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Medicinal activity of *Vitex negundo* L. (Family: Lamiaceae) leaves extract: Assessment of phytochemical and pharmacological properties

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Abstract
The present study was performed to evaluate phytochemical groups, analgesic and neuropharmacological activities of ethanol extract of leaves of *Vitex nigundo* (Lamiaceae). Preliminary phytochemical screening of ethanol extract exhibited the presence of glycosides, flavonoids, carbohydrates, tannins, steroids and terpeniods. Here, the study of analgesic activity of *Vitex nigundo* was performed by acetic-acid induced writhing, Tail immersion and Hot-plate test. We found moderate activity which is comparable to the reference drug, Diclofenac-Na. We found potential effect in some neuropharmacological activities using swiss albino mice (body weight: 20-25 gm) as animal model. At the doses of 200 and 500 mg/kg body weight, the extract showed profound decrease in exploratory activity in a dose-dependent manner. It also showed sedative effect at the doses of 200 and 500 mg/kg body weight by a significant reduction in gross behavior. The presented phytochemical groups in this plant may be accountable for these pharmacological effects.

Keywords: *Vitex negundo*, phytochemical groups, analgesic activity, neuropharmacological activity

1. Introduction
Vitex negundo belongs to the family of Lamiaceae and grows as small tree with thin grey bark and green leaves. *Vitex negundo*, commonly known as the Chinese chaste tree, five-leaved chaste tree, or horseshoe vitex. It is a large aromatic shrub, densely whitish. It is widely used in folk medicine, particularly in South and South Asia [1]. The common name of *Vitex nigundo* is Nirgundi, Nishinda, Samalu. Whole plants are used in asthma, bronchitis, inflammations, eye diseases, leucoderma, spleen enlargement and painful teething. Dried fruits are used as vermifuge. Seeds are used in cutaneous diseases and leprosy. Flowers are prescribed in cholera, diarrhoea, fever and liver complaints. Leaves are effective in gonorrheal epididymitis, orchitis, vermifuge. Leaves are applied to rheumatic swellings of the joints and in sprains. Smoke from dried leaves relieves catarrh and headache. Root-bark is useful in rheumatism and irritable bladder. Root is beneficial in boils, cholic, dyspepsia, leprosy [2, 3]. The plant is found throughout India, Ceylon- Afghanistan, tropical Africa, Madagascar, China and Philippines. The constituents of this plant are sabinene, linalool, terpinen-4-ol, b-caryophyllene, a-guaine, globulol. Leaves contain alkaloids like nishindine, flavones, luteolin-7-glucoside, casticin, iridoid glycosides. Seeds contain hydrocarbons, β-sitosterol, benzoic acid and phthalic acid, anti-inflammatory diterpene, flavonoids and triterpenoids Aim of the present study was to determine the phytochemicals present in the whole plant and evaluate the analgesic and neupharmacological activities.

2. Materials and Methods
2.1 Collection and Identification
The plant *Vitex nigundo* was collected from Tangail, Bangladesh. The date of collection was October 2017 during daytime. The sample was identified by the experts of Bangladesh National Herbarium, Mirpur, and Dhaka (Accession No.: DACB 45115).

2.2 Preparation of the plant extract
Vitex nigundo leaves were first separated from undesirable materials. They were dried for one and half week in a shaded place. After drying, the plant part was grinded by Blender Machine (NOWAKE, JAPAN). Coarse powder was obtained after grinding. The powdered plant material (250 gm) was extracted with Ethanol (800 mL) by cold extraction method. The extractive was filtered through fresh cotton bed and finally with Whatman no. 1 filters paper.

The volume of the filter was concentrated under fan in 7 days. The amount of extract was 1.08 gm and the yield value was 0.432%.

