PHYTOCHEMICAL SCREENING OF ETHANOL AND CHLOROFORM EXTRACT OF *TRICHODESMA INDICUM (LINN.) R.BR.* USING GAS CHROMATOGRAPHY AND MASS SPECTROMETER

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Trichodesma indicum (Linn.) R.Br. belongs to the famil

ABSTRACTy Boraginaceae reported to possess various bioactive compounds that plays significant role in curing a number of ailments. The objective of the present study is to investigate the phytochemical compound present in the *Trichodesma indicum*whole plant,using Gas Chromatography and Mass Spectrometry (GC-MS) analysis. 25 grams of powdered plant sample was first soaked in 100 ml of ethanol and chloroform solvent separately for 24 hrs. Samples soaked in solvents were sonicated using Ultrasonic Sonicator at 20 pulses for 20 minutes. Resultant samples were then centrifuged at 10000 rpm for 10 minutes, supernatant collected wasanalyzed using JEOL GCMATE II GC- MS system. The mass spectra obtained was compared with the National Institute of Standards and Technology library. The results showed peaks of thirteen compounds from ethanol extract and twelve compounds from chloroform extract. These compounds will be further studied for their pharmacological applications and purification of bioactive individual compounds would support in the discovery of novel drugs.

***Key words*: *Trichodesma indicum,* phytochemical compounds, ethanol, chloroform, GC-MS analysis.**

I. INTRODUCTION

Based on their function and role in the metabolism of plant, phytochemicals are commonly divided into two groups namely primary and secondary metabolites. Primary metabolites of plants commonly comprised of carbohydrates, proteins, amino acids, and chlorophylls.[1,2] While, the most important bioactive secondary metabolites in plants are alkaloids, tannins,flavonoids, and phenolic compounds, etc. these plant-based phytochemicals are reported to exert a broad range of therapeutic activities in humans. Phenolic compounds from plants were reported to increase bile secretion, reduces blood cholesterol[3] and possesses diverse biological activities, for instance, anti-inflammatory, antioxidant, antiulcer[4], antispasmodic, antitumor, and antidepressant activities[5]. Flavonoids from plants stated to possess anti-inflammatory activity, antimicrobial activity, anti-allergic activity, antioxidant activity, and antitumor activity.[6]Tannin present in plant extracts is used as antidiarrhea, diuretics, against stomach and duodenal tumours[7], they also exhibit anti-inflammatory, antiseptic, and antioxidant properties[8]. Alkaloids are major group of plant constituents showing many pharmacological activities that includes antihypertensive effects, antiarrhythmic effect, antimalarial activity, and anticancer actions.[9] Plant secondary metabolite terpenoids reported with medicinal properties such as anticarcinogenic, antimalarial, anti-ulcer, antimicrobial or diuretic activity.[10,11] Saponin present in plants observed to be act as

antiprotozoans, antifungal and antiviral.[12,13,14]Pharmaceutical industry uses phytocompounds from medicinal plants as important source in the process for developmentof noveldrug and therapeutic agents.[15]

Gas Chromatography and Mass Spectroscopy, is the most commonly used and highly compatible technique for the purpose of identification and quantification of known and unknown compounds. It plays a vital role in the identification of the valuable bioactive constituents of long chain hydrocarbons, alcohols, acids, ester etc.,[16]Some phytochemicals produced by plants have antimicrobial activity and used for the development ofnew antimicrobial drugs. It has been shown that *in-vitro* screening methods could provide the needed preliminary observations to select crude plant extracts with potentially useful properties for further chemical and pharmacological investigations.[17] These active principles usually remain concentrated in the storage organs of the plants such as roots, leaves, flowers, bark and etc., considering all these facts about medicinal plants,
T. indicum, an herbal weed is also reported to possess various therapeutic properties.

Trichodesma indicum (Linn) R.Br. belongs to family Boraginaceae. It is commonly known as Indian borage. It has worldwide distribution in India, Afghanistan, Philippines, Africa and Australia. It is usually found as weed on roadsides and wastelands throughout the greater part of India.[18] The whole plant is used to treat fever, dysentery, anorexia, snakebite poison, skin diseases, arthritis and the root is applied to reduce swellings, particularly of the joints; the extract is used to cure dysentery in children, fever and anti-inflammatory drug in folklore medicine.[19] Thus, the present study is aimed to identify the phytochemical constituents of ethanol and chloroform extracts of *T. indicum* plant by GC-MS analysis.

