PHYTOCHEMICAL AND GC-MS ANALYSIS ON LEAVES OF SELECTED MEDICINAL PLANTS IN BORAGINACEAE FAMILY *CORDIA DICHOTOMA L.*

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ABSTRACT

In the present study to investigate the phytochemical compound and GC-MS analysis of Cordia dichotoma leaves. The physicochemical properties such as total ash values (2.5 %) of leave sand maximum amount of extractive value observed in methanol and petroleum ether solvents (87.5 % and 88.25 %). The C. dichotoma methanol extract of the leaves shows the presence in phenolic compound, tannins and phytosterols. The methanolic extract contain Phenol (1.188±0.08 mg/g), Tannins (0.721±0.02 mg/g) and phytosterols (1.787±0.09 mg/g) were highly present and other compounds are absent. GC-MS study the showed that prevailing compounds are 1, 2 –benzenedicaboxylic acid (49.81%), Tris (2, 4 –di-tert-butyphenyl) phosphate (6.80%) and Octacosanal (4.80%), Octadecanoic acid, 2,3- dihydroxypropoyl ester (4.83%), stigmast-5-en-3-ol,(3,beta), stigma-7,22-dien-3-ol, acetate, (3beta., 5.alpha., 22e). out of 5 hits as obtained from NIST mass spectral library. This will enable the use of our own local, rich plant heritage as effective medicines with no side effects.

***Keywords**: Phytochemical, Cordia dichotoma leaves, GC-MS analysis*

INTRODUCTION

The search for natural products to cure diseases represents an area of great interest in which plants have been the most important source. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these plants bioactive chemical constituents are alkaloids; tannins, flavonoids, and phenolic compounds. It is now clear that, the medicinal value of these plants lies in the bioactive phytochemical constituents that produce definite physiological effects on human body. These natural compounds formed the base of modern drugs as we use today .There are more than 35,000 plant species being used in various human cultures around the world form medicinal purpose.

Plants as sources of bioactive compounds continue to play a dominant role in the maintenance of human health. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions. Medicinal plants have

curative properties due to the presence of various complex chemical substances of different composition, which are found as secondary plant metabolites. They are naturally synthesized in the plant body and any part of the plant body may contain active components. Extraction and characterization of several active phytocompounds from these green factories have given birth to some high activity profile drugs. *Heliotropium idicum, Cordia dichotoma and Cordia sebestena* belongs to the family Boraginaceae.

Usually the drugs are collected by traditional practitioners who have inherited Ayurvedic or other herbal practices. Their identification is mostly based on morphological features or other traditionally known characteristics. In such cases, there is a chance of selecting incorrect raw drugs. Therefore, an extensive anatomical and phytochemical screening is needed for each raw drug used in the formulation to avoid any ambiguity and such a study will serve also as a reference for further studies (Vaibhav and Kamlesh, 2007). Physicochemical standards of particular plants which may ensure and maintain its quality, efficacy and safety profile (Laloo *et al.,* 2013). Standardization practices continue today because of its biochemical benefits as well as place in cultural benefits in many parts of the world and have made a great contribution towards maintaining human health (Nasreen and Radha, 2011).

The phenolic compounds found in plants along with several other endogenous metabolites are found as free radical scavenging molecules rich in antioxidant activity (Velioglu *et al.,* 1998). The antioxidant activity of phenolic compounds was found to be mainly due to their scavenging and redox properties, through neutralizing and quenching free radicals. These are non-nutritive chemical that have protected human from various diseases. These phytochemical constituents are the basic source for the establishment of several pharmaceutical industries. The constituents are playing a significant role in the identification of crude drugs (Savithramma *et al.,* 2011).

Cordia dicodoma tree is used traditionally in treatment of dyspepsia, fever, ringworm, ulcers, prolapsed of uterus/vagina, headache, infection of urinary passage, diseases of lungs and spleen. The leaves, fruit, bark and seeds have been reported to exhibit antidiabetic, antiulcer, anti-inflammatory, immune modulator and analgesic activites. *C. dicodoma* is used in Ayurveda and unani system of medicine for treating cold, cough, fever and skin disease. The leaves are useful as an application to ulcers and in headache. The decoction of leaves is used in cough and cold. 50-100ml of leaves decoation, can be taken in dosage. In this present study the leaves of, *Cordia dichotoma* were screened in phytochemical compound and GC-MS analysis.

