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Investigation of the Pharmacological Potential of *Staurogyne zeylanica* Kuntze: *In vitro* and *In vivo* Studies

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Abstract

This research project aims to explore the medicinal properties of *Staurogyne zeylanica* Kuntze, a relatively underexplored plant from the Acanthaceae family. Recent studies suggest that this plant may have a diverse array of therapeutic properties. Methanol extracts of *S. zeylanica* Kuntze demonstrated significant analgesic effects (59.05 %) comparable to morphine, indicating its potential as a pain-relief agent. Although the extracts exhibited hyperglycemic effects, they did not result in significant reductions in blood glucose levels. Central nervous system (CNS) tests suggested mild depressant effects, pointing to possible applications in treating conditions like depression. Additionally, higher doses of the extracts showed promising anti-diarrheal activity (74.29%), significantly reducing diarrhea. The aqueous extract also exhibited notable inhibition of haemolysis (60.5%) in heat-induced condition, suggesting potential protective effects against oxidative stress or cellular damage and low thrombolytic activity (12.41%). These findings underscore the pharmacological potential of *S. zeylanica* Kuntze and highlight the need for further research, including clinical trials, to fully validate and expand its therapeutic applications.

Key words: *Staurogyne zeylanica* Kuntze, acanthaceae, thrombolytic, anti-inflammatory, analgesic, hypoglycemic, anti-depressant

Introduction

Medicinal flora emerges as a critical wellspring of auspicious lead contenders imbued with therapeutic potential. The intricate compounds stemming from plants serve as pioneering building blocks, extensively scrutinized to unearth fresh pharmaceutical prospects (Awan and Aslam, 2014). A substantial share of contemporary medicinal agents traces back to nature's bounty. The World Health Organization (WHO) underscores that roughly 80% of people worldwide rely on herbal treatments, driven by their economic allure and negligible unwanted repercussions, all the while harboring robust pharmacological prowess (Newman and Cragg, 2016; Cragg and Newman, 2013; Sen and Samanta, 2015; Huang *et al.*, 2020). Humans were aware of the therapeutic benefits of plants even in prehistoric times. Many of these herbs have been utilized as effective treatments for millennia in traditional medicine (Mimi *et al.*, 2024). *Staurogyne zeylanica* Kuntze is a plant species that belongs to the Acanthaceae family. It is a small herbaceous plant native to Sri Lanka and India. *S. zeylanica* is an erect herb with trailing branches. The stems are terete and covered in fine pubescence (Alamgir, 2018). *S.* zeylanica is an herbaceous species that typically has flowers and fruits from January to March. It primarily thrives in moist deciduous forests, including plains. Acanthaceae family plants have much potential to explore and study further. This plant family encompasses several remarkable plants that have proven valuable in treating and managing various severe pathogenic, metabolic, genetic and other disorders. These therapeutic plants could form the

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foundational resources for developing intricate pharmaceutical formulations (Alamgir, 2018). By exploring the medicinal potential of *S. zeylanica* can provide insights into its effectiveness in treating various ailments. Studying its bioactive compounds could aid in developing drugs or treatment approaches. In summary, researching *S. zeylanica* is essential to uncover its medicinal potential.

Material and Methods

Collection of plants and preparation of extracts: In February 2021, a plant was collected from Savar, Bangladesh, and its taxonomic identification was confirmed by the Department of Botany at University of Dhaka. To prepare the collected plants for further use, they underwent a cleaning process to eliminate any mud and dust particles. Subsequently, the plants underwent an initial drying phase at room temperature, after which they were placed in an oven where the temperature was carefully maintained below 40 °C. Once completely dried, the plants were finely ground into a powder which was carefully stored and utilized for the entirety of the research endeavors.

