

Chemical and Biological Investigation of *Sanchezia nobilis* Leaves Extract

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ABSTRACT

The project work was designed to investigate the phytochemical and selected pharmacological activities (anti-diarrheal, analgesic, neuropharmacological behavior and anthelmintic) of leaves of *Sanchezia nobilis*. Hook. F. (Family: Acanthaceae). From its phytochemical analysis we found the presence of reducing sugar, combined reducing sugar, phenolic compounds, tannins, flavonoids, carbohydrates, gums, steroids, alkaloids, glycosides and terpenoids. *In vivo* anti-diarrheal activity was substantiated by prolongation of latent period and decrease in total number of stools. The extract produced 62.49% and 74.01% decrease in stool count at the doses of 250 and 500 mg/kg body weight respectively while the standard drug Loperamide decrease in stool count was found to be 87.05% at a dose of 3 mg/kg body weight. The leaves extract produced 32.7% and 41.78% inhibition of writhing at the doses of 250 mg/kg and 500 mg/kg body weight respectively while the standard drug Diclofenac Na was found to be 74.23% at a dose of 25 mg/kg body weight. The extract showed dose dependent CNS depressant activity by reducing the locomotor activity. Higher dose of this extract (500 mg/kg body weight) comparing with standard Diazepam exposed sedative effect potentially. The extract exhibited concentration dependent anthelmintic activity against *Paramphistomum cervi* using Albendazole (15 mg/mL) as standard. The paralysis occurred between 9.00 to 83.91 min and death occurred between 11.33 to 86.33 min which were comparable to standard drug Albendazole. So, the present study concluded that the extract is fortified with significant anti-diarrheal, CNS depression and anthelmintic activity and moderate analgesic activity.

Keywords: *Sanchezia nobilis*, anti-diarrheal, analgesic, neuropharmacological behavior, anthelmintic.

INTRODUCTION

The plants that possess therapeutic properties or exert beneficial pharmacological effect on the living body are generally known as “Medicinal Plant”. According to WHO, A medicinal plant is any plant which in on more of its organ, contains substances that can be used for therapeutic purposes or which is a precursor for synthesis of useful drugs. Medicinal plants may be defined as a group of plants that possess some special properties or virtues that qualify them as article of drugs and therapeutic

agents and are used for medicinal purposes.¹

Medicinal plants are important natural wealth of a country. They play a significant role in providing primary health care services to mainly rural people. They also serve as therapeutic agents and raw materials for the manufacturer of traditional and modern medicines.

Sanchezia nobilis (Acanthaceae) is a perennial evergreen shrub native to the tropical rainforest in central and south America. In the traditional medicine, plants of the genus *Sanchezia* are used as anti-tuberculosis, antitumor, anti-convulsant, cough sedative, and expectorant.^{2, 3} Previous study reported that *S. nobilis* contains benzyl alcohol, cinnamyl alcohol, flavonoid glycosides, matsutake alcohol glycosides, daucosterol, stigmasterol, '5,7-trihydroxy-3',5'-

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dimethoxyflavone, kaempferol-3-O- α -L-arabinofuranoside, and kaempferol-3-O- β -D-glucopyranoside.^{4,5}

Tropical region covers the largest biodiversity for growing plants in the world, which may have medicinal values. This project work was performed on the leaves of a tropical plant *Sanchezia nobilis*. *Sanchezia* is a genus of the family Acanthaceae. It is estimated to contain about 55 to 58 species. Members of this genus are shrubs, rarely small trees or herbs, occurring in the lowlands of tropical South and Central America.⁶ Examples for species well known from cultivation are *S. nobilis*, *S. parvibracteata* and *S. speciosa*. Some of the species are already well-known for their medicinal values & are used extensively but most of plants of tropical forests are unknown whether they possess active constituents or not, so proper scientific screening is required to evaluate these plants.

So, the aim of this project work was to search bioactive metabolites and to evaluate the pharmacological activities of the leaves of this plant.

MATERIALS AND METHOD

Plant collection and identification

For this present investigation the flowering shrub species *Sanchezia nobilis* was collected from Jessore, Khulna, during April, 2018. The species was identified by experts at Bangladesh National Herbarium, Mirpur, Dhaka, where the voucher specimen was no. **46857 DACB** which was submitted for future reference.

