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RESEARCH ARTICLE

Study of the Mizo Medicinal Plant, *Helicia excelsa* on its Phytochemical Components, Antioxidant Property and Antibacterial Activity

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Abstract

Objectives: *Helicia excelsa* (Roxb.) Blume (family Proteaceae) is an evergreen tree of ethno-medicinal importance among the Mizo people of India. The study was planned to unveil the chemical and antibacterial properties. **Methods:** A petroleum ether extract of *H. excelsa* leaf, prepared by Soxhlet extraction, was used to investigate quantitative phytochemical properties using nine different tests. Qualitative antioxidant tests such as 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical-scavenging, total antioxidant, total flavonoid, total phenolic content and reducing power assays were performed using spectrophotometric analysis. Agar well diffusion method was used to study the antibacterial activity against Gram-positive and Gram-negative species. **Findings:** Phytochemical detection tests showed that the plant extract contained alkaloids, amino acids, carbohydrates, flavonoids, glycosides, phenols, phytosterols, proteins, and tannins, while saponin was absent. DPPH scavenging assay indicated that the plant extract had an IC_{50} value of 34.12±2.51. Reducing power assay indicated a concentration-dependent activity on the substrate, potassium ferricyanide. Total antioxidant content showed 9.09±0.321 mg/g ascorbic acid equivalent (AAE), while total phenolic and total flavonoid content have 2.43±0.039 mg/g quercetin equivalent (QE) and 47.6±2.757 mg/g gallic acid equivalent (GAE), respectively. Positive antibacterial activity was seen against Gram-positive *Staphylococcus aureus* and Gram-negative *Salmonella typhimurium*. **Novelty:** The study showed promising results for *H. excelsa* as a valuable medicinal plant that contains bioactive compounds and exhibits antibacterial activity.

Keywords: Helicia excelsa; antibacterial activity; antioxidant activity; bioactive compound; medicinal plant

1 Introduction

A major portion of drug development relies on natural products, including medicinal plants. Most pharmaceutical drugs were developed directly or indirectly from the phytocompounds for the treatment of mild to deadly diseases[1]. Traditionally used medicinal plants offer convenient and easy leads to identifying novel or better therapeutic compounds as they are practically well established[2]. Understanding the importance, the World Health Organization maintains a record of medicinally valuable plants of over 21,000 species. In India, approximately 6% of the discovered medicinal plants are extensively commercialized by pharmaceutical companies as medicines[3].

The importance of natural products is heightened by an ever-decreasing drug efficacy of many prescription medications as a result of drug resistance in the most important pathogens. Bacteria in particular have developed drug resistance to the level of insusceptibility to all classes of available antibiotics, from multi-drug resistance to extensively and extremely drug resistance. This has become a global threat as even mild infections can become fatal in complex diseases[4,5]. In such cases, new synthetic drugs are of little promise since they are prone to resistance, while bioactive compounds from medicinal plants are the better alternatives with their novelty in chemical compounds and different modes of the mechanism of action[6].

The genus *Helicia* (family Proteaceae) is notably a valuable Mizo traditional medicinal plant. *H. nilagirica* is the most common species available and used. It is used in the treatment of stomach ailments, peptic ulcers, indigestion Experiments have been done on these species for various biological properties[7]. *H. excelsa* (Roxb.) Blume is a lesser-known species. The leaves and barks are used for the treatment of stomachache and the seeds for convulsion[8]. In view of the paucity of information on the pharmacological and biological properties, the plant was selected for investigation for the fundamental pharmacological properties.

2 Methodology

2.1 Specimen Collection and Identification

The plant specimen, *Helicia excelsa*, was collected from nearby forests of Aizawl, Mizoram, India, located at 23.7307° N 92.7173° E. Herbaria of the flower, along with the leaves and fruits of were prepared and authentication was done at the Botanical Survey of India, Shillong. A voucher specimen is deposited at the herbarium collection of the Pachhunga University College, with the accession code PUC-H-21-01.

2.2 Preparation of Extract

The healthy leaves of the plant were washed, dried in the shade and ground to coarse powder. The samples were then loaded to Soxhlet apparatus for hot extraction using a solvent, petroleum ether. After the extract (HEP) was concentrated and purified in a vacuum rotary evaporator (Buchi Rotavapor® R-300), it was stored at 4°C until analysis.

