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Research Paper

Estimation of Antioxidant, Analgesic and Cytotoxic Activities of Methanolic Extract of *Saurauia roxburghii* Leaves

Mst. Mahfuza Khatoon*, Gita Karmoker, Shamima Afrose Shima, Md. Rajibul Islam, Mst. Rozina Parul, Md. Abdur Rouf, Anik Kumar Dey

Department of Pharmacy, Faculty of Health Sciences, Gono Bishwabidyalay (University), Savar, Dhaka, Bangladesh

Corresponding Author: * Mst. Mahfuza Khatoon

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Abstract

The present study aimed to investigate the potential antioxidant, cytotoxic, and analgesic activities of the methanolic leaves extract of *Saurauia roxburghii* in different *in vivo* and *in vitro* experimental models.*Saurauia roxburghii* leaves extract exhibited DPPH free radical scavenging activity with an IC_{50} value of 5.847 µg/ml. The total phenolic and flavonoid content were found to be 68.33 ± 3.8188 mg GAE/gm of dried sample and 59.375583 ± 1.80465 mg QE/gm of dried sample, respectively. The total antioxidant capacity was found to be 102.2167 ± 2.41mg AAE/gm of dried sample. Analgesic activity was determined using acetic acid-induced writhing inhibition and hot plate method in a mouse model. The leaves extract, at doses of 100 mg/kg and 200 mg/kg body weight, showed inhibition of the writhing reflex by 28% ($p < 0.05$) and 60% ($p < 0.05$), respectively. In the hot plate method, the extract showed 79.08% ($p < 0.05$) and 55.61% ($p < 0.05$) pain inhibition at doses of 200 mg/kg and 100 mg/kg, respectively (at 90 minutes). The cytotoxicity study of leaves extract was found to be moderately lethal to brine shrimp nauplii with an LC_{50} value of 1.6 µg/ml.Preliminary phytochemical investigation of leaves extract exhibited the presence of steroids, alkaloids, terpenoids, tannins, saponins, flavonoids, glycosides, phytosterols, and phenolic compounds which could be correlated with its observed biological activities. In conclusion, we explored that the methanolic leaves extract of *Saurauia roxburghii* could be a promising source of antioxidant, analgesic, and cytotoxic agent.

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KEYWORDS: *Saurauia roxburghii*, Phytochemical screening, Antioxidant, Analgesic, Cytotoxicity

1. INTRODUCTION

Since ancient times plants have served as the foundation for complex traditional medical systems that continue to offer novel treatment to humanity. [1] Approximately 80% of modern medications are derived, either directly or indirectly, from plants, according to some liberal estimates. [2] Oxidative stress is defined by an imbalance between increased levels of reactive oxygen species (ROS) and a low activity of antioxidant mechanisms [3] which has been marked as harmful because oxygen free radicals attack biological molecules such as lipids, proteins, and DNA. Increased oxidative stress has been incriminated in physiological conditions, such as aging and exercise, and in several pathological conditions, including cancer, neurodegenerative diseases, cardiovascular diseases, diabetes, inflammatory diseases, and intoxications. [4] Antioxidants are molecules that neutralize free radicals, unstable molecules that can harm cells. [5] Antioxidants reduce the painful side effects of radiation and

chemotherapy, thus supporting the beneficial effects of antioxidants in protecting normal cells during treatment and acting as adjuvants in the treatment of certain cancers. [6]
Analgesic agents retard or eliminate algesia or pain by acting on the sensory nervous system without significantly altering consciousness. [7] Synthetic analgesic drugs have proven side and toxic effects. On the contrary, many medicines of plant origin had been used and are in use successfully for a long time without any serious effects. [8]
Saurauia roxburghii is an evergreen tree species belonging to the family Actinidiaceae. This plant species is widely distributed in the coastal forest and hill tracts of Bangladesh. the stems and leaves are extensively used as herbal medicines against numerous severe diseases like asthma, bronchitis, hepatitis B, ulcers, and central nerves depression; and also, in large numbers for the treatment of boils, eczema, epilepsy, fever, gout, and piles. [9] The present study was undertaken to investigate and justify the antioxidant, analgesic and cytotoxic activities of the methanolic leaves extract of *Saurauia roxburghii* plant using in-vitro and in-vivo models.

