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## PHYTOCHEMICAL SCREENING AND GC-MS ANALYSIS OF BIOACTIVE COMPOUNDS PRESENT IN ETHANOLIC EXTRACTS OF LEAF AND FRUIT OF *TRICHOSANTHESIS DIOICA* ROXB.

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### Keywords:

GC-MS analysis, *Trichosanthes dioica*, Bioactive compounds, Ethanol extract, Anti-oxidant

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**ABSTRACT:** Medicinal plants are considered to be the most important in the primary health care system of developing countries. The therapeutic properties of a plant depend upon the biochemical compounds present in the plant parts employed in the preparation of a medicine. *Trichosanthes dioica* Roxb. is being used as popular Ayurveda and Siddha medicine used to treat various diseases. Objective: The objective of this research was to carry out for identification of bioactive compounds from ethanolic extracts of leaf and fruit of *Trichosanthes dioica* Roxb. by Gas chromatography and Mass spectroscopy (GC-MS). Methods: The air-dried leaves and fruits were powdered and separately extracted with ethanol by using a Soxhlet extractor; then, each of the extracts was subjected to qualitative phytochemical analysis and further subjected to a GC-MS instrument. Results: The result of the qualitative analysis of plant extracts has a wide range of active phytochemicals such as alkaloids, flavonoids, glycosides, phenols, saponins, steroids, reducing sugar, tannins, and terpenoids. The GC-MS analysis, twenty two different phytochemical compounds were identified in *T. dioica* leaf extract. The percentage of major bioactive compounds were 2,3-dihydro-benzofuran (38.38%), 2,5,6-tris (4'-methoxyphenyl)-4-propylpyrimidine (20.16%), psi.,psi.-carotene, 1,1',2,2'-tetrahydro-1,1'-dimethoxy-(CAS) (18.83%), 2-lauro-1,3-didecain (11.08%) whereas fifteen phytochemical compounds were identified in *T. dioica* fruit extract. The percentage of major bioactive compounds such as linoleic acid ethyl ester (29.53%), hexadecanoic acid (CAS) (17.14%), 1,2,3-propanetriol (CAS) (4.89%), à-D-glucopyranoside, methyl (CAS) (4.53%), 1,2-cyclopentanedione (4.49%). The identification of bioactive compounds is based on the retention time, peak area, molecular formula, and probability. Conclusion: From the results, it could be concluded that *T. dioica* may have antioxidant, antimicrobial, anti-cancer, anti-diabetic, hypocholesterolemic, and hepatoprotective activities due to the presence of secondary metabolites in the ethanolic extract.

**INTRODUCTION:** Medicinal plants are known to be the main source of drug therapy in traditional medicine.

It is an alternative to Western medicine and is strongly linked to religious beliefs and practices of indigenous cultures<sup>1</sup>. India is blessed with thousands of Ayurvedic and household formulations to treat various disorders, including anxiety, depression, arthritis, high blood pressure, hormonal imbalances, insomnia, migraines, skin problems, and other disorders. The medicinal property of a plant depends upon the physiologically active biochemical compounds

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called secondary metabolites. Plants have an almost limitless ability to synthesize secondary metabolites which present in the plant parts like leaves, fruits, buds, stem, flowers, bark, roots, etc.<sup>2</sup>

Many phytochemical compounds have been utilized by thousands of physicians in their practices and are consumed under medical management by tens of millions of people<sup>3</sup>. Crude plant extracts and medicines manufactured on the values of natural compounds even by pharmaceuticals companies may lead to large-scale exposure of humans to natural products<sup>4</sup>. The major reason for continued use of herbal remedies is their usefulness, easy availability, low price, and moderately less or no toxic property<sup>5</sup>. It can act on the body as powerful as pharmaceutical drugs, and it can start healing itself. As various phytochemicals with pharmacological activity have been isolated from several traditional Indian medicinal plants, it is pertinent to investigate the therapeutic effects of the traditional Indian medicinal plants<sup>6</sup>. GC-MS is the best sensitive technique used for separation and identification of the many structurally complex components that are present in plant extracts.

*Trichosanthes dioica* Roxb. is a dioecious, vine perennial herbal plant that belongs to the family Cucurbitaceae. It is distributed in tropical Asia, Polynesia, and Australia. It is found in the wild in the plains of North India from Punjab to Assam<sup>7</sup>. It is widely cultivated as a vegetable crop in Tripura, Assam, Orissa, West Bengal, Uttar Pradesh, Bihar, and Tamil Nadu<sup>8</sup>. In India, it is otherwise called pointed gourd and green potato. Unripe young fruits are valued by Europeans next to brinjals and potatoes.

