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Isolation, Purification and Characterization of 3-β Acetyl Oleanolic Acid from *Catharanthus pusillus* (Murr) G. Don (Apocynaceae)

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Original Research Article

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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 interpretation of the spectral data, the isolated compound was found to be 3-β acetyl oleanolic acid.

Keywords: *Catharanthus Pusillus*, TLC, spectroscopy.

INTRODUCTION

From time immemorial, man depended on plants as medicine. From a historical perspective, it is evident that the fascination for plants is as old as mankind itself. The plant kingdom represent a rich store house of organic compounds, many of which have been used for medicinal purposes and could serve as lead for the development of novel agents having good efficacy in various pathological disorders in the coming years. Plants are still an independent source of medication in the contemporary health care delivery system. Their role is twofold in the development of medicines and served as a natural blue print for the development of new drugs, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs [1]. An impressive number of modern drugs have been isolated or derived from natural sources, based on their use in traditional medicine [2].

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 spectral analysis like FT IR, ^{1}H NMR and ^{13}C NMR were done to identify the presence of functional groups, protons and carbon atoms respectively.

METHODOLOGY

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, and 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.

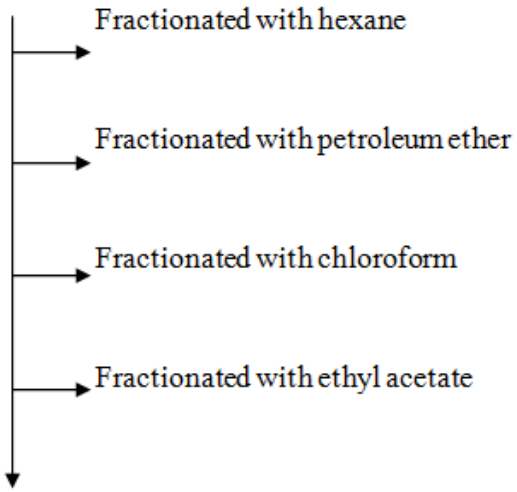
The spectrophotometer systems used were Shimatzu UV spectrophotometer, Shimadzu spectrum 1 FT-IR spectrometer and ESI-MS analysis (TofSpec 2E MALDI time - of flight (TOF) Instrument (Micromass,

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



75% MeOH Extract

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 PTLC, UV-VIS spectrophotometer, FTIR, ESI-MS, MS, H1 NMR, C13 NMR and structure elucidation.

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 meshes). 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 TLC 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

The isolated compound from the whole plant of *Catharanthus pusillus* showed molecular ion peak at 498, corresponding to its molecular ion $[M+H]^{+}$ ion and the molecular formula is $C_{32}H_{50}O_4$. The IR spectrum showed important absorptions attributable to hydroxyl (3222 cm-1), carboxylic (1727cm–1, -COOH) carbonyl functions. The other peaks are at 2945 cm-1 (sp3 C-H), 1680 cm-1 (conjugated C=C), 1253 cm-1 (C–O).The ^{1}H NMR spectra of the isolated compound showed singlet and multiplets at δ 4.45, 5.27, 7.26 due to 1H,1H and 2H of C-3 and C-12. The CNMR spectra shows the C position and δC (ppm) followingly as1- CH_2-38.10; 2 -CH_2 -27.70; 3-CH -81.96; 4-C -37.70; 5-CH -55.30; 6-CH_2 -18.20; 7-CH_2-32.06 ; 8-C -39.20; 9-CH -47.60; 10-C-37.02; 11-CH_2 -23.40; 12-CH-122.6; 13- C-143.6; 14 -C-41.50; 15-CH_2 -27.70; 16-CH_2 -23.55; 17-C-46.60; 18-CH-40.96; 19-CH_2- 45.87; 20-C-30.68; 21-CH_2-33.80; 22-CH_2-32.47; 23-CH-28.06; 24-CH_3-16.67; 25- CH_3-15.40; 26-CH_3-17.20; 27-CH_3- 25.90; 28-C-184.03; 29-CH_3 -3.06; 30-CH-23.60; 31-CH_3-1.29 and 32-C-171.04. Altogether, there are 8 methyl groups, 10 methylene groups, 5 methine groups and 9 quaternary carbons. A carboxylic and ester function groups are appeared at δ 184.03ppm and 171.04ppm, respectively. And, there are 32 carbon, 49 hydrogen, and 3 oxygen atoms with one hydroxyl groups.

