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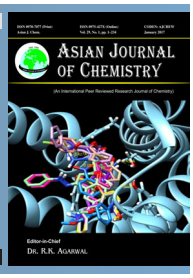

Isolation and Characterization of Bioactive Compounds from Stem of *Nyctanthes arbor-tristis* Linn. and Effect of Different Fractions on Phytopathogens

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Phytochemical investigation of the stem of *Nyctanthes arbor-tristis* (Harsingar) resulted the isolation of 21α-hydroxyfriedel-4-(23)-en-3-one, β-sitosterol, 1-triacontanol, friedel-1-ene-3-one, pelargonic acid and lignoceric acid. Extract/fractions of stem of *Nyctanthes arbor-tristis* were evaluated for their antifungal activity against the two plant pathogenic fungi *viz. Rhizoctonia solani* and *Mycogone perniciosa* and three phytopathogenic bacteria *viz. Xanthomonas axonopodis pv. citri, Pseudovorax sp.* and *Dickeya zeae*. The results revealed that the chloroform and ethyl acetate fractions exhibited maximum % growth inhibition *i.e.* 83.96 and 82.29 % against *Mycogone perniciosa* fungus respectively. Methanol extract was found most effective against *Xanthomonas axonopodis pv. citri* and *Pseudovorax sp.* bacteria with 11 and 3.50 mm inhibition zone at 2000 µg/mL concentration.

Keywords: ***Nyctanthes arbor-tristis,*** **Harsingar, Stem, Bioactive compounds, Antifungal activity, Antibacterial activity.**

INTRODUCTION

Agricultural chemicals have an important role in the production of food and fiber products for increasing population of India. But indiscriminate and continuous uses of these chemicals have posed a great threat to environment and human health, throughout world. Most synthetic chemicals that have been commercialized as pesticides have long persistence in the environment and continuous exposure to these chemicals may cause immunosuppression. Due to the development of resistance in pests, toxicity to non-target organisms and high cost of pesticides attempts are going on to evolve better ones.

Nyctanthes arbor-tristis is among those plants, which have been claimed to possess multiple pharmacological activities [1]. Nyctanthes means 'night flowering' and *arbor-tristis* mean 'the sad tree'. It belongs to family Oleaceae, commonly known as Parijatha (Sanskrit), Harsingar (Hindi) and Night jasmine (English). Its taxonomical classification is: Gamopetalae – Bicarpellatae – Gentianales – Oleaceae – *Nyctanthes arbor-tristis*. It is a terrestrial woody perennial having life span of 5-20 years. It blooms during September to November and becomes leafless during March to May.

It is widely used for the treatment of various diseases and disorder in Ayurvedic medicine. It has also been credited with hepatoprotective, antileishmanial, antiviral and antifungal activities *in vitro* [2]. The plant possesses antibacterial, antioxidant, antiviral, insecticidal and fungicidal activities [2]. Traditionally, the powdered stem bark is used in rheumatic joints pain, in the treatment of malaria and as an expectorant [3]. The tribal people use various parts of *Nyctanthes arbor-tristis* to relieve cough, hiccup, dysentery, snakebite and sores in central India [4,5]. Juice of the leaves is used in enlargement of spleen [6], as digestives [7], antidot to reptile venom, diuretic and diaphoretic [8]. This plant has exhibited different pharmacological properties but it has not attracted much attention for its agrochemical properties. So, it was thought to investigate this plants stem to determine its phytoconstituents as well as their efficacy for antimicrobial activities.

EXPERIMENTAL

The stem of *Nyctanthes arbor-tristis* was collected from the university campus, Chaudhary Charan Singh Haryana Agricultural University, Hisar, India. The stem samples were dried, grinded and then extracted with hot methanol through refluxing for 8 h. The methanolic extract was concentrated on water bath under reduced pressure to get the viscous mass and then it was subjected to column chromatography to carry out isolation of the compounds from stem of *Nyctanthes arbor-tristis*. The eleuotropic series comprising of hexane, benzene, ethyl acetate, methanol and their mixtures was used. Each eluate obtained from column was monitored by thin layer

chromatography (TLC) on silica gel-G plates. The column chromatography of the stem of *Nyctanthes arbor-tristis* afforded six compounds labeled as 1 to 6.

