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RESEARCH ARTICLE

Exploration of the Mizo Traditional Medicine: Pharmacognostic Studies of the Indigenous Medicinal Plant, *Erythrina stricta*

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Abstract

Objectives: The study aimed to understand the range of medicinal applications of *Erythrina stricta* Roxb. (Fabaceae) among the Mizo people of India, and to analyse the pharmacological properties of the bark extract. **Methods:** Ethnomedicinal investigation was conducted among traditional healers and users. The petroleum ether extract of the bark was analysed for its phytochemical components. Antioxidant property was evaluated by DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. Total antioxidant capacity, flavonoid and total phenolic contents of the plant extract were assessed by using the phosphomolybdate method, Folin-Ciocalteu assay and aluminium chloride assay respectively. Antimicrobial activity was tested on two Gram-negative and four Gram-positive bacteria. **Findings:** *E. stricta* was used for the treatment of stomach ulcer, diabetic blisters, intestinal roundworm infection, skin infection, sores and wounds. The plant extract contained phytochemicals like alkaloids, flavonoids, phenols, tannins, amino acids and proteins, phytosterols and triterpenoids. The extract showed concentration-dependent antioxidant activity against DPPH; showing an IC_{50} value of 3.33±0.024 μg/ml compared to 0.23±0.095 μg/ml for the reference butylated hydroxytoluene. The total antioxidant capacity was 12.01±0.33 mg ascorbic acid equivalent per gram (AAE/g) of the dry extract; the total phenolic content was 3.77±0.12 mg gallic acid equivalent per gram (GAE/g) of the dry extract; while the total flavonoid content was 7.75±1.13 mg quercetin equivalent per gram (QE/g) of the dry extract. The extract effectively inhibited the growth of two Gram-positive bacteria, *Bacillus cereus* and *Staphylococcus aureus*, and one Gram-negative species, *Klebsiella pneumoniae*. **Novelty:** *E. stricta* was found to have important medicinal applications and pharmacological potential, and needs systematic conservation and propagation. It showed high antioxidant values, and species-specific antibacterial activities.

Keywords: Erythrina stricta; antioxidant activity; antibacterial activity; medicinal plant; phytochemical

1 Introduction

Species of *Erythrina* L. (family Fabaceae) are trees with various medicinal uses in different traditional systems. They are distributed widely in the tropical and subtropical regions of the world. In Indian traditional system, *E. stricta* Roxb. is used in various ailments such as in the treatment of treat joint pains, rheumatism, itching, epilepsy, and asthma. Several species are known and validated for their antibacterial, anticancer and antiviral activities[(1)]. Some species are used for the treatment of amenorrhea, asthma, general infections, eye problems, female infertility, headache, inflammation, liver diseases, and malaria. Certain species contains compounds that are potent central nervous system depressants, hypotensive, neuromuscular blockers, and sedatives. Their secondary metabolites such as flavonoids and pterocarpans were shown to have anti-inflammatory property[(2,3)]. Alkaloids from some species were demonstrated to have anticonvulsant, anxiolytic, curare-like activity, insecticidal and cytotoxic activities[(4)].

There has been a rapid increase in the spread of drug resistant bacteria which show diminished susceptibility to all types of prescription antibiotics[(5)]. The situation is alarming as higher drug resistance develop in many pathogens. Improvements in drug chemistry or novel compounds are urgently needed to check the problems arising in the clinical management of infections. Medicinal plants are considered one of the best options. Major research attention has progressively turned to traditional medicine in looking for new antimicrobial compounds and their mechanism of action to develop improved medications[(6,7)].

Despite the fact that several species of plant have been experimented for antimicrobial activities in a wide range of microbes, larger number have not been thoroughly investigated[(8)]. Therefore, systematic research into the investigation of medicinal plants for their true chemical nature and biological effects is crucial. This study is an attempt to investigate an indigenously used medicinal plant among the Mizo people to understand its pharmacological potentials.

