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Research Article

Analysis of the Phytochemical and Antibacterial Properties of the Indigenous Mizo Medicinal Plant, *Helicia Excelsa*

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ABSTRACT

Background: *Helicia excelsa* (Roxb.) Bl. is a scarcely known medicinal plant and is native to Southeast Asia. It is most notably used for the treatment of gastric problems in the Mizo traditional medicine in India. **Method:** The leaves of *H. excelsa* were collected from Aizawl, Mizoram, India. An extract was prepared using chloroform. Qualitative phytochemical tests were performed to detect the important phytocompounds. The antioxidant activity was determined by total phenolic content, total flavonoid content, total antioxidant content, DPPH- and ferric-reducing antioxidant power. Antibacterial activity was evaluated by agar well-diffusion method. **Results:** *H. excelsa* leaf contains amino acids, alkaloids, carbohydrates, glycosides, phenols, phytosterols, proteins, and tannins. It showed inhibition in selected Gram-negative and Gram-positive bacteria. The phenol, flavonoid and total antioxidant contents were 4.52±0.09 gallic acid equivalent (GAE mg/g), 64.27±1.04 quercetin equivalent (QE mg/g), 11.39±0.45 ascorbic acid equivalent (AAE mg/g) respectively. IC_{50} value of DPPH-scavenging activity was 5.67±0.36. The ferric ion-reducing power showed concentration-dependent activity. The plant extract showed growth-inhibitory actions against Gram-negative bacterium, *Escherichia coli,* and Gram-positive species, *Bacillus cereus* and *Staphylococcus aureus*. **Conclusion:** *H. excelsa* leaf contains important bioactive compounds that need to be identified. The antioxidant and antibacterial activities support the basis of its medicinal application.

Key words: Antibacterial activity, Antioxidant, *Helicia excelsa*, Plant extract, Mizo traditional medicine.

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INTRODUCTION

Traditional medicine involving medicinal plants and their products serve as the major source of medical treatments for various ailments and the development of pharmaceutical drugs. Since antiquity, the use of plants as a source of therapeutic agents was the mainstay of disease management and have been extended even today by ensuring effectiveness, safety, and benefits of these plants with empirical-based studies.[1,2] Around 400,000 species of vascular plants have been recorded out of which only a small proportion, precisely 17,810 species, are known to be of any medicinal values, according to the latest Royal Botanic Gardens, Kew report in 2016.[3] As a consequence of diminishing drug efficacy of most prescription drugs due to the development of resistance, a need for complementary and novel compounds prompted a big surge in scientific investigations of medicinal plants during the past decade.[4]

Helicia is a group of small trees and shrubs consisting of more than 100 species in the family Proteaceae, and distributed in Asian and Australian regions.[5,6] Only a handful of species are recognised as medicinally useful. *H. nilagirica* is the most well-known species recognised for its sedative, hypnotic, analgesic, and other central nervous system inhibitory effects.[7] It is demonstrated to have anticancer[8] and anti-inflammatory activities.[9] The leaves of *H. cochinchinensis* are used as a tonic and a remedy for cough. Glucosides including ampelopsisionoside, heliciosides A and B, helicidiol, icariside, rhodioloside, and naringenin 5-*O*-β-d-glucopyranoside have been identified as the bioactive components.[10]

Helicia excelsa (Roxb.) Bl. is a rare, medium-sized tree that is found in the Indo-Burma region and largely confined to northeast India.[11] In India, it has been reported as an endangered species.[12] Its medicinal value is not known outside of the Mizo people. In the Mizo traditional medicine, the seeds are used for the treatment of convulsion, the leaves and barks for the treatment of stomach problems and intestinal discomfort (colic).[13] Based on its unique and indigenous application, the plant specimen from Mizoram was selected for investigating the chemical and medicinal properties.

Plant specimen

The plant specimen, *Helicia excelsa* (Figure 1), was collected from nearby forests of Aizawl, Mizoram, India, located at 23.7307° N 92.7173° E. Herbaria of the flower, leaves and fruits were authenticated at the Botanical Survey of India, Government of India, Shillong, India. A voucher specimen is maintained at the herbarium section (accession code PUC-H-21-01) of the Pachhunga University College.

