Indian Journal of Natural Products and Resources
Vol. 12(1), March 2021, pp 145-152

Short Communication

GC-MS phytochemical profiling of leaf extracts of *Aristolochia tagala* Cham., a rare and important ethnomedicinal plant

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Received 30 September 2019; Revised 27 November 2020

The current study was aimed at obtaining the phytochemical profile of leaf extracts of *Aristolochia tagala* Cham., a rare ethnomedicinal plant by GC-MS analysis. The chromatograms of GC-MS analysis of methanol, chloroform, and petroleum ether extracts of leaf showed 39, 37, and 30 peaks respectively. The mass of compounds and fragments recorded were matched with NIST 11 and Wiley 8 libraries for the identification of probable compounds which accounted for a total of 71 different phytochemical compounds present in the tested sample. Some compounds appeared in all the solvent extracts but others were unique to specific solvent extract. Out of 71 recorded compounds, 14 were sesquiterpenes, 10 compounds could be categorized as other terpenoids or compounds containing isoprene units, 4 as steroids, 4 as other aromatic hydrocarbon compounds, fatty acids and their esters accounted for 18 compounds and remaining 21 compounds were other hydrocarbons. Most of these compounds are recorded for the first time in this species. This valuable data can be further employed to know the phytocompounds available in large quantities in this plant which has therapeutic properties that can form the basis for pharmaceutical research to develop drugs for efficient disease control.

Keywords: *Aristolochia tagala*, GC-MS analysis, Leaf extracts, Phytochemicals, Sesquiterpenes, Terpenoids

IPC code; Int. cl. (2015.01)- A61K 36/00, A61K 127/00

Introduction

Ethnomedicinal plants have medicinal properties and are used by various ethnic groups to cure various ailments. They form a part of the traditional system of medicine followed by people from time immemorial. Large sections of the population in India are still relying on traditional herbal medicine, many ethnic groups have their traditional knowledge about the use of plants and other natural resources as medicine. Phytochemicals present in these plants form the basis for their medicinal properties. Tribal knowledge about plants forms the storehouse of information and plays a key role in improving the Ayurvedic Materia Medica[1].

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Aristolochia tagala Cham. is one such rare and important ethnomedicinal plant used by many ethnic tribes in India and worldwide. Tribals of Andaman and Nicobar Islands and Kani tribe settled in Pottamavu and Chembikkunnu tribal settlements in Trivandrum Kerala use this plant to prepare antidote against the scorpion, cobra, krait, and other venomous snake bites[2,3]. Tribal people of Chittagong hill tracts (India and Bangladesh border) use different parts of this plant to cure different ailments like fever, dysentery, rheumatism, toothache, and as an antidote against snake bite[4-6]. Kuruchiya tribe of Kerala use grounded leaves as stomachic[7]. Irula tribe of Nilgiris, Tamil Nadu use the extracts of this plant to treat diarrhoea and vomiting[8]. Chakma tribe of Bangladesh use the infusion and paste of the leaves of this plant to cure abdominal pain, rheumatic pain and tumour[9]. In Indonesia, the leaves are used to resolve swollen limbs and to treat bilious disorders. In Malaysia, the pounded leaves are used to treat fever. In the Philippines, root powder is used to invigorate health, to treat infantile tympanitis, and to promote digestion[10]. Scientific enumeration of the phytochemicals present in such an important ethnomedicinal plant can form a data resource for the isolation of novel compounds and drug discovery research. Hence the present study was aimed at knowing the probable phyto-compounds present in the leaves by employing a cost-effective and less time-consuming method of extraction using three solvents having different polarity and subjecting the extracts to GC-MS analysis.