2 Methodology

2.1 Ethnomedicinal Survey

While investigating the traditional medicinal plants of the Mizo people of India, it was noticed that *Erythrina stricta* was an indigenous medicinal plant with a specific application not known in other traditional medicines. Traditional healers and users were inquired from several villages on the types of medicinal applications.

2.2 Microorganisms

The microorganisms used for the present study were two Gram-negative bacteria, namely *Escherichia coli* (ATCC 10536) and *Klebsiella pneumoniae* (ATCC 10031), and four Gram-positive bacteria such as *Bacillus cereus* (ATCC 13061), *S. aureus* 1(ATCC 700698), *S. aureus* 2 (ATCC 11632) and *Micrococcus luteus* (ATCC 10240) which were maintained by serial sub-culture at DBT-BUILDER National Laboratory, Pachhunga University College, Aizawl, Mizoram.

2.3 Plant Collection and Authentication

The plant specimen of *Erythrina stricta* Roxb. was gathered from a nearby forest of Aizawl, Mizoram, India. A herbarium specimen was submitted and authenticated at the Botanical Survey of India. The voucher specimen was deposited and preserved at the Herbarium Repository of Pachhunga University College (with an accession code PUC-E-21-01).

2.4 Preparation of Extracts

The plant samples were cleaned rigorously with distilled water. The bark was peeled off, chopped into fine pieces and then kept at 50°C in an oven until all the water molecules evaporated. The dried sample was ground into coarse powder in a mixer-grinding machine. Then the ground plant sample was loaded into Soxhlet apparatus for hot continuous extraction. Extraction was done with petroleum ether at room temperature (27±3°C) for 72 hours. After the complete extraction, the product from the solvent was collected and subjected to rotary vacuum evaporator (Buchi Rotavapor® R-300) to get the concentrated extract of the plant sample. A semi-solid extract so obtained was labelled and refrigerated at 4°C for further investigation.

2.5 Phytochemical Analysis

The crude extract of *E. stricta* bark was tested for the presence of alkaloids, flavonoids, phenolics, carbohydrates, glycosides, saponins, tannins, amino acids and proteins, phytosterol, triterpenoids and diterpenes based on standard protocols[(9)]. In summary: alkaloids were tested by Meyer's test, Wagner's test, Hager's test and Dragendroff's test; flavonoids by alkaline

reagent test, lead acetate test, ferric chloride test and Shinoda test; phenolics by ferric chloride test, lead acetate test, potassium dichromate test, iodine test, ellagic acid test and gelatin test; carbohydrates test by Molisch's test, Benedict's test, Fehling's test; glycosides by Liebermann's test, Salkowaski's test, Keller-Kiliani test and Borntrager's test; saponins test by froth and foam tests; tannins by gelatin test, Braymer's test, 10% sodium hydroxide test; amino acid and proteins by Biuret test, Millon's test and ninhydrin test; phytosterols by Salkowski's test, Libermann's test; triterpenoids by Salkowski's test; and diterpenes by copper acetate test.

2.6 Antimicrobial Activity

The antimicrobial activity of the extract was determined by using disk diffusion method [10,11]. Paper discs were prepared by using Whatman filter no 3 in 5 mm diameter. Nutrient agar medium was made in distilled water and then sterilized. Culture plates were prepared by the pour plate method. Dimethyl sulphoxide (DMSO) was used for negative control to ensure that the solvent used for dissolving extract did not have antibacterial activity. For standard reference, ciprofloxacin 2% 1x was used. Bacterial samples were then grown in culture plates maintaining the negative control (with DMSO) and positive control (with standard antibiotic). Then the bacteria were allowed to grow at 37°C in an incubator. After 24 hours, the growth zones of bacteria were measured to determine the relative propagation or inhibition.

2.6.1 ***Minimum Inhibitory Concentration***

The minimum inhibitory concentration of the bark extract of *E. stricta* was determined as the least concentration of the extract sample that effectively inhibited the growth of the test bacteria that included both Gram-positive and Gram-negative species (Table 1).

