Asian Journal of Chemistry; Vol. 33, No. 8 (2021), 1950-1956

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2021.23277

Phytochemical Analysis and Comprehensive Evaluation of Pharmacological Activities, Isolation and Characterization of Bioactive Compound from the Bark of *Sterculia urens* Roxb.

CHAITANYA DARAPUREDDY, K.R.S. PRASAD* and PHANI R.S. CH

Department of Chemistry, Koneru Lakshmaiah Education Foundation, Vaddeswaram-522502, India

*Corresponding author: E-mail: krsprasad_fed@kluniversity.in

Received: 27 April 2021; *Accepted*: 20 July 2021; *Published online*: 26 July 2021; AJC-20451

The objective of the present study is to investigate the phytochemical constituents by qualitative and quantitative analysis, pharmacological activities such as antioxidant, antidiabetic, anti-inflammatory, thrombolytic and antibacterial activities of different crude extracts from bark of *Sterculia urens* Roxb. Further, the preparative HPLC isolation and spectroscopic characterization of the bioactive phytochemical constituents were also carried out. Different solvents such as *n*-hexane, ethyl acetate, methanol and water were used to prepare the crude extracts from the bark using Soxhlet extraction apparatus. DPPH free radical scavenging assay (antioxidant), α-amylase inhibition assay (antidiabetic), albumin denaturation assay (anti-inflammatory), blood clot lysis method (thrombolytic) and well-diffusion method (antibacterial) were performed for the determination of pharmacological activities of the bark extracts. The preparative HPLC analysis was carried for the separation and purification of bioactive compounds and the identification of isolated compounds was carried using ^{1}H NMR, ^{13}C NMR and mass spectroscopy. The quantitative estimation studies confirmed that methanolic extract contains 7.75 ± 0.141 GAE/g of phenolic compounds, 10.47 ± 0.033 mg of QE/g of flavonoids and 8.70 ± 0.047 mg/g of terpenoids. The ethyl acetate extract contains 2.16 ± 0.126 GAE/g of phenolic compounds whereas the aqueous extract contains 16.53 ± 0.055 mg/g of saponins. High DPPH radical scavenging was observed for methanolic extract with IC_{50} concentration of 85.38 ± 0.213 µg/mL. The α-amylase inhibition activity with IC_{50} concentrations of 145.67 ± 1.87, 98.36 ± 0.47 and 194.47 ± 0.55 µg/mL for ethyl acetate, methanol and aqueous extracts respectively. The albumin denaturation inhibition activity was found to be very high for methanolic extract with IC_{50} values of 132.08 ± 0.13 µg/mL which is near to the standard (107.13 ± 0.13 µg/mL). The % clot lysis of the methanolic extract in thrombolytic activity was found to be similar to the 100 µL of streptokinase (62.36 ± 0.140 %). Two terpenoids (One known terpenoid mansonone G and one new terpenoid) were isolated from the methanolic extract using preparative HPLC separation. Three known flavonoids (farrerol, apigenin and 6-hydroxyluteolin) and one new flavonoid were also isolated from the methanolic extract. The results suggested that bark extracts of *Sterculia urens* Roxb. having rich phytochemical constituents with high pharmacological activities.

Keywords: *Sterculia urens* Roxb., Pharmacological activities, Terpenoid, Flavonoids, Preparative HPLC, Biological activities.

INTRODUCTION

Plant derived natural products serves as a huge source of compounds having amazing structural and functional diversity that makes significant contribution in the development of new therapeutic drugs. Many researchers throughout the world are carrying research to explore and isolate the bioactive secondary metabolites from natural resources [1]. In recent years, the exploration of plant based products for control of different diseases has been taken extensive attention and different products were explored from plants. In treatment of many common diseases, plants are utilized since ancient times and these practiced medicines become tradition and is still used as part of habitual treatment of various maladies [2]. Medicines which are derived from the natural products have significant important source as therapeutic agents and 25-30% of medicines available to cure various diseases are derived from natural products [3].

Medicinal plants having diverse assortment of compounds such as tannins, alkaloids, saponins, phenolic compounds and terpenoids that can produce explicit physiological action in human body. These plant derived compounds have high therapeutic performance and very low toxicity. Numerous therapeutically active compounds have been isolated from the various

parts of the plants which can be used for the invention of novel drugs for treatment of different diseases [4]. *Sterculia urens* Roxb. (karaya) is a small to medium size tree belongs to family *Malvaceae*. It is proved to exhibit the antifungal [5], antioxidant and antimicrobial [6] properties. The gum obtained from *S. urens* was used as a laxative and also in the preparation of hydrophilic matrix tablets [7]. In the literature, it was observed that the pharmacological activities of the *Sterculia urens* Roxb. were not explored and hence the present study is aimed for the determination of pharmacological activities and identification of bioactive phytochemicals in bark of *Sterculia urens* Roxb. plant.

EXPERIMENTAL

Collection of plant material: The fresh bark of *Sterculia urens* Roxb. was collected in Paderu village, Visakhapatnam district, India in January 2018. The collected voucher specimen of plant species was identified and authenticated by Dr. Ch. Srinivasa Reddy, Department of Botany, P.B. Siddhartha College of Arts and science Vijayawada, India. The bark was rinsed with little distilled water and then surface cleaned with sterile cotton to remove the dirt on the surface of bark. Then cut into small pieces and dried under shade until constant weight was obtained. The dried sample was grounded into fine powder and preserved in an Amber colour bottle for further use.

Extraction and sample preparation: Soxhlet extraction apparatus was used for the extraction of phytochemical constituents from *Sterculia urens* Roxb. bark as per the procedure described earlier [8]. The extraction was carried by successive extraction with *n*-hexane, ethyl acetate, methanol and water as extraction solvents. The extracts were dried using rotatory vacuum evaporator and the crude extracts were preserved in a freezer at 0 ºC for future analysis [9].