II. MATERIAL AND METHODS

2.1. Collection of Plant Materials

*Trichodesma indicum,*whole plant of was collected during the month of February2016 from the natural habitat of Sirumailur village, Kanchipuram district, Tamil Nadu, India. The plant material was identified and authenticated by Department of Botany, Ramakrishna Mission Vivekananda College, Chennai, India. Initiallycollected plant was washed with running tap water and then with sterile distilled water to remove the unwanted debris and kept for drying under shade for four weeks.

2.2. Preparation of Plant Extracts

Dried plant material was grounded to fine powder using a blender. 25g of powdered leaf material was drenched in 250 ml of ethanol and chloroform in two distinct Erlenmeyer's flasks for 24 hoursand were sonicated separately in an Ultrasonic Sonicator at 20 pulses for 20 minutes. The ethanol and chloroform plant extract were then centrifuged at 10000 rpm for 10 minutes. The supernatant was collected, labelled and stored at 4^{o}C until further use.

2.3. GC-MS analysis

GC-MS analysis of the ethanol and chloroform extract of the plant material was carried out with 2 µl of extract employed on a GC clarus 500 Perkin Elmer system comprising a AOC-20i auto sampler 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×1 EM df, composed of 100% dimethyl polysiloxane), 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 minutes), with an increase of

10°C/minutes, to 200°C/minutes, then 5°C/minutes to 280°C/minutes, ending with a 9 minutes isothermal at 280°C. MS were taken at 70 eV; a scan interval of 0.5 s and fragments from 40 to 550 Da. The MS of the unknown component was compared with the spectrum of the known components stored in The National Institute of Standards and Technology (NIST) library.[20] The name, molecular weight, and structure of the components of the test materials were ascertained.

2.4. Identification of compounds

Interpretation on mass spectrum obtained through GC-MS was conducted by comparing with the database of National Institute Standard and Technology (NIST) library having more than 62,000 known patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST (2008) library. The name, molecular weight and structure of the components of the test materials were ascertained.

III. RESULTS

GC-MS chromatogram of the ethanolic extract (**Figure - 1)** of *T. indicum* showedthe presence of thirteen bioactive components namely Pentadecanoic acid, 14-methyl-, methyl ester, 1-Propen-3-one, 3-(o acetylaminophenyl)-1-(p-methoxyphenyl)-, Hexadecanoic acid, ethyl ester, 13-Hexyloxacyclotridec-10-en-2-one, Phytol, a'-Ketostearic acid, Dasycarpidan-1-methanol,acetate (ester), 2a,3b,5b,6a-Tetramethoxycarbonyl-bicyclo(2,2,2)oct-7-ene, Octadecanedioic acid, dimethyl ester, 2-Heptenoic acid,[6-(4-cyanophenyl)2-naphthyl] ester, (Z,Z)-Octadeca-5,9-dienic acid, picolinyl ester, Glutaric acid monoamide,N-4-carbomethoxyphenyl-,benzoylmethyl ester, N,N-Dibutyl-3-[2-dibutylcarbamoyl (ethylthio)]propionamide**.** The identification of phytochemical compounds was carried out based on their retention time (RT), molecular formula (MF), molecular weight (MW). The % of peak area and compound structure were given in the**Table - 1**.

GC-MS chromatogram of the chloroform extract (**Figure - 2)** of *T. indicum* showed the presence of twelve bioactive components namely Tridecanoic acid, 12-methyl-, methyl ester, 11-Hexadecenoic acid, methyl ester, Hexadecanoic acid, methyl ester, 2,6-Dimethoxy-benzoylamino)-propionic acid, ethyl ester, Hexadecanoic acid, 15-methyl-, methyl ester, 13-Hexyloxacyclotridec-10-en-2-one,11-Octadecenoic acid, methyl ester, (Z)-, Octadecanoic acid, methyl ester, Oxiraneoctanoic acid, 3-octyl-, methyl ester, 4-Hexyl-1-(7-methoxycarbonylheptyl) bicycle(4.4.0)deca-2,5,7-triene, Nonadecanoic acid, 18-oxo-, methyl ester, 9-Octadecenoic acid (Z)-,2-hydroxy-1-(hydroxymethyl)ethyl ester. The phytochemical compounds were identified based on their retention time (RT), molecular formula (MF), molecular weight (MW). The % of peak area and compound structure were represented in **Table - 2**.