MATERIALS AND METHODS

Identified in the herb of local flora

The whole plants of, *Cordia dichotoma* were collected from Nagachi, Thanjavur Dt, Tamil Nadu, India. The plants were identified then there were washed under running tap water to remove the surface pollutants and the whole plants were air dried under shade. The dried sample was powdered and used for further studies.

Extract Preparation

The three plant leaf powder was extracted with 70% methanol and petroleum ether for 24 hours. The extract was stored in refrigerator until used for physicochemical and phytochemical analysis, Trease and Evans (1989) and Harborne (1973, 1984).

Chemicals

Methanol, petroleum ether, alcoholic solution, α-naphthol, concentrated sulphuric acid, 2% copper sulphate, 95% ethanol, potassium hydroxide, ninhydrin solution, hydrochloric acid, hager's reagent, 5% ferric chloride, 10% ammonium hydroxide, magnesium ribbon, glacial acetic acid, acetic anhydride, phenolphthalein, anthrone reagent, phosphate buffer, chloroform, amyl alcohol, folin-denis reagent, sodium carbonate, potassium hexacyanoferrate were used for this study.

Physico Chemical Analysis

Preparation of powder sample

Leaves of, *C. dichotoma, were* shade dried and mechanically powdered after keeping them in oven at 35°C for 24 hrs. These powdered materials were used for further physiochemical and phytochemical analysis. The procedure recommended in Indian pharmacopeia 1960 was followed for calculating the total ash value and extractive value. The percentages of extractive value in different solvents (methanol and petroleum ether) were calculated.

Determination of Total Ash

The powder material (2g) was accurately weighted and placed in a crucible. The material was spread in an even layer and it was ignited to a constant weight by gradually increasing the heat to 500-600C until it was white indicating the absence of carbon. The residual ash was allowed to cool in a desiccator. The content of total ash (in mg) of air-dired material was calculated as follow.

$$\% \text{ of total ash} = \text{weight of ash/weight of sample taken x } 100$$

Determination of Alcohol Soluble Extractive

Accurately weighted powdered leaf material (4gm) was placed in a glass stopper round bottle flask 100 ml. Methanol and petroleum ether was added, it was shaken well and allowed to stand for 1h. A reflux condenser was attached and boiled gently for 1h, and then it was cooled and filtered. The flask was shaken well and filtered rapidly through a dry filter paper. After that, 25ml of the filtrate was transferred to a tarred flat bottomed dish and evaporated to dryness on a water bath. Then the dish was dried at 105c for 6h and cooled in a desiccators and weighted. The content of extractable matter (% w/w) air dried material was calculated as follows:

$$\% \text{ of alcohol soluble extractive} = \text{weight of residues/weight of sample x}100$$

Qualitative phytochemical screening

The whole plant extract of *C. dichotoma,* were analyzed for the presence of major phytochemicals such as Carbohydrates, Proteins, Alkaloids, Saponins, Phenolic compounds, Tannins, Flavonoids, Glycosides, cardiac glycosides, Phytosterols, Fixed oils & fats, and Gums & mucilages according to standard methods (Raaman, 2006).

Carbohydrates - Molish's test (Ramakrishnan *et al.,* 1994)

About 100 mg of the extract was dissolved in 5ml of water and filtered. Two drops of alcoholic solution of α-naphthol was added to 2ml of the filtrate and 1ml of concentrated sulphuric acid was added slowly along the sides of the test tube and allowed to stand. A violet ring indicated the presence of carbohydrates.

Proteins - Biuret test (Gahan, 1984)

The extract (100 mg) was dissolved in 10 ml of distilled water and filtered through whatman No.1 filter paper. 2ml aliquot of the filtrate was treated with one drop of 2% copper sulphate solution. To this , 1ml of 95% ethanol was added, followed by excess of potassium hydroxide pellets. Pink color in the ethanolic layer indicated the presence of proteins.

Amino acids - Ninhydrin test (Yasuma and Ichikawa, 1953)

Two drops of ninhydrin solution was added to 2ml of aqueous filtrate. The presence of amino acids was indicated by the presence of a characteristic purple colour.

Alkaloids - Hager's test (Wagner *et al.,* 1996)

Solvent free extract, 50 mg was stirred with 5ml of dilute hydrochloric acid and filtered. To the filtrate, 2ml of Hager's reagent (saturated aqueous solution of picric acid) was added. A prominent yellow precipitate indicated the test as positive.

