Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
Impact Factor (RJIF): 6.35
www.phytojournal.com
JPP 2026; 15(1): 230-239
Received: 14-11-2025
Accepted: 13-12-2025

Shaikat Mallick
Department of Pharmacy, Stamford University Bangladesh, Siddeswari Road, Dhaka-1217, Bangladesh

Afia Ferdous
Department of Pharmacy, Stamford University Bangladesh, Siddeswari Road, Dhaka-1217, Bangladesh

MD Abdul Mannan
Department of Pharmacy, Stamford University, Siddeswari Road, Dhaka-1217, Bangladesh

MD Azizul Haque
Department of Pharmacy, Stamford University Bangladesh, Siddeswari Road, Dhaka-1217, Bangladesh

MD Shafiqul Islam
Department of Pharmacy, Stamford University Bangladesh, Siddeswari Road, Dhaka-1217, Bangladesh

Corresponding Author:
MD Abdul Mannan
Department of Pharmacy, Stamford University, Siddeswari Road, Dhaka-1217, Bangladesh

Investigation of phytochemical and pharmacological activities of the ethanolic extract of *Nyctanthes arbor-tristis* leaves in swiss albino mice

Shaikat Mallick, Afia Ferdous, MD Abdul Mannan, MD Azizul Haque and MD Shafiqul Islam

DOI: https://www.doi.org/10.22271/phyto.2026.v15.i1c.15727

Abstract
Background: In Bangladesh, *Nyctanthes arbor-tristis* is frequently referred to as "Night Jasmine." Numerous ailments, including sciatica, rheumatism, internal worm infections, chronic fever, laxative, diaphoretic, and diuretic, are treated with the herb. The current study's goal was to use animal models to examine the antinociceptive effects of an ethanolic extract of *Nyctanthes arbor-tristis* leaves (EENA).
Methods: A wide range of mouse models of acute pain, including the hot plate, tail immersion, acetic acid-induced writhing, formalin, and glutamate-induced tests, were used to measure the antinociceptive activity. The conventional medications were administered intraperitoneally as diclofenac sodium (10 mg/kg) and morphine sulphate (5 mg/kg). Oral dosages of 50, 100, and 200 mg/kg of EENA were given, whereas the control group was given deionised water (0.1 mL/mouse, P.O.). These techniques evaluate potential antinociceptive substances or plant extracts using traditional nociception models.
Results: According to the current research, EENA strongly reflected the antinociceptive activity of all chemical and heat-induced pain models in mice. In contrast to thermal-induced nociception, 200 mg/kg showed a substantial (*p<0.05) ability to prolong the response of latency to pain in hot plate and tail immersion tests. The writhing test caused by acetic acid showed inhibitions of 13.31%, 43.42%, and 71.01%, respectively. The extract suppressed 15.00%, 35.00%, and 56.43% of the first phase of formalin-induced nociception. The extract showed 23.28%, 43.84%, and 57.54% licking in the late phase as compared to the control group. The glutamate-induced nociception test was significantly inhibited by the plant extract. 16.38%, 37.93%, and 61.21% of licking was inhibited during the first phase. When compared to the control, another phase resulted in a substantial inhibition of 30.28%, 53.21%, and 64.22%, respectively (***p<0.001).
Conclusions: It was discovered that *Nyctanthes arbor-tristis* leaf ethanolic extract has strong antinociceptive properties. According to the current study, the extract may be used in place of an analgesic medication. To determine the true molecular mechanism of the active ingredients in the leaf extract, more research is required.

Keywords: *Nyctanthes arbor-tristis*, extract, antinociceptive, pain, nociception models

Introduction
Pain is a warning signal that is mainly protective in nature, but it may also be excruciating and debilitating [1]. When the body is subjected to an unpleasant stimuli, pain results. In practice, nonsteroidal anti-inflammatory medications are frequently used to manage pain [2]. Pain arises when the body is exposed to a nociceptive stimulus. The wounded tissue is exposed to endogenous pain-producing substances such histamine, 5-HT, quinine, acetylcholine, lactate, and K+, as well as pain-modifying substances like prostaglandins and leukotrienes. Prostaglandins increase the severity and duration of pain by sensitising afferent nerve terminals to the aglycogenic power of the chemicals that cause pain [3]. The use of these medications as analgesics has not always been effective due to their negative side effects, such as stomach lesions brought on by NSAIDs and tolerance and dependency brought on by opiates. The process of developing new drugs is extensive, costly, time-consuming, and risky [4]. Conversely, a number of plant-based medications have been used for antiquity without any negative side effects. Therefore, it is imperative that new medicinal plants be introduced in order to provide more affordable and effective medications. Plants are a rich natural supply of beneficial substances that might be used as a starting point for the creation of new medications [5].

The effectiveness of plant-based medications used in traditional medicine has received a lot of attention since they are inexpensive, have few adverse effects, and, according to the WHO, around 80% of the world's population still primarily uses plant-based medications [6].

A little, revered ornamental tree is *Nyctanthes arbor-tristis* Linn. The fragrant white blossoms of the shrub are well known across the nation. It is frequently referred to as "Night Jasmine" [7-9]. It is indigenous to Southern Asia, extending from northern India and Southeast Thailand to northern Pakistan and Nepal. It is found in parts of Jammu and Kashmir, Nepal, Bengal, and Tripura, the eastern part of Assam, the central region, and Godavari in the south of India, where it grows in the outer Himalayas [10]. The powdered leaves are traditionally used as an expectorant and to treat malaria and rheumatic joint pain. The bark is used to cure bronchitis and snakebite. In Ayurvedic medicine, the leaves are widely used to treat a variety of conditions, including rheumatism, sciatica, internal worm infections, chronic fever, laxative, diaphoretic, and diuretic [11-12]. To alleviate cough, leaf juice is combined with honey and administered three times a day. Leaf paste is administered with honey to treat diabetes, high blood pressure, and fever [13]. Loss of appetite, piles, intestinal worms, liver and biliary problems, persistent fever, stubborn sciatica, rheumatism, and fever with rigours are all treated with the leaf juice [14]. Alopecia and anthelmintics are two uses for the seeds. The powdered seeds are used to treat skin conditions, piles, and scurfy scalp affections [15]. In addition to mannitol, astringent, resinous substances, ascorbic acid, colouring materials, sugar, and traces of an oily substance, tannic acid, methyl salicylate, carotene, an amorphous resin, and volatile oil, the leaves of *Nyctanthes arbor-tristis* contain an alkaloidal principle known as nyctanthine. 12-16% of the pale yellow brown fixed oil, which contains glucosides of linoleic, oleic, lignoceric, stearic, palmitic acid, and sitosterol, is extracted from seed kernels. Essential oils, colouring materials, mannitol, tannin, and glucose are all found in flowers. Alkaloids, tannins, and glucosides make up roots [16-19]. A glycoside and two alkaloids one soluble in water and the other in chloroform are found in the bark. The oesophageal ciliary motility is stimulated by the water-soluble alkaloid, but not by the chloroform-soluble alkaloid [20]. Through *in vitro* and *in vivo* experiments, the purported traditional medicinal uses have been scientifically proven. The leaves' ethanolic, aqueous, and hydroalcoholic extracts were tested for their antibacterial properties [21]. It was discovered that the water-soluble component of the leaves' alcoholic extract exhibited antihistaminic properties [22]. Researchers have tried to determine whether infusing rats with hot flowers of *Nyctanthes arbor-tristis* has a sedative effect [23]. The leaves' methanolic extract has been shown to have antimalarial, antistress, nootropic, and anxiolytic properties [24-25]. Alcoholic leaf, flower, stem, and fruit extracts were investigated for their anti-inflammatory, analgesic, antipyretic, and ulcerogenic properties [26-27]. The extract has demonstrated antiallergic, antifungal, antihepatotoxic, antitrypanosomial, anti-influenza, and antiedema activity [30-37]. Arbortristosides from its leaves modulate murine peritoneal macrophages and intracellular killing of *Candidia albicans*; consistent depletion of factor-Z in the plasma of soluble protein [28-29].

