

**GC–MS Profiling Of Bioactive Compounds From The Leaf, Stem And Root Methanolic Extract Of *Rubia cordifolia* Linn. (Indian madder)****Sujatha D<sup>1</sup>, Jyothirmai R<sup>1</sup>, Sunitha R<sup>2</sup>, Anusha Bairy<sup>3</sup>, Vaishnavi Anumula<sup>3</sup>, Jyothi P<sup>4</sup>, Christopher Reuben Thammidala<sup>1\*</sup>**<sup>1</sup>Department of Botany, Kakatiya University, Hanumakonda.<sup>2</sup>Department of Botany, Government Degree College (Women's), Karimnagar.<sup>3</sup>Department of Biotechnology, Kakatiya University, Hanumakonda.<sup>4</sup>Department of Microbiology, Chaitanya (Deemed to be University), Hyderabad.<sup>1\*</sup>**Corresponding Author:** Christopher Reuben Thammidala, tcreuben@gmail.com.

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**Abstract**

*Rubia cordifolia* Linn. (Rubiaceae) is an important flowering medicinal plant of coffee family, Rubiaceae. The main objective of this study was, by using Gas Chromatography-Mass Spectrometry (GC-MS) analysis is to find out the bioactive compounds from the methanolic extract of leaf, stem and root of *Rubia cordifolia*. Phytochemicals are chemical compounds produced by plants, that have biological activities such as antibacterial, antioxidant, antifungal, analgesic, anti-depressant, anti-inflammatory and cardioprotective properties. By removing free radicals, they prevent diseases. In GC–MS analysis 15,20 and 15 bioactive compounds are found in the methanolic extract of leaf, stem and root of *Rubia cordifolia* respectively. Diethyl Phthalate, phthalic acid, ethyl isopropyl ester and phthalic acid, ethyl pentyl ester are the bioactive compounds with antimicrobial activity identified in leaf, stem and root methanolic extract of *Rubia cordifolia*. 9,10-Anthracenedione, 2-methyl-(RT-29.228), a chemical from root extract has anticancer property. These bioactive compounds have both pharmacological and biological properties. To combat several diseases in plants and humans it is very essential to isolate and purify these compounds.

**Keywords:** *Rubia cordifolia*, GC-MS analysis, Pharmacological property, Phytochemicals, Methanolic extracts.

**Introduction**

*Rubia cordifolia* is one of the major species in Rubiaceae (coffee) family with diverse phytochemicals. Phytochemicals are secondary metabolites found in plants. These have been used as herbal medicines from several years. Local people used these phytochemicals present in different parts of the plant for healing of certain disorders [1]. Manjistha also known as “Indian Madder” *R. cordifolia* is a perennial

climber that is widely distributed in India and China. It also distributed from Nilgiris and in the Himalayas from Kashmir eastwards and other hilly districts of India [2]. *R. cordifolia* is perennial, hardy climber with long cylindrical roots covered with thin red bark [3]. Leaves are ovate, arranged in four whorls, lower leaves are larger. In ancient period, Manjistha is reputed as immunomodulator and an efficient blood purifier [4], hence it is extensively used against skin, blood and urinary diseases [5,6]. Manjistha has been used in the treatment of different ailments in modern pharmacopoeia [7-10]. These evidences showed that all parts of the plant have medicinal properties. They have pharmacological effect, so great attention was laid on this plant. The prime objective of this present research is to identify several bioactive compounds from the methanolic extract of leaf, stem and root of *R. cordifolia* by GC-MS analysis.

## **Material and Methods**

### **Collection and Preparation of Plant Material**

The leaves, stems, and roots of *Rubia cordifolia* L. were gathered from Paderu, which located 100 km away from Vishakapatnam, Andhra Pradesh. The samples which were collected, washed thoroughly with running water, then dried in the shade followed by homogenization. About 5g powder was taken from dried sample and dissolved in 50 ml of methanol. Then samples were incubated for 48 hours in orbital shaker with 22<sup>0</sup> C temperature at 120 rpm. Samples were extracted by cold maceration method.

