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GC-MS ANALYSIS AND IDENTIFICATION OF BIOACTIVE CONSTITUENTS FROM FLOWERS OF *TODDALIA ASIATICA (RUTACEAE)*

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ABSTRACT

The herbal market is flooded with substitutes and adulterants due to non-availability of the genuine drug and poor knowledge and difficulty in identifying the pure active principle. *Toddalia asiatica* (Rutaceae) is a constituent of traditional systems of medicine used for treating a paradigm of diseases. The flowers were extracted with methanol, the filtered extract was concentrated to dryness and the residue subjected to GC-MS analysis. The results showed the presence of diverse bioactive compounds such as dihydroxyacetone, propylcarbamate, 4H- pyran-4-one, 2,3 - dihydro-3,5-dihydroxy-6-methyl, 5-hydroxymethylfurfural, Dichloropropylphospine,5-Ethyl-2,4-dimethyl isoxazolidine,oleic acid, 4,5- diamino-2-hydroxypyrimidine, cyclopentanone dimethylhydrazone, 4-mercaptophenol, 2,4-dihydroxy-2,5-dimethyl-3(2H)-furan-3-one, Deferoxamine, 1,2,3-propanetriol,1-acetate, butanenitrile,2,3,-dioxo-dioxime,o,o-diacetyl, acetamide,N-butyl, 3-pentanol,2,3,4-trimethyl, 1,3-dioxolane,2-butyl-2-methyl, n-Hexadecanoic acid, Urea,N,N'-dibutyl- N,N'-dimethyl and Cis-13-Octadecenoic acid with varying chemical structures that are specific to floral tissues, this vouches for phytochemical potential of *T.asiatica* flowers and is indicative of their medicinal use.

KEYWORDS: ***Toddalia asiatica*, traditional medicine, GC-MS**

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INTRODUCTION

Toddalia asiatica (Rutaceae) is a constituent of Asian and African traditional Ayurveda and Siddha systems of medicine and folk medicines used for treating unrelated diseases and disorders such as malaria, coughs, fever, influenza, rheumatism, cholera diarrhoea, indigestion, traumatic injury, stomachache, snake bites etc[1,2]. Shade dried flowers collected from Thandarai, Kanchipuram District, Taminadu, India were extracted with methanol, the filtered extract *Toddalia asiatica* (L.) Lam., the commonly called 'Wild orange tree or Orange climber or Forest pepper' is a monotypic genus of Rutaceae is a robust and armed green leaf liana distributed in the tropical and sub-tropical forests of Africa, Madagascar, Sri Lanka, and Himalayan, Nilgiri, Palani and Tirunelveli regions of India [3]. It grows in forested riparian habitats of high rainfall areas with woody, corky, and thorny stems and citrus scented leaves and yellow green flowers. All parts of the plant are claimed to have medicinal value. The leaves, flowers, and roots are used to treat lung diseases, skin diseases, rheumatism, malaria, arthritis, cough and throat pain [4]. In Ayurveda, the aerial parts of *T. asiatica* have been used for the treatment of diabetes and cancer and in Siddha, screening of the root drug Milagaranai Chooranam has validated bactericidal and fungicidal activities of the preparation[5]. Apart from the classified Indian systems of medicine, *Toddalia asiatica* is widely used as a folk medicine where oral traditions prevail especially in rural parts of India. The root and its bark have been used to treat fever, malaria, cholera, diarrhoea and [6]. The Kani tribals in southern India use the leaves of *Toddalia asiatica* mixed with coconut oil and three other plants apply externally to cure skin diseases [7]. The roots are chewed for toothache and the fruits are chewed for the treatment of coughs. The leaves are used to treat lung diseases and rheumatism, and the root bark extracts are used as a tonic during convalescence and constitutional debility. *Toddalia asiatica* is also used to treat nasal and bronchial pains, stomachache, venomous snake bites, haemostatics,and rituals [8]. Contemporary pharmacological studies elucidated that T. asiatica has significant spasmolytic, antimalarial, antibacterial and antifungal activities.The chemical components of T. asiatica mainly involve alkaloids [9,10] coumarins, benzenoids [11] and their derivatives, among which alkaloids were considered the main bioactive constituents. Extracts of the plant also contained coumarins having anti-plasmodial activity and antiviral activity against H1N1 influenza virus[12]. In view of the diverse uses of *T. asiatica* in mainstream traditional systems of medicine and oral traditions of the ethnic communities in India, the methanol extract of *Toddalia asiatica* was analyzed using GC-MS to identify the long chain and branched chain hydrocarbons, alcohols, acids, esters, etc. which may contribute to the observed activities.

