

Phytochemical Profiling And GC-MS Study Of *Antigonum leptopus* Hook & ARN.

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Abstract: The present study investigated the chemical constituents of a traditionally used ethanobotanical plant *Antigonum leptopus* using a GC-MS approach. Phytochemical analysis of *Antigonum leptopus* revealed the presence of alkaloids, saponium, steroid, phenolic compounds, fatty acids, flavonoids and volatile oils in leaves and tubers. Interestingly, ten phytochemical constituents were analyzed and characterized by GC-MS that confirmed the presence of (1) Glycerin (2.49%), (2) Propane,1,1,3-triethoxy (4.09%), (3) 4H-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl- (14.34%), (4) methyl salicylate (1.14%), (5) 2-furancarboxaldehyde,5-(hydroxymethyl)- (24.45%), (6) dodecanoic acid (1.89%), (7) 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl)ester (6.06%), (8) n-hexadecanoic acid (15.20%), (9) oleic acid (18.02%) and (10) 1,2-Benzenedicarboxylic acid, diisooctyl ester (12.33%). Results from this work indicated useful information on the phytochemistry of *A. leptopus* tuber, which can pave way to further applications and utility in the pharmaceutical and nutraceutical field.

Keywords: *Antigonum leptopus*, GC-MS, Phytochemical component.

Introduction

According to World health organization, medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has been derived from medicinal plants. The medicinal plants are of great important to the health of individual and communities. The medicinal value of these plants lie in some active chemical substances called phytochemical that produce a physiological action on the human body. Phytochemicals are naturally occurring biochemical compounds in plants for color, flavor, smell and texture for pollination and define mechanism. Some plant secondary metabolites such as alkaloids, phenols, tannins, glycosides, terpenoids, saponins, flavonoids and steroids have been implicated in their ability to inhibit the formation of pro-inflammatory signaling molecules such as prostaglandin or leukotrienes¹.

However, such plants should be investigated to better understand their properties, safety and efficiency². The herbal plant *Antigonum leptopus* are widely used by the tribal people as an efficient medicine that cures piles. Therefore, this study is conducted to provide a thorough knowledge on the tribal plant *A. leptopus*. Studies based on the ethnobotanical use of plants have often provided a more efficient method of drug discovery than random screening³⁻⁴. In the present study, we evaluated phytochemical screening of leaf and tuber of *A.*

leptopus on the quantitative and qualitative analysis by using the GC-MS method for the identification of compounds and their activity based on ethanobotanical databases.

Materials and methods

Plant material

The plant materials such as leaves and tubers were collected from the college outskirts region of the campus of H.H. The Rajah's College, Pudukkottai, a distinct headquarters of Tamilnadu state. The vegetative parts, leaves and tubers from *A. leptopus*, belonging to the family Polygonaceae was employed for the analysis. Herbarium specimens of the plant parts were prepared and deposited as voucher specimens in the departmental herbarium for future verification. The fresh leaves and tuber were dried thoroughly under shade and powdered finely and stored in a clean plastic container for phytochemical analysis.

Phytochemical estimation

The phytochemical estimation from fresh leaves and tuber powder of *A. leptopus* were quantitatively determined by adopting standard protocols viz., Chlorophyll⁵, carotenoid⁶, total soluble sugars⁷ and total soluble starch⁸ using glucose as standard, total soluble proteins⁹ by using bovine serum albumin as standard, total free amino acids¹⁰ using leucine as standard, total phenols¹¹ using catechol as standard, hydroxy phenols¹² using catechol as standard. All experiments were repeated three times for precision and values were expressed in mean \pm standard deviation in terms of fresh leaves and air dried tuber.

Preparation of plant extracts

Twenty gram of tuber powder was soaked in 50 ml of absolute alcohol overnight for alcoholic extraction. It was filtered through Whattmann filter paper No. 41 along with 2gm sodium sulfate which had been wetted with absolute alcohol to remove the sediments and traces of water in the filtrate. Then the filtrates were concentrated by bubbling nitrogen gas into the solution and reduce the volume to 1 ml. The extract contained both polar and non-polar phytochemical components.

GC-MS analysis

The plant sample (tuber extract) was analyzed in a Perkin Elmer GC Clarus 500 MS system for different components present in the extract, under the following conditions: column– dimethyl polysiloxane DB-1 fused silica capillary column (30m x 0.25 mm x 0.1 μ m of film thickness); carrier gas – helium (1ml / min); injector temperature –250 $^{\circ}$ C; detector temperature - 200 $^{\circ}$ C; column temperature – 35-180 $^{\circ}$ C at 4 $^{\circ}$ C / min – then 180 – 250 $^{\circ}$ C at 10 $^{\circ}$ C / min; MS electron impact 70 eV.

Identification of compound

Interpretation on mass spectrum of GC-MS was conducted using the database of National Institute Standard and Technology (NIST Ver.2.1) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were identified. The activity of the tuber extract of biochemical compound was compared with Dr. Duke's Phytochemical and Ethnobotanical Databases.