The plant is being used as popular Ayurveda and Siddha medicine used to treat constipation problems, Bilious fever, convalescents, cancer-like conditions, bronchitis, and diuretics and to improve appetite and digestion. The fruits are used as a therapy for spermatorrhoea, cooling and labor women laxative<sup>9</sup>, diuretic, antiulcerous property<sup>10</sup>. There are many other pharmacological applications include antidiabetic<sup>11, 12</sup>, cholesterol-lowering activity<sup>13, 14</sup>, anti-inflammatory<sup>15</sup>, anti-bacterial<sup>16</sup>, antifungal<sup>17</sup>, hepatoprotective<sup>18</sup>, antioxidant<sup>19</sup>, and wound healing activities<sup>20</sup>. Hence, the present study was aimed to find the phytochemicals /

bioactive compounds present in the ethanolic extracts of leaf and fruit of *T. dioica* Roxb. by qualitative photochemical screening and Gas chromatography and mass spectrometry analysis.

## MATERIALS AND METHODS:

### Collection and Authentication of Plant

**Materials:** Fresh, healthy, young leaves and unripe fruits of *Trichosanthes dioica* Roxb. (*T. dioica*) used for the investigation were collected from SKM Herbal Research Centre, Erode, Tamil Nadu, India. The plant was authenticated by Dr. V.R. Mohan, Department of Botany, V.O. Chidambaram College, Tuticorin. The voucher specimen (No. VOCB 2307) was deposited in Ethnopharmacology Unit, Research Department of Botany, V.O. Chidambaram College, Tuticorin, Tamil Nadu.

**Preparation of Plant Extracts:** Freshly collected leaves and fruits of *T. dioica* were washed with running tap water and the fruits were cut into small pieces. Both fruits and leaves were dried under shade for two weeks. The properly dried leaves and fruits were powdered separately in an electric blender. The coarsely powdered materials were kept in airtight containers to avoid the effect of humidity and then stored at room temperature until use. About 500 g of dry powdered samples were extracted with ethanol (95%) by continuous hot percolation using Soxhlet extractor for 24 h. The extract was filtered through Whatman no.41 filter paper separately, and the extracts were concentrated in vacuum at 60 °C using a rotary evaporator to evaporate the ethanol from it. To evaporate the remaining solvent, the extracts were kept in an oven at a temperature of 40-50 °C for 8 h. Dark brown colour residues were obtained. The residues were kept separately in airtight containers and stored at 4 °C for further use.

**Preliminary Phytochemical Analysis:** The ethanolic extracts were used for qualitative identification of various secondary metabolites were carried out by using standard methods<sup>21</sup>.

**Gas Chromatography-Mass Spectroscopy (GC-MS) Analysis:** Gas Chromatography-Mass Spectrometry (GC-MS) plays a key role in the analysis of unknown components of plant origin. The chemical composition of ethanolic extracts of leaf and fruit of *T. dioica* were subjected to GC-MS analysis.

GC-MS analysis of these extracts were carried out using the equipment 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 condition: Equipped with a column Elite-1, fused silica capillary column (30 m × 0.25 mm ID × 1 μm df, composed of 100% dimethyl polysiloxane), operating in electron impact mode at 70 eV; helium gas (99.999%) was used as carrier gas at a constant flow rate of 1 ml/min and an injection volume of 2 μl was employed (split ratio of 10:1). The injector temperature is set at 250 °C, and the ion-source temperature is 280 °C. The oven temperature was programmed from 110 °C (isothermal for 2 min), with an increase for 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 seconds, and fragments from 45 to 450 Da. Total GC running time was 36 min. The relative percentage amount of each component was calculated by comparing its

average peak area to the total areas. Software adapted to handle mass spectra, and chromatograms was a Turbo mass ver. 5.0.

Interpretation on mass spectrum of GC-MS was conducted using the database of National Institute of 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 and the results obtained have been tabulated<sup>22</sup>.

**Statistical Analysis:** All the qualitative test/analysis was performed in triplicate.

## RESULTS:

**Preliminary Phytochemical Analysis:** The distribution of different phytochemical constituents in ethanolic extracts of leaf and fruit of *T. dioica* were evaluated qualitatively and shown in **Table 1**.

**TABLE 1: PRELIMINARY PHYTOCHEMICAL ANALYSIS OF ETHANOLIC EXTRACTS OF LEAF AND FRUIT OF *T. DIOICA***

S. no.	Phytochemical constituents	Test/ Reagents	Result	
			<i>T. dioica</i>	
			Leaf	Fruit
1	Alkaloids	Wagner's test, Dragendorff's test	+	+
2	Coumarins	Alcoholic NaOH	+	+
3	Flavonoids	Shinoda's test	+	+
4	Glycosides	Anthrone + Conc. H <sub>2</sub> SO <sub>4</sub>	+	+
5	Phenols	Lead acetates test	+	+
6	Proteins and Free amino acids	NaOH and copper sulphate, Ninhydrin test	+	+
7	Quinones	Conc. H <sub>2</sub> SO <sub>4</sub>	+	-
8	Saponins	Foam Test	+	+
9	Steroid	Acetic acid + Chloroform + Conc. H <sub>2</sub> SO <sub>4</sub>	+	+
10	Reducing sugars	Fehling's Test	+	+
11	Tannins	Basic lead acetate	+	+
12	Terpenoids	Noller's Test	+	+
13	Fixed oil	Spot test	+	-

Note: + = Present, - = Absent

From **Table 1**, it was clear that a lot of active biochemical compounds like alkaloids, flavonoids, glycosides, phenols, tannins, saponins, steroids, coumarin, and terpenoids were present in the ethanol extracts of leaf and fruit of *T. dioica* whereas fruit of *T. dioica* revealed the absence of quinines and fixed oil.

