

Phytochemical and Trace elements Variation in different traditional medicinal plant species of Peren District, Nagaland

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Abstract

The utilization of medicinal plants for unconventional health therapy is increasingly gaining significant recognition and popularity on a global scale. Screening of phytochemicals plays a major role in discovering many bioactive compounds significant to human health. Many parameters and conditions such as physiological conditions, soil condition and the type of extracts correspond to significant changes in phytochemical compositions. Thereby, the current study aim to assess phytochemical, and trace element contents in eight traditionally-used medicinal plants from Benreu village, Nagaland along with the corresponding soil profile. The studied plants also exhibited variations in the levels of trace element content, viz. Fe, Zn, Mn, and Cu. Variable soil nutrient levels were also observed in soil samples for N, P and K. High values of flavonoids and phenols were observed, especially in *Ageratina adenophora* (820.39 ± 0.97 mg QE/100 g dw) and *Erigeron karvinskianus* (115.75 ± 0.36 mg GAE/100 g dw), respectively. This study, through qualitative screening and quantitative evaluations, provides essential baseline data on the phytochemical contents and essential elements present in commonly-used medicinal plants along with soil profile for the traditional treatment of various ailments.

INTRODUCTION

Plants represent diverse range of phytochemical compounds such as alkaloids, flavonoids, phenolics, tannins, saponins, steroids, glycosides and terpenes (Shaikh and Patil 2020). These phytochemicals are naturally synthesized in the bark, leaves, stem, root, flower, fruits, seeds etc. (Solomon and Onuoha 2013). Globally indigenous populations depend on plant resources for traditional medicine, with herbal remedies playing a key role in rural healthcare (Agidew 2022). In Nagaland, numerous traditionally-used plants are vital for both food and medicine, forming an integral part of indigenous therapeutic practices. The practice of traditional indigenous medicine, confined to small tribal and geographical regions known as "little traditions," serves as a valuable repository of knowledge concerning the medicinal properties of botanical sources (Mothana and Lindequist, 2005). Among the Naga tribal community, phytotherapy is extensively embraced as a fundamental approach for addressing a diverse range of physical, physiological, mental, and social ailments (Jamir and Tsurho 2017). The utilization of traditional medicine for health promotion is influenced by various factors, including the cost of western medications, challenges in accessing healthcare facilities in rural areas (WHO 2005).

Nagaland is home to 16 significant ethnic groups, viz. Angami, Ao, Chakhesang, Chang, Kachari, Khamniungan, Konyak, Kuki, Lotha, Phom, Pochury, Rengma, Sangtam, Sumi, Yimchunger, and Zeliang. Peren district is home to the Zeliang ethnic group; till date the local inhabitants predominantly continue to place their trust in traditional healing approaches that involve the utilization of medicinal plants, serving as their primary means of healthcare (Jamir et al. 2022). Moreover, the district is a reservoir of natural vegetation where virgin forests are still found. Peren district is situated between 25'33'55" N latitude and 93'20'E-94'00'42"E longitude with altitude of 1445 MSL. The study location, Benreu village, lies at an altitude of 1950 MSL surrounded by Jalukie tehsil towards west, Kohima tehsil towards east, Kuhuboto tehsil towards North and Dhansiripar towards west. Studies on traditional medicinal plants of this region

have been limited in comparison to its diverse natural vegetation; there has not been much foray into the area of phytochemical studies and more specifically into the realm of pharmacognosy. In order to encourage the appropriate utilization of herbal medicine and assess their viability as a reservoir for new pharmaceuticals, a more focused and comprehensive investigation into medicinal plants with entrenched folkloric recognition becomes indispensable. The study, therefore, was taken up to ascertain the popular medicinal plants available in Benreu village thorough qualitative and quantitative evaluations of their natural constituents in their natural habitat.

MATERIALS AND METHODS

Selection of plants

In this study, standard tests were employed to qualitatively screen for phytochemicals in eight medicinally important plants of Benreu village, viz. *Artemisia nilagirica*, *Erigeron karvinskianus*, *Ageratina adenophora*, *Houttuynia cordata*, *Plantago major*, *Persicaria chinensis*, *Drymaria cordata* and *Pseudognaphalium luteoalbum*.

A. nilagirica (Indian wormwood; family Asteraceae) is an aromatic perennial herb found in hilly regions of India. This plant has a long history of traditional use in treating epilepsy, digestive issues, morning sickness, irregular menstrual cycles. Besides, it has been reported to cure typhoid, renal problems, bronchitis, and malaria (Suresh et al. 2011).

E. karvinskianus (Mexican fleabane; family Asteraceae) is a small perennial herb, found abundantly in Nagaland as weed. The plant is used in the traditional system of medicine. Its leaves are applied externally to treat skin diseases. It has traditionally been employed for addressing issues such as indigestion, enteritis, epidemic hepatitis, and hematuria (Rajalakshmi et al. 2016).

A. adenophora (Crofton weed; family Asteraceae) is a perennial used in traditional medicine to treat various ailments, including fever, diarrhea, and respiratory infections (Zhang et al. 2013). Among the tribal people of Meghalaya and Nagaland states in India, a decoction of leaves is administered to cure stomach aches (Kumar 2002).

H. cordata (Fishwort; family Saururaceae) is a herbaceous perennial plant and serves as both a medicinal and edible herb. A decoction of this plant is administered internally to treat a variety of ailments, including cancer, coughs, dysentery, enteritis, and fever. Externally, used in treating snakebites and skin disorders (Seal 2011).

P. major (Common plantain; Plantaginaceae) is a perennial medicinal plant, used in traditional medicine for its various medicinal properties, including anti-inflammatory, antimicrobial, antiviral, analgesic, and wound healing effects. It also used to treat a variety of ailments, including coughs, colds, fever, asthma, bronchitis, and diarrhea. It has also been used externally for wound healing and as a poultice for insect bites and stings (Zhakipbekov et al. 2023).

