



## GCMS ANALYSIS OF METHANOLIC EXTRACTS OF DIFFERENT PARTS OF *JUSTICIA ADHATODA* L.

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The current study aims to determine phytochemical profile of the plant *Justicia adhatoda* via usage of Gas Chromatography-Mass Spectrometry (GC-MS) analytical technique. The study shows results pertaining to GC-MS analysis of different parts of *Justicia adhatoda*, including, flowers, leaves, root and stem. The results of the study show presence of 24 peaks corresponding to different phytocompounds in GC-MS analysis of *Justicia adhatoda* flowers. Similarly, GC-MS analysis of leaves, root and stem of *Justicia adhatoda* revealed presence of 36, 29 and 29 peaks respectively, corresponding to different phytocompounds. Detailed literature survey showed that most of the bioactive phytocompounds present in flowers, leaves, root and stem of the plant are reported to be antioxidant, anti-inflammatory and antimicrobial in nature. Therefore, the study concludes with a message that presence of bioactive phytocompounds in different parts of *Justicia adhatoda* imparts its pharmacological attributes and its potential applications in amelioration of chronic oxidative and inflammatory conditions.

**Keywords:** Antioxidant, Anti-inflammatory, Antimicrobial, Gas Chromatography-Mass Spectrometry, *Justicia adhatoda*.

### Introduction

*Justicia adhatoda*, commonly known as the Malabar nut or adhatoda, is a perennial shrub plant belonging to the Acanthaceae family. The plant is indigenous to tropical parts of Asia, such as India, Sri Lanka, and certain parts of Southeast Asia. The plant is characterized by presence of white or purplish flowers, stout stems and broad green leaves. *Justicia adhatoda* can grow up to a height of 1.5 meters and holds significant medicinal and cultural importance. *Justicia adhatoda* is recognised of extreme medicinal importance by various medicinal systems, such as Ayurveda and Unani. Different parts of the plant *Justicia adhatoda* have been employed for diverse pharmacological applications, such as usage as anti-inflammatory, antimicrobial, antitussive, expectorant, and bronchodilator effects<sup>1,2</sup>.

The medicinal and pharmacological attributes of the plant *Justicia adhatoda* may be attributed to presence of several bioactive key secondary metabolites and bioactive phytocompounds that play a crucial role as antioxidant, anti-inflammatory and antimicrobial agents. It is due to presence of these properties that *Justicia adhatoda* is used for treatment of a number of chronic ailments characterized by presence of oxidative stress and inflammatory outburst, such as, asthma, bronchitis, cough, cardiovascular disorders, neurodegenerative disorders and several other ailments. The medicinal attributes of *Justicia adhatoda* may be attributed to its phytochemical composition, comprising of bioactive alkaloids, flavonoids, tannins, as well as essential oils. For instance, presence of an alkaloid vasicine provides plant's bronchodilator activity, while antioxidant activities are contributed by flavonoids. Therefore, it is critically important to

understand the plethora of specific compounds present in different parts of the plant in order to substantiate the traditional medicinal uses of the plant and also explore its potential in modern therapeutics<sup>3-7</sup>.

Considering this, the current study has been drafted to accurately identify and quantify the presence of bioactive compounds in *Justicia adhatoda* via usage of Gas Chromatography-Mass Spectrometry (GC-MS) analytical technique. The objective of the current study is to conduct a detailed GC-MS analysis of the different parts of the plant *Justicia adhatoda*, with an aim to identify as well as characterize presence of bioactive compounds present in each plant tissue. The study attempts to characterize and identify phytochemical profile of flowers, leaves, roots, and stems of *Justicia adhatoda*, and accordingly assess the variation in chemical composition as well as potential implications for usage of plant in therapeutics. Apart from determination of therapeutic applications of the plant, the exploration of *Justicia adhatoda*'s phytochemical profile is important for advancement of our understanding of plant-based resources in modern healthcare system. Therefore, insights gained from this study would not only enhance our understanding of medicinal usage of plants but also substantiate research endeavours aimed at isolation of specific phytocompounds from plants for usage in pharmaceutical industry.