The various chemicals used were of LR grade. The melting points were determined on Ganson Electrical Melting Point Apparatus. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker Avance II 400 NMR Spectrometer in $CDCl_3$ and DMSO-d_6 using TMS as internal standard. The chemical shift values are expressed in δ units while *J* value in Hz. The IR spectra were recorded on FTIR Perkin Elmer Infrared Spectrophotometer. Mass spectra were recorded on VG- 70S 11250J GC-MS-DS Mass Spectrometer. All spectroscopic studies were done at SAIF Panjab University, Chandigarh, India.

21α-Hydroxyfriedel-4-(23)-en-3-one (**1**) was obtained on elution with benzene:hexane (1:9) and crystallized from benzene: hexane (1:1), 35 mg, m.p.: 264-266 °C (lit. m.p.: 265-267 °C) [9]. It responded to brown colour in Liebermann-Burchard reaction. IR (KBr, ν_{max}, cm^{-1}): 3390, 2918, 1680, 1620, 1460, 1378. ^{1}H NMR (δ, $CDCl_3$): 0.77 (s, 3H, 1xCH_3), 0.83 (s, 3H, 1xCH_3), 0.89 (s, 3H, 1xCH_3), 0.99 (s, 3H, 1xCH_3), 1.09 (s, 3H, 1xCH_3), 1.19 (s, 3H, 1xCH_3), 0.81-1.24 (m, 19H, 9xCH_2, 1xCH), 2.21 (t, 2H, CH_2CO), 3.72 (s, 1H, CHO<u>H</u>), 5.90 (m, 1<u>H</u>, =C<u>H</u>). LCMS (*m/z*, relative intensity): 440 (M^+, 6.5), 412 (8.90), 406 (13.40), 382 (21.5), 380 (82.03), 352 (100), 331 (14.76), 285 (8.9), 271 (6.9).

β-Sitosterol (**2**) was obtained on elution with benzene:hexane (1:3) and crystallized out from benzene, 30 mg, m.p.: 137-139 °C (lit. m.p.: 136-137 °C) [10]. It responded to Liebermann-Burchard reaction. IR (KBr, ν_{max}, cm^{-1}): 3420, 2932, 1451, 1370, 1060, 928. ^{1}H NMR (δ, $CDCl_3$): 0.71 (s, 3H, C_{18}-CH_3), 0.79 (d, *J* = 7.0 Hz, 6H, 2xCH_3, C_{26}-CH_3, C_{27}-CH_3), 0.81 (t, *J* = 7.0 Hz, 3H, C_{29}- CH_3), 0.90 (d, *J* = 7.0 Hz, 3H, C_{21}-CH_3), 1.24 (s, 3H, C_{19}-CH_3), 2.27-1.45 (m, 29H, 11x-CH_2, 7xCH-), 5.12 (m, 1H, C_3), 5.33 (1H, br, =CH, C_6). LCMS (*m/z*, relative intensity): 414 (M^+, 5.6), 412 (10.76), 408 (21.35), 382 (80.5), 355 (100), 341(26.10), 201 (15.76), 173 (14.30)

1-Triacontanol (**3**) was obtained on elution with benzene: hexane (1:1) and recrystallized from acetone, 28 mg, m.p.: 85-86 °C (lit. m.p.: 87-88 °C) [9]. It gave positive test to jones reagent to prove presence of primary alcohol. IR (KBr, ν_{max}, cm^{-1}): 3418, 2917, 2849. ^{1}H NMR (δ, $CDCl_3$): 0.89 (t, 3H, 1xCH_3), 1.10-1.60 (m, 60H, 30xCH_2), 3.47 (t, 2<u>H</u>, 1xC<u>H</u>$_2$-OH), 3.65 (s, 1<u>H</u>, CH_2-O<u>H</u>). LCMS (*m/z*, relative intensity): 438 (M^+, 8), 437 (25), 423 (33), 421 (100), 393 (17), 361 (27).