2. MATERIALS AND METHODS

Plant Material and Extraction: The leaves of *Saurauia roxburghii* was collected from the Sylhet district of Bangladesh and identified by the authority and experts of the National Herbarium, near Botanical Garden, Mirpur in Dhaka, Bangladesh. The fresh leaves were dried and reduced into coarse powder with the help of a mechanical grinder. About 650 gm of powder was extracted by maceration over 7 days with 2 L methanol solvent. The whole suspension was then filtered by a piece of clean, white cotton cloth and then filtered by filter paper. The obtained filtrates were evaporated by Rotary evaporator (Bibby RE-200, Sterilin Ltd., UK), connected with water bath and temperature was maintained below 40°C. The crude extract was collected after complete evaporation (Yield value 2.43%) and used for experimental purposes.

Phytochemical screening: Standard qualitative procedures were followed to identify the phytochemicals like saponins, tannins, glycosides, flavonoids, steroids, carbohydrate, terpenoids, sterol, phytosterol, amino acid, phenolic compound and alkaloids of Saurauia roxburghii leaves extract using standard protocols. Then, the extract was used for pharmacological screening.
In-Vitro Antioxidant Activity.

DPPH Radical Scavenging Activity: Free radical scavenging activity of various compounds can be determined by measuring the change in absorbance of DPPH (1,1-Diphenyl-2-picrylhydrazyl radical) at 517 nm by the spectrophotometric method described by Braca A *et al.*, (2001). [10] Ascorbic acid was used as reference. 1 ml of plant extract or standard of different concentrations (400µg/ml to 6.25µg/ml) were taken in different test tubes. 2ml of 0.004% DPPH solution was added to each test tube to make the final volume 3 ml. The mixture was incubated in room temperature for 30 minutes in a dark place and then the absorbance was measured at 517 nm. The DPPH scavenging ability was calculated by the following equation:
DPPH radical scavenging activity (%) = (1 − Absorbance of sample /Absorbance of control) × 100
Where, Absorbance of control is the absorbance of DPPH radical + methanol; Absorbance of sample is the absorbance of DPPH radical + sample extract/standard. Total Antioxidant Capacity Assessment: The total antioxidant activity of the extract can be evaluated by the phosphomolybdenum method according to the procedure of Prieto P et al., (1999). [11] The assay is based on the reduction of Mo(VI) - Mo(V) by the extract and subsequent formation of green PO/Mo (V) complex at acidic pH. Ascorbic acid was used as the reference. 1 ml of plant extract or standard of different concentrations (400µg/ml to 3.125µg/ml) were taken in different test tubes and mixed with 3 ml reagent solution and incubated at 95°C for 90 minutes to complete the reaction. The absorbances of the solutions were measured at 695 nm using a spectrophotometer against blank after cooling to room temperature.

Reducing Power Capacity Assessment: The reducing power was determined according to the method previously described by Oyaizu, 1986. [12] In this assay, the yellow color of the solution changes to various shades of green and blue depending on the reducing power of antioxidant samples. Ascorbic acid was used as the reference. Plant extract or standard of different concentration solutions (400µg/ml to 3.125µg/ml) were taken in different test tubes. 2.5ml of phosphate buffer (0.2M) & 2.5ml potassium ferricyanide (1%) were added into the test tubes and the mixture was incubated for 20 min at 500 C to complete the reaction. Then 2.5ml of trichloro acetic acid (10%) solution was added into the test tubes. The total mixture is centrifuged at 3000rpm for 10 min and 2.5ml of supernatant solution was mixed with 2.5ml of distilled water. 0.5ml of ferric chloride (0.1%) solution was added to the diluted reaction mixture. Then the absorbance of the solution is measured at 700 nm using a spectrophotometer against blank.

Determination of Polyphenolic Content
Determination of Total Flavonoids: The content of total flavonoids of *saurauia Roxburghii* was determined by aluminum chloride colorimetric method. [13] Quercetin was used as standard. 1 ml of plant extract (400µg/ml) or standard of different concentrations (400µg/ml to 0.78µg/ml) solution were taken in different test tubes and mixed with 200µl of 10% aluminnium chloride solution, 200µl of 1M potassium acetate solution and 5.6 ml of distilled water was mixed with the reaction mixture and incubated for 30 minutes at room temperature to complete the reaction. Then the absorbance of the solution was measured at 510 nm using a spectra photometer against blank. The Total content of flavonoid compounds in plant extract was expressed as mg quercetin equivalent (QE) per gram of dried extract.

