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G Anburaj M Marimuthu
Research Scholar, PG and Research Department of Chemistry, A.V.V.M. Sri Pushpam College (Autonomous), Thanjavur, Tamil Nadu, India

V Rajasudha
Research Scholar, PG and Research Department of Chemistry, A.V.V.M. Sri Pushpam College (Autonomous), Thanjavur, Tamil Nadu, India

Dr. R Manikandan
PG and Research Department of Chemistry, A.V.V.M. Sri Pushpam College (Autonomous), Poondi-613503, Thanjavur, Tamil Nadu, India

Correspondence
G Anburaj M Marimuthu
Research Scholar, PG and Research Department of Chemistry, A.V.V.M. Sri Pushpam College (Autonomous), Thanjavur, Tamil Nadu, India

Phytochemical screening and GC-MS analysis of ethanolic extract of *Tecoma stans* (Family: *Bignoniaceae*) Yellow Bell Flowers

G Anburaj, M Marimuthu, V Rajasudha and Dr. R Manikandan

Abstract
Aim: Flowers of *Tecoma stans* Linn popularly known as "yellow bell flowers" contain flavonoids. Flavonoids have been established to have antidepressant activity of the possible phytochemical components from the ethanolic extracts of *Tecoma stans* (flowers). Among the phytochemical screening of these two plant extracts showed that the plant was rich in alkaloids, flavonoids, phenols, saponins and quinones. This study was extended by analyzing the potent bioactive compounds in the ethanolic extract of *Tecoma stans* (flowers) using GC-MS. The analysis revealed that *Tecoma stans* (flowers) extracts 25 compounds were identified in the flowers ethanol extract. Medicinal potential of these compounds needs further research on microbial aspects to develop safe drug.

Keywords: *Tecoma stans* (flowers) Phytochemicals, GC-MS, Bio active compounds.

1. Introduction
Plants have been used in virtually all cultures as a source of medicine. Plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and wellbeing. Traditional medicine using plant extracts continues to provide health coverage for over 80% of the world's population, especially in the developing world (WHO, 2002). Genetic biodiversity of traditional medicinal herbs and plants is continuously under the threat of extinction as a result of growth hexploitation, environment-unfriendly harvesting techniques, loss of growth habitats and unmonitored trade of medicinal plants [1].
A survey of literature on *Tecoma stans (Family: Bignoniaceae*) popularly known as Yellow Bells or Ginger Thomas revealed that alkaloids, steroids, saponins, anthraquinones and flavonoids, tannins, terpenes, phytosterols, phenols, and glycosides constitute major classes of phytoconstituents of this plant. Pharmacological reports revealed that it is having antidiabetic, anticancer, antioxidant, antispasmodic, antimicrobial, and antifungal, properties, and extensively used in the treatment of diabetes. It is a fast growing evergreen plant with 20-30 ft in height, having moderate growth and yellow flowers. Leaves are green compound, imparipinnate, and lanceolate with serrate margin. Fruits are elongated and clustered. Ginger thomas leaves, bark and roots contains biologically active chemicals, and extracts from those tissues are in use as traditional folk medicines. Plant is in use through Mexico, India and Central America for diabetes, roots for diuretic and urinary disorder control. *Tecoma stans* was also investigated for antifungal effect in roots. Standardization of a plant is first requirement for its use in herbal medicines [2]

2. Materials and Methods
2.1 Preparation of plant material
The rhizomes of the *Tecoma stans (Family: Bignoniaceae*) were collected, washed, shade dried and powdered. The powder was preserved in air sealed polythene cover for further evaluation.

2.2 Preparation of plant extract
The dried powdered tubers were defatted with ethanol (30 to 40 °C) by hot extraction method in a soxhlet apparatus. The defatted powder materials were further extracted with ethanol and concentrated extracts were used for the analysis.

2.3 Qualitative evaluation of phytochemicals
The different types of secondary metabolites such as contains Alkaloids, Carbohydrates, Tannins,

Saponins and phenolic compounds. Flavonoide and coumarins present in the crude ethanol tuber extract were detected through the preliminary phytochemical studies using different standard tests [3].