Results

The present study carried out on the plant samples revealed the presence of medicinally active constituents. Quantitative estimation of the biochemical compounds of the plant parts such as leaf and tuber was studied and summarized in Table 1. The content of pigments like total chlorophyll (1.452 \pm 0.06), chlorophyll a (0.772 \pm 0.33), chlorophyll b (0.681 \pm 0.02), and carotenoid (0.632 \pm 0.05) in leaves and the components like total soluble sugars (312.31 \pm 12.76), total soluble starch (107.12 \pm 3.5), total soluble proteins (31.21 \pm 1.04) and free amino acids (76.32 \pm 4.49) as well as phenols (42.41 \pm 2.34) and total hydroxyphenols (156.13 \pm 5.73) in tubers was comparatively higher than that of leaves.

The presence of secondary metabolites in the leaf and tuber of the *A. leptopus* investigated were summarized in Table 2. The results show that the presence of saponin, steroid / triterpenoid, phenolic compounds, fatty acid, flavonoids and volatile oils were present in leaf and tuber of the plant. Tannins and glycosides were absent in leaf and tuber respectively, whereas alkaloids were present in the tuber.

Table 1. Basic biochemical components in leaves and tubers of *Antigonum leptopus* Hook.&Arn.

S.No	Basic biochemical components	Leaf (mg g ⁻¹ f.w.)	Tuber (mg g ⁻¹ d.w.)
1	Chl. <i>a</i>	0.772± 0.33	-
	Chl. <i>b</i>	0.681± 0.02	-
	Total chl.	1.452± 0.06	-
2	Carotenoids	0.632± 0.05	-
3	Total soluble sugar	47.72± 2.49	312.31± 12.76
4	Total soluble starch	42.51± 2.54	107.12± 3.5
5	Total soluble proteins	13.83± 0.65	31.21± 1.04
6	Total free amino acids	18.22± 4.71	76.32± 4.49
7	Total phenols	20.32± 1.29	42.41± 2.34
8	Hydroxy phenols	20.71± 1.43	156.13± 5.73

Table 2. Qualitative analysis of phytochemicals from *Antigonum leptopus* Hook. & Arn.

S.No.	Name of the compounds	Plant parts	
		Leaf	Tuber
1	Alkaloids	-	+
2	Tannin	-	-
3	Saponin	+	+
4	Triterpenoid	+	+
5	Phenolic compounds	+	+
6	Fatty acid	+	+
7	Flavonoids	+	+
8	Glycoside	-	-
9	Volatile oils	+	+

+ = Presence of constituent

- = Absence of constituent

GC-MS chromatogram of the alcoholic extract of *A. leptopus* showed ten peaks indicating the presence of ten compounds. The chemical compounds and their activity in the alcoholic extract of *A. leptopus* are presented in Table 3, 4 and Figure 1. The ten phytochemical constituents characterized by GC-MS peak are as follows: (1) Glycerin (2.49%), (2) Propane,1,1,3-triethoxy (4.09%), (3) 4H-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl- (14.34%), (4) methyl salicylate (1.14%), (5) 2-furancarboxaldehyde,5-(hydroxymethyl)- (24.45%), (6) dodecanoic acid (1.89%), (7) 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl)ester(6.06%), (8) n-hexadecanoic acid(15.20%), (9) oleic acid (18.02%) and (10) 1,2-Benzenedicarboxylic acid, diisooctyl ester(12.33%). The properties of the compounds and their bioactivities based on Dr. Duke's phytochemical and Ethnobotanical databases are listed below.

Compound 1 was detected as Glycerin and its molecular formula was assigned to be C₃H₈O₃ compound nature was alcohol group, based on the high-resolution GC-MS spectrum. The spectral features and physicochemical properties revealed 1 to be Glycerol. This compound showed RT as 4.07, molecular wt as 92; and peak area as 2-49% (Figure 2.A). It showed the following bioactivities, based on Dr. Duke's phytochemical and Ethnobotanical databases: Hygroscopic action, anti-inflammatory and antimicrobial properties and also used as softening agent and preservative. It had the following other synonyms: 1. 1,2,3-Propanetriol, 2. Glycerol, 3. Glycerine, 4. Glyceritol, 5. Glycyl alcohol, 6. Glyrol, 7. Glysanin, 8. Osmoglyn, 9. Propanetriol, 10. Trihydroxypropane, 11. Synthetic glycerin, 12. 90Technical glycerin, 13. Dagalax and 14. Glycerin anhydrous.

Compound 2 was detected as Propane, 1,1,3-triethoxy- and its molecular formula was assigned to be C₉H₂₀O₃, compound nature was found to be belong to ether compound, based on the high-resolution GC-MS spectrum. The spectral features and physicochemical properties revealed 2 to be α-Ethoxypropionaldehyde diethyl acetal. This had 5.93 RT, 176 molecular wt. as 4.09 peak area % (Figure 2.C). It showed the following bioactivities, based on Dr. Duke's phytochemical and Ethnobotanical databases: No activity was reported so far. It had the following other synonyms: 1. α-Ethoxypropionaldehyde diethyl acetal; 2. 3-Ethoxypropionaldehyde

diethyl acetal; 3. Propionaldehyde, 3-ethoxy-, diethyl acetal; 4. 1,1,3-Triethoxypropane; 5. Propane, 1,3,3-triethoxy- 6. 1,3,3-Triethoxypropane.