Extraction

The collected plants were dried by shade drying to ensure the active constituents free from decomposition. 300 gm of *S. nobilis* powder was taken in clean, flat-bottomed glass containers and soaked in 1500 ml of 96% ethanol and kept for a period of 14 days. The filtrate obtained (ethanol extract) was evaporated using rotary evaporator and dried. 17.19 gm. crude extract semisolid was obtained from 300 gm. of dried powder material. So, the obtained yield was 5.73%.

Animals

Young Swiss albino mice aged 4-5 weeks, average weight of 25-30 gm were used for pharmacological experiments. The mice were purchased from the animal house of Jahangirnagar University, Savar, Dhaka-1342. They were kept in the animal house of the Pharmacy Discipline, Khulna University, under standard laboratory condition (relative humidity (55-60)%, room temperature (25 \pm 2)⁰C and 12 hours (light: dark cycle) for period of 14 days prior to performing the experiment. The animals were provided with standard rodent food and tap water.

Drugs

The standard drugs loperamide, diclofenac sodium, diazepam and albendazole were purchased from local pharmacy shops in Khulna, Bangladesh.

Phytochemical test

The crude extract was subjected to preliminary phytochemical screening for the detection of major functional groups.⁷ We have done some phytochemical tests to detect the major phytochemical groups such as, Benedict's test, Fehling's test, ferric chloride test, flavonoid test, saponin test, Molish's test, sulphuric acid test, Meyer's test, Dragendroff's test, glycosides test, terpenoids test, xanthoprotein test and acidic compounds test. Then, the extract was screened for biological effects.

Determination of antidiarrheal test

Antidiarrheal activity was tested using the model of castor oil induced diarrhea in mice.⁸ All the mice were screened initially by giving 0.5 mL of castor oil and only those showing diarrhea were selected for the experiment. The test animals were randomly chosen and divided into four groups having five mice in each group. All reagents and samples were dissolved in DMSO. Control group received 1% tween 80 at the dose of 10 mg/kg body weight whereas, positive control group received the standard antimotility drug, loperamide at the dose of 50 mg/kg body weight as oral suspension. Group I and group II were considered as test groups and were treated with ethanol

extract of *S. nobilis* at the oral dose of 250 and 500 mg/kg body weight. In this study, the control vehicle and extract were administered orally 1 hour prior to oral administration of castor oil at the dose of 0.5 mL. Individual animal of each group was placed in separate cages having adsorbent paper beneath and examine for the presence of defecation after 4 hour of castor oil administration. The number of defecations, the number of diarrheal feces and percentage of inhibition of diarrheal feces were calculated.⁹

Determination of analgesic activity

The analgesic activity of *S. nobilis* was investigated using acetic acid induced writhing method in mice.^{10,11} Experimental animals were randomly selected and divided into four groups denoted as control group, positive control group and test group I and test group II consisting of five (05) mice in each group. Test samples, positive and negative control solution were given orally by using feeding needle. All reagents and samples were dissolved in DMSO. Control group received 1% Tween-80 at the dose of 10 mg/kg body weight and Positive control group received Diclofenac sodium at the dose of 25 mg/kg body weight. Test group I and Test group II were treated with test sample at the dose of 250 and 500 gm/kg body weight. A thirty minutes interval was given to ensure proper absorption of the administered substances. Then the writhing inducing chemical, acetic acid solution (0.7%) was administered intra-peritoneal to each of the animal of a group. After an interval of 5 minutes, which was given for absorption of acetic acid, number of writhing was counted for up to 15 minutes. The animals do not always perform full writhing. The incomplete writhing was taken as half-writhing, so two half-writhing were taken as one full writhing.