2.3 *Qualitative Phytochemical Screening*

The plant extract was subjected to phytochemical detection according to standard procedure[9]. In brief: the presence of alkaloids was tested by Dragendroff's test, Hager's test, Meyer's test, and Wagner's test; flavonoids by alkaline reagent test, lead acetate test, ferric chloride test, Shinoda's test, and Zn-HCl reduction test; phenolics by ferric chloride test, lead acetate test, potassium dichromate test, iodine test, ellagic acid test, and gelatin test; carbohydrates test by Benedict's test, Fehling's test, and Molisch's test; glycosides by Liebermann's test, Salkowaski's test, Keller-Kiliani test, Borntrager's test and Legal's test; saponins test by froth and foam tests; tannins by gelatin test, Braymer's test, and 10% sodium hydroxide test; amino acid and proteins by Biuret test, Millon's test, ninhydrin test, and xanthoproteic acid test; phytosterols by Liebermann-Burchard's test, Salkowski's test, and acetic anhydride test.

2.4 Quantitative Phytochemical Analysis

2.4.1 ***Total Phenolic Content***

The total phenolic content of the plant extract was estimated by Folin-Ciocalteu assay based on the method of Singleton *et al.* with slight change using gallic acid as standard reference[10]. The stock solution was made by dissolving in distilled water at a concentration of 1 mg/ml, and from that, 100 μl of the plant extract and gallic acid with six concentrations (viz. 10, 20, 40, 60, 80, 100 μg/ml) were taken into test tubes. After mixing them with ten-fold diluted Folin-Ciocalteu reagent, they were left standing for 3 minutes. Then, 4 ml of 0.7M sodium carbonate was added to each tube and incubated at ambient temperature.

After 1 hour, the absorbance was taken at 765 nm in a UV-vis spectrophotometer.

The absorbance values of gallic acid at varying concentrations were plotted to give a standard curve. The total phenolic content of the samples was determined from the calibration curve, expressing the final values as milligrams of gallic acid equivalent per gram (GAE mg/g) of the sample.

2.4.2 ***Total Flavonoid Content***

The total flavonoid content was estimated by using quercetin as a standard using aluminum chloride assay based on the method of Zhishen et al. with slight modification[11]. 1 mg/ml of stock solution prepared for all the extracts and quercetin. To 100 μl of the plant extracts and different concentrations of quercetin, 2 ml distilled water was added. After keeping them still for 5 minutes, 0.3 ml of 10% aluminum chloride with 3 ml of 5% sodium nitrite were mixed to the solution. To all the solutions, 2 ml of sodium hydroxide was then added. The total volume of each sample was made to 10 ml using distilled water. After incubating for 1 hour, the absorbance was taken at 510 nm.

The absorbance values of quercetin plotted on graph to yield a standard curve, with which the total flavonoid content was estimated and finally given as milligrams of quercetin equivalent per gram (QE mg/g) of the sample.

2.4.3 ***Total Antioxidant Content***

The total antioxidant activity was estimated based on phosphomolybdate assay using ascorbic acid[12]. Ascorbic acid was prepared in varying concentrations. 100 μl of the plant extracts and each of the different concentrations of ascorbic acid were mixed with 3 ml of a reagent containing ammonium molybdate, sodium phosphate and sulphuric acid. After incubating the mixture at 95°C for 90 minutes, it was allowed to cool down at room temperature. The absorbance was measured at 695 nm.

The absorbance values of ascorbic acid were used to plot a calibration curve. The total antioxidant activity was determined from the standard values and expressed as milligrams of ascorbic acid equivalent per gram (AAE mg/g) of the sample.

2.5 Assessment of Antioxidant Property

2.5.1 ***DPPH Scavenging Assay***

The ability to scavenge DPPH free radical was performed by using the method of Blois with some modifications[13]. An antioxidant compound, Butylated hydroxytoluene (BHT), was used as a standard reference. The stock solution for HEP and BHT was diluted to 1 mg/10 ml. Different concentrations, i.e., 10, 20, 40, 60, 80 and 100 μg/ml were made for both BHT and the plant extract. The concentrations prepared were made up to 5 ml using methanol. 1 ml of 0.1 mM DPPH was added to each tube. A blank sample was also maintained by preparing the mixture through the same procedure but without the extract. After 30 minutes incubation at room temperature, the samples were recorded for their absorbance at 517 nm. Using the following equation, the percentage of inhibition (I) was determined:

I% = (Absorbance control − Absorbance sample)/Absorbance control × 100

Free radical scavenging activity by DPPH was expressed by IC_{50} which was calculated using the fitted line, i.e., Y = a*X + b,

$IC_{50} =$ (50 - b)/a

Where, a = slope, b = intercept.

