

Phytochemical Analysis of Bioactive Compounds from *Calophyllum inophyllum* L., Leaf Extract Using GC-MS Analysis.

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ABSTRACT

Phytoconstituents are the natural bioactive compounds found in plants. These phytoconstituents work with nutrients and fiber to form an integrated part of defense system against various diseases and stress conditions. The present study involves phytochemical analysis of *Calophyllum inophyllum* leaf extract obtained using methanol as solvent by Gas Chromatography and Mass Spectrometry (GC-MS) method. The GC-MS analysis showed peaks of nine different phytochemical compounds namely Linoleic acid (32.25%), Methyl Oleate (32.25%), Phytol (17.82%), Methylisostearate (11.96%), Diphenylmethane (8%), 2-Phenazinamine (8%), Adenanthin (1.47%), Carbazole (1.45%), 5-Aminomethyl-dibenzosuberane (0.28%). These different phytochemical have been found to possess a wide range of activities, which may help in the protection against incurable diseases.

Keywords: *Calophyllum inophyllum*, GC-MS analysis, phytoconstituents, Methanol, Linoleic acid.

INTRODUCTION

The plant kingdom represents a rich source of organic compounds, many of which have been used for medicinal and other purposes¹. Higher plants as sources of bioactive compounds continue to play a dominant role in the maintenance of human health. Plants are rich sources in secondary metabolites with interesting biological activities². In fact, many of the currently available drugs were derived either directly or indirectly from the plants.

The genus *Calophyllum* belongs to the family Clusiaceae and is composed of about 180-200 different species confined to the warm humid tropics of the world³. *Calophyllum inophyllum* L., is a tree that can grow 8-20 meter tall with a broad spreading crown of irregular branches which exudes white latex when bruised. The leaves have opposite arrangements, and are petiolate, thick and shiny with numerous parallel secondary veins. The flower is 25 mm wide and occurs in racemose or paniculate inflorescences consisting of 4 - 15 flowers⁵.

The fresh fruit and its oil were used externally against rheumatism, in topical infection and seborrhea in human adult. The dried leaf and its decoction used to cure rheumatism, skin infections, cuts and sores¹. The fresh leaf's infusion are used to cure bacterial infection, fungal infection and as vermifuge/pediculicide. Kashman and Patil reported that (+)- calanolide A and inophyllum B isolated from *Calophyllum lanigerum* Miq. and *Calophyllum inophyllum* L. showed strong activity against Human Immuno Deficiency virus type 1 [HIV-1]⁶. Five bioactive compounds isolated from *Calophyllum inophyllum* L., leaves namely calophyllic acid and isocalophyllic acid mixture, 3-oxofriedelin-28-oic acid,

canophyllic acid, amentoflavone and shikimic acid showed dose dependent lipid lowering activity in *in vivo* experiments⁷. Mass spectrometry, coupled with chromatographic separations such as Gas chromatography is normally used for direct analysis of components existing in traditional medicines and medicinal plants.

Phytochemicals are responsible for medicinal activity of plants⁸. In recent years GC-MS studies have been increasingly applied for the analysis of chinese medicinal plants as this technique has proved to be a valuable method for the analysis of non polar components and volatile essential oil, fatty acids and lipids⁹. The present study is to identify the bioactive compounds from the methanol leaf extract of *Calophyllum inophyllum* using GC-MS analysis.