Saponins - Frothing test (Kokate, 1999)

The extract (50mg) were diluted with distilled water and made up to 20ml. The suspension was shaken in a graduated cylinder for 15 minutes. A 2cm layer of foam indicated the presence of saponins.

Phenolic compounds - Ferric chloride test (Mace, 1963)

About 50 mg of the extract was dissolved in 5ml of distilled water. To this, few drops of neutral 5% ferric chloride solution was added. Phenolic compounds were indicated by the presence of dark green colour.

Tannins - Potassium hydroxide test (Williamson *et al.,* 1996)

The extract (0.5g) were added into 10ml of freshly prepared 10% potassium hydroxide (KOH) in a beaker and shaken to dissolve. A dirty precipitate indicated the presence of tannin.

Flavonol glycosides - Magnesium and hydrochloric acid reduction (Harborne, 1998)

The extracts (50mg) were dissolved in 5ml alcohol and few fragments of magnesium ribbon were added. Concentrated hydrochloric acid was added drop wise into the test tube. Development of pink or crimson colour indicated the presence of flavonol glycosides.

Cardiac glycosides - Keller Killiani test (Ngbede *et al.,* 2008)

Total 100mg of extracts were dissolved in 1ml of glacial acetic acid containing one drop of ferric chloride solution. This was then under layered with 1ml of concentrated sulphuric acid. A brown ring obtained at the interface indicated the presence of a deoxy sugar characteristic of cardinolides.

Phytosterols - Libermann and Burchard's test (Finar, 1986)

About 50mg of extracts were dissolved in 2ml of acetic anhydride. To this, one or two drops of concentrated sulphuric acid were added slowly along the sides of the test tube. An array of colour changes showed the presence of phytosterols.

Gums and mucilages - Absolute alcohol test (Whistler and BeMiller, 1993)

The extracts (100 mg) were dissolved in the 10 ml of distilled water and to this 25 ml of absolute alcohol were added with constant stirring. White or cloudy precipitate indicates the presence of gums and mucilages.

Quantitatively analysis of Phytochemicals compounds

Carbohydrate test - Anthrone test

2ml plant extract were added in 1ml of water and 4ml anthrone reagent was added in test tubes then heat and kept in a boiling water bath for 10 minutes after cool it rapidly, color would change to blue green. The carbohydrates measure the optical density of this color at 630 nm using spectrophotometer.

Protein test - Biuret method

In this method, the reagent dilute copper sulfate solution at alkaline pH react with peptides proteins. This complex undergoes a blue to violet color transformation that requires five minutes to complete. The protein is quantitated at 540nm.

Alkaloids test

To 1ml of methanolic extract 5ml pH 4.7 phosphate buffer was added and sample solution shake a mixture with 4ml of chloroform. The extracts were collected in 10ml volumetric flask and then diluted to adjust to volume with chloroform. The absorbance of the complex in chloroform was measured at 470nm.

Saponins test

Methanolic and petroleum ether extract was dissolved in 80% methanol, 2ml of vanillin in ethanol was added, mixed well and 2ml of 72% sulphuric acid solution was added, mixed well and heated on a water bath at 60°C for 1min, absorbance was measured at 544 nm against reagent blank. Diosgeninis used as a standard material and compared the assay with diosgenin equivalents.

Phenol test

The extract was boiled with 50ml of ether for the extraction of the phenolic component for 15 minutes. 5ml of the extract was pipette into a 50ml flask, then 10ml of distilled water was added. 2ml of ammonium hydroxide solution and 5ml of concentrated amyl alcohol were also added. The sample were made up to make and left to react for 30 minutes for colour development. This was measured at 505nm in a spectrophotometer.

Tannins test

2ml of extract was taken in test tube. Then 5ml of Folin – Denis reagent added in side test tube after 10ml of sodium carbonate solution were added and few drop of water mixed and the solution shaken well then take the solution at 700nm of spectrophotometrically.

Phytosterols test

1ml of methanolic extract of steroid solution was transferred into 10ml volumetric flasks. Sulphuric acid (4N, 2ml) and iron(III) chloride (0.5% w/v,2ml), were added followed by potassium hexacyanoferrate(III) solution 5ml. The mixture was heated in a water-bath maintained at 70±20c for 30 minutes with occasional shaking and diluted to the make with distilled water. The absorbance was measured at 780nm against the reagent blank.

