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Phytochemical Analysis, Pharmacological Activities, Isolation and Characterization of Bioactive Compounds from the Roots of *Sterculia urens* Roxb.

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The present study was intended to explore the pharmacological significance of the crude root extract of *Sterculia urens* Roxb. Further the bio-active compounds were isolated and characterized using chromatographic and spectroscopic techniques. Soxhlet extraction apparatus was utilized for isolation of the chemical constituents from the root using a series of solvents such as *n*-hexane, ethyl acetate, methanol and water. The pharmacological activities such as inhibition of DPPH radical, α-amylase enzyme activity, albumin denaturation along with antibacterial and thrombolytic activities. The isolation of purified bioactive constituents was carried using preparative HPLC technique and the purified compounds were characterized using spectroscopic techniques like NMR, IR and mass. Among the crude root extracts, methanolic extract shows high DPPH radical scavenging activity with IC_{50} concentration of 26.74 ± 0.08 µg/mL. The IC_{50} concentrations in α-amylase enzyme inhibition activity was 263.96 ± 0.90, 127.73 ± 1.23 and 223.54 ± 4.76 µg/mL, respectively for ethyl acetate, methanol and water extracts, respectively. The methanolic extract shows high albumin denaturation inhibition assay than other extracts with IC_{50} concentration as 137.09 ± 0.20 µg/mL, which is very close to standard ascorbic acid. The methanolic extract also shows high % clot lysis than other extracts and results were comparable with 100 µL of streptokinase standard. The preparative HPLC followed by spectral analysis confirm that two known alkaloids (Sterculinine I & II) and three known flavonoids (gossypetin, apigenin and 6-hydroxyluteolin) were purified and characterized from the root methanol of *Sterculia urens* Roxb. The purified and identified compounds were reported for the first time in *Sterculia urens* Roxb.

Keywords: *Sterculia urens* Roxb., Preparative HPLC, Sterculinine, Gossypetin, Apigenin, 6-Hydroxyluteolin.

INTRODUCTION

Plants like spices, fruits, vegetables, medicinal herbs, *etc*. have been utilizing to reduce various infections/diseases since ancient period. Now, in modern era, very high number of pharmaceutical synthetic drugs is available, but these synthesis drugs may cause harmful side effects. Hence, people are preferred to use traditional medicine with no or very less side effects [1]. Plants have different variety of medicinally active compounds called secondary metabolites, which are having capability to cure various diseases and are utilized as antiviral, anti-inflammatory, antibacterial, analgesic, anticancer, *etc*. agents [2].

Now a day, researchers is focusing to develop/identify new drugs to enhance the effectiveness of a medicine to cure disease, due to development of resistance to the antibiotics by the pathogens and various free radicals causes oxidative stress in the human body [3]. The crude plant extracts obtained from medicinal plants will become best choice for investment of new pharmaceuticals, which cure various diseases with very less side effects [4].

Sterculia urens Roxb., is traditionally called as karaya plant belongs to Malvaceae. It is medium to small tree having pharmacological activities such as antifungal [5], antimicrobial and antioxidant activities [6]. The vegetative gum obtained as exudate from the trees of *Sterculia urens* Roxb. is utilized as denture adhesive, laxative, emulsifier and thickener in foods as well as hydrophilic matrix tablets preparation [7]. The pharmacological significance and phytochemical composition of the *Sterculia urens* Roxb., was not adequately evaluated in literature. In view of this, the present study intended to explore the pharmacological activities along with chemical composition of *Sterculia urens* Roxb. roots.

EXPERIMENTAL

Collection of plant roots: The fresh roots of *S. urens* was collected from Paderu (village), Visakhapatnam (district) in January 2020. The dirt and sand from the collected roots were cleaned with little amount of water and then with sterile cotton. The cleaned and dry roots were sliced to paces and then dried in shade until achieving constant weight. The dried roots were stored in Amber bottle for further study.