MATERIALS AND METHODS

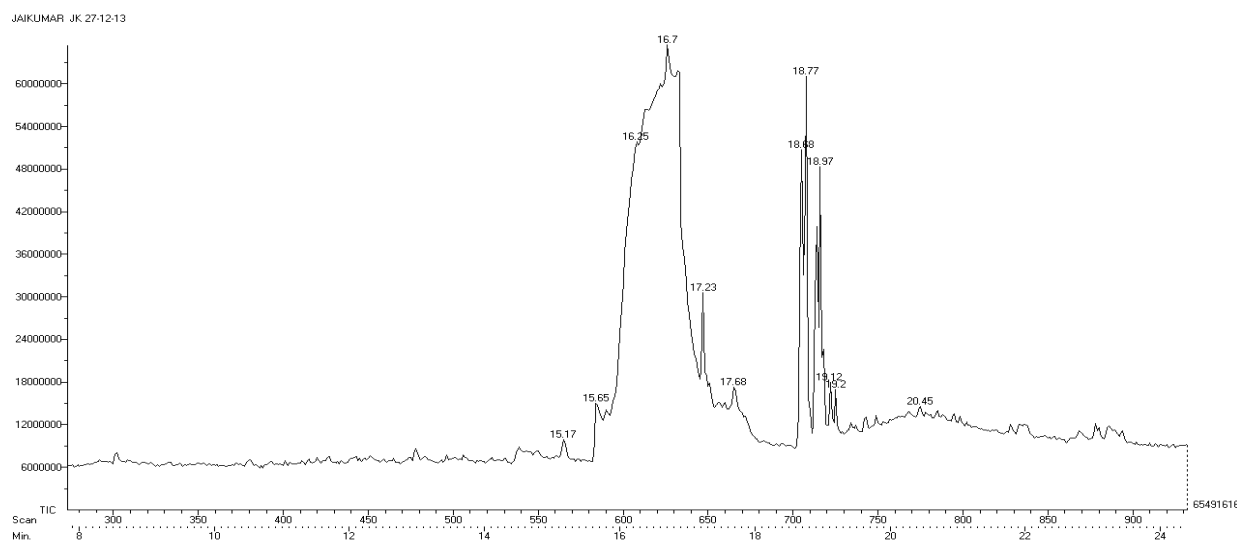
Collection of Plant Material: The leaves of *Calophyllum inophyllum* were collected during month of September 2013, from Rajiv Gandhi Salai (OMR), in Chennai, Tamil nadu, India. The plant material was identified and authenticated by Department of Botany, Ramakrishna Mission Vivekananda College, Chennai, India. Herbarium voucher submitted.

Preparation of Leaf Extract: The fresh leaves were collected and washed with running tap water, chopped into small pieces and then kept in shade dry for 30 days and then grounded using electric blender. 50 gm of powdered leaves were extracted with 300ml of methanol in soxhlet apparatus for 6 hours. The extract was then concentrated at reduced pressure using rotary evaporator and stored in vials at 4°C until further analysis¹⁰.

GC-MS Analysis: GC-MS analysis was carried out on a

Table 1: GC-MS profile of methanol leaf extract of *Calophyllum inophyllum*.

S.NO.	Retention Time(RT)	Name of the compounds	Molecular Formula	Molecular weight	Peak area%
1.	15.17	Adenanthin	C ₂₆ H ₃₄ O ₉	490.54	1.47%
2.	15.65	Carbazole	C ₁₂ H ₉ N	167.20	1.45%
3.	16.25	Diphenyl methane	C ₁₃ H ₁₂	168.23	8.0%
4.	16.48	2-Phenazinamine	C ₁₂ H ₉ N ₃	195.21	8.0%
5.	17.27	5-Aminomethyl-dibenzosuberane	C ₁₆ H ₁₇ N	223.32	0.28%
6.	18.72	Linoleic acid	C ₁₉ H ₃₄ O ₂	294.47	32.25%
7.	18.80	Methyl Oleate	C ₁₉ H ₃₆ O ₂	296.48	32.25%
8.	19.12	Phytol	C ₂₀ H ₄₀ O	296	17.82%
9.	19.20	Methyl isostearate	C ₁₉ H ₃₈ O ₂	298.50	11.96%

Figure 1: GC-MS Chromatogram of methanolic extract of *Calophyllum inophyllum*.

GC Calrus 500 Perkin Elmer System comprising an AOC-20 I auto sampler. Gas chromatography interfaced to a Mass spectrometer (GC-MS) instrument employing with column Elite-1 fused silica capillary column 30 X 0.25mm x 1µm df composed of 100% Dimethyl poly siloxane, operating in electron impact mode at 70eV; helium (99.999%) was used as carrier gas at a constant flow of 1 ml per minute with injection volume of 0.2µl was employed (split ratio of 10:1) injector temperature maintained at 250°C, with ion-source temperature 280°C. The oven temperature was programmed from 110° C (Isothermal for 2 min) with an increase of 10°C per-minute to 200°C, then with 5°C increase per-minute upto 280°C ending with a 9min hold isothermal at 280°C. Mass spectral analysis was taken at 70eV; a scan interval of 0.5 seconds Mass scan fragments from 45 to 450 Da. Total GC running time is 36min and total MS running time is also 36min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The mass-detector used in this analysis was Turbo-Mass Gold-Perkin-Elmer, and the software adopted to handle mass spectra and chromatograms was a Turbo-Mass ver-5.2, chromatogram were compared again NIST database for compound identification.

RESULTS

GC-MS is one of the significant method used to identify the constituents of volatile matter, long and branched chain hydrocarbons, alcohols, acids, esters etc. The GC-MS analysis of *Calophyllum inophyllum* leaves showed the presence of nine compounds namely Methyl isostearate (RT-19.2), Phytol (RT-19.12), Methyl Oleate (RT-18.8), Linoleic acid (RT-18.72), 5-Aminomethyl-dibenzosuberane (RT-17.27), 2-Phenazinamine (RT-16.48), Diphenylmethane (RT-16.25), Carbazole (RT-15.65), Adenanthin (RT-15.17). The identification of the compounds were confirmed based on the peak area, retention time and molecular formula. The active principle with their Retention time (RT), Molecular formula, Molecular weight (MW) and Peak Area in percentage are presented in Table 1. and Fig 1. The individual fragmentations of the components are illustrated in Fig 2A-2I.

