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Gas chromatography - Mass spectroscopy analysis of Bioactive compounds from Methanolic leaf Extract of *Macrosolen parasiticus* (L.) Danser

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Abstract

Macrosolen parasiticus (L.) Danser is a Hemiparasitic mistletoe plant, belongs to Loranthaceae family and is also known as Parasite Honeysuckle. The present investigation was carried out to determine the possible bioactive phytoconstituents from leaves of *M. parasiticus* using GC-MS analysis. The GC-MS analysis of the methanolic leaf extract revealed that, the presence of sixteen phytoconstituents, with valuable biological activities. The identified phytoconstituents were 4H-1-Benzopyron-4-one,2,3-dihydroxy-2-phenyl-,(S)- (45.03%) was found as major compound followed by 1,6-Anhydro-beta-D-glucopyranose (15.42%), Hexadecanoic acid, 2-hydroxy-1- (hydroxy methyl)ethyl ester (11.60); 9-Hexadecenoic acid (6.21%); Oleic acid (3.82%); (5beta)Pregnane-3,20beta-diol,14alpha,18alpha-[4-methyl-3-oxo-(1-oxa-4-azabutane-1,4-diyl)]-,diacetate (2.78%); 11-Octadecenoic acid, methyl ester (2.48%); 9,12-Octadecadienoic acid [ZZ] (2.10%); Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester(2.04%); 1,2,3,4,5-Cyclopentanepentol (1.99%); n-Hexadecanoic acid (1.82%); d-Mannose (1.20%); E-3-Pentadecen-2-ol (1.15%); Adenosine,4'-de(hydroxymethyl)-4'-[N-ethylaminoformyl]- (0.95%); 4alpha-Phorbol 12,13-didecanoate (0.84%) and Agaricic acid (0.49%). The results confirm the presence of bioactive phytoconstituents which are known to exhibit medicinal significance as well as pharmacological activities.

Medicinal plants are the more important therapeutic aid, for the alleviating the ailments of humankind.[16] Nowadays, the plant derived drug is called “Green Medicine”, these are safe and more defendable than the costly synthetic drugs. Nature has been very

rich botanical wealth and a huge number of plants grow in different parts of our country.[8] In India, usage of several medicinal plants used to cure specific diseases from the ancient times. In rural areas of the developing countries, they continue to be used as the principal source of medicine. About 80% of the people in developing countries use medicines for the health care.[28] World Health Organization (WHO) noted that a significant part of the world's population depends on traditional medicine for primary care.[17]

The medicinal benefit of plants lies in naturally occurring phytochemical constituents, that produce a positive physiological action on the human body.[19] Secondary metabolites are produced within plants, which are of great pharmacological importance having variation in molecular structure and their property.[5] GC-MS is the best technique to identify the bioactive phytoconstituents of long chain hydrocarbons, alcohols, steroids, acids, alkaloids, esters, amino and nitro compounds etc.[14] It can be an interesting tool for testing the amount of some active principles in herbs used in drugs, cosmetics, food or pharmaceutical industry, environmental and forensic applications.[26] It combines two analytical techniques to a single method of analyzing blend of chemical compounds. Gas chromatography splits the components of the mixture and mass spectroscopy analyzes each of the components distinctly.

Macrosolen parasiticus (L.) Danser a Hemiparasitic Mistletoe plant, belongs to Loranthaceae family, used in traditional veterinary medicine and as a leaf paste to eradicate ticks.[20] It grows extensively in the Western Ghats of India, on peepal, mango, neem and jackfruit trees.[7]

However, phytochemical investigation of *Macrosolen parasiticus* revealed the presence of alkaloids, flavonoids, saponins, tannins, phenolic compounds, sterols, terpenoids, glycosides, cardiac glycosides, carbohydrates, fixed oils and fats. It has has been previously reported to have antioxidant activity[18] and anticancer activity.[23,24] Keeping this in view, the present study has been undertaken to investigate GC-MS analysis to identify the phytoconstituents present in methanolic leaf extracts of *Macrosolen parasiticus*.