Determination of neuropharmacological behavior

Neuropharmacological activity of *S. nobilis* was investigated using open field model in mice. Experimental animals were randomly selected and divided into four

groups denoted as control group, positive control group and test group I and test group II consisting of five (05) mice in each group. All reagents and samples were dissolved in DMSO. Control group received 1% Tween-80 at the dose of 10 mg/kg body weight and Positive control group received Diazepam at the dose of 1 mg/kg body weight. Test group I and II were treated with test sample at the dose of 250 and 500 gm/kg body weight, respectively. After respective treatment, animals were placed individually in one of the corners of the square and the number of squares visited by the animals was counted for 3 min on 0, 30, 60, 90 and 120 min during the study period. The experiments were carried out in a sound attenuated room.¹²

Determination of anthelmintic activity

Anthelmintic activity of *S. nobilis* was determined according to Hossain *et al.*¹³ For this test four petridishes were taken denoted as Control group, Positive control group and test group I, test group II, test group III and test group IV consisting of six parasites (*Paramphistomum cervi*) in each Petri dish. All reagents and sample were dissolved in phosphate buffer saline (PBS). 10 mL of 0.1 % Tween-80 in PBS as negative control, albendazole at the dose of 150 mg/10 mL as positive control and suspension of the ethanol extract at the dose of 250 and 500 mg/10 mL was taken in different petridishes. Time taken for paralysis for each parasite (*Paramphistomum cervi*) was recorded when no movement was observed unless shaken vigorously. Time taken for death for each parasite was recorded after evaluating that the parasites did not move when shaken vigorously, dipped in warm water (50°C) or subjected to external stimuli. Anthelmintic activity is expressed as the time required for paralysis and death of parasites as compared to control.

STATISTICAL ANALYSIS

Student's t-test was used to determine significant differences between the control group and test group.

RESULTS**Phytochemical screening**

The phytochemical screening of the extract indicated the presence of some secondary metabolites of reducing sugar, combined reducing sugar, phenolic compounds, tannins, flavonoids, gum, alkaloids, steroids, glycosides, and terpenes. [Table 1]

Antidiarrheal activity

Antidiarrheal activity of *S. nobilis* was tested by castor oil induced diarrhea in mice. The extract caused an increase in latent period (143.0 min and 190.0 min) i.e., delayed the onset of diarrheal episode at the dose of 250 and 500 mg/kg body weight (b.wt), respectively as compared to the standard drug loperamide, where the mean latent period was 199.9 min [Figure 1]. Latent period means time between the receipt of dose and onset of diarrhea. Percent inhibition of defecation for *S. nobilis* at the doses 250 and 500 mg/kg b.wt was 62.49 ($p < 0.0002$) and 74.012 ($p < 0.0003$) respectively whereas loperamide showed 87.05% ($p < 0.0001$) inhibition of defecation [Figure 2]. [Table 2]

Analgesic activity

The ethanol extract of *S. nobilis* exhibited significant inhibition of writhing reflex by 32.7% ($p < 0.03$) and 41.78% ($p < 0.02$) at the doses of 250 and 500 mg/kg body weight respectively while the standard drug Diclofenac sodium exhibited significant inhibition of writhing reflex

by 74.23% ($p < 0.008$) at a dose of 25 mg/kg body weight [Figure 3] [table 3].

Neuropharmacological behavior

The number of squares crossed by mice at 0 min, 30 min, 60 min, 90 min & 120 min was 142.5, 103.7, 77.2, 64.5 & 35.8 respectively at the dose of 250 mg/kg. The number of squares crossed by mice at 0 min, 30 min, 60 min, 90 min & 120 min was 117.7, 93.0, 68.8, 36.6, 12.4 respectively at the dose of 500 mg/kg.

Diazepam exerted a sedative effect i.e., the number of squares crossed by mice at 0 min, 30 min, 60 min, 90 min and 120 min, were 79, 58.2, 46.4, 29.6 & 8.0 respectively [Figure 4] and [Table 4].

Anthelmintic activity:

The crude extract of *S. nobilis* showed less significant anthelmintic effect and dose dependent decrease in paralysis time and death time of the parasite. The time taken for paralysis at 25, 50, 100 and 200 mg/mL concentration were approximately 83.91, 34.83, 21.15, and 9.00 min respectively [Figure 5].

The time taken for death at 25, 50, 100 and 200 mg/mL concentration was approximately 86.33, 37.33, 23.83, and 11.33 min respectively [Figure 6].

Here, standard (Albendazole) showed approximately 6.58 min for paralysis and 8.15 min for death at the dose of 15 mg/mL against the parasite [Table 5].

