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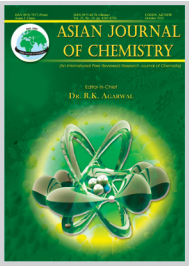

Phytochemical and Biological Investigations of *Saurauja roxburghii*

MD. ALIM UDDIN CHOWDHURY[1], MD. ATIAR RAHMAN[1,*], MARUFA ZAHAN[1], MD. HOSSAIN SOHRAB[2], CHOWDHURY MD. MONIRUL HASAN[1] and YUNUS AHMED[3]

[1]Department of Biochemistry and Molecular Biology, University of Chittagong, Chittagong-4331, Bangladesh
[2]Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka-1205, Bangladesh
[3]Chittagong University of Engineering and Technology, Chittagong, Bangladesh

*Corresponding author: Fax: +88 031 726310; Tel: +88 01711709084; E-mail: atiarh@yahoo.com

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Two compounds were isolated from the *n*-hexane soluble fraction of a petroleum ether extract of the *Saurauja roxburghii* leaf. The structures of the isolated compounds were elucidated as β-amyrin (**1**) and stigmasterol (**2**), by high field NMR analyses as well as by comparison with structurally related compounds. In *in vitro* thrombolytic assay, 100 µL methanol extract of *Saurauja roxburghii* showed 26.09 % blood clot lytic activity. Promising antibacterial activity of *Saurauja roxburghii* extract against **4** gram positive and **6** gram negative bacteria were observed in disc diffusion technique. In *in vitro* antioxidant assay, both the extract and ascorbic acid (standard antioxidant) showed very good DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity with IC_{50} value 3.34 µg/mL for extract and 0.05 µg/mL for ascorbic acid.

Key Words: β-Amyrin, Stigmasterol, Thrombolytic, Antioxidant, DPPH.

INTRODUCTION

The aim of the study was to delineate a phytochemical and pharmacological effect of *Saurauja roxburghii* leaf extract. *Saurauja roxburghii* (SR) is an evergreen medium size tree belonging to the Dilleniaceae family found in very rare locations especially in hillsides of world. This is a plant with a long history of traditional medicinal uses in several countries of the world. Different parts of this plant have been used by local tribes of Chittagong, Bangladesh in treating various diseases like asthma, bronchitis, paralysis, ulcers, piles, sinuses, scabies, eczema and warts. It is believed that detailed information as presented in the review on its phytochemistry and various biological properties of the extracts and the constituents might provide incentive for proper evaluation of the use of the plant in medicine and in agriculture although uses of some of these species are based on old and new experiences and clinical data. An attempt has been taken to study systematically the chemical constituents and biological effects of *Saurauja roxburghii*, found in Bangladesh.

EXPERIMENTAL

Fresh leaves of *Saurauja roxburghii* were collected from Hillside of the University of Chittagong, Bangladesh and were taxonomically identified by a Dr. Mostafa Kamal Pasha, Professor, Department of Botany, University of Chittagong, Bangladesh. A voucher specimen that contains the identification characteristics of the plant has been deposited in the Bangladesh National Herbarium, Dhaka (DACB Accession no. 32567), for future reference.

Extract preparation: The fresh plant leaves of *Saurauja roxburghii* were chopped into small pieces, air dried at room temperature (23 ± 5) ºC for about 10 days, ground into powder (1 kg) and extracted with 2.5 L pure methanol for 15 days at room temperature with occasional stirring. Filtered methanol was concentrated under reduced pressure below 50 ºC through rotatory vacuum evaporator (RE200 Sterling, UK). The concentrated extract (48 g blackish-green coloured) was preserved at 4 ºC for further use.

Qualitative phytochemical screening: *Saurauja roxburghii* leaf extract was subjected to preliminary phytochemical screening for the identification of chemical constituents according to Brindha and Saraswathy[1]. Essentially, alkaloid was tested with Dragendroff's and Mayer's, Wagner's reagent, cardiac glycosides with Keller-Killani test and Baljet test. Anthraquinone-glycosides were tested by aglycone test, O-glycosides test, C- glycosides test, flavonoids with H_2SO_4, ethyl acetate and ammonia solution, tannins with $FeCl_3$ and terpenoid with salkowski test, carbohydrates with molisch test, steroids by Liberman Burchard`s test. Test for phlobatannins

with 1 % aqueous hydrochloric acid and saponin using frothing test.

