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ISOLATION AND CHARACTERIZATION OF TWO ALKALOIDS FROM THE ETHANOLIC EXTRACT OF *NYCTANTHES ARBOR-TRISTIS* L. LEAVES

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ABSTRACT:
The objective of the present study was to isolate and characterize phytoconstituents from the ethanolic extract of *Nyctanthes arbor-tristis* L. leaves. By of the ethanolic extract of *Nyctanthes arbor-tristis* L., two alkaloidal compounds fractionated and isolated by column chromatography and further structures of these compounds were determined on the basis of spectral and chemical studies. It comprised two novel compounds i.e. 1- (8-Hydroxy-7-((4-nitrophenyl) (phenyl amino) methyl) quinoline-3-yl) propan-2-one and 2- (8-Hydroxy-7-((4-nitrophenyl) (phenyl amino) methyl) quinoline-3-yl) acetic acid. Structures of all the isolated compounds were elucidated by spectroscopic methods like IR, NMR and Mass spectrometry.

Keywords: *Nyctanthes arbor-tristis*, Alkaloids, Extraction and Isolation, Spectroscopy

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INTRODUCTION

Nyctanthes arbortristis Linn (Oleaceae)(NAT) is one of the well known medicinal plant. It is commonly called as nyctanthes means night flowering and arbortristis means as it loses its brightness during day time. It is common wild hardy large shrub or small tree, native to India, distributed wild in sub-Himalayan regions and southwards to Godavari. It is also planted in Indian gardens for ornamental purpose due to its highly fragrant flowers [1-2]. It is a shrub or small tree up to 10 m in height with gray to greenish rough bark with stiff whitish hairs. Leaves are opposite, ovate, acute or acuminate, entire or with few large distant teeth, short bulbous hairs rounded or slight cuneate. Flowers are small, delightful fragrant, sessile, slender, and hairy; corolla glabrous, orange colored and lobes are white. Fruits are a capsules of 1-2 m in diameter, long and broad, compressed, 2 celled separating into 2 flat one seeded carpels, reticular veined and glabrous. Leaves are responsible for some CNS activities like hypnotic, tranquilizing and local anesthetics [3-5] and antiasthmatic activity [6]. The decoction of leaves is extensively used by Ayurvedic physicians for the treatment of arthritis, obstinate sciatica, malaria, intestinal worms and as a tonic, cholagogue and laxative[7-8]. β-Sitosterol isolated from N. arbortristis leaves showed analgesic and anti-inflammatory activity [9]. Iridoid glucoside isolated from this plant has antileishmanial activity[10]. Ethanolic flower extract of this plant is used for the synthesis of gold nanoparticles[11]. Seeds, leaves and flower extract of this plant showed CNS depressant activity[12]. Arbortristoside-A isolated from seeds possesses anti-inflammatory and analgesic activity[13]. Leaf and fruit extracts are useful in the treatment of arthritis[14]. Arbortristoside A and arbortristoside C isolated from plant showed antiviral activity[15]. The water-soluble portion of the alcoholic extract of leaves of *N.*

arbortristis (NAT) has been reported to possess Anti-inflammatory activity in a variety of experimental models[16].

MATERIAL AND METHODS

Plant material: NAT Leaves ware collected in the Third week of September 2008 from the Shasthri Nagar, Jodhpur, Rajasthan. Plant was authenticated from Botanical Survey of India, Jodhpur. Vide their letter no. BSI/AZC/A-19014/SG-i/Tech.2008 dated 26 Aug. 2008. The specimen was deposited at herbarium.

Preparation of column

Activated silica gel (60-120 mesh size, Central Drug House, New Delhi) was used as adsorbent for column chromatography. Heating at 120ºC for one hour in hot air oven activated it. The activated silica gel was poured in a beaker and thin slurry of it was made with solvent system, chloroform: methanol (95: 5) and chloroform: benzene (70:30) to prepare two columns. The bottom of glass column was plugged with glass wool and the silica gel slurry was poured into the column. The solvent was allowed to drip at the rate of 80 drops/minute and a level of 5 cm of the solvent was maintained at the top of the silica gel layer. The silica gel was allowed to pack until there was no further shrinkage of the column and no air bubble inside the column.

Separation and collection of different fractions

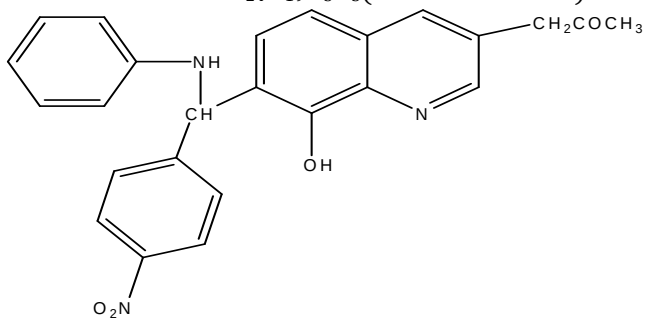
Dried ethanol extract was completely mixed with activated dried silica gel (3 gm extract + 15 gm silica gel) and poured in to each column. The column was developed by allowing the respective solvent system to elute the material at the rate of 80 drops per minute. The fractions were collected separately from each column in the conical flasks with a time gap of 10 minutes per fraction. In all 15 fractions were from each column collected at 10 minutes interval and they were numbered as A1-A15 and B1-B15. TLC was performed for each fraction using the respective solvent systems chloroform: methanol (95:5) and chloroform: benzene (70:30), the spot obtained by TLC were viewed under UV light and their Rf values were recorded. Those elute having same no. of spot at similar Rf values were combined together (fraction 1- 5, 6- 10 and 11-15. Two different compounds, A-1 from chloroform: methanol (95:5) fraction and A-2 from chloroform: benzene (70:30), were isolated and characterized.

