Research Article

The gas chromatography–mass spectrometry study of one medicinal plant, *Aristolochia indica*

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ABSTRACT

Objective: Verification of the herbal plants by latest scientific tools toward understanding the molecular mechanism underlying their medicinal role is the need of the hour. **Methods:** Gas chromatography–mass spectrometry study of water extracts of the leaves of one herbal plant, *Aristolochia indica* was undertaken by standard procedures and its medicinal activity was verified with the biomolecules present in it. **Results:** The presence of some important biomolecules such as 2-propenoic acid, ethenyl ester, oxalic acid, cyclobutyl ethyl ester, phosphoric acid, 2-chloroethenyl dimethyl ester, pentanoic acid, 2-methylcyclohexyl ester, cis-, butylphosphonic acid, butyl cyclohexylmethyl ester, octadecanoic acid, 2,3-dihydroxypropyl ester, acetic acid, cesium salt and 2-Fluoro-6-trifluoromethylbenzoic acid, 4-nitrophenyl ester, and some others indicate represents the medicinal roles of this plant as is reported ethnopharmacologically. **Conclusions:** The results and discussion above clearly indicate that the various molecules present in *A. indica* leaf extracts do have medicinal roles ascribed to it and further work in this regard is on.

KEY WORDS: 2,3-dihydroxypropyl ester, 2-chloroethenyl dimethyl ester, 2-methylcyclohexyl ester, 2-Propenoic acid, Ethenyl ester, Acetic acid, *Aristolochia indica*, Butyl cyclohexylmethyl ester, Butylphosphonic acid, Cis-, Cyclobutyl ethyl ester, Gas chromatography–mass spectrometry, Octadecanoic acid, Oxalic acid, Pentanoic acid, Phosphoric acid

INTRODUCTION

Plants are the sources of medicines, and all the modern medicines owe their origin to plants. Herbal medicines, although age old, require thorough efficacy evaluation to prove their ethno pharmacological basis. This validation requires the use of latest technological advancements. Gas chromatography–mass spectrometry (GC-MS) is one such method where the plants or herbal medicines can be analyzed to understand the biomolecules present therein. This knowledge can go a long way in understanding the mechanism of action of these medicines toward curing a certain disease. There are reports on this aspects and the present work is a step further in this direction, in which one medicinal plant, *Aristolochia indica* leaf extract was subjected to GC-MS analysis.[1-17]

A. indica is a creeper with various medicinal roles. Ethnopharmacologically, it is used to treat cholera, leprosy, skin disease, menstrual problems, and antidote for snakebites. The medicinal role of this plant has been reviewed thoroughly.[18] There are reports of its antibacterial, anticancer, anti-inflammatory, antipyretic, antidiabetic, and antivenom.[19-24] The plant is shown to have good mast cell stabilizing, anti-inflammatory, and antipruritic activity.[25] An enzyme from *A. indica* is reported to help in removal of amyloid coaggregates thus having a potential to be used for central nervous system diseases such as Parkinson's and Alzheimer.[26] A related species, *Aristolochia tagala* was found to have compounds which showed apoptotic activity in HeLa cells.[27] The present study deals with the GC-MS analysis of water extract of leaves of *A. indica* and to understand the medicinal roles of the molecules indicated in the report.

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METHODS

The plant, *A. Indica* was collected from the nearby hills at Chengalpattu, Tamil Nadu. The plant was identified by a qualified botanist at Chennai. The water ethanol extract of the shade dried leaves was collected

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after 48 h of soaking. The extract was evaporated and the dried powder was used for GC-MS analysis by standard procedures.

GC-MS Procedure

Instrument: GC (Agilent: GC: (G3440A) 7890A. MS/MS: 7000 Triple Quad GCMS) was equipped with MS detector.

Sample Preparation

About 100 ml sample was dissolved in 1 ml of suitable solvents. The solution was stirred vigorously using vortex stirrer for 10 s. The clear extract was determined using GC for analysis.

GC-MS Protocol

Column DB5 MS (30 mm × 0.25 mm ID ×0.25 µm, composed of 5% phenyl 95% methylpolysiloxane), electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1 ml/min injector temperature 280°C; auxilary temperature: 290°C ion-source temperature 280°C.

The oven temperature was programmed from 50°C (isothermal for 1.0 min), with an increase of 40°C/min, to 170°C C (isothermal for 4.0 min), then 10°C/min to 310°C (isothermal for 10 min) fragments from 45 to 450 Da. Total GC running time is 32.02 min. The compounds are identified by GC-MS Library (NIST and WILEY).

