stored overnight and filtered. The filtrate was then analyzed for inorganic components using an Atomic Absorption Spectrophotometer (AAS).

Total phenolic and flavonoid content

The total phenolic content was estimated using the Folin-Ciocalteu assay. In contrast, the total flavonoid content was determined using the Aluminum Chloride method, as described by Olufunmiso *et al*., 2011 (19). The total phenolic and flavonoid contents were quantified using standard curves of gallic acid and quercetin equivalent, respectively. Results were expressed as mg of standard equivalent per gram of the sample.

DPPH free radical scavenging assay

The antioxidant activity of the *Z. rhetsa* extract was evaluated using a modified Blois method (20), employing 2,2-diphenyl-1-picrylhydrazyl (DPPH) as the target free

radical and Butylated Hydroxytoluene (BHT) as the standard antioxidant. Various concentrations of plant extract and BHT (10-100 µg/mL) were prepared and mixed with DPPH solution. After incubation, the absorbance was measured at 517 nm. The scavenging activity was calculated as a percentage using the formula:

$$\text{Scavenging activity (\%)} = \frac{\text{Abs control - Abs of plant extract x 100}}{\text{Abs of control}}$$

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The chemical components of the plant extract were analyzed using a Thermo Scientific TRACE 1300 ISQ LT GC-MS system. The extract, dissolved in acetonitrile, was separated on a TR-5MS column using a programmed temperature gradient. Helium was used as the carrier gas, and the sample was injected with a 1:50 split ratio. Mass spectrometry was conducted with 70 eV electron ionization. The resulting chromatogram, generated using Xcalibur software, was analyzed with the Wiley Registry and NIST databases to identify compounds based on their molecular characteristics and retention times.

Determination of Antinutritional

The level of total tannin and saponins were determined using the method outlined by Sakung *et al.,* 2022 (21). The total phytate content was assessed according to the method described by Olabimtan *et al*., 2020 (22). Additionally, the total oxalate and alkaloid levels were measured using the method developed by Adeniyi *et al.*, 2009 (23).

Statistical analysis

The arithmetic mean, standard error and graph plotting were performed using SPSS version 16.0, while Microsoft Excel was utilized to evaluate the total phenolic and flavonoid content, as well as the inhibition percentages of DPPH.

Results

Qualitative phytochemical tests

The phytochemical analysis of methanol extracts from *Z. rhetsa* confirmed the presence of flavonoids, tannins, phytosterols, and glycosides, as shown in Table 1. These compounds are considered to possess a variety of beneficial properties. However, this analysis did not detect other compounds such as alkaloids, carbohydrates, saponins, reducing sugars, proteins and amino acids.

Quantitative analysis

The nutritional composition of *Z. rhetsa* is presented in Table 2. Estimates indicate that 1g of the sample contains 0.033g of fat, equivalent to 0.4086 kcal. Additionally, 1g of plant material includes 0.00011g of carbohydrates, which amounts to 0.00044 kcal. Furthermore, it was found that 1 g of the plant sample contains 0.054g of proteins, contributing 0.216kcal. As a result, the overall calorific values obtained from these components were calculated as 51.344 kcal/100g of the sample.

Table 1: Phyto group detected from methanol extract of *Z. rhetsa*

Phytochemical Group	Chemical Test	Indication
Alkaloids	Mayer's test	Absent
	Dragendroff's test	Absent
	Wagner's test	Absent
	Hager's test	Absent
Flavonoid	Alkaline reagent test	present
	Lead acetate test	Absent
	Shinoda test	Absent
Tannins	Ferric chloride test	Absent
	Potassium Dichromate test	Present
Phytosterols	Lead acetate test	Absent
	Salkowski reaction	Absent
	Liebermann Burchard	Present
Saponins	Foam test	Absent
Carbohydrates	Benedict's test	Absent
	Fehling's test	Absent
	Molisch's test	Absent
	Barfoed's test	Absent
Reducing sugars	Fehling's test	Absent
	Benedict's test	Absent
Protein and amino acid	Biuret test	Absent
	Benedict's test	Absent
Glycosides	Liebermann's test	Present
	Keller Kiliani test	Absent
	Legal's test	Absent

Mineral content analysis

The edible parts of plants contain minerals such as aluminum, iron, manganese, nickel and zinc in varying concentrations. The mineral analysis of *Z. rhetsa* revealed that manganese has the highest concentration at 481.356 mg/100g. Conversely, nickel was found at the lowest concentration, measuring 1.614 mg per 100 g, while copper was not detectable. These findings are illustrated in Table 3.