P. chinensis (Chinese knotweed; Polygonaceae) is an annual herb widely distributed all over India. The plant is traditionally used to treat fever, whooping cough, chest diseases, wounds, to treat gastritis, reduce inflammation, enhance blood circulation, manage dysentery, as a diuretic and in controlling hemorrhages (Sarmah et al. 2014).

D. cordata (Chickweed; Caryophyllaceae) is characterized by its heart-shaped leaves, is a small herbaceous plant. The plant is extensively distributed in moist locations across the tropical regions of Africa, Asia, and the Americas, where its diverse applications in agriculture and traditional medicine have been documented (Burkill 1985). In North East India, it has a history of use as an antidote, appetizer, depurative, emollient, febrifuge, laxative, and stimulant for humans and animals (Saklani and Jain, 1994).

P. luteoalbum (Jersey cudweed; Asteraceae) is a commonly and widely distributed weed, found in all continents. *P. luteoalbum* has been used in traditional medicine to treat various ailments, such as respiratory infections, gastrointestinal disorders, skin conditions, cancer, epilepsy, flux, stomach ache, and malaria (Kumar et al. 2012; Rahmatullah et al. 2010).

Collection and identification of plant material

Fresh plants materials were collected from Benreu village of Peren district, Nagaland, India (Fig. 1). The ethnobotanical information of the selected eight traditional plants were recorded including the vernacular names, utilized plant parts, and medicinal applications through verbal interactions and interviews with local practitioners and village elders with traditional knowledge and expertise in their utilization (Table 1). These plants commonly occur in cultivated land, forest, along the roadside, in shady and moist places. The plants collected were taxonomically identified and authenticated at Botanical Survey of India (BSI), Eastern Regional Centre, Shillong, Meghalaya.

Soil sampling and determination of N, P, and K

Soil samples were collected from 30 cm depth. The samples were air-dried after removing extraneous matter like pebbles and plant roots. Subsequently, the samples were ground carefully to prevent mixing and sifted using a 2.0 mm sieve and then stored in sealed plastic bags for soil analysis. The available nitrogen content in the soil was determined using alkaline potassium permanganate method, as described by Subbiah and Asija (1956). Phosphorous availability was extracted using a solution consisting of 0.03 N NH_4F in 0.025 N HCl. This procedure is primarily intended for soils with a moderate to strongly acidic pH range and is determined using the Brays and Kurtz method (1945). The available potassium content in the soil was extracted using neutral normal ammonium acetate (pH 7.0), and the potassium content in the resulting extract was determined using flame photometer (Ikon instrument, Digital and Microprocessor) as described by Jackson (1945). The soil pH was assessed in a 1:2 soil-water suspension using pH meter, following the method described by Richards (1954).

Preparation of plant extract for Phytochemical screening

The selected plant materials were thoroughly washed in clean water 2–3 times to remove the dust particles, followed by drying in room temperature for 3–4 days and then dried in an oven at 50°C to a constant weight. The dried plant sample were then ground to powdered form and stored at 4°C until analysis. Powdered samples were extracted in 5 different absolute solvents i.e., Methanol, Aqueous, Ethyl Acetate, Chloroform and Dichloromethane. Twenty grams of samples were dissolved in 250 ml of each solvent, respectively. The mixture was heated on a hot plate with continuous stirring at 30–40°C for 20 min. After 24 h, the plant materials were filtered through Whatman filter paper 1, and the filtrate was used for testing the presence of secondary plant constituents, *viz.* alkaloids, terpenoids, steroid, triterpenoids, saponins, flavonoids, tannin, glycoside, carbohydrates, quinones, anthracquinones and xanthoproteins and validated using standard methods (Savithramma et al. 2011, Harborne 1998, Velavan 2015, Sofowora 1993, Evans 2009).

QUANTITATIVE ANALYSIS

Extraction of plant materials

One gram of each dry sample was extracted overnight in 5 mL of 80% methanol. The extract was centrifuged at 5000 rpm for 10 min, and the supernatant was taken for the estimations.

Determination of Total Flavonoid content

The total flavonoid concentration in plant extract was measured by the aluminium chloride colorimetric assay following the method of Kusirisin et al. (2009). An aliquot of extract (0.2 ml) was taken in a test tube and made up to 3 ml with distilled water. To this, 150 μL of 5% NaNO_2 (w/v) was added and incubated for 5 min at room temperature followed by the addition of 150 μL of 10% AlCl_3 solution. After incubation for another 6 min at room temperature, 1 ml of 1 M NaOH was added immediately and vortexed. Absorbance was measured against the blank at 510 nm using spectrophotometer (Shimadzu UV-1700, Japan). Total flavonoids content was expressed as mg Quercetin equivalents (QE) 100 g^{-1} dw.

Determination of Total Phenol content

Total phenol content was estimated through Folin-Ciocalteu reagent as per the method described by Singleton et al. (1999). An aliquot (0.2 ml) of the plant extract was made up to 3 ml with distilled water. Subsequently, 1 ml of DMSO and 10% of 1 ml of Folin-Ciocalteu reagent were added to the mixture and vortexed. After a 3-minute interval, 3 ml of 7.5% Na_2CO_3 solution was added, and the tubes incubated at room temperature for 2 h. Absorbance was measured at 760 nm using a spectrophotometer (Shimadzu UV-1700, Japan). Total phenolic content was determined using gallic acid standard curve and expressed as mg gallic acid equivalents (GAE) 100 g^{-1} dw.

Micronutrients analyses

Trace elements (Fe, Zn, Mn, Cu) were analyzed as per the method of (Audu and Lawal 2006). One gram of plant sample consisting of viable plant tissue was digested in di-acid (HNO_3 and HClO_4 ; 3:1 ratio) and kept on digestion block for 3 h at 85°C until the solution turns clear. The resulting solutions were subsequently diluted to 50 ml with distilled water and filtered with Whatman No.42 filter paper. The filtrates were then subjected to elemental analysis using atomic absorption spectroscopy (Thermo Scientific Ice 3000 SERIES), utilizing a mixture of acetylene and air in the flame. The accuracy of the instrument was established through calibration, involving the use of high purity grade standards specific to the respective elements.

