Original Article

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HARMALINE FROM *PASSIFLORA FOETIDA*

*Krishnaveni A, Santh Rani Thaakur
*Institute of Pharmaceutical Technology,
Sri Padmavathi Mahila Viswavidyalayam (Women's University),
Tirupati, Andhra Pradesh, India - 517502.

Abstract

The present experimental study was carried through extraction and characterization of the hydrpalcoholic extract of leaf of *Passiflora foetida*. The leaf material was defatted further extracted with hydroalcohol, concentrated in vacuo. Column chromatography was adopted for the separation of phytoprinciple present in the *Passiflora foetida*. Column chromatragraphy was packed with silica gel by wet method, solvents were eluted over in the increasing order of polarity. Considerable amount of chloroform fractions were collected identified by thin layer chromatography, pooled, subjected to physical, chemical and spectral methods. Spectral techniques includes ultraviolet, H_1 NMR and mass spectrum were used. Harmaline, a betacarboline alkaloid was identified and its structure was confirmed by spectroscopic methods (including ultraviolet, H_1 NMR and mass spectra) as harmaline (7-methoxy, 3, 4 -dihydro betacarboline). Harmaline is found to have monoamaine oxidase inhibitory effect, useful for the antidepressant activity. The present method is simple, rapid, economic and effective method used for the separation of harmaline from the *Passiflora*. Synthetic approaches for the harmaline are costly and time consuming; hence this alternate procedure can be adopted for the plant source. Beta carboline alkaloid acts against stress and proved protective role in oxidative stress. The above specified reports evidenced scientifically that harmaline was present in *Passiflora foetida and* envisages additional research towards its other pharmacological action.

Key words: Betacarboline, Harmaline, Passifloraceae, *Passiflora foetida*.

Introduction

Passiflora foetida belongs to Passifloraceae, is commonly named as stinking passion flower used by the Gond tribe of India to treat cancer [1]. The plant is used as sedative; its prepartions are used to treat asthma, giddiness, menstrual and nervous disorders [2-5]. The plant also exhibited insect deterrent activity, hypoglycemic, antiproliferative, antibacterial, antioxidant and antimelanogenesis[6-11].Previous studies revealed the isolation of passifloricins, alpha pyrones and vitexin from leaves of *Passsiflora foetida* [12, 13].

Author for Correspondence:
Krishnaveni A,
Institute of Pharmaceutical Technology,
Sri Padmavathi Mahila Viswavidyalayam
(Women's University), A.P, India – 517 502.
Email: akrishnaveni72@rediffmail.com

Preliminary phytochemical studies revealed the presence of alkaloids, tannins and flavanoids [14]. The present investigation was performed to identify and isolate harmaline from the alcoholic extract of *Passiflora foetida*.

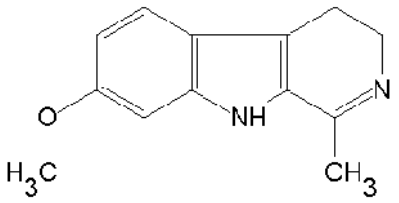

Figure 01: Structure of Harmaline

Plant Material

Leaves of *Passiflora foetida* were collected from the foot hills of Tirumala, Tirupathi, Andhra Pradesh, India during the year 2006 was identified by Dr.Madhav

Shetty, Taxonomist, Dept. of Botany, S.V.University, Tirupathi, Andhra Pradesh, India. The voucher specimen was kept in our college for further reference.

Apparatus used

Column chromatography (1:50cm) silica gel (Column chromatography grade,S.D.fine chemicals, Mumbai, India. Spectral analysis were recorded using UV-Visible spectrophotometer (UV-1800 Shimadzu), H_1 NMR (AV Bruker AVIII, DMSO, 500 MHz,) were recorded. Co-TLC was performed using silica gel precoated plates (Merck, Germany) with the mobile phase ethanol: chlororform : acetic acid(40:60:6).Mass spectra was analysed by EIMS at 70eV using JEOL insertion probe.