Table 1: Results of phytochemical screen

Metabolites	Reducing sugar	Combined	Phenolic	Tannins	Flavonoids	Saponin	Gum	Alkaloids	Steroids	Glycosides	Xanthoproteins	Terpinoids	Acidic
Extract of <i>Sanchezia nobilis</i>	+	+	+	+	+	-	+	+	+	+	-	+	-

“+” indicates Presence; “-” indicates Absence

Table 2: Effect of *S. nobilis* on castor oil induced diarrhea in mice

Animal group	Treatment	Latent period (min) (Mean ± SD)	% inhibition of defecation (Mean ± SD)
Control	1% tween-80 solution in DMSO (10 mL/kg)	33.5 ± 4.384	
Positive control	Loperamide (3 mg/kg)	199.9 ± 7.212	87.05 ± 1.6829*
Test group I	Extract (250 mg/kg)	143.0 ± 4.243	62.49 ± 0.1980**
Test group II	Extract (500 mg/kg)	190.0 ± 14.142	74.015 ± 1.8173***

Values are expressed as mean ± SD (Standard Deviation); *P<0.0001, **P<0.0002, ***P<0.0003 vs control

Latent period means time between the receipt of dose and onset of diarrhea

Table 3: Effect of *S. nobilis* on acetic acid induced writhing in mice

Animal group	Treatment	Average writhing (mean ± SD) (%)	% inhibition of writhing (mean ± SD)
Control	1% tween-80 solution in DMSO (10 mL/kg)	34.7±2.97 (100%)	
Positive control	Diclofenac Na (25 mg/kg)	8.9±1.56 (25.77%)	74.225 ± 1.9728*
Test group I	Extract (250 mg/kg)	23.3±0.707 (67.30%)	32.6950 ± 3.7265**
Test group II	Extract (500 mg/kg)	20.1±0.424 (58.22%)	41.78 ± 3.5638***

Values are expressed as mean ± SD (Standard Deviation); *P<0.008, **P<0.03, ***P<0.02 vs control

Table 4: Effect of *S. nobilis* on neuropharmacological behavior of mice by open field model

Animal group	Treatment	No. of square crossed by mice (average ± SD)				
		0 min	30 min	60 min	90 min	120 min
Control	1% tween-80 solution in DMSO (10 mL/kg)	78.9 ± 19.386	83.3 ± 35.771	110.3 ± 42.93	101.2 ± 80.609	89.0 ± 3.39
Positive control	Diazepam (1 mg/kg)	79.0 ± 3.317	58.2 ± 2.58	46.40 ± 8.583	29.60 ± 2.702	8.0 ± 1.58
Test group I	Extract (250 mg/kg)	142.7 ± 22.538	103.7 ± 32.322	77.20 ± 22.577	64.5 ± 29.321	35.8 ± 26.32
Test group II	Extract (500 mg/kg)	117.7 ± 11.378	93.0 ± 20.034	68.8 ± 28.924	36.6 ± 23.072	12.4 ± 2.07

Values are expressed as mean ± SD (Standard Deviation)

Table 5: Paralysis time and death time at different concentration of *S. nobilis*

Animal group	Treatment	Time taken for paralysis (min) Average time ± SD	Time taken for death (min) Average time ± SD
Control	0.1 % Tween-80 in PBS	---	---
Positive control	Albendazole (15 mg/kg)	6.5850±0.1202	8.1500±0.4950
Test group I	Extract (25 mg/kg)	83.9150±2.7082	86.3350±2.3547
Test group II	Extract (50 mg/kg)	34.8350±1.1809	37.3350±0.9405
Test group III	Extract (100 mg/kg)	21.1500±2.6163	23.8350±1.1809
Test Group IV	Extract (200 mg/kg)	9.0000 ± 1.6546	11.3350 ± 1.8880

Values are expressed as mean ± SD (Standard Deviation)