Compound 3 was detected as 4H-Pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl- and its molecular formula was assigned to be $C_6H_8O_4$ and compound nature was found to be Pyran ring, based on the high-resolution GC-MS spectrum. The spectral features and physicochemical properties revealed 3 to be 4H-Pyran-4-one, 2, 3-dihydro-3,5-dihydroxy-6-methyl-. This had 7.58RT, 144 molecular wt and 14.34 % peak area (Figure 2.B). It showed the following bioactivities, based on Dr. Duke's phytochemical and Ethnobotanical databases: No activity was reported. It had the following other synonym: 3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one .

Compound 4 was detected as Methyl Salicylate and its molecular formula was assigned to be $C_8H_8O_3$, based on the high-resolution GC-MS spectrum. The spectral features and physicochemical properties revealed 4 to be Methyl Salicylate. This compound showed RT as 8.78, molecular wt. as 152; and peak area as 1.14% (Figure 2.D). It showed the following bioactivities, based on Dr. Duke's phytochemical and Ethnobotanical databases: antipyretic, anti-inflammatory, analgesic, antiseptic, pesticide, insecticide, cancer-preventive carminative and perfumery. It had the following other synonyms: 1.Benzoic acid, 2-hydroxy-methylester; 2.Salicylic acid, methyl ester; 3.o-Hydroxybenzoic acid, methyl ester;4.Analgit; 5.Betula;6.Betula oil; 7.Betula Lenta; 8.Exagien; 9.Flucarmit;10.Gaultheria oil;11.Gaultheria Oil, artificial; 12.Gaultheriaoil; 13.Methylhydroxybenzoate; 14.Methyl2-hydroxy; benzoate;15.Oil of Wintergreen.

Compound 5 was detected as 2-Furan carboxaldehyde, 5-(hydroxymethyl)-and its molecular formula was assigned to be $C_6H_6O_3$ compound nature was lauric acid, based on the high-resolution GC-MS spectrum. The spectral features and physicochemical properties revealed 5 to be 2-Furancarboxaldehyde, 5-(hydroxymethyl)-. This compound showed RT as 9.03, molecular wt as 126; and peak area as 24.45% (Figure 2.E). It showed the following bioactivities, based on Dr. Duke's phytochemical and Ethnobotanical databases: antimicrobial, preservative. It had the following other synonyms: 1.2-Furaldehyde, 5-(hydroxymethyl)-; 2.5-Hydrxoyethylfurfural; 3.Hydroxymethylfurfurole; 4.HMF;5.5-(Hydroxymethyl) Furfurole;6.5-(Hydroxymethyl)-2-formylfuran; 7.5-(Hydroxymethyl)-2-furaldehyde; 8.5-(Hydroxymethyl)-2-furancarboxal; 9. 5-(Hydroxymethyl)-2-furfural; 10. 5-(Hydroxymethyl) -2 -furfuraldehyde; 11. 5-(Hydroxymethyl)furan-2-aldehyde;12.5-Hydroxymethyl)furfural;13.5-Hydroxymethylfuraldehyde;14.5-Oxymethylfurfurole; 15.5-Hydroxymethylfurfuraldehyde;

Compound 6 was detected as Dodecanoic acid and its molecular formula was assigned to be $C_{12}H_{24}O_2$ compound nature was lauric acid, based on the high-resolution GC-MS spectrum. The spectral features and physicochemical properties revealed 6 to be Dodecanoic acid. This compound showed RT as 17.41, molecular wt as 200; and peak area as 1.89%. It showed the following bioactivities, based on Dr. Duke's phytochemical and Ethnobotanical databases: antioxidant, antibacterial, COX-1 & COX-2 inhibitor,antiviral hypcholesterolmic, candidicide.

Compound 7 was detected as 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester and its molecular formula was assigned to be $C_{16}H_{22}O_4$ compound nature was Plasticizer compound, based on the high-resolution GC-MS spectrum. The spectral features and physicochemical properties revealed 1 to be 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester. This compound showed RT as 23.72, molecular wt as 278 and peak area as 6.06% (Figure 2.F). It showed the following bioactivities, based on Dr. Duke's phytochemical and Ethnobotanical databases: No activity was reported. It had the following other synonyms: 1.Phthalic acid, diisobutyl ester; 2.Diisobutyl phthalate; 3.Hexaplas M/1B; 4.Isobutyl phthalate.

Compound 8 was detected as n-Hexadecanoic acid and its molecular formula was assigned to be $C_{16}H_{32}O_2$ compound nature was Palmitic acid, based on the high-resolution GC-MS spectrum. The spectral features and physicochemical properties revealed 8 to be n-Hexadecanoic acid. This compound showed RT as 25.35, molecular wt as 256; and peak area as 15.20% (Figure 2.G). It showed the following bioactivities, based on Dr. Duke's phytochemical and Ethnobotanical databases: Antioxidant Hypcholesterolemic; Nematicide; Pesticide; Lubricant; Antiandrogenic; Flavor; Hemolytic; 5-Alpha reductase inhibitor. It had the following other synonyms: 1.Hexadecanoic acid;2.n-Hexadecoic acid;3.Palmitic acid;4.Pentadecanecarboxylic acid;5.1-Pentadecanecarboxylic acid;6.Cetylic acid;7.Emersol 140; 8. Emersol 143; 9. Hexadecylic acid; 10. Hydrofol ; 11. Hystrene 8016;12. Hystrene 9016; 13. Industrane 4516; 14.Prifrac 2960 ; 15.Glycon P-45 ; 16. Prifac 2960; 17. Univol U332.

