International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2018; 6(2): 821-826

Received: 12-01-2018
Accepted: 15-02-2018

Sumona Kumari
Department of Chemistry and Biochemistry, College of Basic Sciences and Humanities, Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana, India

Rajvir Singh
Department of Chemistry and Biochemistry, College of Basic Sciences and Humanities, Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana, India

Jyoti Punia
Department of Chemistry and Biochemistry, College of Basic Sciences and Humanities, Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana, India

Meera
Department of Chemistry and Biochemistry, College of Basic Sciences and Humanities, Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana, India

Correspondence
Sumona Kumari
Department of Chemistry and Biochemistry, College of Basic Sciences and Humanities, Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana, India

Investigation of chemical constituents and antifungal activity of flowers of *Nyctanthes arbor-tristis*

Sumona Kumari, Rajvir Singh, Jyoti Punia and Meera

Abstract
Phytochemical investigation of the flowers of *Nyctanthes arbor-tristis* (Harsingar) resulted the isolation of methyl tetracosanoate, octacosane, tetrapentacontane, 3β-hydroxy olea-12-ene-28-oic acid, β-sitosterol and stigmastan-3-en-6-ol. Extract/fractions of flowers of *Nyctanthes arbor-tristis* were evaluated for their antifungal activity against the two plant pathogenic fungi *viz. Rhizoctonia solani* & *Mycogone perniciosa.* The results revealed that flowers extract/ fractions of *Nyctanthes arbor-tristis* were found more active against *Rhizoctonia solani* than *Mycogone perniciosa*. Chloroform fraction of flowers was found highly active against both *Rhizoctonia solani* and *Mycogone perniciosa.* It showed 89.33 % growth inhibition against *Rhizoctonia solani* and 77.50 % growth inhibition against *Mycogone perniciosa* at 2000 µg/ml concentration.

Keywords: *Nyctanthes arbor-tristis*, Harsingar, Flower, Antifungal activity, *Rhizoctonia solani*, *Mycogone perniciosa*

Introduction
Agricultural crops are constantly exposed to infection by pathogens such as fungi, bacteria and viruses. These phytopathogens are widely distributed in nature, and their infectious processes result in major losses of various economically important crops. The losses would have been even more if pests were not controlled effectively by the pesticides. Injudicious application of synthetic chemicals have resulted in emergence of many new problems like pest resurgence, destruction of non-target organisms, phytotoxicity [1], environmental pollution and cases of accidental poisoning [2]. Also the presence of residues of these toxic chemicals in the food supply and their consequent accumulation in human beings lead to adverse health effects. Natural plant products seem to provide a viable solution to the environmental problems caused by synthetic pesticides and many researchers are trying to identify effective natural products to replace synthetic pesticides. Because they are easily degradable, less persistent in the environment and eco-friendly in nature.

Nyctanthes arbor-tristis is the plant, which have multiple biological and pharmacological activities [3]. It is also known as Parijatha (Sanskrit), Harsingar (Hindi) and Night jasmine (English). According to Hindu mythology, it is a sacred tree, one of the five trees that exist in heaven, which was came out of ocean during samundra manthan. It is a native of India, flourishing wild in sub-Himalayan regions and forests of central India. 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 usually a small tree growing to 10 m tall, with grey bark and having highly fragrant flowers, which bloom at night and fall off before sunrise. Thus, during the day the plant loses all its brightness and hence called "tree of sadness". The flowers are produced in clusters of two to seven together, with individual flowers opening at dusk and finishing at dawn; they have white corolla with an orange centre. Flowers are used for worship in temples [4].

Flowers have also been shown to possess immunostimulant activity by activating the cell mediated immune system [5]. Rengylone isolated from the ethanolic extract of flowers of *N. arbor-tristis* and its acetate derivative exhibited *in-vitro* antiplasmodial activity against *Plasmodium falciparum*, *Leishmania donovvani* and *Entamoeba histolytica* [7]. Ratnasooriya *et al.* (2005) examined the sedative potential of hot infusion of flowers in rats. The infusion had a moderate dose dependant (3.7 to 18.7 mg/kg) conscious sedative activity in male rats. Sandhya *et al.* (2016) investigated the antioxidant potential of methanolic extract of *N. arbor-tristis* flowers.

The total phenolic and flavonoid content for *N. arbortristis* was 4.7910±0.005mg TAE/g and 12.2543±0.0015mg Quercetin equivalents (QE)/g DW. Flowers of *Nyctanthes arbor-tristis* are astringent, carminative stomachic, used in ophthalmic purposes and gout treatment, as a tonic for hair in preventing greying of hairs and hairfall [9, 10, 11]. Various pharmacological/biological activities shown by flowers of Nyctanthes arbor-tristis and our search [12] for natural antimicrobial encouraged us to investigate *Nyctanthes arbor-tristis* flowers for its chemical constituents and antifungal activity.

