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Phytochemical Screening, Antioxidant and Antibacterial Activity of Some Medicinal Plants Grown In Sylhet Region

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Abstract: *Five medicinal plants such as Melastoma malabathricum, Mimosa pudica, Ricinus communis, Solanum torvum, Alocasia macrorrhiza were employed to investigate the presence of various phytochemicals using different biochemical tests, to determine total phenolic content based on Folin-Ciocaltaeus reagent method, total flavonoid contents using aluminium chloride colorimetric method, antioxidant potentiality according to DPPH assay and antibacterial efficacy by disc diffusion assay of the selected medicinal plants. The phytochemical screening exhibited that the extracts were enriched with the existence of alkaloid, steroids, phenols, terpenoids, flavonoids, saponin, quinones, coumarins etc. Presence of phytochemicals varied from plant to plant. Quantitative analysis of phenolics revealed that Melastoma malabathricum contained maximum phenolics compound counting 76.29 mg/g gallic acid equivalent (GAE) of dry leaves powder. Total flavonoids content was found to be highest in Ricinus communis extract amounting 23.23mg/g quercetin equivalent(QE) of dry leaves powder. Mimosa pudica and Melastoma malabathricum extract possessed the highest free radical scavenging activity against DPPH. IC_{50} values, obtained by DPPH activity, was very high in Heliotropium indicum (228.58µg/ml). In contrast, lowest value of IC_{50} was found in Mimosa pudica (32.46µg/ml) which was followed by Melastoma malabathricum (48.90 µg/ml) and Ricinus communis (54.84µg/ml). IC_{50} values were shown inversely proportional to the free radical scavenging activity. Furthermore, antibacterial activities of methanolic extracts of selected plant's leaves were analyzed against five clinically significant organisms (Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella sp, Staphylococcus aureus). Comparing six medicinal plants, the crude extract of Melastoma melabathricum and Solanum torvum exhibited potent antibacterial activity against all studied bacterial strains (11-16.75 mm zone of inhibition). Other four medicinal plant's extracts showed antibacterial activity against different bacterial strains. To broaden this study, further in vivo models are essential for proving the effectiveness of leaves as candidate drug.*

Keywords: *Medicinal plant, phytochemical, antioxidant, antibacterial activity*

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I. Introduction

Our planet is enriched with medicinal plants virtually in all cultures as a repository of medicine (Singh, 2015). The use of herbs as complementary and alternative medicine has increased dramatically in the last 20–25 years (Rios and Recio, 2005). Around 80% of individuals from developing countries relies chiefly on local medicinal plants as their therapeutic aid for mitigating ailments of various diseases (WHO, 2002). Several ethnobotanical survey-based research in Bangladesh suggest that traditional medicinal plants are frequently used especially by impoverished peoples, village dwellers, tribes and ethnic communities as preventive and curative to treat various diseases(Ocvirk, 2013; Rahmatullah, 2010; Khan, 2015). The creation of antibiotic resistant microorganism that has to propel scientist towards the discovery of antibacterial drug. The emergence of multiple drug-resistant strains of microorganisms is increased due to the rashness use or malmanagement of antibiotics and many antibiotics have lost their effectiveness against microorganism (Chowdhury et al., 2015).

Unbalanced generation of free radicals creates abnormal physiological conditions that lead oxidative damage to cells by degrading lipids, proteins and nucleic acids biomolecule (Percival,1998), and in a consequence, overexpression of oncogenes, mutagens formation, induction of atherogenic activity, or inflammation occur. Oxidative stress is suggested to play a pivotal role in the development of many chronic

diseases like cardiovascular diseases, neurodegeneration, cancers, immune disorders, diabetes, aging, and others (Liu, 2013). Oxidative damage caused by reactive oxygen species is repaired by protection mechanisms of antioxidant which convert continuously generated free radicals into less harmful molecules intercepting radical chained reactions (Wichi, 1998). Plant products have been a part of phytomedicines since the early stage of time. Medicinal value can be derived from barks, leaves, flowers, roots, fruits, seeds from various plants (Criagg, 2001). The therapeutic value of plants lies in natural substances that produce a definite physiological action on the human body (Edeoga et al., 2005)

Plant-derived bioactive compounds have a protective role in minimizing oxidative stress. A large number of plant's crude extract contained high oxidative capacity and a remarkable amount of total phenolic compounds (Kahkonen et al., 1999). Natural phytochemicals derived from medicinal plants have gained a lot of recognition in the treatment and management of various diseases in the past two decades (Dyana JP, 2012). Indeed, many studies have been demonstrated that phytochemical constituents in plants like flavonoids, polyphenols, tannins, carotenoids, and phenolic terpenes exert antioxidant activities by quenching free radical production in the body (Mathew, 2015; Ghosh et al., 2013 and Shetty et al, 2008). Use of plant-derived drugs in medical practice has shown that they are relatively non-toxic, safe and even free from serious side effects (Momin, 1987). Hence, scientific research is focused on antimicrobial and antioxidant activities of a number of plant extracts in order to explore an alternative therapy against different types of microorganisms and oxidative reactions (Marasini, 2015). The current study was designed to examine the therapeutic value by analyzing the presence of various phytochemicals, by evaluating the antioxidant and antibacterial activity of few local medicinal plants in Sylhet region.