Aproximately 500 g of finely powdered sample was soaked in an airtight flat-bottom container with 1.5 l of distilled methanol for 20 days, with occasional shaking. The cold extraction method involved placing the methanol-soaked sample in a flat-bottomed container, where it was stirred frequently. The resulting methanolic solution was filtered through cotton, followed by additional filtration using Whatman filter paper. The filtered solution was then concentrated using a rotary vacuum evaporator, yielding 13.67 g of crude extract. The crude methanol extract was obtained by evaporating the solution at temperatures below 40°C. For in-vivo bioactivity evaluation, the crude extract was administered at three different doses: 200 mg/kg, 400 mg/kg, and 600 mg/kg body weight. The extract (5.0298 g) was subjected to fractionation using a modified Kupchan Method. After adding 140 ml and 60 ml of n-hexane. The n-hexane layer was separated and the aqueous layer was treated with dichloromethane and ethyl acetate. The solvents were evaporated and the weights of the fractions were recorded for further analysis.

Chemicals: All the solvents and laboratory-grade reagents that were used throughout the investigation procured from Merck. Complimentary samples of aspirin, morphine (morphine G), and glibenclamide were kindly provided by Square Pharmaceuticals Ltd. And Gonoshasthaya Pharmaceuticals Ltd., Bangladesh. Pure sodium thiosulphate ($Na_2S_2O_3$) was supplied by Incepta Pharmaceuticals Ltd., Bangladesh. high-quality analytical-grade chemicals and reagents were purchased from Beximco Pharmaceuticals Ltd. Furthermore a vial of freeze-dried Altepase (Streptokinase) with a commercial potency of 15,000,000 I.U. was obtained from Beacon Pharmaceutical Ltd.

Animals: The researchers employed Swiss albino mice weighing between 20 to 25 grams, regardless of gender, obtained from the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B). The mice were encased in a controlled environment with a temperature of 26±2°C and subjected to a 12 hrs light/dark cycle each day for one week, allowing them to acclimate to typical laboratory conditions. All procedures carried out during the experiments were in compliance with established protocols and guidelines.

Analgesic activity: To assess the central analgesic activity of *S. zeylanica* in mouse, the tail immersion test was employed. Mice were categorized into different groups: positive control, negative control (standard drug morphine), and test groups receiving methanol soluble extracts at dosages of 200, 400 and 600 mg/kg body weight. Following proper weight measurement, each group was treated accordingly. The time taken by mice to react to tail immersion in warm water (55ºC) was recorded after 30 min intervals of post-administration for 90 min. The outcomes were contrasted with those of the unaffected group (Aziz *et al.*, 2019).

Peripheral analgesic activity: To investigate the peripheral analgesic effects of *S. zeylanica* in mice, the formalin-induced lick test was conducted. Similar

group divisions were established as in the previous test. Test samples (Methanol soluble extracts at a dosages of 200, 400, 600 mg/kg body mass), standard aspirin (50 mg/kg) and control were given orally, followed by subcutaneous injection of formalin (1%) after 30 minutes. Biting and licking reactions were recorded during the initial 5 minutes post-formalin injection (Demsie *et al*., 2019). The percentage of formalin that inhibits the licking reaction was measured using the formula:

$$\% \text{ Reduction of Licking} = \frac{(\text{Negative control mean responses} - \text{Test group mean responses}}{\text{Negative control mean responses}} \times 100\%$$

Hypoglycemic activity assesment: The hypoglycemic activity of *S. zeylanica* was tested through an oral glucose tolerance test (OGTT). Mouse were grouped into negative control, standard and test groups, each comprising 3 mouse. At the start (zero-hour), oral administration of test samples, standard glibenclamide (10 mg/kg body weight) and control took place. After 60 minutes of plant sample administration, all groups of mice were given a 10% solution of glucose (2 mg/kg total body weight) orally. Blood glucose levels were then measured using a glucometer at 30 min, 60 min and 120 minutes after giving glucose (Mansur, Siddiqi, & Saha, 2018).