Preparation of extract

The leaves were separated and washed, shade dried and ground into pieces. The ground leaves were then extracted in a Soxhlet apparatus using chloroform as the solvent. The crude extract was concentrated by recovering the solvent in a vacuum rotary evaporator (Buchi Rotavapor® R-300). The resultant extract was stored in a refrigerator at 4°C for further tests.

Qualitative phytochemical tests

The plant extracts were qualitatively tested in nine different chemical groups according to standard

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protocols.[14] In brief, alkaloids were tested by Dragendroff's test, Hager's test, Mayer'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 lead acetate test, ferric chloride test, potassium dichromate test, iodine test, ellagic acid test, and gelatin test; carbohydrates test by Molisch's test, Benedict's test, and Fehling's test; glycosides by Liebermann's test, Salkowski'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 Salkowski's test, Liebermann-Burchard's test, and acetic andydride test.

Quantitative analysis of total phenolic content

Total phenolic content of the plant extract was determined by Folin-Ciocalteu assay according to the method of Singleton *et al.* with slight modification.[15] Gallic acid was used as a standard reference. From the stock solution (prepared as 1 mg/ml) of both the plant extract and gallic acid, 100 µl of the plant extract and different concentrations of gallic acid (10, 20, 40, 60, 80, and 100 µg/ml) were transferred to separate test tubes. A ten-fold diluted Folin-Ciocalteu reagent was added to each tube and allowed to stand for 3 minutes. After adding 4 ml of 0.7 M sodium carbonate solution, they were incubated at room temperature for 1 hour. The absorbance was then taken at 765 nm by using a UV-Vis spectrophotometer.

The absorbance values of gallic acid at different concentrations was used to plot a calibration curve, from which the total phenolic content of the plant extracts was determined and expressed as milligrams of gallic acid equivalent per gram (GAE mg/g) of the dried extract.

Total flavonoid content

Total flavonoid content of the extract was estimated by a standard protocol provided by Zhishen *et al.*[16] From the stock solution prepared in 1 mg/ml for the plant extract and quercetin as a reference compound, 100 µl of the extract and different concentrations of the standard (10, 20, 40, 60, 80, and 100 µg/ml) were pipetted out into test tubes. The total volume of each sample made was up to 1 ml by adding distilled water. The mixture was allowed to undergo reaction for 5 minutes. Then, 3 ml of 5% sodium nitrite, followed by 0.3 ml of 10% aluminium chloride and 2 ml of sodium hydroxide were added to sample. The final volume of each mixture was then made up to 10 ml with distilled water and left at room temperature for 1 hour. The absorbance was then taken at 510 nm.

The absorbance values of different concentrations of quercetin was used to plot standard graph and the total flavonoid content was estimated and expressed as milligrams of quercetin equivalent per gram (QE mg/g) of the dried extract.

Total antioxidant content

The total antioxidant activity was determined by phosphomolybdate reaction using ascorbic acid as a standard antioxidant.[17] A series of concentrations of ascorbic acid (10, 20, 40, 60, 80, and 100 µg/ ml) and 100 µl of the plant extract were taken into test tubes from the stock solution (1 mg/ml). The volume of each solution was made up to 1 ml using distilled water. 3 ml of a reagent solution containing sulphuric acid, sodium phosphate and ammonium molybdate was added to each test tube and incubated at 95°C for 90 minutes. After allowing to cool at room temperature, the absorbance was measured at 695 nm.

The calibration curve was plotted from the absorbance values of ascorbic acid at different concentrations. The total antioxidant activity was presented as milligrams of ascorbic acid equivalent per gram (AAE mg/g) of the dried extract.

DPPH-scavenging assay

Antioxidant activity based on DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical-scavenging activity was assessed by the modified method of Blois.[18] A stable free radical, DPPH, was used as a substrate which have hydrogen accepting capability to scavenger molecules, i.e. antioxidant. Butylated hydroxytoluene (BHT) was used as a reference standard. The plant extract and BHT were prepared in different concentrations (10, 20, 40, 60, 80 and 100 µg/ml). To each sample, 1 ml of 0.1 mM DPPH was added. Absorbance was measured at 517 nm after 30 minutes incubation.

