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Analysis of Bioactive constituents from the Ethanolic leaf extract of T*abebuia rosea* (Bertol.) DC by Gas Chromatography – Mass Spectrometry

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Abstract: *Tabebuia rosea* is one of the medicinally important plants belonging to the family Bignoniaceae. *Tabebuia* sp are native to tropical rain forests throughout Central and South America. The herbal products obtained from the bark of tabebuia trees are called "taheebo", "lapacho", "pau d'arco", and "ipe roxo". Traditionally, taheebo has been used for treating ulcers, syphilis, gastrointestinal problems, candidiasis, cancer, diabetes, prostatitis, constipation, and allergies. In the present study the ethanolic leaf extract of *Tabebuia rosea* has been subjected to GC-MS analysis. The major chemical constituents are 2-furancarboxaldehyde, 5-hydroxy methyl (19.39%), 2-deoxy, D-erythropentose (11.01%), Santolina triene(8.28%), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl (6.07%), 7-Quinolinol (6.01%), phenol, 2-(2-methyl propyl) (5.41%) and Cinnamadehyde (2.42%). Thus the extract of *Tabebuia rosea* was characterized by various types of active compounds such as aromatic aldehydes (21.81%), sugar (11.01%), aromatic compounds (7.28%), terpenoids (8.3%), quinone (6.01%), alkanes (6.35%), phenolics (6.85%) and flavonoid (6.07%).
Keywords: *Tabebuia rosea*, phytochemicals, GC-MS analysis, (5-hydroxy methyl), 2-furancarboxaldehyde, santolina triene, cinnamaldehyde, 2,3-dihydro-3,5-dihydroxy-6-methyl, 4H-Pyran-4-one, Quinolinol.

INTRODUCTION

Natural products have played an important role in the development of drugs and drug leads for various diseases including cancer[1]. The secondary metabolites from natural sources are good candidates for drug development because being elaborated within the living systems, they are perceived to exhibit more similarities to drugs and show more biological friendliness than totally synthetic drugs[2]. Thus a search for anticancer compounds from medicinal plants is on a rise.

Tabebuia rosea (Bertol.) DC. Commonly known as "Pink Trumpet Tree" can grow up to 15 meter and well known for its beautiful flowers. The timber is widely used for general construction and carpentry in many European countries. The fruits are green, long and bean pod -like with a length of 20-40 cm (8-16 inch). The fruits turn dark brown when ripe and contain flat, heart-shaped seeds with tiny wings. The graceful beauty is a treat for the eyes, but the tree has medical uses as well. Tea made from the leaves and bark is known to have a fever-reducing effect[3].

Taheebo is reported to be an astringent, anti-inflammatory, antibacterial, antifungal, diuretic, and laxative[4-8].

The *Tabebuia roseae* ethanolic leaf extract is said to have remarkable antimicrobial activity against a wide range of gram positive and gram negative bacteria[9]. The essential oil of *Tabebuia rosea* leaf and bark is reported to be cytotoxic which may be due to the presence of *o-xylene (2.13%), 2,4-dimethylhexane (1.03%), methyl cyclohexane (53.13%), methyl benzene (12.75%), 3-Pentene-2-one(0.11%)*[10]. The earlier investigations on the phytochemical constituents of *Tabebuia rosea* leaves in our lab revealed the presence of saponins, tannins, phenolic acids, flavonoids and alkaloids[11]. Also the alkaloid extract from *Tabebuia rosea* leaves is preferentially said to be cytotoxic to human T-cell leukemia (MOLT-4) cells in a dose and time dependent manner with the absence of genotoxicity[12]. This work reports the active constituents in the ethanolic leaf extract of *Tabebuia rosea* by Gas chromatography-mass spectrometry (GC-MS).

MATERIALS AND METHODS

Collection of plant material

Mature healthy leaves were collected from the tree found in the Centre of Biodiversity and forest studies, Madurai Kamaraj University, Madurai, India. The collected plant materials were botanically authenticated by the Director, Centre for Biodiversity and Forest Studies, Madurai Kamaraj University, Madurai, India.

Preparation of powder and extract

Leaves (1KG) were shade dried, powdered and extracted with ethanol for 6-8 hours using soxhlet apparatus. The extract was then filtered through muslin, evaporated under reduced pressure and vacuum dried to get the viscous residue. The ethanolic extracts of the plant was used for GC-MS analysis.

GC –MS ANALYSIS

Preparation of extract

2 µl of the ethanolic extract of *Tabebuia rosea* was employed for GC/MS analysis.