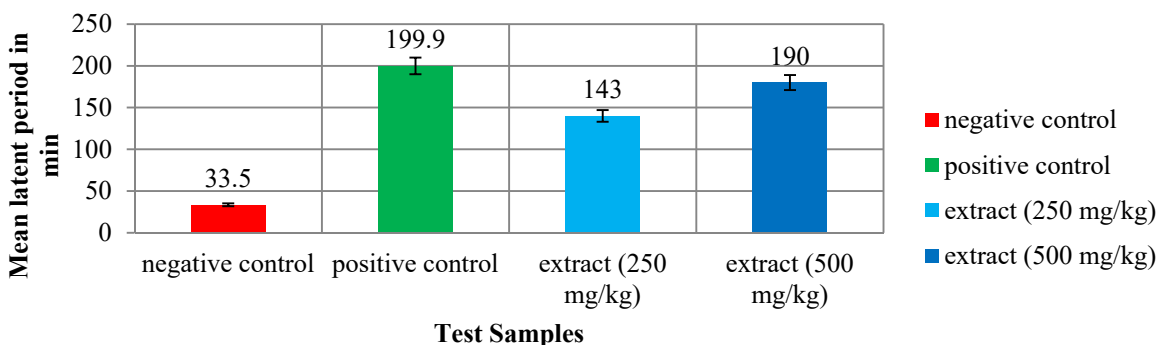


Fig 1: Effect of ethanolic extract of *S. nobilis* on prolongation of the latent period in castor oil-induced diarrheal episode in mice.

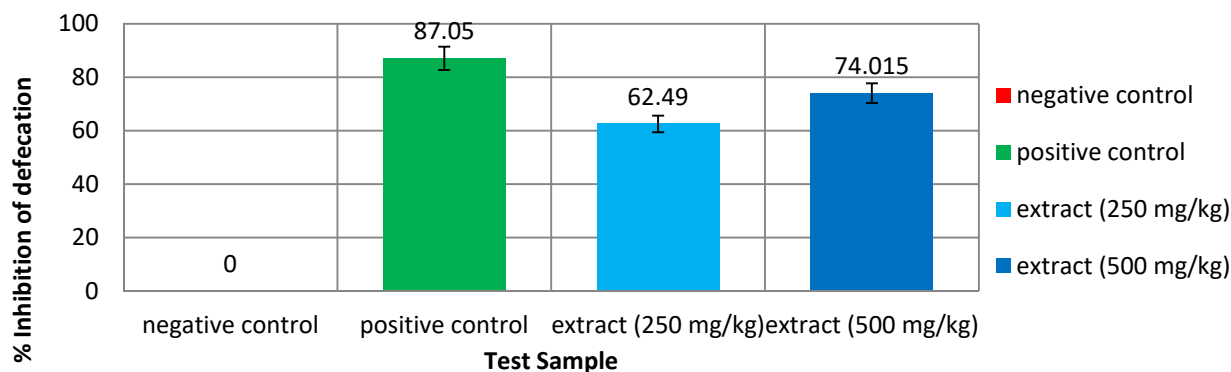


Fig 2: Percent inhibition of defecation by the ethanolic extract of *S. nobilis* in castor oil-induced diarrheal episode in mice.

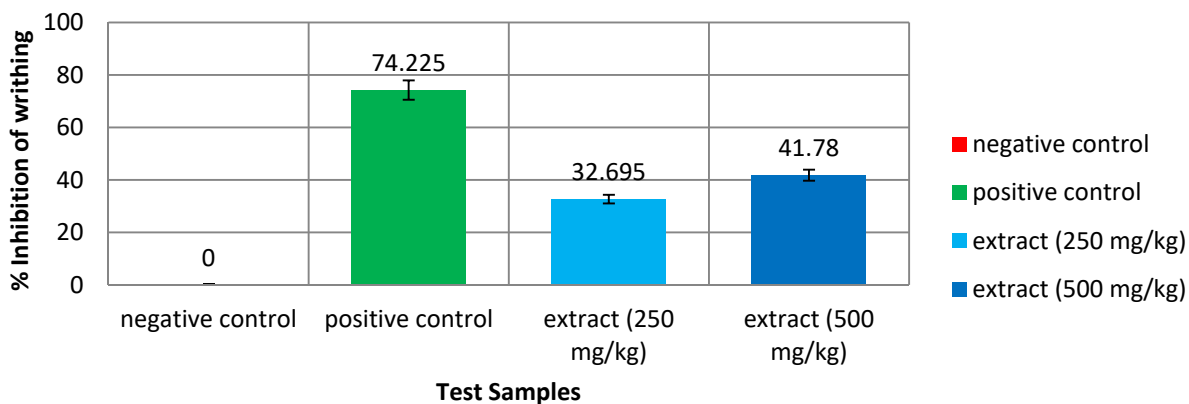


Fig 3: Percent inhibition of writhing vs. treatment with Diclofenac Na and *S. nobilis* ethanolic extract on acetic acid-induced writhing in mice.