Materials and Methods

Collection of the plant material

The field survey was conducted in the Western Ghats of India (covering the areas of Hassan, Dakshina Kannada, and Chikkamagaluru districts of Karnataka). *A. tagala*, a climber, was identified in the Charmadi and Bisle Ghat Forest areas. Plant specimen for herbarium and fresh healthy leaves for extraction were collected from Bisle ghat forest area (Sakleshpur taluk, Hassan district). The collected plant specimen was pressed and the prepared herbarium was submitted to BSI–Pune for the authentication (Accession No. 136269). The fresh and healthy leaves

collected were washed under running tap water. Excess water was removed with a clean cotton cloth and dried in shade under room conditions. The dried leaves were powdered and used for extraction.

Extraction

Ten grams of the leaf powder was treated with 50 mL of each solvent (viz., methanol, chloroform, and petroleum ether) separately in closed containers at 50 °C (in a water bath) for four hours and then filtered with Whatman No. 1 filter paper. Residues collected were discarded and the solvent with plant extract was collected and evaporated on a hot water bath to get semi-solid crude plant extract. These were submitted to the Kerala Forest Research Institute, Thrissur District, Kerala, India for GC-MS analysis.

GC-MS analysis

GC-MS (Shimadzu GC-MS QP2010S) analysis was carried out under the following conditions: carrier gas: helium; column: Rxi-5Sil MS (30 m length, 0.25 mm ID, 0.25 µm thickness); Oven temperature: 80° C; injection temperature: 260° C for methanolic and chloroform leaf extracts (coded as B1 and B2 respectively) and 280° C for petroleum ether leaf extract (coded as B3), split ratio for B1 was 50.0; and 100.0 for B2 and B3. Ion source temperature: 200° C and interface temperature: 280° C, oven temperature programme for B1: 80° C (hold for 2 minutes) raised to 280: rate 5° C/min: 280° C ending with an isothermal at 280° C for 6 minutes; run time: 48 min; oven temperature programme for B2: 80 °C (hold for 2 minutes) raised to 200° C: gradually at a rate 5°C/min, isothermal at 200° C for 6 minutes, further raised to: 260° C at a rate of 5° C/min ending with an isothermal at 260° C for 2 minutes; run time: 46 min and oven temperature programme for B3: 80° C raised to 280° C: gradually at a rate 5° C/min and ending with isothermal at 280° C for 5 minutes; run time: 45 min. GC-MS solutions software was used operating the instrument. These different temperature programmes were selected based on the type of solvents used for extraction. For the ionization of electrons, 70eV was used. Scan range was 50-500 m/z. The mass of the compounds and the fragments recorded were matched with NIST 11 and Wiley 8 libraries for the identification of probable compounds present in the extract. PubChem database and other research articles were cross referred to the structure and activities of the detected compounds[11-14].

Results and Discussion

In the present study, leaves of *A. tagala* were extracted with three different solvents namely methanol, chloroform and petroleum ether to know all the possible compounds with different polarities or affinities towards different solvents. These crude extracts were subjected to GC-MS analysis. The chromatograms obtained are presented in Fig. 1-3. The chromatograms of GC-MS analysis of methanol, chloroform and petroleum ether extracts of leaf showed 39, 37, and 30 peaks respectively.

The mass of compounds and fragments recorded were matched with NIST 11 and Wiley 8 libraries for the identification of probable compounds which accounted for a total of 71 different phytochemical compounds present in the tested samples. Some compounds appeared in all the solvent extracts but

