

# Screening of Phytochemicals and Identification of Chemical constituents of *Pongamia pinnata* by GC-MS

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**Abstract:** The flowers of *Pongamia pinnata* was extracted with methanol was subjected to preliminary phytochemical analysis. Flavonoids, alkaloids, carbohydrates, tannins, lignins, saponins, glycosides and fixed oil and fats were found to be present. The methanolic crude extract further fractionated with three different solvents, i.e. benzene, dethyl ether and ethyl acetate and the chemical composition of the ethyl acetate extract was determined by GC/MS analysis. The present investigation revealed the following major compounds from *P.pinnata*. In this analysis 30 bioactive phytochemical constituents were identified. Based on the peak area and molecular weight, the dibutyl phthalate and oleic acid was observed as the major constituents.

**Keywords:** *Pongamia pinnata*, GC-MS, Phytochemicals, Chemical constituents.

## Introduction

The aim of this work was to characterize the chemical composition of flowers extract of *Pongamia pinnata*. *Pongamia pinnata* (Linn.) is a medium sized glabrous tree popularly known as karanja in hindi, Indian Beech in English and Pongam in Tamil<sup>1</sup>. Most of the Indian system of traditional medicine Ayurveda and Siddha used to treat *P.pinnata* for various kinds of diseases including diabetes mellitus<sup>2</sup>. The seeds are reported to contain on an average about 28 – 34% oil with high percentage of polyunsaturated fatty acids<sup>3</sup>. Historically, *Pongamia* has been used as folk medicinal plant, particularly in Ayurvedha and Siddha systems of Indian medicine<sup>4</sup>. All parts of the plant have been used as a crude drug for the treatment of tumours, piles, skin diseases, itches, abscess, painful

rheumatic joints wounds, ulcers, diarrhoea etc<sup>4,5</sup>. More recently, the effectiveness of *P. pinnata* as a source of biomedicines has been reported<sup>6</sup> specifically as antimicrobial and therapeutic agents. Nature produces a large number of chemical compounds those structures and properties have fascinated by organic chemists to describe the chemistry of these compounds<sup>7</sup>.

## Materials and Methods

The fresh flowers of *Pongamia pinnata* was collected from the local gardens of S.T.E.T. Women's College, Mannargudi and voucher specimens are deposited in the STET Herbarium at the Department of Botany and Microbiology, S.T.E.T. Women's College, Mannargudi.

### Extraction and Fractionation

The flowers were extracted with 85% EtOH (4 x 500 ml) under reflux. The alcoholic extract was concentrated in vacuo and the aqueous concentrate successively fractionated with benzene (3 x 250 ml), peroxide – free Et<sub>2</sub>O (3 x 250 ml) and EtOAc (4x 250 ml). The yellow colour solid was obtained from the crude extracts.

### Phytochemical screening

Phytochemical components were analyzed qualitatively by the method of Harborne(1973).

### GC-MS

The ethyl acetate extract was subjected to GC-MS analysis. GC analysis was performed on GC Clarus 500 Perkin Elmer systems under the following conditions. Injector temperature 250°C, using a capillary column Elite 5 (100% Dimethyl poly siloxane). Oven temperature was a programme from 110-280°C at 5°C / min. with initial hold of 2 min. the detector was mass detector turbo mass Gold Perkin Elmer, software – Turbo mass. 1µl ethyl acetate extract was injected into GC-MS, which split the component 10:1.

The spectrums of the unknown component was compared with the spectrum of the known components stored in the NIST and MS libraries and ascertain the name, molecular weight and structure of components of the test materials. The results obtained were interpreted.

## Results

### Phytochemical screening

The phytochemical active compounds of *Pongamia pinnata* were qualitatively analyzed and the results are presented in Table-1. In methanolic extract of *Pongamia pinnata* contained alkaloids, flavonoids, carbohydrates and glycosides, saponins and tannins, lignins and fixed oil and fats, but did not contain any proteins based on the presence or absence of colour changes.

