

Research Article

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Phytochemical and pharmacological profiling of *Trewia nudiflora* Linn. leaf extract deciphers therapeutic potentials against thrombosis, arthritis, helminths, and insects

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Abstract: The objective of the current study was to examine the phytochemical and *in vitro* thrombolytic, anti-arthritis, anthelmintic, and insecticidal effects of *Trewia nudiflora* (TN) methanolic leaf extract with its methanol (MTN), chloroform (CTN), and ethyl acetate (ETN) fractions. *Pheretima posthuma* and *Tribolium castaneum* were employed for evaluating the antihelmintic and insecticidal properties, respectively. All the tested extracts showed the presence of copious potential constituents in phytochemical analysis. Among all extracts, MTN extract exhibited the utmost clot lysis ($35.95 \pm 4.81\%$)

property compared to standard streptokinase (SK) ($53.77 \pm 7.52\%$). All samples displayed striking protein denaturation activity in a dose-dependent manner ($100\text{--}500\text{ }\mu\text{g/mL}$), where the highest inhibition was observed for MTN ($67.26 \pm 6.39\%$ at $500\text{ }\mu\text{g/mL}$). Each extract demonstrated considerable anthelmintic activity at $25\text{--}75\text{ mg/mL}$ dose ranges. ETN showed the strongest anthelmintic activity at the highest dose. Among all samples, the CTN extract displayed the utmost mortality rate (77.22%) in the insecticidal test. The results of the study suggest that *T. nudiflora* leaf extracts may have potential against thrombosis, arthritis, helminths, and insects, which warrants the necessity of extensive isolation and identification of bioactive compounds to develop newer effective drugs upon preclinical and clinical investigations.

Keywords: *Trewia nudiflora*, thrombolytic, anti-arthritis, anthelmintic, insecticidal activity

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1 Introduction

From ancient times, nature has been considered a source of healing materials, and based on their traditional use in various diseases, a remarkable amount of contemporary medications have been developed from this natural origin. It is believed that natural products, especially medicinal herbs, can treat a number of human ailments [1,2]. According to the World Health Organization, roughly 80% of the world's population, especially those living in vast rustic zones of developing countries, depends on herbal medicines for their primary health care; nearly 7,000 enlisted Western pharmacopeia's medicinal compounds are derived from plants [3].

There has recently been a lot of interest in extracting plant bioactive chemicals to assess their pharmacological

effects for primary health care in both developed and emerging nations due to the vast biological and therapeutic value, high safety margins, and low cost of herbal treatments [3,4]. Many natural chemicals that are produced by mangroves and aquatic and tropical plants showed therapeutic activities. They also act as essential therapeutic agents and essential raw materials for the preparation of conventional and modern medicine (analgesic, antipyretic, thrombolytic, anthelmintic, etc.) [5]. Numerous earlier reports and studies suggested that *in vitro* screening might be used as a first step in the process of identifying different promising mangrove and aquatic and tropical plant crude extracts with therapeutic qualities, opening the door to more in-depth pharmacological research [6]. These details and information inspired us to use the *Trewia nudiflora* (Euphorbiaceae) plant to open up new possibilities for medicinal research.

T. nudiflora is a fast-growing, soft, wooded, versatile, dioecious plant found in most parts of Bangladesh and throughout South Asia [7,8]. The extract and decoction of different plant parts are used for the removal of bile and phlegm, as a cerebroprotective agent in different blood and neuronal disorders, for wound healing, hyper locomotion, flatulence, gout, rheumatism, tumor, and fungal growth inhibition [7–11]. Previous phytochemical studies on the seed and bark of *T. nudiflora* revealed the presence of valuable bioactive components like cardenolides, triterpenoids, diterpenoids, alkaloids, and nitrogen-containing compounds, lignans, neolignans, flavonoids, ellagic acid derivatives, phenylpropanoid glucosides, α -tocopherol, cucurbitacin-trewinine, scopoletin, and indole-3-carboxylic acid, triglycerides [7–12].

The numerous ethnopharmacological uses and phytoconstituents of *T. nudiflora* and few *in vitro* studies of the plant parts provoked us to investigate the phytochemical and *in vitro* pharmacological properties (anti-arthritis, thrombolytic, insecticidal, and anthelmintic criteria) of methanol (MTN) extract of leaves and its different solvent fractions of this plant species, as there is currently no literature available on these activities.

