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Research Article

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

Chaitanya Darapureddy[a], K R S Prasad[b]*, Phani R S Ch[c]

[a,b,c] Department of Chemistry, Koneru Lakshmaiah Education Foundation, Vaddeswaram, Guntur District, Andhra Pradesh, India

Abstract

The present study is 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 utilised for isolating the chemical constituents from the root extract using a series of solvents such as n-hexane, ethyl acetate (EA), methanol (ME) and water (WA). The pharmacological activities such as inhibition of DPPH radical activity, α-amylase enzyme activity, albumin denaturation along with anti-bacterial and thrombolytic activities. The isolation of purified bio-active constituents was carried using preparative HPLC technique and the purified compounds were characterized using spectroscopic techniques like NMR and Mass spectroscopy. 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 for EA, ME, WA extracts respectively. The ME 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 ME 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 characterised from the root ME 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, purification of compounds, alkaloids, flavonoid, characterization*

Introduction

Plants like spices, fruits, vegetables, medicinal herbs etc., have been utilizing to reduce various infections/diseases since ancient time. Now, in this modern era, very high number of pharmaceutical synthetic drugs are 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 that are having capability to cure various diseases and are utilised as anti-inflammatory, antibacterial, antiviral, analgesic, anticancer etc., agents[2].

Now a day, researchers are 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 that 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 anti-fungal[5], anti-microbial and anti-oxidant activities[6]. The vegetative gum obtained as exudate from the trees of *Sterculia urens* Roxb., is utilised 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.

Materials And Methods:

Collection of Plant roots:

The fresh roots of *S. urens* were collcted in Paderu (village), Visakhapatnam (district) in January 2018. The dirt and sand from the collected roots were cleaned with little amount of water and then with sterile cotton. Then the cleaned and dry roots were sliced to paces and then dried in shade until achieving constant weight. Then 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., 2014[8]. The extraction was carried in continuous process using a series of solvents such as n-hexane, EA, ME, WA solvents. The extracts were dried with rotatory vacuum evaporator and the obtained dried crude dried 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 procedure reported by Harborne 1973[10], Erum et al., 2015[11] and Rondon et al., 2018[12]. The colour change in each specified test confirms the presence of the studied phytochemical constituent and positive in the identification test were further studied for quantitative determination.

Quantitative determination of Phytochemicals:

The folin-ciocalteu spectrophotometric method reported by Hafiza et al., 2017[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 summarised as gallic acid equivalent (GAE) present in gram of root extract. Sandip et al., 2018[14] reported aluminum chloride method was utilised for the quantitative evaluation of total flavonoids in extracts using quercetin standard and results summarised as quercetin equivalent (QE)/gram*S urens* root extract.Abiola et al., 2016[15] reported 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. Batool et al., 2019[16] reported 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.

Evaluation of Pharmacological activities:

Antioxidant activity:

The anti-oxidant activity of crude root extracts of *S urens* was evaluated using performing DPPH free radical scavenging assay and the experiment was conducted based on procedure reported by Djacbou et al., 2014[17]. Anti-oxidant activity of the crude extracts was confirmed by calculating the 50 % (IC_{50}) inhibition concentration of each extract and results were compared with ascorbic acid standard.

Anti-diabetic activity:

The anti-diabetic activity of the crude root extracts of *S urens* was evaluated by performing α-amylase enzyme inhibition assay as per *Shettar et al*., 2017[18 reported methodology. Anti-diabetic activity of the crude extracts was confirmed by calculating the 50 % (IC_{50}) inhibition concentration 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 study based on Syed et al., 2018[19] reported procedure. Anti-inflamatory activity of the crude extracts was confirmed by calculating the 50 % (IC_{50}) inhibition concentration of each extract and 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 as per procedure reported by 2018[20]. Streptokinase (30,000 I.U.) and methanol were selected as positive and negative controls respectively.

Anti-bacterial activity:

The anti-bacterial activity of root extracts of *S urens* was evaluated by performing agar plate well diffusion method based on procedure reported by Sahar et al., 2020[21]. In this study, two-gram negative bacterial such as *Pseudomonas aeruginosa* (MTCC – 1748), *Escherichia coli* (MTCC – 294) and two gram-positive bacteria such as *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 results summarised asmillimetre (mm) of inhibition zone observed for each sample studied.