2.3 Experimental Animal

For the experiment, both sexes of *Swiss Albino* mice of 3-4 weeks of age, weighing between 20 to 25 gm were collected from the Jahangirnagar University, Saver, Dhaka, Bangladesh. Animals were maintained under Standard Environmental conditions. Temperature (24.0 ± 1.00^0C), Relative humidity (55-65% and 12 hrs. light /12 hrs. dark cycle). The mice were given a week rest to get over the food and water restrictions incurred during transit and to get themselves adapted with the new environment of the laboratory, before being employed in any experiment.

2.4 Phytochemical Screening

The crude extract was subjected to preliminary phytochemical screening for the detection of major functional groups like alkaloids, reducing sugar, tannins, glycosides, flavonoids, carbohydrates, saponins etc. by standard method [4].

2.5 Test for Analgesic Activity

2.5.1 Acetic Acid Induced Writhing Test

The antinociceptive activity of the samples was studied using acetic acid-induced writhing model in mice [5]. The animals were divided into control, positive control, and test groups with five mice in each group. The animals of test groups received test samples at the doses of 200 and 500 mg/kg body weight. Positive control group received standard drug diclofenac-Na at the dose of 10 mg/kg body weight and negative control group was treated with DMSO at the dose of 10 mL/kg body weight. Test sample and DMSO were administered orally 30 min before intraperitoneal administration of 0.6% acetic acid but diclofenac-Na was administered 15 min before injection of acetic acid. After an interval of 5 min, the mice were observed for specific contraction of body referred to as 'writhing' for the next 10 min [6].

2.5.2 Tail Immersion Test

The procedure is based on the observation that morphine like drugs selectively prolong the reaction time of the typical tail withdrawal reflex in mice. The animals of the control, positive control and test groups were treated with Diclofenac-Na (5 mg/kg body weight), DMSO (0.02 mL/kg body weight) and test samples at the doses of 200 mg/kg and 500 mg/kg body weight respectively. 1 to 2 cm of the tail of mice was immersed in warm water kept constant at 55^0C. The reaction time was the time taken by the mice to deflect their tails. The first reading was discarded and the reaction time was recorded as a mean of the next three readings. A latency period of 20 sec was defined as complete analgesia and the measurement was stopped when the latency period exceeded to avoid injury to mice. The latent period of the tail-flick response was taken as the index of antinociception and was determined at 0, 30, 60, 90 and 120 min after the administration of the test drugs and standard [7].

2.5.3 Hot- plate test

Hot plate test was used to measure the response latencies based on the procedure describe by Eddy and Leimbach [8]. In this experiment hot plate was maintained at 50 ± 05^0C. The reaction time was recorded for animals pre-treated with DMSO (0.02 mL/kg 30 min before orally) as control or sample (200 mg/kg b.w. And 500 mg/kg b.w., 30 min before) Diclofenac-Na (5 mg/kg b.w. intrperitonially, 15 min before) which has used as a positive control group.

Animals were placed into the hot plate chamber and the time of latency was defined as the time period between the zero point, when the animal was placed on the hot plate surface and the time when animal licked its back paw or jumped off to avoid thermal pain. The latent period of response was taken as the index of antinociception and was determined at the pre-treatment. 30, 60, 90 and 120 min after the administration of the test drug and standard in the order to minimize the damage on the animal paws. The cut off time was taken as 20 seconds.

2.6 Neuropharmacological Test

2.6.1 Hole cross test

The most consistent behavioral change is a hyperemotional response to novel environmental stimuli. The aim of this study was to characterize the emotional behavior of mice using the hole-board test. The number of head-dips in the hole-board test in single-housed mice was significantly greater. A steel partition was fixed in the middle of a cage having a size of 30×20×14 cm. A hole of 3cm diameter was made at a height of 7.5cm in the center of the cage. Movement of the animals through the hole from one chamber to the other was counted for 3 minutes in this test. The observations were made on 0, 30, 60, 90 and 120 minutes after oral administration of the test drugs [9, 10].

