

PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL ACTIVITIES OF *NYCTANTHES ARBOR-TRISTIS* L.

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Abstract

The plant *Nyctanthes arbor-tristis* L. or Night jasmine belongs to the family Oleaceae. The plant is known as Seik-Phalu in Myanmar. It grows in tropical and subtropical region. The specimens were collected from Banmaw University Campus, Kachin State, during the flowering and fruiting periods. The morphological characters, preliminary phytochemical test and antimicrobial activities were studied. The plant used in traditional medicines as stomachic, carminative, intestinal astringent, expectorant, in biliousness, piles, various skin diseases and hair tonic. In morphological study, the plant was perennial small tree, quadrangular branches, opposite and decussate, simple leaves and fragrant flowers. Preliminary phytochemical test revealed the presence of alkaloid, α -amino acid, glycoside, flavonoids, reducing sugar, phenolic compound, saponin, starch, tannin and carbohydrate. The different solvent extracts were prepared for antimicrobial activities tests and their inhibitory zones were also evaluated. The antimicrobial activities were carried out by agar well diffusion method on seven types of test microorganisms. Methanol extract of leaves showed significant antimicrobial activity against *Bacillus pumilus*, *Bacillus subtilis* and *Staphylococcus aureus*. Ethanol extracts of leaves showed the significance against on *Bacillus pumilus* and *Xanthomonas oryzae*. Watery extracts showed moderate activities on *Bacillus pumilus* and *Bacillus subtilis*, petroleum ether extracts exhibited against only on *Bacillus subtilis* and chloroform extracts of leaves showed the best activities on *Candida albicans*.

Keywords *Nyctanthes arbor-tristis* L., Phytochemical test, Antimicrobial activities

Introduction

Natural products and traditional medicines are of great importance. They have own advantages, such as chemical structures, biological activities and antimicrobial activities. Since prehistoric times, humans have used natural products, such as plants, animals, microorganisms, and marine organisms for the treatment of diseases. According to fossil records, the human use of plants as medicines may be traced back of least 60,000 years (Shi *et al.*, 2010).

The plant *Nyctanthes arbor-tristis* L. or Night jasmine belongs to the family Oleaceae. *Nyctanthes arbor-tristis* L. is native to India. It is also widely distributed in Bangladesh, Indo-Pak subcontinent and South-East, tropical and sub-tropical South East Asia. It grows in the Indo-Malayan region and distributed across Terai tracts as well as Myanmar, Ceylon and Thailand (Kirtikar and Basu, 1975). The common names of the plant are night jasmine, coral jasmine (English) and Sihau (Hindi). It has highly fragrant and brilliant flowers. The flowers start falling after midnight and by the day break. The generic name *Nyctanthes* has been coined from two Greek words Nykhta (Night) and Anthos (flower).

The plants are used as traditional medicine for women related problems, such as provoke menstruation, for treatment of scabies and other skin diseases and hair tonic (Jain and Pandey, 2016). The bitter leaves juice is used to expel roundworms and threadworms in children, chronic fever, malarial fever, obstinate sciatica and rheumatism. The leaves decoction is used for the treatment of arthritis and malaria. The young leaves are used as female tonic and in alleviating gynecological problems (Bhalakiya and Modi, 2019).

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The aim and objectives of the present research were to verify the morphological characters of *Nyctanthes arbor-tristis* L., to know the phytochemical constituents, medicinal uses and to examine the antimicrobial activities of leaves.

Materials and Methods

Botanical Studies

The specimens used in this research were collected from Banmaw University Campus, Kachin State. They were collected especially during the flowering and fruiting period from July to October in 2021. The collected fresh specimens of the plants were identified by using literatures of Lawrences, 1964; Backer, 1965; Hundley and Chit Ko Ko, 1987; Dassanayake and Clayton, 2000 and Kress *et al.*, 2003.

Chemical Studies

The collected leaves of *Nyctanthes arbor-tristis* L. were washed with distilled water thoroughly and dried at room temperature under the shade for 2-3 weeks. The dried samples were pulverized by grinding with a blender to get fine powder and stored in air tight container. For preliminary phytochemical test, the powdered leaves were tested for alkaloids, α -amino acid, carbohydrates, flavonoids, glycoside, phenolic compound, reducing sugar, saponin, starch and tannin.

