

HPTLC AND GC-MS Analysis of Methanolic Extracts of *Samanea Saman* (*Albizia Saman*) (Jacquin) F. Mueller

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Abstract: *Samanea saman* (*Albizia saman*) is a wide-canopied tree with a large symmetrical crown. It usually reaches a height of 25 m (82 ft) and a diameter of 40 m. The leaves fold in rainy weather and in the evening, hence the name "rain tree" and "five o'clock tree" (Pukul Lima) in Malay. In the present investigation, the bioactive methanolic fraction was subjected to HPTLC and GC-MS analysis. GC- MS analysis revealed the presence of six compounds from the methanolic fraction of the *Samanea saman* (1) 9-Octadecenoic acid (Z) methyl ester, (2) Dodecanoic acid, 10-methyl, methyl ester, (3) 13- Hexyloxyacylotridec-10-en-2-one, (4) 15-Octadecenoic acid, methyl ester (5) Diethyl -1- (8 -amino -1- naphthyl) -1,2,3 triazole -4,5 -dicarboxylate were identified from the methanolic extract from the leaves of *Samanea saman*. The presence of these compounds was confirmed by NIST library values. HPTLC analysis concludes the presence of four major phytoconstituents which was eluted at R_f 0.67; 0.79; 0.85 & 0.90 respectively.

Key words: *Samanea saman* (*Albizia saman*); HPTLC analysis; GC- MS analysis.

I. Introduction

Medicinal plants play a vital role in rescuing the various ailments caused in the human arena. Natural product could be a potential drug for human and live stock and their analogues can acts as an intermediate for the synthesis of useful drugs. Folklore claims stated that there were many unsung biological potentials were present in the plant *Samanea saman* (*Albizia saman*) which belong to the family fabaceae, sub family Mimosoideae. *Samanea saman* (*Albizia saman*) is an ever green umbrella-shaped canopy, which grows up to the height 15-25m and it is a native to South America, Columbia, Mexico, Fiji and also cultivated in the plains of India. The synonym is a rain tree. The parts of the plant possess various medicinal properties like antioxidant, antiulcerogenic, antimicrobial, analgesic, antitubercular and cytotoxic activities [1-3]. The present work has been undertaken to evaluate the phytoconstituents present in the leaf extracts using Gas chromatography and mass spectrometer analysis.

II. Material and Methods

Plant Materials

The leaves of *Samanea saman* (*Albizia saman*) were collected from Turkapally –Village, Shamirpet –Mandal, Ranga reddy district. Care was taken to select the healthy plants and for normal organs (i.e. leaves, flowers, fruits and seeds).The required parts were cut and removed from the plant after proper identification and authentication has done by the National Institute of Herbal research, Chennai, Tamil Nadu (PARC/2014/858).

Extract Preparation and column chromatographic analysis

The air –dried leaves of *Samanea saman* was thoroughly washed with water and then shade dried. The shade dried leaves were being pulverized in to coarse powder . The coarse powder of the dried leaves were subjected to cold maceration based on increasing polarity (Hexane, Chloroform and Methanol). The extracts were concentrated using a rota vap *in vacuo*. The corresponding percentage of extracts were 0.5%, 1.2% & 1.6%. The extracts were subjected for biological screening to explore the unsung biological potentials. Bioactive methanolic extract (1.6%) of *Samanea saman* was fractionated over a silica gel (100-200 mesh) column by eluting with solvents of increasing polarity. The fractions obtained were subjected to TLC analysis. The similar fractions were been combined as monitored by the TLC. The obtained fractions were subjected to phytochemical analysis to explore their phytoconstituents. The fraction (ethyl acetate : methanol 80:20) were concentrated using a rota vap *in vacuo*. The obtained brown oily liquid were subjected to HPTLC analysis and GC- MS analysis to explore the bioactive phyto constituents.

