Phytochemical screening studies on *Melia orientalis* by GC-MS analysis

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ABSTRACT

Background: *Melia orientalis* (MO) is an important Ayurvedic medicinal plants. The plant part such as leaves and roots are traditionally used for the treatment of diabetes, edema, traumatic swelling, skin diseases, oligospermia and bleeding disorders. **Objective:** To investigate the phytochemical identification of ethanol leaf extract of MO. **Materials and Methods:** The fresh leaves of MO (1000g) were collected and shade dried at room temperature for 30 days and the dried leaves were made into a fine powder. The ethanol leaf extract obtained was dried and used for phytochemical identification by GC-MS analysis. **Results:** The phytochemical screening studies have been carried out and identified ten chemical constituents present in the leaf extract of MO. **Conclusion:** Thus, our results show that MO possess important phytocomponents such as phytol, squalene and stigmasterol.

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INTRODUCTION

Traditional knowledge of medicinal plants has always guided the search for new cures. In spite of the advent of modern high throughput drug discovery and screening techniques, traditional knowledge systems have given clues to the discovery of valuable drugs.[1] Traditional medicinal plants are often cheaper, locally available and easily consumable, raw or as simple medicinal preparations. Nowadays, traditional medicinal practices form an integral part of complementary or alternative medicine. Although their efficacy and mechanisms of action have not been tested scientifically in most cases, these simple medicinal preparations often mediate beneficial responses due to their active chemical constituents.[2]

Melia orientalis Linn is an evergreen shrub growing up to a height of three meters. It is widely distributed in dense evergreen forests of India, especially in Western Ghats.[3]

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The plant belonging to the family of Meliaceae are generally reported to contain triterpenoids and tetranortriterpenoids as chemotaxonomic markers.[4] The medicinal properties of plants are widely used in the treatment of edema, traumatic swelling, skin diseases, diabetes, worms, oligospermia and bleeding disorders. It is also effective for the treatment of snake and cobra poison.[5] The daily intake of half ounce of leaf juice is to make a permanent resistance against cobra poison. Thus the aim of our present study is to investigate the phytochemical identification by GC-MS analysis.

MATERIALS AND METHODS

Plant collection and preparation of the extract

Fresh leaves of *Melia orientalis* (MO) were collected from Trivandrum district, Kerala, India. The plant specimen was authenticated by Mrs. Padmaja, an expert in the field of Botany and the specimen was deposited in Ayurveda Research Institute for Mother and Child Health Care (ARIMCHC), Trivandrum. The fresh leaves of MO (1000g) were shade dried at room temperature (28 ± 2°C) for 30 days and the dried leaves was made into a fine powder (particle size-0.25mm) by using electric blender. 20g of the powdered leaves was soaked in absolute ethanol for 12 h. The extract was then

filtered through Whatmann filter paper No. 41 along with 2g sodium sulphate to remove the sediments and traces of water in the filtrate. Before filtering, the filter paper along with sodium sulphate was wetted with absolute ethanol. The filtrate was then concentrated by bubbling nitrogen gas into the solution. The extract contained both polar and non-polar phytocomponents of the plant extract was used.

GC-MS analysis

GC-MS analysis was carried out at Indian Institute of Crop Processing Technology (IICPT), Thanjavur, India, GC Clarus 500 Perkin Elmer system and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: Column Elite-1 fused silica capillary column (30mm x 0.25mm ID x1µmdf, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70ev; Helium (99.999%) was used as carrier gas at a constant flow of 1 ml/min and an injection volume of 2µl was employed (Split ratio of 10:1); Injector temperature 250°C; Ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min) with an increase of 10°C/min, to 200°C, then 5°C/min to 280°C, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70ev; a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC running time was 36 min.

Identification of components

Interpretation of mass spectrum GC-MS was conducted using the database of National Institute Standard and Techniques (NIST). WILEY 8 and FAME having more than 65,000 patterns. The spectrum of the unknown components stored in the NISTO8s, WILEY8 and FAME library. The name, molecular weight, molecular formula and structure of the component of the test material was ascertained.[6] The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a GC-MS solution Ver.2.53.

RESULTS

The phytochemical constituents present in the leaves of MO were reported in Table 1. The GC-MS analysis of plant extract revealed the presence of thirteen chemical compounds (Phytochemical constituents) that could contribute the medicinal properties of the plant. The identification of the active principles present in the leaf

Table 1: Phytocomponents identified in the ethanol leaf extract of *Melia orientalis* by GC-MS analysis

S.No	RT	Name of the compound	Molecular formula	MW	Peak area %
1	11.36	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	4.54
2	14.63	Phytol	$C_{20}H_{40}O$	296	12.09
3	20.46	1,2-Benzenedicarboxylic acid , diisooctyl ester	$C_{24}H_{38}O_4$	390	29.62
4	22.75	Eicosane, 2-methyl-	$C_{12}H_{44}$	296	7.60
5	24.29	Squalene	$C_{30}H_{50}$	410	10.93
6	25.48	Nonadecane, 2-methyl-	$C_{20}H_{42}$	282	6.07
7	28.20	Heptacosane	$C_{27}H_{56}$	380	4.96
8	30.51	Stigmasterol	$C_{29}H_{48}O$	412	5.17
9	31.69	τ-Sitosterol	$C_{29}H_{50}O$	414	16.74
10	32.03	Cholest-5-en-3-ol,24-propylidene-3, (3β)-	$C_{30}H_{50}O$	426	2.27

