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IDENTIFICATION OF BIOACTIVE COMPOUNDS IN *LOESENERIELLA ARNOTTIANA* WIGHT ROOT BY GC-MS ANALYSIS

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ABSTRACT

Loeseneriella arnottiana is commonly used in the folklore medicine for treating diabetes mellitus. Preliminary phytochemical analysis of root extract revealed the presence of tannins, flavonoids, saponins, glycosides, steroids, terpenoids, and alkaloids. N- hexane extract of *L. arnottiana* root was subjected to Gas Chromatography coupled with Mass Spectroscopy (GC-MS) for the identification of components thereon. GC-MS data indicates the presence of forty low polar components. Out of the 13 identified constituents, beta-amyrin (14.92%), squalene (9.97%), alpha-amryin (9.14%), benzenamine (1.51%), nonadecane (1.28%), 1-methyl-4-(1-methylethyl)-3-[1-methyl-1-(4-methylpentyl)-5-methylheptyl]cyclohexene (1.05%), octadecane (1.04%) were the major constituents. The remaining six constituents 11-Octadecenoic acid, methyl ester (0.91%), hexanedioic acid (0.89%), eicosane (0.55%), hexadecanoic acid, methyl ester (0.54%), 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione (0.38%), methyl stearate (0.37%) were found in trace amounts. Some of these constituents are known to possessanti-inflammatory, antidiabetic, antioxidant, anticancer and antimicrobial properties and hence *L. arnottiana* can be used as an active therapeutic ingredient.

KEYWORDS: *Loeseneriella arnottiana*, GC-MS analysis, n-hexane extract, amyrins, antioxidant, antidiabetic.

INTRODUCTION

Plants are useful in healing as well as for curing of human diseases because of the presence of phytochemical constituents that produce a definite physiological action on the human body. Phytochemicals are naturally occurring in the plants that have defence mechanism and protect from various diseases. Plants are a rich source of secondary metabolites with interesting biological activities. In general, these secondary metabolites are an important source with variety of structural arrangements and properties.

Loeseneriella arnottiana (*Hippocratea arnottiana)* belongs to the family Celastraceae (Hippocrateaceae). The family comprises about 88 genera with 1,300 species of vines, shrubs and small trees. Most of the representatives of this family are shrubs and some are climbers by their branchlets, twisting round their supports. *L. arnottiana* is a climbing shrub distributed in West Coast and the Western Ghats from South Canara to Malabar and Travancore and is used by folklore practitioners as they possess various pharmacological properties.

The literature shows that the ethanolic root extract of a related species *Hippocratea africana* has antidiabetic and hypolipidaemic activities[1], analgesic, anti-inflammatory, antipyretic, antidiarrhoel and antinuclear activities.[2,3] The roots and stems of *Salacia reticulata* and the roots of *S. oblonga,* have been extensively used for the initial stages of diabetes in the Ayurvedic system of traditional medicine.[4,5] Two Sri Lankan plants (*Salacia reticulata* and *Salacia prinoides*) are being patented as having pharmaceutical potential in producing anti-diabetic drugs.[6] Therefore, with the hypothesis that the plant *Loeseneriella arnottiana* possesses pharmacological properties as evident from folklore medicine and literature on related species of the plant, a GC-MS study was carried out on *L. arnottiana* root extract to identify the bioactive compounds contributing to its pharmacological activity.

In recent years Gas chromatography – Mass Spectrum (GC-MS) studies have been increasingly applied for the analysis of medicinal plants as this technique has proved to be a valuable method for the analysis of essential oil, alcohols, acids, esters, alkaloids, steroids, amino and nitro compounds with great success.

MATERIALS AND METHODS

Plant material: The roots of *Loeseneriella arnottiana* plant were collected from Bantamale Reserve Forest, Sullia taluk, Karnataka, India and authenticated.

Preparation of extract

The roots were washed under running tap water, dried in oven at 40°C and coarsely powdered in a grinder. The sample was extracted with methanol in soxhlet extractor. The extract obtained was dried in water bath at 60°C.