METHODOLOGY

Preparation of plant materials

The flowers of T. *asiatica* collected from Thandarai**,** Kanchipuram District**,**TamilNadu**,**India and were identified and authenticated by Prof.N.Raaman, Centre for Advanced Studies in Botany, Madras University.

Preparation of plant extract

The collected flowers were thoroughly washed with distilled water and dried under shade. The dried plant material was pulverized and about 250 gram of powdered material was extracted with methanol at room temperature for 72 h. The extract was filtered through Whatman No. 1 filter paper and concentrated to dryness. The final residue obtained was then subjected to GC-MS analysis.

Gas chromatography–mass spectrometry (GC–MS)

For GC-MS analysis, the samples were injected into a HP-5 column (30 m X 0.25 mm i.d with 0.25 µm film thickness), Agilent technologies 6890 NJEOL GC Mate II GC-MS model. Chromatographic conditions were as follows: helium as carrier gas, flow rate of 1 mL/min; the

injector operated at 200°C and column oven temperature programmed to 50-250°C at a rate of 10°C/min injection mode. MS conditions were as follows: ionization voltage of 70 eV; ion source temperature of 250°C; interface temperature of 250°C; mass range of 50-600 mass units.

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. Name, molecular weight and structure of the components of the test materials were ascertained from the library.

RESULTS

GC-MS analysis

The compounds in the methanol extract of the flowers were identified using mass spectrometry attached with GC. The GC-MS chromatogram of T*. asiatica* is presented in Fig.1. The compounds were eluted as a function of retention time in the GC and the heights of the peaks indicated the relative concentrations of the components present in the plant. The mass spectrometer analyzed the compounds eluted at different times to identify the nature and structure of the compounds.

