JCHR (2020) 10(4), 353-363

Journal of Chemical Health Risks

www.jchr.org

ORIGINAL ARTICLE

Isolation of Cerebroside from *Gynura procumbens* Leaves and Biological Activities of the Leaves Extracts

Md. Saddam Hossain, Md. Maniruzzaman*, M.M.R. Chowdhuary, Junaid Uddin Ahmed, Md. Mizanur Rahman Badal, Mohammad Abu Yousuf

Department of Chemistry, Khulna University of Engineering & Technology, Khulna-9203, Bangladesh

(Received: 6 September 2020 Accepted: 26 December 2020)

KEYWORDS

Gynura procumbens;
Cerebroside;
Antimicrobial activity;
Artemia salina;
Cytotoxicity;
Brine shrimp lethality bioassay

ABSTRACT: Several plants have been used in folk medicine to treat various types of diseases. *Gynura procumbens* (*G. procumbens*), a medicinal plant is widely used in traditional treatment of many aliments due to the presence of potent bioactive components. In this study, *G. procumbens* leaves were extracted with three different polarities of solvents viz., ethyl acetate (EA), methanol (MeOH) and methanol-water (MeOH-H_2O). A cerebroside and a pure compound were isolated from the EA and MeOH crude extracts respectively. Antibacterial, antifungal activities and cytotoxicity of the crude extracts of *G. procumbens* leaves were investigated using six bacterial strains (three gram positive and three gram negative), one fungal strain and brine shrimp lethality bioassay, respectively. The EA extracts showed moderate antimicrobial activity, whereas MeOH and MeOH-H_2O extracts exhibited very minute sort of sensitivities. The MeOH-H_2O extract showed a significant cytotoxic activity having LC_{50} of 3.98 µg/mL on the other hand EA and MeOH exhibited mild and moderate cytotoxic activities having LC_{50} values of 63.10 and 4.47 µg/mL, respectively. These results demonstrate the presence of potential bioactive components in the studied crude extracts of the *G. procumbens* leaves.

INTRODUCTION

Medicinal plants have been considered as the most common source of therapeutic agents, especially antimicrobial agents and still many of today's drugs are plant-derived natural products or their derivatives [1]. These plants are being more accepted and trusted to be more effective than synthetic pharmaceuticals products. Other advantages of herbal medicines are less expensive, easily accessible to remote communities, comparatively safe to use and biodegradable. Medicinal plants are mostly used in Asia, Latin America, and Africa whereas minimal side effects are reported [2, 3]. Recently, pharmaceutical companies are focusing on the pharmaceutical lead drugs from natural sources which are known to be biocompatible and economical. The rising incidence in multidrug resistance amongst pathogenic microbes has further necessitated the need to search for newer antibiotic sources.

Gynura procumbens (*G. procumbens*), a fast-growing evergreen herb belongs to the Asteraceae family, locally known as diabetics plant, and traditionally used as a medicinal plant. This herb is widely found in South East Asian countries, including Indonesia, Thailand, and Malaysia. *G. procumbens* leaves are traditionally used for the treatment of migraines, constipation, rash, diabetes mellitus, eruptive fevers, hypertension, and cancer [4-7].

*Corresponding author: mzaman_103@chem.kuet.ac.bd (Md. Maniruzzaman)
DOI: 10.22034/jchr.2020.1908719.1174

It has been reported that *G. procumbens* leaf extracts have anti-herpes simplex virus [8], anti-hyperglycaemic [9, 10], anti-inflammatory [11, 12], blood hypertension reduction capabilities [13-15], anti-hyperglycemic [16], anti-cancer [17] antiproliferative [18, 19], antioxidative [20, 21], and anti-ulcerogenic [22] properties due to the presence of alkaloids, terpenes, flavonoids, steroids, phenolics, flavonoids, etc. [1,4,9]. It has been found that methanol extract of *G. procumbens* leaves has antimycobacterial activity against *M. tuberculosis* [23]. Phytochemical screening results of *G. procumbens* extract reveal the presence of flavonoids and terpenes. Several studies have reported flavonoids and terpenes compounds as anti *M. tuberculosis*. There was positive correlation between the flavonoid and terpenes content and antimycobacterial activity [23]. It has been investigated the antimicrobial activities of DCM and EA crude extracts of *G. procumbens* leaves exhibiting mild activity on the other hand *n*-hexane and MeOH extracts did not exhibit any sort of activity [24]. This indicates that the antimicrobial activity of *G. procumbens* leaves extracts depends on the polarity of the solvent. Different solvent extracts may possess different pharmacological potential to treat various diseases. Therefore, *G. procumbens* can be introduced as a new potential natural source of compounds with numerous pharmacological activities which can be exploited for the development of novel therapeutic agents [5]. Since *G. procumbens* has a wide variety of biological activities, it may be a promising candidate for the isolation of bioactive compounds.

In this study, two known compounds were isolated from the EA and MeOH crude extracts of *G. procumbens* leaves followed by elucidating the structure with the help of spectroscopic evidences and previously reported data. Then antimicrobial activity of EA, MeOH and MeOH-H_2O extracts were investigated against six bacterial and one fungal strains. Finally, the cytotoxicity of the studied extracts was assessed through brine shrimp lethality bioassay.

MATERIALS AND METHODS

The Analytical grade *n*-hexane (HX), ethanol (EtOH) and chloroform from Merck (Darmstadt, Germany), acetone and dimethyl sulfoxide (DMSO) from Fisher Scientific Co. (Leicestershire, UK), methanol (MeOH) from Sigma (Steinheim, Germany), dichloromethane (DCM) and ethyl acetate (EA) from Daejung (Korea) and LobaChemie (India), respectively, were purchased and used without any further purification. Distilled water was used in this study to prepare all stock solutions. The melting point was measured by melting point apparatus (Stuart's electrothermal, Model no. SMP 30). FT-IR spectrum was measured on FT-IR Shimadzu-8400 over the frequency range from 4000-400 cm^{-1} using KBr pellets. Both ^{1}H-NMR and ^{13}C-NMR spectra were recorded with an AVANCE Bruker NMR spectrometer at 400 MHz using DMSO as a solvent and TMS as an internal standard.

