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Short Communication

Methanolic extraction and isolation of bioactive chemicals from *Pithecellobium dulce* leaves by column chromatography and GC-MS studies

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Abstract

Pithecellobium dulce is a species family Fabaceae, that is native to the India mostly Maharashtra area of Vidarbha. Pithecellobium dulce found secondary metabolism. It is used as medicinal plant. The methanolic extract of leaves was obtained by Soxhelt extractor followed by concentration in rotary evaporator. Separation of bioactive chemicals was carried out by column chromatography while studies by GC-MS which shows presence of following bioactive chemicals Squalene,9-Octadecenoic acid(Z)-,2-hydroxy-13propanediyl ester; 9 9-Octdecenoic acid,1,2,3-propanetriyl ester.

Keywords: Soxhelt extraction, Column chromatography, GC-MS.

Introduction

Some substances synthesized by plants are necessary for their fundamental activities whereas others, called secondary metabolites. The Pithecellobium dulce plant will be selected on the basis of intensive review and ethno pharmacologic information for convenience, the plant materials will be classify on the basis of Genera. Pithecellobium dulce is a species of flowing plant in pea family Fabaceae. It is used as medicinal plant. The extract of leaves is used for gall ailment to prevent miscarriage and seed is used for clean ulcers[1]. The leaves of species will be collected from Chikhaldara Valley and from the nearby region of Amravati City. Collection will be made according to the plan of study.

The leaves extract of each species will be prepared by standard procedure using soxhlet extractor and extract will be used for further investigations. Column chromatography is a method used to isolate individual bioactive chemical compounds from mixtures of compound. It is used for preparative applications on scales from micrograms up to kilograms. The main advantage of column chromatography is relative low cost and disposability of stationary phase used in the process. The latter prevents cross-contamination and stationary phase degradation due to recycling[2].

Material and methods

Collection of plant material: The fresh leaves of Pithcellobium dulce were collected from Melghat region Dist-Amravati (Maharashtra) in the month July 2015 and the Authentication of plant was confirmed by botanist (Prof.S.K Tippat, Department of Environment Science, Art, Commerce and Science College Amravati).

Preparation of plant extract: The plant were dried over ambient temperature and the dried sample were grind properly and dried powder sample was extracted in Soxhlet extractor by using solvent Methanol at 65ºC, extracts were concentrated by gradually evaporating the respective solvent on rotary evaporator. The concentrated extract was collected in sterile bottles and kept in a cool and dark place prior to analysis[3].

Isolation of bioactive chemicals by column chromatography: Column chromatography was performed on a classic 20cm. long and 2cm. diameter glass column packed with silica gel G Merck, Germany. The concentrated extract of *P. dulce* (20 mL) was applied to the column by use of a pipette and the column was eluted sequentially with 90% Benzene and 10% Ethanol. Each fraction collected was tested prior GC-MS study[4].

GC-MS Analysis of *Pithecellobium dulce:* Gas Chromatography: Gas chromatography of the plant extract was carried out on a 6890 Gas chromatography model 5765 equipped with direct injector and split ratio set to 10:1 (DB-5) (5% phenyl polysioxane, 30m length 250u internal diameter; 0.25um film coating) fused capillary column. Helium was carrier gas at 1.0 ml min. The oven temperature program was to start at 35°C hold for 2min then temp at 20°C per min to 300°C and hold for 5 min. Injector and detector temperature were 220°C and 230°C respectively. Injection size was 0.02 ul neat[5].

Gas chromatography and mass spectroscopy: A JEOL GCmate II benchtop double-focusing magnetic sector mass spectrometer operating in electron ionization (EI) mode with TSS- 2000 software was used for all analyses. Low-resolution mass spectra were acquired at a resolving power of 1000 (20% height definition) and scanning from m/z 25 to m/z 700 at 0.3 seconds per scan with a 0.2 seconds inter-scan delay. High

resolution mass spectra were acquired at a resolving power of 5000 (20% height definition) and scanning the magnet from m/z 65 to m/z 750 at 1 second per scan.

Identification of chemical constituents: Identification of the chemical constituents was done on the basis of retention index (RI) using a mass spectra library search NIST and by comparing the mass spectral and retention data with literature[6]. The relative amount of individual component were calculated based on the GC peak area (FID response) without using a correction factor.