Qualitative phytochemical analysis: The phytochemical evaluation of the crude ethyl acetate, methanol and aqueous bark extracts of *Sterculia urens* Roxb. was carried as per the standard procedures [10-12]. The colour change as per the procedure described in each studied test indicates the positive test, which confirms the presence of the studied compound in the extract and no change in colour indicates negative test and confirms the absence of the studied compound in the extracts.

Quantitative Phytochemical analysis: Folin-Ciocalteu reagent assay method as per the procedure described by Noreen *et al*. [13] was performed for the determination of total phenolic compounds in the bark extracts using gallic acid as standard and the results were expressed as mg gallic acid equivalent (GAE)/g of extract. The total flavonoid content in the bark extracts was determined using aluminum colorimetric method as per the procedure described by Pawar & Dasgupta [14]. The known flavonoid quercetin was used as standard and quantification results were expressed in terms of mg quercetin equivalent (QE)/g of plant extract. The total alkaloids present in the bark extracts were determined using bromocresol green spectrophotometric method [15] using atropine standard and the results were expressed in terms of mg atropine equivalent (AE)/g of the plant extract. The non-spectrophotometric quantitative analysis was used for the determination of terpenoids and saponins in the bark extract of *S. urens* Roxb. [16] and the results were expressed in % yield per gram of the extract.

Pharmacological activities

Antioxidant activity by DPPH free radical scavenging assay: The antioxidant activity of different solvent extracts of *Sterculia urens* Roxb. was studied by DPPH free radial scavenging assay method [17]. In a typical experiment, 3 mL of *S. urens* Roxb. bark extracts in various concentrations was mixed with 2 mL of methanolic DPPH (0.1 mmol/L) solution. The content was incubated in dark at room temperature for 30 min. Then after incubation, the optical density (OD) of the solution was measured at 517 nm using double UV-visible spectrophotometer (TECHOMP UV 2301, Japan). The radical scavenging activity of the bark extracts was calculated using eqn. 1:

$$\text{Radical scavenging activity (\%)} = \frac{OD_{control} - OD_{sample}}{OD_{control}} \times 100 \quad (1)$$

The effective concentration of bark extracts that scavenged the DPPH radicals by 50 % (IC_{50}) was calculated by the interpolation from linear regression analysis of the obtained results. The ascorbic acid (AA) was considered as reference standard and from the obtained IC_{50} values, the AA equivalent (AA_{EQ}) was calculated using eqn. 2:

$$AAEQ = \frac{IC_{50,AA}}{IC_{50,extract}} \quad (2)$$

Antidiabetic activity by α-amylase inhibition assay: Antidiabetic activity of the bark extracts of *S. urens* Roxb. was determined as per the methodology reported by Shettar *et al*. [18]. In brief, 0.5 mL different concentrations of bark extract were mixed with 0.5 mL (0.5 mg/mL) α-amylase solution with sodium phosphate buffer (pH 6.9). Then the reaction mixture was incubated for 10 min at room temperature and 0.5 mL (1%) starch solution in sodium phosphate buffer was added. The resulting solution was incubated for 10 min at room temperature, then heated on a waterbath at 100 ºC for 5 min. Then the reaction was terminated by adding 1 mL of dinitrosalicylic acid colour reagent and then cooled to room temperature. The final volume in all the test tubes were made up to 10 mL and the absorbance was measured at 540 nm. Similar experiment was performed by replacing extract and α-amylase with buffer solution and considered as blank. The control solution was prepared by replacing extract with buffer. The standard drug acarbose is considered as standard and the α-amylase inhibition activity was calculated using eqn. 3:

$$\text{Inhibition (\%)} = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100 \quad (3)$$

Anti-inflammatory activity by inhibition of albumin denaturation assay: The bark extracts of *Sterculia urens* Roxb. were screened for its anti-inflammatory activity by inhibition of albumin denaturation assay as per the method described by Murthuza & Manjunatha [19] using diclofenac as standard. In brief, 1 mL of the selected concentration of bark extract was mixed with 1 mL of albumin (1mM) solution in phosphate buffer. The reaction mixture was incubated at room temperature for 10 min. Then, the turbidity of the reaction mixture was

measured spectrophotometrically at 660 nm. The % albumin denaturation inhibition activity was calculated using eqn. 4:

$$\text{Inhibition (\%)} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100 \quad (4)$$

Thrombolytic activity by blood clot lysis method: The thrombolytic activity of the bark extracts of *S. urens* Roxb. was evaluated as per the procedure described by Imam *et al.* [20]. In this study, 1 mL of blood sample was taken in a sterile, pre-weighed microcentrifuge tube. The tubes were incubated at room temperature till the formation of blood clot. The serum formed in the tubes were discarded carefully without disturbing the clot and the weight of the clot in each tube was determined. Then in each tube, 100 µL of selected concentrations of plant extract was added and the tubes were incubated at 37 ºC for 90 min. The lysis clot in the tube and the formation of fluid in each tube was observed. The fluid formed in each tube was discarded carefully without disturbing the clot and then the weight of clot after clot disruption was determined and the % clot lysis was calculated using eqn. 5:

$$\text{Clot lysis (\%)} = \frac{\text{Weight of released clot}}{\text{Clot weight}} \times 100 \quad (5)$$

Streptokinase and methanol was selected as positive and negative control, respectively in this study. The same procedure described for the plant extract was performed for 100 µL of both positive and negative controls.