**Gas Chromatography-Mass Spectroscopy (GC-MS) Analysis:** Different phytochemical/bioactive compounds of the ethanolic extracts of leaf and

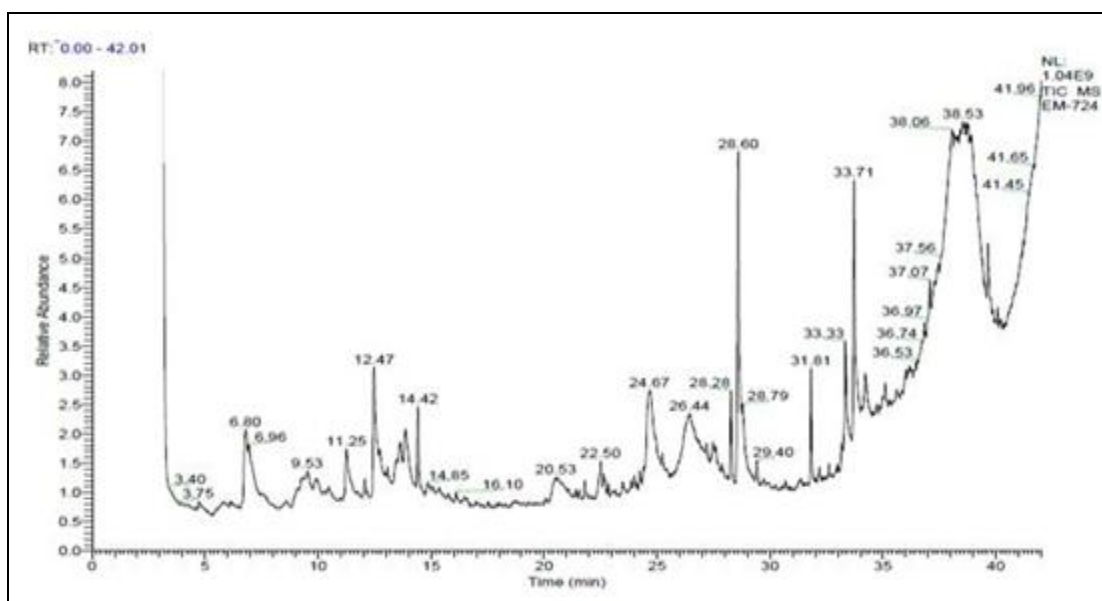
fruit of *T. dioica* were analyzed by using GC-MS. The chromatograms of the extracts were shown in **Fig. 1** and **2** and summarized in **Tables 2** and **3**.

GC-MS chromatogram of *T. dioica* leaf extract showed 22 peaks which indicated the presence of 22 different bioactive/phytochemical compounds **Fig. 1**. The results revealed that the percentage of major bioactive compounds viz., 2,3-dihydro-benzo furan (38.38%), 2,5,6-tris (4'-methoxyphenyl)-4-propylpyrimidine (20.16%), psi., psi.-carotene, 1,

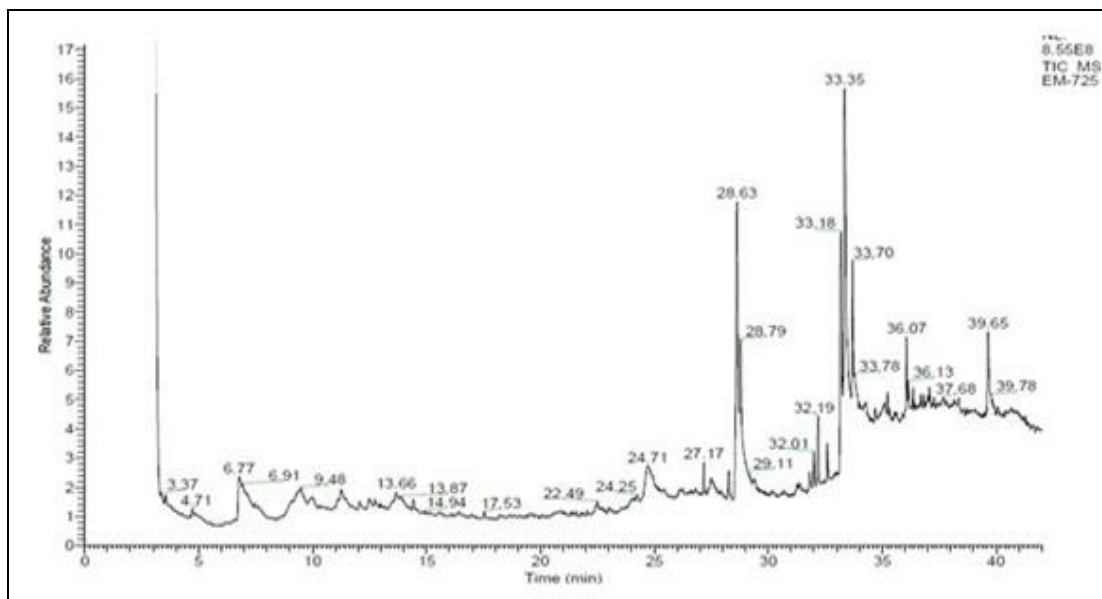
1',2,2'-tetrahydro-1,1'-dimethoxy-(CAS) (18.83%), 2-lauro-1, 3-didecain (11.08%), n-hexadecanoic acid (8.70%),  $\alpha$ -methyl-D-mannopyranoside (6.19%), 9,12,15-octadecatrienoic acid, (Z,Z,Z)- (5.67%), methyl-9,9,10,10-D4-octadecanoate (4.72%), 1,2-cyclopentanedione (4.14%), desulphosinigrin (3.80%), 9,12-octadecadienoic acid (Z,Z)- (3.29%), lactone G (2.11%) and 2-propyl-tetrahydropyran-3-ol (2.00%) were found as the major compounds in the ethanolic extract of leaf of *T. dioica* **Table 2**.

