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Phytochemical Screening and Bioactivity Determination of Ethyl Acetate and Methanolic Extracts of Leaf and Bark of the Plant *Nyctanthes arbortristis* L.

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ABSTRACT

The present study targeted to evaluate the phytoconstituents of *Nyctanthes arbortristis* Linn., and its biological activity. By solvent extraction procedure, a total of four extracts were designated from the leaf and bark of the plant using two solvents (leaf extract in ethyl acetate and methanol: NALE & NALM; bark extract in ethyl acetate and methanol: NABE & NABM). Phytochemical screening was conducted by qualitative analysis and thin-layer chromatography. Parallelly, antioxidant property (by DPPH free radical scavenging method) and antimicrobial activity (by disk diffusion method) were also investigated to determine bioactivity. The presence of alkaloids and glycosides was detected by qualitative phytochemical assay of the extracts. Furthermore, TLC successfully observed the versatility of the compound's presence, such as phenolic compounds, different alkaloids, and glycosides. In DPPH assay, methanolic extracts are highly capable of scavenging the radicals than the ethyl acetate extracts. Surprisingly, IC50 for NABM (1.69 µg/ml) is less than ascorbic acid (3.58µg/ml), which is an exploration of excellent antioxidant potential of the plant. But any extracts showed no positive result in the antimicrobial test against gram-positive or gram-negative bacteria, even against yeast.

Keywords: Phytoconstituents, Thin layer chromatography, Alkaloids, Glycosides, and Free radical scavenging.

INTRODUCTION:

Phytochemicals, labeled as primary or secondary metabolites, primarily focus on their function in the plants' metabolism. Studies have discovered a broad array of functional capability on human physiology of secondary phytochemicals such as alkaloids, terpenes, flavonoids, curcumins, saponins, phenolics, flavonoids, and glucosides (Makkar *et al*., 2007). A wide range of secondary metabolites is found in the plant family Oleaceae having high level of pharmacological values (Huang *et al*., 2019). But, little is known regarding the chemistry and pharmacology of *N. arbortristis* Linn., (Night jasmine) of this family.

It is a shrub of 10m long with flowers of high fragnance which bloom at night and fall off before sunrise. N. arbor-tristis is indigenous to India, widely distributed in South East Asia, and also cultivated in tropical and subtropical regions globally (Rani *et al*., 2012). Earlier studies on this plant revealed that several iridoid glycosides (Sing *et al*., 1995; Tuntiwachwuttikul *et al*., 2003, Khanapur *et al*., 2014), three carotenoid glucosides (Mathuram and Kundu, 1991), phenyl-propanoid glycoside (Tuntiwachwuttikul *et al*., 2003), desrhamnosylverbascoside (Mathuram *et al*., 1997), rengyolone (Tuntiwachwuttikul *et al*., 2003), 6ß hydroxylloganin, nyctanthoside (Jensen

and Nielsen, 1982) were isola-ted from the plant. *N. arbortristisis* a mythological plant and has high therapeutic values used in Homeopathic, Sidha, Unani, and Ayurveda (Rani *et al*., 2012). The commonly used case highlights its anti-helminthic and antipyretic effects (Khanapur *et al*., 2014). Furthermore, the isolated pure compounds possess significant bioactivity like anti-inflammatory, antiallergic, immune-modulatory, anticancer, antiviral, and antileishmania (Agrawal and Pal, 2013; Shukla *et al*., 2011). Therefore, the present investigation has an aim to determine the diversity and bioactivity of several plant parts using the solvents of different polarity.

MATERIALS AND METHODS:

Collection and drying of the plant - The leaves and bark of *N. arbortristis* were collected from several household gardens in Noakhali, Bangladesh, and identified by botanists. The leaves and bark are dried in room temperature and humidity. It is then heated at 60°C in an oven for 10 hours for the final drying before the grinding.

Extraction of phytochemicals - Each powdered portion was soaked into ethyl acetate and methanol for 1st 7 days and 2nd 7 days. After every seven days, the filtrates were subjected to cold extraction procedures using a rotary evaporator. The final concentrated four extracts preserved in fridge labeling NALE, *N. arbortistis* leaf in ethyl acetate; NALM, *N. arbortistis* leaf in methanol; NABE, *N. arbortistis* bark in ethyl acetate; NABM, *N. arbortistis* bark in methanol.

Phytochemical screening

Detection of alkaloids - The extracts were dissolved individually in dilute Hydrochloric acid. Then alkaloid detection was carried out treating the sample with Mayer's reagent (Potassium Mercuric Iodide), Wagner's reagent (Iodine in Potassium Iodide), and Dragendorff's reagent (solution of Potassium Bismuth Iodide). The formation of turbidity or precipitation scored positive for alkaloids (Kaur and Arora, 2009).

