

# Isolation of Plasticizer Compound 1, 2-Benzenedicarboxylic acid in Leaf Extract of *Andrographis paniculatas*

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**ABSTRACT:** The main objective of the present investigation was to identify and characterize the compound 1,2-Benzenedicarboxylic acid obtained from the leaves of *Andrographis paniculata* (Burm. F) Nees (Acanthaceae) by using the GC-MS. 100g powdered leaf sample is extracted in soxhlet apparatus with 1.5 ml methanol and filtered through No.1 whattman paper and the extract was concentrated to dryness. The obtained brown residues were chromatographed by column chromatography over silica gel, eluted with methanol-acetone (60:40) ratio. The compound detection employed the NIST ver.2.0 – year 2005 library. The GC-MS analysis of collected crude extract revealed the presence of thirty different phytochemical compounds. The spectrum profile of GC-MS results as a major compound confirmed the presence of 1, 2-benzenedicarboxylic acid as a major compound with retention time 34.113. The results of the present study shows the presence of potential of elasticity plasticizer compound of 1,2-benzenedicarboxylic acid a major traditional source in the medicinal plant *Andrographis paniculata*. Therefore, this compound may use to for the study on various as preventing cell damage caused by various environmental factors.

**KEYWORDS:** *A.paniculata*, 1, 2-benzenedicarboxylic acid, GC-MS, Medicinal plants.

## I. INTRODUCTION

Medicinal plants are expensive gift from nature to human. The approval of traditional medicine as an alternative form of health care and the improvement of microbial resistance to the existing antibiotics has lead researchers to scrutinize the antimicrobial compound (Sumathi 2010) [1]. Herbal medicines are safer than synthetic medicines because the phytochemicals in the plant extract target the biochemical pathway. Medicinal plants have been used all over the world for the treatment and prevention of various ailments, particularly in developing countries where infectious diseases are endemic and modern health facilities services are inadequate (Zaidan et al., 2005) [2]. Plant-based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, etc. (Gordon 2001) ) [3]. . There is growing awareness is correlating the phytochemical constituents of a medicinal plant with its pharmacological activity (Prachayasittikul et al., 2008) [4]. . The active components of *A.paniculata* are diterpene lactones collectively known as andrographolides found in the aerial parts of the plant. The main chemical constituents is andrographolide (AP), a bitter colourless crystalline compound and other major bitter diterpene lactone constituents is 14-deoxy-11,12-didehydroandrographolide (DIAP). The andrographolides isolated from the aerial parts of *A.paniculata* have liver disorders including treatment of hepatitis (Sharma et al 1992, Tang et al 1992, Holt et al 1998) [5-7]. With this background in the present investigation, an attempt was made to find suitable antiproliferation, and elasticity containing compound further medicinal plant *A.paniculata*.