Table 2: Nutritional composition of *Z. rhetsa*

Plant	Parameters						Nutritive value kcal/100g
	Moisture (%)	Ash (%)	Crude fiber (%)	Carbohydrates (w/w)	Protein (w/w)	Fat (w/w)	
Zanthoxylum rhetsa	52.5%	56.5%	15.5%	0.00011	0.054	0.033	51.344

Table 3: Analysis of mineral content of *Z. rhetsa*

Minerals	(mg/100g of DW.)
Aluminum	19.486 ± 2.21
Copper	0±0
Iron	17.9 ± 0.98
Manganese	481.356± 4.37
Nickel	1.614 ± 0.02
Zinc	7.446± 0.87

Results are the mean values and standard error of the mean (SEM) of three replicates of the same sample.

Total phenolic and flavonoid content

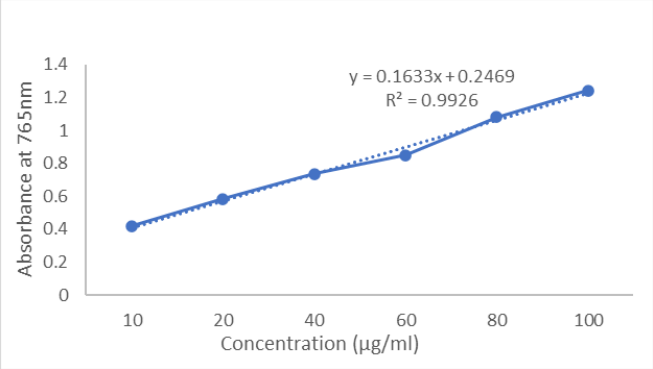

Fig.1. Standard graph of Gallic acid for estimation of Total Phenolic Content.

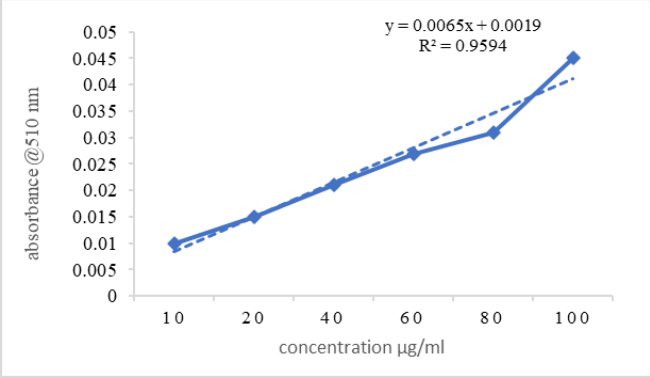

Fig.2. Standard graph of Quercetin for estimation of Total Flavonoid Content

The quantification of phenolics and flavonoid content in the *Z. rhetsa* extract, performed via the Folin-Ciocalteu assay and the Aluminum Chloride method, is illustrated in Fig. 1 and 2, respectively. The total phenolic content was determined to be 8.28 mg GAE/g and the flavonoid concentration was estimated to be 43.04 mg QE/g.

Table 4: DPPH scavenging activity of BHT and methanol extract of Z. rhetsa

S. No	Concentration (µg/mL)	Inhibition percentage (µg/mL)	
		BHT	Methanol extract
1	10	40.93	26
2	20	47.68	35
3	40	70.46	53
4	60	83.31	72
5	80	89.4	83
6	100	91.52	89
	IC_{50}	17.07	38.43

The antioxidant activity of the sample was evaluated using the DPPH assay, with the percentage of inhibition serving as a measure of antioxidant potency. Higher percentages of inhibition indicated greater activity, as shown in Table 4. Butylated hydroxytoluene was used as the standard and the values at different concentrations, ranging from 10 µg/mL to 100 µg/mL, were compared.

GC-MS Analysis

Following the GC-MS analysis, several components were identified in the GC fraction of the *Z. rhetsa* essential oil extract. Fig. 3 presents the GC-MS chromatogram of the essential oil extract of *Z. rhetsa* leaves. The active compounds in the essential oil extract of *Z. rhetsa* leaves, their retention time (RT), molecular formula, molecular weight (MW) and relative abundance are listed in Table 5.

