

PHYTOCHEMICAL SCREENING, TOXICITY EVALUATION, AND ANTIOXIDANT ACTIVITY OF STROBILANTHES REPTANS (RED FLAME) LEAF EXTRACT

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ABSTRACT

Strobilanthes reptans (Red Flame), a member of the family *Acanthaceae*, is traditionally utilized in local medicinal practices but remains scientifically underexplored. This study aimed to evaluate the phytochemical composition, antioxidant potential, and toxicity profile of the ethanolic leaf extract of *S. reptans*. Qualitative phytochemical screening revealed the presence of flavonoids, phenolics, tannins, alkaloids, saponins, and steroids. Antioxidant activity was assessed using the DPPH radical scavenging assay, yielding an IC₅₀ value of 101.3 ppm, indicating strong free radical scavenging capacity. Toxicity evaluation via Brine Shrimp Lethality Assay (*Artemia salina*) produced an LC₅₀ value of 223.9 ppm, classifying the extract as moderately toxic. The findings demonstrate that *S. reptans* contains bioactive secondary metabolites with significant antioxidant activity and measurable cytotoxic potential. These results support its potential for further pharmacological investigation and development as a natural therapeutic resource.

Keywords: *Strobilanthes reptans*, phytochemical screening, antioxidant activity, DPPH assay, brine shrimp lethality assay, medicinal plants.

I. INTRODUCTION

Plants have long served as primary sources of therapeutic agents due to their diverse bioactive secondary metabolites. Numerous plant species demonstrate antioxidant, anti-inflammatory, antimicrobial, and cytoprotective properties that contribute to disease prevention and management. The Philippines, recognized for its rich biodiversity, harbors many plant species with potential medicinal value that remain insufficiently investigated.

Strobilanthes reptans, an ornamental plant belonging to the family *Acanthaceae*, is widely cultivated in tropical regions of Southeast Asia. Although related species have shown promising pharmacological properties, limited scientific data exist regarding the biological activities of *S. reptans*. Thus, this study aims to provide baseline experimental data on its phytochemical composition, antioxidant activity, and cytotoxic potential.

II. MATERIALS AND METHODS

Fresh leaves of *S. reptans* were collected at Kitcharao, Agusan del Norte, Philippines. The leaves were then washed, air-dried for 7-days, grind into semi-powder and packed in a sealed bag. Extraction was performed using ethanol through maceration and rotary evaporation. Qualitative phytochemical screening was conducted following standard laboratory procedures at the Chemistry Department of MSU-IIT-Iligan.

Extraction of Plant Material

Simple Alcohol Extraction - Approximately 200 g of the ground plant material were weighed and transferred into a 1000 mL Erlenmeyer flask. Ethanol (or methanol) was added until the solvent level was approximately 1 inch above the surface of the plant material. The mixture was allowed to soak for 48 hours to ensure the complete extraction of the phytochemical components present in the plant material. After the extraction period, the mixture was filtered, preferably using suction filtration, to separate the alcoholic extract from the solid residue and maximize recovery of the extract. The filtrate was then subjected to rotary evaporation at a temperature below 50°C to remove the alcohol solvent and obtain the crude extract. Evaporation was continued until approximately 90% of the solvent had been removed. The resulting crude extract was collected and used for phytochemical screening, while the recovered alcohol solvent was reserved for possible further purification.

Serial Extraction with Solvents of Increasing Polarity - In cases where serial extraction was performed, the plant material was successively extracted using solvents of increasing polarity to ensure the isolation of compounds with varying chemical properties. The solvents were applied in the following order: hexane, dichloromethane, acetone, and ethanol or methanol, allowing for the progressive extraction of nonpolar to more polar phytochemical constituents.

Phytochemical Screening

1. Saponins (Froth Test)

Two milliliters (2 mL) of the plant extract were placed in a test tube, and 2 mL of distilled water were added. The mixture was shaken vigorously for approximately 30 seconds. The formation of a stable froth measuring about 2 cm in height that persisted for at least 30 minutes indicated the presence of saponins.