Preparation of root extracts: Soxhlet extraction apparatus was used to extract the phytochemical compounds from roots of *S. urens* and experiment was performed as described by Redfern *et al*. [8]. The extraction was carried in continuous process using a series of solvents such as *n*-hexane, ethyl acetate, methanol and water solvents. The extracts were dried with rotatory vacuum evaporator and the obtained dried crude extracts were stored in a refrigerator for further use [9].

Qualitative determination of phytochemicals: The qualitative identification of phytochemicals in the crude root extracts of *S. urens* was performed based on the literature procedures [10-12]. The colour change in each specified test confirms the presence of the studied phytochemical constituents and positive in the identification test were further studied for the quantitative determination.

Quantitative determination of phytochemicals: The Folin-Ciocalteu spectrophotometric method [13] was followed for the determination of total phenolic content in the crude root extracts of *S. urens* using gallic acid as standard and results were summarized as gallic acid equivalent (GAE) present in gram of root extract. Aluminum chloride method was applied for the quantitative evaluation of total flavonoids in extracts using quercetin standard and results summarised as quercetin equivalent (QE)/gram *S. urens* root extract [14]. Bromocresol green reagent method was adopted for the quantitative evaluation of total alkaloids by considering atropine as standard and results summarised as mg of atropine equivalent (AE) per gram *S. urens* root extract [15]. A non-spectroscopic method was adopted for the determination of saponins and terpenoids content in the root extract of *S. urens* and results reported as percentage per gram extract [16].

Evaluation of pharmacological activities

Antioxidant activity: The antioxidant activity of crude root extracts of *S. urens* was evaluated by performing DPPH free radical scavenging assay and the experiment was conducted by Sylvie *et al*. [17]. The antioxidant activity of the crude extracts was confirmed by calculating the 50 % inhibition concentration (IC_{50}) of each extract and results were compared with ascorbic acid standard.

Antidiabetic activity: The antidiabetic activity of the crude root extracts of *S. urens* was evaluated by performing α-amylase enzyme inhibition assay [18]. The antidiabetic activity of the crude extracts was confirmed by calculating the 50 % inhibition concentration (IC_{50}) of each extract and results were compared with acarbose standard.

Anti-inflammatory activity: The anti-inflammatory activity of the root extracts of *S. urens* was evaluated by performing the inhibition of albumin denaturation method [19]. The anti-inflamatory activity of the crude extracts was confirmed by calculating the 50 % inhibition concentration (IC_{50}) of each extract and the results were compared with diclofenac standard.

Thrombolytic activity: Thrombolytic activity of the crude root extracts of *S. urens* was determined by blood clot lysis method [20]. Streptokinase (30,000 I.U.) and methanol were selected as positive and negative controls, respectively.

Antibacterial activity: The antibacterial activity of root extracts of *S. urens* was evaluated by performing agar plate well diffusion method [21]. In this study, Gram-negative bacteria *viz. Pseudomonas aeruginosa* (MTCC-1748), *Escherichia coli* (MTCC-294) and two Gram positive bacteria *viz. Staphylococcus aureus* (MTCC-1430), *Bacillus subtilis* (MTCC-1427) were selected. The standard drug gentamycin was considered as positive control whereas the distilled water selected as negative control and the results summarized as millimetre (mm) of inhibition zone observed for each sample studied.

Isolation compounds: A semi-preparative HPLC analysis was performed for the purification of compounds in the crude root extracts of *S. urens*. The analysis was carried on semi-preparative HPLC (Shimadzu, Japan) system coupled with plunger type pump (LC-20A), Rheodyne® type sample injector (7725I), Waters XBridge (250 mm × 19 mm; 5 µm) preparative column, programmable UV-visible detector (SPD20A). The column eluents were monitored, and equipment was controlled using LabSolutions software (Shimadzu, Japan). The extracts at a concentration of 50 mg/mL were filtered through 0.2 µ nylon membrane filter and used for isolation study.