2.5.2 ***Determination of Reducing Power***

The reducing power of the plant extract was evaluated by a modified protocol developed by Oyaizu using ascorbic acid as a reference compound[14]. From the prepared stock solution of 1 mg/10 ml of the extract dissolved in distilled water, increasing concentrations, namely, 10, 20, 40, 60, 80 and 100 μg/ml were taken in test tubes. The final volume of each was made to 5 ml using distilled water. To each tube, 2.5 ml of 6.6 pH buffer and 2.5 ml of 1% potassium ferricyanide were added. The reaction was allowed to run at 50°C and then terminated after 30 minutes by adding 2.5 ml of 10% trichloroacetic acid. The mixtures were centrifuged for 10 minutes at 3000 rpm. 2.5 ml of the supernatant was taken into another tube to which 2.5 ml of distilled water was added. To the solution, 0.5 ml of freshly prepared 0.1% of ferric chloride solution was added and incubated for 10 minutes. A blank sample was also made using the same steps but without adding the extract. The absorbance of all the solutions was read at 700 nm.

2.6 Assessment of Antibacterial Activity

The antimicrobial property of HEP was performed by a well diffusion method using bacteria representing Gram-positive and Gram-negative species[15]. The bacterial strains used were *Bacillus cereus*, *Enterococcus faecalis*, *Salmonella typhimurium*,

Staphylococcus aureus 1, *S. aureus* 2, *Escherichia coli*, and *Klebsiella pneumoniae*. Three concentrations i.e., 200, 100 and 50 mg/ml were prepared for the extract. 1% ciprofloxacin as standard antibiotic and 1% dimethyl sulphoxide (DMSO) were employed as positive and negative treatments respectively. Each culture plate contained 50 μl each of the concentrations and 5 μl of ciprofloxacin and 10 μl of DMSO. The culture plate was then incubated at 28°C. After 24 hours the zones of bacterial growth and inhibition appeared around each well and was measured by a digital caliper.

3 Results and Discussion

3.1 Qualitative Phytochemical Tests

Quantitative phytochemical detection of the petroleum ether extract of *H. excelsa* leaves was carried out using nine different chemical tests as shown in Table 1. The presence of alkaloid, flavonoid, phenol, carbohydrate, glycoside, tannin, protein and amino acids and phytosterols was confirmed, while saponin was absent.

Table 1. Phytochemical assessment of the petroleum ether extract of *H. excelsa* leaves.

Phytochemicals	Name of test	Extract indication
Alkaloid	1. Dragendroff's test	+
	2. Hager's test	-
	3. Meyer's test	+
	4. Wagner's test	+
Flavonoid	1. Alkaline test	-
	2. Lead acetate test	+
	3. Ferric chloride test	+
	4. Shinoda's test	-
	5. Zn-HCl reduction test	-
Phenolic	1. Lead acetate test	-
	2. Ferric chloride test	+
	3. Potassium dichromate test	-
	4. Iodine solution test	-
	5. Ellagic acid test	+
	6. Gelatin test	+
Carbohydrate	1. Molisch's test	-
	2. Benedict's test	+
	3. Iodine test	-
	4. Fehling's test	-
Glycoside	1. Liebermann's test	+
	2. Salkowski's test	-
	3. Keller-Kiliani test	-
	4. Borntrager's test	-
	5. Legal's test	-
Saponin	1. Froth test	-
	2. Foam test	-
Tannin	1. Braymer's test	-
	2. Gelatin test	-
	3. 10% NaOH	+
Proteins and amino acid	1. Biuret test	-
	2. Millon's test	+
	3. Ninhydrin test	-
	4. Xanthoproteic acid test	-
Phytosterol	1. Salkowski test	-
	2. Liebermann-Burchard's test	+
	3. Acetic anhydride test	+

+ = present; – = absent

The petroleum ether extract of *H. excelsa* leaves was found to contain major bioactive compound groups such as alkaloids, amino acids, carbohydrates, flavonoids, glycosides, phenols, proteins, phytosterols and tannins; but saponin was not detected. These natural secondary metabolites from different plants and microbes are responsible for different medicinal applications,

with their biological and pharmacological activities ranging from anticancer, antiparasitic, anti-inflammatory, and antimicrobial activities [16,17]. The present study indicates the presence of such compounds that could be the underlying principle for the medicinal usage of *H. excelsa* in the Mizo traditional medicine.