### **Gas Chromatography and Mass Spectroscopic (GC- MS) Analysis.**

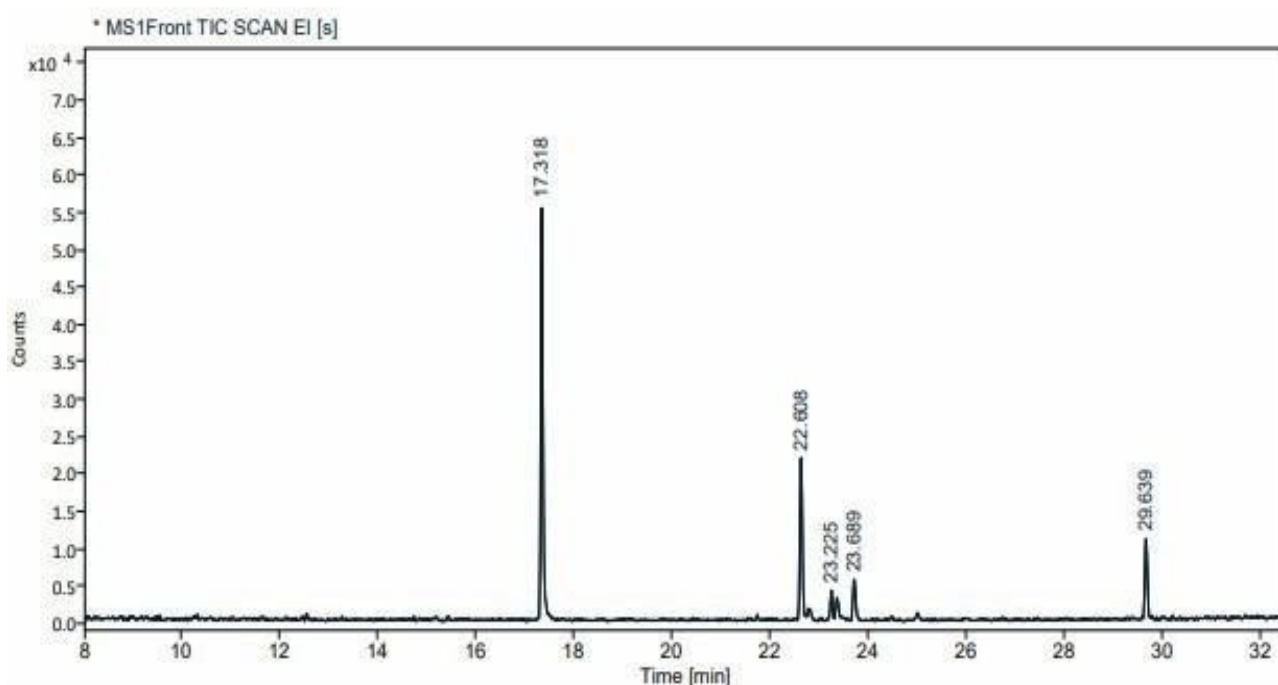
Gas chromatography and mass spectrometry (GC-MS) was used to determine the quantitative and qualitative compounds of organic chemicals present in the given sample. From the methanolic extract of leaf, stem and root of *Rubia cordifolia*, bioactive compounds were evaluated by using GC-MS (Agilent8890GC system). Helium was used as the gaseous carrier, with 1 $\mu$ l of injector volume and a flow rate of 1 ml/min at a temperature of 75°C. The oven temperature was 350°C. The total running time of GC-MS for leaf, stem and root methanolic extract of *Rubia cordifolia* was 53.5 minutes respectively. Based on RT value, peak area, molecular weight and molecular formula bioactive compounds were identified. By comparing their generated MS spectrum patterns and the MS spectra at SAIF-IIT Madras phytochemicals were identified.

## **Results and Discussion:**

GC-MS profiling of methanol extracts of *Rubia cordifolia* leaves, stem, and root showed the presence of different possible organic molecules with distinct biological activities. Phytoconstituents in extracts were detected using multiple parameters, including retention time (RT) and the molecular formula of the compounds. 15 compounds were found in leaf extract, 20 compounds in stem extract, and 15 compounds in root extracts by using GC-MS.