Plant materials

G. procumbens leaves were collected from Bezerdanga Bazar, Khulna, Bangladesh and these were identified by a botanist Dr. A.M.M. Golam Adam, Associate Professor, Department of Botany, Jagannath University, Dhaka-1100, Bangladesh. After collection, the leaves were washed thoroughly with distilled water to ensure the removal of dirt prior to the drying process. Then the leaves were dried and grinded to finely powder.

Extraction and isolation

The powdered leaves were soaked with *n*-hexane (HX) (2 L) at room temperature for 24 hours followed by 8 hours of reflux at 35 °C. This process was repeated two times and combined HX extract was filtered through a Whatman filter paper (No. 1). The extract was concentrated using a rotary evaporator at below 40 °C. The residual powdered leaves were extracted similarly with EA, MeOH and MeOH-H_2O respectively. A portion of these crude extracts was taken for the antibacterial, antifungal screening and cytotoxicity investigations. The remaining extract was further separated gradually with an increment of solvent polarity using silica gel column chromatography. After separating from column chromatography, the TLC study of EA crude extract showed a single spot (R_f value = 0.78) in EA-EtOH (8 ꞉ 2) mixture, designated as GPEA. Similarly, for MeOH crude extract designated as, GPM was obtained having R_f

value of 0.76. Then melting point, FT-IR and NMR spectra of the obtained samples were recorded.

Microorganisms

The antibacterial activity of each extracts was tested against three gram positive (G^+) *Bacillus cereus, Listeria monocytogenes and Staphylococcus aureus*, respectively and three gram negative (G^-) *Escherichia coli, Salmonella typhimurium* and *Citrobacter freundii*, respectively bacterial strains. The antifungal activity of each extracts was also tested against *Trichodarma harzianum* fungal strain. All of the microorganisms were collected from Centre for Advanced Research in Sciences (CARS), University of Dhaka, Dhaka-1000, Bangladesh.

Antimicrobial screening

Antibacterial and antifungal activities of the studied crude extracts were tested in vitro by the widely accepted Kirby–Bauer disc diffusion method [25]. Sterile nutrient agar media (Difco Laboratories, Lawrence, KS, U.S.A.) having pH range 7.2 to 7.4 was used as a basal medium for the test bacteria followed by streaking with a sterile cotton swab of the 24 hours' liquid cultures containing microorganisms. On the other hand, sterile potato dextrose agar (PDA) (Scharlau Chemi SA, U.S.A.) was used as basal medium for the fungal growth. Oxoid™ Antimicrobial Susceptibility discs (Thermo Fisher Scientific, USA) having extraction concentration of 300 µg/disc were placed gently onto the pre-inoculated agar plates. Each crude extract was dissolved with acetone and evaporated completely before application. Blank disc impregnated with acetone followed by evaporation. Ciprofloxacin hydrochloride at 25µg/disc, as standard for antibacterial test, Miconazole at 50 µg/disc, as standard for antifungal test, were used as negative and positive control, respectively. After 24 hours of incubation at 37 °C aerobically, inhibitory activity was measured (in mm) as the diameter of the observed inhibition zone. These experiments were performed in triplicate for each extract and values are presented as average ± SDs.

Cytotoxicity assay

Cytotoxicity of the studied crude extracts of *G. procumbens* leaves was investigated using Brine shrimp lethality bioassay [26]. During experiment, 2 g of each of the extracts was dissolved in 2.0 mL volume of DMSO to make the desired concentration of the prepared solution. Then a series of solutions of lower concentrations were prepared from the solution by serial dilution with DMSO and the concentrations obtained for each sample were 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.563 and 0.781 µg/mL. Vincristine sulphate (VS) was taken as the positive control. Then the solutions were taken in the pre-marked vials containing ten live brine shrimp nauplii in 9 mL simulated seawater. After 48 hrs, the vials were inspected using a magnifying glass and the number of lived nauplii in each vial was counted. From this data, lethality percentage (%) of the brine shrimp nauplii was calculated for each concentration. Lethal concentration for 50 % mortality i.e., LC_{50} was determined by using probit analysis.

RESULTS AND DISCUSSION

Identification of the isolated compounds

The compounds, GPEA and GPM were idendified using FT-IR, ^{1}H-NMR and ^{13}C-NMR spectral data, and determined as 1-O-*β*-D-glucopyranosyl-2-[2′-hydroxylignocenoyl-amino]-10-octadecene-3, 4-diol [27,28] and *β*-sitosterol-3-O-*β*-D-glucopyranoside [29].

The isolated compound, GPEA obtained was white amorphous powder; soluble in DMSO; R_f value = 0.78 (in 100% acetone); m. p. = 126-128 °C; IR (KBr), υ_{cm-1}: 3337 (OH), 3218 (NH), 2917 and 2848 (CH), 1728 (C=O), 1544 (C–N), 1620 (>C=C<), 1276, 1164, 1067 and 1022 (glycosidic C–O) cm^1. The ^{1}H- and ^{13}C-NMR data of GPEA (Table 1, Figure1) indicates that it contains a sugar moiety, an amide linkage and aliphatic hydrocarbon long chains, suggesting the glycosphingolipid nature of GPEA.