Determination of Total Phenolic content: The content of total phenolic content of *saurauia Roxburghii* leaves extract was

determined according to (Wolfe et al., 2003) [14] in which Folin-ciocalteu reagent (FCR) was used as an oxidizing agent and Gallic Acid (GA) was used as standard. 1 ml of plant extract (400µg/ml) or standard of different concentrations (400µg/ml to 0.78µg/ml) solution was taken in different test tubes. 5 ml of Folin-Ciocalteu (Diluted 10-fold) reagent solution was added to each test tube. 7.5% Sodium carbonate solution (4 ml) was added and the mixtures were incubated for 30 minutes at room temperature and the test tubes containing extract solution were incubated for 1 hour at room temperature to complete the reaction. Then the absorbance of the solution was measured at 765 nm using a spectrophotometer against blank. The Total content of phenolic compounds in plant extract was expressed as mg Gallic acid equivalent (GAE) per gram of dried extract.

Determination of Cytotoxic Activity

Brine Shrimp Lethality Bioassay: Brine-shrimp lethality bioassay was followed to assess cytotoxic activity which is described by McLaughlin et al. [15, 16] Solution of different concentrations was prepared with the extract by using dimethyl sulfoxide (DMSO) as solvent. 10 shrimps were taken in each test tube and solution of different concentration applied on it and the volume of liquid was adjusted by saline water. For negative control, a test tube with saline water was kept for observation with 10 shrimps under the same condition with the test sample. Vincristine sulfate was used as positive control at the concentrations of 10, 5, 2.5, 1.25, 0.625, 0.325 and 0.157 µg/ml. After an incubation period of 24h at room temperature, the number of viable naupliis was counted using a magnifying glass. The percent of mortality of the brine shrimp nauplii was calculated for every concentration to determine LC_{50} (lethal concentration).

Determination of Analgesic Activity

Acetic acid-induced Writhing Test: The experimental animals were randomly divided into four groups consisting of 4 mice in each group. The groups were denoted from Group I to group- IV. The test samples (100 and 200 mg/kg dose), control(1% tween 80 in water) and Standard (Diclofenac sodium 10 mg/kg) were administered orally with the help of a feeding needle at the beginning of the experiment. After 30 minutes, 0.7% acetic acid was injected intraperitonally into each of the animals of all the groups to create a pain sensation. Approximately 5 minutes after the injection of acetic acid, the number of writhes (abnormal contraction or stretches) was counted for the next 10 minutes and recorded. The recorded number of acetic acid-induced writhes that occurred in the standard and test group compared with those in the control group. [17]

Hot Plate Test: The hot plate test was used to measure the response latencies according to Wolfe and MacDonald, 1944 method. [18] The temperature of the hot plate (model 7280; Ugo Basile Italy) was maintained at 55±2°C. Animals were placed in a Perspex cylinder on a heated surface and the time between placement of the animal on the hot plate the occurrence of discomfort, indicated by either licking of the paws or jumping off the surface, was recorded as response latency. Mice with baseline latencies of more than 10s were eliminated from study; the cut-off time for hot plate latency was set at 15s. The latency of discomfort was measured at 0, 30, 60 and 120 min after test solution administration.

Statistical Analysis:. Statistical analysis between group comparison was done by using one way ANOVA with post hoc Dunnett test. Values with **$p<0.05$ were considered significant. All analyses were carried out in triplicates. Results were expressed as mean value ± SD (n = 3).

3. RESULTS AND DISCUSSION

Phytochemical screening: Preliminary phytochemical investigation exhibited the presence of steroids, alkaloids, terpenoids, tannins, saponins, flavonoids, phytosterols and phenolic compound and the absence of amino acid in the plant extract.

Table 1: Result of phytochemical screening of *S. roxburghii*.

Phytochemical groups	Observed result
Steroid	+
Amino acid	-
Flavonoids	+
Carbohydrate	+
Glycoside	+
Phenolic compound	+
Terpenoids	+
Tanins	+
Saponin	+
Alkaloid	+
Sterol	+
Phytosterol	+

'+' presence; '-' absence

DPPH Radical Scavenging Activity:

Table 2: Determination of %RSA and IC_{50} value of Ascorbic acid and *S. roxburghii* leaves extract different concentrations.

