

## Research Article



## GC-MS Analysis of Bioactive Components from Methanol Leaf Extract of *Toddalia asiatica* (L.)

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### ABSTRACT

Medicinal plants are sources of important therapeutic aids for alleviating human ailments. *Toddalia asiatica* (L.) is one of the important medicinal plants belonging to family Rutaceae and commonly called as Jangli Mirachi in Marathi. Traditionally leaves are used in the treatment of stomach ache, rheumatism, skin diseases and snake bite. The present investigation was carried out to determine the chemical components in the leaves of *T. asiatica* by GC-MS technique. The major phytocomponents found in the leaves are 2,2,6- Trimethyl- 2H, 5H pyrano (3,2-C) quinolin-5-one; Butylated hydroxytoluene; Cycloheptasiloxane tetradecamethyl; Nerolidyl acetate; Spathulenol; Acetic acid, dodecyl ester; 2-Naphthalenemethanol, decahydro alpha; n-Hexadecanoic acid and Dibutyl phthalate. Many of these phytocomponents have antibacterial, antifungal, antifouling, immunomodulatory and antitumor activities which justify the folklore use of leaves in traditional system to cure various ailments.

**Keywords:** Ailments, Leaf, Phytocomponents, *Toddalia asiatica* (L.)

### INTRODUCTION

Medicinal plants have been used by human beings since early ages in traditional medicine due to their therapeutic potential and the search on medicinal plants had led to the discovery of novel drugs used against many diseases. Higher plants as a source of bioactive compounds continue to play a dominant role in the maintenance of human health.<sup>1</sup> Plants are rich source of secondary metabolites with interesting biological activities. In recent years Gas Chromatography - Mass Spectroscopy (GC-MS) studies have been increasingly applied for the analysis of medicinal plants as this technique has proved to be a valuable method for the analysis of various chemical compounds. *Toddalia asiatica* is a monotypic genus of flowering plant in the Rutaceae family containing single species.<sup>2</sup> *T. asiatica* is distributed throughout India up to 2500m. Locally it is known as Jangli Mirachi and common English name is Orange climber. It is large shrub with sharp recurved straight prickles. Traditionally leaves are used for the treatment of abdominal pains, malaria and to stimulate appetite.<sup>3</sup> The leaves are also used for lung diseases and rheumatism. Ethanobotanical studies of Kolhapur District reveals that leaves of *T. asiatica* are used in the Treatment of snake bite, skin diseases, stomach ache and rheumatism. Since there are no reports on the phytochemical aspects of leaves of *T. asiatica*, it was chosen as the subject of this study. Hence the objective of the present study is to identify the phytochemical constituents with the aid of GC-MS technique.

### MATERIALS AND METHODS

#### Plant Material

Plant material was collected from the forest of Kolhapur district, Maharashtra, India and identified with the help of local flora.<sup>4</sup>

#### Preparation of plant extract

Preparation of plant extract was done by following the method of Anwar (2010).<sup>5</sup> The leaves were dried and pulverized to powder in a mechanical grinder. Required quantity of plant sample was weighed, transferred to flask, treated with the methanol until the powder was fully immersed, incubated over night and filtered through a Whatman No.41 filter paper. Filtrate is then concentrated till dry residue was remained. After weighing the residue, respective amount of methanol was added to make the final solution. This solution was further used for GC-MS analysis.

#### GCMS analysis of bioactive compounds from sample

The methanol leaf extract obtained from sample was subjected to Gas Chromatography and Mass Spectroscopy for the determination of bioactive volatile compounds. Some of the important features are summarized below.