II. Materials and Methods

Collection of plant sample

The leaves of six medicinal plants were collected from different location in and around Sylhet Agricultural University campus and Tilagor Eco Park, Sylhet. On the basis of morphological characteristics and monograph of leaves, flowers and roots, plant samples were identified and confirmed in department of Plant and Environmental Biotechnology, Sylhet Agricultural University.

Preparation of leaves

The leaves of the selected plants were separated using new scissor and dusts were removed by washing under running tap water, finally rinsed with distilled water. The plant samples were dried in sunbathing for 48 hours to remove water content of leaves and the dry leaves were crushed by electric grinder machine to prepare dry leaves powder. Then, the dried powder was stored in plastic container for experimental use.

Preparation of water, ethanol and methanol extract:

Water, ethanol and methanol solvents were used to prepare three different extract. For the preparation of aqueous, ethanol and methanol extract of each individual plant sample, 5g of dry leaves powder were homogenized with respective solvent using mortar and pestle for 15 minutes. Respective solvent (Water/Ethanol/methanol) was further added to the blended paste in 250 ml conical flask and adjusted to 200ml with different solvent. After that, the sample containing flask was kept at room temperature for 72 hours. Then it was filtered with markin cloth followed by filtering with Whatman's No.1 filter paper. The extract was ready for phytochemical testing.

Phytochemical screening test of the plant

Terpenoids Test: 5 ml of extract was mixed with 2 ml of $CHCl_3$ in a test tube. 3 ml of concentrated H_2SO_4 was carefully added to the mixture to form a layer. An interface with a reddish brown coloration was formed for the presence of terpenoids (Trease and evans, 2002).

Flavonoids Test: Sodium hydroxide test and shinoda test were used for the presence of flavonoid. Precipitation of yellow colouration formed from the addition of 2ml 10% aqueous sodium hydroxide solution indicates the presence of flavonoids. Yellow colour turns into colorless on addition of dilute hydrochloric acid. In case of shinoda test, the addition of few pieces of magnesium chips along with 2 drops of conc. HCl in extract creates red or pink colour that shows the presence of flavonoids (Peach and Tracey, 1956).

Alkaloids Test: A reddish-brown or orange red precipitation after the addition of few drops of Wagner's reagents or Dragendorff's reagent considered as the positive test for alkaloids (Harborne, 1998; Trease and Evans, 2002) .

Test for Tannins: Brownish green or a blue-black colouration obtained from the summation of 2-3 drops of 5% ferric chloride solutions to the extract shows the presence of tannin (Ciulci, 1994).

Sterols (Salkowski's test): 2ml of extract was mixed in 2ml of chloroform. Then 2ml concentrated Sulphuric acid was carefully added to form a layer.A reddish brown coloration of the interface was formed to show positive results for the presence of sterols (Sheel et al, 2014)

Phenolic compound test: 3 drops of this freshly prepared mixture produced from equal amount of 1% ferric chloride solution and 1% potassium ferrocyanide was added to extract. After filtering this solution, a positive result shows the formation of a bluish-green color (William and Wenn, 1972)

Cardiac Glycosides (keller-kiliani test): Concisely, 2 ml of extract was treated with 1 ml of glacial acetic acid containing one drop of ferric chloride solution pursued by addition of 1 ml of concentrated sulphuric acid. Appearance of purple ring beneath the brown ring while the formation of a greenish ring in the acetic acid layer is considered as indicative for cardiac glycoside (Harborne, 1998).

Test for Saponins (Froth test): Briefly, 2.5 ml extract was added to 10 ml of sterile distilled water in a test tube. The test tube was closed with cap and shaken vigorously for about 30 second. It was then allowed to stand for half an hour. Honeycomb froth indicated the presence of saponins (Harborne, 1998).

Anthraquinone Test: 1ml benzene in combination with 1ml of 10% ammonia treated with the extract produces pink, red or violet colouration that indicates the presence of anthraquinones (Trease and Evans, 1989).

Test for Quinones and Coumarins: Blue green or red precipitations confirms the presence of quinones after adding 10% NaOH into the test sample whereas yellow color developed from the same test indicates the presence of coumarin (Harborne, 1998).

Determination of Total Phenolic Content

The total phenolic content (TPC) was determined using gallic acid as a standard, according the method described by Keskin-Sasic et al. (2012) with slight modification. 10mg pure standard gallic acid was mixed with 80ml distilled water in a100ml volumetric flask and final volume (100ml) was adjusted by dropwise addition of distilled water to get the standard gallic acid concentration 0.1mg/ml. Serial Dilutions was performed to prepare varying concentration (12.5, 25, 50, 75, 100 µg/ml) of gallic acid. Blank solution comprises 0.5 ml FCR, 1ml 7.5% Na_2CO_3 and 5.5 ml distilled water to prepare blank solution. 2N commercially available FCR reagent was diluted at a ratio of 1:10 with distilled water. To prepare 7.5% sodium carbonate solution (Na_2CO3), 7.5 g Na_2CO_3 was mixed well with distilled water and harmonized to make the volume upto 100ml. The reaction mixture was made by mixing 0.5 ml FCR reagent, 1 ml extract or different concentration of standard, 1 ml 7.5% Na_2CO_3 (after 3 minutes) and 4.5 ml distilled water. It was then kept at room temperature for at 20 minutes to complete whole reaction. The blue colour intensity was recorded at 680 nm against the reagent blank. Finally, the content of total phenolic compounds was determined using a reference curve of gallic acid concentration.