# International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 5, Issue 4, April 2016

## II. RELATED WORK

Kalaivani et al., (2012) [8] reported that the 13 different compounds from the ethanolic extract of *A. paniculata* leaves and presence of 1,2-benzenedicarboxylic acid (1.17%) retention time 24.60 and other major compounds namely Retinoic acid, methyl ester [9-11], Androstan-17-one, 3-ethyl-3-hydroxy-, (5 $\alpha$ ), Phytol. Kalaiselvan et al., (2012) [12], noted that the 19 different phytochemical compounds from the ethanolic extract of plant bark of *Andrographis paniculata* and presence of 1,2-benzenedicarboxylic acid, diundecylestero (1.52%), retention time (21.45) other major compounds 2,5-octadecadiynoic acid, methyl ester, 10-12-pentacosadiynoic acid, 1,2,3,5-cyclohexanetetrol, (1a,2a,3a,5a). Somerita panda and Bandyopadhyay (2013) reported that the GC-MS study on methanolic leaf extract of *Andrographis paniculata* namely there Cyclopentadecanone, 2-Hydroxy, Phytol, 2beta-hydroxy-9-oxoverrucosane, Naphthalene, Stigmast-5-en-3-ol, Eicosanoic acid. Our results were 1,2-Benzenedicarboxylic acid found as the major component in the column chromatography fraction of leaves of *A. paniculata*. 1,2-Benzenedicarboxylic acid is suggested to be plasticizer compound and it may acts as an antimicrobial and antifouling agent. Rasika and Torane et al (2012), reported that the isolation and Characterisation of 1, 2 Benzenedicarboxylic acid, a bioactive Compound from hexane extract of leaves of *Ehretia laevis*. Previous studies determined the chemical profile of chloroform extracts of *A. paniculata* leaves using GC-MS (Roy et al 2010) [13]. They observed the presence of phenols, aromatic carboxylic acids and esters in the chloroform extract of *A. paniculata*. The therapeutically important active principle andrographolide was observed in the aerial parts of *A. paniculata* (Chem and Liang 1982; Siripong et al., 1992; Rastogi and Mehrotra 1998; Rastogi and Mehrotra 1999; Jain et al., 2009). Other therapeutic compounds include 14-deoxy-11-oxoandrographolide, 14-deoxy-11,12-didehydroandrographolide, non-bitter compound neoandrographolide, homoandrographolide, andrographosterol, andrographane, andrographosterin, andrograpanin,  $\alpha$ -sitosterol, stigmastrol, apigenin-7,4-di-O-methyl ester, 5-hydroxy 7,8,2,3-tetramethoxy flavones, monohydroxytrimethyl flavones, andrographnin, dihydroxy-di-methoxyflavones, panicolin, andrographoneo, andrographoside, andropaniculosin A; andropani-culoside A, andrograpanin, isoandrographolide and skullcaflavone. Shen et al., (2006) [14-19] reported six entlabdanediterpenoids, i.e. 3-O-beta-D-glucopyranosyl-14,19-dideoxyandrographolide, 14-deoxy-17-hydroxyandrographolide, 19-O-(beta-D-apifuranosyl(1-2)-beta-D-lucopyranosyl)-3, 14-dideoxyandrographolide, 3-O-beta-D-glucopyranosyl andrographolide, 12S-hydroxyandrographolide and andrographatoside from the aerial parts of the plant.

## III. MATERIALS AND METHODS

### A. COLLECTION AND PREPARATION OF PLANT MATERIAL

The leaves of *Andrographis Paniculata* were collected from Thanjavur district (Tamil Nadu, India). The leaves were washed thoroughly for 3 times in running tap water to remove soil particles and adhered debris and finally with sterile distilled water. The leaves were cut, shade dried, ground into fine powder and stored in air tight polythene bags until use.

### B. EXTRACTION OF PLANT MATERIAL

The shade dried and powdered leaves of *Andrographis Paniculata* (100g) were successively extracted with methanol (1L). The methanol extract was concentrated to dryness and obtained 21.60g residue. Further fractions were separated from the obtained residues using the solvent methanol-acetone (6:4 ratio) through column chromatography on silica gel 60-120 mesh.

### C. GC-MS ANALYSIS

GCMS is an effective method utilized for some applications which has high affectability and specificity. For the most part its application is situated towards the particular recognition and potential distinguishing proof of mixes in light of the sub-atomic mass in a mind boggling blend. The point of the present study is to build up a fast strategy for the quantitative determination of natural mixes in herbs by GC-MS method. It is accordingly fundamental that endeavours ought to be made to acquaint new restorative plants with create less expensive medications. Plants still speak to a vast undiscovered wellspring of fundamentally novel aggravates that may serve as lead for the advancement of novel medications. The GC-MS analysis was carried out on a GC clarus 500 Gas chromatography equipped with Elite-5 capillary column (5% phenyl 95% dimethyl polysiloxane) (30m x 0.25mm ID x 0.25 $\mu$ m df) and was detector

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turbomass gold of the company. Helium was used as carriers gas at a constant flow rate of 1ml/min, split ratio of 10:1 and injector temperature 250°C. The oven temperature programmed 110°C with an increase of 10°C/min, to 200°C/min, then 5°C/min to 280°C/min. Mass spectra were taken at. 70ev; scan interval of 0.2 and fragments from 45-450Da.

