IJBPAS, August, 2012, 1(7): 964-972

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

NARINGENIN, A FLAVONONE FROM THE STEM OF *NYCTANTHES ARBOR-TRISTIS* LINN.

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ABSTRACT

Flavonoids, one of the most important secondary metabolite products occurring in plants are considered to have bioactive effect on human health . They serve as antioxidant, anti-inflammatory, carbohydrate metabolism promotor and immune system modulator. They act as scavengers for free radical which are responsible to cause variety of diseases by damaging proteins , lipids , membranes and nucleic acids. The present study deals with the phytochemical screening of the ethanolic extract of the stem of *Nyctanthes arbor-tristis*. The ethanolic extract was fractionated with different solvents viz. petroleum ether, ethyl acetate and butanol.The ethylacetate fraction was chromatographed over Si-gel column, which resulted in the isolation of Naringenin. Naringenin, a flavonone is reported to possess antiviral, antioxidant, anticarcinogenic activities. The structure of the isolated compound was elucidated on the basis of melting point determination and using various spectroscopic techniques (IR,UV,NMR and Mass) and confirmed with available data.

Keywords: *Nyctanthes* arbor-*tristis* , Naringenin, Bioflavonoid, Flavonone

INTRODUCTION

Naringenin is a plant bioflavonoid classified as flavonone. Main sources of naringenin are citrus fruits and tomatoes **[1, 2]**. In grapefruit, naringenin is principally present in glycosidic form as naringenin-7-rhamnoglucoside (naringin) **[3]**. Naringenin is found to show tyrosinase inhibitory activity **[4]**, antiviral activity **[5]**, antioxidant,anticarcinogenic and blood lowering lipid activities **[6]**. It has been isolated from many plants like stem of *Allamanda cathartica* **[4]**, *Gonio thalamus velutinus* **[5]** and *Premna fulva* **[7]**, aerial part of *Tanacetum parthenium* **[8]**, leaves of

Impatients bicolor in the form of its glycoside **[9]**, leaves,barks and fruits of *Ficus benjamina* **[10]**.

Nyctanthes arbor-tristis (family-Oleaceae) commonly known as Harsingar or Night Jasmine, is extensively used by Ayurvedic physicians for the treatment of various diseases **[11]**. Pharmacological investigations of the plant *N. arbor-tristis* show that it possesses powerful antioxidant activity and is effective as scavengers of H_2O_2 and free radicals **[12, 13, 14, 15]**. The overall antioxidant activity of *N. arbor-tristis* might be attributed to the polyphenolic contents and other phytochemical constituents. These polyphenolic compounds from natural products which are potent antioxidants have the capacity to improve food quality and stability and can also work to terminate free radical chain reactions in biological processes **[16, 17]**. Furthermore, the natural phenolic and flavonoid compounds can replace the synthetic material in foods or medicines which are responsible to carcinogenicity **[18]**. Earlier phytochemical studies on *N. arbor- tristis* resulted in the isolation of a number of iridoid glucosides from the leaves **[19, 20, 21]**, and seeds **[22, 23, 24]**. A flavonol glucoside Naringenin-4′-O-β-glucopyranosyl-α-xylopyranoside has been isolated from the ethanolic extract of the stem **[25]**. Recently we isolated a flavonol glucoside Astragalin from the aqueous methanolic extract of the stem part of the plant **[26]**. Looking towards the multiple biological effects of flavonoids that are beneficial to human health,the present investigation led to the phytochemical analysis of the ethanolic extract of the stem part of *N. arbor-tristis* which gave positive test for the presence of phenolic and flavonoid compounds and resulted in the isolation of Naringenin, a flavonone.

MATERIALS AND METHODS

Plant Material

The plant *N.arbor-tristis* was collected locally and identified by Dr.TVS Dhaka, Head, Department of Botany DAV College Muzaffarnagar (UP), India. The stem part was washed and dried in shade at room temperature and mechanically crushed.

Reagents and Chemicals

All organic solvents petroleum ether, chloroform, ethyl acetate, ethanol, butanol and inorganic reagents $FeCl_3$, CH_3COOH, NaOH, $NaHCO_3$ used were of Qualigens analytical grade. Column chromatography was carried out using Qualigens Silica gel (60-120 mesh). Thin layer chromatography was done using Merck TLC plate (8″x8″).