RESULTS AND DISCUSSION

The results of the GC-MS analysis of the leaf extracts of *A. indica* are tabulated in Table 1. The medicinal roles of each compound indicated in GC-MS are indicated in Table 2. Figure 1 represents the GC-MS profile of leaf extracts of *A. indica.* From Table 2, it is evident that some molecules such as 2-Propenoic acid, ethenyl ester, oxalic acid, cyclobutyl ethyl ester, phosphoric acid, 2-chloroethenyl dimethyl ester, pentanoic acid, 2-methylcyclohexyl ester, cis-, butylphosphonic acid, butyl cyclohexylmethyl ester, octadecanoic acid, 2,3-dihydroxypropyl ester, acetic acid, cesium salt and 2-Fluoro-6-trifluoromethylbenzoic acid, and 4-nitrophenyl ester have medicinal properties such as acidifier, arachidonic acid inhibitor, and increase aromatic amino acid decarboxylase activity. Molecules such as catechol, myo-Inositol, and 4-C-methyl-, 2-methyl-3-(3-methyl-but-2-enyl)-2-(4-methyl-pent-3-enyl)-oxetane have medicinal roles such as catechol-O-methyltransferase inhibitor, methyl-donor, methylguanidine inhibitor. The molecules myo-inositol, 4-C-methyl- have properties such as myo-neuro-stimulant, myocardiocontractant, myocardiodepressant, myoprotective, catechol-O-methyltransferase inhibitor, and Methyl-Donor, Methylguanidine inhibitor. Another molecule, 4-Ethyl-2-hydroxycyclopent-2-en-1-one, has the properties such as decrease endothelial leukocyte adhesion, endothelium-derived relaxing factor

Table 1: Gas chromatography–mass spectrometry profile of *Aristolochia indica* showing retention time, name of the compound, molecular formula, peak area, peak height, and molecular mass

Retention time	Name of molecule	Formula	Area	Height	Mass
3.8046	2-Propenoic acid, ethenyl ester	$C_5H_6O_2$	33,965,487	2,713,107	96
3.8468	2,2,4-Trimethyl-3-pentanone	$C_8H_{16}O$	64,564,609	6,978,819	128.1
3.9757	Oxalic acid, cyclobutyl ethyl ester	$C_8H_{12}O_4$	9,228,928	945,944	172.1
4.3456	Catechol	$C_6H_6O_2$	34,317,576	6,401,308	110
4.3715	Silanol, trimethyl-, acetate	$C_5H_{12}O_2Si$	19,930,708	2,169,627	132.1
4.4454	Phthalan	C_8H_8O	11,761,294	1,247,282	120.1
4.5419	Silanol, trimethyl-, acetate	$C_5H_{12}O_2Si$	20,093,918	4,315,202	132.1
5.022	2-Propenamide	C_3H_5NO	36,558,353	2,111,294	71
5.2414	Cyclobuta[1,2-d: 3,4-d']bis[1,3]dioxole, tetrahydro-, (3a.alpha.,3b.alpha.,6a.alpha.,6b.alpha.)-	C_6H_8O4	5,991,383	983,895	144
5.2633	2-Phenyl-1,3-oxazol-2-ine	C_9H_9NO	7,161,821	1,658,473	147.1
5.309	Ethyl mesitylglyoxylate	$C_{13}H_{16}O_3$	17,613,850	2,028,986	220.1
5.6316	1,3,5,7-Tetroxane	$C_4H_8O_4$	9,246,182	663,272	120
5.9563	Phosphoric acid, 2-chloroethenyl dimethyl ester	$C_4H_8ClO_4P$	3,051,4033	5,119,496	186
6.3811	Pentanoic acid, 2-methylcyclohexyl ester, cis-	$C_{12}H_{22}O_2$	40,243,784	4,579,974	198.2
6.5056	Urea, propyl-	$C_4H_{10}N_2O$	15,241,5612	9,141,221	102.1
6.6975	Myo-inositol, 4-C-methyl-	$C_7H_{14}O_6$	99,088,366	9,639,428	194.1
6.7122	Benzene, 1-(butylthio)-4-methyl-	$C_{11}H_{16}S$	9,732,062	2,004,212	180.1
6.7614	Phenol, 2,5-bis (1-methylethyl)-, acetate	$C_{14}H_{20}O_2$	7,269,963	1,645,608	220.1
6.8825	3-Oxabicyclo[4.1.0]heptan-2-one, 4,4,7,7-tetramethyl-	$C_{10}H_{16}O_2$	134,621,215	1,096,9688	168.1
6.9183	9-Oxabicyclo[4.2.1]nonan-2-ol, acetate	$C_{10}H_{16}O_3$	108,670,803	10,238,904	184.1
6.956	Phosphorus P4	P_4	8,065,052	6,425,731	123.9
7.0641	4-Ethyl-2-hydroxycyclopent-2-en-1-one	$C_7H_{10}O_2$	12,452,550	1,619,722	126.1

