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PRELIMINARY PHYTOCHEMICAL AND GC MS STUDY OF ONE MEDICINAL PLANT *CARISSASPINARUM*

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ABSTRACT

The knowledge of scientific validation of the efficacy of medicinal plants in the light of modern research parameters is of great importance in understanding the plant's role as medicine. To understand the presence of different phytochemicals present in a medicinal plant a number of techniques are available. The present study is one step in this direction which deals with the preliminary phytochemical and Gas Chromatography Mass Spectroscopy analysis of different leaf extracts of one medicinal *Carissa spinarum*. This plant has wide medicinal and edible properties. It was observed that flavonoids, tannins, saponins and steroids were present in Methanolic extract. Flavonoids, alkaloids, saponins and steroids were present in hexane fraction where as alkaloids, saponins, proteins and triterpenoids were present in aqueous extract. The GC MS analysis of hexane and aqueous leaf extracts indicated the presence of some important biomolecules such as Hexadecanal, 2-(1-Cyclohexenyl) cyclohexanone, Phytol, Squalene, Vitamin E, Octasilo-xane, 1, 1, 3, 3, 5, 5, 7, 7, 9, 9, 11, 11, 13, 13, 15, 15-hexadecamethylz, 1-Monolinoleoylglycerol trimethylsilyl ether, .beta.-Sitosterol, .alpha.-Amyrin, Lupeol, 1-Monolinoleoylglycerol trimethylsilyl ether and Lup-20(29)-en-3-ol, acetate, (3.beta.)-, Catechol, N-Benzyl-2-phenethylamine, Resorcinol, 2-Methyl-9-.beta.-d-ribofuranosylhypoxanthine, Paromomycin and 3-O-Methyl-d-glucose etc. correspond well with the reported medicinal roles of *C. spinarum*. Further work is in progress towards understanding this plant's role as a medicine.

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INTRODUCTION

The role of plants and plant parts as sources of medicine is immense. Most of the present day medicines have plant origin. The knowledge of the active principle and medicinal compounds in plants is continuously being updated through various biochemical methods. [1-6] There are a number of methods such as Thin layer Chromatography, HPLC, HPTLC, NMR, XRD, FTIR etc. which are used in the analysis of various biochemical and chemical parameters of plants. The present study deals with the phyto-chemical and GC MS analysis of the various leaf extracts of one such medicinal plant, *Carissa spinarum* L. Since not many reports are there this plant, the present work was undertaken.

Carissa spinarum is an evergreen shrub with spines, white tubular flowers and edible berries.

Ethnobotanically, this plant is used in Indian subcontinent for the treatment of worms in animals and humans, wounds, malaria, dysentery, sexual weakness and for snake bites [7]. There are various reports of its medicinal roles such as anticonvulsant, hepatoprotective, antiarthritic, antibacterial, antioxidant [8-12]. Leaves are used to treat anticancer, jaundice, hepatitis, antinociceptive and antidiabetic [13, 17]

The present study deals with the phytochemical screening and GC MS analysis of hexane and aqueous leaf extracts of *Carissa spinarum*.

MATERIALS AND METHODS

Collection of Samples

Fresh leaves of *Carissa spinarum* were collected from the hilly areas near Chengalpattu, 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.

Preparation of Extracts

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

Phytochemical 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 [18-20].

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 plant 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 plant leaf extract was added was 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 plant 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 2ml 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% CuSO₄ 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 2ml of chloroform and 3ml of con. H₂SO₄ to form a monolayer of reddish brown coloration of the interface was showed to form 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.H₂SO₄ was added on the sides 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 analysis was performed by standard procedures.

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 micro litre 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.

The results obtained were tabulated and the medicinal values of the important compounds present were collected from various sources such as Dr. Duke's Phytochemical and Ethnobotanical data base, NIST Chemical Library and available literature.

RESULTS

The phytochemical analysis results of Methanol, Hexane and Water extracts of leaves of *C. spinarum* are tabulated in Table 1.

Table 1. Preliminary phytochemical constituents of Methanol, Hexane and Aqueous extracts of *Carissa spinarum*.

Sl. No	Phytochemical	Methanol	Hexane	Aqueous
1	Flavonoids	+	+	-
2	Alkaloid	-	+	+
3	Tannin	+	-	-
4	Saponin	+	+	+
5	Triple Sugars	-	-	-
6	Amino Acid	-	-	-
7	Anthraquinons	-	-	-
8	Proteins	-	-	+
9	Glycosides	-	-	-
10	Triterpenoids	-	-	+

Legend: (+) = Present; (-) = Absent

The GC MS report of Hexane fraction is shown in Figure 1 and Table 2. The GC MS report of water extract is shown in Figure 2 and Table 3.