Preliminary phytochemical screening

Freshly prepared extracts of the roots of *L. arnottiana* were subjected to preliminary phytochemical screening as per method of Harbone.[7]

GC-MS analysis

Sample preparation

The methanolic root extract was then extracted successively with the help of separating funnel using solvents such as n-hexane, ethyl acetate, butanol and water in the increasing order of polarity. The n-hexane extract obtained was used for GC-MS analysis.

GC-MS method

GC-MS analysis was carried out using Perkin Elmer Turbo Mass Spectrophotometer (GC-MS-5975C, AGILENT, USA) equipped with an auto sampler XLGC. The column used was Perkin Elmer Elite - 5 capillary column (dimethyl polysiloxane, 30m × 0.25mm) with a film thickness of 0.25mm. The carrier gas used was Helium at a flow rate of 1.5ml/min. 1µl sample injection volume was utilized. The injector temperature was maintained as 250°C. The oven temperature was programmed initially at 70°C for 3 minutes and then programmed to increase to 300°C at a rate of 10°C. Total run time was 35 minutes. The MS transfer line was maintained at a temperature of 240°C. MS was recorded using electron spray ionization at 70eV and data was evaluated using total ion count (TIC) for compound identification and quantification. The spectra of the components were compared with spectral database of known components in the GC-MS library (NIST-11). Measurement of peak areas and data processing were carried out by Turbo-Mass-OCPTVS-Demo SPL software.[8]

RESULTS AND DISCUSSION

The preliminary phytochemical analysis of *Loeseneriella arnottiana* root extract revealed the presence of phytoconstituents such as tannins, flavonoids, saponins, glycosides, terpenoids, and alkaloids (Table1).

Table 1: Qualitative phytochemical analysis of *Loeseneriella arnottiana* root

Terpenoids	+
Alkaloids	+
Saponins	+
Flavonoids	+
Glycosides	+
Tannins	+
Resins	-

+ present, - absent.

GC-MS analysis revealed the presence of 40 low polar constituents (Fig.1).The components present in the n- hexane extract *L. arnottiana* by GC-MS is listed in table 2. Out of forty constituents 27 could not be identified as the mass fragmentation showed similarity below 80%. A major compound eluted at RT 30.085 min was beta-amyrin and it accounted for 14.92%. The identified compounds possess many biological properties. Out of the 13 identified constituents, beta-amyrin (14.92%), squalene (9.97%), alpha-amryin (9.14%), benzenamine (1.51%), nonadecane (1.28%), 1-methyl-4-(1-methylethyl)-3-[1-methyl-1-(4-methylpentyl)-5-methylheptyl]cyclohexene(1.05%), octadecane (1.04%) were the major constituents. The remaining six constituents 11-Octadecenoic acid, methyl ester (0.91%), hexanedioic acid (0.89%), eicosane (0.55%), hexadecanoic acid, methyl ester (0.54%), 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione (0.38%), methyl stearate (0.37%) were found in trace amounts. Table 3 shows list of the major phytocompounds and its biological activities obtained through the GC-MS.

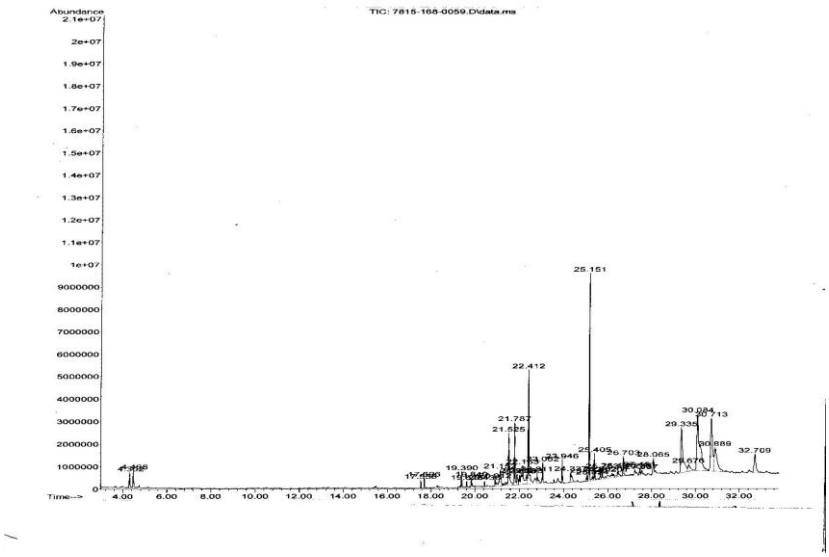

Fig. 1: Total Ion Chromatogram (TIC) of n-hexane extract of *Loeseneriella arnottiana* root