### Material and Methods

#### *Collection and processing of plant materials :*

Root, stem, leaves and flowers of the selected plants were collected from Jaipur, Rajasthan, India. Those were shade dried, and grinded to make fine powder. 1 gm of plant material was soaked in methanol and kept for 24 hours. Those were filtered and sent for characterization.

#### *Gas Chromatography and Mass Spectrum analysis :*

The extracts and the standards were analysed by GC-MS technique of Hewlett-Packard 6890/5973 operating at 1000 eV ionization energy, equipped with utilizing Agilent 7890A/5975C GC HP-5. Capillary column (phenyl methyl siloxane, 25 m × 0.25 mm i.d.) with Helium (He) gas was utilized as carrier with split ratio 1:5. Temperature of oven was set at 100 °C (for 3 minutes) to 280 °C at 1-40°C/min; detector temperature, 250 to 280°C; carrier gas, He (0.9 ml/min). Retention indices were determined by utilizing retention times of samples that were injected under the same chromatographic conditions. The substances of the standard and plant samples were identified by comparing their mass spectra and retention time with those given in literature and by comparing with the mass spectra of the Wiley library (National Institute of Standards and Technology data bank) or with the published mass spectra.

### Results

#### *GC-MS analysis of Justicia adhatoda root :*

GC-MS analysis of *Justicia adhatoda* roots yielded a total of 29 peaks, corresponding to different phytocompounds, that include, 2,2-Dimethoxybutane-; Glyceraldehyde-; Dihydroxyacetone-; 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-on-; 3-Pentanone, 2,4-dimethyl-; Maltol-; Dodecane-; 5-Hydroxymethylfurfural-; 3,5-Diisopropoxy-1,1,1,7,7,7-hexamethyl-3,5-; 9-methylheptadecane-; 3-Hydroxyphenylacetic acid, 2TMS derivative-; Heneicosane-; Octadecane, 1-chloro-; Hexadecane, 1-iodo-; Deoxypeganine-; Carbonic acid, decyl nonyl ester-; Benzimidazole-5-carboxamide, 1-ethyl-2-meth-; Benzenepropanoic acid, 3,5-bis(1,1-dimethyle-; n-Hexadecanoic acid-; 1H-Pyrrolo[2,1-b]quinazolin-9-one, 3-hydroxy-; 9,12-Octadecadien-1-ol, (Z,Z)-; Triacotane, 1-iodo- and Octacosan-14-one (Figure 1; Table 1).





Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl); Squalene and Eicosane (Figure 2; Table 2).

*GC-MS analysis of Justicia adhatoda leaves :*

GC-MS analysis of *Justicia adhatoda* leaves yielded a total of 36 peaks, corresponding to different phytochemicals, including, Ethane, 1-chloro-1-fluoro-; Isopropyl alcohol; Ethylene glycol, TMS derivative; acetic acid; Ethanol; 2-(2-methoxyethoxy)-; Propanal; 2,3-dihydroxy-, (S)-; Cyclopentane, 1-acetyl-1,2-epoxy-; Decane, 3,7-dimethyl-; glycerin; 2,2-Dimethoxybutane; Cyclopentane, 1-acetyl-1,2-epoxy; Heptane, 5-ethyl-2-methyl-; benzoic acid; Benzofuran, 2,3-dihydro-; 1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-; Dodecane, 4,6-dimethyl-; Phenol, 3,5-bis(1,1-dimethylethyl)-; eicosane; Acetic acid, 17-(4-hydroxy-5-methoxy-1,5-dimethyl)-; eicosane; Docosanoic acid, docosyl ester; Z-(13,14-Epoxy) tetradec-11-en-1-ol acetate; Pyrimidine, 5-hydroxy-4-phenyl-; Neophytadiene; 2-Octylcyclopropene-1-heptanol; 3,7,11,15-Tetramethyl-2-hexadecen-1-ol-; 1H-[1] Pyridine-3-carbonitrile, 4-ethyl-2-oxo; Hexadecanoic acid, methyl ester; Mono(2-ethylhexyl) phthalate; 1H-Pyrrolo[2,1-b]quinazolin-9-one, 3-hydroxy; 9,12,15-Octadecatrienoic acid, methyl ester; phytol; Hexadecanoic acid, 14-methyl-, methyl ester; 9,12,15-Octadecatrienoic acid, (Z,Z,Z)-; squalene and (2R,3R,4aR,5S,8aS)-2-Hydroxy-4a,5-dimethyl-3 (Figure 3; Table 3).