Isolation compounds:

The 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 X 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 was used for isolation study.

The purification of flavonoids in the methanolic root extract was performed based on procedure reported by Ines et al., 2020[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., 2017[23] and the isolated crude alkaloid fraction was subjected to preparative HPLC purification as per procedure reported by Atlabachew et al., 2017[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 characterised using spectroscopic studies such as NMR and mass spectroscopy. The interpretation of the data obtained from all the spectral studies confirms the molecular structure of the purified compound.

Results:

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, EA, ME and WA solvents respectively. The ME extract shows high quantity of extract than the other solvent studied. The results observed during the preliminary qualitative analysis study for root extracts of *S urens* was tabulated in table 1.

S. No	Test studied	EA extract	ME extract	WA extract
1	Terpenoids	+	-	-
2	Flavonoids	-	++	-
3	Saponins	-	-	+
4	Steroids	+	+	-
5	Cardiac glycosides	+	++	-
6	Proteins	-	-	-
7	Carbohydrates	-	-	-
8	Monosaccharides	-	-	-
9	Reducing sugars	-	-	-
10	Phenolic compounds	+	++	-

11	Alkaloids	-	++	-
12	Coumarins	-	-	+
13	Anthraquinones	+	+	-
14	Glycosides	-	+	+

− indicates absence; + indicates low concentration; ++ indicates high concentration.

Table 1: Results obtained in phytochemical screening studies

The quantitative analysis was carried for the compounds that give positive test in qualitative analysis. In the quantitative study, it was calculated that EA extract contains 3.91±0.053 GAE/g and ME extract contains 17.52±0.105 GAE/g of phenolic compounds. The ME extract contains 15.76±0.060 QE/gram extract of flavonoids and 5.96±0.063 AE/g of alkaloids. The WA 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, EA, ME and WA extracts. The IC_{50} concentration of ME extract was observed to be very low and is very close to the ascorbic acid standard that proves that ME extract having high DPPH radical inhibition activity.

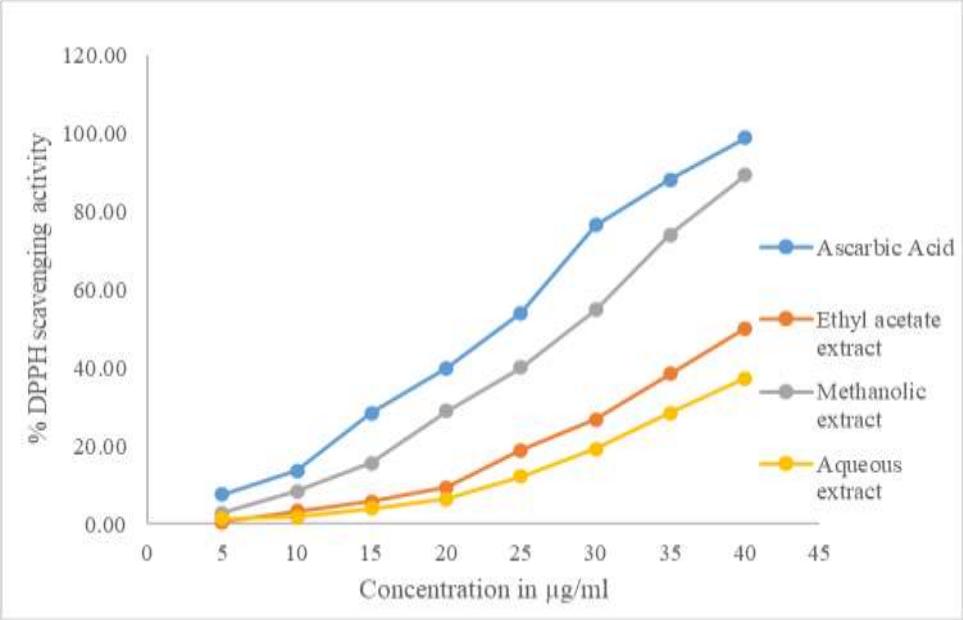


Fig 1: Antioxidant activity results

A wide concentration range of 2.34 to 300 µg/mL (Fig. 2) was studied for the evaluation of the anti-diabetic 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, EA, ME and WA extracts respectively. The results proved that the IC_{50} concentration of ME was observed to be very close to the acarbose and hence having potential α-amylase inhibition activity than EA and WA extracts.

