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Reverse phase HPLC estimation of antioxidants and antimicrobial activities of *Clerodendrum colebrookianum* Walp

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Abstract
Methanol crude leaf extract and its solvent fraction were used to evaluate the antioxidant activity, total phenolics, total flavonoid and antimicrobial activity. Solvent fractionation was done by liquid-liquid partition with hexane, ethyl acetate, *n*-butanol and water and get fraction of hexane, ethyl acetate, *n*-butanol and residual water respectively. Antioxidant activity was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Reducing power. For antimicrobial activity, samples were tested for its potent antimicrobial activity against gram-positive bacteria such as *Bacillus cereus* and *Staphylococcus aureus,* and gram-negative bacteria *viz*. *Escherichia coli,* and *Pseudomonas aeruginosa.* Among the fractions, *n*-butanol fraction which had the highest polyphenolic content showed an appreciable DPPH radical scavenging activity (EC_{50} = 43.49 ± 1.95 µg/mL) as compared to the others fractions. The content of gallic acid, rutin, quercetin and kaempferol in extracts were evaluated by high performance liquid chromatography (HPLC) method. Ethyl acetate fraction showed remarkable effect on *B. cereus* at low concentration MIC, 1.5 mg/ml. Phytochemical screening showed the presence of different types of compound like alkaloids, carbohydrate, phenols and flavonoids and saponin, which might be responsible for antioxidant and antimicrobial activity.

Keywords: *Clerodendrum colebrookianum* Walp, antioxidant activity, antimicrobial activity, total phenols, total flavonoids, HPLC.

Introduction
Plant has been used as source of medicine for the treatment of various ailments since time immemorial [1]. Medicinal plants are the excellent source of natural products and phytochemicals which possess many properties like antioxidant, anticancer, anti-inflammatory and antimicrobial. Natural antioxidants can protect the human body from free radicals. A free radicle is defined as any atom or molecule that contains one or more unpaired electrons. Reactive oxygen species (ROS) is composed of both oxygen radicals, such as superoxide anion (O_2^-), hydroxyl (OH^-), peroxyl (RO_2^-), and hydroperoxyl (HO_2^-) radicals, and certain nonradical oxidizing agents, such as hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), and ozone (O_3), that can be converted easily to into radicals [2]. Free radical, which are normally produced in numerous biochemical reactions in the body lead to oxidative stress thereby causing damage to cell membranes, lipids, proteins and nucleic acids [3] have been implicated as mediators of many human diseases such as cancer [4], atherosclerosis [5], inflammation and heart diseases [6]. Antioxidants are agents which scavenge the free radical or prevent the oxidation of easily oxidable substrates [7]. Among the various natural antioxidants, polyphenols are reported to be active with redox properties, which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers [8, 9]. Northeast region of India is full of natural resources especially in medicinal and aromatic plants, which are extensively used by the traditional healer. *Clerodendrum colebrookianum*, Walp (Family, Verbenaceae) is one of such important medicinal plants, widely used by the local people of this region as a home remedy for high blood pressure [10], helminthic infactions [11], and most popularly known as “Pakkom” in Mising, and “Nefafu” in Assamese.

Materials and Methods
Chemicals
Folin-Ciocalteu phenol reagent were purchased from Merck and 2, 2-diphenyl-1-picrylhydrazyl (DPPH), sodium carbonate (Na_2CO_3), sodium hydroxide (NaOH), disodium

hydrogen phosphate (Na_2HPO_4), sodium dihydrogen phosphate (NaH_2PO_4), aluminum chloride, potassium ferricyanide [$K_3Fe(CN)_6$], trichloroacetic acid (TCA), ferric chloride ($FeCl_3$), and rutin were procured from Himedia Laboratories Pvt. Ltd, Mumbai, India. Gallic acid, catechin, quercetin, kaempferol, and sodium nitrite ($NaNO_2$) were purchased from Sigma Co. (St. Louis, MO, USA). Ascorbic acid was obtained from Sisco Research Laboratories Pvt. Ltd, Mumbai, India and Methanol obtained from Merck Company, Darmstadt, Germany. HPLC grade solvent were purchased from Rankem (Avanto™), Avantor Performance Materias India Limited, India.

