Original Article

Chemical Investigation of the Riparian Tree Species *Madhuca insignis* (Radlk.) H. J. Lam. (Sapotaceae) by GC-MS Profiling

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ABSTRACT

Objective: The present study aims to investigate the various phytochemicals present in the tree species *Madhuca insignis* by employing GC-MS profiling.
Methodology: The different parts of the tree species *viz.,* leaves, bark, roots, fruits and seeds was analysed by GC-MS profiling. Identification was based on the molecular structure, molecular mass and calculated fragments interpreted using NIST library.
Results: GC-MS profiling has revealed a total of 31 known compounds, the different classes identified ranged from fatty acid derivatives, steroids, terpenoids, hetrocyclic compounds, caratenoids to phenolic compounds. The identified compounds were reviewed to posses biological functions such as antimicrobial, antidiabetic, antitumor, anti-inflammatory activity and many more.
Conclusion: This study is a first time report of chemical profiling done in the riparian tree species *Madhuca insignis* (Radlk.)H.J.Lam to harness its medicinal potential by studying its active phyto-components.

Keywords: *Madhuca,* GC-MS profiling, phytocompounds, medicinal properties.

INTRODUCTION

Throughout centuries, natural products and their derivatives from plants have been an undeniable source for human medicine. Developments during the 19th and 20th century in the areas of modern medicine and pharmaceutical industry were mostly based on the leads from medicinal plants and their derivatives[1]. The medicinal

functions of the plant are normally attributed to the rich reservoir of secondary metabolites found in them[2]. Knowledge on the chemical constituents of these medicinal plants found in the form of secondary metabolites is required not only for the discovery of newer therapeutic molecules but also will help in identifying the new sources of economic phytochemicals for the synthesis of complex and targeted drug systems[3]. In the last century alone, about 121 pharmaceutically formulated products were reported[4]. Plants, with reported ethnopharmacological uses, have been the primary sources of health care products for early drug discovery[5]. However currently researchers relay largely on initial phytochemical screening which is an effective bioactivity-guided isolation method. Some of the classic examples are the important anticancer agents, paclitaxel from *Taxus brevifolia* and camptothecin from *Camptotheca acuminate*[6].

Madhuca insignis is a riparian tree species belonging to the family sapotaceae, found distributed only in southern India namely in the states of Karnataka[7-11] and Kerala[12] . There are various reports on the pharmacological uses of the other species of *Madhuca* also found in India[13-16], but until now there has been no study undertaken on the chemical profile or the medicinal activity of *M. insignis* hence this present study aims to investigate the various phytochemical compounds using GC-MS profiling. GC-MS studies have been widely used for analysis of compounds in medicinal plants[17]. Also in the recent years this technique has proved to be a reliable and valuable technique for analysis of various non-polar compounds, fatty acids, lipids, essential oils and alkaloids[18]. In this study the various parts of *M.insignis* is being chemically profiled for the first time and this work would be a key to identify compounds of therapeutic importance in health care sector.

MATERIALS AND METHODS

Collection of Plant material

Leaves, roots, bark, fruits and seeds of *M.insignis* were identified and collected from the region of Dakshin kannada, Karnataka, India during the study period 2013-14.

Sample Preparation

The various parts (leaf, bark, root, fruit and seed) of *M.insignis* were washed thoroughly in running tap water 2-3 times in order to remove impurities like dust, soil and debris; followed by washing with distilled water prior to drying. The different samples (leaf, bark, root, fruit and seed) were shade dried for nearly 30 days and powdered. 25g of the various powered samples (leaf, bark, root, fruit and seed) were then soaked in 10 ml 95% methanol for 12 hrs and then concentrated to 1ml by rotary evaporator at 65^0C. The extracts were then filtered using whatman filter paper. In case of seed and fruit, prior to extraction de-fatting was done using Soxilate apparatus. The cure extracts were then analysed using GC-MS.

GC-MS Analysis

The GCMS analysis was carried out on Variant 4000 GC MS MS. The column used was DH5 factor 4 manufactured variant 30 m X 0.2 mm with a film thickness of 0.25mm. The carrier gas composed of Helium at a flow rate of 1ml/min. 1.5µl sample injection volume was utilized. The injector port temperature was maintained at 270^0C. The oven temperature was programmed initially at 50^0C for 4 min, then an increase to 240^0C at the rate of 5^0C/min, hold for 2mins, followed by 260^0C at the rate of 5 ^{0}C, hold for 12mins. The total run time was 60 mins. The MS transfer line was maintained at a temperature of 280^0C and the ion source temperature at 200^0C. The trap temperature was 190^0C with the split ratio of 1:20. GCMS was analysed using

electron impact ionization at 70eV. The data was evaluated using total ion count and the detector was operated in scan mode from 50-550 amu.