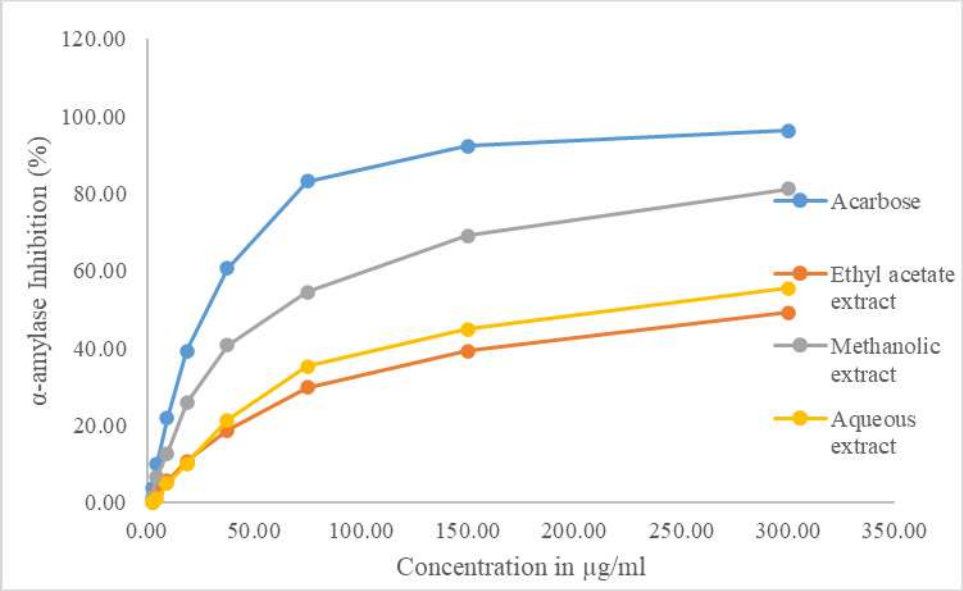


Fig 2: Antidiabetic activity results

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 ME extract shows IC_{50} values at a lowest concentration of 137.09±0.20 µg/mL confirms that the ME having high activity among other root extracts of *S urens*. The IC_{50} concentration of standard was observed to be 107.13±0.13µg/mL whereas 210.74±1.81 and 190.86±0.28 µg/mL was observed for EA extract and ME extract respectively.