Antibacterial activity by agar plate well diffusion method: The antibacterial activity of bark extracts of *S. urens* Roxb. was studied by agar plate well diffusion method [21]. In this study, two Gram-positive bacteria namely *Bacillus subtilis* (MTCC 1427) and *Staphylococcus aureus* (MTCC 1430); two Gram-negative bacteria namely *Escherichia coli* (MTCC 294) and *Pseudomonas aeruginosa* (MTCC 1748) were selected. In a sterile petri dish, 10 mL of Mueller- Hinton agar medium was poured as a basal layer followed with 15 mL of seeded medium previously inoculated with selected bacterial suspension (100 mL of medium/1 mL of 10^7 CFU) to attain 10^5 CFU/mL of medium. Then wait till the complete solidification of medium in the petri plate and wells were prepared using sterilized stainless-steel cork borer. In each well, 25 µL of selected concentration of stem extracts and gentamycin (standard) were loaded with sterile micro-pipette. Simultaneously in a separate petri dish, water was loaded and served as negative control and plates were grown at 37 ºC for 24 h. The zone of inhibition of standard and bark extracts was measured in mm by comparing with negative control.

Isolation and purification of compounds with semi-preparative scale HPLC: The isolation and purification of bioactive phytochemical constituents in the bark extracts of *S. urens* Roxb. was carried on loop load Semi-preparative HPLC-UV system (Shimadzu, Japan) equipped with LC-20A module pumps, UV-visible detector (SPD20A), Rheodyne® valve (7725I) injector and fraction collector (FRC-10A). The separation of phytoconstituents was carried in Waters XBridge BEH C18 OBD Preparative column (250 mm × 19 mm; 5 µm). The HPLC system was controlled and monitored by using Lab-Solutions software (Shimadzu, Japan). The extract was reconstituted with solvent at a concentration of 50 mg/mL and then filtered through 0.2 µ nylon membrane filter and the filtered sample used for preparative HPLC separation of compounds in the extract.

The preparative HPLC separation of phenolic compounds present in the methanolic bark extract was carried according to the procedure described by Jallali *et al.* [22] with slight modifi-cation. The mobile phase consists of 0.025% TFA as solvent A and acetonitrile as solvent B at a flow rate of 3 mL/min in gradient elution. The gradient elution program was: 10% B (0-5/min), 10-100% B (5-55/min), 100% B (55-60/min), 100-10% B (60–65/min). Flow rate was fixed at 3/mL/min and the column eluents were monitored using UV detector at 280 nm wavelength. The terpenoids separated from the methanolic bark extract in quantitative estimation were used for the isolation of terpenoid compounds using preparative HPLC. The method conditions for the separation of terpenoids was adopted from Morin *et al.* [23] and Hu *et al.* [24]. The separation was carried using aqueous MeOH (82%) as mobile phase at a flow rate of 10 mL/min and UV detection was monitored at 220 nm.

The retention time of the resultant chromatograms were monitored for the identification of number of compounds separated in the extract. Each peak in the chromatogram represents compound and were collected as separate fraction and each fraction contains one compound. The collected fractions were concentrated under vacuum to evaporate the solvent and the residue was freeze dried and stored in an air-tight amber glass container until further use.

Characterization of isolated compounds: The compounds isolated from the bark extracts of *S. urens* Roxb. were characterized using spectroscopic studies such as ^{1}H & ^{13}C NMR, FT-IR and mass for each purified compound. The integral values in each spectral study were interpreted and structure of the isolated pure compound was elucidated.

RESULTS AND DISCUSSION

The present study is aimed to evaluate the phytochemical composition and pharmacological activities of *Sterculia urens* Roxb. bark. The extraction of phytochemicals from bark was carried in continuous extraction process using Soxhlet extraction apparatus. The volatile organic solvents such as *n*-hexane, ethyl acetate, methanol and aqueous solvents were used for extraction of phytochemical constituent's in successive extraction method with increase in polarity of extraction solvents. The extract obtained from the non-polar solvent *i.e.* *n*-hexane was discarded and further study was carried with ethyl acetate, methanol and water solvent extracts. The phytochemical screening confirmed that ethyl acetate extract shows positive for flavonoids, cardiac glycosides, phenolic compounds and anthraquinones. The methanolic extract shows positive for terpenoid, flavonoids, steroids, cardiac glycosides, phenolic compounds, alkaloids, coumarins and glycosides whereas the aqueous extract shows the positive test for saponin and steroids (Table-1).

TABLE-1
PHYTOCHEMICAL SCREENING RESULTS OF BARK EXTRACTS OF *Sterculia urens* Roxb

Test studied	Ethyl acetate extract	Methanol extract	Aqueous extract
Terpenoids	–	++	–
Flavonoids	+	++	–
Saponins	–	–	++
Steroids	–	+	+
Cardiac glycosides	+	++	–
Proteins	–	–	–
Carbohydrates	–	–	–
Monosaccharides	–	–	–
Reducing sugars	–	–	–
Phenolic compounds	+	++	–
Alkaloids	–	++	–
Coumarins	–	+	–
Anthraquinones	+	–	–
Glycosides	–	+	–

++ Indicates high concentration; + Indicates low concentration; – Indicates absence.

The percentage yield of the different solvent extracts obtained from bark of *S. urens* Roxb. was found to be 5.61 ± 0.015, 12.56 ± 0.082, 18.83 ± 0.097 and 8.46 ± 0.106 for *n*-hexane, ethyl acetate, methanol and aqueous extracts, respectively. The results confirmed that high percentage yield was obtained for the methanolic extract followed by ethyl acetate extract.

The quantitative estimation of the phenolic compounds present in ethyl acetate and methanolic bark extracts of *S. urens* Roxb. was studied by Folin-Ciocalteu reagent assay using gallic acid as standard. It was estimated that ethyl acetate extract contains 2.16 ± 0.126 GAE/g of extract whereas the methanolic extract contains 7.75 ± 0.141 GAE/g of extract. The preliminary screening results confirm the presence of flavonoids in ethyl aceate and methanolic extracts and studied for the quantitative estimation by following aluminum colorimetric method using quercetin standard and results were expressed in QE/gram of plant extract. The high quantity of flavonoids was quantified in methanolic extract where in 10.47 ± 0.033 mg of QE/g of extract of flavonoids were estimated whereas ethyl acetate extract contains 4.00 ± 0.034 mg of QE/g. The methanolc bark extract was tested positive for alkaloids and hence studied for the quantitative estimation using atropine and the results were expressed in mg of AE/g of each extract. The methanolic extract contains 8.78 ± 0.079 mg of AE/g of alkaloids.