Collection of plant materials :

The fresh leaves of *Macrosolen parasiticus* (L.) Danser growing on *Mangifera indica* was collected from Western ghats region of Karnataka, India. The plant was identified with the help of Flora of Presidency of Madras (Gamble, 1935) and a voucher specimen is deposited in the herbarium, Department of Applied Botany, Kuvempu University, Shankaraghatta, Shivamogga district of Karnataka, India, with voucher specimen number (KU/AB/RN/KPS-01).

Preparation of the extracts :

The fresh leaf samples were washed under running tap water, shade dried about 20-25 days, and mechanically powdered. The powdered material (leaves) was subjected to Soxhlet extraction by using methanol. Obtained crude extract collected is concentrated using a rotary flash evaporator under reduced pressure and controlled temperature. Stored at 4° C in air tight vials and used for further studies.

Gas chromatography and mass spectroscopic analysis :

GC-MS analysis of the methanolic leaves extracts of *Macrosolen parasiticus* was performed using the equipment Thermo GC-Trace Ultra Version: 5.0, Thermo MS DSQ II. The equipment has a DB 35 – MS Capillary Standard non-polar column with dimensions of 30 mm × 0.25 mm ID × 0.25 µm film. The carrier gas used is Helium with at low of 1.0 ml/min. The injector was operated at 250°C and the temperature of the oven was programmed as follows: 60°C for 15 min, then gradually increased to 280 °C at 3 min.

Identification of components :

GC-MS detection of phytoconstituents from methanolic leaf extracts of *Macrosolen parasiticus* was based on the computer evaluation of mass spectra of samples through NIST (National Institute Standard And Technology) having more than 62000 patterns, the spectrum of unknown constituent was compared with spectrum of the known component store in the NIST library. The name, structure and molecular weight of the components of the test material were ascertained.

RESULTS

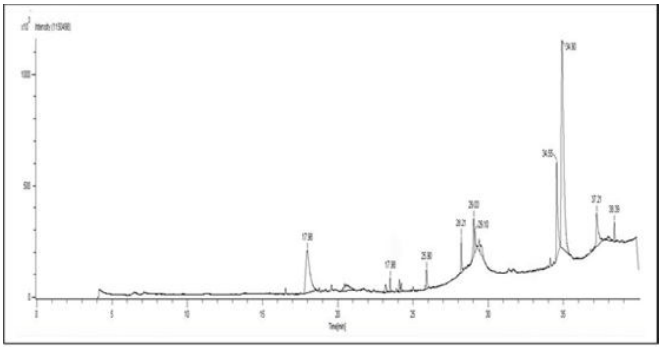

Figure 1: GC-MS chromatogram of methanolic leaf extract of *Macrosolen parasiticus* (L.) Danser.

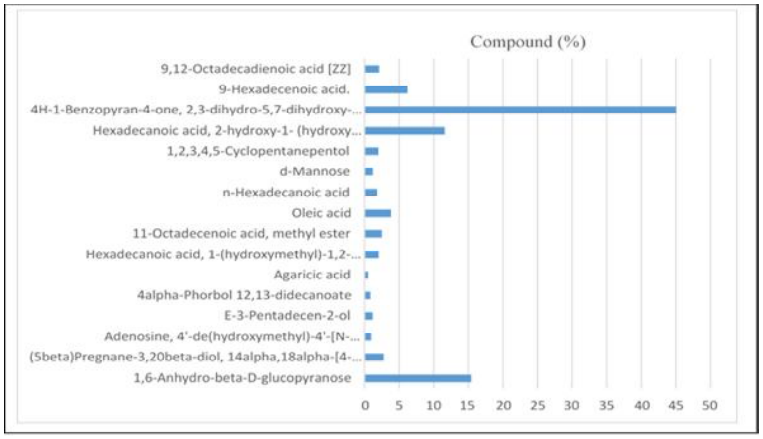

Figure 2: Gas chromatography and mass spectroscopy of crude *Macrosolen parasiticus* (L.) Danser. Methanolic leaf extract showing percentage of different compounds