2. Cyanogenic Glycosides

One milliliter (1 mL) of the plant extract was placed in a 20 mL test tube, and 4–5 drops of chloroform were added. A picrate paper strip was suspended above the solution without touching the liquid. The test tube was placed in a hot water bath for about 5 minutes. The development of a red coloration on the picrate paper indicated the presence of cyanogenic glycosides.

3. Alkaloids

Two milliliters (2 mL) of the plant extract were acidified with 2 mL of 2 M hydrochloric acid (HCl) and filtered to obtain a clear solution. Three to five (3–5) drops of Wagner's reagent were added to one portion of the filtrate, while 3–5 drops of Mayer's reagent were added to another portion. The formation of a brown precipitate with Wagner's reagent or a white precipitate with Mayer's reagent indicated the presence of alkaloids.

4. Flavonoids

Two milliliters (2 mL) of the plant extract were dissolved in 5 mL of 80% ethanol. One milliliter (1 mL) of concentrated hydrochloric acid (HCl) was added, and the mixture was heated in a water bath for 5 minutes. The development of a red coloration indicated the presence of flavonoids.

5. Tannins

Two milliliters (2 mL) of the plant extract were mixed with 2–3 drops of 1% ferric chloride (FeCl_3) solution. The appearance of a blue-black or greenish-black coloration indicated the presence of tannins.

6. Phenolic Compounds

Two milliliters (2 mL) of plant extract were mixed with 2 mL of distilled water, followed by the addition of 3–5 drops of 10% ferric chloride (FeCl_3) solution. The formation of a bluish-black coloration confirmed the presence of phenolic compounds.

7. Steroids (Liebermann–Burchard Test)

One milliliter (1 mL) of the plant extract was dissolved in 2 mL of chloroform. One milliliter (1 mL) of acetic anhydride was added, followed by the careful addition of 1–2 drops of concentrated sulfuric acid (H_2SO_4) along the side of the test tube. The development of a blue-green or green coloration indicated the presence of steroids.

8. Anthraquinones (Borntrager's Test)

Two milliliters (2 mL) of plant extract were mixed with 5 mL of benzene and shaken thoroughly. The benzene layer was separated and treated with 1 mL of 10% ammonia solution. The formation of a reddish-pink coloration in the ammoniacal layer indicated the presence of anthraquinones.

9. Terpenoids (Salkowski Test)

Two milliliters (2 mL) of plant extract were mixed with 2 mL of chloroform. Two milliliters (2 mL) of concentrated sulfuric acid (H_2SO_4) were carefully added along the side of the test tube to form a separate layer. The appearance of a reddish-brown coloration at the interface indicated the presence of terpenoids.

Antioxidant Test

Determination of Antioxidant Activity Using DPPH Radical Scavenging Assay

(IC₅₀)

The antioxidant activity of the plant extract was determined based on its ability to scavenge the stable free radical DPPH (2,2-diphenyl-1-picrylhydrazyl). When the purple DPPH solution reacts with antioxidant compounds present in the extract, it is reduced to a yellow-colored compound known as diphenylpicrylhydrazine. This reaction results in a decrease in absorbance, which can be measured spectrophotometrically. The antioxidant capacity of the extract was expressed as the IC₅₀ value, defined as the concentration required to inhibit 50% of the DPPH free radicals.

A 0.1 mM DPPH solution was prepared in methanol and stored in a dark container to prevent degradation. Various concentrations of the methanolic extract of *Strobilanthes reptans* were prepared at 10, 50, 100, 250, and 500 µg/mL. One milliliter (1 mL) of the DPPH solution was pipetted into each test tube, followed by the addition of 1 mL of the prepared extract solution at different concentrations. The mixtures were gently mixed to ensure proper reaction. A solution containing 1 mL of DPPH in methanol without extract served as the control. All samples were then incubated in the dark at room temperature for 30 minutes to allow the reaction to occur.