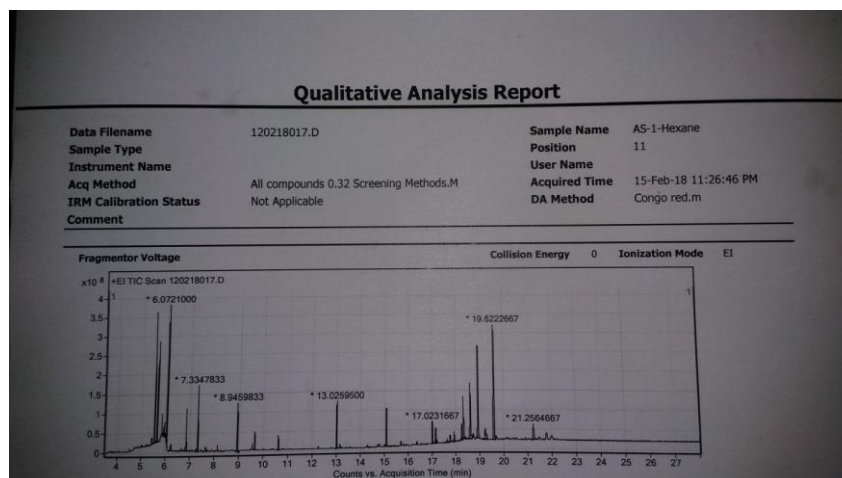


Figure 1. The GC MS results of Hexane fraction of *Carissa spinarum*.

Table 2. The GC MS results of Hexane fraction of *Carissa spinarum* indicating the Retention value, type of compounds, their molecular formulae, percent peak values and medicinal roles. The medicinal roles of the biomolecules are indicated after referring Dr. Duke's Phytochemical and Etnobotanical Data bases. [21].

Sl No.	RT	Compound	Mol. Formula	Percent Peak Value	Medicinal Properties
1.	5.5481167	Cyclohexanone, 2-cyclohexylidenez	C ₁₂ H ₁₈ O	13.95	Not Known
2	5.8666667	[1,1'-Bicyclohexyl]-2-one	C ₁₂ H ₂₂	1.24	Not Known
3	5.9571333	8-Hexadecenal, 14-methyl-, (Z)-	C ₁₇ H ₃₂ O	0.79	Not Known
4	6.0721	[1,1'-Bicyclohexyl]-2-one, 1'-hydroxyz	C ₂₁ H ₂₈ Cl ₂ N ₂ O ₂	15.57	Not Known
5	6.86555	2-(1-Cyclohexenyl)cyclohexanone	C ₁₂ H ₁₈ O	2.92	Not Known
6	7.3347833	Hexadecanal	C ₁₆ H ₃₂ O	4.49	Protein dimerization role, neuropeptide
7	8.9459833	2-(1-Cyclohexenyl)cyclohexanone		3.6	5-alpha reductase inhibitor, alpha agonist, alpha amylase inhibitor, Testosterone 5 alpha reductase inhibitor, antiamyloid beta, beta blocker, ER beta binder, beta adrenergic receptor blocker,
8	9.6583167	Hexadecanal		1.24	Protein dimerization role, neuropeptide
9	10.6231667	Oxirane, hexadecylz		1.03	Adhesive
10	13.02595	Phytol		4.22	Antimicrobial Antiinflammatory Antioxidant Diuretic
11	15.0933167	1,19-Eicosadiene		3.3	Not Known
12	17.0231667	Bis(2-ethylhexyl) phthalate	C ₂₄ H ₃₈ O ₄	2.13	Not Known
13	17.16075	Squalene		1.23	Monooxygenase inhibitor, biochemical precursor in the preparation of steroids, natural moisturizer, used in cosmetics Skin ointments, Steroid Precursor
14	17.647	Heptacosane	C ₂₇ H ₅₆	0.33	Not Known
15	17.77515	Vitamin E	C ₂₉ H ₅₀ O ₂	0.71	Antioxidant, Anticancer, antidote, antitumor, cytochrome P450 2E1 inhibitor, endocrine tonic
16	17.93535	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethylz		1.02	Antimicrobial
17	18.2633	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethylz		1.5	Antimicrobial