GC-MS Analysis

Preparation of plant extract

20g of powdered plant material was soaked in 50ml of absolute alcohol overnight and then filtered through whatmann filter paper No.41 along with 2 g of sodium sulphate to remove the sediments and traces of water in the filtrate, before filtering. The filter paper along with sodium sulphate was wetted with absolute alcohol. The filtrate was then concentrated by bubbling nitrogen gas into the solution and reduced the volume to 1ml. the extract contain both polar and non-polar phytocomponents of the plant material used.

GC-MS Condition

GC-MS was performed with Hewlett-Packed (HP) 7683/5975 equipment conditions modified from a methods published by Frankel *et al.,* (1995). Compounds were separated on a 30m × 250 µm × 0.25 µm capillary column coated with a 0.25 µm film of HP-5-MS (J and W Scientific, Folson, USA) sample were injected with split ratio of 10:1 helium was used as carrier gas at 1.0 ml min^{-1}. The column temperature was maintained at 100 °C for 1 minute, after injection then increased at 10 min^{-1} to 275 °C which was sustained for 20 minutes. The time required for chromatography of one sample 40 minutes.

Analysis of the phytocomponents of leaves in, *C. dichotoma*, using GC-MS techniques.

One micro liter of the filtrate was injected into the GC-column. There the sample get evaporated and carried away by the carrier gas, helium and it get segregated into individual components. The sample fraction coming out of the column was let into the mass detector and the mass spectrum of each component was recorded. The mass spectrum of the unknown component was compared with the known spectrum of NIST library and the components were identified.

Statistical Analysis

The results obtained in the present investigation were subject to statistical analysis like Mean $(\bar{x})$ and Standard Deviation (SD) by Zar (1984).

$$Mean\ (\bar{x}) = \frac{\sum x}{N}$$

$$\text{Standard Deviation } (\delta) = \sqrt{\frac{\sum (x - \bar{x})^2}{N - 1}}$$

Where,

Add together all the values of X and $\sum x$

N – Total number of observation

RESULTS

This present study was carried out on phytochemical and GC-MS analysis on leaves of *C. dichotoma*. The findings of results were presented in this chapter.

Physico Chemical Analysis

The physicochemical properties such as total ash values and solvent extractive value were analyzed. The investigated results were presented *C. dichotoma ash value* (2.5 %) of leaves. The *C. dichotoma* powder leaves of maximum amount of extractive value observed in methanol and petroleum ether solvents (87.5 % and 88.25 %).

Qualitative Phytochemical screening the leaves of *C. dichotoma*

In these investigation carbohydrates, protein, alkaloids, saponin, tannins and phytosterols showed positive results and amino acids, phenolic compound, flavonol glycosides, cardiac glycosides and gum mucilages were absent in *C. dichotoma*. Leaves of petroleum ether extract investigated results were presented in Table - 1. The *C. dichotoma* methanol extract of the leaves shows the presence in phenolic compound, tannins and phytosterols.

Quantitative analysis of phytochemical compounds in *C. dichotoma*

phytochemical constituents of the leaves of petroleum ether extract of *C.dichotoma* were quantitatively estimated. The investigated results were present in Table – 2 and Fig. – 1. Amount of phytosterol and tannin is equal in both extract (1.787±0.08; 1.747±0.09 and 0.750±0.02; 0.721±0.02). Compare the phytochemical compounds in methanolic and petroleum ether extract. The petroleum ether contain phytosterol (1.747±0.02 mg/g) and protein (1.642±0.02 mg/g) content maximum level present compare than other phyto compounds. The methanolic extract contain Phenol (1.188±0.08 mg/g), Tannins (0.721±0.02 mg/g) and phytosterols (1.787±0.09 mg/g) were highly present and other compounds are absent.

Table – 1 Phytochemical screening of the leaves of *C. dichotoma*

S. No.	Phytochemicals Name	*Heliotropium indicum*		
		Control	Methanol	Petrolium ether
1	Carbohydrates	-	+	+
2	Protein	-	-	-
3	Amino acids	-	-	-
4	Alkaloids	-	+	-
5	Saponins	-	+	+
6	Phenolic compound	-	+	-
7	Tannins	-	+	+
8	Flavonol glycosides	-	-	-
9	Cardiac glycosides	-	-	-
10	Phytosterols	-	+	+
11	Gums and mucilages	-	-	-

