PHYTOCHEMICAL AND BIOLOGICAL SCREENING OF THREE SELECTED ETHNOMEDICINAL PLANTS FROM MIZORAM, INDIA

Pratima Khandayataray, Meesala Krishna Murthy * and Dibyaranjan Samal
Department of Biotechnology, Academy of Management and Information Technology (AMIT), Plot No. 67, VidyaVihar, IID Center, Near Barunei Hills, Khurda - 752057, Odisha, India.

ABSTRACT: This research explores *Achyranthes bidentata* L., *Adhatoda zeylanica* and *Aeginetia indica* L's pharmacological potential. Antibacterial disc assay (20 mg/ml), antifungal disc assay (20 mg/ml) and antioxidant DPPH/reducing energy assay (50 µg/ml, 100 µg/ml and 200 µg/ml) were tested for biological operations of plant extracts (aqueous, methanol and acetone leaf extracts). Significant alkaloid content (378.37 ± 35.02 mg/g) was identified in *Aeginetia indica* L. with the lowest tannin content in *Achyranthes bidentata* L. (13.37 ± 3.15 mg/mg). *Aeginetia indica* L. methanol extract (99.28 ± 10.47%) disclosed significant antioxidant potential ($p<0.05$). Significant antibacterial and antifungal activity was demonstrated against *Escherichia coli* (22.80 ± 5.06 mm), *Pseudomonas aeruginosa* (22.80 ± 5.06 mm), *Bacillus subtilis* (25.40 ± 5.21 mm), *Candida albicans* (19.40 ± 2.05 mm) and *Aspergillus niger* (19.80 ± 1.48 mm) from *Aeginetia indica* L. methanol extracts. All plant methanol extracts showed significant antibacterial activity (22.80 ± 5.06 mm - 25.40 ± 5.21 mm) and antifungal activity (19.40 ± 2.05 mm - 19.80 ± 1.48 mm) very close to standard medicines whereas radical DPPH scavenging potential (47.28 ± 1.87% - 99.28 ± 10.47%) and ferric energy reduction capability with absorbance values (0.341-0.587) are very near to normal ascorbic acid. The present research supports the comprehensive use of these plants in ethnomedicine as well as promoting elaborate *in-vivo* research, isolation of pure therapeutic compounds, and plant-based drug formulation.

Keywords:

Achyranthes bidentata L., *Aeginetia indica* L., Anti-bacterial, Anti-fungal, Phytocompound screening

INTRODUCTION: Using plants for health care is as old as human civilization. However, the earliest records used for the treatment of various diseases were found in ancient Hindu scripture Rig Veda, 3500 B.C. Since 1800 B.C., Indian elders recognized the importance of plants as medicines and the treatment of many plants was documented in many scriptures of the Vedic period.

Many books, including Atharvaveda (1200 BC), Charak Samhita, and Shushrut Samhita (1000-500 BC), reviewed the remedies of more than 700 medicines [1]. Sumerian clay tablets were written in the written form of medical treatment almost four thousand years ago when they met with various diseases [2].

Sumerian plants have prescribed for toothache, turmeric for blood clots, endogenous organic roots plants for the treatment of gallstones and raw garlic for circulatory disorders. Despite the development of modern medicines, natural products have grown globally because therapeutic options for various diseases are due to nontoxic or less toxic and less known side effects than modern generics [3, 4].

Plants are rich sources of bioactive compounds and serve as an important raw material for the production of drugs. Plants synthesize some secondary metabolites, such as alkaloids, glycosides, tannins, volatile oils, and can have a great potential for biological activity and maybe a therapeutical agent for therapeutic

purposes. Testing of antimicrobial susceptibility can be used for drug detection, development, and therapeutic outcome.

After the "golden era" revolution, when all groups of major antibiotics (macrolides, cephalosporins, aminoglycosides, and tetracyclines) were found, but nowadays, resistance to antibiotics is an increasingly serious threat to the health of the world's population. The situation of bacterial pathogens today is very different, and the need for powerful new antimicrobial agents has never been greater [5]. The therapeutic properties of plants are due to the production of secondary metabolites produced in response to different environmental conditions or as protective mechanisms. Various factors such as temperature, season, light, and climate change, humidity, radiation, salinity, nutrient deficiency, pathogens influence, and heavy metal stress, and control the quality and quantity of phytochemicals in plants. The production and accumulation of polyphenols usually increase as a result of biotic or abiotic stress signals. Cold stress or winter stimulates the production of soluble sugars and nitrogen compounds [6]. The areas are responsible for providing the necessary conditions for the sustainable production of bioactive agents in plants [7]. It is, therefore important to consider the plant's area and climate when describing its medicinal capacity. Ethnomedicine is practiced for health care by many ethnic groups living around the world and since ancient times

India has local traditional knowledge of plants. This traditional health knowledge has been passed on from generation to generation for thousands of years. With the emergence of modern medicine, however, there is a decline in the traditional, alternative, and complementary systems and ethnomedicinal practices, which shows the need to revitalize these systems, especially with the necessary documentation and research behind them [8]. It is, therefore, practical to test ethnomedicinal plants for the development of new drugs by studying new chemical components and also to establish a scientific basis for their traditional use. Therefore, three ethnomedicinal plants were selected from Mizoram in this study.