CNS Anti-depressant activity: The sodium thiosulphate-induced sleeping time test: The potential antidepressant effects of the methanol fraction derived from the *S. zeylanica* plant in Swiss albino mice were assessed using the sodium thiosulphate-induced sleeping time test. The mice were categorized into negative control, standard and test groups, each comprising 5 mice. The standard sodium thiosulphate injection (200 mg/ml), was mixed with saline water to achieve a volume of 10 ml, resulting in a dosage of 25 mg/kg total weight. To initiate sleep, all groups received an intraperitoneal administration of sodium thiosulphate at a dosage of 25 mg/kg body mass, followed by the recording of sleep onset and total sleep duration for both the control and treatment groups. This method aimed to estimate the potential anti-depressant properties of the extract of *S. zeylanica* by observing its impact on sleep patterns in mice (Brand-Williams *et al.* 1995).

Anti-diarrheal activity: The anti-diarrheal activity of the methanol fraction from the *S. zeylanica* plant was assessed using a mice study involving castor oil-induced diarrhea. In this procedure, each mouse was given 1 ml of highly pure analytical-grade castor oil to induce diarrhea. The count of fecal stools produced by every mouse was documented. Through a comparison of observations from the experimental groups with the control group, the anti-diarrheal potential of the samples was evaluated. The control group received 10 ml/kg of a vehicle orally. The positive control group was administered 50 mg/kg of loperamide orally (Shoba and Thomas, 2001).

Thrombolytic activity: The thrombolytic activity of various fractions from the methanol fraction of *S. zeylanica* whole plant was tested using streptokinase (SK) as a standard. Venous blood was collected, clots were formed and solutions were added to induce clot lysis. After incubation, weight differences were measured. Streptokinase and various controls were used for comparison. We employed the subsequent formula to calculate the degree of clot dissolution (Mahfuz *et al.*, 2019).

$$\% \text{ Clot lysis} = \frac{\text{Total Weight of released clot}}{\text{Total Weight of blood clot}} \times 100\%$$

Erythrocyte membrane stabilization: A method to assess *in vitro* anti-inflammatory activity, was employed. Three tubes were labeled positive, negative and test groups. They received phosphate buffer, hyposaline and human erythrocyte suspension. Aspirin and distilled water were added to standard and control tubes, while plant samples were added to the test group. After incubation and centrifugation, absorbance of the supernatants was evaluated to determine anti-inflammatory potential through the percentage of inhibition regarding hemolysis induced by a hypotonic solution (Uddin *et al.*, 2016).

$$\% \text{ inhibition of hemolysis} = \frac{\text{AControl} - \text{ATest}}{\text{AControl}} \times 100\%$$

Here, A = Measurement of absorbance specific to each group.

Statistical analysis: Mean ± SEM were presented for experimental data. Statistical analysis was performed using Student's t-test with Graph Pad Software, USA.When the p-value was less than 0.05 ($p<0.05$), the result was considered significant.This analysis aimed to evaluate the hypoglycemic potential of *S. zeylanica* extracts by assessing their effect on glucose tolerance in mice.

Here, "N" represents the average amount of licking and biting responses for each respective group.

These analgesic activity assessments help gauge the potential pain-relieving effects of *S. zeylanica* extracts through central and peripheral mechanisms in mice.

Results and Discussion

Central analgesic activity: The percentage of time elongation for tail immersion was calculated by comparing the tail immersion duration of each mouse to that of the control group. The mean immersion time for each group was then determined. The formula used to calculate the percentage of time elongation is as follows:

% Time elongation = (Average duration of tail flicking for test samples - Average duration of tail immersion for the control group)/Average duration of tail flicking for the control group

This calculation allows for the assessment of the central analgesic effect of each group. A higher percentage of time elongation indicates a greater central analgesic effect. The central analgesic activity of the test samples was compared to that of morphine to evaluate their effectiveness.

According to the figure 1, the crude extracts of plant *S.* zeylanica at doses of 600 and 400 mg/kg demonstrate significant central analgesic activity (Rafi *et al.*, 2023). On the other hand, doses of 200 mg/kg shows central analgesic activity to a lesser extent after 30 minutes comparing to morphine. And after 60 minutes, at doses of 600, 400 and 200 mg/kg has extremely significant central analgesic activity. The data (Figure 1) shows that at 90 minutes the central analgesic activity of the methanolic extracts of plant *S. zeylanica* at a dose of 600, 400 and 200 mg/kg became stronger.