Plant material and preparation of extract

The leaves of *Clerodendrum colebrookianum,* was collected from Dhemaji District, Assam. The fresh leaves was washed thoroughly with tap water and dried under shade and ground into coarse powder. The powdered plant material was finally soaked in the solvent methanol for 4 days with intermittent shaking[12]. The extract was filtered through Whatman filter paper No.-1. The process was repeated three times to assure exhaustive extraction. The filtrates were concentrated in a rotatory evaporator (Buchi rotavapor, R-215) at 45 °C to get the methanol extracts and kept at 4 °C in refrigerator. From that dried extract, fractionation was carried out using liquid-liquid extraction. The methanol extract (CLM) was suspended in water and extracted successively with hexane, ethyl acetate and butanol to give hexane (CLHF), ethyl acetate (CLEF), *n*-butanol (CLBF) and residual water (CLWF) fractions respectively. The dried extracts were used for analysis of phenolic content, flavonoids, antioxidant and antimicrobial assays.

Phytochemical screening

The screening of preliminary phytochemicals in *Clerodendrum colebrookianum* crude extract and solvent fractions was done to ascertain the presence of bioactive components, namely alkaloids, carbohydrates, phenols, flavonoids, tannin, glycosides and saponins [13].

Estimation of total phenolic content

The concentration of phenolics in plant extracts was determined using Folin-Ciocalteu reagent [14] with slight modification. Briefly 100 µL samples (1mg/mL)/ blank or standard gallic acid (various concentration 1-100 µg/mL) was mixed with 500 µL Folin-Ciocalteu reagent (10% v/v) in 2 mL assay tube and held at room temperature for 10 min. Then 400 µL of 7% Na_2CO_3 solution was added. The reaction mixture was shaken vigorously and thereafter incubated at 40 °C for 30 min. transferred 300 µL samples, standard and blank solution from respective assay tubes to clear 96 well microplate and absorbance of each well was determined at 760 nm in Thermo scientific Multiskan spectrum reader against blank. The concentration of the total phenolics contents was calculated by using an equation obtained from gallic acid calibration curve. The assays were carried out in triplicate and the results are expressed as mean values ± standard deviations and expressed as gallic acid equivalents milligram per gram of extract.

Estimation of total flavonoid content

The content total flavonoids of samples were measure by the method of aluminum chloride ($AlCl_3$), colorimetric assay as previously described [15]. To the 2 mL test tubes 400 µL of water and 100 µL of plant extract (1 mg/mL) or standard (various concentration 1-100 µg/mL) were added. To this 30 µL of 5% sodium nitrite and at 5 min 30 µL of 10% aluminum chloride were added. After 6 min, 200 µL of 1 M sodium hydroxide was added and the volume made up to 1 mL with water (240 µL). After 15 minutes absorbance was measured at 510 nm. The total flavonoids were calculated from Calibration curve of catechin (1-100 µg/mL) plotted by using the same procedure and total flavonoids was expressed as catechin equivalents in milligrams per gram sample.

Antioxidant activity

DPPH radical scavenging assay

Radical scavenging activity of plant extracts were determined by colorimetric assay using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical as source of free radical according to the method of Blois [16], with a slight modification. Briefly, a 0.1 mM solution of DPPH radical solution in methanol was prepared. In a clear 96 well plate 100 µL of standard Ascorbic acid or sample in various concentration (1-100 µg/mL) and 100 µL methanol/water was transferred and then 200 µL of DPPH solution was added. The reaction mixture were left for 30 min at room temperature in dark. The absorbance of each 96 well was measured at 517 nm in Thermo Multiskan reader. This activity is given as percent DPPH scavenging and is calculated as

$$\%\ DPPH\ scavenging = \left(\frac{Control\ absorbance - Extracts\ absorbance}{Control\ absorbance}\right) \times 100$$

Reducing power assay

Total reducing power was determined as described [17]. Briefly, one mL of test extracts (1-100 µg/mL) were mixed with 2.5 mL of Sodium phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide (1%) and then the mixture was incubated at 50° C for 20 min. Afterwards, 2.5 mL of trichloro acetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. Finally, 2.5 mL of the supernatant was mixed with 2.5 mL of distilled water followed by addition of 0.5 mL $FeCl_3$ (0.1%). The absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power, and the EC_{50} value is the effective concentration at which the absorbance was 0.5 for reducing power.