The purification of flavonoids in the methanolic root extract was performed based on procedure reported by Jallali *et al*. [22] with slight modification. The mobile phase comprises 0.025% aqueous triethyl amine as solvent A and pure acetonitrile as solvent B pumped in gradient elution at 3.0 mL/min flow rate. The gradient program set as 0-5 min (10% solvent B), 5-55 min (10-100% solvent B), 55-60 min (100% solvent B), 60-65 min (100-10% solvent B). The column eluents were monitored at 280 nm using UV detector. The column fractions at identified retention time was collected using fraction collector.

The alkaloids present in the methanolic root extract of *S. urens* was isolated using procedure reported by Maria *et al*. [23] and the isolated crude alkaloid fraction was subjected to preparative HPLC purification as per procedure reported by Atlabachew *et al*. [24]. The mobile phase comprises 0.3% aqueous phosphoric acid at pH 1.7 as mobile phase A and 10% aqueous acetonitrile as mobile phase B in gradient elution at 9 mL/min. The gradient programme set as 20 min (0-70 % solvent B). The column eluents were monitored at 220 nm using UV detector. The column fractions at identified retention time was collected using fraction collector.

Characterization of isolated compounds: The purified alkaloid and flavonoid compounds were characterized using spectroscopic studies such as NMR, Fourier-transform infrared spectroscopy (FT-IR) and mass spectroscopy. The interpretation of the data obtained from all the spectral studies confirms the molecular structure of the purified compound.

RESULTS AND DISCUSSION

The present study is aimed to explore the pharmacological properties and the isolation of bioactive compounds from the root extract of *S. urens*. The ethyl acetate extract shows the presence of terpenoids, steroids, cardiac glycosides and phenolic compounds. The methanolic extract shows positive tests for cardiac glycosides, alkaloids, flavonoids, steroids, glycosides, anthraquinones and phenolic compounds whereas the aqueous extract gives positive results for coumarins, glycosides and saponins. The quantitative estimation of phytochemical compounds present in the root extract of *S. urens* confirmed that the methanolic extract contains 15.76 ± 0.060 mg of QE/g of flavonoids, 5.96 ± 0.063 AE/g of alkaloids and 17.52 ± 0.105 GAE/g of phenolic compounds. The ethyl acetate extract having 3.91 ± 0.053 GAE/g of phenolic compounds whereas the water extract having 2.54 ± 0.036 mg/g of saponins. This proved that the methanolic extract contain significantly high quality and quantity of phytochemical constituents and results were in argument with the literature available for *S. urens* [6] as well as the same genera [25,26].

The % of extracts obtained during the solvent extraction was found to be 3.71 ± 0.035, 8.38 ± 0.040, 12.46 ± 0.076 and 9.56 ± 0.026 for *n*-hexane, ethyl acetate, methanol and water solvents, respectively. The methanol extract shows high quantity of extract than the other solvents studied. The results observed during the preliminary qualitative analysis study for root extracts of *S. urens* is shown in Table-1.

TABLE-1
RESULTS OBTAINED IN PHYTOCHEMICAL SCREENING STUDIES OF *S. urens*

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

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

The quantitative analysis was carried for the compounds which give positive test in a qualitative analysis. In the quantitative study, it was calculated that ethyl acetate extract contains 3.91 ± 0.053 GAE/g and methanol extract contains 17.52 ± 0.105 GAE/g of phenolic compounds. The methanolic extract contains 15.76±0.060 QE/g extract of flavonoids and 5.96 ± 0.063 AE/g of alkaloids, while the water extract confirms the presence of 2.54 ± 0.036 mg/g of saponins.

The DPPH radical scavenging activity was studied in the concentration range of 5-40 µg/mL (Fig. 1) for standard as well as crude root extracts of *S. urens*. The IC_{50} concentrations was calculated as 22.20 ± 0.03, 44.41 ± 0.13, 26.74 ± 0.08 and 57.37 ± 0.09 µg/mL, respectively for standard, ethyl acetate, methanol and water extracts. The IC_{50} concentration of methanolic extract was observed to be very low and very close to the ascorbic acid standard, which proves the methnolic extract having high DPPH radical inhibition activity [27].