Extraction and Isolation

Dried and crushed stem of *N, arbor-tristis*(5 kg) was extracted with ethanol for 24 hours. After evaporation of solvent in

vacuum ,dark green coloured mass (62 gm) was obtained which was redissolved in little ethanol and treated with animal charcoal to remove colouring impurities and filtered .The filtrate was concentrated under vacuum and the residue was partitioned successively with petroleum ether, ethylacetate, and butanol to afford the corresponding fractions. These fractions were analysed for the presence of phenolic and flavonoid contents. The ethylacetate fraction gave positive test .This fraction was subjected to column chromatography on Si gel column and eluted with solvent systems of increasing polarities to obtain eighteen fractions (Fr-1 to 18).Fractions showing similar colour and same behaviour on TLC plate were added together to provide five fractions Fr-A, B, C, D,& E. These fractions were tested for phenolic and flavonoid content and Fr-D (pale yellow coloured) eluted with ethylacetate- methanol, gave positive test for the same. This fraction was evaporated under reduced pressure and concentrated. Concentrated residue was redissolved in little methanol and filtered. The filtrate was kept in refrigerator whereby pure crystals (1.90 mg, yellow coloured) were obtained. Purity of the compound was checked by TLC and melting point determination.

Test for Phenolic and Flavonoid Compound

The presence of phenol was determined by reacting test solution with neutral $FeCl_3$ solution and that of flavonoid by reacting with Mg/HCl **[27, 28]**.

Degradation of Naringenin

Naringenin was dissolved in minimum amount of ethanol (5 ml) and refluxed with (10 ml); of 50% ethanolic KOH solution at 100˚ C for six hours. It was allowed to cool and acidified with HCl and extracted with ether. The ethereal layer was washed with water and successively shaken with 50% $NaHCO_3$ and 50% NaOH.

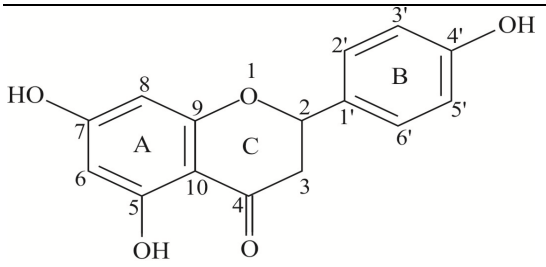
RESULT AND DISCUSSION

Phenolic and Flavonoid Compound

The test solution gave dark brown colouration with neutral $FeCl_3$ and red with Mg/HCl showing the presence of phenolic and flavonoid group in the compound.

Chromatographic Analysis

The purity of the pale yellow substance(1.90 mg), naringenin **(Figure 1)** was confirmed on the basis of its behaviour on TLC plate. The purified compound appeared on TLC plate as deep purple spot (R_f=0.88) with butanol:acetic acid:water (4:1:5 V/V) as developing phase and changing to greenish purple with ammonia vapours **[29].** The mp of the compound was observed to be 250-252˚C (Lit-250-251˚C) **[30]**.



Naringenin
(5, 7, 4′-trihydroxy flavonone)

Figure 1: Structure of Naringenin

Degradation Products of Naringenin

$NaHCO_3$ layer gave p-hydroxy benzoic acid (mp-212˚C) and NaOH layer gave phloroglucinol (mp-219˚C).These degradation products of naringenin suggest that the flavone is 5,7,4′-trihydroxy flavonone i.e naringenin. Proposed degradation pathway for the degradation of naringenin is presented in **Figure-2**. The first step is of C-ring fission forming an chalcone intermediate which on further cleavage can release three proposed possible products i.e phloroglucinol from ring A and p-coumaric acid and p-hydroxy benzoic acid from ring B ,but the melting point determination values suggest the degradation product of naringenin to be phloroglucinol and p-hydroxy benzoic acid.It indicates that the C-O bond in the C-ring undergoes an initial fission to generate a chalcone structure and that the C-2, C-3 bond is concomitantly reduced to a double bond **[31]**.

Spectral Analysis

The spectral data (IR, UV, NMR, Mass) of the compound are as follows: The IR [(KBr), υ_{max} cm^1], indicated the presence of absorbance bands at 3307 (–OH); 1641(>C = O).The appearance of bands in the UV [(MeOH), λ_{max}] at 326 and 289 nm represents the compound as the plant phenol **[32]**. The ^1H NMR [400MHz,CD_3COCD_3, δ(ppm), J/Hz] spectrum of the compound shows the singlet signal at 12.18,9.56 and 8.42 indicating the protons of hydroxyl groups (5-OH,7-OH,4′-OH).The proton at position 3 resonated as two signals 2.69(3-Hα,d, J=17.0) and 3.40(3-Hβ,dd,J=17.0,13.0) .The peaks at 5.94 (6-H, J=2.04) and 5.90 (8-H, J=1.8) show the pattern due to1,2,3,5 tetra-substituted ring A. The doublet peaks appeared at 7.38(2′-&6′H, J=8.6) while the other peaks appeared at 6.88 (3′& 5′-H,J=8.26) assignable to aromatic protons of ring B. The ^{13}C NMR [(400 MHz, CD_3COCD_3, δ (ppm)] spectrum shows the presence of fifteen carbons which were in agreement by comparison with literature values **[33, 34]**. Consisting of a signal at 197.2 (carbonyl >C=O carbon,4-C); six quaternary carbons at 165.2,165.9,162.9,163.2,128.9,158.6(5-C,7-C,9-C,10-C,1′-C,4′-C) .The seven methine carbons were at 115.9 (x2),129.9 (x2),97.8,96.1 & 80.1(3′&5′-C,2′&6′-C,6-