*GC-MS analysis of Justicia adhatoda flowers :*

GC-MS analysis of *Justicia adhatoda* flowers yielded a total of 24 peaks, corresponding to different phytochemicals, including, Ethanethiol, 2-(diethylboryloxy)-; 1,2-Ethanediamine, N-propyl-; Acetic acid; 2-Propanone-1-hydroxy; Glycerine; 6-Oxa-bicyclo [3.1.0] hexan-3-one; Cyclopentane, 1-acetyl-1,2-

epoxy-; Decane, 3,7-dimethyl; 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-; 2,4-Dinitrophenylhydrazine of ribose tetrabate; Oxalic acid, isohexyl neopentyl ester; 1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-; Octane, 2-methyl-; Eicosane, 1-iodo; Heptadecane, 2,6,10,15-tetramethyl; Benzimidazole-5-carboxamide, 1-ethyl-2-methyl-; Tetradecanoic acid, 12-methyl-, methyl ester; Benzenepropanoic acid, 3,5-bis (1,1-dimethylethyl)-; n-Hexadecanoic acid; 1,6-Nonadien-3-ol, 3,7-dimethyl-; Octanoic acid, 4,6-dimethyl-, methyl ester; 2-Methylhexacosane and Heptadecane, 8-methyl- (Figure 4; Table 4).

**Discussion**

The GC-MS analysis of different parts of *Justicia adhatoda* revealed presence of a huge plethora of phytochemicals, possessing several properties, such as, antimicrobial efficacy, anti-inflammatory property and antioxidant properties. The current study shows data pertaining to GC-MS analysis of different parts of *Justicia adhatoda*, including, flowers, leaves, roots and stem. The study showed the following key findings:

- GC-MS analysis of *Justicia adhatoda* flowers yielded a total of 24 peaks.
- GC-MS analysis of *Justicia adhatoda* leaves yielded a total of 36 peaks.
- GC-MS analysis of *Justicia adhatoda* roots yielded a total of 29 peaks.
- GC-MS analysis of *Justicia adhatoda* stem yielded a total of 29 peaks.

The results show disparity in the number of peaks corresponding to different plant parts, which translates to variation in the number of phytochemicals among the different plant parts. The leaves contained highest number of peaks corresponding to highest number of phytochemicals (36), followed by roots, stem (29) and flowers (24) with the lowest number of phytochemicals. This disparity in phytochemical composition of different plant parts may occur owing to variability in factors such as tissue type,