Identification of compounds

Identification was based on the molecular structure, molecular mass and calculated fragments. Interpretation on mass spectrum GC-MS was conducted using NIST (National institute Standard and Technology) 2007 and Wiley 2009[19]. The name, molecular weight and the structure of the components was ascertained through this method.

RESULTS AND DISCUSSION

GC-MS analysis was carried out on methanol extracts of leaf, bark, root, fruit and seed revealing the presence of a number of phytocomponents that may be contributing to the medicinal nature of *M.insignis*. The relative percentage of each of the identified components was calculated by comparing its average peak area to the total area. The obtained mass spectrum was interpreted by comparison with the compounds present in the NIST database. In order to identify compounds by this method 95% spectral identity is required. The compound predication and identification is based on Dr. Dukes Phytochemical and Ethno botanical databases by Dr. Jim Duke of ARS/USDA[19]. The phytocompounds identified, their structure and their biological activity are listed in the table 1.

The GC- MS profiling has revealed a total of 31 known compounds pertaining to different parts of *M.insignis* such as leaf, bark, root, fruit and seed with the help of NIST library. The different classes of compounds identified ranged from fatty acid derivatives, steroids, terpenoids, hetrocyclic compounds, phenolic compounds, caratenoids to phenolic compounds. The identified compounds were found to posses many biological functions such as antimicrobial, antidiabetic, antitumor, anti-inflammatory activity and many more. Previous studies on other species of *Madhuca* have also reported similar biological functions; in *M.longifolia* the bark has been reported to have antidiabetic and antioxidant activity, seeds -antioxidant activity, leaf – antimicrobial and antioxidant activity[13]. Biological functions such as antidiabitic, antitumor, antibacterial activity have been reported in yet another related species *M.indica*[16], these biological functions can be attributed to the presence of various phytocompounds such as steroids, terpenoids, fatty acid derivatives and phenolic compounds. Steroids such as stigmasta, lanosterin and terpenoids such as amyrin and betulin have been previously reported in one of the most widely researched species of *Madhuca* namely *M.longifolia*[20], which have also been also reported in this present study.

Our results suggest that further work is needed to locate the active principles from *M.insignis* which could result in the discovery of newer and much active compounds. This species can be a potential hub to discover bioactive products that may serve leads for the development of the new pharmaceuticals that would address therapeutic needs. These bioactive compounds in addition of being developed directly as drugs can also serve as effective prototype drug models for chemical synthesis of newer medicines.

CONCLUSION

GC-MS is one of the robust techniques to obtain chemical fingerprints of different plant species, which would help in accessing and harnessing the potential medicinal value of documented and most importantly unexplored plants such as *Madhuca insignis*. The present study reveals the presence of phytocompounds with