2.6.2 Open Field Test

Open Field Test is clearly the most frequently used of all behavioural tests in pharmacology and neuroscience. Despite the simplicity of the apparatus, however, open field behaviour is complex. Consequently, it has been used to study a variety of behavioural traits, including general motor function, exploratory activity and anxiety-related behaviours. Open-field behavioral assays are commonly used to test both loco motor activity and emotionality in rodents. The test group received leaf extract of *Vitex nigundo* at the doses of 200 mg/kg and 500 mg/kg body weight orally whereas the control group received vehicle water [11].

2.6.3 Mouse light/dark box test

In light/dark box test the method was carried out as described by Hascoet and Bourinet [12] which consisted of a fully automated box monitored. An open-topped rectangular box (46×27×30 cm high) was divided into a small (18×27 cm) area and a large (27×27 cm) area with an opening door (7.5×7.5 cm) located in the centre of the partition at floor level. The small compartment was painted black and illuminated by a dim red light (60 W; 4 lx), whereas the large compartment was painted white and brightly illuminated with a 60-W (400 lx) light source. The compartments were equipped with infrared beam sensors (four in the white area, three in the black one). Each mouse was tested by placing it in the center of the white area, facing away from the dark one, and was allowed to explore the novel environment for 5 min and thereby enabling the detection of locomotion in each zone, time spent in each zone, latency of the first crossing from one compartment to the other, and shuttle crossings between both compartments. The data for these four parameters were directly collected. This test exploited the conflict between the animal's tendency to explore a new environment and its fear of bright light [12, 13].

3. Result and Discussion

The therapeutic benefits of medicinal plants provide us with traditional remedies from the ancient periods [14, 15]. *Thunbergia* genus were used traditionally for managing fever, asthma, bronchitis, inflammations, eye diseases, leucoderma, spleen enlargement and painful teething and numerous disorders. In the preliminary phytochemical analysis of leaves of *V. nigundo* extract exhibited the presence of glycosides, flavonoids, carbohydrates, tannins, steroids and terpeniods (Table 1).

Table 1: Result of Phytochemical group test

Phytochemical Groups	Result
Glycosides	+
Flavonoids	+
Carbohydrates	+
Saponins	-
Tannins	+
Reducing sugar	-
Steroids	+
Terpenoids	+
Gums	-

Here, + indicates Presence; – indicates Absence

The present study has established analgesic potential of *Vitex nigundo* using acetic acid-induced writhing test for visceral pain by central activity. Acetic acid-induced writhing is a highly sensitive and useful method for screening peripherally acting analgesic drugs. At the dose of 500 mg/kg body weight of *V. nigundo* extract caused antinociception against chemical induced pain in mice (Table 2). The sample was found to exhibit significant writhing response inhibitory effect.

Table 2: Effect of the *Vitex nigundo* on acetic acid-induced writhing in mice

Treatment	Dose (mg/kg b.w.)	Mean ± SEM	% of Inhibition
Control	0.1 mL/Mouse	31.2±4.92	0.00
Positive control	10 mg/kg	5.4±2.88	82.7
Sample	200 mg/kg	18.3±17.83	41.35
Sample	500 mg/kg	12.8±7.16	58.97

Values are expressed as Mean ±SEM (n=***5***); [Control (DMSO 0.1 mL/Mouse), Positive Control (Diclofenac-Na 10 mg/kg b.w.)

In our experiments, *Vitex nigundo* exhibited significant ($p<$***0.05)*** analgesic activity in tail immersion test (Table 3). It seems possible that the 500 mg/kg b.w. dose of the extract have more potent analgesic effect.