Isolated compound

TLC: Colourless substance**,** R_f value was 0.87 (Figure 1).

UV spectrum: λmax observed at 236, 314 nm. (Figure 2).

IR: The IR spectrum showed important absorptions at 1253 cm^{-1} (Figure 3)

Fig- 1: TLC of isolated compound

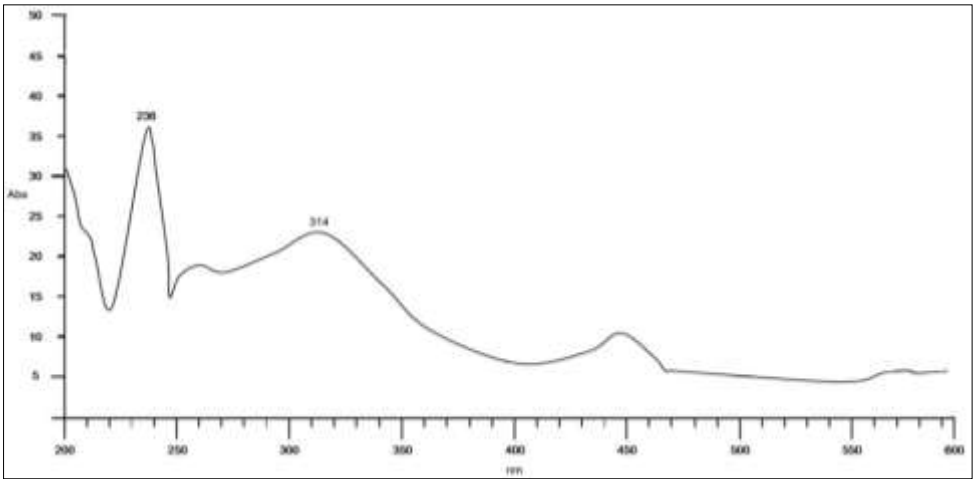


Fig-2: UV-VIS Spectrum of isolated compound

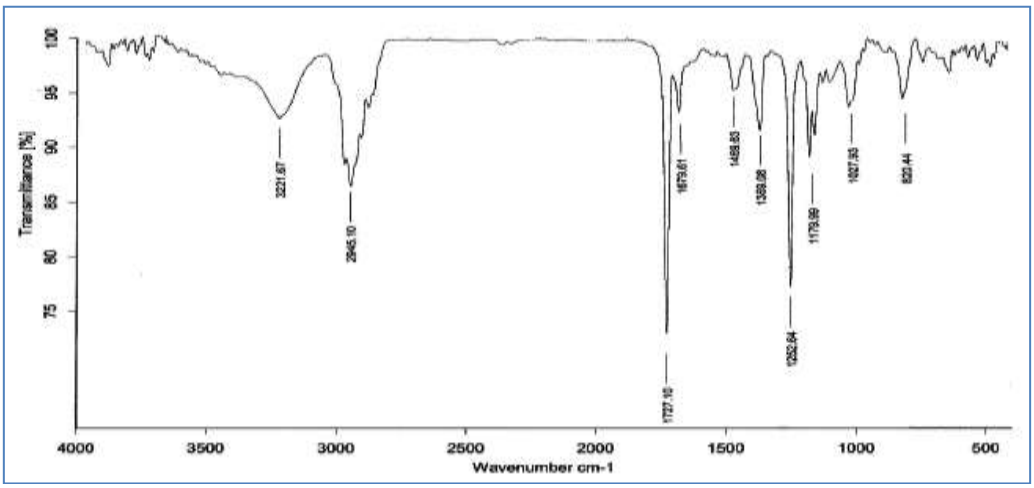


Fig-3: FT-IR Spectrum of isolated compound

Mass Spectrum

The high resolution EI-MS showed the [M+] at m/z = 498 revelaed the isolated substance molecular formula $C_{32}H_{50}O_4$. (Figure 4).

