

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

Clark Aldrin Dave A. Ibao, Jelian Mae A. Baguinaon, Precious Amor Basadre, Ghian Carlos
M. Añober, Queenzebel Bullos, Romel B. Alierta

¹Science Department, Kitcharao National High School, Kitcharao, Agusan del Norte,
Philippines

Corresponding Author: Clark Aldrin Dave A. Ibao

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 herb 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 Purok-2, crossing, Kitcharao, Agusan del Norte, washed, air-dried for 7 days, grind into semi-powder, and pack in a sealed bag. Extraction was performed using ethanol through maceration and rotary evaporation. Qualitative phytochemical screening was conducted following standard laboratory procedures. Antioxidant activity was evaluated using the DPPH radical scavenging assay. Percentage inhibition was calculated and IC₅₀ values were determined through linear regression analysis. Cytotoxicity was assessed using the Brine Shrimp Lethality Assay (*Artemia salina*), and LC₅₀ values were calculated based on 24-hour mortality data.

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

Table 2.

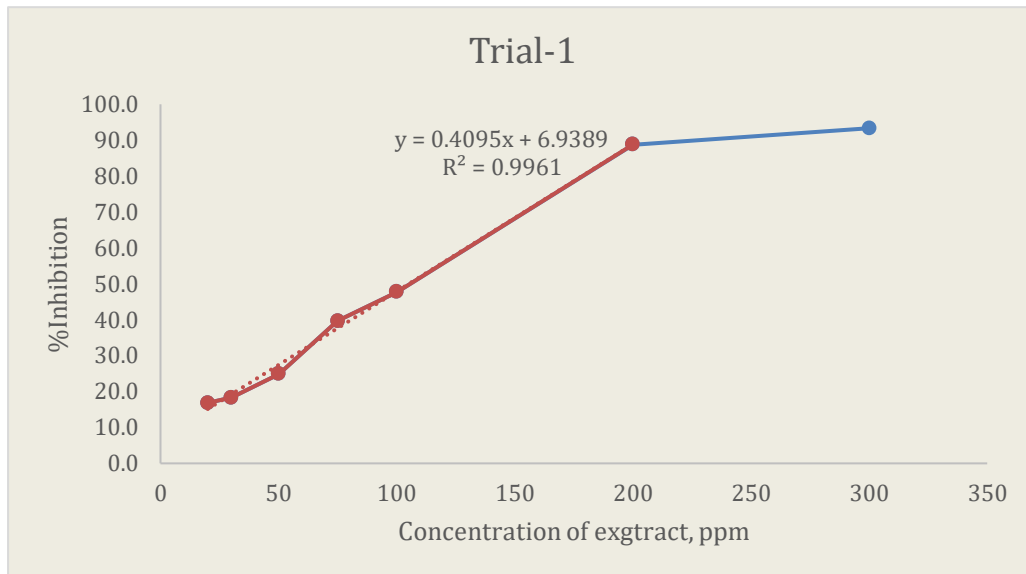
% Percent of Inhibition			
<i>Strobilanthes reptans</i>			
ppm extract	I	II	III
20	16.8	15.1	15.8
30	18.2	18.6	17.9
50	24.9	28.4	27.7
75	39.6	43.2	43.5
100	47.7	52.6	53.3
200	88.8	90.2	90.9
300	93.3	93.0	93.3

Table 3.

	I	II	III
IC ₅₀	105.1553114	99.73632905	99.07869392
Mean	101.3 ppm		
SD	3.3 ppm		
	Strong antioxidant		

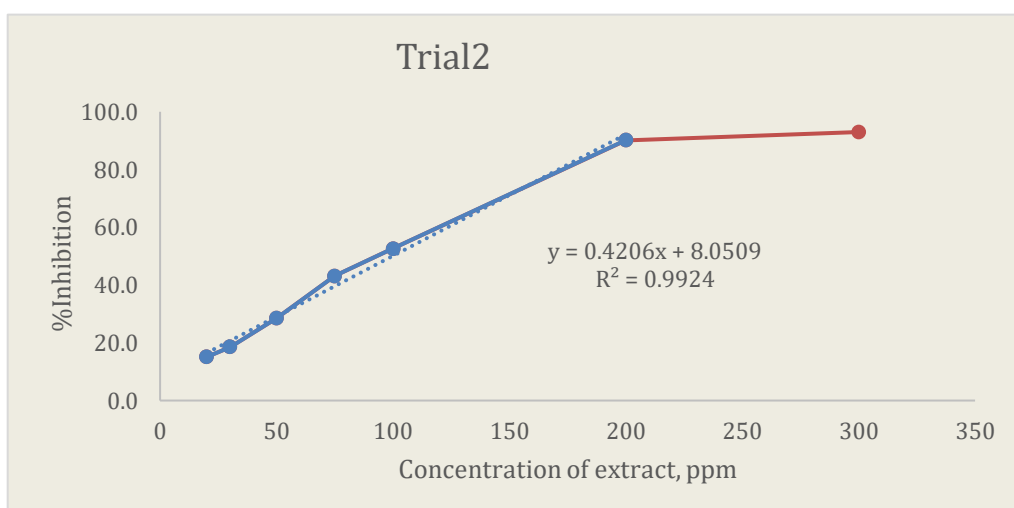
The ethanolic leaf extract exhibited strong radical scavenging activity with an IC₅₀ value of 101.3 ppm.