The edible part of the plant specimen contained various antinutritional factors, as shown in Table 6. All results are expressed as g/g. Among them, the oxalate content was the highest at 0.6818g/g and tannin was found to have the lowest concentration at 0.002491g/g.

Discussion

Flavonoids possess antioxidant properties and are known to prevent cell damage, offering anticancer and anti-inflammatory effects (24). Tannins are well-known antimicrobial agents with antioxidant potential and are active ingredients in medicine and beverages (25). However, certain compounds can impact health either positively or negatively. Phytosterols are plant-derived molecules that resemble cholesterol and are naturally present in plant-based meals. They facilitate the reduction of cholesterol absorption and decrease plasma LDL cholesterol concentrations (26).

The nutritional value of many wild edible plants consumed by local populations remains largely unknown, making it crucial to determine their nutrient content. Our research aims to clarify the amount of nutrients and compounds in these plants. Shivprasad *et al*., 2016 reported various wild edible plant species, noting that the total calorific value of *Z. rhetsa* fruit was 20.6740 kcal/100g, which is lower than the present study's findings (27). In comparison, the calorific value of *Z. rhetsa* leaves is lower than that of *Alocasia fornicata*, which has a calorific value of 81.71 kcal/100g and *Trevesia palmata*, with a calorific value of 86.12 kcal/100g (28). The nutritional composition of these often-overlooked plants suggests they can be a valuable source of nutrients during times of famine. Analyzing a selected population of wild edible plants reveals that their moisture, ash, carbohydrate, protein, fat, fiber and energy content align with the Recommended Dietary Allowance (RDA) levels.

Mineral elements are essential for human health, playing a critical role in regulating bodily functions such as enzyme activity, bone formation, nerve signaling, muscle contraction and blood clotting. Nutritional guidelines emphasize the importance of consuming a diet rich in these essential elements to support physical development and

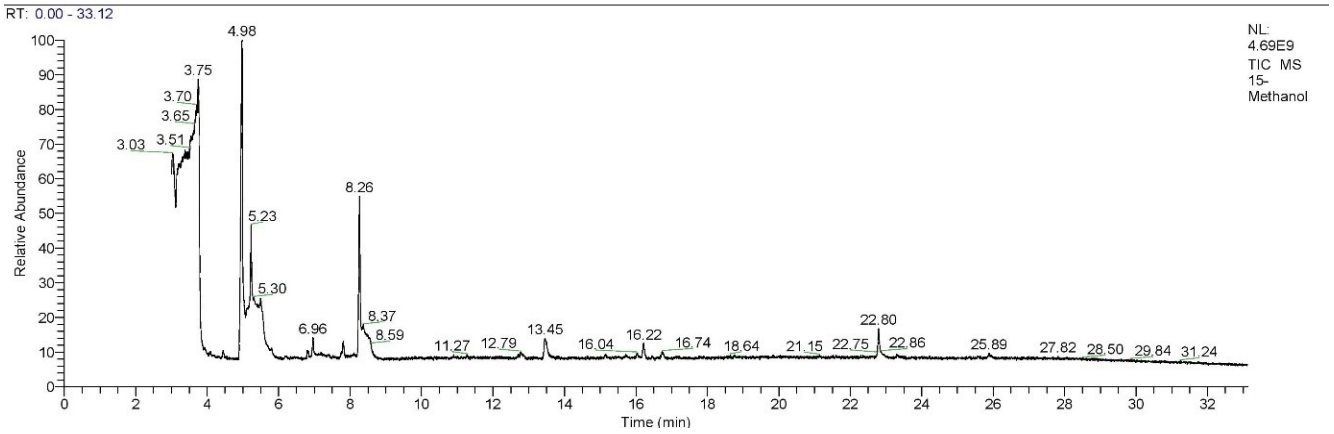

Fig 3. GC-MS chromatogram of essential oil extract of *Z. rhetsa.*

Table 5: Compounds identified from essential oil extract of *Z. rhetsa* using Gas Chromatography-Mass Spectrometry.

