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PRELIMINARY PHYTOCHEMICAL AND GC MS ANALYSIS OF DIFFERENT EXTRACTS OF *PSOPHOCARPUS TETRAGONOLOBUS* LEAVES.

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Running Title: Phytochemcial and GC MS study of ***PSOPHOCARPUS TETRAGONOLOBUS*** leaves

Abstract:

The present study deals with the phytochemical and GC MS analysis of on medicinal plant, Psophocarpus tetragonolobus, which is commonly used in Ayurveda and sidhha forms of medicinal practice. Ethanol, methanol, hexane and water extracts of leaves of P. tertagonolobus were studied for the presence of various phytochemicals present therein. The GC MS analysis of methanol and aqueous extracts of P. tetragonolobus leaves was performed. In the ethanol fraction flavonoids, saponins and cardiac glycosides were present. In the methanol extract tannins, anthraquinones and steroids were present. In hexane fraction, flavonoids, alkaloids and triterpenoids were present. The water extract contained alkaloids, saponins, amino acids and anthraquinones. The GC MS results indicated the presence of some important biomolecules such as Catechol, N-Benzyl-2-phenethylamine, Arbutin, Tetracyclo[6.3.2.0(2,5).0(1,8)]tridecan-9-ol, 4,4-dimethyl-, Tetracyclo[6.3.2.0(2,5).0(1,8)]tridecan-9-ol, 4,4-dimethyl-, Diepicedrene-1-oxide, 1-Heptatriacotanol, Phytol, Stigmasterol in the aqueous fraction of leaves. In the Methanol fraction N-Benzyl-2-phenethylamine, Phenol, 3-methyl-5-(1-methylethyl)-, methylcarbamate, Phenol, 2,4-bis(1,1-dimethylethyl)-, 10-Methyl-8-tetradecen-1-ol acetate, 3-O-Methyl-d-glucose, 17-Octadecynoic acid, methyl ester, Hexadecanoic acid, methyl ester, Oleic Acid, 1-Monolinoleoylglycerol trimethylsilyl ether, .beta.-Sitosterol, Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-, alpha.-Amyrin were present. These biomolecules indicate the medicinal activities which are being claimed in the ethnobotanical practice.

Key words: ***Psophocarpus tetragonolobus,*** *Phytochemical, GC MS, Catechol, Catechol, Arbutin, Phytol, Stigmatsterol*

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INTRODUCTION:

The need to understand the potential medicinal roles of plants is the need of the hour, particularly after the emergence of drug resistant microorganisms and wide ranging side effects of the present molecular medicines. The use of alternative therapies based on the ethnobotanical knowledge and background could be a great support to medical science. The search for new plant based drugs is gathering momentum and many reports in this regard is a great sign. Ayurveda and Sidhha forms of medicines have a great repository of such knowledge which is under focus in the present days. [1-7] The present study deals with the phytochemical and GC MS analysis of the leaves of one herbal plant, *Psophocarpus tetragonolobus.*

Psophocarpus tetragonolobus is a tropical leguminous plant. Most of the plant parts are edible and thus it is known as "poor man's food". The leaves are used as salads or cooked as vegetable. The flowers, roots and pods are eaten raw or cooked. The seeds are cooked and taken. This plant has high nutrition value containing Vitamin A, Vitamin C, Calcium, Iron, proteins and fats. Although used as a food plant, it has a number of medicinal roles which are being reported only recently. Some scholarly articles on the medicinal properties are available. Lee *et al*, 2011, have reported that the fruits of this plant have anti-inflammatory, antioxidant and antinociceptive roles. [8] Sasidharan *et al*, 2008, reported the antimicrobial activity of various parts of this plant like, root, stem, leaves and pod. [9] Apart from being a rich source of lectins, *P. tetragonolobus* also contains erucic acid (an antitumor medication) and polyunsaturated fatty acids that can be used to treat acne and eczema. [10] The leaves are boiled to make a decoction, and it is used as a lotion to cure smallpox. [11] Ethnobotanically, the aqueous extract of the leaves are given to pregnant ladies after 5th month in combination with other plant leaves to keep the fetus healthy. The present study deals with the phytochemical and GC MS analysis of the leaves of this plant.