Compound 9 was detected as Oleic Acid, and its molecular formula was assigned to be $C_{18}H_{34}O_2$ compound nature was Fatty acid, based on the high-resolution GC-MS spectrum. The spectral features and physicochemical properties revealed 9 to be Oleic Acid, ethyl ester. This compound showed RT as 29.14, molecular wt. as 282; and peak area as 18.02% (Table3, 4 and Figure). It showed the following bioactivities, based on Dr. Duke's phytochemical and Ethnobotanical databases: Antiinflammatory; Antiandrogenic. It had the following other synonyms: 1.9-Octadecenoic acid (Z)-;2.ë(Sup9)-cis-Oleic acid;3.cis-ë(Sup9)-Octadecenoic acid;4.cis-Oleic Acid;5.cis-9-Octadecenoic Acid;6.Emersol 211;7.Emersol 220 White Oleic Acid;8.Emersol 221 Low Titer White Oleic Acid;9.Oelsauere;10.Oleine 7503;11.Pamolyn 100;12.Red oil.

Compound 10 was detected as 1,2-Benzene dicarboxylic acid, diisooctyl ester and its molecular formula was assigned to be $C_{24}H_{38}O_4$ compound nature was Plasticizer compound, based on the high-resolution GC-MS spectrum. The spectral features and physicochemical properties revealed 1 to be 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester. This compound showed RT as 35.67, molecular wt as 390 and peak area as 12.33% (Figure 5.H). It showed the following bioactivities, based on Dr. Duke's phytochemical and Ethnobotanical databases: No activity was reported and so further research has to be carried out with respect to this compound.

Table 3. Isolation and characterization of phytochemical components from alcoholic tuber extract of *Antigonum leptopus* Hook. & Arn. (GC-MS analysis)

No	RT	Name of the compound	Molecular Formula	MW	Peak Area %
1	4.07	Glycerin	$C_3H_8O_3$	92	2.49
2	5.93	Propane, 1,1,3-triethoxy-	$C_9H_{20}O_3$	176	4.09
3	7.58	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144	14.34
4	8.78	Methyl Salicylate	$C_8H_8O_3$	152	1.14
5	9.03	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	$C_6H_6O_3$	126	24.45
6	17.41	Dodecanoic acid	$C_{12}H_{24}O_2$	200	1.89
7	23.72	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	$C_{16}H_{22}O_4$	278	6.06
8	25.85	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	15.20
9	29.14	Oleic Acid	$C_{18}H_{34}O_2$	282	18.02
10	35.67	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	12.33

Table 4. Characterization of phytochemical components from alcoholic tuber extract of *Antigonum leptopus* Hook. & Arn., displaying their bioactivities

No	Name of the compound	Compound nature	Activity**
1	Glycerin	Alcohol	Antimicrobial, Preservative
2	Propane, 1,1,3-triethoxy-	Ether compound	No activity reported
3	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	Pyran compound	No activity reported
4	Methyl Salicylate		Antipyretic, Antiinflammatory, Analgesic, Antiseptic, Pesticide, Insectifuge, Cancer-preventive, Carminative, Perfumery
5	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	Aldehyde	Antimicrobial Preservative
6	Dodecanoic acid	Lauric acid	Antioxidant, Antibacterial, COX-1 & COX-2 inhibitor, Antiviral, Hypocholesterolemic, Candidicide.
7	1,2-Benzenedicarboxylic bis(2-methylpropyl) ester	acid, Plasticizer compound	No activity reported
8	n-Hexadecanoic acid	Palmitic acid	Antioxidant, Hypocholesterolemic Nematicide, Pesticide, Lubricant, Antiandrogenic, Flavor, Hemolytic 5-Alpha reductase inhibitor
9	Oleic Acid	Fatty acid	Antiinflammatory, Antiandrogenic Cancer preventive,

				Dermatitigenic Hypocholesterolemic, 5-Alpha reductase inhibitor, Anemiagenic Insectifuge, Flavor
10	1,2-Benzenedicarboxylic diisooctyl ester	acid,	Plasticizer compound	No activity reported

**Source: Dr.Duke's Phytochemical and Ethnobotanical Databases

Table 5. *A. leptopus* biochemical compounds identified form different plants and their uses.