Tests for glycosides - Keller-Killiani test: Dissolved plant extracts (100 mg) in absolute methanol (2 ml) were treated with glacial acetic acid (2 ml). Then, 5% of ferric chloride solution (1ml) and concentrated sulfuric acid (1ml) were added. The appearance of a reddish-brown ring indicated the presence of cardiac glycoside (Harborne, 2005).

Borntrager test - Filtrate of 100mg plant extracts in chloroform (5 ml) was shaken with an equal volume of 10% NH_4OH. The appearance of pink-violet or red color in the bottom layer is positive for anthraquinones (Raaman, 2006).

Test for steroids

Salkowski's Test - Filtrates of extracts with chloroform were treated concentrated sulfuric acid. The appearance of reddish-brown color at the interface after shaking indicates steroids' presence (Joshi *et al*., 2013).

Liebermann Burchard's test - Extracts in chloroform were treated with acidic anhydride, boiled, cooled and followed by concentrated sulfuric acid addition. Formation of greenish transient color scores positive for steroids (Raaman, 2006).

Test for Saponins - 20 ml diluted extracts were shaken in a graduated cylinder for 15 minutes. A one-centimeter layer of foam indicated the presence of saponins (Raaman, 2006).

Thin Layer Chromatography (TLC)

Detection of major phytochemicals was also carried out by pre-coated (Kiesel gel 60 PF254) TLC plate (Wagner *et al*., 2004). Mix solvents (Toluene: Ethyl acetate = 90%: 10%) is used to plate development and observed under visible light as well as UV light (254 nm and 365 nm). The number and diversity of colors with different Rf differentiate the types of compounds present in the extracts.

Free Radical Scavenging Activity (DPPH Method) - The hydrogen-donating abilities of extracts were examined through the method described by Khanapur *et al*., 2014, with a little bit modification. Briefly, 1.6 mg of different extracts was dissolved in methanol to get a mother solution. Serial dilution of each 2 ml mother and addition with 2 ml 2, 2-diphenyl-1-picrylhydrazyl in methanol (20 µg/ml) got a total of 4 ml mixture of different sample concentration from 200.0 to 0.78125 µg/ml. Methanol (95%) and ascorbic acid (same concentrations as a sample) were used as blank and reference. The UV absorbance was taken at 517 nm after 30 minutes' incubation at a dark place. 50% of DPPH free radical scavenging concentration. (IC_{50}) has been measured using the regression equation attain from the values of at least five consequent dilutions.

Antimicrobial Test - The antibacterial potential of the four extracts of *N. arbortristis* was determined by the disc diffusion method described by Santoyo *et al.*, 2005 against two gram-positive bacteria (*Staphylococcus aureus*, *Enterococcus* sp.), three gram-negative bacteria (*Escherichia coli*, *Salmonella typhi*, *Pseudomonas* sp.), and one fungus (*S. cerevisiae*). Each disc of plant extracts contained 40µg and the zone of inhibition compared to ciprofloxacin (20µg/disc) and ketoconazole (20 µg/disc).

RESULTS AND DISCUSSION:

In the study, ethyl acetate (medium polar) and methanol (polar) were used to extract both polar and slightly polar compounds of *N. arbortristis* leaf and bark. A total of four samples were designated as NALE, NALM, NABE, and NABM. The samples showed distinguishable features in phytochemical screening and antioxidant activity, which indicates the impact of the solvent's polarity and the unequal distribution of phytoconstituents in leaf and bark.