Relative abundance	RT	Name	Molecular formula	Molecular weight (Da)
70.99	3.03	2 undecanone	$C_{11}H_{22}O$	170
80.17	4.98	Vinyl decanoate	$C_{12}H_{22}O_2$	198
43.76	5.23	2 tridecanone	$C_{13}H_{26}O$	198
58.59	6.96	Caryophyllene oxide	$C_{15}H_{24}O$	220
58.72	8.26	Dodecanoic acid, ethenyl ester	$C_{14}H_{26}O_2$	226
49.80	8.37	Dodecanoic acid, pentafluorophenyl ester	$C_{18}H_{23}F_5O_2$	366
47.15	11.27	Cyclopentadecanone, 4-methyl-	$C_{16}H_{30}O$	238
81.98	12.79	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	$C_{17}H_{24}O_3$	276
76.83	13.45	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256
29.67	16.04	2-Hydroxy-1,1,10-trimethyl-6,9-epidioxydecalin	$C_{13}H_{22}O_3$	226
55.26	16.22	Phytol	$C_{20}H_{40}O$	296
14.92	16.74	1-Hexadecyn-3-ol, 3,7,11,15-tetramethyl	$C_{20}H_{38}O$	294
72.14	22.80	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	$C_{19}H_{38}O_4$	330
52.62	22.86	Glycerol 1-palmitate	$C_{19}H_{38}O_4$	330
57.95	25.89	Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{21}H_{42}O_4$	358
63.78	27.82	Silicic acid, diethyl bis(trimethylsilyl) ester	$C_{10}H_{28}O_4Si_3$	296

Table 6: Oxalate, Phytate, Tannin, Saponin and Alkaloid content of Zanthoxylum rhetsa

Sr. no	Name of plant species	Edible part	Antinutritional factors				
			Saponin (g/g)	Tannin (g/g)	Phytate (g/g)	Alkaloid (g/g)	Oxalate (g/g)
1	*Zanthoxylum rhetsa*	Young stem and leaf	0.07	0.002491	0.1148	0.0288	0.6818

maintain physiological functions. Inadequate mineral intake can hinder growth patterns and lead to permanent nutritional deficiencies (29).

Among the tested elements, manganese was the highest and is known to enhance hemoglobin formation. It plays a crucial role as a multi-enzyme activator, vital for energy production and maintaining the immune system (30). Manganese deficiency can lead to bone abnormalities, glucose intolerance, skin issues, weakness, and fatigue (31). Additionally, zinc intake positively influences the growth of some stunted children and protects against childhood diseases, such as diarrhea (32). Zinc deficiency can result in growth failure, malnutrition, diarrhea, pneumonia, a compromised immune system, increased infant mortality, nervous system dysfunction and abnormalities in fetal development (33).

The production of hemoglobin relies on iron and iron deficiency can lead to anemia, a common condition associated with hookworm infection (34). Renthlei *et al.* 2016 reported the mineral composition of *Z. rhetsa* leaves from the northeastern region of India, noting varying concentrations of minerals: copper (1.34 mg/100g), iron (16.19 mg/100g), manganese (28.59 mg/100g) and zinc (2.84 mg/100g) (35). According to Kuladip *et al.,* ripened fruit of *Z. rhetsa* collected from Kolhapur District, Maharashtra, contained the following minerals concentrations: copper (1.2 mg/100g), iron (2.2 mg/100g), manganese (3.1 mg/100g) and zinc (2 mg/100g) (36). Our study revealed that the leaves of *Z. rhetsa* had a higher overall concentration of minerals than previous research, which can be attributed to variations in geography and environment. Once regarded as an important source of essential elements, this plant is presently recognized as a potential provider of critical minerals in dietary planning.

Plants rich in phenols and flavonoids are potent antioxidants, primarily by scavenging free radicals. Numerous studies have established a strong positive correlation between a plant's phenolic content and its antioxidant activity (37). In this study, the total phenolic content (TPC) of the methanol extract was found to be 8.28 mg GAE/g, which is higher than the 6.01 mg GAE/g reported by Tukun (38) but lower than the 9.45 mg GAE/g reported by Ramesh (39). The total flavonoid content of the methanol extract was determined to be 43.04 mg QE/g, significantly exceeding the 1.52 mg QE/g found in the methanol extract of *Z. rhetsa* bark studied by Ramesh (39).