Quantitative phytochemical test: All reagents and solvents were reagent grade. Open column chromatography and medium-pressure column chromatography (FMI pump system) were performed with silica gel (kiesel gel 60, particle size 70-230; Merck Inc., Germany, and Merck silica gel 60, 230-400 ASTM mesh, respectively). Analytical thin-layer chromatography (TLC) and preparative thin-layer chromatography was accomplished by using plates precoated with Merck silica gel 60 F254 (0.25 mm and 0.5 mm thickness, respectively). ^{1}H nuclear magnetic resonance (NMR) spectra (400 MHz) were recorded on an AMX400 FT-NMR spectrometer (Biospin, Bruker, AG, Switzerland) using tetramethylsilane as internal standard. The crude extract was chromatographed over silica gel using *n*-hexane with gradually increasing proportions of EtOAc to 100 % EtOAc and finally with MeOH as eluent giving fractions 1-19. Fractions 14, 15 and 19 were chosen for further analysis to isolate the compounds by repeated preparative thin-layer chromatography separation. Fraction 14 (SR-14, 10 mg) and 19 (SR-19, 8.0 mg) were separated by preparative thin-layer chromatography (petroleum ether/ diethyl-ether, 50:50) to give pure compounds of each. The isolated pure compounds were then characterized using various spectroscopic techniques.

***In vitro* thrombolytic activity:** To the commercially available lyophilized streptokinase (SK) vial (Durakinase, Dongkook Pharma. Co. Ltd. South Korea) of 15, 00,000 I.U., 5 mL sterile distilled water was added and mixed properly. This suspension was used as a stock from which 100 µL (30,000 I.U.) was used for *in vitro* thrombolysis.

Specimen: Whole blood (4 mL) was drawn from healthy human volunteers (n = 20) without a history of oral contraceptive or anticoagulant therapy (using a protocol approved by the Institutional Ethics Committee of Central India Institute of Medical Sciences, Nagpur). 500 µL of blood was transferred to each of the three previously weighed microcentrifuge tubes to form clots.

Herbal preparation: 100 mg of *Saurauja roxburghii* leaf extract was suspended in 10 mL distilled water and the suspension was shaken vigorously on a vortex mixer. The suspension was kept overnight and decanted to remove the soluble supernatant, which was filtered through a 0.22-micron syringe filter. 100 µL of this aqueous preparation of herbs was added to the micro centrifuge tubes containing the clots to check thrombolytic activity.

Clot lysis: 4 mL venous blood drawn from healthy volunteers was distributed in three different pre weighed sterile microcentrifuge tube (0.5 mL/tube) and incubated at 37 ºC for 45 min. After clot formation, serum was completely removed without disturbing the clot and each tube having clot was again weighed to determine the clot weight (clot weight = weight of clot containing tube - weight of tube alone).

To each microcentrifuge tube containing pre-weighed clot, 100 µL extract of *Saurauja roxburghii* was added. As a positive control, 100 µL of streptokinase and as a negative non thrombolytic control, 100 µL of distilled water were separately added to the control tubes numbered. All the tubes were then incubated at 37 ºC for 1.5 h and observed for clot lysis. After incubation, fluid released was removed and tubes were again weighed to observe the difference in weight after clot disruption. Difference obtained in weight taken before and after clot lysis was expressed as percentage of clot lysis. The experiment was repeated 20 times with the blood samples of 20 volunteers.

Statistical analysis: The significance between % clot lysis by streptokinase and herbal extract by means of weight difference was tested by the paired *t*-test analysis. Data are expressed as mean ± standard error of mean. The significance of difference between means was determined by student's *t*-test values of $p < 0.05$ were considered significant whereas $p < 0.01$ and $P < 0.001$ as highly significant.