Table-1. List of identified phyto-compounds in crude leaf methanolic extract of *Macrosolen parasiticus* (L.) Danser by GC-MS analysis

Sl no	Retention time (RT)	Average Percentage	Chemical compound present	Molecular formula	Molecular weight
1	17.98	15.42	1,6-Anhydro-beta-D-glucopyranose	$C_6H_{10}O_5$	162
2	20.45	2.78	(5beta) Pregnane-3,20beta-diol, 14alpha, 18alpha-[4-methyl-3-oxo-(1-oxa-4-azabutane-1,4-diyl)]-, diacetate	$C_{28}H_{43}NO_6$	489
3	23.18	0.95	Adenosine, 4'-de(hydroxymethyl)-4'-[N-ethylaminoformyl]-	$C_{20}H_{22}N_6O_6$	442
4	23.48	1.15	E-3-Pentadecen-2-ol	$C_{15}H_{30}O$	226
5	24.09	0.84	4alpha-Phorbol 12,13-didecanoate	$C_{40}H_{64}O_8$	672
6	24.22	0.49	Agaricic acid	$C_{22}H_{40}O_7$	416
7	25.90	2.04	Hexadecanoic acid, 1-(hydroxymethyl)-1, 2-ethanediyl ester.	$C_{35}H_{68}O_5$	568
8	28.21	2.48	11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296
9	29.03	3.82	Oleic acid	$C_{18}H_{34}O_2$	282
10	29.10	1.82	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256
11	29.40	1.20	d-Mannose	$C_6H_{12}O_6$	180
12	29.47	1.99	1,2,3,4,5-Cyclopentanepentol	$C_5H_{10}O_5$	150
13	34.55	11.60	Hexadecanoic acid, 2-hydroxy-1-(hydroxy methyl)ethyl ester	C19H38O4	330
14	34.90	45.03	4H-1-Benzopyran-4-one, 2,3-dihydro-5, 7-dihydroxy-2-phenyl-, (S)-	$C_{15}H_{12}O_4$	256
15	37.21	6.21	9-Hexadecenoic acid.	$C_{16}H_{30}O_2$	254
16	38.39	2.10	9,12-Octadecadienoic acid [ZZ]	$C_{18}H_{32}O_2$	280

Medicinal plants are the important resources of new drugs. Many of the modern medicines are manufacture indirectly from the medicinal plants. They have contributed number of ingredients to fight against various diseases and illness. The analysis and extraction of plant materials play an essential role in the development, modernization and quality control of herbal formulations. Studying of medicinal plants also facilitates to understand plant toxicity and also helps to protect human and animals from natural poisons. Hence the present study was undertaken to find out the bioactive phytoconstituents present in the methanolic leaf extract of *M. parasiticus* by using Gas chromatography and Mass spectroscopy. The active principles with their retention time (RT), molecular formula,

molecular weight (MW), concentration (peak area %) are presented in Table 1, Figure 1 and Figure 2. Which shows the presence of sixteen peaks indicating presence of sixteen bioactive phytochemical compounds in the methanolic leaf extract of *M. parasiticus*.