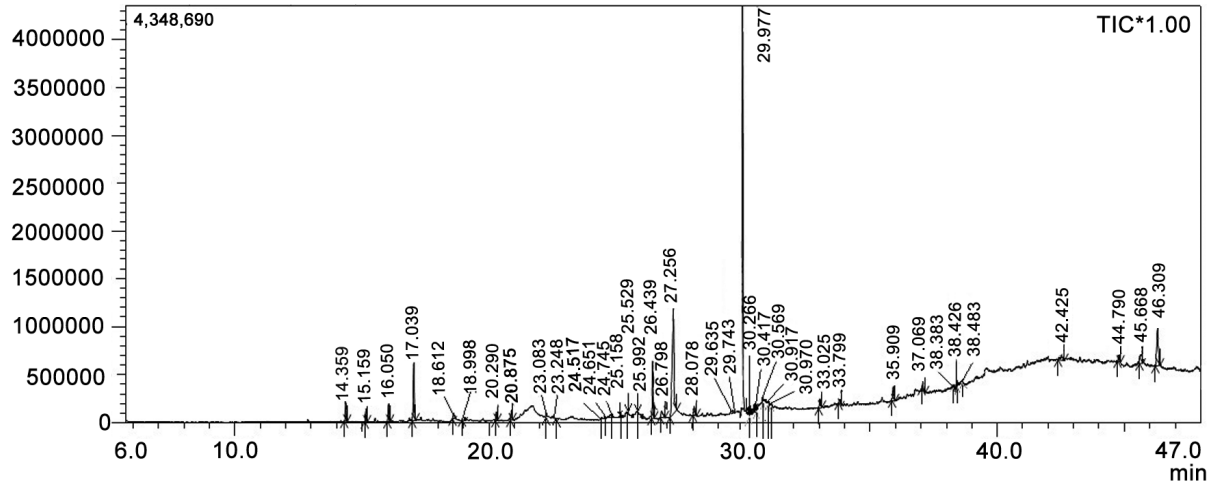

Fig. 1 — Chromatogram of methanolic leaf extract of *Aristolochia tagala* Cham.

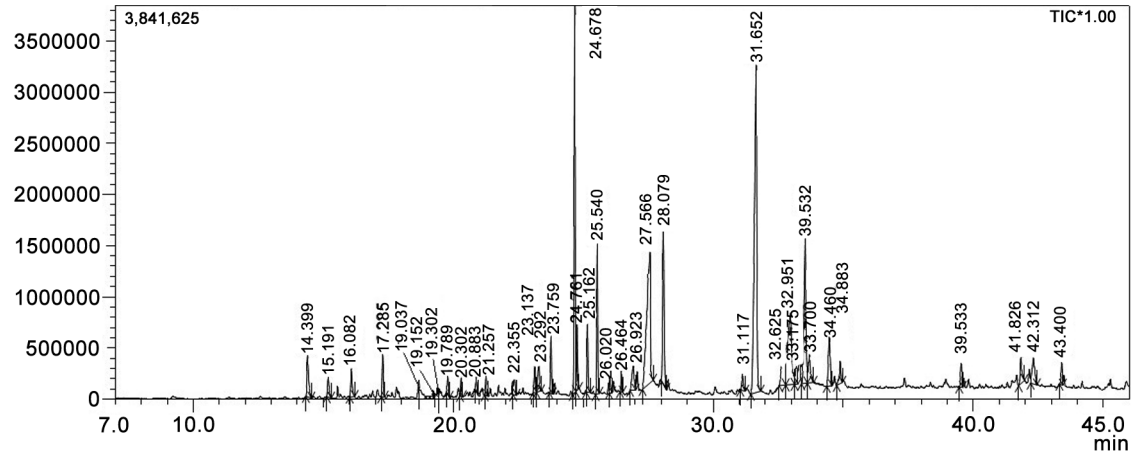

Fig. 2 — Chromatogram of chloroform leaf extract of *Aristolochia tagala* Cham.