Materials and Methods

Collection of the plant materials

The flowers of *Nyctanthes arbor-tristis* were collected from the university campus, Chaudhary Charan Singh Haryana Agricultural University, Hisar, India in the month of October-November. The plant materials were immediately brought to laboratory washed thoroughly with water and dried under shade before processing.

Chemicals and Equipments

The various chemicals used were of LR grade. Chemicals used during this work were petroleum ether, hexane, benzene, ethyl acetate, methanol, chloroform, pyridine, acetic anhydride, potassium hydroxide, magnesium acetate, glacial acetic acid, sulphuric acid and ferric chloride. Required chemicals and solvents were obtained from SD-Fine chemicals Ltd. (SDFCL), CDH (P) Ltd., Ranbaxy fine chemicals Ltd and Hi-Media chemicals. Sterile disposable Petri dishes used during analysis were of Tarsons Products Pvt. Ltd.

The melting points were determined on Ganson Electrical Melting Point Apparatus. ^{1}HNMR and [13] CNMR 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.

Extraction and isolation of compounds

The flower samples were dried, grinded and then extracted with hot methanol through refluxing for eight hours. The methanolic extract was concentrated on rotary evaporator to get the crude mass and then it was subjected to column chromatography to carry out isolation of the compounds from flowers 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 flowers of *Nyctanthes arbor-tristis* afforded six compounds labeled as 1 to 6.

Antifungal Activity

Sterilization of the apparatus

During activity analysis, all the glassware used were cleaned with chromic acid followed by washing with mild liquid detergent and finally with distilled water. The glasswares were dried, packed and sterilized in hot air oven at 180ºC for two hours. Media and other heat sensitive required items were sterilized in autoclave at 121˚C for 15-20 mins.

Test Organisms

The extract/ fractions from the flowers of *Nyctanthes arbor-tristis* were screened for their toxicity against two plant pathogenic fungi, *Rhizoctonia solani* and *Mycogone perniciosa. 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*.

Preparation of Fractions

The crude methanolic extract of flowers of *N. arbor-tristis* was mixed with silica gel (60 – 120 mesh) and fractionated successively with the solvent of increasing polarity viz. hexane, benzene, chloroform, ethyl acetate and acetone. Each solvent was mixed with methanolic extract, kept for 24 hours and concentrated under reduced pressure to obtain the resultant fraction, labeled accordingly. The procedure was repeated three times. Crude methanolic extract and all fractions were evaluated for their antifungal activity against phytopathogenic fungi.

Test of fungi toxicity

Amongst the several methods available, poisoned food technique [13, 14] 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 hrs 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.

Results

Phytochemical Investigation

The column chromatography of the flowers of *Nyctanthes arbor-tristis* afforded six compounds labeled as 1 to 6.

Methyl tetracosanoate (1) was obtained on elution with hexane as a white solid, 40mg, m.pt. 59-61°C (lit. 58-60°C) [15]. It was crystallized from benzene: hexane (1:1). The hydroxamic acid test for this compound hinted the presence of ester. Its Rf value was found 0.88 in benzene: hexane (3:7). The molecular formula $C_{25}H_{50}O_2$ was deduced from m/z 382 $[M]^+$ by its LC-MS. IR υ_{max}^{KBr} (cm^{-1}): 1732, 2850, 2918. ^{1}HNMR (δ, $CDCl_3$): 0.89 (t, J=8.0Hz, 3H, 1xCH_3), 1.10-1.56 (m, 42H, 21xCH_2), 2.36 (t, J=8.0Hz, 2H, 1xCH_2CO), 3.50 (s, 3H, 1xOCH_3). LCMS (m/z, relative intensity): 382 (M^+, 21), 350 (90), 302 (100), 258 (80), 209 (61), 150 (38.5), 100 (20), 50 (1.1).

Octacosane (2) was obtained as waxy solid on elution from hexane and recrystallized from chloroform (12 mg), m.p. 60-61°C (lit. m.p. 60-62°C) [16]. Its Rf value was found to be 0.67 in benzene: hexane (3:7). Molecular formula $C_{28}H_{58}$ was deduced from its LCMS. IR υ_{max}^{KBr} (cm^{-1}): 2948, 2924, 1448,

1237, 1215, 1076, 780. ^{1}HNMR (δ, $CDCl_3$): 0.86 (t, J=7.5Hz, 6H, 2 x CH_3), 1.25-1.30 (m, 52H, 26 x CH_2). LCMS (m/z, relative intensity): 394 (M^+), 370 (11.5), 359 (8.5), 279 (20.0), 201 (100), 167 (31.0), 132 (25.0).

