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Gas chromatography: Mass spectroscopic analysis of phytocomponents of *Pongamia pinnata* in methanol

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Abstract
Phytocomponants in *Pongamia pinnata* (Karanja) root methanolic extract was attempted using Gas chromatography mass spectrometry (GCMS) analysis. Four major components were identified as 6(3-Methyl-3-cyclohexenyl)-2-Methyl-2,6-Heptadienol (RT 15.67 min), 5-Phenyl- 2-Pentenal, (RT 22.72 min), 3-(phenoxymethyl)-1,2,3-benzotriazine-4(3H)-one, (RT 24.62 min) and, 2-((3, 5-Dinitrobenzoyl) Amino)-Benzoic acid (RT 28.37 min). The identified compounds have biological activities.

Keywords: Adsorption, azo dye, industrial wastes, kinetics, Langmuir isotherm

Introduction
Evolution has led to emergence of some very complex plant physiologies where primary and secondary metabolites act together for some state-of-art plant-based activities such as living alone, protection, reproduction, symbiosis, parasitism, antagonism and many more. Due to this complex physiology, different plant parts *i.e.*, root, stem, leaves, flowers, fruits may contain different genre and levels of natural chemicals or "phytochemicals". More than thousand known phytochemicals of secondary metabolite origin. These secondary metabolites have shown a complex structures and thousands of derivatives have come up from a basic metabolite. Still search for newer compounds with wider biological activity is on and plants have given a good opportunity for researchers in this direction. Examples of plant based biologically active secondary metabolites are different types of alkaloids, tannins, phenols & polyphenols, flavonoids, anthroquinone, cardiac glycosides etc [1]. The structure-function relationship is quite common in plant secondary metabolites, hence prediction of biological activities based on their structure is common phenomenon [2].
Karanja (*Pongamia pinnata* (L.) Panigrahi) is a common tree of tropical and subtropical conditions, including Central India. Various parts of tree have commercial importance and used as fooder, fuel, dye pigments etc. Beyond this, all parts of Karanja tree (roots, flowers, leaves, bark) are used for medicinal purposes. Karanja is widely used in managing constipation as it helps to improve gut motility and has a laxative property. It might also be used for piles due to its astringent and anti-inflammatory properties [3].
This plant is still underexplored for their medicinal properties in light of modern-day techniques. There is great need to re-explore different parts of in order to make full use of this plants for medicinal, industrial and other purposes. Present study uses GC MS technique to identify phytocomponants in root of *P. pinnata* extracted in methanol to correlated its biological activity.

Materials and methods
Collection of plant materials
Plant material, root of Karanja (*Pongamia pinnata*) was collected by guidelines of good agricultural practices (GACP) for medicinal plants from Narsinghpur region of MP India. The plant was authenticated State Forest Research Institute (SFRI), Jabalpur (MP).

Processing of plant materials
Collected samples of Karanja (*Pongamia pinnata*) root was cleaned, packed in jute bags and brought to Lab, after washing thoroughly in running water fresh roots were cut into small pieces and dried at room temp.

Shade dried plant samples of above plant roots were powdered using power grinder mill and stored in air-tight polythene bags for chemical analysis.

Extraction of plant materials

Powdered plant samples of Karanja (*Pongamia pinnata*) root subjected to successive extraction with methanol using Soxhlet apparatus. 2 kg of dried plant powder of each plant part was extracted in 2.5 lit of methanol in successive manner for 30 hrs. Solvent were evaporated to dryness to yield respective extracts which were used for pharmacological and GC-MS analysis.

GC-MS analysis of plant extracts

Methanol extracts of roots of *P. pinnata* was subjected to chemical analysis by using GC-MS instrument (Perkin Elmer, USA) with Turbo Mass. Compounds were separated on capillary column (PE-5MS 30m length x 0.25mm inner diameter x 0.25µm film thickness). Oven temp was programmed with initial isothermal temp of 75°C for 5 min and then increased up to 280 °C at rate of 10ºC per min and finally hold for 15 min. Sample (1µl) was injected by keeping the injection port at 250 °C. Mass detection was done via electro-ionization source set as 220 °C. He gas with 99.999% purity was used as carrier gas at 1 ml/min flow rate. Molecular weight range was set at 22 to 620 amu. Molecular weight, Molecular ion peak, fragmentation pattern and number of hits were used to identify name of compounds by comparison with NIST library [4].