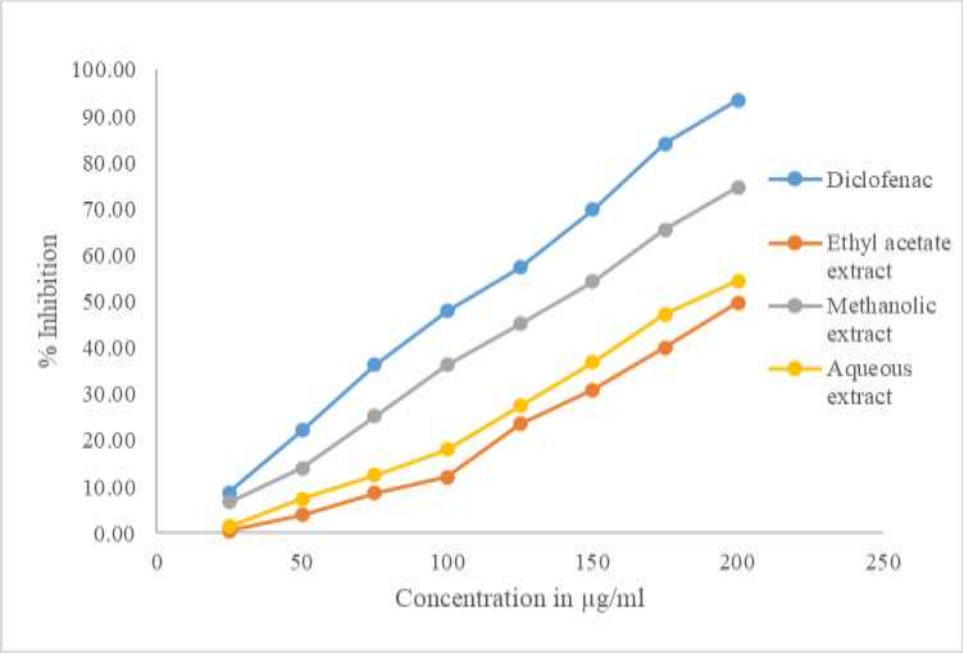


Fig 3: Anti-inflammatory activity results

The thrombolytic activity of crude root extracts of *S urens* was evaluated by *in-vitro* clot lysis study and results achieved were summarised in table 2. The % clot lysis at 10 mg/mL concentration of root extracts was observed to be 11.16±5.009, 48.76±2.656& 15.32±3.149 for EA, ME and WA extracts respectively whereas the standard 100 µL of standard

streptokinase shows 62.36±0.140 % of clot lysis. The results proved that ME having high clot lysis potential and hence having high thrombolytic activity than other root extracts in the study.

S No	Concentration studied (mg/mL)	% clot lysis		
		EA extract	ME extract	WA extract
1	2	1.01±0.229	3.86±1.553	1.52±0.497
2	4	2.14±1.568	10.22±1.045	3.14±0.912
3	6	5.01±2.295	21.76±5.225	6.63±1.394
4	8	7.81±3.274	31.17±7.309	10.94±2.237
5	10	11.16±5.009	48.76±2.656	15.32±3.149
Treatment	% of clot lysis			
Streptokinase	62.36±0.140 (positive control)			
Blank	0.53±0.020 (Negative control)			

Results expressed as mean ± SD (n=3)

Table 2: *In-vitro* clot lysis activity results

The anti-bacterial activity of crude root extracts of *S urens* and standard gentamycinwas studied at a concentration of 1, 10 and 50 µg/mL (Table 3). In the studied samples, EA extract doesn't show any zone of inhibition against the studied bacteria at 1 & 10 µg/mL concentrations. The WE 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 and standard shows zone of inhibition against studied bacteria proves that the extracts have capability to inhibit the growth of the studied bacteria.

S No	Extract/sample	Growth inhibition zone observed in mm			
		B. subtilis	***S. aureus***	***E. coli***	***P. aeruginosa***
1	EA at 1 µg/mL	-	-	-	-
2	EA at 10 µg/mL	-	-	-	-
3	EA at 50 µg/mL	4.53±0.06	4.10±0.10	5.27±0.06	5.60±0.10
4	ME at 1 µg/mL	-	-	2.80±0.17	3.43±0.15
5	ME at 10 µg/mL	2.67±0.12	3.20±0.10	5.87±0.06	5.47±0.21
6	ME at 50 µg/mL	4.67±0.15	5.63±0.15	8.70±0.20	7.80±0.17
7	AE at 1 µg/mL	-	-	-	-

8	AE at 10 µg/mL	-	-	3.57±0.12	3.80±0.17
9	AE at 50 µg/mL	5.17±0.25	5.17±0.25	6.20±0.10	6.53±0.15
10	GM at 1 µg/mL	4.70±0.20	4.13±0.15	5.33±0.15	3.73±0.21
11	GM at 10 µg/mL	6.77±0.15	6.00±0.10	8.37±0.15	8.77±0.15
12	GM at 50 µg/mL	11.30±0.20	10.57±0.21	12.53±0.06	13.37±0.25

GM = Gentamycin standard

Results expressed as average± SD (n=3)

Table 3: Anti-bacterial activity results

The biological activates confirms that the root ME extract having high activities and qualitative, quantitative phytochemical assay proved that ME 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 ME extract was purified using semi-preparative HPLC analysis.

