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Evaluation of Analgesic, Antioxidant, Cytotoxic and Thrombolytic Potential of *Stachytarpheta cayennensis* (Rich.) Vahl Leaves

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Abstract

The current study was undertaken for the preliminary investigation of the analgesic, cytotoxic, antioxidant, and thrombolytic properties of the methanolic extract of *Stachytarpheta cayennensis* (Rich.) Vahl leaves. Both the central and peripheral analgesic action of the crude extract of methanol were significant and the analgesic effect increases with the increasing of dose and time with the pronounced effect at 600 mg/kg body weight. According to the brine shrimp lethality bioassay, the pet-ether soluble fraction (PESF) displayed notable cytotoxic action showing LC_{50} value (5.33 µg/ml) among the fractions as compared to the standard vincristine sulfate (0.452 µg/ml) and crude extract of the leaves contained a total phenolic content ranging from 18.68-145.98 mg of GAE/gm while revealing moderate thrombolytic activity.

Key words: *Stachytarpheta cayennensis*, analgesic, thrombolytic, cytotoxic.

Introduction

The family Verbenaceae (also known as the vervain or verbena family) is a flowering plant of tropical latitudes. Plants in this family contain trees, shrubs, herbs, or lianas (tropical vines) which is noticeable for different characteristic parts such as spikes, heads, or small flowers forming a cluster. Many of those flowers have an aromatic fragrance. The family Verbenaceae is a diverse group of ornamental species including about 32 genera and around 800 species of plants, most of which occur in the tropics (Cardoso *et al.*, 2021). Out of all, approximately 21 genera and 125 species are distributed throughout India (Cardoso *et al*., 2021; Marx *et al*., 2010).

Stachytarpheta cayennensis (Rich.) Vahl, also known as blue snakeweed is an evergreen herb that lasts for an infinite time. It is extended mostly to tropical America, the Caribbean, and Mexico. It also grows through tropical South America to southern Brazil. In Bangladesh, it is an ornamental plant mostly found in Cox's Bazar area (Meva *et al*., 2019). In several Latin American countries, extract of the plant is recognized as a treatment for easing the symptoms of malaria. It is also helpful for the treatment of pain, dysentery, and liver diseases. Tea made from the leaves is popularly used to control blood sugar levels by people in Peru and other Latin countries (Guimarães *et al.*, 2023; Okokon *et al.*, 2008; Olayode *et al*., 2020). Alkaloids, terpenoids,

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tannins, saponins, glycosides, gluconic acid, steroids, and phenolic compounds were the reported phytochemicals isolated from *S. cayennensis* (Adebajo *et al*., 2007; Ho and BO, 2005; Onofre *et al*., 2015). The present experiment was designed to explore different pharmacological actions of crude methanolic extract of *S. cayennensis* such as analgesic, thrombolytic, cytotoxic, and antioxidant activities.

Materials and Methods

Collection of plant materials and extraction: Fresh leaves of this plant were collected from the hillside of Kumira, situated in Chittagong in November 2020, the period when the plant grows the most. The plant was identified by an experienced professional at the Bangladesh National Herbarium (BNH) and issued an accession number (DACB86934). The plant materials had undergone two steps of processing during which extraneous and undesired substances were removed. At first, the rotten and insect-infested leaves were withdrawn by hands immediately after collection of the leaves. Again, the soil or dust had been removed by sieving through a net aided by a flow of air from an electric fan before the plant materials went dried.

The plant materials were crushed with a grinder to form powder (450 g) which was soaked in methanol (2 L) at room temperature for almost 15 days. The resultant mixture was subjected to filtration employing fresh cotton and eventually, filtered by Whatman filter paper number 1. The crude extract was weighed properly after concentrating the filtrate using a rotary evaporator. A portion of the collected concentrate of crude extract (8 gm) was fractioned using modified Kupchan Partition, a solvent-solvent partitioning method (Ramjan *et al*., 2014). As per the method, three organic solvents and distilled water were used for the fractionation procedure and the resultant fractions (pet-ether soluble fraction PESF: 2.50 gm, DCM soluble fraction DCMSF: 2.35 gm, ethyl acetate soluble fraction EASF: 1.25 gm, aqueous soluble fraction AQSF: 1.50 gm) of the methanolic crude extract (MESF) were dried through evaporation and stored in a refrigerator for the further analysis.

Drugs and chemicals: Methanol, streptokinase (Beacon Pharmaceuticals Limited, Bangladesh), normal saline (Beximco Pharmaceuticals Limited, Bangladesh), diclofenac sodium (Square Pharmaceuticals Limited), tween-80 (BDH chemicals, UK) were used to conduct the experiment. Besides, other reagents utilized for the study were of analytical grade.