Results And Discussion

TIC (Fig 1) is methanol extract of Karanja (*Pongamia pinnata*) root. TIC shows many peaks, which could not be separated, may be due to higher number of compounds eluting from column at same time. Chromatogram shows sharp, and distinct peaks at retention times of 15.69, 22.75, 24.66 and 28.39 min. The detailed mass in these areas is then calculated.

Mass spectrum & Fragmentation peaks of compound appearing at RT of 15.67 min (Fig 2). Compound was identified as (3-Methyl-3-cyclohexenyl)-2-Methyl-2,6-Heptadienol based on major fragmentation peaks appearing the GC MS profile. The fragmentation pattern of compound is given below.

Mass spectrum & fragmentation pattern of compound appearing at RT of 22.72 min (Fig 3) in GC of root of Karanja. Compound was identified as 5-Phenyl-2-Pentenal based on major fragmentation peaks appearing the GC MS profile and comparison with NIST library.

Mass spectrum & fragmentation pattern of compound appearing at RT of 24.62 min (Fig 4) in GC of root of Karanja. Compound was identified as 3-(phenoxymethyl)-1,2,3-benzotriazine-4(3H)-one, based fragmentation peaks appearing in MS profile and comparison with NIST library.

Mass spectrum & fragmentation pattern of compound appearing at RT of 28.37 min (Fig 5) in GC of root of Karanja. Compound was identified as, 2-((3, 5-Dinitrobenzoyl) Amino)- Benzoic acid based on fragmentation peaks appearing GC MS profile and comparison with NIST library.

Various parts of *P. pinnata*, especially leaves, are known to possess antihelmintic activities [5]. Our study with root extracts of this plants identified a compound (3-Methyl-3-cyclohexenyl)-2-Methyl-2,6-Heptadienol, which was also identified in plants of *Juniperus species* (Family Cupressaceae) and has been shown to have wormicidal activity against *Schistosoma mansoni* worms and molluscicidal activity against *Biomphalariaalexandrinasnails* [6]. Nagarathna *et al*. (2009) have shown diuretic activity of methanolic extracts P. *pinnata* leaves [7]. Compound identified was 3-(phenoxymethyl)-1,2,3-benzotriazine-4(3H)-one, is a benzotriazenesulfonamide compound, which have shown to possess diuretic activity [8]. Compounds, 5-Phenyl-2-Pentenal and, 2-((3, 5-Dinitrobenzoyl) Amino)-Benzoic acid could not be correlated with literature for any biological activity.

Conclusion

The present study investigated the phytocomponants of root of *P. pinnata* using GC MS in methanol extract concludes that root of P. pinnata has biologically active phytocomponents which have similar biological activities as of leaves of plant.

