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Ethnobotanical validation and phytochemical profiling of *Glinus oppositifolius* (L.) Aug. DC used by indigenous tribes of Mizoram

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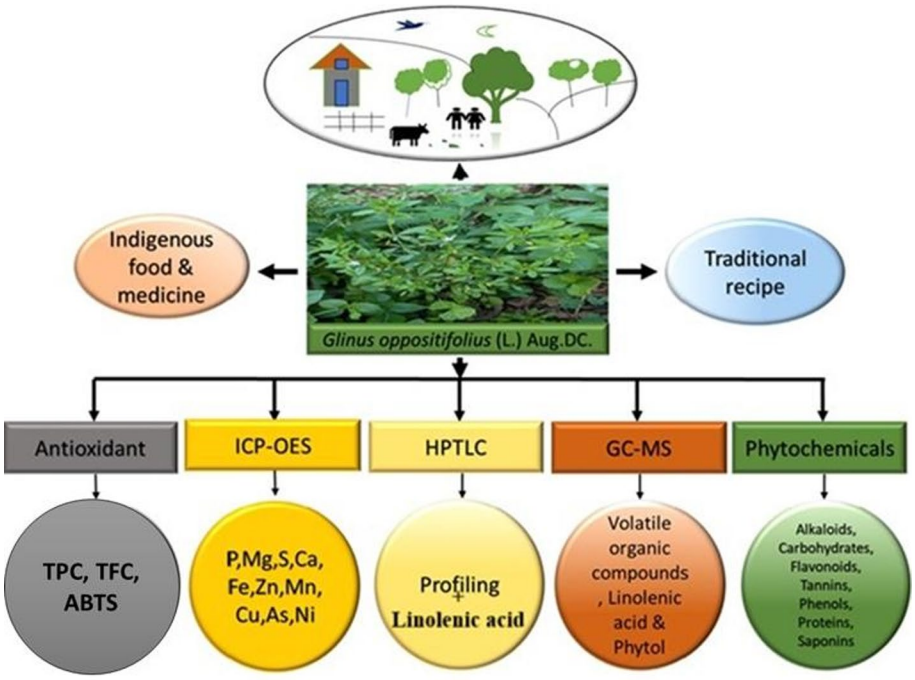
Abstract

Glinus oppositifolius (L.) Aug. DC, a wild edible vegetable (WEV) deeply rooted in the diets and ethnomedicinal traditions among the six ethnic tribes of Mizoram, viz., *Hmar*, *Paihte*, *Ralte*, *Lai*, *Mara*, and *Lusei*, is culturally and nutritionally significant. However, despite its long-standing use, scientific understanding of its health benefits remains limited, and further scientific investigation is needed to validate these aspects and support its sustainable utilization. This study aims to fill these pivotal gaps by profiling the bioactive and phytochemical properties of *G. oppositifolius* (L.) Aug. DC, thereby reinforcing its potential nutritional and therapeutic applications. Traditional recipes and ethnomedicinal practices among the six tribes were documented before the plants were harvested. Whole plants of *G. oppositifolius* (L.) Aug. DC, collected in February 2024, were extracted with methanol using a Soxhlet apparatus and analysed for their phytochemical composition. The analysis revealed a diverse array of metabolites, including alkaloids, flavonoids, terpenoids, tannins, saponins, steroids, phenols, carbohydrates, and fats and oils. Quantitative assays indicated a total phenolic content (TPC) of 52.39 mg gallic acid equivalent (GAE)/g and a total flavonoid content (TFC) of 1.10 mg GAE µg/mL. Antioxidant evaluation showed moderate ABTS radical scavenging activity (IC_{50} = 113.7 µg/mL). GC-MS identified linolenic acid and phytol as major volatile compounds, while HPTLC quantified linolenic acid at 157.3 µg per 30 mg sample. ICP-OES revealed elevated potassium levels (356.624 mg/kg), confirming its nutritional importance and potential contribution to cardiovascular health. Ethnobotanical surveys documented varied culinary and medicinal applications, including management of hypertension, diabetes, and support for lactation within the indigenous tribes of Mizoram. These findings validated the traditional uses of *G. oppositifolius* (L.) Aug. DC and provide data for its bioactivity and phytochemical composition, and emphasise its potential as a nutritionally and therapeutically valuable plant in Mizoram, encouraging further research and sustainable utilization.

Keywords WEVs, Ethnic tribes, *G.oppositifolius* (L.) aug. DC, Bioactivities, Phytochemicals

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Graphical abstract



1 Introduction

Wild edible vegetables (WEVs) are vital to food security and health, particularly in resource-constrained communities, owing to their nutrient-rich profiles and therapeutic bioactive compounds [1, 2]. In the Indian subcontinent, around 9,500 wild plant species supply vital resources such as food, medicine, fodder, fibre, fuel, essence, and cultural significance to nearly 53 million tribal people belonging to 550 distinct groups, mainly in the northeastern regions of India [3]. One of the seven northeastern states, Mizoram, a biodiversity hotspot, is known for its abundant flora and diverse ethnic makeup, which both contribute to its rich cultural character [4]. The state is made up of six prominent ethnic tribes, viz., the *Hmar*, *Paihte*, *Ralte*, *Lai*, *Mara*, and *Lusei*, [5] each having distinct food traditions, passed down through generations, emphasising the integral role of WEVs in sustaining livelihoods and health [4, 6–9].

Plants produce diverse secondary metabolites by modifying core metabolic pathways. Based on biosynthesis, these plant metabolites are classified into three major groups: phenolic groups (containing benzene rings and often linked to simple sugars), terpenes and steroids (composed of carbon and hydrogen), and nitrogen-containing compounds [10]. Plants rich in a variety of secondary metabolites have been shown to have tremendous therapeutic and medicinal potential [11]. Accordingly, plant extracts have been used for the treatment of various ailments, displaying antioxidant, antimicrobial [12], antifungal [13, 14], and anti-inflammatory effects [15].

Glinus oppositifolius (L.) Aug. DC., a WEV belonging to the family Molluginaceae, is a tropical and subtropical herbaceous species [16]. The genus *Glinus* comprises approximately 8–10 species, of which 4–5 are reported in India [17]. Traditionally native to Mali, the plant has long been recognized for its immunomodulatory and therapeutic