Assay for antibacterial activity: Antibacterial activity of plant extract was determined by disc diffusion method as described by Bauer *et al.*[2]. Three gram positive bacteria (*Bacillus subtilis, Staphylococcus aureus, Bacillus megaterium* and *Bacillus cereus*) and **6** gram negative bacteria (*Salmonella typhi, Salmonella paratyphi, Pseudomonas aeruginosa, Vibrio cholera, Shigella dysenteriae* and *Escherichia coli*) were used for the present study. All the test bacteria were collected from Department of Microbiology, University of Chittagong, Bangladesh. Dried filter paper discs (4 nm in diameter) impregnated in known amount of test substances (1 mg/disc, 2 mg/disc and 3 mg/disc concentration) were placed on Mueller-Hinton agar medium uniformly seeded with the test organisms. Tetracycline discs (30 µg/disc) soaked in respective solvent were used as positive control. These plates were then kept at low temperature (4 ºC) for 2 to 4 h to allow maximum diffusion of compound. The diffusion occurred according to the physical law that controls the diffusion of molecules through agar gel[3]. The plates were then incubated at 37 ºC for 24 h to allow maximum growth of the microorganisms. If the test materials have any antibacterial activity, it will inhibit the growth of the microorganisms giving the distinct zone around the disc called zone of inhibition. The antibacterial activity of the test material was determined by measuring the diameter of the zones of inhibition in millimeter with transparent scale.

Determination of MIC: The minimum inhibitory concentration (MIC) value of *Saurauja roxburghii* extract against 10 test bacteria were determined by micro and macrodillution broth technique[4] using Mueller-Hinton medium. During MIC experiment, *Saurauja roxburghii* extract of 50-500 ppm concentrations were used.

***In vitro* assay of antioxidant activity:** The antioxidant activity of *Saurauja roxburghii* extract was assessed in comparison to standard antioxidant ascorbic acid (Sigma, Germany) on the basis of scavenging effect of the stable 2,2- diphenyl-1-picrylhydrazyl (DPPH) free radical according to established method described by Brand-William[5] with slight modifications. Plant extract with different concentrations (20, 40, 60, 80, 100, 200, 400 and 800) µg/mL and ascorbic acid in (20, 40, 60, 80, 100, 200, 400 and 800) µg/mL concentration solutions were prepared in methanol. 2,2-Diphenyl-1-picrylhydrazyl solution (0.004 %) was prepared in methanol and 3 mL of this solution was mixed with diluted 5 mL of extract and standard solution separately. The mixtures were kept in dark for 0.5 h to measure the absorbance at 517 nm using UV-Visible spectrophotometer (UV 3600, Shimadzu Corporation, Japan). Lower absorbance of the reaction mixture indicated higher free radical-scavenging activity.

RESULTS AND DISCUSSION

The present study carried out on the plant sample revealed the presence of medicinally active constituents. The phytochemical characters investigated are summarized in Table-1 alkaloids, glycosides, O-glycosides, flavonoids, terpenoids, carbohydrates, steroids, reducing sugar tannins, phlobatannins and saponin are present in this plant whereas cardiac glycosides are absent.

TABLE-1
QUALITATIVE PHYTOCHEMICAL SCREENING OF SECONDARY METABOLITES OF *Saurauja roxburghii* LEAF EXTRACT

Constituents	Observation
Alkaloids	+
Glycosides	+++
Cardiac glycosides	++
Anthraquinone-glycosides	+++
Flavonoids	+++
Terpenoids	++
Carbohydrates	+
Steroids	+++
Tanins	++
Phlobatanins	+++
Saponins	+

N.B. "+++" = very abundant; "++" = abundant; " +" = traces

Characterization of SR-14 as β-amyrin: Compound SR-14 (Fig. 1): It was isolated from the column fraction of petroleum ether extract by elution with petroleum ether: 65-70 % dichloromethane. It was obtained as colourless crystal. It appeared as dark quenching spot on the TLC plate under UV light at 254 nm and also exhibited blue fluorescence at 365 nm (R_f = 0.54; toluene/10 % ethyl acetate). Compound **SR-14** (10 mg) was soluble in dichloromethane, chloroform and ethyl acetate. It appeared as a purple spot on the TLC plate when sprayed with vanillin-sulfuric acid spray reagent, followed by heating at 110 ºC, for 5 min.