Graphical Analysis.



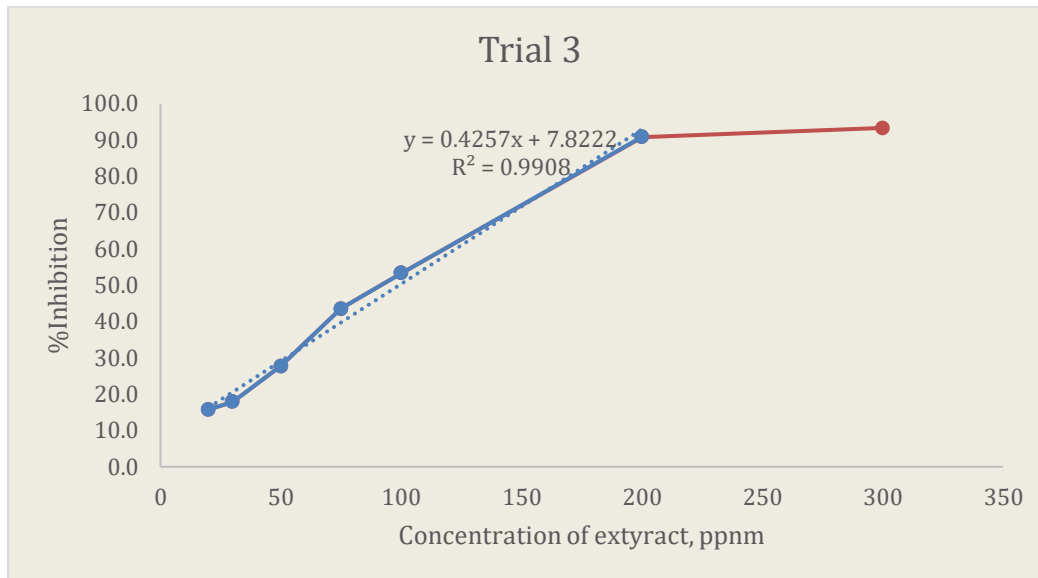
TRIAL 1. Inhibition Percent

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

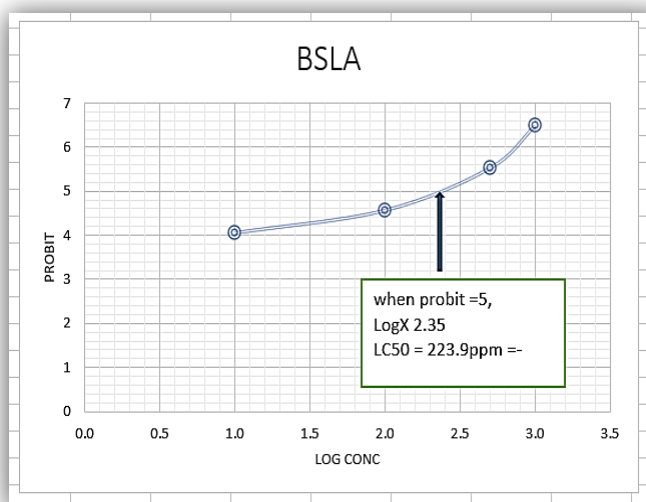
Trial 3 also showed excellent linearity ($R^2 = 0.9908$). The % inhibition rose rapidly with concentration and stabilized near 90–92% at higher doses. This trial further validates the concentration-dependent antioxidant effect of the extract.

Brine Shrimp Lethality Assay (BSLA)

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

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



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.

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.