2 Materials and methods

2.1 Plant material and extraction

Fresh leaves of *T. nudiflora* were collected from Natore district, Bangladesh (December 2019). The plant was identified and authenticated by a taxonomist of the Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh (DACB

Accession No: 4500). After washing and cleaning, the collected leaves were dried under shade for 7 days and crushed into a coarse powder. Then, the powder was preserved in an airtight vessel in a dark, cool, and dry place until the inception of the experiments. Five hundred grams of powder was taken into a clean glass vessel and soaked in 2 L 95% MTN for 7 days with random shaking and stirring. After 1 week, the entire mixture was filtered through a clean cotton bed, followed by Whatman filter paper number 1 (Bibby RE200, Sterilin Ltd, UK). This filtrate was concentrated using a rotary vacuum evaporator at 40°C under decreased pressure to produce the crude methanolic extract. In this study, we followed a modified Kupchan solvent–solvent partitioning method to separate constituents based on their polarity [13]. Around 5 g of the concentrated extract of *T. nudiflora* was partitioned with chloroform (CTN) and ethyl acetate (ETN). Later, all fractioned (CTN and ETN) samples were evaporated for drying before being used for advanced analysis.

2.2 Drugs and chemicals

All the chemicals were of analytical reagent grade collected from Sigma Chemical Co. (St. Louis, MO, USA). Standard drugs were collected from different pharmaceutical companies such as streptokinase (SK) (Popular Pharmaceuticals Ltd, Bangladesh); diclofenac sodium, and albendazole (Square Pharmaceuticals Ltd, Bangladesh).

2.3 Phytochemical analysis

The MTN leaf extract of *T. nudiflora* and its different soluble fractions were analyzed for the presence of various bioactive constituents via standard procedures, such as carbohydrate by the Molish test; tannin and phenol by the ferric chloride test; alkaloid by the Wagner and Meyer tests; saponin by the foam test; resin by the copper acetate test; flavonoids by Akinseye et al. method; steroids by the Salkowski's test; and fixed oil by the filter paper test [13–16].

2.4 *In vitro* thrombolytic activity

In vitro thrombolytic potentialities of the tested extracts were evaluated by the method described by Anjum et al. [17]. Ten healthy human volunteers without a history of

hematological problems or anticoagulant medication were chosen to provide blood samples (4 mL of venous blood). A consent paper was taken from each volunteer providing the information of the research project as well as the purpose of the research. Aliquots of blood were transferred to a number of pre-weighed sterile Eppendorf tubes (1 mL/tube) and were incubated at 37°C for 45 min to clot blood. Blood serum was entirely removed from every tube without unsettling the clot and reweighed to measure the mass of the clot. Two Eppendorf tubes were marked as negative and positive controls and filled with streptokinase (100 µL) and distilled (100 µL), respectively. All tubes were then incubated for an additional 90 min at 37°C to check for clot lysis. The discharged fluid was discarded after incubation, and the tubes were reweighed to determine the weight difference following clot disruption. The difference between weight measurements taken before and after clot lysis was expressed as a percentage of clot lysis [17]:

$$\% \text{ Clot lysis} = (\text{Weight of clot lysis} / \text{Weight of clot before lysis}) \times 100.$$

2.5 *In vitro* anti-arthritic activity

The modified Naz *et al.* method [18] was followed to evaluate anti-arthritic activity. In separate sterile tubes, 5% aqueous solution of bovine serum albumin (0.45 mL) and 0.05 mL of various sample extracts at different concentrations (100–500 g/mL) were mixed. Protein denaturation was started in the tubes by incubating them for 30 min at 37°C before being heated to 57°C (for 3 min). Once the tubes had cooled to room temperature, 2.5 mL of phosphate buffer (pH 6.3) was added, and then absorbance measurements were made (at 660 nm). Here, we used 0.05 mL of distilled water solution as a control and diclofenac sodium as a reference drug, and the outcome was obtained from the following formula:

$$\% \text{ inhibition} = \frac{V_c - V_t}{V_t} \times 100,$$

where V_t = absorbance of test samples and V_c = absorbance of control.