### GC-MS

*P.pinnata* , flowers revealed the presence of medicinal active constituents. In the GC-MS analysis, 30 bioactive photochemical compounds were identified in the ethyl acetate extract of *P.pinnata*. The identification of photochemical compounds is based on peak area, molecular weight and molecular formula. Dibutyl phthalate (C<sub>16</sub> H<sub>22</sub>O<sub>4</sub>) with RT 18.49 has peak area 23.87, oleic acid (C<sub>18</sub>H<sub>34</sub>O<sub>2</sub>) with RT 21.92, Nonadecene (C<sub>19</sub>H<sub>38</sub>) RT 14.93, 1-Docosene (C<sub>22</sub>H<sub>44</sub>) with (RT 18.83) and oxalic acid, allyl hexa decyl ester (C<sub>21</sub>H<sub>38</sub>O<sub>4</sub>) with RT 10.77, respectively. The minimum amount of constituents present in the flower extracts were Cyclopentane, 1-hexyl-3-methyl-(C<sub>12</sub>H<sub>24</sub>) and 1-cyclohexylnonene has the peak area 0.25 and 0.19 respectively. The results are presented in Table 2 and Figure 2.

**Table-1:Phytochemical constituents of *Pongamia pinnata* fresh flowers**

S. No.	Phytochemical constituent	Methanolic Extract
1.	Alkaloids	+
2.	Flavonoids	+
3.	Carbohydrates	+
4.	Glycosides	+
5.	Fixed oil and fats	+
6.	Saponins	+
7.	Steroids	+
8.	Tannins	+
9	Proteins	-

+ = Presence, - = Absence

**Table2:Phytochemical constituents of ethyl acetate extract of *Pongamia pinnata* (GC-MS)**

No.	RT	Name of the Compound	Molecular	Molecular weight	Peak Area
1	3.47	2-Hexyl-1-octanol	C <sub>14</sub> H <sub>30</sub> O	214	1.7
2	3.68	Cyclohexane, 1,1'-(1,2-dimethyl- 1,2-ethanediyl) bis-	C <sub>16</sub> H <sub>30</sub>	222	1
3	4.12	Cyclohexane, hexyl-	C <sub>12</sub> H <sub>24</sub>	168	1.34
4	4.55	Decane 2,3,5,8-tetramethyl-	C <sub>14</sub> H <sub>30</sub>	198	0.4
5	5.6	Cyclohexane,1,2,4,5-tetraethyl – (1 $\alpha$ ,2 $\alpha$ ,4 ,5 $\alpha$ )-	C <sub>14</sub> H <sub>28</sub>	196	0.35
6	5.92	Cyclohexane, 1,5-diethyl-2,3- dimethyl-	C <sub>12</sub> H <sub>24</sub>	168	0.58
7	6.64	1-Tridecanol	C <sub>13</sub> H <sub>28</sub> O	200	4.98
8	6.78	Tridecane 6-methyl-	C <sub>14</sub> H <sub>30</sub>	198	1.87
9	7	1,7-Dimethyl-4-(1-methylethyl) cyclodecane	C <sub>15</sub> H <sub>30</sub>	210	1.13
10	7.18	Cyclopentane, 1-hexyl-3-methyl-	C <sub>12</sub> H <sub>24</sub>	168	0.25
11	7.66	1-Cyclohexylnonene	C <sub>15</sub> H <sub>28</sub>	208	0.19
12	7.8	Cyclohexane, octyl	C <sub>14</sub> H <sub>28</sub>	196	1.17
13	8.55	Undecane, 3,8-dimethyl-	C <sub>13</sub> H <sub>28</sub>	184	0.71
14	10.61	Acetic Acid, trifluoro-3,7-dimethyloctylester	C <sub>12</sub> H <sub>21</sub> F <sub>3</sub> O <sub>2</sub>	254	1.02
15	10.77	Oxalic acid, allylhexadecyl ester	C <sub>21</sub> H <sub>38</sub> O <sub>4</sub>	354	11.13
16	10.9	Nonadecane	C <sub>19</sub> H <sub>40</sub>	268	1.6
17	12.12	Cyclohexane, Undecyl-	C <sub>17</sub> H <sub>34</sub>	238	1.14
18	12.57	8-Pentadecanone	C <sub>15</sub> H <sub>30</sub> O	226	3.27
19	12.97	Sulfurous acid, hexyl pentadecyl ester	C <sub>21</sub> H <sub>44</sub> O <sub>3</sub> S	376	1.02
20	14.78	Tridecane, 3-methylene-	C <sub>14</sub> H <sub>28</sub>	196	0.94
21	14.93	1-Nonadecene	C <sub>19</sub> H <sub>38</sub>	266	13.62
22	16.35	n-Heptadecylcyclohexane	C <sub>23</sub> H <sub>46</sub>	322	0.7
23	18.49	Dibutylphthalate	C <sub>16</sub> H <sub>22</sub> O <sub>4</sub>	278	23.87
24	18.83	1-Docosene	C <sub>22</sub> H <sub>44</sub>	308	13.12
25	20.41	10-Nonadecanone	C <sub>19</sub> H <sub>38</sub> O	282	1.75
26	21.92	Oleic Acid	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	282	19.35
27	27.13	Z-10-Pentadecen-1-ol	C <sub>15</sub> H <sub>30</sub> O	226	1.85
28	28.12	1,2-Benzenedicarboxylic acid, diisooctyl ester	C <sub>24</sub> H <sub>38</sub> O <sub>4</sub>	390	1.33
29	31.99	6,11-Dimethyl-2,6,10- dodecatrien-1-ol	C <sub>14</sub> H <sub>24</sub> O	208	0.52
30	35.69	Oxalic acid, allyl octadecyl ester	C <sub>23</sub> H <sub>42</sub> O <sub>4</sub>	382	3.26