Tetrapentacontane (3) was obtained on elution with benzene: hexane (1:19) as a waxy solid, 10mg, m.p. 95 °C. It was crystallized from chloroform as white waxy solid. Its Rf value in benzene: hexane (1:1) was found to be 0.65. The molecular formula $C_{54}H_{110}$ was deduced from m/z, 759 [M^+] by its LCMS. IR υ_{max}^{KBr} (cm^{-1}): 2951, 2921, 1441, 1238, 1215, 1076, 799. ^{1}HNMR (δ, $CDCl_3$): 0.87 (t, J=7.5Hz, 6H, 2 x CH_3), 1.25-2.17 (m, 104H, 52 x CH_2). LCMS (m/z, relative intensity): 759(M^+), 701 (22), 589 (23.5), 506 (88.5), 469 (51.5), 445 (12.5), 365 (95), 301 (100), 274 (21.5), 163 (28.0), 149 (34.5), 85 (23).

3β-hydroxy olea-12-ene-28-oic acid (4) was isolated on elution with benzene: hexane (1:14) and crystallized out from benzene to get colourless needles, 20mg, m.pt. 302-303°C (lit. 310°C) [17]. Compound responded to brown colour in Liebermann-Burchard reaction. Its Rf value was found to be 0.72 in benzene: hexane (1:3) solvent. The molecular formula $C_{30}H_{48}O_3$ was deduced from m/z 456 (M^+) by its LCMS. IR υ_{max}^{KBr} (cm^{-1}): 3350, 2930, 2850, 1660, 1470, 1380, 1370, 1200, 275 cm^{-1}. ^{1}HNMR (δ, $CDCl_3$): 0.68 (s, 3H, 1xCH_3), 0.78 (s, 3H, 1xCH_3), 0.82 (s, 3H, 1xCH_3), 0.98 (s, 3H, 1xCH_3), 1.04 (s, 3H, 1xCH_3), 1.09 (s, 3H, 1xCH_3), 1.15 (s, 3H, 1xCH_3), 1.30-2.30 (m, 22H, 4xCH & 9xCH_2), 4.21 (t, J= 4.0Hz, 2H, CH_2CO). ^{13}CNMR (δ, $CDCl_3$): 28.80 (C-1), 27.40 (C-2), 80.12 (C-3), 36.48 (C-4), 50.39 (C-5), 18.18 (C-6), 32.00 (C-7), 35.65 (C-8), 45.43 (C-9), 37.01 (C-10), 21.45 (C-11), 29.03 (C-12), 31.52 (C-13), 41.75 (C-14), 24.15 (C-15), 28.20 (C-28), 20.40 (C-29), 20.40 (C-29), 180.01 (C-30, COOH). LCMS (m/z, relative intensity): 456 (M^+, 11.5), 302 (18.5), 266 (41.5), 270 (9.50), 190 (5), 120 (25.5).

β-sitosterol (5) was obtained on elution with benzene: hexane (1:9) and crystallized out from benzene: hexane (1:1), 24mg, m.p. 134-136°C (lit. m.p.136-137°C) [18]. It responded to Liebermann-Burchard reaction. The appearance of green colour indicated the presence of steroid. Its Rf value was 0.74 in benzene: hexane (3:7). LCMS data showed M^+ peak as molecular mass of the compound HF-5 to be 414 with molecular formula $C_{29}H_{50}O$. IR υ_{max}^{KBr} (cm^{-1}): 3427, 2932, 2850, 1463, 1380, 1052, 958. ^{1}HNMR (δ, $CDCl_3$): 0.78 (s, 3H, C_{18}-CH_3), 0.82 (d, J=7.0Hz, 6H, 2xCH_3, C_{26}-CH_3, C_{27}-CH_3), 0.89 (t, J=7.0Hz, 3H, C_{29}-CH_3), 0.99 (d, J= 7.0Hz, 3H, C_{21}-CH_3), 1.17 (s, 3H, C_{19}-CH_3), 2.22-1.44 (m, 29H, 11xCH_2, 7xCH), 5.12-5.14 (m, 1H, C_3-H), 5.35 (br, 1H, =CH, C_6-H). LCMS (m/z, relative intensity): 414 (M^+, 8.5), 393 (14.7), 371 (57), 355 (32.0), 301 (28.6), 274 (13.4), 223 (16.5), 175 (7.6).