From the ^{1}H-NMR spectrum, signals obtained at δ_H 4.02-4.03, 4.04-4.10, 3.60-3.80, 3.90 and 3.86-3.89 indicates the presence of five protons of the sugar moiety. The signal observed at δ_H 5.10 is for an anomeric proton. The signals at δ_H 8.16 for (–NH–) proton, 4.86 for (–CH_2–)

protons and 0.86 for two terminal–CH_3 protons were observed. The signals recorded at δ_H 1.50-1.60 and 1.24-1.35 represents the presence of long-chain hydrocarbon.

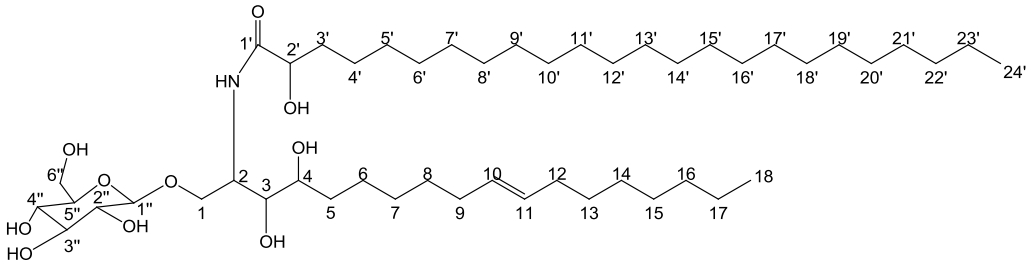

Figure 1. Structure of GPEA (1-O-*β*-D-glucopyranosyl-2-[2′-hydroxylignocenoyl-amino]-10-octadecene-3, 4-diol).

From the ^{13}C-NMR spectrum of GPEA, four carbon signals bearing hydroxyl groups (δ_C 67.1, 73.8, 66.2) and a double bond (δ_C 130.4 and 130.6) were observed. The signal observed at δ_C 175.2 illustrates the presence of an amide carbonyl group. An anomeric carbon signal was recorded at δ_C 101.3. Two terminal–CH_3 groups were obtained at δ_C 14.4. The signals observed at δ_C 101.3, 75.8, 69.7, 70.9 and 63.1 indicates that the sugar moiety in the compound GPEA is a *β*-glucopyranoside. Signals obtained at δ_C 24.9, 22.6, 28.9-29.5 suggests the presence of long-chain alkanes. The signal at δ_C 42.6 represented the carbon signal linked to the amide groups and this indicates that two long chain aliphatic moieties are connected by an amide bond. The obtained FT-IR, ^{1}H-NMR and ^{13}C-NMR spectral data were compatible with the reported data [27,28] of 1-O-*β*-D-glucopyranosyl-2-[2′-hydroxylignocenoyl-amino]-10-octadecene-3, 4-diol.

Table 1. ^{13}C-NMR and ^{1}H-NMR spectral data of the compound GPEA

Position of Carbon	^{13}C-NMR Peak δ_C in ppm	Types of Carbon	Position of Proton	^{1}H-NMR Peak δ_H in ppm	Types of Proton
C-1	67.1	CH_2	H-1	4.86 (d)	2-H
C-2	42.6	CH	H-2	5.30 (m)	1-H
C-3	73.8	CH	H-3	4.37 (brs)	1-H
C-4	66.2	CH	H-4	4.11 (brs)	1-H
C-5	33.4	CH_2	H-5	2.015(m)	2-H
C-6	28.9-29.5	CH_2	H-6	1.24-1.35 (m)	2-H
C-7	28.9-29.5	CH_2	H-7	1.24-1.35 (m)	2-H
C-8	28.9-29.5	CH_2	H-8	1.50-1.60 (m)	2-H
C-9	24.9	CH_2	H-9	2.27 (m)	2-H
C-10	130.4	CH	H-10	5.56 (dt)	1-H
C-11	130.6	CH	H-11	5.61 (dt)	1-H
C-12	24.9	CH_2	H-12	2.01(m)	2-H
C-13	28.9-29.5	CH_2	H-13	1.50-1.60 (m)	2-H
C-14	28.9-29.5	CH_2	H-14	1.24-1.35 (m)	2-H
C-15	28.9-29.5	CH_2	H-15	1.24-1.35 (m)	2-H
C-16	31.8	CH_2	H-16	1.24-1.35 (m)	2-H
C-17	22.6	CH_2	H-17	1.50-1.60 (m)	2-H
C-18	14.4	CH_3	H-18	0.86 (t)	3-H
C-1′	175.2	C	-	-	-
C-2′	66.2	CH	H-2′	4.71 (m)	1-H
C-3′	33.4	CH_2	H-3′	2.01 (m)	2-H
C-4′	21.2	CH_2	H-4′	1.24-1.35 (m)	2-H
C-5′	28.9-29.5	CH_2	H-5′	1.24-1.35 (m)	2-H

C-6′	28.9-29.5	CH_2	H-6′	1.24-1.35 (m)	2-H
C-7′	28.9-29.5	CH_2	H-7′	1.24-1.35 (m)	2-H
C-8′	28.9-29.5	CH_2	H-8′	1.24-1.35 (m)	2-H
C-9′	28.9-29.5	CH_2	H-9′	1.24-1.35 (m)	2-H
C-10′	28.9-29.5	CH_2	H-10′	1.24-1.35 (m)	2-H
C-11′	28.9-29.5	CH_2	H-11′	1.24-1.35 (m)	2-H
C-12′	28.9-29.5	CH_2	H-12′	1.24-1.35 (m)	2-H
C-13′	28.9-29.5	CH_2	H-13′	1.24-1.35 (m)	2-H
C-14′	28.9-29.5	CH_2	H-14′	1.24-1.35 (m)	2-H
C-15′	28.9-29.5	CH_2	H-15′	1.24-1.35 (m)	2-H
C-16′	28.9-29.5	CH_2	H-16′	1.24-1.35 (m)	2-H
C-17′	28.9-29.5	CH_2	H-17′	1.24-1.35 (m)	2-H
C-18′	28.9-29.5	CH_2	H-18′	1.24-1.35 (m)	2-H
C-19′	28.9-29.5	CH_2	H-19′	1.24-1.35 (m)	2-H
C-20′	28.9-29.5	CH_2	H-20′	1.24-1.35 (m)	2-H
C-21′	28.9-29.5	CH_2	H-21′	1.24-1.35 (m)	2-H
C-22′	31.8	CH_2	H-22′	1.24-1.35 (m)	2-H
C-23′	22.6	CH_2	H-23′	1.50-1.60 (m)	2-H
C-24′	14.4	CH_3	H-24′	0.86 (t)	3-H
C-1″	101.3	CH	H-1″	5.10 (d)	1-H
C-2″	75.8	CH	H-2″	4.02-4.03 (m)	1-H
C-3″	69.7	CH	H-3″	4.04-4.10 (m)	1-H
C-4″	70.9	CH	H-4″	4.04-4.10 (m)	1-H
C-5″	69.7	CH	H-5″	3.60-3.80 (m)	1-H
C-6″	63.1	CH_2	H-6a″	3.90 (m)	2-H
			H-6b″	3.86-3.89 (m)	
			N-H	8.16	1-H