Statistical analysis

The experiment was performed in triplicates and the results were expressed as mean $\pm$ SE. For all tests, $P < 0.05$ was considered to indicate a statistically significant difference.

RESULTS

Folklore use of *A. nilagirica*, *E. karvinskianus*, *A. adenophora*, *H. cordata*, *P. major*, *P. chinensis*, *D. cordata* and *P. luteoalbum* plants used by the traditional healers and villagers of the study area along with the plant parts utilized is presented in Table 1. The estimated soil parameters pH, N, P and K is presented in Table 2. The soil of the site is acidic with a mean pH of 5.48. The mean nitrogen content was 200.52 kg ha⁻¹, available phosphorus was 2.19 kg ha⁻¹, and potassium amounted to 473.19 kg ha⁻¹.

Phytochemical screening test

The results of phytochemical screening analysis of the eight selected plant species are shown in Table 3. All the plants showed the presence of alkaloids, terpenoids, steroid, triterpenoids, saponins, flavonoids, tannin, glycoside, carbohydrates, quinones and xanthoproteins. However, anthraquinone was absent in all the plants studied. Among the solvents, methanol is observed as the most promising solvent for all the studied plant species.

Total flavonoids and phenols content

A comparative study of the total flavonoid and phenol content on methanolic plant extract was also estimated (Table. 4, Fig. 4 and Fig. 5). The total flavonoid content ranged between (820.39 $\pm$ 0.97 to 524.34 $\pm$ 1.68 mg QE/100g dw). Total phenol content ranged between (115.75 $\pm$ 0.32 to 25.9 $\pm$ 0.27 mg GAE/100 g dw).

Micronutrient analyses

Mineral content determined was based on plant dry weight. While, the highest amount of Fe was found in *H. cordata* (3.645 $\pm$ 0.03 mg/100 g) and lowest in *D. cordata* (0.692 $\pm$ 0.03 mg/100 g); Zn was found highest in *P. major* (0.087 mg/100 g) and lowest in *D. cordata* (0.028 mg/100 g); Mn was found highest in *P. chinensis* (0.091 mg/100 g) and *D. cordata* was not detected for Mn. Cu was deemed negligible in *H.*

cordata, *A. adenophora*, *D. cordata* and *P. luteoalbum*. Elemental accumulation in different plant species depends on several factors, including the plant species, type of soil, fertilization method, and environmental conditions (Bengtsson et al. 2003). The present study findings indicated that the selected medicinal plants are a significant source of essential minerals (Table.5, Fig.5).

DISCUSSION

The study aimed to evaluate the nutritional, phytochemical, and mineral composition of selected medicinal plants for therapeutic uses from Benreu village, Nagaland. The findings of the current study emphasize that organic solvent extracts showcase comparably enhanced levels of health-alleviating phytochemicals. This phenomenon can be attributed to the fact that the active principles responsible for these effects were either polar or non-polar in nature and were effectively extracted solely through organic solvent mediums (Patel et al. 2011; Truong et al. 2019,). These observations propose that organic solvent extraction can serve as a suitable approach to validate the therapeutic properties of medicinal plants, also supported by other studies (Galvez et al. 2005; Kumar et al. 1997; Ashagrie et al. 2023). The choice of solvent significantly affects predicting bioactive compounds from plant material. While, water is a common choice for traditional healers (Parekh et al. 2005), the study showed that methanol led to more consistent secondary metabolite presence compared to chloroform, ethyl acetate, dichloromethane, and water. This is attributed to the compounds polarity extracted by each solvent. Methanol extraction of dried plant material as a reasonable preparation method has also been highlighted (Vongsak et al. 2013).

A. nilagirica revealed the presence of biologically active phyto-constituents, viz. alkaloids, terpenoids, flavonoids, glycoside, tannins, saponins and steroids. Previous study has also reported similar result in *A. nilagirica* (Pradeep et al. 2014). *A. nilagirica* is reported to have antimalarial, antioxidant, antiparasitic, antiepileptic, antidiabetic, antiemetic, anticancer, gastroprotective, anthiasthmatic, insecticidal and antiviral activities, and also against COVID-19 (Perumal and Pradeep 2008; Aparna and Duraiswamy 2009; Suresh et al. 2011; Bisht et al. 2021).

E. karvinskianus screening also highlighted abundance of alkaloids, flavonoids, tannins, saponins, steroids, terpenes and glycosides, and this corroborates similar report on *E. karvinskianus* (Belho et al. 2022). *Erigeron* spp. is reported to exhibit antifungal, antibacterial, anti-inflammatory and antioxidant properties (Li et al. 2005, Asongalem et al. 2004). Considering the plant's historical application in treating painful conditions like dysmenorrhea and inflammatory diseases such as gout, cystitis, nephritis, and rheumatic arthritis, it became valuable to confirm its antinociceptive and anti-inflammatory properties in rats and mice (De las Heras et al. 1998).

A. adenophora boasts a diverse range of bioactive compounds and therapeutic effects, encompassing anti-inflammatory, analgesic, antipyretic, antioxidant, antibacterial, antifungal, and even anticancer properties (Tripathi et al. 2018). In our study, the presence of xanthoproteins, alkaloids, steroids and triterpenoids was confirmed in *A. adenophora*; similar observation is also reported by (Mazumder et al. 2013). *A. adenophora* was tested for cytotoxicity in human lung cancer in three tumor cell lines (MCF 7,

Hep G₂, HeLa) *in vitro*, and exhibited significant antitumor activity against the cell lines (Tiwary et al. 2015). The methanolic extract of *A. adenophora* leaf samples, collected from Mizoram, has been found to induce hepatotoxicity in albino mice (Katoch et al. 2000). Another study reported the wound healing potential of *A. denophora* both in excision and incision wound models (Negi et al. 2011).