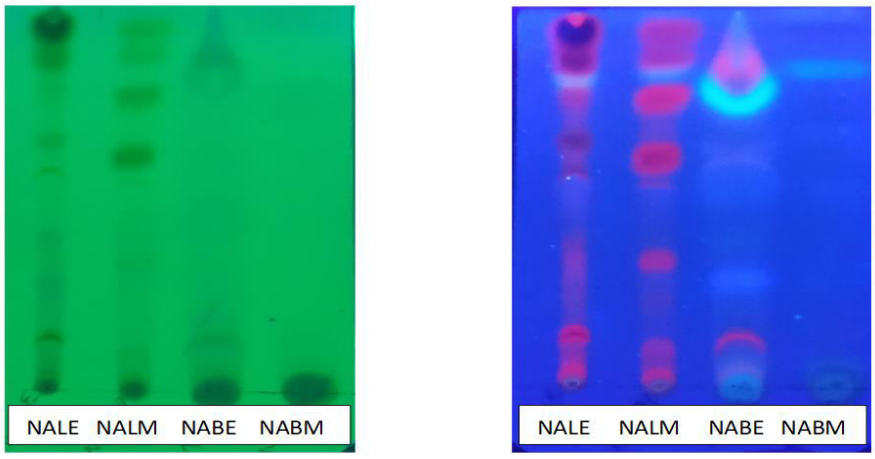
Qualitative Phytochemical screening

Table 1: Level of phytoconstituents' presence in samples

Tests	Samples			
	NALE	NALM	NABE	NABM
Test for Alkaloids:				
Mayer's Test	-	+	-	-
Wagner's Test	++	++	++	++
Dragendorff's reagent	-	-	-	-
Tests for glycosides:				
Cardiac glycosides (Keller-Killiani test)	++	++	++	++
Anthraquinone glycosides (Borntrager's test)	-	-	+	-
Tests for steroids:				
Liebermann-Burchard test	-	-	-	-
Salkowski test	-	-	-	-
Tests for Saponins:				
Foam test	-	-	-	-

+ slightly present, ++ highly present, -absent

TLC Screening



A. 254 nm B. 365 nm

Fig 1: Screening of NALE, NALM, NABE, NABM by thin-layer chromatography (TLC) at (A) 254 nm and (B) 365 nm UV light.

Table 2: List of color development in TLC plate and probable compounds of the samples

Test Sample	Visual color	UV light at 254nm	UV light at 365nm	Remarks on probable compounds
NALE	Green, Dark	Dark, Dark green, Bluish	Red, Black, Light, Brownish, Light red	Chlorophyll, Phenolics, Quinolone, Glycosides, Anthocyanin
NALM	Green	Two dark, Light, Bluish	Red, Sky blue, light	Chlorophyll, Phenolics, Anthraquinone, Glycosides
NABE	Brown, Yellow	Two dark, Light,	Red, Bluish, Blue	Quinolone, Anthraquinone, Flavonoids, Phenolics, Terpenoids, Glycosides
NABM	Brown	Two dark Light	Blue, Reddish	Phenolics, Glycosides, Quinolone

In Wagner's test and Keller-Killiani test, all samples explored the presence of high-level alkaloids and cardiac glycoside. NABE had a slight presence of anthraquinone glycosides. Test for steroids and saponin presented negative results in every case.

TLC screening showed several spots in both long and short of UV light for the samples except NABE in the solvent system (Toluene: Ethyl acetate = 90%: 10%). The developed plates represented the presence of different types of probable compounds mentioned in **Table 2**. All samples had a chance to have alkaloids, phenolic compounds, and glycosides.

The qualitative phytochemical screening tabulated in **Table 1** show the presence of alkaloids and cardiac glycosides to a large extent. Alkaloids represent a range of heterocyclic compounds with broad biological activity such as anti-inflammatory, antipyretic, neuroactive, antitumor, anticancer, and immune-modulatory effects (Roberts, 2013). Cardiac glycosides are a unique type of compound and are effectively used in cardiac failure. It also has antitumor and antiviral activity (Morsy, 2017). Furthermore, the multi-colored TLC screening spots revealed the plant as a repository of a good amount of phytoconstituents.

Antioxidant test

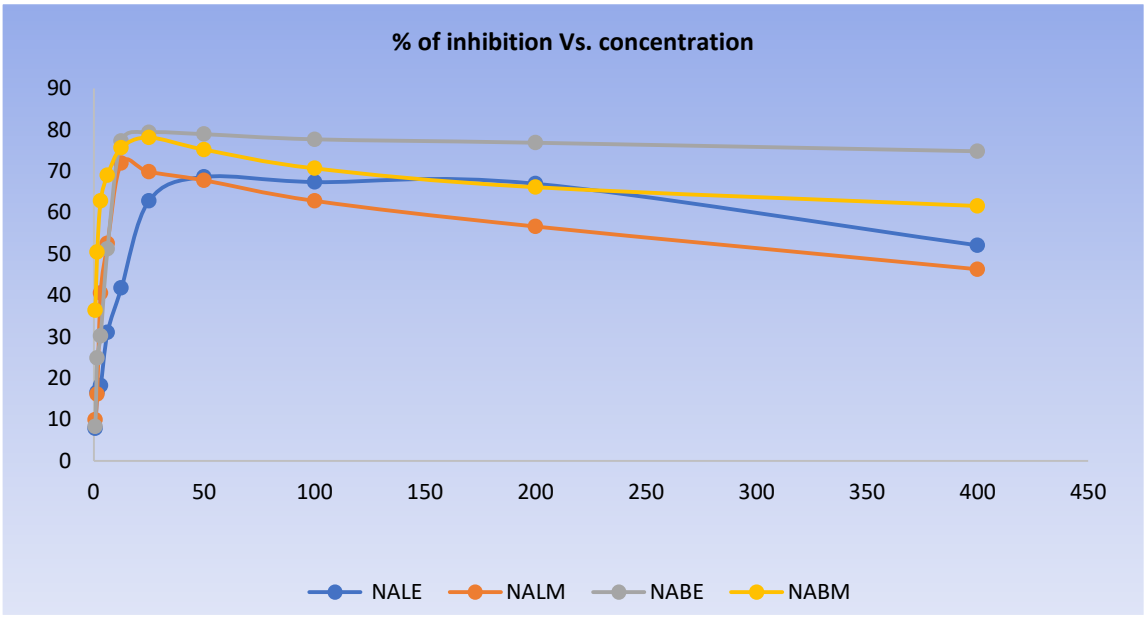


Fig 2: DPPH radical scavenging activity curve of leaf and bark extract of the plant *N. arbortristis*.

