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Original Article

Isolation of a phosphoglyceride from *Pongamia pinnata (L.) Pierre* pods

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ABSTRACT

Aim of the present study was to perform the extraction, isolation and phytochemical screening of methanolic extract of *Pongamia pinnata (L.) Pierre*. The methanolic extract was subjected to preliminary phytochemical screening and then fractionated to silica gel column chromatography for the isolation of phytoconstituents. The isolated compound was characterized on the basis of spectral analysis. Fractionation of methanolic extract by column chromatography furnished a compound named phosphoglyceride. The structure of phosphoglyceride was elucidated as 1-octadec-9'-enoate, 2-tetradecanoate 3-phosphate.

1. INTRODUCTION

Pongamia pinnata (L.) Pierre (Papilionaceae) is the genus having only one species. *P. Pinnata*, commonly known as Karanja, is distributed throughout India in tidal and beach forest. It is used medicinally in India, China, Australia and Philippine Island. In Indian traditional system of Medicine, different parts of *P. Pinnata* have been used for bronchitis, whooping cough, rheumatic joints and quench dipsia in diabetes [1,2]. *P. Pinnata* is a medium-sized glabrous tree with short bole and spreading crown up to 18 m high or sometime even more and 1.5 m in girth. Bark is grayish green or brown, smooth or covered with tubercles, leaves are imparipinnate, leaflets 5-7, ovate or elliptic. Flowers are lilac or white tinged with pink or violet and fragrant and in axillary racemes. Pongaglabol, a hydroxyfuranoflavone and aurantiamide acetate, a rarely occurring modified phenylalanine dipeptide have been isolated together with four furanoflavone, karanjin, lancheolatin B, kanjone and pinnatin [3], two new prenylated b-diketone pongagallone A and B [4], five flavonoids: Pongamone A, pongamone B, pongamone C, pongamone D, pongamone E from stems [5]. *P. Pinnata* showed significant anti-inflammatory analgesic and antiulcerogenic [6, 7]. *P. Pinnata* flower showed significant antihyperglycemic and anti-lipidperoxidative, antioxidant property and hypoglycemic effect [8, 9, 10].

2. EXPERIMENTAL

2.1 Material and methods

All the chemicals and reagents were obtained from S.D. fine chemicals and were of analytical grade. Sodium sulfate was used as drying agent for various solvents used to run the column. All the weighing was done on a single pan meter balance. Melting points were determined on perfit melting apparatus. Ultraviolet spectra were recorded on Lambda Bio 20 spectrometer in methanol. Infra-red spectra were recorded on Bio-Red FTIR spectrophotometer using KBr pellets; ν_{max} values are given in cm^{-1}. ^{1}HNMR spectra were screened on advance DRY 400, Bruker spectrospin 400 MHz instrument using $CDCl_3$ as solvent and TMS as an internal standard. Chemical shift values are given in d (ppm) scale and coupling constants (J) in Hz. Notations used throughout as s = singlet, d= doublet, dd = double doublet, t = triplet, m = multiplet and brs = unresolved broad singlet. ^{13}C FT-NMR spectra were recorded on advance DRY 400, Bruker spectrospin 100 MHz n 5 mm spinning tubes at 27°C (Figure 3). Mass spectra's were scanned by effecting electron Impact ionization at 70 eV on a JEOL-JMS-DX303 instrument equipped with direct inlet probe system. The m/z values of the more intense peaks were mentioned and the figures in brackets attached to

each m/z value indicate relative intensities with respect to the base peak.

2.2. Plant Material

The pods of *P. Pinnata* were collected from Jamia Hamdard campus in October and were identified by Dr. Javed Ahmad, Department of Botany, Jamia Hamdard. A specimen for further reference has been retained.

2.3. Extraction

The pods of *P. Pinnata* were dried in an oven at a temperature below 45ºC for 2-3 days and coarsely powdered. The ground pods (2.2 kg) were extracted exhaustively first with hexane and then with methanol. The methanolic extract was concentrated under reduced pressure to yield (160g, 7.27%) dark brown, viscous syrupy mass.