After incubation, the absorbance of each sample was measured at 517 nm using a UV–Visible spectrophotometer. The percentage of radical scavenging activity was calculated using the standard inhibition formula. A graph of percentage inhibition versus extract concentration was plotted to determine the IC₅₀ value of the plant extract. The obtained IC₅₀ value was then compared with that of standard antioxidants such as Ascorbic acid or Butylated hydroxytoluene.

Interpretation:

Lower IC₅₀ value → Higher antioxidant activity.

Higher IC₅₀ value → Lower antioxidant activity.

A lower IC₅₀ value indicates higher antioxidant activity, while a higher IC₅₀ value indicates lower antioxidant activity.

Toxicity Test

Determination of Toxicity Using the Brine Shrimp Lethality Assay (BSLA)

The toxicity of the plant extract was evaluated using the Brine Shrimp Lethality Assay, a simple bioassay that measures the lethality of test substances against *nauplii* of *Artemia salina*. This assay estimates the toxicity of plant extracts based on the mortality rate of the organisms after exposure to different concentrations of the extract. The toxicity level is expressed as the LC₅₀ value, which represents the concentration of the extract required to cause 50% mortality of the test organisms.

Artificial seawater was prepared by dissolving 35–38 g of sea salt in 1 L of distilled water. Brine shrimp cysts were hatched in the artificial seawater under continuous illumination at a temperature of 25–30 °C with gentle aeration. After 24–48 hours, newly hatched *nauplii* were collected, and only active and freely swimming *nauplii* were selected for the experiment.

The plant extract was dissolved in a suitable solvent such as dimethyl sulfoxide (DMSO), ethanol, or methanol, ensuring that the final solvent concentration did not exceed 1% in the test solution. Serial dilutions of the extract were prepared in artificial seawater to obtain concentrations ranging from 10 µg/mL to 1000 µg/mL.

For the toxicity test, ten (10) *nauplii* were transferred into each test vial containing 1–5 mL of artificial seawater. The prepared extract solutions at different concentrations were then added to the vials. Each concentration was tested in triplicate to ensure reliability of the results. A control group containing seawater with the solvent (≤1%) but without the extract was also prepared.

The test samples were incubated for 24 hours at 25–30 °C under light conditions without feeding or aeration. After the incubation period, the *nauplii* were examined, and the number of live and dead organisms was recorded. *Nauplii* were considered dead when they showed no movement even after gentle agitation of the container. The percentage mortality was calculated for each concentration and corrected using the control mortality when necessary.

$$\text{PercentMortality} = \frac{\text{Total Number of Nauplii}}{\text{Number of Dead Nauplii}} \times 100$$

The LC₅₀ value was determined by plotting the percentage mortality against the logarithm of the extract concentration and analyzing the data using probit analysis. The LC₅₀

value was reported together with the 95% confidence interval to indicate the toxicity level of the plant extract.

Interpretation of Toxicity

The toxicity of the extract was interpreted based on the LC_{50} value as follows:

$LC_{50} \leq 10 \mu\text{g/mL}$ – Extremely toxic

$LC_{50} = 10\text{--}100 \mu\text{g/mL}$ – Highly toxic

$LC_{50} = 100\text{--}250 \mu\text{g/mL}$ – Moderately toxic

$LC_{50} = 250\text{--}1000 \mu\text{g/mL}$ – Low toxicity

$LC_{50} > 1000 \mu\text{g/mL}$ – Non-toxic

The method followed the procedure described by Mai et al. (2024) in the study titled “Optimization of Brine Shrimp Lethality Test for In Vivo Toxicity Evaluation of Poisonous Plant Species Collected from Quang Tri Province.”