Stigmastan-3-en-6-ol (6) was obtained as colourless crystalline compound (20mg) on elution with ethyl acetate: benzene (1:3) and recrystallized from ethyl acetate, m.p. 137°C. Its Rf value was found to be 0.64 in ethyl acetate solvent. Its positive response towards Liebermann-Burchard reaction indicated that this compound could be a sterol. M^+ peak of LCMS provide the information regarding molecular mass of compound HF-6 to be 414 and molecular formula $C_{29}H_{50}O$. IR υ_{max}^{KBr} (cm^{-1}): 3362, 2957, 2951, 2920, 1621, 1465. ^{1}HNMR (δ, DMSO-d_6): 0.68 (s, 3H, 1xCH_3), 0.76 (s, 3H, 1xCH_3), 0.83 (s, 3H, 1xCH_3), 0.85 (s, 3H, 1xCH_3), 0.94 (s, 3H, 1xCH_3), 0.96 (s, 3H, 1xCH_3), 1.24-2.38 (m, 27H, 9xCH_2, 9xCH), 3.02 (s, 1H, C_6-OH), 4.80 (m, 1H, C_4-C=CH), 4.30 (m, 1H, C_3-C=CH). LCMS (m/z, relative intensity): 414 (M^+, 100), 192 (6.5), 309 (19.5), 273 (41.5), 268 (10.5), 123 (26.75).

Antifungal activity of flower of *Nyctanthes arbor- tristis* (Harsingar)

A perusal of the activity data presented in table 1 revealed that chloroform fraction exhibited highest antifungal activity with EC_{50} value 425 and 89.33 % inhibition at 2000 µg/ml concentration followed by benzene fraction with 84.67 % inhibition at 2000 µg/ml against *Rhizoctonia solani*. Comparatively, moderate activity was shown by ethyl acetate and acetone fractions of flowers of *Nyctanthes arbor-tristis* with 82.92 and 80.42 % inhibition at 2000 µg/ml concentration against tested fungi. Hexane fraction of flowers of *Nyctanthes arbor-tristis* showed 75.42 % inhibition at highest tested concentration. Methanol extract exhibited lowest antifungal activity with 1055 EC_{50} value and 64.38 % inhibition at 2000 µg/ml against *Rhizoctonia solani* fungus.

Table 1: Antifungal activity of flower of *Nyctanthes arbor- tristis* (Harsingar) against *Rhizoctonia solani*

Extract/Fractions	Growth Inhibition (%)			
	250µg/ml	500µg/ml	1000µg/ml	2000µg/ml
Hexane	28.33±2.57	36.67±3.32	55.00±1.02	75.42±0.29
Benzene	40.44±0.63	48.67±4.25	70.44±1.91	84.67±2.37
Chloroform	37.11±2.20	55.78±1.13	80.22±3.10	89.33±5.76
Ethyl acetate	36.88±4.92	50.83±0.78	59.58±4.34	82.92±1.56
Acetone	13.54±4.82	27.50±4.54	52.50±2.22	80.42±2.12
Methanol	31.67±0.78	37.29±2.81	49.17±3.62	64.38±1.35

All values are mean ±S.D.

The activity data presented in table 2 revealed that chloroform and acetone fractions of flowers of *Nyctanthes arbor- tristis* showed maximum inhibition i.e. 77 % inhibition at 2000 µg/ml concentration. Comparatively, moderate activity was shown by hexane and benzene fractions with 68.33 and 66.67 % inhibition at 2000 µg/ml concentration against tested fungi. Further, ethyl acetate fraction exhibited 62.50 % inhibition at 2000 µg/ml concentration against *Mycogone perniciosa*. Methanol extract of flowers of *Nyctanthes arbor- tristis* exhibited lowest antifungal activity with EC_{50} value 2525 and 47.08 % inhibition at 2000 µg/ml concentration.

Table 2: Antifungal activity of flower of *Nyctanthes arbor- tristis* (Harsingar) against *Mycogone perniciosa*

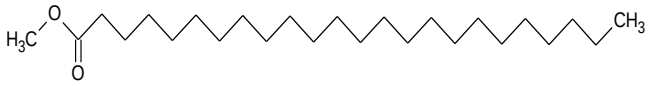
Extract/Fractions	Growth Inhibition (%)			
	250 µg/ml	500 µg/ml	1000 µg/ml	2000 µg/ml
Hexane	30.21±0.29	42.50±2.55	58.54±1.64	68.33±3.40
Benzene	16.67±2.52	42.71±3.32	59.17±2.12	66.67±0.78
Chloroform	45.00±2.84	55.00±3.19	63.33±1.28	77.50±1.84
Ethyl acetate	34.58±2.41	48.75±3.06	57.92±2.57	62.50±2.55
Acetone	38.33±1.56	54.38±2.84	66.67±2.41	77.29±3.08
Methanol	32.50±1.35	35.21±2.52	41.46±1.64	47.08±1.56

All values are mean ±S.D.