3. Phytochemical Evaluation of Powdered Flower Extract of *Tecoma stans*

The powdered flower sample of *Tecoma stans* was taken and phytochemical screening was done to check the phytoconstituents present using standard reagents.

a) Carbohydrates

Molisch's test

About 2ml of powdered flower extract was mixed with 0.2 ml of alcoholic solution of α- naphthol 10% in addition to 2 ml of sulphuric acid, a bluish violet zone is formed this indicates the presence of carbohydrates and /or glycosides.

b) Alkaloids

About 0.2 g of the powdered flower extracts was warmed with 2% H2S04 for two minutes. It was filtered and few drops of Dragendroff's reagent were added. Orange red precipitate indicates the presence of alkaloids.

c) Tannins

Small quantity of powdered flower extract was mixed with water and heated on water bath. The mixture was filtered and ferric chloride was added to the filtrate. A dark green solution indicates the presence of tannins.

d) Glycosides

The powdered flower extract was hydrolyzed with HCl solution and neutralized with NaOH solution. A few drops of Fehling's solution A and B were added. Red precipitate indicates the presence of glycosides.

e) Saponins

About 0.2 g of the powdered flower extract was shaken with 5ml of distilled water and then heated to boil. Frothing (Appearance of creamy miss of small bubbles) shows the presence of saponins.

f) Flavonoids

Powdered flower extract of about 0.2 g was dissolved in diluted NaOH and HCl was added. A yellow solution that turns colourless, indicates the presence of flavonoids.

g) Steroids (LB test)

2 ml of acetic anhydride was added to 0.5 g of the powdered flower of each with 2 ml of H2 S04.The colour changed from violet to blue or green in samples indicating the presence of Steroids.

h) Proteins

To the powdered flower extract, 5% NaOH and 1% copper sulphate solution were added. Violet color produced shows the presence of proteins.

i) Amino acids

The powdered flower was treated with Million's reagent. Red colour showed the presence of amino acids.

j) Phenolic compounds

Small quantities of powdered flower samples were taken separately in water and test for the presence of phenolic compounds was carried out by using reagents like 5% ferric chloride solution, 1% gelatin solution containing 10% NaCl and 10% lead acetate.

k) Gums and Mucilage

A small quantity of powdered flower extracts were added separately to 25ml of absolute alcohol with stirring and filtered. The precipitate was dried in air and examined for its swelling properties. No swelling was observed indicates the absence of gums and mucilage [4].

Table 1: Phytochemical evaluation of powdered flower extract of *Tecoma stans*

Extracts	Alkaloids	Flavonoids	Saponins	Tannins	Phenols	Steroids	Anthraquinones
Methanol	+	+	+	+	+	+	+
ethanol	+	+ -	+	+	+	+	+
Water	+	+	+	+	+	+	+
Ethyl Acetate	-	-	-	-	+	+	-

3. 1 Gc Ms Analysis

GC MS analysis was carried out on Shimadzu 2010 plus comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer instrument employing the following conditions: column RTX 5Ms (Column diameter is 0..32mm, column length is 30m, column thickness 0.50µm), operating in electron impact mode at 70eV; Helium gas (99.999%) was used as carrier gas at a constant flow of 1.73 ml /min and an injection volume of 0.5 µI was employed (split ratio of 10:1) injector temperature 270 ºC; ion-source temperature 200 ºC. The oven temperature was programmed from 40 ºC (isothermal for 2 min), with an increase of 8 ºC/min, to 150 ºC, then 8 ºC/min to 250 ºC, ending with a 20 min isothermal at 280 ºC. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 40 to 450 Da. Total GC running time is 51.25min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a Turbo Mass Ver 5.2.0 (Srinivasan *et al*., 2013) [5]

3.2 Identification of components

Interpretation on GCMS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The 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 (Dr. Dukes, 2013 [6]

4. Results

GC-MS is one of the best techniques to identify the constituents of volatile matter, long chain, branched chain hydrocarbons, alcohols acids, esters etc. The GC-MS analysis of *Tecoma stans* (Family: *Bignoniaceae*) revealed the presence of seventeen compounds (phytochemical constituents) that could contribute the medicinal quality of the plant. The identification of the phytochemical compounds was

confirmed based on the peak area, retention time and molecular formula. The active principles with their Retention time (RT), Molecular formula, Molecular weight (MW) and peak area in percentage are presented in Table and Fig 1 & 2. The first compound identified, Propane, 1,1,3-Triethoxy, 1'-Hydroxy-4,3'-Dimethyl-bicycl, Cyclobutanecarboxylic Acid, Decyl Ester, L-(+)-Ascorbic Acid 2,6-Dihexadecanoate, Hexadecanoic Acid, Ethyl Ester, 9,12-Octadecadienoic Acid (Z,Z).The phytochemicals identified through GC-MS analysis showed many biological activities relevant to this study are listed in Table 3. The biological activities listed are based on Dr. Duke's Phytochemical and Ethnobotanical Databases created by Dr. Jim Duke of the Agricultural Research Service/USDA.