Quantitative Assays

3.2.1 *Total Phenolic Content*

The total phenolic content of the plant extract was calculated from the absorbance values of varying concentrations using the standard curve of gallic acid activity (Figure 1). The total phenolic content of *H. excelsa* leaves was 2.43±0.039 GAE mg/g.

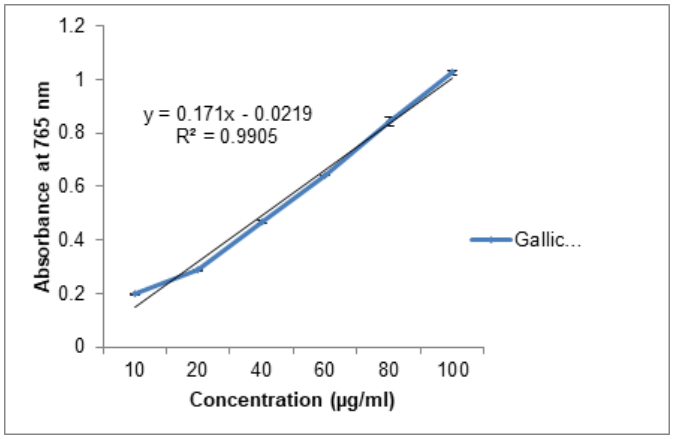

Fig 1. Gallic acid standard curve for total phenolic content assay. Linear black line indicates the linear graph. Values represented in means ± standard deviation (n=3)

3.2.2 *Total Flavonoid Content*

The total flavonoid content of *H. excelsa* leaves was calculated from the standard calibration curve prepared from the absorbance values of quercetin at different concentrations measured at 510 nm (Figure 2). The total flavonoid content of HEP was found to be 47.6±2.757 QE mg/g.

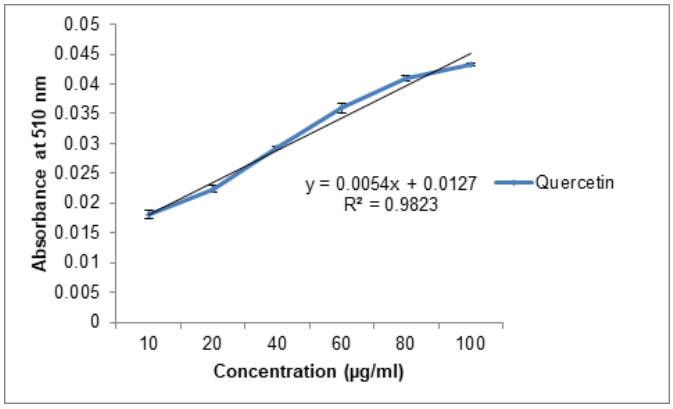

Fig 2. Quercetin standard curve for total flavonoid content assay. Linear black line indicates the linear graph. Values represented in means ± standard deviation (n=3)

3.2.3 *Total Antioxidant Content*

The absorbance values of quercetin as standard antioxidant were used to plot a calibration curve and from which the total antioxidant content was calculated for *H. excelsa* leaf extract (Figure 3). HEP showed 9.09±0.321 AAE mg/g as its total

antioxidant content.

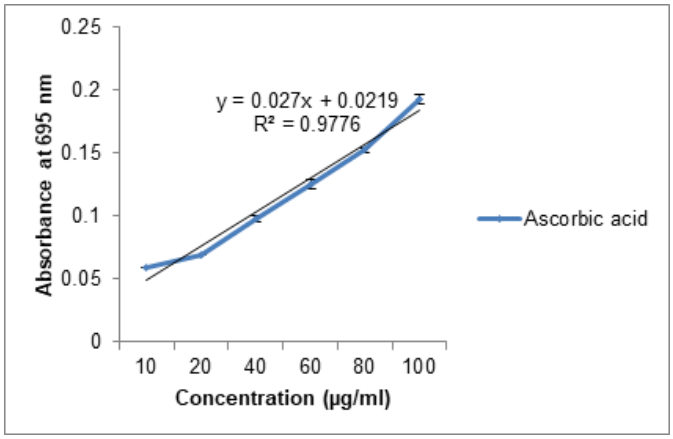

Fig 3. Ascorbic acid standard curve for total antioxidant content assay. Linear black line indicates the linear graph. Values represented in means ± SD (n=3)

3.3 Antioxidant Activity

3.3.1 ***DPPH Scavenging Activity***

DPPH free radical-scavenging assay was used to determine the antioxidant reaction of *H. excelsa* leaf extract. From different concentrations, i.e., 10, 20, 40, 60, 80 and 100 μl, a concentration-dependent DPPH scavenging activity was observed (Figure 4). The IC_{50} value calculated by standard formula showed inhibition value of 34.12±2.51 for HEP while that of the standard BHT was 5.67±0.21, indicating the plant showed antioxidant activity although, it was higher for BHT.