The current study was conducted in light of folk claims regarding *Nyctanthes arbor-tristis* in order to explore the plant's strong antinociceptive effects and demonstrate the phytochemical and pharmacological potential of ethanolic extract using a variety of animal models.

Methods

Plant material and extraction

The Principal Scientific Officer of the Bangladesh National Herbarium in Mirpur, Dhaka, Bangladesh, verified the authenticity of the *Nyctanthes arbor-tristis* leaves that were gathered for this study from Bagharpara in Jashore, Bangladesh. For future use, the number is placed in the herbarium. For five days, the leaves were left out of direct sunlight and allowed to dry at room temperature. The plant samples were blended into a fine powder and then soaked in ethanol for seven days. The extract was then gathered, and a rotary evaporator was used to remove all of the solvent. All experimental investigations were conducted using the 9.80 g extract (Yield 3.92% w/w) that was obtained.

Animals

For this investigation, Swiss albino mice (20-25 g) of both sexes were employed. The animals were acquired from Jahangirnagar University's Pharmacology Laboratory in Savar, Dhaka, Bangladesh. They were kept in polyvinyl cages with soft wood bedding and standard environmental conditions, including room temperature (25±2 ˚C), relative humidity (55-65%), and a 12-hour light/dark cycle. The Institutional Animal, Medical Ethics, Biosafety, and Biosecurity Committee (SUB/IAEC/17.02) of Stamford University Bangladesh gave its approval to the study protocol. International guidelines for the care and use of laboratory animals were followed, and the institutional animal ethical committee approved the set of rules used for the animal experiment.

Drugs and treatments

As standard medications, morphine sulphate (5 mg/kg) and diclofenac sodium (10 mg/kg) were used in hot plate, tail immersion, acetic acid-induced writhing, formalin, and glutamate-induced nociception tests. The drugs were injected intraperitoneally (I.P.) 15 minutes prior to the experimental mice in each experiment. The animals in the control group received deionised water (0.1 mL/mouse, P.O.), while the ethanolic extract of *Nyctanthes arbor-tristis* was given orally 30 minutes prior to the experiments (apart from the hot plate and tail immersion tests) at doses of 50, 100, and 200 mg/kg.

Phytochemical analysis

Using recognised techniques, the extract was qualitatively screened for alkaloids, flavonoids, saponins, tannins, cardiac glycosides, carbohydrates, reducing sugars, proteins, terpenoids, and steroids [38].

Test for alkaloids

A test tube was filled with 0.2 ml of diluted hydrochloric acid and 2 ml of the test sample. Mayer's reagent (1 ml) was then added. Alkaloids were recognised by the precipitate's yellowish buff colour.

Test for flavonoids

A little quantity of the plant extract solution was mixed with a few drops of strong hydrochloric acid. The presence of flavonoids is immediately indicated by the production of a red colour.

Test for saponins
In a test tube, 5 ml of the test sample and 5 ml of deionised water were vigorously shaken. The presence of saponins is indicated by the formation of stable foam.

Test for tannins
A test tube was filled with about 5 ml of the test sample and 1 ml of a 10% lead acetate solution. The presence of tannins was shown by the production of a yellow precipitate.

Test for cardiac glycosides
A test tube was filled with 2 ml of the test sample, 1 ml of distilled water, and a few drops of sodium hydroxide solution. When cardiac glycosides are present, a yellow hue develops.

Test for carbohydrates
A test tube was filled with 2 ml of the test sample and 2 ml of concentrated sulphuric acid. At the interphase of the two layers, a crimson or reddish violet ring was seen. It shows that there are carbohydrates present.

Test for reducing sugar
Fehling's solutions and 5 ml test sample were placed to a test tube, which was then brought to a boil for a few minutes. The lack of reducing sugars is shown by the absence of brick red precipitation.

Test for proteins
A few drops of strong nitric acid solution were added to a 1 ml test sample. Proteins are indicated by the yellow colour form.

Test for terpenoids
In a test tube, 2 ml of the test sample were dissolved in 2 ml of chloroform and evaporated until completely dry. After that, 2 ml of concentrated sulphuric acid were added, and the mixture was heated for around two minutes. Terpenoids are indicated by a greyish hue.

Test for steroids
In a test tube, 1 ml of chloroform was used to dissolve 10 mg of extract. 1 ml of strong sulphuric acid was then added. The presence of steroids is indicated by the development of a reddish brown colour.

Acute toxicity test
The test mice were split up into six groups, each with five mice. The control group was given deionised water (0.1 mL/mouse, P.O.), while the mouse groups were given *Nyctanthes arbor-tristis* leaf extract orally at doses of 100, 200, 500, 1,000, 1,500, and 2,000 mg/kg body weight. Immediately after dosing, the animals were observed continuously for the first 4 hrs for any behavioral changes. Thereafter, they were then kept under observation up to 14 days to find out the mortality [39].

Antinociceptive activity
Hot plate test
As stated by Woolfe and McDonald [40], the hot-plate test was used to measure antinociceptive activity. It is not harmful to the skin, but the mice's paw is extremely sensitive to heat. Hot plate delay was the reaction that manifested as paw withdrawal. After that, each animal was put on Eddy's hot plate, which was maintained at a temperature of 52±0.5° C. The latency time was measured at 0, 30, 60, 90, and 120 minutes following the administration of the test solution, and those that demonstrated a reaction time of less than 20 seconds were put on the hot plate. The control group was given 0.1 mL of deionised water per mouse, P.O. The standard medication was morphine sulphate (5 mg/kg, I.P.). *Nyctanthes arbor-tristis* ethanolic extract was administered to the test groups at doses of 50, 100, and 200 mg/kg body weight (P.O.). At the aforementioned times, the latency was noted. The% analgesic activity was estimated using the following method [41].

Percentage of protection = (Drug latency-Base line latency / Base line latency) × 100.

Tail immersion test
The purpose of the tail immersion test was to assess *Nyctanthes arbor-tristis*'s central antinociceptive activity. It was documented by Cha, *et al*. [42] with minor modifications. Two-thirds of the animal's tail was submerged in hot water that was 54±1 °C after it was handled carefully. Every mouse functioned as its own controller. A stopwatch was used to measure the animal's reaction time when it was administered extract (50, 100, and 200 mg/kg, P.O.), control (0.1 ml/mouse, P.O.), or morphine sulphate (5 mg/kg, I.P.) at 0, 30, 60, 90, and 120 minutes. The cut-off time was set at 20 s to prevent tissue damage. The following formula was used to determine the results as a percentage of the maximal possible effect (%MPE).