The isolated compound, GPM obtained white powder, soluble in DMSO, R_f value = 0.76 (in EA-EtOH (8:2) mixture, and m.p. = 292-294 ^oC. IR (KBr), υ_{cm-1}: 3397 (OH), 2959 and 2868 (CH), 1641 (>C=C<), 1245, 1165, 1073 and 1023 (glycosidic C–O), 1465 and 1367 (CH bending in gem-dimethyl group), 799 (CH stretching of >C=C–H) cm^1. The ^1H- and ^{13}C-NMR data of GPM (Table 2, Figure 2) indicates the presence of aglycone and sugar moieties in the structure of GPM.

Table 2. ^{13}C-NMR and ^1H-NMR spectral data of the compound GPM

Position of Carbon	^{13}C-NMR Peak δ_C in ppm	Types of Carbon	Position of Proton	^1H-NMR Peak δ_H in ppm	Types of Proton
C-1	37.3	CH_2	H-1	1.90 (m)	2H
C-2	29.7	CH_2	H-2	1.79-2.16 (m)	2H
C-3	77.4	CH	H-3	3.13-3.06 (m)	1H
C-4	38.8	CH_2	H-4	2.89 (m)	2H
C-5	140.9	C	-	-	-
C-6	121.9	CH	H-6	5.34 (d)	1H
C-7	31.9	CH_2	H-7	0.79-1.51 (m)	2H
C-8	31.9	CH	H-8	1.01-1.82 (m)	1H
C-9	50.1	CH	H-9	1.01-1.82 (m)	1H

C-10	36.7	C	-	-	-
C-11	23.1	CH_2	H-11	0.79-1.51 (m)	2H
C-12	40.6	CH_2	H-12	0.79-1.51 (m)	2H
C-13	42.3	C	H-13	-	-
C-14	56.9	CH	H-14	1.01-1.82 (m)	1H
C-15	24.3	CH_2	H-15	0.79-1.51 (m)	2H
C-16	28.1	CH_2	H-16	0.79-1.51 (m)	2H
C-17	55.9	CH	H-17	1.01-1.82 (m)	1H
C-18	12.2	CH_3	H-18	0.66-0.68 (d)	3H
C-19	19.4	CH_3	H-19	0.96 (s)	3H
C-20	35.9	CH	H-20	1.47 (m)	1H
C-21	19.1	CH_3	H-21	1.01 (d)	3H
C-22	33.8	CH_2	H-22	0.90-1.01 (m)	2H
C-23	29.7	CH_2	H-23	0.90-1.01 (m)	2H
C-24	45.6	CH	H-24	0.99 (m)	1H
C-25	29.2	CH	H-25	1.79 (m)	1H
C-26	20.2	CH_3	H-26	0.92 (d)	3H
C-27	19.6	CH_3	H-27	0.90 (d)	3H
C-28	25.9	CH_2	H-28	1.24 (m)	2H
C-29	12.3	CH_3	H-29	0.83 (t)	3H
C-1´	101.3	CH	H-1´	4.88-4.87 (d)	1H
C-2´	73.9	CH	H-2´	3.67-3.63 (t)`	1H
C-3´	77.4	CH	H-3´	4.24-4.22 (dd)	1H
C-4´	70.6	CH	H-4´	4.24-4.22 (dd)	1H
C-5´	77.3	CH	H-5´	3.13-3.06 (m)	1H
C-6´	61.6	CH_2	H-6´	4.43 (dd)	2H

The ^{1}H-NMR spectrum exhibits five tetrahydropyran protons at δ_H 4.88-4.87, 3.67-3.63, 4.24-4.22 and 3.13-3.06. The signals recorded at δ_H 0.66-0.68 and 0.92 represent two angular methyl signals. The signal obtained at δ_H 4.43 indicates the presence of a methylene group. Two secondary methyl group protons give signals at δ_H 0.92 and 0.90 exhibiting the presence of olefinic double bond. Signal obtained at δ_H 4.43 indicates a methylene group. Ten aglyconic methylene protons give signals at δ_H 2.89, 1.79-2.16, 1.90, 1.24, 0.90-1.01, and 0.79-1.51 whereas seven aglyconic methine protons give signals at δ_H 1.79, 1.47, 1.01-1.82, and 0.99.