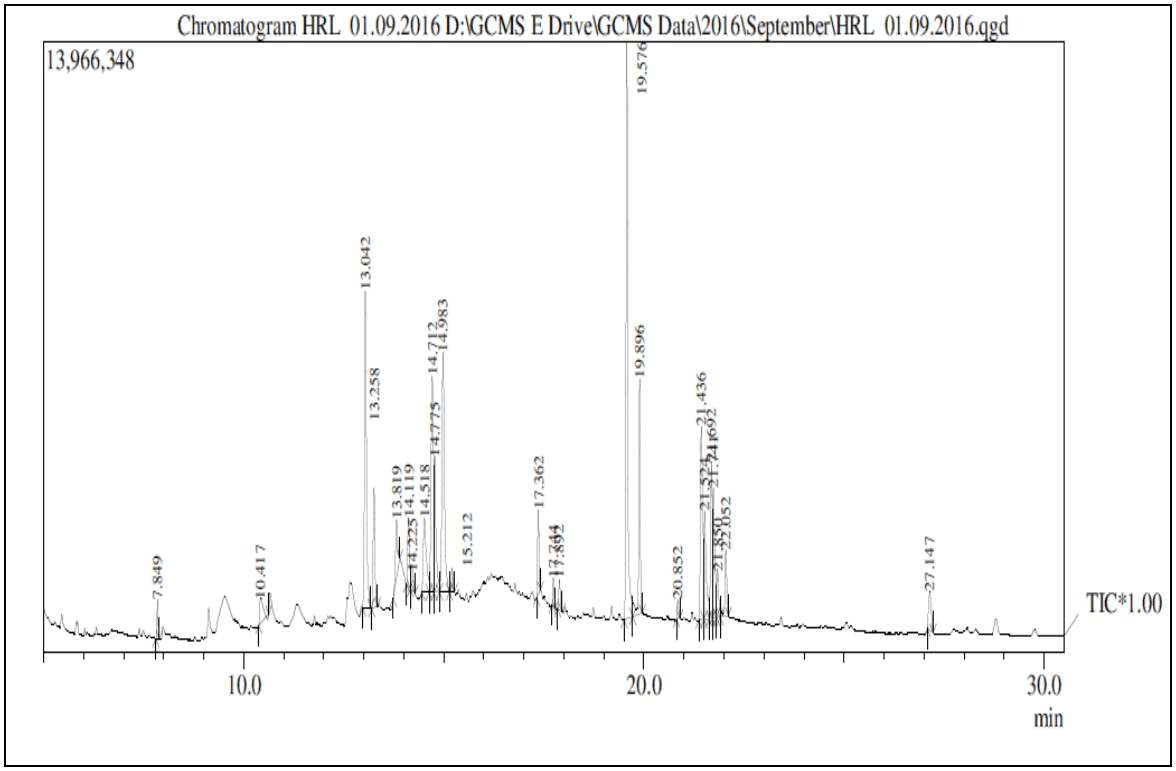

Fig 1: GC MS chromatogram of plant extract

Table 2: Bioactive compounds identified in the plant extract by GC-MS study.