MATERIALS AND METHODS:

Collection of Samples:

Fresh leaves of *Psophocarpus tetragonolobus* were collected from Karaikudi, Tamil Nadu, India. The Leaves were thoroughly washed to remove any dust and impurities and shade dried. The dried leaves were ground to fine powder and the powder was used for the analysis.

Preparation of Extracts:

500 grams of dried powder of *Psophocarpus tetragonolobus* was packed in separate round bottom flasks for sample extraction using ethanol, methanol, hexane and distilled water as solvents. The extraction was conducted with 750 ml of the solvent for a period of 72 hours. At the end of the extraction the solvents were concentrated under reduced pressure and the crude extracts were stored in refrigerator.

Phyto-chemical Analysis:

The extracts prepared were analyzed for the presence of alkaloids, saponins, tannins, steroids, flavonoids, anthraquinones, triple sugars, amino acids, proteins, glycosides and reducing sugars as per standard protocols [12-14].

Test for Alkaloids:

About 0.5 g of the prepared residue was dissolved in 2 N Hydrochloric acids. The mixture was filtered and the filtrate was divided into two equal portions. One portion was treated with a few drops of Mayer's reagent and the other was treated with equal amount of Dragendorff's reagent respectively. The appearance of creamish precipitate and orange precipitate respectively, indicated the presence of alkaloids.

Test for Saponins:

About 0.5 g of the leaf extract was vigorously shaken with water in a test tube and then heated to boil. Frothing was observed which was taken as evidence for the presence of the Saponins.

Test for Tannins:

About 0.5 g of leaf extract was added in 10 ml of water in a test tube and filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or blue-black coloration.

Test for Steroids:

2 ml of acetic anhydride was added to 2 ml of leaf extract along with 2 ml Conc. sulphuric acid. The color change from violet to blue or green is observed for the presence of steroids.

Test for Flavonoids:

2 ml of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution few drops of conc. Hydrochloric acid was added and the red color was observed for flavonoids and orange color for flavones.

Test for Anthraquinones:

About 0.5 g of extract was taken in a dry test tube and 5 ml of chloroform was added and shaken for 5 min. The extract was filtered and the filtrate shaken

with equal volume of 10% of ammonia solution. A pink violet or red color in the ammonical layer was observed for the presence of anthraquinones.

Test for Cardiac Glycosides:
0.2 g of extract was dissolved in 1 ml of glacial acetic acid containing 1 drop of ferric chloride solution. This was then under layered with 1ml of concentrated sulphuric acid. A brown ring obtained at the interface indicated the presence of cardiac glycosides.

Test for Amino acids:

To 2ml of sample was added to 2 ml of Ninhydrin reagent and kept in water bath for 20 minutes. Appearance of purple color indicated the presence of amino acids in the sample.

Test for Proteins:
To 2 ml of the extract solution, 1ml of 40% NaOH solution and 1 to 2 drops of 1% $CuSO_4$ solution was added. A violet color indicated the presence of peptide linkage of the molecule.

Test for Tri-Terpenoids
5 ml of each extract was added to 2 ml of chloroform and 3 ml of con. H_2SO_4 to form a monolayer of reddish brown coloration of the interface indicated positive result for the tri-terpenoids.

Test for Triple Sugar:
To 2 ml of extract 2 drops of Molisch's reagent was added and shaken well. 2ml of con. H_2SO_4 was added on the side of the test tube. A reddish violet ring appeared at the junction of two layers immediately, indicated the presence of triple sugars.

The GC MS process is mentioned hereunder: Instrument: Gas chromatography (Agilent:GC:(G3440A) 7890A. MS MS:7000 Triple Quad GCMS,) was equipped with Mass spectrometry detector .

GC-MS Principle: In gas chromatography Helium gas is used as stationary phase. The principle of separation in GLC is partition. For GLC the compound should be volatile. They have to be heated to higher temperature and converted in to vapors in injector portion and mixed with gaseous mobile phase then the components are separated according to their partition co-efficient. In GLC the Mass spectrometer is used as detector. Mass Spectrometer is used for the determination of molecular weight of the compound and also for their structure elucidation. The compounds are identified by GC-MS Library (NIST & WILEY).
Sample Preparation: 100 microlitre sample Dissolved in 1 ml of suitable solvents. The solution stirred vigorously using vortex stirrer for 10 seconds. The clear extract was determined using gas-chromatography for analysis.
GC-MS protocol: Column DB5 MS (30mm×0.25mm ID ×0.25 µm , composed of 5% phenyl 95% methyl poly siloxane), Electron impact mode at 70 eV; Helium (99.999%) was used as carrier gas at a Constant flow of 1ml/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 10min) fragments from 45 to 450 Da. Total GC running time is 32.02 min.