Instruments and chromatographic conditions

GC-MS analysis was carried out on a GC clarus 500 Perkin Elmer system comprising a AOC-20i autosampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: column Elite-1 fused silica capillary column (30 × 0.25 mm ID ×1EM df, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1ml/min and an injection volume of 0.5 EI was employed (split ratio of 10:1) injector temperature 250°C; ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C/min, to 200°C/min, then 5°C/min to 280°C/min, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 40 to 550 Da.

Identification of components

Interpretation on mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

Figure1: GC-MS Chromatogram of ethanolic extract of the whole plant of *Tabebuia rosea*

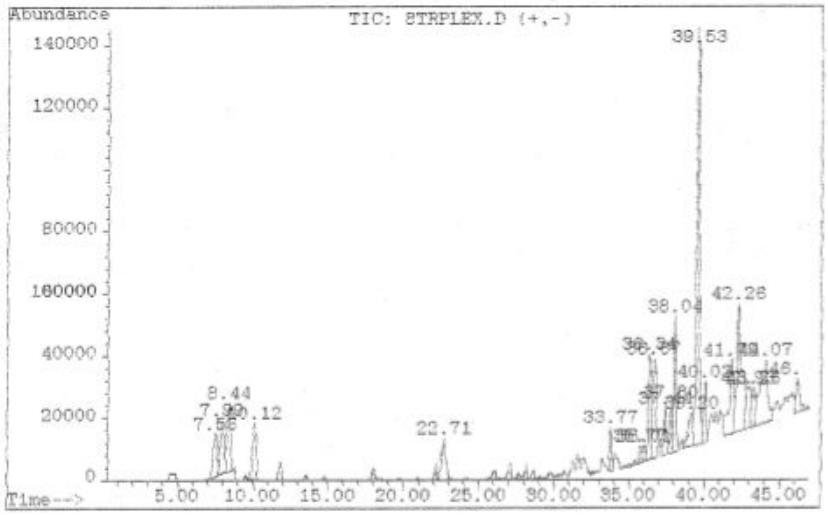


Table1: Phytocomponents identified in the ethanolic extracts of the whole plant of *Tabebuia rosea* by GC-MS.

No	RT	Name of the compound	Molecular Formula	MW	Peak Area(%)
1.	10.12	Cyclopentane, methyl-	C_6H_{12}	84.17	3.46
2.	22.71	1,6:2,3-dianhydro-4-o-acetyl-beta-d-allopyranose	$C_8H_{10}O_5$	186.16	3.22
3.	33.77	3-hydroxy phenyl acetylene	C_8H_6	102.13	1.44
4.	36.01	Cyclohexaneethanol,4-methyl-beta-methylene-trans	$C_{10}H_{16}O$	152.24	0.73
5.	36.33	4H-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl	$C_6H_8O_4$	144.13	6.07
6.	36.67	7-Quinolinol	C_9H_7NO	145.16	6.01
7.	37.44	Cinnamaldehyde	C_9H_8O	132.16	2.42
8.	37.80	Benzene, 1-methyl-2-nitro-	$C_7H_7NO_2$	137.14	1.79
9.	38.04	Benzofuran, 2,3-dihydro-	C_8H_8O	120.15	3.18
10.	39.20	Cyclohexane, 1-ethyl-4-methyl,cis	C_9H_{18}	126.24	2.89
11.	39.34	2-Furancarboxaldehyde,5(hydroxymethyl)	$C_6H_6O_3$	126.11	19.39
12.	40.03	9-Borabicyclo(3.3.1)nonane,9-(1-methylbutyl)	$C_8H_{15}B$	244.03	2.57
13.	41.79	Phenol,2-(2-methylpropyl)-	$C_{10}H_{14}O$	150.22	5.41
14.	42.26	D-erythro pentose,2-deoxy	$C_5H_{10}O_4$	134.13	11.01
15.	42.93	Oxirane, hexadecyl-	$C_{18}H_{36}O$	268.48	3.34
16.	43.25	1-hexacosanol	$C_{26}H_{54}O$	382.71	2.03
17.	44.07	Santolina triene	$C_{10}H_{16}$	136.23	8.28
18.	46.16	2-methyl Benzoic acid	$C_8H_8O_2$	136.2	2.31

Table 2: Activity of phyto-components identified in the ethanolic extracts of the whole plant of *Tabebuia rosea* by GC-MS analysis.