properties. It has been widely employed to alleviate joint pain, reduce inflammation, treat fever, and prevent malaria, as well as stimulate wound healing [18]. Extensive pharmacological investigations have validated its diverse bioactivities. Antioxidant activity evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, where the IC_{50} values for stems, leaves, and flowers were 9.523 µg/ mL, 32.89 µg/ mL, and 23.07 µg/ mL, respectively, compared to IC_{50} of vitamin C (1.698 µg/ mL), indicating moderate antioxidant activity against DPPH free radicals [19]. Puzon et al. [20] reported that the plant extracts (water, ethanol, chloroform, and ethyl acetate) demonstrated antibacterial activity against *Bacillus cereus*, *B.subtilis*, *Enterobacter aerugenes*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus, and S.epidermidis* using the disc diffusion method. Notably, the ethyl acetate extract inhibited *B.cereus* and *B.subtilis* at 2 mg/ disc [20]. They further reported that leaf and stem extracts (chloroform, ethanol, and methanol) showed activity against methicillin-resistant *S. aureus*, vancomycin-resistant *Enterococcus*, extended-spectrum ß-lactamase-producing and carbapenem-resistant *Enterobacteriaceae*, and metallo-ß-lactamase-producing *P.aeruginosa* and *Acinetobacter baumannii* through disc diffusion, minimum inhibitory concentration, and bactericidal concentration assays. This study highlighted the plant's antibacterial activity [20]. Antifungal activity of the plant was investigated by Zhang et al. 2019 [21] who isolated new 25 triterpenoids and triterpenoid saponins, along with 12 known compounds from the whole plants of *G.oppositifolius* (L.) Aug. DC, which were tested against *Microsporum gypseum* and *Trichophyton rubrum.* The result showed that triterpenoid compounds: glinusopposide B, glinusopposide Q, glinusopposide T, and glinusopposide U showed strong inhibitory activities against *M.gypseum* (MIC_{50} 7.1,6.7, 6.8 and 11.1 µM, respectively) and *T.rubrum* (MIC_{50} 14.4, 13.4, 11.9, and 13.0 µM, respectively) [21]. A previous study conducted by Chen et al. [22] isolated 20 compounds, along with new 3 triterpenoids, from the aerial part of the plant. Among the isolated compounds, spergulagenin D, 6,8-dimethyl-5,7,4'-trihydroxyflavone, and trans-ferulic acid showed effective inhibitory effects on LPS-induced TNF-α production in RAW 264.7 cells with notable IC_{50} values (18.35 ± 1.34, 17.56 ± 1.41, and 14.27 ± 1.29 µM, respectively. This showed the plant's potential for anti-lipidemic activity [22]. In rodent models, the plant methanol extracts (500 g/kg) reduced diarrheal symptoms by approximately 86% compared to controls, highlighting their anti-diarrhoeal potential [23]. Furthermore, aqueous and methanol extracts (200 and 400 mg/kg) significantly improved lipid profiles on streptozotocin- and Triton-induced hyperlipidemic rats by reducing triglycerides, total cholesterol, Low-Density Lipoprotein (LDL), and very low-density (VLDL) while increasing high-density lipoprotein (HDL) levels, suggesting anti-hyperlipidemic properties [24].

Although a growing body of research acknowledges the nutritional and therapeutic benefits of WEVs, much of the existing evidence remains anecdotal, fragmentary, or methodologically inconsistent, thereby necessitating rigorous scientific validation [25, 26]. Within Mizoram, *G.oppositifolius* (L.) Aug. DC occupies a distinctive niche in the local culinary and medicinal landscapes. Surveys conducted among the six ethnic tribes of Mizoram confirm that the plant is a linchpin of traditional diets and local medicine, particularly for managing hypertension, blood levels, and lactation in nurturing women. Although the plant occasionally finds mention in regional ethnobotanical records, botanical journals, and historical botanical literature, scientific exploration of its properties within the state has been minimal. Much of the available information remains

descriptive rather than analytical, leaving its nutritional and pharmacological attributes insufficiently understood. Its broad use highlights its importance to culture and health, but its potential for commercial and medicinal uses is limited by the lack of comprehensive bioactivity and phytochemical data in this region.

This study intends to address these critical deficiencies by establishing foundational, scientifically based data on the plant's bioactivity and phytochemical properties to promote sustainable use, ensure quality control, and facilitate greater incorporation into local food and healthcare frameworks. To the best of our knowledge, this is the first study to systematically investigate the bioactivity profiling and phytochemical characterisation of *G.oppositifolius* (L.) Aug. DC in Mizoram.

2 Methodology

2.1 Ethnobotanical study of *G.oppositifolius* (L.) Aug. DC

2.1.1 Traditional culinary and medicinal preparations of G.oppositifolius (L.) Aug. DC among the six ethnic tribes of Mizoram

An ethnobotanical survey was conducted in February 2024, strategically aligned with the seasonal availability of *G.oppositifolius* (L.) Aug. DC, to document its traditional culinary and medicinal applications among six ethnic tribes of Mizoram, viz., *Hmar*, *Paihte*, *Ralte*, *Lai*, *Mara*, and *Lusei.* Participants, comprising elders and traditional cooks from each community, were selected. Ethical compliance was ensured through the prior acquisition of informed consent. The names of dishes and methods of preparation were initially recorded in the respective native languages of each community. Subsequently, a translator rendered these accounts into English. Each documented recipe was accompanied by photographic evidence and a detailed English description. This process guaranteed that the culinary customs would be accurately and completely preserved.

2.2 Ethnobiological study of *G.oppositifolius* (L.) Aug. DC

2.2.1 Collection and processing of plant materials

Whole plants of *G.oppositifolius* (L.) Aug. DC were harvested from Lunglei, Mizoram, India (Lat 22.907961° Long 92.764412°) (Fig. 1) in February 2024 and catalogued in Mizoram University and assigned the accession number MZUBOT0366. The plant material was shade-dried at ambient temperature (25–30 °C) for 3 weeks to preserve



Fig. 1 *G.oppositifolius*(L.) Aug. DC (Lunglei, Mizoram)

thermolabile compounds, with adequate ventilation to prevent microbial contamination. The dried material was milled into a fine powder with a particle size of approximately 0.5–1 mm to ensure uniform extraction efficiency. The fine powder was stored in airtight, transparent plastic containers at 4 °C to prevent degradation until extraction [27].

2.3 Preparation of extracts

A total of 264.879 g of powdered plant material was extracted using methanol in a Soxhlet apparatus at 60–80 °C [22]. The extract was concentrated in a rotary evaporator to form a paste, which was stored in an airtight container at 4 °C for subsequent biochemical analysis.

2.4 Analysis

2.4.1 Screening of phytochemicals

Preliminary phytochemical analyses followed established protocols to detect alkaloids, terpenoids, carbohydrates, fats and oils, flavonoids, phenols, saponins, and tannins. For the analysis of alkaloids, terpenoids, carbohydrates, fats and oils, flavonoids and phenols, the procedures established by Harborne [28] and Siddiqui [29] were followed, and for the analysis of saponin and steroids, the procedures established by Dubale et al. [30] were followed.

2.5 Evaluation of antioxidant compounds

2.5.1 Total phenolic content (TPC)

TPC was determined using a modified Folin-Ciocalteu method [31]. Initially, 1 µg/mL of stock solution was prepared, and a standard solution was prepared by dissolving 10 µg of gallic acid in 100 mL of water to achieve a concentration of 100 µg/mL. Gallic acid solutions ranging from 50 µg/mL to 500 µg/mL were prepared from stock solution. Then, 100 µL of plant extract (20 µg/mL) was mixed with 900 µL of water and 500 µl of 50% Folin-Ciocalteu reagent (1 N). Following incubation for 5 min, 1.5 mL of 7% $NaCO_3$ was added, and the total volume was adjusted to 10 mL with Milli-Q water. The optical density was measured at 725 nm and 760 nm. Using a gallic acid standard curve, the TPC was calculated and expressed as milligrams of gallic acid equivalents per gram (mg GAE/g) of dry extract [31].

2.5.2 Total flavonoid content (TFC)

TFC was determined using the aluminium chloride method [32]. Firstly, 500 µl of plant extract solution was mixed with 1.5 mL of methanol, 0.1 mL of aluminium chloride, and 0.1 mL of potassium acetate. The total volume was adjusted to 5 mL with distilled water. The mixture was incubated at room temperature (22–23 °C) for 30 min. Upon incubation, the optical density (OD) was measured at 420 nm and 430 nm, using a UV-VIS spectrophotometer. As a control, a blank sample was made using the same procedure without adding the plant extract. Then, TFC was calculated using the quercetin standard curve and expressed as mg (GAE)/g of dry extract [32].

