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Isolation and identification of terpenoid compounds from the folklore medicinal plant, *Acacia caesia* (L.) willd. leaves

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Abstract
This paper reports on the isolation and identification of terpenoid compounds from the folklore medicinal plant, *Acacia caesia* (L.) Willd. (Mimosaceae). The methanol extract was purified with suitable mobile phase using column chromatography. The two terpenoid compounds were identified from column purified fractions on the basis of Thin Layer Chromatography followed by Mass Spectral data of ^{1}H NMR, GC-MS and LC-MS were determined. The data revealed that the presence of diterpene group of N - octadecane and straight-chain structural isomer of saturated aliphatic hydrocarbon of N - eicosane compounds were reported in the study plant species.

Keywords: terpenoid, medicinal plant, diterpene group, N - eicosane

Introduction
Plants naturally synthesize several carbon compounds, basically for physiologic functions or for use as chemical weapons against disease organisms, insects and predators (Fatope, 1995) [5]. The investigation of plants for bioactive secondary metabolites is an area which most plant scientists have recently focused with an aim of discovering new clinically useful and commercially important plant products (Dewick, 1997) [4]. The genus of *Acacia* (Mimosaceae) is known a rich source of secondary metabolites such as alkaloids, cyanogenic glycosides, cyclitols, fatty acids and seed oils, fluoroacetate, gums, non-protein amino acids, terpenes (including essential oils, diterpenes, phytosterol and triterpene genins and saponins), hydrolyzable tannins, flavonoids and condensed tannins (Seigler, 2003) [11]. Generally terpenoids are an important volatile part of plants and play vital role in traditional herbal remedies. They are used as antibacterial, antineoplastic, anti-carcinogenic, antimalarial, anti-ulcer and hepaticidal and diuretic and other pharmaceutical functions (Rodriguez, 2004; Bertea, *et al*., 2005) [10, 3].
The species, *Acacia caesia* (L.) Willd. which has not previously been chemically investigated entirely, is a straggling shrub distributed in tropical regions of India. The leaves are used by local peoples to treat some diseases like bronchitis (asthma) and cold and skin problems. In the present study, we describe the isolation and structural elucidation of a terpenoid compounds from the leaves of this species. The methanol extract of leaves was subjected to column chromatography on silica gel eluted with suitable mobile phase. Further, the responded column purified fraction was analyzed through 1H NMR, GC-MS and LC-MS to know the molecular formula and weight of the study compounds. The structures of the known compounds were established on the basis of spectral data, NMR spectra and by comparison with authentic literatures.

Materials and Methods
Collection of Plant material and extraction
The healthy leaves were collected from Chennimalai hills and shade dried for 10 days. The powdered leaves (100 g) of *A. caesia* were extracted in a soxhlet apparatus (500 ml) for 4 days with methanol.

Isolation and identification of Terpenoid compounds
The dried methanol extract (36.20g) was dissolved in a minimum amount of methanol and 10g of silica gel (particle size 0.063-0.200 nm (60-120 mesh ASTM) (E. Merck)) was added to it. It was further dried in a desiccator to remove completely the traces of the solvent. The dried material was then packed (wet packing) on top of a short column of silica gel (100g) and then the column was developed with petroleum ether followed by petroleum ether with benzene in the ratio of 3:1. The fractions are washed with methanol.

Thin Layer Chromatography (TLC)
The TLC studies were performed by using silica gel-G as stationary phase in the chromatographic plates of 15x5 cm with 3 mm thickness. The appropriate mobile phases were attempted for the identification of terpenoids according to Wagner *et al.* (1984) [14]. The plates were sprayed with vanillin- phosphoric acid reagent and heated at 1100C for 5min. The appearance of blue coloured spot indicates the presence of terpenoid compounds.

Proton Nuclear Magnetic Resonance (1H NMR) Spectroscopy
Column purified fraction (II) was dissolved in deutero-chloroform ($CDCl_3$) and analyzed by proton NMR. 1H-NMR spectra were recorded in CDCl3 (300.1318534 MHz) using a av 300 spectrometer, equipped with an Indy Silicon Graphics computer. Free induction decay (FID) was fourier transformed with LB of 0.3 Hz.

GC-MS analysis
Five ml of methanol extract was evaporated to dryness and reconstituted in 2 µl methanol. Chromatographic separation was carried out with CE GC 8000 top MSMD 8000 Fyson instrument with Db 35 mr column (10 m x 0.5 mm, 0.25 µm film thickness). Heating programs were executed from 100 -

250 oC at 3 minutes by using the helium was used as a carrier gas with a flow rate of 1 ml/min in the split mode (1:50).