**Table 1: Phytochemicals identified in methanolic extract of roots of *Justicia adhatoda* by GC-MS analysis.**

Peak	R.Time	Area %	Height %	Name
1	1.579	10.51	18.23	Isopropyl Alcohol
2	3.168	0.86	1.40	2,2-Dimethoxybutane
3	4.316	0.63	0.80	Glyceraldehyde
4	5.508	0.70	1.05	Dihydroxyacetone
5	7.364	0.63	1.33	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one
6	8.640	0.38	0.70	3-Pentanone, 2,4-dimethyl-
7	9.374	1.63	2.92	Maltol
8	11.219	8.55	7.35	Benzoic acid
9	11.944	0.38	0.64	Dodecane
10	12.396	1.12	1.13	5-Hydroxymethylfurfural
11	15.167	0.93	1.31	3,5-Diisopropoxy-1,1,1,7,7,7-hexamethyl-3,5-bis
12	15.630	0.53	0.75	9-methylheptadecane
13	16.401	18.35	5.71	Sucrose
14	16.740	0.43	0.69	3-Hydroxyphenylacetic acid, 2TMS derivative
15	18.925	0.44	0.66	Heneicosane
16	20.441	0.73	0.85	Octadecane, 1-chloro-
17	21.076	0.67	0.81	Hexadecane, 1-iodo-
18	21.350	0.45	0.31	Decane, 1-iodo-
19	22.174	2.29	1.86	Deoxypeganine
20	22.890	0.57	0.29	Carbonic acid, decyl nonyl ester
21	23.172	17.36	22.28	Benzimidazole-5-carboxamide, 1-ethyl-2-methyl
22	23.513	2.06	3.97	Benzenepropanoic acid, 3,5-bis(1,1-dimethyl-4-hydroxy-
23	23.820	3.65	4.40	n-Hexadecanoic acid
24	24.838	1.75	2.80	1H-Pyrrolo[2,1-b]quinazolin-9-one, 3-hydroxy-2,3-dihydro-
25	25.720	2.34	1.66	9,12-Octadecadien-1-ol, (Z,Z)-
26	26.979	14.59	8.03	-
27	28.697	3.28	3.20	Triacontane, 1-iodo-
28	31.524	1.60	2.23	Octacosan-14-one

environmental conditions and developmental stage.

The flowers of the plant showcased presence of a number of antioxidant biocompounds, including, Ethanethiol, 2-(diethylboryloxy)-; 6-Oxa-bicyclo [3.1.0] hexan-3-one<sup>8</sup>; Cyclopentane, 1-acetyl-1,2-

epoxy<sup>9</sup> ; 1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-; Octane, 2-methyl-; Eicosane, 1-iodo<sup>10</sup> ; Tetradecanoic acid, 12-methyl-, methyl ester<sup>11</sup>; 2-Methylhexacosane<sup>12</sup>; and Heptadecane, 8-methyl-<sup>13</sup>. Similarly, a number of anti-inflammatory compounds

**Table 2: Phytochemicals identified in methanolic extract of stem of *Justicia adhatoda* by GC-MS analysis.**

Peak	R.Time	Area %	Height %	Name
1	1.517	2.24	3.91	Ethyl2-((methylamino)carbonyl)hydrazinecarboxylate
2	1.580	14.69	27.71	Isopropyl Alcohol
3	1.745	2.22	2.77	Propane, 2,2-dimethoxy-
4	1.779	3.82	3.46	Acetic acid
5	2.819	1.90	4.48	Glycerin
6	5.503	1.90	1.52	Dihydroxyacetone
7	7.359	2.59	2.78	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one
8	11.185	1.77	1.09	1,2-Ethanediol, monobenzoate
9	11.317	1.10	1.06	phenol, 2-amino-4-[[4-chlorophenylthio]methyl]-
10	15.644	0.81	0.53	Hexane, 2,2,3,3-tetramethyl-
11	16.359	7.89	3.06	1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-
12	18.201	0.73	0.60	Oxalic acid, 6-ethyloct-3-yl ethyl ester
13	18.808	1.31	0.86	Dichloroacetic acid, 4-pentadecyl ester
14	20.869	1.57	0.53	tert-Hexadecanethiol
15	21.074	1.58	0.99	Nonadecane
16	22.876	1.21	0.58	Octacosanal
17	23.171	36.26	30.92	1H-[1]Pyridine-3-carbonitrile, 4-ethyl-2-oxo-2,5,6,7-tetrahydro-
18	23.411	3.07	2.22	Decanoic acid, methyl ester
19	23.508	1.81	2.28	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl
20	23.817	2.08	2.20	Undecanoic acid, pentyl ester
21	24.830	1.57	1.62	1H-Pyrrolo[2,1-b]quinazolin-9-one, 3-hydroxy-2,3-dihydro
22	25.520	0.88	0.89	-
23	26.089	2.29	0.78	-
24	26.680	1.06	0.57	Docosyl nonyl ether
25	29.250	0.97	0.57	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester
26	31.737	0.90	0.72	Squalene
27	32.467	1.80	1.29	Eicosane