Friedel-1-ene-3-one (**4**) was obtained on elution with ethyl acetate: benzene (1:19) and recrystallized from ethyl acetate: benzene (1: 9), 25 mg, m.p.: 230-232 °C (lit. m.p.: 228-230 °C) [9]. It gave positive test for Liebermann-Burchard reaction. IR (KBr, ν_{max}, cm^{-1}): 2923, 2854, 1718, 1463. ^{1}H NMR (δ, $CDCl_3$): 0.80 (s, 3H, 1xCH_3), 0.82-1.01 (m, 21H, 7xCH_3), 1.18-2.32 (m, 23H, 3xCH, 10xCH_2), 4.56 (d, *J* = 11.0 Hz, 1H, 1x=CH), 5.32 (d, *J* = 11.0 Hz, 1H, 1xCO-CH). LCMS (*m/z*, relative intensity): 424 (M^+, 7.5), 191 (5.6), 301 (21.0), 300 (11.5), 278 (40.5), 273 (9.10), 271 (26.5), 123 (100).

Pelargonic acid (**5**) was obtained as yellow oil (20 g) on elution with ethyl acetate: benzene (1: 3), crystallized out with ethyl acetate. It gave positive $FeCl_3$ test to confirm it as acid. IR (KBr, ν_{max}, cm^{-1}): 3373, 2921, 1716, 1602, 1454, 1217. ^{1}H NMR (δ, DMSO-d_6): 0.90 (t, *J* = 8.0 Hz, 3H, 1xCH_3), 1.11-1.54 (m, 10H, 5xCH_2), 3.49 (t, *J* = 8.0 Hz, 2H, 1xCH_2CO), 3.76 (s, 1H, CO-OH). LCMS (*m/z*, relative intensity): 158 (M^+, 5.6), 140 (809), 120 (11.50), 110 (15.9), 95 (100).

Lignoceric acid (**6**) was obtained on elution with ethyl acetate and was crystallized from methanol, 30 mg, m.p.: 84-86 °C. IR (KBr, ν_{max}, cm^{-1}): 3390, 2918, 2850. ^{1}H NMR (δ, DMSO-d_6): 0.90 (t, *J* = 8.0 Hz, 3H, 1xCH_3), 1.11-1.55 (m, 42H, 21xCH_2), 3.52 (t, *J* = 8.0 Hz, 2H, 1xCH_2-CO), 3.75 (s, 1H, CO-OH). ^{13}C NMR (δ, DMSO-d_6): 32.2 (CH_2), 29.9 (CH_2), 29.6 (CH_2), 22.8 (CH_2), 14.3 (CH_3, C-1, C_{24}). GCMS (*m/z*, relative intensity): 368 (M^+, 3.5), 280 (5.0), 130 (10), 120 (25), 97 (100).

Bioevaluation/antimicrobial activity

Test organisms: The extract/fractions from the stem of *Nyctanthes arbor-tristis* were screened for their toxicity against two plant pathogenic fungi, *Rhizoctonia solani* and *Mycogone perniciosa* and three phytopathogenic bacteria, *Xanthomonas axonopodis pv. citri, Pseudovorax sp.* and *Dickeya zeae. Rhizoctonia solani* causes various plant diseases such as collar rot, root rot, damping off and sheath blight. *Mycogone perniciosa*, causes wet bubble disease in *Agaricus bisporus*. In phytopathogenic bacteria, *Xanthomonas axonopodis pv. citri* is causal agent of citrus canker and *Dickeya zeae* causes soft rot of maize and banana.