In our investigation *H. cordata* is rich in flavonoids, saponins, alkaloids, tannins and steroids. Recently, the inhibitory activity of phytochemicals derived from *H. cordata* against SARS-CoV-2 virus was highlighted (Das et al. 2022). The Naga tribes of North-east India have employed its leaves as a medicinal remedy for treating intestinal infections (Yadav and Temjenmongla, 2011). In light of this traditional usage, its potential anti-parasitic properties were explored through an investigation involving albino rats infected with *Hymenolepis diminuta*, a zoonotic cestode, highlighting robust anthelmintic effects (Vijaya and Yadav, 2016).

P. major was found rich in flavonoids, alkaloids, tannins, carbohydrates and terpenoid. This corroborates a combination of bioactive elements such as carbohydrates, flavonoids, tannins, and trace quantities of alkaloids reported (Lukova et al. 2017). Similarly, research has indicated that *P. major* possesses the ability to promote wound healing, antioxidant activity, and acts as an agent against ulcers, diabetes, diarrhea, inflammation, cancer, pain sensitivity, bacterial infections, and viruses (Adom et al. 2017). The presence of tannins, flavonoids, and alkaloids in *P. major* may account for its antidiarrheal effect; tannins demonstrate antidiarrheal effects by forming protein tannates, thereby reducing intestinal secretion (Tripathi 1994; Mukherjee et al. 1995).

This study reports the presence of flavonoids, alkaloids, tannins and saponins in *P. chinensis*. Studies have confirmed different components of the plant for their potential antibacterial, antifungal, anti-inflammatory, hepatoprotective, anti-tumor, anti-diarrheal, gastro-protective, cytotoxic, antioxidant, antimicrobial, and anthelmintic activities (Manasa et al. 2016). Caffeic acid, quercetin and kaempferol derived from *P. chinensis* exhibited high anti-inflammatory efficacies in both *in vitro* and *in vivo* in mice model (Hossen et al. 2015). Besides the evidence of *in vivo* antitumor effects of *P. chinense* against human hepatocellular carcinoma cell line, Bel-7404, the study highlights the potential of distinct individual compounds, each possessing varying chemopreventive abilities to treat existing cancers (Manasa et al. 2016). The antidiarrheal effect of the plant extract in mice models was found associated with two specific polyphenols, namely ellagic acid and corilagin (Xiao et al. 2013).

D. cordata revealed the presence of alkaloids, flavonoid, saponins, and terpenoids in the study. It was also reported that in the initial phytochemical analysis of the methanolic extract from *D. cordata*, researchers identified secondary metabolites such as alkaloids, flavonoids, phenols, tannins, and saponins, along with the presence of steroids and terpenoids (Bhattacharyya et al. 2019). This medicinal plant holds significant importance, boasting a range of remarkable phytoconstituents and exhibiting numerous pharmacological activities including analgesic, antibacterial, anti-convulsant, anti-inflammatory, antipyretic, antitussive, anxiolytic, and cytotoxic effects (Kashyap et al. 2014). A study demonstrated by (Mukherjee et al. 1997) on significant antibacterial effectiveness of methanolic extract of

D. cordata against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Bacillus pumilis*, and *Pseudomonas aeruginosa*. Successful clinical outcomes for treating sinusitis with *D. cordata* in Manipur is reported (Singh 2017).

In our analysis *P. luteoalbum* was found rich in alkaloids, flavonoids, carbohydrates, terpenoids, tannins and saponins. Pharmacological investigation reported that *P. luteoalbum* exhibited antibacterial, antifungal, antioxidant, and anti-inflammatory properties (Al-Snafi 2019). Methanolic extract of *P. luteoalbum* leaves exhibited significant cytotoxicity, particularly against gastric and breast cancer cell lines (Akter et al. 2014). Methanolic extract demonstrated the highest DPPH scavenging activity in fractions extracted from its aerial parts (Uddin et al. 2017).

Phenolic compounds have been recognized as antioxidants agents that help to stop harmful effects of free radicals in our bodies (Samatha et al. 2012). Flavonoids, characterized by their hydroxyl content, account for the radical scavenging effects or chelating process observed in the majority of plants (Giri et al. 2022). Similar to our study, the maceration of dried leaves using 80% methanol also yielded the highest amount of the plant extract (Tessema Desta et al. 2022). The amount of total phenolics content vary in different plants and range from 25.9 to 115.75 mg GAE/100 g dw (Table.4). The highest levels are detected in *E. karvinskianus* and *A. adenophora* (98.88 and 115.75 mg GAE/100 g dw, respectively), and the lowest levels are detected in *A. nilagirica* and *P. chinensis* (25.9 and 98.88 mg GAE/100 g dw, respectively). The phenol content obtained with *A. adenophora* was higher than the value of 30.0 mg GAE/100 g reported from Aizawl (Lallianrawna et al. 2013). Similarly, the values obtained with *E. karvinskianus* are higher than the value of 89.9 mg GAE/100 g reported by (Zhang et al. 2020).