2.6 Anthelmintic test

The modified Duvey technique was used to check the anthelmintic activity of *T. nudiflora* extracts [19]. Bangladeshi earthworms (*Pheretima posthuma*) were chosen in this investigation because they resemble the human intestinal roundworm parasite in terms of anatomy and physiology [20]. All worms were collected from damp

soil, cleansed to remove any feces, and acclimated to the laboratory environment before performing the test. The experiment was conducted using freshly made standard and test solutions. The extracts were examined in a Tween 20 (1%) solution that had been diluted with sterile normal saline at dosages ranging from 20 to 75 mg/mL. Almost equally sized earthworms were split into five different groups (each contained six worms) and released into the experiment formulations (30 mL). The first group was considered as the control group which was treated with Tween-20 added normal saline. The second group was marked as standard and received the reference drug Albendazole (20 mg/mL). Groups 3–5 were treated with different fractionates along with the crude extract of *T. nudiflora* at various concentrations (20–75 mg/mL). The worms were considered paralyzed when they stopped moving, with the exception of when they were violently shaken, and dead when they stopped moving even after being shaken vigorously or submerged in hot water (50°C), which was followed by the fading of their body colors.

2.7 Insecticidal activity test

Tribolium castaneum (stock cultures were collected from the Crop Protection and Toxicology Laboratory at Sher-e-Bangla Agricultural University, Dhaka, Bangladesh) was used to check the insecticidal activity of the tested samples at six different concentrations (2.5, 5, 10, 20, 40, and 50 mg/mL). Sample solutions were prepared by dissolving the extracts in the appropriate solvents. The solutions were poured separately on the base of each petri dish (60 mm) and left in open air for evaporation of the solvents. To each petri dish, six insects were placed and kept at room temperature in a safe place, including the control [21]. Then, three replicates of the control and treated solutions were set up. After 30 min interval, the mortality of the insects was observed. Then, data collection was done after exposure of 1, 2, 4, 8, 12, and 48 h. A simple-functional microscope was used to identify the natural movement of organs, and for the confirmation of death, frequent use of a warm needle was used upon lack of movement. The mortality rate of the *T. castaneum* adults was measured by the following formula [21]:

$$\text{Pr} = \frac{PO - Pc}{100 - Pc} \times 100,$$

where Pr = Amended mortality (%); Po = Observed mortality (%); Pc = Control mortality (%), often called natural mortality (%).

2.8 Statistical analysis

Three repetitions of each *in vitro* experiment were performed. SPSS software version 19.0 was employed to conduct the statistical analysis. The results were expressed as mean $\pm$ standard error of mean (SEM), and the values of *P* were calculated using Student's *t*-test and one-way analysis of variance (ANOVA) followed by Dunnett's *post hoc* multiple comparison test. Results with a value of less than $p < 0.05$ were regarded as noteworthy.

3 Results

3.1 Phytochemical screening

Table 1 lists the classes of phytoconstituents that were found through primary phytochemical tests on *T. nudiflora* leaf extracts.

3.2 Thrombolytic activity

The crude extracts of *T. nudiflora* exhibited remarkable thrombolytic activity (Table 2). Our research revealed that after 90 min of incubation (at 37°C), 100 μ L of streptokinase (a positive control for SK) demonstrated $53.77 \pm 7.52\%$ clot lysis. On the contrary, negative control distilled water showed the presence of negligible clot lysis percentage ($11.22 \pm 1.16\%$). All tested samples showed a promising thrombolytic effect. Among them, tested, MTN

Table 2: Effects of *T. nudiflora* leaves extracts on *in vitro* clot lysis

Sample	% of Lysis
Water	11.22 ± 1.16
Streptokinase (SK)	$53.77 \pm 7.52^*$
MTN	$35.95 \pm 4.81^*$
CTN	$25.54 \pm 3.11^*$
ETN	$24.69 \pm 2.61^*$

Note: The values are given as mean $\pm$ SEM ($n = 3$). ANOVA was used to evaluate the data, and then Dunnett's *t*-test was performed, $*p < 0.001$ compared with negative control.

showed the utmost percentage of clot lysis ($25.54 \pm 3.11\%$). In comparison to the negative control, other fractions also showed potential clot lysis property that was statistically significant ($p < 0.001$).

3.3 Anti-arthritic activity

Five different concentrations of leaf extracts (100–500 μ g/mL) were used for the anti-arthritic activity test. The obtained result from the experiment is given in Table 3. All plant extracts inhibited protein denaturation dose-dependently, with a remarkable effect perceived at the utmost concentration (Table 3). A significant inhibition percentage was found for MTN ($67.26 \pm 6.39\%$ at 500 μ g/mL). On the other hand, ETN ($43.39 \pm 4.87\%$ at 100 μ g/mL) showed the lowest inhibition.