Antifungal activity: Amongst the several methods available, poisoned food technique [11] which is the most common was used for testing antifungal activity. The extract/fractions were evaluated at four concentrations *viz*. 250, 500, 1000 and 2000 µg/mL. The test fungi were grown on potato dextrose agar (PDA) medium. The required amount of extract/fractions dissolved in 1 mL of DMSO was incorporated aseptically into 99 mL aliquots of sterilized potato dextrose agar cooled at 45 °C, mixed and poured into Petri dishes and allowed to solidify. Each dish was inoculated centrally with a 5 mm mycelial disc cut from the periphery of 2-3 days old fungal colonies. Inoculated Petri plates were incubated in the dark at 25 ± 2 °C for 48-72 h and colony diameters were measured periodically till the control dishes were nearly completely covered with fungus growth. Three replicates were used for each concentration of the extract/fractions together with three dishes containing only the solvent and no toxicant. The degree of inhibition of growth was calculated from the mean differences between treatments and the control as percentage.

Antibacterial activity: The zone inhibition method [12] was followed for screening the extract/fractions for their antibacterial activity 250, 500, 1000 and 2000 µg/mL concentrations. The bacterial suspension was prepared from 48 h old culture. The medium was melted and cooled to 45 °C, needed medium was poured aseptically in sterilized Petri plates and rotated gently for even distribution of the medium and was allowed to solidify. The circular paper discs of 10 mm diameter were prepared from Whatman's filter paper No. 1. The discs were kept in a Petri plate and autoclaved at 15 lbs pressure for 20 min. These were dipped in different concentrations of the extract/fractions and were transferred aseptically to Petri plates containing media and bacterial suspension spread over the surface. Each concentration was replicated three times. Such

Petri plates were inverted and kept at 5 °C for two h for better diffusion of the chemicals in agar medium. Later on, the Petri plates were incubated at 25 ± 2 °C for 48 h. The zone of inhibition for each concentration was recorded in mm after 48 h of incubation.

RESULTS AND DISCUSSION

Compound **1** was obtained on elution with benzene: hexane (1:9) as white solid, 35 mg, m.p.: 264-266 °C (lit. m.p.: 265-267 °C) [9]. The appearance of absorption band at 1680 cm^{-1} confirms the presence of carbonyl group. Other absorptions appeared at 3390, 2918, 1460 and 1378 cm^{-1} in their IR spectra. LCMS and elemental analysis suggested the molecular mass to be 440 and molecular formula to be $C_{30}H_{48}O_2$ for compound **1**. In ^{1}H NMR spectra of compound **1** in $CDCl_3$, a triplet at 2.21 for methylene attached to >C=O. Six signals as singlet at 0.77, 0.83, 0.89, 0.99, 1.09 and 1.19 δ integrating for three protons each were assigned for six methyl groups. A multiplet in the range of 0.81-1.24 δ, representing nineteen protons could be due to nine methylene and one methane functionality. A singlet centered at 3.72 δ integrating one proton could be assigned proton of hydroxyl group, which is attached to CH. The MS fragmentation pattern lends further support to the proposed structure. Based upon above data and literature survey, this compound could be characterized as 21α-hydroxy-friedel-4-(23)-en-3-one (**1**).