Mizoram is a small state in India and is located in its northeastern region. It is one of the 25 hottest spots of the mega biodiversity in the world, where more than 400 species of plants are found. The native population of Mizoram uses these plants reasonably for their health needs [9]. *Achyranthes bidentata* L. (Amaranthaceae) is an herb and traditionally crushed leaves juice used for the treatment of poisoned leach bite [9]. *Adhatoda zeylanica* (Acanthaceae) is an herb and traditionally used for the treatment of chronic bronchitis, anti-diarrhea, antirheumatism, insecticidal, anti-periodic, antithelminthic, antiseptic, antigornorrhic and antispasmodic [9]. *Aeginetia indica* L. (Orobanchaceae) is an ephemeral and traditionally used for the treatment of mumps, inflammatory glands and for fertility [9].

To evaluate the pharmacological significance of these selected plants, qualitative and quantitative phytocompound screening, antibacterial, antifungal and antioxidant assays were performed. As far as we know, for the determination of the pharmacological properties of selected experimental species, studies in this study have not been reported elsewhere for analyzing the three solvent analyzes of new and different polarities.

MATERIALS AND METHODS:

Sampling and Identification: *Achyranthes bidentata* L., *Adhatoda zeylanica* and *Aeginetia indica* L. were collected from the Champhai district of Mizoram in the year 2018. All plants were identified by the elders of the Mizo community and certified by the Department of Horticulture and Aromatic Medicinal Plants, Mizoram University, Aizawl, India. Collected plant samples are stored in the herbarium of the department of zoology, Mizoram University, Aizawl, India. The details about these ethnomedicinal plants such as local/Mizo name, family, habitat, and voucher number are given in **Table 1**.

Preparation of Extracts of Plants: Mature and healthy leaves of *Achyranthes bidentata* L., *Adhatoda zeylanica* and *Aeginetia indica* L. were gathered, air-dried and grinded. Three distinct polarity index solvents, water, methanol, and acetone, were used to extract by cold maceration method at room temperature for 48 h. The individual extracts were filtered using a single layer of gauze cloth and a Whatman filter paper no. 42 (125 mm), and dried using a rotary evaporator (Sigma-Aldrich) at 30 °C ± 2. For further experimental procedures, dried extracts were kept in the refrigerator at 4 °C.

Phytochemical (Qualitative and Quantitative) Testing: According to Raman, (2006); Gupta *et al*., (2013), various biochemical experiments have been used with certain modifications [10, 11].

Detection of Alkaloids: In 1 ml of each plant extract, 2-3 drops of Dragendroff's reagent were added and the formation of orange-brown color demonstrates the existence of alkaloids.

Detection of Amino Acids: When boiled with 0.2% ninhydrin solution, the test solution would lead in the formation of violet color, indicating the existence of free amino acids.

Detection of Anthraquinones: Few drops of 2% of HCl have been added to samples, and red color precipitate indicates the anthraquinone presence.

Detection of Cardiac Glycosides: Add 2 ml of acetic acid and two drops of $FeCl_3$ + 1 ml of H_2SO_4 in a 1 ml sample and the formation of a brown ring at the interface attesting to the presence of cadiacidal glycosides.

Detection of Carbohydrates: The test solution is mixed with a few drops of Benedict's regent and warmed in a water bath, which is observed for a reddish-brown formation showing positive results for the presence of carbohydrate.

Detection of Flavonoids: To the sample, 1 ml of NaOH (2N) has been added, and the yellow color showed the presence of flavonoid.

Detection of Phenols: 1 ml of 5% $FeCl_3$ was added to samples, and the blue or green indicates the presence of phenols.

Detection of Phlobatannins: A few drops of ammonia were added to the samples, and the pink deposit appearance refers to phlobatannins.

Detection of Proteins: The test solution was treated with 10% NaOH solution and 2 drops of 0.1% $CuSo_4$ solution and observed to form a purple/pink color.

Detection of Saponins: 20 ml of distilled water was added to the samples, and the dilute solution shaken vigorously. The formation of the foam layer refers to saponins.

Detection of Steroids: Added 1 ml of chloroform and a few drops of H_2SO_4 and a brown ring or bluish brown ring appearance suggests the existence of steroids.