2.6 Determination of 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) free radical scavenging activity

With slight modification of the method described by Seal et al. [33] the ABTS free radical scavenging activity was assessed. The ABTS radical was produced by mixing 7 mM ABTS with 2.45 mM potassium persulfate ($K_2S_2O_8$) and incubating at room temperature (22–23 °C) for 14 h. A working solution was prepared from the stock solution by diluting 1 mL of the ABTS radical solution with 60% methanol to achieve an absorbance of 0.699 ± 0.01 at 734 nm. Plant extract solutions ranging from 10 µg/mL to 1000 µg/mL were prepared, and 100 µL of each was mixed with 100 µL of ABTS radical solution in a 96-well plate. After 3 min, the OD was measured at 734 nm. Gallic acid (20 µg/mL to 200 µg/mL) was used as the positive control [33]. The inhibition % was calculated using the formula: $[(A_{0_}A_1)/A_0]$ x 100, where A_0 is the absorbance of the control and A_1 is the absorbance of the extract or standard. The IC_{50} value was determined by plotting the inhibition % against the logarithm of the concentration [18].

2.7 Gas chromatography mass spectrometry (GC-MS) analysis for prediction of subcomponents

2.7.1 Instrumentation

The plant extracts were analysed using a Thermo Scientific ISQ LT model with X-Caliber software. The oven temperature was ramped at 10 °C/minute to 350 °C. The mass spectrometer was operated to detect ions between 10 and 1000 atomic mass units (a.m.u) and was thoroughly calibrated. Compounds were identified by comparing mass spectra with a linked library database.

2.8 High-Performance thin layer chromatography (HPTLC)

2.8.1 Instrumentation and reagents

The CAMAG HPTLC system used comprised VisionCATS 3.2 SP2 software, LINOMAT V automatic sample applicator, a twin-trough glass chamber, visualizer, and scanning densitometer CAMAG scanner 3. Aluminium-based silica gel plate 60 F_{254} (Merck, Mumbai) with a particle size of 5–10 µm and dimensions 20 cm x 10 cm was utilised as the stationary phase. All solvents were of analytical grade. A 100 µL syringe (Hamilton, Switzerland) was used for sample application. Linolenic acid purchased from *Sigma-Aldrich* (L2376-500 µg) was used as the standard.

2.8.2 Sample Preparation

30 mg of the methanolic extract of *G.oppositifolius* (L.) Aug. DC. was dissolved in 1mL of methanol solution, and was vortexed and filtered using a 0.45 µm pore size syringe filter.

2.8.3 Standard Preparation

Linolenic acid (1 µg) was dissolved in 1 mL of methanol to prepare a 1 µg/mL. Serial dilutions were performed to create a working stock solution of 250 µg/mL. The HPTLC analysis was conducted using a Merk HPTLC Silica gel 60 F_{254} aluminium plate (20 × 10 cm) with the following parameters: application position Y: 8.0 mm, X: 16.0 mm; track distance: 12.0 mm; band length: 10.0 mm; and solvent front development to 70.0 mm. Standard stock solutions (1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 µL), and sample stock

(2, 4, 6, 8, and 10 µL) were applied. The isocratic HPTLC was performed using an optimised mobile phase: n-hexane: ethyl acetate: formic acid (6:4:0.2 v/v/v) with a 20-minute saturation using a Whatman filter paper with 10 mL mobile phase in the front trough and 20 mL mobile phase in the rear trough, making it a total volume of 30 mL. After development, the plate was dried at room temperature (22–23 °C) and derivatised using anisaldehyde-sulfuric acid reagent, followed by drying in the oven at 100 °C for 3 min. Scanning was done at 450 nm with the following settings: single wavelength, Single λ as scanner type, optimised for light sensitivity, absorbance mode, 20 mm/s scanning speed, 100 µm/step data resolution, slit dimension of 6 × 0.45 mm (micro), and a tungsten lamp.

2.8.4 Detection

The same scanner parameters were used to perform spectrum scanning with a deuterium and tungsten lamp in the wavelength range of 190–450 nm.

2.9 Inductively coupled plasma optical emission spectroscopy (ICP-OES)

2.9.1 Instrumentation

The iCAP 7000 with autosampler (AS) was powered on and warmed up for at least 30 min, as recommended by the manufacturer. The argon gas supply was checked and ensured to be properly connected and flowing. The sample introduction system, including the nebulizer, spray chamber, and torch, was inspected and cleaned to prevent contamination or blockages. The instrument was calibrated using standard solutions for each metal of interest to guarantee accurate measurements [34].

2.9.2 Method parameters

For UV analysis, parameters were adjusted following [35] with minor modifications. Exposure time was set to 15 s for both radial and axial configurations, with an RF power of 1150 W, and nebuliser gas flow at 0.5 L/minute for both configurations. For VIS analysis, exposure time was set to 5 s, with the same RF power (1150 W) and a 0.5 L/minute nebuliser gas flow for both radial and axial setups. Additionally, cool gas flow was set to 12 L/minute, and auxiliary gas flow to 0.5 L/minute for both configurations. Samples were introduced to the ICP-OES instrument via an autosampler, and emission spectra were used to determine metal concentrations by converting emission intensities with calibration curves, with corrections applied for background signals or interferences.

3 Results

3.1 Traditional culinary and medicinal preparations of *G.oppositifolius* (L.) Aug. DC among the six ethnic tribes of Mizoram

The traditional recipes of the six ethnic tribes of Mizoram are highlighted below (Fig. 2).

3.1.1 Lai ethnic group

Bakhate is a local name of *G. oppositifolius* (L.) Aug. DC among the *Pawi* tribe. The main method of preparing *Bakhate* is to cook it with tomatoes. The tomatoes were first fried for about 5–10 min, then once they were cooked, 200–500 g *Bakhate* was added. *Bakhate* can also be served as "bai", a broth-like accompaniment. The preparation process for '*Bakhate bai*' includes boiling a little amount of water for 10–15 min. Following the boiling of the water, minced 5 g of garlic is added, followed by the addition of

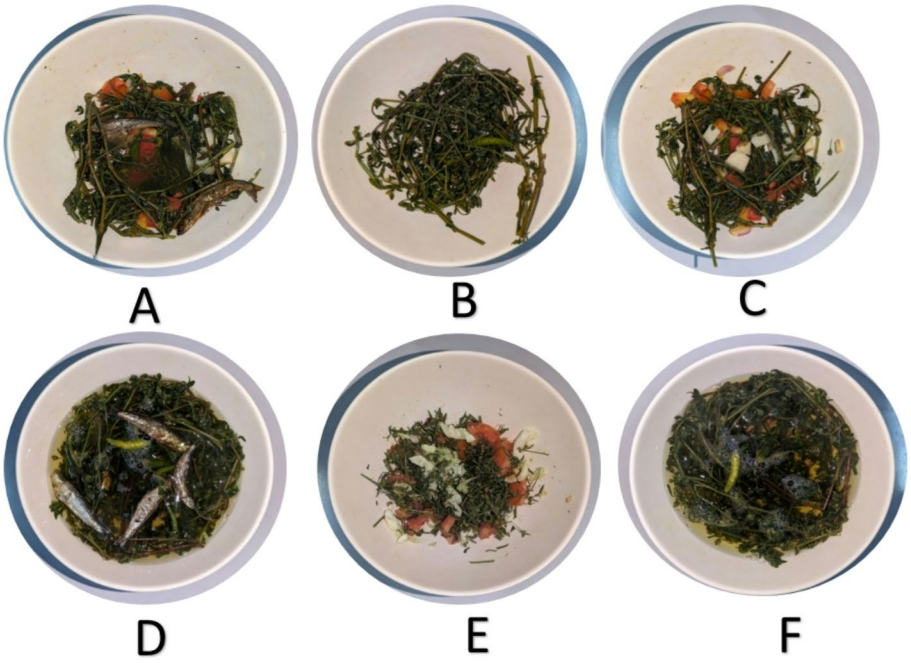


Fig. 2 **a** Different modes of preparation of *G. oppositifolius* (L.) Aug. DC by six different ethnic tribes: *Lusei*, *Lai* ; *Ralte*, *Hmar* **b** *Lai* (**c**); *Paihte* (**d**)*Mara* (**e**); *Lai*, *Hmar* (**f**)

2–3 chillies (depending on the spiciness level) and 2–3 small fermented fish, which is known as "*nghaum*" for flavour. In order to add extra taste, some people also add 1–2 tablespoons of Ajino-moto. 1 tablespoon of salt is added after the *Bakhate* is introduced (Fig. 2a and c)**.** The boiled juice of *Bakhate* is used for managing elevated blood sugar levels, and the juice is also consumed by lactating women to enhance milk production.

