



ISOLATION AND IDENTIFICATION OF TANNINS AND POLYPHENOL FROM THE METHANOL EXTRACT STEMS OF SAMANEA SAMAN(JACQ) MERR.

BY USING TLC, UV, IR AND GC-MS METHODS

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Abstract:

This study was aimed at analysis of potential bioactive constituents of leaves of *Samanea Saman(Jacq) Merr.* Methanol extracts of the leaves were subjected to thin layer chromatography (TLC), UV, IR and Gas chromatography-mass spectroscopic (GCMS) analysis. Total two compounds were isolated by preparative TLC using chloroform: ethyl acetate: methanol (5: 3: 2) as mobile phase. These compounds were found to be tannins and polyphenol by spraying with ferric chloride solution (5%) as spraying reagent. Spectroscopic data of UV, FTIR and GC-MS also confirmed that the functional groups found were matched with the tannins moiety. Analytical studies showed the presences of phenolic and tannins in *Samanea saman* methanolic extract (SSME).

KEYWORDS: *Samanea saman*, UV, IR, TLC, GC -MS

Introduction:

Samanea saman(Jacq) Merr. (Fabaceae), commonly called Rato siris in Gujrati, Vilaiti Siris, Gulabi siris in Hindi and Saman, Rain Tree, Monkey Pod in English, is a native of tropical Africa and Asia. The synonym of the *Samanea saman* is “Monkey pod” which is a fast growing tree that is been introduced to many tropical countries throughout the world from its native habitats in Central America and Northern South America. Even though, it has been planted as an ornamental tree, it has a great value of pastures as shade for the cattle[1]. Rain tree exhibits. several bioactive compounds which produce different medicinal properties such as antioxidants, anti-ulcer, antibacterial, analgesic, antifungal, insecticidal and cytotoxic activities[2]. The crude drug is always been cheaper and readily available in abundance with negligible side effects and it is suitable for the patients of all age groups. The multiple therapeutic

actions of *Samanea saman* are evidently supported by the minimal classical literatures[3]. Hence the present study, review the following pharmacological activities which have recently explored. Phytochemical screening of the leaves of plant *Samanea saman* revealed the presence of tannins, flavonoides, saponins, steroids, cardiac glycosides and terpenoids. Tannins and flavonoides are generally antioxidant in nature. Preliminary phytochemical analysis of stem of *Samanea saman* revealed the presence of tannins. *Samanea saman* is used as antioxidant, laxative, antimalarial, sedative and for the treatment of stomach cancer and sore throat.

Materials and Methods:

The stem of *Samanea saman* Linn were collected from Anand Agriculture University, near Borsad road, Anand, Gujarat, India in the month of may, june and dry stem of plant for phytochemical screening as well as analytical work was procured from Mac ayurcare, Khambhat. The plant was authenticated and confirmed by Dr. A. S. Reddy, Prof. and Head of Botany Dept., Sardar Patel University, Vallabh Vidyanagar. In the college, The stems were dried in shade and stored at 250°C. It was powdered, passed through 60# and stored in air tight bottles. An exhaustive analytical study was carried out using standard methodology [3-13].

Thin layer Chromatographic analysis

TLC was performed on a pre-coated silica gel TLC plates grade F254 (E-Merck, Darmstadt, Germany) to determine the number of compounds present in the plant crude extract. A total of 5 µl (10 mg/ml) of sample was spotted at 1 cm from the bottom of silica gel plates using capillary tubes. Different solvents at various combinations and concentrations were used for metabolites profiling. Development of the chromatogram was done in closed tanks, in which the atmosphere has been saturated with eluent vapor by wetting a filter paper lining. The chromatogram was visualized under Day light and Anisaldehyde in sulphuric acid and Ferric chloride solution (5 %) reagent spray. The Rf values of the compounds were calculated using the following formula [9,11,14].

Rf = distance travelled by the compound/ distance travelled by the solvent front

Total tannin contents

The tannin contents were determined by method of Broadhurst et al., 1978 with slight modification, using catechin as a reference compound. A volume of 400µL of extract is added to 3 mL of a solution of vanillin (4% in methanol) and 1.5 mL of concentrated hydrochloric acid. After 15 min of incubation the absorbance was read at 500 nm. The condensed tannin was expressed as g E.Catechin.100g⁻¹DM.