Identifications were based on the molecular structure, molecular mass and calculated fragmentations. Resolved spectra were identified for phytochemicals by using the standard mass spectral database of WILEY and NIST (Suo and Yang, 2006; Guido *et al.*, 2007) [12, 6].

LC-MS (Liquid Chromatography - Mass spectrometry) (Ardrey, 2003)

Sample Preparation

The column purified fraction (II) was dissolved thoroughly in petroleum ether and stored in a refrigerator.

Chromatography with Mass spectrometry

A mass spectrum was obtained on a Shimadzu LC-MS instrument coupled with an Agilent Zorbax C18 column (5 cm x 2.5 mm, 3µ). A 20 µl of petroleum ether dissolved samples from column purified fractions were injected in analytical liquid chromatography. The chromatographic studies were performed using methanol and 0.5% acetic acid in the ratio of 9:1 as mobile phase. For full scan MS analysis, the spectra were recorded in the range of *m/z* 50 to 600.

Results

The isolation of the terpenoids was carried out from methanol using column chromatography with petroleum ether and benzene at the ratio of 3:1 (Krishnaswamy, 2003) [8]. Investigation of the column elution provided VI semisolid fractions *viz*., 0.13 g, 0.34 g, 0.05 g, 0.08 g, 1.34 g and 0.07 g (Table 1). The TLC results of column purified fractions showed that the fraction (II) gave positive results to terpenoids by developing blue coloured spots at all suitable mobile phases (Table 2 and Fig 1) by spraying the reagent, vanillin phosphoric acid. The 1HNMR ($CDCl_3$, 300.1318534 MHz) spectra of column purified fraction II (colorless white semi - solid) exhibited the signals at δ_H = 0.8 -1.8 ppm displayed for aliphatic regions (Fig. 2). The mass spectral data obtained through GC-MS of the compounds gave molecular formulae C18H38 with the molecular weight of 254 and C20H42 with the molecular weight of 282. They showed that the compounds are tentatively identified as N - octadecane (diterpene) and N - eicosane (straight-chain structural isomer of saturated aliphatic hydrocarbon) respectively (Figs 3 - 4).

The LC-MS mass spectra also support the data of GC by having strong resemblances of molecular weight (Figs. 5 and 6). The results showed the formation of predominant peak for a terpenoid compounds at *m/z* 255 and 282 which confirmed the molecular weight of the compounds I and II are 254 and 282 respectively.

Discussion

As these two compounds are already known and reported earlier (Philip *et al.*, 2006) [9]. But the compounds are reported for the first time in the study species *A. caesia*. The structures of these two compounds have been assigned based on information from literature. The results based on RT/min. agreed with the GC-MS data obtained, and confirmed the presence of the aliphatic groups of the two terpenoids such as N - octadecane and N - eicosane. Philip *et al.* (2006) [9] and Ingram (2007) [7] have noted similar retention time as observed in the present study, 16.592 RT/min. for N - octadecane and 13.099 RT/min. for N-eicosane compounds. Several biological actions have been reported for diterpenes including antibacterial, antifungal, anti-inflammatory, antileishmanial, cytotoxic and antitumor activities (Batista, 1999; Tanaka, 2000) [2, 13].

Conclusion

Medicinal plants are used in the folklore medicine may be an interesting and largely unexplored source for the development of potential new compounds. It is very essential to isolate and characterize the active principle for the beneficial of human being to fight against the dreadful diseases. It is our first attempt to identify the active principles from *A. caesia* and the results revealed that the two compounds are previously established.

Table 1: The percentage yield of column purified fractions of *Acacia caesia.*

Mobile phase	Column purified fractions	Nature of eluents	Percentage yield (g)
Petroleum ether: benzene (3:1)	I	Green semi - solid	0.13
	II	white semi - solid	0.34
	III	white semi - solid	0.05
	IV	white semi - solid	0.08
	V	white semi - solid	1.34
	VI	white semi - solid	0.07

Table 2: Identification of terpenoids with their R_f values by thin layer chromatography from column eluted fractions of *A. caesia.*

Mobile phase for terpenoids	R_f values of column purified fractions					
	I	II	III	IV	V	VI
Petroleum ether: ethyl acetate (4:1)	0.80	0.85	0.75	-	-	0.73
Benzene: ethyl acetate (97:5)	0.90	0.70	-	-	-	-
Hexane: ethyl acetate (3:1)	-	0.60	-	-	-	-
Toluene: ethyl acetete (95:5)	-	0.90	-	-	-	-