**GC-MS Analysis of Leaf Methanolic Extract**

GC-MS analysis of *Rubia cordifolia* Leaf extracts identified 15 bioactive components (Table 1, Fig.1). The bioactive chemicals detected by GC-MS analysis were Diethyl Phthalate, Phthalic acid, ethyl isopropyl ester and Phthalic acid, ethyl pentyl ester which have antimicrobial activity [11-14]. Neophytadiene has antidepressant, anti-inflammatory, antioxidant and cardioprotective properties reported in methanolic extract of *Crataeva nurvala* and *Blumea lacera*, plants. [15,16]. 3,7,11,15- Tetra methyl-2-hexadecen-1-ol has four retention times (RT-22.608, 23.225, 23.689 & 29.639) exhibits antimicrobial activity in *phyllanthus amarus*. [17-19]. The endemic plant species, *Onosma mutabilis* aerial parts contains Cyclopentane,1,2dimethyl-3-(1-methylethenyl)- which has antioxidant activity [20]. The compound Phytol exhibits antinociceptive, antioxidant, antimicrobial, and anti-inflammatory [21-23].



**Fig.1** GC-MS chromatogram of *Rubia cordifolia* leaf methanolic extract.

**Table1:** Phytochemical components identified in *Rubia cordifolia* Leaf methanolic extract using GCMS analysis and their reported biological activities.

S.No	RT value	Name of the compound	Molecular Wt. (g/mol)	Molecular Formula	Area%	Recorded Biological activity
1	17.318	Diethyl phthalate	222.24	C <sub>10</sub> H <sub>10</sub> O <sub>4</sub>	55.37	plasticiser activity and antimicrobial

2	17.318	Phthalic acid, ethyl isopropyl ester	236.26	C <sub>13</sub> H <sub>16</sub> O <sub>4</sub>	55.37	Antimicrobial activity
3	17.318	Phthalic acid, ethyl pentyl ester	385.41	C <sub>15</sub> H <sub>20</sub> O <sub>4</sub>	55.37	Antimicrobial, synergistic activity
4	22.608	3-Methylene-7,11-dimethyl -1- dodecene	208.38	C <sub>15</sub> H <sub>28</sub>	23.41	No activity
5	22.608	Neophytadiene	278.516	C <sub>20</sub> H <sub>38</sub>	23.41	Antidepressant, Antiinflammatory, antioxidant and cardioprotective
6	22.608	3,7,11,15- Tetramethyl - 2- hexadecen -1- ol	296.53	C <sub>20</sub> H <sub>40</sub> O	23.41	Antimicrobial activity
7	23.225	3- Methylene- 7,11-dimethyl -1-dodecene	204.35	C <sub>15</sub> H <sub>28</sub>	2.88	No activity
8	23.225	3,7,11,15- Tetramethyl- 2 hexadecen- 1 ol	296.53	C <sub>20</sub> H <sub>40</sub> O	2.88	Antimicrobial activity
9	23.225	Cyclopentane, 1,2-dimethyl-3-(1methylethenyl)-	154.25	C <sub>10</sub> H <sub>18</sub> O	2.88	Antioxidant activity
10	23.689	3-methylene- 7, 11-dimethyl - 1- dodecene	208.38	C <sub>15</sub> H <sub>28</sub>	6.41	No activity
11	23.689	Neophytadiene	278.5	C <sub>20</sub> H <sub>38</sub>	6.41	Antidepressant, Antiinflammatory, antioxidant and cardioprotective
12	23.689	3,7,11,15-Tetramethyl- 2hexadecen-1ol	296.53	C <sub>20</sub> H <sub>40</sub> O	6.41	Antioxidant, Anti inflammatory
13	29.639	Phytol	296.53	C <sub>20</sub> H <sub>40</sub> O	11.93	Antinociceptive, Antioxidant and Anti-inflammatory
14	29.639	3,7,11,15-Tetramethyl- 2hexadecen-1-ol	296.53	C <sub>20</sub> H <sub>40</sub> O	11.93	Antioxidant, Anti inflammatory
15	29.639	3-Methylene-7,11-dimethyl-1dodecene	208.38	C <sub>15</sub> H <sub>28</sub>	11.93	No activity