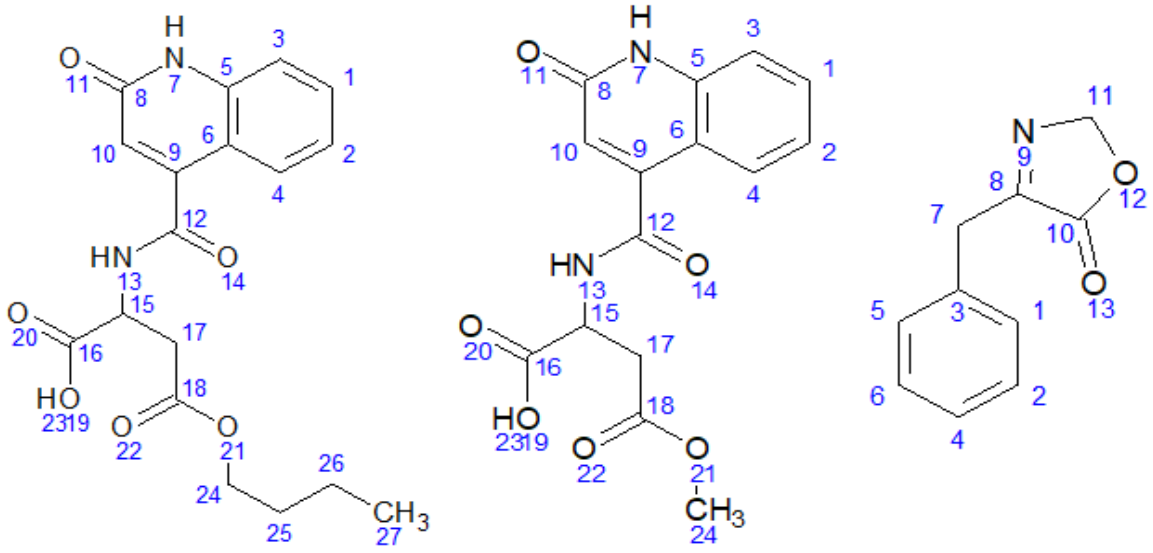
The LC chromatogram of the semi-preparative HPLC isolation of alkaloid fraction shows five peaks. This confirms that five dissimilar alkaloids were present in the crude ME extract. The response of the peak corresponds to individual alkaloid confirms that two alkaloids were found to be quantitatively 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 summarised as

RAF 1: whitepowder; *m.f.*:$C_{18}H_{20}N_2O_6$; UV (CD_3OD, λ_{max}) 227 nm, 384 nm; ^{1}H NMR (500 MHZ, CD_3OD, δ, ppm): δ 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, *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): δ 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: whitepowder; *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): ^{1}H NMR: δ 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, the compound RAF 1 and RAF 2 were confirmed as Sterculinine I & II which are commonly found in plants especially *Sterculia* species. The third isolated alkaloid RAF 3 was confirmed as Oxazolone class alkaloid with IUPAC name *4-benzyl-1,3-oxazol-5(2H)-one*. The structure of the isolated alkaloids derived from the interpretation of the spectral data were given in fig 4.



RAF 1 **RAF 2** **RAF 3**

Fig 4: Molecular structure of isolated alkaloids

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 were identified using spectral analysis. The results observed in spectral analysis was summarised as