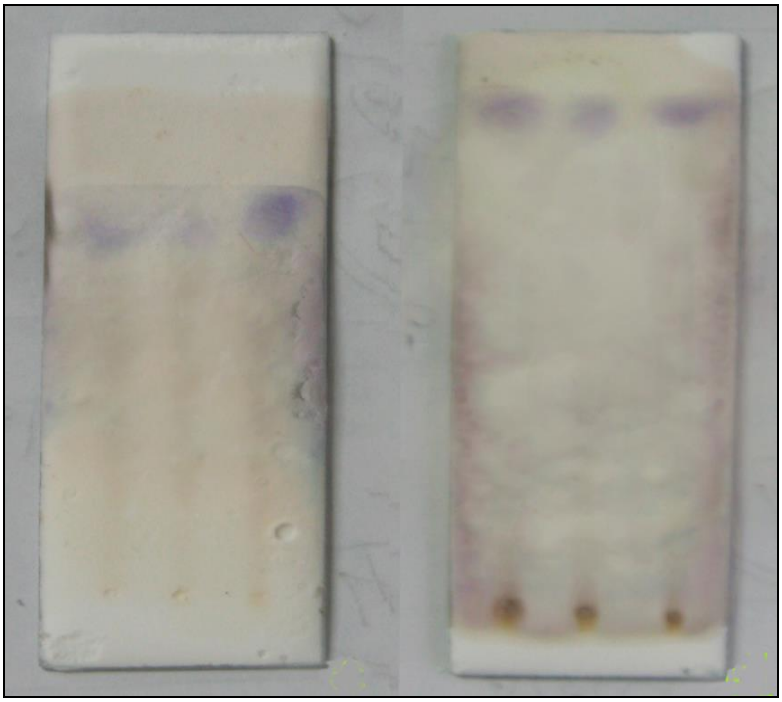

Fig 1: TLC study in column eluted fraction (II) of methanolic xtract of *Acacia caesia* for the confirmation of terpenoids.

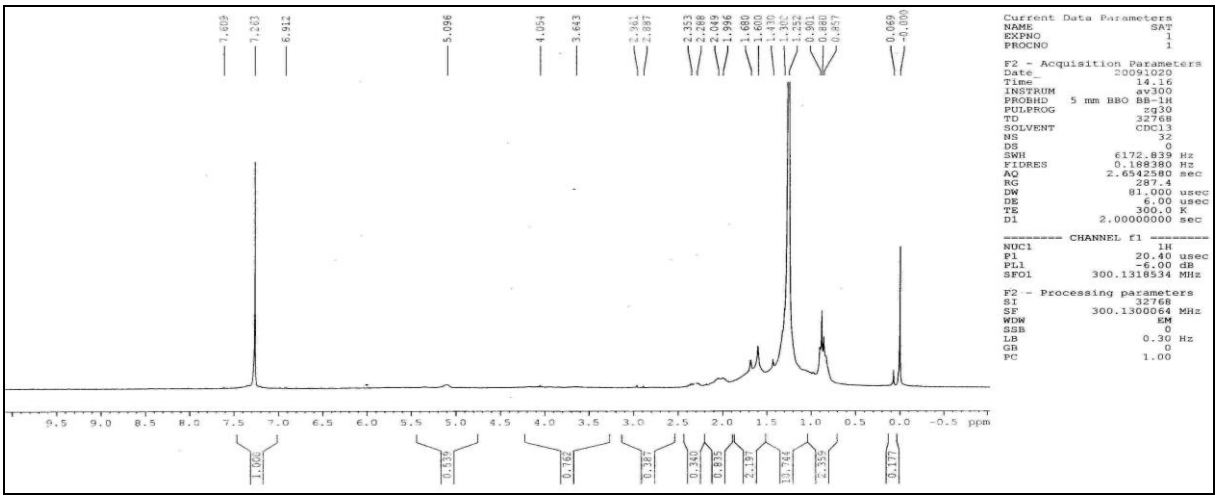

Fig 2: H1 NMR (proton NMR) spectra of the column purified terpenoid compounds of *Acacia caesia* leaves in the Chennimalai population.