S. N	Name of the Compound present	Plant Name	Plant Part	Uses	Reference
1.	Glycerin	<i>Mimosa pudica</i> <i>Promsa serratifolia</i> <i>Alstonia venerata</i> <i>Morinda citrifolia</i>	Leaf Leaf Leaf Leaf	Therapeutic uses Therapeutic uses Rheumatic complaints Antibacterial, antiinflammatory, analgesis antioxidant and antitumor effect	Sridharan <i>et al.</i> (2011) ²⁵ Singh <i>et al.</i> (2011) ²⁶ Sutha <i>et al.</i> (2012) ²⁷ Rivera <i>et al.</i> (2012) ²⁸
2.	Propane,1,1,3 triethoxy	<i>Vitex negundo</i> <i>Wattaka volubilis</i> <i>Andrographis paniculata</i> <i>Caesalpinia sappan</i> <i>Vigna mungo</i> <i>Clitoria ternatea</i> <i>Cadaba trifoliata</i> <i>Cadaba trifoliata</i> <i>Polygala chinensis</i> <i>Naringi crenulata</i> <i>Euphoria longan</i>	Leaf Leaf Leaf Aerial part part Gram Aerial part Leaf Root Whole plant Leaf & bark Leaf	Antioxidant activity Antiinflammatory Upper respiratory infections Antioxidant Liver diseases cancer & diabetes Nephroprotective antioxidant Purgative and Phlogistree Anticancer, anti diabetic, anti inflammatory Cough and bronchitis Dysentery and colic disorders. Amnesia insomnia, aneamia palpitations and neurosis.	Kumar <i>et al.</i> (2010) ²⁹ Vishnusithan and Kanaraj (2012) ³⁰ Kalaivani <i>et al.</i> (2012) ³¹ Sarumathy <i>et al.</i> (2011) ³² Anbuselvi <i>et al.</i> (2012) ³³ Sarumathy <i>et al.</i> (2011) ³² Velmurugan and Kamaraj (2011) ³⁴ Velmurugan <i>et al.</i> (2010) ³⁵ Alagammal <i>et al.</i> (2011) ³⁶ Sarada <i>et al.</i> (2011) ³⁷ Devi <i>et al.</i> (2009) ³⁸
3.	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	<i>Euphoria longan</i>	Leaf	Amnesia insomnia, aneamia palpitations and neurosis.	Devi <i>et al.</i> (2009) ³⁸
4.	Methyl Salicylate	<i>Syzygium caryophyllatum</i> <i>Mallotus philippensis</i>	Leaf & bud Root	Antibacterial, antifungal and antioxidant Skin diseases and rheumatism	Bhuiyan <i>et al.</i> (2010) ³⁹ Velanganni <i>et al.</i> (2011) ⁴⁰
5.	2-Furancarboxaldehyde,5-(hydroxymethyl)	<i>Caesalpinia sappan</i>	Leaf	Emenagogue, emostatic and anti inflammatory	Sarumathy <i>et al.</i> (2011) ³²
6.	Dodecanoic acid	<i>Withania somnifera</i>	Root	Anti inflammatory	Kumar <i>et al.</i> (2011) ⁴¹

7	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	<i>Cadaba trifoliata</i>	Leaf	Purgative Amnesia aneamia palpitations and neurosis. Upper infections	insomnia, respiratory	Velmurugan and Kamaraj (2011) ³⁴ Devi <i>et al.</i> (2009) ³⁸ Kalaivani <i>et al.</i> (2012) ³¹
		<i>Euphoria longan</i>	Leaf			
		<i>Andrographis paniculata</i>	Leaf			
8.	n-Hexadecanoic acid	<i>Alstonia venerata</i>	Leaf	Rheumatic complaints		Sutha <i>et al.</i> (2012) ²⁷
		<i>Cadaba trifoliata</i>	Leaf	Purgative		Velmurugan and Kamaraj (2011) ³⁴
		<i>Cadaba trifoliata</i>	Root	Anticancer, anti diabetic, anti inflammatory		Velmurugan <i>et al.</i> (2010) ³⁵
		<i>Morinda citrifolia</i>	Lead & fruit	Antibacterial, antiinflammatory, analgesis antioxidant and antitumor effect		Rivera <i>et al.</i> (2012) ²⁸
		<i>Euphoria longan</i>	Leaf	Amnesia insomnia, aneamia palpitations and neurosis.		
		<i>Andrographis paniculata</i>	Leaf	Upper respiratory infections		Devi <i>et al.</i> (2009) ³⁸
		<i>Withania somnifera</i>	Root	Anti inflammatory		Kalaivani <i>et al.</i> (2012) ³¹ Kumar <i>et al.</i> (2011) ⁴¹
9.	Oleic Acid	<i>Cadaba trifoliata</i>	Root	Anticancer, anti diabetic, anti inflammatory		Velmurugan <i>et al.</i> (2010) ³⁵
		<i>Polygala chinensis</i>	Whole plant	Cough and bronchitis		Alagammal <i>et al.</i> (2011) ³⁶
		<i>Aloe vera</i>	plant			
10	1,2-Benzenedicarboxylic acid, diisooctyl ester	<i>Polygala chinensis</i>	Whole plant	Cough and bronchitis		Alagammal <i>et al.</i> (2011) ³⁶
		<i>Withania somnifera</i>	Root	Anti inflammatory		Kumar <i>et al.</i> (2011) ⁴¹

Figure 1. GC-MS chromatogram of the alcoholic extract of the tuber of *Antigonum leptobus*