3.1.2 Mara ethnic group

Mahlomapaw is a local name of *G. oppositifolius* (L.) Aug. DC among the *Mara* tribe. It is typically served as a salad. The dish is prepared by mixing shredded or sliced 500 g of *Mahlomapaw*, mixed with 2–3 chillies, 1–2 tablespoons of peanuts, and 5–8 sliced tomatoes, creating a flavourful and crunchy combination. Some variations include additional ingredients such as lime juice and other spices to enhance the overall taste profile. Some people from the tribes also extract only the juice from the *Mahlomapaw* plant. This preparation method involves squeezing the plant (500–600 g) to extract its juice, combining it with chopped chillies and a dash of ajino moto for a distinctive flavour. *Mahlomapaw* is used to make juice, which is historically used to help nursing women produce more milk (Fig. 2e).

3.1.3 Ralte ethnic group

Bakkhate is a local name of *G. oppositifolius* (L.) Aug. DC among *Ralte* tribe. *Bakkhate* is traditionally prepared by boiling the leaves of the plant with 200–300 ml of water, 1–2 tablespoons of salt, 2–4 chillies, 1–2 tablespoons of turmeric, and various spices. This mixture is simmered until the flavours meld together, creating a savory and aromatic broth. Another common variation is frying the leaves with 2–3 tablespoons of groundnuts and peanuts, providing a crunchy texture to the preparation. Some individuals also enhance the flavour of *Bakkhate* by frying it with fermented soybeans and fish,

complemented by the tangy taste of tomatoes (Fig. 2a). Boiled juice of *Bakkhate* is used by the *Ralte* ethnic tribe to control high blood sugar levels.

3.1.4 Paihte ethnic group

Tungkha is a local name of *G. oppositifolius* (L.) Aug. DC among the *Paihte* ethnic tribe. The leaves are traditionally boiled with 200–300 mL of water, 2–3 chillies, and spices to make a fragrant and savoury soup. A distinctive and earthy flavour can be introduced to the dish by boiling the leaves (200–500 g) with 10–15 water snails. Various meats, including pork, beef, and fried fish, are also added in *Tungkha* to enhance its flavour (Fig. 2d). The boiled juice of *Tunghkha* is orally consumed to manage high blood sugar levels and for the treatment of diarrhea.

3.1.5 Lusei ethnic group

Bakkhate is a local name of *G. oppositifolius* (L.) Aug. DC among the *Lusei* tribe. The traditional preparation of *Bakkhate* involves boiling the leaves with water, chillies, and various spices, resulting in a fragrant and savoury soup. The preparation process for '*Bakkhate bai*' includes boiling a little amount of water (100–200 mL) for 10–15 min. Following the boiling of the water, 3–4 minced garlic cloves are added, followed by the addition of 2–3 chillies, 1–2 tablespoons of salt, and masala, in order to add extra taste; some people also add Ajino-moto. *Bakkhate* is also prepared by frying it with a small amount of oil, tomatoes, and fermented fish "*nghaum*". (Fig. 2a). The boiled juice of *Bakkhate* is used for managing elevated blood sugar levels; the juice of *Bakkhate* is also consumed by lactating women to enhance milk production.

3.1.6 Hmar ethnic group

Bakhate is a local name of *G. oppositifolius* (L.) Aug. DC among the *Hmar* tribe. *Bakhate* is traditionally prepared by boiling the leaves of the plant (200–500 g) with 200–300 mL of water, 1–2 tablespoons of salt, 2–3 chillies, 1–2 tablespoons of turmeric, and various spices. Another popular variation involves frying the leaves along with peanuts and groundnuts, adding a crunchy texture to the dish. (Figure 2b and f). The boiled juice of *Bakhate* is used for managing elevated blood sugar levels. The juice of *Bakhate* is also consumed by lactating women to enhance milk production.

3.2 Preliminary phytochemical group testing

Preliminary phytochemical analyses of the methanol plant extract of *G.oppositifolius* (L.) Aug. DC showed positive results for phytochemical compounds such as alkaloids, flavonoids, terpenoids, tannin, saponin, steroids, phenols, carbohydrates, and fats and oils.

3.2.1 TPC

Different concentrations (µg/mL) of the plant extract were tested for the TPC assay at two different wavelengths, 725 nm and 760 nm, respectively. Using this method, a concentration of 1000 µg/mL at 760 nm was found to exhibit the highest phenolic content, i.e., 52.39 mg GAE/g of sample extract (Table 1).

Table 1 TPC assay of *G.oppositifolius* (L.) Aug. DC at 725 nm and 760 nm

Sample	Wavelength (nm)	Conc. (µg/mL)	TPC (mg GAE/g of sample extracts ± SD)
G.oppositifolius (L.) Aug. DC	**725**	5	< 1000
		10	0.067 ± 0.000
		20	0.069 ± 0.001
		40	0.071 ± 0.001
		80	0.084 ± 0.002
		150	0.098 ± 0.001
		300	0.129 ± 0.002
		500	0.153 ± 0.004
		1000	0.264 ± 0.000
	760	5	< 1000
		10	< 1000
		20	< 1000
		40	< 1000
		80	< 1000
		150	< 1000
		300	7.323 ± 0.750
		500	15.145 ± 1.265
		1000	52.390 ± 0.100

Table 2 TFC assay of *G.oppositifolius* (L.) Aug. DC at 420 nm and 430 nm

Sample	Wavelength (nm)	Conc. (µg/mL)	TFC (mg GAE/g of sample extracts ± SD)
G.oppositifolius (L.) Aug. DC	**420**	5	0.154 ± 0.004
		10	0.174 ± 0.005
		20	0.17 ± 0.002
		40	0.177 ± 0.001
		80	0.231 ± 0.008
		150	0.293 ± 0.014
		300	0.424 ± 0.014
		500	0.749 ± 0.229
		1000	1.102 ± 0.036
	430	5	0.133 ± 0.003
		10	0.148 ± 0.004
		20	0.0144 ± 0.002
		40	0.147 ± 0.002
		80	0.176 ± 0.005
		150	0.207 ± 0.010
		300	0.274 ± 0.003
		500	0.434 ± 0.104
		1000	0.589 ± 0.012

3.2.2 TFC

Different concentrations (µg/mL) of the plant extract were tested for their TFC at two different wavelengths, 420 nm and 430 nm. Using this method, a concentration of 1000 µg/mL at 420 nm was found to exhibit the highest flavonoid content, i.e., 1.10 mg (GAE)/g of extract (Table 2).