Concentration (µg/ml)	%RSA (AA)	IC_{50} (µg/ml) AA	%RSA (SRM)	IC_{50} (µg/ml) SRM
6.25	8.356545961	3.655	0.765483646	5.96
12.5	14.7632312		1.670146138	
25	41.50417827		5.845511482	
50	70.75208914		14.12665275	
100	77.71587744		36.11691023	
200	84.67966574		55.25400139	
400	88.02228412		72.86012526	

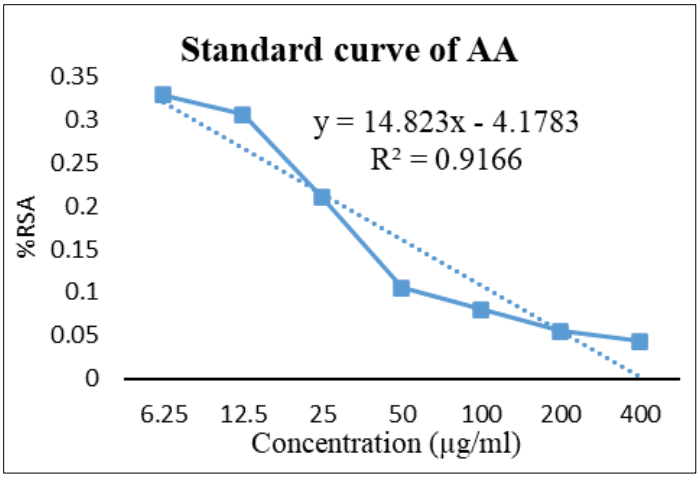


Fig. 1: Standard curve of ascorbic acid

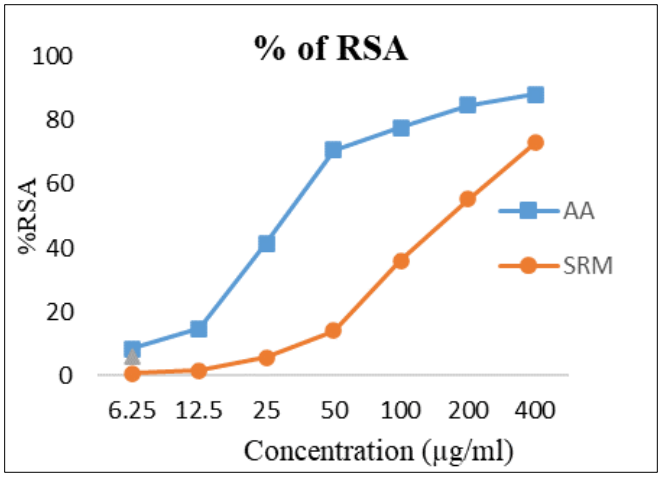


Fig. 2: DPPH scavenging activity of *S. roxburghii* and standard (Ascorbic acid)

The scavenging activity was increased with the increasing concentration of both the standard and sample. DPPH free radical scavenging assay on methanolic extract of *S. roxburghii* leaves showed an IC_{50} (inhibitory conc. 50%) value of 5.96 µg/ml. Whereas, standard ascorbic acid showed an IC_{50} value of 3.655 µg/ml (Table 1).

Total Antioxidant Capacity:

Table 3: Absorbance of Ascorbic acid (AA) at different concentrations

Concentration (µg/ml)	**Absorbance**
3.125	0.041
6.25	0.043
12.5	0.047
25	0.06
50	0.08
100	0.139
200	0.217
400	0.28

Fig. 3: Standard curve of ascorbic acid (AA).

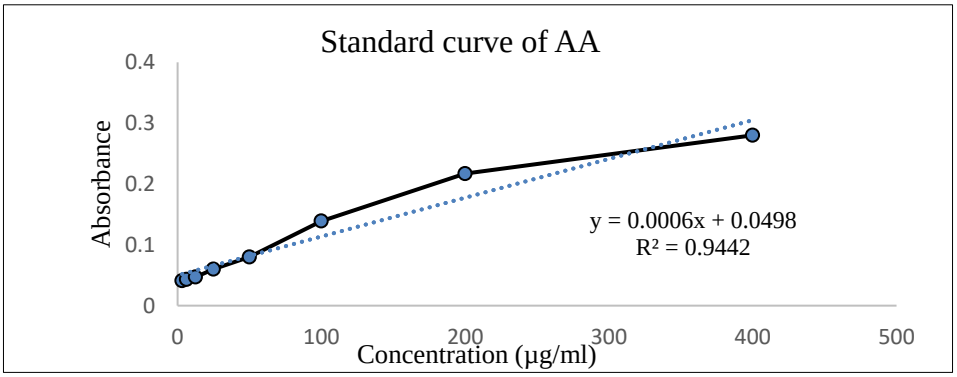


Table 4: Data for the determination of total Antioxidant capacity (TAC) in *Saurauia roxburghii* leaf extract.