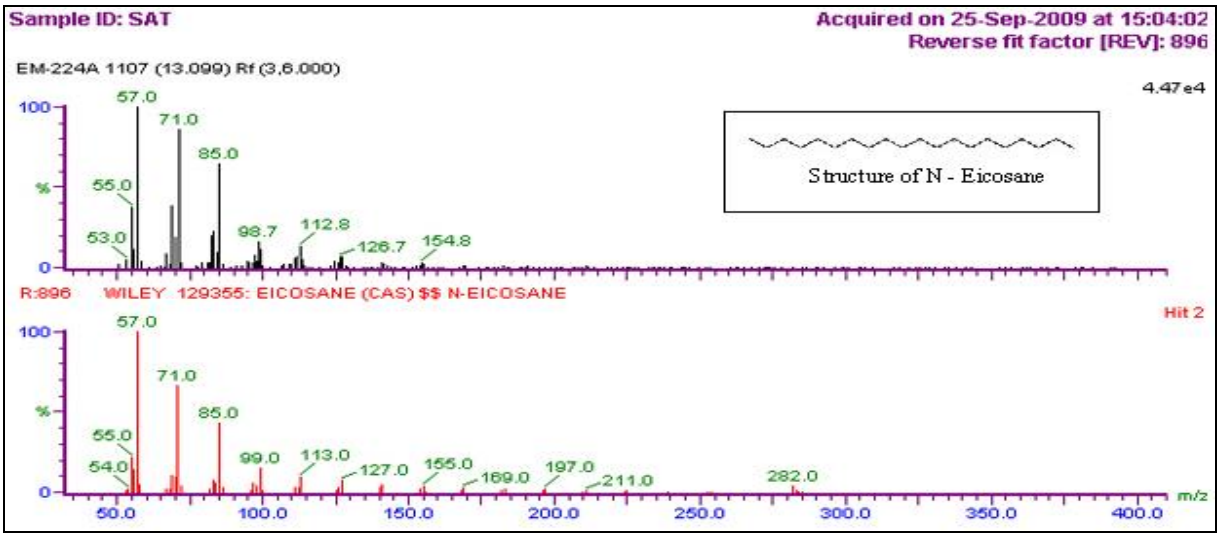

Fig 3: Mass spectra of the column purified terpenoid compounds in the leaves of *A. caesia* in the Chennimalai population.

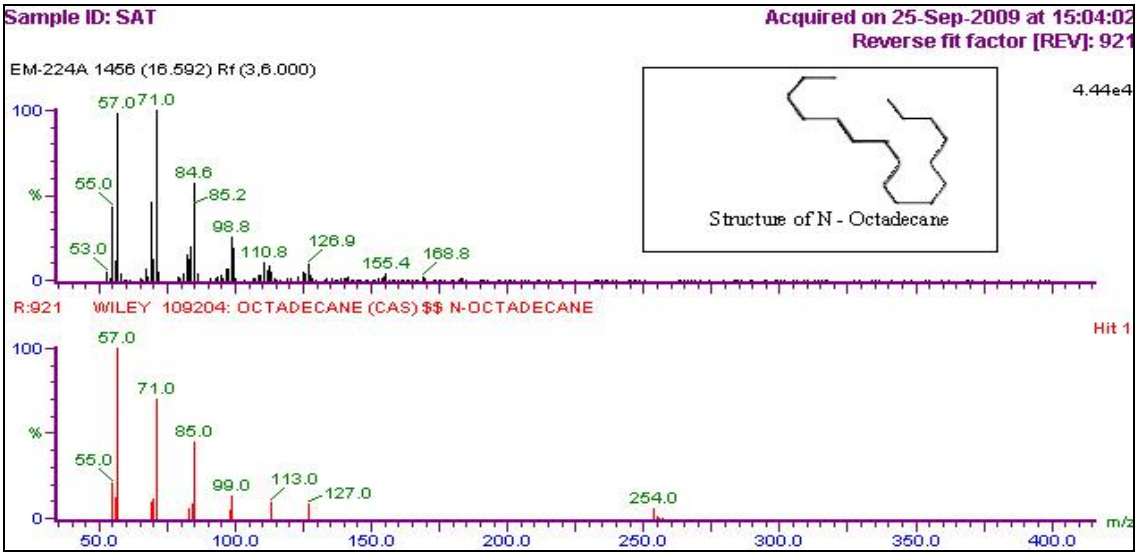

Fig 4: Mass spectra of the column purified terpenoid compounds in the leaves of *A. caesia* in the Chennimalai population.