3.2.3 ABTS antioxidant assay

Different concentrations (µg/mL) of *G.oppositifolius* (L.) Aug. DC were tested for ABTS decolourization assay at 734 nm. The methanol extract was found to have moderate ABTS scavenging ability with IC_{50} value of 113.7 µg/mL.

3.2.4 Gas chromatography-mass spectrometry (GC-MS)

GC-MS study of the *G. oppositifolius* (L.) Aug. DC methanol extract produced two notable peaks in its chemical composition (Fig. 3). The most abundant compound was Linolenic acid (9,12,15-Octadecatrienoic acid), methyl ester, which was detected at retention time 18.12 min and phytol at retention time 18.22 min.

3.2.5 HPTLC

The developed HPTLC plates of the methanolic extract of *G.oppositifolius* (L.) Aug. DC showed no detectable bands under 254 nm (Fig. 4a), while distinct bands were observed under 366 nm after derivatization with anisaldehyde-sulfuric acid reagent (Fig. 4b) and under white light (Fig. 4c). The chromatographic technique shows the R_f of standard Linolenic acid and the methanol extract of *G.oppositifolius* (L.) Aug. DC to be 0.655 ± 0.005 (Table 3; Fig. 5 and Fig. 6) using n-hexane: Ethyl acetate: Formic acid (6:4:0.2 v/v/v) as the suitable solvent system for development of the plate and achieve better separation. The standard calibration curve of Linolenic acid gave calibration function $y = 5.865 \times 10^{-8}x + 2.337 \times 10^{-2}$, Coefficient of variation CV = 1.80%, and Correlation coefficient $R = 0.999228$ (Table 4); with LOD and LOQ value of 299.9 ng and 771.8 ng respectively (Fig. 8a and b). The calibration curve of standard + *G.oppositifolius* (L.) Aug. DC gave calibration function $y = 5.256 \times 10^{-9}x + 2.096 \times 10^{-3}$, Coefficient of variation CV = 1.79%, and Correlation coefficient $R = 0.998787$; with LOD and LOQ value of 196.3 ng and 505.1 ng, respectively. The Lambda max (λ_{max}) of Linolenic acid and methanol extract

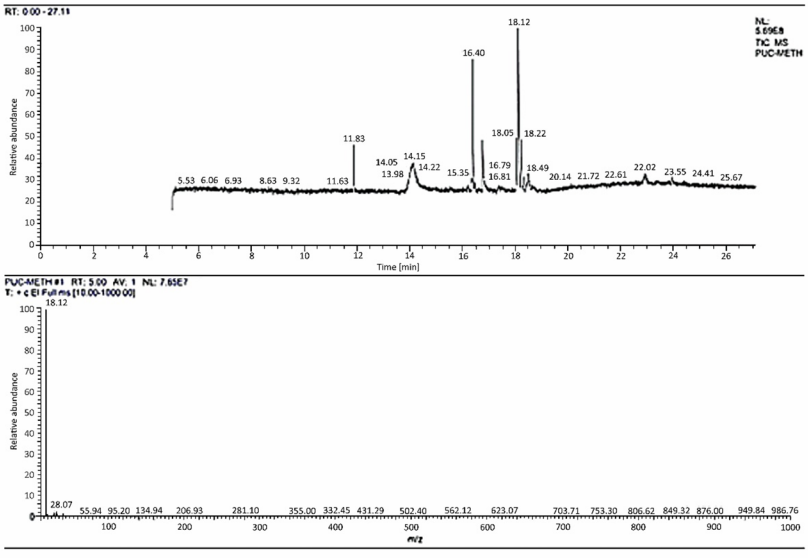


Fig. 3 GC-MS result of methanol extract of *G.oppositifolius* (L.) Aug. DC showing two prominent peaks (Linolenic acid (9,12,15-Octadecatrienoic acid), methyl ester, detected at retention time 18.12 min and phytol at retention time 18.22 min)

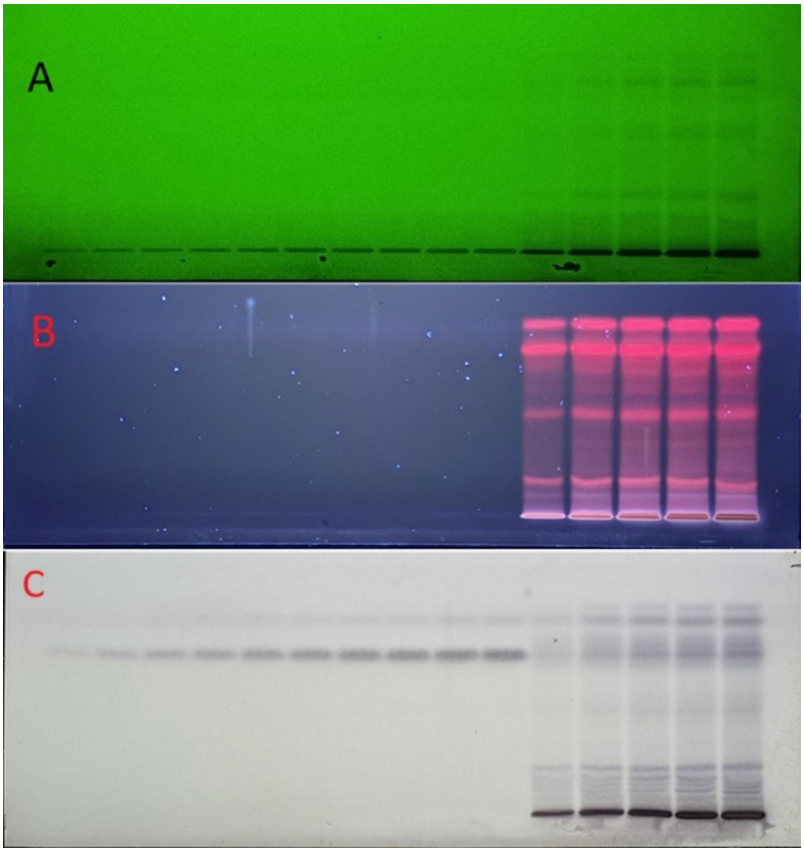


Fig. 4 **a**: Chromatographic bands of *G.oppositifolius* (L.) Aug. DC not visible under 254 nm, and **b** under 366 nm, after derivatization with Anisaldehyde sulfuric acid, **c** detection under white light. Lanes 1–10 represent linolenic acid standard applied at 1,2,3,4,5,6,7,8,9, and 10 µL, respectively. Lanes 11–15 represent the methanolic extract of *G.oppositifolius* (L.) Aug. DC applied at 2,4,6,8, and 10 µL, respectively. Co-migration of sample bands with the linolenic acid standard at R_f 0.655 ± 0.005 confirms the presence of linolenic acid in the extract

Table 3 R_f of all the peaks (Substance liolenic acid Std (R_f 0.655+/-0.005)

Substance Linolenic acid std (R_f 0.655 +/- 0.005):			
Track	R_f	X (mm)	Y (mm)
1	0.660	16.0	48.9
2	0.658	28.0	48.8
3	0.653	40.0	48.5
4	0.655	52.0	48.6
5	0.655	64.0	48.6
6	0.655	76.0	48.6
7	0.653	88.0	48.5
8	0.652	100.0	48.4
9	0.655	112.0	48.6
10	0.656	124.0	48.7
11	0.655	136.0	48.6
12	0.660	148.0	48.9
13	0.656	160.0	48.7
14	0.655	172.0	48.6
15	0.660	184.0	48.9