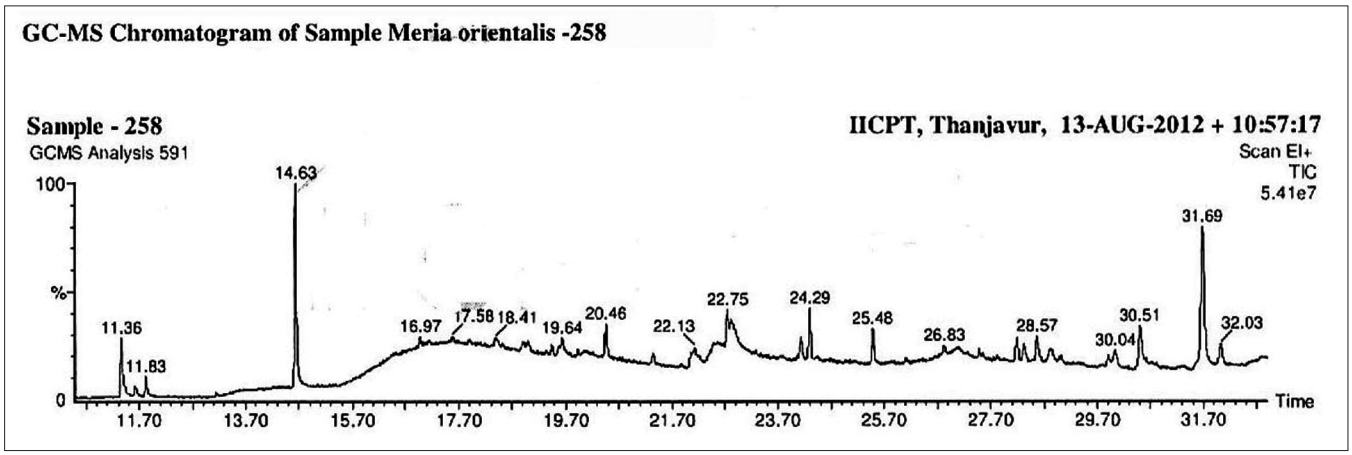

Figure 1: GC-MS analysis of ethanolic leaf extract of *Melia orientalis*

extract was confirmed based on the peak area, retention time, molecular formula, molecular weight and peak area in percentage were shown in Table 1 and Figure 1. The first compound identified with less retention time (11.36min) was 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, whereas Cholest-5-en-3-ol, 24-propylidene-3, (3β) was the last compound which took longest retention time (32.03 min) to identify. The phytocomponents identified by GC-MS analysis showed many biological activities of ethanol leaf extracts of MO was presented in Table 2. The biological activities listed are based on Dr.Duke's phytochemical and Ethanobotanical databases.

DISCUSSION

Free radicals-induced oxidative damage is involved with various human diseases such as cardiovascular diseases, neural disorders such as Alzheimer's disease and Parkinson's disease, diabetes and cancer.[7] Antioxidants are compounds that help to inhibit the oxidative reactions caused by free radicals thereby preventing or delaying damage to the cells and tissues.[8] Their mechanism of action includes scavenging reactive oxygen and nitrogen free radical species, metabolizing lipid peroxides to non-radical products, chelating metal ions to prevent generation of free radicals, etc.[9] Endogenous antioxidants such as ascorbic acid, vitamin-E, uric acid, thiols, and bilirubin present in extracellular fluids acts as a primary defense system that protects against oxidative damage.[10] The aim of our present study is to investigate the phytochemical identification of ethanol leaf extracts of MO by GC-MS analysis and the results indicated a concentrated dependent antioxidant ability of MO. The phytochemical screening studies have been carried out by GC-MS analysis and identified the chemical constituents present in the leaf extracts of MO. The gas chromatogram shows the relative concentrations of various compounds getting eluted as a function of retention time. The heights of the peak indicate the relative concentrations of the components present in the plant extract. The mass spectrometer analyzes the compounds eluted at different times to identify the nature and structure of the compounds. These mass spectra are fingerprint of that compound which was identified from the NIST library databases. The compounds which were identified by GC-MS analysis such as 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, Phytol, 1,2-Benzenedicarboxylic acid , diisooctyl ester, Eicosane, 2-methyl, Squalene, Nonadecane, 2-methyl, Heptacosane, Stigmasterol, τ-Sitosterol and Cholest-5-en-3-ol, 24-propylidene-3.

Table 2: Phytocomponents identified and their biological activities of *Melia orientalis*

S.No	Name of the compound	Therapeutic activity
1	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Antimicrobial and Anti-inflammatory
2	Phytol	Antimicrobial, Anti-inflammatory and Anticancer
3	1,2-Benzenedicarboxylic acid , diisooctyl ester	Antimicrobial and Antifouling
4	Squalene	Anti-atherosclerosis and Steatosis, Anti-bacterial, Antioxidant, Anti-tumor, Cancer preventive, Pesticide, Immunostimulant, Chemoprevention and Lipid inhibitor
5	Stigmasterol	Thyorid inhibitory effect, antiperoxidative and Hypoglycemic effects

CONCLUSION

Thus, from the results obtained that phytochemical screening studies have been identified the presence of phytol, squalene, stigmasterols and τ-sitosterol. These compounds possess important biological activity such as antimicrobial, anti-inflammatory, anticancer, antioxidant, immunostimulator, thyroid inhibitory effect, lipid inhibitors, antiperoxidative and hypoglycemic effect.

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