RESULTS:

The phytochemical analysis results of Ethanol, Methanol, Hexane and Water extracts of leaves of *Psophocarpus tetragonolobus* are tabulated in Table 1.

Table 1. A preliminary phytochemical constituent of Ethanol, Methanol, Hexane and Aqueous extract of *Psophocarpus tetragonolobus*

SL. NO	PHTOCHEMICALS	ETHANOL	METHANOL	HEXANE	WATER
1	FLAVONOIDS	+	-	+	-
2	ALKALOIDS	-	-	+	+
3	TRI TERPENOIDS	-	-	+	-
4	SAPONINS	+	-	-	+
5	TANINS	+	+	-	-
6	TRIPLE SUGAR	-	-	-	-
7	AMINO ACID	-	-	-	+
8	ANTHROQUINONES	-	+	-	+
9	STEROIDS	-	+	-	-
10	PROTEINS	-	-	-	-
11	CARDIAC GLYCOSIDES	+	-	-	-

(+) = Present; (-) = Absent

The GC MS report is shown in Figure 1. The results of GC MS are tabulated in Table 2 indicating the retention time, mol. formula, peak percentage value and reported medicinal roles.

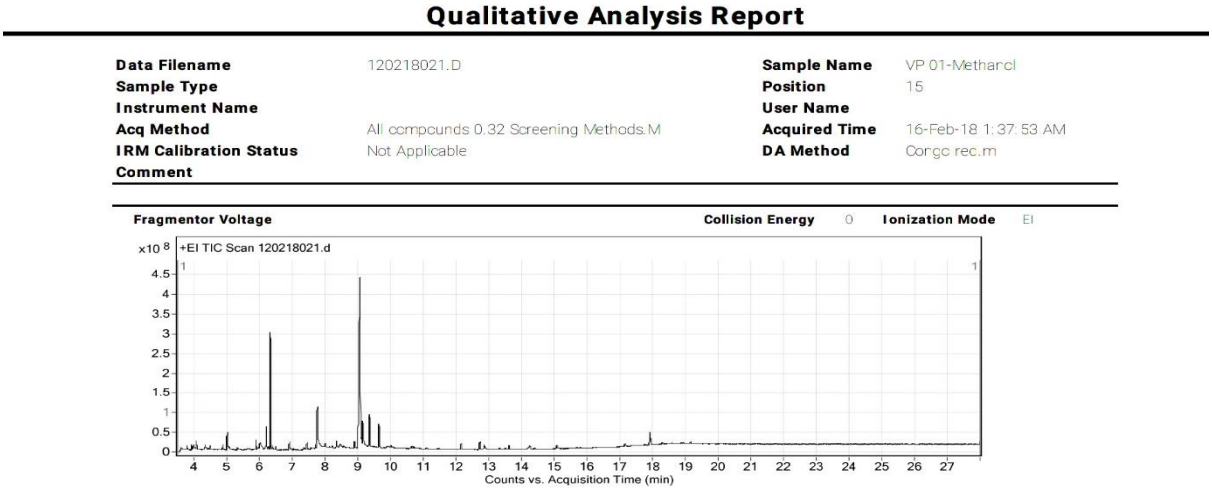

Fig. 1: GC MS graph of Psophocarpus tetragonolobus (Aqueous fraction VP 01, Charged with methanol)

Table 2: Indicating the retention time, mol. formula, peak percentage value and reported medicinal roles of various compounds present in the aqueous extracts of leaves of Psophocarpus tetragonolobus