Table 3: Effect of the *Vitex nigundo* by tail withdrawal reflex in mice

Treatment	Dose (mg/kg b.w.)	Response Times (in seconds) Mean + SEM				
		0 min	30 min	60 min	90 min	120 min
Control	0.1 mL/mouse	4.84±0.50	3.57 ±0.26	2.65±0.33	3.8±0.397	4.33±0.878
Diclofenac-Na	5 mg/kg	3.25±0.79	2.66±0.22	4.08±0.53	3.35±0.45	5.59±1.13
Sample	200 mg/kg	3.29±0.66	2.47±0.49	4.88±***0.***90	3.05±0.32	4.73±***0.***42
Sample	500 mg/kg	3.32±0.37	2.41±0.20	3.25±***0.***23	3.1±***0***.42	4.05±***0.***95

Values are expressed as Mean ±SEM (n=***5***); [Control (DMSO 0.1 mL/Mouse), Positive Control (Diclofenac -Na, 5 mg/kg b.w.)

The sample displayed a potent and dose dependent analgesic activity (Table 4). The significant analgesic response was confirmed from hot plate test where reflex time was notably increased.

Table 4: Effect of the *Vitex nigundo* by Hot Plate test in mice

Test Group	Dose (mg/kg b.w.)	0 min	30 min	60 min	90 min	120 min
Control	0.1 mL/mouse	10.74±2.5	7.50±1.39	9.01±1.81	7.05±1.88	8.33±1.98
Positive Control	5 mg/kg	4.80±0.47	9.70±3.06	5.70±1.04	13.07±3.19	11.54±1.09
Sample	200 mg/kg	3.6±0.92	2.8±0.37	5.2±1.77	5.7±0.99	8.7±2.62
Sample	500 mg/kg	7.24±1.66	3.65±0.53	6.85±1.09	6.7±0.54	13.3±3.35

Values are expressed as Mean ± SEM, (n=5) [Control: DMSO (0.1 mL/mouse) Positive Control: Diclofenac -Na (5 mg/kg b.w.)

The phytochemical groups may exert analgesic activity by inhibiting the synthesis, release, and/or antagonizing the action of pain mediators at the target sites. The recognized phytochemical groups namely flavonoids and tannins in *V. negundo* leaves extract may be responsible for analgesic activity both centrally and peripherally [16, 17]. The extract revealed potential analgesic activity displayed by dose dependent inhibition of writhing, and reflex time as compared to control group. The feasible mechanism may be the inhibition of prostaglandins (PGE_2 and $PGE_{2\alpha}$) and bradykinin synthesis or anatomization the action of these substances [18] and by increasing hot plate latency as well as tail withdrawal response. The fundamental antinociceptive activity of *V. negundo* may due to its effect on μ-opioid receptors of spinal as well as the supraspinal system. *V. negundo* containing compounds may lessen the activity of adenylyl cyclase which is accountable for the Ca^{2+} influx. The antinociceptive effect exerts through hyperpolarization of the nerves by decreasing cAMP level, K+ efflux, and subsequently.

The most important step in evaluating drug action on CNS is to perceive its effect on locomotors activity of the animal model. The activity is a measure of the level of excitability of CNS and any decrease may be closely related to sedation resulting from depression of the central nervous system. From the results of hole cross test it shows that activity is increased when the concentration of sample is increased (Table 5).

Table 5: Effect of methanol extract of the leaf of *Vitex nigundo* on Hole Cross test

Group	Route of Administration	Observation				
		0 min	30 min	60 min	90 min	120 min
Control	Oral	10.8±1.65	7±0.83	4± 0.31	2.6±0.4	3.4±1.02
Diazepam	i.p	6.4±1.12	3.4±0.50	3.6± 0.81	3.4±1.02	1.8±0.86
VN -1	Oral	16.2±1.49	8.6±1.50	7.8±0.48	8.6±1.07	8±1.22
VN -2	Oral	13±1.41	10.8±1.59	5.8±1.24	5.6±0.92	4.2±1.06

Values are expressed as Mean±SEM (n=***5***); [Control (DMSO, 0.1 mL/Mouse), Positive Control (Diazepam, 1 mg/kg b.w.), VN-1 = *Vitex nigundo* (200 mg/kg b.w.), Vn- 2 = *Vitex nigundo* (500 mg/Kg b.w.)]