(*Contd...*)

Table 1: (*Continued)*

Retention time	Name of molecule	Formula	Area	Height	Mass
7.2591	2-Methyl-3-(3-methyl-but-2-enyl) -2-(4-methyl-pent-3-enyl)-oxetane	$C_{15}H_{26}O$	63,263,050	9,025,973	222.2
7.3073	Butylphosphonic acid, butyl cyclohexylmethyl ester	$C_{15}H_{31}O_3P$	48,653,146	9,677,362	290.2
8.4246	O-Isobutyl methylphosphonothiolate	$C_5H_{13}O_2PS$	20,789,237	2,047,723	168
8.4379	2-Methyl-3-methoxy-4H-pyran-4-one	$C_7H_8O_3$	34,231,496	2,858,827	140
8.9056	Tridecanoic acid, methyl ester	$C_{14}H_{28}O_2$	48,393,939	10,080,777	228.2
12.175	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	$C_{19}H_{38}O_4$	434,549,673	12,350,892	330.3
12.271	Cyclobutanone, 2-tetradecyl-	$C_{18}H_{34}O$	25,631,152	1,871,808	266.3
14.121	Octadecanoic acid, 2,3-dihydroxypropyl ester	$C_{21}H_{42}O_4$	222,231,636	10,701,912	358.3
17.659	2,2-Dimethyl-propyl 2,2-dimethyl-propanesulfinyl sulfone	$C_{10}H_{22}O_3S_2$	11,645,495	2,051,781	254.1
22.351	2,4 (1H,3H)-Pyrimidinedione, 6-iodo-5-methyl-	$C_5H_5IN_2O_2$	8,007,528	1,980,794	251.9
22.786	Acetic acid, cesium salt	$C_2H_3CsO_2$	157,294,343	980,160	191.9
22.853	2-Ethyl-3,5-dimethylpyridine	$C_9H_{13}N$	826,930,451	1,764,060	135.1
23.072	N-(1,1-Dimethyl-2-propynyl)-N, N-dimethylamine	$C_7H_{13}N$	377,736,471	1,434,723	111.1
23.473	2-Fluoro-6-trifluoromethylbenzoic acid, 4-nitrophenyl ester	$C_{14}H_7F_4NO_4$	12,451,795	963,278	329
23.562	p-Toluic acid, 4-nitrophenyl ester	$C_{14}H_{11}NO_4$	106,278,506	523,443	257.1
24.2	2,4 (1H,3H)-Pyrimidinedione, 6-iodo-5-methyl-	$C_5H_5IN_2O_2$	209,156,456	2,038,617	251.9
24.356	2-Fluoro-6-trifluoromethylbenzoic acid, 4-nitrophenyl ester	$C_{14}H_7F_4NO_4$	25,991,232	1,028,971	329