HPLC analysis

The HPLC analysis was performed with A Shimadzu LC-20AD HPLC system (Shimadzu, Japan) with a binary solvent delivery system (LC-20AD), a Rheodyne injector with 20 µL sample loop and DAD detector (SPD-M 20 A). Chromatographic separation was performed using a reverse phase column (Capcelll Pack C-18, MGII, 5µm, 250 mm x 4.6 mm) with an extended guard column. The mobile phase consisted of methanol-acetonitrile-water (40:15:45, v/v/v) containing 1.0% acetic acid with isocratic elution for 30 minutes. Shimadzu LC solution software was used for the data acquisition and processing. The diode array detector was set at an acquisition range from 240 - 800 nm. The flow rate was 1 mL/min and injection volume for all samples and standard solutions was 20 µL. The analytes peaks were identified by matching their retention times and UV spectra with those of the reference standards and the peaks were confirmed by spiking the sample with a small amount of the standards.

Determination of antimicrobial activities

The antimicrobial properties of *Clerodendrum colebrookianum* were investigated against gram negative bacterial strain; Escherichia coli ATCC-11229, gram positive bacterial strains; *Bacillus cereus* MTCC-430, *Staphylococcus aureus* ATCC-11632, *Pseudomonas aeruginosa* ATCC-15442. All strains were sub-cultured on Luria Bertani (LB) Agar at 37° C for 24 h before use. Then these activated bacteria and fungi were aseptically transferred to a Luria Bertani broth medium, and cultured in an incubator at 37° C for 24 h. The density of each microorganism was further adjusted to a turbidity equivalent to 0.5 on the McFarland scale before use.

Measurement of inhibition zone diameter

The agar diffusion method [18, 19] with some modification was used to evaluate the antimicrobial. The plant extract was allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The sterile liquid LB agar was autoclaved and then poured 20 – 25 mL of heated LB agar into each petriplate. After the liquid agar was cooled down and solidified, 100 µL of bacterial suspension were uniformly spread onto the surface with the help of sterile L-spray rolled to make a microbial lawn. The plates were punched to make well of 6 mm diameter using a sterile metallic cylinder and 30 µL (3 mg/well) of plant extracts dissolved in dimethyl sulfoxide (DMSO; 100 mg/mL) and 10 µL of standards (10 µg/well) were added into each well. Then the bacteria were incubated at 37° C for 24 h. Gentamycin for bacteria was used as positive controls and Dimethyl sulfoxide was used as a negative control. Antimicrobial activities were evaluated by measuring the diameters of inhibition zone. Each sample were repeated in triplicate.

Determination of minimum inhibitory concentration (MIC)

The minimum inhibition concentration (MIC) is the lowest concentration of antimicrobial agent at which no growth of microorganism was observed. MIC was determined by broth microdilution method using 96-well plates [20]. All the extracts were dissolved in 10% Dimethyl sulfoxide in order to make stock solution of 10 mg/mL. In a 96-well plates 100 µL of nutrient broth were dispensed into each well. A 100 µL of *Clerodendrum colebrookianum* extracts initially prepared at the concentration of 10 mg/mL was added into the first wells of each column. Then, a serial two-fold dilution was performed into seven consecutive wells of each column. Lastly, 100 µL of microbial inoculum (10^6 CFU) diluted in 0.9% saline water was pipetted into each wells. 100 µL of LB broth with or without 100 µL of inoculum served as control. The plates were then covered with sterile covers and sealed with a sterile parafilm and incubated at optimum conditions for each pathogen. As reference antibiotic, gentamycin was employed. After incubation, 40 µL of a 0.4 mg/mL nitroblue trotetrazolium chloride (NBT) indicator was added to visualize growth inhibition in each well, followed by 20 min incubation at 37° C. Bacterial growth in the wells was indicated by a blue colour, whereas clear wells indicated inhibition of growth by the tested sample. The minimum inhibitory concentration was defined as the lowest concentration of the test sample where there is no visible microbial growth (as indicated by colour). The MIC value was recorded as the mean concentration of triplicates.

Statistical analysis

The obtained antioxidant and antimicrobial results were stated in mean ± SD for three replicates. Statistical analysis was performed and graphs were obtained using Microsoft Excel (Microsoft office 2010).

Results and Discussions

Phytochemical screening

The phytochemicals screening conducted on the methanol crude extract and it derived fractions of *Clerodendrum colebrookianum,* exhibited the presence of alkaloids, carbohydrates, flavonoids, phenolic compounds, saponins, and phytosteroids (Table 1). It is possible that the presence of phytoconstituents might be responsible for the bioactivity of the plant extracts [21].