GC-MS chromatogram of *T. dioica* fruit extract showed 15 peaks which indicated the presence of 15 phytochemical constituents. **Fig. 2** and **Table 3** revealed that the percentage of major bioactive

compounds such as linoleic acid ethyl ester (29.53%), hexadecanoic acid (CAS) (17.14%), 1,2,3-propanetriol (CAS) (4.89%),  $\beta$ -D-glucopyranoside, methyl (CAS) (4.53%), 1,2-cyclopentanedione (4.49%), hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester (CAS) (4.09%), 9,12-octadecadienoic acid (Z,Z)-, methyl ester (3.59%), 9,12,15-octadecatrienoic acid, ethyl ester, (Z,Z,Z)- (2.88%), 2,4-dimethyl-1-pentanol (2.85%), 9, 12, 15-octadecatrienoic acid, ethyl ester, (Z,Z,Z)- (2.83%) and 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (2.58%) were found as the major compound in the ethanolic extract of fruit of *T. dioica*.



**FIG. 1: GC-MS CHROMOTOGRAM OF ETHANOLIC EXTRACT OF *T. DIOICA* LEAF**



**FIG. 2: GC-MS CHROMOTOGRAM OF ETHANOLIC EXTRACT OF *T. DIOICA* FRUIT**

**TABLE 2: ACTIVITY OF BIOACTIVE COMPOUND IDENTIFIED IN ETHANOLIC EXTRACT OF *T. DIOICA* LEAF**

S. no.	Name of the Compounds	Retention Time (min)	Peak Area %	Molecular Formula	Probability	Reported Biological activity
1	Isopropyl palmitate	5.82	0.96	C <sub>19</sub> H <sub>38</sub> O <sub>2</sub>	52.14	Penetration enhancer of drugs in skin <sup>23</sup>
2	1,2-Cyclopentanedione	6.82	4.14	C <sub>5</sub> H <sub>6</sub> O <sub>2</sub>	19.83	Antioxidant activity <sup>24</sup>
3	Desulphosinigrin	9.53	3.80	C <sub>10</sub> H <sub>17</sub> NO <sub>6</sub> S	14.35	Antimicrobial activity <sup>25</sup>
4	3-Amino-5-methyl-1,2,4-triazine 2-oxide	9.92	1.05	C <sub>4</sub> H <sub>6</sub> N <sub>4</sub> O	73.37	No reported activity
5	2-Propyl-tetrahydropyran-3-ol	11.27	2.00	C <sub>8</sub> H <sub>16</sub> O <sub>2</sub>	18.31	Anti-allergenic and Anti-bacterial activity <sup>26</sup>
6	Isopropyl palmitate	12.08	0.46	C <sub>19</sub> H <sub>38</sub> O <sub>2</sub>	15.25	Penetration enhancer of drugs in skin <sup>23</sup>
7	2,3-Dihydro-benzofuran	12.49	38.38	C <sub>8</sub> H <sub>8</sub> O	38.38	Antifungal, Antiproliferative activity <sup>27</sup>
8	4-Vinyl-2-methoxy-phenol	14.44	1.61	C <sub>9</sub> H <sub>10</sub> O <sub>2</sub>	17.02	Antimicrobial, Antioxidant, Anti-inflammatory and Analgesic activity <sup>28,29</sup>
9	Hexadecanoic acid, 2,3-dihydroxypropyl ester (CAS)	14.87	0.42	C <sub>19</sub> H <sub>38</sub> O <sub>4</sub>	50.55	Emulsifying agent
10	Lactone G	20.53	2.11	C <sub>5</sub> H <sub>8</sub> O <sub>4</sub>	81.29	No reported activity