Detection of Tannins: 2 ml of 5% $FeCl_3$ was added to specimens, and tannins were formed in the dark blue or greenish-black.

Detection of Terpenoids: In samples, 2 ml of chloroform and 1 ml of concentrated H2SO4 were added and the existence of terpenoids was confirmed by brown ring formation at the interface.

Quantitative Phytochemical Screening: Quantitative estimation of total alkaloids, flavonoids, phenols, proteins, saponins, and tannins were performed by following the previously reported methods [12, 13].

Alkaloid quantification: C_2H_5OH added 2.5 grams of each plant powder to 100 ml of 10% CH_3COOH. It was filtered after 4 h and filtrate lowered to $1/4^{th}$. Up to precipitation, NH_4OH was added dropwise. Precipitates have been cleaned and filtered with diluted NH4OH. The residue gathered has been dried and weighed. The following formula was used to estimate total alkaloid content.

% of total alkaloid = (Final weight / Initial weight) × 100

Flavonoid Quantification: In 25 ml of 80% aqueous methanol, 2.5g of each plant powder were added. The solution was filtered, evaporated, and weighed to dryness. The following formula has been used to estimate total flavonoid concentration.

% of total flavonoid = (Final weight / Initial weight) × 100

Saponins Quantification: In 25 ml of 20% aqueous ethanol, 2.5g of each plant powder were added. The mixture has been decreased and filtered at 55 °C. A concentrated solution has been incorporated into a separate funnel. Then 5ml of diethyl ether was added to the funnel, and now the entire solution was strongly shaken. Two distinct layers have been created, the aqueous layer has been gathered, and the ether layer has been removed. 10ml of butanol was added, and 5 ml of 5% aqueous NaCl washed over the whole mixture. The solution was heated and oven-dried at 40 °C for constant weight. The total content of saponins was estimated using the formula below:

% of total saponins = (Final weight / Initial weight) × 100

Phenol Quantification: Various concentrations of Gallic acid (10-100 µg/ml) in methanol have been prepared. It took 0.5 ml of separate Gallic acid concentrations and added 2.5 ml of 10 fold diluted Folin's reagent and 2 ml of 7.5% Na_2CO_3 to these Gallic acid concentrations.

Incubated solutions for 30 min and read absorbance at 760 nm. Like Gallic acid, 0.5 ml of the sample was treated. The complete phenolic content was determined by placing the absorbance value in the standard curve equation, where y shows the sample absorption at 760 nm, and x shows the total phenolic content.

Tannins Quantification: 0.1 ml was taken from various Gallic acid concentrations, and in addition to these Gallic acid concentrations, 0.5 ml Folin's reagent and 1 ml of 35% Na_2CO_3 were added. Solutions were allowed to incubate for 30 min, and a UV-Vis spectrophotometer was used to read absorbance at 725 nm. Like Gallic acid, 0.1 ml of the sample was treated. The complete content of tannins was determined by placing the absorbance value in the standard curve equation where y is the sample absorption at 725 nm, and x is the total content of tannins.

Protein Quantification: The concentration of protein was estimated using the Bradford technique (1976) based on Coomassie Blue binding of protein and measurement of protein-dye complex absorbance.

Anti-microbial Studies:

Anti-bacterial and Anti-fungal Activity: For the antibacterial activity, the technique of disc diffusion was adopted [14]. Ciprofloxacin was used as a standard at a concentration of 10 mcg/disc. The filter paper impregnated with extracts (in each extract at a concentration of 20 mg/ml separately) and ciprofloxacin discs were placed aseptically on the seeded agar medium (Bio-zone Research Technologies Pvt. Ltd., Chennai), which was already swabbed with the test organisms (*Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis*) and incubated at 37 °C for 24 h. The inhibition area in mm has been evaluated.

The studied plant extracts antifungal activity was determined by disc diffusion technique against *Candida albicans* and *Aspergillus niger*. Ketoconazole was used as a standard at a concentration of 10 mcg/disc. The filter paper disc impregnated with various extracts (20 mg/ml) individually and ketoconazole disc were placed aseptically on the seeded sabouraud dextrose agar medium (SDA) (Bio-zone Research Technologies Pvt. Ltd., Chennai) which was already swabbed with the test organisms and incubated at 37 °C for 48 h. The zone of inhibition (in mm) was measured and recorded.

Antioxidant Activity: Free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay and reducing power assay were carried out using reported techniques [15, 16]. To determine their antioxidant activity,

various concentrations (50 µg/ml, 100 µg/ml, and 200 µg/ml) of plant extracts were used. Ascorbic acid (Sigma-Aldrich) has been used for positive control.