were also found in flowers of *Justicia adhatoda*, including, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-; 2,4-Dinitro phenylhydrazone of ribose tetrabe<sup>14</sup> ; 1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-; Octane, 2-methyl-; n-Hexadecanoic acid<sup>15</sup> ; and Heptadecane, 8-methyl-<sup>16</sup>. The reported antimicrobial bio compounds included 6-Oxa-bicyclo [3.1.0] hexan-3-

one; Cyclopentane, 1-acetyl-1,2-epoxy-; Decane, 3,7-dimethyl; 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-; 2,4-Dinitro phenylhydrazone of ribose tetrabe; Oxalic acid, isohexyl neopentyl ester; 1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-; Heptadecane, 2,6,10,15-tetramethyl; Tetradecanoic acid, 12-methyl-, methyl ester; and n-Hexadecanoic acid<sup>17</sup>.

**Table 3: Phytochemicals identified in methanolic extract of leaves of *Justicia adhatoda* by GC-MS analysis.**

Peak	R.Time	Area %	Height %	Name
1	1.433	53.02	39.64	Ethane,1-chloro-1-fluoro-
2	1.584	13.64	27.57	Isopropyl Alcohol
3	1.745	1.58	1.83	Ethylene glycol,TMS derivative
4	1.789	2.44	1.99	Acetic acid
5	1.885	0.43	0.92	Ethanol,2-(2-methoxyethoxy)-
6	2.257	0.28	0.55	Propanal,2,3-dihydroxy-,(s)-
7	2.827	0.82	2.27	Glycerin
8	3.169	0.94	1.13	2,2-Dimethoxybutane
9	9.398	0.58	0.22	Cyclopentane,1-acetyl-1,2-epoxy-
10	9.928	0.86	0.60	Heptane,5-ethyl-2-methyl-
11	11.218	2.42	1.39	Benzoic acid
12	12.295	0.28	0.24	Benzofuran,2,3-dihydro-
13	16.188	1.17	0.66	1,3-prpanediol,2-(hydroxymethyl)-2-nitro-
14	17.150	0.23	0.23	Dodecane,4,6-dimethyl
15	17.448	0.30	0.53	Phenol,3,5-bis(1,1-dimethyl)-
16	17.890	0.32	0.36	Eicosane
17	19.344	0.34	0.32	Acetic acid, 17-(4-hydroxy-5-methoxy-1,5-dim
18	21.086	0.89	0.49	Eicosane
19	21.270	0.42	0.32	1-Decanol,2-hexyl-
20	21.394	0.50	0.39	3-Eicosane,(E)-
21	21.558	0.56	0.37	Docosanoicacid,docosyl ester
22	21.736	0.52	0.35	Z-(13,14-Epoxy)tetradic-11-en-1-ol acetate
23	22.210	0.32	0.27	Pyrimidine,5-hydroxy-4-phenyl-
24	22.334	0.95	1.03	Neophytadiene
25	22.645	0.59	0.42	2-Octylcyclopropene-1-ol
26	22.880	0.55	0.48	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
27	23.146	5.82	6.06	1H-(1)Pyridine-3-carbonitrile,4-ethyl-2-oxo-
28	23.420	0.38	0.56	Hexadecanoic acid, methyl ester
29	23.812	0.75	1.00	Mono(2-ethylhexyl)phthalate
30	24.837	0.99	1.12	1H-Pyrrolo(2,1-b)quinazolin-9-one,3-hydroxy
31	25.391	0.23	0.41	9,12,15-Octadecatrienoic acid,(Z,Z,Z)-
32	25.521	1.70	2.49	Phytol
33	25.663	0.28	0.40	Hexadecanoic acid, 14-methyl-,methyl ester
34	25.781	0.28	0.32	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-
35	31.741	2.20	1.89	Squalene
36	32.011	2.41	1.20	(2R,3R,4aR,5S,8aS)-2-Hydroxy-4a,5-dimethyl