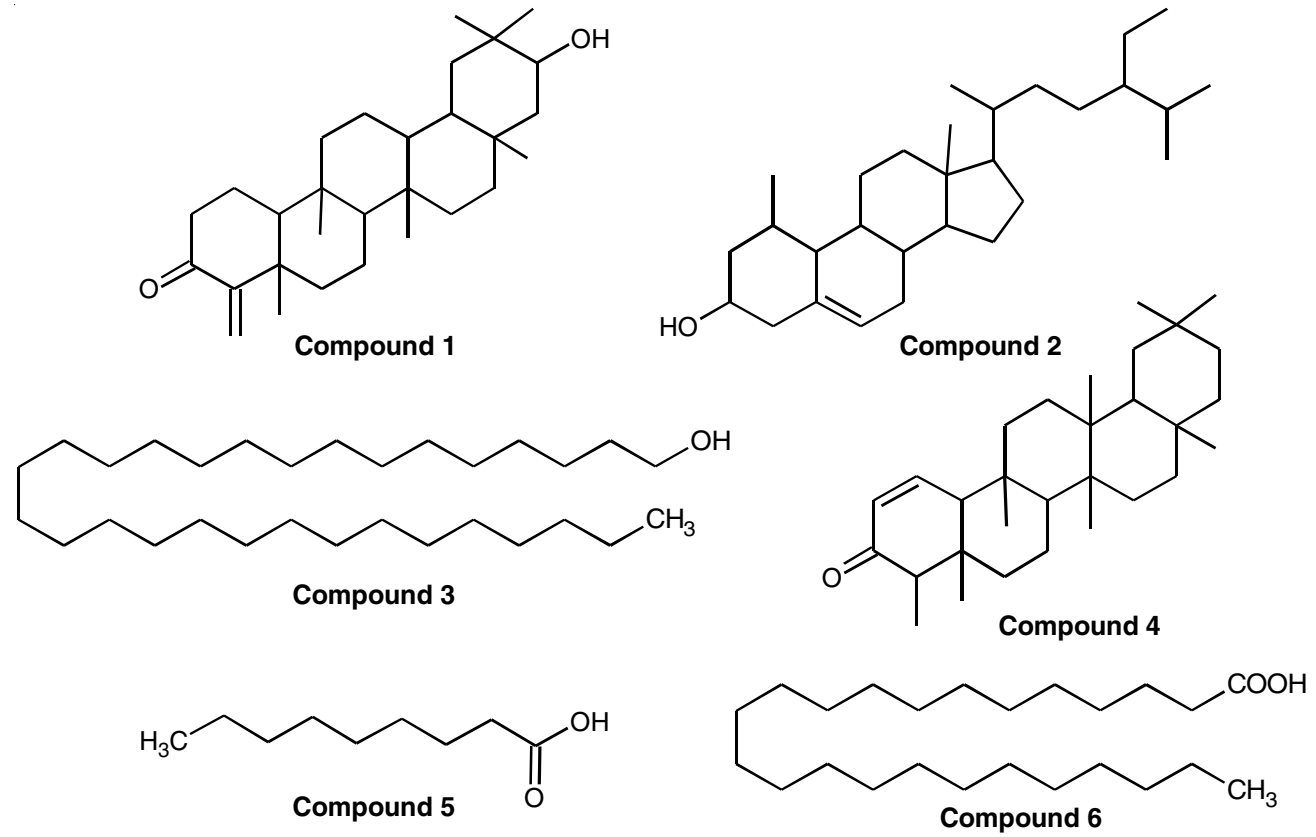
Compound **2** was obtained on elution with benzene: hexane (1:3) and crystallized out from benzene, m.p.: 137-139 °C (lit. 136-137 °C) [10]. A green colour in Liebermann-Burchard reaction suggested that compound to be a steroid. The IR of the compound showed a peak at 3420 cm^{-1} indicating a hydroxyl group in the compound. LCMS data showed M^+ peak as molecular mass of the compound **5** to be 414 with molecular formula $C_{29}H_{50}O$.The ^{1}H NMR spectra of the compound **2** in $CDCl_3$ exhibited a broad signal at 5.33 δ for one proton which was assignable to an olefinic proton. A multiplet centered at 5.12 δ integrating one proton, could be a proton α-position to a hydroxyl group. Appearance of a multiplet in the range of 2.27-1.45 δ representing twenty nine protons hinted the presence of seven methines and eleven methylenes. A singlet at 1.24 δ representing for three protons could be due to methyl group at C-19 position. A doublet centered at 0.90 δ with J = 7.0 Hz integrating three protons suggested the presence of a methyl group at C-21 and a triplet at 0.81 δ (J = 7.0 Hz) representing three protons was assignable to the methyl group at C-29 position. A doublet centered at 0.79 δ with J = 7.0 Hz integrating for six protons indicates the presence of two methyl groups positioned at C-26 and C-27, respectively. Presence of a singlet at 0.71δ for three protons was assignable to another methyl group at C-18 position. The MS fragmentation pattern lends further support to proposed structure. Based upon above data the identity of compound **2** was characterized as β-sitosterol. A comparison with literature data confirms its identity as β-sitosterol (**2**).