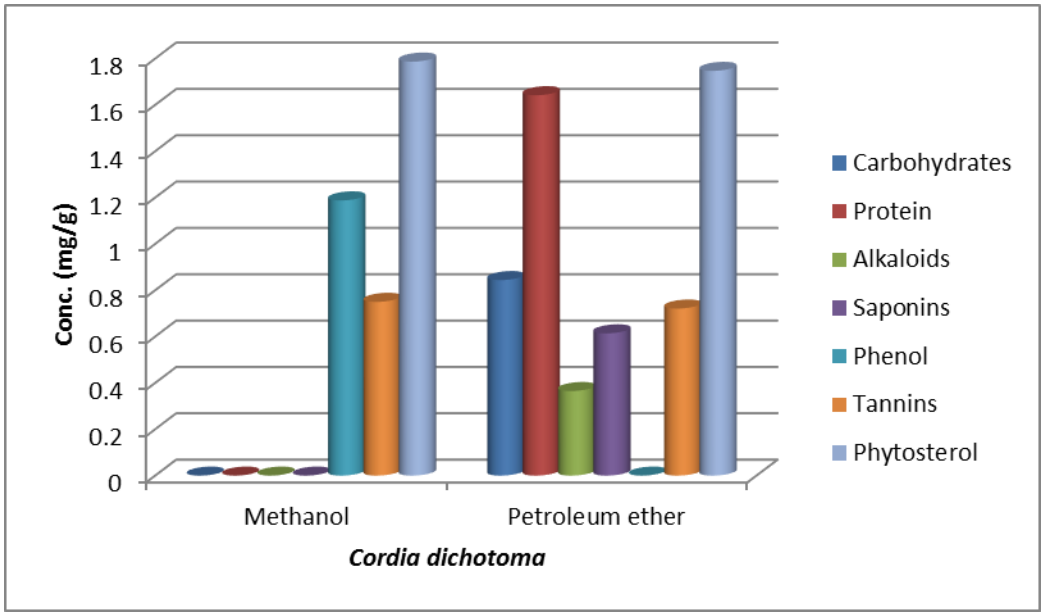
(+) present; (-) absent.

Table – 2 Quantitative analysis of phytochemical compounds in *Cordia dichotoma*

S. No.	Phytochemicals Name	*Cordia dichotoma*	
		Methanol	Petroleum ether
1	Carbohydrates (mg/g)	-	0.844±0.08
2	Protein (mg/g)	-	1.642±0.02
3	Alkaloids (mg/g)	-	0.365±0.05
4	Saponins (mg/g)	-	0.614±0.07
5	Phenol (mg/g)	1.188±0.05	-
6	Tannins (mg/g)	0.750±0.02	0.721±0.02
7	Phytosterol (mg/g)	1.787±0.08	1.747±0.09

Values are expressed in Mean ± Standard deviation; n=3

Fig. –1 Quantitative analysis of phytochemical compounds in *Cordia dichotoma*



Analysis of Phytochompound by Gas Chromatography-Mass Spectral technique

C. dichotoma (Leaves) phytocompounds were analyzed by Gas Chromatography-Mass Spectral technique. The investigated results were presented in Table-3 fig – 2.

Identification of Components

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unkown components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained. The biological activities listed in (Table-9)

are based on Dr. Duke's phytochemical and Ethanobotanical Databases by Dr. Jim Duke of the Agricultural Research Service/USDA.

C. dichotoma (Leaves) contain different compounds were present in the table 10 and Fig 6. *C.dichotoma* was Subjected to GC-MS study for identification of medicinal properties, According to the results, the Phytocomponents are screened, and most of the medicinal properties, such as high level prevailing compounds are 1, 2 –benzenedicaboxylic acid (49.81%), Tris (2, 4 –di-tert-butyphenyl) phosphate (6.80%) and Octacosanal (4.80%), Octadecanoic acid, 2,3- dihydroxypropoyl ester (4.83%), STIGMAST-5-EN-3-OL,(3,BETA), stigma-7,22-dien-3-ol, acetate, (3beta., 5.alpha., 22E). Out of 5 Hits as obtained from NIST08 mass spectral library, Hit11 matched compound 16 and was identified as Eicosane, Hit 19 matched compound 22 and was identified as 1-Acetoxynonadecane, Hit26 matched compound 30 and was identified as 1,2-BENZENEDICCARBOXYLIC ACID and the respective molecular formula, molecular weight and structure were considered for identification. Thus, this type of GS-MS analysis is the first step towards understanding the nature of active principles in this medicinal plant and this type of study will be helpful for further detailed study.