## D. NMR- ANALYSIS

In the NMR- spectroscopic analysed and the result was discussed by  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded on a Bruker Avance AV-500 instrument (Bruker Co., Switzerland) at 500 MHZ. CD3OD was used as a solvent and DMS was used as an internal standard. Chemical shifts ( $\delta$ ) were expressed in ppm shift. The structure for compound interpretation was determined by  $^1\text{H}$  NMR and the  $^{13}\text{C}$  NMR studies carried out at the CECRI, Karaikudi, Tamilnadu, India.

## E. IDENTIFICATION OF COMPONENTS

Interpretation of Mass Spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library, and the name, molecular weight and structure of the components of the test materials were determined.

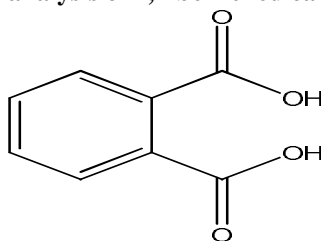
## III. RESULTS AND DISCUSSION

The phytochemical constituents present in the leaves of *A.paniculata* were analyzed GC-MS. The analysis shown a 30 different compounds namely Propane (21.10), 3,6-Dipropyl 1,2,4,5 – tetrazine (23.08), Phytol (24.22), n-Hexadecanoic acid (25.63), Pentnoic acid (27.37), Tridecnicacid, methyl ester (28.38), Tyroline-n-oxide(29.19),12-hydroxy-9-octadecenoicacid(29.85),Methyl-9 ol (30.25), Methyl heptadecanoate (30.39),Silane(31.20), Tetradeconoic acid (33.53), 2, 6 – Dimethyl – 2 – hepton – 4 – one (33.90) , Heptadecane – 2 – methyl (34.05), 1, 2 Benzenedicrboxylic acid (34.37) , Octanoic acid (34.70), Disulfide, dioctyl (35.34), 1Propanone (38.45), 1Heptanol (38.85), Tetracosanoicacid, methylester (39.35) , 2 , 4, 4 – Trimethylpentan - 1-ol(40.68), 9 – decen - 3-one,2,2,6,6-tetramethyl(41.70), Alpha-tocopherol (Vitamin E)(42.02), 1-Pentadecanol(44.41),2(E)-2-nonnen-1-ol(45.44), 4(R-S)-vinyl-6(S)-iodimethyl9undecenal(46.46),Oxirane(48.52) The retention time, molecular weight, molecular formula, relative percentages of the compounds were also measured in leaves of *A.paniculata* (Table.1).The obtained residue of maximum concentration was further separated by column chromatography by using silica gel with the solvent methanol: acetone(60:40) and the isolated fraction were analysis by GC-MS the GC-MS analysis shown the 97.44% one major compounds namely 1,2-benzendicarboxylic acid retention time 34.11. The nature of 1,2-benzendicarboxylic acid was plasticizer compound and therapeutic activity were antimicrobial and antifouling agent. Other 10 different phytochemical constituents namely Propane(26.17), Naphthalene(27.72),2,2,3,3,4,4,4-Heptofluorobutanoate(28.71),1-Propene,3-chloro(29.20), Butanenitrile(30.47), (R,Z)-12-hydroxy-9-octadecenoic acid(33.97), Peroxide bis (1,1-dimethyle(31.54), 1-Phenanthrenicarboxylic acid(33.97), 3,6-Dihydroxypregan-2-one(35.18), Ethanone (35.71). The retention time molecular weight, molecular formula and relative percentage of the compound were noted in (Table.2).

## A. Analysis of $^1\text{H}$ NMR spectrum of 1,2-benzenedicarboxylic acid

The -OH protons gives the signal at 8.23 ppm as a singlet. The aromatic protons are resonates in the region of 7.41 and 7.59 ppm.