DISCUSSION

Traditional medicines are prepared from a single plant or combination of more than one plant. Indian contribution to herbal market and emphasis on novel research is continuously increasing. Phytochemical constituents are responsible for pharmacological activities of plant species¹¹. The phytochemical analysis of leaf extract of *Calophyllum inophyllum* showed presence of secondary metabolites such as Alkaloids, Carbohydrates, Glycosides,

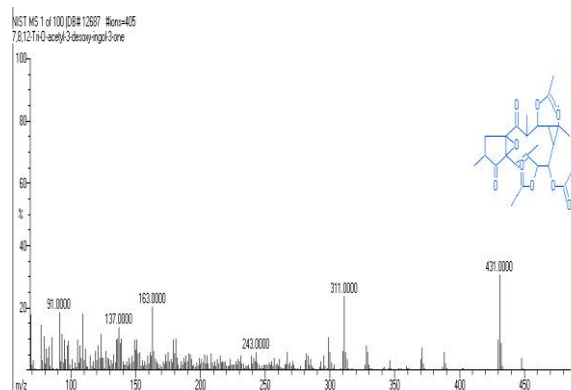


Figure 2A: Adenanthin

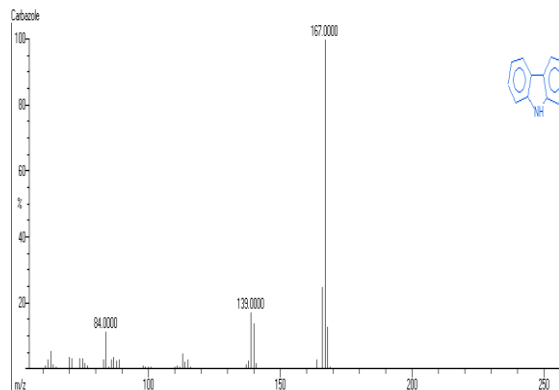


Figure 2B: Carbazole

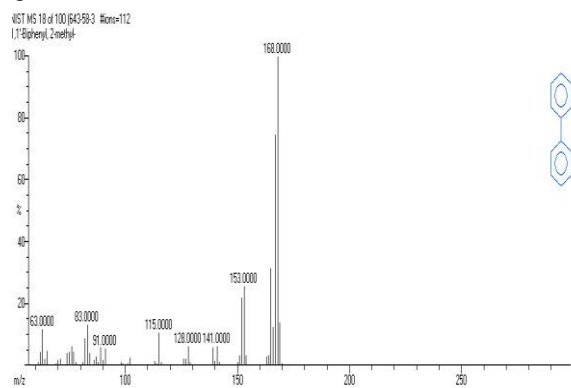


Figure 2C: Diphenylmethane

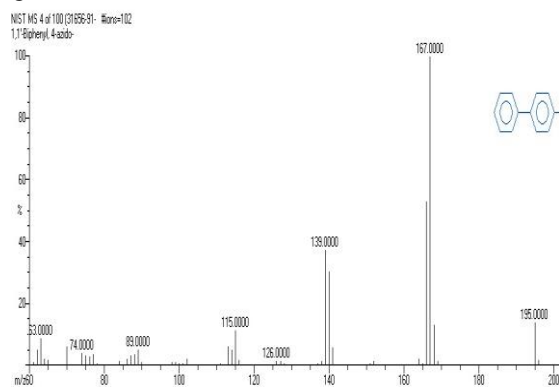


Figure 2D: 2-Phenazinamine

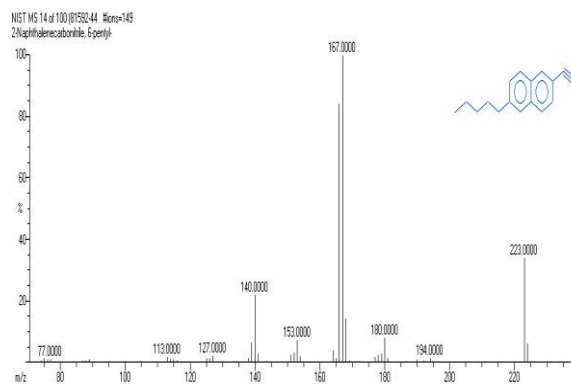


Figure 2E: 5-Aminomethyl-dibenzosuberane

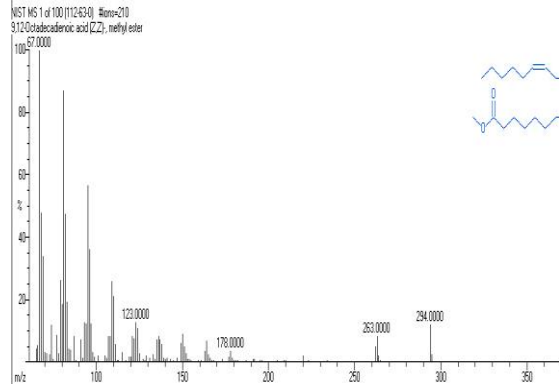


Figure 2F: Linoleic acid

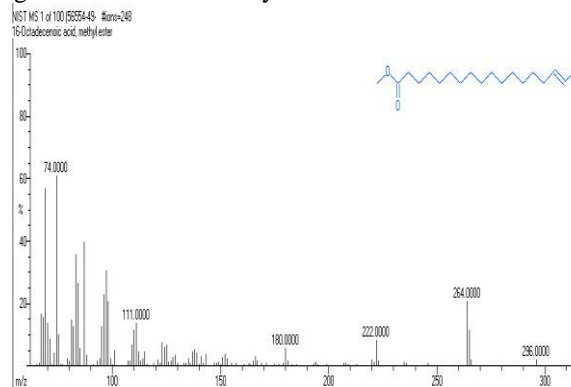


Figure 2G: Methyl Oleate

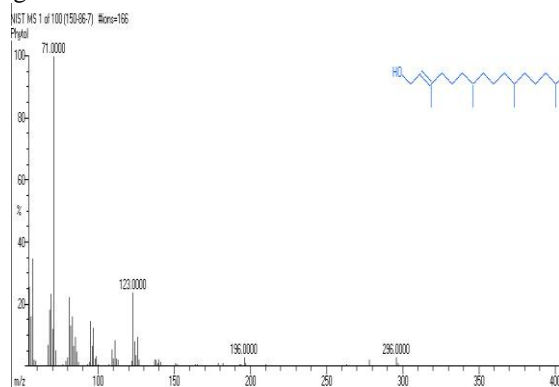
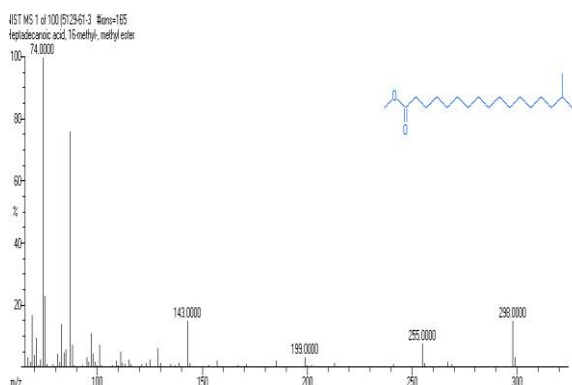


Figure 2H: Phytol

Saponins, Phenols, Tannins, Flavanoids, Proteins, Steroids and Coumarins. These secondary metabolites possess antioxidant and anticancer activities¹².

Earlier reports of *Calophyllum inophyllum* leaf extract using alcoholic solvents by Malarvizhi 2011 yielded