Peripheral analgesic activity: The statistical analysis of the data confirmed that the raw extract exhibited notable peripheral pain-relieving properties at 200, 400 and 600 mg/kg doses with percent inhibition of writhing within the range of 34.29% to 59.05% for *S. zeylanica.*

Hyperglycemic activity: The study evaluated the potential blood glucose-lowering activity of the methanolic extract of *S. zeylanica* at different doses, including 600, 400 and 200 mg/kg.

Despite the initial hypothesis and the potential therapeutic properties associated with the plant, the administration of the methanolic extract at these particular doses did not lead to a significant decrease in blood glucose levels compared to the control group.

CNS anti-depressant activity: The methanolic extract of *S. zeylanica* at 200, 400 and 600 mg/kg dose was subjected to a sodium thiosulphate-induced sleeping time test. The impact on the central nervous system (CNS) caused by various doses of the methanol extract from the *S. zeylanica* plant resulted in a slight enhancement of the sedative effect induced by diazepam sodium. This effect appeared to be dose-dependent, implying a subtle CNS antidepressant activity profile.

Anti-diarrheal activity: The methanolic extract of *S. zeylanica* was tested for anti-diarrheal properties using a castor oil-induced test, and the resulting data was recorded. The methanol fraction of plant *S. zeylanica* exhibited very statistically significant 74.29% reduction of diarrhea at dosage of 600 mg/kg and 60% reduction of diarrhea at dosage of 400 mg/kg. On the other hand 200 mg/kg compared to the standard loperamide 42.86% has no quite statistically significant anti-diarrheal activity.

Membrane stabilizing activity: Under hypotonic solution-induced conditions, the n-hexane soluble fraction (HSF) demonstrated the highest thrombolytic activity at 56.59 %, surpassing the 7.80 % activity of acetyl salicylic acid (ASA) (Figure 6, table 1). In the

heat-induced condition, the aqueous soluble fraction (AQSF) displayed the highest thrombolytic activity at 60.5%, whereas acetyl salicylic acid (ASA) exhibited a higher activity of 84.41 % (Figure 7, table 2).

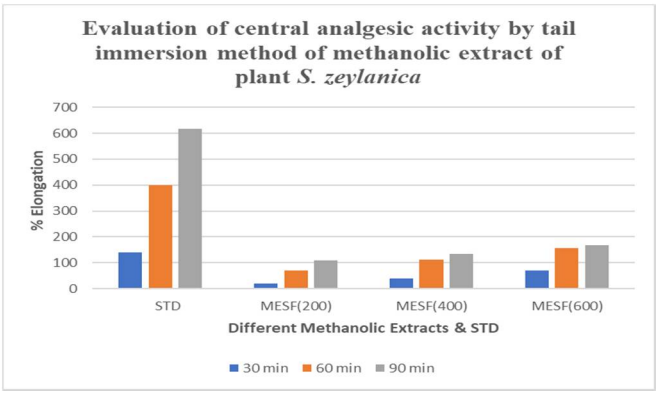

Figure 1. Comparison of the percentage of time that each sample's tail elongated during immersion.

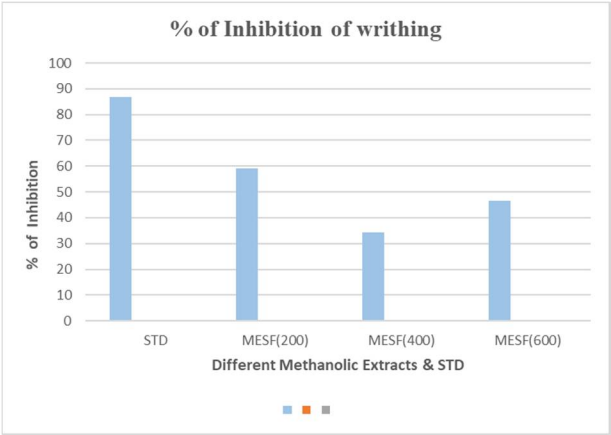

Figure 2**.** Comparison of % inhibition of writhing responses.