HPTLC analysis

HPTLC is a modern adaptation of TLC with improved versatility, separation efficiency and detection limits. It is a reliable method for quantization of nanogram level in a complex formulation. Camag TLC scanner 3” Scanner

3 -070408 “ S/N 070408 (1.14.21) was used for detection and CAMAG Linomat 5 sample applicator was used for the application of the tracks [4].

Sample preparation: The bioactive methanolic fraction (20µL) of *Samanea saman* were loaded in the CAMAG Linomat 5 sample applicator. The sample were coated in the aluminum sheets pre-coated with silica gel G₂₅₄ (Merck), 0.2mm layer thickness. The slit dimensions 5mmx0.45mm and scanning speed of 20mm/sec was employed.

Mobile phase: The chromatogram was developed using N-hexane: Ethyl acetate (60:40 v/v) as a mobile phase.

Detector: Densitometric scanning was performed on CAMAG TLC scanner at 420nm operated by WINCATS software version 1.14.21.

GC-MS analysis

GC-MS analysis [5] was carried out on a perkin elmer clarus 680 GC-MS instrument employing the following condition : column elite -5MS (30.0m, 0.25mm ID, 250µm, operating electronic impact mode; helium was used as a carrier gas at a constant flow and split ratio is 10:1; injector temperature is 250 ° C; flow rate is 1mL/min; oven temperature is 60° C for 3 min, ramp 20 ° C/min to 500 ° C hold for 6 min. The total run time is 35.00 min. The molecular weight and structure of the compounds were ascertained by interpretation using the data base of National Institute of Science and Technology (NIST).

III. Results and Discussion

HPTLC analysis

Conventional extraction of methanolic *Samanea saman* adopted in this work reveals the presence of 4 bioactive components with the corresponding R_f values 0.67, 0.79, 0.85 & 0.90 respectively. The % of area of the corresponding peaks were been tabulated in the Table 1.[Figure 1].

GC-MS analysis

The GC-MS chromatogram of methanolic *Samanea saman* extract gave 6 peaks which are shown in Figure 2. The active principle with their retention time, molecular weight and structure are shown in Table 2. Six compounds were detected in the methanolic extract of *Samanea saman*. The GC spectral study revealed the presence of six compounds include : **(1)** 9-octadecenoic acid- RT 17.12, **(2)** Dodecanoic acid,10 – methyl – methyl ester- RT 18.5, **(3)** 13-Hexyloxacyclotridec-10-en-2-one – RT 18.83, **(4)** 15- octa decenoic acid methyl ester - RT 20.52, **(5)** Diethyl 1-(8-amino -1-naphthyl) -1,2,3 triazole -4,5 –dicarboxylate – RT 22, **(6)** N.N – [1,4 –butanediyl bis [ethylimino] -3,1 propane diyl] bis[N-ethyl acetamide]- RT 24.1 respectively.

IV. Conclusion

The conventional method of extraction (Cold maceration) is a simple, inexpensive and also avoids the decomposition of thermo labile substances which are bioactive in nature. In the present study, four major components were been identified from the methanolic extract of *Samanea saman* by HPTLC analysis. GC-MS analysis identifies the presence of six compounds in which N.N – [1,4 –butanediyl bis [ethylimino] -3,1 propane diyl] bis [N- ethyl acetamide] was first reported in the leaf extract of *Samanea saman*. The present analytical study would explore the unsung bio molecules and provide a new arena for the discovery of modern drug molecules.

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Table 1 : HPTLC data of Methanolic extract of *Samanea saman*

Peak No	Peak strat	Peak position	Max position	Max height	Max %	End position	End height	Area	Area %
1	0.67 Rf	0.2 AU	0.72 Rf	155.2 AU	19.56%	0.78 Rf	0.6 AU	4843.3 AU	20.36%
2	0.79 Rf	0.3 AU	0.83 Rf	182.1 AU	22.95%	0.85 Rf	76.4 AU	4836.1 AU	20.33%
3	0.85 Rf	76.5 AU	0.88 Rf	243.9 AU	30.74%	0.90 Rf	164.0 AU	7693.9 AU	32.35%
4	0.90 Rf	165.2 AU	0.92 Rf	200.2 AU	25.23%	0.97 Rf	2.1 AU	6253.9 AU	26.30%