Preliminary Phytochemical Test of *Nyctanthes arbor-tristis* L. Leaves

The preliminary phytochemical tests were carried out according to Vogel, 1956; British Pharmacopoeia 1968, Marini Bettolo *et al.*, 1981; Robinson 1983 and Central Council for Research in Unani Medicine, 1987.

Test for Alkaloid

One gram of powdered sample was boiled for about 20 minutes with 20ml of 10% HCl and filtered. The filtrate tested with Wagner's reagent. The precipitate formed on the addition of the reagent indicated the presence of alkaloid (Robinson, 1983).

Test for α -Amino acid

One gram of powdered sample was boiled with 10ml of distilled water for 20 minutes and then filtered. And then, a few drops of each filtrate was spotted on a filter paper using a capillary tube, allowed it to dry and sprayed with ninhydrin reagent. The filter paper was dried at room temperature and then kept it in oven at 110°C for a few minutes after which the purple colour appears due to the presence of α -amino acids (Marini Bettolo *et al.*, 1981).

Test for Glycoside

One gram of powdered sample was heated in a glass test tube with 10ml of distilled water on the water-bath for 20 minutes. The mixture was filtered and 10% basic lead acetate solution was added drop-wise to the filtrate. Pale yellow precipitate was observed which showed the presence of glycoside (Marini Bettolo *et al.*, 1981).

Test for Carbohydrate

One gram of powdered sample was boiled with 10 ml of distilled water for 20 minutes and filtered. The filtrate was introduced into a test tube and a few drops of 10% α -naphthol was added and shaken. The test tube was then inclined at an angle of 45° and concentrated sulphuric acid was added slowly along the side of the inner tube. A red ring was formed between the two layers, showing the presence of carbohydrate (Marini Bettolo *et al.*, 1981).

Test for Flavonoids

Dried powdered sample one gram was extracted with methanol and filtered. When the methanolic extract was treated with a few drops of concentrated hydrochloric followed by a small piece of magnesium. The solution was boiled for a few minutes. The appearance of dark green colour indicates the presence of flavonoid (Central Council for Research in Unani Medicine, 1987).

Test for Reducing Sugar

One gram of powdered sample was boiled with dilute sulphuric acid and filtered. The filtrate was then neutralized with dilute sodium hydroxide solution. When the resulting solution was treated with Benedict's solution, it furnished yellow precipitates, indication the presence of a reducing sugar (Marini Bettolo *et al.*, 1981).

Test for Starch

One gram of dried powdered sample was boiled with 10 ml of distilled water for about 20 minutes. It was then filtered and two drops of iodine solution were added to the filtrate. Reddish brown precipitate was formed which indicate the presence of starch (Central Council for Research in Unani Medicine, 1987).

Test for Saponin

One gram of powdered sample was boiled with 10 ml of distilled water for about 20 minutes and filtered. The filtered and the filtrate shaken vigorously with distilled water for a few minutes. Marked fothing which lasted for about half an hour to take place, indicating the presence of saponin (Marini Bettolo *et al.*, 1981).

Test for Tannin

One gram of powdered sample was boiled with 10ml of distilled water for about 20 minutes and filtered. The filtrate was treated with a few drops of 1% ferric chloride solution. If a bluish black or yellowish brown colour resulted indicating the presence of tannins (Marini Bettolo *et al.*, 1981).

Test for Phenolic compound

One gram of powdered sample was boiled with 10 ml of distilled water for 20 minutes and filtered. The filtrate was treated with neutral 5% ferric chloride solution, it gave dark green colour, indicating the presence of phenol groups (Marini Bettolo *et al.*, 1981).

Antimicrobial Activities of Different Solvent Extracts of *Nyctanthes arbor-tristis* L. Leaves

The antimicrobial activity test on different solvent extracts of powered sample was done by agar well diffusion method described in Medicinal Microbiology (Cruickshank, 1975).