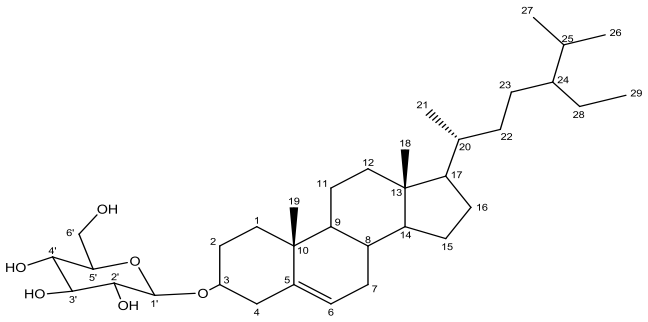

Figure 2. Structure of *β*-sitosterol-3-O-*β*-D-glucopyranoside

From ^{13}C-NMR spectrum, δ_C at 20.2 and 19.6 for two separate terminal methyl carbons, δ_C 12.2, 19.4 and 19.1 for the angular methyl carbons were observed. The signals at δ_C 31.9, 36.7 and 56.9 are due to the carbons that were fused in the proposed β-sitosteryl-D-glycoside [29]. Similarly, signals observed at δ_C 50.1 and 42.3 are the carbons that were fused in the proposed skeleton. The signals at δ_C 37.3, 29.7, 77.4, 38.8, 31.9, 23.1, 40.6, 24.3 and 28.1 for the cyclohexyl and cyclopentyl carbons, δ_C 35.9, 33.8, 29.7, 45.6, 29.2, 25.9 and 12.3 for the carbons constituting the side chain of six carbons in the cyclopentyl ring were noticed. The signals obtained at δ_C 73.9, 77.4, 70.6, 77.3 and 61.6 are due to the carbons of the sugar moiety. Moreover, the >C=C< carbons at δ_C 140.9 and 121.9 and tetrahydropyran carbon at δ_C 101.3 were observed. The obtained FT-IR, ^{1}H-NMR and ^{13}C-NMR spectral data were compatible with the previous reported data of β-sitosterol-3-O-β-D-glucopyranoside [29].

Antimicrobial screening

The crude extracts of *G. procumbens* leaves exhibited antimicrobial activity against bacterial strains and the fungi strain (Table 3 and Figure 3).

According to the results obtained from the disc diffusion assay given in Table 3, EA crude extract of leaves showed the highest activity against *Citrobacter freundii* (12 mm), shown in inset (a) of Figure 3, followed by *Bacillus cereus* (11 mm), *Staphylococcus aureus* (11 mm), shown in inset (b) of Figure 3, and *Salmonella typhimurium* (8 mm). However, MeOH extract exhibited moderate antibacterial activity against *Citrobacter freundii* (8 mm) and *Staphylococcus aureus* (6 mm) and did not show any antibacterial activity against all the rest G^+ and G^- bacteria. Similarly, MeOH–H_2O extract exhibited moderate antibacterial activity against *Citrobacter freundii* (10 mm) and *Staphylococcus aureus* (8 mm) and did not show any antibacterial activity against all the rest G^+ and G^- bacteria.

Table 3. Antimicrobial activity of the leaf extracts of *Gynura procumbens*

Test Microorganisms	**Diameter of zone of inhibition (mm ± SD) (n = 3)**				
	EA	MeOH	MeOH-H_2O	Ciprofloxacin hydrochloride	Miconazole
Gram-positive bacteria (G^+)					
Bacillus cereus	11±0.5	-	-	30 ± 0.5	
Listeria monocytogenes	-	-	-	40 ± 0.5	
Staphylococcus aureus	11±0.5	6±0.5	8±0.5	32 ± 0.5	
Gram-negative bacteria (G^-)					
Escherichia coli	-	-	-	35 ± 0.5	
Salmonella typhimurium	8±1	-	-	30 ± 0.5	
Citrobacter freundii	12±1	8±0.5	10±1	35 ± 0.5	
Fungi					
Trichodarma harzianum	8±1	-	-		33.0 ± 0.5

'-' indicates no activity

The antifungal activity of the leaf extracts was tested against one fungal species, *Trichoderma harzianum* (Table 3). Interestingly, only EA extract showed activity against *Trichoderma harzianum* having inhibition zone of 8 mm and the other extracts were unchanged i.e., inactive. The EA extract of *G. procumbens* leaves shown a significant antibacterial activity, followed by MeOH and MeOH–H_2O extracts against both G^+ and G^- bacteria. This result suggests that the leaf extracts of *G. procumbens* can be used for the treatment of bacterial diseases. Moreover, the studied crude extracts have promising inhibition activity towards G^- bacteria, *Citrobacter freundii* (Figure 3). This indicates the presence of natural phytotoxic chemicals which can penetrate through the lipopolysaccharide membrane of the G^- bacteria [30,31]. On the other hand, broad

inhibition activity towards the G^+ bacteria were not observed probably because of specific activity towards the bacterial strains and relatively thick continuous cell wall [30]. However, only EA extract shows antifungal activity which indicates that the EA extract contains some phytotoxic chemicals which can penetrate through the cell membrane of the microorganisms.