Processing of the plant material

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

Extraction and Isolation

Powdered leaf of *Passiflora foetida* was extracted with 70% alcohol by Soxhlet extraction process after defatting. The extract was further concentrated in vacuo. About 5 gram of the extract was placed over column chromatography packed with silica gel eluted with various solvents ranging from petroleum ether to methanol [10]. The phytoconstiutent (compound 1, R_f 0.66,10 mg) (figure1) was obtained from chloroform fractions were separated based on TLC studies. The isolated compound was yellowish brown crystals, subjected to chemical and spectral methods. The structure of the compound was established in accordance with the previous literature.

Spectroscopic data

UV absorption band (lambda maxima, in methanol) was found at 270 and 375 nm was identical with that of harmaline. H_1NMR (AV-BrukerAVIII, DMSO, 500MHz) δ 2.07 (1H, s, J=3, methyl group), δ 2.98 (1H, ddd, J=13.3, 9.66, 4.33, **H-6**), δ 6.35(1H, ddd, J=13.30, 4.70,1.64. **H-12**), δ 3.43(1H,ddd,13.24, 9.62, 4.70 **H-14)**, δ3.55 1H, ddd, ,13.24, 4.42, 1.66, **H-5**), δ 3.71(3H,s,OCH3), δ 6.22 (1H, dd J=8.49, 1.51, **H-10**), ,δ7.52(1H,dd,J=8.49,1.43,**H-9**),δ 3.05(1H, dd,J=13.297, 4.650, **H-3**).

EI-MS (70eV, m/z)

The fragmentation and fragments of the mass spectra were 214(M^+100%), 202(10%), 199.9(53%), 186 (10%), 170(37%), 153(10%), 143(10%), 128(4%), 115(6.4%), 107(8%), 90(4%), 84(5%) and 74(5%).

Result and discussion

The isolated compound showed positive response with specific harmaline alkaloidal test and exhibited UV absorption band showed the maxima absorbance at 270 and 375 nm indicated the aromatic band of alkaloids. The H_1 NMR spectrum added the confirmation of alkaloidal structure and depicted the presence of protons at position H-14, H-6, H-5, H-10, H-12 and H-9. It showed three doublets (one doublelet of doublet at δ 6.35, 6.22, 7.52 (1H, dd J=8.49,1.51,H-12; 1H, dd J=8.49, 1.51, H-10; 1H,dd, J=8.49,1.43,H-9) and two doublets of doublet at δ 2.98, 3.05, 3.43, 3.55 (1H, ddd J=13.3, 9.62, 4.42 H-6), 1H, ddd, J=13.30, 4.70, 1.64, H-3),1H, ddd J=13.24, 9.62, 4.70, H-14, 1H, ddd J=13.24, 4.42, 1.64, H-5) and singlet at δ 2.07 and δ 3.71(1H, s J=3, H-14and 3H, s, H-7,OCH_3). The Mass spectra show the fragmentation pattern 214(100%), 202(5%), liberation of carbon atom 199.9(20%), 186(20%), 170(11.6%), 153 (9.6%), 143(8%), 128(0.4%), 114(8.38%), 106(10%), 90(9%), 84 and 75(5%) indicated the presence of molecular base peak (M+) at 214(M^+100%) and the fragments indicated the presence of one hydrogen atom, another hydrogen atom, one imino (NH group), one oxygen atom, one hydroxyl atom(OH molecule), five hydrogen atoms, one methyl group, one nitrogen atom, four hydrogen atoms, one oxygen atom, three hydrogen atom respectively. It also revealed the empirical formula $C_{13}H_{14}N_2O$. Therefore, UV, H-NMR and EI-MS data led to the identification of compound **1** as harmaline (3,4 dihydro,7-methoxy betacarboline) and its structure was depicted in figure1. The derived spectra was a marked pattern of betacarboline alkaloids especially harmaline. The structure of the isolated compound was confirmed as harmaline and was in good agreement with the earlier literature.

Conclusion

Harmaline belonging to beta carboline group reported to possess monoamine oxidase inhibitory activity and acting as antidepressant drug. The present method

may be helpful to separate the harmaline on large scale separation from *Passiflora foetida*. Hence, this compound (harmaline) being reported for first time the leaves of *Passiflora foetida*. A further investigation may be envisaged to investigate the mechanism antidepressant activity. The present article provides an additional evidence to the earlier report identified by the team.

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