### Spectral analysis of 1,2-benzenedicarboxylic acid



1,2-benzene dicarboxylic acid

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**Table: 1. Phytoconstituents of leaf Extract of *A. paniculata* (Burm. F.) Nees**

S.No	R.Time	Compound	M.W	Peak area%	M.formula
1.	21.101	Propane, 2-Nitorpropane	89	0.17%	C <sub>3</sub> H <sub>7</sub> O <sub>2</sub>
2	23.081	3,6-Dipropyl 1,2,4,5-tetra azine	166	0.14%	C <sub>8</sub> H <sub>14</sub> N <sub>4</sub>
3	23.226	Phytol	296	58.53%	C <sub>22</sub> H <sub>42</sub> O <sub>2</sub>
4	25.639	n-Hexadecanoic acid	256	8.03%	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>
5	27.374	Pentnoic acid, 4-methyl	116	2.61	C <sub>6</sub> H <sub>12</sub> O <sub>2</sub>
6	28.381	Tridecnic acid, Methyl ester	228	0.24%	C <sub>14</sub> H <sub>28</sub> O <sub>2</sub>
7	29.193	Tyrosine-n-oxide	85	0.27%	C <sub>4</sub> H <sub>17</sub> NO
8	29.852	12-hydroxy-9-Octadecenoic acid	298	0.75%	C <sub>18</sub> H <sub>34</sub> O <sub>3</sub>
9	29.936	Methyl-9-octadecenoate	296	1.46%	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>
10	30.010	9,12,15-Octadecatrienoic acid (Z,Z,Z)	292	1.59%	C <sub>19</sub> H <sub>32</sub> O <sub>2</sub>
11	30.259	3,7,11,15-Tetramethyl hexadec-2-n 1-ol	296	2.14%	C <sub>20</sub> H <sub>40</sub> O
12	30.394	Methylheptadecanoate	284	2.54%	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>
13	31.203	Silane	368	0.20%	C <sub>23</sub> H <sub>48</sub> O <sub>2</sub>
14	33.534	Tetradecanoic acid	256	1.38%	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>
15	33.900	2,6-Dimethyl-2-hepton-4-one	140	0.51%	C <sub>9</sub> H <sub>16</sub> O
16	34.056	Heptadecane-2-methyl	254	1.04%	C <sub>18</sub> H <sub>38</sub>
17	34.377	1,2-Benzenedicarboxylic acid	312	12.37%	C <sub>19</sub> H <sub>20</sub> O <sub>4</sub>
18	34.708	Octanoic acid	158	0.13%	C <sub>9</sub> H <sub>18</sub> O <sub>2</sub>
19	35.349	Disulfide, dioctyl	290	0.22%	C <sub>16</sub> H <sub>34</sub> S <sub>2</sub>
20	38.452	1-Propanone	98	0.29%	C <sub>6</sub> H <sub>10</sub> O
21	38.857	1-Heptanol	116	0.26%	C <sub>7</sub> H <sub>16</sub> O
22	38.064	Octane, 1,2-Dibromo	270	0.14%	C <sub>8</sub> H <sub>16</sub> Br <sub>2</sub>
23	39.358	Tetracosanoic acid, methyl ester	382	1.04%	C <sub>25</sub> H <sub>50</sub> O <sub>2</sub>
24	40.682	2,4,4-Trimethylpentan-1-ol	130	0.18%	C <sub>8</sub> H <sub>18</sub> O
25	41.700	9-decen-3-one, 2,2,6,6-tetramethyl	210	0.30%	C <sub>14</sub> H <sub>26</sub> O
26	42.028	Alpha-tocopherol (Vitamin E)	430	0.29%	C <sub>29</sub> H <sub>50</sub> O <sub>2</sub>
27	44.419	1-pentadecanol	228	0.68%	C <sub>15</sub> H <sub>32</sub> O
28	45.444	2(E)-2-nonen-1-ol	142	1.61%	
29	46.468	4(R-S)-vinyl-6(S)-10-dimethyl-9-undecenal	222	0.21%	C <sub>15</sub> H <sub>26</sub> O
30	48.524	Oxirane	116	0.35%	C <sub>6</sub> H <sub>12</sub> O <sub>2</sub>