Biological activity

The existence of various bioactive chemical compounds proves the use of this plant for various ailments by traditional medical practitioners. Thus the plant studied can be used as a potential source of new useful drugs. The phytochemical characterization of the extracts, the isolation of bioactive components and their biological activity are necessary for future studies. The presence of various bioactive compounds justifies the use of leaves of *cordia dichotoma* for various ailments by traditional practitioners. Biological activities present in tables (4).

Table – 3 Analysis the Phytochompound by GC-MS techniques - methanolic leaf extracts of *Cordia dichotoma*

Peak#	R.Time	Area	Area%	Name
1	8.110	56010	0.20	Nonane, 5-Butyl-
2	12.33	93572	0.33	Hexadecane
3	13.63	94974	0.33	Hexadecane
4	15.50	99892	0.35	Heptadecane
5	16.27	10360	0.37	Hexadecane
6	17.29	89830	0.32	Octadecane
7	18.48	80505	0.28	Benzene, (1-Ethylundecyl)-
8	19.00	70003	0.25	Heptadecane
9	19.19	82006	2.89	Benzene, (1-Methyldodecyl)-
10	19.46	28066	0.99	Hexadecanoic Acid, Methyl Ester
11	19.97	16276	0.57	Eicosane
12	20.35	24909	0.88	N-Hexadecanoic Acid
13	22.77	13458	0.47	2-Methyltetracosane
14	22.96	34410	1.21	Phytol

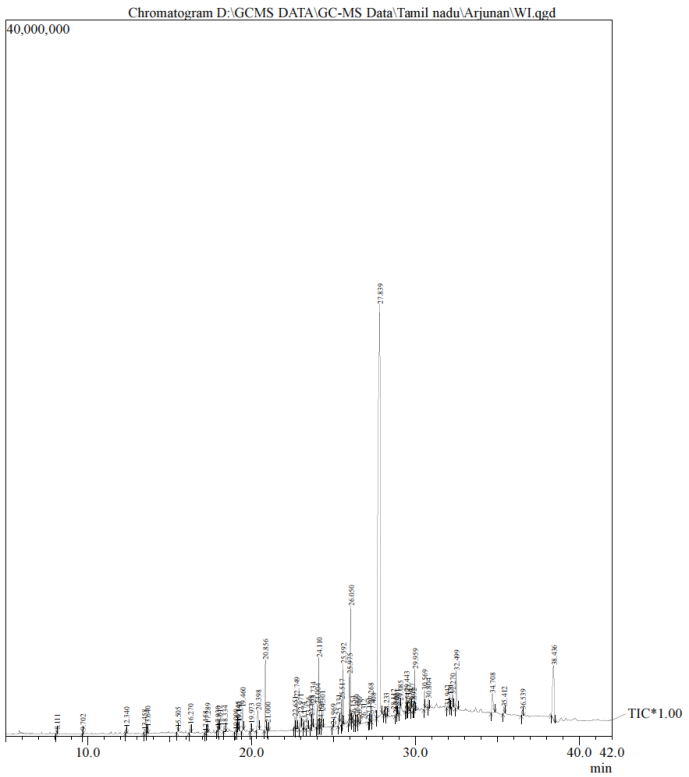
15	23.17	79621	0.28	Octadecanoic Acid, Methyl Ester
16	23.67	13304	0.47	Eicosane
17	24.10	57534	0.20	
18	24.19	70028	0.25	Heneicosane
19	24.30	35972	1.27	1-Acetoxynonadecane
20	25.33	27201	0.96	Oxiraneoctanoic Acid, 3-Octyl-, Methyl Ester, Cis-
21	26.15	11235	0.40	Tetracosane
22	26.54	17010	0.60	1-Acetoxynonadecane
23	26.97	51396	0.18	
24	27.07	61506	0.22	1,1,1,3,3,3-Pentaisopropyldisiloxane
25	27.19	20712	0.73	2,3-Dihydroxypropyl Icosanoate, 2TMS Derivative
26	27.26	10319	0.36	1,2-Benzenedicarboxylic Acid
27	27.35	31650	0.11	Pentacosane, 13-Phenyl-
28	27.40	22224	0.78	Octadecane, 1-Chloro-
29	27.48	31148	1.10	1-(4-Undecylphenyl)Ethanone
30	27.80	14133	49.81	1,2-Benzenedicarboxylic Acid
31	27.90	28402	0.10	Tetracyclo[6.1.0.0(2,4).0(5,7)]Nonane, 3,3,6,6,9,9-
32	28.10	13173	0.46	Behenyl Chloride
33	28.24	11393	0.40	
34	28.34	66192	0.23	Tetracyclo[6.1.0.0(2,4).0(5,7)]Nonane, 3,3,6,6,9,9-
35	28.81	11280	0.40	3,3,6,6,9,9-Hexaethyltetracyclo[6.1.0.0~2,4~.0
36	28.90	17803	0.63	1h-Indole-3-Ethanamine
37	29.02	28690	0.10	Benzene, (1-Propylheptadecyl)-
38	29.09	13189	0.46	Tetradecane
39	29.14	30106	1.06	
40	29.34	13698	4.83	Octadecanoic Acid, 2,3-Dihydroxypropyl Ester
41	29.59	13033	0.46	Benzene, 2-(1-Decylundecyl)-1,4-Dimethyl-
42	29.77	82350	0.29	Heptacosane, 1-Chloro-
43	29.90	57352	0.20	Tetracyclo[6.1.0.0(2,4).0(5,7)]Nonane, 3,3,6,6,9,9
44	29.96	19403	0.68	Squalene
45	30.15	53010	0.19	Henicosanal
46	30.32	89854	0.32	Benzene, Hexadecylpropyl-
47	30.57	55068	0.19	Heneicosane
48	30.62	67409	0.24	Pentafluoropropionic Acid, Hexadecyl Ester
49	31.66	37063	1.31	Docosanal
50	32.12	34348	1.21	Hexacosane
51	32.26	46828	1.65	Stigmasta-7,22-Dien-3-Ol, Acetate,
52	33.60	67031	2.36	Heptacosanal
53	34.17	80653	0.28	Hexacosane
54	34.71	62537	2.20	Stigmast-5-En-3-Ol, (3.Beta.)-
55	36.29	13611	4.80	Octacosanal
56	38.46	19279	6.80	Tris(2,4-Di-Tert-Butylphenyl) Phosphate
57	40.18	27667	0.98	Octacosanal
		28372	100.0	