The inhibition curve was prepared by calculating percentage inhibition activity from the equation:

$$I\% = [(A_C - A_S)/A_C] \times 100$$

where A_C is the absorbance of the control, and A_S the absorbance of the extract/BHT.

IC_{50} values calculated from Prism GraphPad version 8.0.2 was used to represent the antioxidant activity. The log dose was determined from the concentrations, 1, 0.5, and 0.25 mg/ml of the extract.

Ferric-reducing antioxidant power (FRAP) assay

The reducing power of *H. excelsa* extract was determined based on the ability of electron transfer from ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}).[19] Varying concentrations of the extract and ascorbic acid, *viz.* 10, 20, 40, 60, 80 and 100 µg/ml were prepared in different test tubes. To each sample, 2.5 ml of 6.6 pH buffer, 2.5 ml of 1% potassium ferricyanide were added and incubated at 50°C for 30 minutes. A blank solution was also prepared by following the same procedure but without the extract or ascorbic acid. After the reaction was complete, 2.5 ml of 10% trichloroacetic acid was added to all the samples and centrifuged at 3000 rpm for 10 minutes. 2.5 ml supernatant was measured out from each sample, diluted with 2.5 ml distilled water, and mixed with 0.5 ml of freshly prepared 0.1% of ferric chloride. After 10 minutes, the absorbance was taken at 700 nm against the blank solution.

Antibacterial test

H. excelsa extract was tested for antibacterial activity using well-diffusion method.[20] The bacterial strains used were Gram-negative bacteria, *Escherichia coli* (ATCC 10536), and Gram-positive species, *Bacillus cereus* (ATCC 13061) and *Staphylococcus aureus* (ATCC 700698). The bacteria were grown in Mueller-Hinton agar medium. Different concentrations, i.e., 200, 100, and 50 mg/ml, of the plant extracts were prepared by serial dilutions. 50 µl of each sample was loaded to the media in culture plates. A standard antibiotic, 1% ciprofloxacin was used as positive control, and 1% dimethyl sulphoxide (DMSO) was used as negative control. The bacteria were allowed to grow for 24 hours at 28°C in a microbiological incubator. The growth zones or zones of inhibition were measured for all the samples. The mean antibacterial activities were compared by Tukey's multiple comparison test using Prism GraphPad. The mean difference was considered significant at *p* value less than 0.5.

RESULTS

Quantitative phytochemical tests

The phytocompounds detected in the chloroform extract of *H. excelsa* leaf are shown in Table 1. The chemical group of alkaloids, phenolic, carbohydrate, glycoside, tannin and phytosterol were tested positive, while showing the absence of flavonoid, saponin and protein and amino acid groups.

Table 1: Phytochemical groups detected from the leaf of *H. excelsa*.

Phytochemical	Name of test	Extract indication
Alkaloid	1. Hager's test	+
	2. Wagner's test	-
	3. Mayer's test	+
	4. Dragendroff's test	+
Flavonoid	1. Alkaline test	-
	2. Lead acetate test	-
	3. Ferric chloride test	-
	4. Shinoda's test	-
	5. Zn-HCl reduction test	-
Phenol	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 test	+
Protein and amino acid	1. Biuret test	-
	2. Millon's tet	-
	3. Ninhydrin test	-
	4. Xanthoproteic acid test	-
Phytosterol	1. Salkowski's test	-
	2. Liebermann-Burchard's test	+
	3. Acetic andydride test	+

+ for presence; and – for absence of a particular compound group

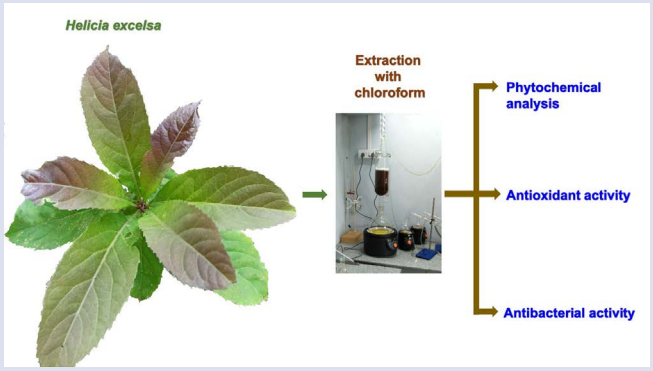

Figure 1: Helicia excelsa from Aizawl, India, and workflow of the analysis.