Isolation of compounds by Preparative TLC:

Two compounds were isolated by preparative TLC using chloroform: ethyl acetate: methanol (5: 3: 2).The compounds were scrap from the plate and dissolve in to the methanol and concentrate to dryness[9,11,14].

FTIR analysis

Dried powder of methanolic extract was used after performing KBr pelleting. The sample was loaded onto FTIR spectroscope (Shimadzu, IR Affinity Japan) in a scan range of 400-4000⁻¹ cm[9,11,14].

UV Visible analysis:

UV-Vis Spectrophotometry was done using Shimadzu UV 1800 Double beam Spectrophotometer. (6) Sample analysis was conducted at room temperature. System calibration was automatically programmed in the Spectrophotometer. UV probe software provided by Shimadzu is used for analysis and interpretation of spectral data. Raw materials' extracts are diluted randomly initially and tested for UV-VIS spectral analysis. Total spectrum analysis from 4000 nm to 400 nm was done as it facilitates to understand various peaks arising due to multiple components present in the extract. By this we identified the main constituent present in methanolic extract [9,11,14].

GC-MS analysis

The extract was subjected to GC MS analysis to identify the various bioactive compounds present. The sample was analyzed in Perkin Elmer- Clarus-600 instrument using software Turbomass 5.2 version. Capillary standard non-polar column (30 m X 0.25 mm, 0.25 mm film thickness) was used. The volume of injected specimen was 1 μ l of methanol extract, injector temp. 220°C with a split ratio of 25:1 Carrier gas Helium, Solvent Delay=3.00 min, source Temp=180°C, oven temperature program initial temp 60°C for 5 min, ramp 7°C/min to 300°C, hold 15 min, Scan: 50 to 600Da, ionization energy 70 eV, in the electronic ionization mode. The identification of compounds was done using computer matching of mass spectra with those of standards (Mainlib, Replib and NIST library). The name, molecular weight and the structure of the components of the test materials were ascertained [9,11,14].

Results and discussion

Thin Layer Chromatography of the extracts obtained in successive extraction:

TLC of the methanolic extract of *Samanea saman* stem shows present of Phytosterol and tannins in solvent system Hexane:Ethyl acetate (6 : 4) and Chloroform: Ethyl acetate: Methanol(5:3:2).

Thin layer chromatographic analysis revealed the presence of diverse potent biomolecules in the plant extract. Solvent system of Hexane:Ethyl acetate(6 : 4) and Chloroform: Ethyl acetate:Methanol(5:3:2) gave a better separation of compounds (Table1). Methanolic extract revealed 3 spots and 3 spots with R_f values of 0.76 (violet color), 0.66 (orange color), 0.45(pink color) and 0.90 (blue color) 0.75 (blue color) 0.65 (brown color) respectively. This study shows present of two components that are Phytosterol and Tannins.

Table 1: Thin Layer Chromatography of the extracts obtained in successive extraction

Phytochemical Constituents	Solvent System for TLC	P.Ether Extract (60° – 80°C) (R_f and color spot)	Detection
Phytosterol	Hexane: Ethyl acetate (6 : 4)	$R_f = 0.76$ (violet color) $R_f = 0.66$ (orange color) $R_f = 0.45$ (pink color)	Anisaldehyde in sulphuric acid

Table 2: Thin Layer Chromatography of the extracts obtained in successive extraction

Phyto-chemical Constituents	Solvent System for TLC	Acetone Extract (R_f and color spot)	Methanol Extract (R_f and color spot)	Detection
Tannins	Chloroform: Ethyl acetate: Methanol (5:3:2)	$R_f = 0.90$ (blue color) $R_f = 0.75$ (blue color) $R_f = 0.65$ (brown color)	$R_f = 0.70$ (brown color) $R_f = 0.45$ (brown color)	Ferric chloride solution (5 %)

DETERMINATION OF TOTAL TANNIN CONTENT:

The total tannin content in *Samanea saman* stem was found to be **0.15%**.

ISOLATION OF COMPOUND BY PREPARATIVE TLC:

Two compounds were isolated by preparative TLC using chloroform: ethyl acetate: methanol (5: 3: 2). The compounds were scraped from the plate and dissolved in methanol and concentrated to dryness which is used for the different instrumental. The R_f value and color spot of these compounds were given in following Table 3.