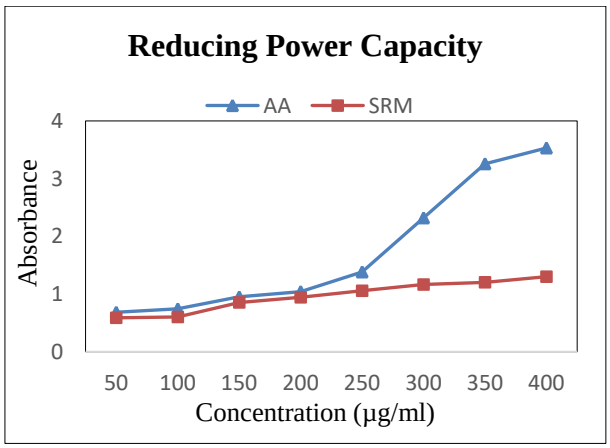
Sample solution (µg/ml)	Wt. of dry extract per g/ml	Absorbance	Ascorbic acid Conc.(C) (µg/ml)	Ascorbic acid Conc.(C) (mg/ml)	TAC as AAE, A=(c*v)/m (mg/g)	mg AAE/g of dried sample (Mean value ± SD)
400	0.0004	0.075	42	0.042	105	102.2167 ± 2.41
400	0.0004	0.074	40.33	0.04033	100.825	
400	0.0004	0.074	40.33	0.04033	100.825	

Values are mean of triplicate experiments and are represented as 'Mean'. The total antioxidant capacity of *Saurauia roxburghii* (Table 4) was 102.2167±2.41mg of AAE/gm of dried extract at a concentration of 400 µg/mL.

Reducing Power Assessment

Table 4: Determination of Fe^{3+} reducing power capacity of Ascorbic Acid and *S. roxburghii* at different concentrations.

Concentration (µg/ml)	Absorbance (AA)	Absorbance (SRM)
3.125	0.684	0.588
6.25	0.743	0.602
12.5	0.951	0.853
25	1.041	0.943
50	1.382	1.056
100	2.32	1.165
200	3.257	1.202
400	3.531	1.301



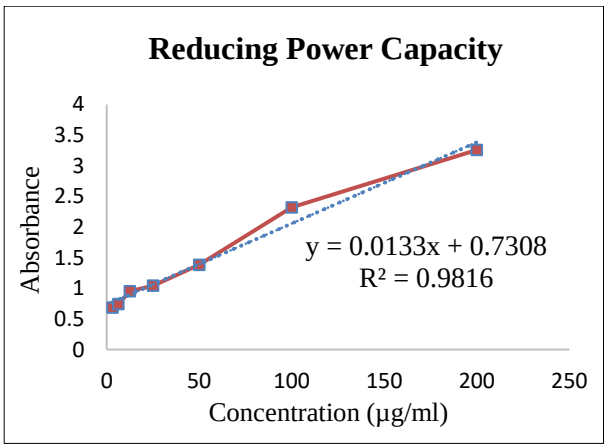


Fig. 5: Stantard curve of ascorbic acid.

Fig. 6: Comparison of Reducing Power Capacity of *S. roxburghii* with Ascorbic acid.

The results suggested that *S. roxburghii* leaves extract had quite good but not significant reducing capacity to that of the standard, ascorbic acid. The reducing capacity of both extracts is concentration-dependent.

Total flavonoid content:

