Research Article

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PHYTOCHEMICAL STUDIES OF *ARGYREIA NERVOSA*

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Abstract

Argyreia nervosa (AN) is commonly known as elephant creeper found in Assam and Bihar locally named as Vriddhara. There are many pharmacological studies of *Argyreia nervosa* are reported there is least number of phytochemical reports are available on this plant. This article is the first detailed phytochemcial investigation on this species. Hydroalcoholic extract of AN were fractionated using column chromatography the compounds were isolated, identified and elucidated by physical and spectral methods. Indole alkaloids namely ergometrine and flavanoidal constituent such as isoquercetin were isolated from the hydroalcoholic extract of *Argyreia nervosa.* Due to the presence of biologically active phytorinciple such as ergometrine and isoquercetin this species may be used as a major source of pharmaceutical purpose.

Key words: *Argyeia nervosa*, Concolvulaceae, Ergometrine, Isoquercetin.

Introduction

Argyreia nervosa (AN) is soft woolly climber with hardy woody stalk bearing soft silky heart shaped, large leaves, acute apex and cordate base. Flowers are purple, silky pubescent and wooly corolla white calyx, tomentose outside with glabrous ovary inside. Fruits are globose and indehiscent berry [1, 2]. In India, the leaves are used to prevent conception by the tribals of Rajasthan, whereas in Assam and Bihar leaves are used as vegetable[3]. Roots are used as an appetitiser, anti-inflammatory, aphrodisiac, expectorant, cardiotonic and brain-tonic. Roots are used to treat obesity, diabetes, anemia, ulcers, wounds, synovitis and gonohorrea [2] . Leaves are used to treat boils, swellings, ringworm infestations, externally to treat itches, eczema, wounds and skin diseases [4]. The literature revealed the presence of nineteen indole alkaloids, lysergic acid, isolysergic acid and ergoline alkaloids[5,6]. Presence of ergometrine, caffeic acid, ethyl caffeate, N-formylloline alkaloids, flavanoidal sulphates and argyroside were identified [7,8,9]. The plant proved antimicrobial activity, antifungal and phytotoxic activity, anti- inflammatory, aphrodisiac, antidiarrhoeal, nootropic effect, immunomodulatory, analgesic and anti inflammatory effect [10-17]. The plant showed effect on central nervous system, anticonvulsant and anti obesity effect [18-20].

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The previous research showed that no report availiable on the isolation of compounds so for. The present investigation identified the presence of alkaloids especially indole group and flavanoids from AN.

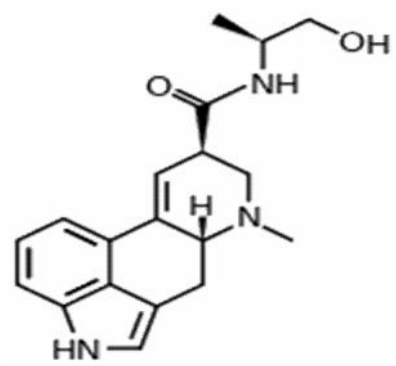

Figure 01: Structure of ergometrine

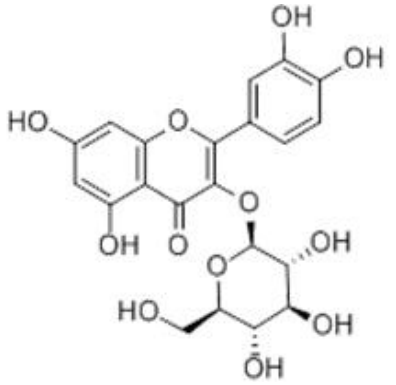

Figure 02: Structure of isoquercetin

Plant materials

The leaves of *Argyreia nervosa were* collected from the foot hills of Tirumala, Tirupati, Andhra Pradesh. All the plants were authenticated by Dr. Madhava Chetty, Professor, Dept of Botany, S.V. University, Tirupati. Voucher specimens were preserved in the Institute of Pharmaceutical Technology, Sri Padmavati Mahila Visvavidyalayam, Tirupati, Andhra Pradesh, India.

Apparatus required

UV-Spectra (Systronics), IR Spectra (Perkin Elmer) spectra were recorded. ^{1}H NMR spectra was obtained (AVIII Bruker, 500 MHz), ^{13}C NMR (AVIII Bruker, 500 MHz) spectra were recorded using the solvents $CDCl_3$ and MeoD. Electro Spray Ionization mass spectra were recorded using HP 1100 MSD.

Processing of the material

The collect plant material was dried in shade, coarsely powdered and subjected to extraction and stored in air tight container for further use.