*A standard t-test was performed to compare the test samples with the positive control, and the statistical significance of the data was computed.

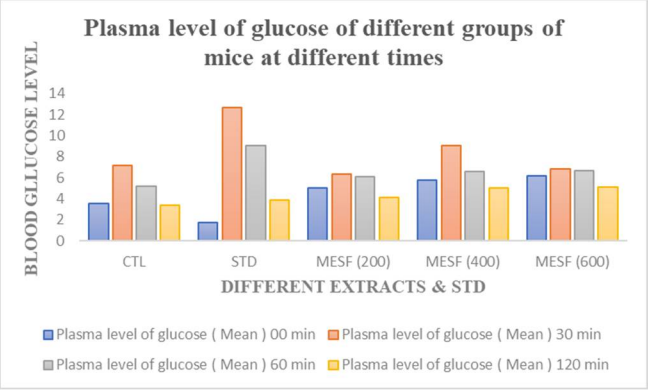

Figure 3. The variations in the plasma glucose levels among distinct groups of mice at various time points.

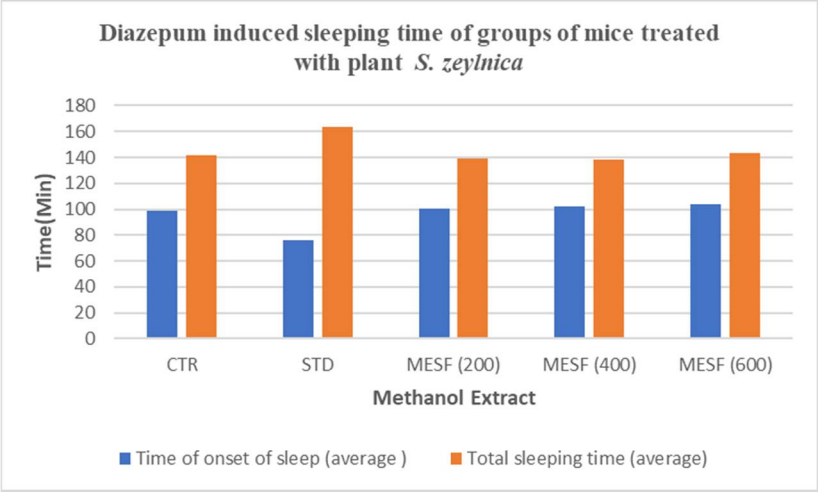

Figure 4. Sodium thiosulphate-induced sleeping time.

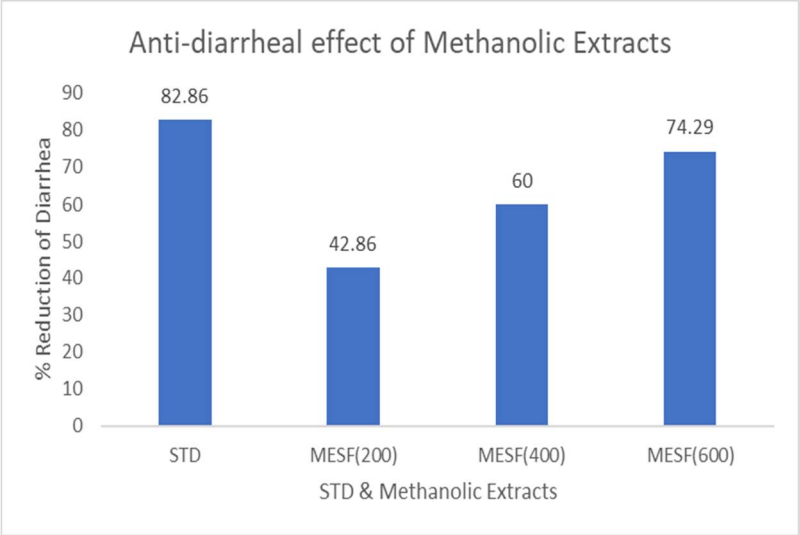

Figure 5. Anti-diarrheal effect of methanol fraction of *S. zeylanica* Kuntze on castor oil (1 ml/mice) induced diarrhea in mice.