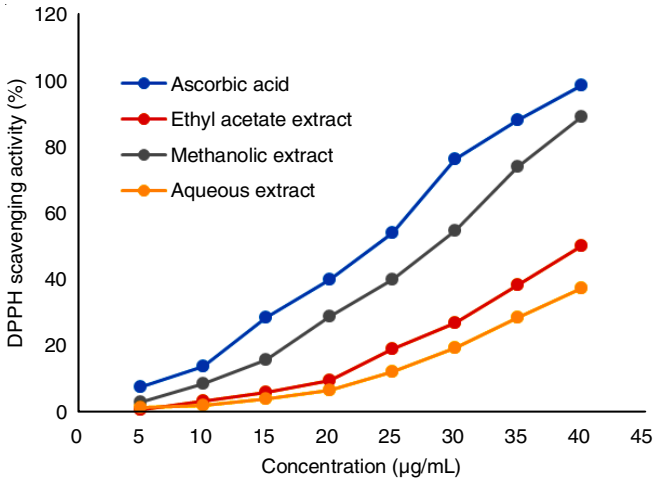

Fig. 1. Antioxidant activity data of *S. urens*

A wide concentration range of 2.34 to 300 µg/mL (Fig. 2) was studied for the evaluation of the antidiabetic activity of the root extracts of *S. urens*. The IC_{50} concentration was calculated as 71.51 ± 0.08, 127.73 ± 1.23, 263.96 ± 0.90 and 223.54 ± 4.76 µg/mL for standard acarbose, ethyl acetate, methanol and water extracts, respectively. The results proved that the IC_{50} concentration of methanol extract was observed to be very close to the acarbose and hence having potential α-amylase inhibition activity than ethyl acetate and water extracts.

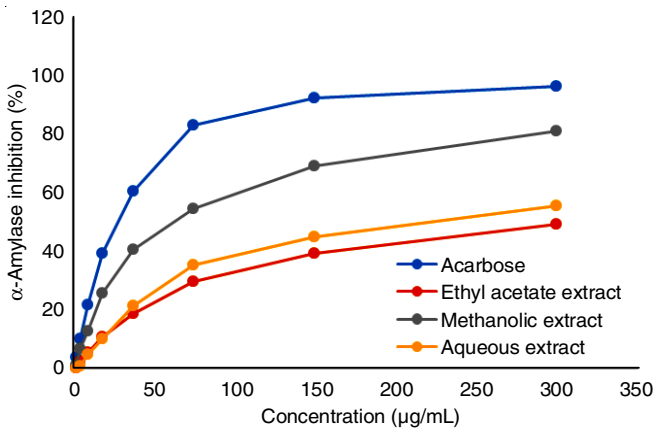

Fig. 2. Antidiabetic activity data of of *S. urens*

Albumin denaturation inhibition assay was studied in the concentration range of 25-200 µg/mL for crude root extracts of *S. urens* and standard diclofenac. The results (Fig. 3) proved that the methanolic extract shows IC_{50} values at a lowest concen-

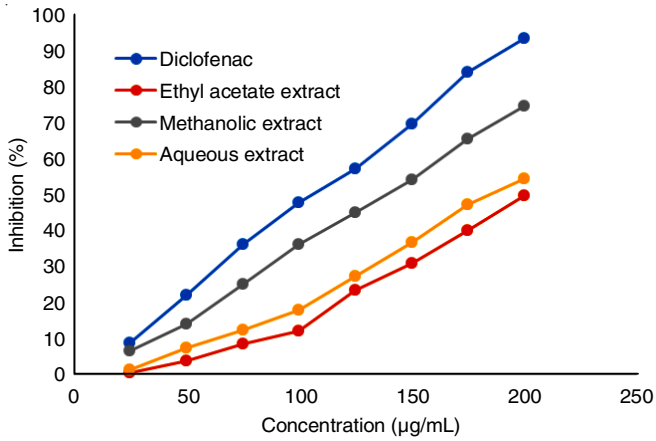

Fig. 3. Anti-inflammatory activity results of *S. urens*

tration of 137.09 ± 0.20 µg/mL confirms that the methanol extract having high activity among other root extracts of *S. urens*. The IC_{50} concentration of standard was observed to be 107.13 ± 0.13 µg/mL whereas 210.74 ± 1.81 and 190.86 ± 0.28 µg/mL was observed for ethyl acetate and methanolic extracts, respectively.