The non-spectrophotometric method was used for the quantitative estimation of terpenoids in methanolic extract and saponins in aqueous extract. The methanolic extract contains 8.70 ± 0.047 mg/g of terpenoids while the aqueous extract contains 16.53 ± 0.055 mg/g of saponins. The results of the quantitative estimation of different solvents bark extracts of *S. urens* Roxb. confirm that the methanolic extract contains high amount of phenolic compounds and aqueous extract contains high amount of saponins.

The results of the quantitative analysis confirm that the phytochemical compounds were present in high quantity in the extracts. The quantitative analysis of the present study is supported by the available literature with the same plant [6] and various studies in the same genera [25,26].

The DPPH free radical scavenging activity of different solvent extracts of *S. urens* Roxb. bark was studied in the concentration range of 5-40 µg/mL and the results were compared with standard ascorbic acid. The results confirmed that the inhibition activity was observed to very high in methanolic extract whereas ethyl acetate extract shows very less activity. At very high concentration studied *i.e.* at 40 µg/mL, the DPPH radical inhibition was observed to be 98.77 ± 0.102, 46.14 ± 0.329, 85.38 ± 0.213 and 54.06 ± 0.516%, respectively for ascorbic acid standard, bark ethyl acetate, methanolic and aqueous extracts, respectively. The IC_{50} was observed to be 22.20 ± 0.028, 48.51 ± 0.208, 27.76 ± 0.057 and 45.72 ± 0.045 µg/mL, respectively and the results are shown in Fig. 1. The results confirmed that the methanolic extracts shows the high activity and the results are in argument towards earlier reports suggesting the use of methanol extract for the radical scavenging activity analysis [27].

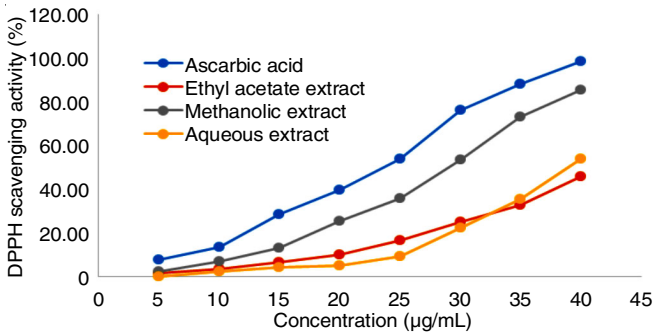

Fig. 1. DPPH radical scavenging activity results

α-Amylase inhibition assay: The α-amylase is the key target enzymes in the digestion of carbohydrates such as starch to oligosaccharides. The inhibition of these enzymes is an important therapeutic strategy to manage postprandial blood glucose peaks [28]. In this perspective, several antidiabetic drugs are being probed in an endeavor to produce potent enzyme inhibitors. Nonetheless, many of them have proved to induce unfavorable side effects [29].

α-Amylase inhibition assay was carried for the determination of antidiabetic activity of the bark extracts of *S. urens* Roxb. using acarbose as standard. The α-amylase inhibition activity of methanolic extract was found to be very high than ethyl acetate and aqueous extracts. At a concentration of 75 µg/mL, the α-amylase inhibition assay was found to be 82.96 ± 0.247, 48.64 ± 0.119, 72.52 ± 0.137 and 34.32 ± 0.342 for standard acarbose, ethyl acetate, methanol and aqueous extracts, respectively. A high concentration *i.e.* at 300 µg/mL, the α-amylase inhibition assay was found to be 96.20 ± 0.119, 80.15 ± 0.137, 91.46 ± 0.119 and 64.73 ± 0.247 for the standard acarbose, ethyl acetate, methanol and aqueous extracts, respectively. The 50% inhibition concentration (IC_{50}) was found to be 71.51 ± 0.08, 145.67 ± 1.87, 98.36 ± 0.47 and 194.47 ± 0.55 for standard acarbose, ethyl acetate, methanol and aqueous extracts, respectively. Fig. 2 gives the comparative α-amylase inhibition assay results of barks extracts of *S. urens* Roxb.

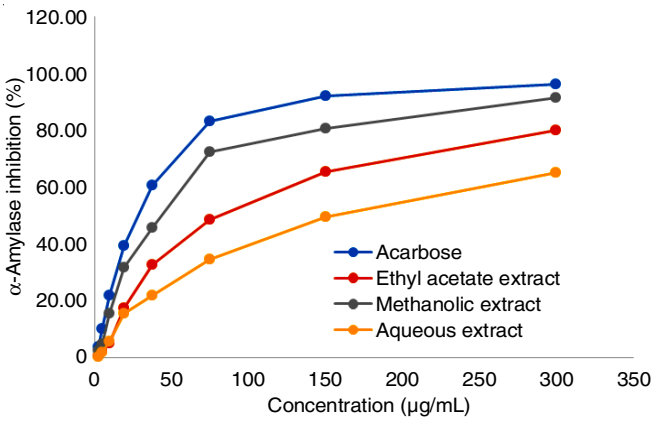

Fig. 2. α-Amylase inhibition assay

Albumin denaturation assay: The albumin denaturation inhibition assay was performed for the determination of anti-inflammatory activity of different solvents bark extracts of *S. urens* Roxb. and the results observed in the study is represented in Fig. 3. The assay was performed in the concentration range of 25-200 µg/mL and the drug diclofenac was used as standard. Among the extracts studied, the albumin denaturation inhibition activity was found to be very high for methanolic extracts with IC_{50} values of 132.08 ± 0.13 µg/mL which is close to the standard (107.13 ± 0.13 µg/mL). The ethyl acetate and aqueous bark extracts were found to be less albumin denaturation inhibition activity with IC_{50} at 184.22 ± 0.19 and 217.50 ± 0.33 µg/mL, respectively. This confirmed that ethyl acetate and aqueous extracts show less while the methanolic extract shows the high anti-inflammatory activity.