Table 1: Qualitative phytochemical screening of methanol crude extract and its derived fractions from *S. pulcherrima.*

Phytochemical test	Extracts of *Sarcochlamys pulcherrima*				
	CLM	**CLHF**	**CLEAF**	**CLBF**	**CLWF**
Alkaloids					
a. Mayer's reagent	+	-	+	+	+
b. Wagner's reagent	-	-	+	-	-
c. Hager's reagent	+	+	+	-	+
Carbohydrates and glycosides					
a. Molish's test	+	+	+	+	-
b. Fehling's test	+	-	-	+	+
c. Barfoed's test	-	-	-	+	+
d. Benedict's test	+	+	+	-	-
e. Borntrager's test	-	+	+	+	-
f. Legal's test	+	+	+	-	+
Saponins					
Foam test	+	-	+	+	+
Phenolic compound and flavonoids					
a. Ferric chloride test	+	+	+	+	+
b. Lead acetate test	+	-	+	+	+
c. Alkaline reagent	+	+	-	-	+
d. Magnesium and hydrochloric acid reduction	-	-	+	+	-
Proteins and amino acids					
a. Milon's reagent	+	+	+	-	+
b. Ninhydrin reagent	+	+	+	+	+
Phytosteroids					
Liebermann-burchard's test	+	-	+	+	-

Total phenolic and flavonoid contents

Total phenolic content is expressed as milligrams of gallic acid equivalent per gram of the extract or fraction (mg GAE/g extract or fraction) and was calculated from the regression equation of the calibration curve (R^2 =0.9923, y = 0.008*x* + 0.0511). The total phenolic content of methanol crude extract is to be found 55.27 ± 0.86 mg GAE/g dried extract. The total phenolic content of the different solvent fractions of *Clerodendrum colebrookianum* was solvent dependent and it is ranged from 38.76 ± 0.59 to 138.04 ± 0.94 mg GAE/g dried extract (Table 2). *n*-Butanol fraction showed highest total phenolic content. Total flavonoids content of methanol extract and its derived solvent fractions from *Clerodendrum colebrookianum* are shown in Table 2. Total flavonoids content was calculated from the regression equation of calibration curve (R^2 = 0.9936, y = 0.0022x + 0.0293), expressed as milligram catechin equivalent per gram of extract or fraction (mg CTE/g extract or fraction), varied from 14.71 ± 4.23 to 112.31 ± 3.52 mg CTE/g dried extract (Table 2). The flavonoids content of solvent fractions were in descending order of *n*-butanol fraction > ethyl acetate fraction > hexane fraction > residual water fraction.

Antioxidant activity

The results of the antioxidant activity determined by two test assays, viz. - DPPH radical scavenging and reducing power activity are shown in Table 2. In the present study, the capacity of the extract samples to scavenge DPPH radical was determined on the basis of their concentration providing 50% inhibition (EC_{50}). *n*-Butanol fraction of the plant extract exhibited the highest radical scavenging activity with an IC_{50} value of 43.49 ± 1.95 µg/mL followed by methanol crude extract (EC_{50} = 168.89 ± 8.89 µg/mL), hexan fraction (EC_{50} = 36.05 ± 0.191 µg/ml), water fraction (219.55 ± 5.76 µg/mL). Ethyl acetate fraction showed the least radical scavenging activity. Fig. 1. (A) showed the radical scavenging ability of *Clerodendrum colebrookianum* crude extract and fractions on DPPH in different concentrations. The radical scavenging activity of the leaf extract and different solvent fractions of *Clerodendrum colebrookianum* may be due to its phenolic compounds.

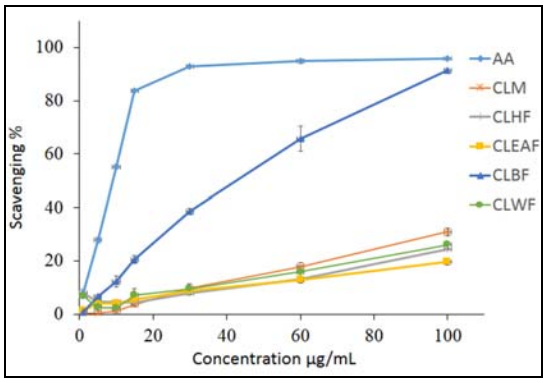

Fig 1: DPPH radical scavenging capacity of crude methanol extract and its solvent fraction from *Sarcochlamys pulcherrima*. Each value is expressed as mean ± standard deviation (n = 3). AA, Ascorbic acid; SPM, methanol crude extract; SPHF, hexane fraction; SPEAF, ethyl acetate fraction; SPBF, n-butanol fraction; SPWF, water fraction.