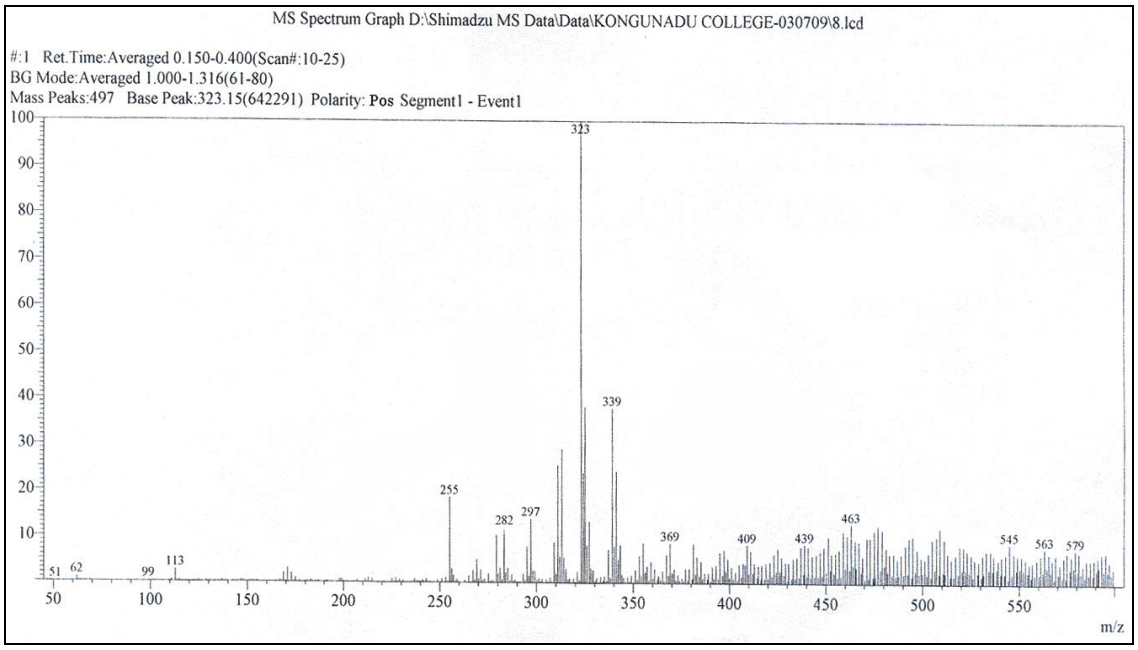

Fig 5: LC - MS mass spectra (positive polarity) of the column purified terpenoid compounds of *A. caesia* leaves in Chennimalai population.

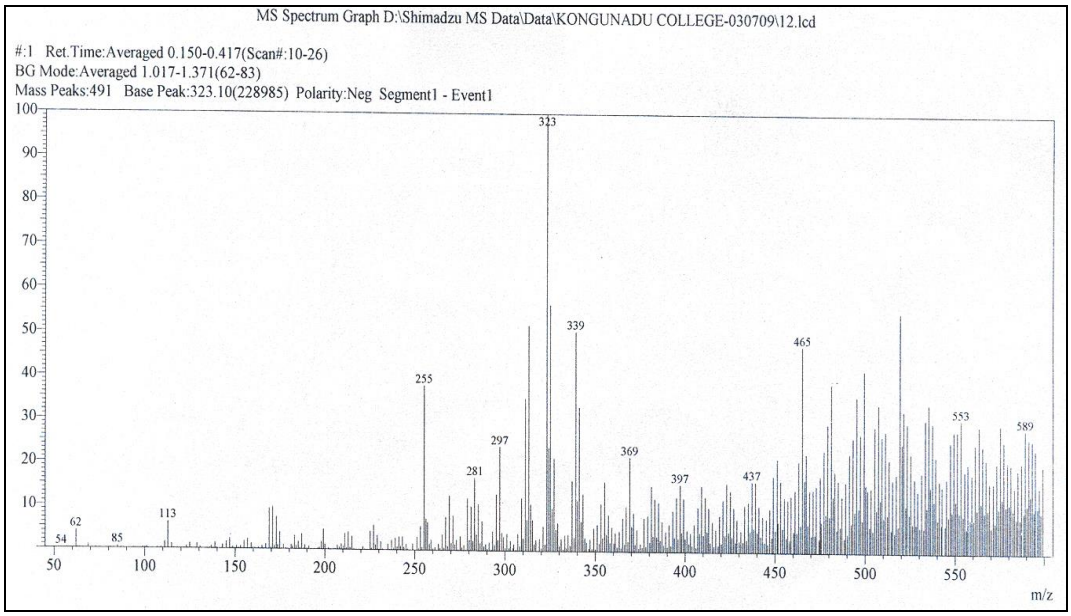

Fig 6: LC - MS mass spectra (negative polarity) of the column purified terpenoid compounds of *A. caesia* leaves in Chennimalai population.

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