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Isolation, Purification and Characterization of (*R*)-Petranine from *Catharanthus pusillus*(Murr.) G.Don(Apocynaceae)

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Abstract: ***The whole plant of Catharanthus pusillus of family Apocynaceae was subjected to isolation and identification of chemical constituents. The extract was purified and isolated by column chromatography and thin layer chromatography (TLC). The isolated compound was then subjected to UV spectrum, FTIR for identification of functional groups and ^{1}HNMR and ^{13}C-NMR for identification of protons and carbon atoms. ESI-MS was done to identify the molecular weight of the isolated compound. From the spectra obtained from FT-IR, ^{1}H-NMR, ^{13}CNMR and ESI-MS, the isolated compound was found to be (R)-petranine with the molecular weight of 285***

Keywords: *Catharanthus Pusillus* , TLC, spectroscopy

1. Introduction

Plants are utilized as therapeutic agents since time immemorial in both organized and unorganized form.The healing properties of many herbal medicines have been recognized in ancient culture[1].Natural products chemistry has always been concerned with nature and natural phenomena and, as a consequence, biologically active metabolites. Natural products research remains one of the main means of discovering bioactive compounds. Since it is little known about the etiology of many human, animal and plant diseases, it is difficult to design potentially active molecules for their treatment, and therefore leads from natural sources will continue to be sought [2].The importance of natural products, and particularly of plant-derived natural products, as a source of molecular diversity for drug discovery research and development may appear to be self-evident. Plants have a strong biological and ecological rationale to produce bioactive compounds.

Catharanthus pusillus belonging to family Apocynaceae is known with various names in India and all over the world. It is widely used as various treatments of diseases and traditionally used as herbal medicine [3]. The roots, leaves and latex of these plants are used to treat skin and liver diseases, leprosy, dysentery, worms, ulcers, tumor and ear aches. The leaf powder of *C. pusillus* were mixed with coconut oil and used for treat the antidandruff activity and also used to kill the lice [4].

The present study deals with the extraction and characterization of the methanolic extract of whole plant of *C. pusillus.*The characterization of the extract includes the isolation and purification using the column and thin layer chromatography.The isolated compounds from TLC were subjected to various instrumental analysis like IR, H NMR and C NMR were done to identify the prescence of functional groups,protons and carbon atoms respectively.

2. Experimental

Materials and Reagents

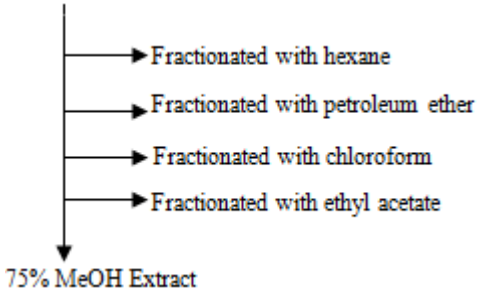
The whole plant of *Catharanthus pusillus* were collected from Pechiparai, Kanayakumari District, Tamil Nadu. With the help of local flora, voucher specimens were identified and preserved in the Ethnopharmacology unit, Research department of Botany, V.O. Chidambaram College, Thoothukudi, Tamil Nadu for further references.

Hexane, petroleum ether, chloroform, ethyl acetate, acetone, methanol and ethanol were of analytical grade procured from Merck. Column chromatography was performed on column (length 50 & diameter 150 mm), silica gel (60-120 mesh) and Merck TLC readymade sheets 20 x 20 cm.

The spectrophotometer systems used were Shimatzu UV spectrophotometer, Shimadzu spectrum 1 FT-IR spectrometer and ESI-MS analysis (TofSpec 2E MALDI time - of flight (TOF) Instrument (Micromass, Manchester, UK). 1H-NMR and 13C-NMR spectra were recorded on Brucker spectrometer using CDCl3 as solvent and TMS as internal standard. The observed chemical shifts (8) were recorded in ppm and the coupling constants (J) were recorded in Hz.