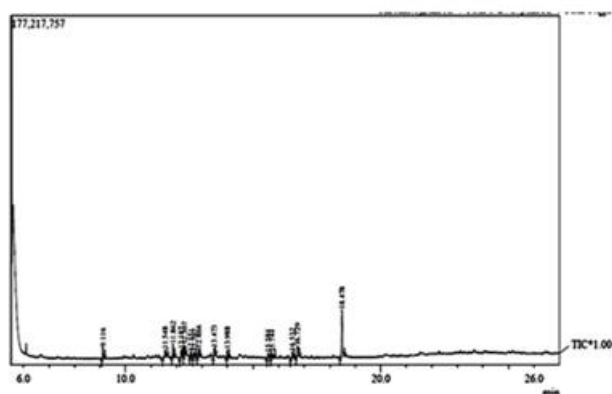
GC-MS analysis of the sample was carried out using Shimadzu Make QP-2010 with non polar 60 M RTX 5MS Column. Helium was used as the carrier gas and the temperature programming was set with initial oven temperature at 40°C and held for 3 min and the final temperature of the oven was 480°C with rate at 10°C [min.sup.-1]. 2 µL sample was injected with split less mode. Mass spectra was recorded over 35 - 650 amu range with electron impact ionization energy 70 eV. The



total running time for a sample was 45 min. Quantitative determinations were made by relating respective peak areas to TIC areas from the GC-MS.

### Identification of phytoconstituents

Interpretation on 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 spectrum of known component stored in NIST library. Quantitative determinations were made by relating respective peak areas to TIC areas from the GC-MS. The name, molecular weight, retention time and peak area percentage of the test materials was ascertained.



**Figure 1:** GC-MS Chromatogram of methanol leaf extract of *Toddalia asiatica* (L.)

### RESULTS AND DISCUSSION

GC-MS chromatogram of the methanolic leaf extract of *T. asiatica* shows 15 peaks indicating presence of fifteen compounds (Figure 1). The compounds identified are presented in table 1. It is revealed that 2,2,6 - Trimethyl-2H, 5H-pyrano (3,2-c) quinolin-5-one (31.42%) is found as the major compound and the fourteen minor compounds such as Cyclohexasiloxane, dodecamethyl (7.06%); Butylated Hydroxytoluene (4.57%); Cycloheptasiloxane, tetradecamethyl (6.58%); 1-Cyclohexene-1-butanol, alpha, 2, 6, 6-tetramethyl (4.62%); Nerolidyl acetate (5.84%); Spathulenol (2.36%); Acetic acid, dodecyl ester (4.67%); 2-Naphthalenemethanol, decahydro-alpha (5.19%); Cyclooctasiloxane, hexadecamethyl (3.89%); 1, 2-Benzenedicarboxylic acid, butyl 8-methylnonyl ester (3.75%); Cyclononasiloxane, octadecamethyl (2.41%); Dibutyl phthalate (3.55%) and n-Hexadecanoic acid (10.11%).

The medicinal and biological activities of the Phytochemicals identified in the leaves of *T. asiatica* are presented in table 2. Phenolic compounds Butylated Hydroxytoluene have activities like antibacterial and antifungal. Acidic compounds like Nerolidyl acetate and Dibutyl Phthalate and 1, 2-Benzenedicarboxylic acid, butyl 8-methylnonyl ester have antibacterial and antifouling activities. Sesquiterpene Spathulenol has antimicrobial, immunomodulatory and antitumor activities. These

Phytochemicals are responsible for various pharmacological action of the leaves of the plant.