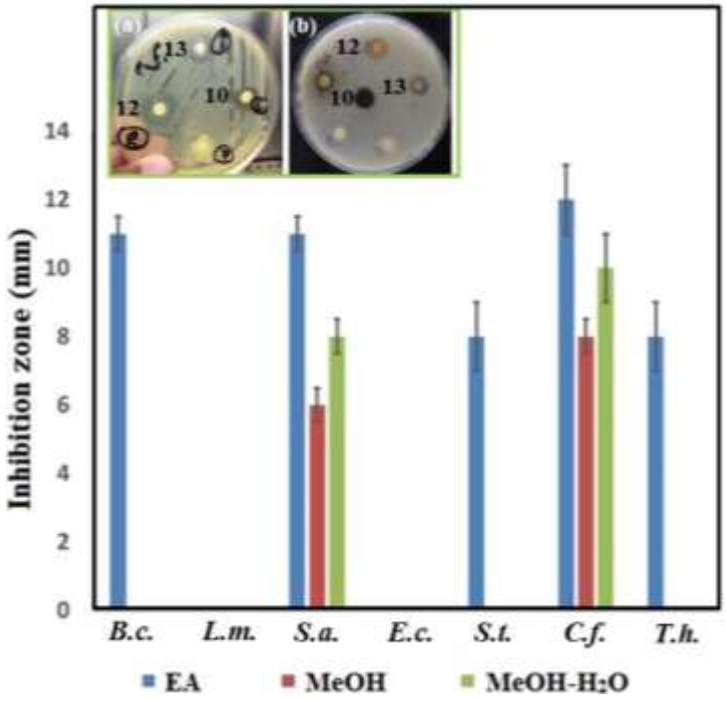

Figure 3. Inhibition zone of the crude extracts of *G. procumbens* leaves against the tested bacterial and fungal strains. *B.c.* (*Bacillus cereus*), *L.m.* (*Listeria monocytogenes*), *S.a.* (*Staphylococcus aureus*), *E.c.* (*Escherichia coli*), *S.t.* (*Salmonella typhimurium*), *C.f.* (*Citrobacter freundii*), *T.h.* (*Trichodarma harzianum*). (Inset: Antibacterial activity of extracts (a) 13(12), 10(8), 12(10) against *Citrobacter freundii* and (b) 13(11), 10(6), 12(8) against *Staphylococcus aureus*)

Cytotoxicity analysis of the crude extracts

The cytotoxic activities of the studied extracts are summarized in Tables 4 and 5. The LC_{50} values obtained from brine shrimp lethality bioassay were 63.10, 4.47, and 3.98 µg/mL, for EA, MeOH and MeOH-H_2O extracts, respectively. Compared to the positive control (VS, LC_{50} 0.36 µg/mL), all the extracts showed promising brine shrimp cytotoxic activity.

Table 4. Effect of ethyl acetate (EA), methanol (MeOH), methanol-H_2O (MeOH-H_2O) extracts of *Gynura procumbens* leaves and positive control vincristine sulphate on brine shrimp

Conc. (µg/mL)	Log C	% of Mortality			Probits of Mortality (%)			LC_{50} (µg/mL)			Vincristine Sulphate				
		EA	MeOH	MeOH -H_2O	EA	MeOH	MeOH -H_2O	EA	MeOH	MeOH -H_2O	Conc. µg/mL	Log C	% of Mortality	Probits of Mortality (%)	LC_{50} µg/mL
200	2.301	60	100	100	5.25	7.37	7.37	63.10	4.47	3.98	20	1.300	100	7.37	0.36
100	2.000	60	80	90	5.25	5.84	6.28				10	1.000	90	6.28	
50	1.699	50	70	70	5	5.52	5.52				5	0.698	80	5.84	
25	1.398	40	60	60	4.75	5.25	5.25				2.50	0.698	80	5.84	
12.5	1.097	30	60	60	4.48	5.25	5.25				1.25	0.096	70	5.52	
6.25	0.796	20	50	50	4.16	5	5				0.625	–0.204	60	5.25	
3.125	0.495	20	50	50	4.16	5	5				0.313	–0.488	40	4.75	
1.563	0.194	10	40	40	3.72	4.75	4.75				0.156	–0.806	30	4.48	
0.781	–0.107	10	30	30	3.72	4.48	4.48				0.078	–1.107	30	4.48	
											0.039	–1.409	20	4.16	

Table 5. The results of cytotoxicity of ethyl acetate (EA), methanol (MeOH), methanol-H_2O (MeOH-H_2O) extracts of *Gynura procumbens* leaves and positive control vincristine sulphate (VS) on brine shrimp

Samples	LC_{50} (µg/mL)	Regression Equation	[a]R^2
EA	63.10	y = 0.7077x + 3.7181	0.979
MeOH	4.47	y = 1.0089x + 4.3243	0.8209
MeOH-H_2O	3.98	y = 1.0532x + 4.3131	0.8576
VS	0.36	y = 1.0444x + 5.4516	0.9314

[a]Log (conc.) vs. probit correlation coefficient

The cytotoxicity of the studied extracts was found to be dose dependent and the mortality rate of the brine shrimp increases with the concentration of extracts (Table 4). It is interesting to observe the cytotoxicity of the extracts also depended on the polarity of the extraction solvent. Cytotoxic activity was one order of magnitude higher for MeOH-H_2O extracts compared to the EA extracts. This reflected the presence of polar bioactive active compounds at the Gynura procumbens Leaves [26].

At the experimental condition, the positive control groups showed nonlinear Probits of mortality at higher concentrations and linear rates at lower concentrations (Figure 4). Similar trends were observed for the MeOH and MeOH-H_2O extracts indicating the analogous mode of action compared to the positive control (Figure 4). On the other hand, for low polar EA extracts the Probit of mortality was linearly related to the log concentration thus the cytotoxic activity is concentration depended for this solvent system.