Extraction and isolation

Dried plant material were ground to coarse powder was defatted with petroleum ether(60-80°C,4 hrs) using Soxhlet extractor, further with extracted hydroalcohol (70%v/v) till the exhaustion of the material. The extract was evaporated under reduced pressure and the residue was subjected to column chromatography. The column was prepared in ethyl acetate and left overnight, the column contents were eluted with gradient elution starting with pet ether: toluene followed by chloroform, ethyl acetate, methanol and water (90:10, 70:30, 50:50, 30:70 and 10:90). Chloroform: Ethyl acetate (80:20) and ethyl acetate: Methanol (20:80) eluted the compound AN-1(20 mg, Figure 1) ergometrine and AN-2 isoquercetin(15mg, Figure 2) was identified by physical, chemical and spectral studies. The structure was identified with in accordance with the previous literature.

Spectroscopic data of AN-1

Amorphous, UV spectrum $\lambda_{max:}$ 215. FT-IR: V_{max} cm^{-1}: 3358, 2268, 1644, 1435, 1262, 1161, 1052, 991 and 701. ^{1}H NMR (500 MHz,$CDCl_3$)::δ 1.06 (s, J= 4.13Hz,1H, $CONH_2$), (s, J=0.25, H in N-CH_3), 8.05 (dd, J=1.59-CHCHNH) , 7.034 (s, J=1.00, H-3),

7.313 (dd, J=1.00, H-4) 7.44 (dd, J-1.74, H-5) presence of indole group, 7.91 (dd, J=1.72) 7.84 (dd, J=3.27), 7.66 (dd, J= 3.07) 7.42 (dd, J=1.74, H-6) 8.53 (s, J=1.59, H-6 '') 7.63 (dd, J=3.0, H- 7), 7.70 (dd, J= 1.72) 7.76 (dd, J=2.02) . ^{13} C NMR 500 MHz, $CDCl_3$): δ 148.89 (C- 1), 138.79 (C adjacent to NH), 125.984 (C- 2) 124.35(C=C), 117.73 (C- 4), 113.71 (C-3). EISM (70 eV, m/z (relative intensity): 348 (M^+) 346, 340, 322, 310, 299.9, 280, 272, 257, 249, 230, 222, 206, 191, 183, 168, 155, 149, 137, 123. The molecular was deduced as C_{19} H $_{23}$ N $_3$ O_2. The data was compared and upon close proximity with the earlier literature, the compound AN-1 was found to be ergometrine [21,22].

Spectroscopic data of AN-2

Yellow, amorphous, UV spectrum λ_{max} : 286 and 334 nm, upon addition of NaOH (248 and 343 nm), $AlCl_3$ 3(255, 378 and 347 nm), HCl (242, 305, 319 and 390 nm), CH_3CooNa (270, 296 nm) and H_3Bo_3 305, 326, 364 and 251, 279 nm. FT-IR, V_{max} cm^{-1}: 3407, 2921 1663 , 1610 , 1562, 1521, 1450, 1407, 1382, 1319, 864, 841, 824 , 796, 722, 681, 1263,1199 , 1169, 1131, 1092 , 1014 , 941, 639, 603 and 492. ^{1} H NMR spectrum (500MHz, MeoD): δ 5.001 (s, J=1.00, H-1''), 3.324 (s, J=1.00, H-2'' NH group), 7.75 (dd, J=1.10,H-2' aromatic group), 3.28 (s, J=1.00, H-3'' hydroxide group), 3.331 (s, J=1.00, H- 4'' ether group), 3.334(s, J=1.00, H-5'' alkyl protons) 6.87 (s, J=1.00, H-5') and 6.17 (s, J=1.03,H-6 aromatic group, 3.337 (s, J=1.00, H- 6'' amino group), 7.45 (dd J=1.07, H-6') and 6.37 (s, J=1.00, H-8, phenol group). ^{13} C NMR (500MHz, MeoD): δ 146.6 (C-2), 35.8 (C-3) 161.0 (C-5), 97.8 (C-6), 164.12 (C-7), 156.83 (C-8), 93.7 (C-9), 103.12 (C-10), 76 (C-4), 122.75 (C-1'), 114.6 (C-3&5'), 147.37, (C-4'), 114.5 (C-4'&5') 144.82 (C-2'). EISM (70 eV, m/z (relative intensity): 487 (M+Z), 466, 406, 351, 297, 274, 247, 213, 193, 163, 146,108.