**GC–MS Analysis of Stem Methanolic Extract**

20 distinct compounds were identified in stem methanolic extract of *Rubia cordifolia*. 1,3- Dicyclohexylurea (RT-3.406) has antimicrobial activity [24]. Other three compounds which show high area percentage (73.50%) Diethyl phthalate, Phthalic acid, ethyl 2- pentyl ester have antimicrobial activity [12,25,14]. Phthalic acid, hept-3-yl isobutyl ester and Phthalic acid, hex-3-yl isobutyl ester (PAEs) are a class of lipophilic chemicals widely used as plasticizers and also, they possess as antiviral, antiinflammatory and

anti-tumor activities [26]. Neophytadiene is a diterpene found in *Crataeva nurvala* and *Blumea lacera* is reported with anxiolytic-like activity, antidepressant and sedative properties [15]. Phytol is a compound found abundantly in photosynthetic organisms, show a wide range of bioactivities such as antianxiety, cytotoxic, metabolism-modulating, antioxidant, antimicrobial, antitumour, autophagy and apoptosis inducing, antinociceptive, anti-inflammatory, immune-modulating, and antimicrobial effects [21,22,23,18,27]. Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester compound reported antimicrobial activity in bark extracts of *Psychotria dalzellii* [28,29].

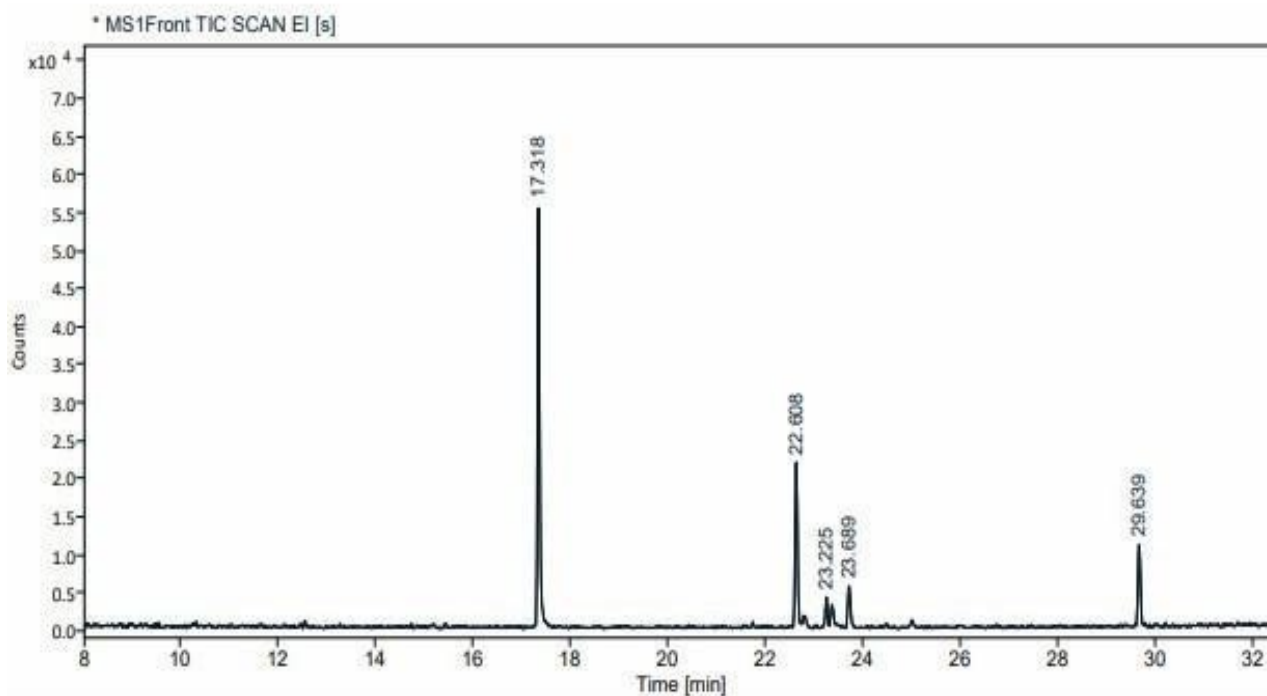


Fig.2. GC-MS chromatogram of *Rubia cordifolia* stem methanolic extract.