Table – 4 Biological activity of some of the phytocomponents identified in the methanolic leaf extracts of *Cordia dichotoma*

S. No.	R. Time	Name of the compound synonym	Biological activity
1	27.804	1,2 Benzenedicarboxylic Acid	Antiarthritic, Anticancer, Anti inflammatory, Antialzhemeran, 5 lipoxygenase inhibitor
2	29.342	Octadecanoic acid, 2,3-dihydroxy propyl ester	Cosmetic, Flavor, Hypocholesterolemic, metastatic
3	31.669	Docosanal	Anti parkinsonian, Antioxidant
4	32.127	Hexacosane	Antidiabetic, Antiatherosclerotic
5	32.269	Stigmasta-7,22-dien-3-ol, acetate(3 beta., 5 alpha., 22E	Antiulcerogenic, Antithrombotic
6	36.296	Octacosanal	Antineuritic , Antipasmodic
7	38.462	Tris(2,4-di-tert-butylphenyl) phosphate	Antiproliferent

*Source Dr. Dukes phytochemical and ethano botanical databases (online database)

Fig 2 Chromatogram of methanolic leaf extracts of *Cordia dichotoma*



DISCUSSION

In this present study the leaves of *C. dichotoma* were screened for phytochemicals and analyzed by GC-MS. The investigated results were discussed with previous theoretical and statistical reports in this chapter.

Hamburger and Hostettmann, (1991) reported that the total number of plant chemicals may exceed 400,000 and out of it more than 10,000 are secondary metabolites whose major role in plant is defensive in nature. Thus, plant based secondary metabolites, which have defensive role may be exploited for the management of storage pest. However, the most species of higher plants have never been described surveyed. Their chemical or biologically active constituent which is potential to be used as new sources of commercially valuable pesticides remain to be discovered (Balandrin *et al.,* 1985). This is mainly due to the lack of information on the screening/evaluation of diverse plants for their antibacterial potential.

In these investigation carbohydrates, protein, alkaloids, saponin, tannins and phytosterols showed positive results in *C. dichotoma* leaves petroleum ether extract. Many substances may be antimicrobial, but only a few of them will be potential therapeutic agents for the simple reason that mammalian cells are more sensitive to chemical inhibition than microbial cells (Sivakumar and Alagesaboopathi, 2006). Moreover emphasized the need for toxicity testing of drugs derived from medicinal plants because the crude products obtained from such cheaper sources are often associated with a large number of compounds that have discomforting abilities (Ramdas *et al.*, 2006). Hence the herbal drugs have to be subjected to extensive pharmacological, toxicological and clinical tests to confirm the prescribed status. Thus the ethnobotanical approach will be like a search for molecular diversity subjecting a wide variety of new molecules from plant sources and testing them with as many different tests as possible (Muhammad and Muhammad, 2005).