Compound **3** was obtained as colourless solid on elution with benzene:hexane (1:1) and crystallized from acetone, m.p.: 85-86 °C (lit. 87-88 °C) [9]. The IR absorption at 3418, 2917 and 2849 cm^{-1} indicated the presence of OH and CH_2 groups. M^+ peak of LCMS provide the information regarding molecular mass of compound **3** to be 438 and molecular formula $C_{30}H_{62}O$. The ^{1}H NMR spectra of this compound in $CDCl_3$ displayed a triplet for terminal methyl group at 0.89 δ. Other methylene groups appeared as multiplet in the range of 1.10 to 1.60 δ. CH_2 group attached to OH functionality appeared at 3.47 δ. ^{1}H NMR spectra displayed a singlet at 3.65 δ, integrating for one proton confirmed the presence of –OH group. The presence of OH group includes the possibility of proposed structure. The MS fragmentation pattern lends further support to proposed structure. Based upon above data and comparison with the literature data, the compound was characterized as 1-triacontanol (**3**).

Compound **4** was obtained on elution with ethyl acetate: benzene (1:19), m.p.: 230-232 °C (lit. 228-230 °C) [9]. The compound responded to Liebermann-Burchard reaction and gave brown colour indicating the presence of terpenoid. The absorptions at 1718, 2923 and 2854 cm^{-1} in its IR spectra confirmed the presence of >C=O and CH_2 groups. The molecular ion peak at *m/z* 424 (M^+, 7.5 %) in LCMS confirmed the proposed structure. Deduced molecular formula of compound **4** is $C_{30}H_{48}O$. In ^{1}H NMR spectra of compound **4** in $CDCl_3$, a broad singlet at 0.80 δ indicated the presence of three protons of methyl group. Twenty one protons of another seven methyl group could be picked up as multiplet in the range of 0.82-1.01 δ. Methylene protons (2H) and three methine protons (3H) appeared in the range of 1.18-2.32 δ as multiplet. Two doublets at 4.56 and 5.32 δ with coupling constant (J = 11.0 Hz) integrating one proton each were assignable protons of =CH and CO-CH moieties, respectively. The data indicated the compound to be friedel-1-ene-3-one (**4**). The MS fragmentation pattern lends further support to proposed structure. A complete agreement of the data of compound **4** with literature data of friedel-1-ene-3-one established the identity of compound **4** to be friedel-1-ene-3-one.

Compound **5** was obtained on elution with ethyl acetate: benzene (1:3) as yellow oily compound. LCMS data showed M^+ peak as molecular mass of the compound **5** to be 158 with molecular formula $C_9H_{18}O_2$. The IR (KBr, ν_{max}, cm^{-1}) indicated the presence of absorption bands at 3373 (OH), 2921 (CH_2) and 1716 for >C=O moieties. ^{1}H NMR spectra of compound **5** in DMSO-d_6 exhibited a triplet for terminal methyl group at 0.90 δ (J = 8.0 Hz). A multiplet at 1.11-1.54 δ integrating for ten protons was assignable of five methylene groups. Protons of CH_2 group attached to –COOH showed a triplet at 3.49 δ with coupling constant 8.0 Hz. A singlet at 3.76 δ integrating for one proton of OH group indicated that the compound could be pelargonic acid. The MS fragmentation pattern lends further support to proposed structure. A complete agreement of the data of compound **5** with literature data of pelargonic acid established the identity of compound **5** to be pelargonic acid.