%MPE = [(Post drug latency-pre drug latency)/(Cut off period-pre drug latency)] × 100

Acetic acid-induced writhing test
The acetic acid-induced writhing test was conducted using a slightly modified version of the procedure outlined by Zakaria *et al*. [43]. Deionised water (0.1 mL/mouse, P.O.), diclofenac sodium (10 mg/kg, I.P.), or ethanolic extract of *Nyctanthes arbor-tristis* (50, 100, and 200 mg/kg body weight, P.O.) were administered to the five mice. In order to induce a typical stretching reaction, all mice received an intraperitoneal injection of 0.6% acetic acid thirty minutes later. Mice were placed in separate cages five minutes after receiving an injection of acetic acid, and the number of writhes in each group was tallied and meticulously documented for thirty minutes. The% suppression of writhes was used to calculate the analgesic activity.

Formalin-induced paw licking test
With a few minor adjustments, the test was conducted as previously reported by Zakaria *et al*. [43]. The right hind paw's sub-plantar area received an injection of 20 µL of 2.5% formalin. Five groups of five mice each were created from the mice (n=5). Sixty minutes before to the formalin injection, mice were given either ethanolic extract of *Nyctanthes arbor-tristis* (50, 100, and 200 mg/kg body weight, P.O.), diclofenac sodium (10 mg/kg, I.P.), or deionised water (0.1 mL/mouse, P.O.). Each mouse was put in its own observation room and kept under observation for thirty minutes. One measure of the pain response was the duration of the injected paws licking and biting reactions. The analgesic impact was assessed in two stages. The first five minutes following formalin injection were used to document the early phase (0-5 min), while the final 15 minutes were used to record the late phase (15-30 min).

Glutamate-induced nociception

The technique outlined by Beirith *et al.* [44] was used to assess the involvement of glutamate receptors. The mice were given ethanolic extract of *Nyctanthes arbor-tristis* (50, 100, and 200 mg/kg, P.O.), deionised water (0.1 mL/mouse, P.O.) to the control group, and diclofenac sodium (10 mg/kg I.P.) to the reference group. 20 µL (10 µmol/paw) of glutamate was injected into the ventral surface of the mice's right hind paw 30 minutes after the treatments. The analgesic impact was assessed in two stages. The first 5 minutes following glutamate injection were used to capture the early phase (0-5 min), and the final 15 minutes were used to record the late phase (15-30 min). As a sign of nociception, their behaviour response injected paw licking was noted.

Statistical analysis

The data were shown as mean ± SEM (standard error of mean). Using the Statistical Package for the Social Sciences (SPSS) software (version 18.00), one-way analysis of variance (ANOVA) and Dunnett's post hoc test were used for statistical analysis. *p<0.05 compared to the control was deemed statistically significant.

Results

Phytochemical screening

To identify the primary active moiety, a preliminary phytochemical test was performed on the ethanolic extract. Alkaloids, carbohydrates, flavonoids, glycosides, protein, saponins, terpenoids, steroids, and tannins were found in the ethanolic leaf extract of *Nyctanthes arbor-tristis*, according to the results of many chemical tests.

Acute toxicity test: The ethanolic extract of *Nyctanthe arbor-tristis* leaves did not exhibit any toxic symptoms, physical abnormalities, or behavioural changes over the 14-days observation period. Additionally, when the extract was given at a dose of 2000 mg/kg body weight, there was no mortality within 72 hours. Up to a maximum dosage of 2000 mg/kg, the extract was deemed safe.

Table 1: *Nyctanthes arbor-tristis* ethanolic extract preliminary qualitative phytochemical screening (EENA)

Extract	EENA
Alkaloid	+
Flavonoid	+
Glycoside	+
Carbohydrate	+
Steroid	+
Tannin	+
Reducing Sugar	-
Saponin	+
Terpenoids	+
Proteins	+

EENA=Ethanolic Extract of *Nyctanthes arbor-tristis*; (+): Present; (-): Absent

Antinociceptive activity

Hot plate test

In Figure 1 and Table 2, the ethanolic extract of *Nyctanthes arbor-tristis* showed a substantial antinociceptive effect at dosage of 200 mg/kg (*p<0.05). At 200 mg/kg, the maximum reaction time of 16.80±0.86 s was recorded after 120 minutes. Furthermore, compared to the control group, morphine (5 mg/kg) demonstrated a substantial antinociceptive effect (*p<0.05).

Table 2: Antinociceptive effects of leaf extract of *Nyctanthes arbor-tristis* on hot plate test

Treatment	Dose (mg/kg)	Latency of nociceptive response (in seconds)				
		0 min	30 min	60 min	90 min	120 min
Control	0.1 ml/ mouse	04.20±0.20	06.00±0.44	08.80±0.37	10.60±0.87	13.80±0.73
Morphine	5	06.00±0.70	08.20±0.37	11.60±0.51*	14.20±0.86*	17.00±0.70*
EENA	50	04.60±0.24	05.80±0.37	08.60±0.74	10.40±0.51	12.00±0.83
EENA	100	06.40±0.74	07.40±1.03	09.60±0.67	13.20±0.66	15.80±0.37
EENA	200	06.60±0.60	07.80±1.06	11.80±0.58*	13.80±0.80*	16.80±0.86*

Values are presented as mean ± SEM (N=5). EENA=Ethanolic extract *of Nyctanthes arbor-tristis;* *p<0.05 compared with the control group (Dunnett's test).

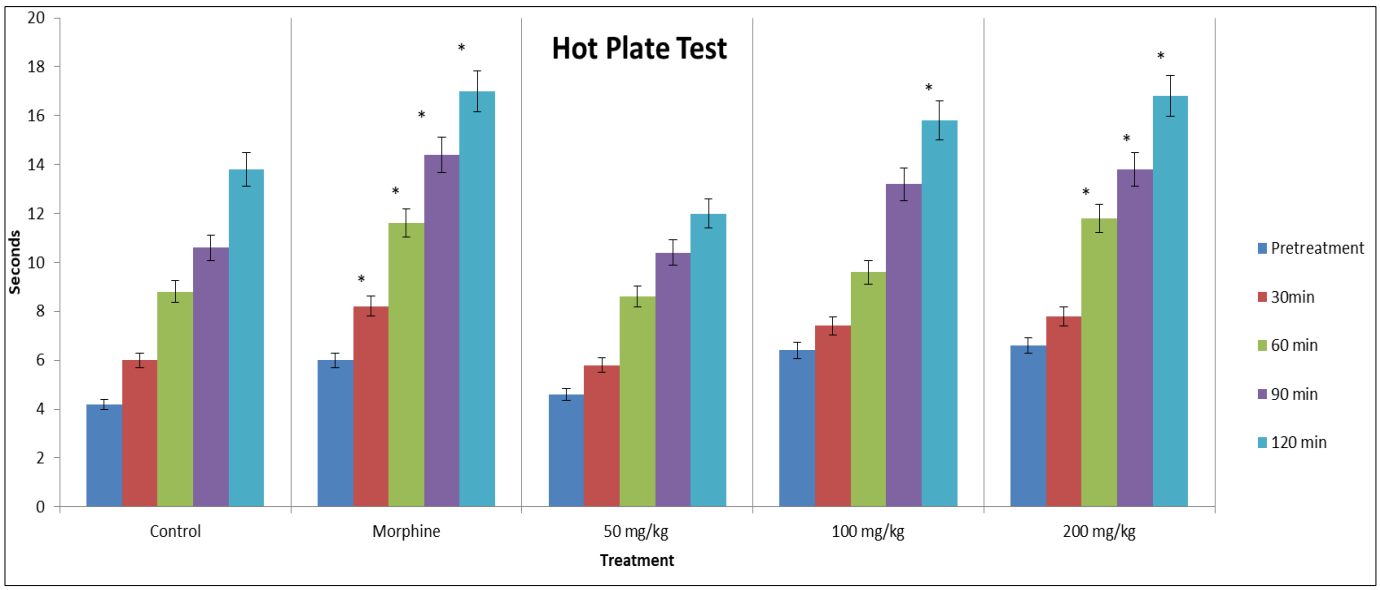

Fig 1: Antinociceptive effect of *Nyctanthes arbor-tristis* leaves extract and morphine in hot plate test, values are presented as mean± SEM (N=5). * p<0.05 compared with the control group (ANOVA followed by post hoc Dunnett's test).