The reducing power assay is simple and direct method to assay antioxidant power. The antioxidants can transform $Fe_3 + (CN)_6$ into $Fe_2 + (CN-)_6$, so that the reducing power can be monitored by measuring Prussian blue formation at 700 nm. Increased absorbance is correlated with the reducing power of the extracts. A dose-response relationship was found in reducing ability of plant extract (Fig. 1.B), the reducing capacity is increasing with increased in concentration of extract. The reducing ability of methanol extract and different fractions was demonstrated in Fig 1. The reducing power ranking order was CLBF > CLHF > CLM > CLEAF > CLWF. Ascorbic acid as the positive control (EC_{50} = 100.268 ± 1.14 µg/mL). The reducing properties are generally associated with the presence of reductones [22].

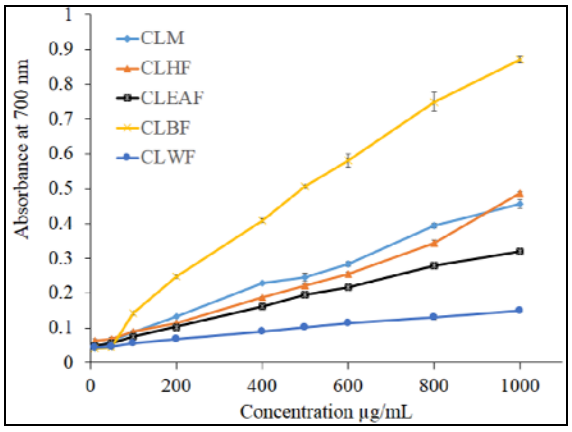

Fig 1: Reducing power of crude methanol extract and its solvent fraction from *Sarcochlamys pulcherrima*. Each value is expressed as mean ± standard deviation (n = 3). AA, Ascorbic acid; SPM, methanol crude extract; SPHF, hexane fraction; SPEAF, ethyl acetate fraction; SPBF, n-butanol fraction; SPWF, water fraction.

Table 2: Total phenolics content, total flavonoids content and antioxidant activity of the extract of *Sarcochlamys pulcherrima*.

Sample	TPC (mg/g GAE)	TFC (mg/g CTE)	DPPH EC_{50}[a] (µg/mL)	Reducing power EC_{50}[b] (µg/mL)
Methnol extract	55.27 ± 0.86	43.67 ± 8.343	168.89 ± 8.89	309.89 ± 4.32
Hexane fraction	38.76 ± 0.59	47.35 ± 11.84	208.87 ± 2.35	1176.79 ± 19.80
Ethyl acetate fraction	85.27 ± 4.17	92.56 ± 24.73	353.57 ± 4.25	180.75 ± 9.87
n-Butanol fraction	138.04 ± 0.94	112.31 ± 3.52	43.49 ± 1.95	162.65 ± 8.52
Aqueous fraction	46.37 ± 0.87	14.71 ± 4.23	219.55 ± 5.76	918.51 ± 18.89

AA			8.91 ± 0.017	100.27 ± 1.14

Each value is expressed as mean ± standard deviation (n = 3).
[a] EC_{50} (µg/mL): effective concentration at which 50% of DPPH radicals are scavenged.
[b] EC_{50} (µg/mL): effective concentration at which the absorbance is 0.5.
TPC: total phenolics content, TFC: total flavonoids content, AA: Ascorbic acid

HPLC analysis

In order to determine the various components with antioxidant and antimicrobial activities, an HPLC method was developed to quantify rutin, gallic acid, quercetin and kaempferol in methanol crude extract and its different solvent fractions of *Clerodendrum colebrookianum*. The HPLC chromatograms of extracts and tested compound are shown in Fig. 2. Peak of gallic acid was present in all chromatograms, eluting at a retention time (Rt) 2.89. The retention time (Rt) of rutin, quercetin and kaempferol were 3.89, 8.37 and 13.08 respectively. Quantification was done via a calibration curves with standards (external standard method). The amount of compound detected in the analyzed samples is shown in Table 3. results are expressed as mg/g dry extract. The content of gallic acid (1.5446 ± 0.01) represent the highest amount of phenolic substance, followed by kaempferol (1.21 ± 0.01 mg/g), qurcetin (0.870 ± 0.10 mg/g), and rutin (0.119 ± 0.03 mg/g) in the methanol crude extract. Among the solvent fractions the content of rutin in CLBF shows the highest amount (37.798 ± 1.06 mg/g) followed by gallic acid in CLWF (10.19 ± 0.31 mg/g), kaemperol in CLEAF (6.681 ± 0.38 mg/g) and quercetin in CLEAF (1.813 ± 0.07).