Free Radical DPPH Scavenging Activity: 1.5 ml of samples were mixed with DPPH solution 1.5 ml (0.1mM). Ascorbic acid was the standard. By mixing 1.5 ml of methanol and 1.5 ml (0.1mM) DPPH solution, a negative control was prepared. At room temperature in the dark, all solutions were incubated for 30 min, and absorbance was read at 517 nm. The calculation of percentage inhibition using the formula given below:

% of inhibition = $(A_0 - A_1 / A_0) \times 100$

Where A_0 = absorbance of the control and A_1 = absorbance of the sample.

Reducing Power Activity: The respective plant extract (0.5 ml) was mixed with 0.5ml of phosphate buffer and 0.5 ml of potassium ferricyanide. For 20 minutes, the solution was evaporated at 50 °C. It added to 0.5 ml of 10% trichloroacetic acid after cooling. The solution was centrifuged for 10 min at 3000 rpm, and 1.5ml of distilled water and 0.1ml of 0.1% $FeCl_3$ were added to the supernatant. The absorbance was read at 700 nm after 10 minutes of incubation. High absorbance value shows elevated power reduction.

Statistical Analysis: All the experiments were performed five times (N=5). The values were displayed as the mean ± standard error mean (S.E.M) in each group. One way ANOVA experiments were conducted to calculate the value of *p*. It was regarded that the *p*-value less than or equal to 0.05 was significant.

RESULTS:

Evaluation of Qualitative and Quantitative Phytocompounds: All types of well-known plant components such as alkaloids, tannins, saponins, steroids, phlobatannins, terpenoids, flavonoids, cardic glycosides, anthraquinones, carbohydrates, polyphenols, proteins, and amino acids were present on *Achyranthes bidentata* L., *Adhatoda zeylanica*, and *Aeginetia indica* L. respectively. Alkaloids and tannins were present in all extracts of all three plants, **Fig. 1**. Phlobatannins and cardiac glycosides were absent in aqueous extract of all three plants, **Fig. 1A, D,** and **G**. Methanol extract of *Achyranthes bidentata* L. contained all types of phytocompounds, whereas terpenoids and steroids were absent in methanol extract of *Adhatoda zeylanica*, and *Aeginetia indica* L. **Fig. 1B, E,** and **H**. Cardiac glycosides, terpenoids, saponins, steroids, proteins, and amino acids were absent in acetone extract of all three plants **Fig. 1C, F,** and **I**.

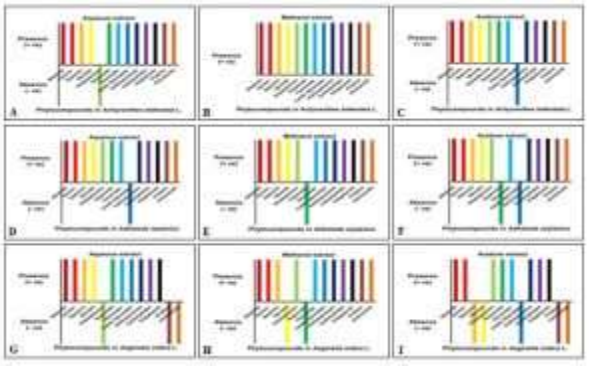

FIG. 1: QUALITATIVE PHYTOCHEMICAL SCREENING IN SELECTED THREE ETHNOMEDICINAL PLANTS. PHYTOCOMPOUNDS IN AQUEOUS (A, D AND G), METHANOL (B, E AND H) AND ACETONE (C, F AND I) EXTRACTS OF *ACHYRANTHES BIDENTATA* L., *ADHATODA ZEYLANICA* AND *AEGINETIA INDICA* L. RESPECTIVELY

Quantitative analysis of all three plants was revealed that alkaloids content was more in *Aeginetia indica* L. (378.37 ± 35.02) followed by *Adhatoda zeylanica* (302.20 ± 30.92) and *Achyranthes bidentata* L. (92.80 ± 23.53) **Fig. 2A**. Phenol concentration was present excessively in *Aeginetia indica* L. (158.96 ± 15.28)

followed by *Adhatoda zeylanica* (85.79 ± 7.48) and *Achyranthes bidentata* L. (45.86 ± 5.3) **Fig. 2B**. Flavonoids and tannins concentration were found extra in *Aeginetia indica* L. (85.68 ± 7.89 and 187.87 ± 25.48) than to *Adhatoda zeylanica* (25.91 ± 4.14 and 73.38 ± 7.11) and *Achyranthes bidentata* L. (15.17 ± 2.01 and 13.37 ± 3.15) **Fig. 2C** and **E** whereas saponins and proteins concentration were more in *Adhatoda zeylanica* (54.86 ± 7.27 and 70.72 ± 7.46 compared to *Achyranthes bidentata* L. (38.59 ± 5.94 and 13.37 ± 2.15) and *Aeginetia indica* L. (15.85 ± 2.05 and 35.89 ± 5.87) **Fig. 2D** and **F**. One way ANOVA was done among the ethnomedicinal plants as well as the values were showed in mean ± standard error mean (S.E.M). Different letters indicate significant ($p < 0.05$) variation among the study plants **Fig. 2**. All concentrations of phytocompounds expressed in mg/g.