III. RESULTS AND DISCUSSIONS

Phytochemical Screening

NO.	Secondary Metabolites	Presence in Leaf Extract
1	Flavonoids	(+++)
2	Alkaloids	(+++)
3	Phenolics	(+++)
4	Saponins	(+++)
5	Tannins	(+++)
6	Steroids	(+++)
7	Terpenoids	(+)

Phytochemical analysis confirmed the presence of flavonoids, phenolics, tannins, alkaloids, saponins, and steroids. These secondary metabolites are widely recognized for their antioxidant and therapeutic properties.

Antioxidant Activity (DPPH Radical Scavenging Assay)

Table 1.

	Absorbance at 517nm		
<i>Strobilanthes reptans</i>			
ppm extract	I	II	III
0	0.285	0.285	0.285
20	0.237	0.242	0.240
30	0.233	0.232	0.234
50	0.214	0.204	0.206
75	0.172	0.162	0.161
100	0.149	0.135	0.133
200	0.032	0.028	0.026
300	0.019	0.02	0.019

The table presents the absorbance values at 517 nm obtained from the DPPH Assay used to evaluate the antioxidant activity of the methanolic extract of *Strobilanthes reptans*. Measurements were conducted in triplicate (I, II, and III) at extract concentrations ranging from 0 to 300 ppm. The control (0 ppm) showed a constant absorbance of 0.285, representing the DPPH solution without the extract. A progressive decrease in absorbance was observed as the extract concentration increased, indicating enhanced free radical scavenging activity. The lowest absorbance values were recorded at 300 ppm, demonstrating the strongest antioxidant effect of the extract at higher concentrations.

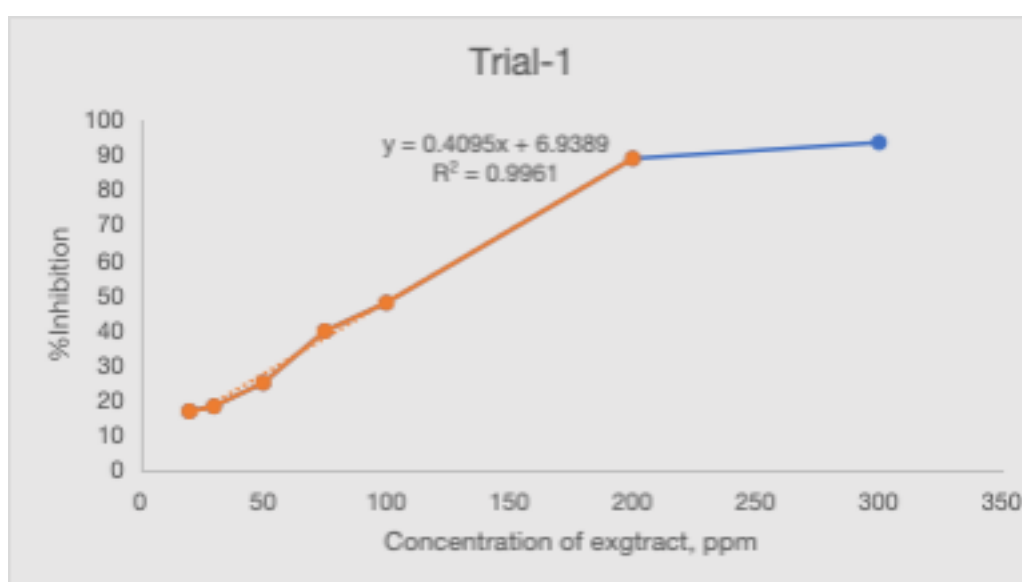
Table 2.