On the other hand, other studies have reported higher phenol content in *A. nilagirica* and *P. chinensis* (Rao et al. 2006; Sarmah et al. 2014). These variations could be attributed to certain physiological and ecological functions, which help the plants to adapt at different environmental conditions (Holopainen et al. 2018). In fact, there have been reports indicating that the quantity of total phenolics varies in relation to plant families and varieties (Kaur and Kapoor 2004; Tlili et al. 2013). The studied medicinal plants are rich in flavonoids (Table 4). The total content of flavonoids varies between 820 and 524 mg QE/100 g dw. The highest flavonoid values are similarly detected in *A. adenophora* and *E. karvinskianus* (820.39 and 791.64 mg QE/100 g dw, respectively), while the lowest values are detected in *P. chinensis* and *A. nilagirica* (524.34 and 545.73 mg QE/100 g dw, respectively). The result obtained with *A. adenophora* is three times higher than the value reported for the same species collected in Aizawl (Lallianrawna et al. 2013). Furthermore, it is important to highlight that the examined plants possess elevated concentration of flavonoids in contrast to other medicinal plants sourced from Kottakkal (Sulaiman and Balachandran, 2012). The level of total phenolic compounds in all the studied plants is higher compared with other traditional medicinal plants from China, such as *Artemisia capillaries*, *Ecliptica prostrate*, *Juglans regia*, *Morus alba*, *Plantago asiatica* and *Polygonum orientale* (Cai et al. 2004) or from Nigeria, such as *Acalypha wilkesiana*, *Azadiracta indica* and *Solanum scabrum* (Anokwuru et al. 2011). The high phenolic and flavonoids content in our findings can, therefore, support and validate the traditional applications of the studied plants in the realm of traditional medicine for therapeutic uses as recorded for Benrue village.

The soil pH was found to be acidic in nature with a mean pH of 5.48. Acidity of soil can be attributed to two primary factors: the high rainfall in the NE region, which leads to the leaching of basic cations from soil colloids, and the gradual decomposition of organic matter, resulting in a continuous release of low molecular acids (Singh and Munth, 2013). In Nagaland, land use is shaped by local socio-economic requirements, with certain regions prioritizing subsistence farming, while others pursue more lucrative endeavors such as plantations. Land use differs across districts because farmers adjust their practices based on local soil conditions, fertility, terrain, altitude, and proximity to their villages. Consequently, land use choices are influenced by specific factors at the local and site levels (Mishra and Francaviglia, 2021). Soil serves as a primary reservoir of essential nutrients required for plant growth, with nitrogen (N), phosphorus (P), and potassium (K) being the three key nutrients. In tropical soils, nitrogen frequently becomes a limiting factor for plant growth, indicating its significance on soil fertility (Maithani et al. 1998). In the present study the amount of available nitrogen $200.52 \text{ kg ha}^{-1}$ was determined. Also surface changes due to the removal of live vegetation and forest floor litter can significantly impact nitrogen levels, rendering them susceptible to erosion, runoff, and the leaching of mineral nitrogen unabsorbed by plant roots (McGrath et al. 2001). Also, a recurring observation is the common practice of burning biomass and debris in paddy fields can result in reduction of nitrogen stocks. The available phosphorous content in Benreu village was detected at 2.19 kg ha^{-1} , which is low. The limited available phosphorus may be attributed to the existing acidic soils which increases phosphorus fixation, and thereby the high insolubility of phosphorus in soil. Besides, factors such as plant uptake, erosion, and leaching, can also collectively contribute to the reduced phosphorus content in soil samples from the region (Gao and Li, 2023). In the present study potassium content was $473.19 \text{ kg ha}^{-1}$. The increased soil potassium levels could potentially be a result of the various tree species thriving in the forest, possibly accounting for the higher levels of exchangeable potassium. These forest soils maintain their natural potassium balance due to the undisturbed ecosystem and the absence of residue removal practices that might deplete potassium. (Reichardt et al. 2020). With respect to fertility status of soil in Benreu village, Nagaland, the soil analysis results indicate a lower availability of soil nutrients, which can be attributed to the non-use of chemical fertilizers in the study area.

Dietary mineral elements are necessary in trace amounts to promote and facilitate the efficient functioning of human health (Radha et al. 2021). The Fe content in the samples was in the range of 0.69 ± 0.03 – $3.65 \pm 0.03 \text{ mg/100g}$. The concentration levels of iron in some medicinal plants from Khonoma village, Nagaland, ranges from 71.46 mg/100g – 163.4 mg/100g (Anal and Chase, 2016). Some medicinal plants of East Africa recorded Fe content in the range from 1.53 mg/100g – 38.04 mg/100g (Omolo et al. 2001). The World Health Organization (WHO) recommends iron levels of 200 mg/100 g in medicinal plants (WHO 1998).

Zinc is essential for all organisms and serves as an important trace element with defined roles in metabolism, growth, and development by Singh and Garg (1997). The Zn content in the study range from 0.032 ± 0.01 to $0.091 \pm 0.01 \text{ mg/100g}$. The World Health Organization (WHO) recommends a limit of 500 mg/100 g for zinc in medicinal plants (Khan et al. 2008).

Copper (Cu) serves as a vital cofactor for numerous enzymes, playing a crucial role in metabolism and maintaining the integrity of blood vessels, epithelial and connective tissues, and skin. In the study, Cu values of $0.003 \pm 0.01 - 0.018 \pm 0.01$ mg/100g was observed in *A. nilagirica*, *H. cordata*, *P. major* and *P. chinensis*. Cu was not detected in *E. karvinskianus*, *A. adenophora*, *D. cordata* and *P. luteoalbum*, and is therefore deemed negligible. A study examined in some medicinal importance plants of Pakistan on essential trace elements, reported that Cu content was negligible in *Azadiracta indica* and *Aloe barbedensis* (Ansari et al. 2004). Another study reported on medicinal plants collected from geographically diverse locations within India, detected the range of copper from 0.096 mg/100g – 0.073 mg/100g (Sadhu et al. 2015). The current results indicate that the Cu concentrations in all traditional herbal products tested are below the limit permitted by the WHO, which is 100 mg/100g (Olusola et al.2021).

Manganese (Mn) is essential for the growth of both plants and animals. The range of Mn varied from 0.018 ± 0.01 mg/100g – 0.289 ± 0.01 mg/100g, while it was negligible in *D. cordata*. Similar study reported the range of manganese varies between 1.69 mg/100g – 13.6 mg/100g (Lesniewicz et al. 2006). Another study examined on Mn content of some medicinal plants of Tamil Nadu and found to be negligible in *Hibiscus cannabinus* plant (Subramanian et al. 2012). The World Health Organization has established a maximum permissible limit of Mn in medicinal plants at 2000 mg/100g (WHO 1998).