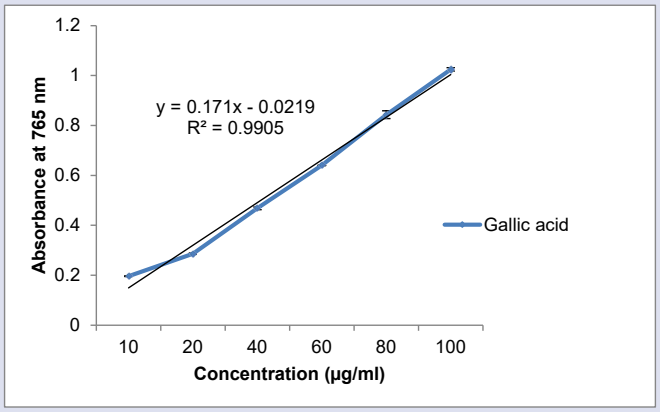

Figure 2: Standard curve of gallic acid for total phenol assay. Linear black line represents the linear graph. Values in means ± standard error of means (*n*=3)

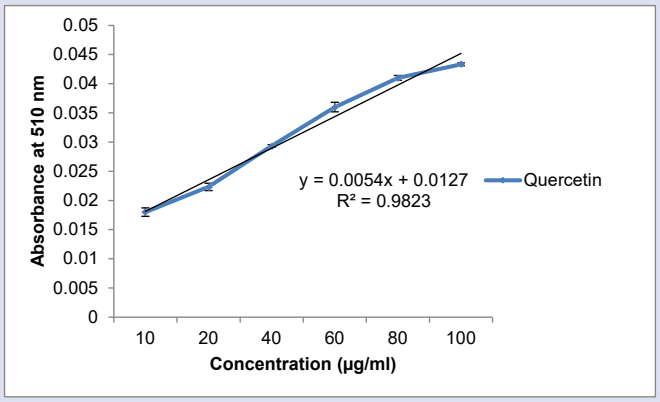

Figure 3: Standard curve of quercetin for total flavonoid assay. Linear black line represents the linear graph. Values in means ± standard error of means (*n*=3)

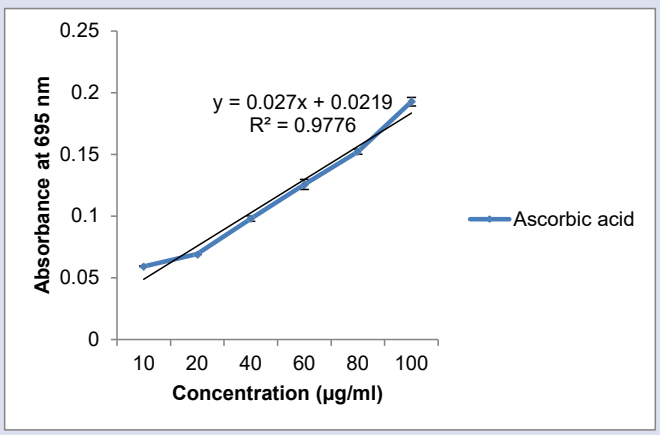

Figure 4: Standard curve of ascorbic acid for total antioxidant assay. Linear black line represents the linear graph. Values in means ± standard error of means (*n*=3)

Total phenolic content

The total phenolic content of the *H. excelsa* extract was determined from the gallic acid calibration curve (Figure 2). The total content of phenol present in the plant extract was found to be 4.52±0.09 GAE mg/g.

Total flavonoid content

The total flavonoid content was calculated from the absorbance values of different concentrations of quercetin (Figure 3). The flavonoid content was determined to be 64.27±1.04 QE mg/g.

Total antioxidant content

The total antioxidant for *H. excelsa* extract was calculated from the standard calibration curve plotted for ascorbic acid and was expressed as milligram of ascorbic acid equivalent per gram of the dried extract (Figure 4). The antioxidant content was 11.39±0.45 AAE mg/g.