18	18.32925	1-Monolinoleoylglycerol trimethylsilyl ether		4.1	Antimicrobial Antiinflammatory Antiasthma, Diuretic	Antioxidant Antiarthritic
19	18.6176333	Heptacosane	C ₂₇ H ₅₆	6.21	Not Known	
20	18.9455833	.beta.-Sitosterol		10.66	17-beta hydroxysteroid dehydrogeanse inhibitor, beta 2 receptor agonist, beta adrenergic receptor blocker, Skin ointments, Steroid Precursor	
21	19.2452833	.alpha.-Amyrin		2	5 alpha reductase inhibitor, alpha amylase inhibitor, alpha glucosidase inhibitor, Antibacterial, Antioxidant, Potential antiplatelet components, Hypoglycemic, Hypolipidemic effects, Sedative action, and Hepatoprotective activities	
22	19.6222667	Lupeol	C ₃₀ H ₅₀ O	15.26	Anticancer, chemopreventive and anti-inflammatory properties, Antimalarial Antiflu Antiviral Antioxidant Anti-inflammatory Antiperoxidant Antitumor	antiprotozoal, and anti-inflammatory properties, Antioxidant Anti-inflammatory
23	21.2564667	1-Monolinoleoylglycerol trimethylsilyl ether	C ₂₇ H ₅₄ O ₄ Si ₂	2.51	Antimicrobial Antiinflammatory Antiasthma, Diuretic	Antioxidant Antiarthritic
24		Lup-20(29)-en-3-ol, acetate, (3.beta.)-	C ₃₂ H ₅₂ O ₂	15.26	Antitumor, Lupus generating, 17-beta hydroxysteroid dehydrogeanse inhibitor, beta 2 receptor agonist, beta adrenergic receptor blocker	

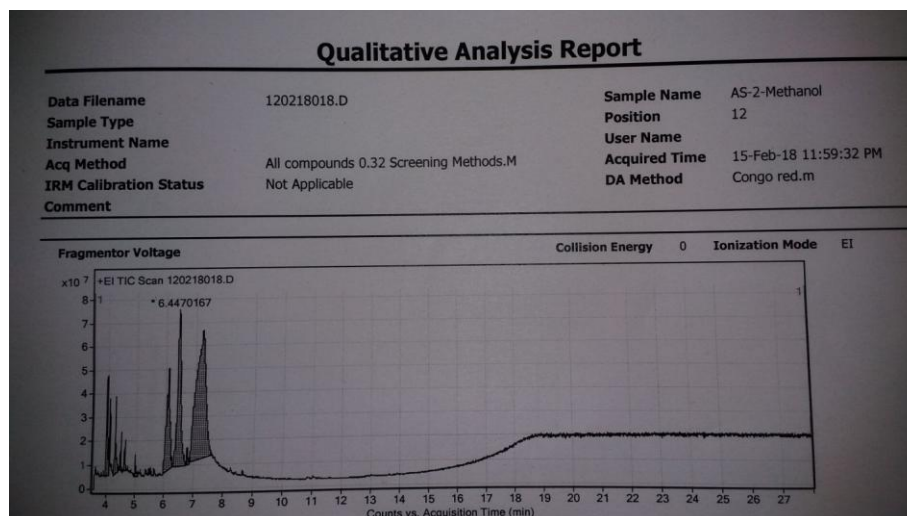


Figure 2. GC MS analysis results of *Carissa spinarum* water extract (Charged with Methanol).

Table 3. The GC MS results of water fraction of *Carissa spinarum* indicating the Retention value, type of compounds, their molecular formulae, percent peak values and medicinal roles.