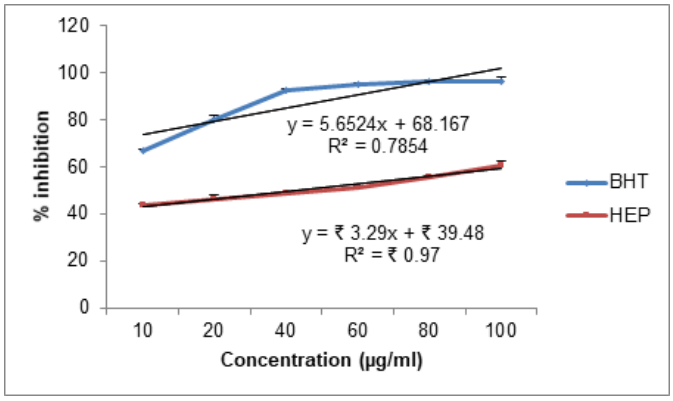

Fig 4. Percentage inhibition of DPPH scavenging by *H. excelsa* leaf extract (HEP) and BHT. Linear black line indicates the linear graph. Values represented in means ± SD (n=3)

3.3.2 ***Reducing Power***

H. excelsa leaf extract at different concentrations (viz. 10, 20, 40, 60, 80 and 100 μl) showed positive reducing power against potassium ferricyanide in concentration-dependent activity. The standard antioxidant, ascorbic acid showed higher activity that the plant extract (Figure 5).

Highly reactive chemicals like free radicals including reactive oxygen species (ROS) are the chemical by-products from aerobic respiration and inflammatory reactions, and are detrimental to cellular functions when they are produced in excess. Excessive free radicals can cause genomic mutations, irreparable oxidative aberrations of vital proteins (such as in oxidation and peroxidation), glycans and fatty acids (advanced lipoxidation and glycation) that lead to promotion of diseases or premature

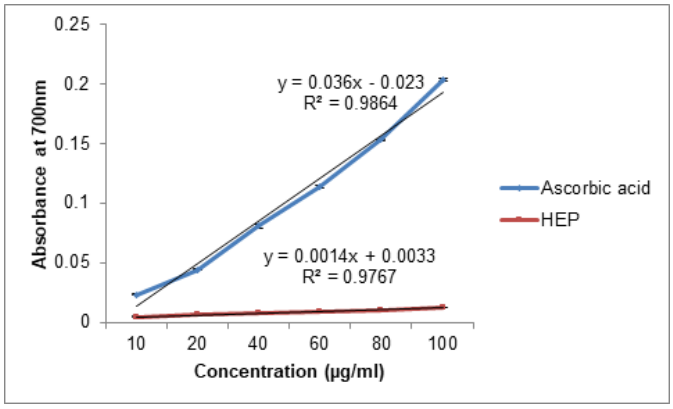

Fig 5. Potassium ferricyanide reducing activity of ascorbic acid and HEP. Linear black line indicates the linear graph. Values represented in means ± SD (n=3)

cell death[18]. They tend to attack vital macromolecules including nucleic acids, lipids, and proteins as a consequent of oxidative stress[19]. Antioxidant molecules present inside the body or acquired through food play critical roles in balancing the actions of the free radicals, by preventing or reducing the detrimental effects of cellular oxidative stress[20].

Synthetic antioxidants can cause tissue toxicity, cell damage, inflammation, and atherosclerosis both in humans and animals. For this reason, attention has been drawn towards the use of naturally occurring antioxidants in plants containing high bioactive antioxidants which have strong potential to scavenge free radicals, thereby preventing debilitating diseases[21]. The present findings also indicate that *H. excelsa* is a rich source of antioxidant compounds.

3.4 Assessment of Antibacterial Activity

The antibacterial activity of HEP was evaluated against seven selected bacteria such as *B. cereus*, *E. faecalis*, *S. typhimurium*, *E. coli*, *S. aureus* 1, *K. pneumoniae* and *S. aureus* 2 as shown in Figure 6. Among the seven test microorganisms, the different concentrations of the extract showed activity only against two bacteria, *S. typhimurium*, a Gram-negative species, and *S. aureus* 1, a Gram-positive species. HEP produced inhibition zones of 3.44 mm at 100 mg/ml and 16.93 mm at 50 mg/ml against *S. typhimurium*; while they were 3.66 mm, 1.66 mm and 4.66 mm at 200, 100 and 50 mg/ml against in *S. aureus* 1.