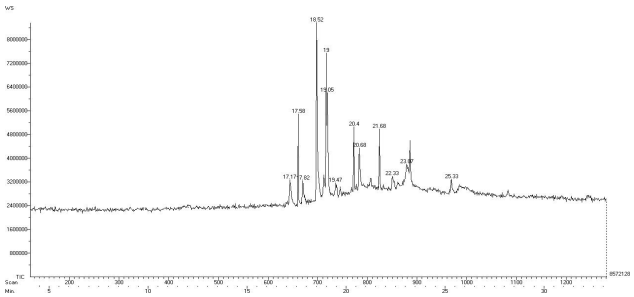
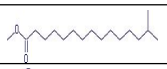
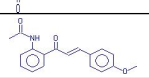
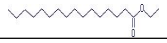
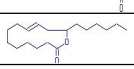
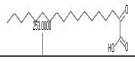
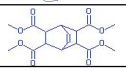
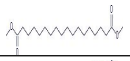
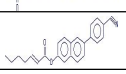
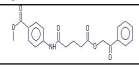


Figure - 1: GC-MS Chromatogram of ethanol extract of*T. indicum*

Table - 1: Phytocomponents identified in the ethanol extract of *T. indicum*

S. No.	RT	Name of the Compound	MF	MW	PA (%)	Molecular Structure
1	17.17	Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$	270.45	5.27	
2	17.58	1-Propen-3-one, 3-(o acetylaminophenyl)-1-(p-methoxyphenyl)-	$C_{16}H_{15}NO_2$	253.29	4.09	
3	17.82	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284.47	4.18	
4	18.52	13-Hexyloxacyclotridec-10-en-2-one	$C_{18}H_{32}O_2$	280.45	17.36	
5	19.0	Phytol	$C_{20}H_{40}O$	296.53	21.03	
6	19.05	a'-Ketostearic acid	$C_{18}H_{34}O_3$	298.46	21.03	
7	19.47	Dasycarpidan-1-methanol,acetate (ester)	$C_{20}H_{26}N_2O_2$	326.44	1.34	
8	20.40	2a,3b,5b,6a-Tetramethoxycarbonyl-bicyclo(2,2,2)oct-7-ene	$C_{16}H_{20}$	340.32	5.06	
9	20.68	Octadecanedioic acid, dimethyl ester	$C_{20}H_{38}O_4$	342.51	4.71	
10	21.68	2-Heptenoic acid,[6-(4-cyanophenyl)2-naphthyl] ester	$C_{24}H_{21}NO_2$	355.4	3.71	
11	22.33	(Z,Z)-Octadeca-5,9-dienic acid, picolinyl ester	$C_{24}H_{37}NO_2$	371.6	2.66	
12	23.07	Glutaric acid monoamide,N-4-carbomethoxyphenyl-,benzoylmethyl ester	$C_{21}H_{21}NO_6$	383.4	7.47	
13	25.33	N,N-Dibutyl-3-[2-dibutylcarbamoyl(ethylthio)]propionamide	:$C_{22}H_{44}N_2O_2S$	400.7	2.02	

RT- Retention Time, MF-Molecular formula, MW- Molecular weight, PA- Peak Area

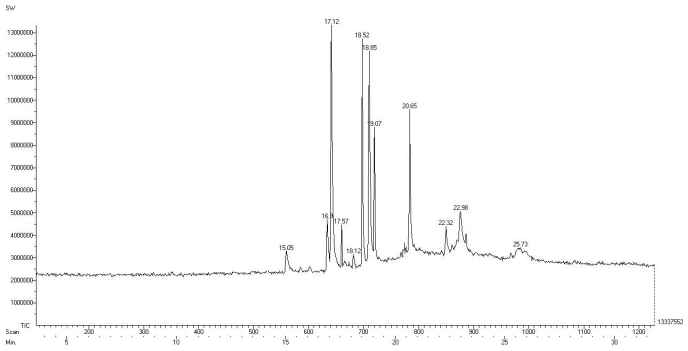
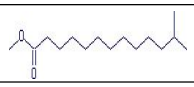
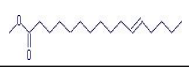
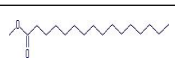
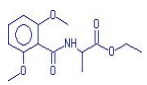
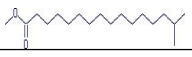
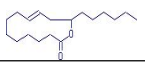
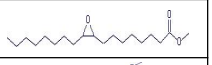
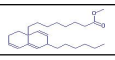
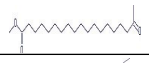
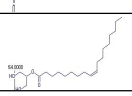