The effect of synthetic and herbal anti-inflammatory agents on the stabilization of erythrocyte membrane, which can be extrapolated to the stability of lysosomal membrane, has been extensively studied. The results indicate that the extracts tested showed significant protection against both hypotonic solution and heat-induced haemolysis of human erythrocytes. The observed activity was comparable with the standard anti-inflammatory drug such as acetyl salicylic acid. Flavonoids, tannins and saponins have been reported to exhibit stabilizing effects on lysosomes and erythrocyte membranes in previous studies.

Thrombolytic activity: Positive control with 100µl SK (30,000 I.U.) led to 39.33% clot lysis after 90-minute 37°C incubation. Negative control had minimal clot lysis (1.47%). Difference between positive and negative controls was statistically significant. Aqueous soluble fraction (AQSF) of *S. zeylanica*'s crude leaf extract exhibited highest thrombolytic activity (12.41%), followed by n-hexane soluble fraction (5.92%), Ethyl acetate soluble fraction (3.24%), DCM soluble fraction (2.68%) and methanol soluble fraction (3.36%) with moderate thrombolytic activity.

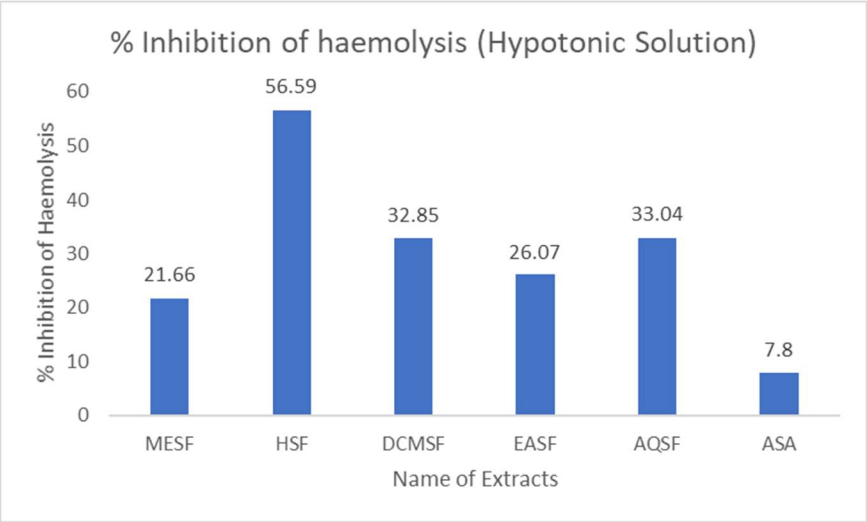

Figure 6. % Inhibition of haemolysis of different extractives of *S. zeylanica* on hypotonic solution induced condition.

Table 1. Effect of various extractives of *S. zeylanica* of hypotonic solution induced haemolysis of erythrocyte membrane.

Sample code	Absorbance	Concentration	% Inhibition of haemolysis (Hypotonic solution induced)
Hypotonic medium		50 Mm	
MESF	2.079	2 mg/ml	21.66
HSF	1.152	2 mg/ml	56.59
DCMSF	1.782	2 mg/ml	32.85
EASF	1.962	2 mg/ml	26.07
AQSF	1.777	2 mg/ml	33.04
ASA	2.448	0.10 mg/ml	7.80
Control	2.654		

Table 2. Effect of various extractives of *S. zeylanica Kuntze* of heat-induced haemolysis of erythrocyte membrane.