Extraction

100g of whole plant powder of *Catharanthus pusillus* were extracted with 90% methanol using soxhlet apparatus and concentrated for further using simple distillation method. The concentrated plant extract liquid fractionated with the solvents hexane, petroleum ether, Chloroform, Ethyl acetate. Altogether, 5 fractions were obtained and used for separation of pure isolates using column chromatography method. Conc. 90% MeOH extract redissolved in 75% MeOH



Column chromatography
60-120 mesh size silica gel was dissolved in the low polarity solvent hexane and tightly packed in 50 X 150 mm glass column up to 100 mm height without air bubbles. Then the experimental extracts were loaded individual glass columns and fractionated with solvents hexane, petroleum ether, chloroform, ethyl acetate, acetone and methanol at various proportion of solvent mixture.

Screening of purity for column chromatography fractions using TLC
15 ml of fractions were collected using each solvents and the collected fractions were screened for purity using thin layer chromatography (Merck TLC Readymade sheets 20 X 20 cm) with appropriate solvent systems (Petroleum ether : Hexane :Chloroform : Ethyl acetate Acetone : methanol : ethanol 7 : 1 : 1 : 0.5 : 0 : 0.5 : 0.5).

Preparative TLC:
The closely mixture fractions was re-separated using PTLC. The mixture fractions were spotted on TLC for separation individual components and scraped using sterile needles and dissolved in methanol. Then centrifuged at 10,000 rpm. The supernatant was taken for further characterization like TLC, UV-VIS spectrophotometer, FTIR, ESI-MS, MS, H1 NMR, C13 NMR and structure elucidation.

3. Results and Discussion

The ethyl acetate fraction was purified using chloroform and methanol as eluent in the combination of 9:2 by silica gel column chromatography (60-120 mesh). The isolated fractions 35 to 78 showed colourless and they are showed mixture of compounds along with major spots in screening of purity on TLC under iodine vapour visualization. Consequently, flash column was used to separate the pure compound with silica gel column (200 mesh). The mobile solvent is ethyl acetate:methanol in the ratio of 9:1. The fractions 21 to 68 are show similar pattern on TLC under iodine vapour with few major spots. The PTLC carried out for one of the major spot and re-isolation by scraping, dissolving in pure methanol, followed by centrifuge at 10,000 rpm for 10 minutes. The supernatant was concentrated and screened for purity and appear single spot on TLC under iodine vapour. It was taken for further characterization. *Viz.*, TLC, UV scanning, FTIR/IR, MS/ESIMS/EIMS, ^{1}H NMR, ^{13}C NMR.

TLC
Colourless substance and the compound gave spot showed Rf value 0.95.(Fig.1)

Figure 1: TLC of isolated comound

UV-VIS: λmax was found 216 nm.(Fig.2)

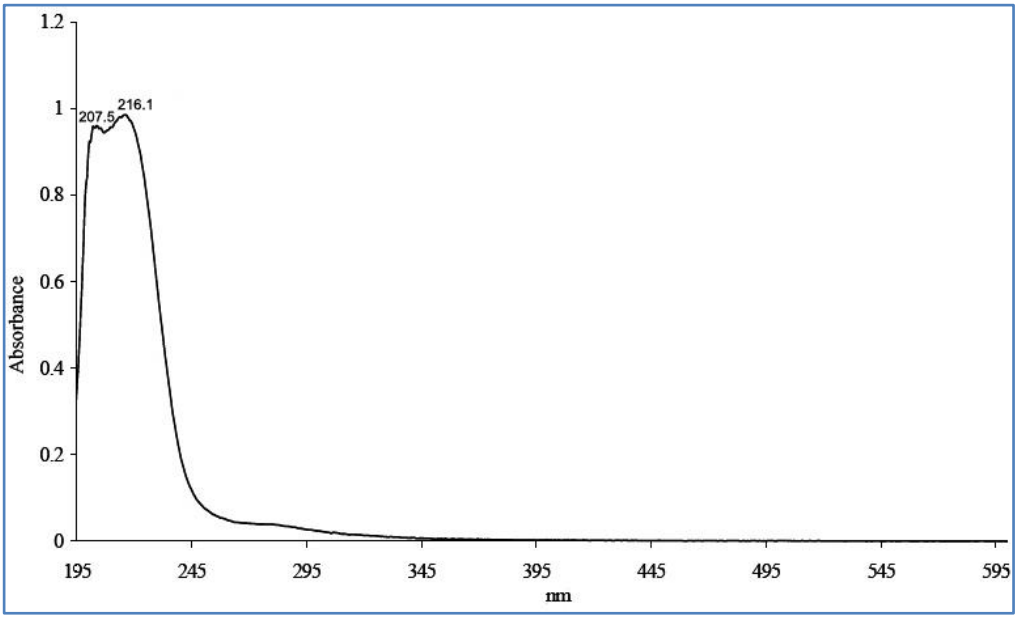


Figure 2: UV-VIS spectrum of isolated comound

FTIR spectrum

The molecular peak 2692, 2969, 2854, 1718, 1458, 1385, 1232, 1155 and 1045 .(Fig.3)