Discussion

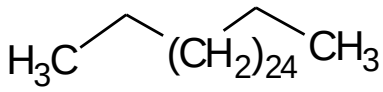
Compound (1) was obtained on elution with hexane as a white solid (40mg). It was crystallized from benzene: hexane (1:1). The presence of ester was determined by reacting compound with hydroxylamine in the presence of sodium hydroxide (hydroxamic test). The purity of the compound was confirmed on the basis of its behavior on TLC plate as deep yellow spot (Rf= 0.88) with iodine as developing phase. The m.p. of the compound was observed to be 59-61°C (lit. 58-60°C[15]).

LCMS data showed M^+ peak as molecular mass of compound (1) to be 382 with molecular formula $C_{25}H_{50}O_2$. The IR [KBr, υ_{max} cm^{-1}] indicated the presence of absorption bands at 1732 (>C=O), 2850 (CH_2) and 2918 (CH_2). The ^{1}HNMR ($CDCl_3$) spectra of compound exhibited the triplet signal (J=8.0Hz) at 0.89 δ for terminal methyl group. A multiplet observed at 1.10-1.56 δ describes the 42 protons of methylene group. A triplet peak with J=8.0 Hz appeared at 2.36 Hz assignable to 2 protons of methylene attached to carbonyl functionality. Compound (1) showed a singlet at 3.50 δ indicating the protons of methoxy group. All above discussed spectral data were in agreement with the literature data of methyl tetracosanoate[15] identify this compound (1) to be methyl tetracosanoate.



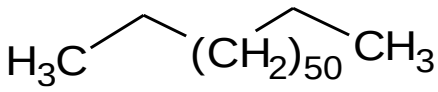
1

Compound (2) was obtained as a waxy solid in hexane solvent system. It was recrystallized from chloroform (12 mg), m.p. 60-61°C (lit. m.p. 60-62°C[16]). Rf value of the compound (2) in benzene: hexane (3:7) solvent was found to be 0.67. Its molecular formula $C_{28}H_{58}$ was deduced from LCMS, 394 (M^++2). Absence of functional groups absorptions in their IR spectra indicated that the compound is simple alkane. The ^{1}HNMR spectrum of compound (2) in $CDCl_3$ exhibited a multiplet in the range of 1.25-1.30 δ, integrating fifty two protons for twenty six methylenes. A triplet centered at 0.86 δ with J=7.5Hz integrating six protons of two terminal methyl groups confirmed the presence of two methyl group in compound (2). This data suggested the compound (2) to be octacosane. The mass fragmentation and elemental analysis of compound (2) supported the proposed structure. Thus the compound (2) settled as octacosane.



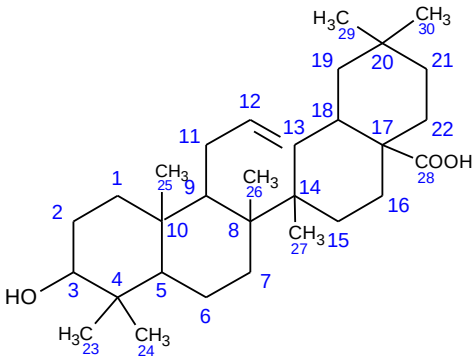
2

Compound (3) was obtained on elution with benzene: hexane (1:19) as a waxy solid. It was crystallized out from chloroform as white waxy solid (10mg), m. p. 95°C. Rf value of the compound (3) was found to be 0.65 in benzene: hexane (1:1) solvent. The IR spectrum of the compound (3) did not show the presence of functional groups. The LCMS of the compound indicated its molecular wt. 758 and molecular formula to be $C_{54}H_{110}$. The ^{1}HNMR spectrum of the compound in $CDCl_3$ showed a multiplet in the range from 1.25-2.17 δ for 104 protons, representing fifty two methylenes. A triplet (J=7.0Hz) was observed at 0.87 δ, integrating for six protons, indicated the presence of two terminal methyl groups. The observed spectroscopic and analytical data were identical to literature data for tetrapentacontane. The mass fragmentation pattern and elemental analysis of compound (3) supported the proposed structure. The compound (3) therefore settled as tetrapentacontane.