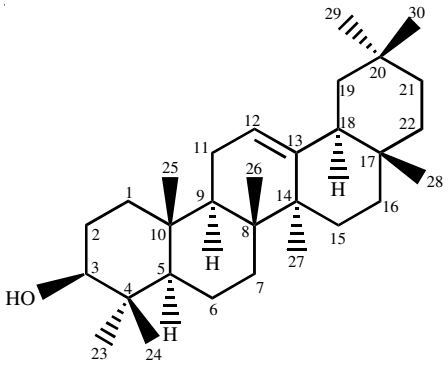

Fig. 1. Chemical structure of **SR-14**

The ^{1}H NMR spectra of compound **SR-14** displayed the characteristic vinylic proton resonance as a triplet (J = 3.7 Hz) at δ 5.12 and the oxymethine proton signal as a doublet (J = 11.5, 5.0 Hz). In addition, the ^{1}H NMR spectrum showed signals for eight methyl groups at δ 1.07, 1.00 (3H × 2), 0.95, 0.91, 0.86, 0.79 (3H × 2). These ^{1}H NMR data suggested the β-amyrin type carbon skeleton for **SR-14**. Comparison of these data with published values for β-amyrin[6] established the identity of **SR-14** as β-amyrin. Although has previously been reported from many plants[7] this is the first report of its isolation from *Saurauja roxburghii*. The ^{1}H NMR data of **SR-14** (Table- 2) were found to be identical to those reported for the compound β-amyrin[8].

TABLE-2
COMPARISON BETWEEN THE ^{1}H NMR SPECTRAL DATA OF SR-14 AND β-AMYRIN (400 MHz, $CDCl_3$)

Protons	SR-14	β-amyrin
	δH (mult. J in Hz)	δH (mult, J in Hz)
12-H	5.12 (1H, t, J = 3.2 Hz)	5.12 (1H, t, J = 3.7 Hz)
3-H	3.21 (1H, m)	3.23 (1H, dd, J = 11.0,5.0 Hz)
25-CH_3	1.07 (3H, s)	1.07 (3H, s)
26-CH_3	1.00 (3H, s)	1.00 (3H, s)
27-CH_3	1.00 (3H, s)	0.99 (3H, s)
28-CH_3	0.95 (3H, s)	0.95 (3H, s)
23-CH_3	0.91 (3H, s)	0.80 (3H, s)
24-CH_3	0.86 (3H, s)	0.80 (3H, s)
29-CH_3	0.79 (3H, s)	0.79 (3H, s)
30-CH_3	0.79 (3H, s)	0.79 (3H, s)

Characterization of SR-19 as stigmasterol: Compound SR-19 (Fig. 2): It was isolated from the column fraction of petroleum ether extract by elution with petroleum ether: 90 % dichloromethane. It was not visible under UV light. **SR-19** (8 mg) was obtained as white needle shaped crystal. Spraying the developed plate with vanillin-sulphuric acid followed by heating at 110 ºC for several minutes gave purple colour. The compound was identified as stigmasterol by comparing the ^{1}H NMR data (Table-3) with those reported for the compound[9-11].

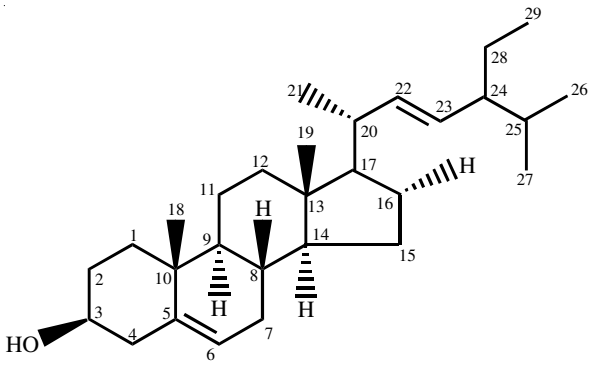

Fig. 2. Chemical structure of **SR-19**

The ^{1}H NMR spectra (400 MHz, $CDCl_3$) of **SR-19** (Table-3) showed two one proton multiplets at δ 3.52 and δ 5.34 typical for H-3 and H-6 of a steroidal nucleus. The olefinic protons H-22 and H-23 appeared as characteristic downfield signals at δ 5.16 and δ 5.03 respectively in the ^{1}H NMR spectrum. Each of the signals was observed as double doublet, which indicated coupling with the neighboring olefinic and methine protons. The ^{1}H NMR spectrum displayed two three-proton singlets at δ 1.00 and δ 0.67 assignable for 19-Me and 18-Me respectively. In addition, two doublets at δ 0.82 and δ 0.84 could be ascribed to the two methyl groups at C-25, which are 27-Me and 26-Me respectively and another three-proton doublet at δ 0.91 for 21-Me. On the other hand, one three-proton triplet at d 0.80 could be assigned to the primary methyl group