Conclusion

Findings of this study provide some scientific support for the use of traditional medicinal plants in the treatment of several ailments. The phytochemical studies revealed the presence of alkaloids, terpenoids, steroid, triterpenoids, saponins, flavonoids, tannin, saponins, glycoside, carbohydrates, quinones, and xanthoproteins in all the plant species. According to the findings, the qualitatively-identified phytochemicals are bioactive in nature with inherent medicinal properties. All the plants were also found to have trace elements within the permissible levels. The soil profile in Benreu village, Nagaland indicates low N and P, emphasizing the need for sustainable land use and soil management to restore its health and support sustainable yield for the local agricultural community. The data also indicates that medicinal plants in Benreu village may serve as sources of essential elements (Fe, Zn, Cu, and Mn). To corroborate our qualitative findings, *E. karvinskianus*, *A. adenophora* and *P. luteoalbum* species exhibited more antioxidant potential as indicated by their phenol and flavonoid contents. Nevertheless, additional pharmaceutical research and clinical trials is required to explore and validate the metabolic regulation, *in vivo* antioxidant and anti-inflammatory potential, and biological characteristics of these phytochemicals to conclusively highlight their therapeutic effects. Recording traditional knowledge, conducting scientific screenings, and comprehensive evaluations serve as the fundamental groundwork for contemporary ethnopharmacological studies, with the potential to drive the eventual creation of innovative pharmaceuticals. It is important to note that this study represents an initial validation effort within the broader context of traditional knowledge concerning other medicinal plants.

Declarations

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Authors contribution

TP planned the study, executed the workplan and drafted the manuscript. HV and PR provided the technical guidance. LS and AD assisted in carrying out the workplan. CA contributed to the experiments and revised the manuscript. All authors contributed to the article and approved the submitted version.