Determination of Total Flavoind Content

A standard Aluminium chloride colorimetric protocol was followed to estimate total flavonoid content (Chang et al., 2002). Quercetin solutions of various concentrations were used to make the standard calibration curve. 10mg of quercetin was dissolved in 100ml methanol and then serial dilution was performed to make different concentration (12.5, 25, 50, 75,100 µg/ml) of standard compound quercetin using methanol. To perform the assay for the estimation of total flavonoid content, firstly 3 ml methanol was taken to 1ml extract of different plant samples or 1ml of varying concentration of standard separately in test tubes. Then, 0.2ml of 10% aluminium chloride solution and 0.2ml of 1M potassium acetate solution and finally 5.6 ml distilled water were unified into each separated test tube containing standard or solely different plant extract and mixed well. After filtering all the prepared solutions through Whatman no-1 filter paper, their absorbance was measured. Sample blank was prepared in a similar way by replacing aluminium chloride solution with distilled water. Each test tube was incubated at room temperature for at least 30 minutes to complete reaction. The intensity of yellow color was measured at 420 nm against the suitable blank. Absorbance against concentration was plotted to prepare the calibration curve. Total flavonoid content was expressed as mg of quercetin equivalent (QE) per gram of dry leaves powder (DLP).

Determination of anti-oxidant activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) Scavenging Assay:

Active antioxidant ability of plant extracts was determined by virtue of DPPH free radical scavenging assay as the method described by Brand-William et al. (1995) and Susanti et al. (2007) with slight modifications. DPPH becomes colorless or pale yellow when neutralized by the chemical reaction.

The readiness of DPPH solution and preparation of standard ascorbic acid: 4.0mg dark-violate colored DPPH powder was dissolved in 100ml of 95% methanol in order to prepare 0.004%(w/v) deep violate DPPH solution which was kept in a dark condition at room temperature. Varying concentration (25, 50, 75, 100ug/ml) of ascorbic acid solution was prepared from the stock solution of 0.1 mg/ml concentration in methanol. The L-ascorbic acid was highly dissolved in methanol and water as compared to other solvents (Shalmashi and Eliassi, 2008)

Preparation of Plant extract and control: 5mg of dry leaves powder was vortexed for 20minutes in 10ml methanol to make 0.5mg/ml concentration. It was then left at room temperature for 48 hours in soaking

condition. After that it was filtered and the filtrate was used for serial dilution to prepare varying concentration (25, 50, 75, 100ug/ml) of the solution. 3ml DPPH and 1ml methanol solution were used as control.
Procedure of DPPH radical scavenging activity: 1ml of each extract or standard at various concentrations (100, 75, 50, 25µg/mL) were added to 3 ml of freshly prepared DPPH solution (0.004%) in methanol. The resulting mixture was allowed to stand for 30 min in a dark place and absorbance was recorded at 520 nm. The degree of decolorization of DPPH is proportional to the scavenging efficiency of the extract.
The following equation was used to calculate the percentage of inhibition of DPPH free radical scavenging activity:

Percentage(%) of inhibition = [($ABS_{CONTROL}$ −ABS_{SAMPLE}) / $ABS_{CONTROL}$] × 100;
Where, $ABS_{CONTROL}$ denotes absorbance of DPPH + methanol and ABS_{SAMPLE} denotes the absorbance of DPPH + extract/standard
IC_{50} value was determined from the plotted graph of percentage of radical scavenging ability against various concentration of extract, where IC_{50} value denotes the effective concentration of extract necessary for 50% diminution of initial DPPH radical concentration.