attached to C-28 (*i.e.*, Me-29). These ^{1}H NMR spectral features are characteristics of a steroidal carbon skeleton of stigmasterol. Therefore, on the basis of these spectral data **SR-19** was identified as stigmasterol. Although it is a known compound but this is the first report of its occurrence in the genus *Saurauja*.

TABLE-3
COMPARISON BETWEEN THE ^{1}H NMR SPECTRAL DATA OF **SR-19** (400 MHz, $CDCl_3$) AND STIGMASTEROL

Protons	**SR-19**	Stigmasterol
	δH (mult. *J* in Hz)	δH (mult. *J* in Hz)
3	δ 3.52 m	δ 3.52
6	δ 5.34 m	δ 5.32
22	δ 5.16 dd (*J* = 15.0, 6.5 Hz)	δ 5.15
23	δ 5.03 dd (*J* = 15.0, 9.0 Hz)	δ 5.03
18-CH_3	δ 0.67s	δ 0.65
19-CH_3	δ 1.00s	δ 1.00
21-CH_3	δ 0.91d	δ 0.90
26-CH_3	δ 0.84d	δ 0.83
27-CH_3	δ 0.82d	δ 0.83
29-CH_3	δ 0.80t	δ 0.81

In vitro* clot lysis:** In this experiment, addition of 100 µL streptokinase as positive control showed 26.09 % clot lysis. Clot when treated with 100 µL sterile distilled water as negative control gives only 9.40 % clot lysis (Table-4). The mean difference in clot lysis percentage between positive and negative control was significant (P value < 0.001). On the other hand, after treatment of clots with 100 µL *Saurauja roxburghii* methanol extract showed 26.09 % clot lysis activity and when compared with negative control (water) the mean clot lysis % difference (Table-4) was significant (***P value < 0.001). Statistical representation of the effective clot lysis percentage by *Saurauja roxburghii* extract, positive thrombolytic control (Streptokinase) and negative control (sterile distilled water) is tabulated in Table-4.

TABLE-4
COMPARING THE DATA OF % OF CLOT LYSIS USING THE STUDENT'S t-TEST

Mean % of clot lysis		
Negative control (water)	Positive control (streptokinase)	*Saurauja roxburghii* extract
3.166 ± 0.494	37.03 ± 1.37***	26.09 ± 1.79***
t calculated 18.11 8.98; t tabulated 3.67 3.67; student's t test degrees of freedom 28, 28; P value < 0.001 < 0.001		
Here, all values are expressed as MEAN ± SEM (n = 15); ***P< 0.001 significant compared to negative control		

This study shows *in vitro* dissolution of clots by streptokinase, assayed by an *in vitro* clot lysis model[12]. Most of the *in vitro* methods that were conventionally or currently applied to study thrombolysis have certain limitations. Some involve complex computation and mathematical skills that to give only theoretical prediction of the outcome and most are expensive to be performed in a laboratory. In context with the current scenario of developing a clot lysis model that would be easy to perform and cost effective too, should be materialized. It is concluded that this plant extract possesses mild to moderate thrombolytic activity *in vitro* when tested on human blood.

Keeping this idea in the prime focus, weight of the clot before lysis and after lysis was considered as appropriate determinant of calculating clot lysis percentage. Blood collected by the standard protocol of venipuncture was allowed to form clots naturally and the weight difference obtained before and after lysis was noted and clot lysis percentage was calculated. In other methods, there are different parameters to analyze the extent of clot lysis. For example, radiolabelling of factors that are actively involved in clot lysis mechanism, MRI, ultrasound frequency, turbidity determination using microtiter plate reader (euglobulin lysis test), study of fibrinolytic activity by circulating fibrinolytic enzymes and monitoring the effect by calculating shear rate. All these methods are sophisticated but complicated whereas, this model is simple and easy to perform and one can even visually observe the lysis of clots.