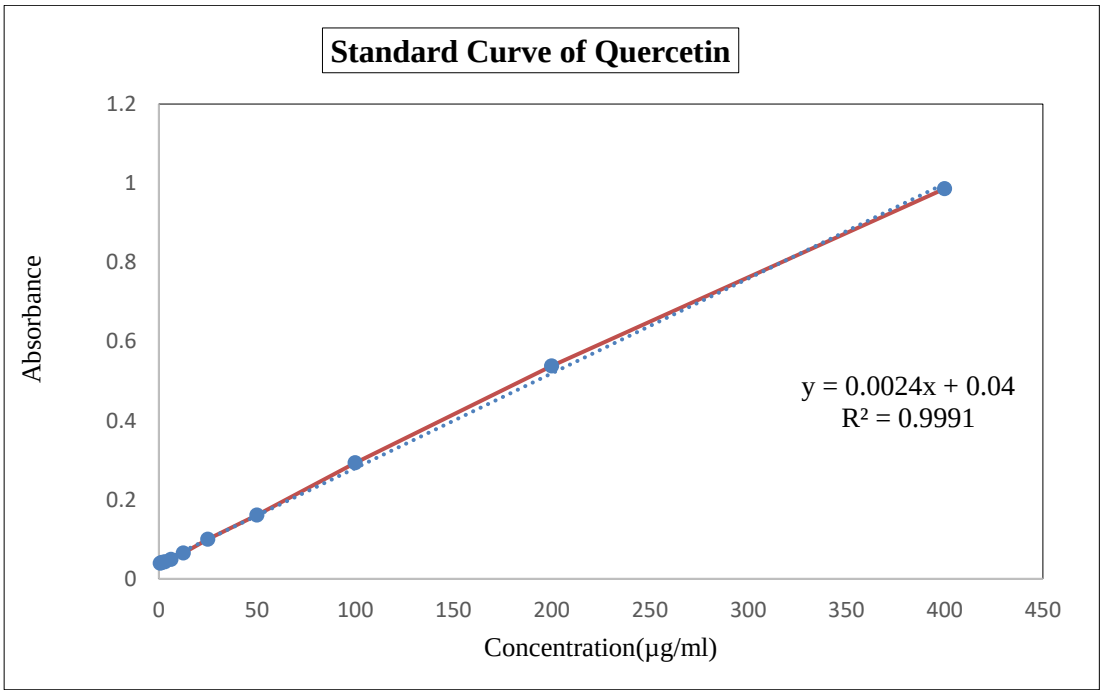


Fig. 7: Standard curve of Quercetin

Table 5: Absorbance of Quercetin at different concentrations.

Concentration (µg/ml)	Absorbance
0.78	0.039
1.56	0.041
3.125	0.043
6.25	0.049
12.5	0.065
25	0.1
50	0.161
100	0.293
200	0.538
400	0.986

Table 6: Data for the determination of total Flavonoid content (TFC) in *Saurauia roxburghii* leaf extract.

Sample solution (µg/ml)	Wt. of dry extract per g/ml	Absorbance	Quercetin Conc.(C) (µg/ml)	Quercetin Conc.(C) (mg/ml)	TFC as QE, A=(c*v)/m (mg/g)	mg QE/g of dried sample (Mean value ± SD)
400	0.0004	0.095	22.9167	0.0229167	57.29175	59.375583 ± 1.80465
400	0.0004	0.098	24.167	0.024167	60.4175	
400	0.0004	0.098	24.167	0.024167	60.4175	

Values are the mean of triplicate experiments and are represented as 'Mean'. The flavonoid content of *Saurauia roxburghii* (Table 6) was 59.376 ± 1.80mg of QE/gm of dried extract at a concentration of 400 µg/mL.

Total phenolic content:

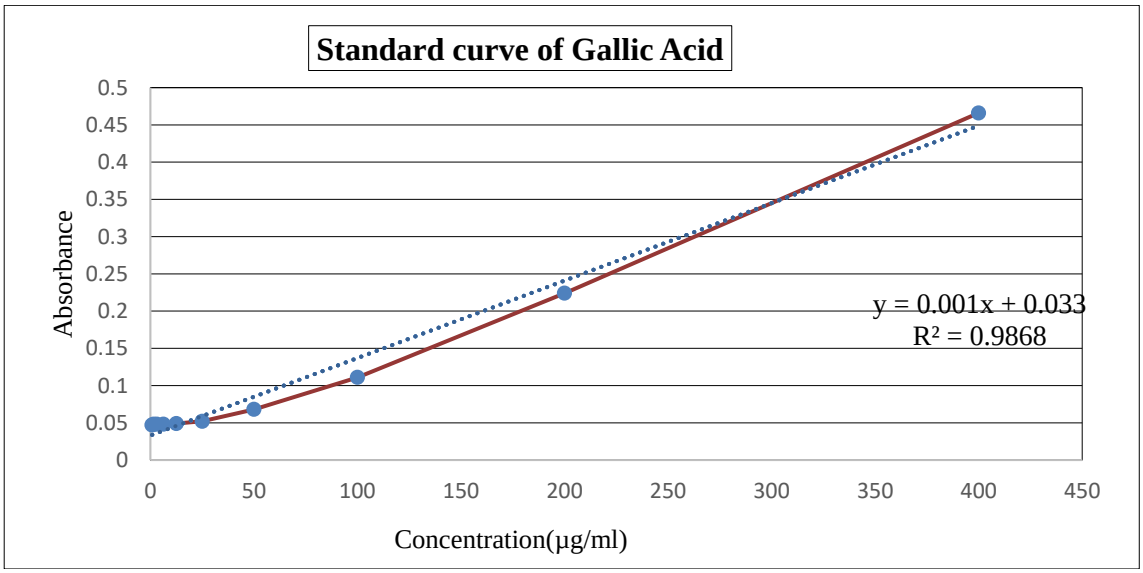


Fig. 8: Standard curve of Gallic acid.