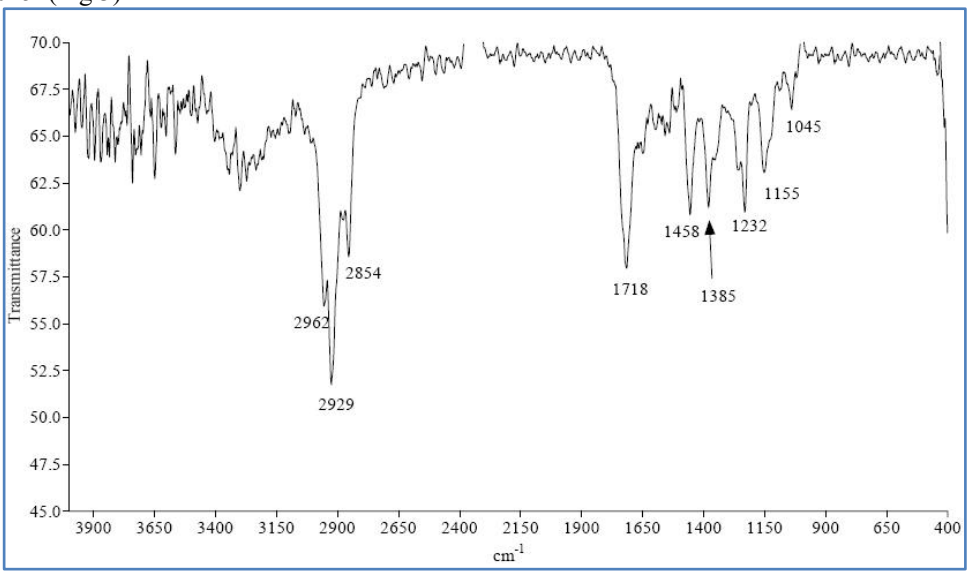


Figure 3: FT-IR spectrum of isolated comound

EISMS-MS

MS spectrum shows molecular ion peak *m/z* 238.3, 286.3 [M + H]+ and 288, 352.1 .(Fig.4)

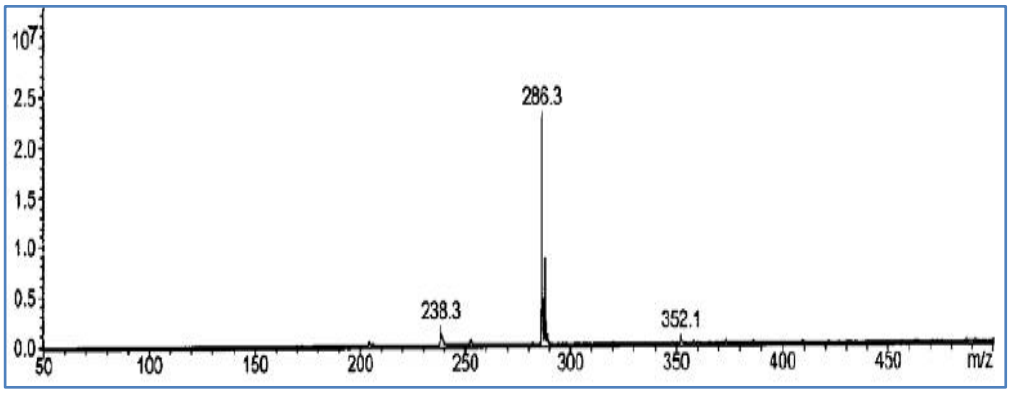


Figure 4:UV-VIS spectrum of isolated comound

H NMR

Proton NMR spectrum of (*R*)-petranine chemical shift values most useful shift values are those for the protons on C-2, C-6, C-7, C-8 and C-9 are 5.82, 2.46 (2.64), 4.90, 5.28 and 4.86. In 1H-NMR spectrum, the presence of single proton at 6.13 ppm and the six protons at C-15 (1.90 ppm, 3H) and C-14 (1.98 ppm, 3H) with *J*-coupling values (*J*=7 Hz, *J*=1.4 Hz) is typical *J*coupling values of chemical shifts for substituted double-bond in angeloyl. .(Fig.5)