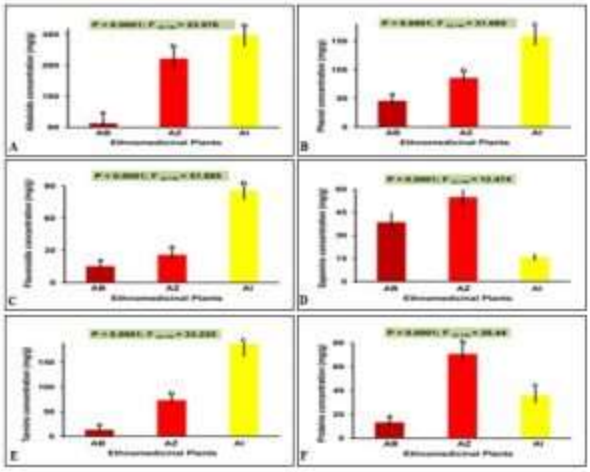

FIG. 2: QUANTITATIVE PHYTOCHEMICAL SCREENING IN SELECTED THREE ETHNOMEDICINAL PLANTS. ALKALOID CONCENTRATION (A), PHENOL CONCENTRATION (B), FLAVANOID CONCENTRATION (C), SAPONINS CONCENTRATION (D), TANNINS CONCENTRATION (E) AND PROTEINS CONCENTRATION (F) OF *ACHYRANTHES BIDENTATA* L., *ADHATODA ZEYLANICA,* AND *AEGINETIA INDICA* L. RESPECTIVELY. THE VALUES WERE EXPRESSED AS MEAN ± STANDARD ERROR MEAN (N=5). ONE WAY ANOVA WAS PERFORMED; DIFFERENT ALPHABETS SHOW SIGNIFICANT DIFFERENCE AMONG THE PLANTS. P- VALUE IS < 0.05. AB, AZ AND AI *DENOTEACHYRANTHES BIDENTATA* L., *ADHATODA ZEYLANICA* AND *AEGINETIA INDICA* L. RESPECTIVELY. ALL PHYTOCOMPOUNDS CONCENTRATION HAS BEEN EXPRESSED IN mg/g.

DPPH Scavenging and Reducing Power Assays: Methanol extract of three plant species showed higher antioxidant potential (Free radical DPPH removal activity) than in their respective aqueous and acetone extract. The highest antioxidant activity was tested for *Aeginetia indica* L. methanol extract (99.28% ± 10.47), which is very close to standard activity (99.45% ± 10.68), *i.e.* ascorbic acid, aquatic extraction of *Achyranthes bidentata* L. showed lower antioxidant activity (25.54% ± 1.54). All extracts of *Aeginetia indica* L. showed the potential of greater antioxidant potential than two other plant extracts **Fig. 3A, B,** and **C**. The values were expressed in mean ± standard error mean (S.E.M).

Increasing absorption is an indication of the increase in reducing power activity in plant extracts. The highest absorption values were tested in the methanol extract of the *Aeginetia indica* L. (0.587, 0.687, and 0.859 absorbance values with respect to the extract concentrations 50 µg/ml, 100 µg/ml and 200 µg/ml), which shows the greatest reduction among all plant extracts. The lowest reduction is possible due to the aqueous extract of *Achyranthes bidentata* L. **Fig. 3D** among all plant extracts of the lowest absorption value (0.085,

0.124 and 0.131 absorbance values with respect to the extract concentrations 50 µg/ml, 100 µg/ml and 200 µg/ml). Ascorbic acid used as a standard.