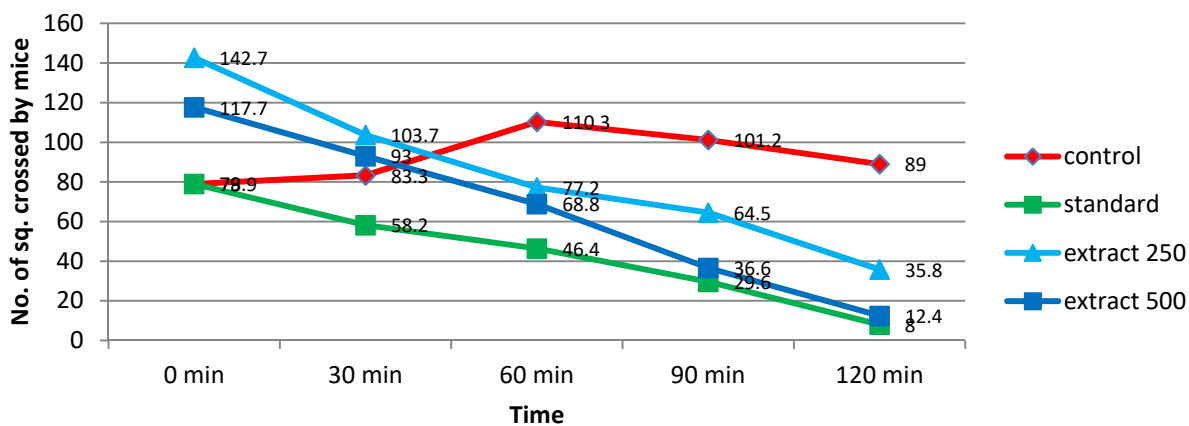


Fig 4: Comparison among different doses of *S. nobilis* ethanolic extract with the standard

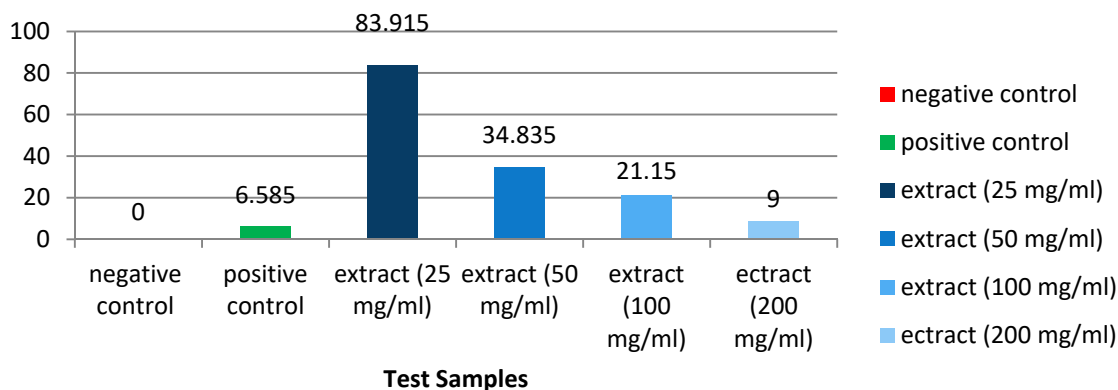


Fig 5: Figureical representation of the paralysis time by ethanolic extract of *S. nobilis*.