RT	Name of the compound	Compound Nature	**Activity
10.12	Cyclopentane, methyl-	alkane	Precursorfor cyclopentane monoterpenoid synthesis
22.71	1,6:2,3-dianhydro-4-o-acetyl-beta-d-allopyranose	Sugar glycoside	Preservative
33.77	3-hydroxy phenyl acetylene	Phenol	Antibacterial
36.01	Cyclohexaneethanol,4-methyl-beta-methylene-trans	Alkane ethanol	Antibacterial
36.33	4H-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl	Flavonoid	Antimicrobial,anti-inflammatory,anti-proliferative
36.67	7-Quinolinol	Heterocycle quinoline	Metal chelator, anti-fungal
37.44	Cinnamaldehyde	Phenolic aldehyde	Flavorant,anti-cancer antimicrobial
37.80	Benzene, 1-methyl-2-nitro-	Aromatic compound	
38.04	Benzofuran, 2,3-dihydro-	Coumaran	Antihelminthic,anti-inflammatory,anti-diarrhoeal
39.20	Cyclohexane, 1-ethyl-4-methyl,cis	Alkane	
39.34	2-Furancarboxaldehyde, 5(hydroxymethyl)	Aldehyde	Antimicrobial, Preservative

40.03	9-Borabicyclo(3.3.1)nonane,9-(1-methylbutyl)	Organo borane	Antimicrobial
41.79	Phenol,2-(2-methylpropyl)-	Phenol	Antibacterial
42.26	D-erythro pentose,2-deoxy	Sugar	Preservative
42.93	Oxirane, hexadecyl-	Epoxide	Adhesives
43.25	1-hexacosanol	Fatty alcohol	Antibacterial
44.07	Santolina triene	Terpenoid,essential oil	Cytotoxic, Anti-fungal, Antibacterial, Anti-inflammatory
46.16	2-methyl Benzoic acid	Aromatic carboxylic acid	Antimicrobial

Figure 2: The mass spectrum analysis and structure of 2-Furancarboxaldehyde, 5(hydroxymethyl) (19.39%)

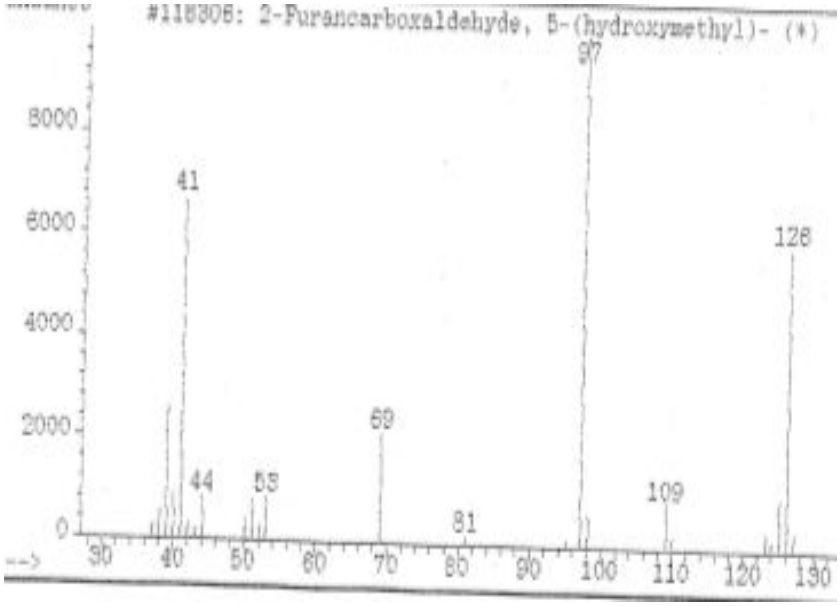


Figure 3: The mass spectrum analysis and structure of D-erythro pentose, 2-deoxy (11.01%)