C,8-C, and 2-C) . The methylene carbon signal appeared at 43.9 (3-C). The mass spectrum indicated the presence of a molecular ion peak at m/z 272 which corresponded to the molecular formula $C_{15}H_{12}O_5$.

The peaks assigned in the spectral data corresponded to those reported in earlier studies **[32, 35] (Table-1)**.

CONCLUSION

The present investigation was carried out on the ethanolic extract of the stem of *Nyctanthes arbor tristis*, a well documented medicinal plant which resulted in the isolation of a bioflavonone, Naringenin. Flavonoids are plant phenolic compound which are considered beneficial to human health. So, the isolated compound may be further explored for pharmacological activity to design a modern drug from this plant.

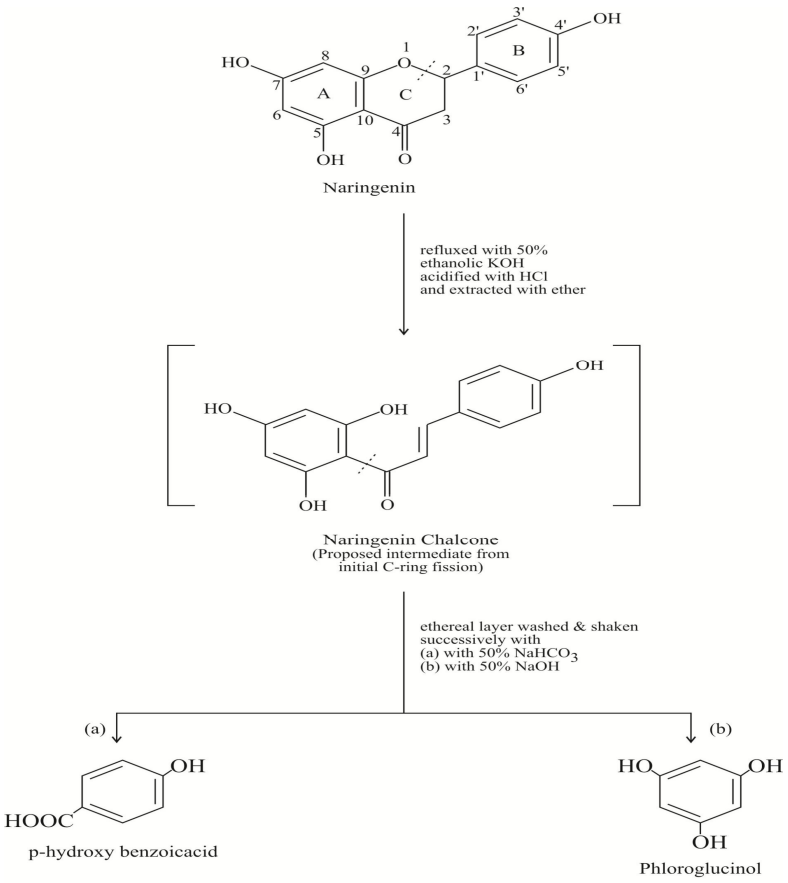


Figure 2: Degradation Pathway of Naringenin

Table 1: ^{13}C and ^{1}H NMR Spectral Data of Naringenin

C/H Position	□C(ppm)	□□ (ppm)	J (Hz)
2	80.1	5.48 dd	2.74, 12.96
3	43.9	2.69,3.40 dd	17.1, 13.0
4	197.2		
5	165.2		
6	97.8	5.94 d	2.04
7	165.9		
8	96.1	5.90 s	1.8
9	162.9		
10	163.2		
1′	128.9		
2′	129.9	7.38 d	8.26
3′	115.9	6.88 d	8.26
4′	158.6		
5′	115.9	6.88 d	8.26
6′	129.9	7.38 d	8.26
5-OH		12.18 s	
7-OH		9.56 s	
4′-OH		8.42 s	

ACKNOWLEDGEMENT

The authors are thankful to **Dr. TVS Dhaka**, Head, Department of Botany, DAV College, and Muzaffarnagar, India for identifying the plant material and **Dr. M.P Dhobal**, Department of Chemistry, University of Rajasthan, Jaipur, India for providing the necessary facilities to carry out this research work.

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