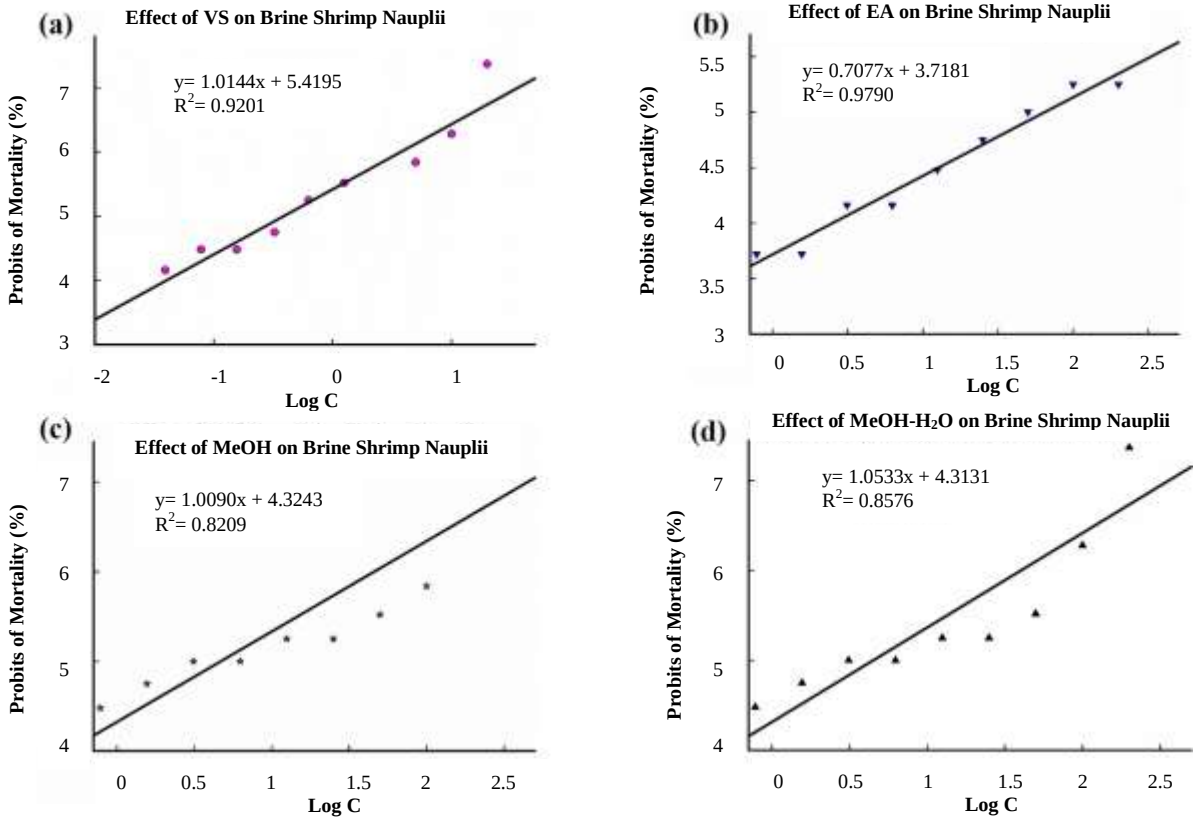

Figure 4. The correlation between Log C and probit of mortality percentages of (a) vincristine sulphate (VS), (b) EA, (c) MeOH and (d) MeOH-H_2O to *Artemia salina*

CONCLUSIONS

In this study, two compounds a cerebroside, 1-O-*β*-D-glucopyranosyl-2-[2′-hydroxylignocenoyl-amino]-10-octadecene-3, 4-diol from ethyl acetate and a *β*-sitosterol-3-O-*β*-D-glucopyranoside from methanol crude extracts of *Gynura procumbens* leaves have been isolated. Among the EA, MeOH, and MeOH-H_2O crude extracts, EA crude extract showed highest antimicrobial activity against most of the taken bacterial and fungal strains. On the other hand, the MeOH and MeOH-H_2O extracts exhibited almost similar antimicrobial activities towards all the G^+ and G^- bacterial and fungal strains. EA, MeOH, and MeOH-H_2O extracts of *Gynura procumbens* leaves exhibited cytotoxicity towards brine shrimp nauplii having LC_{50} values of 63.10, 4.47, and 3.98 μg/mL. Among the studied crude extracts, MeOH-H_2O extract was found to show the highest toxicity towards brine shrimp nauplii. This study clearly indicates that the studied crude extracts contain some potential bioactive components. Further investigations are needed to identify the active compounds present in the leaf extract responsible for the antimicrobial properties.

ACKNOWLEDGEMENTS

The authors strongly acknowledge Khulna University of Engineering & Technology for financial assistance. The authors are also grateful to Wazed Miah Science Research Centre (WMSRC), Jahangirnagar University, Savar, Dhaka, Bangladesh for supporting the NMR analysis. The authors are also thankful to Dr. Md. Latiful Bari, Chief Scientist & Head, Food Analysis and Research Laboratory, Centre for Advanced Research in Sciences (CARS), University of Dhaka, Bangladesh, for the investigations of antimicrobial activities.

Conflict of interests

The authors declare that there has no conflict of interest.

REFERENCES

1. Atanasov A.G., Waltenberger B., Pferschy-Wenzig E.M., Linder T., Wawrosch C., Uhrin P., Temml V., Wang L., Schwaiger S., Heiss E.H., Rollinger J.M., Schuster D., Breuss J.M., Bochkov V., Mihovilovic M.D., Kopp B., Bauer R., Dirsch V.M., Stuppne H., 2015. Discovery and resupply of pharmacologically active plant-derived natural products: A review Biotechnol Adv. 33(8), 1582–1614.

2. Doughari J.H., 2006. Antimicrobial activity of *Tamarindus indica Linn*. Trop J Pharm Res. 5(2), 597–603.

3. Maghrani M., Zeggwagh N.A., Michel J.B., Eddouks M., 2005. Antihypertensive effect of *Lepidium sativum L*. in spontaneously hypertensive rats. J Ethnopharmacol. 100(1–2), 193–197.

4. Dash P.R., Mou K.M., 2016. A comprehensive review on *Gynura procumbens* leaves. Int J Pharmacognosy. 3(4), 167–174.

5. Tan H.L., Chan K.G., Pusparajah P., Lee L.H., Goh B.H., 2016. *Gynura procumbens*: An overview of the biological activities. Front Pharmacol. 7, 52.

6. Rohin M.A.K., Jumli M.N., Ridzwan N., Baig A.A., Latif A.Z.A., Hadi N.A., 2018. Effect of *Gynura procumbens* extracts on anti-proliferative activity and its associated morphological changes of human glioblastoma multiforme cell line (U-87). Pharmacogn J. 10(3), 492–496.