The result revealed that, 4H-1-Benzopyron-4-one,2,3-dihydroxy-2-phenyl-,(S)- (45.03%), was found as major component followed by 1,6-Anhydro-beta-D-glucopyranose (15.42%), Hexadecanoic acid, 2-hydroxy-1-(hydroxy methyl)ethyl ester (11.60); 9-Hexadecenoic acid (6.21%); Oleic acid (3.82%); (5beta) Pregnane-3,20beta-diol, 14alpha,18alpha-[4-methyl-3-oxo-(1-oxa-4-azabutane-1,4-diyl)]-,diacetate (2.78%); 11-Octadecenoic acid, methyl ester (2.48%); 9,12-Octadecadienoic acid [ZZ] (2.10%); Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester(2.04%); 1,2,3,4,5-Cyclopentanepentol (1.99%); n-Hexadecanoic acid (1.82%); d-Mannose (1.20%); E-3-Pentadecen-2-ol (1.15%); Adenosine,4'-de(hydroxymethyl)-4'-[N-ethylaminoformyl]-(0.95%); 4alpha-Phorbol 12,13-didecanoate (0.84%) and Agaricic acid (0.49%) in the methanollic leaf extract of *M. parasiticus*. Due to the presence of above mentioned phytocompounds in the leaf methanoloic extract of *M. parasiticus* may be used in various pharmaceutical and industrial applications.

GC-MS analysis of the methanolic leaf extract of *M. parasiticus* shows the presence of bioactive molecules, in that presence all the molecules were represented as carbohydrates and fatty acid moieties. Among the identified phytocompounds 11 compounds were known for its biological activities and remaining 5 compounds were unknown for its biological activities.

Sodde *et al.*[24] found that *M. parasiticus* has substantial anticancer activity against Ehrlich's Ascites Carcinoma and MCF-7 breast cancer cells.[23,24] Puneetha *et al*. earlier reported that it exhibits antioxidant properties.[18] Major recognised chemicals with anti-cancer and antioxidant effects were identified in this investigation. Quattrocchi reported that it has been used in traditional veterinary medicine and as a leaf paste to eradicate ticks.[20] The compound n-Hexadecanoic acid have a potent mosquito larvicidal property.

Major compound 4H-1-Benzopyron-4-one,2,3-dihydroxy-2-phenyl-,(S)- is a Flavonoid fraction (Pinocembrin) and have the properties of antimicrobial, anti-inflammatory, anticancer and neuroprotective Activity.[22] n-Hexadecanoic acid exhibits antioxidant, hypocholesterolemic, nematicidal, pesticidal, antiandrogenic, hemolytic, 5-alpha reductase inhibitor and anti-Inflammatory activities which was reported by the earlier workers.[15,4] However Oleic acid is a fatty acid that has antifungal, antioxidant, apoptotic activity.[2,9] 9,12-Octadecadienoic acid [ZZ] is a fatty acid (linoleic acid) and have a medicinal properties of anti-inflammatory, antibacterial, antiarthritic, hepatoprotective, anti-histaminic, anticoronary activity.[5] 4alpha-Phorbol 12,13-didecanoate and Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester have antimicrobial properties.[11,13] Furthermore, Agaricic acid have a properties of antimicrobial and cytototoxic activity.[10]

11-Octadecenoic acid, methyl ester is a Fatty acid methyl ester exhibits antibacterial

property.[12] Hexadecanoic acid, 2-hydroxy-1-(hydroxy methyl)ethyl ester have a properties of antimicrobial and antioxidant activity.[5,25] 9-Hexadecenoic acid have a properties of anti-inflammatory protective effects on hepatic steatosis and insulin signaling in murine.[3] d-Mannose a carbohydrate shows good immunostimulatory, anti tumor and antibacterial activity.[27,1]

In the present study, 16 phytocompounds were identified from the leaf methanolic extract of *Macrosolen parasiticus* (L.) Danser by GC-MS analysis. Among the identified compounds, majority of compounds have the role in antioxidant, antimicrobial, anticancer and antiiflammatory effects. The presences of various bioactive compounds in the leaves of *Macrosolen parasiticus* (L.) Danser shows their medicinal importance. However, isolation of individual phytochemical compounds and subjecting it to biological activity, will definitely give fruitful results. So it is recommended as a plant for phytopharmaceutical importance. However, further clinical trial is required to prove its efficacy.

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Conflict of interest :

The authors declare no conflicts of interest.

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