seventeen compounds¹³, in which two compounds are found similar to present study namely Linoleic acid, Phytol and seven different compounds were observed namely Adenanthin, Carbazole, Diphenylmethane, 2-Phenazinamine, 5-Aminomethyl-dibenzosuberane,



Methyl Oleate, Methyl isostearate. The difference in plant components from previous study might arise from solvent variation whereas, the present study of methanolic leaf extract exhibited important bioactive compounds which possess various biological activities.

Phytol is one among the nine compounds of the present study. Nanadagopalan 2015 reported the presence of Phytol in the leaves of *Kirganelia reticulata* aerial parts, which was found to be effective in different stages of arthritis¹⁴. The compound Linoleic acid is used against Antiinflammatory, Nematicide, Insectifuge, Hypocholesterolemic, Cancer preventive, Hepatoprotective, Antihistaminic, Antiacne, Antiarthritic, Antieczemic. Potterat 2005 has reported that a new carbazole alkaloid, named clausine Z, has been isolated from the stems and leaves of *Calophyllum excavata*¹⁵.

From the present study it is concluded that the bioactive compounds thus identified from GC-MS analysis of the methanol leaf extract of *Calophyllum inophyllum* has justified the use of leaf extract for various ailments by traditional practitioners, further studies on isolation and identification of individual constituents are recommended.

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