Determination of anti-bacterial activity

10 grams of powdered leaves of each plant were weighted and then mixed in 100 ml of the 80% methanol for 72 hours. The supernatant phase was harvested through filtration by Whatman no. 1 filter paper. Autoclaved petridish containing the filtered solution was heated in an oven to evaporate methanol solvent and the remaining dried crude extract was collected and all the dried plant extract were dissolved in DMSO to prepare a final concentration of 50 mg/ml. The pure cultures of five strains of different microbes (*Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniaea, Pseudomonas areoginosa, Salmonella sp*) were collected from the Department of Genetic Engineering and Biotechnology, Shahjalal University of Science and Technology, Sylhet. A standard agar disc diffusion protocol (also known as Kirby-Bauer method) was used to assess the antibacterial potential of different plant extract as the method developed by Bauer *et al.*(1966). Stock culture of test bacteria was cultivated in nutrient broth medium at 37^0C for at least 22 hours. An inoculating loop was used to transfer test bacteria from broth to test tube containing 5 ml sterile distilled water. The inoculum was added until the final bacterial concentration was adjusted to 0.5 Mcfarland turbidometry to attain the optimum turbidity (Burt and Reinderd, 2003). A cotton swab was used for spreading the dried surface of Muller-Hinton agar plate with bacterial suspension. 5mm sterile filter paper discs were prepared using punching machine. Prior to inoculation, these discs were impregnated with 100mg/ml concentrated solution of plant extract. An autoclaved forceps was used to transfer these discs onto solidified agar medium. This was done for all kinds of plant extracts. 37^0C for 24 hours was maintained as an optimum growth condition for test bacteria. DMSO was used as negative control and gentamycin was used as positive control for comparing the results. After 24 hours of incubation, each plate was examined. There was the uniformly circular zone of inhibition on the surface. The diameter of the complete zone of bacterial growth inhibition (judged by unaided eye) was measured and recorded in millimeter.

Data processing

Data obtained from experiments were processed carefully; mean and standard deviation were calculated using Microsoft excel 2007 software. Linear regression analysis was used to calculate the IC_{50} values.

III. Results

Phytochemical screening

The result unveils the presence of medically active compounds in the aqueous, methanolic and ethanolic extract of dry leaves powder in six different plant species such as *Malastoma malabathricum* (MM), *Mimosa pudica* (MP), *Ricinus communis* (RC), *Solanum torvum* (ST), *Alocasia macrorrhiza*(AM), *Heliotropium indicum* (HI). Terpenoids were present in all extracts of studied medicinal plants except aqueous extract of ST and HI, methanolic extract of ST and AM, ethanolic extract of ST and RC. Phenolic compounds were found present in all plant's extract whereas tannins were absent only in ethanolic and methanolic extract of HI. Quinones were absent in all plant samples except *Melastoma malabithricum* extract. All plant species exhibited the presence of flavonoid compounds in all extract of plant samples. There were no saponins present in the aqueous, methanolic and ethanolic extract of MP and methanolic extract of HI. Coumarins were detected in five medicinal plants out of six different plants. Higher presence of steroids observed in extract of *Ricinus communis* as compare to other plant's extract. Comparatively better presence of terpenoids was found in the extract of *Mimosa pudica*. Cardiac glycosides were found to be present in three plant extract out of six plant extract. Anthraquinone was absent in the aqueous extract of five medicinal plant except *Melastoma malabathricum*. Both ethanolic and methanolic extract of *Melastoma malabathricum* and *Ricinus communis*

plant contained anthraquinone. As given in table 2, it was found that cardiac glycosides were present in both ethanolic and methanolic extract of three medicinal plant extracts out of six medicinal plant extract. However, the presence of diverse kinds of phytochemicals was confirmed in phytochemical evaluation tests in all the crude extracts of six medicinal plants as presented in table 1 and table 2.

Table-1: Preliminary phytochemical analysis of screened medicinal plant species (aqueous extract)

Phyto-chemicals	**Test Name**	**MM**	**MP**	**RC**	**ST**	**AM**	**HI**
Terpenoids	$CHCl_3$ test	+ +	+	+	-	+ +	-
Flavonoids	Pb-acetate test	+ +	+	+ +	+	+ +	++
	NaOH test	+	+	+ +	+	+ +	++
	Shinoda test	+ +	+	+ +	+	+	++
Alkaloids	Wagner's test	+ +	++	++	++	+++	++
	Dragen droff's test	+	+	++	+++	+++	++
Tannins	$FeCl_3$ test	+	+ + +	+	+++	+ +	++
Sterols	Salkowski's test	+	+	+ +	-	+ +	+
Phenols	Potassium ferrocyanide test	+ + +	+ + +	+	+++	+ ++	+++
Saponins	Froth test	+ + +	-	+ +	+	+	++
Quinones	NaOH test	+ +	-	-	-	-	-
Coumarins	NaOH test	-	+ + +	+ +	++	+ +	++
Cardiac glycoside	Keller-kellani	+	+	+	-	-	-
Anthraquinone		+	-	-	-	-	-

[MM=*Melastoma malabathricum* MP=*Mimosa pudica* RC=*Ricinus communis* ST=*Solanum torvum* AM=*Alocasia macrorrhiza* HI=*Heliotropium indicum* (+) = indicates low concentration (++) = shows moderate concentration (+++) = shows high concentration (-) = indicates absence]

Table-2: Preliminary phytochemical analysis of screened medicinal plant species (Methanol and ethanol extract)