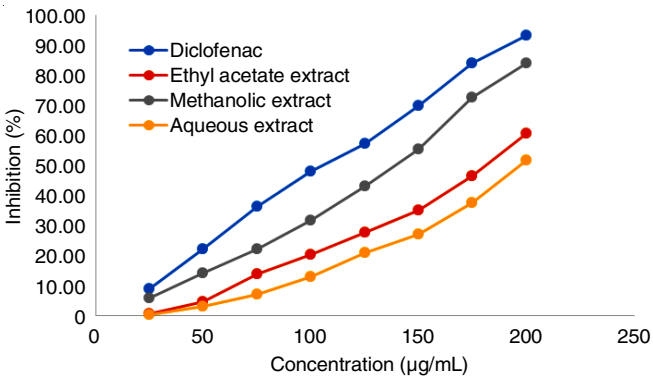

Fig. 3. Anti-inflammatory activity by inhibition of albumin denaturation assay

Thrombolytic activity: The effect of bark extracts of *S. urens* Roxb. on *in vitro* clot lysis are shown in Table-2. The results confirmed that the % of clot lysis was 62.36 ± 0.140% when 100 µL of strepto-kinase (30,000 I.U.) was used as a positive control. Distilled water is used as negative control and shows the negligible (0.53 ± 0.020%) lysis in the blood clot. The methanolic extract at 10 mg/mL showed more significant clot lysis *i.e.* 51.72 ± 0.031%. At a high dose of 10 mg/mL, the % clot lysis for ethyl acetate and aqueous extracts were found to be 16.93 ± 0.040 and 18.91 ± 0.025%, respectively, which confirmed that the activity was very less in ethyl acetate and aqueous extracts. However, all the extracts showed zone inhibition at a very low concentration of 1 µg/mL, which confirmed that the extracts exhibit antibacterial activity.

TABLE-2
EFFECTS OF DIFFERENT CONCENTRATION OF BARK EXTRACTS OF BOTH *Sterculia urens* Roxb ON *in vitro* CLOT LYSIS

Concentration (mg/mL)	% of Clot lysis observed		
	Ethyl acetate extract	Methanolic extract	Aqueous extract
2	1.23 ± 0.021	5.64 ± 0.036	2.03 ± 0.021
4	3.95 ± 0.010	11.45 ± 0.036	4.13 ± 0.020
6	7.66 ± 0.044	27.71 ± 0.030	8.17 ± 0.021
8	11.46 ± 0.049	39.53 ± 0.017	13.36 ± 0.050
10	16.93 ± 0.040	51.72 ± 0.031	18.91 ± 0.025

Negative control (water) and positive control (Streptokinase)
Treatment % of clot lysis
Streptokinase: 62.36 ± 0.140; Blank: 0.53 ± 0.020

All the value expressed as mean ± SD (n = 3)

Agar plate well diffusion method: The bark extracts of *S. urens* Roxb. were screened for the determination of antibacterial activity by agar plate well diffusion method. Gentamycin standard and plant extracts at the concentrations of 1, 10 and 50 µg/mL were studied against two Gram-positive and two Gram-negative bacteria and the results are presented in Table-3. The results confirmed that all the plant extracts were potentially effective in suppressing the growth of studied bacteria with variable potency. Among the bacteria studied, the extracts show more potent against the growth of Gram-negative bacteria. The ethyl aceate and methanolic extracts at a very low concentration of 1 µg/mL also showed the zone of inhibition against the growth of Gram-negative bacteria *i.e. Escherichia coli* and *Pseudomonas aeruginosa*. Moreover, the aqueous extract at low concentration of 1 µg/mL doesn't show zone of inhibition against the studied bacteria. At a concentration of 10 and 50 µg/mL, all the extracts having potential growth inhibition zone against all the bacteria.

HPLC analysis: The results observed in the pharmacological activities confirmed that the methanolic extract shows the dominant activity and the quantitative analysis confirmed the presence of high amount of phenolic compounds, flavonoids and terpenoids. Hence, the flavonoids and terpenoids in the methanolic bark extract were separated, purified using preparative HPLC analysis and the structure elucidation of the purified compounds was carried using spectral analysis.

The preparative HPLC analysis of the isolated terpenoid fraction shows four peaks corresponds to four different terpenoids in the isolate fraction. Among the four compounds, two compounds were found to be quantitatively very less while other two compounds were enough isolated and designated as **BTF-1** and **BTF-2**. These two isolated compounds were characterized by UV, mass, ^1H & ^{13}C NMR spectroscopic techniques.

BTF-1: White-off crystal; *m.f.:* $C_{15}H_{16}O_3$; UV (CD_3OD, λ_{max}) 263 nm; ^1H NMR (500 MHZ, CD_3OD, δ ppm): 1.36 (6H, d, J = 7.0 Hz), 2.27 (3H, s), 2.62 (3H, s), 3.83 (1H, sept, J = 7.0 Hz), 6.87 (1H, s), 7.53 (1H, s), 10.61 (1H, s); ^{13}C NMR (500 MHZ, CD_3OD, δ ppm): 123.57 (C-1), 135.85 (C-2), 180.85 (C-3), 138.67 (C-4), 182.57 (C-5), 136.18 (C-6), 120.50