Extraction of crude drugs

Five grams of powder was soaked with 50 ml of different solvents such as methanol, ethanol, acetone, water and pet-ether for about three days and thoroughly shake. The mixture was filtered and evaporated.

Cultivation of Test Organisms

Bacillus pumilus (IFO 905771), *Bacillus subtilis* (IFO 90571), *Candida albicans* (NITE 09542), *Micrococcus luteus* (NITE 83297), *Pseudomonas* sp. (IFO 94307), *Staphylococcus aureus* (ATCC 12877) and *Xanthomonas oryzae* were used. They were inoculated into the nutrient broth and transferred into nutrient agar media.

Preparation of Plates for Antimicrobial Activities Test

The antimicrobial activities were performed by agar-well diffusion method. Nutrient agar was prepared according to method described by Cruickshank, 1975. Nutrient agar was boiled and 20-25 ml of the medium was poured into a test-tube and plugged with cotton wool and autoclaved at 121 °C for 15 minutes. Then, the tubes were cooled down to 30-35°C and poured into sterilized petridishes and 0.01 ml of spore suspension were also added into the dishes. The agar was allowed to set for 30 minutes after with 8 mm plate agar well was made with the help of sterilized cork borer. After that, about 0.1ml of sample was introduced into the agar-well and incubated at 37°C for 24-48 hrs. The inhibition zone showed around the agar-well indicating the presence of anti-microbial activity. The extent of antimicrobial activity was measured from the zone of inhibition diameter. The results were shown in Table 2, Figures 4 - 5.

Results

Morphological Characters of *Nyctanthes arbor-tristis* L.

Scientific name	- <i>Nyctanthes arbor-tristis</i> L.
Myanmar name	- Seik-Phalu
English name	- Night Jasmine
Family	- Oleaceae
Flowering and fruiting period	- July to October
Parts used	- Leaves

Perennial small tree, about 5 m in high. Stems cylindrical, woody with grey or greenish-white bark; branches quadrangular. Leaves simple, opposite and distichous, petiolate, exstipulate; lamina ovate-lanceolate, 8.3-14.3 x 2.7-8.6 cm, rounded at the base, entire or with a few large distant teeth at the margin, acuminate at the apex, dark green above and pale green beneath, coriaceous, hirsute. Inflorescences axillary or terminal cluster cymes, 3-7 flowered. Flowers white with orange-red center, about 3.4 cm across at anthesis, bracteate, ebracteolate, sessile, complete, bisexual, regular, actinomorphic, 6-merous, cyclic, hypogynous, delicate fragrant. Calyx 6 lobes, narrowly campanulate, greenish yellow, ribs present, glabrous with and ciliate without; tube about 0.9cm long; lobes obscurely. Corolla salverform, glabrous; tube 1-1.1 cm long, orange colour, lobes unequally obcordate, cuneate, 1-1.3 cm long, white with an orange-red center, glabrous. Stamens 2, epipetalous, inserted near the top of the corolla tube; filaments very short, adnate to the corolla tube, bright orange, glabrous; anthers oblong, about 0.2 cm long, pale yellow, ditheous, dorsifixed, dehiscence longitudinal slit. Ovary superior, globose, about 0.15 cm in diameter, yellowish green, glabrous, carpels 2, bicarpellary, syncarpous, bilocular with one ovule in each locule, basal placentas; style terete, about 0.2 cm long, white, glabrous; stigma bilobed. Fruits capsule, obcordate, 1.5-2.5x1.2-1.7cm, one seeded.