Tail immersion test

Figure 2 and Table 3 display the extract's and morphine's antinociceptive activity in the tail immersion test. The extract's effects were more noticeable 120 minutes at the dosages of 100, and 200 mg/kg. At 200 mg/kg, the maximum reaction time of 7.80±0.58 s was recorded after 120 minutes. When compared to the control, morphine (5 mg/kg) demonstrated a substantial anti-nociceptive effect at all observation periods (*p<0.05).

Table 3: Antinociceptive effects of leaf extract of *Nyctanthes arbor-tristis* extract on tail immersion test

Treatment	Dose (mg/kg)	Response Time (in seconds)				
		0 min	30 min	60 min	90 min	120 min
Control	0.1ml/mouse	1.80±0.20	2.20±0.20	3.20±0.58	4.20±0.37	4.80±0.37
Morphine	5	2.80±0.37	4.20±0.37*	5.80±0.49*	6.60±0.51*	8.20±0.37*
EENA	50	1.60±0.24	2.40±0.24	3.00±0.31	4.80±0.67	5.20±0.37
EENA	100	2.20±0.20	3.00±0.44	4.20±0.37	5.20±0.20	6.40±0.24*
EENA	200	2.40±0.24	3.60±0.51	5.40±0.24*	6.40±0.51*	7.80±0.58*

Values are presented as mean ± SEM (N=5). EENA=Ethanolic extract of *Nyctanthes arbor-tristis;* * p<0.05 compared with the control group (Dunnett's test)

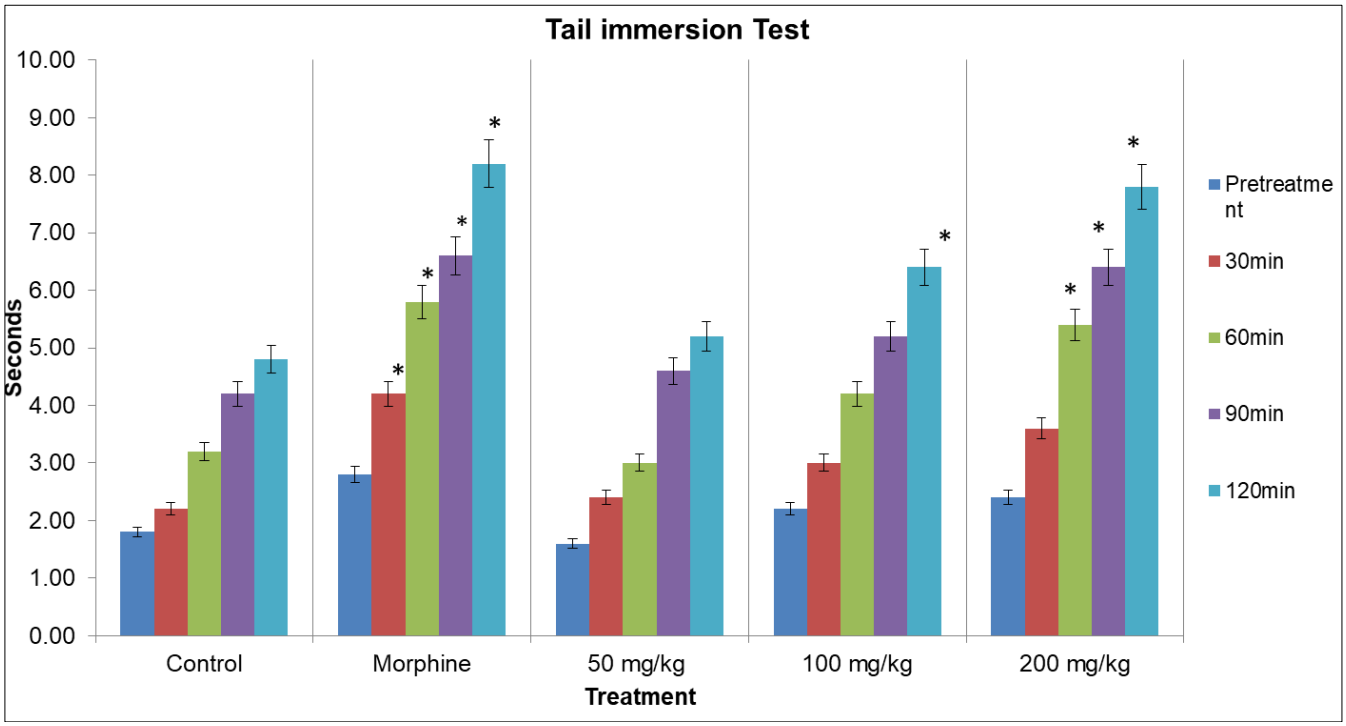

Fig 2: Antinociceptive effect of *Nyctanthes arbor-tristis* leaves extract and morphine in tail immersion test. Values are presented as mean± SEM (N=5), * p<0.05 compared with the control group (ANOVA followed by post hoc Dunnett's test)

Acetic acid induced-writhing test

In comparison to the control, the extract significantly (***p<0.001) and dose-dependently reduced the number of writhes (Figure 3 and Table 4). At 50, 100, and 200 mg/kg, the extract's inhibition rates of writhing were 13.31%, 43.42%, and 71.01%, respectively. Diclofenac sodium was inhibited by 80.11%.

Table 4: Antinociceptive effects of leaf extract of *Nyctanthes arbor-tristis* extract on acetic acid-induced abdominal writhing test

Treatment	Dose (mg/kg)	Mean ± SEM	% of Inhibition
Control	0.1 ml/mouse	71.40±0.60	0.00
Diclofenac Sodium	10	14.20±0.37***	80.11
EENA	50	61.90±0.81***	13.31
EENA	100	40.40±0.81***	43.42
EENA	200	20.70±1.09***	71.01

Values are expressed as Mean ± SEM (N=5); EENA=Ethanolic extract *of Nyctanthes arbor-tristis;* *** p<0.001 compared with the control group (Dunnett's test)

** p<0.01 compared with the control group (Dunnett's test).

* p<0.05 compared with the control group (Dunnett's test).