11	à-Methyl-D-mannopyranoside	24.67	6.19	C <sub>7</sub> H <sub>14</sub> O <sub>6</sub>	29.91	Anti-microbial activity <sup>30</sup>
12	Methyl-9,9,10,10-D4-octadecanoate	26.42	4.72	C <sub>19</sub> H <sub>34</sub> D <sub>4</sub> O <sub>2</sub>	16.23	Cell membrane stability
13	Pluchidiol	28.28	1.59	C <sub>13</sub> H <sub>20</sub> O <sub>2</sub>	62.59	Antioxidant and Anti-inflammatory activity
14	n-Hexadecanoic acid	28.60	8.70	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	83.80	Antioxidant, Hypocholesterolemic, Anti-inflammatory property, 5-Alpha reductase inhibitor activity <sup>31,32</sup>
15	(-)-Loliolide	29.42	0.38	C <sub>11</sub> H <sub>16</sub> O <sub>3</sub>	66.16	Anti-diabetic, Antidepressive, Antioxidant activity <sup>33</sup>
16	Phytol	31.81	1.73	C <sub>20</sub> H <sub>40</sub> O	58.83	Antimicrobial, Anticancer, Anti-inflammatory Antioxidant, Anti-diabetic, Anti-diuretic properties, Neuroprotective, Antidepressant, Anticonvulsant activity <sup>34,35,36,37</sup>
17	9,12-Octadecadienoic acid (Z,Z)-	33.35	3.29	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>	27.24	Anti inflammatory, Anti-arthritis, Antiacne, Anti-histaminic, Anti-eczemic, Anti-androgenic, Anti coronary, Anticancer, Antihypercholesterolemic, Heptaoprotective, 5-Alpha reductase inhibitor activity <sup>38,39</sup>
18	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	33.53	5.67	C <sub>18</sub> H <sub>30</sub> O <sub>2</sub>	33.35	Normal growth and development, Anticancer, Anti-diabetic, Anti-atherosclerotic, Anti-hypertensive, Antimicrobial activity <sup>40,41</sup>
19	Psi.,psi.-Carotene, 1,1',2,2'-tetrahydro-1,1'-dimethoxy-(CAS)	37.07	18.83	C <sub>42</sub> H <sub>64</sub> O <sub>2</sub>	0.51	Nutrient, Antioxidant, Cytotoxic activity
20	2-Lauro-1,3-dodecoic	38.08	11.08	C <sub>35</sub> H <sub>66</sub> O <sub>6</sub>	32.57	Antioxidant, Anti-allergy, Antimicrobial, Anti-dandruff property <sup>42</sup>
21	2,5,6-Tris(4'-methoxyphenyl)-4-propyl pyrimidine	38.55	20.16	C <sub>28</sub> H <sub>28</sub> N <sub>2</sub> O <sub>3</sub>	44.17	No reported activity
22	Pentanedioic acid, bis-dodecylamide	41.42	0.49	C <sub>29</sub> H <sub>58</sub> N <sub>2</sub> O <sub>2</sub>	21.93	Antimicrobial activity

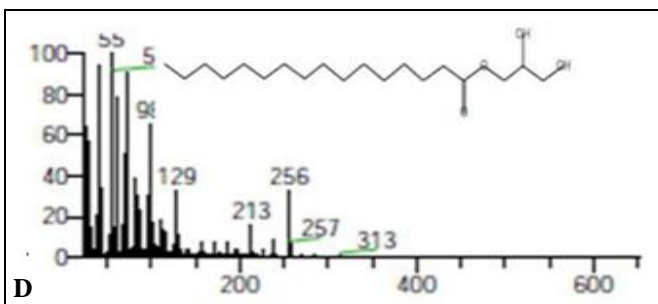
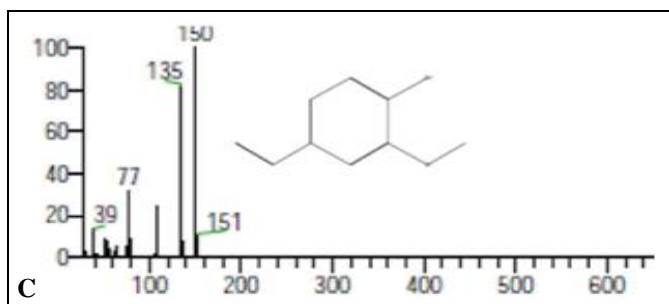
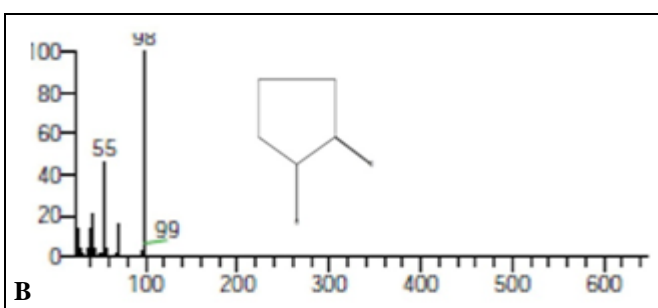
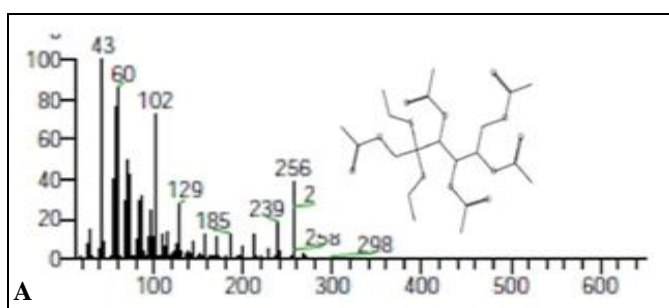
**TABLE 3: ACTIVITY OF BIOACTIVE COMPOUND IDENTIFIED IN ETHANOLIC EXTRACT OF *T. DIOICA* FRUIT**