	% Percent of Inhibition		
<i>Strobilanthes reptans</i>			
	I	II	III

	16.8	15.1	15.8
	18.2	18.6	17.9
	24.9	28.4	27.7
	39.6	43.2	43.5
	47.7	52.6	53.3
	88.8	90.2	90.9
	93.3	93.0	93.3

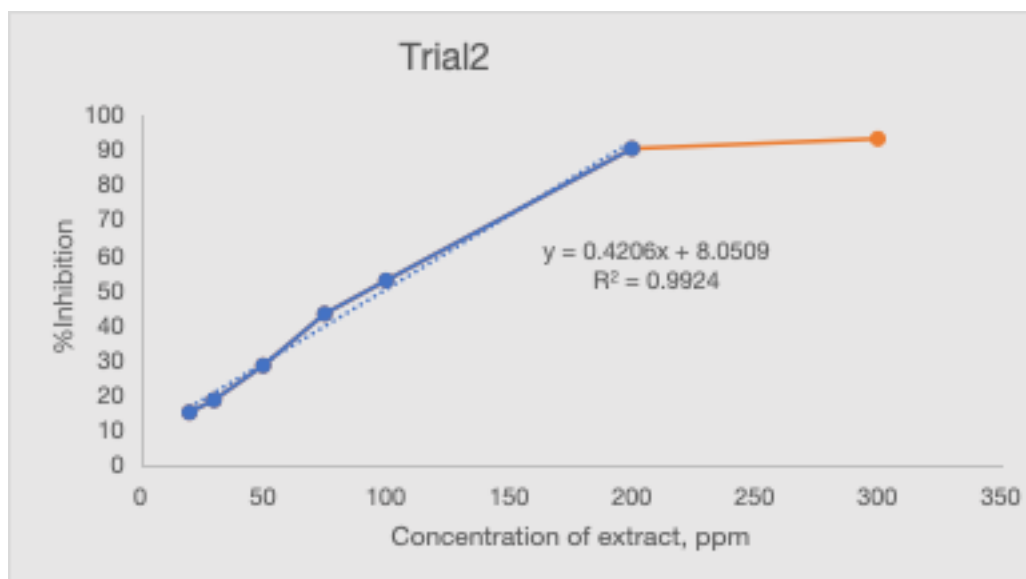
Graphical Analysis.

TRIAL 1. Inhibition Percent



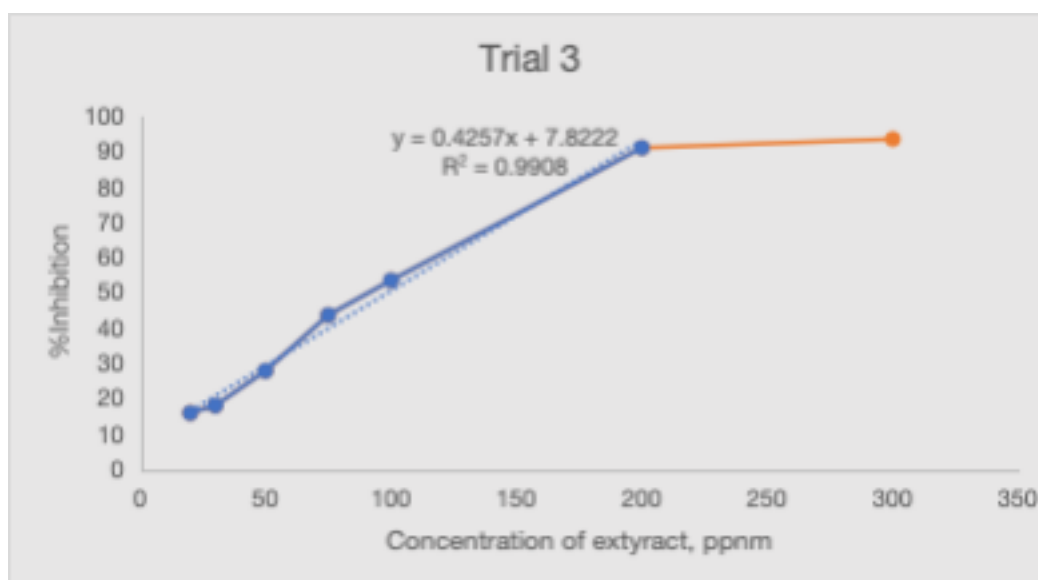
The antioxidant activity increased steadily with extract concentration, showing a strong linear relationship ($R^2 = 0.9961$). At 200 ppm, inhibition reached nearly 90%, and only a slight rise was observed beyond this point.