Free radicals are directly linked with various disease symptoms, making antioxidants crucial for maintaining optimal health by neutralizing these radicals and supporting the body's antioxidant defense. To meet the growing demand for natural antioxidants such as polyphenols, researchers are increasingly focusing on medicinal plants, which are rich in antioxidants and offer therapeutic benefits. The antioxidant properties of phenolic substances are attributed to their ability to disrupt oxidative and nitrosative processes at the cellular level. These substances can regulate cellular signaling pathways by modifying enzymatic activity, thereby influencing cell survival or death. (40)

Payum *et al*., 2013 reported an IC_{50} value of 306 ± 4.21 µg/mL for the methanol extract of Z. rhetsa shoots (41). In contrast, our study found that the methanol extract of Z. rhetsa leaves exhibited a significantly higher free radical scavenging activity, with an IC_{50} value of 38.43 ± 0.192 µg/mL. The standard antioxidant used in our study had an IC_{50} value of 17.07 µg/mL, indicating it possessed greater scavenging activity than the plant extract. Generally, lower IC_{50} values correspond to higher scavenging activity. Our results demonstrate that the *Z. rhetsa* extract has considerable antioxidant properties and flavonoid content, with increasing inhibition percentages at higher concentrations. Additionally, the extract is rich in nutrients and minerals, providing protective benefits to the human body.

These compounds exhibit diverse biological effects, from broad antimicrobial properties to specific actions such as reducing inflammation and inhibiting particular enzymes. This broad spectrum of activities suggests potential drug development and medical treatment applications. Identifying these and other bioactive substances lays a foundation for further scientific investigation and potential product development in medical and cosmetic industries. Compounds currently labeled as having "no reported activity" represent unexplored areas in science. Future research may reveal hidden properties or applications for these seemingly inactive substances, broadening our understanding of their roles and uses.

Plants significantly impact human life, providing nourishment, medicinal benefits, environmental balance, and cultural value. Interest in traditional and herbal medicines has grown recently, driven by concerns about availability, safety and lower side effects than synthetic drugs. Guil *et al.,* 1997 highlighted that rural populations commonly consume nutrient-rich wild edible plants, though their nutritional use is sometimes limited by toxic and antinutritional compounds (42). Plants produce secondary metabolites that contribute nutritional benefits and are defined against various challenges (43). According to Karr *et al*., 2024 oxalates and their by-products impair calcium absorption and contribute to kidney stone formation, negatively impacting human nutrition and health (44). Saponins affect the absorption of nutrients like cholesterol and glucose through physicochemical interactions and are noted for their hypocholesterolemic effects (45). Jambunathan and Singh reported that tannins inhibit digestive enzyme function, making their presence undesirable in nutrition, even in small amounts (46). Phytic acid decreases the bioavailability of essential minerals like iron, calcium and phosphorus, as these nutrients are less absorbable when bound to phytic acid (47). Alkaloids, which plants produce in response to al stress, exhibit significant biological activities and structural diversity. Previous studies reported lower levels of oxalate (0.0006ate (0.0002 g/g) and tannin (0.00002 g/g), with no detectable saponins. In contrast, our findings revealed higher concentrations: oxalate at 0.6818 g/g, phytate at 0.1148 g/g, tannin at 0.002491 g/g, saponin at 0.07 g/g and alkaloid at 0.0288 g/g.

Conclusion

The study revealed that *Z. rhetsa* leaves are rich in nutrients, possess strong antioxidant properties, and contain various minerals that support overall health. This wild edible plant demonstrates significant nutritional potential due to its protein, carbohydrates, fats, minerals, antioxidant compounds and other beneficial compounds. The antioxidant activity and the presence of phenolic acids and flavonoids augment its nutritional value, rendering it attractive for medicinal uses. However, as the plant also contains antinutritional factors, consuming large amounts, particularly those with higher levels of these compounds, may not be advisable.

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Authors' contributions

Lalhumhima led the study, taking charge of its conception, design, data collection, analysis and interpretation of results. J. Lalbiaknunga, Lalbiakdika and Vanlalhruaii Ralte collaborated on the analysis and interpretation of the findings. J. Lalbiaknunga and Vanlalhruaii Ralte worked together to prepare the initial draft of the manuscript. All authors participated in reviewing the results and gave their approval for the final version of the manuscript before submission.

Compliance with ethical standards

Conflict of interest: The authors state they have no conflicts of interest.

Ethical issues: None

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