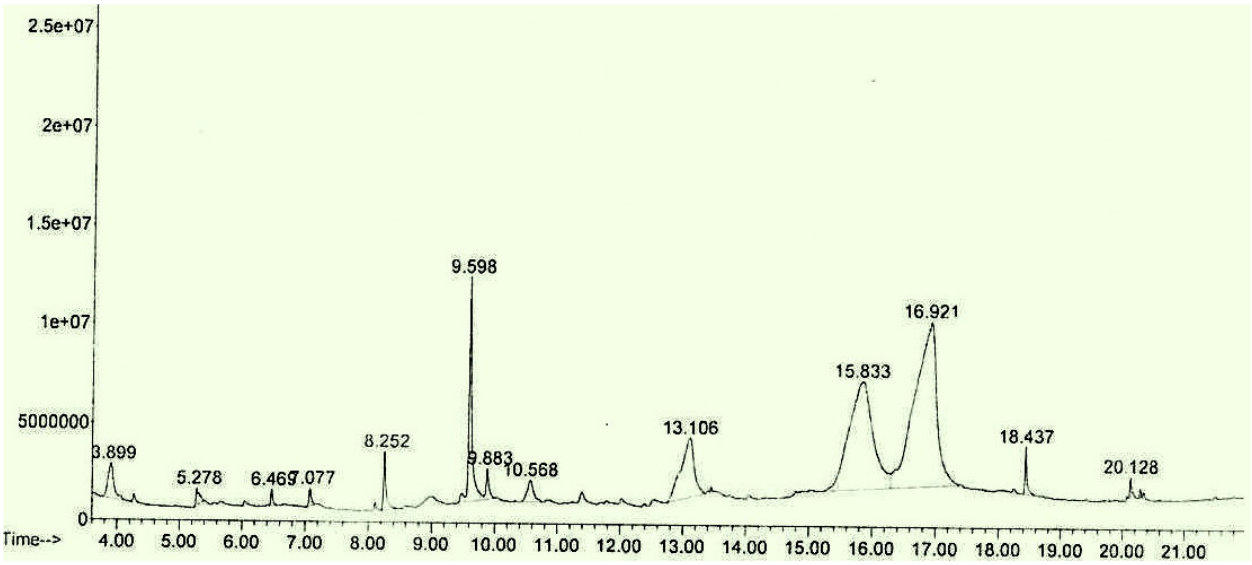
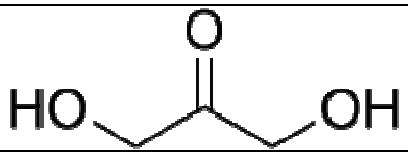
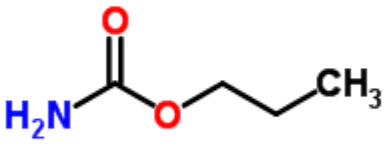
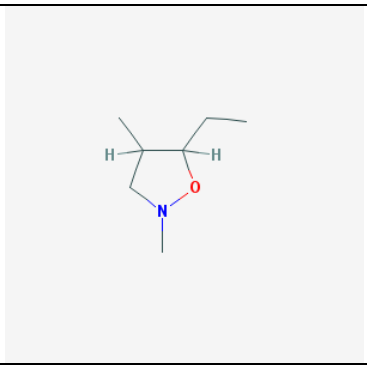
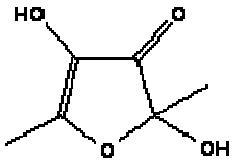
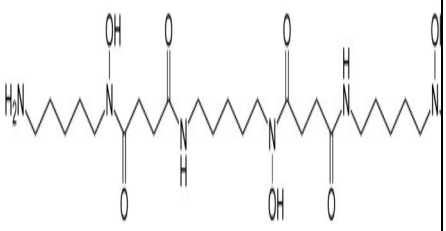


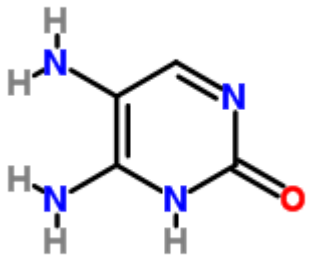
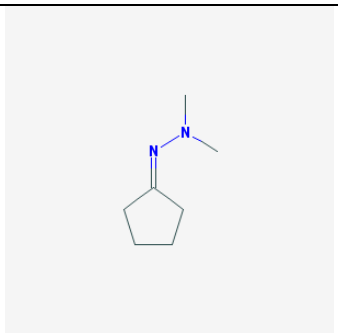
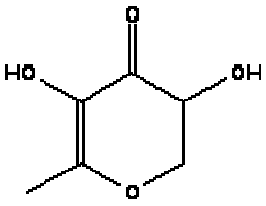
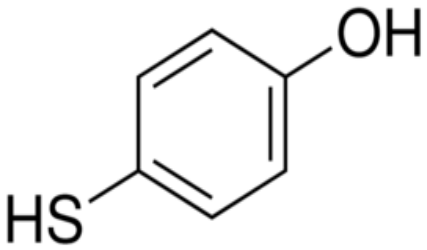
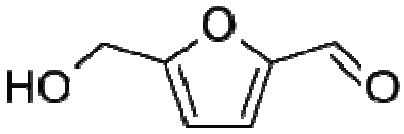
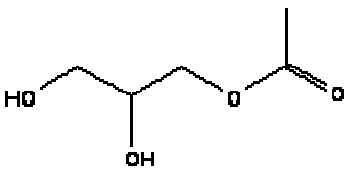
Figure 1
GC-MS Chromatogram of methanolic leaf extract of Toddalia asiatica