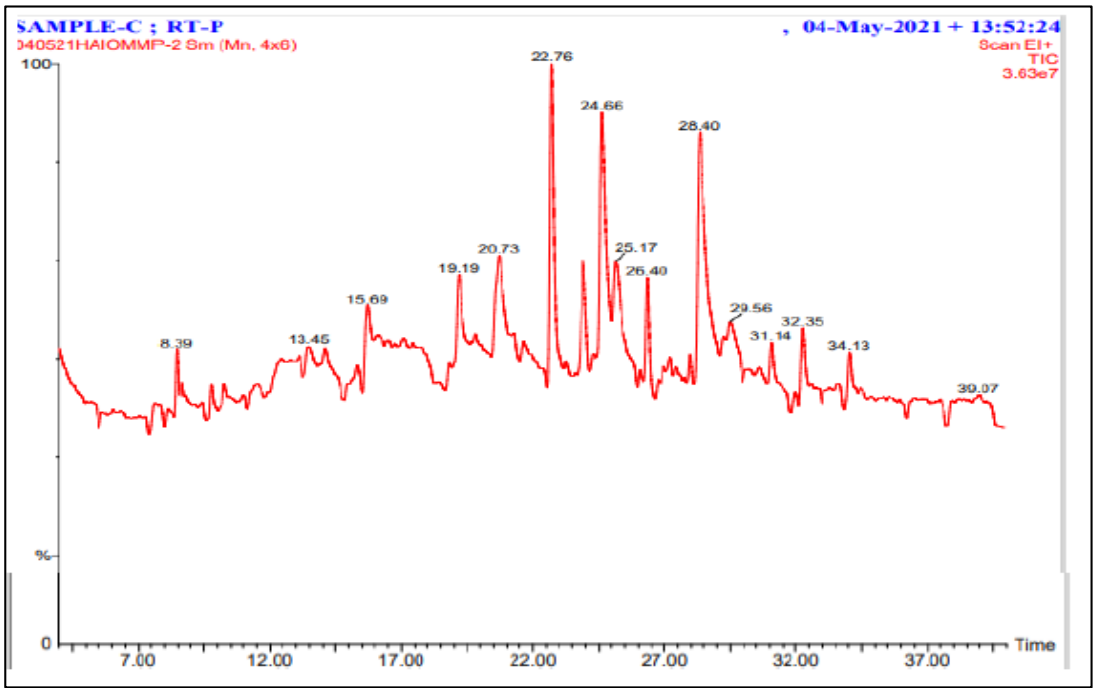

Fig 1: TIC of GC-MS analysis of *P. pinnata* (Karanja) roots methanol extract.

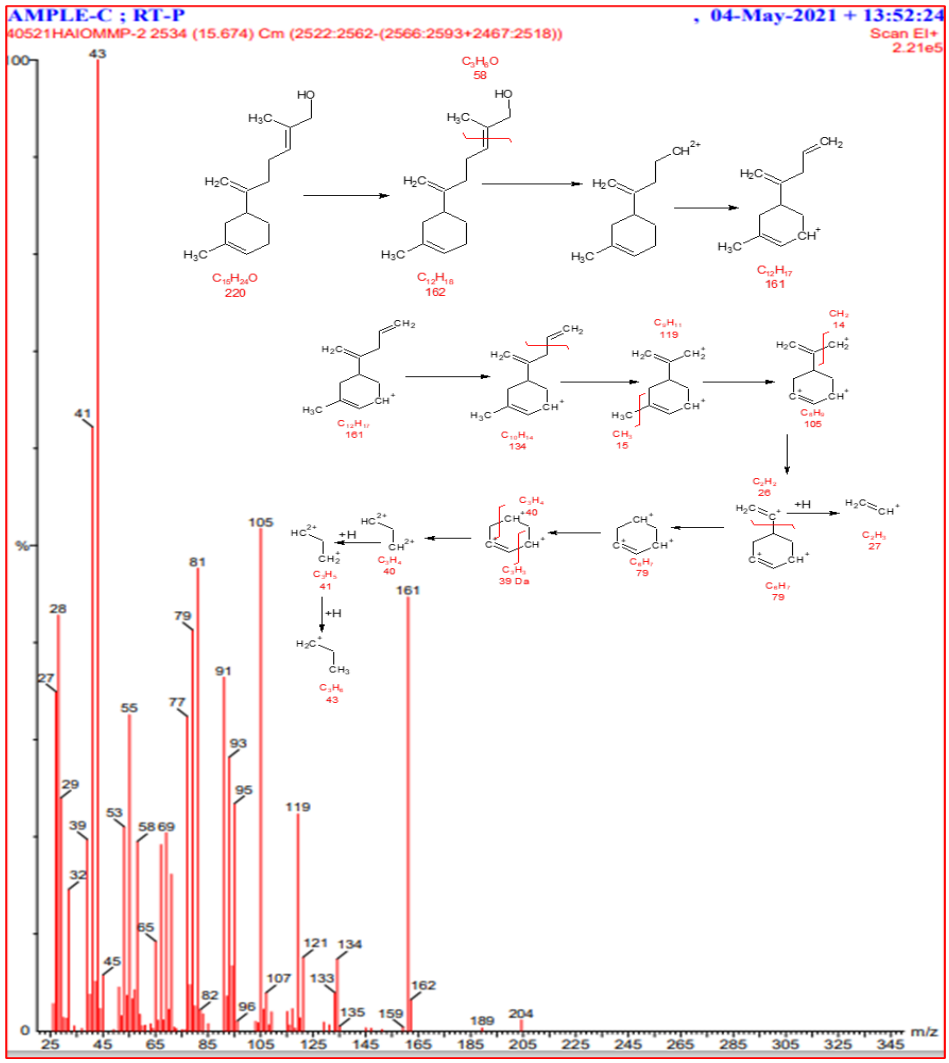

Fig 2: Mass spectrum and fragmentation pattern of (3-Methyl-3-cyclohexenyl)-2-Methyl-2,6-Heptadienol of *Pongamia pinnata* roots extract.