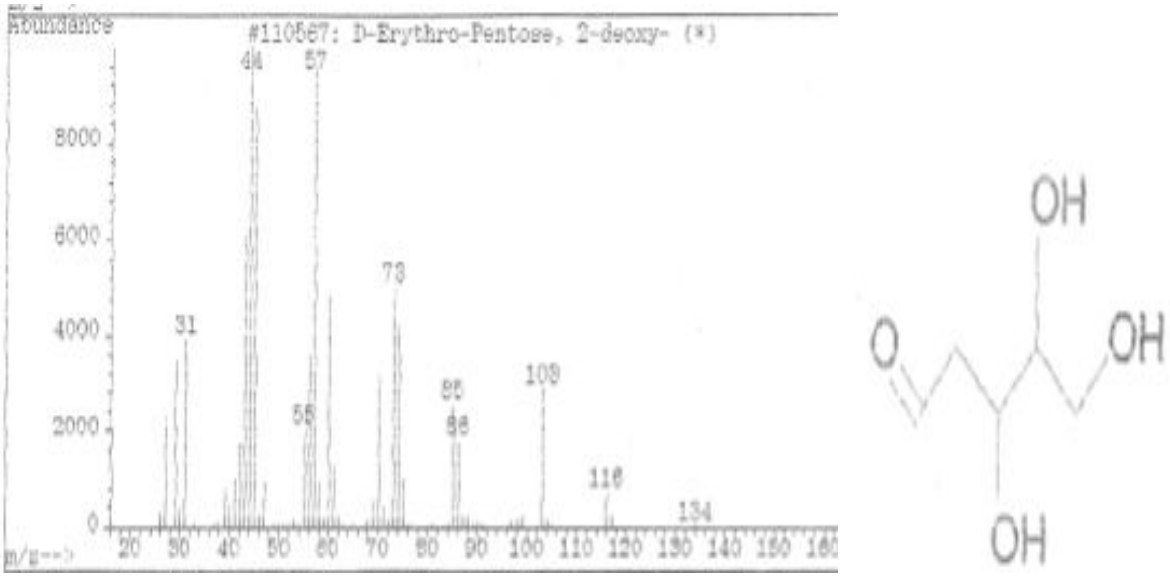


Figure 4: The mass spectrum analysis and structure of Santolina triene (8.28%)

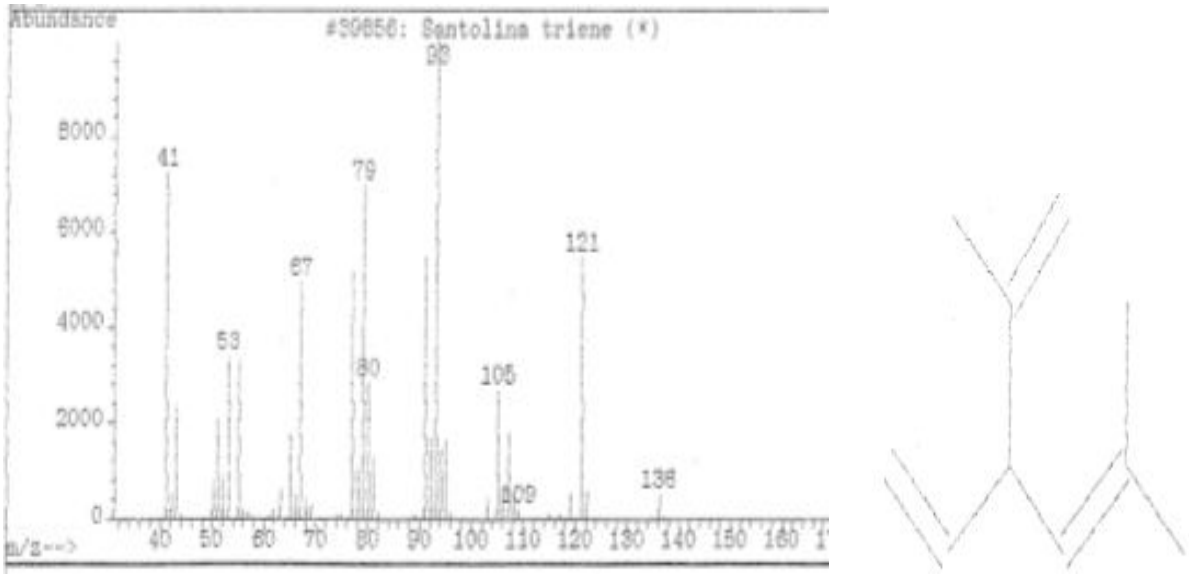
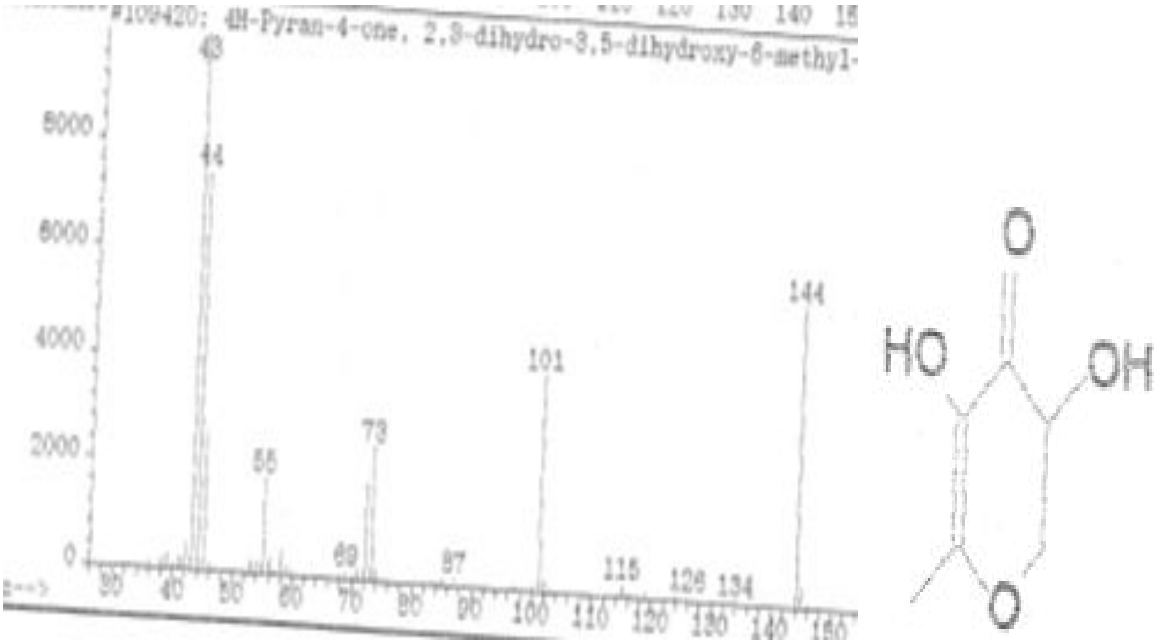