Antibacterial activity: On the basis of the result obtained in this present investigation, we bring to a close that the methanol extract of *Saurauja roxburghii* had significant *in vitro* antibacterial activity. This implied that the gram positive bacteria were equally susceptible to the extract like the gram negative bacteria. Possibly because of the presence of active compounds which inhibit the process of growth of both tested gram negative bacteria like *Salmonella typhi*, *Salmonella paratyphi, Pseudomonas aeruginosa, Vibrio cholerae, Shigella dysenteriae* and *E. coli* and gram-positive bacteria *Bacillus subtilis, Staphylococcus aureus, Bacillus cereus* and *Bacillus megaterium* (Table-5). The zone of inhibition was almost equal to the standard (Table-5). It showed that the test organisms were sensitive to the plant extract even better than the standards tetracycline and ampicillin in some cases. All the bacterial strains demonstrated significant degree of sensitivity to the plant extract.

The preliminary phytochemical screening showed the presence of various secondary metabolites in this plant. The flavonoids are antimicrobial[13] and the presence of flavonoids in this plant shows strong antimicrobial activity. Other secondary metabolites present in this plant may also shows antibacterial activity.

Bacillus megaterium, Bacillus subtilis and *E. coli* were the most susceptible bacteria, an observation that may be attributed to the presence of single membrane of the organism, which makes it more accessible to permeation by active principles of the extract of *Saurauja roxburghii.* The results of present research highlights, the fact that the organic solvent extracts exhibited greater antimicrobial activity because the antimicrobial principles were either polar or non-polar and they were extracted only through the organic solvent medium[14,15]. The present observation suggests that the organic solvent extraction was suitable to verify the antibacterial properties of medicinal plants and they are supported by many investigators[16-18]. The present study justifies the claimed uses of *Saurauja roxburghii* in the traditional system of medicine to treat various infectious diseases caused by the microbes. This study also encourages cultivation of the highly valuable plant in large-scale to increase the economic status of cultivars in the country. The obtained results may provide a support to use of the plant in traditional medicine. Based on this, further chemical and pharmacological investigations to isolate and identify minor chemical consti-

TABLE-5
IN VITRO ANTIBACTERIAL ACTIVITY OF *Saurauja roxburghii* EXTRACT

Test organism	Diameter of zone of inhibition (mm)				
	Saurauja roxburghii extract			Tetracycline (50 µg/disc)	Ampicillin (50 µg/disc)
	1 mg/disc	2 mg/disc	3 mg/disc		
Gram positive organism					
Bacillus subtilis	9	11	12	15	8
Stapphyloccus aureus	9	11	11	Nil	Nil
Bacillus cereus	9	10	11	16	Nil
Bacillus megaterium	15	16	19	20	44
Gram negative organism					
Salmonella typhi	10	10	10	16	Nil
Salmonella paratyphi	8	10	11	20	8
Pseudomonas aeruginosa	9	10	10	20	8
Vibrio cholerae	9	10	10	20	Nil
Shigella dysenteriae	10	11	11	15	7
Escherichia coli	9	16	18	8	22

TABLE-6
MINIMUM INHIBITORY CONCENTRATION (MIC) OF CRUDE EXTRACT FROM *Saurauja roxburghii* AGAINST 10 BACTERIAL TESTS BACTERIA

Bacterial strain	Bacterial growth in Mueller-Hinton broth (*Saurauja roxburghii* extract concentration ppm)						
	50	100	200	300	400	500	MIC (ppm)
Gram-positive organism	-	-	+	+	+	+	200
Bacillus cereus	-	+	+	+	+	+	100
Bacillus subtilis	-	-	+	+	+	+	200
Bacillus megaterium	-	-	+	+	+	+	200
Staphylococcus aureus							
Gram-negative organism							
Escherichia coli	-	+	+	+	+	+	100
Pseudomonas aeruginosa	-	-	+	+	+	+	200
Salmonella typhi	-	-	+	+	+	+	200
Salmonella paratyphi	+	+	+	+	+	+	50
Shigella dysenteriae	+	+	+	+	+	+	50
Vibrio cholerae	-	-	+	+	+	+	200

(+) = growth; (-) = No growth

tuents in *Saurauja roxburghii* and to screen other potential bioactivities may be recommended.