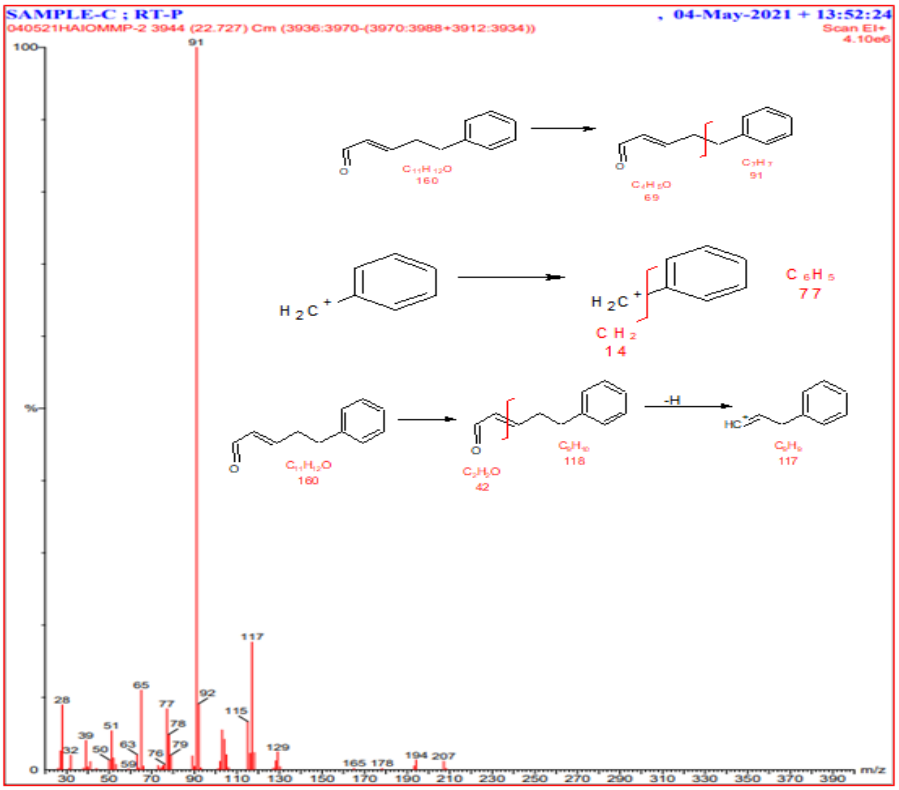

Fig 3: Mass spectrum and fragmentation pattern of, 5-Phenyl-2-Pentenal of *Pongamia pinnata* roots methanol extract.