Habit



Ventral View of Leaves



Dorsal View of Leaves

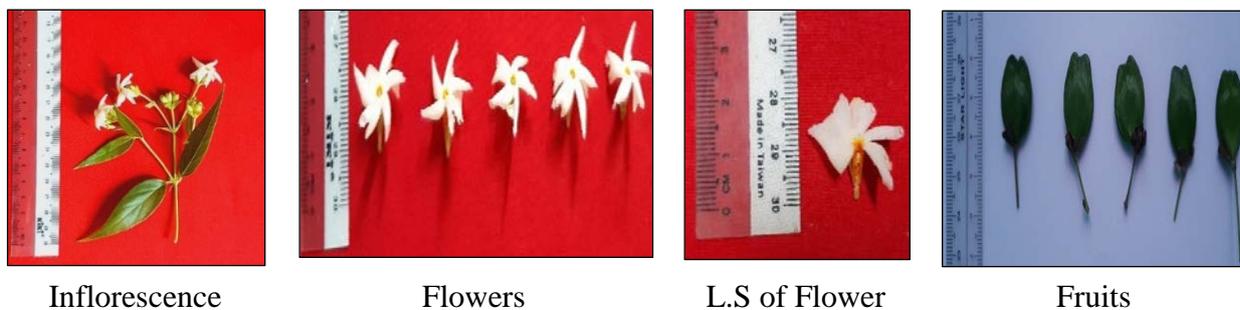


Figure 1 Morphological Characters of *Nyctanthes arbor-tristis* L.

Preliminary Phytochemical Test of *Nyctanthes arbor-tristis* L. Leaves

The results of preliminary phytochemical test of air-dried powdered leaves from *Nyctanthes arbor-tristis* L. indicated that alkaloid, α -amino acid, glycoside, flavonoids, reducing sugar, phenolic compound, saponin, starch, tannin and carbohydrate were found to be present. Among them, the amount of precipitate from glycoside was highest than the other tests.



Figure 2 Phytochemical Test of *Nyctanthes arbor-tristis* L. Leaves

Table 1 Preliminary Phytochemical Test of Leaves of *Nyctanthes arbor-tristis* L.

No.	Test	Extract	Test reagent	Observation	Results
1.	Alkaloid	10% HCl	Wagner's reagent	Brown ppt	+
2.	α -amino acid	H ₂ O	Ninhydrin reagent	Purple	+
3.	Glycoside	H ₂ O	10% lead acetate solution	Pale yellow ppt	+
4.	Carbohydrates	H ₂ O	10% α -naphthol (H ₂ SO ₄ Conc:)	Red ring	+
5.	Flavonoids	MeOH	H ₂ SO ₄ Conc: + Magnesium	Dark green	+
6.	Reducing sugar	H ₂ O	Benedict's solution	Furnished Yellow	+
7.	Starch	H ₂ O	Iodine solution	Reddish Brown ppt	-
8.	Saponin	H ₂ O	Distilled water	Foaming	+
9.	Tannin	H ₂ O	1% FeCl ₃ solution	Bluish Black	+
10.	Phenolic compound	H ₂ O	5% FeCl ₃ solution	Dark Green	+

Key to the table (+) = present (-) = absent (ppt) = precipitate

Antimicrobial Activities of Different Solvent Extracts of *Nyctanthes arbor-tristis* L. Leaves

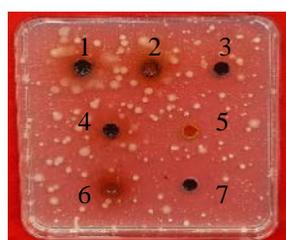
In this study, ethanol extracts of leaves showed the significant against on , *Bacillus pumilus* and *Xanthomonas oryzae*, moderate against on *Bacillus subtilis*, least against on *Candida albicans*, *Staphylococcus aureus* and gave the negative results on *Micrococcus luteus* and *Pseudomonas* sp. Methanol extract of leaves showed significant against on *Bacillus pumilus*, *Bacillus subtilis*, and *Staphylococcus aureus*, moderate against on *Xanthomonas oryzae* and negative results on all other test organisms. Watery extracts showed moderate activities on *Bacillus pumilus*, *Bacillus subtilis* and gave the negative results on all other test organisms. Ethyl acetate extracts of leaves exhibited negative results on *Candida albicans* and *Micrococcus luteus*, moderate activities exhibited on other test organisms. Pet-ether extracts exhibited against only on *Bacillus subtilis*. Acetone extract revealed the negative result on *Micrococcus luteus*, moderate against on *Candida albicans* and the best activities on all other test organisms. Chloroform extracts of leaves showed the moderate activities on *Bacillus pumilus*, *Bacillus subtilis* and the best activities on all other test organisms.