FIGURE 1 HPTLC analysis of Methanolic extract of *Samanea saman*

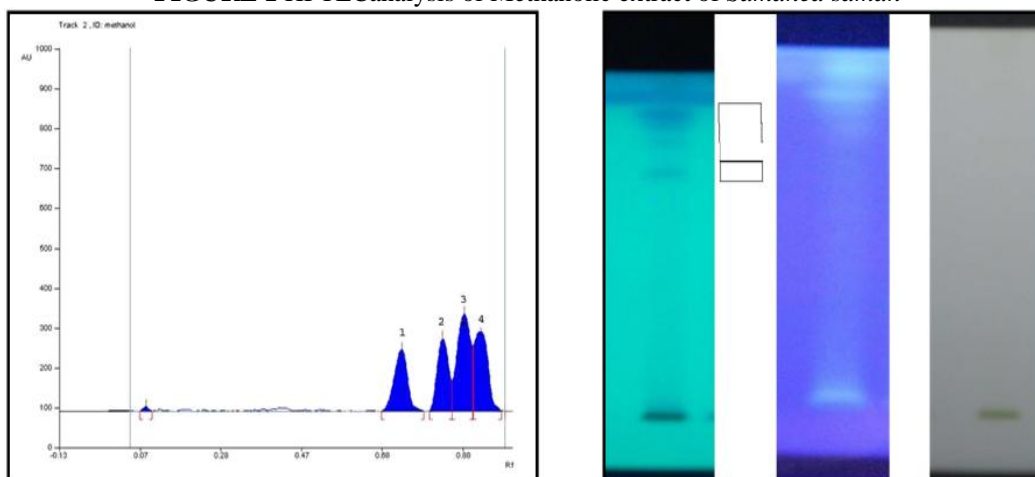
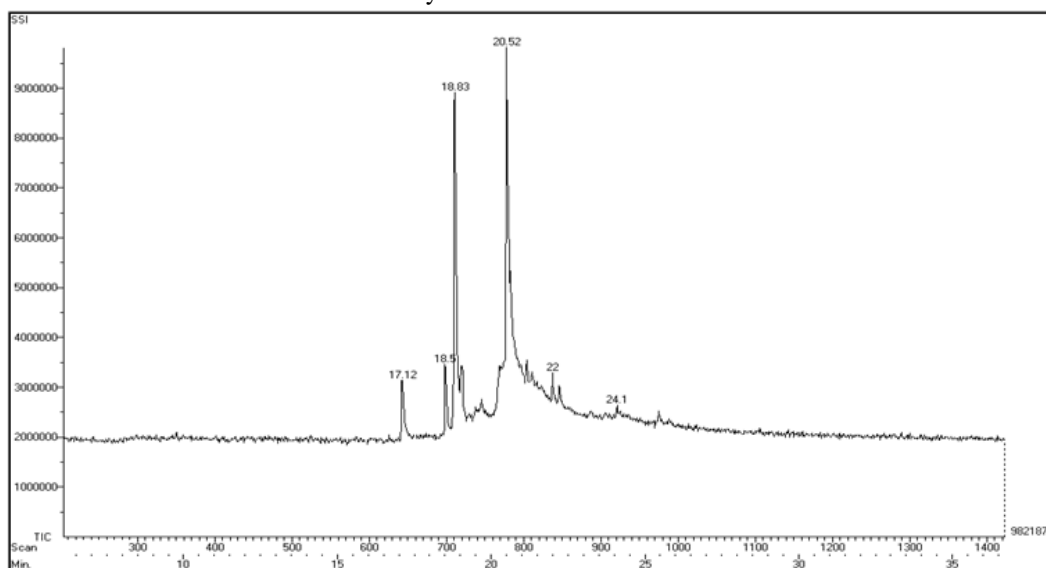


FIGURE 2 GC-MS Analysis of Methanolic extract of *Samanea saman*



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