3.4 Anthelmintic activity

The outcomes of the anthelmintic test of various extracts of *T. nudiflora* as well as albendazole (reference drug) are tabulated in Table 4. In comparison to the reference medication, all compounds demonstrated dose-dependent earthworm paralysis and death, as well as considerable anthelmintic action at all dosages. In comparison to the reference medicine, MTN extract at a dose of 75 mg/mL took slightly less time to kill earthworms and slightly longer to cause paralysis.

3.5 Screening of insecticidal activity

Figure 1 displays the outcomes of the insecticidal activity of different extracts of *T. nudiflora*. We noticed a dose-

Table 1: Screening of bioactive phytoconstituents of *T. nudiflora* leaf extracts

Phytoconstituents	MTN	CTN	ETN
Carbohydrate	+++	+++	+++
Glycoside	+++	+++	+++
Tannin	+++	++	+++
Alkaloid	+++	++	+++
Saponin	++	+	++
Resin	++	+	++
Phenol	+++	++	+++
Flavonoid	+++	+	+++
Steroid	++	++	++
Fixed oil	—	—	—

Note: “+” specifies the existence and “—” demonstrates that no phytochemical group is present. Bioavailability key: (+++)ve = strong intensity, (++)ve = moderate intensity, (+)ve = weak intensity, (–)ve = absence.

Table 3: Anti-arthritic activity of *T. nudiflora* leaf extracts at different doses

Sample	% of inhibition				
	Dose ($\mu\text{g/mL}$)				
	100	200	300	400	500
Diclofenac sodium	65.46 $\pm$ 1.01	73.41 $\pm$ 1.54	80.13 $\pm$ 1.62	83.42 $\pm$ 6.69	88.45 $\pm$ 4.05
MTN	49.02 $\pm$ 3.34*	54.89 $\pm$ 3.75*	60.32 $\pm$ 3.79*	49.69 $\pm$ 7.00*	67.26 $\pm$ 6.39*
CTN	46.52 $\pm$ 4.18*	49.45 $\pm$ 4.72*	56.65 $\pm$ 5.55*	50.65 $\pm$ 7.76*	63.61 $\pm$ 4.41*
ETN	43.39 $\pm$ 4.87*	53.89 $\pm$ 4.19*	61.31 $\pm$ 2.76*	63.61 $\pm$ 4.42*	66.26 $\pm$ 7.02*

Note: The values are given as mean $\pm$ SEM ($n = 3$). ANOVA was used to evaluate the data, and then Dunnett's *t*-test was performed, * $p < 0.01$ compared with standard.

Table 4: Anthelmintic activity of *T. nudiflora* leaves extracts at different doses

Treatment	Dose (mg/ml)	Elapsed time until paralysis (min)	Time taken for death (min)
Control			
Standard	20	39.67 $\pm$ 1.53	48.00 $\pm$ 2.64
MTN	25	59.67 $\pm$ 6.51*	70.33 $\pm$ 7.51*
	50	46.33 $\pm$ 2.51*	65.33 $\pm$ 5.03*
	75	40.66 $\pm$ 4.04*	51.00 $\pm$ 1.73*
CTN	25	61.67 $\pm$ 7.63	70.00 $\pm$ 7.21*
	50	60.00 $\pm$ 7.21	67.67 $\pm$ 7.37*
	75	51.67 $\pm$ 3.78*	56.33 $\pm$ 1.52*
ETN	25	58.67 $\pm$ 7.37*	67.00 $\pm$ 6.55*
	50	52.00 $\pm$ 4.35*	60.67 $\pm$ 4.61
	75	51.33 $\pm$ 3.21*	59.67 $\pm$ 8.62

Note: The values are given as mean $\pm$ SEM ($n = 3$). ANOVA was used to evaluate the data, and then Dunnett's *t*-test was performed, * $p < 0.001$ compared with standard drug.

dependent increase in their insecticidal potency. The CTN fraction showed the highest death rate (77.22%).