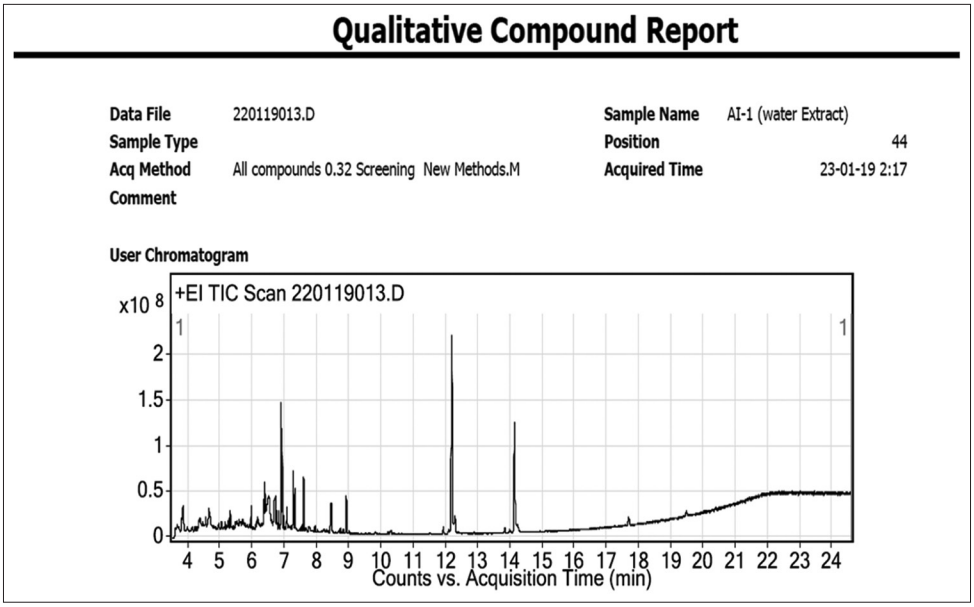

Figure 1: The gas chromatography–mass spectrometry graph of leaf extracts of *Aristolochia indica*

promoter, endocrine-tonic, enteromotility- enhancer, and ergotamine-enhancer. The molecule, namely O-Isobutyl methylphosphonothiolate is reported to have medicinal values such as aldehyde oxidase inhibitor, anticancer (oral), antitumor (ovary), catechol- O-methyltransferase inhibitor, decrease glutamate oxaloacetate transaminase, downregulation of nuclear and cytosol androgen, increase osteocalcin, inhibit destruction of glycosaminoglycans, inhibit production of tumor necrosis factor, ionic channel opener, NADH-oxidase inhibitor, and NADH–ubiquinone oxidoreductase inhibitor. Thus, it is clear that the molecules as found in the GC-MS profile of *A. indica* represent the medicinal roles ascribed too it ethnopharmacologically as well as scientific reports.

Table 2: The medicinal roles of each molecule shown in the gas chromatography–mass spectrometry profile of *Aristolochia indica* leaf extracts