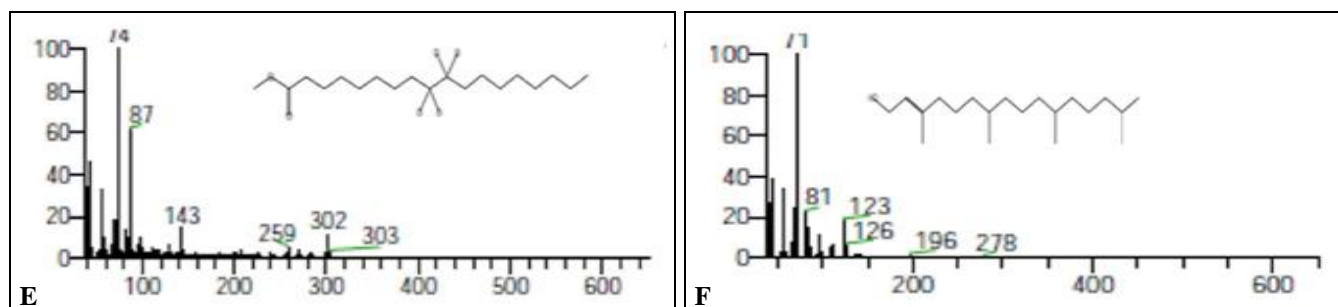
S. no.	Name of the Compounds	Retention Time (min)	Peak Area %	Molecular Formula	Probability	Reported Biological activity
1	9-Octadecenoic acid (Z)- (CAS)	4.73	0.80	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	38.56	Stroke, Acute neurologic disorder, Respiratory failure, Anemia treatment
2	1,2-Cyclopentanedione	6.82	4.49	C <sub>5</sub> H <sub>6</sub> O <sub>2</sub>	31.80	Antioxidant activity <sup>24</sup>
3	1,2,3-Propanetriol (CAS)	9.45	4.89	C <sub>3</sub> H <sub>8</sub> O <sub>3</sub>	33.35	Antimicrobial, Antiviral properties
4	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	11.26	2.58	C <sub>6</sub> H <sub>8</sub> O <sub>4</sub>	91.54	Antioxidant activity
5	2,4-Dimethyl-1-pentanol	13.68	2.85	C <sub>7</sub> H <sub>16</sub> O	11.10	No reported activity

6	(1S,6R)-3,7,7-Trimethylbicyclo[4.1.0]hept-3-en-5-one	14.43	0.42	C <sub>10</sub> H <sub>14</sub> O	17.40	No reported activity
7	2-Piperidinomethyl-tetrahydrofuran	22.49	1.02	C <sub>10</sub> H <sub>19</sub> NO	22.18	Polymerization
8	à-D-Glucopyranoside, methyl (CAS)	24.71	4.53	C <sub>7</sub> H <sub>14</sub> O <sub>6</sub>	23.77	Antioxidant property
9	Hexadecanoic acid, methyl ester (CAS)	27.17	0.91	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	76.96	Antioxidant, Antimicrobial, anti-hypercholesterolemic property, Antiandrogenic 5-Alpha reductase inhibitor activity <sup>43</sup>
10	Hexadecanoic acid (CAS)	28.63	17.14	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	83.47	Antioxidant, Hypocholesterolemic, 5-alpha reductase inhibitors activity <sup>44</sup>
11	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	32.19	3.59	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	17.02	Anti-cancer <sup>45</sup>
12	Linoleic acid ethyl ester	33.35	29.53	C <sub>20</sub> H <sub>36</sub> O <sub>2</sub>	17.88	Hypocholesterolemic, Anti-arthritis, Anti-acne, Hepatoprotective
13	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	33.70	2.88	C <sub>20</sub> H <sub>34</sub> O <sub>2</sub>	20.03	Anti-histaminic, Anti-coronary, Anti-eczemic, 5-Alpha reductase inhibitor activity <sup>46</sup>
14	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	36.07	2.83	C <sub>20</sub> H <sub>34</sub> O <sub>2</sub>	63.91	Normal growth and development, Anti-diabetic, Anti-atherosclerotic, Antihypertensive, Anti-cancer, Anti-inflammatory, Hypocholesterolemic, Hepatoprotective, Anti-histaminic, Antieczemic, Antiacne, Antiandrogenic, Antiarthritic, Anticoronary, 5-Alpha reductase inhibitor activity <sup>47</sup>
15	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester (CAS)	39.65	4.09	C <sub>19</sub> H <sub>38</sub> O <sub>4</sub>	73.21	Normal growth and development, Anti-diabetic, Anti-atherosclerotic, Antihypertensive, Anti-inflammatory, Hypocholesterolemic, Hepatoprotective, Anti-histaminic, Antieczemic, Antiacne, Antiandrogenic, Antiarthritic, Anticoronary, 5-Alpha reductase inhibitor activity <sup>47</sup>

The GC-MS study of the ethanolic extracts of leaf and fruit of *T. dioica* shown the presence of lots of

phytochemical constituents which strength contribute to the medical bioactive of the plant.