Table 2 Inhibition Zone Exhibited by Different Solvent Extract of *Nyctanthes arbor-tristis* L. Leaves

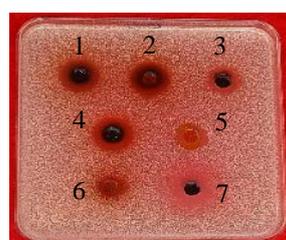
No.	Microorganisms	Inhibition Zones (mm)						
		H ₂ O	MeOH	EtOH	EtOAC	CHCl ₃	P.E	Ace
1.	<i>Bacillus pumilus</i>	15	20	20	15	15	-	18
2.	<i>Bacillus subtilis</i>	10	18	18	15	15	12	20
3.	<i>Candida albicans</i>	-	-	12	-	22	-	15
4.	<i>Micrococcus luteus</i>	-	-	-	-	18	-	-
5.	<i>Pseudomonas</i> sp.	-	-	-	12	20	-	20
6.	<i>Staphylococcus aureus</i>	-	17	15	14	18	-	17
7.	<i>Xanthomonas oryzae</i>	-	15	20	13	17	-	18

Key to the Table

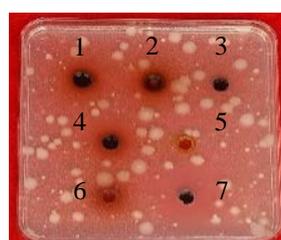
MeOH - Methanol EtOH - Ethanol P.E - Pet-ether CHCl₃ - Chloroform
 Ace - Acetone H₂O - Watery EtOAC -Ethylacetate Agar well = (8 mm)



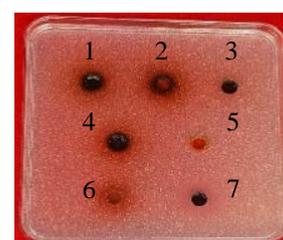
Bacillus pumilus



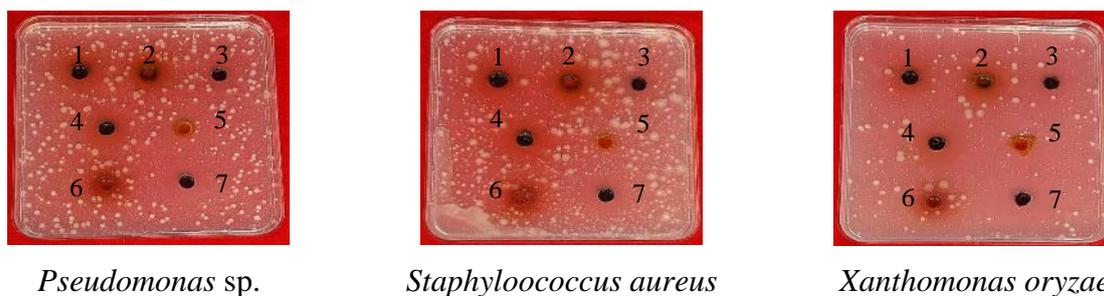
Bacillus subtilis



Candida albicans



Micrococcus luteus



1. Ethanol Extract 2. Methanol Extract 3. Ethylacetate Extract 4. Acetone Extract
5. Pet-ether Extract 6. Watery Extract 7. Chloroform Extract

Figure 3 Antimicrobial activities of leaves from *Nyctanthes arbor-tristis* L.

Discussion and Conclusion

Herbal medicines, also referred to as botanical medicine or phytomedicine. According to "The World Health Organization", 80% of people in some Asian and African countries rely on herbal medicines for their primary health care. Pharmacologist, botanists, microbiologists and chemists have been used various plants for treatment of infectious diseases. Plants are rich in a variety of secondary metabolites. The quality control of herbal medicine became important as the use of a variety of medicinal plants. Therefore, the plant materials are the most important for human health care.