Table 7: The absorbance of Gallic Acid at different concentrations.

Concentration (µg/ml)	Absorbance
0.78	0.047
1.56	0.048
3.125	0.048
6.25	0.048
12.5	0.049
25	0.052
50	0.068
100	0.111
200	0.224
400	0.466

Table 8: Data for the determination of total phenolic content (TPC) in *S. roxburghii* extract.

Sample solution (µg/ml)	Wt. of dry extract per (g/ml)	Absorbance	GAE Conc.(C) (µ/ml)	GAE Conc.(C) (mg/ml)	TPC as GAE, A=(c*v)/m (mg/g)	mg GAE/g of sample (Mean value ± SD)
400	0.0004	0.062	29	0.029	72.5	68.33 ± 3.8188
400	0.0004	0.059	26	0.026	65	
400	0.0004	0.06	27	0.027	67.5	

Values are mean of triplicate experiments and are represented as 'Mean'. The phenolic content of *Saurauia roxburghii* (Table 8) was 68.33 ± 3.82mg of GAE/gm of dried extract at a concentration of 400 µg/mL.

Cytotoxic Activity by Brine Shrimp Lethality Bioassay:

Table 9: Effect of Vincristine Sulfate (standard) on mortality of Brine Shrimp nauplii at different concentrations.

Concentration (µg/ml)	Log conc.	No. of nauplii taken (N_0)	No. of nauplii alive (N_1)	No. of nauplii dead	% of Mortality = $(N_0-N_1)/N_0 \times 100$	LC_{50} (µg/ml)
0.157	0.804100348	10	8	2	20	0.259
0.313	0.504455662	10	8	2	20	
0.625	0.204119983	10	7	3	30	
1.25	0.096910013	10	6	4	40	
2.5	0.397940009	10	4	6	60	
5	0.698970004	10	3	7	70	
10	1	10	1	9	90	

Table 10: Effect of *Saurauia roxburghii* leaves extract on mortality of Brine Shrimp nauplii at different concentrations**.**

Concentration (µg/ml)	Log conc.	No. of nauplii taken (No)	No. of nauplii alive (N_1)	No. of nauplii dead	% of Mortality = (No-N1)/No × 100	LC50 (µg/ml)
6.25	0.795880017	10	8	2	20	1.6
12.5	1.096910013	10	6	4	40	
25	1.397940009	10	5	5	50	
50	1.698970004	10	5	5	50	
100	2	10	4	6	60	
200	2.301029996	10	3	7	70	
400	2.602059991	10	2	8	80	
800	2.903089987	10	1	9	90	

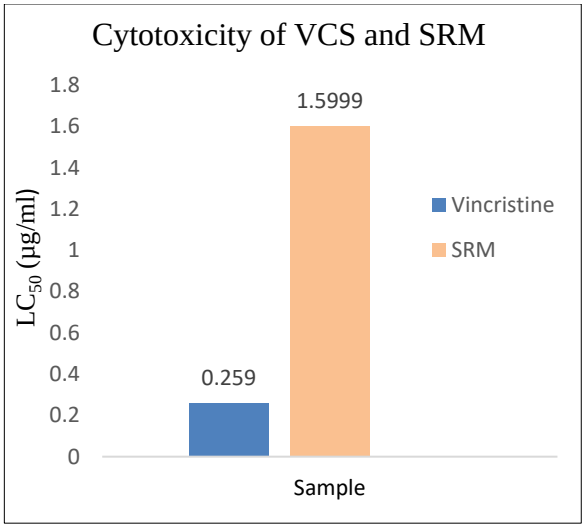


Fig. 9. Calibration curve of vincristine.