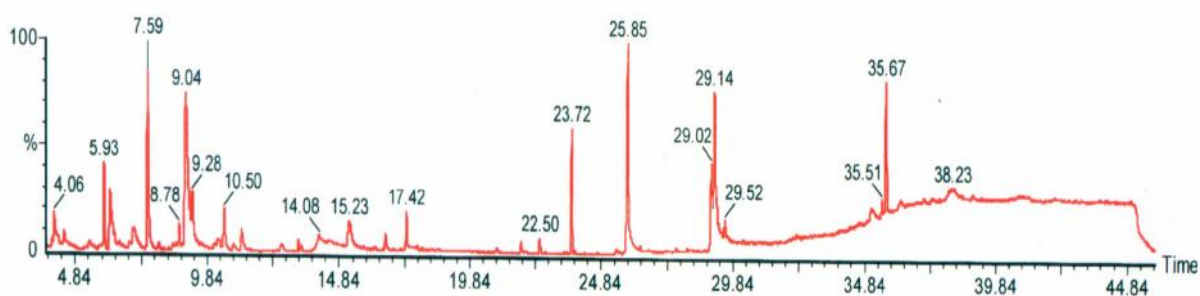
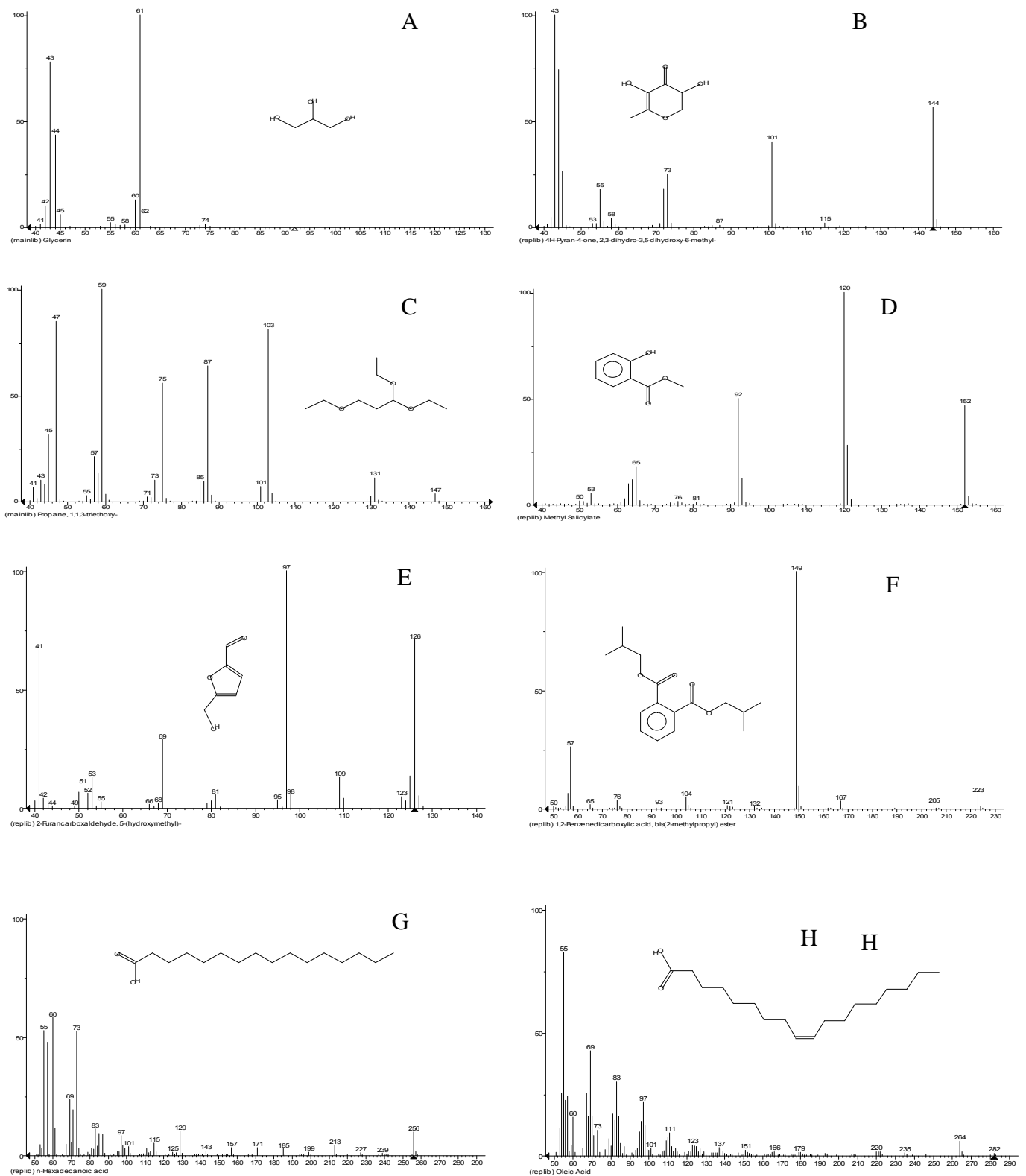


Figure 2. Chemical structure as well as spectrum of a compound viz., **A.** Glycerin 1, **B.** 4H-Pyran-4-one, 2, 3-dihydro-3,5-dihydroxy-6-methyl, **C.** Propane, 1,1, 3-triethoxy, **D.** Methyl Salicylate, **E.** 2Furan carboxaldehyde, 5- (hydroxymethyl), **F.** 1,2-Benzene dicarboxylic acid, bis(2-methylpropyl) ester, **G.** n-Hexadecanoic acid, **H.** Oleic Acid



Discussion

Glycerin, Methyl Salicylate, Oleic Acid, Dodecanoic acid, n-Hexadecanoic acid, 2-Furancarboxaldehyde and 5-(hydroxymethyl)- have more bioactivities while Propane, 1,1,3-triethoxy, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, 1,2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester, 1,2-Benzenedicarboxylic acid and diisooctyl ester had no activity which was confirmed from Dr. Duke's phytochemical and Ethanobotanical databases (Table 4).