4 Discussion

From the beginning, human and plants interactions have shared most influential factors in human civilization, specifically in the field of medicine [13,22,23]. Till now, 80% population of developing countries are dependent on herbal formulation [24–27]. Currently, the phytopharmacological investigation has led to the discovery of many plant-based medications that have received food and drug administration approval and are proving to be the most effective treatments for a variety of ailments [28].

T. nudiflora is a tropical plant distributed in South Asia with vast traditional use [29,30]. Many studies reported the existence of my chemical constituents such as alkaloids,

polyphenols, steroids, saponins, glycosides, flavonoids, triglycerides, lignan, etc., in different parts of *T. nudiflora*, and these had been confirmed in part by our own findings. The extracts of various plant parts exhibited a number of *in vivo* pharmacological activities [10,11,14,29]. However, only a few studies had been conducted on its *in vitro* properties, which motivated us to conduct different *in vitro* pharmacological activities of the leaves of this plant [30,31]. In this study, we checked the *in vitro* thrombolytic, anti-arthritic, anthelmintic, and insecticidal activities of the methanolic leaf extract along with its different fractionate and discovered that it would be a valuable source for developing novel medications.

Thrombosis is a crucial occurrence in arterial diseases linked to myocardial infarction, anoxia, hypertension, stroke, and venous thromboembolic disorders, which are responsible for a substantial portion of the global mortality rate.

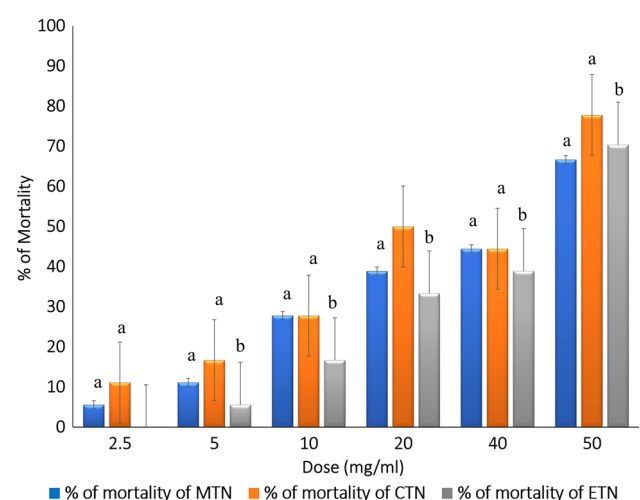


Figure 1: Insecticidal effects of leaves of *T. nudiflora*. The values were presented as mean $\pm$ SEM. The outcomes were obtained using the Student's *t*-test, where *a* and *b* were reported as $p < 0.05$, and $p < 0.01$ vs control, respectively.

The term “thrombolysis” refers to the medical procedure of pharmacologically dissolving or lysing blood clots inside a blood vessel [32]. Many thrombolytic drugs are now used in clinical settings to dissolve blood vessel clots that have already formed. Some of them have natural origins, while others have had their chemical constituents modified using recombinant technology to increase their efficacy and site specificity. Unfortunately, all of them still have a number of drawbacks, including a high risk of hemorrhage and a severe anaphylactic reaction that can be catastrophic [32–35]. Because of this reason, scientists are still searching for newer, safer, and effective plant-based drugs for thrombolytic remedies. Furthermore, epidemiologists have recently conducted several studies with plant and natural products and reported that natural thrombolytic/fibrinolytic agents can minimize the risk of thrombosis compared to synthetic products [18,33–36]. In our study, the crude methanolic leaf extract of *T. nudiflora* and its fractions showed significant thrombolytic properties. Here, the thrombolytic properties of plant extracts were checked against reference standard streptokinase. We found that adding water to the clot did not cause clot lysis during a comparison between positive and negative controls. However, the inclusion of the examined extracts demonstrated a substantial clot lysis. MTN had the highest rate of clot lysis among the extracts evaluated ($35.95 \pm 4.81\%$). Since tannin, flavonoids, alkaloids, saponins, and phytosterols were confirmed by the phytochemical analysis of the experimented crude extracts, and it was assumed that these bioactive constituents might be in charge of clot lysis action which was reported previously [34–42].