Phyto-chemicals	**Test Name**	**MM**		**MP**		**RC**		**ST**		**AM**		**HI**	
		Mth	*Eth*	*Mth*	*Eth*	*Mth*	*Eth*	*Mth*	*Eth*	*Mth*	*Eth*	*Mth*	*Eth*
Terpenoids	$CHCl_3$ test	+ +	++	+++	+++	+ +	-	-	-	-	++	++	++
Flavonoids	Pb-acetate test	+ +	++	++	++	+ ++	+++	+	+	+ +	++	+	++
	NaOH test	+	++	++	++	+ +	+	+	+	+	+	++	+
	Shinoda test	+ +	++	++	++	+ +	++	+	+	+	+	+	+
Alkaloids	Wagner's test	+ +	++	++	+	+	-	++	++	++	+++	+	+
	Dragendroffs test	+ +	+	+	+	+	-	+++	+++	++	+++	+	+
Tannins	$FeCl_3$ test	+	++	+ +	++	+ +	++	+++	+++	+	++	-	-
Sterols	Salkowski's test	+	++	+ +	++	+ ++	+	-	-	-	+	-	-
Phenols	Potassium ferrocyanide test	+ + +	+++	+ + +	+++	++	++	+++	+++	+ ++	+++	+	+
Saponins	Froth test	+	++	-	-	+	+	+	+	+	+	-	+
Quinones	NaOH test	+ +	+++	-	-	-	-	-	-	-	-	-	-
Coumarins	NaOH test	-	-	+	+	+	+	++	++	-	++	+	+
Cardiac glycoside	Keller-kellani	+	+	+	+	++	+	-	-	-	-	-	-
Anthraquinone		+	+	-	-	+	+	-	-	-	-	-	-

[MM=*Melastoma malabathricum* MP=*Mimosa pudica* RC=*Ricinus communis* ST=*Solanum torvum* AM=*Alocasia macrorrhiza* HI=*Heliotropium indicum* (+) = indicates low concentration (++) = shows moderate concentration (+++) = shows high concentration (-) = indicates absence, Met=Methanol extract, Eth= Ethanol extract]

Total phenolic and total flavonoid contents:

Follin-ciocalteau reagent and aluminium chloride were used to determine total phenolic and total flavonoid contents respectively in different plant sample. Total phenolic contents were determined in terms of gallic acid equivalent(GAE), while total flavonoid contents of the extracts were expressed in term of quercetin equivalent (QE).The selected flora were rich in phenolics content. In present study, the methanolic extract of *Melastoma malabathricum* possessed highest quantity of total phenolic compounds, having value 76.29±0.80 mg GAE/g dry leaves powder(DLP) followed by *Mimosa pudica* extract (59.96±0.70 mg GAE/g DLP), *Solanum torvum* fraction (37.88±0.20 mg GAE/g DLP), *Ricinus communis* (33.08±0.93 mg GAE/g DLP) while the lowest amount of total phenolic content (15.79±0.69 mg GAE/g DLP) was observed in *Alocasia macrorrhiza* extract preceded by *Heliotropium indicum* extract which was account for 27.45±1.23mg GAE/g DLP as enlisted in the table 3

The selected flora contained reasonable amount of flavonoids content. The extract of *Ricinus communis* had the highest concentration of flavonoids content (23.23 ± 0.56) mg QE/g DLP followed by *Alocasia macrorrhiza* extract (11.90±0.45mg QE/g DLP), whereas lowest concentration of total flavonoids was observed in the extract of *Heliotropium indicum* (1.50±0.79 mg QE/g DLP) which was preceded by the result of *Melastoma malabathricum* extract(1.7±0.79mg QE/g of DLP) . Other plant samples contained a noticeable amount of total flavonoids that are shown in table-3 and figure-1.

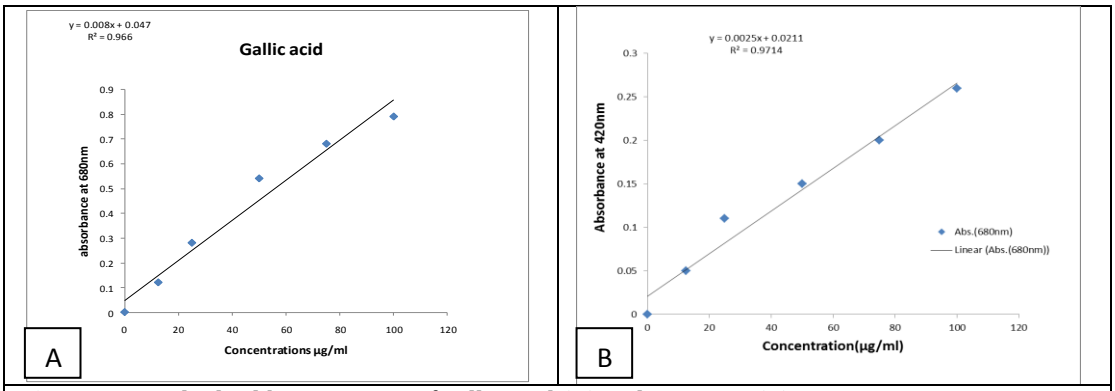

Figure 1: Standard calibration curve of gallic acid (A) and quercetin (B)