TRIAL 2. Inhibition Percent



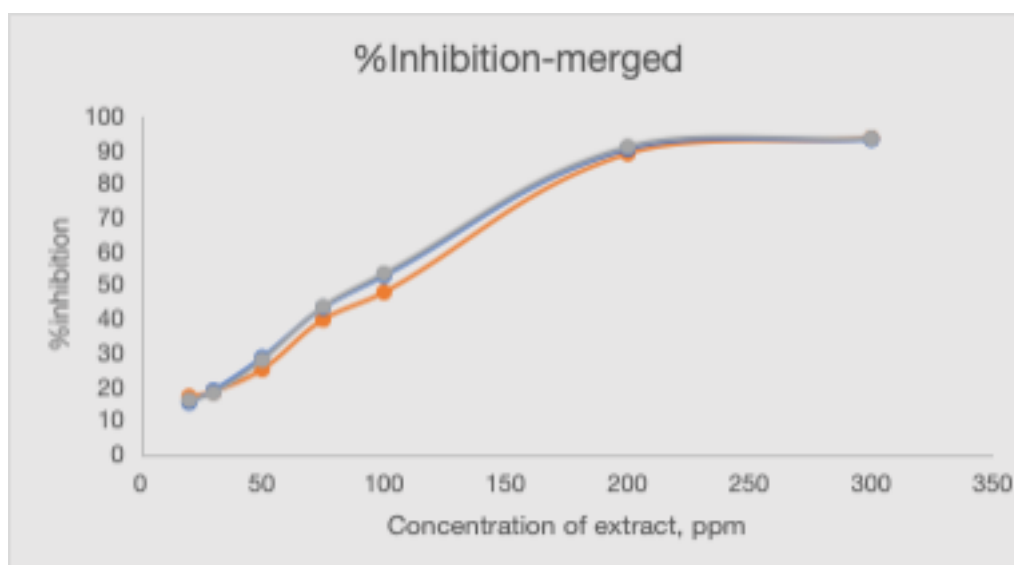
A similar trend was observed ($R^2 = 0.9924$), confirming high linearity and consistency. The percentage inhibition increased proportionally up to 200 ppm, where it approached 90%. Beyond this level, the increase plateaued, suggesting that maximum antioxidant potential was nearly achieved.

TRIAL 3. Inhibition Percent



Trial 3 also showed excellent linearity ($R^2 = 0.9908$). The % inhibition rose rapidly with concentration and stabilized near 90–92% at higher doses. This trial further validates the concentration-dependent antioxidant effect of the extract.

Inhibition Percent-merged



The percent of the merged inhibition curve showed strong linearity, with antioxidant activity increasing sharply with concentration and stabilizing around 90% at higher doses, confirming the concentration-dependent effect of the extract.

Discussion:

The calculated IC_{50} value of *Strobilanthes reptans* leaf extract was 101.3 ppm, classifying it as a strong antioxidant. The IC_{50} value represents the concentration required to inhibit 50% of DPPH radicals — a lower IC_{50} indicates a stronger antioxidant capacity.

Toxicity Test

Brine Shrimp Lethality Assay (BSLA)

<i>Strobilanthes reptans</i>					
	No. of survivors			Number of Survivor	Number of Lethality
ppm Extract	I	II	III		% Percent of Lethality

0	10	10	10		10.00	0.00	0%
10	8	9	8		8.33	1.67	17%
100	7	6	7		6.67	3.33	33%
500	4	2	3		3.00	7.00	70%
1000	0	1	1		0.67	9.33	93%

Table Description

The table presents the results of the Brine Shrimp Lethality Assay (BSLA) for the methanolic extract of *Strobilanthes reptans* at concentrations ranging from 0 to 1000 ppm, measured in triplicate (I, II, III). The number of surviving *nauplii* decreased as the extract concentration increased, resulting in a corresponding rise in lethality from 0% at 0 ppm to 93% at 1000 ppm. These results indicate that the extract exhibits dose-dependent toxicity, with higher concentrations causing greater mortality of *Artemia salina nauplii*.