known biological functions for the first time thereby showcasing *M.insignis* as a potential phytopharmaceutical candidate, and further substantiating the requirement of evaluating and harnessing the pharmacological potential of *M.insignis* in the sector of healthcare.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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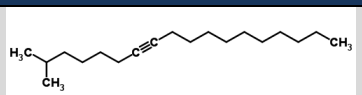
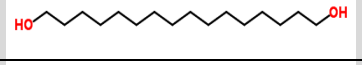
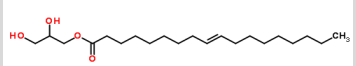
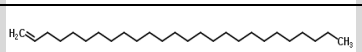
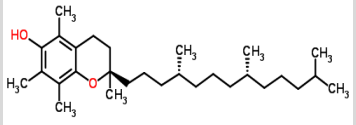
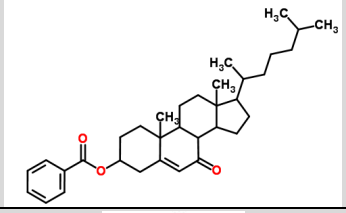
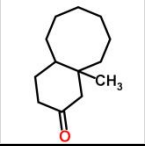
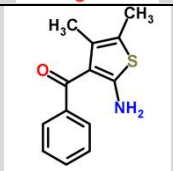
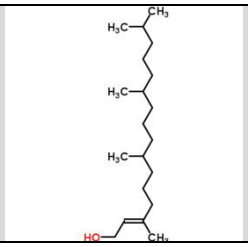
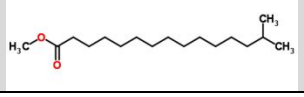
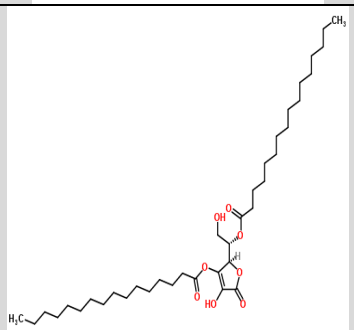
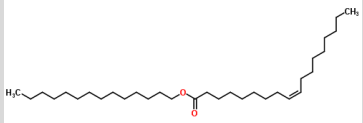
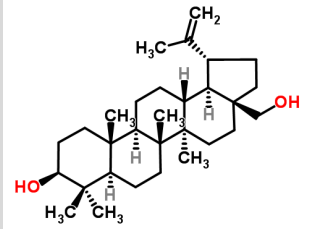
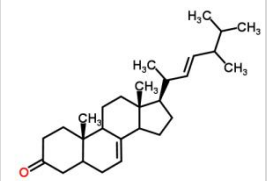
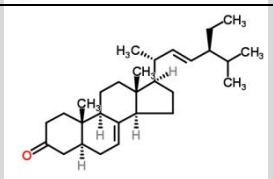
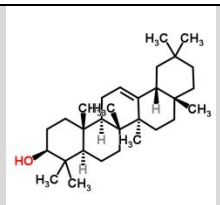
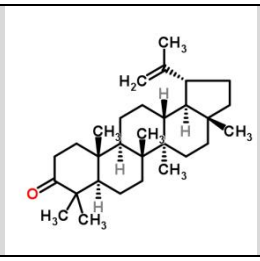
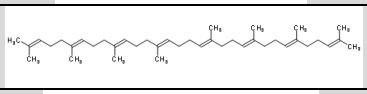
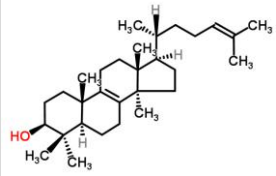
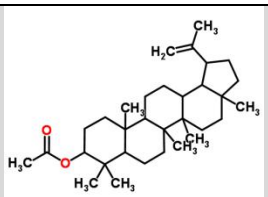
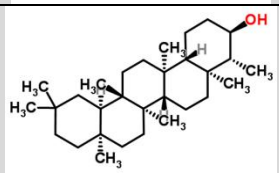
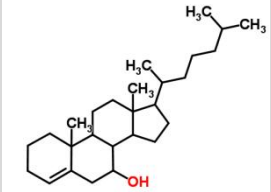
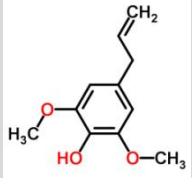
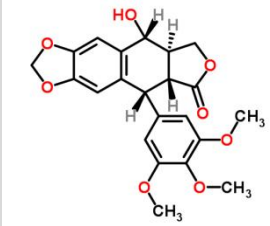
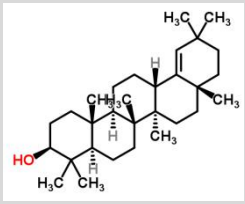
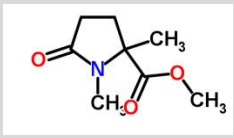
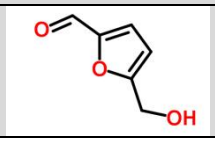
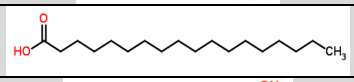
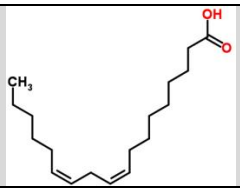
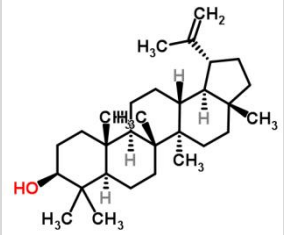
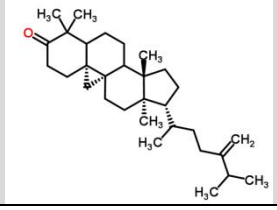
Table 1. Phytocompounds identified in various parts of *M.insignis*