Table 3: Determination of total phenolic and total flavonoid contents

Plant sample	Total phenolic content in mg GAE/ g DLP (mean ± SD)	Total flavonoids content in mg QE/ g DLP (mean ± SD)
Melastoma malabathricum	76.29 ±0.80	1.7±0.79
Mimosa pudica	59.96±0.70	7.86±0.66
Ricinus communis	33.08±0.93	23.23±0.56
Solanum torvum	37.88±0.20	2.76±0.29
Alocasia macrorrhiza	15.79±0.69	11.90±0.45
Heliotropium indicum	27.45±1.23	1.50±0.79
Each value represents mean ± SD of three replicates DLP= Dry Leaves Powder, GAE= gallic acid equivalent, QE= quercetin equivalent		

Antioxidant activity:

Free radical scavenging activities of six plant extracts were assessed through DPPH radical assay. Disappearance of DPPH radical was occurred due to the effect of antioxidants. Different concentration of plant extract fades the purple appearance into yellow colour which was determined by measuring the absorbance at 520nm. As is given in table 4, the result of DPPH rapid scavenging test illustrated that methanolic extract of *Mimosa pudica* and *M. malabathricum* showed very fast reaction with high intensity DPPH radical. From the table 5, it was revealed that *Mimosa pudica* extract exhibited highest antioxidant activity (IC_{50}= 32.46 ± 0.35) which was followed by *Melastoma malabathricum* (IC_{50}= 48.90 ± 1.92) while *H. indicum* extract had the lowest scavenging effect (IC_{50}= 228.58 ± 5.33) preceded by the antioxidant activity of *Alocasia macrorrhiza*. Study showed that IC_{50} values were decreased with the increase of free radical quenching activity indicating inverse relationship.

Table-4: % of SCV (free radical scavenging activity) in different plants sample in different concentrations

Name of plant extract/standard	Concentration (µg/ml)			
	25	**50**	**75**	**100**
M. malabathricum	40.28 ± 1.85	46.45 ± 0.71	62.55 ± 1.94	80.10 ± 1.28
M. pudica	49.29 ± 0.71	56.40 ± 1.01	63.98 ± 0.52	83.88 ± 0.89
R. communis	35.07 ± 0.73	43.13 ± 1.04	62.55 ± 1.01	77.26 ± 1.26
S. torvum	12.32 ± 0.78	33.96 ± 1.18	43.61 ± 1.85	57.07 ± 1.83
A. macrorrhiza	8.05 ± 1.57	12.32 ± 0.78	18.41 ± 2.3	27.49 ± 0.95
H. indicum	2.37 ± 1.65	9.95 ± 2.43	16.11 ± 1.58	19.44 ± 1.73
Ascorbic acid	57.82 ± 1.57	68.25 ± 0.78	81.05 ± 0.66	87.21 ± 1.33
Each value represents mean ± standard deviation of three independent experiments				

Table-5: IC_{50} values of different plants sample

Studied Sample	**IC_{50} values**
Ascorbic acid	3.01 ± 0.92
Melastoma malabathricum	48.90 ± 1.92
Mimosa pudica	32.46 ± 0.35
Ricinus communis	54.84 ± 0.91
Solanum torvum	85.74 ± 2.98
Alocasia macrorrhiza	192.86 ± 7.45
Heliotropium indicum	228.58 ± 5.33
Each value represents mean ± standard deviation of three replicates	

Antibacterial activity: To evaluate antibacterial efficacy, discs containing methanolic extract of six plants were applied on to the medium inoculated with five different bacterial pathogens. Varying degree of antibacterial potentiality of six medicinal plant's extracts is presented in Table 6 and figure 2. In general, the mean zone of inhibition produced by all extracts varied from 7.25mm to 16.75mm. Gentamicin (10 µg) was used as standard positive control that showed diameter of zone of inhibition ranging from 18.25mm-21.75mm against all the tested organisms. The result showed that most potent antibacterial activity against *E.coli*, *Klebsiella pneumoniae* and *S. aureus* was obtained from *Solanum torvum* extract. *Melastoma malabathricum* exhibited most potent anti-bacterial activity against *Salmonella sp.* with the zones of inhibition of 16.75mm followed by *Pseudomonas aeruginosa* (14.5mm). *Heliotropium indicum* extract had the lowest inhibitory response to the most of the tested bacteria. Taking zone of inhibition into consideration, *Mimosa pudica* extract possessed highest inhibitory activity against *Pseudomonas* (14.75mm) followed by *Klebsiella* (10.5mm) whereas *Ricinus communis* had the strong inhibitory effect on *Klebsiella sp*. (12.25mm) followed *by S. aureus* (10.75mm). *Alocassia macrorrhiza* extract was more effective in inhibiting the growth of bacteria *Salmonella sp*. followed by *Pseudomonas* with inhibitory zone of 14.5mm and 11.5mm respectively.