Table 1. Bacterial strains used for the antimicrobial assay.

Bacterial strains	Type strain	Gram stain
Escherichia coli	ATCC 10536	Negative
Klebsiella pneumoniae	ATCC 10031	Negative
Bacillus cereus	ATCC 13061	Positive
Staphylococcus aureus 1	ATCC 700698	Positive
Micrococcus luteus	ATCC 10240	Positive
Staphylococcus aureus 2	ATCC 11632	Positive

2.7 Antioxidant Activity

2.7.1 ***DPPH Radical Scavenging Assay***

The antioxidant activity of the plant extract was determined by using DPPH (2,2-Diphenyl-1-picrylhydrazyl) free radical scavenging assay according to the method reported by von Gadow *et al.* [12]. Stock solution (1 mg/ml) of the extract of *E. stricta* bark was prepared in methanol from which different concentration was carried out as 10, 20, 40, 60, 80 and 100 μg/ml and the volume were made up to 3ml each with methanol respectively. Then 0.5 ml of the methanolic solution of 0.1 mM DPPH was added for different concentration of the sample. 1 ml of DPPH methanolic solution and 3 ml of methanol were used as control and blank sample that contained the same amount of methanol. The solutions prepared were incubated at 37°C for 30 min. Butylated hydroxyltoluene (BHT) was used as a reference antioxidant. The level or reduction of DPPH was measured by taking the absorbance at 517 nm against a blank sample using UV-visible spectrophotometer. Each test was done in triplicate. Free radical scavenging activity was estimated as percentage as follows:

% DPPH radical-scavenging = [(Absorbance of control — Absorbance of test Sample) / (Absorbance of control)] x 100

The percentage of DPPH inhibition was plotted for the plant extracts at varying concentrations to determine the minimum concentration required to inhibit the DPPH free radical by 50% (IC_{50}).

2.7.2 ***Total Antioxidant Activity***

The total antioxidant activity of *E. stricta* bark extract was estimated based on the reaction with phosphomolybdate and using ascorbic acid as a reference antioxidant [13]. In brief, 0.1 ml of the sample solution was prepared in distilled water, mixed with 3 ml of a reagent composed of 4 mM ammonium molybdate, 28 mM sodium phosphate, and 0.6 M sulphuric acid. After incubating at 95°C for 90 min, the absorbance was recorded at 695 nm.

2.7.3 ***Total Flavonoids Content***

The total flavonoid content was evaluated based on colorimetric assay using aluminium chloride reaction [14]. 1 ml of the plant extract (prepared in 1 mg/ml) was mixed with 2 ml of distilled water in a test tube. After 5 min, 3 ml of 5% sodium nitrite and 0.3 ml of 10% aluminium chloride solution were added. After 6 min, 2 ml of 1.0 M sodium hydroxide was added and left for 1 hour. A series of concentrations of quercetin (viz. 10, 20, 40, 60, 80 and 100 μg/ml) was prepared according to the same steps as that of the extracts. The absorbance values of the samples were measured against a blank concentration at 510 nm. The total flavonoid content was determined from the calibration curve of quercetin. The determination of the total flavonoid content of the sample was done in triplicate for each concentration.

2.7.4 ***Total Phenolic Content***

Folin-Ciocalteu reaction was used for the estimation of the total phenolic content of *E. stricta* bark extract. Gallic acid was used as a reference compound [15]. 1 ml of the plant extract (1 mg/ml) and 5 ml of Folin-Ciocalteu reagent were mixed in a test tube. After 3 min, 4 ml of 0.7 M sodium carbonate solution was put into the mixture and left at room temperature for 1 hour. Different concentrations of gallic acid (i.e., 10, 20, 40, 60, 80 and 100 μg/ml) were made accordingly. The absorbance of the samples were read against the blank sample at 765 nm. Using the calibration curve of gallic acid, the total phenolic content was determined. All tests were carried out in triplicate for each sample.