The thrombolytic activity of crude root extracts of *S. urens* was evaluated by *in-vitro* clot lysis study and results achieved are summarised in Table-2. The % clot lysis at a concentration of 10 mg/mL concentration of root extracts was observed to be 11.16 ± 5.009, 48.76 ± 2.656 and 15.32 ± 3.149 for ethyl acetate, methanol and water extracts respectively whereas the standard 100 µL of standard streptokinase shows 62.36 ± 0.140 % of clot lysis. The results proved that the methanolic extract having high clot lysis potential and hence having high thrombolytic activity than other root extracts in the study.

TABLE-2
in vitro CLOT LYSIS ACTIVITY RESULTS OF *S. urens*

Conc. (mg/mL)	Clot lysis (%)		
	Ethyl acetate extract	Methanol extract	Water extract
2	1.01 ± 0.229	3.86 ± 1.553	1.52 ± 0.497
4	2.14 ± 1.568	10.22 ± 1.045	3.14 ± 0.912
6	5.01 ± 2.295	21.76 ± 5.225	6.63 ± 1.394
8	7.81 ± 3.274	31.17 ± 7.309	10.94 ± 2.237
10	11.16 ± 5.009	48.76 ± 2.656	15.32 ± 3.149

Streptokinase = 62.36 ± 0.140 (positive control)
Blank = 0.53 ± 0.020 (negative control)
Results expressed as mean ± SD (n = 3)

The antibacterial activity of crude root extracts of *S. urens* and standard gentamycin was studied at a concentration of 1, 10 and 50 µg/mL (Table-3). In the studied samples, ethyl acetate extract doesn't show any zone of inhibition against the studied bacteria at 1 and 10 µg/mL concentrations. The water extract at a concentration of 1 µg/mL for studied bacteria and 10 µg/mL against gram negative bacteria doesn't shows any zone of inhibition. The methanolic extract and standard gentamycin shows potential inhibition against the growth of studied bacteria at a very low concentration of 1 µg/mL. At a very high concentration of 50 µg/mL, all the extracts of *S. urens* and standard shows zone of inhibition against studied bacteria proves that the extracts have capability to inhibit the growth of the studied bacteria.

The biological activates confirmed that the methanolic extract having high activities and qualitative, quantitative phytochemical assay proved that methanol extract contains flavonoids and alkaloids in comparatively high quantities than other type of chemical constituents. In view of this, the flavonoids and alkaloids in the methanolic extract was purified using semi-preparative HPLC analysis.

The LC chromatogram of the semi-preparative HPLC isolation of alkaloid fraction shows five peaks. This confirms that five dissimilar alkaloids were present in the crude methanol extract. The response of the peak corresponds to individual alkaloid confirms that two alkaloids were found to be quantitatively very less and takes difficulty to isolate the purified fractions. The peaks corresponding to three compounds shows high response and hence were purified. The purified fractions were names as **RAF 1, RAF 2** and **RAF 3**. The purified compounds were identified and structure elucidated by correlating the spectral data, and the results observed in spectral analysis was summarized as follows:

RAF 1: White powder; m.f.: $C_{18}H_{20}N_2O_6$; UV (CD_3OD, λ_{max}): 227 nm, 384 nm; ^1H NMR (500 MHz, CD_3OD, δ ppm): 0.92 (3H, t, J = 7.1 Hz), 1.41 (h, J = 7.1 Hz), 1.60 (quint, J = 7.1 Hz), 2.64 (d, J = 6.3 Hz), 4.08 (t, J = 7.1 Hz)), 4.52 (1H, t,