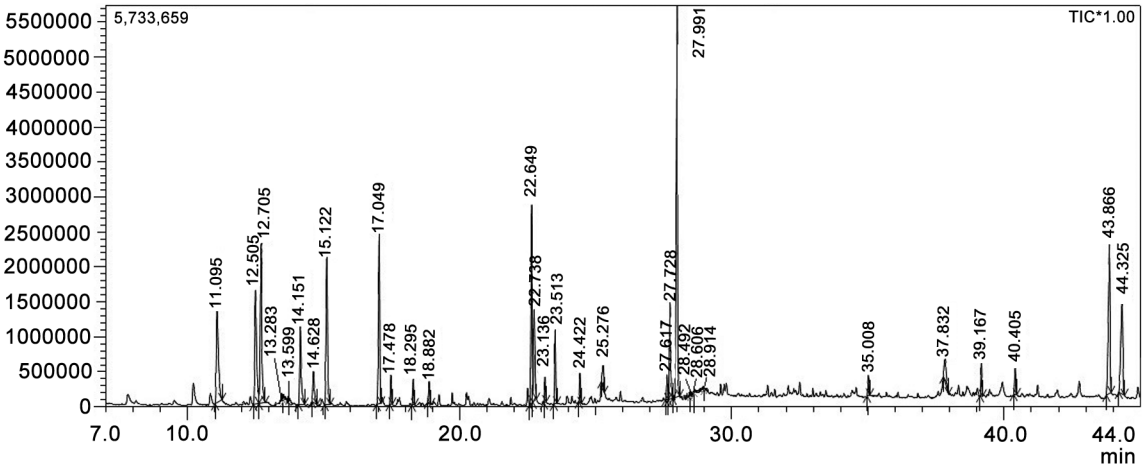

Fig. 3 — Chromatogram of petroleum ether leaf extract of *Aristolochia tagala* Cham.

others were unique to specific solvent extract. Among these 71 recorded compounds, 14 were sesquiter penoids, 10 compounds could be categorized as other terpenoids or compounds containing isoprene units, 4 as steroids, 4 were other aromatic hydrocarbon compounds, fatty acids and their esters accounted for 18 compounds and 21 compounds were other hydrocarbons. Out of total 71 compounds (Table 1), 11 were seen to be present in all the three solvent extracts, of which 5 were sesquiterpenes, 2 were other terpenes, 1 was from another aromatic hydrocarbon group, and 3 were fatty acids or their esters. Based on the percentage of peak area, it could be said that sesquiterpeness were more in concentration in petroleum ether extract. The major compounds were phytol, followed by neophytadiene, a sesqueterpene. Isospathulenol was found only in the methanolic extract, retinal and epiglobulol only in chloroform extract, and bicycloelemene in petroleum ether extract only. The present report also shows a detailed fatty acid composition of *A. tagala* leaves.

In general, for extraction, a wide range of solvents are used which are widely grouped as polar, mid-polar, and non-polar solvents. Further, the polarity of the solvents within each of these groups vary from one another. Polar solvents like water, ethanol, methanol etc., can be used to extract carbohydrates, polyphenols, flavonoids, tannins, saponins, or other compounds with polar groups (like, -OH, -COOH, =O etc), while non-polar solvents like chloroform, petroleum ether or hexane are usually employed to extract terpenes, waxes, oils and certain resins[15,16]. The genus *Aristolochia* is known to be a source of a wide range of terpenes, terpenoids, and sesquiterpene compounds because of which they are used in traditional medicines[17,18]. Hence in the present study, one polar and two non-polar solvents were used. Consequently, 14 sesquiterpene compounds and 10 other compounds containing terpenes or terpenoids or isoprene units were recorded in the leaf extracts of *A. tagala*.

Table 1 — Compounds found in the leaf extracts of *Aristolochia tagala* Cham.