Table2: Phytochemical components identified in *Rubia cordifolia* stem methanolic extract using GC-MS analysis and their reported biological activities.

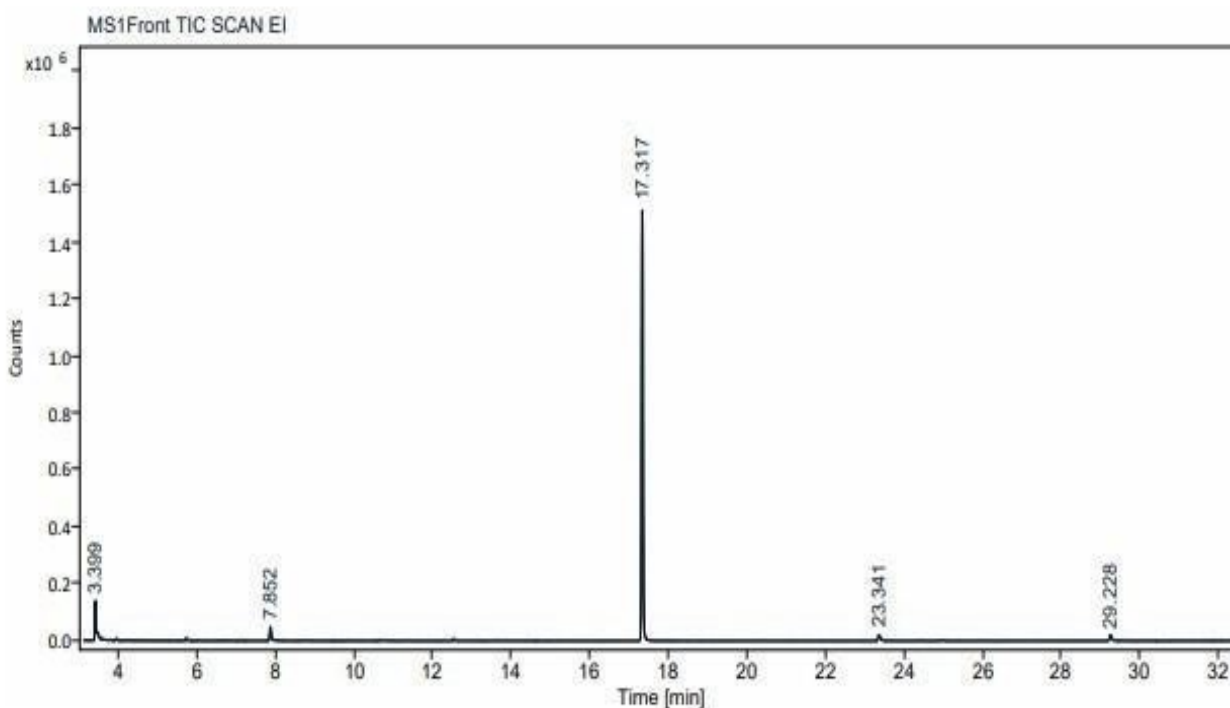
S.No	RT Value	Name of the compound	Molecular Wt. (g/mol)	Molecular Formula	Area %	Recorded Biological activity
1	3.406	Glycerin	92.09	C <sub>3</sub> H <sub>8</sub> O <sub>3</sub>	5.30	Antimicrobial and antiviral activity
2	3.406	1,3- Dicyclohexylurea	224.34	C <sub>13</sub> H <sub>24</sub> N <sub>2</sub> O	5.30	Antimicrobial activity
3	3.406	Tetramethylphosphonium cation	171.02	C <sub>4</sub> H <sub>12</sub> Brp	5.30	Antitumour, anticancer activity