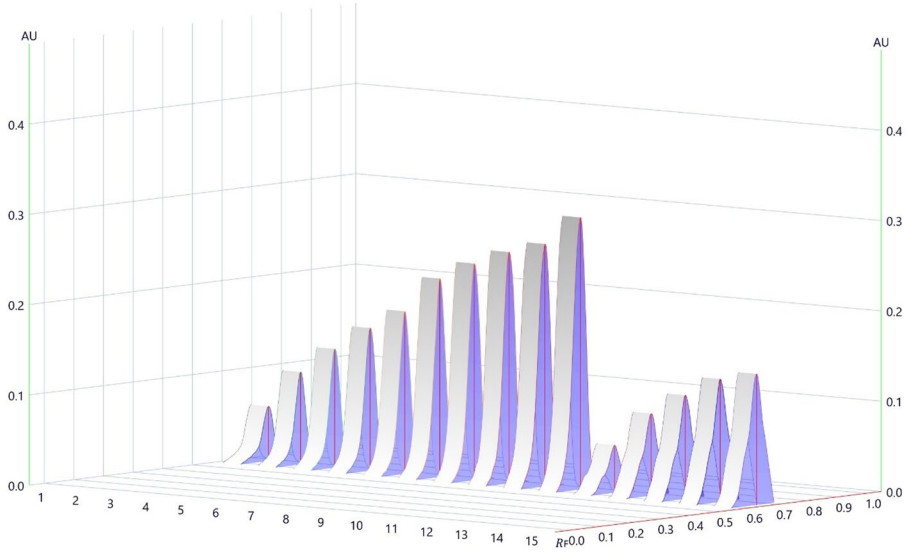


Fig. 5 Scanning results: Linolenic acid and *G.oppositifolius* (L.) Aug. DC peak display

of *G.oppositifolius* (L.) Aug. DC at 366 nm from the spectrum data were found to be 220–221 nm with sample to standard correlation of 0.965535675 (Fig. 7). The quantified amount of Linolenic acid in methanol extract of *G.oppositifolius* (L.) Aug. DC was found to be 157.3 µg in 30.00 mg sample.

Details for Fig. 8(a): Regression mode: Linear-2, Coefficient of variation (CV): 1.80%.

Correlation coefficient: $R = 0.999228$, Limit of detection (LOD): 299.9 ng, Limit of quantification (LOQ): 771.8 ng.

Details for Fig. 8(b): Regression mode: Linear-2, Coefficient of variation (CV): 1.79%, Correlation coefficient: $R = 0.998787$, Limit of detection (LOD): 196.3 ng, Limit of quantification (LOQ): 505.1 ng.

3.3 Quantitative analysis of elements via ICP-OES

Elemental analysis via ICP-OES revealed that *G.oppositifolius* (L.) Aug. DC contains the following elements in the following order of abundance (Table 5):

K > Mg > S > Ca > Fe > P > Zn > Mn > Cu > As > Ni, and the average total extractable metal concentrations were in the order 356.624 > 15.422 > 11.716 > 3.822 > 1.177 > 0.951 > 0.698 > 0.231 > 0.166 > 0.052 > 0.041 mg/kg, respectively.

4 Discussion

Phytochemical screening of the methanolic extract of *G.oppositifolius* (L.) Aug. DC revealed phenols, alkaloids, saponins, flavonoids, terpenoids, carbohydrates, steroids, fats and oils, consistent with Adhikari et al. [36]. These diverse bioactive compounds collectively confirm the multifaceted therapeutic potential of the plant, such as its anti-inflammatory, anti-cancer, antimicrobial, antifungal, antioxidant, hormone-regulating, anti-diarrheal, analgesic, cardiovascular health-promoting properties [37–50]. As a macronutrient in the diet, fat absorbs and holds onto flavours in food, making the plant not only a medicinal powerhouse but also a valuable dietary component [51].

The antioxidant activity of *G.oppositifolius* (L.) Aug. DC can be attributed to the TPC and TFC measured at 52.39 mg (GAE) /g and 1.10 mg (GAE) /g, respectively, align with

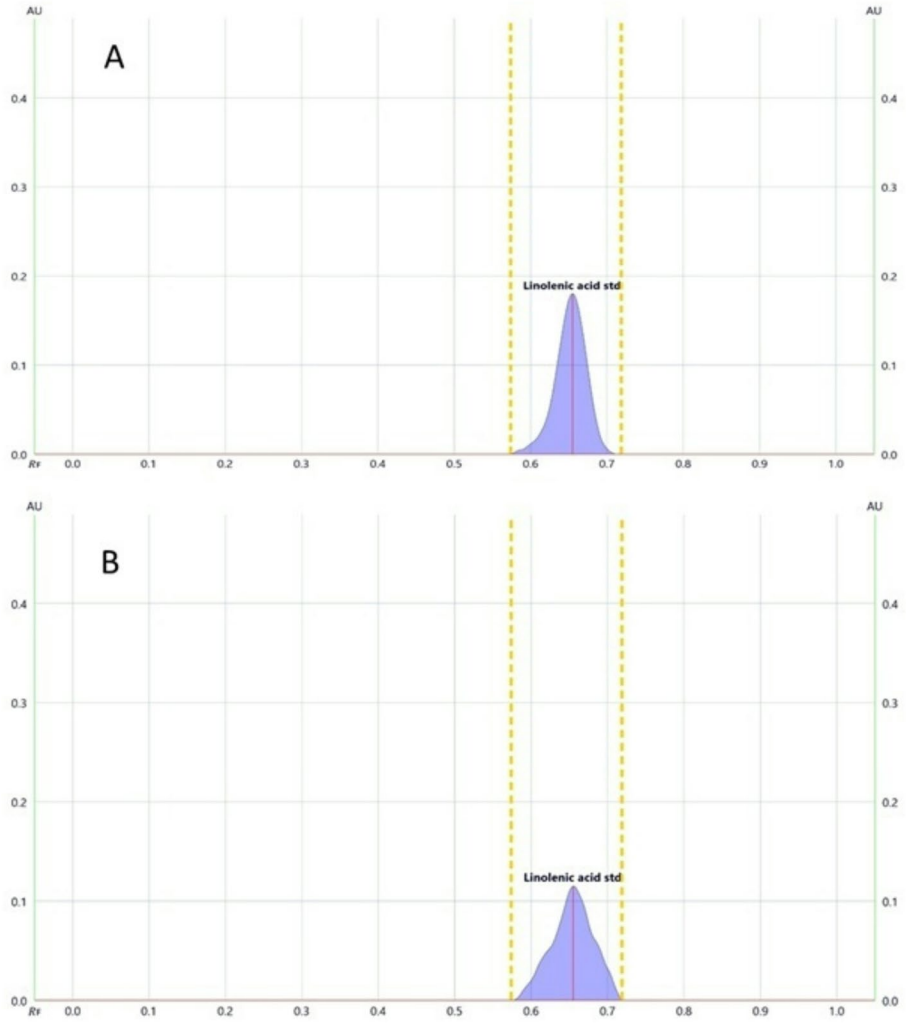


Fig. 6 **a** Linolenic acid peak at R_f 0.655; **b***G.oppositifolius* (L.) Aug. DC peak at R_f 0.655

Table 4 Spectrum correlation data

Substance	Reference spectrum	Correlation
Linolenic acid	R_f 0.655, Sub. Linolenic acid	0.999
Linolenic acid	R_f 0.655, Sub. Linolenic acid	0.966

the findings of Abdullah et al. [52] who reported TPC and TFC contents of 3.39 ± 0.03 mg (GAE)/g and 1.86 ± 0.03 mg (QE)/g, respectively, in the ethanol extract of the same plant. Phenolic and flavonoid compounds are well known for their ability to neutralize free radicals by donating hydrogen atoms to free radicals, contributing to the overall antioxidant potential of the plant [53]. The antioxidant capacity was further evaluated using ABTS assay, which is based on the electron-donating ability of antioxidants to reduce radicals [54]. In the ABTS assay, radical scavenging activity was observed with an IC_{50} of 113.7 µg/mL, consistent with the findings of Vasincu et al. [55], who reported ABTS radical scavenging activity of aqueous and ethanol extracts with an IC_{50} of 276.76 ± 1.52 µg/mL and 174.32 ± 1.49 µg/mL, respectively. The observed ABTS radical scavenging activity can be directly linked to the high phenolic and flavonoid content reported (52.39 µg GAE/g and 1.10 µg GAE/g, respectively.