The extract of the leaves of *Vitex nigundo* not significantly decreased the locomotors activity as showed by the results of the open field test.

Table 6: Effect of leaf extract of *Vitex nigundo* on Open Field test

Group	Route of Administration	Observation				
		0 min	30 min	60 min	90 min	120 min
Control	Oral	63±4.70	12.8±2.95	14.4±5.46	19.4±2.65	24.8±7.57
Positive control	i.p	43.2±5.20	17.8±5.40	36.2±12.88	24.4±7.31	18.4±5.57
Group -1	Oral	60.2±15.60	43.6±8.50	34.8±9.93	25.4±9.91	22.2±8.66
Group-2	Oral	81.8±11.48	44.8±7.49	48.2±1.65	35.8±3.70	23±7.96

The mice of Group-1 (200 mg/kg b.w.) spent maximum time in light area and the mice of Group-2 (500 mg/kg b.w.) spent maximum time in dark area. Number of entry in dark area is greater than in light area of both VN-1 and VN-2 (Table 7).

Table 7: Effect of the *Vitex nigundo* leaves on Light/dark box test

Group	Route of Administration	No. of entry in Light area	Time in Light area (seconds)	No. of entry in Dark area	Time in Dark area (seconds)
Control	Oral	4.4±1.63	60.2±16.83	4.2±1.59	239.8±16.83
Positive Control	i.p	1.6±0.50	1.44±64.97	1.6±0.40	1.55±64.97
VN-1	Oral	8±1.64	47±15.67	7±1.64	252.2±15.67
VN-2	Oral	10±0.83	17.6±2.92	9.2±0.91	282.4±2.92

Values are expressed as Mean ±SEM (n=***5***); [Control (DMSO, 0.1 mL/Mouse), Positive Control (Diazepam, 1 mg/kg b.w.), VN-1 = *Vitex nigundo* (200 mg/kg b.w.), VN-2 = *Vitex nigundo* (500 mg/Kg b.w.)]

In open field test, the locomotion effect of *V. negundo* showed that decreased number of squares crossed by mice was dose-dependent activity at second (30 min) to fifth (120 min) during observation. According to hole cross test, the behavioral state of mice diminished the number of hole cross (from second to fifth observation) convinced us about the sedative activity of *V. negundo*. These study also demonstrated that the sedative response of *V. negundo* effect almost similar to diazepam. The dose-dependent sedative effects also observed in the thiopental sodium-induce sleeping test. This validated model suggests that the activity of TS on gamma-aminobutyric acid (GABA) mediated hyperpolarization of CNS [19]. Light-dark box test is another popular evaluation approach due to its methodological simplicity. *V. negundo* treated mice selected light part to spend more time instead of dark part of the light-dark box in a dose-dependent manner. Time spent in light part express the anxiolytic-like behavior of mice. Diazepam treated mice also showed same effects when compared with control. The presented phytochemical groups namely glycosides, flavonoids, and tannins in *V. negundo leaves* extract may be responsible for CNS depressant activity [20].

4. Conclusion

Based on the result of the present study, it can be concluded that the leaves of crude plant extracts of *Vitex nigundo* possesses potential analgesic property and moderate neoropharmacological property. Various phytochemical constituents like alkaloids, glycosides, flavonoids, carbohydrates, tannins and steroids present in the plant, as evident from phytochemical analysis. At the dose of 500 mg/kg body weight, notable analgesic activity was observed from writhing, hot plate and tail immersion test. Therefore, the present study provides the evidences to upkeep the traditional use of this plant in folk medicine. But further research is desired to find out its individual phytoconstituents in order to familiarize this plant part in pharmaceutical industries for emerging semi-synthetic and synthetic drugs with similar or better therapeutic properties for the well-being of human.

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