Table-1: Major Bioactive chemical in column fraction 1st

Sr. No	Rention Time	Name of chemical constituent	Molecular Formula	Molecular Weight	Peak Area %
1.	25.83	Squalene	$C_{30}H_{50}$	410.39	14.39
2.	29.47	9Octadecenoic acid (Z), 2hydroxy1,3propanediylester	$C_{39}H_{72}O_5$	620.54	24.12
3.	29.89	9Octadecenoic acid (Z), 2hydroxy1,3propanediylester	$C_{39}H_{72}O_5$	620.54	19.54

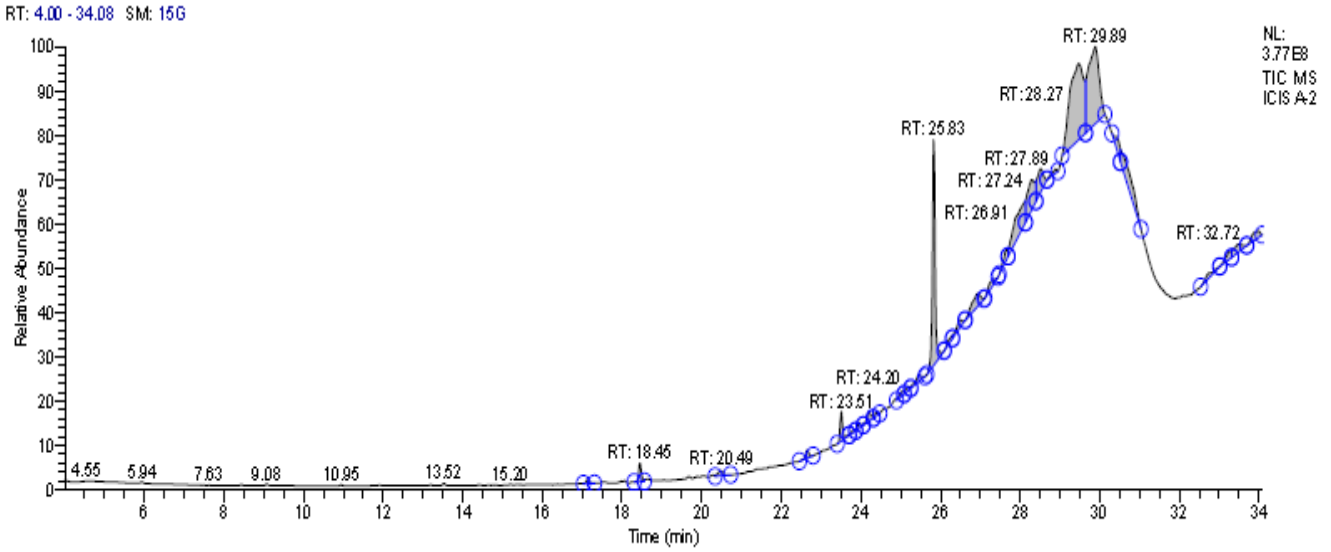

Figure-1: Gas Chromatogram of Column fraction-1

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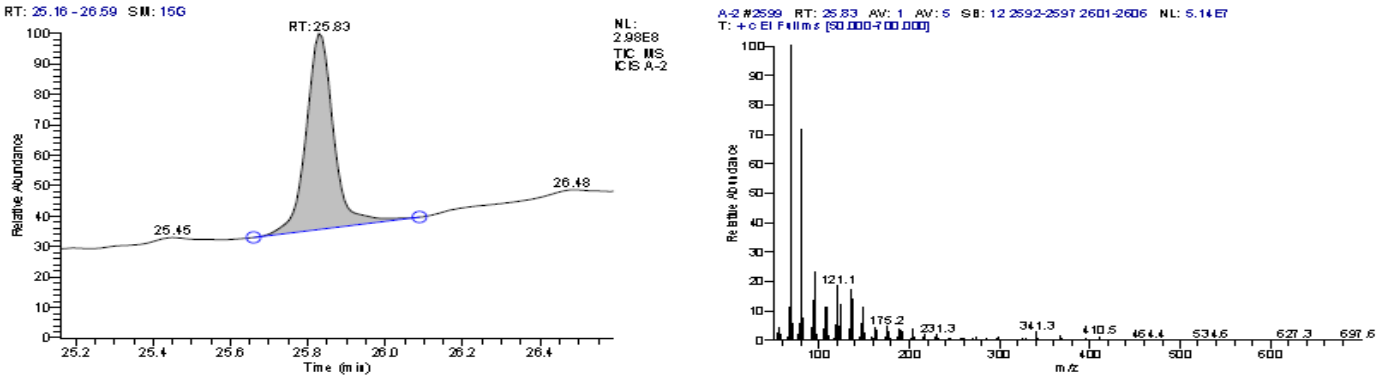
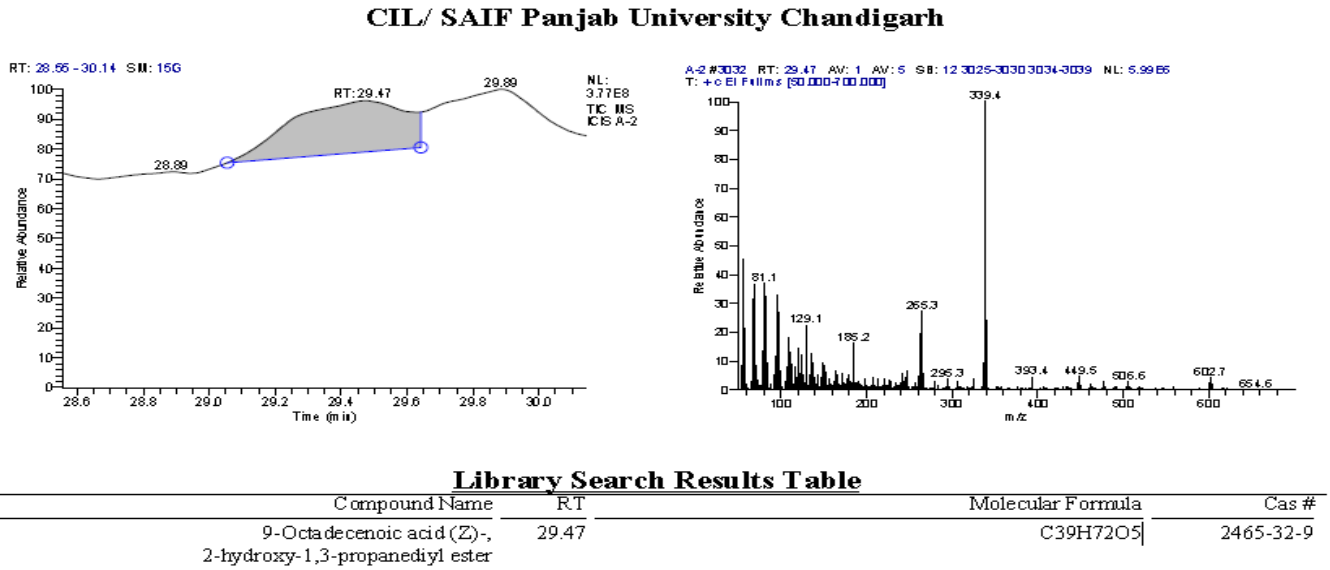