TABLE-3
ANTIBACTERIAL ACTIVITY RESULTS OF *S. urens*

Extract/sample	Growth inhibition zone observed in mm			
	B. subtilis	*S. aureus*	*E. coli*	*P. aeruginosa*
Ethyl acetate at 1 µg/mL	–	–	–	–
Ethyl acetate at 10 µg/mL	–	–	–	–
Ethyl acetate at 50 µg/mL	4.53 ± 0.06	4.10 ± 0.10	5.27 ± 0.06	5.60 ± 0.10
Methanol at 1 µg/mL	–	–	2.80 ± 0.17	3.43 ± 0.15
Methanol at 10 µg/mL	2.67 ± 0.12	3.20 ± 0.10	5.87 ± 0.06	5.47 ± 0.21
Methanol at 50 µg/mL	4.67 ± 0.15	5.63 ± 0.15	8.70 ± 0.20	7.80 ± 0.17
Atropine equivalent at 1 µg/mL	–	–	–	–
Atropine equivalent at 10 µg/mL	–	–	3.57 ± 0.12	3.80 ± 0.17
Atropine equivalent at 50 µg/mL	5.17 ± 0.25	5.17 ± 0.25	6.20 ± 0.10	6.53 ± 0.15
Gentamycin at 1 µg/mL	4.70 ± 0.20	4.13 ± 0.15	5.33 ± 0.15	3.73 ± 0.21
Gentamycin at 10 µg/mL	6.77 ± 0.15	6.00 ± 0.10	8.37 ± 0.15	8.77 ± 0.15
Gentamycin at 50 µg/mL	11.30 ± 0.20	10.57 ± 0.21	12.53 ± 0.06	13.37 ± 0.25

Results expressed as average ± SD (n = 3)

J = 6.3 Hz), 7.06 (1H, s), 7.21 (ddd, *J* = 7.9, 7.4, 1.2 Hz), 7.29 (1H, ddd, *J* = 7.8, 1.4, 0.6 Hz), 7.89 (1H, ddd, *J* = 7.9, 1.4, 0.5 Hz), 7.42 (1H, ddd, *J* = 8.6, 7.4, 1.4 Hz), 11.91 (1H, s), 11.11 (1H, s), 12.21 (1H, s); ^{13}C NMR (500 MHz, CD_3OD, δ ppm): 131.16 (C-1), 122.27 (C-2), 115.75 (C-3), 126.05 (C-4), 139.15 (C-5), 116.28 (C-6), 161.39 (C-8), 145.78 (C-9), 119.76 (C-10), 166.60 (C-12), 49.17 (C-15), 171.55 (C-16), 35.52 (C-17), 170.60 (C-18), 64.71 (C-24), 30.45 (C-25), 18.62 (C-26), 13.81 (C-27).

RAF 2: White powder; m.f.: $C_{15}H_{14}N_2O_6$; UV (CD_3OD, λ_{max}): 227 nm, 384 nm; ^{1}H NMR (500 MHZ, CD_3OD, δ ppm): 2.65 (d, *J* = 6.3 Hz), 3.62 (3H, s), 4.51 (1H, t, *J* = 6.3 Hz), 6.56 (1H, s), 7.21 (ddd, *J* = 7.9, 7.4, 1.2 Hz)), 7.29 (1H, ddd, *J* = 7.8, 1.4, 0.6 Hz), 7.89 (1H, ddd, *J* = 7.9, 1.4, 0.5 Hz), 7.42 (1H, ddd, *J* = 8.6, 7.4, 1.4 Hz), 11.91 (1H, s), 11.11 (1H, s), 12.21 (1H, s); ^{13}C NMR (500 MHz, CD_3OD, δ ppm): 131.16 (C-1), 122.27 (C-2), 115.75 (C-3), 126.05(C-4), 139.15 (C-5), 116.28 (C-6), 161.39 (C-8), 145.78 (C-9), 119.67 (C-10), 166.59 (C-12), 49.29 (C-15), 171.64 (C-16), 36.25 (C-17), 170.94 (C-18), 52.13 (C-24).