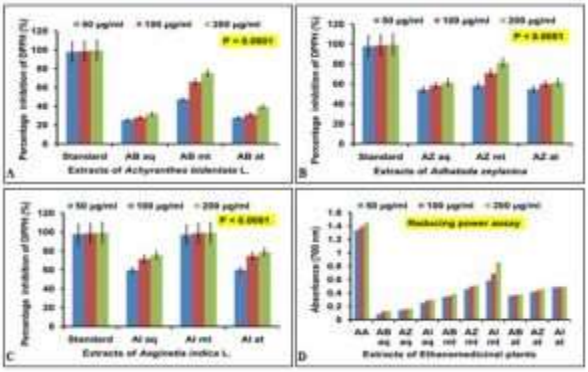

FIG. 3: PERCENTAGE INHIBITION OF DPPH (%) ACTIVITY (A, B AND C) AND REDUCING POWER ACTIVITY (D) OF *ACHYRANTHES BIDENTATA* L., *ADHATODA ZEYLANICA* AND *AEGINETIA INDICA* L. WERE MEASURED WITH VARIOUS CONCENTRATIONS (50 µg/mL, 100 µg/mL AND 200 µg/mL) ALONG WITH ASCORBIC ACID AS A STANDARD. WHERE AB, AZ, AND AI, DENOTES*ACHYRANTHES BIDENTATA* L, *ADHATODA ZEYLANICA* AND *AEGINETIA INDICA* L. RESPECTIVELY AS WELL AS AQ, MT AND AT REFERS TO AQUEOUS, METHANOL AND ACETONE EXTRACTS RESPECTIVELY. THE VALUES WERE EXPRESSED AS MEAN ± STANDARD ERROR MEAN (N = 5)

Anti-bacterial and Anti-fungal Activity: All plant extracts tested in this study showed anti-bacterial and fungal activity **Fig. 4** and **5**. *Aeginetia indica* L. methanol extract showed great inhibitory activity (mm) against *Escherichia coli* (22.8 ± 5.06, **Fig. 4A**), *Pseudomonas aeruginosa* (22.8 ± 5.06, **Fig. 4B**), *Bacillus subtilis* (25.4 ± 5.21, **Fig. 4C**), *Candida albicans* (19.4 ± 2.05, **Fig. 5A**) and *Aspergillus niger* (19.8 ± 1.48, **Fig. 5B**) followed by methanol extracts of *Adhatoda zeylanica* and *Achyranthes bidentata* L. **Fig. 4** and **5**. Aqueous extract of *Achyranthes bidentata* L. showed low anti-bacterial and fungal activity against *Escherichia coli* (6.2 ± 1.24, **Fig. 4A**), *Pseudomonas aeruginosa* (4.2 ± 0.05, **Fig. 4B**), *Bacillus subtilis* (9.3 ± 1.01, **Fig. 4C**), *Candida albicans* (5.6 ± 0.08, **Fig. 5A**) and *Aspergillus niger* (8.5 ± 1.05, **Fig. 5B**) followed by aqueous extracts of *Adhatoda zeylanica* and *Aeginetia indica* L. **Fig. 4** and **5**. Acetone extracts of studied plants were showed moderate antibacterial and fungal activity. The values were expressed in mean ± standard error mean (S.E.M).

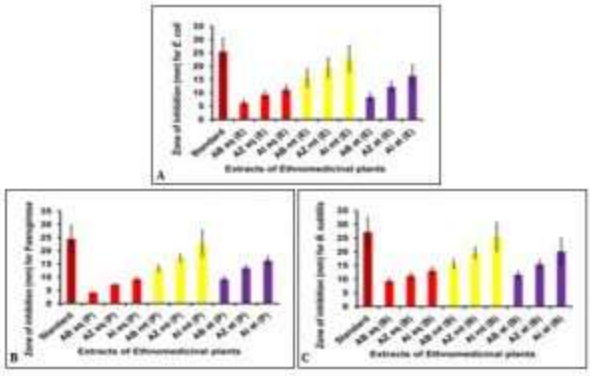

FIG. 4: ZONE OF INHIBITION (mm) OF *ESCHERICHIA COLI* [E; (A)], *PSEUDOMONAS AERUGINOSA* [P; (B)] AND *BACILLUS SUBTILIS* [B; (C)] OF *ACHYRANTHES BIDENTATA* L, *ADHATODA ZEYLANICA* AND *AEGINETIA INDICA* L. ALONG WITH STANDARD

DRUG (CIPROFLOXACIN, 0.01 mg/mL). WHERE AB, AZ, AND AI, *DENOTESACHYRANTHES BIDENTATA* L, *ADHATODA ZEYLANICA,* AND *AEGINETIA INDICA* L. RESPECTIVELY AS WELL AS AQ, MT AND AT REFERS TO AQUEOUS, METHANOL, AND ACETONE EXTRACTS RESPECTIVELY. THE VALUES WERE EXPRESSED AS MEAN ± STANDARD ERROR MEAN (N=5)

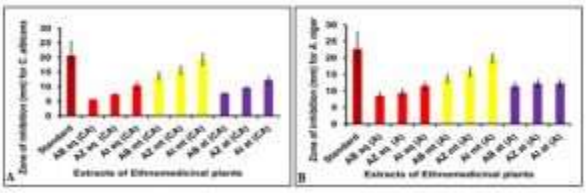

FIG. 5: ZONE OF INHIBITION (mm) OF *CANDIDA ALBICANS* [CA; (A)] AND *ASPERGILLUS NIGER* [A; (B)] OF *ACHYRANTHES BIDENTATA* L., *ADHATODA ZEYLANICA* AND *AEGINETIA INDICA* L. ALONG WITH STANDARD DRUG (KETOCONAZOLE, 0.01 mg/mL). WHERE AB, AZ, AND AI, DENOTES*ACHYRANTHES BIDENTATA* L., *ADHATODA ZEYLANICA* AND *AEGINETIA INDICA* L. RESPECTIVELY AS WELL AS AQ, MT AND AT REFERS TO AQUEOUS, METHANOL, AND ACETONE EXTRACTS RESPECTIVELY. THE VALUES WERE EXPRESSED AS MEAN ± STANDARD ERROR MEAN (N=5)