## Discussion

### Phytochemical screening

The phytochemical analysis of the methanolic extract of *P.pinnata* flowers based on the presence or absence of colour changes indicated the presence and absence of various constituents. Methanolic extract of *P.pinnata* contained alkaloids, flavonoids, carbohydrates and glycosides, saponins and tannins, lignins and fixed oil and fats, but did not contain any proteins is in accordance with the findings by<sup>8</sup>, who reported that their studies on comparative phytochemical studies on the leaves of *Aegle marmelos* and *Feronia elephantum*.

### Identification of phytochemical constituents by GC-MS

The GC-MS revealed the presence of 30 compounds were isolated. The fatty acid were present in higher amount and other compounds were isolated in very low quantity. The maximum amount of the component is Dibutylphthalate (C<sub>16</sub>H<sub>22</sub>O<sub>4</sub>) was recorded with RT 18.49, and has peak area 23.87%, followed by oleic acid (C<sub>18</sub>H<sub>34</sub>O<sub>2</sub>) with (RT 21.92) peak area 19.35, 1-Docosene (C<sub>22</sub>H<sub>44</sub>) with (RT 18.83) and oxalic acid, allyl hexa decyl ester (C<sub>21</sub>H<sub>38</sub>O<sub>4</sub>) with RT 10.77. The remaining compound is 2-Hexyl-1-octanol (C<sub>14</sub>H<sub>30</sub>O), Cyclohexane, 1,1'-(1,2-dimethyl-1,2-ethanediyl) bis-(C<sub>16</sub>H<sub>30</sub>), Cyclohexane, hexyl-(C<sub>12</sub>H<sub>24</sub>), Decane2,3,5,8-tetramethyl (C<sub>14</sub>

