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Original Research Article



Preliminary phytochemical screening and anti-diarrhoeal activity of methanolic root extract of *Strychnos colubrina* L.

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Abstract

Strychnos colubrina L., a large, climbing shrub, belongs to Loganiaceae, represented by more than 200 species across the globe while with 7 and 5 species in India and the Eastern Ghats respectively. It is used by adivasis and rural communities in Eastern Ghats of Andhra Pradesh for diarrhoea, dysentery, stomach disorders, tumours, mania, and chicken pox etc. This is rare and endemic to the forests and under-explored species. The present work is focussed on phytochemical screening and qualitative analysis which revealed the presence of active compounds in high concentration viz., alkaloids, flavonoids, steroids, catecholic compounds, phenols, reducing sugars, saponin, phytosterols, and tri-terpinoids. The folk claims especially on anti-diarrhoeal activity is justified with the help of rich phytochemical composition.

Keywords *Strychnos colubrina*, Phytochemical Screening, Anti-diarrhoeal activity.

Introduction

Plants are essential components to help all human societies in all respects and play a major role in the operation of ecosystems (Harborne, 1984; Biswas et al., 2002). The early man's way of life was completely dependent on plant society (Farnsworth & Soejarto, 1991). Diarrhoea is usually the symptom of gastrointestinal infection, caused by a variety of viral, parasitic, and pathogenic bacteria. As per UNICEF report, diarrhoeal diseases account for nearly 1.3 million deaths a year among children less than five years of age making them the second most common cause of child death worldwide. Over half of the deaths occur in just five countries in the third world like India, Nigeria, Afghanistan, Pakistan, and Ethiopia. According to World Health Organisation (WHO), diarrhoeal disease is responsible for killing over 5 Lakhs (5,25,000) children every year around the globe.

Materials and Methods

Plant collection, identification, and processing

Strychnos colubrina roots were collected from Rapur forest (Nellore district), Eastern Ghats of Andhra Pradesh, during 2018 and identified using flora of the Eastern Ghats, Flora of Nellore district, and Gamble Vol-II and also a comparison with authentic specimens housed in SKU (Ananthapuramu), MH (Coimbatore) and CAL (Howrah). Voucher specimens were deposited in Sri Krishnadevaraya University Herbarium (SKU) Ananthapuramu.

Extraction procedure

Strychnos colubrina root powder was used in the crude form as 90 % of methanolic extract, prepared using standard methods (Sudhira, et al., 2015).

The root material was shade dried at room temperature, powdered, and stored till further use. The powders were subjected to the solvent extraction process individually (Francis & Suseem, 2016; John et al., 2015). *Strychnos colubrina* root powder 300 gm was extracted with 1100 ml methanol using Soxhlet apparatus yielded 38.3g crude extract.

Procurement of Animal and maintenance

Swiss albino mice, weighing 18–30 g, 6 to 8 weeks of age were procured from Sri Venkateswara Enterprises, Bangalore. Standard pellet diet was purchased from Sai durga feeds and foods Bangalore. Ethical clearance for the handling of experimental animals was obtained from the committee, constituted for the purpose (1889/GO/Re/S/16/CPCSEA).

Drugs and chemicals

Castor oil (SD Fine), 0.9% sodium chloride solution (normal saline) charcoal meal (10% activated charcoal in 5% gum acacia), 2% (v/v) aqueous tween 80 and loperamide (Waksman Selman pharmaceuticals Pvt. Ltd) were used.

Acute oral toxicity test

An acute oral toxicity test was conducted according to the OECD 425 guideline on five female Swiss albino mice at a single oral limit dose of 2000 mg/kg body weight (OECD, Guidelines Slovak Republic, 2015). Five female Swiss albino mice were randomly selected for the test. One animal was the first dose at the limit test dose and survived in the 24-hour follow-up. Then, the other four additional animals were sequentially dosed at 2000 mg/kg so that a total of five animals were tested. The animals were observed individually for signs of toxicity at least once in the first 30 minutes after dosing, periodically during the first 24 hours, and daily for an additional 13 days, for a total of 14 days.