1H NMR 400 MHz, δppm

δ 4.45 (, m), 5.27 (, S), 7.63 (, S) 2.46 (2H,), 1.98 (34, 7, 1.90 (3H) Figure 5.

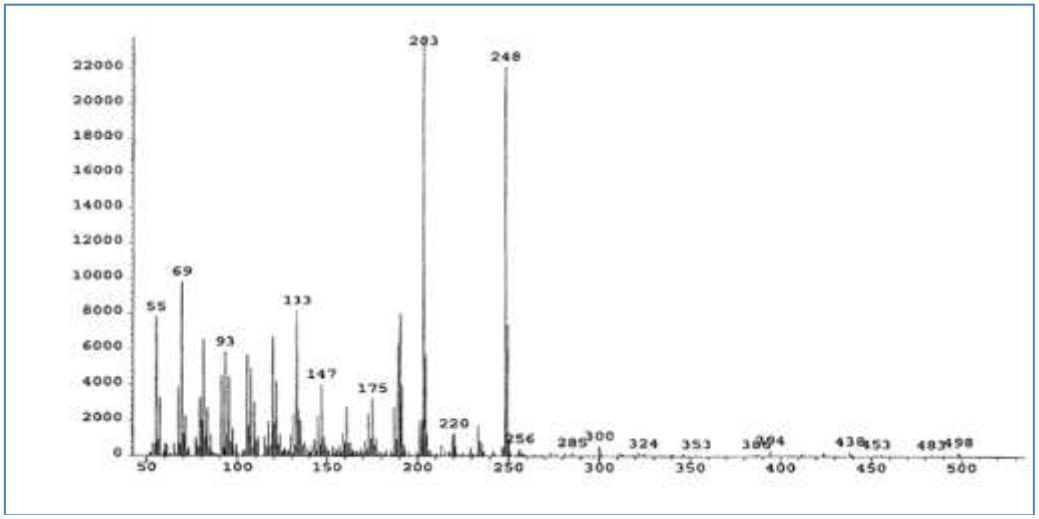


Fig-4: MS Spectrum of isolated compound

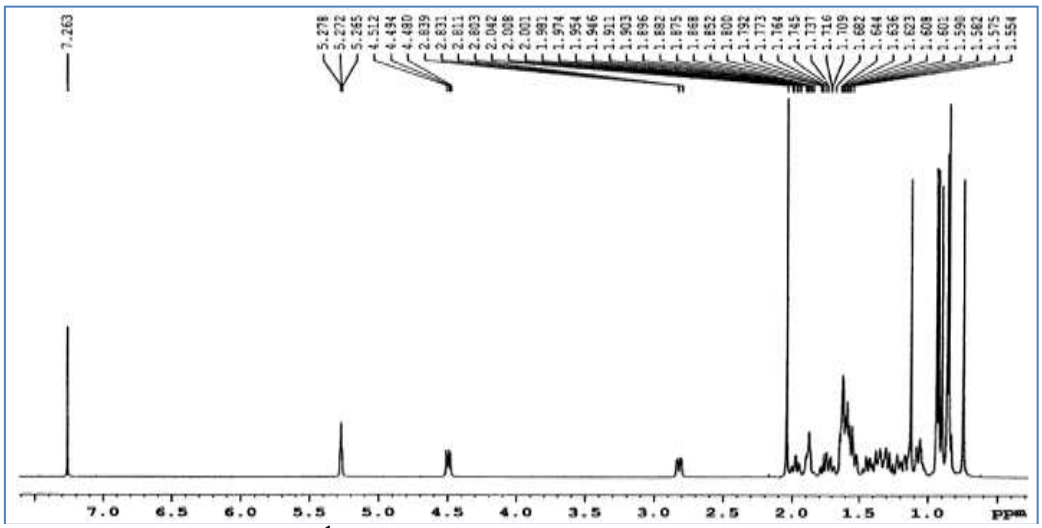


Fig-5: ^{1}H NMR Spectrum of isolated compound

^{13}C NMR 400 MHz δppm

δ 122.58, 143.63, 171.04 and 184.03 (Figure 6).