Peak#	R. Time	Area%	Name of the compound	Molecular Formula	Molecular Weight
1	7.849	0.72	Propane, 1,1,3-Triethoxy-	$C_9H_{20}O_3$	176
2	10.417	1.34	5-Hydroxymethylfurfural	$C_6H_6O_3$	126
3	13.042	10.48	1'-Hydroxy-4,3'-Dimethyl-bicycl	$C_{14}H_{20}O_2$	220
4	13.258	3.25	9-Oxabicyclo[3.3.1]Nonan-2-One	$C_8H_{12}O_3$	156
5	13.891	2.10	1,10-Decanediol	$C_{10}H_{22}O_2$	174
6	14.119	1.98	1,2,3,4,7,7a-Hexahydro-2,4,7-Trimethyl-6H	$C_{11}H_{17}NO$	179
7	14.225	0.95	Tropane, 2-Acetyl-2,3-Methylene-	$C_{11}H_{17}NO$	179
8	14.518	3.84	5-Undecanol, 2-Methyl	$C_{12}H_{26}O$	186
9	14.712	8.89	6-Dodecanol	$C_{12}H_{26}O$	186
10	14.775	3.98	Silacyclopentane, 1,1-Dimethyl	$C_6H_{14}Si$	114
11	14.983	10.06	Cyclobutanecarboxylic Acid, Decyl ester	$C_{15}H_{28}O_2$	240
12	15.212	0.57	Propanamide, 3-(1-Piperazinyl)-	$C_7H_{15}N_3O$	157
13	17.362	0.57	Tetradecanoic Acid	$C_{14}H_{28}O_2$	228
14	17.744	0.55	Tetradecanoic Acid, Ethyl Ester	$C_{16}H_{32}O_2$	256
15	17.744	0.80	2(4h)-Benzofuranone, 5,6,7,7atetr	$C_{11}H_{16}O_3$	196
16	19.576	16.73	L-(+)-Ascorbic Acid 2,6-Dihexadecanoate	$C_{38}H_{68}O_8$	652
17	19.896	5.80	Hexadecanoic Acid, Ethyl Ester	$C_{18}H_{36}O_2$	284
18	20.852	0.41	N-Nonadecanol-1	$C_{19}H_{40}O$	284
19	21.436	7.82	9,12-Octadecadienoic Acid (Z,Z)	$C_{18}H_{32}O_2$	280
20	21.524	3.87	Ethyl (9z,12z)-9,12-Octadecadien	$C_{20}H_{36}O_2$	308
21	21.692	4.50	Octadecanoic Acid	$C_{18}H_{36}O_2$	284
22	21.741	4.47	N-Propyl 9,12-Octadecadienoate	$C_{21}H_{38}O_2$	322
23	21.850	1.39	9,12,15-Octadecatrienoic Acid, Ethyl Ester	$C_{20}H_{34}O_2$	306
24	22.052	1.98	Octadecanoic Acid, Ethyl Ester	$C_{20}H_{40}O_2$	312
25	27.147	1.61	Hexatriacontane	$C_{36}H_{74}$	506

Table 3: Activity of some of the phyto components identified in the extract by GC-MS.

S. No	R. Time	Name of the compound	Biological activity**
1.	17.36	Tetradecanoic acid	Antioxidant, Lubricant, Hypercholesterolemic, Cancer-preventive, Cosmetic
2.	19.86	Hexadecanoic Acid, Ethyl Ester	Antioxidant, hypocholesterolemic, Anti androgenic, hemolytic, Alpha reductase inhibitor.
3.	19.57	l-(+)-Ascorbic acid 2,6-dihexadecanoate	Vitamin C, Antioxidant, Immunomodulator
4.	20.85	N-Nonadecanol-1	Antiinflammatory, Hypocholesterolemic, Cancer preventive, Hepatoprotective, Nematicide, Insectifuge Antihistaminic, Antiarthritic, Anticoronary, Antieczemic Antiacne, 5-Alpha reductase inhibitor Antiandrogenic,
5.	21.43	9,12-Octadecadienoic Acid (Z,Z)-	Hypocholesterolemic, 5-Alpha reductase inhibitor, Antihistaminic, Insectifuge, Antieczemic, Antiacne
6.	21.85	9,12,15-Octadecatrienoic Acid, (Z,Z,Z)	Hypocholesterolemic, Nematicide Antiarthritic, Hepatoprotective, Anti androgenic, Nematicide 5-Alpha reductase inhibitor, Antihistaminic Anticoronary, Insectifuge, Antieczemic Anticancer
7	22.05	Octadecanoic acid	Cosmetic, Flavor, Hypocholesterolemic, Lubricant, Perfumery, Propecic, Suppository

**Dr. Duke's Phytochemical and Ethno botanical Databases, Phytochemical and Ethnobotanical Databases.

5. Conclusion

Preliminary phytochemical screening of the rhizomes of the *Tecoma stans (Family: Bignoniaceae* are (shown in the Table 2) investigation concluded that the stronger extraction capacity of ethanol has been produced number of active constituents responsible for many biological activities. So that those might be utilized for the development of traditional medicines and further investigation needs to elute novel active compounds from the medicinal plants which may be created a new way to treat many incurable diseases. 1,1,3-Triethoxy, 1'-Hydroxy-4,3'-Dimethyl-bicycl, Cyclobutanecarboxylic Acid, Decyl Ester, L-(+)-Ascorbic Acid 2,6-Dihexadecanoate, Hexadecanoic Acid, Ethyl Ester, 9,12-Octadecadienoic Acid (Z,Z).The phytochemicals identified through GC-MS analysis showed many biological activities relevant to this study are listed in (Table 3) however, require further testing.

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