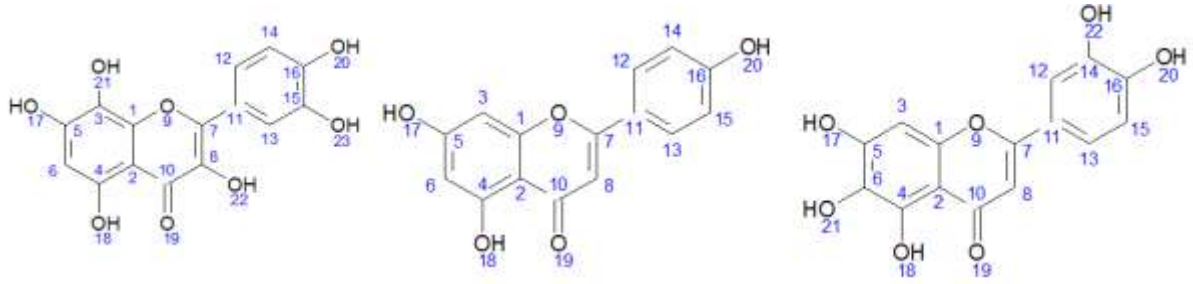
RFF 1: yellow crystalline powder; *m.f.*:$C_{15}H_{10}O_8$; UV (CD_3OD, λ_{max}) 286 nm, 374 nm; ^1H NMR (500 MHZ, CD_3OD, δ, ppm): δ 5.55 (1H,s), 6.40 (1H, s), 6.81 (1H, dd, *J* = 8.4, 0.5 Hz), 7.34 (1H, dd, *J* = 1.8, 0.5 Hz), 7.27 (1H, dd, *J* = 8.4, 1.8 Hz), 8.08 (1H,s), 9.10 (1H,s), 12.24 (1H,s), 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; ^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).

RFF 3: 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 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 Hydroxyluteolin respectively which are known flavonoids. The molecular structures of the isolated flavonoids were given in fig 5.



RFF 1 **RFF 2** **RFF 3**

Fig 5: Molecular structure of isolated Flavonoids

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 EA extract shows the presence of terpenoids, steroids, cardiac glycosides and phenolic compounds. The ME extract shows positive tests for cardiac glycosides, alkaloids, anthraquinones, flavonoids, steroids, glycosides and phenolic compoundswhereas the aqueous gives positive results for coumarins, glycosides and saponins.The quantitative estimation of phytochemical compounds present in the root extract of *S urens* confirms that the ME extract contains 15.76±0.060 mg of QE/gram of flavonoids, 5.96±0.063 AE/g of alkaloids and 17.52±0.105 GAE/g of phenolic compounds. The EA extract having 3.91±0.053 GAE/g of phenolic compounds whereas the WA extract having 2.54±0.036 mg/g of saponins. This proved that the ME 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 IC_{50} concentrations in DPPH radical scavenging assay 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 ascorbic acid, EA, ME and WA extracts. This proved that the phytochemicals in the root extracts having significant role in the inhibition of free radical activity. The findings in the present study are in correlation with the reports available in literature[27].

The root extracts of *S urens* shows potential inhibition on the activity of α-amylase with IC_{50} concentrations of 71.51±0.08, 263.96±0.90, 127.73±1.23 and 223.54±4.76 µg/mL for standard drug, EA, ME and WA extracts respectively. The anti-inflammatory activity, thrombolytic activity and anti-microbial activity was observed to be significantly high for ME extract whereas comparatively less for EA and WA extracts. These activity of the methanolic extract are in close argument with corresponding standards proved that root ME extract of *S urens*having high pharmacological activities.

The semi preparative HPLC isolation followed by spectral characterization confirms that two well-known alkaloids namely Sterculinine I & II were isolated and identified from the root

ME extract which were reported in *S. lychnophora* by Ru-Feng et al., 2003[28]. Our findings reports the two alkaloids (Sterculinine I & II) for the first time in *S urens*. One Oxazolone class alkaloid with IUPAC name *4-benzyl-1,3-oxazol-5(2H)-one* was also isolated from the root methanolic extract which is reported in the leaf extract of *S. urens* by Nanadagopalan et al., 2015[29]. Two known flavonoids Apigenin and 6-Hydroxyluteolin were isolated in the root ME extract and were reported in the leaves of *Sterculia colorata*[30]. One known flavonoid Gossypetin was isolated in the root ME extract and were reported in *Sterculia diversifolia* by Fazle Rabbi et al., 2020[31].

Conclusion:

It can be concluded that, the root extracts of *S urens* shows quantitatively high number of phytochemical constituentsthat shows significantly high pharmacological activities. Among the extracts, ME shows significantly dominant activities than other extracts. Two well-known alkaloids namely 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(2H)-one* was also isolated from the root methanolic extract. Three known flavonoids (Gossypetin, Apigenin and 6-Hydroxyluteolin) were isolated from the methanolic extract for the first time in *S urens* Roxb.

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