**Table 4: Phytochemicals identified in methanolic extract of flowers of *Justicia adhatodaby* GC-MS analysis.**

Peak	R.Time	Area %	Height %	Name
1	1.580	33.65	53.67	Isopropyl Alcohol
2	1.712	3.66	2.34	Ethanethiol,2-(diethylboryloxy)-
3	1.745	1.89	3.26	1,2-Ethanediamine,N-propyl-
4	1.778	5.29	3.75	Acetic acid
5	2.241	1.13	1.08	2-Propanone,1-hydroxy-
6	2.819	4.57	7.06	Glycerin
7	6.155	1.10	0.08	6-Oxa-bicyclo(3.1.0)hexan-3-one
8	9.375	1.63	1.07	Cyclopentane,1-acetyl-1,2-epoxy-
9	9.911	1.64	0.09	Decane,3,7-dimethyl-
10	10.802	2.62	1.66	4H-Pyran-one,2,3-dihydro-3,5-dihydroxy-6
11	11.164	5.90	2.85	2,4-Dinitrophenylhydrazone of Ribonasetetrabe
12	14.240	1.33	0.88	Oxalic acid,isoheptyl neopentyl ester
13	16.265	10.14	2.88	1,3-propanediol,2-(hydroxymethyl)-2-nitro-
14	17.130	1.22	0.89	Octane,2-methyl-
15	20.445	1.27	1.03	Eicosane,1-iodo-
16	21.075	1.29	0.88	Heptadecane.2,6,10,15-tetramethyl-
17	23.149	7.49	4.84	Benzimidazole-5-carboxamide,1-ethyl-2-nitro-
18	23.407	1.27	1.20	Tetradecanoic acid ,12-methyl-,methylester
19	23.500	1.37	1.24	Benzenepropanoic acid ,3,5-bis(1,1-dimethylether
20	23.802	3.38	2.20	n-Hexadecanoic acid
21	25.523	2.56	1.27	1,6-Nonadien-3-ol,3,7-dimethyl-
22	25.657	1.95	0.98	Octanoic acid,4,6-dimethyl-methylester,(4S)
23	30.761	1.49	1.66	2-Methylhexacosane
24	32.463	2.15	1.60	Heptadecane,8-methyl-

Similarly, leaves of the plant *Justicia adhatoda* also showed presence of a large number of phytochemicals, including, several antioxidant molecules, such as, Ethane, 1-chloro-1-fluoro<sup>18</sup> ; Heptane, 5-ethyl-2-methyl<sup>19</sup> ; Dodecane, 4,6-dimethyl-; Phenol, 3,5-bis(1,1-dimethylether)<sup>20</sup> ; Eicosane<sup>21</sup> ; and Docosanoic acid, docosyl ester<sup>22</sup> . The identified anti-inflammatory compounds included Dodecane, 4,6-dimethyl-; Eicosane; Neophytadiene<sup>23</sup> ; and 3,7,11,15-Tetramethyl-2-hexadecen-1-ol<sup>24</sup> . Similarly, a number of antimicrobial compounds were also reported, including, Dodecane, 4,6-

dimethyl-; Eicosane ; Neophytadiene<sup>23</sup>; and 3,7,11,15-Tetramethyl-2-hexadecen-1-ol<sup>24</sup> . Additionally, 9,12,15-Octadecatrienoic acid, methyl ester<sup>25</sup>; and Hexadecanoic acid, 14-methyl-, methyl ester<sup>26</sup> .

Just like flowers and leaves, roots of the plant showed presence of antioxidant compounds, including, 2,2-Dimethoxybutane<sup>27-28</sup>; Dodecane; 3,5-Diisopropoxy-1,1,1,7,7,7-hexamethyl-3,5-; Hexadecane, 1-iodo- ; n-Hexadecanoic acid<sup>15</sup> . The identified anti-inflammatory compounds included Dodecane ; 3,5-Diisopropoxy-1,1,1,7,7,7-hexamethyl-3,5- ; Hexadecane, 1-iodo- ; 9,12-Octadecadien-

1-ol, (Z,Z)- ; Triacontane, 1-iodo-. The identified antimicrobial compounds in plant roots were Dihydroxyacetone ; Maltol<sup>29</sup> ; Heneicosane ; Octadecane, 1-chloro- ; Deoxypeganine; 1H-Pyrrolo[2,1-b]quinazolin-9-one, 3-hydroxy ; 9,12-Octadecadien-1-ol, (Z,Z)-.