RAF 3: White powder; m.f.: $C_{15}H_{10}O_8$; UV (CD_3OD, λ_{max}): 227 nm, 258 nm; ^{1}H NMR (500 MHz, CD_3OD, δ ppm): 3.66 (2H, s), 4.77 (2H, d, *J* = 11.7 Hz), 7.26 (dddd, *J* = 7.7, 1.5, 1.2, 0.5 Hz), 7.29 (tdd, *J* = 7.7, 1.8, 0.5 Hz); ^{13}C NMR (500 MHZ, CD_3OD, δ ppm): 128.97 (C-1), 128.96 (C-2), 137.01 (C-3), 127.33 (C-4), 128.97 (C-5), 128.96 (C-6), 35.06 (C-7), 161.53 (C-8), 169.25 (C-10), 81.67 (C-11).

The results of the spectral interpretation, compounds **RAF 1** and **RAF 2** were confirmed as sterculinine I & II, which are commonly found in plants especially in *Sterculia* species. The third isolated alkaloid **RAF 3** was confirmed as oxazolone class alkaloid with IUPAC name 4-benzyl-1,3-oxazol-5(2*H*)-one. The structure of the isolated alkaloids derived from the interpretation of the spectral data is given in Fig. 4.

The LC-chromatogram observed during the semi-preparative analysis of flavonoids shows four peaks corresponds to four different flavonoids. Among the four peaks observed, the peak intensity of one was observed to be very less and three peaks was observed to be enough quantity to isolate and purity the compound. Hence, three fractions were collected and designated as **RFF 1**, **RFF 2** and **RFF 3**. The isolated flavonoid individual fractions were dried and identified using spectral analysis. The results observed in spectral analysis are summarized as

RFF 1: Yellow crystalline powder; m.f.: $C_{15}H_{10}O_8$; UV (CD_3OD, λ_{max}): 286 nm, 374 nm; ^{1}H NMR (500 MHz, CD_3OD, δ ppm): 5.55 (1H,s), 6.40 (1H, s), 6.81 (1H, dd, *J* = 8.4, 0.5 Hz), 7.34 (1H, dd, *J* = 1.8, 0.5 Hz), 7.27 (1H, dd, *J* = 8.4, 1.8 Hz), 8.08 (1H, s), 9.10 (1H,s), 12.24 (1H,s), 13.19 (1H,s); 144.91 (C-1), 102.77 (C-2), 125.43 (C-3), 152.72 (C-4), 153.50 (C-5), 98.72 (C-6), 147.25 (C-7), 136.57 (C-8), 177.84 (C-10), 122.63 (C-11), 121.12 (C-12), 115.16 (C-13), 115.88 (C-14), 145.49 (C-15), 148.53 (C-16).

RFF 2: Yellow crystalline powder; m.f.: $C_{15}H_{10}O_5$; UV (CD_3OD, λ_{max}): 273 nm, 381 nm; ^{1}H 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).

RFF 3: Yellow amorphous powder; m.f.: $C_{15}H_{10}O_7$; UV (CD_3OD, λ_{max}): 258 nm, 347 nm; ^{1}H 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 interpretation of all the spectral data obtained for each isolated fraction confirms that the compound **RFF 1**, **RFF 2** and **RFF 3** were identified as gossypetin, apigenin and 6-