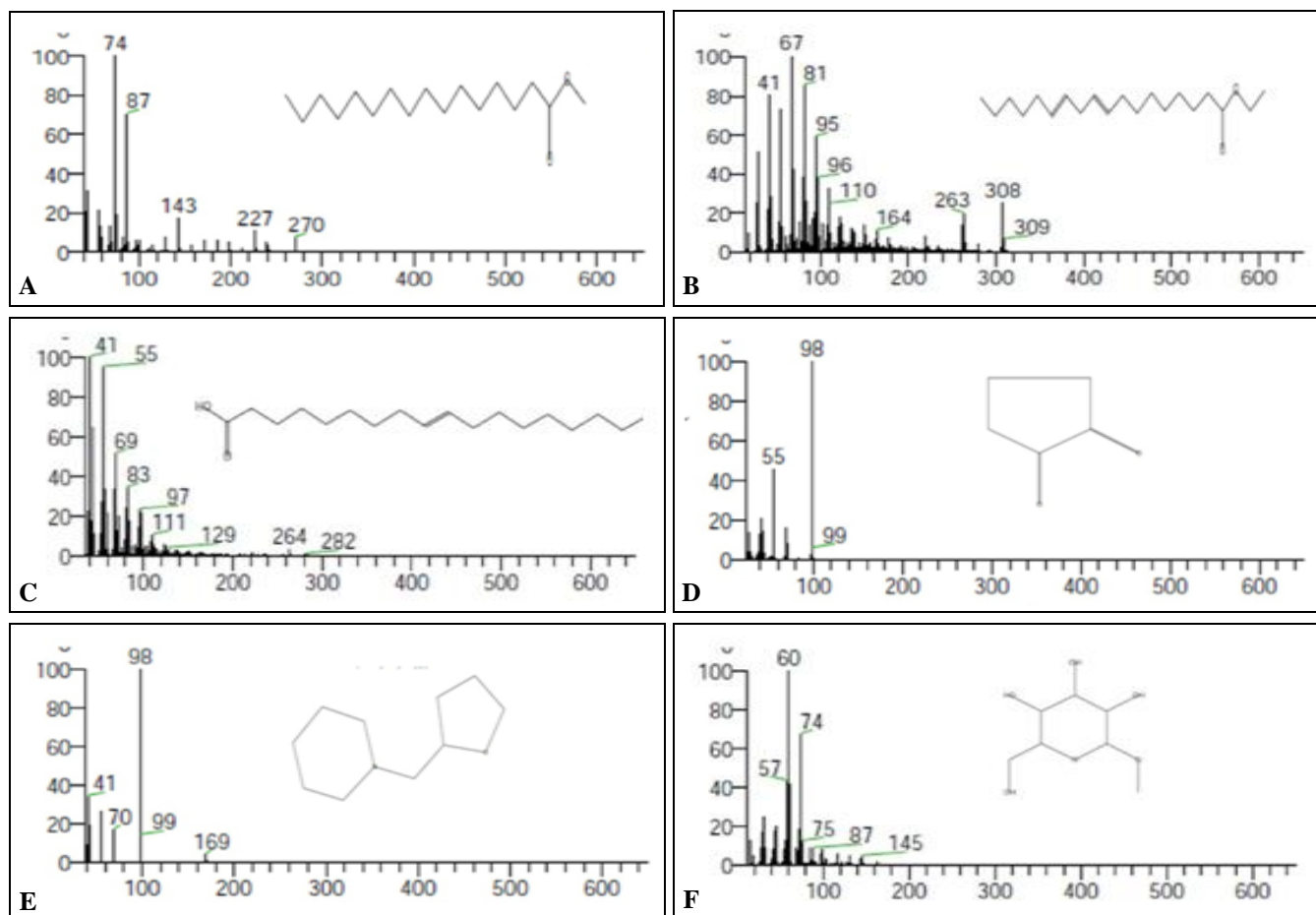




**FIG. 3: GC-MS MASS SPECTRA OF A FEW COMPOUNDS DETECTED FROM ETHANOLIC EXTRACT OF *T. DIOICA* LEAF.** (a) Mass spectra of Isopropyl Palmitate ( $C_{19}H_{38}O_2$ ), (b) Mass spectra of 1,2-Cyclopentanedione ( $C_5H_6O_2$ ), (c) Mass spectra of 4-vinyl-2-methoxy-phenol ( $C_9H_{10}O_2$ ), (d) Mass spectra of Hexadecanoic acid, 2,3-dihydroxypropyl ester (CAS) ( $C_{19}H_{38}O_4$ ), (e) Mass spectra of Methyl-9,9,10,10-D4-Octadecanoate ( $C_{19}H_{34}D_4O_2$ ), (f) Mass spectra of Phytol ( $C_{20}H_{40}O$ )

GC-MS mass spectra detected some compounds from ethanolic extracts of *T. dioica* leaf and fruit. Mass matching of the spectrums of prominent

compounds present in the experimental samples was done with the spectrum of standard compounds of NIST library as shown in **Fig. 3 (a-f)** and **4 (a-f)**.