The present study revealed that the above chemical compounds exhibits high bioactivity. The potential antinoceptive effect by methanolic extract of *A. leptopus* root could be due to different nociceptive stimuli¹³. Biochemicals and phytochemicals such as saponins, terpenoids, flavonoids, tannins, steroids, and alkaloids have anti-inflammatory effects¹⁴⁻²⁰. Some polycyclic glycosides, flavonoids, tannins, and alkaloids have hypoglycemic²¹. The anti-edematogenic mechanism of action of *A. leptopus* may also be due to prostaglandin synthesis inhibition as described for the anti-inflammatory mechanism of aspirin-like drugs²². Primary metabolites, for example, sugars, proteins, lipids, and starch are of prime importance and essentially required for growth of plants. The studies of primary metabolites have been carried out in some plants in the past such as *Balanites aegyptiaca*, *Cissus quadrangularis*, *Eclipta alba* and *Nerium indicum*²³. The identified compounds from *A. leptopus* were found to have unique bioactivity from earlier reports (Table 5).

The phytochemical screening of *A. leptopus* showed that the leaves and tuber were rich in alkaloids, saponin, steroid/triterpenoid, phenolic compounds, fatty acid, flavonoids and volatile oils. The present investigation on biochemical compounds of *A. leptopus* (Glycerin, Methyl Salicylate, Oleic Acid, Dodecanoic acid, n-Hexadecanoic acid, 2-Furancarboxaldehyde and 5-(hydroxymethyl)) show medicinal activity as well as physiological activity²⁴. The evaluated *A. leptopus* being used as traditional medicine must be taken forward to complete phytochemical analysis so as to study in depth the individual potential compounds which will pave way to clinical leads.

Acknowledgements

The authors are grateful to the Indian Institute of Crop processing technology (IICPT), Thanjavur, for providing the (GC-MS) laboratory facilities.

References

1. Polya G.M, Biochemical Targets of Plant Bioactive Compounds. A Pharmacological Reference Guide to Sites of Action and Biological Effects, CRC Press, Florida, 2003,520-524
2. Arunkumar S. and Muthuselvam M., Analysis of phytochemical constituents and antimicrobial activities of *Aloe vera* L. aganst clinical pathogens, world J. Agric. Sci., 2009, 5(5),572–576.
3. Slish D.F., Ueda H., Arvigo R. and Balick M.J., Ethnobotany in the search for vasoactive herbal medicines, J. Ethnopharmacol., 1999, 66,159–165.
4. Khafagi I.K. and Dewedar A., The efficiency of random versus ethno-directed research in the evaluation of Sinai medicinal plants for bioactive compounds, J. Ethnopharmacol., 2000, 71,365–376.
5. Arnon D.I., Copper enzymes in isolated chloroplast. Polyphenol oxidase in *Beta ulgaris*, Plant Physiol., 1949, 24,1-15.
6. Goodwin T.W., Carotenoids, Hand Book of Plant Analysis. Peach K. and Tracey M.V., eds, Springer Verlag, Berlin, 1954, 3,272-331.
7. Dubois M., Gills K.A., Hamilton J.K., Reberts P.A. and Smith F.C., Colorimetric method for the determination of sugars and related substances, Anal. Chem., 1956, 28,250- 256.
8. McCready R.M., Guggolz J., Silvierra V. and Owens H.S., Determination of starch and amylose in vegetables. Application to peas, Anal. Chem., 1950, 22, 1156-1158.
9. Lowry O.H., Rosenberg N.J., Farr A.L. and Randall R.J., Protein measurement with the folin – phenol reagent, J. Biol. Chem., 1951, 193, 265-275.
10. Troll W. and Canon R.K., A modified photometric ninhydrin method for the analysis of aminoacids, J. Biol. Chem., 1953, 200,803-811.
11. Swain T. and Hillis W.E., The phenolic constituents of *Prunus domestical* L. The quantitative analysis of phenolic compounds, J. Sci. Food Agro., 1959, 10,63–58.