Result and Discussion

The isolated compound AN-1 and AN-2 showed the positive response with specific indole alkaloidal and flavanoidal reagents respectively. The derived UV absorption spectra of AN-1 and AN-2 showed the maxima at 215, 286 and 334 nm respectively, identified the presence of indole alkaloids and aromatic flavanoid. Upon addition of shift reagents to AN-2 showed the maxima NaoH (248,343nm), sodium acetate(270,296nm), aluminium chloride(255, 378, 347nm), boric acid (305,364, 326, 251, 278nm), hydrochloric acid (242,305,319,390) nm.

The ^{1}H NMR of AN-1 showed the presence of protons in $CONH_2$, N-methyl group, at position 3(indole group),6"(hydroxide group). It showed the singlet at δ 1.06, 1.09, 7.034, 8.53 (IH, J=4.13Hz), (1H J=0.25 Hz), (H-3J=1.00 Hz), (H-6" J=1.59 Hz) respectively. It showed ten doublets indicating the presence of proton in CHCHNH (N-methyl group, dd, J=1.59) at δ 8.05, at position 4,5, at δ 7.44,(dd, J=1.00), δ 7.43 (dd, J-1.74 indole group) . H- 5" δ7.91,7.84,7.66 (dd, J=1.72 ;dd, J=3.27; dd, J= 3.07)- amino group, H-6 δ 7.42 (dd, J=1.74 aromatic group) ; H-7 δ 7.63; δ 7.70 (dd, J= 1.72 Phenolic Hydroxyl ; dd, J=3.0 Indole substituted group) at δ 7.76 (dd, J=2.02) indicated the presence of indole substituted group.

The ^{13}C NMR of AN-1 showed the presence of carbon at position 1, 2, 3, C=C and amino (CONH) exhibited at δ 149,125.96,113.71, 117.73,124.35 and 139.79 respectively.

The mass spectrum showed the fragments and fragmentation at 348 (M^+), m/z 346, 340, 322, 310, 299.9, 280, 272, 257, 249, 230, 222, 206, 191, 183,168, 155,149,137, 123,107, showed the presence of hydrogen atom, three hydrogen atom, NH_4 molecule, one carbon atom, five hydrogen atoms, HF, four hydrogen atoms, methyl group, four hydrogen atoms, HF, four hydrogen atoms, oxygen atom, methyl group, , four hydrogen atoms, methyl group, nitrogen group, three hydrogen's, carbon group, NH and NH_2 group respectively.. The molecular formulae was deduced as C_{19} H $_{23}$ N $_3$ O_2.The ^{1}H NMR of AN-2 showed the presence of protons at position 1'' ,2'', 3'',4'',5',6'' and 6.It showed the singles at δ5.001, 3.324, 3.28, 3.337, 3.334 6.87, 6.17, 3.338, 6.37 at J=1.00. In addition, presence of protons at position 8, 6, 5', 2' 6', in δ 6.37, 6.87, 7.75 and 7.45 at J=1.00 and J=1.07 respectivly. The ^{13}C NMR of AN-2 showed the presence of carbon at position 2, 3, 4, 5, 6, 7 , 8 ,9, 10, 1'', 2'', 3''&5'', 4'' and 4''& 5'' at 146.6,35.8,76,161.0,97.8,164.12, 156.83,93.7,103.13,122.75,144.82,114.6,143.37 and 114.5 respectively. The mass spectrum showed the fragments and fragmentation of 487 (M+Z), m/z 466, 406, 351, 297, 274, 247, 213, 193, 163, 146 and108 showed the presence of H_3O atom, CH_2COOH+H, C_4H_7, C_4H_6, C=CH, C_2H_3, two hydroxyl group, HF, two methyl group, hydroxyl group and C_3H_2 group respectively. The molecular formulae was deduced as $C_{21}H_{20}O_{12}$. The ^{1}H NMR and ^{13}C NMR and AN-2 confined the presence of indole nucleus and flavanoidal structure. The derived mass spectra of AN-1 and AN-2 confirmed the fragments of the spectra are relevant to ergometrine (Figure 1) and isoquercetin (Figure 2) respectively. The derived mass spectra of of AN-1 and AN-2 showed remarkable pattern of (indole compound) ergometrine and flavanoidal structure (isoquercetin), further lead to the identification of compound AN-1 as ergometrine and AN-2 as isoquercetin, those structure were in close proximity with the earlier literature.

Conclusion

The phytochemical studies revealed the presence of ergometrine belonging to the class of indole alkaloid reported to possess oxytocic effect, anti ageing and contraception. These compounds were being reported for the first time in this plant *Argyreia nerosa*. The current method may be fruitful and easily adaptable for the seperation of ergometrine and isoquereti‌n on l;arge scale . The present article provides scientific credit to its ethnocliam and support to its earlier report. A further investigation a may be provoked to investigate the pharmacological activites of the compound. Further research is in progress.

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