Name	B1 RT	B1 Area%	B2 RT	B2 Area%	B3 RT	B3 Area%
Compounds that were found in all three solvent-leaf extracts						
β-caryophyllene	15.159	0.64	15.191	0.68	13.283	2.10
α-caryophyllene	16.050	0.93	16.082	1.02	14.151	3.75
(-)-Spathulenol	18.998	1.98	19.037	6.94	17.049	6.65
Neopytadiene	24.651	7.07	24.678	9.30	22.649	6.60
Hexahydrofarnesyl acetone	24.745	2.61	24.761	1.58	22.738	3.64
Phytol, acetate	25.158	1.58	25.540	3.42	23.136	0.72
Hexadecanoic acid, methyl ester	26.439	3.14	26.464 & 28.079	0.48 & 5.23	24.422	0.88
Hexadecanoic acid	27.256	10.49	27.566	11.36	25.276	01.05
9,12,15-octadecatrienoic acid, methyl ester, (Z,Z,Z)-	29.743	06.48	31.117	0.64	27.728	03.84
Phytol	29.977	25.06	31.652	19.33	27.991	15.33
Naphthalene, 1-(phenylmethoxy)	30.917	00.51	33.175	0.94	28.914	01.93
Compounds common to methanolic and chloroform extracts						
Tetradecanoic acid	23.083	0.72	23.137	0.84		
(-)Loliolide	23.248	0.55	23.292	1.67		
Octadecanoic acid	30.97	0.83	33.700	1.52		
Compounds common to methanolic and petroleum ether extracts						
β-elemen-(2)	14.359	0.97			12.505	6.22
Bicyclogermacrene	17.039	3.17			14.628	1.62
Juniper camphor	20.875	0.50			18.882	0.76
Methyl octadeca-9,12-dienoate	29.635	1.13			27.617	0.70
β-sitosterol	38.383	4.19			37.832	1.48
α-Tocopherol	46.309	3.40			44.325	5.34
Compounds common to chloroform and petroleum ether extracts						
9-eicosyne			25.162	1.67	23.513	2.29
Palmitaldehyde, diallyl acetal			32.625	2.62	28.492	0.81
Tetratetracontane			42.312	1.6	43.866	7.85
Compounds found only in methanolic extract						
Dodecanoic acid	18.612	0.40				
Isospathulenol	20.290	0.45				
2-Hexadecene, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-	24.517	0.27				
Hexadecanal	25.529	2.38				
Cyclopropaneoctanoic acid, 2-hexyl-, methyl ester	25.992	0.28				
cis-Vaccenic acid	26.798	0.71				
2-Butenal, 2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	28.078	0.63				
Octadecanoic acid, methyl ester	30.266	0.99				
9,12-octadecadienoic acid	30.417	0.69				
9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	30.569	8.27				
Octanoic acid, 2-dimethylaminoethyl ester	33.025	0.55				
cis-1,2-Cyclododecanediol	33.799	0.34				
3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester	35.909	0.91				
Hexadecanal	37.069	0.47				
Card-20(22)-enolide, 3,5,14,19-tetrahydroxy-, (3.beta.,5.beta.)-	38.426	1.39				
.gamma.-Sitosterol	38.483	3.09				
1-Hentetracontanol	42.425	0.60				
γ-Tocopherol	44.790	0.48				
2-tert-butyl-4,6-bis(3,5-di-tert-butyl-4-hydroxybenzyl)phenol	45.668	1.14				
Compounds found only in chloroform extract						
trans-Z-α-Bisabolene epoxide			14.399	1.66		
2,4-Ditert-butylphenol			17.285	1.20		
B-caryophyllene epoxide			19.152	0.54		

(*contd.*)

Table 1 — Compounds found in the leaf extracts of *Aristolochia tagala* Cham. (*contd.*)

	B1		B2		B3	
Name	RT	Area%	RT	Area%	RT	Area%
E-14-Hexadecenal			19.302	1.07		
Humulene epoxide 2			19.789	0.42		
Retinal			20.302	0.42		
Epiglobulol			20.883	0.38		
Retinal			21.257	0.47		
cis-1-Chloro-9-octadecene			22.355	0.61		
E-15-Heptadecenal			23.759	1.31		
7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione			26.020	0.44		
9-Hexadecenoic acid			26.923	1.32		
(Z,Z)-6,9-CIS-3,4-EPOXY-NONADECADIENE			32.951	7.07		
Ethyl (9z,12z)-9,12-octadecadienoate			33.532	6.71		
Octadecanoic acid, ethyl ester			34.460	2.22		
Heptadecyl acetate			34.883	0.86		
1-Heneicosanol			39.533	0.72		
Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester			41.826	1.11		
Heneicosyl heptafluorobutyrate			43.400	0.67		
Compounds found only in petroleum ether extract only						
Bicycloelemene					11.095	5.67
Tetradecane					12.705	7.63
Butanoic acid, 3-methyl-, octyl ester					13.599	0.57
Octadecane, 1-chloro-					15.122	6.64
Hexadecane					17.478	0.87
Ethyl iso-allocholate					18.295	0.88
cis,cis,cis-7,10,13-Hexadecatrienal					28.606	1.73
1,2-benzenedicarboxylic acid					35.008	0.60
Squalene					39.167	0.97
Pentatriacontane					40.405	0.88