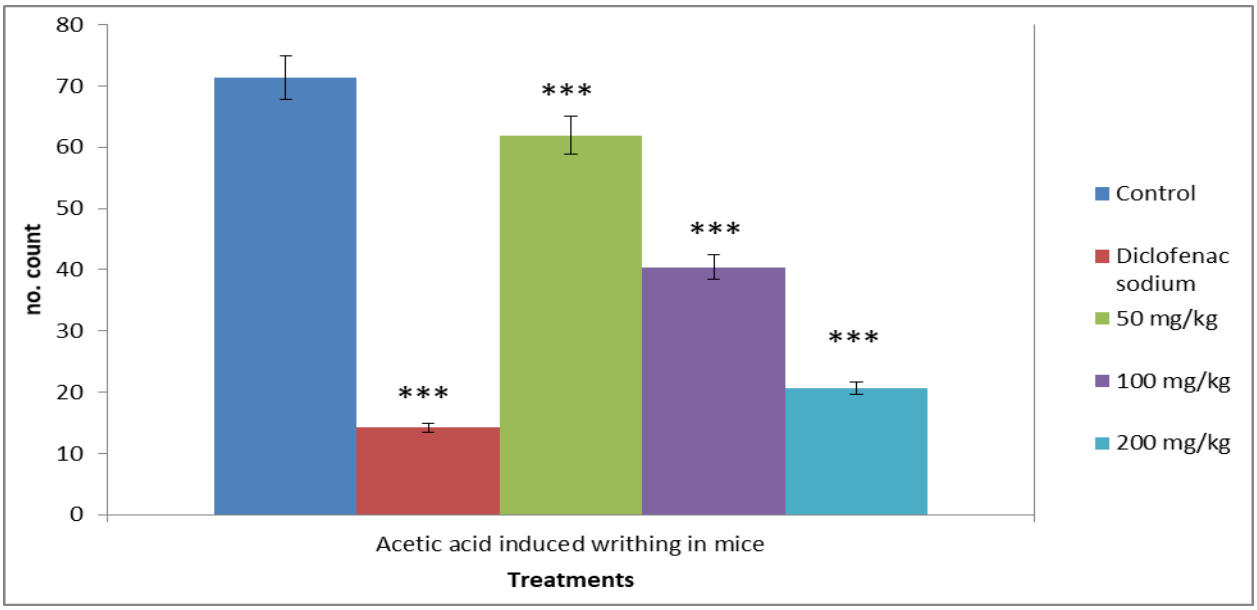

Fig 3: Antinociceptive effect of *Nyctanthes arbor-tristis* leaves extract in acetic acid-induced writhing. All values are presented as mean± SEM (N=5). *** $p<0.001$ compared with the control group (ANOVA followed by post hoc Dunnett's test)

Formalin test

When compared to controls, the extract at all dosages (50, 100, and 200 mg/kg, P.O.) significantly (***$p<0.001$) decreased the amount of time the mice spent licking the injected paws in both the early and late stages. Compared to the earlier period, the impact seems to be greater in the late phase. For the first phase, the extract suppressed 15.00%, 35.00%, and 56.43% of licking. The extract showed 23.28%, 43.84%, and 57.54% licking in the late phase as compared to the control group. Diclofenac sodium dramatically reduced pain in both the early and late periods by 63.57% and 73.97%, respectively.

Table 5: Antinociceptive effects of *Nyctanthes arbor-tristis* leave extract in formalin-induced nociception test.

Treatment	Dose (mg/kg)	Licking of the hind paw			
		Early phase	% of inhibition	Late phase	% of inhibition
Control	0.1ml/mouse	28.00±0.84	0.00	14.60±0.24	0,00
Diclofenac sodium	10	10.20±0.37***	63.57	03.80±0.58***	73.97
EENA	50	23.80±0.97**	15.00	11.20±0.58***	23.28
EENA	100	18.20±0.66***	35.00	08.20±0.37***	43.84
EENA	200	12.20±0.37***	56.43	06.20±0.58***	57.54

Values are presented as mean± SEM (N=5). EENA= Ethanolic extract of *Nyctanthes arbor-tristis;*
***$p<0.001$ compared with the control group (Dunnett's test)
**$p<0.01$ compared with the control group (Dunnett's test).
*$p<0.05$ compared with the control group (Dunnett's test)

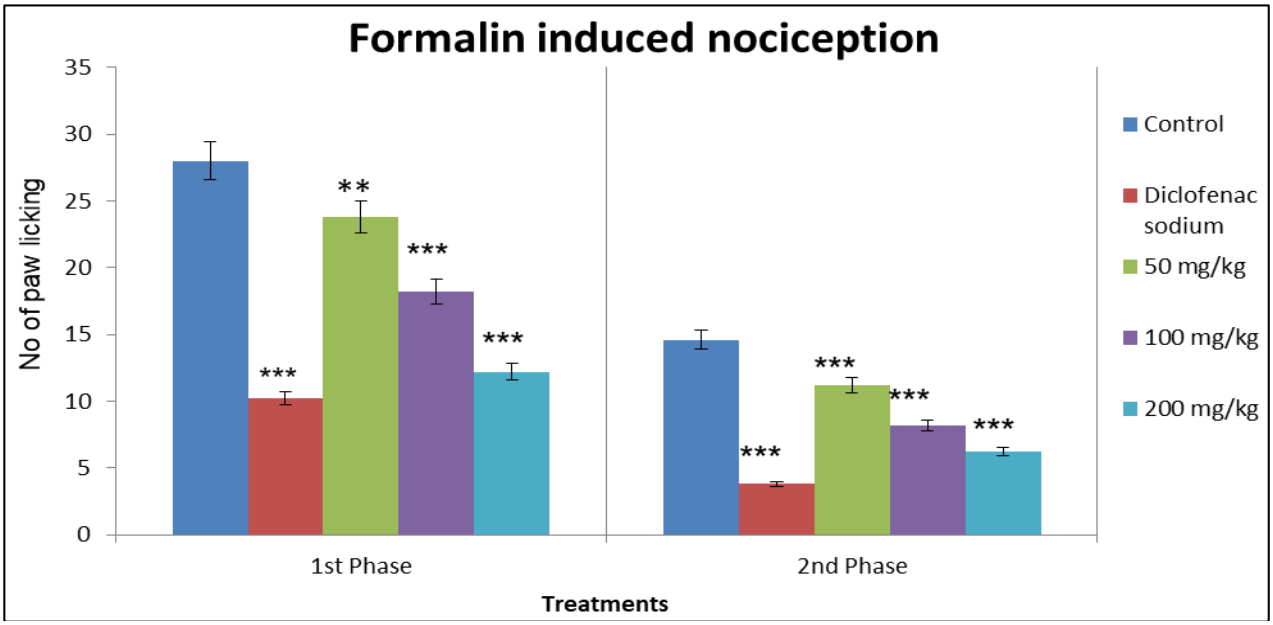

Fig 4: Antinociceptive effects of *Nyctanthes arbor-tristis* leave extract in formalin-induced nociception. Values are presented as mean± SEM (N=5). ***$p<0.001$ compared with the control group (ANOVA followed by post hoc Dunnett's test)

Glutamate-induced nociception

Fig. 5 and Table 6 show the outcomes of the glutamate-induced test. At early phase 16.38%, 37.93%, and 61.21% licking, the extract demonstrated a strong and dose-dependent antinociceptive effect. In the latter stage, the impact manifests as 30.28%, 53.21%, and 64.22% licking, respectively. In this test, diclofenac sodium (10 mg/kg) showed a considerable (***p<0.001) antinociceptive activity of 71.55% and 81.56% inhibition. Compared to the earlier period, the impact seems to be greater in the late phase.

Table 6: Antinociceptive effects of *Nyctanthes arbor-tristis* leave extract in glutamate-induced nociception test

Treatment	Dose (mg/kg)	Licking of the hind paw			
		Early phase	% of inhibition	Late phase	% of inhibition
Control	0.1ml/mouse	46.40±0.75	0.00	21.80±0.97	0,00
Diclofenac sodium	10	13.20±0.49***	71.55	06.20±0.58***	81.56
EENA	50	38.80±0.86***	16.38	15.20±0.37***	30.28
EENA	100	28.80±0.86***	37.93	10.20±0.58***	53.21
EENA	200	18.00±1.00***	61.21	07.80±0.58***	64.22

Values are presented as mean± SEM (N=5). EENA= Ethanolic extract of *Nyctanthes arbor-tristis*, *** p<0.001 compared with the control group (Dunnett's test).
** p<0.01 compared with the control group (Dunnett's test).
* p<0.05 compared with the control group (Dunnett's test).