Table 3.

Sample No.	R_f Value	Color of Spot	
		In Day light	5% FeCl ₃ solution
1	0.45	Brown	
2	0.70	Brown	

FTIR analysis was used to identify the functional group of active components based on peak values in the region of infrared radiation. FTIR spectrum was analyzed (Coats et al., 2000) and the results of FTIR spectrum

profile was illustrated in the Fig.2. By the spectra its conformed that separated constituents are Phytosterol and tannins. Spectroscopic data of UV, FTIR and GC-MS also confirmed that the functional groups found were matched with the tannins moiety.

GC-MS analysis

The results of GCMS analysis was illustrated in Fig. 3 that shows present of Phytosterol and tannins . Spectroscopic data of GC-MS also confirmed that the functional group found were matched with the tannins moiety.

FTIR:

FTIR analysis was used to identify the functional group of active components based on peak values in the region of infrared radiation. FTIR spectrum was analyzed (Coats et al., 2000) and the results of FTIR spectrum profile was illustrated in the Fig.2 and tabulated in Table 2. Spectroscopic data of FTIR confirmed that the functional groups found were matched with the tannins moiety.

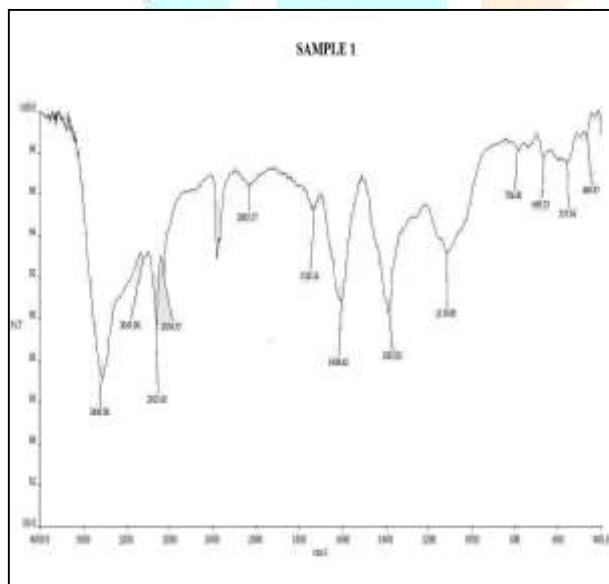


Figure.1 IR Spectrum of Sample 1

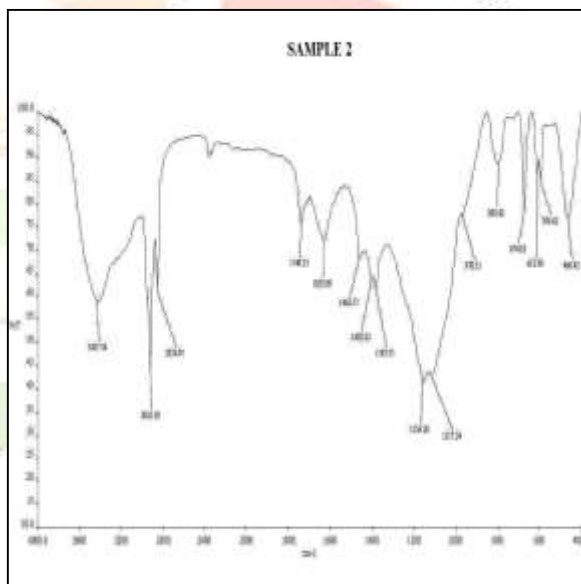


Figure.2 IR Spectrum of Sample 2

UV

This study of UV-Vis spectrum from 400-4000 nm of extracts will make us understand number peaks i.e. chemicals present in a sample. Spectroscopic data of UV confirmed that the functional groups found were matched with the tannins moiety.