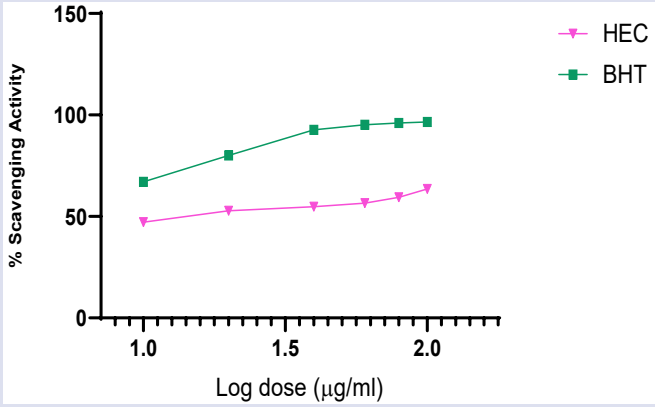

Figure 5: Percentage inhibition of DPPH by *H. excelsa* extract and BHT. Values in means ± standard error of means (*n*=3)

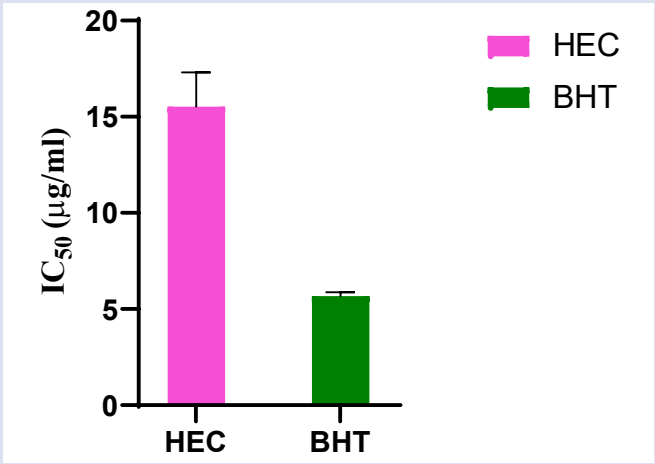

Figure 6: Comparison of IC_{50} of *H. excelsa* extract and BHT. Values in means ± standard error of means (*n*=3)

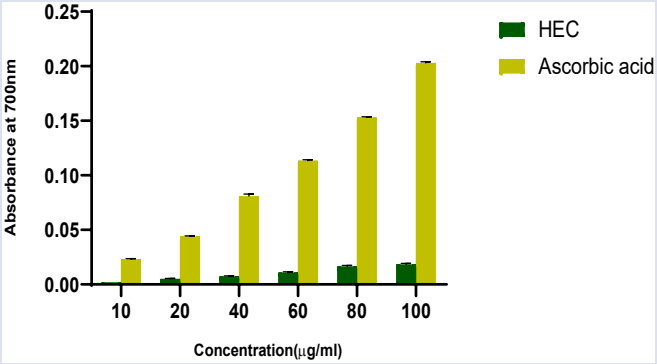

Figure 7: Ferric-reducing activity of *H. excelsa* extract and ascorbic acid. Values in means ± standard error of means (*n*=3)

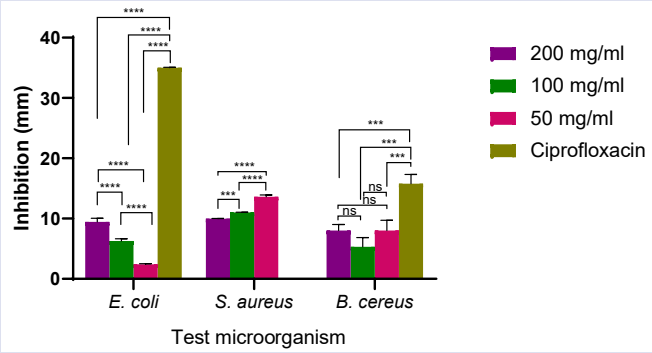

Figure 8: Comparison of the inhibition of *H. excelsa* extract against *E. coli*, *S. aureus* and *B. cereus*. Values in means ± standard error of means (*n*=3). ****p<0.0001, ***p<0.002, ns = not significant, i.e., p>0.05

DPPH-scavenging activity

The antioxidant activity of *H. excelsa* extract estimated using DPPH-scavenging assay is shown in Figure 5. Percentage inhibition activity showed concentration-dependent scavenging activity for of the plant extract. The IC_{50} value of BHT at 15.52±3.08 was lower than that of the plant extract at 5.67±0.36 (Figure 6), indicating that the plant has less antioxidant power.