Sl. NO	COMPOUND NAME	COMPOUND NATURE	COMPOUND STRUCTURE	PART FOUND IN	R/T	ACTIVITY
1.	2-methyl-7-octadecyne	Aliphatic Polyacetylene		Leaf	30.927	Antimicrobial Antioxidant
2.	1,16-Hexadecanediol	Aliphatic Alcohol		Leaf	43.197	Antifungal
3.	2,3-Dihydroxypropyl elaidate	Aliphatic Ester		Leaf	50.625	Antimicrobial Antifungal
4.	1-hexacosene	Aliphatic Hydrocarbon		Leaf	51.692	Anti diabetic
5.	Alpha Tocopherol/ Vitamin E	Methylated phenol		Leaf Bark Root Fruit	55.422	Antioxidant
6.	7-Oxocholest-5-en-3-yl benzoate	Steroid derivative		Leaf	57.977	Antibacterial Antitumor
7.	10a-Methyldecahydrobenzo[a]cyclooctene-2(1H)-one	Biocyclo Alkane		Bark	28.574	Antiandrogenic
8.	2-Amino-3-benzoyl-4,5-dimethylthiophene	Heterocyclic Amine		Bark	29.536	Angiogenesis stimulator

9.	3,7,11,15-Tetramethylhexadec-2-en-1-ol	Aliphatic Alcohol		Bark	31.79	Antimicrobial Antioxidant Anti-inflammatory
10.	Methyl 14-methylpentadecanoate	Aliphatic Ester		Bark Fruit	32.791	Antimicrobial
11.	(1S)-2-Hydroxy-1-[(2R)-4-hydroxy-5-oxo-3-(palmitoyloxy)-2,5-dihydro-2-furanyl]ethyl palmitate	Fatty Acid Derivative		Bark Fruit Seed	33.587	Dermatologic effect Anti-inflammatory
12.	Oleic acid	Fatty Acid Ester		Bark Fruit Seed	36.016	Hypotensive effect
13.	Betulin	Terpenoid		Bark Fruit	36.703	Anti-inflammatory Antitumor
14.	Ergosta-7,22-dien-3-one	Steroid		Bark	44.542	Anti-inflammatory Apoptotic effect
15.	Stigmasta-7,22-dien-3-one	Steroid		Bark Root	44.668	Antihyperglycemic
16.	α/ β-amyrin	Terpenoid		Bark Root Fruit Seed	45.108	Antihyperglycemic Hypolipidemic

17.	Lupenone	Terpenoid		Bark Root Fruit	46.528	Antitrypanosomal Antimalarial
18.	Lycopersene	Caratenoid		Bark	48.059	Sedative
19.	Lanosterin	Steroid		Bark	48.785	Neuroprotective Antitumor
20.	Lup-20(29)-en-3-yl acetate	Terpenoid		Bark Root Fruit Seed	53.698	Anti-inflammatory Antioxidant Antihypersensitive
21.	Friedelan-3α-ol	Terpenoid		Bark Root Fruit	54.661	Antimicrobial Antiproliferative
22.	Cholest-4-en-7-ol	Steroid		Bark Fruit	55.051	Steroid drugs for obesity/ liver diseases
23.	2,6-Dimethoxy-4-allylphenol	Phenolic compound		Root	28.602	Antimicrobial
24.	Podophyllotoxin	Lignan		Root	47.533	Antirheumati, Antiviral, Antitumor

25.	Germanicol	Terpenoid		Root Fruit Seed	59.632	Antimicrobial Anti-inflammatory
26.	Methyl 1,2-dimethyl-5-oxo-2-pyrrolidinecarboxylate	Hetrocyclic compound		Fruit Seed	18.962	Anxiolytic Antimicrobial
27.	5-(Hydroxymethyl)-2-furaldehyde	Hetrocyclic compound		Fruit	24.272	Anti-inflammatory
28.	Stearic acid	Fatty acid derivative		Fruit	42.176	Tablet/ capsule lubricant
29.	Linoleic acid	Fatty acid derivative		Fruit Seed	42.313	Antiglycemic
30.	β-Viscol	Terpenoid		Fruit	50.003	AntimalarialAphrodisiacAntidiabetic
31.	24-Methylene-9,19-cyclolanostan-3-one	Steroid derivative		Fruit	50.151	Improves hyperglycemia