Table 3: Mean content of rutin, gallic acid, quercetin and kaempferol (mg/g[a] ± SD) quantified by HPLC in extracts of *S. pulcherrima*.

	CLM	CLHF	CLEAF	CLBF	CLWF
Rutin	0.119 ± 0.03	0.197 ± 0.08	ND	37.798 ± 1.06	ND
Quercetin	0.870 ± 0.097	0.786 ± 0.00	1.813 ± 0.07	0.913 ± 0.043	ND
Gallic acid	1.5446 ± 0.01	2.204 ± 0.01	1.614 ± 0.03	2.00 ± 0.17	10.19 ± 0.31
Kaempferol	1.212 ± 0.01	0.397 ± 0.01	6.681 ± 0.38	0.367 ± 0.01	ND

Values were determined from integration of HPLC signals and response factors calculated from standards, Each value is expressed as mean ± standard deviation (n = 3).
[a] = Dry weight of the extract of *S. pulcherrima*. ND = Not detected.

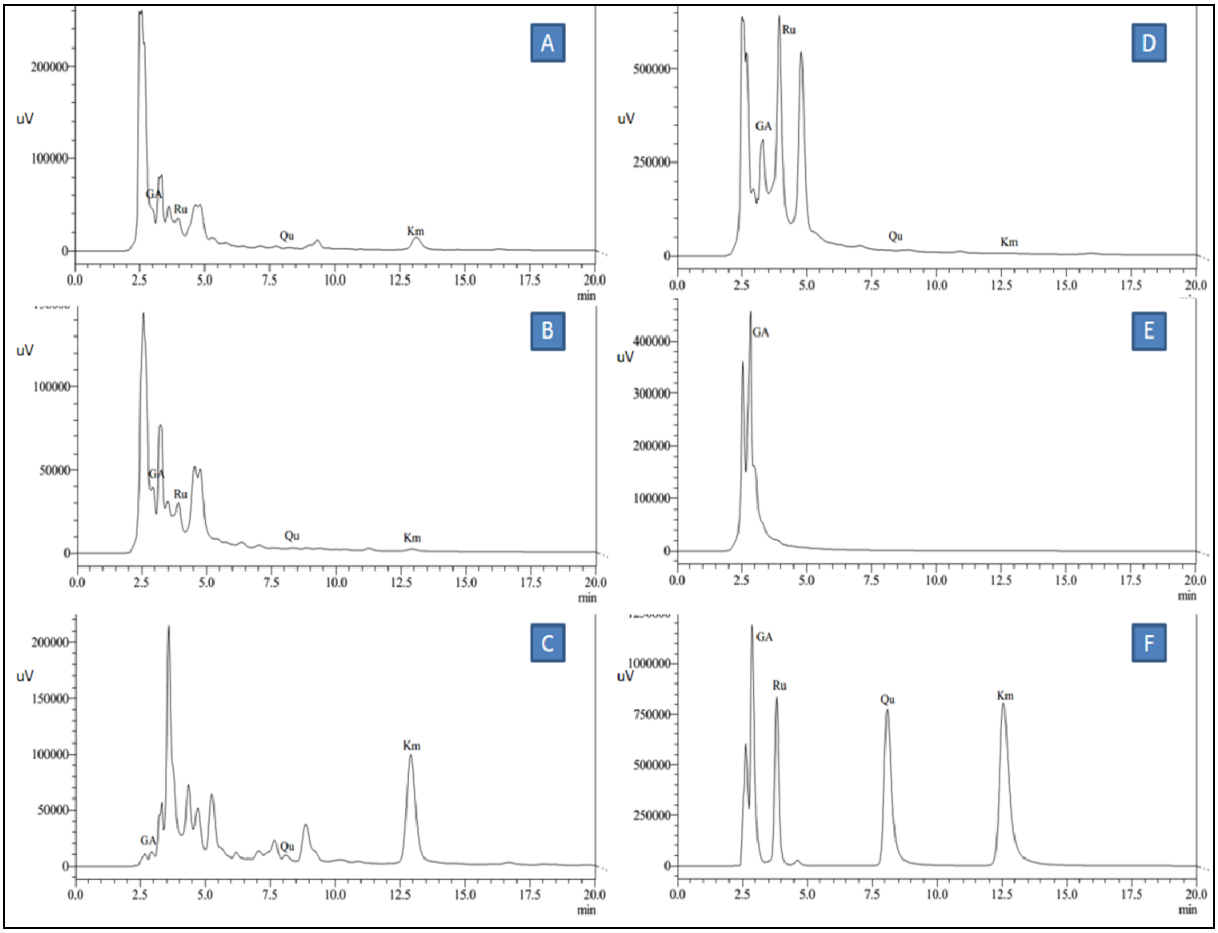

Fig 2: HPLC chromatogram of standard mixture and plant extracts. (A) methanol crude extract; (B) hexane fraction; (C) ethyl acetate fraction; (D) n-butanol fraction; (E) water fraction; (F) standard mixture. GA, gallic acid; Ru, rutin; Qu, quercetin; Km, kaempferol.

Antimicrobial activity

The antimicrobial activities of *Clerodendrum colebrookianum* methanol crude extract and fraction extracts against gram positive bacteria and gram negative bacteria were investigated and their potency were quantitatively assessed by the presence or absence of inhibition zones and zone diameter (table 4) and MIC values (table 5). The results indicated that the *n*-butanol fraction had highest antimicrobial activity against all bacterial species. However, the hexane fraction showed no antimicrobial activity against any of the bacterial strains in the present study. The maximum inhibition zone (11 mm) was observed in *n*-butanol fraction and lowest MIC value 1.25 mg/ml was found against *B. cereus*. Water fraction exhibited the least antimicrobial activity with MIC value range 5-10 mg/mL, against all the strains. This result shows that high polar fractions in the plant are having good antimicrobial activities.