TABLE-3
ANTIBACTERIAL ACTIVITY RESULTS

Sample	Zone of growth inhibition observed (mm)			
	Bacillus subtilis	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*
EAE at 1 µg/mL	–	–	2.70 ± 0.10	2.20 ± 0.10
EAE at 10 µg/mL	3.67 ± 0.06	3.13 ± 0.06	4.83 ± 0.06	5.67 ± 0.06
EAE at 50 µg/mL	5.60 ± 0.10	4.73 ± 0.06	7.20 ± 0.10	8.83 ± 0.06
ME at 1 µg/mL	2.23 ± 0.15	2.20 ± 0.10	3.87 ± 0.15	3.23 ± 0.15
ME at 10 µg/mL	4.60 ± 0.10	5.27 ± 0.06	7.73 ± 0.12	7.20 ± 0.10
ME at 50 µg/mL	6.90 ± 0.10	7.90 ± 0.20	9.77 ± 0.15	10.93 ± 0.15
AE at 1 µg/mL	–	–	–	–
AE at 10 µg/mL	2.93 ± 0.15	2.53 ± 0.15	3.53 ± 0.06	4.30 ± 0.20
AE at 50 µg/mL	4.93 ± 0.15	4.30 ± 0.10	6.03 ± 0.15	6.50 ± 0.20
GM at 1 µg/mL	4.70 ± 0.20	4.13 ± 0.15	5.33 ± 0.15	3.73 ± 0.21
GM at 10 µg/mL	6.77 ± 0.15	6.00 ± 0.10	8.37 ± 0.15	8.77 ± 0.15
GM at 50 µg/mL	11.30 ± 0.20	10.57 ± 0.21	12.53 ± 0.06	13.37 ± 0.25

EAE = Ethyl acetate extract; ME = Methanolic extract; AE = Aqueous extract; GM = Gentamycin standard.
All the value expressed as mean ± SD (n = 3)

(C-7), 162.49 (C-8), 145.87 (C-9), 133.24 (C-10), 27.52 (C-11), 21.28 (C-12), 21.28 (C-13), 23.13 (C-14), 15.85 (C-17).

BTF-2: White off crystal; *m.f.*: $C_{14}H_{16}O_4$; UV (CD_3OD, λ_{max}) 267 nm; ^1H NMR (500 MHZ, CD_3OD, δ ppm): 1.22 (3H, d, *J* = 6.6 Hz), 2.62 (3H, s), 2.30 (3H, s), 3.18 (1H, dd, *J* = 15.1, 10.2 Hz), 3.31 (1H, dd, *J* = 15.1, 3.3 Hz), 3.16 (1H, dq, *J* = 10.2, 6.6 Hz), 3.64 (1H, s), 4.42 (1H, td, *J* = 10.2, 3.3 Hz), 6.77 (1H, s), 9.76 (1H, s); ^{13}C NMR (500 MHZ, CD_3OD, δ ppm): 128.07 (C-1), 141.36 (C-2), 203.27 (C-3), 33.35 (C-4), 49.87 (C-5), 70.20 (C-6), 119.15 (C-7), 163.12 (C-8), 143.43 (C-9), 121.63 (C-10), 205.76 (C-11), 31.68 (C-13), 21.47 (C-14), 12.91 (C-18).

Compound **BTF-1** was confirmed as mansonone G, which is a known sesquiterpenoid found in plants,whereas compound **BTF-2** was also confirmed as sesquiterpenoid which is not reported previously in the literature and the IUPAC name of the compound was assigned as 5-acetyl-3,6-dihydroxy-2,8-dimethyl-3,4-dihydronaphthalen-1(2*H*)-one (Fig. 4).

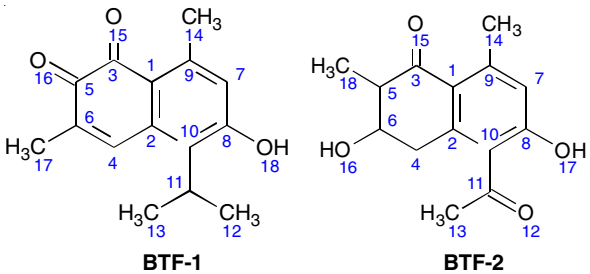

Fig. 4. Terpenoids isolated from the bark methanolic extract of *Sterculia urens* Roxb

The preparative HPLC separation of flavonoids confirmed that six compounds were identified in the sample and among the six fractions, the percentage composition of the two fractions was found to be very less hence the two compounds were not purified. Four different compounds were isolated in the extract and the isolates were named as BFF-1, BFF-2, BFF-3 and BFF- 4. These four isolated compounds were also characterized using UV, mass, ^1H & ^{13}C NMR spectroscopy techniques.

BFF-1: Yellow amorphous powder; *m.f.:* $C_{17}H_{14}O_5$; UV (CD_3OD, λ_{max}) 278 nm, 385 nm; ^1H NMR (500 MHz, CD_3OD, δ ppm): 2.12 (3H, s), 2.29 (3H, s), 6.79 (1H, s), 6.95 (2H, ddd, *J* = 8.3, 1.1, 0.5 Hz), 7.86 (2H, ddd, *J* = 8.3, 1.8, 0.5 Hz), 8.57 (1H, s), 9.18 (1H, s), 10.63 (1H, s); ^{13}C NMR (500 MHz, CD_3OD, δ ppm): 152.65 (C-1), 106.68 (C-2), 102.08 (C-3), 157.06 (C-4), 163.20 (C-5), 103.70 (C-6), 160.54 (C-7), 103.49 (C-8), 182.28 (C-10), 123.60 (C-11), 128.93 (C-12, C-13), 116.07 (C-14, C-15), 161.46 (C-16), 8.32 (C-21), 8.02 (C-22).

BFF-2: Yellow crystalline powder; *m.f.*: $C_{15}H_{10}O_5$; UV (CD_3OD, λ_{max}) 273 nm, 381 nm; ^1H NMR (500 MHZ, CD_3OD, δ ppm): 6.28 (1H, d, *J* = 2.0 Hz), 6.73 (1H, s), 6.53 (1H, d, *J* = 2.0 Hz), 6.95 (2H, ddd, *J* = 8.3, 1.1, 0.5 Hz), 7.86 (2H, ddd, *J* = 8.3, 1.8, 0.5 Hz), 8.57 (1H, s), 10.9 (1H, s), 11.8 (1H, s); ^{13}C NMR (500 MHZ, CD_3OD, δ ppm): 159.09 (C-1), 104.85 (C-2), 94.79 (C-3), 161.45 (C-4), 164.61 (C-5), 99.40 (C-6), 164.34 (C-7), 104.13 (C-8), 183.85 (C-10), 122.88 (C-11), 128.50 (C-12, C-13), 116.07 (C-14, C-15), 161.46 (C-16).