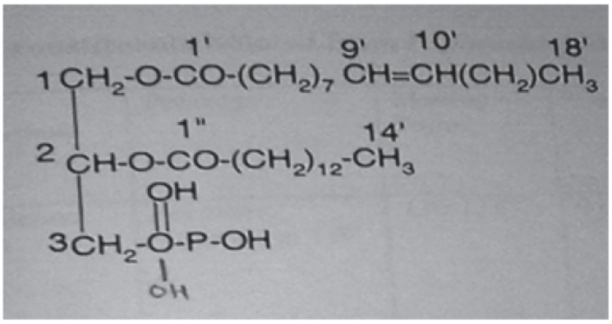

Fig. 1. 1-octadec-9'-enoate, 2-tetradecanoate 3-phosphate

3. RESULTS AND DISCUSSION

Elution of the column with chloroform-methanol (8.5:1.5) mixture furnished dull yellow amorphous mass compound recrystallized from methanol, 100 mg. Rf: 0.90 Mp: 140-160° C IR Vmax: 3409, 2923, 2852, 1740, 1644, 464, 1258, 1091, 1025, 807, 721 cm-1 ^{1}HNMR (DMSO-d6): 5.19 (2H, brs, H-9', H-10'), 3.50 (1H, m, H-2), 3.47 (1H, d, *J*= 6.8 Hz, H_2-3a), 3.45 (1H, d, *J*=6.8 Hz, H2-3b), 3.31 (2H, brs, H_2-1), 2.62 (2H, brs, H_2-2'), 2.13 (2H, brs, H_2-2"), 1.87 (4H, brs, H_2-8', H_2-11'), 1,43 (2H, brs, H_2-2), 1.12 (4H, brs, 22 x CH_2), 1.14 (3H, t, *J*=7.2 Hz, Me-14"), 0.74 (3H, t, *J*=6.8 Hz, Me-18') ^{13}CNMR (DMSO-d6): 171.32 (C-1,' C-1") 72.45 (C-2), 63.60 (C'-1, C-3), 57.23 (C-2', C-2"), 31.81 (CH_2) 29.66 (CH_2) , 29.44 (CH_2), 29.23 (CH_2), 27.13 (CH_2), 27.59 (CH_2), 18.42 (Me-14") 14.13 (Me'-18') EIMS m/z : 346 $[M]^+$ ($C_{35}H_{67}O_8$ P). Alkaline hydrolysis of Phosphoglyceride (30mg) was performed by heating with alkaline ethanolic solution for 1hr. It was acidified with dilute HCl, extracted with $CHCl_3$, concentrated add chromatographed on TLC with authentic sample of myristic and oleic acid.

Compound was obtained as a yellow amorphous mass from chloroform: methanol (8.5:1.5) eluents. Its IR spectrum showed distinctive absorption band for hydroxyl groups (3409 cm-1), ester groups (1740 cm-1), unsaturation (1644 cm-1) and long aliphatic chain (807,721 cm-1) (Figure 6). Its mass spectrum, exhibited a molecular ion peak at m/z 646 corresponding to a phosphoglyceride, $C_{35}H_{67}O_8P$ (Figure 4). The ^{1}HMR spectrum of compound (Figure 2) displayed a two- proton broad signal at δ 5.19 assigned to vinylic H-9' and H-10'. A one- proton multiplets at δ3.50 was ascribed hydroxymethine H-2 proton. Two one-proton doublets at δ 3.47 (*J* = 6.8 Hz) and 3.45 (*J* = 6.8 Hz) and a two-proton broad signal at δ 3.31 were attributed to oxygenated methylene H_2-3 and H_2- 1 protons, respectively. Two broad signal at δ 2.62 and 2.13 both integrated for two-proton were accounted to H_2-2' and H_2-2' methylene proton, respectively. Adjacent to the ester groups. Two three- proton triplets at δ. 1.14 (*J* = 7.2 Hz) and 0.74 (*J* = 6.8 Hz) were associated with the terminal H_3-14"and H_3-18' primary methyl protons. The remaining methylene protons resonated at δ 1.87 (4H), 1.43 (2H) and 1.12 (44 H Alkaline hydrolysis of compound yielded myristic and oleic acid (TLC comparable. On the basis of spectral data analysis and chemical reaction (Figure 5). The structure of compound has been formulated as 1-octadec-9'-enoate, 2-tetradecanoate 3-phosphate (Figure 1).