Figure - 2: GC-MS Chromatogram of chloroform extract of*T. indicum*

Table - 2: Phytocomponents identified in the chloroform extract of *T. indicum*

S. No.	RT	Name of the Compound	MF	MW	PA (%)	Molecular Structure
1	15.05	Tridecanoic acid, 12-methyl-, methyl ester	$C_{15}H_{30}O_2$	242.40	3.34	
2	16.90	11-Hexadecenoic acid, methyl ester	$C_{17}H_{32}O_2$	268.44	3.32	
3	17.12	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.45	28.90	
4	17.57	2,6-Dimethoxy-benzoylamino)-propionic acid, ethyl ester	$C_{14}H_{19}NO_5$	281.30	1.20	
5	18.12	Hexadecanoic acid, 15-methyl-, methyl ester	$C_{18}H_{36}O_2$	284.48	0.61	
6	18.52	13-Hexyloxacyclotridec-10-en-2-one	$C_{18}H_{32}O_2$	280.45	14.71	

7	18.85	11-Octadecenoic acid, methyl ester, (Z)-	$C_{19}H_{36}O_2$	296.48	19.02	
8	19.07	Octadecanoic acid, methyl ester	$C_{19}H_{34}O_2$	294.47	7.03	
9	20.65	Oxiraneoctanoic acid, 3-octyl-, methyl ester	$C_{19}H_{36}O_3$	312.49	11.09	
10	22.32	4-Hexyl-1-(7-methoxycarbonylheptyl) bicycle(4.4.0)deca-2,5,7-triene	$C_{28}H_{34}N_2O$	414.00	2.75	
11	22.98	Nonadecanoic acid, 18-oxo-, methyl ester	$C_{20}H_{38}O_3$	326.52	6.53	
12	25.73	9-Octadecenoic acid (Z)-,2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{21}H_{42}O_4$	358.55	1.44	

RT- Retention Time, MF- Molecular formula, MW- Molecular weight, PA- Peak Area

IV. DISCUSSION

Since most of the existing commercial drugs are prone to induce different side effects, on the other hand plant-based compounds could be useful in meeting the demand for newer drugs with minimal side effects.[21] Plant components that tend to possess therapeutic property to be used for producing newer drugs can be identified and characterized using GC-MS analytic technique. Thus GC-MS analysis of ethanol and chloroform extract of whole *T. indium* plant was performed. In the previous study, it was reported that the GC-MS analysis of methanolic leaf extract of *T. indium* revealed the presence of twenty bio active compounds whereas in the present study ethanol extracts of *T. indicum* whole plant exhibited the presence of one similar compound namely Phytol and twelve divergent compounds.[22] In the case of chloroform extract, two similar compounds namely Hexadecanoic acid, methyl ester; Octadecanoic acid, methyl ester and ten divergent compounds were identified.

Study reported that the GC-MS analysis of hydro methanolic extract of *T. indium* whole plant that revealed the occurrence of twenty-four bioactive compounds[23] while in the present study, ethanol and chloroform extracts of *T. indicum* whole plant extracted using ultrasonication exhibited the presence of overall twenty-five compounds. In sustenance to the pharmacological property said to be possessed by *T. indicum*, the identified phytochemical compounds in the present study said to have many biological properties for instance, Octadecanedioic acid, dimethyl ester has been reported to decrease blood cholesterol and possess anticancer, antifungal and antioxidant activity.[24] Similarly, Phytol and Hexadecanoic acid, methyl ester reported to contain anti-inflammatory activities, antischistosomal, antioxidant effects and antimicrobial activity[25,26,27]; Dasycarpidan-1-methanol, acetate (ester) have antimicrobial activity[28]; Nonadecanoic acid, 18-oxo-, methyl ester reported to exhibit antibacterial activity.[29]Tridecanoic acid methyl ester was evidenced to play a significant role as anti-enteric compound against enteropathogenic Gram-positive and Gram-negative bacteria related to several gastrointestinal diseases.[30]

V CONCLUSION

The presence of various bioactive components validates the use of the *T. indicum*as remedy for various ailments by traditional practitioners. The present study revealed the presence of phytocomponents withsignificant pharmacological activity in *T. indicum* that suggest that the furtherevaluation of these components maycontributeto the broad-spectrum application of the plant in the field of medicine. This type of phytochemical analysis of the medicinal plants have significance in both research institutes and pharmaceuticals companies for the manufacturing of the new drugs for treatment of various diseases in future.

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