Compound **6** was obtained on elution with ethyl acetate and crystallized from methanol, 30 mg, m.p.: 84-86 °C. The absorptions at 3390, 2918 and 2850 cm^{-1} in its IR spectra confirmed the presence of –OH and -CH_2 groups. LCMS and microanalysis suggested the molecular mass to be 368 and molecular formula to be $C_{24}H_{48}O_2$ for compound **6**. The ^{1}H NMR spectra of the compound **6** in DMSO-d_6 showed a triplet



Structures of compounds **1-6**

for three protons of terminal methyl group at 0.90 δ. There was a multiplet for 42 protons in the range of 1.11-1.55 δ indicating the presence of 21 methylenes. Protons of -CH_2 group attached to -COOH appeared as triplet at 3.52 δ integrating two protons. A singlet centered at 3.75 for one proton of -OH group confirmed the presence of OH group in compound **6**. The ^{13}C NMR data and MS fragmentation pattern lends further support to the proposed structure. Spectroscopic data was in agreement with the literature data of 1-tetracosanoic acid (lignoceric acid), established the identity of compound **6** to be lignoceric acid.

Antimicrobial activity

Antifungal activity of extract/fractions of stem of *Nyctanthes arbor-tristis*: Antifungal activity of methanol extract, hexane, benzene, chloroform, ethyl acetate and acetone fractions of stem of *Nyctanthes arbor-tristis* was tested against *Rhizoctonia solani* and *Mycogone perniciosa* at 250, 500, 1000 and 2000 µg/mL concentrations. A perusal of the activity data presented in Table-1 revealed that hexane fraction exhibited highest activity with 79.80 % inhibition at 2000 µg/mL concentration followed by ethyl acetate having 77.92 % inhibition at 2000 µg/mL concentration against *Rhizoctonia solani* fungus. Moderate activity was shown by benzene and chloroform fractions with 74 % and 75 % inhibition against tested fungi. Although methanol extract remains highly active at lowest tested concentration with 52.50 % inhibition at 250 µg/mL concentration. Acetone fraction exhibited lowest antifungal activity with 65.42 % inhibition at 2000 µg/mL concentration.

The data presented in Table-2 revealed that maximum inhibition *i.e.* 83.96 % inhibition at 2000 µg/mL was shown by chloroform fraction followed by ethyl acetate with 82.29 % inihibition against *Mycogone perniciosa*. Moderate activity was shown by acetone fraction and methanol extract with 73.55 and 78.75 % inhibition at 2000 µg/mL against tested fungi. Lowest inhibition was shown by benzene fraction with 69.58

TABLE-1
ANTIFUNGAL ACTIVITY OF STEM OF *Nyctanthes arbor-tristis* (HARSINGAR) AGAINST *Rhizoctonia solani*

Extract/ Fractions	Growth of inhibition (%)			
	250 µg/mL	500 µg/mL	1000 µg/mL	2000 µg/mL
Hexane	47.92±2.90	59.79±0.78	67.71±2.90	79.80±2.30
Benzene	37.08±2.12	52.30±2.41	63.75±1.02	74.80±2.81
Chloroform	45.21±0.59	53.12±1.84	61.67±2.06	75.42±0.59
Ethyl acetate	41.46±2.90	55.62±3.19	62.71±1.06	77.92±2.81
Acetone	28.33±2.30	37.30±1.93	46.67±1.28	65.42±2.95
Methanol	52.50±2.70	62.50±1.77	71.46±1.28	76.25±2.70

All values are mean ± S.D.

TABLE-2
ANTIFUNGAL ACTIVITY OF STEM OF *Nyctanthes arbor-tristis* (HARSINGAR) AGAINST *Mycogone perniciosa*

Extract/ Fractions	Growth of inhibition (%)			
	250 µg/mL	500 µg/mL	1000 µg/mL	2000 µg/mL
Hexane	22.30±0.78	37.08±1.56	53.55±1.93	61.05±1.18
Benzene	34.58±2.90	51.67±1.56	63.12±1.35	69.58±2.57
Chloroform	48.12±3.68	62.08±2.81	75.21±3.12	83.96±1.28
Ethyl acetate	33.55±1.93	45.63±1.35	60.21±2.99	82.29±1.47
Acetone	31.05±1.64	45.00±2.04	58.55±2.81	73.55±2.52
Methanol	33.33±3.08	47.92±1.64	55.83±2.12	78.75±1.84

All values are mean ± S.D.