BFF-3: Yellow crystalline powder; *m.f.*: $C_{18}H_{16}O_6$; UV (CD_3OD, λ_{max}) 267 nm, 336 nm; ^1H NMR (500 MHZ, CD_3OD, δ ppm): 2.02 (3H, s), 2.42 (3H, s), 3.93 (3H, s), 6.33 (1H, s), 6.59 (1H, d, *J* = 1.7 Hz), 6.90 (1H, d, *J* = 1.7 Hz), 8.34 (1H, s), 8.36 (1H, s), 8.95 (1H, s), 9.53 (1H, s); ^{13}C NMR (500 MHz, CD_3OD, δ ppm): 157.22 (C-1), 105.09 (C-2), 93.92 (C-3), 160.36 (C-4), 163.62 (C-5), 108.86 (C-6), 154.60 (C-7), 123.67 (C-8), 182.74 (C-10), 123.50 (C-14), 145.61 (C-15), 123.12 (C-16), 146.85 (C-17), 130.40 (C-18), 111.90 (C-19), 21.35 (C-22), 56.28 (C-23), 7.68 (C-24).

BFF-4: Yellow amorphous powder; *m.f.:* $C_{15}H_{10}O_7$; UV (CD_3OD, λ_{max}) 258 nm, 347 nm; ^1H NMR (500 MHz, CD_3OD, δ ppm): 6.56 (1H, s), 5.59 (1H, s), 6.89 (1H, dd, *J* = 8.4, 0.5 Hz), 6.92 (1H, s), 7.42 (1H, dd, *J* = 1.7, 0.5 Hz), 7.44 (1H, dd, *J* = 8.4, 1.7 Hz), 8.09 (1H, s), 8.29 (1H, s), 10.13 (1H, s): ^{13}C NMR (500 MHz, CD_3OD, δ ppm): 152.60 (C-1), 103.63 (C-2), 94.14 (C-3), 147.51 (C-4), 153.27 (C-5), 129.56 (C-6), 164.53 (C-7), 103.78 (C-8), 182.47 (C-10), 122.36 (C-11), 113.92 (C-12), 119.55 (C-13), 146.18 (C-14), 115.93 (C-15), 149.83 (C-16).

The spectral analysis of the isolated compounds confirmed that compounds **BFF-1**, **BFF-2** and **BFF-4** were confirmed as farrerol, apigenin and 6-hydroxyluteolin, respectively which are known flavonoids. However, the isolated compound **BFF-3**

was confirmed as an unknown flavonoid with IUPAC name 5,7-dihydroxy-3-(2-hydroxy-3-methoxy-5-methylphenyl)-4*H*-chromen-4-one, which is not reported in previous literature. The molecular structures of the isolated flavonoids are given in Fig. 5.

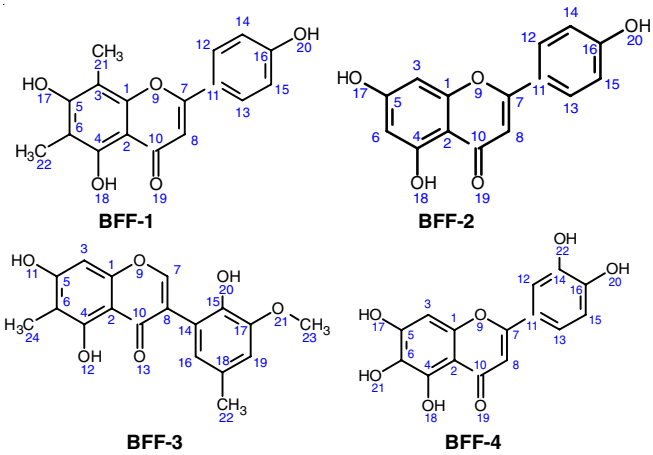

Fig. 5. Flavonoids isolated from the bark methanolic extract of *Sterculia urens* Roxb