4	17.315	Diethyl phthalate	222.24	C <sub>10</sub> H <sub>10</sub> O <sub>4</sub>	73.50	plasticiser and antimicrobial activity
5	17.315	Phthalic acid, ethyl isopropyl ester	374.85	C <sub>15</sub> H <sub>20</sub> O <sub>4</sub>	73.50	Antibacterial activity
6	17.315	Phthalic acid, ethyl 2-pentyl ester	278.51	C <sub>15</sub> H <sub>20</sub> O <sub>4</sub>	73.50	Antibacterial activity
7	22.609	Neophytadiene		C <sub>20</sub> H <sub>38</sub>	2.60	Antidepressant effects, Anti-inflammatory, antioxidant and cardioprotective
8	22.609	3,7,11,15-Tetramethyl-2hexadecen-1-ol	296.53	C <sub>20</sub> H <sub>40</sub> O	2.60	Antimicrobial activity
9	22.609	3-Methylene-7,11-dimethyl-1-dodecene	208.38	C <sub>15</sub> H <sub>28</sub>	2.60	No activity
10	23.343	Phthalic acid, hept-4-yl isobutyl ester	320.42	C <sub>19</sub> H <sub>28</sub> O <sub>4</sub>	12.60	Anti-inflammatory activity
11	23.343	Phthalic acid, hept-3-yl isobutyl ester	320.42	C <sub>19</sub> H <sub>28</sub> O <sub>4</sub>	12.60	Anti-inflammatory activity
12	23.343	Phthalic acid, hex-3-yl isobutyl ester	166.13	C <sub>6</sub> H <sub>4</sub> (CO <sub>2</sub> H) <sub>2</sub>	12.60	Antimicrobial activity
13	29.640	Phytol	296.53	C <sub>20</sub> H <sub>40</sub> O	1.12	Antinociceptive, Antioxidant Activity and Anti-inflammatory activity
14	29.640	3,7,11,15-Tetramethyl-2hexadecen-1-ol	296.53	C <sub>20</sub> H <sub>40</sub> O	1.12	Antimicrobial and antioxidant activity
15	29.640	2-hexadecen-1-ol,3,7,11,15tetramethyl- acetate	280.5	C <sub>22</sub> H <sub>44</sub> O <sub>2</sub>	1.12	Antimicrobial activity
16	37.006	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	330.50	C <sub>19</sub> H <sub>38</sub> O <sub>4</sub>	1.97	Antimicrobial activity
17	37.006	Glycerol 1-palmitate	330.50	C <sub>19</sub> H <sub>38</sub> O <sub>4</sub>	1.97	No activity
18	37.688	Phthalic acid, di(2-propylpentyl)ester	250.29	C <sub>24</sub> H <sub>38</sub> O <sub>4</sub>	2.92	
19	37.688	Phthalic acid, di(oct-3-yl)ester	390.55	C <sub>24</sub> H <sub>38</sub> O <sub>4</sub>	2.92	No activity

20	37.688	Phthalic acid, octyl 2- propyl pentyl ester	348.47	C <sub>21</sub> H <sub>32</sub> O <sub>4</sub>	2.92	No activity
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**GC-MS Analysis of *Rubia cordifolia* Root Extract:**

15 bioactive compounds were identified in methanolic root extract of *Rubia cordifolia*. 1,3Dicyclohexylurea with (RT-3.399) shows antimicrobial activity [24], Methyl salicylate compound with RT value 7.852 exhibits pathogen resistance [30-32]. Phthalic acid, ethyl 2-pentyl ester with RT value 17.317 reported antimicrobial, anticancer and anti-toxic properties in *Physalia angulate* [33]. Another compound Diethyl phthalate with RT value 17.317 used as cosmetic ingredient, plasticizer and shows antimicrobial activity [11,12]. Phthalic acid, ethyl isopropyl ester with a peak area of 87 % shows antimicrobial activity and synergistic efficacy in extracted essential oils of stem bark extract of *Spondias mombin* [13]. Phthalic acid, hex-3-yl isobutyl ester shows the antimicrobial activity [30]. Phthalic acid, hept-4-yl isobutyl ester exhibits anti-inflammatory activity in methanolic extract of dried leaves of *Chenopodium album* and *Stellaria media* [34]. Antifungal and antimicrobial activity reported from the chloroform extract of *Sansevieria cylindrica* [35]. 9,10Anthracenedione,2-methyl- and 9,10-Anthracenedione,1-methyl (RT29.228) exhibits anticancer activity [36].