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Tables

Tables 1 to 5 are available in the Supplementary Files section

Figures

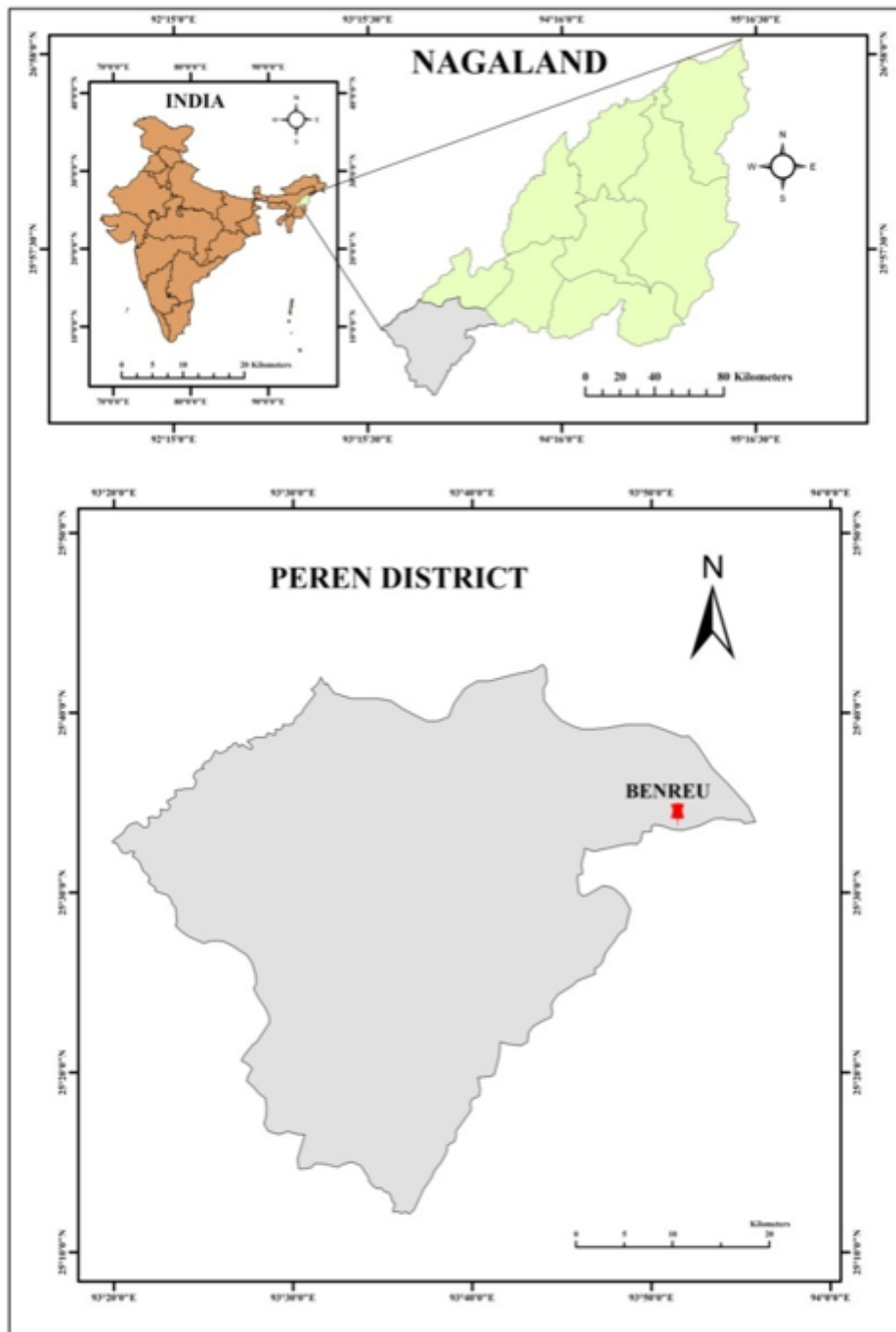


Figure 1

Location of study area of Benreu village, Peren District, Nagaland

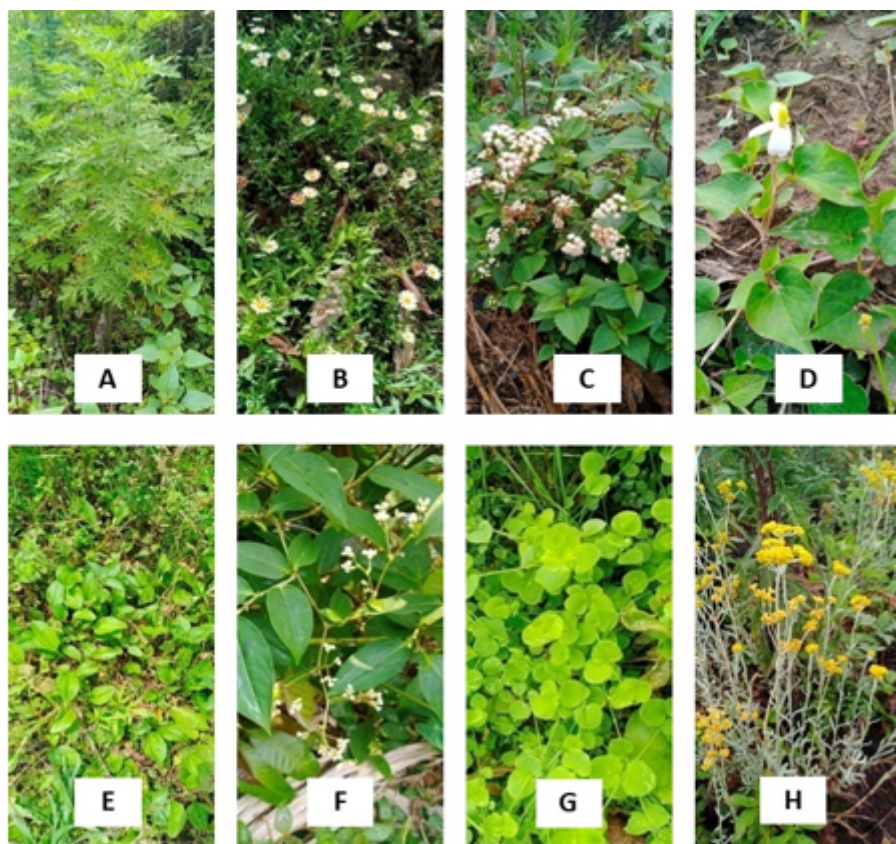


Figure 2

Collected medicinal plants recorded from Benreu village

A. *Artemisia nilagirica*; **B.** *Erigeron karvinskianus*; **C.** *Ageratina adenophora*; **D.** *Houttuynia cordata*; **E.** *Plantago major*; **F.** *Persicaria chinensis*; **G.** *Drymaria cordata*; **H.** *Pseudognaphalium luteoalbum*