Sl No	Retention Time	Compound	Mol. Formula	Mol Wt	% Peak Value	Medicinal Role
1	3.9952167	Catechol	C6H6O2	110	0.64	As estrogen backbone, stimulant
2	4.0856833	N-Benzyl-2-phenethylamine	C15H17N	211	0.67	Neuro modulator, Cognitive enhancer, mood enhancer, weight loss agent, works like serotonine
3	4.3664833	Arbutin	C12H16O7	272	0.90	beta-D-glucopyranoside of hydroquinone which inhibits tyrosinase activity in melanocytes of the skin and used a medicine to reduce over pigmentation.
4	5.0336167	Tetracyclo[6.3.2.0(2,5).0(1,8)]tridecan-9-ol, 4,4-dimethyl-	C15H24O	220	3.35	Oligosaccharide provider
5	5.9061333	Caryophyllene oxide	C15H24O	220	1.06	Nitric oxide synthatase inhibitor.
6	6.0361667	Tetraacetyl-d-xylonic nitrile	C14H17NO9	343	2.83	Smart drug, 17 beta hydroxysteroid dehydrogenase inhibitor, Alcohol dehydrogenase inhibitor, anticancer, antidote, CNS depressant, coronary dialator, decongestant.
7	6.2208333	1-Naphthalenol, 1,2,3,4,4a,7,8,8a-octahydro-1,6-dimethyl-4-(1-methylethyl)-, [1S-(1.alpha.,4.alpha.,4a.beta.,8a.beta.)]-	C15H26O	222	2.68	Not known
8	6.34145	2-Naphthalenemethanol, decahydro-.alpha.,.alpha.,4a-trimethyl-8-methylene-, [2R-(2.alpha.,4a.alpha.,8a.beta.)]-	C15H26O	222	13.98	Not known

9	6.9218833	5-tert-Butylpyrogallol	C10H14O3	182	1.25	Not known
10	7.4570667	2-Naphthalenemethanol, decahydro-.alpha.,.alpha.,4a-trimethyl-8-methylene-, [2R-(2.alpha.,4a.alpha.,8a.beta.)]-	C15H26O	222	1.14	Not known
11	7.7755167	Diepicedrene-1-oxide	C15H24O	220	7.87	Nitric oxide synthatase inhibitor.
12	8.3634667	3H-Naphtho[2,3-b]furan-2-one, 4-hydroxy-4a,5-dimethyl-3-methylene-3a,4,4a,5,6,7,9,9a-octahydro-	C15H22O2	234	1.27	Not known
13	8.9137	1-Heptatriacotanol	C37H360	536	1.08	Enzyme inhibitor, anti-hypercholesterolemic effects [15]
14	9.0795333	3H-Naphtho[2,3-b]furan-2-one, 4-hydroxy-4a,5-dimethyl-3-methylene-3a,4,4a,5,6,7,9,9a-octahydro-	C15H22O2	234	41.25	Not known
15	9.1172333	Ethyl 6,9,12,15-octadecatetraenoate	C20H32O2	304	2.64	Not known
16	9.1624667	1-Heptatriacotanol	C37H76O	536	3.16	Enzyme inhibitor, anti-hypercholesterolemic effects [15]
17	9.3754167	1,4-Hexadien-3-one, 5-methyl-1-[2,6,6-trimethyl-2,4-cyclohexadien-1-yl]-	C16H220	230	4.59	Catechol O methyl Transferase Inhibitor, methyl donor, methyl guanidine inhibitor
18	9.6599667	Phytol	C20H40O	296	3.56	Antimicrobial; Anti-inflammatory, Anticancer; Diuretic
19	12.16825	5-(7a-Isopropenyl-4,5-dimethyl-octahydroinden-4-yl)-3-methyl-pent-2-en-1-ol	C20H34O	290	0.84	5 alpha reductase inhibitor, ACE inhibitor, Acetyl cholin antagonist, ACTH genic
20	12.7430333	cis-1-Chloro-9-octadecene	C18H35Cl	286	1.1	Not known
21	12.8655333	1-Heptatriacotanol	C37H76O	536	0.92	Enzyme inhibitor, anti-hypercholesterolemic [15]
22	13.63065	Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate	C16H28O3	268	0.98	Increase zinc bio-availability, oligosaccharide provider, encephalopathic, endocrine tonic, enterorelaxant.
23	15.0893	2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol	C30H52O	428	0.57	Not Known
24	17.9351667	Stigmasterol	C29H48O	412	2.07	Precursor of progesterone act as intermediate in the biosynthesis of androgens, and estrogens Antiosteoarthritic, antihypercholestrolemic, cytotoxic, antitumor, hypoglycaemic, antimutagenic, antioxidant, anti-inflammatory, Analgesic [16]