Figure 5: The mass spectrum analysis and structure of 4H-pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl (6.07%)



RESULTS AND DISCUSSION

GC-MS analysis

GC-MS chromatogram of the ethanolic extract of *Tabebuia rosea* is given in (Figure1). On comparison of the mass spectra of the constituents with the NIST library, twenty peaks were obtained, out of which eighteen phytoconstituents were characterized and identified (Table 1). The retention times (RT) are in minutes. The various phytochemicals which contribute to the medicinal activity of the plant are listed in (Table 2).

The active components present in the ethanolic extract of *Tabebuia rosea* are grouped as follows, aromatic aldehydes (21.81%), sugar (11.01%), aromatic compounds (7.28%), terpenoids (8.3%), quinone (6.01%), alkanes (6.35%), phenolics (6.85%) and flavonoid (6.07%). The major constituent was found to be 2-Furancarboxaldehyde, 5(hydroxymethyl)

at retention time of 39.34. The sugar, 2-deoxy-D-erythro pentose is found in next higher concentration at the retention time of 42.26. The flavonoid component, 4H-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl is found at the retention time of 36.35. The monoterpenoid, santolina triene is found at the retention time of 44.07. The heterocycle quinoline, 7-Quinolinol is found at retention time of 36.67. Phenolic compounds such as 3-hydroxy phenyl acetylene and phenol, 2-(2-methyl propyl) are found at retention time of 33.77 and 41.79 respectively. Methyl cyclopentane reported in essential oil of leaf and bark is also found in ethanolic leaf extract at retention time of 10.12. Cinnamaldehyde, a phenolic aldehyde is found at retention time of 37.44.

The major phytochemical constituent's present in ethanolic extract of *Tabebuia rosea* are presented as mass spectra and compound structures are in (Figure 2 to Figure 5). They were identified as 2-Furancarboxaldehyde, 5(hydroxymethyl) (19.39%), D-erythro pentose, 2-deoxy (11.01%), Santolina triene (8.28%) and 4H-pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl (6.07%) respectively.

CONCLUSION

The present study has been found useful, where a variety of active compounds have been found in ethanolic extract, instead going for essential oils. The presence of various bioactive compounds (identified as aromatic aldehydes, alkanes, quinone, alcohols, sugar, monoterpenoids, phenolics, flavonoid) justifies the use of the whole plant for various ailments by traditional practitioners. It could be concluded that *Tabebuia rosea* leaf ethanolic extract of plant is of phytopharmaceutical importance. However, isolation of individual phytochemical constituents and subjecting it to biological testing will definitely give fruitful results.

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