Minimum inhibitory concentration (MIC): The minimum inhibitory concentration value of the *Saurauja roxburghii* plant extract varied between 50 and 200 ppm against the test bacteria. The crude extract of plant showed the lowest minimum inhibitory concentration value (50 ppm) against *Salmonella paratyphi* and *Shigella dysenteriae* (Table-6).

***In vitro* antioxidant activity:** A simple method utilizing the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical has been developed to determine the antioxidant activity of natural products. IC_{50} value of extract and ascorbic acid were found 3.34 µg/mL and 0.05 µg/mL respectively (Table-7). The free radical scavenging action of *Saurauja roxburghii* methanol extract showed the highest scavenging activity (96.47 %) at 400 µg/mL. Such a stronger scavenging action effectively shows the therapeutic potential of natural medicinal plants as an antioxidant in reducing free radical induced tissue injury. 2,2-Diphenyl-1-picrylhydrazyl stable free radical method is a sensitive way to determine the antioxidant activity of plant extracts[19-21]. The odd electron in the 2,2-diphenyl-1-picrylhydrazyl free radical gives a strong absorption maximum at 517 nm and is purple in colour. The colour turns from purple to yellow as the molar absorptivity of the 2,2-diphenyl-1-picrylhydrazyl radical at 517 nm reduces from 9660 to 1640 when the odd electron of 2,2-diphenyl-1-picrylhydrazyl radical becomes paired with hydrogen from a free radical scavenging antioxidant to form the reduced 2,2-diphenyl-1-picrylhydrazyl-H. The resulting decolourization is stoichiometric with respect to number of electrons captured. Ascorbic acid was used as a standard and it is acting as a chain breaking antioxidant impairs with the formation of free radicals in the process of formation of intracellular substances throughout the body, including collagen, bone matrix and tooth dentine[22,23]. The quantitative determination of ascorbic acid in plant extract shows that they are good source of ascorbic acid. A striking pathological change resulting from ascorbic acid deficiency is the weakening of the endothelial wall of the capillaries due to a reduction in the amount of intercellular substance. Therefore, a clinical manifestation of scurvy hemorrhage from mucous membrane of the mouth and gastrointestinal tract, anemia, pains in the joints can be related to the association of ascorbic acid and normal connective tissue metabolism[24,25].

Plant phenolics constitute one of the major groups of compounds acting as primary antioxidants or free radical terminators. The phenols contain hydroxyls that are responsible

for the radical scavenging effect mainly due to redox potential[26]. The high phenolic content in *Saurauja roxburghii* can explain its high free radical scavenging activity. This study reveals that tested plant material have significant antioxidant activity and free radical scavenging activity. The result of the present study suggests that selected plants can be used as a source of antioxidants for pharmacological preparations which is very well evidenced by the present work.

TABLE-7
DPPH FREE RADICAL SCAVENGING ACTIVITY OF *Saurauja roxburghii (SR)* EXTRACT AND ASCORBIC ACID

Test material		Scavenging activity (%)		IC_{50} value (µg/mL)	
SR Extract	Ascorbic acid	*SR* Extract	Ascorbic acid	*SR* extract	Ascorbic acid
Concentration (µg/mL)					
10		54.22	73.59		
15		64.78	77.11		
20		77.11	93.83		
40		78.87	95.24		
60		80.63	95.95	3.34	0.05
80		82.39	96.48		
100		84.15	97.01		
200		96.30	97.18		
400		96.47	97.36		

Conclusion

In summary, phytochemical and biological studies reveal that *Saurauja roxburghii* methanol extract has significant antibacterial, antioxidative and moderate thrombolytic effect. We believe that different active secondary metabolites including those identified in the extract might play to operate in a synergistic manner. However, further studies are necessary to reveal the mechanisms behind these effects. This report may serve as a footstep on the aspect therapeutic research.