REFERENCES:

- Baboo, R. C., & Sirajudheen, M. K. (2024). *A review on Hemigraphis colorata*. <https://doi.org/10.20959/wjpr202413-32788>
- Balasubramaniam, G., Sekar, M., & Badami, S. (2020). Pharmacognostical, physicochemical, and phytochemical evaluation of *Strobilanthes kunthianus* (Acanthaceae). *Pharmacognosy Journal*. <https://www.phcogj.com/article/1170>
- Ban, W. K., Lim, F. I., Khong, H. Y., & Phung, J. H. Y. (2022). Wound healing, antimicrobial, and antioxidant properties of *Clinacanthus nutans* (Burm. f.) Lindau and *Strobilanthes crispus* (L.) Blume extracts. *Molecules*, 27(5), 1722. <https://doi.org/10.3390/molecules27051722>

- Bisso, N. E., Olounladé, A. P., Agbangnan, D. C. P., Dossa, C. P. A., Lalèyè, A., & Sohounhloué, D. K. C. (2022). Phytochemical screening, antioxidant, and antimicrobial activities of seven underinvestigated medicinal plants. *Evidence-Based Complementary and Alternative Medicine*, 2022, 1998808. <https://doi.org/10.1155/2022/1998808>
- Chen, C.-S., Loke, C.-F., Poh, T.-V., Fong, E.-L., Lai, O.-M., & Tan, S.-P. (2024). Phytochemical screening and photocytotoxicity of *Strobilanthes crisp*a (L.) Blume (Acanthaceae) leaf extracts. *Phytochemicals Paper* 2024.
- Devasahayam Arokia Balaya, R., Palollathil, A., Kumar, S. T. A., Chandrasekaran, J., Upadhyay, S. S., Parate, S. S., ... & Prasad, T. S. K. (2024). Role of *Hemigraphis alternata* in wound healing: Metabolomic profiling and molecular insights into mechanisms. *Scientific Reports*, 14(1), 3872. <https://doi.org/10.1038/s41598-024-54352-x>
- Dewi, R. S., Sandhiutami, N. M. D., Sarsono, D. A., & Cuinita, D. P. (2023). Antioxidants and antihyperlipidemia test of ethanol extract of Indonesian plant Sambang Getih leaves (*Hemigraphis bicolor* Boerl.) in hyperlipidemia mice. *Open Access Macedonian Journal of Medical Sciences*, 11(A), 92–98. <https://doi.org/10.3889/oamjms.2023.10962>
- Elangovan, M., Ramachandran, D., & Rajesh, K. (2021). Green synthesis of silver nanoparticles using flower extract of *Hemigraphis colorata* as reducing agent and its biological activity. *Letters in Applied NanoBioScience*, 10, 2646–2654. <https://doi.org/10.33263/LIANBS104.26462654>
- Feba, A. (2022). *In vitro* evaluation of alginate films modified with chitosan and *Hemigraphis colorata* for wound healing applications. <http://localhost:9494/xmlui/handle/123456789/1642>
- Giri, S., & Saha, P. (2025). Anti-inflammatory flavonoids found in medicinal plants of Acanthaceae family. *Indian Journal of Natural Products and Resources (IJNPR)*, 16(2), 209–226.
- Gomathi, R., Rejila, R., Remya, R. S., Sharmi, S. S., & Raj, D. S. (2024). Phytochemical investigation of *Hemigraphis colorata* leaf extract. *LIPS Research Journal*, 1(2), 11–

18. <https://lipsresearch.org/wp-content/uploads/lips-journal/vol01iss02/lips-vol01iss02-p11.pdf>

Gurjar, V. K., & Pal, D. (2024). Classification of medicinal plants showing anti-viral activity, classified by family and viral infection types. In D. Pal (Ed.), *Anti-viral metabolites from medicinal plants* (Reference Series in Phytochemistry). Springer. https://doi.org/10.1007/978-3-031-12199-9_3

Joy, T., Krishnan, A. G., Chinnamma, M., & Bhasker, S. (2024). Comparative analysis of tender and mature *Hemigraphis colorata* leaves on TGF- β activity in wound healing and molecular characterisation of plant extracts: TGF- β in wound closure. *Indian Journal of Experimental Biology*, 62(10), 815–823. <https://doi.org/10.56042/ijeb.v62i10.1199>

Koshy, J., & Sangeetha, D. (2022). *Hemigraphis alternata* leaf extract incorporated agar/pectin-based bioengineered wound dressing materials for effective skin cancer wound care therapy. *Polymers*, 15(1), 115. <https://doi.org/10.3390/polym15010115>