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Table: 2.GC-MS analysis of major fraction isolated by column chromatography

S.No	R.Time	Compound	M.W	Peak area%	M.formula
1	26.176	Propane,2-nitro	89	0.13%	C <sub>3</sub> H <sub>7</sub> NO <sub>2</sub>
2	27.726	Indacen-1(2H)-one	256	0.79%	C <sub>18</sub> H <sub>24</sub> O
3	28.716	2,2,3,3,4,4,-heptafluorobutanoate	630	0.06%	C <sub>18</sub> H <sub>20</sub> F <sub>14</sub> O <sub>8</sub>
4	29.203	1-Propene,3-Chloro	76	0.04%	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>
5	30.474	Mehane,isocyano	41	0.02%	C <sub>2</sub> H <sub>3</sub> N
6	31.770	(R,Z)-12hydroxy,-9-octadecenoic acid	298	0.21%	C <sub>18</sub> H <sub>34</sub> O <sub>3</sub>
7	31.547	Di-tert-butylperoxide	146	0.01%	C <sub>8</sub> H <sub>18</sub> O <sub>2</sub>
8	33.972	1Phenanthrenecarboxylicacid	314	0.64%	C <sub>21</sub> H <sub>30</sub> O <sub>2</sub>
9	<b>34.113</b>	<b>1,2-Benzenedi carboxylic acid</b>	<b>312</b>	<b>97.44%</b>	<b>C<sub>19</sub>H<sub>20</sub>O<sub>4</sub></b>
10	35.188	3,6-Dihydroxypregan-2-one	334	0.17%	C <sub>21</sub> H <sub>34</sub> O <sub>3</sub>
11	35.712	Ethanone	152	0.50%	C <sub>10</sub> H <sub>16</sub> O