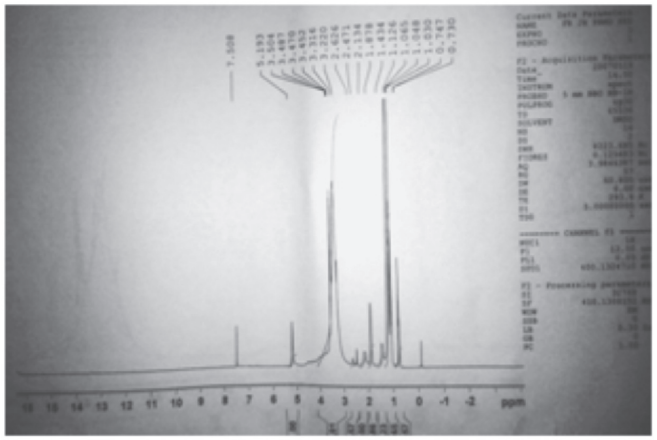

Fig. 2. ^{1}HNMR of phosphoglycerides

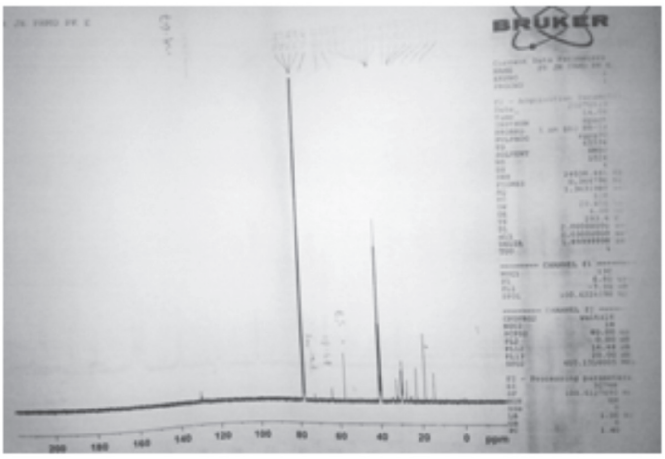

Fig. 3. ^{13}C NMR spectra of Phosphoglycerides

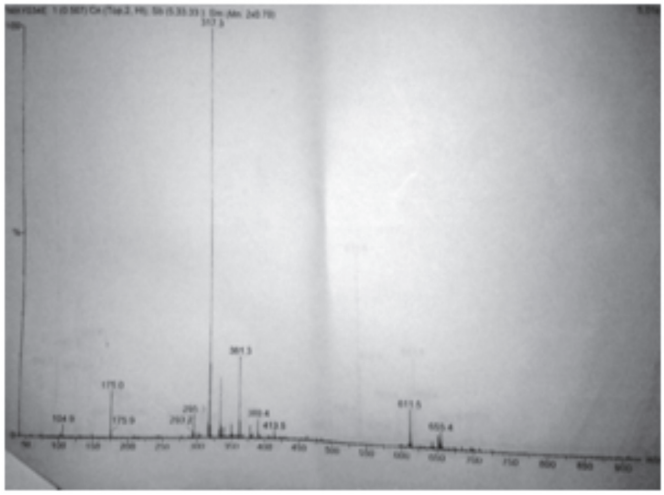

Fig. 4. Mass Spectra of Phosphoglycerides

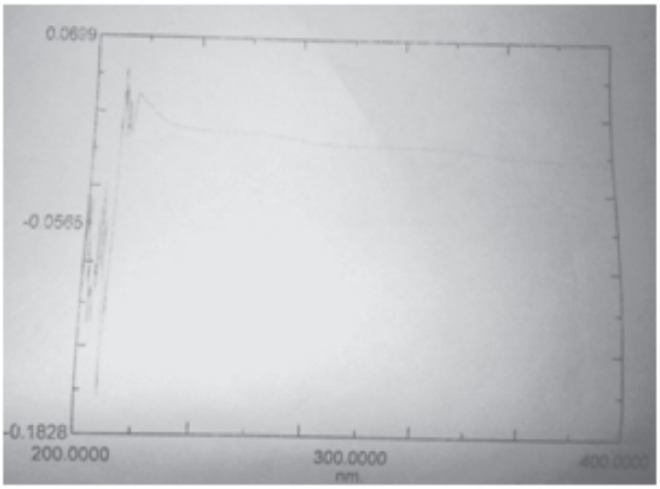

Fig. 5. UV spectra of Phosphoglycerides

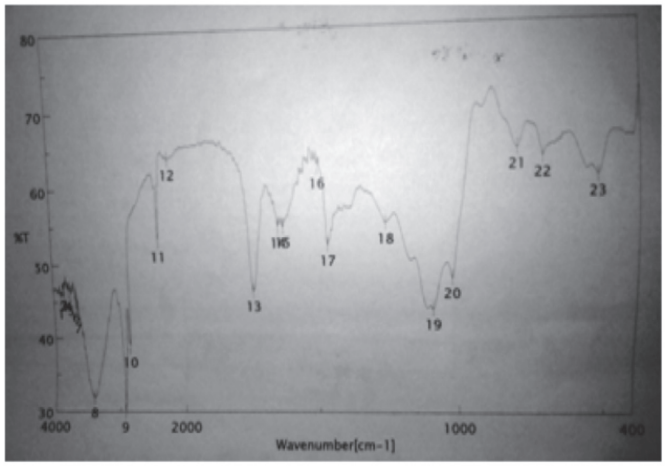

Fig. 6. IR spectra of Phosphoglycerides

4. CONCLUSION

Pongamia Pinnata has been used traditionally to cure various ailments like skin diseases, urinary tract infections and hypoglycemia in ayurvedic system of medicine. *P pinnata* is the source diverse type of phytoconstituents that can subjected for column chromatography for the isolation of new molecule as source of modern medicine.

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