DISCUSSION: This is the first-ever report on aqueous, methanol, and acetone extracts of *Achyranthes bidentata* L., *Adhatodazey lanica* and *Aeginetia indica* L. were collected from the Mizoram. These plant extracts have been utilized for the study of various biological parameters. Qualitative analysis of *Achyranthes bidentata* L. has no previous reports. However, our results were similar to previous reports [17]. Who have done a phytochemical analysis of various parts of *Achyranthes aspera* revealed the presence of different secondary metabolites. As well as the methanol extract of *Achyranthes bidentata* L. showed the presence of carbohydrate and saponins in the present study while these results were not similar to previous study [18]. This is due to the geographical variation of the sampling site. Sharif *et al*., (2018) collected from Bangladesh while we collected from Mizoram, India [18].

In the current study, *Adhatoda zeylanica* plant extracts also showed the presence of various phytochemicals such as alkaloids, tannins, saponins, steroids, flavonoids, carbohydrates, polyphenols, proteins, and amino acids. Our present study results were agreed with past study of Tandel *et al*., (2018) [19]. In the past study, the authors have conducted experiments on ethanol extract of *Adhatoda zeylanica* plant leaves, and they found the presence of alkaloids, tannins, steroids, carbohydrates, polyphenols, and proteins like present study results. Phytochemical screening of *Aeginetia indica* L. showed the presence of different secondary metabolite in this study while no work has done extensive manner on qualitative phytochemical screening of *Aeginetia indica* L. However, our results were similar to the previous study revealed the presence of flavonoids in methanol extract of *Aeginetia indica* L.[20]

No previous reports regarding quantitative phytochemical screening of selected study plants in this research while as per Kumari *et al*., (2018); Ahmed *et al*., (2013) the phytochemical quantification of aqueous, methanol, chloroform, and hexane extracts of *Amaranthus viridis* belongs to the family Amaranthaceae (*Achyranthes bidentata* L's family) showed greater concentration of saponins and lower concentration of tannins whereas in the current study alkaloid content is more and tannins content is less showed little deviation from the past studies due to two different plants from the same family collected from different locations [21, 22]. *Adhatoda vasica* contains more alkaloids reported by Khan *et al*., (2011) [23]. *Adhatoda zeylanica* contains more alkaloids as same as *Adhatoda vasica* while the quantity is varied due to the

different species and collected from different geographical locations. So, our results were agreed with Khan *et al*., (2011) [23].

Methanol extracts of *Achyranthes bidentata* L., *Adhatoda zeylanica,* and *Aeginetia indica* L. were showed greater antioxidant potential against free radicals (DPPH) whereas methanol extract of *Aeginetia indica* L. showed highest antioxidant activity is similar to the standard molecule is ascorbic acid. Similarly, very low antioxidant properties against DPPH were observed in aqueous extract of *Achyranthes bidentata* L. Amongthree studied plants, *Aeginetia indica* L. showed greater antioxidant properties against DPPH. Previous studies reported that secondary metabolites in ethnomedicinal plants act as an antioxidant because of their strength to remove free radicals similarly antiviral, anti-inflammatory, antioxidant, antimicrobial, antimutagenic, and chemopreventive activities [24]. In the current study, the presence of secondary metabolites could be the reason showed greater antioxidant properties against DPPH.

Absorbance increase indicates higher reductive potentials. Higher absorbance value was observed in methanol extract of *Aeginetia indica* L. whereas lowest absorbance value was observed in aqueous extract of the *Achyranthes bidentata* L. which means that methanol extract of *Aeginetia indica* L. showed greater reducing potential as compared to aqueous extract of *Achyranthes bidentata* L. All methanolic extracts of all plants showed greater reducing power as compared to the other plant extracts. Among three plants, *Aeginetia indica* L. was expressed highest reducing potentials due to the highest absorbance value. According to Jayanthi and Lalitha (2011), greater absorbance means greater reduction of power, which in turn leads to greater antioxidant properties [25]. According to previous report [26], potential reduction increases, while an increase in the concentration of extracts similarly in this study also observed an increase in the reducing activity due to the increase in the concentration of the extract.