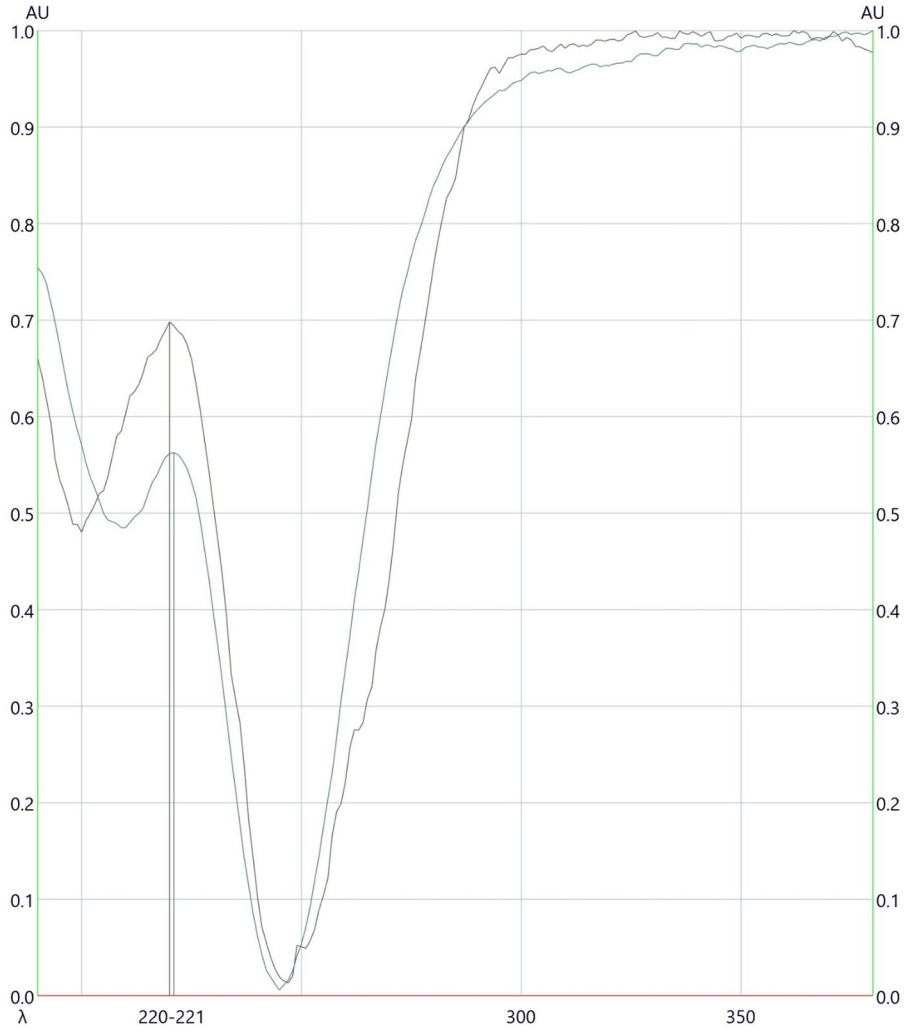


Fig. 7 Linolenic acid + *G.oppositifolius* (L.) Aug. DC Spectra showing Lambda max (λ_{max}) at 220–221 nm

GC-MS analysis detected the presence of linolenic acid (LA) and phytol in the plant extract. Das et al. [56] previously reported the occurrence of linoleic acid through Liquid Chromatography-Mass Spectrometry (LC-MS) analysis, however, to the best of our knowledge, no recent study has documented the detection of linolenic acid using GC-MS. It was further observed that Phytol was previously reported by Kannan and Kandavel [57] through GC-MS. LA, an essential plant-derived fatty acid, serves as a precursor for long-chain n-3 fatty acids, including eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA), essential fatty acids for brain development, cardiovascular function, and inflammatory modulation [58, 59]. Phytol, a chloroplast lipid precursor, can form fatty acid phytyl esters (FAPEs) under stress, and its derivatives, including phytanol, phytanyl amine, and phytanyl mannose, activate immune responses via chemokine and cytokine induction [60, 61–62]. HPTLC fingerprinting quantified linolenic acid at 157.3 µg in a 30.00 mg sample, consistent with GC-MS findings. Together, the GC-MS, and HPTLC analyses substantiate the potential role of *G.oppositifolius* (L.) Aug. DC in promoting cardiovascular health and providing therapeutic benefits in metabolic disorders, particularly diabetes.

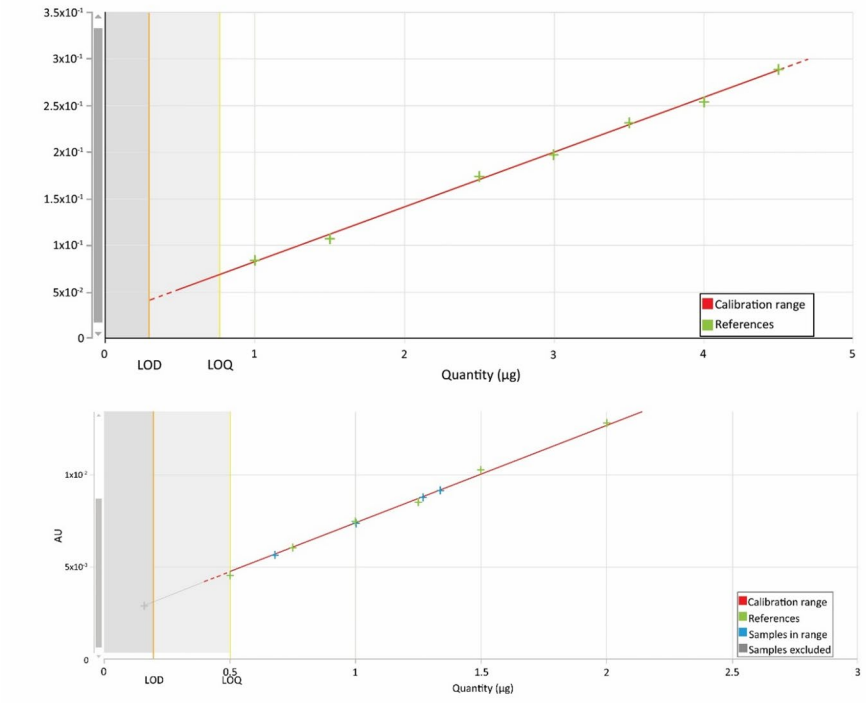


Fig. 8 **a** Calibration curve of standard, **b** Calibration curve of standard + sample

ICP-OES analysis confirmed that heavy metals were within World Health Organisation (WHO) permissible limits, with trace Zn (0.698 mg/kg), Mn (0.231 mg/kg), and Ca (3.822 mg/kg), which were significantly lower than the levels reported by Adhikari et al. (2023) [36] (205.55 mg/kg, 115.70 mg/kg, and 3.44 mg/kg, respectively), who sourced the plant sample from Kolkata, India [36]. These differences likely arise from lower soil metal bioavailability in Mizoram's rural environment compared to Kolkata's urban setting with higher industrial and agricultural pollution, alongside potential variations in plant growth stage or analytical methods [60]. The plant's potassium was found to be abundant (356.624 mg/kg), consistent with its known role in maintaining vascular smooth muscle and endothelial function, blood pressure regulation, and reducing the severity of hypertensive complications such as stroke [63–67] Thus, this may validate the role of *G.oppositifolius* (L.) Aug. DC. in supporting cardiovascular function and managing hypertensive disorders.