**FIG. 4: GC-MS MASS SPECTRA OF A FEW COMPOUNDS DETECTED FROM ETHANOLIC EXTRACT OF *T. DIOICA* FRUIT.** (a) Mass spectra of Hexadecanoic acid, methyl ester (CAS) ( $C_{17}H_{34}O_2$ ), (b) Mass spectra of Linoleic acid ethyl ester ( $C_{20}H_{36}O_2$ ), (c) Mass spectra of 9-Octadecenoic acid (Z)-(CAS) ( $C_{18}H_{34}O_2$ ), (d) Mass spectra of 1,2-Cyclopentanedione ( $C_5H_6O_2$ ), (e) Mass spectra of 2-piperidinomethyl-tetrahydrofuran ( $C_{10}H_{19}NO$ ), (f) Mass spectra of à-D-Glucopyranoside, methyl (CAS) ( $C_7H_{14}O_6$ )

**DISCUSSION:** Qualitative screening is very important to determine the phytochemical compounds present in herbal plants. This procedure is a simple preliminary pre-requisite before going for detailed phytochemical investigation<sup>48</sup>.

In India, tribal and rural populations are commonly using the local plant's crude extract for medicinal and other purposes. Crude extracts and medicines manufactured on the principles of natural compounds, even by pharmaceutical companies,

may lead to large-scale exposure of humans to natural products. The first step towards this goal is the biological and phytochemical screening of plant extracts from traditional preparations used in popular medicine<sup>49, 50</sup>. The phytochemicals are well known to have curative activity against several human problems such as diuretics, skin diseases<sup>51</sup>, hypercholesterolemia<sup>52</sup> and hyperglycemic disorders<sup>53, 54</sup> and could suggest the folk use of the medicinal plants.

GC-MS analysis is one of the first steps towards understanding the nature of active principles in medicinal plants and to decide whether the plant species has any individual compound or group of compounds. The spectrum profile of GC-MS confirmed the presence of main components with their retention time. The heights of the peak show the relative concentrations of the components present in the extracts. In comparison of the mass spectra of the constituent with the NIST library, the phytoconstituents were characterized and identified.

In the present study, among the identified phytochemicals in the investigated samples, n-Hexadecanoic acid, hexadecanoic acid, methyl ester (CAS), hexadecanoic acid, 1,2-Cyclopentanedione, 4-Vinyl-2-methoxy-phenol, Pluchidiol, (-)-Loliolide, Phytol, 2-Lauro-1,3-dodecoic acid, 1,2-Cyclopentanedione, 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one,  $\alpha$ -D-Glucopyranoside, methyl (CAS), Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester (CAS) and Psi., psi.-Carotene, 1,1',2,2'-tetrahydro-1,1'-dimethoxy-(CAS) have the antioxidant property. Desulphosinigrin, 2-Propyl-tetrahydropyran-3-ol, 4-Vinyl-2-methoxy-phenol,  $\alpha$ -Methyl-D-mannopyranoside, Phytol, 9, 12,15-Octadecatrienoic acid, (Z,Z,Z)-, 2-Lauro-1,3-dodecoic acid, Pentanedioic acid, bis-dodecylamide and 2-Lauro-1,3-dodecoic acid have the antimicrobial activity. 4-Vinyl-2-methoxy-phenol, Pluchidiol, 9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-n-Hexadecanoic acid, Phytol, 9,12-Octadecadienoic acid (Z,Z)-, 9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)- have anti-inflammatory activity. 9, 12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-, Linoleic acid ethyl ester, Hexadecanoic acid (CAS), Hexadecanoic acid, methyl ester (CAS), 9,12-Octadecadienoic acid (Z,Z)- have hypocholesterolemic activity.

Phytol, (-)-Loliolide, 9, 12, 15-Octadecatrienoic acid, (Z,Z,Z)- and 9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)- may play an important role in the prevention and treatment of diabetes. 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- 9,12-Octadecadienoic acid (Z,Z)-, methyl ester, 9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)- and Phytol may play a significant role in the treatment of cancer. Using Dr. Duke's phytochemical and ethnobotanical database (online), the biological activity of the identified phytochemicals was ascertained<sup>55</sup>.

**CONCLUSION:** Based on the results obtained in the present investigation, it may be concluded that the biological activities of the identified phytochemicals used for anti-microbial, anti-inflammatory, anti-diabetic, hepatoprotective, anti-hypercholesterolemic, and anti-cancer activities. Therefore, *T. dioica* is recommended as a source of phytopharmaceutical value.

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