Compound	Medicinal role
2-Propenoic acid, ethenyl ester	Acidifier, Arachidonic acid inhibitor, Increase Aromatic Amino acid Decarboxylase activity
2,2,4-Trimethyl-3-pentanone	Not Known
Oxalic acid, cyclobutyl ethyl ester	Acidifier, Arachidonic acid inhibitor, Increase Aromatic Amino acid Decarboxylase activity
Catechol	Catechol-O-Methyltransferase inhibitor, Catecholaminogenic, Catecholaminolytic
Silanol, trimethyl-, acetate	Not known
Phthalan	Not known
2-Propenamide	Not known
Cyclobuta[1,2-d: 3,4-d']bis[1,3]dioxole, tetrahydro-, (3a.alpha.,3b.alpha.,6a.alpha.,6b.alpha.)-	Not known
2-Phenyl-1,3-oxazol-2-ine	Not known
Ethyl mesitylglyoxylate	Not known
1,3,5,7-Tetroxane	
Phosphoric acid, 2-chloroethenyl dimethyl ester	Acidifier, Arachidonic acid inhibitor, Increase Aromatic Amino acid Decarboxylase activity
Pentanoic acid, 2-methylcyclohexyl ester, cis-	Acidifier, Arachidonic acid inhibitor, Increase Aromatic Amino acid Decarboxylase activity
Urea, propyl-	Urealytic, Urenase inhibitor
Myo-inositol, 4-C-methyl-	Myo-neuro-stimulant, Myocardiocontractant, Myocardiodepressant, Myoprotective, Catechol-O-methyltransferase Inhibitor, Methyl-Donor, MethylGuanidine Inhibitor
Benzene, 1-(butylthio)-4-methyl-	Not known
Phenol, 2,5-bis (1-methylethyl)-, acetate	Not known
3-Oxabicyclo[4.1.0]heptan-2-one, 4,4,7,7-tetramethyl-	Not known
9-Oxabicyclo[4.2.1]nonan-2-ol, acetate	Not known
Phosphorus P4	Anti-cAMP-phosphodiesterase, Anticancer (Pancreas, Pharynx, Prostate, Breast), Antimitral-valve-prolapse,
4-Ethyl-2-hydroxycyclopent-2-en-1-one	Decrease Endothelial Leukocyte Adhesion, Endothelium-Derived Relaxing Factor Promoter, Endrocrine-Tonic, Enteromotility-Enhancer, Ergotamine-Enhancer
2-Methyl-3-(3-methyl-but-2-enyl) -2-(4-methyl-pent-3-enyl)-oxetane	Catechol-O-MethylTransferase Inhibitor, Methyl-Donor, MethylGuanidine Inhibitor
Butylphosphonic acid, butyl cyclohexylmethyl ester	Acidifier, Arachidonic acid inhibitor, Increase Aromatic Amino acid Decarboxylase activity
Tridecanoic acid, methyl ester	Catechol-O-MethylTransferase Inhibitor, Methyl-Donor, MethylGuanidine Inhibitor, Acidifier, Arachidonic acid inhibitor, Increase Aromatic Amino acid Decarboxylase activity
O-Isobutyl methylphosphonothiolate	Aldehyde oxidase inhibitor, Anticancer (Oral), Antitumor (Ovary), Catechol-O-MethylTransferase Inhibitor, Decrease Glutamate Oxaloacetate Transaminase, Down regulation of nuclear and cytosol androgen, Increase Osteocalcin, Inhibit Destruction of Glycosaminoglycans, Inhibit Production of Tumor Necrosis Factor, ionic channel opener, NADH-oxidase inhibitor, NADH–ubiquinone oxidoreductase inhibitor
2-Methyl-3-methoxy-4H-pyran-4-one	Catechol-O-MethylTransferase Inhibitor, Methyl-Donor, MethylGuanidine Inhibitor, 11B-HSD-Inhibitor, 17-beta-hydroxysteroid dehydrogenase Inhibitor, 5-HETE-Inhibitor, 5-HT-Inhibitor, Anti-HIV-Integrase, Aryl-Hydrocarbon-Hydroxylase-Inhibitor
Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	17beta hydroxysteroid dehydrogenase Inhibitor, 17-beta-hydroxysteroid dehydrogenase Inhibitor, Testosterone-Hydroxylase-Inducer, Acidifier, Arachidonic acid inhibitor, Increase Aromatic Amino acid Decarboxylase activity
Cyclobutanone, 2-tetradecyl-	Not known
Octadecanoic acid, 2,3-dihydroxypropyl ester	Acidifier, Arachidonic acid inhibitor, Increase Aromatic Amino acid Decarboxylase activity
2,2-Dimethyl-propyl 2,2-dimethyl-propanesulfinyl sulfone	Not known
2,4 (1H,3H)-Pyrimidinedione, 6-iodo-5-methyl-	Iodothyronine Deiodinase Inhibitor, 11B-HSD-Inhibitor, Hemagglutinator, Anti-HIV-Integrase, 11B-HSD-Inhibitor, 12-Lipoxygenase-Inhibitor, 17-beta-hydroxysteroid dehydrogenase

(*Contd...*)

Table 2: (*Continued*)

Compound	Medicinal role
	Inhibitor, 5-Alpha-Reductase-Inhibitor, 5-HT-Inhibitor, Aryl-Hydrocarbon-Hydroxylase-Inhibitor, Hematonic,
Acetic acid, cesium salt	Acidifier, Arachidonic acid inhibitor, Increase Aromatic Amino acid Decarboxylase activity
-Ethyl-3,5-dimethylpyridine	Smart Drug, (+)-Inotropic, (-)-Chronotropic, 11B-HSD Inhibitor, 12-Lipoxygenase Inhibitor, 17-beta-hydroxysteroid dehydrogenase-Inhibitor, 5-Alpha-Reductase Inhibitor, 5-HT-Inhibitor, 8-HT Inhibitor, 5-Lipoxygenase Inhibitor
N-(1,1-Dimethyl-2-propynyl)-N, N-dimethylamine	Not known
2-Fluoro-6-trifluoromethylbenzoic acid, 4-nitrophenyl ester	Acidifier, Arachidonic acid inhibitor, Increase Aromatic Amino acid Decarboxylase activity
p-Toluic acid, 4-nitrophenyl ester	Acidifier, Arachidonic acid Inhibitor, Increase aromatic amino acid decarboxylase activity, Adrenalin-pressor, Anti-cAMP-phosphodiesterase, Anticancer (prostate, pancreas, and pharynx), Antidote

CONCLUSIONS

The results and discussion, as mentioned above, indicate the potential of *A. indica* as a medicine for various ailments as claimed ethno medically and also by modern research reports.

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