Nyctanthes arbor-tristis L. is perennial small tree, about 5 m in high. Stems cylindrical, woody with grey or greenish-white bark; branches quadrangular. Leaves simple, opposite and distichous, petiolate, exstipulate; lamina ovate-lanceolate, rounded at the base, entire or with a few large distant teeth at the margin, acuminate at the apex, dark green above and pale green beneath, coriaceous, hirsute. Inflorescences axillary or terminal cluster cymes, 3-7 flowered. Flowers white with orange-red center, across at anthesis, bracteate, ebracteolate, sessile, complete, bisexual, regular, actinomorphic, 6-merous, cyclic, hypogynous, delicate fragrant. Calyx 6 lobes, narrowly campanulate, greenish yellow, ribs present, glabrous with and ciliate without; tube about 0.9cm long; lobes obscurely. Corolla salverform, glabrous; tube orange colour, lobes unequally obcordate, cuneate, white with an orange-red center, glabrous. Stamens 2, epipetalous, inserted near the top of the corolla tube; filaments very short, adnate to the corolla tube, bright orange, glabrous; anthers oblong, pale yellow, ditheous, dorsifixed, dehiscence longitudinal slit. Ovary superior, globoid, yellowish green, glabrous, carpels 2, bicarpellary, syncarpous, bilocular with one ovule in each locule, basal placentas; style terete, white, glabrous; stigma bilobed. Fruits capsule, obcordate, one seeded.

In Ayurvedic medicine, *Nyctanthes arbor-tristis* L. leaves were used for the treatment of sciatica, chronic fever, rheumatism, internal worm infections, laxative, and diuretic. Leaf juice is mixed with honey and given thrice daily for the treatment of cough. Paste of leaves is given with honey for the treatment of fever, high blood pressure and diabetes (Meshram *et al.*, 2012).

In this research, the preliminary phytochemical analysis of the extracts of leaves contained alkaloid, α -amino acid, glycoside, flavonoids, reducing sugar, phenolic compound, saponin, starch, tannin and carbohydrate were found to be present. These data were agreed with Jain and Pandey, 2016. They stated that the presence of secondary metabolites such as tannins, saponins, alkaloids, flavonoids, steroids, phenolic compounds, reducing sugar and carbohydrate. Rani *et al.*, 2012 stated that alkaloids, phenolics, tannins, flavonoids, glycosides and saponins.

In the antimicrobial activities test, ethanol extracts of leaves showed the significant against on *Bacillus pumilus* and *Xanthomonas oryzae*, moderate against on *Bacillus subtilis*, least against on *Candida albicans*, *Staphylococcus aureus* and gave the negative results on *Micrococcus luteus* and *Pseudomonas* sp. Methanol extract of leaves showed significant against on *Bacillus pumilus*, *Bacillus subtilis*, and *Staphylococcus aureus*, moderate against on *Xanthomonas oryzae* and negative results on all other test organisms. Watery extracts showed moderate activities on *Bacillus pumilus*, *Bacillus subtilis* and gave the negative results on all other test organisms. Ethyl acetate extracts of leaves exhibited negative results on *Candida albicans* and *Micrococcus luteus*, moderate activities exhibited on other test organisms. Pet-ether extracts exhibited against only on *Bacillus subtilis*. Acetone extract revealed the negative result on *Micrococcus luteus*, moderate against on *Candida albicans* and the best activities on all other test organisms. Chloroform extracts of leaves showed the moderate activities on *Bacillus pumilus*, *Bacillus subtilis* and the best activities on all other test organisms. Priya and Ganjewala, 2007, Shrivastava and Bharadwaj, 2018 stated that methanolic extract of leaves exhibited significant antibacterial activity against *Staphylococcus aureus*, *Staphylococcus epidemis*, *Samonella typhi*, *Samonella paratyphi*. Meshram *et al.*, 2012 stated that chloroform extract showed both antibacterial and antifungal activity, the petroleum ether and ethanol extracts possess only antibacterial activity.

The present study was undertaken to investigate the preliminary phytochemical analysis of leaves, to check the secondary metabolites and antimicrobial activities of *Nyctanthes arbor-tristis* L., help in other experimental analysis, to distribute the knowledges and advandages of traditional medicines for local people.

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