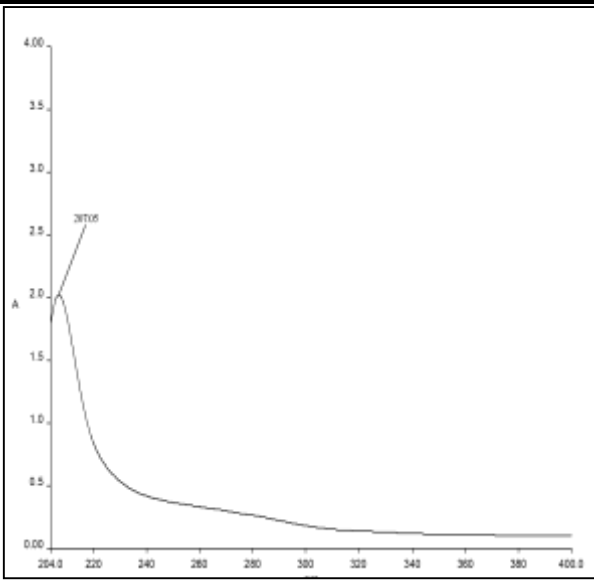


Figure.3 U.V. Spectrum of Sample 1

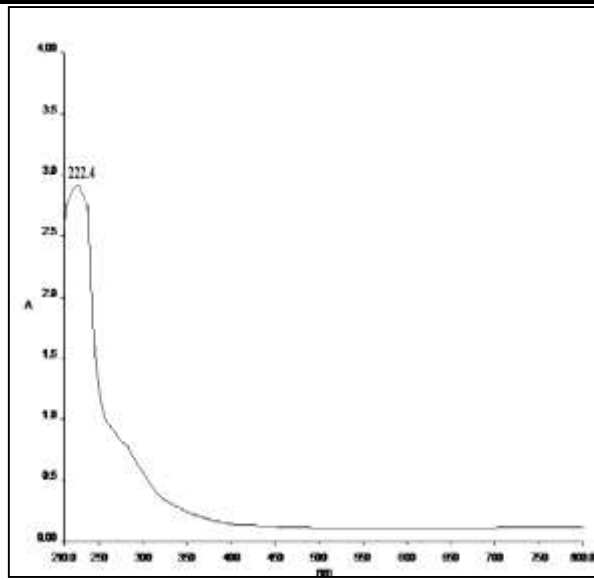


Figure.4 U.V. Spectrum of Sample 2

GC MS

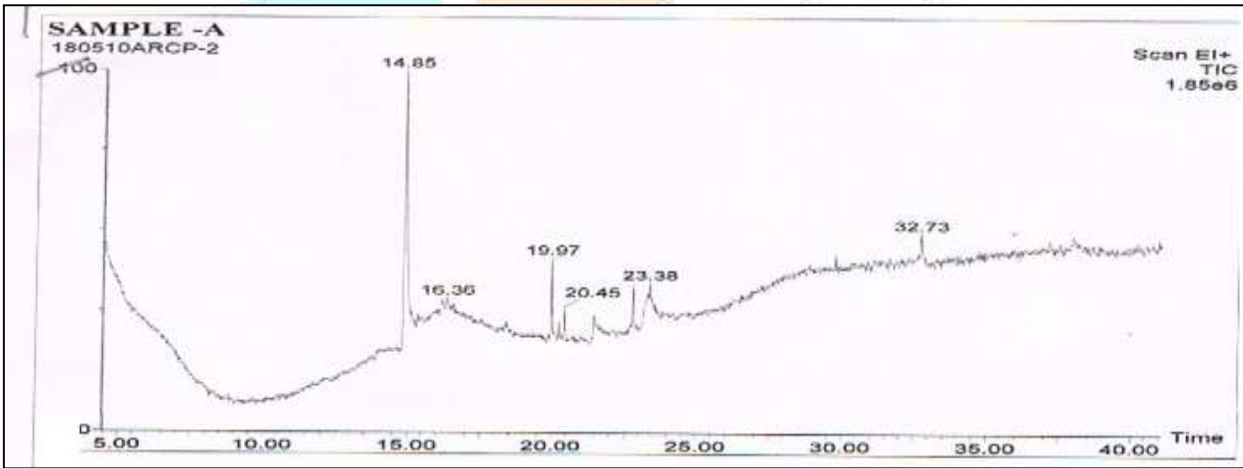


Figure.5 GC-MS of Sample 1

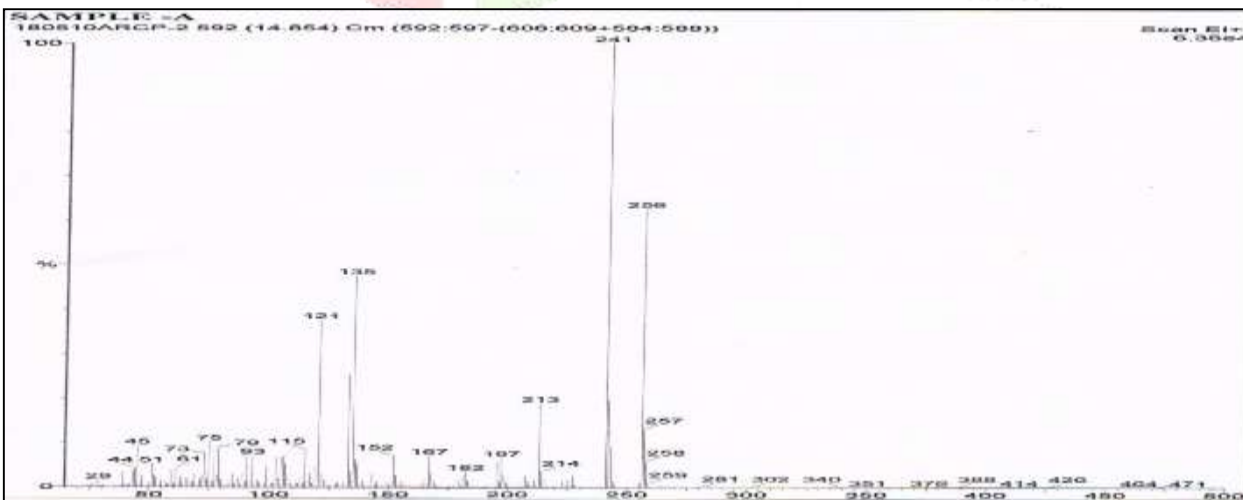


Figure.6 GC-MS of Sample 1