Further, the solvents were chosen such that they have moderate boiling points, (unlike ether, dichloromethane, or acetone which have low boiling points and can evaporate even before the compounds are transferred) and do not have high reactive groups (like DMSO or ethyl acetate which have functional groups that can interact with other phyto-compounds at varying temperatures.)

Soxhlet method of extraction is most preferred and industrially used; however, in this method, the heat labile compounds in the extract collected in the flask may get degraded due to heat exposure for longer duration[15,16]. Hence in the present work, the powdered drug was treated with solvent in a closed container at 50 °C for just 4 hours, so that the increased temperature enhances the compound transfer from powder to solvent and at the same time, since the duration of heat exposure is less, most of the compounds (including certain heat-sensitive compounds) are extracted without deteriorating them. Thus it can be utilized in future research to successfully extract a large number of sesquterpene/terpene compounds and additional modifications like sequential extraction with series of solvents, further purification by column chromatography using solvent mixtures of different grades can help in the isolation of targeted elite compounds.

In the past, very few LCMS, HPTLC, or GCMS analyses of this plant have been carried out by other researchers to know the phytochemical composition. Hadem *et al*. fractionated the methanolic root extract of *A. tagala* using column chromatography with various gradients of methanol-chloroform solvents. They have tested the obtained three major fractions for their antioxidant activity and have confirmed that anthocynidin 3-glycosides, 6-hydroxylated flavonols, flavones and chalcones are the probable compounds present in them by conducting HPTLC analysis[19]. Recently Hadem & Sen conducted HR-LCMS analysis of the aqueous-methanolic root extract of the same plant. They enlisted 21 compounds of which aristolactams, aristolactone, aristolochic acid-I, 3-Oxo-ishwarane, β-sitosterol, stigmasterol etc were the major compounds[19,20]. In both these articles, they have demonstrated that methanolic root extract has polar compounds which are responsible for antioxidant and anticancer potentials of the extract. Except for β-sitosterol and stigmasterol, the present GCMS analysis recorded 69 different compounds in the leaves.

Similarly, Deepa *et al* conducted antimicrobial experiments and GCMS analysis of the essential oil obtained from areal parts of *A. tagala*. They have reported moderate antimicrobial activity and recorded 7 compounds, out of which 5 were sesquiterpeness viz., β-caryophyllene, caryophyllene oxide, α-humulene, germacrene-A and bicyclogermacrene[21]. The present work is in accordance with this report, in which total of 14 sesqiterpenes were found (viz., α & β- caryophyllenes, β-caryophyllene epoxide, α-humulene epoxide, bicyclogermacrene, spatulenol, isospathulenol, neophytadiene, hexahydrofarnesyl acetone, β-elemene, juniper camphor, α-bisabolene epoxide, epiglobulol and bicycloelemene), apart from these other 10 compounds (viz., Phytol and its acetate, loliolide, 2-Butenal, 2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl) or boronol, α, DL- α and γ - tocopherols, retinal, 7,9-Di-tert-butyl-1-oxaspiro(4,5) deca-6,9-diene-2,8-dione, squalene) were either terpenoids or compounds containing isoprene units.

A few other researchers[22-24], have isolated certain compounds worked on certain specific compound isolation from this plant; however, no other research work shows detailed enumeration of the phyto-compound composition of the leaves of *A. tagala*. Further, it can be inferred that phytochemical composition of roots differs from that of leaves, similarly, essential oils may have only a few fragrant sesquiterpenes/terpenes but may lack other compounds like steroids. Parallelly solvent used for the extraction determines the type of compounds extracted from the different parts of the plant. Hence, no single work can be considered as an exclusive report that can show all the compounds present in the plant.