Table 2: Compounds identified from n-hexane extract of *L. arnottiana* root by GC-MS analysis

Number	RT	Compound	Match	% Area
1	4.301	---	-	-
2	4.471	---	-	-
3	17.538	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	91	0.38
4	17.964	Hexadecanoic acid, methyl ester	98	0.54
5	19.388	11-Octadecenoic acid, methyl ester	99	0.91
6	19.626	Methyl stearate	96	0.37
7	19.841	---	-	-
8	20.436	---	-	-
9	20.911	---	-	-
10	21.149	---	-	-
11	21.528	---	-	-
12	21.788	---	-	-
13	21.906	Hexanedioic acid	93	0.89
14	22.018	---	-	-
15	22.166	---	-	-
16	22.330	---	-	-
17	22.412	---	-	-
18	22.813	1-methyl-4-(1-methylethyl)-3-[1-methyl-1-(4-methylpentyl)-5-methylheptyl]cyclohexene	90	1.05
19	23.065	---	-	-
20	23.949	---	-	-
21	24.366	---	-	-
22	25.056	Eicosane	89	0.55
23	25.153	Squalene	99	9.97
24	25.324	---	-	-
25	25.405	---	-	-
26	25.665	---	-	-
27	25.754	---	-	-
28	26.200	Benzenamine	94	1.51
29	26.453	Octadecane	92	1.04
30	26.705	---	-	-
31	27.233	Nonadecane	90	1.28
32	27.448	---	-	-
33	27.537	---	-	-
34	28.065	---	-	-
35	29.335	---	-	-
36	29.667	---	-	-
37	30.085	Beta-Amyrin	98	14.92
38	30.709	Alpha-Amyrin	98	9.14
39	30.888	---	-	-
40	32.708	---	-	-

RT- Retention time, --- Unidentified compounds.

Table 3: Compounds identified from n-hexane root extract of *L arnottiana* by GC-MS analysis, their nature and biological activities

Name of the compound	Nature of compound	Activity
Beta- Amyrin	Triterpene	Anti-inflammatory,antimicrobial Antihyperglycemic
Alpha – Amyrin	Triterpene	Anti-inflammatory, antimicrobial Antihyperglycemic
Squalene	Triterpene, Vitamin E	Prevents bad cholesterol, antioxidant, immunostimulant, antibacterial, anticancer activity
Benzenamine	Aromatic amines	Antimicrobial
Nonadecane	Alkane hydrocarbon	Antioxidant
1-methyl-4-(1-methylethyl)-3-[1-methyl-1-(4-methylpentyl)-5-methylheptyl]cyclohexene	Alkene	Unknown
Octadecane	Alkane hydrocarbon	Anticancer, antioxidant and antimicrobial activities
11- octadecanoic acid, methyl ester	Stearic acid	Antiviral, antibacterial and antioxidant activities
Hexanedioic acid, methyl ester	Palmitic acid methyl ester	Antioxidant, hypocholesterolemic, anti-inflammatory effects
Eicosane	Alkane	Antitumor activity
Hexadecanoic acid, methyl ester	Palmitic acid	Antioxidant,anti-inflammatory hypocholesterolemic Antidiabetic activity
7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	Ketone	Antioxidant
Methyl stearate	Fatty acid methyl esters	Antidiarrheal, cytotoxic and antiproliferative activities

The active constituents of medicinal plant or the metabolic products of plant cells are closely linked to human growth and general health. The amyrins are a pair of closely related natural chemical compounds of the triterpene class designated as α-amyrin and β-amyrin with the chemical formula $C_{30}H_{50}O$. They are commonly found in medicinal plants and known to have a number of biological effects.[9] α, β-amyrins have been shown to exhibit various pharmacological activities *in vitro* and *in vivo* conditions against various health-related conditions such as inflammation, microbial, fungal, and viral infections and cancer cells. α-amyrin in hexane extracts of *Bursera simaruba* (L.) Sarg. leaves showed anti-inflammatory effect on adjuvant-carrageenan-induced inflammation in rats.[10] β-amyrins are responsible for the anti-inflammatory potency of *Ligustrum* spp. which is used by Chinese physicians to prevent and cure hepatitis and chronic bronchitis.[11]