3 Results and Discussion

3.1 Ethnomedicinal uses

Mizo people often resort to traditional medicinal plants and use them to collect various herbal parts from the forests for various ailments and one of such is the bark of *E. stricta*. The plant is usually found in low land, fallow areas and along the roadside. Our investigation showed that the bark of this tree has been traditionally utilized by the Mizo people for more than a decade as a remedy for stomachache and ulcers, diabetic blisters, threadworm infection, skin infection, sores and different types of wounds. It was reported from the traditional healers and users that consumption of the decoction of the bark after meal for several months can heal stomach ulcers and diabetic blisters. There have been no documented records on this plant for such medicinal purposes.

The bark of *E. stricta* has been a part of the Mizo traditional medicine as a remedy for stomachache and ulcers, diabetic blisters, threadworm infection, skin infection, sores and wounds. With a knowledge of these traditional usages, the plant offers a valuable resource for pharmacological investigations, as it is well established that plants are the sources of many of the most important pharmaceutical drugs. More than 100 drugs are developed from plant products [16]. Therefore, it is crucial to explore the hidden sources in the botanical arena for better and new drugs [17].

3.2 Phytochemical detections

The phytochemical determination from the *E. stricta* bark extract revealed the presence of important secondary metabolites including alkaloids, flavonoids, phenols, tannins, amino acids and proteins, phytosterols and triterpenoids. Among the phytochemical test, test for carbohydrates, glycosides, saponins and diterpenes did not show any result in the extract of the plant sample (Table 2). These phytocompounds detected in the extract may be responsible for the biological activities shown by *E. stricta* outlying the reason for its use as a traditional medicine by the Mizo tribe.

Table 2. Phytochemical compounds of the petroleum ether extract of *E. stricta* bark

Sl. no.	Phytochemicals	Name of test	Extract indication
1.	Alkaloids	Meyer's test	+
		Wagner's test	+
		Hager's test	+
		Dragendroff's test	+
2.	Flavonoids	Alkaline reagent test	-
		Lead acetate test	+
		Ferric chloride test	-
		Shinoda test	-
3.	Phenols	Lead acetate test	+
		Potassium dichromate test	-

Continued on next page

Table 2 continued

		Gelatin test	+
4.	Carbohydrates	Molisch's test	-
		Benedict's test	-
		Fehling's test	-
5.	Glycosides	Liebermann's test	-
		Salkowaski's test	-
		Keller-Kiliani test	-
		Borntrager's test	-
6.	Saponins	Froth test	-
		Foam test	-
7.	Tannins	Gelatin Test	+
		Braymer's test	-
		10% NaOH test	+
8.	Amino acids and proteins	Biuret Test	+
		Millon's test	+
		Ninhydrin test	-
9.	Phytosterols	Salkowski's test	+
		Liebermann's test	+
10.	Triterpenoids	Salkowski's test	+
11.	Diterpenes	Copper acetate test	-

Indicators: (+) present, (-) absent.

An extract of *E. stricta* was found to contain alkaloids, flavonoids, phenols, tannins, phytosterols and triterpenoids. All these are known to be secondary metabolites of plants that are bioactive compounds. Specific compounds among these groups are responsible for specific biological and pharmacological activities ranging from anticancer, anthelmintic, anti-inflammatory, and antimicrobial activities [18]. Our findings suggest that specific molecules among these compounds could be the basis of the ethnomedicinal usage of *E. stricta*.

3.3 Antioxidant activity

Free radicals are molecules with unpaired electrons that are highly reactive and unstable, the natural by-product of chemical processes of cellular metabolism [19]. They attack essential macromolecules including lipids, proteins and nucleic acid leading to cell damaging, homeostatic disruption and initiation of chromosomal defects. Various numbers of medicinal plants contain antioxidant which are substances capable of delaying and protecting the development of rancidity mainly caused by oxidation at low concentration [20]. Therefore, antioxidant molecules play a crucial role in neutralizing the free radicals and reactive oxygen species (ROS) inside the cell by reducing the damage caused by oxidation.