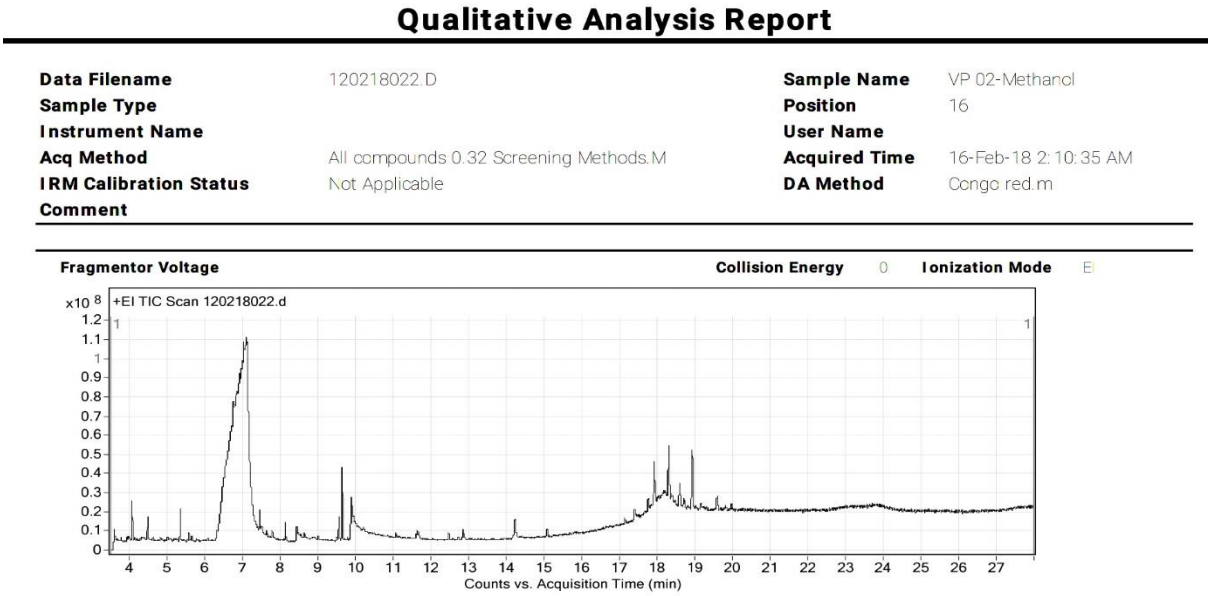

Fig. 2: Indicating the retention time, mol. formula, peak percentage value and reported medicinal roles of various compounds present in the aqueous extracts of leaves of *Psophocarpus tetragonolobus* (methanol fraction – VP 02)

Table3: Indicating the retention time, mol. formula, peak percentage value and reported medicinal roles of various compounds present in the methanol extracts of leaves of *Psophocarpus tetragonolobus.*

Sl No	Retention Time	Compound	Mol. Formula	Mol Wt	% Peak Value	Medicinal Role
1	4.0875667	N-Benzyl-2-phenethylamine	C15H17N	211	0.55	Anaphylactic, antitumor, decrease norepinephrine production, GABA-nergic, increase NK cell activity, Inhibit tumor necrosis factors, NADH oxidase inhibitor
2	4.49465	Phenol, 3-methyl-5-(1-methylethyl)-, methylcarbamate	C12H16NO 2	206	0.37	Oligosaccharide provider, Catechol O methyl Transferase Inhibitor, methyl donor, methyl guanidine inhibitor
3	5.3577833	Phenol, 2,4-bis(1,1-dimethylethyl)-	C14H22O	206	0.46	Oligosaccharide provider, Catechol O methyl Transferase Inhibitor, methyl donor, methyl guanidine inhibitor
4	5.591466	10-Methyl-8-tetradecen-1-ol acetate	C17H32O2	268	0.1	Oligosaccharide provider, Catechol O methyl Transferase Inhibitor, methyl donor, methyl guanidine inhibitor
5	7.1123833	3-O-Methyl-d-glucose	C14H22O	206	85.42	Catechol O methyl Transferase Inhibitor, methyl Donor, Glucose- 6-phosphate inhibitor, anticancer, anti tumor, inhibits Production of tumor necrosis factor
6	7.4705167	17-Octadecynoic acid, methyl ester	C19H34O2	294	0.34	Catechol O methyl Transferase Inhibitor, methyl Donor, Arachidonic acid inhibitor, Urine acidifier, Inhibit Uric acid production