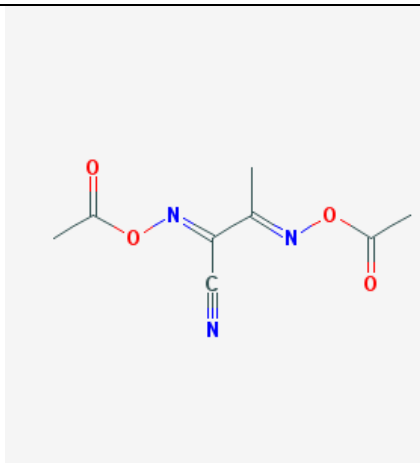
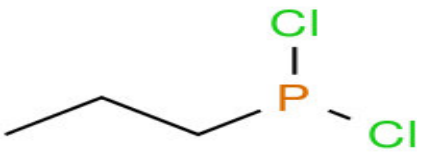
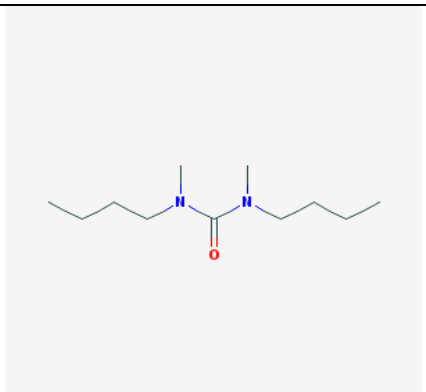
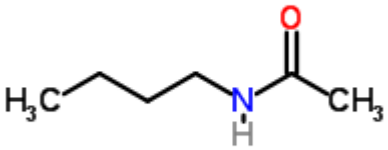
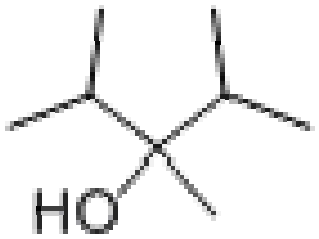
In GC-MS, the large compounds fragmented into small ones giving rise to appearance of peaks at different m/z ratios. These mass spectra are fingerprint of the compounds which were identified from the data library. The compounds eluted and identified are listed in Table 1.

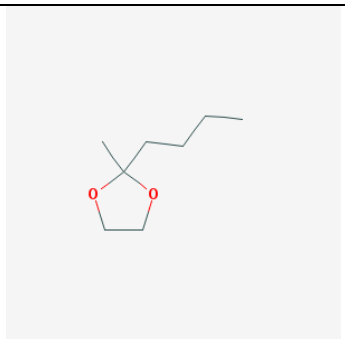
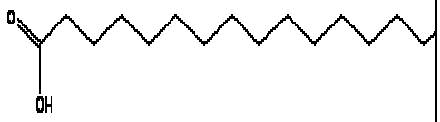
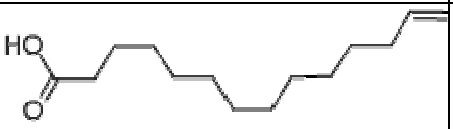
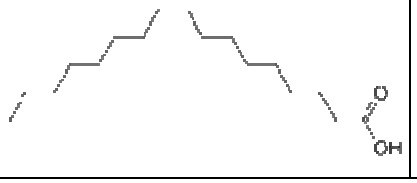
Table1

List of compounds detected using GC-MS analysis in the flower extracts of Toddalia asiatica

No	Structures	Name of the compound	Molecular formula	Molecular weight
1		Dihydroxyacetone	$C_3H_6O_3$	90.08
2		Propyl carbamate	$C_4H_9NO_2$	103.119 797
3		Isoxazolidine, 5-ethyl-2,4-dimethyl	$C_7H_{15}NO$	129.200 1
4		2, 4-Dihydroxy-2, 5-dimethyl-3(2H)-furan-3-one	$C_6H_8O_4$	144.125 3
5		Deferoxamine	$C_{25}H_{48}N_6O_8$	560.68