**Table 1:** Phyto components identified in leaf extract of *Toddalia asiatica* (L.)

RT	Name of compound	MF	MW	Peak area %
9.116	Cyclohexasiloxane, dodecamethyl	C <sub>12</sub> H <sub>36</sub> O <sub>6</sub>	444	7.06
11.548	Butylated Hydroxytoluene	C <sub>15</sub> H <sub>24</sub> O	220	4.57
11.862	Cycloheptasiloxane, tetradecamethyl	C <sub>14</sub> H <sub>42</sub> O <sub>7</sub>	518	6.58
12.187	1-Cyclohexene-1-butanol, alpha, 2, 6, 6-tetramethyl	C <sub>14</sub> H <sub>24</sub> O	208	4.62
12.307	Nerolidyl acetate	C <sub>17</sub> H <sub>28</sub> O <sub>2</sub>	264	5.84
12.551	Spathulenol	C <sub>15</sub> H <sub>24</sub> O	220	2.36
12.707	Not identified	-	-	3.96
12.866	Acetic acid, dodecyl ester	C <sub>14</sub> H <sub>28</sub> O <sub>2</sub>	228	4.67
13.473	2-Naphthalenemethanol, decahydro-alpha	C <sub>15</sub> H <sub>26</sub> O	222	5.19
13.988	Cyclooctasiloxane, hexadecamethyl	C <sub>16</sub> H <sub>48</sub> O <sub>8</sub>	592	3.89
15.584	1, 2-Benzenedicarboxylic acid, butyl 8-methylnonyl ester	C <sub>22</sub> H <sub>34</sub> O <sub>4</sub>	362	3.75
15.744	Cyclononasiloxane, octadecamethyl	C <sub>18</sub> H <sub>54</sub> O <sub>9</sub>	666	2.41
16.532	Dibutyl phthalate	C <sub>16</sub> H <sub>22</sub> O <sub>4</sub>	278	3.55
16.729	n-Hexadecanoic acid	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256	10.11
18.478	2,2, 6-Trimethyl-2H, 5H-pyrano(3,2-c) quinolin-5-one	C <sub>15</sub> H <sub>15</sub> N O <sub>2</sub>	241	31.42

Sarada *et al.* (2011)<sup>6</sup> carried out GC-MS determination of bioactive components of *Naringi crenulata* which belong to family Rutaceae. The result obtained from the GC-MS analysis of ethanol leaf extract of above plant reveals presence of seventeen different bioactive compounds. The major compounds were Caryophyllene (12.22%); Propane 1,1,3- triethoxy (11.76%); Octane 3,5 - dimethyl (10.86%); Cyclohexane 1,3,5 trimethyl- 2-octodecyl (4.98%); Cholesta 8, 24 - dien-3-01, 4 methyl- (3a,4a) (4.98%) and Sumatriptan (3.17%). In present study GC-MS analysis of methanol leaf extract of *Toddalia asiatica* belonging to family Rutaceae show presence of fifteen compounds such as Cyclohexasiloxane, dodecamethyl; Butylated hydroxytoluene; Cycloheptasiloxane, tetradecamethyl; 1-Cyclohexene-1-butanol, alpha-2, 6, 6 - tetramethyl; Nerolidyl acetate; Spathulenol; Acetic acid dodecyl ester; 2-Naphthalence methanol, decahydroalpha; Cyclooctasiloxane, hexadecamethyl; 1,2-

Benzenedicarboxylic acid, butyl 8-methylnonyl ester; Cyclonona siloxane, octadecamethyl; Dibutyl phthalate; n-Hexadecanoic acid and 2, 2, 6-Trimethyl-2H, 5H-pyrano (3,2-c) quinolin-5-one. The major compound were 2, 2, 6-Trimethyl-2H, 5H – pyrno (3,2-c) quinolene -5- one (31.42%); n-Hexadecanoic acid (10.11%) and Cyclohexasiloxane, dodecamethyl (7.06).

**Table 2:** Medicinal / Biological activity of compounds identified in leaf extract of *Toddalia asiatica* (L.)

Name of compound	Compound nature	Medicinal / Biological activity
Butylated Hydroxytoluene	Phenolic compound	Antibacterial, antifungal, food additive, preservative. <sup>7</sup>
Nerolidyl acetate	Acidic compound	Antibacterial <sup>8</sup>
Spathulenol	Sesquiterpene	Antimicrobial, immunomodulatory and antitumor <sup>9</sup>
1,2-Benzenedicarboxylic acid, butyl 8-methylnonyl ester	Acidic compound	Antimicrobial and antifouling <sup>8</sup>
Dibutyl phthalate	Plasticizer compound	Antibacterial, antifouling <sup>10</sup>
n-Hexadecanoic acid	Palmitic acid	Antioxidant, hypocholesterolemic, nematocidal, pesticidal, hemolytic, antiandrogenic, hemolytic, 5-alpha reductase inhibitor <sup>11</sup>

## CONCLUSION

In present study fifteen chemical constituent have been identified from methanol leaf extract of the *Toddalia asiatica* by GC-MS analysis. The presence of various

bioactive compounds justifies the use leaves of the plant for various ailments by traditional practitioners.

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