Synthetic antioxidants can be health hazards as they cause cell toxicity, cellular damage, inflammation, and atherosclerosis both in humans and animals [21]. For this reason, attention has been drawn towards the use of naturally occurring antioxidant in plants containing high bioactive flavonoids, which have a strong potential to scavenge free radicals. Species of *Erythrina* are found to have high antioxidant properties [3]. Our study also showed that *E. stricta* is potentially a good source of such beneficial antioxidant compounds.

3.3.1 ***DPPH scavenging assay***

The antioxidant activity of *E. stricta* bark extract was estimated by DPPH radical scavenging assay as shown in Figure 1.

In DPPH radical-scavenging activity, the IC_{50} (the concentration of plant sample required to inhibit DPPH by 50%) values of the plant extract were worked out from the standard graph and was found to be 0.26 for BHT and 3.32 for *E. stricta* extract.

3.3.2 ***Total antioxidant activity***

The assessment of total antioxidant activity was done by the method of phosphomolybdate reaction using ascorbic acid as standard reference. Figure 2 shows the calibration curve of ascorbic acid as standard showing $y = 0.027x + 0.0219$ and $R^2 = 0.9776$ from which the total antioxidant activity was calculated. The total antioxidant activity of petroleum ether extracts of *E. stricta* was 12.01±0.33 mg ascorbic acid equivalent per gram (AAE/g) of the dry extract of the plant. The antioxidant activity shown by the plant may be due to the presence of flavonoids, phenols, tannins and triterpenoids in the plant extract indicating that the plant possesses significant antioxidant activity.

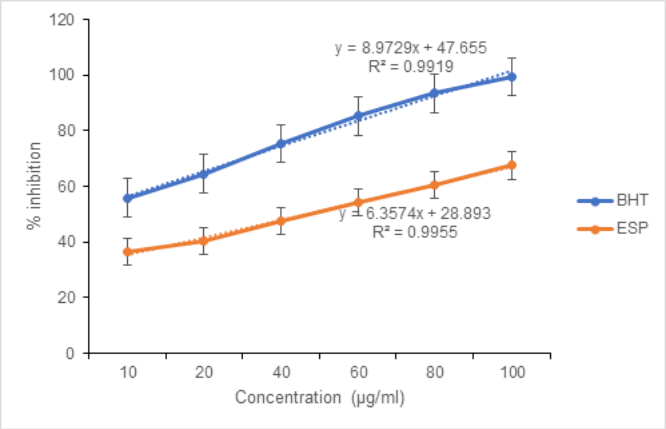

Fig 1. The percentage of inhibition plotted against the plant extract concentration (μg/ml) using BHT as standard reference. Free radical scavenging activity of the petroleum extract of *E. stricta* bark in comparison with standard butylated hydroxytoluene. The values are expressed as means ± standard deviation (n=3).

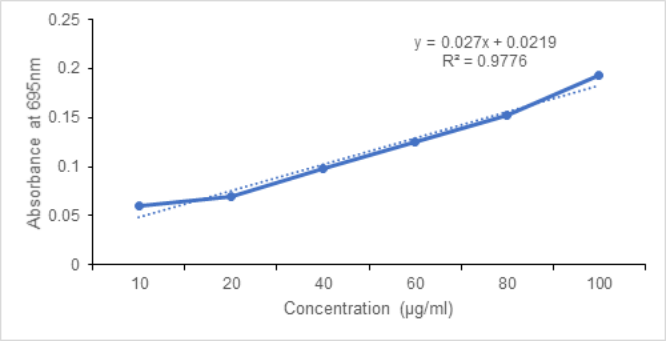

Fig 2. Total antioxidant activity of *E. stricta* bark extract using phosphomolybdate method. The values are expressed as mean ± standard deviation (n=3).