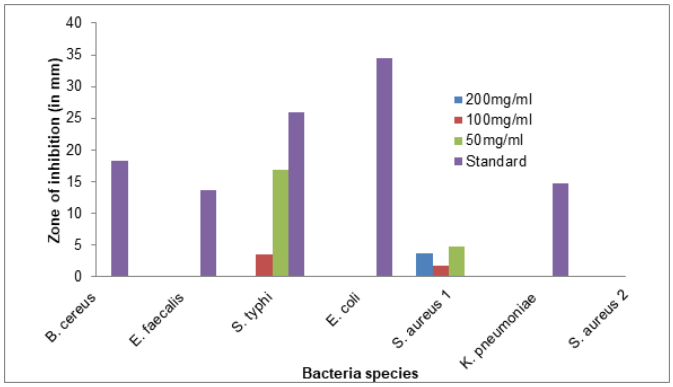

Fig 6. Antibacterial activity of *H. excelsa* extract (HEP) against *B. cereus*, *E. faecalis*, *S. typhimurium*, *E. coli*, *S. aureus* 1, *K. pneumoniae* and *S. aureus* 2

There are now hundreds of antibiotics available for various bacterial infections; but the tragedy is the rampant nature of antibiotic resistance in the most important pathogens. Most of these medications are synthetic or semi-synthetic compounds. There is not a single antibiotic that is safe and free from the resistance phenomenon, and a complete solution to the problem is not in sight[22,23]. It is estimated that more than a thousand species of plants possess therapeutic antimicrobial activities, and over 30,000 antimicrobial compounds exist in nature. For effective management of the antibiotic resistance, it is therefore a

promising attention to search for medicinal plants that are in use for the lead antibacterial compounds with novelty in chemistry and action [8].

The present findings on the beneficial properties of *H. excelsa* indicate that the plant is a promising source of antibacterial compounds. The leaf extract effectively caused inhibition of the propagation of both Gram-positive and Gram-negative bacteria, while further indicating that the antibacterial component is acting as species specific. This specificity is predictable as different species of bacteria respond to drugs through different cellular mechanisms and pathways. For example, plant metabolites like alkaloids, terpenoids, and flavonoids are demonstrated to show antibacterial activities only against a particular species or specific group of bacteria since their target drug-receptive cellular molecules are different [24]. It is therefore important medicinal plants are evaluated and their bioactive compounds identified to obtain new and effective antibacterial compounds [3], and as such *H. excelsa* offers a positive candidate for an antibacterial source.

4 Conclusion

Helicia excelsa is known in Mizo traditional medicine as a curative for different ailments, but remains relatively unknown on scientific grounds. The leaf extract was determined to contain important phytocompounds including amino acids, alkaloids, carbohydrates, flavonoids, glycosides, phenols, phytosterols, proteins, and tannins. It also exhibited a high antioxidant property as determined by estimations of flavonoid content, total antioxidant, total phenolics, and DPPH free radical-scavenging activities. The data indicate that it is a beneficial source of antioxidant molecules that can have beneficial health effects. It showed species-specific antibacterial activity against Gram-positive *Staphylococcus aureus* and Gram-negative *Salmonella typhi* out of the selected bacteria. The findings suggest that the plant may serve as a source of medicinal compounds and warrant further investigation to identify the exact bioactive compounds and pharmacological actions. The study also raised the need for awareness into the conservation and propagation of the medicinal plant for commercial and research purposes.

5 Declaration

Presented in 4th Mizoram Science Congress (MSC 2022) during 20th & 21st October 2022, organized by Mizoram Science, Technology and Innovation Council (MISTIC), Directorate of Science and Technology (DST) Mizoram, Govt. of Mizoram in collaboration with science NGOs in Mizoram such as Mizo Academy of Sciences (MAS), Mizoram Science Society (MSS), Science Teachers' Association, Mizoram (STAM), Geological Society of Mizoram (GSM), Mizoram Mathematics Society (MMS), Biodiversity and Nature Conservation Network (BIOCONE) and Mizoram Information & Technology Society (MITS). The Organizers claim the peer review responsibility

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