**Fig.3.**GC-MS chromatogram of *Rubia cordifolia* root methanolic extract.

**Table3:** Phytochemical components identified in *Rubia cordifolia* root methanolic extracts using GC-MS analysis and their reported biological activities.

S.No	RT Value	Name of the compound	Molecular Wt. (g/mol)	Molecular Formula	Area %	Recorded Biological activity
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1	3.399	Glycerin	92.09	C <sub>3</sub> H <sub>8</sub> O <sub>3</sub>	6.81	No activity
2	3.399	1,3- Dicyclohexylurea	224.348	C <sub>13</sub> H <sub>24</sub> N <sub>2</sub> O	6.81	Antimicrobial activity
3	3.399	Tetramethylphosphonium cation	171.018	C <sub>4</sub> H <sub>12</sub> BrP	6.81	No activity
4	7.852	Methyl salicylate	152.1494	C <sub>8</sub> H <sub>8</sub> O <sub>3</sub>	2.50	Pathogen resistance
5	7.852	Benzoic acid,2-(1methylpropyl)oxy-,methyl ester	122.12	C <sub>6</sub> H <sub>5</sub> COOH	2.50	No activity
6	7.852	Benzoic acid,2-(isopropyl)oxy -,methyl ester	194.2271	C <sub>11</sub> H <sub>14</sub> O <sub>3</sub>	2.50	No activity
7	17.317	Diethyl Phthalate	222.24	C <sub>10</sub> H <sub>10</sub> O <sub>4</sub>	87.80	Antimicrobial activity
8	17.317	Phthalic acid, ethyl 2- pentyl ester	385.4104	C <sub>15</sub> H <sub>20</sub> O <sub>4</sub>	87.80	Antimicrobial, syngistic activity
9	17.317	Phthalic acid, ethyl isoporpyl ester	240.29	C <sub>13</sub> H <sub>16</sub> O <sub>4</sub>	87.80	Antimicrobial
10	23.341	Phthalic acid, hex-3-yl isobutyl ester	166.14	C <sub>8</sub> H <sub>6</sub> O <sub>4</sub>	1.67	Anti-microbial
11	23.341	Phthalic acid, hept-3-yl isobutyl ester	320.4232	C <sub>19</sub> H <sub>28</sub> O <sub>4</sub>	1.67	Anti-inflammatory
12	23.341	Phthalic acid, hept-4-yl isobutyl ester	320.423	C <sub>19</sub> H <sub>28</sub> O <sub>4</sub>	1.67	Anti-inflammatory, antimicrobial and antifungals
13	29.228	9,10- Anthracenedione,2-methyl -	208	C <sub>14</sub> H <sub>8</sub> O	1.23	Anticancer
14	29.228	9,10- Anthracenedione,-1methyl-	208	C <sub>14</sub> H <sub>8</sub> O	1.23	Anticancer
15	29.228	9-Keto-4-fluorenylethylene oxide	222	C <sub>15</sub> H <sub>10</sub> O <sub>2</sub>	1.23	No activity



**Conclusion:**

In GC-MS analysis of *Rubia cordifolia* 50 compounds were identified, with 15 compounds in leaf, 20 compounds in stem and 15 in root methanolic extract. The dominant compounds of leaf, stem and root are Diethyl phthalate, Phthalic acid, ethyl isopropyl ester and Phthalic acid, ethyl 2- pentyl esters. *Rubia cordifolia* has several bioactive chemicals. These chemicals have several therapeutic qualities. Some compounds which are found in leaf, stem and root of *Rubia cordifolia* have not yet been reported. GCMS revealed the presence of anthraquinones, having strong antioxidant activity, and anticancer activity. More research is needed on this plant to get more benefits.

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