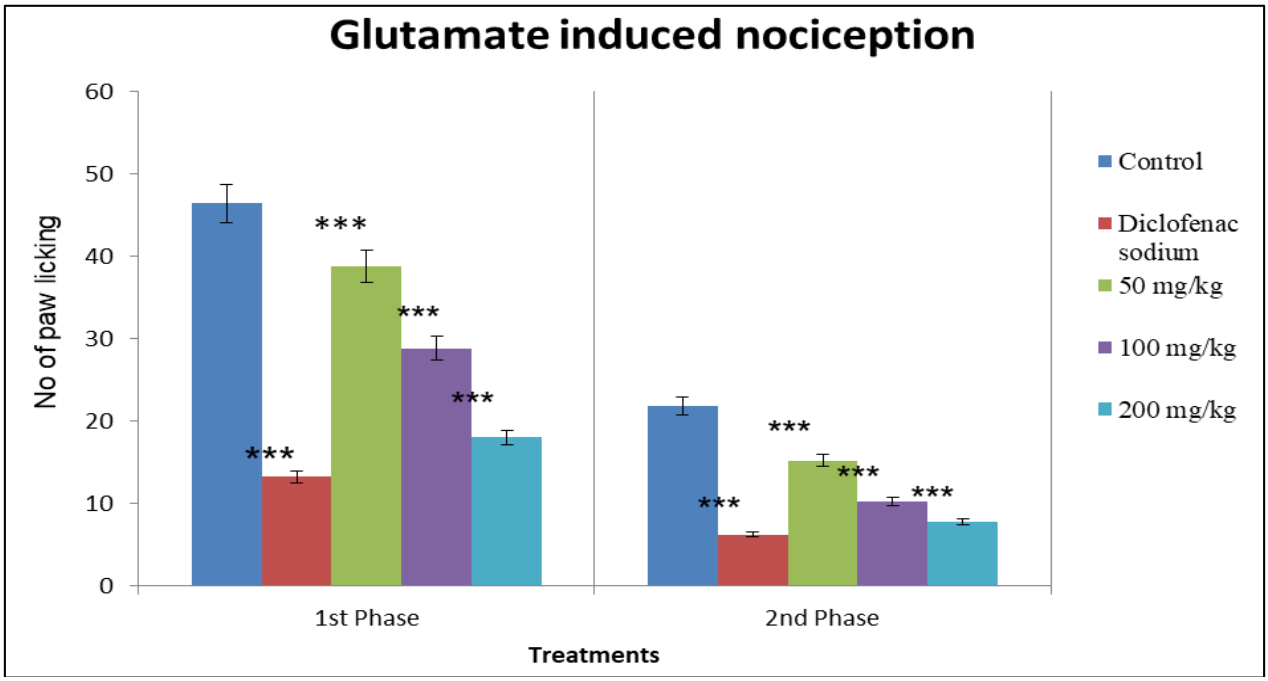

Fig 5: Antinociceptive effects of *Nyctanthes arbor-tristis* leave extract in glutamate-induced nociception test. All values are presented as mean± SEM (N=5). *** p<0.001 compared with the control group (ANOVA followed by post hoc Dunnett's test)

Discussion

The study looked on the antinociceptive properties of *Nyctanthes arbor-tristis* leaf ethanolic extract. A wide range of mouse models of acute pain, including the hot plate, tail immersion, acetic acid-induced writhing, formalin, and glutamate-induced tests, were used to measure the antinociceptive activity. These techniques evaluate potential antinociceptive substances or plant extracts using traditional nociception models.

The hot plate model, which measures mice's pain threshold in response to heat, is frequently used to study medications that exhibit central mechanism analgesia. This test was selected because it is sensitive to potent analgesics and has little tissue damage because of a cutoff period of 20 seconds, which is frequently employed to keep mice safe [45]. *Nyctanthes arbor-tristis* ethanolic extract demonstrated significant central analgesic efficacy at doses of 200 mg/kg. The presence of alkaloids, which are known to have analgesic effects by interfering with pain-enhancing neurotransmitters in the central nervous system, or their interaction with other receptors located in supra-spinal regions might be the cause of this [46]. It was discovered that the extract and morphine had different onsets of action. At dosages of 200 mg/kg, the extract took 120 minutes to begin working, whereas morphine took 60 minutes. This indicates that the extract's action has been postponed. The amount of time it takes for the medication to reach the target location after entering the central compartment, or it may be connected to active analgesic metabolites. The primary mechanism of the extract's analgesic action was assessed using the tail immersion technique. It is well known that mice's pain threshold for pressure and heat is increased by centrally acting analgesics [47]. On the other hand, narcotic participation is indicated by tail immersion, a thermally generated nociception [48]. Opioid-μ receptors are more responsive to thermal nociceptive testing, while opioid-k receptors are more sensitive to non-thermal tests [49]. The findings from these two models imply that the extract's antinociceptive effect could not substantially entail a central mechanism of action.

Oral administration of *Nyctanthes arbor-tristis* extract resulted in a statistically significant suppression of writhes as compared to the control, according to an antinociceptive

assessment utilising the acetic acid induced writhing test. Since any medication that reduces the writhing number exhibits analgesia by blocking prostaglandin production, a peripheral method of pain suppression, this is a sign of the extract's peripheral analgesic efficacy [50-51]. The abdominal writhing response may be inhibited by muscle relaxants and other medications, despite this method's high sensitivity to central and peripheral analgesic medications [52]. This leaves room for results to be misinterpreted.

The formalin test was used to further assess and elucidate the potential mechanism of the extract's antinociceptive action. There are two different stages of intense licking behaviours in the formalin test. Formalin's peripheral stimulation is assumed to be the primary source of C-fiber activation during the early period (the first five minutes). After 15 to 30 minutes, there is another licking frenzy that appears to be connected to the inflammatory reaction that formalin causes. We refer to this stage as inflammatory [53-54]. Both stages of the formalin reaction were blocked by the extract's antinociceptive effect. But in the latter stage, the extract's impact was more noticeable. Serotonin, histamine, bradykinin, and prostaglandins are released during the late phase of inflammation, which might at least partially sensitise the central nociceptive neurones [55]. It thus suggests that the extract's peripheral effect may have contributed to its antinociceptive efficacy.

Glutamate-induced paw licking was significantly inhibited by the ethanolic extract of *Nyctanthes arbor-tristis*. Comparable to diclofenac, 200 mg/kg of extract showed the greatest suppression of paw licking. When plant extract was administered, the nociceptive response brought on by intraplantar glutamate injection was significantly and dose-dependently suppressed. N-methyl-d-aspartate (NMDA) receptor activation, NO release, and several NO-derived compounds are major mediators of the glutamate-induced nociceptive response, which appears to include the peripheral, spinal, and supraspinal sites of action. NO is a crucial neurotransmitter that plays a role in the nociceptive process and helps the dorsal horn of the spinal cord establish central sensitisation. The analgesic activity of the plant extract may be attributed to suppression of NO release or blockage of NMDA receptors, as indicated by the inhibition of glutamate-induced nociception [56-58].