Conclusion

The results of the study indicated the extracts obtained from the bark of *Sterculia urens* Roxb. have shown significant biological activities such as DPPH free radical scavenging assay, α-amylase inhibition assay, inhibition of albumin denaturation assay, thrombolytic activity and antibacterial activity. Among the extracts studied, the methanolic extract showed the exceptional activities. One known terpenoid (mansonone G) which is not reported *S. urens* Roxb. and unknown terpenoid (5-acetyl-3,6-dihydroxy-2,8-dimethyl-3,4-dihydronaphthalen-1(2*H*)-one) was isolated from methanolic extract. One known flavonoid (farrerol), two known flavonoids (apigenin and 6-hydroxyluteolin) but not reported in *S. urens* Roxb. and one unknown flavonoid (5,7-dihydroxy-3-(2-hydroxy-3-methoxy-5-methylphenyl)-4H-chromen-4-one) was isolated from the methanolic bark extract of *Sterculia urens* Roxb. first time. The presence of large number of biological compounds may be responsible for the exceptional biological activities of the methanolic extract.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. A. Bhargava, P. Shrivastava and A. Tilwari, *Future J. Pharm. Sci.*, **7**, 1 (2021); https://doi.org/10.1186/s43094-020-00150-x
2. X.X. Huang, M. Bai, L. Zhou, L.L. Lou, Q.B. Liu, Y. Zhang, L.Z. Li and S.J. Song, *J. Agric. Food Chem.*, **63**, 7252 (2015); https://doi.org/10.1021/acs.jafc.5b02835
3. A.G. Atanasov, B. Waltenberger, E.M. Pferschy-Wenzig, T. Linder, C. Wawrosch, P. Uhrin, V. Temml, L. Wang, S. Schwaiger, E.H. Heiss, J.M. Rollinger, D. Schuster, J.M. Breuss, M.D. Mihovilovic, B. Kopp, V. Bochkov, R. Bauer, V.M. Dirsch and H. Stuppner, *Biotechnol. Adv.*, **33**, 1582 (2015); https://doi.org/10.1016/j.biotechadv.2015.08.001
4. N. Akhtar, Ihsan-ul-Haq and B. Mirza, *Arab. J. Chem.*, **11**, 1223 (2018); https://doi.org/10.1016/j.arabjc.2015.01.013
5. T. Kanoje and K. Sharma, *Int. J. Dev. Res.*, **7**, 14185 (2017).
6. M.O. Ilomuanya, R.F. Elesho, A.N. Amenaghawon, A.O. Adetuyi, V. Velusamy and A.S. Akanmu, *Future J. Pharm. Sci.*, **6**, 1 (2020); https://doi.org/10.1186/s43094-019-0015-8
7. M.M. Gouda, R.A. Shabaraya and S.M.S. Kumar, *Int. Curr. Pharm. J.*, **1**, 376 (2012); https://doi.org/10.3329/icpj.v1i11.12064
8. J. Redfern, M. Kinninmonth, D. Burdass and J. Verran, *J. Microbiol. Biol. Educ.*, **15**, 45 (2014); https://doi.org/10.1128/jmbe.v15i1.656
9. A. Kathirvel and V. Sujatha, *Arab. J. Chem.*, **9**, 1435 (2016); https://doi.org/10.1016/j.arabjc.2012.03.018
10. J.B. Harborne, Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis, Chapman & Hall Ltd.: London (1973).
11. E. Iqbal, K.A. Salim and L.B.L. Lim, *J. King Saud Univ. Sci.*, **27**, 224 (2015); https://doi.org/10.1016/j.jksus.2015.02.003
12. R. Maria, M. Shirley, C. Xavier, S. Jaime, V. David, S. Rosa and D. Jodie, *J. King Saud Univ. Sci.*, **30**, 500 (2018); https://doi.org/10.1016/j.jksus.2017.03.009
13. H. Noreen, N. Semmar, M. Farman and J.S.O. McCullagh, *Asian Pac. J. Trop. Med.*, **10**, 792 (2017); https://doi.org/10.1016/j.apjtm.2017.07.024
14. S.S. Pawar and D. Dasgupta, *J. King Saud Univ. Sci.*, **30**, 293 (2018); https://doi.org/10.1016/j.jksus.2016.09.002
15. A.M. Adeosun, S.O. Oni, O.M. Ighodaro, O.H. Durosinlorun and O.M.Oyedele, *J. Taibah Univ. Med. Sci.*, **11**, 1 (2016); https://doi.org/10.1016/j.jtumed.2015.11.006
16. R. Batool, M.R. Khan, M. Sajid, S. Ali and Z. Zahra, *BMC Chem.*, **32**, 32 (2019); https://doi.org/10.1186/s13065-019-0549-z
17. D.D. Sylvie, P.C. Anatole, B.P. Cabral and P.B. Veronique, *Asian Pac. J. Trop. Biomed.*, **4**, 625 (2014); https://doi.org/10.12980/APJTB.4.201414B168
18. A.K. Shettar, M.K. Sateesh, B.B. Kaliwal and A.B. Vedamurthy, *S. Afr. J. Bot.*, **111**, 202 (2017); https://doi.org/10.1016/j.sajb.2017.03.014
19. S. Murthuza and B.K. Manjunatha, *Beni-Suef Univ. J. Basic Appl. Sci.*, **7**, 719 (2018); https://doi.org/10.1016/j.bjbas.2018.10.001
20. I. Hasan, S. Hussain, S. Millat, N. Sen, A. Rahman, A. Rahman, S. Islam and M.R. Moghal, *J. Tradit. Complement. Med.*, **8**, 107 (2018); https://doi.org/10.1016/j.jtcme.2017.03.005
21. S.J. B. Dalir, H. Djahaniani, F. Nabati and M. Hekmati, *Heliyon*, **6**, e03624 (2020); https://doi.org/10.1016/j.heliyon.2020.e03624
22. I. Jallali, P. waffo Téguo, A. Smaoui, J.-M. Mérillon, C. Abdelly and R. Ksouri, *Arab. J. Chem.*, **13**, 2680 (2020); https://doi.org/10.1016/j.arabjc.2018.06.020
23. P.H. Morin, M. Caude, H. Richard and R. Rosset, *J. Chromatogr. A*, **363**, 37 (1986); https://doi.org/10.1016/S0021-9673(00)88991-8
24. B.-Y. Hu, D.-P. Qin, S.-X. Wang, J.-J. Qi and Y.-X. Cheng, *Molecules*, **23**, 3239 (2018); https://doi.org/10.3390/molecules23123239
25. T.Y. Kristoferson Lulan, S. Fatmawati, M. Santoso and T. Ersam, *Free Radic. Antioxid.*, **8**, 96 (2018); https://doi.org/10.5530/fra.2018.2.15
26. T. Murningsih, P. Praptiwi, L. Liana and A.M. Fathoni, *Nusantara Bioscience*, **11**, 44 (2019); https://doi.org/10.13057/nusbiosci/n110108
27. U.P.N. Kannaian, J.B. Edwin, V. Rajagopal, S.N. Shankar and B. Srinivasan, *Heliyon*, **6**, e03411 (2020); https://doi.org/10.1016/j.heliyon.2020.e03411
28. U. Etxeberria, A.L. de la Garza, J. Campión, J.A. Martínez and F.I. Milagro, *Expert Opin. Ther. Targets*, **16**, 269 (2012); https://doi.org/10.1517/14728222.2012.664134
29. M.C.N. Picot and M.F. Mahomoodally, *Pharm. Biol.*, **55**, 864 (2017); https://doi.org/10.1080/13880209.2016.1277765