Similarly, stem of the plant *Justicia adhatoda* also showed presence of a large number of phytochemicals, including, several antioxidant molecules, such as, Propane, 2,2-dimethoxy-<sup>10</sup> ; 1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-<sup>9</sup>; Oxalic acid, 6-ethyloct-3-yl ethyl ester; tert-Hexadecanethiol<sup>11</sup> ; Nonadecane ; Undecanoic acid, pentyl ester ; Squalene<sup>30</sup> ; Eicosane<sup>10</sup>. The identified anti-inflammatory compounds in stem were 3-1,2-Ethandiol, monobenzoate; Oxalic acid, 6-ethyloct-3-yl ethyl ester<sup>31</sup>; Dichloroacetic acid, 4-pentadecyl ester ; tert-Hexadecanethiol<sup>11</sup>; Nonadecane ; Octacosanal; Undecanoic acid, pentyl ester; Squalene<sup>30</sup>. The identified antimicrobial compounds in stem were Dihydroxyacetone; 3-1,2-Ethandiol, monobenzoate; 1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-<sup>9</sup> ; Dichloroacetic acid, 4-pentadecyl ester; Nonadecane ; Decanoic acid, methyl ester ; Undecanoic acid, pentyl ester; 1H-Pyrrolo [2,1-b]quinazolin-9-one, 3-hydroxy; Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl); Squalene<sup>30</sup>.

The antimicrobial activity of the phytochemicals may occur owing to several mechanistic approaches, including, disruption of bacterial cell membrane leading to compromised membrane integrity. Additionally, compounds such as Benzenepropanoic acid may trigger disruption of bacterial cellular metabolism, while Squalene is capable of integrating into microbial cell membranes, triggering cellular lysis in bacteria. The synergistic effects of combination of these phytochemicals may further augment their antimicrobial efficacy and contribute to bacterial cell lysis<sup>32</sup>.

Talking about anti-inflammatory nature of the phytochemicals, compounds

such as Benzimidazole derivatives, Squalene, and Phenolic compounds have been reported to alter inflammatory pathways by inhibition of pro-inflammatory cytokines, thereby exerting anti-inflammatory effect. For instance, *Squalene* has been reported to exert anti-inflammatory properties by inhibiting of activation of nuclear factor kappa B (NF- $\kappa$ B), which further alters the expression of inflammatory mediators. This mechanism of phytochemicals plays a crucial role in management of conditions characterized by presence of chronic inflammation. In addition to this, acetic acid has also been reported to modulate production of inflammatory mediators such as prostaglandins as well as leukotrienes. Reduction in level of these mediators, can aid in alleviation of symptoms associated with various inflammatory conditions<sup>33</sup>.

The antioxidant potential of *Justicia adhatoda* holds paramount importance in augmenting its therapeutic efficacy. Presence of phytochemicals such as Phenolic acids, Squalene, as well as Flavonoids, all of which act as potent free radical scavenger and aid in neutralization of reactive oxygen species (ROS) play a crucial role in amelioration of oxidative stress in ailments such as cancer, neurodegenerative disorders, and cardiovascular diseases. Presence of compounds like squalene, hexadecenoic acid and phytol further augment the antioxidant potential of *Justicia adhatoda* and contribute to its lipid peroxidation efficacy<sup>34, 35</sup>.

Concluding the findings of the study in a nutshell, the study highlights the rich tapestry of phytochemicals present in the plant *Justicia adhatoda*, wherein each phytochemical exhibits potent antimicrobial, antioxidant and anti-inflammatory attribute. The findings of the study underscore the potential of *Justicia adhatoda* in serving as a novel therapeutic agent to ameliorate the anarchical situation arising due to growing antimicrobial resistance and depleting pool of conventional antimicrobials.

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