From the authors' previous work[25], it is evident that methanolic leaf extract of *A. tagala* has high anti-proliferative potential, but the methanolic extract of leaves differ phyto-chemically from that of roots. This proves that *A. tagala* has many different phyto-compounds with similar anticancer activity, which can be further isolated and screened for the best results. Thus, the current GCMS phytochemical profile of *A. tagala* can serve as a database for future work on isolation of such active principles.

A comparative study of the compounds (reported in the present work) with other literature shows that most of them are responsible for various medicinal properties. α-Caryophyllene, β-Caryophyllene, β-Caryophyllene epoxide are known to inhibit mutagenicity[14] and also possess anti-inflammatory, anti-cancerous, and anti-bacterial properties[26-28]. Neophytadiene has analgesic, antipyretic, anti-inflammatory, antimicrobial, and antioxidant properties[29]. Spathulenol, isospathulenol, β-elemene, bicycloelemene, humulene epoxide have anticancerous properties[30-32]. Bicyclogermacrene and epiglobulol are good flavouring agents and also known to have antimicrobial activity[33,34]. Phytol and its acetate have been shown to possess antioxidant, anti-inflammatory, anticancer, and antidiuretic properties[29,35]. Squalene is used as emollient in the skincare products and is also known to possess antioxidant and antitumour properties[29,36,37].

In the present work, 4 phytosterols were recorded (β and γ -sitosterols, Card-20(22)-enolide, 3,5,14, 19-tetrahydroxy-, (3.beta.,5.beta.) or Strophanthidol and ethyl iso-allocholate). Phytosterols, in general, are considered as efficacious cholesterol-lowering agents. Sitosterols may possess anti-inflammatory and antimicrobial activities[35,38]. As mentioned earlier very few reports are found concerning the detailed illustration of the phyto-compounds. One such review article shows the occurrence of poly cis-prenols of 10, 11, and 12 chain length compounds in this plant[22]. Similarly, another review mentions the presence of aristolodin, aristolochine, and ishwarol in *A. tagala* collected from north and central Vietnam[39]. However in-depth studies on the phytochemical constituents of *A. tagala* have not been carried out so far[40].

On the other hand, literature survey revealed few research experiments accounting for the GC-MS phytochemical profiling of essential oils extracted from *Aristolochia indica* L.[41-43], which is also a medicinal twiner with similar medicinal properties[40] and is a sister species of *A. tagala*. Jirovetz *et al.*[43] have shown the presence of β-caryophyllene, α-humulene, ishwarone, caryophyllene oxide-I, ishwarol, ishwarane, aristolochene, linalool, and α-terpinolene in the essential oil of areal parts of the *A. indica*. Some of these compounds were also recorded in *A. tagala* in the present study, which shows similarities in the chemical composition of these two species accounting for similarities in their medicinal properties.

Conclusion

The current research work presents a detailed phytochemical profile obtained by GC-MS analysis of leaf extracts of *A. tagala* which is a rare and important

medicinal plant used to cure various ailments in traditional health care systems. The present study revealed the presence of a large number of aromatic compounds including compounds containing isoprene units, terpenes, terpenoids, and sesquiterpene compounds which are known to possess a wide range of pharmaceutical properties accounting for the scientific validation of the plant's curative properties. It also provides a detailed database of bioactive compounds present in this plant that can be isolated, purified and utilized for novel drug designing and subjected to clinical research to fight against dreaded diseases.

Acknowledgement

The authors express sincere gratitude to the Department of Science and Technology – Science and Engineering Research Board (DST-SERB), Government of India, New Delhi for funding (SB/EMEQ-249/2014) the current research work.

Conflict of interest

Authors declare that there is no conflict of interests.

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