Characterization of isolated Compound

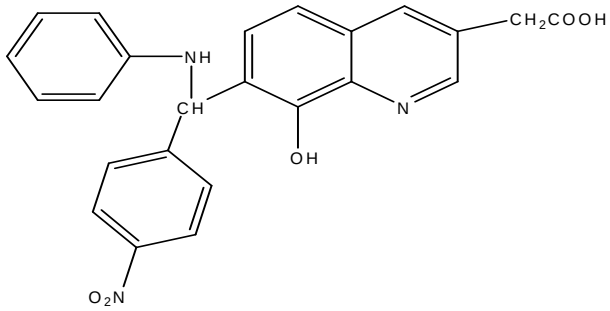
Structures of isolated compounds A-1 and A-2 were established based on IR, 1HNMR and mass spectral studies. The IR spectrum of sample was recorded on Shimadzu FTIR spectrophotometer by using potassium bromide disc technique. Finely powdered sample (1mg) was mixed with dried finely powered potassium bromide (200mg). The mixture was triturated thoroughly for about 2 minutes and was transferred to die and press system (hydraulic pressure was used). The powder was compressed (under hydraulic pressure) for 1 min at the pressure of 10 tons per square inch. The pressure was then released and the disc was recovered from die system. This transparent disc was scanned using FT-IR Spectrophotometer. ^{1}H-NMR spectra were recorded on Bruker NMR spectrophotometer in deuterium substituted $CDCL_3$using TMS as internal standard (Chemical shift in δ ppm). HPLC:MASS has been performed on Agilent 6410 Triple Qudrapole system with solvent system of 0.1% Formic Acid in Water and 0.1% Formic Acid in Acetonitrile as mobile Phase. Water-X-Bridge 150 X 4.6 mm column has been used for study.

RESULTS AND DISCUSSION

Compound A-1 was identified as 1- (8-Hydroxy-7-((4-nitrophenyl) (phenyl amino) methyl) quinoline-3-yl) propan-2-one having molecular formula $C_{25}H_{21}N_3O_4$ (M.P. 250 - 253^{0}C) where as compound A-2 was identified as 2- (8-Hydroxy-7-((4-nitrophenyl) (phenyl amino) methyl) quinoline-3-yl) acetic acid having molecular Formula $C_{24}H_{19}N_3O_5$(M.P.195ºC-200ºC).



Compound A-1



Compound A-2

^{1}H-NMR spectral data of compound **A-1:** $COCH_3$ (3H,singlet δ 0.7), $COCH_2$ (2H,singlet δ 2.2), NH (1H,doublet δ 4.12), OH (1H,singlet δ 4.85), CH (1H,singlet δ 5.28), Ar-NH (5H,multiplet δ 6.48-7.19), quinoline (4H,multiplet δ 7.25-7.48), Ar-CH (4H,multiplet δ 7.58-7.87). **Compound A-2:** $COCH_2$ (2H,singlet δ 1.72), NH (1H,doublet δ 3.95), OH (1H,singlet δ 4.64), CH-Benzene (1H,singlet δ 5.32), Ar-

NH (5H,multiplet δ 6.32-7.49), quinoline (4H,multiplet δ 6.82-6.97), Ar-CH (4H,multiplet δ 7.23-7.55), OH carboxylic (1H,singlet δ 9.95).

IR spectral data of compound **A-1:** 3319 cm^{-1}(OH), 3204.06 cm^{-1}(NH_2), 3045.89 cm^{-1}(Ar-CH), 2900 cm^{-1} (CH_2), 1725 cm^{-1} (C=O), 1539 cm^{-1} (NO_2), 2860.43 cm^{-1} ,2839.22 cm^{-1} (CH_2), 1444 cm^{-1} (OCH_3), 864 cm^{-1} , 812 cm^{-1} , 777.31 cm^{-1} (Ar-CH out of plane deforming). **A-2:** 3431.36 cm^{-1}(OH), 3380.45 cm^{-1}(NH_2), 3031.15 cm^{-1}(Ar-CH), 2868.87 cm^{-1} (CH_2), 1684 cm^{-1} (C=O), 1528 cm^{-1} (NO_2), 1313 cm^{-1} ,1228 cm^{-1} (C-O Str.), 781.89 cm^{-1} , 769 cm^{-1}, 743 cm^{-1} (Ar-CH out of plane deforming).

Mass spectral data of compound **A-1:** *m/z:* 427 (M^+), 412, 371, 340, 324, 323, and 295. The molecular weight of the compound is 427 and the mass spectral data matching the same as 427 m/e it shows that the m+ peak. **A-2:** *m/z:* 429 (M^+), 428, 400, 387, 370, 354, 337, 316, 278, 148. The molecular weight of the compound is 429 and the mass spectral data matching the same as 429 m/e it shows that the m+ peak.

CONCLUSION

Hypothesis about is that compound no. 2 is structurally resembles to Indomethacin which is an established potent NSAIDs. The SAR study of Indomethacin is indicating that the difference b/w N and acetic acid is two carbons in nucleus are necessary for binding to COX-1 & 2. By binding of COX-1 & 2 it inhibit the prostaglandin synthesis which causes inflammation. Structure of Compound two also showing the difference b/w N and acetic acid is two carbons in nucleus. So it may also inhibit the prostaglandin synthesis.

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