4.1 Future directions

To build on our findings and tackle the gaps we have spotted, future research should dive deeper with a mix of curiosity, precision, and a nod to the vibrant traditions of the six ethnic tribes of Mizoram. Here are some paths to explore, blending scientific rigour with a human touch to bring this plant's potential to life: (1) The reported lactation-enhancing properties, documented across the *Lai*, *Hmar*, and *Lusei* tribes, represent a novel finding requiring demanding validation. In vivo studies in animal models, followed by controlled clinical trials in humans, are critical to confirm these effects and identify bioactive compounds, potentially flavonoids or essential fatty acids like linolenic acid, that may modulate prolactin secretion or mammary gland function. Such studies should include dose-response analyses and mechanistic investigations to establish efficacy and

Table 5 Average total amount of elemental concentrations in *G.oppositifolius* (L.) Aug. DC

Sl. no	Micro-elements/ heavy metals	Blank sample conc. (mg /kg)	STD1 (mg /kg)	STD2 (mg /kg)	STD3 (mg /kg)	Wavelength (nm)/ Order	Heavy metal concentration (mg /kg)	R^2	BEC (mg /kg)	LoD (mg /kg)
1	Sulphur (S)	0.000	1.005	3.009	5.995	180.731 {486}	11.720	1.000	0.110	0.018
2	Iron (Fe)	0.000	0.622	2.401	6.362	259.940 {130}	1.177	0.974	0.015	0.001
3	Zinc (Zn)	0.000	1.162	2.796	6.075	213.856 {457}	0.698	0.997	0.003	0.000
4	Copper (Cu)	0.000	1.072	2.743	6.116	324.75 {104}	0.166	0.996	0.006	0.001
5	Manganese (Mn)	0.000	1.101	2.784	6.091	257.610 {131}	0.231	0.997	0.000	0.001
6	Nickel (Ni)	0.000	1.116	2.792	6.085	221.647 {452}	0.041	0.997	0.001	0.000
7	Calcium (Ca)	0.000	1.209	2.711	6.110	393.366 {86}	3.822	0.993	0.047	0.055
8	Potassium (K)	0.000	1.009	2.608	6.194	766.490 {44}	356.624	0.991	0.920	0.621
9	Magnesium (µg)	0.000	1.150	2.759	6.096	279.553 {120}	15.422	0.996	0.013	0.014
10	Phosphorus (P)	0.000	0.468	2.324	6.42	177.495 {490}	0.951	0.964	0.000	0.004
11	Arsenic (As)	0.000	0.890	2.905	6.066	189.042 {478}	0.052	0.999	-0.003	0.007

safety, positioning *G.oppositifolius* (L.) Aug. DC. as a potential natural galactagogue for maternal health, particularly in resource-limited settings. (2) Limited studies have explored the antifungal activity of the plant, warranting further research to evaluate its potential as a plant-derived antifungal agent to validate ethnobotanical applications and develop novel therapeutic agents. (3) Environmental influences on the plant's phytochemical and mineral content merit systematic exploration. The elevated potassium content (356.624 mg/kg) suggests soil-specific enrichment in Mizoram, but variation due to geographic location or seasonal factors was not assessed. (4) Comparative studies across different regions in Mizoram and beyond, coupled with analyses of harvest timing, could quantify the impact of environmental variables on phytochemical profiling and mineral concentrations. In Mizoram, promoting sustainable cultivation and processing of *G.oppositifolius* (L.) Aug. DC. can improve food security and create income opportunities for tribal communities through a small-scale herbal enterprise. This will strengthen the regional bioeconomy and preserve culturally significant plant knowledge.

5 Conclusion

G. oppositifolius (L.) Aug. DC, long fused into the culinary and medicinal traditions of Mizoram's six ethnic tribes, harbours a diverse repertoire of bioactive compounds, solidifying its antioxidant, anti-inflammatory, and anticancer properties. The presence of linolenic acid and phytol, alongside minerals such as potassium, further heightens its nutritional significance and therapeutic relevance, particularly in maintaining cardiovascular health and modulating oxidative and inflammatory pathways. Within the context of Mizoram, where WEVs remain readily accessible, promoting the use and conservation of *G. oppositifolius* (L.) Aug. DC could enhance nutrition and public health across these tribal populations. Systematic documentation, sustainable harvesting, and increased awareness of its health-promoting properties can strengthen food security, alleviate malnutrition, and preserve culturally significant plant knowledge. This study provides a scientific foundation for further research, standardisation of bioactive compounds, and translational applications, ensuring that the traditional and nutritional value of the plant is recognised, conserved and effectively utilised.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1007/s44187-026-00810-6.

Supplementary Material 1

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Plant collection

The *G. oppositifolius* (L.) Aug. DC collected from Lunglei, Mizoram, India, in February 2024 complies with the Indian Biodiversity Act, 2002, and the Mizoram Forest Department guidelines (Mizoram Forest Act, 1955). Prior approval from local authorities was secured, ensuring sustainable harvesting. The species is not listed as endangered on the IUCN Red List (IUCN, 2025) or Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) (CITES, 2025), nor is it classified as a quarantine organism under the International Plant Protection Convention. Collection practices followed the Nagoya Protocol for fair benefit-sharing.

Author contributions

Lalenpuii Zorem: Conducted ethnobotanical surveys, data collection, conducted phytochemical analysis, antimicrobial test, antioxidant assays, and drafted manuscript.Beirachhitha Bohia: Conducted phytochemical analysis, performed antioxidant assays, and antimicrobial testLalrokimi: Performed antioxidant assays and data interpretationRosie Lalmuanpuii: Designed experiments and reviewed manuscript preparationFelicia Lalremruati: Assisted in antimicrobial test, designed experiments, and reviewed manuscript preparationAwmpuizeli Fanai: Assisted

in literature review, manuscript editing, and preparation.Nancy Lalhriatpuii: Contributed to manuscript editing and data validation.C.Lalmuansangi: Performed HPTLC analysis and data interpretation.Lalhriatpuii Tochhawng: Assisted in manuscript editing and preparation.H.Lalruatsanga: Supervised ethnobotanical studies and provided critical inputs. Prashant Kumar Singh: Provided critical inputs and reviewed the manuscript.Zothanpuia: Conceptualized the study, designed experiments, supervised experiments, reviewed the manuscript, and finalized the manuscript.

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Data availability
Data will be available from the corresponding author upon request.

Declarations

Ethics approval and consent to participate
All procedures performed involving human participants were approved by the Ethics Committee of Pachhunga University College in accordance with the ethical standards of the institutional and/or national research committee. All the participants are above the age of 16. Written informed consent was obtained from all participants following the guidelines of the International Society of Ethnobiology.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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