3

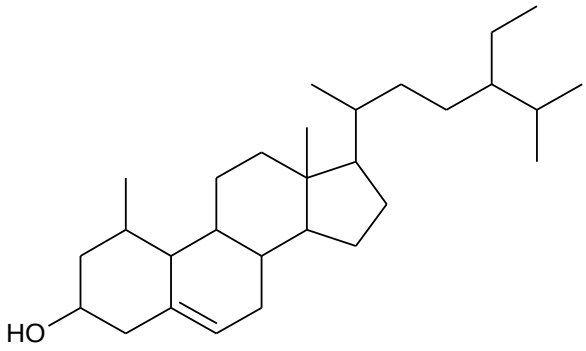
Compound (4) was obtained on elution with benzene: hexane (1:14) solvent system. It was crystallized out from benzene to get colourless needles, 20mg, m.p. 302-303°C (lit. 310°C[17]). Its Rf value was found to be 0.72 in benzene: hexane (1:3) solvent. It gave brown colour in Liebermann-Burchard reaction indicating that compound is terpenoid. Its molecular formula $C_{30}H_{48}O_3$ was deduced from LCMS, 456 (M^+) and elemental analysis. Absorption at 1660 and 3350 cm^{-1} in their IR spectra confirmed the presence of C=O and OH functionality in compound (4). The ^{1}HNMR spectra of this compound in $CDCl_3$ exhibited singlets at 0.68, 0.78, 0.82, 0.98, 1.04, 1.09 and 1.15 δ for seven methyl group indicating the presence of methyl groups at positions C-10, C-23, C-24, C-26, C-27, C-29 and C-30 respectively. The appearance of a triplet at 4.21δ for CH_2-CO moiety integrating two protons with J=4.0Hz assigned the structure. A multiplet in the range from 1.30-2.30 δ integrating for 22 protons indicating the presence of nine methylene and four methines. The mass fragmentation pattern of compound (4) further supported the proposed structure. Our data ^{1}HNMR for the compound is in perfect assignment with literature data of 3β-hydroxy olea-12-ene-28-oic acid and confirmed the identity of compound (4) to be 3β-hydroxy olea-12-ene-28-oic acid.



4

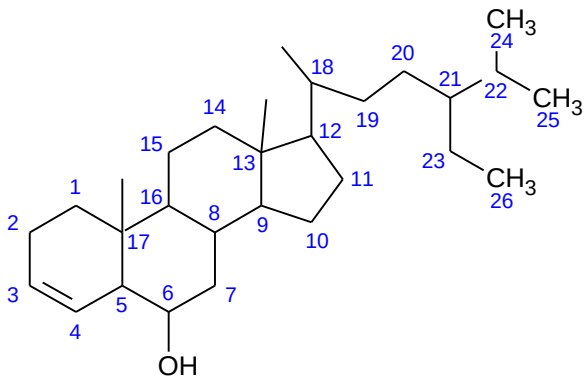
Compound (5) was obtained on elution with benzene: hexane (1:9) and crystallized out from benzene: hexane (1:1), m.pt. 134-136°C (lit. 136-137°C[18]). It responded to Liebermann-Burchard reaction, which suggested that compound could be a steroid. Its Rf value was 0.74 in benzene: hexane (3:7). The IR of the compound showed a peak at 3427 cm^{-1} indicating a hydroxyl group in the compound. Other absorption peaks were at 2932, 2850, 1463, 1380, 1052 and 958 cm^{-1} in its IR spectrum. The LCMS and elemental analysis suggests the molecular mass to be 414 and molecular formula $C_{29}H_{50}O$. The ^{1}HNMR spectra of the compound in $CDCl_3$ exhibited a broad signal at 5.35 δ for one proton which was assignable to an olefinic proton. A singlet at 1.17 δ representing for three protons could be due to methyl group at C-19 position. A multiplet centered at 5.12-5.14 δ integrating one proton, could be a proton α-position to a hydroxyl group. Appearance of a multiplet in the range of 2.22-1.44 δ representing twenty nine protons hinted the presence of seven methines and eleven methylenes. A doublet centered at 1.11 δ with J=7.0Hz integrating three protons suggested the presence of a methyl

group at C-21 and a triplet at 0.89 δ (J=7.0Hz) 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 0.78δ for three protons was assignable to another methyl group at C-18 position. The data of this compound (5) was fully matched with the data of β-sitosterol[18] and the identity of compound (5) as β-sitosterol was confirmed.