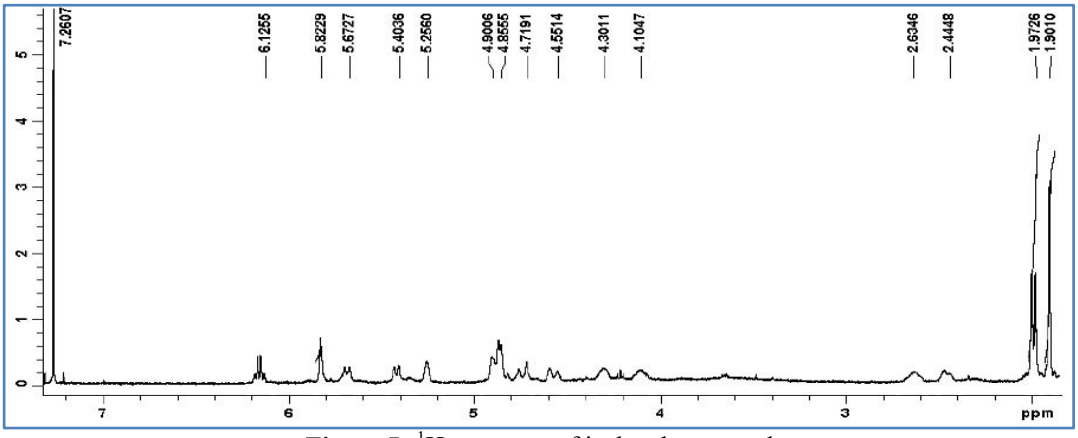


Figure 5: [1]H spectrum of isolated comound

^{13}C NMR

In the double-bond region of 13C-NMR, quaternary C-12 (at 126.9 ppm) and primary C-13 (at 140.3 ppm) were noticed. In fact, two quaternary carbons in 13C-NMR (126.9 ppm, 134.4 ppm) appeared. The two methyl groups of angeloyl at C-14 and C-15 are trans to each other. The main peaks of C-6 protons in 1H-NMR can be easily identified from its typical value at 2.3 ppm with found values at (H-6a = 2.44 ppm and H-6b = 2.63 ppm).

Also coupling between C-6 and C-7 (H-7 =4.90 ppm) and between C-7 and C-8 (H-8 =5.26 ppm) were also observed. The values of protons at C-3 found (H-3a =4.55 ppm and H-3b =4.72 ppm dd, *J* = 16.4 Hz), the long range coupling (*J*=16.4 Hz) enhance the presence of five membered ring. The cyclic bridge between the oxygen at C-7 and the nitrogen atom in the presence of methylene group C-17 between them. The 1H-NMR show the values of C-17 protons are H-17a =5.40 ppm doublet, H-17b =5.68 ppm doublet, those values confirm that C-17 is connected to oxygen. 13C-NMR of C-17 (68.8 ppm) is the other evidence of C-17 chemical environment. This assignment was suggested by the chemical shift values of C-3, C-5 and C-8 protons and carbons to the down field from its typical values of C- 3 (60 ppm), C-5 (53 ppm) and C-8 (76 ppm); On C-3, C-5 and C-8 to its values (C-3 = 70.9 ppm, C-5 = 63.5 ppm, C-8 = 87.7 ppm). .(Fig.6)