Tannic acid, methyl salicylate, amorphous glucosider, mannitol, amorphous resin, ascorbic acid, carotene, and a trace of volatile oil have all been found in an extract of *Nyctanthes arbor-tristis* leaves [59]. Flavonoids have been shown to have a strong anti-inflammatory impact and to inhibit prostaglandin synthetase, more precisely endo-peroxidase. Given that prostaglandins play a role in pain perception and that flavonoids block them, it is possible that *Nyctanthes arbor-tristis*'s analgesic activity results from its inhibition of PG production [60-61]. Additionally, oleanolic acid possesses anti-inflammatory properties. They have been shown to have a significant impact on the cyclooxygenase 2 enzyme as well as interferon of inducible nitric oxide synthase. The acid may have analgesic effects because of its impact on the cyclooxygenase 2 enzyme. Another substance with a potent analgesic action is methyl salicylate, which is also found in trace amounts in the leaves of *Nyctanthes arbor-tristis*. Methyl salicylate is converted to salicylate, a recognised NSAID, within the body. Although the exact mechanism is unknown, traditional medicine practitioners also utilised benzoic acid, another component of the plant, as an analgesic in the early 20th century [62].

Conclusion

When *Nyctanthes arbor-tristis* leaf ethanolic extract was evaluated using eddy's hot plate, tail immersion, acetic acid-induced writhing, formalin-induced nociception, and glutamate-induced tests, it was discovered to have strong antinociceptive action. According to the current study, the extract may be used in place of an analgesic medication. This study served as the first stage in screening the plant extract, and it opens the door for more focus and investigation to pinpoint the active ingredients in charge of the pharmacological and biological effects. To determine the true molecular mechanism of the active ingredients in the leaf extract, more research is required. One of the biggest obstacles to ensuring the safety component before further scientific information is presented is the dearth of toxicity studies.

Declarations

Ethics approval and consent to participate

The Stamford University Bangladesh Institutional Animal, Medical Ethics, Biosafety, and Biosecurity Committee (SUB/IAEC/17.02) accepted the study protocol. The institutional animal ethics committee accepted the set of guidelines used for animal experiments, and they were handled in compliance with international standards for the use and care of lab animals.

Competing interests

There are no conflicts of interest disclosed by the writers. The paper's writing and content are solely the authors' responsibility.

Funding

Each author contributed a portion of their own funds to this study.

Acknowledgements

The authors express their gratitude to Professor Dr. Bidyut Kanti Datta, Chairman of the Pharmacy Department of Stamford University Bangladesh, for granting them access to the Pharmacology and Phytochemistry Laboratory.

Authors' information

Department of Pharmacy, Stamford University Bangladesh, 51, Siddeswari Road, Dhaka-1217, Bangladesh

References

1. Tripathi KD. Essentials of Medical Pharmacology. 7th Ed. New Delhi: Jaypee Brothers Medical Publishers; 2013, p. 453-454.
2. Mersky H. Pain terms: A list with definitions and notes on usage. Pain. 1979;6(3):249-252.
3. Bianchi C, Franceschini J. Experimental observations on Haffner's method for testing analgesic drugs. Br J Pharmacol. 1954;9(3):280-284.
4. Di Masi JA, Hansen RW, Grabowski HG. The price of innovation: New estimates of drug development costs. J Health Econ. 2003;22(2):151-185.
5. Vittalrao AM, Shanbhag T, Kumari M, Bairy KL, Shenoy S. Evaluation of antiinflammatory and analgesic activities of alcoholic extract of *Kaempferia galanga* in rats. Indian J Physiol Pharmacol. 2011;55(1):13-24.
6. Kumara NK. Identification of strategies to improve research on medicinal plants used in Sri Lanka. WHO Symposium; University of Ruhuna; 2001.

7. Rout GR, Mahato A, Senapati SK. *In vitro* clonal propagation of *Nyctanthes arbor-tristis* Linn: A medicinal tree. Hortic Sci. 2007;34(2):84-89.
8. Vats M, Sharma N, Sardana S. Antimicrobial activity of leaves extracts of *Nyctanthes arbor-tristis* Linn. (OLEACEAE). Int J Pharmacogn Phytochem Res. 2009;1:12-14.
9. Suresh V, Jaikumar S, Arunachalam G. Antidiabetic activity of ethanol extract of leaves of *Nyctanthes arbor-tristis* Linn. Res J Pharm Biol Chem Sci. 2010;1(4):311-317.
10. Wealth of India: A Dictionary of Indian Raw Materials and Industrial Products. New Delhi: National Institute of Science Communication, CSIR; 1997, Vol. 7.
11. Kirtikar KR, Basu BD. Indian Medicinal Plants. 2nd Ed. Vol. 2. Allahabad: LM Basu; 1993.
12. Tuntiwachwuttikul P, Rayanil K, Taylor WC. Chemical constituents from the flowers of *Nyctanthes arbor-tristis*. ScienceAsia. 2003;29:21-30.
13. Nawaz MMAH, Hossain M, Karim M, Khan M, Jahan R, Rahmatullah M. An ethnobotanical survey of Jessore district in Khulna division, Bangladesh. Am Eurasian J Sustain Agric. 2009;3(2):238-243.
14. Nair R, Kalariya T, Chanda S. Antibacterial activity of some selected Indian Medicinal Flora. Turk J Biol. 2005;29:41-47.
15. Narendhirakannan RT, Smeera T. In-vitro antioxidant studies on ethanolic extracts of leaves and stems of *Nyctanthes arbor-tristis* L. (Night-flowering jasmine). Int J Biol Med Res. 2010;1:188-192.
16. Nadkarni AK. Indian Materia Medica. Vol. I, 3rd Ed. Mumbai: Popular Prakashan Pvt Ltd; 1982, p. 857-858.
17. Kirtikar KR, Basu BD. Indian Medicinal Plants. Vol. VII. New Delhi: Sri Satguru Publications; 2000, p. 2110-2113.
18. Girach RD, Aminuddin SA, Siddiqui PA, Khan SA. Ethnomedicinal studies on Harsinghar (*Nyctanthes arbor-tristis* L)-A less known medicinal plant in Unani medicine. Hamdard Med. 1994;37(2):60-66.
19. Jain SK. Dictionary of Indian Folk Medicines and Ethnobotany. New Delhi: Deep Publications; 1991, p. 132.
20. Wealth of India. A Dictionary of Indian Raw Materials and Industrial Products. Vol. VII. New Delhi: National Institute of Science Communication, CSIR; 1997, p. 69-70.
21. Khandelwal KR, Kadam SS, Singhama. Antibacterial activity of the leaves of *Nyctanthes arbor-tristis* Linn. Indian J Nat Prod. 1999;15:18-20.
22. Saxena RS, Gupta B, Lata S. Tranquillizing, antihistaminic and purgative activity of *Nyctanthes arbor-tristis* leaf extract. J Ethnopharmacol. 2002;81(3):321-325.
23. Ratnasooriya WD, Ayakody ADI, Hettiarachchi, Dharmasiri MG. Sedative effects of hot flower infusions of *Nyctanthes arbor-tristis* on rats. Pharm Biol. 2005;43(2):140-146.
24. Deshmukh VK, Juvekar AR. Antianxiety and nootropic activity of *Nyctanthes arbor-tristis* leaves. Planta Med. 2006;72(11).
25. Badam L, Rao TLG, Wagh UV. *In vitro*, Antimalarial activity of fresh leaf juice of *Nyctanthes arbor-tristis* Linn. Indian J Parasit. 1987;11(1):13-17.
26. Patel UM, Patel KM, Patel MP, Bhavasar GC. Anti-inflammatory activity of *Nyctanthes arbor-tristis*. Indian J Nat Prod. 1999;15:18-20.
27. Saxena RC, Gupta B, Saxena KK, Srivastava VK, Prasad DN. Analgesic, antipyretic and ulcerogenic activity of *Nyctanthes arbor-tristis* leaf extracts. J Ethnopharmacol. 1987;19(2):193-200.
28. Gyanchandrani A, Khan ZK, Maitra SC. Arbortristosides modulate murine peritoneal macrophages for phagocytosis and intracellular killing of Candida albicans. Pharm Biol. 2000;38(5):340-352.
29. Paul BN, Saxena AR. Depletion of tumour necrosis factor-alpha in mice by *Nyctanthes arbor-tristis*. J Ethnopharmacol. 1997;56(2):153-158.
30. Gupta PP, Srimal RC, Tandon JS. Antiallergic activity of some traditional Indian medicinal plants. Int J Pharmacog. 1993;31(1):15-18.
31. Bhawmic BN, Choudhury BK. Antiallergic activity of some traditional Indian medicinal plants. Indian Bot Rept. 1982;1(2):164-165.
32. Singh CJ, Singh BG. Antifungal activity of some plant extracts against dermatophytes and related keratinophilic fungi. Adv Plant Sci. 1997;10(2):249-251.
33. Laghate P, Grampurohit ND, Miranda S. Antihepatotoxic activity of the leaves of *Nyctanthes arbor-tristis* L. Gandhinagar: National Convention on current Herbal Drugs; 2003.
34. Hukkeri VI, Akkikusum S, Sureban RR, Gopalakrishna B, Byahatti VV, Rajendra SV. Hepatoprotective activity of the leaves of *Nyctanthes arbor-tristis* on rats. Indian J Pharm Sci. 2006;68(4):542-553.
35. Talakal TS, Dwivedi SK, Sharma SR. *In vitro* and *in vivo* antitrypanosomal potential of *Nyctanthes arbor-tristis* leaves. Pharm Biol. 2000;38(5):326-329.
36. Rajbhandari M, Wegner U, Julich M, Schopke T, Mentel R. Screening of Nepalese medicinal plants for antiviral activity. J Ethnopharmacol. 2001;74(3):251-255.
37. Than A, Myint MMS, Myint T, Myint W. Antiedema activity of *Nyctanthes arbor-tristis* L, Curcuma Longa L. and Plantago Major L. Myanmar Health Sci Res J. 1996;8(1):36-40.
38. Ghani A. Medicinal Plants of Bangladesh with Chemical Constituents and Uses. 2nd Ed. Dhaka: The Asiatic Society of Bangladesh; 2003. p. 331-332.
39. Lorke D. A new approach to acute toxicity testing. Arch Toxicol. 1983;54(4):275-287.
40. Woolfe G, MacDonald AD. The evaluation of the analgesic action of pethidine hydrochloride (Demerol). J Pharmacol Exp Ther. 1944;80(3):300-307.
41. Malairajan P, Geetha G, Narasimhan S, Veni KJK. Analgesic activity of some Indian medicinal plants. J Ethnopharmacol. 2006;106(3):425-428.
42. Cha DS, Eun JS, Jeon H. Anti-inflammatory and antinociceptive properties of the leaves of *Eriobotrya japonica*. J Ethnopharmacol. 2011;134(2):305-312.
43. Zakaria ZA, Mustapha S, Sulaiman MR, Jais AMM, Somchit MN, Abdullah FC. The antinociceptive action of aqueous extract from *Muntingia calabura* leaves: The role of opioid receptors. Med Princ Pract. 2007;16(2):130-136.
44. Beirith A, Santos AR, Calixto JB. Mechanisms underlying the nociception and paw oedema caused by injection of glutamate into the mouse paw. Brain Res. 2002;924(2):219-28.