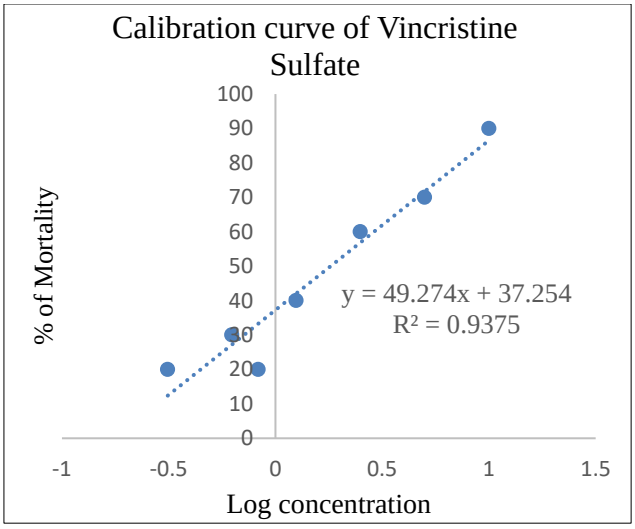


Fig. 10. LC_{50}(µg/mL) values of SRM and standard, VCS.

LC_{50} was found to be 1.5999 µg/mL for leaf extract whereas, positive control vincristine sulfate showed 0.259 µg/mL.

Analgesic test

Acetic acid-induced Writhing Method: Leaves extract at both dose levels produced significant ($p<0.05$) and dose-dependent analgesic effect (Table 11). Maximum inhibition of writhing responses exhibited by the extract (60%) at the dose of 200mg/kg body weight which was comparable with that of the standard drug diclofenac sodium (80%).

Table 11: Effect of methanolic extract of *S. roxburghii* on acetic acid-induced writhing in mice.

Group	Treatment	No. of Writhing				Mean of Writhing ± SEM	% inhibition
		a	b	c	d		
1	1% Tween-80 (Control)	10	17	11	12	12.5± 1.55456	
2	Diclofenac Sodium (STD)	3	4	2	1	2.5± 0.64550**	80%
3	SRM (100mg/kg)	10	6	9	11	9±1.08012	28%
4	SRM (200mg/kg)	3	4	3	10	5±1.68325**	60%

Values are expressed as the **mean ± SEM** (n=4) for all tested doses. Values with **$p<0.05$ were considered significant.

Hot Plate Method: The result demonstrated that the analgesic activity of standard (Diclofenac sodium) and crude extract was dose-dependent ($p<0.05$) and the activity increased for a certain period (30-90 Minutes) and then started to decline after 120 minutes. The leaf extract showed the highest inhibition at 90 minutes (79.08%), at a dose of 200 mg/kg body weight, which is very close to the standard drug (89.80%) used in the experiment.

Table 12: Analgesic activity of SRM and PWM by Hot plate method.

Group	Treatment	Time to response				
		0 Minutes	30 Minutes	60 Minutes	90 Minutes	120 Minutes
1	Saline water (Control)	5.35 ± 0.25981	4.875 ± 0.41708	4.85 ± 0.34034	4.9 ± 0.26141	4.85 ± 0.28723
2	Diclofenac Sodium (STD)	6.075 ± 0.29262	8.6 ± 0.29721**	9.325 ± 0.23936**	9.3 ± 0.27988**	9.075 ± 0.19311**
3	SRM (100mg/kg)	4.85 ± 0.15546	6.275 ± 0.35208	7.425 ± 0.43277**	7.625 ± 0.39449**	7.175 ± 0.22127**
4	SRM (200mg/kg)	5.1 ± 0.52122	7.275 ± 0.18875**	8.65 ± 0.32787**	8.775 ± 0.40697**	8.175 ± 0.22127**

Values are expressed as the **mean ± SEM** (n=4) for all tested doses. Values with **p<0.05 were considered significant.

Table 13: Percentage increase in reaction time or pain threshold inhibition by STD and SRM.

Group	Treatment	% of elongation (Percentage increase in reaction time or pain threshold inhibition)			
		30 Minutes	60 Minutes	90 Minutes	120 Minutes
1	Diclofenac Sodium (STD)	76.41%	92.28%	89.80%	87.11%
2	SRM (100mg/kg)	28.72%	53.09%	55.61%	47.94%
3	SRM (200mg/kg)	49.23%	78.35%	79.08%	68.56%

4. CONCLUSION

The in vitro study revealed that the methanolic leaves extract of *Saurauia roxburghii* is a potential source of polyphenols, which has shown significant antioxidant, and cytotoxic activities. Also showed its ability to suppress abdominal writhes and increase the pain threshold inhibition confirming the analgesic property of the extract. These studies justify the traditional use of this plant in various diseases associated with free radicals. However, further studies are needed to investigate and isolate the bioactive compounds of the plant and work on cell lines (diabetic cells, cancer cells) for a better understanding of their pharmacological properties.

5. Acknowledgment

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6. CONFLICTS OF INTEREST

All the authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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