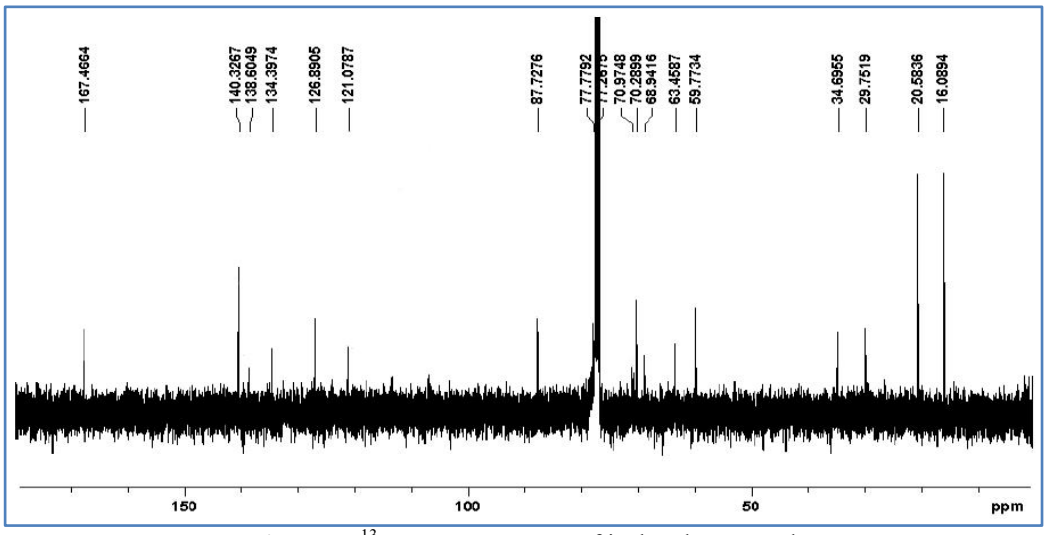


Figure 6: [13]C NMR Spectrum of isolated comound

Depending on the spectral data and reference literature, the isolated compound is (R)-petranine.

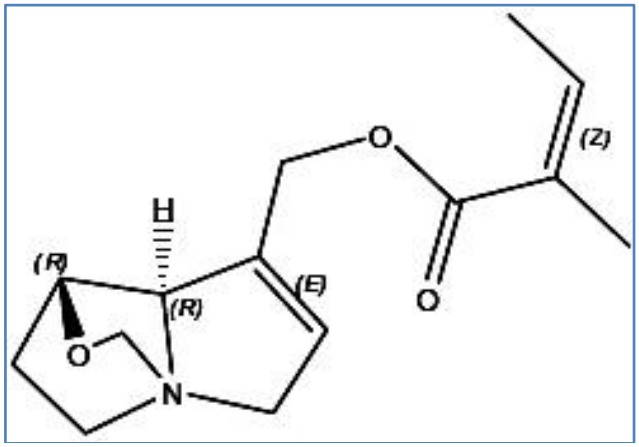


Figure 7: Structure of isolated comound

One important and well-known class of naturally occurring chemicals in foods is the pyrrolizidine alkaloids. Pyrrolizidine alkaloids (PAs) are found in plants growing in most environments all over the world. The potential number of PAs-containing species is as high as 6000, or 3% of the world's flowering plants [5]. (*R*)-petranine (pyrrolizidine alkaloids) is a novel compound isolated from the *Catharanthus pusillus* and it is supposed to have some medicinal activity.

4. Conclusion

The developed method is useful for isolation, purification and characterization of (*R*)-petranine (pyrrolizidine alkaloids) found in *Catharanthus pusillus.* . The method does not require any elaborate treatment and tedious extraction procedure for isolation and purification. It is simple, precise and reproducible approach for further characterisation.

5. Acknowledgement

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References

[1] Rajeshwari Sivraj,Balakrishnan A, Thenmozhi M and Venkatesh R.Preliminary phytochemical screening of Aegle marmelos, Ruta graveolens, Opuntia dellini, Euphorbia royleana and Euphorbia antiquorum, internal journal of Pharmaceutical sciences and research 2(1) : 146-150, 2011.
[2] Steven MC, Russell JM. Bioactive natural products, detection, isolation and structural elucidation. CRC Press; 1993.
[3] Don G. *Catharanthus Roseus* In: Ross I.A (Ed), "Medical Plants of The World", Human Press, Totowa, NJ. 1999; 109-118
[4] Balaji rao, N.S. Rajasekhar, D. and Chengal raju, D (1996).Folklore Remedies for Dandruff from Tirumala hills of Andhra Pradesh. *Ancient Science of life.* **1996;** XV (4), P:296 -300.
[5] Coulombe RA. Pyrrolizidine alkaloids in foods. Advances in Food and Nutrition Research 2003; 45: 61-99.