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REFERENCES

1. P. Brindha and A. Saraswathy, Phytochemical Comparison of Pentatropis, Oldenlandia and Plumeria, In: Proceeding National Seminar on Recent Trends in Natural Products Chemistry, Bharathidasan University, Tiruchirappalli, India (1981).
2. A.W. Bauer, E. Kirby, E.M. Sherris and M. Turk, *Am. J. Clin. Path.*, **45**, 493 (1966).
3. C. Thornsberry, A.L. Barry, R.N. Jones, C.N. Baker and R.E. Badal, *J. Clin. Microbiol.*, **15**, 769 (1982).
4. N.R. Jones, L.A. Barry, L.T. Gavan and J.A. Washington II, in eds.: E.H. Lennette, A. Bellows, W.J. Hausler Jr. and H.J. Shadomy, Susceptibility Tests: Microdilution and Macrodilution Broth Procedures. In Manual of Clinical Microbiology, Am. Soc. Microb. Washington DC, edn. 4 pp. 972-976 (1985).
5. W.B. Williams, M.E. Cuvelier and C. Berset, *Lebensm. Wiss. Technol.*, **28**, 25 (1995).
6. E.R.C.I.L. Dilek and M.K. Sakar, *Turk. J. Chem.*, **28**, 133 (2004).
7. M. Abbas, A. Disi and S. Al-Khalil, *Jord. J. Pharm. Sci.*, **2**, 131 (2009).
8. K. Ara, Ph.D Thesis, Isolation and Characterization of Antibacterial Compounds from Species Belonging to Burseraceae Family, Department of Pharmaceutical Chemistry, University of Dhaka, Dhaka, Bangladesh (2006).
9. S.A. Chowdhury, H.M. Sohrab, M.R. Haque, M.H. Choudhury and M.A. Ori, *Pharm. Exp. Med*., **8**, 97 (2008).
10. R.I. Khan, Natural Product: A Laboratory Guide, Academic Press, NY, USA, edn. 2 (1991).
11. M.D. Greca, M. Monaco and L. Previtra, *J. Nat. Prod.*, **53**, 1430 (1990).
12. S. Prasad, R.S. Kashyap, J.Y. Deopujari, H.J. Purohit, G.M. Taori and H.F. Daginawala, *Thromb. J.*, **4**, 14 (2006).
13. T.P.C. Tim and A.J. Lamb, *Int. J. Antimicrob. Ag.*, **26**, 343 (2005).
14. C. Mohanasundari, D. Natarajan, K. Srinivasan, S.A. Umamaheswari, and A. Ramachandran, *Afr. J. Botechnol.*, **6**, 2650 (2007).
15. J.S. Britto and J. Swamy, *Bot. Club.*, **18**, 81 (2001).
16. T. Krishnakumar, C.E. Ranjini and V.K. Sasidharan, *J. Life Sci.*, **11**, 14 (1997).
17. E. Natarajan, S. Senthilkumar, T.F. Xavier and V. Kalaiselvi, *J. Trop. Med. Plants*, **4**, 9 (2003).
18. D. Natarajan, J.S. Britto, K. Srinivasan, N. Nagamurugan, C. Mohanasundari and G. Perumal, *J. Ethnopharmacol.*, **102**, 123 (2005).
19. II. Koleva, T.A. van Beek, J.P. Linssen, A. de Groot and L.N. Evstatieva, *Phytochem. Anal.*, **13**, 8 (2002).
20. P.K. Suresh, S. Sucheta, V.D. Sudarshana, P. Selvamani and S. Latha, *Afr. J. Biotec.*, **7**, 1826 (2008).
21. S.R. Kanatt, R. Chander and A. Sharma, *Food Chem.*, **100**, 451 (2007).
22. R.E. Beyer, *J. Bioenerg. Biomem.*, **26**, 349 (1994).
23. F. Aqil, I. Ahmad and Z. Mehmood, *Turk. J. Biol.*, **30**, 177 (2006).
24. P. Veeru, M.P. Kishor and M. Meenakshi, *J. Med. Plants Res.*, **3**, 608 (2009).
25. D. Malencic, O. Gasic , M. Popovic and P. Boza, *Phytother. Res.*, **14**, 546 (2000).
26. C.A. Rice-Evans, N.J. Miller and G. Paganga, *Trends in Plant Sci.*, **4**, 152 (1997).