FRAP assay

The ferric ion-reducing power of *H. excelsa* extract was compared with standard ascorbic acid prepared in different concentrations, both of which showed concentration-dependent antioxidant activity. Ascorbic acid exhibited higher activity than the plant extract at all concentrations tested as shown in Figure 7.

Antibacterial activity

The evaluation of *H. excelsa* extract for susceptibility of bacterial growth, performed by well-diffusion method against selected bacteria, is shown in Figure 8. At incremental concentrations, i.e., 1, 0.5, and 0.25 mg/ml, the plant extract showed inhibition zone (in mm) of 9.46±0.34, 6.29±0.20, and 2.42±0.06 mm for *E. coli*, 9.97±0.04, 11±0.03, and 13.63±0.17 mm for *S. aureus*; and 8±0.58, 5.33±0.89, and 8±1.00 for *B. cereus*. Ciprofloxacin at 1 mg/ml showed more potent activity producing inhibition zones of 35.02±0.29 mm for *E. coli* and 15.77±0.89 mm for *B. cereus*. However, the antibiotic was ineffective against *S. aureus*.

The mean differences of antibacterial efficacy indicate that the plant extract showed significant (p>0.05) activity against *E. coli* and *S. aureus* at different concentrations.

DISCUSSION

Plants in the diet are the major nutraceuticals, serving as the source of both our nutritional requirements and therapeutic uses. Either as part of the foodstuff or food supplements, they constitute a major ingredient to normal cellular metabolism and to a remedy of several cellular disorders.[21,22] In this study, we detected phytocompounds including amino acids, alkaloids, carbohydrates, glycosides, phenols, phytosterols, proteins, and tannins from *H. excelsa* leaves. In investigating the medicinal properties of plants, it is a critical and preliminary step to identify the broad groups of compounds present for further investigation of the bioactive molecules. As different species and geographical varieties can contain different phytochemicals, it is further essential to have a screening test for individual group of

compounds. For example, phenols, alkaloids, flavonoids, saponins, steroids, and tannins are diverse phytocompounds from which different medicinal sources and nutritional supplements have been identified.[23,24] In this study, major phytocompounds including alkaloids, glycosides, phenols, phytosterols, and tannins were detected from *H. excelsa* leaves, indicating the plant as a rich source of nutraceutical compounds.

Normal cellular metabolism in humans and other animals continuously produces intracellular oxidants such as free radicals or reactive oxygen species (ROS) in the cells that tend to be highly toxic as they cause disruption of cell function and to cell death due to damaging DNA, RNA, proteins, and lipids.[25] In addition, external factors such as ultraviolet and environmental X-rays exposure, ozone, air pollutants and smoking add to the free radical-damaging effects leading to many chronic diseases such as cancer, heart diseases and diabetes.[26] The body requires external sources of antioxidants to completely neutralise the heavy oxidation effects. Synthetic antioxidants are not a reliable remedy as they are always associated with adverse toxic effects. Therefore, plants are known to be the most employable and effective antioxidants as they are already part of the diet and are natural remedies to different ailments with little or no toxicity.[27] Analysis of *H. excelsa* by different parameters showed that the plant is a valuable source of antioxidant molecules.

There is a mounting global concern in antimicrobial medication as a result of rapid development of drug resistance in the most important pathogens. In fact, multiple drug resistance has become the biggest threat to the management of infectious diseases.[28] A search for novel drugs is demanding as ever. Plants are the major component among the potential sources of new antimicrobial drugs, and a number of plant-specific antimicrobial compounds have been identified.[29] The present findings reveal that *H. excelsa* is a good source of antibacterial compounds as its extract is highly effective against both Gram-positive and Gram-negative bacteria.

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, 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 antibacterial activity against both Gram-positive and Gram-negative bacteria. Therefore, the findings indicate that the plant is a promising source of bioactive compounds and needs further investigation to acknowledge its true potential in new drug discovery.

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CONFLICTS OF INTEREST

None declared.

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