TRIAL 1

Lethality increased with extract concentration, showing a clear dose-dependent pattern ($R^2 = 0.9832$). Mortality began at 17% (10 ppm) and rose steadily, reaching 93% at 1000 ppm. This indicates significant cytotoxic activity at higher concentrations.

TRIAL 2

A similar trend was observed with consistent linearity ($R^2 = 0.9754$). Mortality progressed gradually across concentrations, confirming that the extract's toxicity intensified as the dose increased.

TRIAL 3

Trial 3 also demonstrated high correlation ($R^2 = 0.9817$). The brine shrimp mortality rate rose rapidly from moderate to high concentrations, supporting the concentration-dependent toxicity of the extract.

Log Concentration	Probit
1.0	4.05
2.0	4.56
2.7	5.52
3.0	6.48

LC50	223.9 ppm
	Moderately Toxic

LC₅₀ Determination

The LC₅₀ value of the methanolic extract of *Strobilanthes reptans* was estimated using probit analysis, based on the mortality of *Artemia salina nauplii* at different concentrations. The analysis yielded an LC₅₀ of 223.9 ppm, indicating that the extract is moderately toxic according to the standard toxicity classification. The probit values increased with the logarithm of the extract concentration, confirming a dose-dependent toxicity trend.

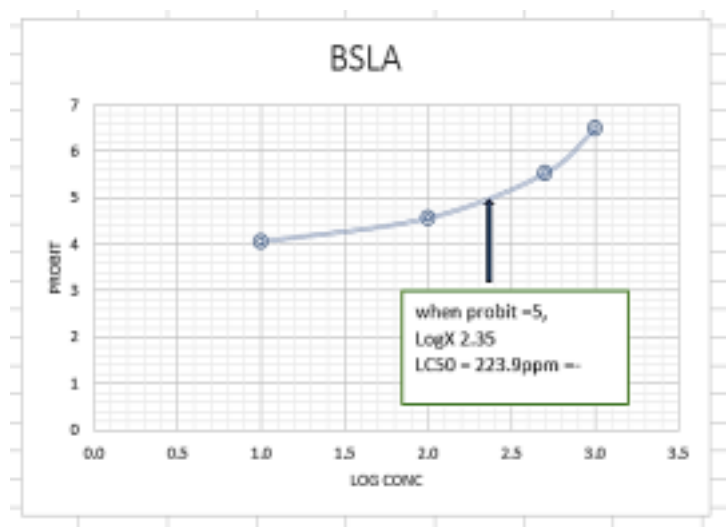


Figure Description

The figure presents the probit analysis plot for the Brine Shrimp Lethality Assay (BSLA) of the methanolic extract of *Strobilanthes reptans*. The x-axis represents the logarithm of the extract concentration (ppm), while the y-axis represents the corresponding probit values.

calculated from the mortality of *Artemia salina nauplii*. The plotted points show a positive linear relationship between concentration and probit, confirming dose-dependent toxicity. The intersection of the regression line with the probit value corresponding to 50% mortality indicates an LC₅₀ of 223.9 ppm, classifying the extract as moderately toxic.

Toxicity testing revealed an LC₅₀ value of 223.9 ppm, indicating moderate cytotoxicity. The observed biological activities may be attributed to the synergistic effects of the identified phytochemicals.

IV. CONCLUSION

The present study demonstrates that *Strobilanthes reptans* leaf extract contains bioactive phytochemicals with significant antioxidant activity and moderate cytotoxic effects. These findings provide foundational scientific evidence supporting its potential pharmacological relevance. Further studies involving compound isolation, quantitative analysis, and in vivo investigations are recommended.

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DECLARATION

Ethical Approval: All experimental procedures were conducted following standard laboratory guidelines.