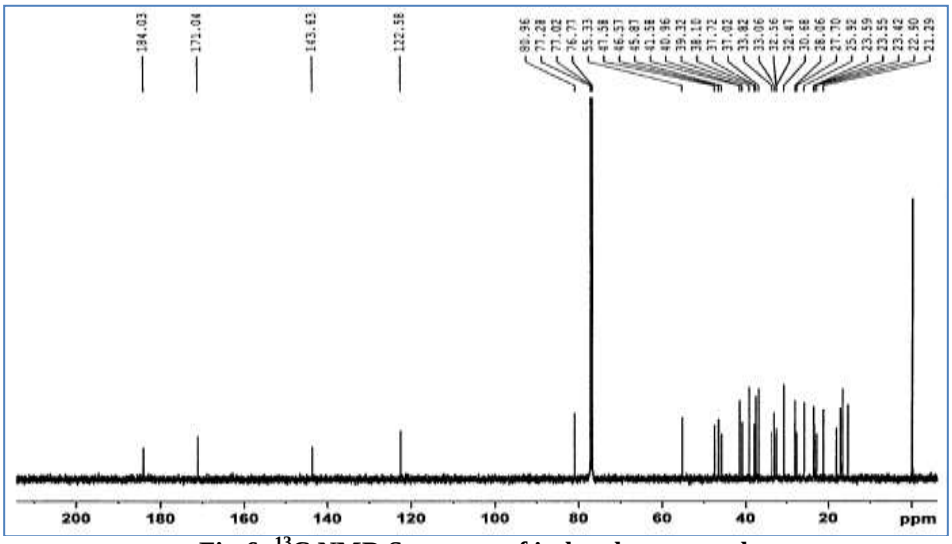


Fig-6: ^{13}C NMR Spectrum of isolated compound

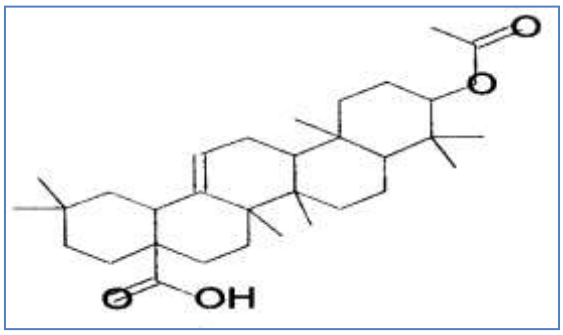


Fig-7: Chemical structure of isolated compound 3β-acetyl-Oleanolic acid

CONCLUSION

Depending on the spectral data and literature reference, the isolated compound is 3β-acetyl-oleanolic acid.

3β-acetyl-oleanolic acid is a derivative of oleanolic acid and it is relatively non –toxic, hepatoprotective and exhibits antitumour and antiviral properties [5].It is a power inhibitor of cellular inflammatory process

REFRENCES

1. Hammer, K. A., Carson, C. F., & Riley, T. V. (1999). Antimicrobial activity of essential oils and other plant extracts. *Journal of applied microbiology*, *86*(6), 985-990.
2. Cragg, M. G., Newman, D.J. (1999). J Nat Prod; 60: 52-60.
3. Don, G. (1999) *Catharanthus Roseus* In: Ross I.A (Ed), "Medical Plants of The World", Human Press, Totowa, NJ. 109-118.
4. Rao, N. B., Rajasekhar, D., & Raju, D. C. (1996). Folklore remedies for dandruff from Tirumala hills of Andhra Pradesh. *Ancient science of life*, *15*(4), 296.
5. Liu, J. (1995). Pharmacology of oleanolic acid and ursolic acid. *Journal of ethnopharmacology*, *49*(2), 57-68.