In our study, all extracts of *Achyranthes bidentata* L, *Adhatoda zeylanica,* and *Aeginetia indica* L. were showed anti-bacterial and anti-fungal activities against studied pathogens. The methanol extract of *Aeginetia indica* L. was showed greater anti-bacterial and anti-fungal activities against studied pathogens. Lowest anti-bacterial and anti-fungal activities against studied pathogens were observed in aqueous extract of *Achyranthes bidentata* L., whereas methanol extracts of all plants showed the highest anti-bacterial and anti-fungal activities against studied pathogens. *Aeginetia indica* L. was showed great anti-bacterial and anti-fungal potentials compared to two other plants.

Jayapriya and Gricilda Shoba, (2015) has reported that anti-microbial activities of leaves of *Justicia adhatoda* (Linn) against *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, and *Candida albicans* [14]*.* Our results were agreement with Jayapriya and Gricilda Shoba, (2015) study [14]. Alkaloids, phenols, and tannins presence can contribute to the stronger anti-microbial properties in *Aeginetia indica* L. compared to two other plants, have a lower amount of alkaloids, phenols, and tannins.

Saboo *et al*., (2013); Kazmi *et al*., (2019) have observed great anti-fungal activity whereas in this study also observed great anti-fungal activity although we have some limitations with previous studies because of the reason behind it can be the sampling sites from different locations play an important role in consisting of different phytocompounds [26, 27].

The greater antioxidant, antimicrobial, and reducing power activities depend upon the phytocompounds present in the extracts of selected study plants such as *Achyranthes bidentata* L., *Adhatoda zeylanica* and *Aeginetia indica* L. Alkaloids, phenols and tannins presence can contribute to the stronger antioxidant, antimicrobial, and reducing power properties in *Aeginetia indica* L. compared to two other plants have lower amount of alkaloids, phenols, and tannins. Other studies also reported that alkaloids,

phenols and tannins presence play a major role in antioxidant, antimicrobial, and reducing power properties [24].

CONCLUSION: The plants selected in this study have all the vital phytocompounds. These compounds were accountable for important antioxidant and anti-microbial activities as exposed to this research. The current research enlightenments *in-vivo* studies to study the important antioxidant and anti-microbial activities, and similarly, this study encourages the road map to isolate important compounds from these plants as well as the development of novel drugs to treat various diseases.

ETHICAL MATTER: NA

ACKNOWLEDGEMENT: All authors were grateful to the Department of Zoology, Mizoram University, for instrument facility.

CONFLICTS OF INTEREST: Authors declared that there is no conflict of interest

AUTHORS CONTRIBUTIONS: MKM designed and coordinated the study. MKM and PK carried out the experiments, biochemical analysis, and data analysis and wrote the manuscript. The plant collection and extract preparation were prepared by DS. All authors read and approved the final manuscript.

REFERENCES:

1. Dash B and Sharma BK: Charak Samhita. Chaukhamba Sanskrit Series Office, Varanasi, India. Third Edition 2001.
2. Kong JM, Goh NK, Chia LS and Chia TF: Recent advances in traditional plant drugs and orchids. Acta Pharmacologica Sinica 2003; 24: 7-21.
3. Jagetia GC and Venkatesha VA: Effect of mangiferin on radiation induced micronucleus formation in cultured human peripheral blood lymphocytes. Environmental and Molecular Mutagenesis 2005; 46: 12-21.
4. Cordell GA: Sustainable medicines and global health care. Planta Medica 2011; 77: 1129-38.
5. Balouiri M, Sadiki M and Ibnsouda SK: Methods for in vitro evaluating antimicrobial activity: A review. Journal of Pharmaceutical Analysis 2016; 6: 71-79.
6. Ramakrishna A and Ravishankar GA: Influence of abiotic stress signals on secondary metabolites in plants. Plant Signaling & Behavior 2011; 6(11): 1720-31.
7. Acharya SN, Basu SK, Banik SD and Parasad R: Genotype X environment interactions and its impact on use of medicinal plants. Open Nutraceuticals Journal 2010; 3: 47-54.
8. Rao RR: Traditional knowledge and sustainable development, Key role of Ethnobiologist. Journal of Ethnobotany 1996; 8: 14-24.
9. Rai PK and Lalramnghinglova H: Ethnomedicinal plant resources of Mizoram, India: Implication of traditional knowledge in healthcare system. Ethnobotanical Leaflets 2010; 14: 274-05.
10. Raman N: Phytochemical Technique. New Indian Publishing Agencies. New Delhi, 2006: 19.
11. Gupta A, Joshi A and Dubey B: Comparative pharmacognostical and phytochemical evaluation of two species of Cyathocline. International Journal of Biomedical Research 2013; 4(10): 538-45.
12. Bradford M: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 1976; 72: 248-54