3.4 Total phenolic content

The total phenolic content of *E. stricta* extract was expressed as mg of gallic acid equivalent per gram (GAE/g) calculated using the values obtained from the standard calibration curve of gallic acid. From the calibration curve (Figure 3), the amount of total phenolic content was found to be 3.77±0.12 mg (GAE/g) of the dry extract of the plant. Phytochemical analysis showed the presence of flavonoids, phenols, tannins and triterpenoids which are phenolic compounds. These compounds have biologically active components responsible for the antioxidant and free radical-scavenging activities of the plant.

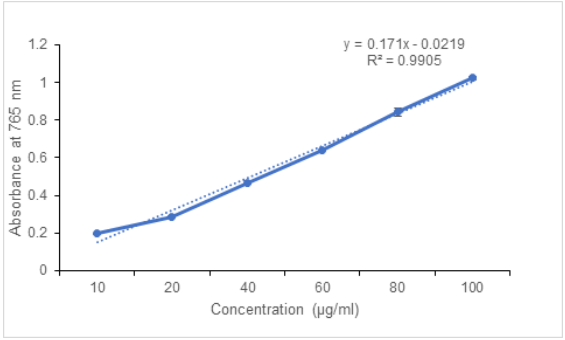

Fig 3. Total phenolic content of *E. stricta* bark extract; The values are expressed as mean ± standard deviation (n=3).

3.5 Total flavonoid content

The total flavonoid content of the plant extract was evaluated by colorimetric assay expressing using the aluminium chloride. The result was given as mg quercetin equivalent per gram (QE/g) of the extract calculated from the values obtained from the calibration curve of the standard quercetin (Figure 4) . The amount of total flavonoid content was found to be 7.75±1.13 mg (QE/g) of the dry extract.

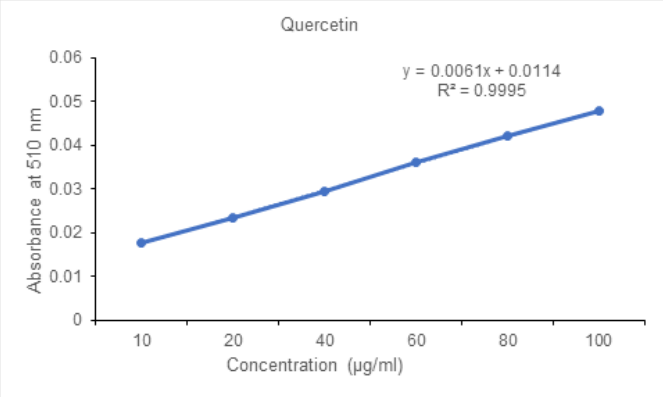

Fig 4. Total flavonoid content of *E. stricta* bark extract. The values are expressed as mean ± standard deviation (n=3).

3.6 Antibacterial activity

The antibacterial activity of the bark extract of *E. stricta* was assessed by determining the relative zones of inhibition and comparing it with that of ciprofloxacin (Table 3). The zones of inhibition of bacterial growth on the agar plates showed that the plant extract possessed antibacterial activity both on selected Gram-positive and Gram-negative strains, but not all the species. The plant extract showed high activity against the bacterial strain *B. cereus*, *K. pneumoniae* and *S. aureus* 1, while it failed to show any inhibitory activity on *E. coli*, *M. luteus* and *S. aureus* 2. In this study, it was found that the antibiotic ciprofloxacin solution did not show inhibitory activity on *S. aureus* 1 while the plant extract effectively inhibited the bacterial growth at different concentrations.