% inhibition at 2000 µg/mL concentration followed by hexane fraction having 61.05 % inhibition at 2000 µg/mL concentration against *Mycogone perniciosa* fungus.

Antibacterial activity of extract/fractions of stem of *Nyctanthes arbor-tristis:* Antibacterial activity of methanol extract, hexane, benzene, chloroform, ethyl acetate and acetone fractions of stem of *Nyctanthes arbor-tristis* was tested against *Xanthomonas axonopodis pv. citri*, *Pseudovorax sp.* and *Dickeya zeae* at 250, 500, 1000 and 2000 µg/mL concentrations. The perusal of activity data (Tables 3-5) revealed that methanol extract was found most active against *Xanthomonas axonopodis pv. citri* and *Pseudovorax* with 11 and 3.50 mm inhibition zone at 2000 µg/mL concentration. Benzene and chloroform fractions inhibited the growth of *Xanthomonas axonopodis pv. citri* up to 8.50 mm at highest tested concentration followed by ethyl acetate fraction with 8.13 mm inhibiton zone. Ethyl acetate and acetone fractions were found most active against *Dickeya zeae* with 8.83 and 8.17 mm inhibition zone at 2000 µg/mL concentration. Hexane fraction exhibited lowest inihibition zone *i.e.* 2 mm against *Xanthomonas axonopodis pv.citra,* while other fractions showed moderate toxicity towards phytopathogens.

TABLE-3
ANTIBACTERIAL ACTIVITY OF STEM OF *Nyctanthes arbor-tristis* (HARSINGAR) AGAINST *Xanthomonas axonopodis pv. citri*

Extract/ Fractions	Growth of inhibition (%)			
	250 µg/mL	500 µg/mL	1000 µg/mL	2000 µg/mL
Hexane	a	a	1.33±0.58	2.00±1.00
Benzene	a	4.67±1.53	7.87±2.01	8.50±1.32
Chloroform	a	a	3.00±1.00	8.50±0.50
Ethyl acetate	a	a	3.83±0.76	8.13±1.80
Acetone	a	a	1.50±1.32	3.60±1.22
Methanol	5.33±0.76	7.83±1.26	9.67±0.58	11.00±1.00

All values are mean ± S.D; a: No growth inhibition

TABLE-4
ANTIBACTERIAL ACTIVITY OF STEM OF *Nyctanthes arbor-tristis* (HARSINGAR) AGAINST *Pseudovorax sp.*

Extract/ Fractions	Growth of inhibition (%)			
	250 µg/mL	500 µg/mL	1000 µg/mL	2000 µg/mL
Hexane	a	0.83±0.76	1.50±0.50	3.00±1.00
Benzene	a	1.17±1.26	1.33±0.58	2.17±0.76
Chloroform	a	a	a	2.00±1.00
Ethyl acetate	a	1.00±0.00	1.33±0.58	2.17±0.29
Acetone	a	0.67±0.58	2.50±0.50	2.83±1.26
Methanol	a	0.33±0.58	1.67±0.76	3.50±0.50

All values are mean ± S.D; a: No growth inhibition

TABLE-5
ANTIBACTERIAL ACTIVITY OF STEM OF *Nyctanthes arbor-tristis* (HARSINGAR) AGAINST *Dickeya zeae*

Extract/ Fractions	Growth of inhibition (%)			
	250 µg/mL	500 µg/mL	1000 µg/mL	2000 µg/mL
Hexane	a	1.50±0.50	1.83±0.76	2.53±0.50
Benzene	a	1.00±0.00	3.00±1.00	5.00±1.00
Chloroform	a	0.33±0.58	2.00±1.00	2.50±0.50
Ethyl acetate	a	2.00±0.00	4.53±0.50	8.83±1.04
Acetone	0.67±0.58	2.17±0.29	6.53±1.29	8.17±1.76
Methanol	a	1.50±0.87	2.40±0.36	4.17±1.04

All values are mean ± S.D; a: No growth inhibition

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