Mai, N. C., Van, N. T., Hoe, P. T., Giang, V. H., Ban, N. K., & Linh, T. M. (2024). Optimization of brine shrimp lethality test for in vivo toxicity evaluation of poisonous plant species collected from Quang Tri Province. *Journal of Biology*, 46(1). <https://doi.org/10.15625/2615-9023/18899>

Mohamad Hanafiah, R., Abd Ghafar, S. A., Lim, V., Musa, S. N. A., Yakop, F., & Hairil Anuar, A. H. (2023). Green synthesis, characterization, and antibacterial activities of *Strobilanthes crispus*-mediated silver nanoparticles (SC-AgNPs) against selected bacteria. *Artificial Cells, Nanomedicine, and Biotechnology*, 51(1), 549–559. <https://doi.org/10.1080/21691401.2023.2268167>

Mohanan, D. P., Thazhenandayipurath, V. V., Sreekanth, K., Stanley, J. V., Mathew, D., Radhakrishnan, N., & Krishnankutty, R. E. (2024). Superior activity of Ag-ZnO bimetallic nanoparticles synthesised using *Hemigraphis colorata* (Blume) over ZnO nanoparticles in free and collagen-coated form against wound pathogens. *Journal of Cluster Science*, 35(8), 3065–3079. <https://doi.org/10.1007/s10876-024-02654-5>

- Nasim, N., Sandeep, I. S., & Mohanty, S. (2022). Plant-derived natural products for drug discovery: Current approaches and prospects. *The Nucleus*, 65(3), 399–411. <https://doi.org/10.1007/s13237-022-00405-3>
- Ndezo Bisso, B., Njikang Epie Nkwelle, R., Tchuenguem Tchuenteu, R., & Dzoyem, J. P. (2022). Phytochemical screening, antioxidant, and antimicrobial activities of seven underinvestigated medicinal plants against microbial pathogens. *Advances in Pharmacological and Pharmaceutical Sciences*, 2022, 1998808. <https://doi.org/10.1155/2022/1998808>
- Olmedo, D. A., Vasquez, Y., Morán, J. A., Guerrero De León, E., Caballero-George, C., & Solís, P. N. (2024). Understanding the *Artemia salina* (brine shrimp) test: Pharmacological significance and global impact. *Combinatorial Chemistry & High Throughput Screening*, 27(4), 545–554. <https://doi.org/10.2174/1386207326666230703095928>
- Prabowo, W. C., Kuncoro, H., Irawan, B., Kusuma, S. A. F., & Susilawati, Y. (2024). Botanical and pharmacognostic investigation of *Strobilanthes kalimantanensis*. *Journal of Advanced Pharmaceutical Technology & Research*, 15(3), 144–149. <https://doi.org/10.4103/JAPTR.JAPTR>
- Reshmy, R., Madhavan, A., Arun, K. B., Philip, E., Sindhu, R., Binod, P., ... & Pandey, A. (2022). Chili post-harvest residue-derived nanocellulose composite as a matrix for in vitro cell culture, and *Hemigraphis colorata*-blended nanocellulose extends antimicrobial potential. *Sustainable Chemistry and Pharmacy*, 25, 100584. <https://doi.org/10.1016/j.scp.2021.100584>
- Sarker, S. D., & Nahar, L. (Eds.). (2024). *Computational phytochemistry*. Elsevier. <https://books.google.com.ph/books?hl=en&lr=&id=3k7VEAAQBAJ&oi=fnd&pg=PP1>
- Shalima, N. K., & Kumudhavalli, M. V. (2025). Ecological and therapeutic insights into *Strobilanthes alternata*: A critical review. *American Journal of Psychiatric Rehabilitation*, 28(4), 180–183. <https://doi.org/10.69980/ajpr.v28i4.286>
- Sundaram, V., Sadhasivam, S., Chandrasekaran, S., Raaman, N., & Pandian, A. (2021). *Strobilanthes heyneanus* root extract as a potential source for antioxidant and

antimicrobial activity. *Future Journal of Pharmaceutical Sciences*, 7(1), 91.
<https://doi.org/10.1186/s43094-021-00242-2>

Suneka, S., & Manoranjan, T. (2021). *Brine shrimp lethality assay with selected medicinal plant extracts*. <http://repo.lib.jfn.ac.lk/ujrr/handle/123456789/11039>

Wood, J. R. I., & Scotland, R. W. (2021). A *Strobilanthes* (Acanthaceae) miscellany. *Kew Bulletin*, 76(4), 827–840. <https://doi.org/10.1007/s12225-021-09990-z>