12. Singleton V.L. and Rossi J.A., Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents, *Am. J. Enol. Vitic.*, 1965, 16,144-158.
13. Mamidipalli W.C., Nimmagadda V.R., Bobbala R.K. and Gottumukkala K.M., Preliminary studies on analgesic and anti-inflammatory properties of *Antigonum leptopus* Hook and Arn., roots in experimental models, *J. Health Sci.*, 2008, 54(3),281–286.
14. Liu R.H., Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals¹⁻⁴, *Am. J. Clin. Nutr.* 2003, 78,517–520.
15. Manach C., Regeat F., Texier O., Agullo G., Demigne C. and Remesy C., Bioavailability, metabolism and physiological impact of 4-oxo-flavonoids, *Nutr. Res.*, 1996, 16,517–544.
16. Latha M., Geetha T. and Varalakshmi P., Effect of *Vernonia cinerea* less flower extract in adjuvant-induced arthritis, *Gen. Pharmacol.*, 1998, 31,601–606.
17. Akindele A.J. and Adeyemi O.O., Anti-inflammatory activity of the aqueous leaf extract of *Byrsocarpus coccineus*, *Fitoterapia*, 2007, 78,25-28.
18. Orhan I., Kupeli E., Sener E. and Yesilada E., Appraisal of anti-inflammatory potential of the clubmoss, *Lycopodium clavatum* L. *J. Ethnopharmacol.*, 2006, 107,146–150.
19. Muruganandan S., Srinivasan K., Chandra S., Tandan S.K., Lal J. and Paviprakash V., Anti-inflammatory activity of *Syzygium cumin* bark, *Fitoterapia*, 2001, 72,369-375.
20. Nadkarni K.M., *Indian Materia Medica*, Vol. I. Popular Book Depot, Bombay, 1954,516–518.
21. Cherian S. and Augusti K.T., Insulin sparing action of leucopelargonidin derivative isolated from *Ficus bengalensis* Linn, *Indian J. Exp. Biol.*, 1995, 33,608-611.
22. Ferreria S.H., Moncada S. and Vane J.R., Further experimentation to establish that the analgesic action of aspirin-like drug depends of the inhibition of prostaglandin biosynthesis, *Br. J. Pharmacol.* 1973, 47,48-58.
23. Vijayvergia R. and Kumar J., Quantification of primary metabolites of *Nerium indicum* Mill. *Asian J. Exp. Sci.*, 2007, 21(1),123-128.
24. Sofowara A., *Medicinal plants and Traditional medicine in Africa*. Spectrum Books Ltd: Ibadan, Nigeria, 1993,289.
25. Sridharan S., Vaidyanathan M., Venkatesh K. and Nayagam A.A.J., GC-MS study and phytochemical profiling of *Mimosa pudica* Linn, *J. Pharm. Res.*, 2011, 4(3),741-742.
26. Singh C.R., Nelson R., Krishnan P.M. and Pargavi B., Identification of Volatile Constituents from *Premna serratifolia* L. through GC-MS. *Int. J. Pharm. Tech. Res.* 2011, 3(2),1050-1058.
27. Sutha S., Devi V.K. and Mohan V.R., GC-MS Determination of Bioactive Components of *Alstonia venenata* R.Br, *Res. J. Pharm. Biol. Chem. Sci.* 2012, 3(2), 291-296.
28. Rivera A., Cedillo L., Hernández F., Castillo V., Sánchez A. and Castaneda D., Bioactive constituents in ethanolic extract leaves and fruit juice of *Morinda citrifolia*, *Ann. Biol. Research*, 2012, 3 (2),1044-1049.
29. Kumar P.P., Kumaravel S. and Lalitha C., Screening of antioxidant activity, total phenolics and GC-MS study of *Vitex negundo*. *Afr. Biochem. Res.*, 2010, 4(7),191-195.
30. Vishnusithan K.S. and Kamaraj M., Phytochemical analysis of leaf extracts of *Wattakaka volubilis* linn. (stapf) by GC-MS method, *Int. J. Pharm. Sci. Res.*, 2012, 3(6),1867-1871.
31. Kalaivani C.S., Sathish S.S., Janakiraman N. and Johnson M., GC-MS studies on *Andrographis paniculata* (Burm.f.) Walll. Ex Nees – A medicinally important plant, *Int. J. Med. Arom. Plants*, 2012, 2,69- 74.
32. Sarumathy K., Rajan M.S.D., Vijay T. and Jayakanthi J., Evaluation of phytoconstituents, nephro-protective and antioxidant activities of *Clitoria ternatea*, *J. Appl. Pharm. Sci.*, 2011, 1(5),164-172.
33. Anbuselvi S., Rebecca L.J., Kumar M.S. and Senthilvelan T., GC-MS study of phytochemicals in black gram using two different organic manures, *J. Chem. Pharm. Res.*, 2012, 4(2),1246-1250.
34. Velmurugan P. and Kamaraj M., GC-MS Analysis of *Cadaba trifoliata* Roxb Leaf Extract, Traditional Valuable Plant, *Afr. J. Basic Appl. Sci.*, 2011, 3 (1),6-8.
35. Velmurugan P., Kamaraj M. and Prema D., Phytochemical constituents of *Cadaba Trifoliata* Roxb. root extract, *Int. J. Phyto.*, 2010, 2,379-384.
36. Alagammal M., Soris P.T., Mohan V.R., Chemical Investigations of *Polygala Chinensis* L. by GC-MS, *Sci Res Reporter*, 2011, 1(2),49-52.
37. Sarada K., Margret R.J. and Mohan V.R., GC – MS Determination of Bioactive Components of *Naringi crenulata* (Roxb) Nicolson, *Int. J. Chem. Tech. Research*, 2011, 3(3),1548-1555.
38. Devi P.M., Nagarajan, Christina A.J.M., Meera R. and Merlin N.J., GC–MS Analysis of *Euphoria Longan* Leaves, *Int. J. Pharma. Res. and Develop.* 2009, 1(8),1-4.

39. Bhuiyan M.N.I., Begum J., Nandi N.C. and Akter F., Constituents of the essential oil from leaves and buds of clove (*Syzigium caryophyllatum* (L.) Alston), African J. Plant Sci., 2010, 4(11),451-454.
40. Velanganni J., kadamban D. and Ramamoorthy D., GC-MS analysis of ethanol extract of root of *Mallotus philippensis* (Lam.) Muell. Arg. Var. philippensis, Int. j. pharm. Res. Develop., 2011, 3(7),63-67.
41. Kumar M.S., Kumar D.V., Kumar A.S., Aslam A. and Shajahan A., The Phytochemical Constituents of *Withania somnifera* and *Withania obtusifolia* by GCMS Analysis, Int. J. Pharm. Phytochem. Res. 2011, 3(3),31-34.