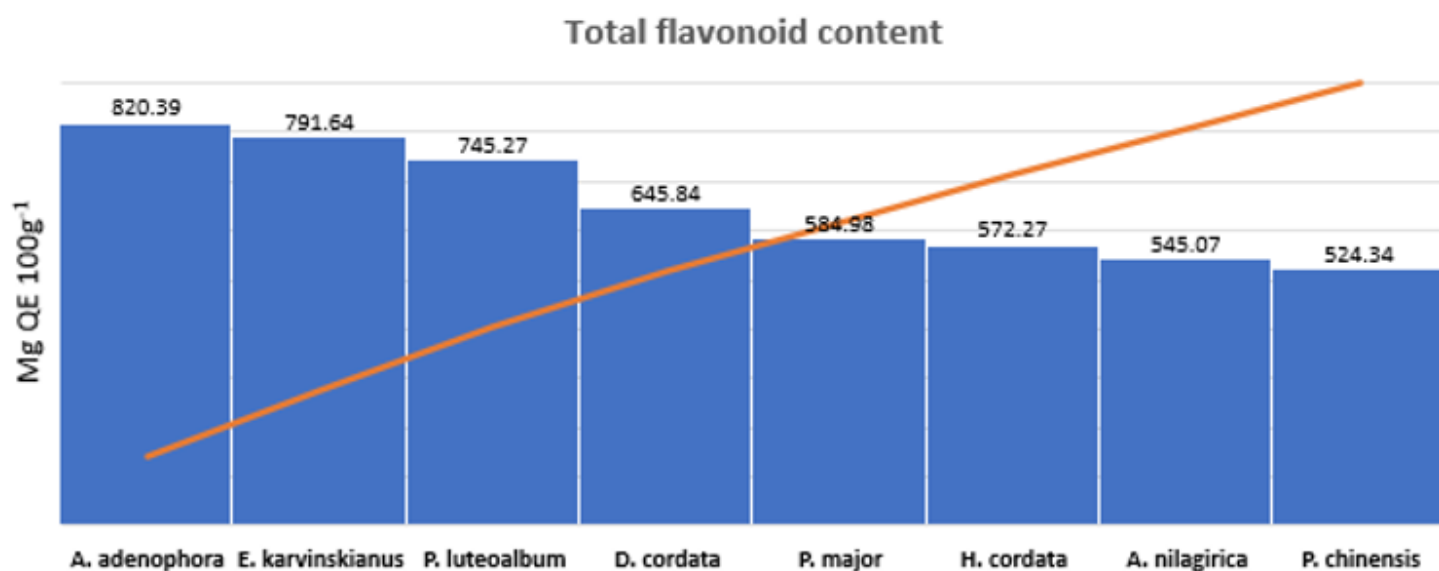


Figure 3

Total flavonoid content of the selected eight medicinal plants

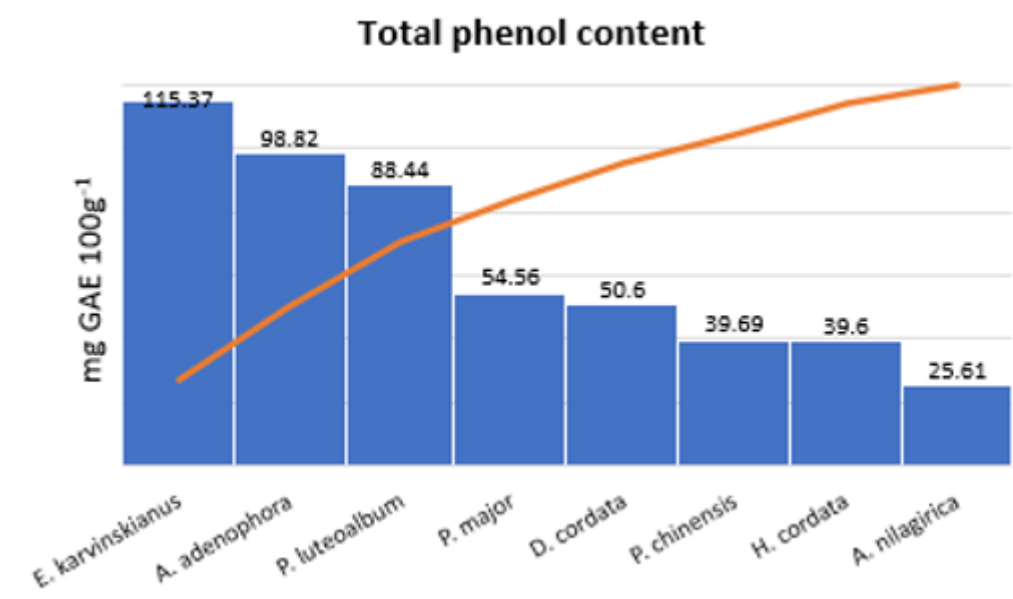


Figure 4

Total phenol content of the selected eight medicinal plants

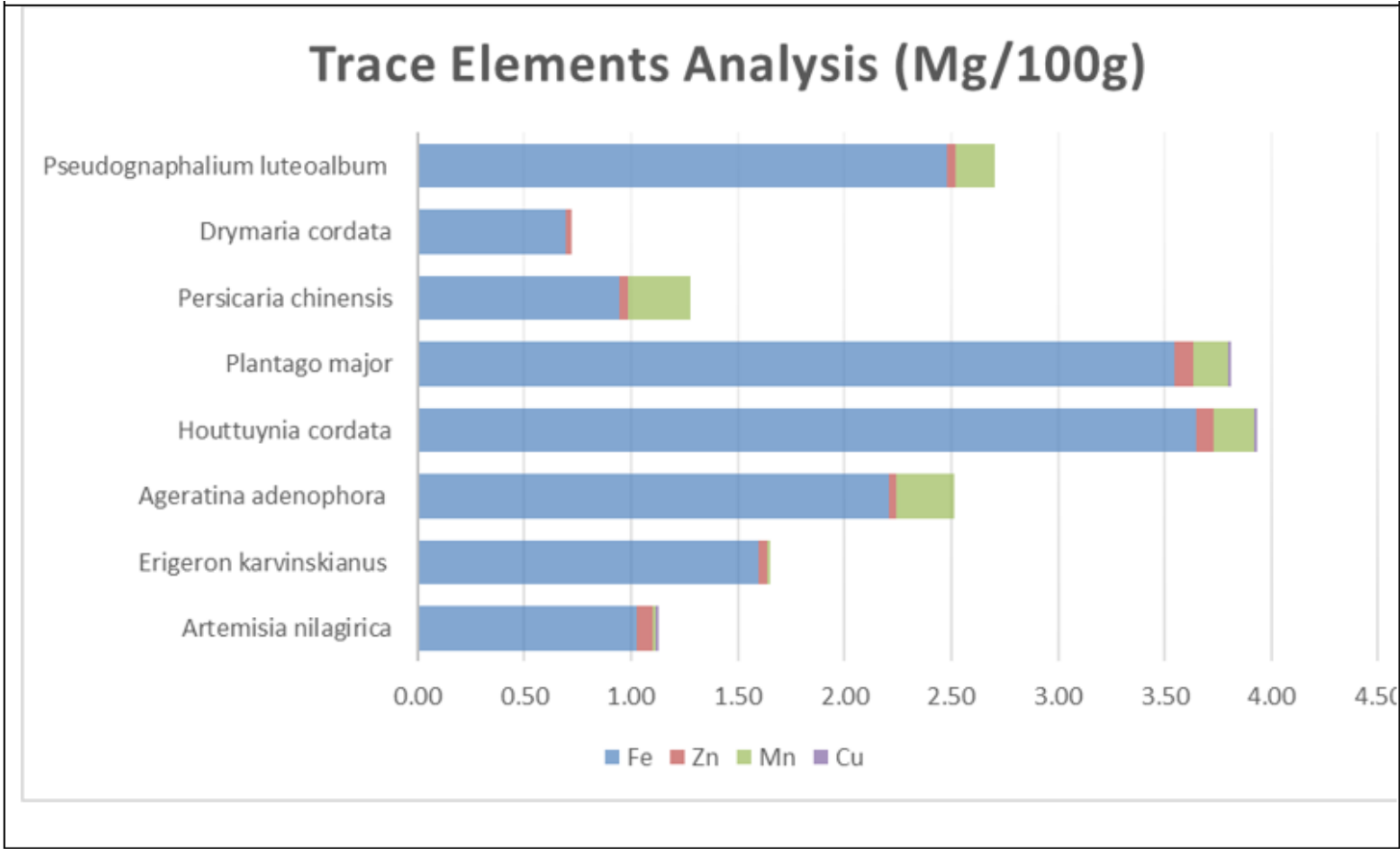


Figure 5

Trace elements of selected medicinal plants

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