Sample code	ABS. Heat	ABS. Cold	Concentration	% Inhibition of haemolysis (Heat induced)
MESF	2.618	0.092	1 mg/ml	1.41
HSF	2.086	0.203	1 mg/ml	23.17
DCMSF	2.224	0.124	1 mg/ml	17.00
EASF	1.815	0.700	1 mg/ml	42.93
AQSF	1.272	0.370	1 mg/ml	60.5
ASA	2.433	1.242	0.10 mg/ml	84.41
Control	2.654			

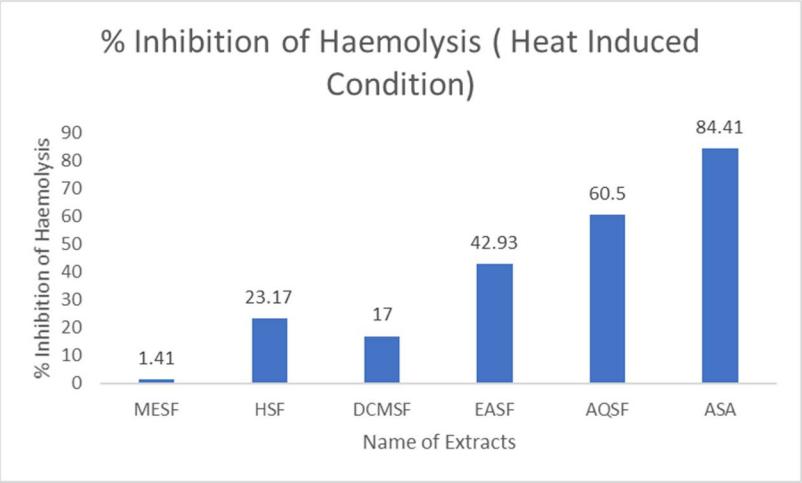

Figure 7. % Inhibition of haemolysis of different extractives of *S. zeylanica* on heat-induced condition.

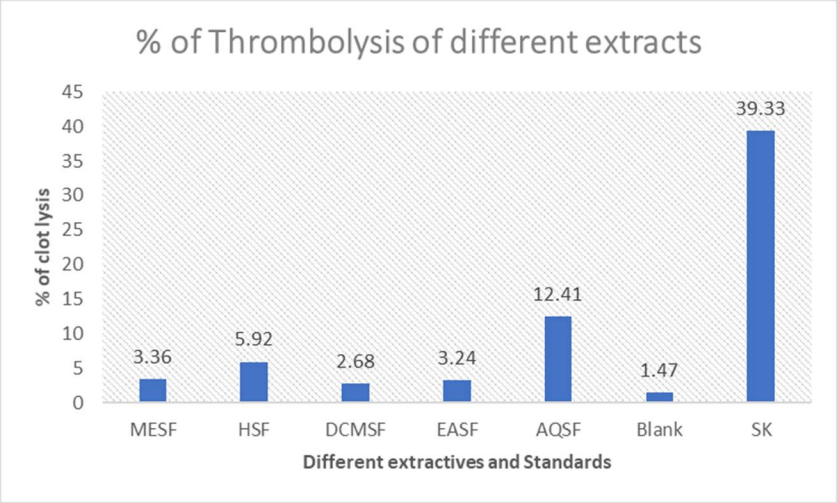

Figure 8. Percentage of thrombolysis of different extracts of *S. zeylanica* Kuntze

Conclusion

This research project investigates the medicinal properties of *Staurogyne zeylanica*. In summary, *S. zeylanica* Kuntze, a relatively understudied plant from the Acanthaceae family, showed promising activity as a medicinal plant with analgesic and anti-diarrheal properties. Aqueous extract exhibited notable inhibition of hemolysis under heat-induced conditions, alongside low thrombolytic activity. Recent studies indicate that this plant possessed a broad range of therapeutic properties. While it demonstrated potent pain relief and anti-diarrheal effects, further research is needed to identify specific compounds and dosages for optimal use. These findings highlight the pharmacological potential of *S. zeylanica* Kuntze and emphasize the need for further research, including clinical trials to fully validate and expand its therapeutic applications.

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