6		4,5- Diamino-2-hydroxypyrimidine	$C_4H_8N_4O_5S$	224.196
7		Cyclopentanone, dimethylhydrazone	$C_7H_{14}N_2$	126.19946
8		4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144.1253
9		4-Mercaptophenol	C_6H_6OS	126.176201
10		5-hydroxymethyl furfural	$C_6H_6O_3$	126.11
11		1,2,3-Propanetriol, 1-acetate	$C_5H_{10}O_4$	134.1305

12		butanenitrile, 2,3- dioxo-,dioxime o,o-diacetyl-	$C_8H_9N_3O_4$	211.17476
13		dichloropropyl phosphine	$C_3H_7Cl_2P$	144.97
14		Urea, N,N'-dibutyl-N,N'-dimethyl-	$C_{11}H_{24}N_2O$	200.32106
15		acetamide, N-butyl-	$C_6H_{13}NO$	115.1735
16		3-pentanol ,2,3,4-trimethyl	$C_8H_{18}O$	130.23

17		1,3-Dioxolane, 2-butyl-2-methyl	$C_8H_{16}O_2$	144.21144
18		n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.4241
19		Cis-13-Octadecenoic Acid	$C_{18}H_{34}O_2$	282.46
20		Oleic acid	$C_{18}H_{34}O_2$	282.46

DISCUSSION

Toddalia asiatica is a traditional medicinal plant widely used in Ayurveda and Siddha preparations in India since ancient times. Most of the pharmacognostical, pharmacological and phytochemical investigations of this woody climber have been carried out using extracts other than flowers. The studies include antimalarial, larvicidal, bactericidal, antifungal, antinociceptive, antioxidant anti-inflammatory, analgesic, anti-inflammatory and antitumor activities. Many of the chemical extracted from the species belong to coumarin, quinoline, triterpenoid, phenanthridine and alkaloid groups [13]. The presence of such bioactive compounds as deferoxamine (used to treat iron poisoning) and oleic acid in the extract validates the use of *T. asiatica* for various ailments by traditional practitioners. Phytochemical scrutiny has been done mostly for extracts of root and root bark [14,15] stem [16] and leaf [17, 18, 19] tissues and not for floral tissues of this species. This report is the first of its kind to analyze the chemical constituents of *T. asiatica* flowers using GC-MS. Scientific testing and authentication of novel compounds from untried tissues of such species with diverse traditional medicinal uses may provide new and effective pharmacological agents. A comparison of the GC-MS profile (Fig. 1) and the individual compounds identified (Table 1) show that the diversity of flower derived chemicals are entirely different from the metabolites reported in root stem and leaf [17,19] tissues of *T. asiatica.* The only metabolite reported common between stem tissue and the flower tissue seems to be hexadeconic acid. Since the functions of a flower are quite different from that of the other parts of the plant, it is not surprising that most of the chemicals vary from those of other parts. The role played by the differing seasonal and environmental regimes in the control of metabolites in different places where the species grows cannot be under estimated. However, examination of the metabolites from different tissues collected from the same place under the identical conditions would greatly help in the elucidation of organ specific synthesis and compartmentalization of the chemicals. As of now, the flowers seem to

have a profile of chemicals different from other organs of *T. asiatica.*

CONCLUSION

It could be concluded that our systematic investigation reveals the potential of *Toddalia asiatica* flowers as a significant source of unique bioactive compounds not reported from root, stem and leaf tissues of natural plants. A GC-MS study on the comparative evaluation of the chemicals from different tissues of the species grown under certain conditions would help resolve the differences in synthesis, partitioning and accumulation of the metabolites in different organs including the flowers as reported herein.

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