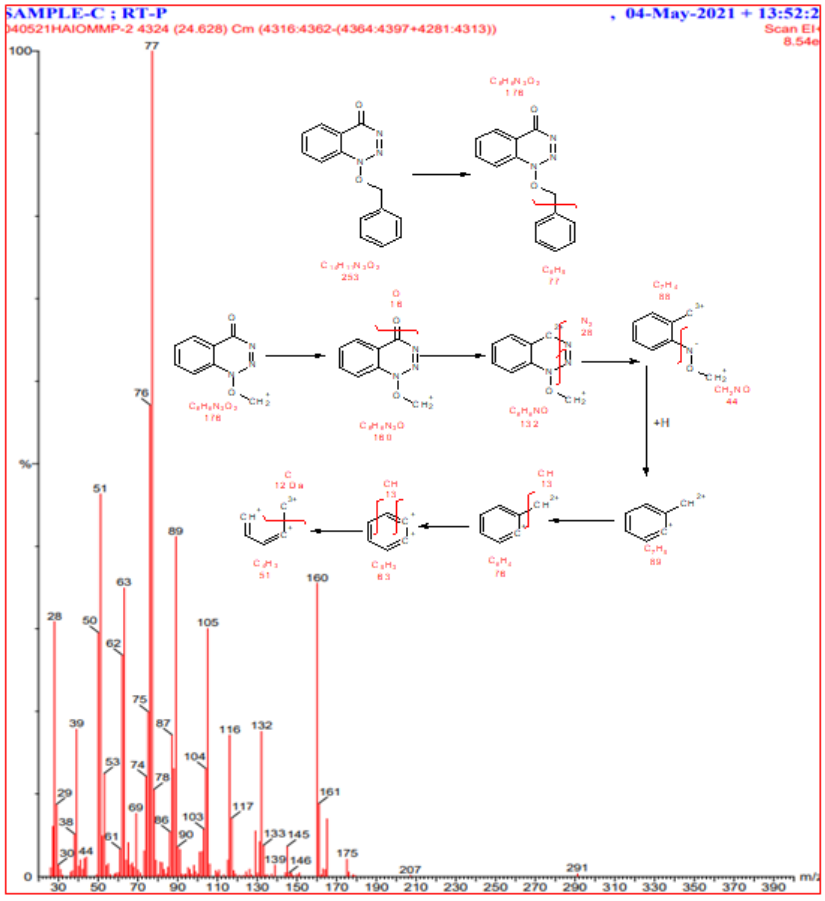

Fig 4: Mass spectrum and fragmentation pattern of 3-(phenoxymethyl)-1,2,3-benzotriazine-4(3H)-one, of *Pongamia pinnata* roots methanol extract.

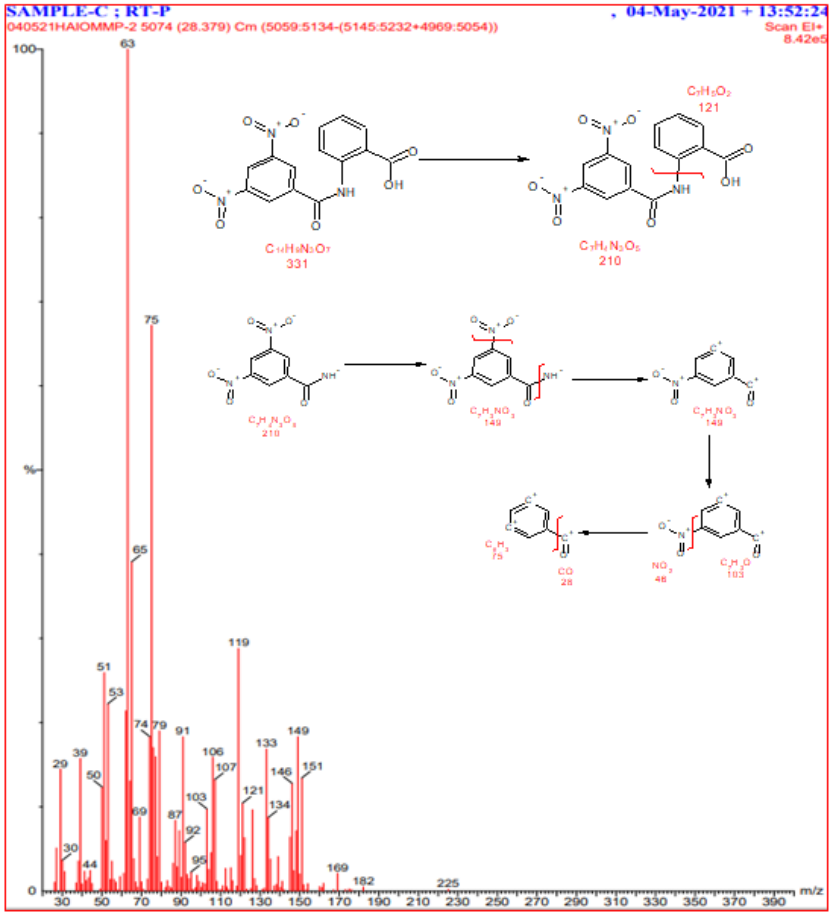

Fig 5: Mass spectrum and fragmentation pattern of, 2-((3, 5-Dinitrobenzoyl) Amino)-Benzoic acid of *Pongamia pinnata* roots methanol extract.

Table 1: Plant Extract and Biological Activity

S. No.	Plant Extract	Compound	Biological Activity
1.	Karanja Root	6(3-Methyl-3-cyclohexenyl)-2-Methyl-2,6-Heptadienol	Schistosomicidal (anti parasites) and molluscicidal Activity
		5-Phenyl-2-Pentenal,	Not Known
		3-(phenoxymethyl)-1,2,3-benzotriazine-4(3H)-one,	Diuretic agent
		2-((3, 5-Dinitrobenzoyl)Amino)- Benzoic acid	Not Known

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