Table-6: Antimicrobial activity of the selected plant sample

Bacterial Isolates	**Zone of inhibition (mm±SD)**						
	Melastoma malabathricum	*Mimosa pudica*	*Ricinus communis*	*Solanum torvum*	*Alocassia macrorrhiza*	*Heliotropium indicum*	Gentamicin (Control)
E. coli	11±0.82	10±0.82	9.75±0.5	15.25±0.5	9±0.82	NZI	20±0.82
Pseudomonas aeruginosa	14.5±0.58	14.75±0.5	10.25±0.96	13.5±0.58	11.5±0.58	6.5±0.58	21.75±0.56
Klebsiella pneumoniaea	12.25±0.96	10.5±0.58	12.25±0.5	15±0.82	9.75±0.5	8.25±0.5	19.5±0.96
Salmonella sp.	16.75±0.5	NZI	10.25±0.96	13±0.82	14.5±1.29	9.75±0.96	18.25±0.5
Staphylococcus aureus	13.75±0.96	8.25±0.5	10.75±0.5	15.5±0.58	9.5±0.58	7.25±0.5	20.75±0.96
Values represent mean (zone of inhibition) of four replicates ± SD, NZI=No zone of inhibition							

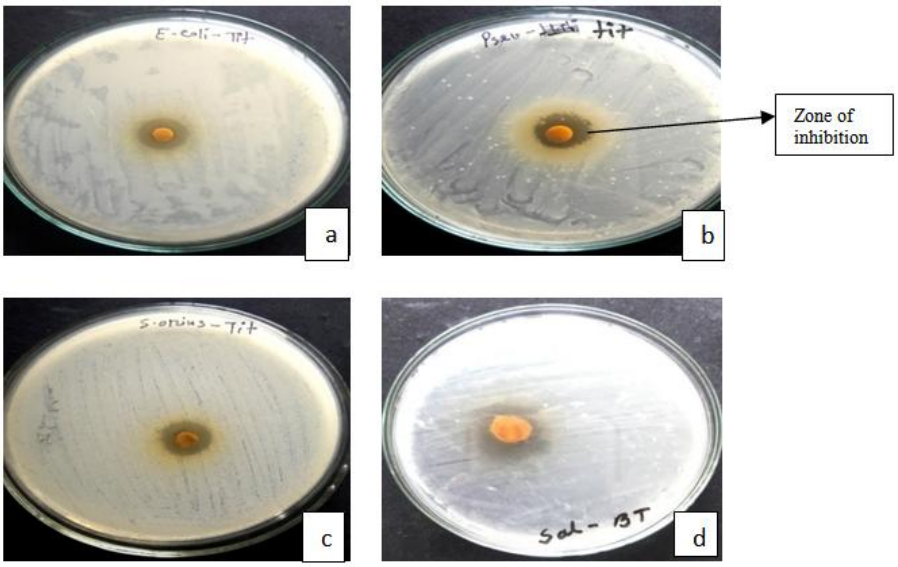

Figure-2: Growth inhibitions zone of *S. torvum* against *E. coli* (a), *Pseudomonas aeruginosa* (b) and *Staphylococcus aureus* (c) and *M. malabathricum* against *Salmonella sp* (d).

IV. Discussion

Phytochemical screening

Medicinal plants contain a structurally diverse group of phytochemicals that have remedial properties for human diseases (Patil, 2009). Although various classes of phytochemicals have been reported having antimicrobial potentiality yet they have not been well-known as therapeutic agents by the medical communal (Gibbons, 2004). In agreement with our results, the phytochemical screening of the leaf extracts of studied plants revealed the presence of phenolics, alkaloid, tannins, steroids, flavonoids, glycosides, saponins and anthraquinones that had variation within the plant species (Danladi , 2015; Zakaria et al., 2011; Kaur et al., 2011; Tamilarasi and Ananthi, 2012; Suurbaar et al., 2017; Sundari et al., 2013; Banik et al., 2014).

Preliminary phytochemical profiling reflects essential information regarding the diversity of different classes of secondary metabolites in the plant extracts. In the present study, qualitative tests for all six crude extracts showed significant indication about the presence of various secondary metabolites. Phytochemical screening plays a pivotal role in the biological activities of the medicinal plant. Various phytochemical tests indicated that saponins, tannins, glycosides, and flavonoids have been recognized in different extracts of six selected plants. Compounds of such classes are known to possess potent antioxidant activity (Lee, 2004). Tannins contribute to the retardation of the growth of many fungi, yeasts, bacteria and viruses (Chung et al., 1998). Saponins are generally used in crude drugs for their detergent properties and also have an inhibitory action on inflammation (Shibata, 1977; Just et al., 1998). These compounds served as natural antibiotics, which assist the body to combat microbial invasion (Santhi et al., 2011). Alkaloids have the analgesic, antispasmodic and antibacterial properties (Malik et al., 2017). Phenolic compounds possess a wide range of biological properties such as, anticarcinogen, antioxidant, antiaging, anti-inflammation, and cardiovascular protection (Han, et al., 2007). Diverse classes of many Steroids are known to possess important biological properties like as anti-inflammatory(Patel and Savjani, 2015), Coumarins have various bioactivities like anti-inflammatory, antimicrobial, anti-cough and anti-arthritis and can be suggested to be helpful for the plants themselves as natural bio-controlling compound and for humans as remedy for hyperproliferative skin diseases (Asif, 2015). Flavonoids possess antibacterial, anticancer, antioxidant, free radical quencing properties and conducive for gaining memory and also contain some other pharmacological properties (Sharma, 2006).