Library Search Results Table

Compound Name	RT	Molecular Formula	Cas #
Squalene	25.83	C30H50	111-02-4

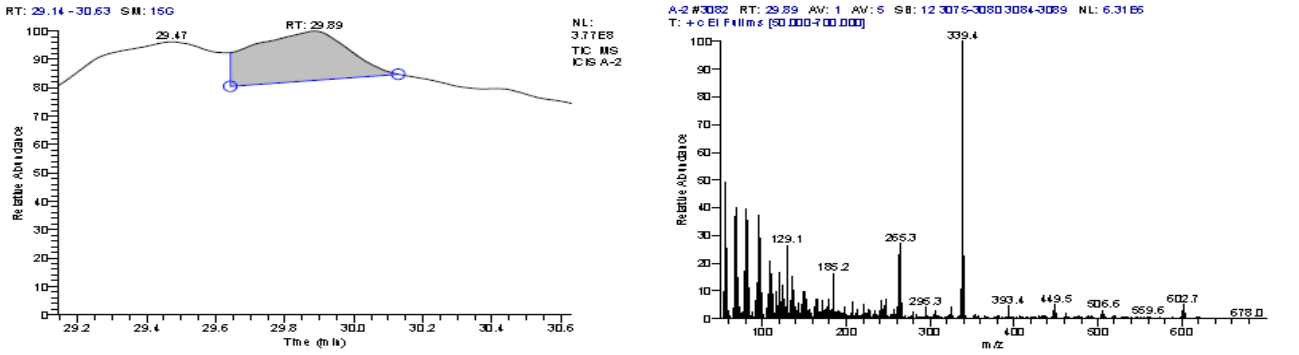
Figure-2: Mass Spectrum of Major peak at R.T.=25.83



Library Search Results Table

Compound Name	RT	Molecular Formula	Cas #
9-Octadecenoic acid (Z)-, 2-hydroxy-1,3-propanediyl ester	29.47	C39H72O5	2465-32-9

Figure-3 Mass Spectrum of Major peak at R.T.- 29.47



Library Search Results Table

Compound Name	RT	Molecular Formula	Cas #
9-Octadecenoic acid (Z)-, 2-hydroxy-1,3-propanediyl ester	29.89	C39H72O5	2465-32-9

Figure-4: Mass Spectrum of Major peak at R.T.-29.89

Result and discussion

The present study was carried out in methanolic extract of *Pithecellobium dulce* followed by column chromatography for isolation of bioactive constituents. The GC–MS of isolated fractions by column chromatography of leaves extract of *Pithecellobium dulce* is shown in Table-1. There are two major bioactive chemicals are found with % peak area Squalene (14.39) and 9Octadecenoic acid (Z), 2hydroxy1,3 propanediylester (29.47). Squalene is a hydrocarbon and a triterpene, and is a natural and vital part of the synthesis of all animal sterols, including hormones, and vitamin D in the human body[7]. Squalene is used in cosmetic, and more recently as an immunologic adjuvant in vaccines. Squalene has been proposed to be an important part of the Mediterranean diet as it may be a chemopreventive substance that protest from cancer[8].

Conclusion

The results presented in this study are the first given informations on the chemical composition of *Pithecellobium dulce* It showed that 9Octadecenoic acid (Z), 2hydroxy1, 3propanediylester and Squalene are the major fraction for the isolated method methods For future works, we will try to structural elucidation by NMR studies.

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