H<sub>30</sub>), Cyclohexane, 1,2,4,5 tetraethyl (1□, 2□□,4□□,5□□) -C<sub>14</sub>H<sub>28</sub>, cyclohexane, 1, 5,-diethyl - 2, 3-dimethyl- (C<sub>12</sub>H<sub>24</sub>), 1-Tridecanol (C<sub>13</sub>H<sub>28</sub>O), 1,7-Dimethyl-4-(1-methyl-ethyl) cyclodecane (C<sub>15</sub>H<sub>30</sub>), Cyclopentane, 1-hexyl- 3-methyl- (C<sub>12</sub>H<sub>24</sub>), Cyclohexane, octyl (C<sub>14</sub>H<sub>28</sub>), Undecane, 3,8-dimethyl- (C<sub>13</sub>H<sub>28</sub>), Acetic Acid, trifluoro-3,7-dimethyloctylester (C<sub>12</sub>H<sub>21</sub>F<sub>3</sub>O<sub>2</sub>), Nanodecane (C<sub>19</sub>H<sub>40</sub>), Cyclohexane, Undecyl-(C<sub>17</sub>H<sub>34</sub>), 8-Pentadecanone (C<sub>15</sub>H<sub>20</sub>O), Sulfurous acid, hexyl pentadecyl ester (C<sub>21</sub>H<sub>44</sub>O<sub>3</sub>S), Tridecane, 3-methylene(C<sub>14</sub>H<sub>28</sub>), n-Heptadecylcyclohexane(C<sub>23</sub>H<sub>46</sub>), 10-Nenadecanone (C<sub>19</sub>H<sub>38</sub>O), Z-10-Pentadecen-1-ol (C<sub>15</sub>H<sub>34</sub>O<sub>2</sub>), 1,2-Benzene dicarboxylic acid, diisooctyl ester(C<sub>24</sub>H<sub>38</sub>O<sub>4</sub>), 6,11-Dimethyl-2,6,10-dodecatrien-1-ol- (C<sub>14</sub> H<sub>24</sub> O), Oxalic acid, allyl octadecyl ester (C<sub>23</sub>H<sub>42</sub>O<sub>4</sub>) is in the agreement with earlier report of Siddhuraju *et al.*, 1995 in the *Tamarindus indica*<sup>9</sup>. Presence of high levels of

unsaturated fatty acids in tribal pulses are nutritionally desirable and also are comparable with some edible legumes like Goa bean and Soybean<sup>10</sup>. In the present investigation, fatty acids were isolated from *P.pinnata* flowers, Similarly Pugalenti *et al.*, (2004) also detected there are 8 fatty acids<sup>11</sup> and Robert Glew *et al.*, (2005), have isolated 17 fatty acids from another plant *Tamarindus indica*<sup>12</sup>.

The present GC-MS work establishes the exclusive presence of some constituents in one variety and these helps in its identification.

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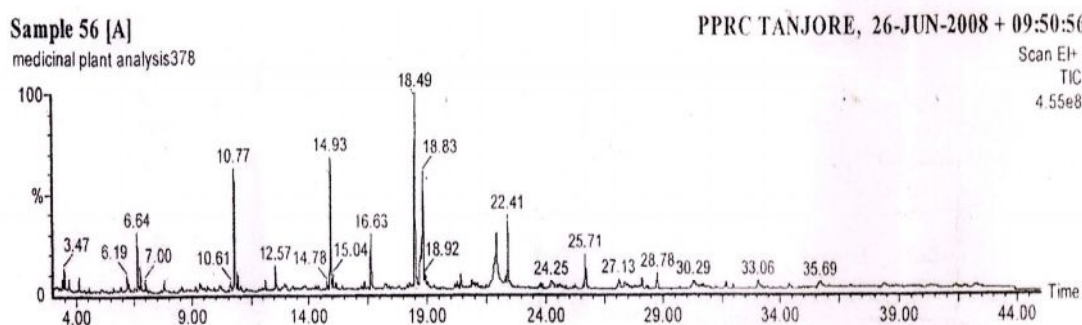


Fig 2 : GC-MS Spectrum of the Phytochemical constituents from EtOAc Fraction of *Pongamia pinnata*

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