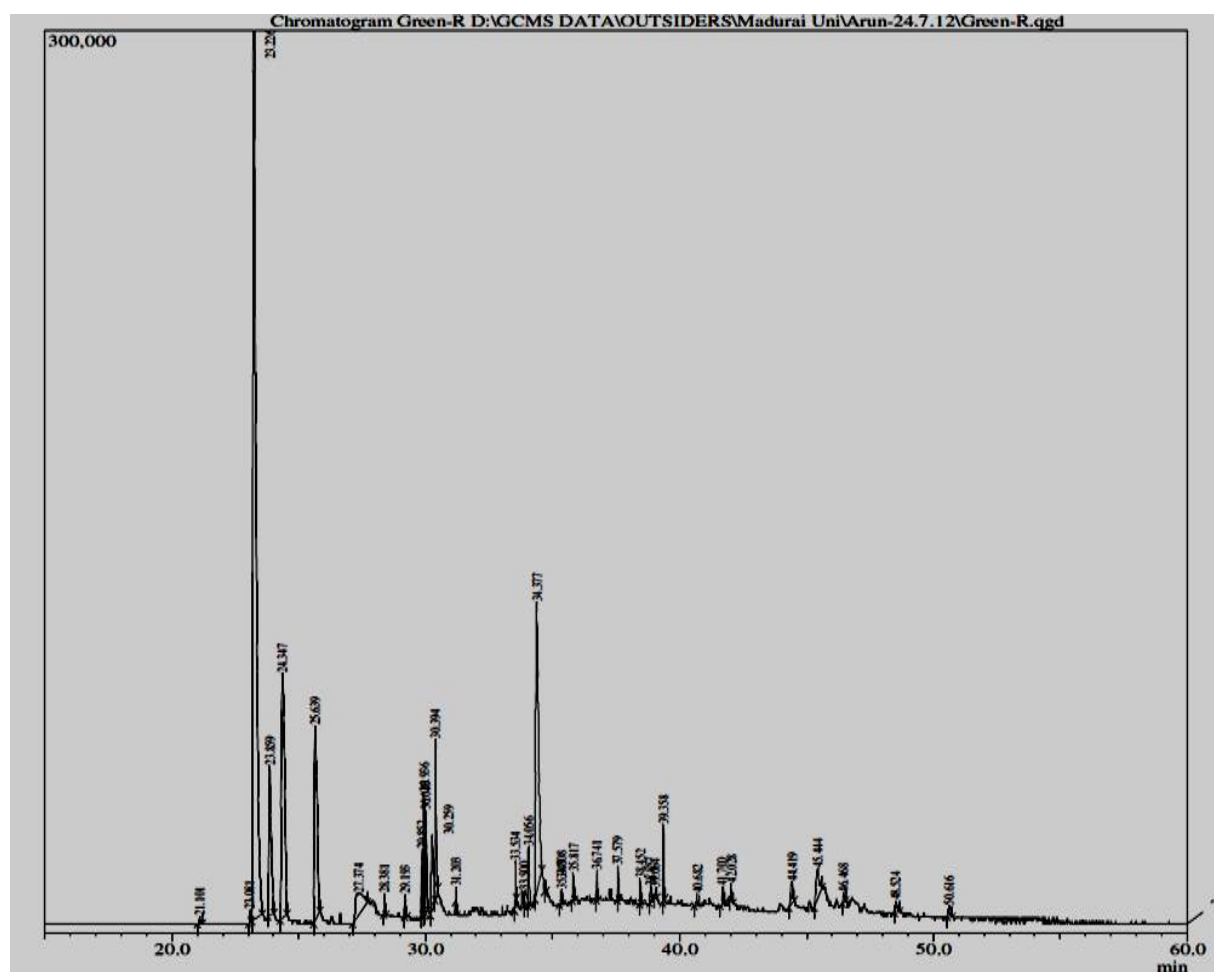
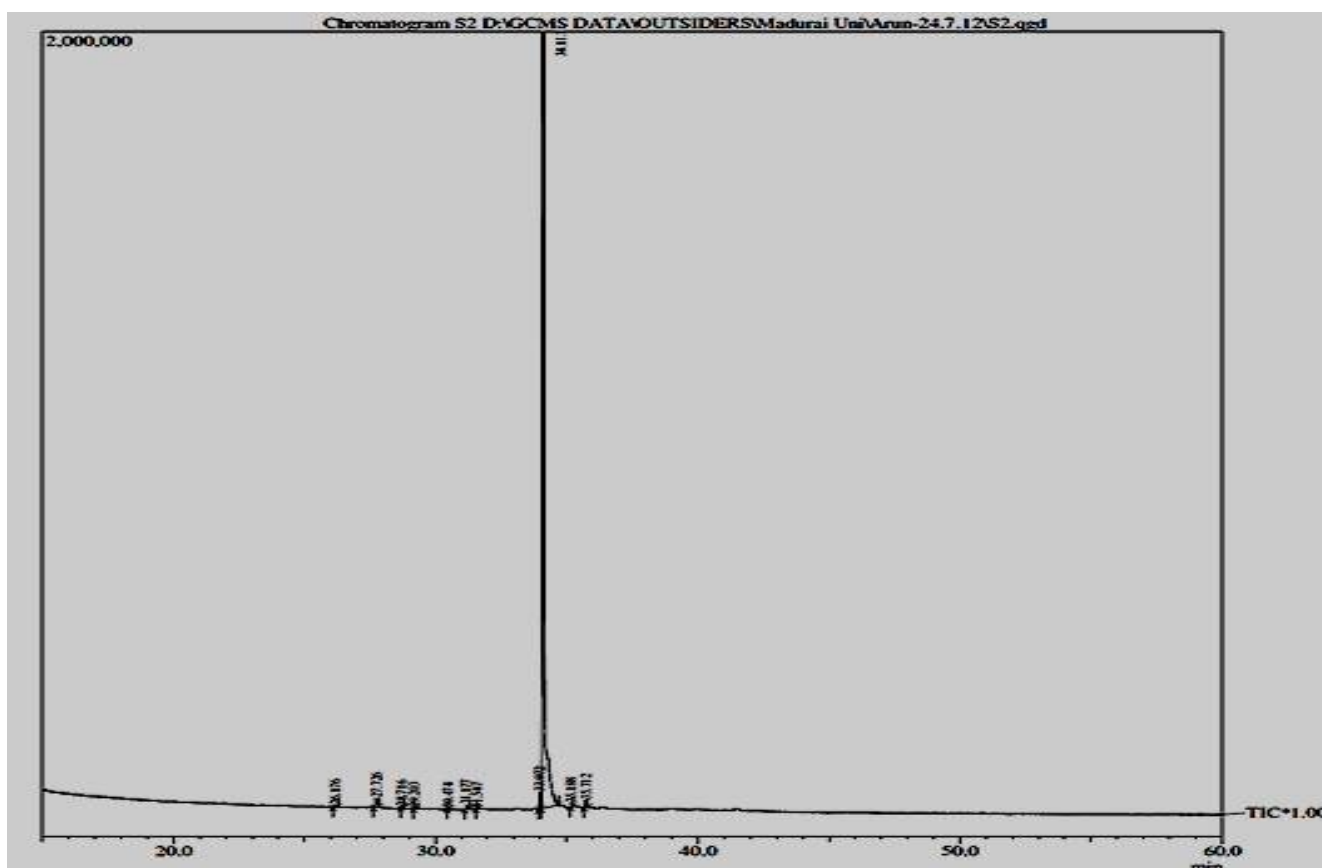