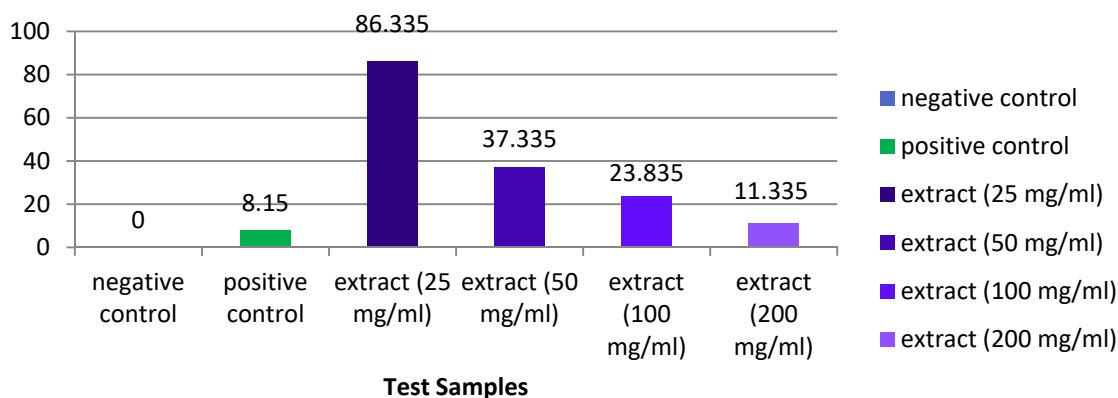


Fig 6: Figureical representation of death time for ethanolic extract of *S. nobilis*

DISCUSSION

The current work presented here was conducted to correlate the presence of phytochemical and pharmacological properties of *S. nobilis*.

Phytochemical screening was conducted to reveal the presence of major functional groups in the crude extract. The ethanol extract of *S. nobilis* was found to be rich in reducing sugars, phenolic compounds, alkaloids, flavonoids, tannins, glycosides, gums, steroids and terpenoids.

Castor oil is made up of 90% ricinoleate, active metabolites which are responsible for the diarrhea inducing properties, which diminishes Na^+ and Cl^- permeability in the intestine; it is also associated with

endogenous stimulation of prostaglandins release. The anti-diarrheal properties of medicinal plants were reported to be due to the presence of tannins, alkaloids, saponins, flavonoids, steroids, terpenoids and reducing sugars.¹⁴

In this investigation, *S. nobilis* extract exhibited anti-diarrheal activity (62.49- 74.02). The effect was comparable to loperamide (87.05%) which is one of the most widely used anti-diarrheal drug and it elicited its activity by antagonizing diarrhea induced by castor oil and prostaglandins, its therapeutic effect could also be due to its antimotility and its anti-secretory properties.¹⁵ Tannins and alkaloids have been known to make the intestinal mucosa more resistant to reduce secretion, therefore, inhibit diarrhea induced by castor oil. These

phytochemical groups are found in leaves of this plant. So, it can be said that the presence of tannins and alkaloids in the plant extract may be responsible for the anti-diarrheal activity.¹⁶

Acetic acid induced writhing test is well proposed method in evaluating the medicinal agents for the analgesic potential. Pain sensation in acetic acid induced writhing paradigm is elicited by producing a localized inflammatory response due to the release of free arachidonic acid from tissue phospholipids via COX, and producing prostaglandins specifically PGE₂ and PGE_{2 α} , and level of lipoxygenase products may also increase in peritoneal fluid.

These prostaglandins and lipoxygenase product cause swelling and agony by the cumulative capillary permeability and liberating endogenous substances that stimulate pain nerve endings. NSAIDs cause inhibition of COX enzyme in the peripheral tissues and affect the transduction mechanism of key afferent nociceptors.¹⁷

Our results of acetic acid-induced abdominal constriction assay demonstrated a prominent reduction in writhing reflex. The analgesic effect observed at 250 mg/kg & 500 mg/kg dose was comparable with the NSAID standard drug diclofenac sodium.

These findings strongly recommend that extracts of *S. nobilis* has peripheral analgesic activity and their mechanisms of action was not clear but may be mediated through inhibition of local peritoneal receptors via cyclooxygenase inhibition.