The percentage of DPPH radical scavenging tendency followed a similar pattern with concentrations (**Fig 2**). The first five doses of every sample had a sharp rise of free radical inhibition, but after that, it had a slight fall of inhibitory activity with the increase of dose (Shahen *et al.*, 2019). The results showed a dose-response relationship from 0.752 µg/ml to 12.5 µg/ml, as mentioned by Khanapur *et al.* (2014).

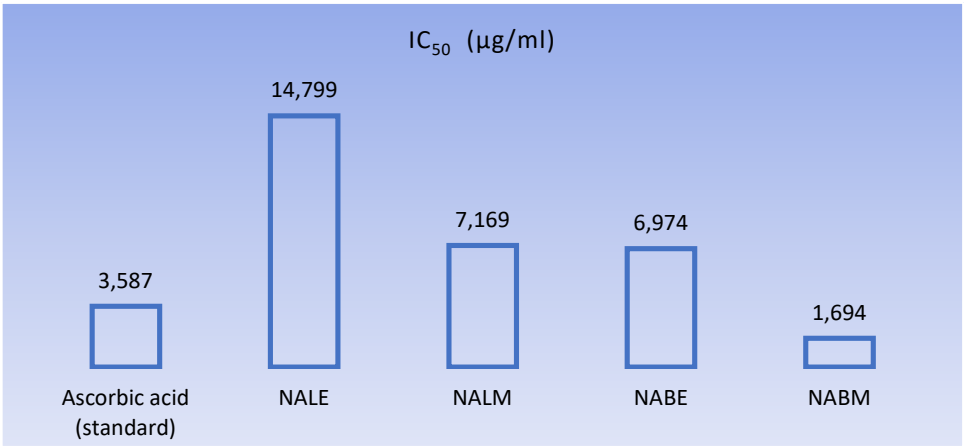


Fig 3: IC_{50} values of standard and samples.

DPPH assay is very sensitive to qualify and quantify the free radical scavenging property, and the present study carried out to determine the plant's antioxidant potential by this method. IC_{50} values of NALE, NALM, NABE and NABM are 14.799 µg/ml, 7.169 µg/ml. 6.974 µg/ml, and 1.694 µg/ml, respectively, where for ascorbic acid; the value is 3.587 µg/ml (**Fig 3**). The extracts of bark showed comparatively better activity than the leaf. Whereas, methanolic extracts are highly capable of scavenging the radicals than the ethyl acetate extracts. IC_{50} of NALM is half of NALE, and for NABM, the value is one-fourth of NABE. So, the polar solvent might had dissolved a few more-polar phytochemicals that had good antioxidant activity. So, the plant might contain polyphenols, carotenoids (Sasikumar *et al.*, 2010). Interestingly, the IC50 values of extracts are not very far from this study's standard (ascorbic acid). NABM shoed more potential than the standard that directly highlights the plant's usefulness as an antioxidant (Rathee *et al.,* 2007).

Antimicrobial test

The mean zone of inhibition of ciprofloxacin (20 µg/disc) against the five test bacteria was 19mm and the inhibitory zone for ketoconazole (20 µg/disc) was 16 mm against yeast. But unexpectedly, the study failed to find any antibacterial or antifungal effect of the samples at the dose 40 µg/disc, though, several studies revealed this activity to a small extent (Ankita *et al.*, 2014; Mosaib *et al.,* 2020; Kumar *et al.,* 2019). Therefore, the research suggests conducting studies on a larger dose than the study.

CONCLUSION:

The present study on ethyl acetate and methanolic extracts of *N. arbortristis* explored a profile of several phytoconstituents and an excellent antioxidant activity. Phytochemical screening marked the high presence of alkaloids and glycosides. Though no antimicrobial activity is observed, the amazing radical scavenging capability of the plant pointed out its potential to use against aging, cancer, cardiac disease. Therefore, the plant is a good source of bioactive compounds and might be a potential drug discovery source.

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CONFLICTS OF INTEREST:

The author (s) declared no potential conflicts of the interest with respect to the research, authorship and/or publication of this article.

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