45. Schaible HG, Ebersberger A, Natura G. Update on peripheral mechanisms of pain, beyond prostaglandins and cytokines. Arthritis Res Ther. 2011;13(2):210.
46. Cadirci E, Suleyman H, Gurbuz P. Anti-inflammatory effects of different extracts from three Salvia species. Turk J Biol. 2012;36:59-64.
47. Singh S, Majumdar DK. Analgesic activity of Ocimum sanctum and its possible mechanism of action. Int J Pharmacogn. 1995;33(3):188-192.
48. Besra SE, Sharma RM, Gomes A. Anti-inflammatory effect of petroleum ether extract of leaves of Litchi chinensis Gaertn. (Sapindaceae). J Ethnopharmacol. 1996;54(1):1-6.
49. Haider S, Nazreen S, Alam MM, Hamid H, Alam MS. Anti-inflammatory and anti-nociceptive activities of Platanus orientalis Linn. and its ulcerogenic risk evaluation. J Ethnopharmacol. 2012;143(1):236-240.
50. Alam MA, Subhan N, Abdul Awal M, Alam MS, Sarder M, Nahar L, Sarker SD. Antinociceptive and antiinflammatory properties of *Ruellia tuberosa*. Pharm Biol. 2009;47(3):209-214.
51. Loganayaki N, Siddhuraju P, Manian S. Antioxidant, anti-inflammatory and anti-nociceptive effects of *Ammannia baccifera* L. (Lythraceae), a folklore medicinal plant. J Ethnopharmacol. 2012;140(2):230-233.
52. Le Bars D, Gozariu M, Cadden SW. Animal models of nociception. Pharmacol Rev. 2001;53(4):597-652.
53. Hunskaar S, Hole K. The formalin test in mice: Dissociation between inflammatory and non-inflammatory pain. Pain. 1987;30(1):103-114.
54. Milano J, Oliveira SM, Rossato MF, Sauzem PD, Machado P, Beck P, *et al*. Antinociceptive effect of novel trihalomethyl-substituted pyrazoline methyl esters in formalin and hot-plate tests in mice. Eur J Pharmacol. 2008;581(1-2):86-96.
55. Verma PR, Joharapurkar AA, Chatpalliwar VA, Asnani A. Antinociceptive activity of alcoholic extract of *Hemidesmus indicus* R.Br. in mice. J Ethnopharmacol. 2005;102(2):298-301.
56. Luiz AP, Moura JD, Meotti FC, Guginski G, Guimarães CL, Azevedo MS, *et al*. Antinociceptive action of ethanolic extract obtained from roots of *Humirianthera ampla* Miers. J Ethnopharmacol. 2007;114(3):355-363.
57. Beirith A, Santos AR, Calixto JB. Mechanisms underlying the nociception and paw oedema caused by injection of glutamate into the mouse paw. Brain Res. 2002;924(2):219-228.
58. Le Bars D, Gozariu M, Cadden SW. Animal models of nociception. Pharmacol Rev. 2001;53(4):597-652.
59. Zaheer SH, Prasad B, Chopra RN, Santapau H, Krishnan MS. The Wealth of India. Vol. VII. New Delhi: Publications and Information Directorate, CSIR; 1966. p. 69-70.
60. Ramaswamy S, Pillai NP, Gopalakrishnan V, Parmar NS, Ghosh MN. Analgesic effect of O-(β-hydroxyethyl) rutoside in mice. Indian J Exp Biol. 1985;23:219-220.
61. Alcazar MJ, Jimenez MI. Flavonoids as anti-inflammatory agents. Fitoterapia. 1988;59:25-38.
62. Havsteen B. Flavonoids: A class of natural products of high pharmacological potency. Biochem Pharmacol. 1983;32(7):1141-1148.