7. Rosidah, Yam M.F., Sadikun A., Ahmad M., Akowuah G.A., Asmawi M.Z., 2009. Toxicology evaluation of standardized methanol extract of *Gynura procumbens*. J Ethnopharmacol. 123(2), 244–249.

8. Nawawi A., Nakamura N., Hattori M., Kurokawa M., Shiraki K., 1999. Inhibitory effects of Indonesian medicinal plants on the infection of herpes simplex virus type 1. Phyther Res. 13(1), 37–41.

9. Akowuah G.A., Sadikun A., Mariam A., 2002. Flavonoid identification and hypoglycaemic studies of the butanol fraction from *Gynura procumbens*. Pharm Biol. 40(6), 405-410.

10. Li W.L., Ren B.R., Min-Zhuo, Hu.Y., Lu, C.G., Wu, J.L., Chen, J., Sun, S., 2009. The anti-hyperglycemic effect of plants in genus *Gynura Cass*. Am J Chin Med. 37(05), 961-966.

11. Iskander M.N., Song Y., Coupar I.M., Jiratchariyakul W., 2002. Antiinflammatory screening of the medicinal plant *Gynura procumbens*. Plant Foods Hum Nutr. 57(3–4), 233–244.

12. Wiart C., 2006. Medicinal plants of the Asia-Pacific: drugs for the future? World Scientific Publishing Co. Pte. Ltd. Singapore.

13. Lam S.K., Idris A., Bakar Z.A., Ismail R., 1998. *Gynura procumbens* and blood pressure in the rat: preliminary study. Asia Pac J of Pharmacol. 13(S1), S14-S15.

14. Kim M., Lee H.J., Wiryowidagdo S., Kim H.K., 2006. Antihypertensive effects of *Gynura procumbens* extract in spontaneously hypertensive rats. J Med Food. 9(4), 587–590.

15. Hoe S.Z., Kamaruddin M.Y., Lam S.K., 2007. Inhibition of angiotensin-converting enzyme activity by a partially purified fraction of *Gynura procumbens* in spontaneously hypertensive rats. Med Princ Pract. 16(3), 203–208.

16. Zhang X.F., Tan B.K.H., 2000. Effects of an ethanolic extract of *Gynura procumbens* on serum glucose, cholesterol and triglyceride levels in normal and streptozotocin-induced diabetic rats. Singap Med J. 41(1), 9-13.

17. Agustina D., Wasito W., Haryana S.M., Supartinah A., 2006. Anticarcinogenesis effect of *Gynura procumbens* (Lour) Merr on tongue carcinogenesis in 4NQO-induced rat. Dent J. (Majalah Kedokteran Gigi). 39(3), 126-132.

18. Lee H.J., Lee B.C., Chung J.H., Wiryowidagdo S., Chun W., Kim S.S., Kim H., Choe M., 2007. Inhibitory effects of an aqueous extract of *Gynura procumbens* on human mesangial cell proliferation. Korean J Physiol Pharmacol. 11(4), 145–148.

19. Ashraf K., Halim H., Lim S.M., Ramasamy K., Sultan S., 2020. *In vitro* antioxidant, antimicrobial and antiproliferative studies of four different extracts of Orthosiphon stamineus, *Gynura procumbens* and *Ficus deltoidea*. Saudi J Biol Sci. 27(1), 417-432.

20. Yam M.F., Sadikun A., Asmawi M.Z., Rosidah., 2008. Antioxidant potential of *Gynura procumbens*. Pharm Biol. 46(9), 616–625.

21. Afandi A., Sadikun A., Ismail S., 2014. Antioxidant properties of *Gynura procumbens* extracts and their inhibitory effects on two major human recombinant cytochrome P450s using a high throughout luminescence assay. Asian J Pharm Clin Res. 7, 36-41.

22. Mahmood A.A., Mariod A.A., Al-Bayaty F., Abdel-Wahab S.I., 2010. Anti-ulcerogenic activity of *Gynura procumbens* leaf extract against experimentally-induced gastric lesions in rats. J Med Plants Res. 4(8), 685–691.

23. Santhosh R.S., Suriyanarayanan B., 2014. Plants: A source for new antimycobacterial drugs. Planta Med. 80(1), 9–21.

24. Rahman A.F.M.M., Asad M.S.A., 2013. Chemical and biological investigations of the leaves of *Gynura procumbens*. Int J Biosci. 3(4), 36–43.

25. Bauer A.W., Kirby W.M., Sherris J.C., Turck M., 1966. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol. 45(4), 493-496.

26. Meyer B.N., Ferrigni N.R., Putnam J.E., 1982. Brine shrimp: A convenient general bioassay for active plant constituents. Planta Med. 45, 31-34.

27. Chen L., Wang J.J., Zhang G.G., Song H.T., Qin L.P., 2009. A new cerebroside from *Gynura divaricata*. Nat Prod Res. 23(14), 1330–1336.

28. Hu J.W., Wu J., Li X.H., Huang B.H., Yuan E., Wu L., Ma G.Q., 2019. A New Cerebroside from the Roots of *Gynura procumbens*. Chem Nat Compd. 55(6), 1053-1055.

29. Jarikasem S., Charuwichitratana S., Siritantikorn S., Chantratita W., Iskander M., Frahm A.W., Jiratchariyakul W., 2013. Antiherpetic effects of *Gynura procumbens*. Evid.-Based Complementary Altern Med. 2013.

30. Silhavy T.J., Kahne D., Walker S., 2010. The bacterial cell envelope. Cold Spring Harb Perspect Biol. 2(a000414), 1–16.

31. Tasdelen F.N., Tanriverdi C.Y., Coban A.Y., Ozatli D., Tanyel E., Durupinar B., Tulek N., 2009. Antimicrobial activity of plant extract Ankaferd Blood Stopper. Fitoterapia. 80(1), 48–50.