Table 3. Antibacterial activity of *E. stricta* bark extract and ciprofloxacin

Sl. No.	Bacterial species	Zone of inhibition (in mm)						Ciprofloxacin 2%, 1x
		125 µg/ml g/disc	250 µg/ml g/disc	500 µg/ml g/disc	600 µg/ml g/disc	700 µg/ml g/disc	800 µg/ml g/disc	
1.	*Bacillus cereus* ATCC 13061	5.63	6.07	6.36	6.78	7.18	8.11	16.7
2.	*Escherichia coli* ATCC 10536	-	-	-	-	-	-	30.66
3.	*Klebsiella pneumoniae* ATCC 10031	-	5.66	5.87	6.73	6.88	7.29	15.09
4.	*Staphylococcus aureus* 1 ATCC 700698	-	-	-	7.83	9.54	9.89	-
5.	*Micrococcus luteus* ATCC 10240	-	-	-	-	-	-	23.17
6.	*Staphylococcus aureus* 2 ATCC 11632	-	-	-	-	-	-	11.24

3.6.1 ***Minimum inhibitory concentration***

The minimum concentration of the plant extract that inhibits the growth of the selected bacterial strains was determined as the MIC of the plant extract in each of the test organisms.

Table 4. MIC of *E. stricta* bark extract on different bacteria

Sl. No.	Bacterial strains	Type Strain	MIC
1	*Bacillus cereus*	ATCC 13061	125 μg/ml
2	*Escherichia coli*	ATCC 10536	-
3	*Klebsiella pneumoniae*	ATCC 10031	250 μg/ml
4	*Staphylococcus aureus* 1	ATCC 700698	600 μg/ml
5	*Micrococcus luteus*	ATCC 10240	-
6	*Staphylococcus aureus* 2	ATCC 11632	-

From Table 4, it was observed that the plant extract is active only in three bacterial strains as indicated by the zones of inhibition. The plant extract was quite effective on *B. cereus* with concentration 125 μg/ml followed by *K. pneumoniae* with concentration 250 μg/ml and 600 μg/ml on *S. aureus* 1.

No currently available antibiotics are safe and free from the problem of drug resistance, and there is no single working solution to the dilemma[22]. More than a thousand plant species are estimated to have therapeutic antimicrobial activities, and over 30,000 plant-derived antimicrobial compounds[23]. To combat the diminishing drug efficacy in pathogenic microbes, it is, therefore, a challenge to look into ethnomedicinal plants that are having well-established applications for the lead pharmaceutical molecules[18].

Our findings indicate that *E. stricta* is an interesting and promising source of antimicrobial compounds. The plant extract effectively inhibited the propagation of two Gram-positive and one Gram-negative bacteria, revealing that the antibacterial component is species specific. This is to be expected since pathogenic bacteria respond to drugs in different ways and with various mechanisms. For instance, different plant secondary metabolites like alkaloids, terpenoids, and flavonoids with demonstrated antimicrobial activities are always effective only against a particular species or specific group of bacteria since they target different drug-receptive cellular molecules[18,24]. Therefore, it is imperative that the research focus is directed to medicinal plants for novel and safe antimicrobials, and as such *E. stricta* offers a promising candidate for further investigations.

4 Conclusion

A survey of Mizo traditional medicine revealed that *Erythrina stricta* is an indigenous medicinal plant among the Mizo people who use the bark for the treatment stomach problems, skin diseases, and helminthiasis. The bark extract was found to contain alkaloids, amino acids and proteins, flavonoids, phenols, phytosterols, tannins, and triterpenoids which are ostensibly responsible for its acclaimed medicinal applications. It possesses a high antioxidant property as shown by estimations of total antioxidant, total phenolics, flavonoid content and DPPH free radical-scavenging activities. It is also a strong antibacterial source and act species-specific against both Gram-positive and Gram-negative bacteria. The results indicate that this particular plant may be an important source in novel drug discovery. Therefore, the present study supports the traditional usage of the medicinal plant, and directly warrants its conservation for further investigations. The evidences clearly indicate that the plant contains bioactive compound(s) that could be potentially employed in drug development, therefore, espouse deeper pharmacological investigations.

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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