Table 4: Antimicrobial activity measured as zone of inhibition at 30 µL (200mg/mL) of crude methanol extract, its derived fractions from *Clerodendrum* and standard antibiotics.

Microbial species	Zone of inhibition (mm)					
	CLM	CLHF	CLEAF	CLBF	CLWF	Gentamycin
B. cereus	7 ± 0.00	-	7,8 ± 0.00	11 ± 0.00	7 ± 0.00	23 ± 0.00
S. aureus	-	-	7 ± 0.00	7 ± 0.00	7 ± 0.00	20,21 ± 0.00
E. coli	7± 0.00	-	-	10 ± 0.00	-	16,17 ± 0.00
Ps. a	8	-	10, 8	11,8	8	23
- = No activity						

Table 5: The MIC values of *S. pulcherrima* extract against the microorganisms tested in microdilution assay

Microbial species	MIC				
	CLM	CLEAF	CLBF	CLWF	Gentamycin
B. cereus	2.5	1.25	2.5	10	5
S. aureus	*	>10	5	5	10
E. coli	10	*	5	*	10
Ps. a	2.5	2.5	5	>10	2.5

MIC: minimal inhibition concentration (mg/mL for sample and µg/mL for Standard).
Each value is expressed as mean ± standard deviation (n = 3).

Conclusions

In the present investigation, comparing to all the fraction *n*-butanol fraction from *Clerodendrum colebrookianum* exhibited strongest radical scavengers in DPPH (43.49 µg/mL) and highest reducing power (162.65 µg/mL). The total phenols and flavonoids content are directly correlate with the antioxidant activity Therefore, the total phenols and total flavonoids are responsible for the antioxidant activity. Estimation of rutin, gallic acid, quercetin and kaempferol in methanol crude extract and its derived fractions are determined by reverse phase HPL analysis and rutin was found to be highest amount in n-butanol fraction which may responsible for high antioxidant activity. The antimicrobial results of this study support several traditional medicinal uses of *Clerodendrum colebrookianum* species in Assam. Methanol crude extracts and its derived fraction from *Clerodendrum colebrookianum* show antimicrobial effects on the tested microorganisms as well as possessed high antioxidant activity. These results justified the traditional use of *Clerodendrum colebrookianum*.

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