Experimental animals: The Swiss Albino mice of both sexes were brought together from the Animal Resource Branch of the Department of Pharmacy, Jahangirnagar University. The average weight of those mice ranges from 25 gm to 35 gm and aged 4-5 weeks. To make them adapt to the environment before employing the tests, the collected mice were kept in the animal house for at least 7 days at the State University of Bangladesh. The controlled temperature and humidity of the animal house were 24.0 ± 1°C and 55-65%, respectively and a cycle of 12 hrs light-12 hrs dark was maintained as well. The mice were fed rodent food formulated by icddr,b as well as water *ad libitum.* The tenet and suggestions of the Federation of European Laboratory Animal Science Association (FELASA) were followed throughout the investigation.

Grouping of mice: To lead the study, twenty mice were distributed into five groups randomly and each group comprised of four mice. Each group was marked as specific name such as positive control (STD), negative control (CTL), and three test batches of mice which were given three different doses of the methanolic extract- 200 (ME 200), 400 (ME 400), and 600 (ME 600) mg/kg of body weight.

Central analgesic activity: To investigate the central analgesic activity, an established method was followed (Pizziketti *et al*., 1985). According to this principle, the negative control and standard groups were given oral tween-80 in saline (1%, 0.1 ml/ 10 mg) and diclofenac sodium (5 mg/kg b.w.), respectively. In addition, three doses of the methanolic extract were received by the experimental animals. To incite pain, the tail tips of the mice were

dipped in hot water. The time required by the mice to take away their tails was recorded at different time intervals (30, 60, and 90 minutes) after the introduction of the test substances. The percent time elongation was calculated using the following formula-

$$\%\ Time\ Elongation = \frac{average\ time\ of\ tail\ flicking\ of\ test\ samples - average\ time\ of\ tail\ flicking\ of\ the\ control\ group}{average\ time\ of\ tail\ flicking\ of\ the\ control\ group}$$

Peripheral analgesic activity: Acetic acid-induced writhing model was imitated to evaluate the activity of peripheral analgesia (Islam *et al*., 2022; Kabir *et al*., 2020). Initially, 0.1 ml of acetic acid was offered peritoneally to individual mice to incite pain sensation. 5 mg/kg b.w. of diclofenac sodium was provided to individual mice of the standard group whereas, the exploratory animals were treated with the extracts. The inhibitory percentage of behavior was enumerated as-

$$\%\ of\ inhibition = \frac{average\ writhing\ of\ control - average\ writhing\ of\ sample}{average\ writhing\ of\ control}$$

Determination of antioxidant activity by the total phenolic content method: Folin-Ciocalteu reagent, an oxidizing agent, was exploited spectrophotometrically to assess the total phenolic content in the plant extractives. The antioxidant properties of the plant extracts were counted utilizing gallic acid as the standard (Harbertson and Spayd, 2006).

Cytotoxicity study: Established method in the literature (Kabir *et al*., 2020) was exerted to insure the toxic prominence of the plant extractives. In a single-day *in vivo* assay, dimethyl sulfoxide (DMSO) solution was exploited in the brine shrimp lethality bioassay to reveal the subsistence of bioactive compounds of the plant extractives against *Artemia salina.* Vincristine sulfate (VS) was used as the positive control. The median lethal concentration (LC_{50}) was enumerated by taking the logarithm of the sample concentration.

Thrombolytic activity: The method reported in established studies (Islam *et al*., 2020) was used to analyze the thrombolytic activity of individual test samples whereas, streptokinase was held as standard.

Statistical analysis: The values are shown like mean ± standard error of the mean (M ± SEM) and one-way ANOVA. For tracing significant differences between the negative control group with standard and test groups, Dunnett's test was assigned. The p values < 0.05 were considered to be significant statistically.

Results and Discussion

The percent elongation of the flicking response of *S. cayennensis* methanolic extract of the leaves exhibited a dose and time-dependent relationship with its desired pharmacological effects. 60 minutes after the administration, the crude extract causes the augmentation of the feedback time at three doses (200-, 400- and 600-mg/kg b.w.) by 90.6%, 126.5%, and 165.81%, respectively (Table 1) as compared to the standard morphine (293.16%). The existent study was sketched to assess central analgesic activity according to the method established by Pizziketti *et al*. (1985) which is believed to be a spinal reflex incited by heat. But it might also intertwine with the higher neural structures. The mechanism involved in pain initiation is due to different intricated pathways which include opiate, serotonergic, descending noradrenergic and dopaminergic systems. The crude extract exerted a significant increment in pain threshold which might be because of the presence of phytochemicals in the plant. It may be the interaction of phytochemicals with the central pathways or appeasement of different types of endogenous

chemicals which are the causative factors of the pain (Mishra *et al*., 2011).