The extract of *S. nobilis* showed CNS depression activity by inhibiting the locomotion activity (less no. of squares crossed by mice at the fixed time duration). Diazepam (1 mg/kg) was used as the standard drug. All of these values were compared with the negative control group. The 500 mg/kg dose of *S. nobilis* extract showed more CNS depressant activity than the 250 mg/kg dose of

extract. In these tests, any agents with sedative properties will produce a decrease in the number of movements, interpreted as a decrease in curiosity of the new environment which is reversed for anxiogenic agents. The tested extracts showed an increase in CNS depressant activity during the 120 minutes of the study. The readings taken 120 minutes after the administration of 500 mg/kg dose, exerted close effect to that of the diazepam. Thus, it can be suggested that, the leaves of *S. nobilis* exert a sedative effect at the tested dose.¹⁸

Helminthiasis is a serious disease in human and poultry farming in South-East Asia. Tannins in several plants have been reported to show anthelmintic property by several investigators.¹⁹ Terpenes (lupeol found in leaves),¹⁹ from several traditional herbal plants, were shown to interfere with energy generation in helminths parasites by uncoupling oxidative phosphorylation or, binding to the glycoprotein on the cuticle of parasite causing death.^{19,20}

Hence further investigations and identification of the active principles might help in the discovery of new lead compounds, effective against various parasitic infections.

CONCLUSION

Preliminary phytochemical screening of *S. nobilis* leaves **ethanolic** extract revealed the presence of reducing sugars, phenolic compounds, flavonoids, tannins, glycosides, alkaloids, terpenes, steroids and gums which are valuable for pharmacological active metabolites. The results of the pharmacological investigations rationalize the uses of the plant in traditional medicine. Hence, more research is needed to find out the biologically active constituents in order to introduce this plant to the pharmaceutical industry for developing semi-synthetic and synthetic drugs with similar or better therapeutic properties for the welfare of human being.

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التحقيق الكيميائي والبيولوجي لمستخلص أوراق سانثيزيا نوبيليس

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ملخص

تم تصميم عمل المشروع للتحقيق في الأنشطة الكيميائية النباتية والأنشطة الدوائية المختارة) مضادات الإسهال والمسكنات والسلوك الدوائي العصبي والديدان (لأوراق *Sanchezia nobilis* صنارة صيد) F. الأسرة. (Acanthaceae): من تحليته الكيميائية النباتي وجدنا وجود اختزال السكر، والسكر المختزل، والمركبات الفينولية، والعضف، والفلافونويد، والكربوهيدرات، واللثة، والمنشطات، والقلويدات، والجليكوسيدات، والترينويدات. تم إثبات النشاط المضاد للإسهال في الجسم الحي بإطالة الفترة الكامنة وانخفاض في العدد الإجمالي للبراز. أنتج المستخلص انخفاضاً بنسبة 62.49% و 74.01% في عدد البراز بجرعات 250 و 500 مجم / كجم من وزن الجسم على التوالي بينما وجد أن انخفاض عقار Loperamide القياسي في عدد البراز بلغ 87.05% بجرعة 3 مجم / كجم من وزن الجسم. أنتج مستخلص الأوراق 32.7% و 41.78% تثبيطاً للتوي بجرعات 250 مجم / كجم و 500 مجم / كجم من وزن الجسم على التوالي بينما وجد أن العقار القياسي Diclofenac Na كان 74.23% بجرعة 25 مجم / كجم من وزن الجسم. أظهر المستخلص نشاط مثبط للجهاز العصبي المركزي يعتمد على الجرعة عن طريق تقليل نشاط القاطرات. جرعة أعلى من هذا المستخلص (500 ملغم / كجم من وزن الجسم) مقارنة بالتأثير المهدئ الذي يتعرض له الديازيبام القياسي. أظهر المستخلص نشاط طارد للديدان يعتمد على التركيز ضد *Paramphistomum cervi* باستخدام (Albendazole 15 مجم / مل) كمعيار. حدث الشلل بين 9.00 و 83.91 دقيقة وحدثت الوفاة بين 11.33 إلى 86.33 دقيقة والتي كانت مماثلة للعقار القياسي ألبيندازول. لذلك، خلصت الدراسة الحالية إلى أن المستخلص محصن بمضادات الإسهال، تثبيط الجهاز العصبي المركزي ونشاط طارد للديدان ونشاط مسكن معتدل.

الكلمات الدالة: سانثيزيا نوبيليس، مضاد للإسهال، مسكن، سلوك دوائي عصبي، طارد للديدان.

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