Castor oil-Induced diarrhoea in Mice

30 mice fasted overnight and divided into five groups as follows: Group 1: control group mice received 1ml 2 % (v/v) aqueous Tween 80 orally. Group II, III and IV were treated with methanol extract of *Strychnos colubrina* at the doses Of 100,200,300 mg/kg body weight by oral route, dissolved in 2 % (v/v) aqueous tween 80. Group V (Standard group) mice of this group were treated orally with standard drug loperamide at the dose of 3 mg/kg body weight. After 1 hour all the mice were treated with 1 ml castor oil by oral route and observed for faecal matter. The number of wet faecal droppings was measured for 4 hrs after castor oil administration. Droppings of diarrhoea were collected in transparent plastic dishes placed below the cages. The total number of diarrhoeal faeces of the control group was considered 100% (Biswas et al., 2002; Paliwal et al., 2019)

Statistical analysis

The results were expressed as mean ± SEM. The statistical analysis of data was carried out using one-way ANOVA.

Results and discussion

Preliminary Phytochemical Screening

The preliminary phytochemical screening test of methanolic root extract of *S.colubrina* revealed the following phytochemicals (Table 1).

Table 1. Preliminary screening of secondary metabolites from *S. colubrina* methanolic root extract.

S. No	Secondary metabolite	Present(+) / absent (-)
1	Alkaloids	+++
2	Flavonoids	++
3	Triterpenoids	++
4	Glycosides	+
5	Sterols	+
6	Tannins	+
7	Phenols	+++

+++ = High, ++ = Moderate and + = Low

Acute oral toxicity test

In the oral acute toxicity test using the limit dose of 2000 mg/kg body weight, the methanolic root extract of *Strychnos colubrina* was found to be safe as there was no mortality or remarkable signs of toxicity noted in the 14-day observation of the mice which were used for the test. This result confirmed that the LD₅₀ of the plant extract is greater than 2000 mg/kg.

Table 2. Effect of *S. colubrina* Methanolic root extract on castor oil-induced diarrhoea.

Group	Treatment	No. of et faces	Inhibition (%)
I	2%(v/v) aqueous Tween 80 (1mL/kg) + Castor oil (1mL)	3.80±0.68	-
II	Extract (100 mg/kg)+ Castor oil (1mL)	1.64±0.27*	56.84
III	Extract (200 mg/kg)+ Castor oil (1mL)	1.49 ±0.34*	60.78
IV	Extract (400 mg/kg)+ Castor oil (1mL)	0.68 ±0.25**	82.10
V	Loperamide (3 mg/kg) + Castor oil (1mL)	0.04 ±0.34**	98.94

Data are expressed as mean ± SEM, n = 5. *p < 0.05, **p < 0.01 when compared with vehicle-control.

Effect of the *S. colubrina* methanolic root extract on Castor oil- induced Diarrhoea

On oral administration of *S.colubrina* extracts produce a significant decrease in the severity of diarrhoea. The reduction in number of wet faeces indicates the presence of anti-diarrhoeal activity in the extract as compared with that of control (Table 2).

Discussion

The results revealed that the activity is more pronounced at the dose of 400 mg/kg body weight (Table 2). The percentage of inhibition of the number of wet faeces was 82.10%, $p < 0.01$ at the dose of 400 mg/kg body weight while that of standard drug loperamide (3 mg/kg) was 98.94 % control of castor oil-induced diarrhoea (Mitra et al., 2011).

Conclusion

S. colubrina contains pharmacological active substances with anti-diarrhoeal activity (Kapadia, et al., 2012; Kifle, et al., 2021). The folk claims especially on use of *S. colubrina* for diarrhoea in corroborated with the in vivo physiological observation recorded on Swiss albino mice (Mohan et al., 2021; Perumal et al., 2016). The test species is endemic to the hills of Nellore district (Rapur) and hither to not explored with scientific experimentation. Hence, the present study gains importance and prompted conduct molecular studies to elucidate the structural composition of active principles involved in the said property. Further research is needed to evaluate and purify the extract to find out bio-dynamic molecule responsible for the anti-diarrhoeal activity.

Conflict of Interest

There are no conflicts of interest among the authors in this inquiry.

Ethical Considerations

The study was approved by the institutional ethical committee.

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