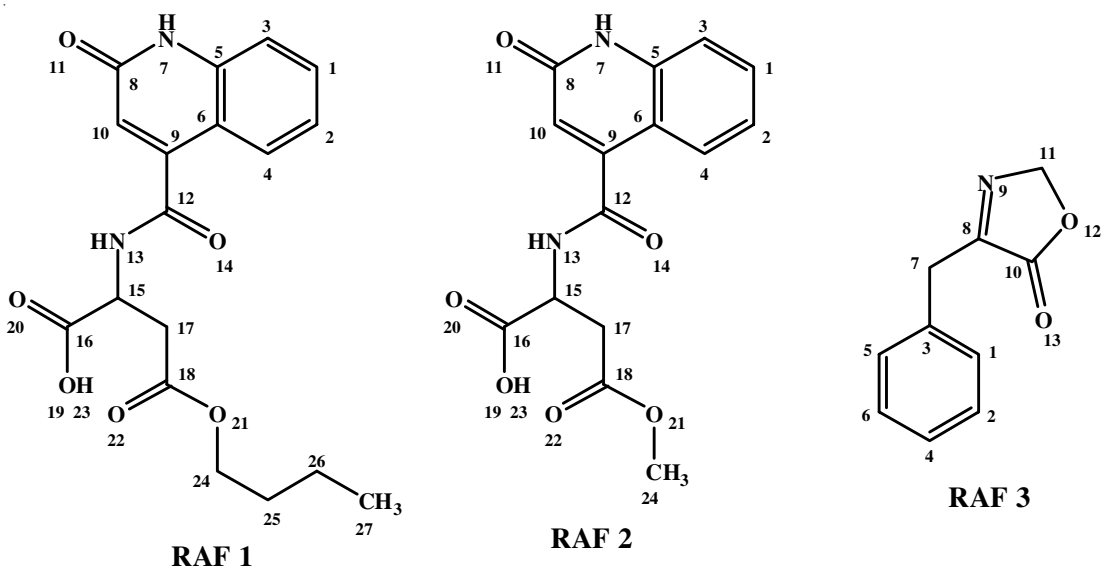

Fig. 4. Molecular structure of isolated alkaloids

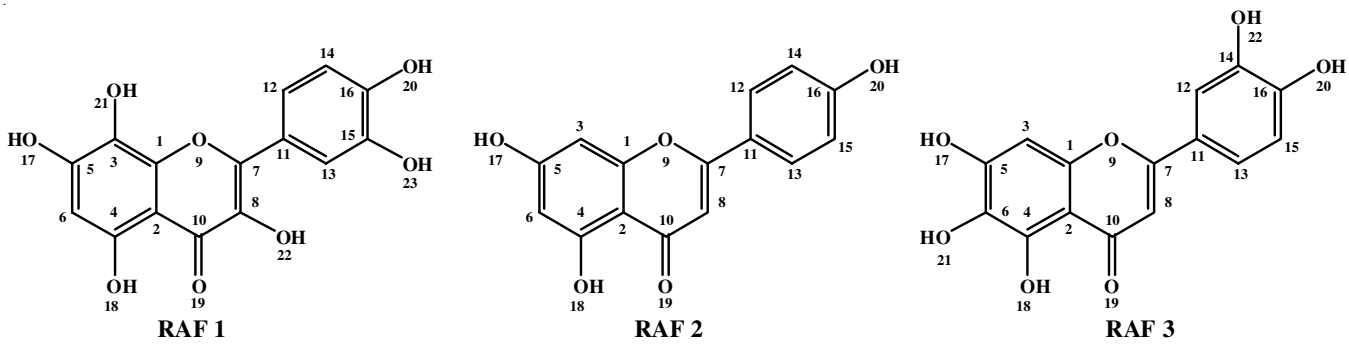

Fig. 5. Molecular structure of isolated flavonoids

hydroxyluteolin, respectively which are known flavonoids. The molecular structures of the isolated flavonoids are given in Fig. 5.

Conclusion

The root extracts of *S. urens* shows high number of phytochemical constituents quantitatively which exhibit significantly high pharmacological activities. Among the extracts, methanol shows significantly dominant activities than other extracts. Two well-known alkaloids sterculinine I & II were isolated and identified from the root methanolic extract which were not reported previously in *S. urens* Roxb. One oxazolone class alkaloid with IUPAC name 4-benzyl-1,3-oxazol-5(2*H*)-one was isolated from the root methanolic extract along with other three known flavonoids (gossypetin, apigenin and 6-hydroxyluteolin) from the methanolic extract for the first time in *S. urens* Roxb.

CONFLICT OF INTEREST

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

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