Whilst standard diclofenac sodium demonstrated 85.25% of inhibition, the crude extract revealed considerable peripheral analgesia at all doses (ME 200, ME 400, and ME600), as indicated by reductions in acetic acid-induced writhing of 32.65%, 61.22%, and 61.22%, respectively (Table 2). As previously reported, abdominal constriction and the boosting of the contiguous peritoneal chemo-sensitive nociceptors may be the cause of the pain brought on by acetic acid. Therefore, the plant extract's ability to exert peripheral analgesic activity may have been influenced by its interaction with these peritoneal receptors.

Table 1. Central analgesic activity of crude methanol extract of *S. cayennensis* leaves.

Animal group	After 30 min		After 60 min		After 90 min	
	M ± SEM	% Elongation	M ± SEM	% Elongation	M ± SEM	% Elongation
CTL	2.54±0.19	-	2.34±0.28	-	2.32±0.39	-
STD	7.09±0.28*	179.13	9.2±0.43*	293.16	16.17±0.60*	596.98
ME 200	3.20±0.45*	25.98	4.46±0.38*	90.6	6.67±0.26*	187.5
ME 400	4.21±0.40*	65.74	5.3±0.28*	126.5	7.40±0.36*	218.96
ME 600	4.61±0.39*	81.49	6.22±0.12*	165.81	9.28±0.95*	300.0

Values are shown as Mean ± SEM (n=4). *p< 0.05 compared to negative control.

Table 2. Peripheral analgesic activity of crude methanol extract of *S. cayennensis* leaves.

Animal group	No. of writhing M ± SEM	% Inhibition
CTL	24.5 ± 1.89	-
STD	4 ± 0.41*	83.67
ME 200	16.5 ± 1.19*	32.65
ME 400	9.5 ± 1.04*	61.22
ME 600	7.5 ± 1.85*	69.38

Values are shown as Mean ± SEM (n=4). *p< 0.05 compared to negative control.

Table 3. Total phenolic content level of crude methanol extract of *S. cayennensis* leaves.

Sample	Total phenolic content (mg of GAE / gm of extracts)
MESF	120.24
PESF	18.68
DCMSF	54.10
EASF	145.98
AQSF	38.82

Values are shown as Mean (n=4).

The results demonstrated that the 200 mg/kg, 400 mg/kg, and 600 mg/kg b.w. doses of the crude methanolic extract of *Stachytarpheta cayennensis* leaves had substantial analgesic action. The experimental data support its conventional use as an analgesic in rheumatoid arthritis. Therefore, the plant can be further investigated to find out new lead compounds (Kabir *et al*., 2021).

The extractives' total phenolic contents were determined to be between 18.68 to 145.98 mg of GAE/gm of extractives (Table 3). Both the methanolic extract and the ethyl acetate soluble fraction (EASF) had substantial phenolic content, whereas the EASF had the highest concentration.

The brine shrimp lethality bioassay of the different fractions indicated the maximum cytotoxic action exerted by the petroleum ether soluble fraction (PESF) with LC_{50} value of 5.33 µg/ml (Table 2) as compared to standard reference VS (0.452 µg/ml). The methanolic extract of the plant expressed slightly higher cytotoxic activity (4.51 µg/ml) than the PESF which provides evidence of the likelihood of the presence of the cytotoxic compound in non-polar fractions of the plant leaves.

Table 4. LC_{50} values for the four soluble fractions and the crude methanolic extract of *S. cayennensis* leaves.

Sample	LC_{50} (µg/ml)
STD	0.452
MESF	4.51
PESF	5.33
DCMSF	6.43
EASF	11.96
AQSF	21.28

Values are shown as Mean (n=4).

Table 5. Thrombolytic activity of methanolic extract of *S. cayennensis* leaves and its Kupchan fractions.

Sample	% Of clot lysis
Water	3.47
STD	63.10*
MESF	55.53*
PESF	4.73
DCMSF	10.32
EASF	14.63
AQSF	17.05

Values are shown as Mean (n=4). *p< 0.05 compared to water.

The crude methanolic extract of *Stachytarpheta cayennensis* leaves together with its organic and aqueous fractions were evaluated for thrombolytic activity by investigating each fraction's ability to lyse blood clots. The samples showed thrombolysis activity ranging from 4.73% to 55.53%, whereas standard streptokinase caused clot lysis of 63.10% (Table 5). The extractives of *Stachytarpheta cayennensis* do have lower clot lysis activity than the standard compound streptokinase. But the crude extract (MESF) showed significant thrombolytic activity when compared to Streptokinase (55.53% and 63.10%, respectively), indicating that it may have therapeutic value for treating various thromboembolic disorders (Islam *et al*., 2022).

Conclusion

To conclude, *in vivo* and *in vitro* studies reflected promising analgesic, cytotoxic and thrombolytic actions which are possibly due to the presence of phytochemicals in the crude extract of the leaves of *S. cyannensis*. Thus, further extensive study is required to isolate the phytochemicals responsible for the aforementioned activities and to demonstrate the probable mechanism of action.

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