In many circumstances, autoantigens can be produced due to tissue protein denaturation, which is a major cause of arthritic conditions [43,44]. There are many factors, such as chemical exposure and heat, that create stress, denature proteins, and provoke the generation of autoantigens, which ultimately adversely affect the joint synovial membrane and cartilage. Another reason for protein denaturation is to an amendment of hydrogen, hydrophobic, disulfide, and electrostatic bonds in proteins [44–47]. Hence, substances that can prevent protein denaturation would be beneficial for the development of anti-arthritic drugs. In this study, all the extracts exhibit dose-dependent bovine serum albumin denaturation. For crude methanolic leaf extract, a significant activity was noticed compared to the other tested fractions at $500 \mu\text{g/mL}$ ($67.26 \pm 6.39\%$). Here, plant extracts were found to have anti-arthritic properties because they contain flavonoids and phenolic chemicals, which were previously claimed in several studies [44–47].

Recently, helminth infection has become a serious concern in both humans and animals, as it is responsible

for creating a chronic and debilitating sickness that can eventually cause death and is also responsible for drug resistance to other diseases [48]. To inhibit helminth infection, studies on natural products such as medicinal herbs are frequently conducted in the medicinal field, as they have some significant bioactive compounds that are novel with no or limited side effects. One of the most important advantages of those medicines is that they are easily accessible to folks in emerging countries and also show great compatibility with human physiology compared to conventional medicinal products. Recently, anthelmintic resistance and toxicity grow attention about drug residues in animal products in the use of plant-based therapies [44,49]. In the anthelmintic investigation, we differentiate the paralysis time and death time of earthworms between plant extracts and standard albendazole. Here, we detected a statistically significant link between extract graded concentrations, exposure time intervals, and adult parasite mortality. The paralysis/death time of the earthworms was inversely correlated with the strength of plant extracts. The MTN extract showed the highest efficacy (paralysis at 40.66 ± 4.04 min and death time at 51 ± 1.73 min) at 75 mg/mL . According to earlier studies, tannins, alkaloids, and saponins are the major bioactive molecules responsible for anthelmintic properties [50–53]. By establishing connections with unbound proteins in the host animal’s digestive tract or with the glycoproteins on the cuticles of earthworms, tannins demonstrate anthelmintic activity that may cause mortality [54]. Saponins mainly cause parallel irritation of the mucus membranes, which ultimately causes paralysis to death. In addition, helminths’ nervous systems and pre-parasitic phases might be directly affected by tannins and alkaloids. Tannins may bind to free proteins in the digestive system or glycoprotein on the parasite’s cuticle and kill them, which is why they have an anthelmintic impact [48,49]. Here, we confirm the above-mentioned bioactive compounds by photochemical screening tests of the extracts. Thus, our findings rationalize the anthelmintic properties of this plant, but an advanced study is required to isolate and characterize the responsible active principle.

Recently, herbal products are gaining attraction for their substantial potential as insecticidal compounds [55]. Our insecticidal study revealed that the mortality rate caused by crude extract and its fractionate was increased in a dose-dependent manner. We noticed that exposure time and dose played a crucial role in influencing susceptibility. Previous literature stated the existence of bioactive compounds in herb extracts, such as flavonoids, carbohydrates, phytosterol, phenol, and saponins, tannins can cause repellent, antifeedant, and toxic effects [56–59],

which were confirmed during the primary phytochemical screening of the experimented plant extracts.

5 Conclusion

In this study, *T. nudiflora* was chosen based on its historical applications and an examination of the literature. It was a primary investigation of thrombolytic, anti-arthritic, anthelmintic, and insecticidal activities of the methanolic and different solvent fractions of the leaf extract, which contain a copious of active phytochemicals. All the studied samples exerted notable protein denaturation activity in a dose-depending manner (100–500 µg/mL), where the methanolic extract of the plant showed the most inhibition ($67.26 \pm 6.39\%$) at 500 µg/mL concentration. ETN fraction of the plant showed the strongest anthelmintic activity at the highest dose. Among all samples, the CTN fraction displayed the utmost mortality rate (77.22%) in the insecticidal test. Finally, it can be concluded that *T. nudiflora* leaf extracts could be potential anti-arthritic, thrombolytic, anthelmintic, and insecticidal properties, which warrant the extensive phytochemical isolation and identification of responsible bioactive compounds to develop newer effective drugs upon preclinical and clinical investigations.

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Conflict of interest: The authors affirm that they have no competing interest.

Ethical approval: The conducted research is not related to either human or animal use.

Data availability statement: The datasets generated during and/or analyzed during this study are available from the corresponding author on reasonable request.

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