7	8.1489333	Hexadecanoic acid, methyl ester	C17H34O2	270	0.34	Catechol O methyl Transferase Inhibitor, methyl Donor, Arachidonic acid inhibitor, Urine acidifier, Inhibit Uric acid production
8	8.45045	Oleic Acid	C18H34O2	282	0.61	Acidifier, Arachidonic acid inhibitor, inhibit production of uric acid, increase aromatic amino acid decarboxylase activity
9	9.5698167	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	C18H30O2	278	0.48	Not known
10	9.6565	Phytol	C20H40O	296	1.52	Antimicrobial; Anti-inflammatory, Anticancer, Diuretic
11	9.9033833	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	C18H30O2	278	3.1	Not known
12	12.8640167	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)-	C18H30O2	278	0.39	Not known
13	14.2397667	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)-	C18H30O2	278	0.71	Not known
14	17.9356333	1-Monolinoleoylglycerol trimethylsilyl ether	C27H54O4 Si2	498	1.23	Antimicrobial, Antioxidant, Antiinflammatory, Antiarthritic, Antiasthma, Diuretic
15	18.3239	.beta.-Sitosterol	C29H50O	414	1.31	A steroid precursor helps reducing LDL, help reduction of prostate hyperplasia, Used in Skin ointments
16	18.6103833	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13 ,15,15-hexadecamethyl-	C16H50O7 Si8	579	0.8	Antimicrobial
17	18.944	.alpha.-Amyrin	C30H50O	426	1.86	Antibacterial, Antioxidant, Potential antiplatelet components, Hypoglycemic, Hypolipidemic, Sedative Hepatoprotective, Alpha reductase inhibitor, Alpha glucosidase inhibitor, Testosteron 5 alpha Reductase inhibitor, Alpha amylase Inhibitor.
18	19.6037	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13 ,15,15-hexadecamethyl-	C16H50O7 Si8	579	0.41	Antimicrobial

DISCUSSION:

The phytochemical analysis of *P. tetragonolobus* leaves show that the ethanol fraction, flavonoids, saponins and cardiac glycosides were present. In the methanol extract tannins, anthraquinones and steroids were present. In hexane fraction, flavonoids, alkaloids and triterpenoids were present. The water extract contained alkaloids, saponins, amino acids and anthraquinones were present. GC MS analysis showed some very important biomoleecules such as Catechol, N-Benzyl-2-phenethylamine, Arbutin, Tetracyclo[6.3.2.0(2,5).0(1,8)]tridecan-9-ol, 4,4-dimethyl-, Caryophyllene oxide, Tetracyclo[6.3.2.0(2,5).0(1,8)]tridecan-9-ol, 4,4-dimethyl-, Diepicedrene-1-oxide, 1-Heptatriacotanol, 1,4-Hexadien-3-one, 5-methyl-1-[2,6,6-trimethyl-2,4-

cyclohexadien-1-yl]-, Phytol, 5-(7a-Isopropenyl-4,5-dimethyl-octahydroinden-4-yl)-3-methyl-pent-2-en-1-ol, Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate and Stigmasterol in the aqueous fraction of leaves. In the Methanol fraction, likewise, some important biomolecules such as N-Benzyl-2-phenethylamine, Phenol, 3-methyl-5-(1-methylethyl)-, methylcarbamate, Phenol, 2,4-bis(1,1-dimethylethyl)-, 10-Methyl-8-tetradecen-1-ol acetate, 3-O-Methyl-d-glucose, 17-Octadecynoic acid, methyl ester, Hexadecanoic acid, methyl ester, Oleic Acid, 1-Monolinoleoylglycerol trimethylsilyl ether, .alpha amyrin, beta.-Sitosterol, Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-, .alpha.-Amyrin etc. The relationship between the claimed medicinal roles and the presence of biomolecules, as indicated by GC MS, seems encouraging and futher work in this required is in process.

CONCLUSION:

It can be seen from the present study that it is high time the molecular efficacy warrants serious study to understand the molecular mechanisms of the medicinal aspects of plants and plant parts, which are already in practice, ethnobotanically.

COMPETING INTERESTS

This is to inform that no conflict of interest exist among the author.

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