Extract from *Calotropis gigantea* was responsible for the inhibition of the lipoxygenase pathway in the arachidonate metabolism. It has a similar mechanism of action as dexamethasone as well as antioxidant and anti-lipoxygenase effects due to the presence of α-amyrin and β-amyrin.[12] α and β- amyrin has the potential to combat acute pancreatitis by acting as an anti-inflammatory and antioxidant agent.[13] An anti-ulcer effect of *Cytocarpa procera* and *Amphipterygium adstringens* was assayed on experimental gastric injury in rats and phytochemical analysis allowed the identification of β-amyrin and β-sitosterol in *A. adstringens* [14] Amyrins are also involved in the biosynthetic pathways of other biologically active compounds such as avenacine, centellosides, glycyrrhizin or ginsenosides.

Beta-amyrin is reported to have antihyperglycemic activity. Pentacyclic triterpenes exert beneficial effects in metabolic disorders. Mice treated with α, β-amyrin (10, 30 and 100mg/ kg, p.o.) had significantly reduced STZ-induced increase in blood glucose, total cholesterol and serum triglycerides. The plasma insulin level and histopathological analysis of pancreas revealed the beneficial effect of α, β-amyrin in the preservation of beta cell integrity.[15]

Literature reports suggest that many plant-derived triterpenoids enhance glucose uptake by acting as insulin mimics and as insulin sensitizers[16], some exhibit alpha-glucosidase inhibition.[17] The mixture of bauerenol, a-amyrin and ß-amyrin from *Carmonaretusa* exhibited 51% analgesic activity and showed 20% anti-inflammatory activity at dosage of 100 mg/kg mouse, while of 250 mg/kg mouse showed a 29% anti-diarrheal activity.[18]

Squalene is a hydrocarbon and a triterpene, is a natural and vital part of the synthesis of all plant and animal sterols, including cholesterol, steroid hormones, and vitamin D in the human body. In humans, about 60 per cent of dietary squalene is absorbed. It is distributed ubiquitously in human tissues, with the greatest concentration in the skin, where it is one of the major components of skin surface lipids. Squalene is not very susceptible to peroxidation and appears to function in the skin as a quencher of singlet oxygen, protecting human skin surface from lipid peroxidation due to exposure to UV and other sources of ionizing radiation.[19]

The presence of squalene in the cells affects the HMG-CoA reductase synthesis rate, which in turn affects the whole synthesis of isoprenoids and cholesterol. This offers possibilities for the prevention and treatment of high levels of “bad cholesterol,” a risk factor for cardiovascular disease.[20] Squalene has properties of an antioxidant and immunostimulant,

acts as antibacterial, antitumor and cancer preventive agent.[21] The administration of squalene at 2% in the diet of rodents 14 days before and 30 days after a lethal total body irradiation with γ-rays was followed by a cellular and systemic radioprotection.[22]

Octadecane is an alkane hydrocarbon which possess various activities such as anticancer, antioxidant and antimicrobial activities[23, 24, 25] , while nonadecane, an alkane hydrocarbon is an antioxidant[26] 7, 9-Di-tert-butyl-1-oxaspiro (4, 5) deca-6, 9-diene-2, 8-dione acts as antioxidant.[27,28] Hexadecanoic acid is an antioxidant and has hypocholesterolemic and anti-inflammatory effects.[29] Eicosane, an alkane shows antitumor activity against the human gastric SGC-7901 cell line[30] Octadecanoic acid esters are reported to be having antiviral, antibacterial and antioxidant activities.[31,32] Methyl stearate is reported to have antidiarrheal and cytotoxic and antiproliferative activities[33,34] whereas benzenamine possess antimicrobial activities.[35]

CONCLUSION

The result of GC-MS analysis specifies that the n-hexane extract of *Loeseneriella arnottiana* root contains various bioactive compounds which have various medicinal properties like antioxidant, antidiabetic, anti-inflammatory, antimicrobial and anticancer properties that can be useful for the treatment of various diseases.

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