Earlier studies have shown the biological activities of phenolics and flavonoids as potent antioxidants and free radical scavengers. It has also been reported that ingestion of polyphenolic compounds from a diet rich in fruits and vegetables daily upto 1 g has inhibitory effects on mutagenesis and carcinogenesis in humans (Benzie and Szeto, 1999).

In this study carbohydrates, protein, saponins, tannin and phytosterols were found in petroleum ether extract *C. sebestena* leaves. At the same time saponins, tannin and phytosterols observed in both extracts. Among the three plants petroleum ether extract has maximum number of phytochemical compounds compare than methanolic extract. Results of present research indicated were supported by the work done by various works. Alcoholic leaf extract was found to have antibacterial effect against the pathogen by Gehlot and Bohra, (2000). Several workers have reported that many plants possess antimicrobial properties including the parts which include; flower, bark, stem, leaf, etc. It has been shown that when solvents like ethanol, hexane and methanol are used to extract plants, most of them are able to exhibit inhibitory effect on both gram positive and gram negative bacteria (Bushra Beegum and Ganga Devi, 2003).

Plants with antioxidant activities have been reported to possess free radical scavenging activity (Das and Pereira, 1990). Free radicals are known as a major contributor to several

clinical disorders such as diabetes mellitus, cancer, liver diseases, renal failure and degenerative diseases as a result of deficient natural antioxidant defense mechanism (Parr and Bolwell, 2000).

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GC–MS analysis of the methanol extracts revealed the presence of various bioactive compounds. The prevailing compounds as per the peak report were Di-n-octyl phthalate (55.46%), Tris (2,4- di-tert-butyl phenyl) phosphate (5.91%) and 912,15-Octadecatrionic acid, ethyl ester (Z,Z,Z) (3.80%), trans,trans-9,12- octadecatrienoic acid, ethyl ester (Z,Z,Z) (1.49%) are best known for their antioxidant properties, which has led to their evaluation in a number of diseases associated with reactive oxygen species (ROS) such as cancer, cardiovascular and neurodegenerative diseases (Ajayi *et al.,* 2011). The 1, 2 –benzenedicaboxylic acid (49.81%), Tris (2, 4 –di-tert-butyphenyl) phosphate (6.80%) and Octacosanal (4.80%), Octadecanoic acid, 2,3- dihydroxypropoyl ester (4.83%), STIGMAST-5-EN-3-OL,(3,BETA), stigma-7,22-dien-3-ol, acetate is present in methanol extract but in different quantities. The three crude extracts did not reveal a common major compound in them. Based on studies, some of the constituents revealed by GC–MS are biologically active compounds.

GC-MS analysis of ethyl acetate extract of Goniothalamus umbrosus revealed the presence of n-Hexadecanoic acid, Hexadecanoic acid, 9, 12 - Octadecadienoic acid, n-hexadecanoic acid, 9, 12, 15-Octadecatrienoic acid, Squalene and Phytol were identified in the ethanol leaf extract of *C. dichotoma*. These reports are in accordance with the result of this study.

The source of many plants (herbs and spices) can be often identified from the peak pattern of the chromatograms obtained directly from headspace analysis. Similarly, particular qualitative and quantitative patterns from a GC analysis will show all the compounds in the leaf extract. *C. dichotoma* have many biological properties which can be used in various purposed to treat many diseases. The compounds identified by the initial qualitative analysis and GCMS analysis have many uses in medical field. Each compounds identified have their unique character to treat various diseases. Further studies needed to reveal its importance in specific field to treat the diseases properly

Conclusion

From this study it is clear that *C. dichotoma* (leaves) indeed exhibit medicinal importance phytochemical compounds. More research needs to be done to unravel the inhibitory effect of this plant. Since this herb had been used for ages traditionally and effectively, it is presumed that side effects should be less. Use of herbs by Indian (south) community is a well-known fact; there is a treasure of herbs that we use daily in our food or in other forms customarily, even without knowing their medicinal benefits. Such use of plant material has always been a tradition, mostly community based that is passed on from one generation to another. In general, lesser known or used herbs and plant materials have to be researched further to study their medicinal properties

especially their antibiotic nature. This will enable the use of our own local, rich plant heritage as effective medicines with probably fewer side effects.

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