5

Compound (6) was obtained as colourless crystalline compound (20mg) on elution with ethyl acetate: benzene (1:3) and recrystallized from ethyl acetate, m.p. 137°C. Its Rf value was found to be 0.64 in ethyl acetate solvent. The compound (6), $C_{29}H_{50}O$ (M^+ at m/z 414) gave positive results to both the tetranitro methane test and Liebermann-Burchard reaction. 6-hydroxy group showed absorption at 3362 cm^{-1} in its IR spectra. The LCMS data of compound suggested molecular mass of compound (6) to be 414. Singlets at 0.68 in the ^{1}HNMR spectrum of (6) in DMSO-d_6 indicated the presence of three protons of one methyl group. Another singlet of methyl group appeared at 0.76 δ integrating for three protons. Presence of two secondary methyl groups at 0.83, 0.85 δ and two other methyl groups at (0.94, 0.96 δ) further corroborated the assigned structure of compound (6). Olefinic resonances were observed at 4.30 and 4.80 δ as multiplets while carbinylic proton observed at 3.02 δ as singlet. A multiplet in the range of 1.24 to 2.38 δ representing twenty seven protons could be assignable to nine methines and nine methylenes of sterol structure. The spectroscopic and analytical data of compound (6) was in full agreement of literature data of stigmastan-3-en-6-ol.



6

Antifungal Activity of Flowers Extract/Fractions of *Nyctanthes arbor-tristis*

Antifungal activity of methanolic extract and various fractions of flowers of *Nyctanthes arbor-tristis* were evaluated against two plant pathogenic fungi viz. *Rhizoctonia solani* and *Mycogone perniciosa* at 250, 500, 1000 and 2000 µg/ml concentrations using poisoned food technique [13, 14]. Data obtained (Fig I and II) revealed that the flowers extract/ fractions of *Nyctanthes arbor-tristis* were found more active against *Rhizoctonia solani* than *Mycogone perniciosa*. However, all the concentrations were significantly different from one another. Irrespective of extract/fractions, 2000 µg/ml concentration proved to be most toxic and 250 µg/ml concentration was found least effective. Chloroform fraction of flowers was found highly active against both *Rhizoctonia solani* and *Mycogone perniciosa*. It showed 89.33 % growth inhibition against *Rhizoctonia solani* and 77.50 % growth inhibition against *Mycogone perniciosa* at 2000 µg/ml concentration. Moderate activity was shown by benzene, ethyl acetate and acetone fractions of flowers against both the tested fungi. Out of all the extract/fractions, methanol extract of flowers exhibited lowest antifungal activity against both the tested fungi. It exhibited 64.38 % growth inhibition against *Rhizoctonia solani* and 47.08 % growth inhibition against *Mycogone perniciosa* at 2000 µg/ml concentration. Hexane fraction of flowers of *Nyctanthes arbor-tristis* showed 75.42 % inhibition at highest tested concentration.

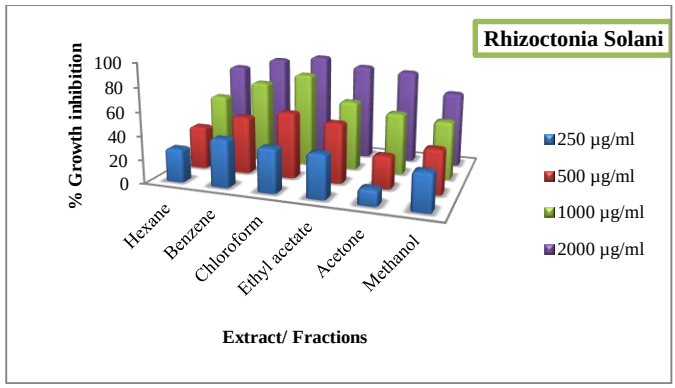


Fig 1: Antifungal activity of flower of *Nyctanthes arbor- tristis* (Harsingar) against *Rhizoctonia solani*

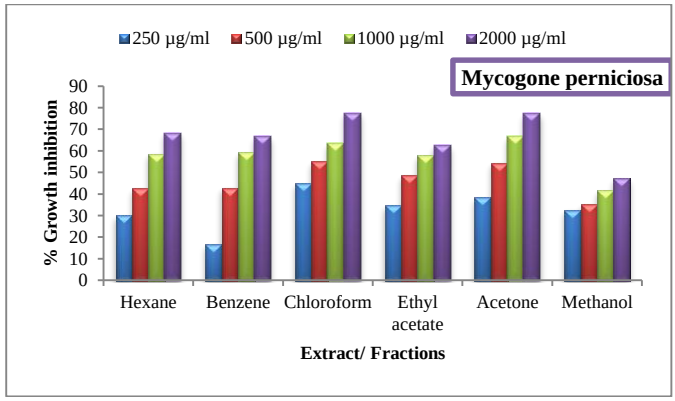


Fig 2: Antifungal activity of flower of *Nyctanthes arbor- tristis* (Harsingar) against *Mycogone perniciosa*