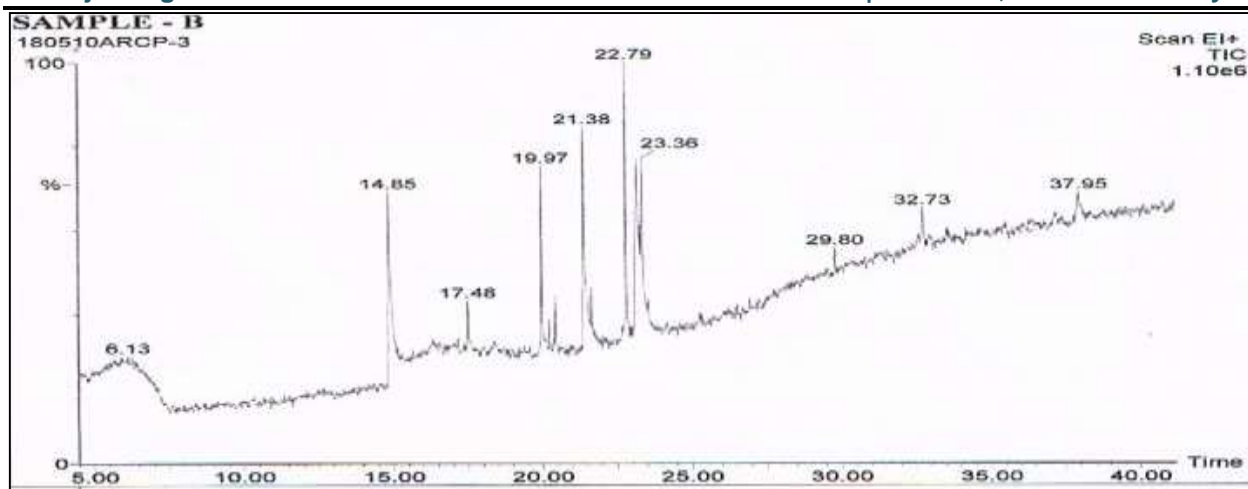


Figure.7 GC-MS of Sample 2



Figure.8 GC-MS of Sample 2

The results of the present study indicate that the methanolic extract of stems *Samanea saman* have Tannins and Phytosterol which are potent phytochemicals with many biological activities.

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