Sl No	Retention Time	Compound	Mol Formula	Peak %	Medicinal Role
1	3.9876833	Catechol	C ₆ H ₆ O ₂	4.61	Catecholaminolytic, Catecholaminogenic, Catechol-O-Methyl-Transferase-Inhibitor, As estrogen backbone, stimulant,
2	4.0875667	N-Benzyl-2-phenethylamine	C ₁₅ H ₁₇ N	1.5	Anaphylactic, Arylamine-N-Acetyltransferase-Inhibitor, Decrease Norepinephrine Production, GABA-nergic, Increase natural killer cell activity, Inhibit Production of Tumor-Necrosis-Factor, Myo-neuro-stimulant, N-Cholinolytic, NADH-Oxidase-Inhibitor, NADH-Ubiquinone-Oxidoreductase-Inhibitor, Natural monoamine alkaloid, neuromodulator, neurotransmitter
3	4.2873333	Resorcinol	C ₆ H ₄ (OH) ₂	3.43	Antiseptic, disinfectant used in skin diseases like eczema
4	4.49465	2-Methyl-9-.beta.-d-ribofuranosylhypoxanthine	C ₁₁ H ₁₄ N ₄ O ₅	0.77	17-beta-hydroxysteroid dehydrogenase-Inhibitor, Anti-amyloid-Beta, Beta-2-Receptor-Agonist, Catechol-O-Methyl-Transferase-Inhibitor, neuroprotective, cardioprotective, anti-inflammatory and immunomodulatory activities.
5	4.6341167	Phenol, 2,6-dimethoxy-	C ₈ H ₁₀ O ₃	0.73	Food smoking agent used for flavouring food.
6	5.0110167	Paromomycin	C ₂₃ H ₄₇ N ₅ O ₁₈ S	0.85	Paromomycin is an antibiotic used to treat a number of infections including amebiasis, giardiasis, leishmaniasis, and tapeworm infection. It is a first line treatment for amebiasis or giardiasis during pregnancy.
7	6.10215	1,2,3,5-Cyclohexanetetrol, (1.alpha.,2.beta.,3.alpha.,5.beta.)-	C ₆ H ₁₂ O ₄	14.57	Reports not found
8	6.4470167	3-O-Methyl-d-glucose	C ₇ H ₁₄ O ₆	22.54	It is a non-metabolisable glucose analogue that is not phosphorylated by hexokinase and is used as a marker to assess glucose transport by evaluating its uptake within various cells and organ systems
9	7.24985	3-O-Methyl-d-glucose	C ₇ H ₁₄ O ₆	50.22	It is a non-metabolisable glucose analogue that is not phosphorylated by hexokinase and is used as a marker to assess glucose transport by evaluating its uptake within various cells and organ systems

DISCUSSION

The medicinal roles of *Carissa spinarum* such as anticonvulsant, hepatoprotective, antiarthritic, antimicrobial, antioxidant, antipyretic, anticancer, antinociceptive and antidiabetic have been reported by various reporters. The GC MS results as shown in Table 2 and 3 indicate the presence of some very important bio-molecules which match well with above mentioned medicinal roles.

It is interesting to note from the GC MS results that the presence of biomolecules such as Hexadecanal, 2-(1-Cyclohexenyl)cyclohexanone, Phytol, Squalene, Vitamin E, Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethylz, 1-Monolinoleoylglycerol trimethylsilyl ether, .beta.-Sitosterol, .alpha.-Amyrin, Lupeol, 1-Monolinoleoylglycerol trimethylsilyl ether and Lup-20(29)-en-3-ol, acetate, (3.beta.)-, Catechol, N-Benzyl-2-phenethylamine, Resorcinol, 2-Methyl-9-.beta.-d-ribofuranosylhypoxanthine, Paromomycin and 3-O-Methyl-d-glucose etc. correspond well with the reported medicinal roles of *C. spinarum*.

Some of the compounds are present in large quantities such as Hexadecanal (4.49%), 2-(1-Cyclohexenyl)cyclohexanone (3.6%), Phytol (4.22%), Squalene (1.23%), Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethylz (1.5%), 1-Monolinoleoylglycerol trimethylsilyl ether (4.1%), .beta.-Sitosterol (10.66%), .alpha.-Amyrin (2%), Lupeol, 1-Monolinoleoylglycerol trimethylsilyl ether (15.26%), Lup-20(29)-en-3-ol, acetate, (3.beta.)- (15.26%), Catechol (4.6%), N-Benzyl-2-phenethylamine (1.5%), Resorcinol (3.43%), and 3-O-Methyl-d-glucose (50.42%) which indicate important biological roles.

The biological roles of some compounds present in large quantities such as Cyclohexanone, 2-cyclohexylidenez (13.95%), [1,1'-Bicyclohexyl]-2-one, 1'-hydroxyz (15.57%), Heptacosane (6.21%), 1,2,3,5-Cyclohexanetetrol, (1.alpha.,2.beta.,3.alpha.,5.beta.)- (14.57%) are not reported yet and it will be of interest to document them to have a better idea of the medicinal role of *C. spinarum*. There are some molecules which are present in small quantities but they have important medicinal roles such as Vitamin E.

CONCLUSIONS

From the above discussion it is clear that the bio-molecules present as seen in the GC MS pattern of the hexane and water fractions and their biological roles of *Carissa spinarum* indicate that ethnobotanical claims of the medicinal value of this plant is reflected well. Further work to verify the activities are in progress.

Abbreviations:

GC MS : Gas Chromatography Mass Spectroscopy
HPLC : High Power Liquid Chromatography
HPTLC : High Power Thin Layer Chromatography
NMR : Nuclear magnetic Resonance
XRD : X-Ray Diffraction
FTIR : Fourier Transform Infra Red Spectroscopy

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Conflict of Interest Statement:

The authors affirm that no conflict of interest exists among them.

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