Conclusion

It is concluded that *Nyctanthes arbor-tristis* is a valuable plant with multiple pharmacological and biological activities. Methanolic extract of *Nyctanthes arbor-tristis* flowers afforded six compounds labeled as 1 to 6. The methanolic extract and various fractions of flowers of *Nyctanthes arbor-tristis* possess significant antifungal activity against two plant pathogenic fungi viz. *Rhizoctonia solani* and *Mycogone perniciosa in-vitro.*

Acknowledgements
Authors thanks to Professor and Head, Department of Chemistry and Biochemistry and Department of Plant Pathology, CCS Haryana Agricultural University, Hisar for research facilities. Thanks are due to SAIF, Chandigarh for spectral studies and DST, Delhi for financial assistance in the form of INSPIRE fellowship.

References

1. Mercier J, Manker DC. Biocontrol of soil-borne diseases and plant growth enhancement in greenhouse soilless mix by the volatile-producing fungus *Muscodor albus*. *Crop Protection*, 2005; 24:355-362.
2. Eddleston M, Karalliede L, Buckley N, Fernando, Hutchinson G, Isbister G, Konradsen F, *et al*. Pesticide poisoning in the developing world—a minimum pesticides list. *Lancet*, 2002; 12(360):1163-67.
3. Nadkarni KM, *Indian Materia Medica*. Bombay: India. Popular Prakashan, 1976; 1:857.
4. Kirtikar KR, Basu BD. An ICS. *Indian medicinal plants*. Dehradun: Bichen Singh Mahendra Pal Singh, 1984; 2(2):1273.
5. Puri A, Saxena R, Saxena RP, Saxena KC, Srivastava V, Tandon JS. Immunostimulant activity of *Nyctanthes arbor-tristis* L. Journal of Ethnopharmacology. 1994; 42:31-37.
6. Tuntiwachwuttikul P, Rayanil K, Taylor WC. Chemical Constituents from the Flowers of Nyctanthes arbor-tristis. *Science Asia*, 2003; 29(1):21-30.
7. Ratnasooriya WD, Jayakody JRAC, Hettiarachchi ADI, Dharasiri MG. Sedative Effects of Hot Flower Infusion of *Nyctanthes arbo-tristis*. on Rats. *Pharmaceutical Biology*, 2005; 43:140-146.
8. Sandhya S, Aruna SS, Anbumalarmathi J, Umamaheswari K. Journal of Chemical, Biological and Physical Science. 2016; 6(2):415-426.
9. Kiew R, Bass P. *Nyctanthes* is a member of the Oleaceae. Proceedings: Indian Academy of Sciences (Plant Sciences). 1984; 93(3):349-358.
10. Nandkarni AK. Indian Materia Medica. Ed. Dr. K.M. Nadkarni, Popular Prakashan Pvt. Ltd, 1982; 1(3):857-858.
11. Girach RD, Aminuddin SA, Siddiqui PA, Khan SA. Ethnomedicinal studies on Harsinghar (*Nyctanthes arbortristis* L) - A less known medicinal plant in Unani medicine. Hamdard Medicus. 1994; 37(2):60-66.
12. Kumari S, Singh R, Gurav NP, Mehta N. Isolation and Characterization of Bioactive Compounds from Stem of *Nyctanthes arbor-tritis* Linn. and Effect of Different fractions on Phytopathogens. Asian Journal of Chemistry. 2017; 29(4):787-791.
13. Tuit J. Plant pathological methods. Fungi and bacteria Minneapolis. Brugers Publishing Co, 1969.
14. Groves RK, Moore JD. Toximetric studies of fungicides against brown rot organism *Sclerotinia fruticola* and *S. laxa*. *Phytopathology*, 1962; 52:876-880.
15. Buckingham J. Dictionary of natural products. Chapman and Hall, London, 1994.
16. Heilbron I, Bunbury HM. Dictionary of Organic Compounds. Eyre and Spottiswoode, London, 1953, 1-4.
17. Winterstein A, Stein G. Double bond between C-12 and C-13 of oleanolic acid is inert to catalytic hydrogenation. *Z. Physiol. Chem*. 1931; 202:222-231.
18. Heilbron J, Cook AH, Bunbury HM, Hey DH. Dictionary of organic compounds. Eyre and Spottiswoode, London, 1965.