TPC and TFC

In Follin-Ciocalteu method, a reaction between phosphomolybdate and phosphotungstate with all phenolic compounds in the plant extract generates a blue coloration that show maximum light absortion at 760nm (Ramirez-Sanchez et al., 2011; Schofield et al., 2001). To determine flavonoid content, aluminium chloride chlorimetric assay was used. In this procedure, a yellow solution was formed due to the generation of the acid-stable complex by reacting aluminum chloride with adjacent keto or hydroxyl groups of flavones or flavonols (Chang et al., 2002). Methanolic extract of *M. melabathricum* exhibited high antioxidant activity using DPPH assay with the value above 90% and also recorded high TPC value (Mamat et al., 2013; Zakaria et al., 2011). Howlader et al.(2016) reported that methanol solvent proved as best solvent to get increased amount of total phenolic content as compared to other solvents. Previous report suggested that there was a linear co-relation between high total phenolic compound and antioxidant activity (Wu et al., 2006).

Antioxidant Activity

An increase in DPPH scavenging ability was observed with increase in concentration of extracts. A stronger yellow color was developed due to scavenge free DPPH radicals indicates a greater antioxidant potential. This trend is in agreement with a previous study (Ozturk et al., 2011). The capacity of polyphenol components for donating hydrogen atom or electron helps plant extract to show free radical scavenging activity(Shon et al., 2003). Generation of highly effective antioxidants and free radical scavengers in leaf tissue reduce oxidative stress experienced by plants in photosynthesis process and oxidative damage (Bhattacharya et al., 2009). On account of possessing strong antioxidant properties, flavonoids are aquainted with having inhibitory action on lipid peroxidation, free radicals scavenging properties and ferrous ion chelating capabilities (Morel et al., 1994). The *M. malabathricum* leaves possessed potential antioxidant activities that could be imposed to its high total phenolic contents (Zakaria et al., 2011).

Antibacterial Activity

The antimicrobial activity of plant extract may depend on the presence of various phytochemical constituents like flavonoids, phenolics and polyphenols, tannins, terpenoids, sesquiterpenes etc. in the crude extract of six medicinal plants (Shah et al., 2011). Generally, a larger zone of inhibition indicates higher antimicrobial activity.

However, all the studied plant extracts had varying levels of inhibitory action on the bacterial pathogen. A comparatively higher degree of antibacterial potential was found in case of *Solanum torvum* and *M. malabathricum* extract while lower degree of antibacterial activity was displayed in *H. indicum*. Past studied reported that *M. melabathricum* extract recorded maximum zone of inhibition against *E. coli* and *S. aureus* (Choudhury et al., 2011). According to Tomar et al. (2014), the antibacterial activity of methanolic extract of *Mimosa pudica* Leaves showed the highest activity with zone of inhibition against *E.coli*, *Staphylococcus aureus* and showed moderate activity against *Pseudomonas aeruginosa*. Leaves extract of *Ricinus communis* from different solvents exhibited inhibitory and bacteriocidal effects on selected microorganisms such as *E. coli, S. aureus, P. aeruginosa and K. pneumonia* (Suurbaar et al., 2017). The seed protein of *Ricinus communis* exhibits considerable antimicrobial activity against *E. coli, P. aeruginosa* and *S. aureus* (Al-Mamun et al., 2016). Accordind to previous study, antibacterial activity of *Mimosa pudica* (Kaur et al., 2011; Tamilarasi and Ananthi, 2012), *Ricinus communis* (Suurbaar et al., 2017), *Heliotropium indicum* (Osungunna and Adedeji, 2011) were reported earlier to show antibacterial effect whereas the antibacterial activity of *Melastoma malabathricum, Solanum* In Follin-Ciocalteu method, a reaction between phosphomolybdate and phosphotungstate with all phenolic compounds in the plant extract generates a blue coloration that show maximum light absorption at 760nm (Ramirez-Sanchez et al., 2011; Schofield et al., 2001). To determine flavonoid content, aluminium chloride chlorimetric assay was used. In this procedure, a yellow solution was formed due to the generation of the acid-stable complex by reacting aluminum chloride with adjacent keto or hydroxyl groups of flavones or flavonols (Chang et al., 2002). Methanolic extract of *M. melabathricum* exhibited high antioxidant activity using DPPH assay with the value above 90% and also recorded high TPC value (Mamat et al., 2013; Zakaria et al., 2011). Howlader et al.(2016) reported that methanol solvent proved as best solvent to get increased amount of total phenolic content as compared to other solvents. Previous report suggested that there was a linear co-relation between high total phenolic compound and antioxidant activity (Wu et al., 2006).

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V. Conclusion

In conclusion, our present research suggests that these plants have the potential to be used as antioxidant and antibacterial compounds. The current result will provide preliminary finding for the selection of some Sylhetian medicinal plants as their antioxidant and antibacterial potential to conduct further investigation which might be necessary to characterize, isolate, and purify bioactive compounds as nature-derived complementary and alternative medicine having curative properties to treat various disease. Different

toxicological studies in animal model need to be clarified for possible clinical uses of these plants extract in the management of human diseases.

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Competing of interests

The authors declare that they have no competing of interests.

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