Figure 1:GC-MS Chromatogram of methanol leaf extract of *A.paniculata*

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**Figure 2:** 1, 2-benzenedicarboxylic acid was identified from the isolated fraction of leaf extract of *A.paniculata* by GC-MS

In the present investigation, there are 30 different compounds were observed from methanolic extract of *A.paniculata* leaves. Similar, several phytochemical screening studies have also been carried out in different parts of the world using GC-MS (Wu L et al 2010, Vohra A 2011) [20,21] to characterize the chemical profile of leaves of *A.paniculata*. Gas chromatogram analysis of the plant extract suggests that the presence of many compounds in different retention time and molecular weight. The high of the peak indicate the relative concentrations of the components present in the plant. The results pertaining to GC-MS analysis leads to the identification of number of compounds from the GC fractions of the Methanolic extract of *Andrographis Paniculata* from Tanjore district. These compounds were identified through mass spectrometry attached with GC.

## IV. CONCLUSION

In this study suggested that the 1,2-Benzenedicarboxylic acid obtained from the methanolic extract of leaves of *Andrographis paniculata* (Burm. F) Nees (Acanthaceae) by using the GC-MS. *A.Paniculata* leaves are often used as nematicide, fungicide, lubricant, pesticide. *A.paniculata* leaves possess bactericide, antiseptic, antistaphylococci, allergenic, antioxidant, antipyretic, anticancer, anti-inflammatory drug, anticoronary, antiarthritic, antisalmonella, antifouling, antiandrogenic, antitubercular, anticonvulsantdrug, analgesic, antihistaminic, anesthetic, antisecretory, antispermigenic, antitonsilitic, antieczemic, cancer reventive, choleric, contraceptive, chemo preventive,



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hypcholesterolemic, hepatoprotective, immunostimulant, sedative properties. Additionally they reduce blood levels of cholesterol and act as  $5 - \alpha$  enzyme substance, etc. this study enhances the standard usage of *A. Paniculata* that possesses many identified and unknown bioactive compounds. By isolating and identifying these bio-active compounds, new medicine may be developed to treat numerous diseases.

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