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GC-MS analysis of methanolic extract of root of *Millingtonia hortensis* L.f.

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Abstract
The objective of the study was to evaluate the phytochemical compounds present in methanolic root extract of *M. hortensis*. Phytochemical screening was carried out using GC-MS instrument following the standard protocol. GC-MS studies revealed the presence of 37 compounds in root extract of *M. hortensis*. Among them highest peak area (47.99%) was obtained by Guanosine. This study identifies the presence of pharmacologically active compounds in the plant extract which can be constructive for the formulation of novel drugs.

Keywords: *M. hortensis*, GC-MS, methanolic extracts, retention time, novel drugs

Introduction
A medicinal plant can be described as one of the plants having components that may be utilized for therapeutically means or which have inactive forms of chemically & pharmaceutically synthesis processes (WHO, 1977). All around the world, plants are utilized conventionally to cure many diseases, specifically transmissible disorders like diarrhoea, fever, cold. At the same time these are also used for birth control and dental hygiene purposes (Mitscher *et al.*, 1987) [12]. Additionally, many psychologically active substances applied in folk medicine are of plant origin (Deans and Svodoba, 1990) [7]. Traditionally used medicinal plants produce multiple known therapeutic substances (Chopra *et al.*, 1992; Harborne and Baxter, 1995; Ahmed and Beg, 2001) [6, 10, 1].

Gas chromatography mass spectroscopy (GC-MS) is easy and fast technique used for secondary metabolite characterization in plants as well as non-plant species (Robertson, 2005) [15].

M. hortensis, commonly known as Indian cork tree, belongs to family Bignoniaceae. The height of the tree is up to 7-11 meters. It is a versatile tree which can grow in all kinds of soil. Generally, the tree is uses as ornamental. It also has many medicinal properties such as flower buds are used to treat asthma, sinusitis, cholagogue and tonic. Leaves and roots of cork tree are used for anti-asthmatic and antimicrobial properties. Whole plant show antipyretic, antitubercular, antimicrobial, larvicidal, antimutagenic, anticancer and antifungal properties (University of Kerela).

Material and Methods
Collection of plant materials
The root samples of *M. hortensis* L.f. were collected from the University of Rajasthan campus, Jaipur in the month of February. Herbarium samples were deposited and authenticated in the department of Botany, University of Rajasthan.

Preparation of plant material
Roots were rinsed with tap water, and dried under shade at room temperature and ground to fine powder by employing an electrical grinder and stored in air tight containers.

Preparation of sample for GC-MS study
About 10 g of the dried powder was soaked in 100 ml methanol. It was left for 24 hours so that non polar components, volatiles and other constituents if present will get dissolved. The methanol extract was filtered using Whatman No. 1 filter paper and the residue was removed. It was again filtered through sodium sulphate in order to remove the traces of moisture.

GC-MS analysis
The GC-MS analysis was conducted at AIRF (Advanced Instrumentation Research Facility), JNU, Delhi. GC-MS analysis was carried out on a GC-MS QP2010Ultra. The GC/MS instrument had Rtx-5MS column with a dimension of 30× 0.25 mm × 0.25 µmdf, composed of 5% Dipheny, 95% Dimethyl poly siloxane, operated in electron impact mode at 70 eV. The carrier gas used was helium, which had a constant flow rate of 1.21 ml/min and with an injection volume of 1 µl was employed (split ratio of 10:1). The oven temperature was programmed initially at 100^0 C for 2 min, then an increase to 250 at rate of 10^0 C/min for 5 min and then programmed to increase to 280^0C at a rate of 20^0C/min for 21 min. The MS operating conditions were as follows interface Temp. 270.00^0C, Ion Source Temp 230.00^0C, Solvent Cut Time: 3.50 min, Scan Speed 3333, mass scan (m/z)-40-650, and threshold: 1000. GC-MS was analysed using electron impact ionization at 70eV and data was evaluated using total ion count (TIC) for compound identification and quantification. The relative percentage amount of each compound present in the GC-MS spectrum was calculated by comparing the individual compounds average peak area to their total areas. Turbo mass 5.2 software was used to handle mass spectra and chromatograms.

Identification of components
The identification of the components in the extracts was assigned by the comparison of their retention data and mass spectra fragmentation patterns with those stored on the computer library and also with published literatures. NIST08.LIB (Stein, 1990) [17], WILEY8.LIB (Lafferly, 1089) library sources were used for matching the identified components from the plant material.

Results and Discussion

GC-MS is the best technique to identify the bioactive components of long chain hydrocarbons, alcohols, acids used in the analysis of the herbal medicines, there are more significant advantages for GC-MS (Sridharan *et al*., 2011) [16].

The GCMS spectra illustrated the occurrence of variety of chemical components with retention time. The mass spectrometer gives the information about the substance isolated at different interval to recognize the structure and characterization of the biomolecules. The large chemicals break down into small ones which cause peaks to appear at different m/z ratios. These mass spectra are the fingerprint of this compound identifiable from the data library. Composition was estimated on the basis of calculation of GC peak area as a percentage with total areas set to 100%.

GC-MS spectra of root extract of *M. hortensis* revealed that the peaks indicated the occurance of different constituents. The spectra fingerprint of compounds identified using the data library and molecular weight, the name of compounds listed in Table 1. is shown in Figure 1 and the spectral In the methanolic extract of root, 37 compounds were identified. The highest peak area was obtained by Guanosine (47.99%). The other most common compounds are Linoleic acid (8.33%), Glyceraldehyde (6.87%), 5-Hydroxymethylfurfural (5.07%), 3-Diazo-2,4-Pentanedione (4.51%), n-Hexadecanoic acid (4.06%), 1,2,3-Propanetriol, monoacetate (2.07%), etc.

Previously it has been reported that the methanolic extracts of plants possess several biological activities lie anti-oxidant, antifungal, anti-microbial, anti-inflammatory and pesticidal activities (Rahman *et al*., 2014) [14].

Most of these constituents have been found to show interesting biological activity against certain illnesses. The major constituents, Guanosine is a purine nucleoside thought to have neuroprotective properties. It is released in the brain under physiological conditions and even more during pathological events, reducing neuroinflammation, oxidative stress, and excitotoxicity, as well as exerting trophic effects in neuronal and glial cells (Bettio *et al*., 2016) [3].

Linoleic acid (18:2ω6; cis, cis-9,12-octadecadienoic acid) is the most highly consumed PUFA found in the human diet. On consumption, linoleic acid has 4 primary fates. Like all fatty acids, it can be used as a source of energy. Because linoleic acid is an essential nutrient, it is typically provided in enteral, parenteral, and infant formulas where the fat content can vary depending on the specific use. Similarly, topical applications can also provide linoleic acid, helping to treat skin-related disorders related to deficiency (Whelan and Fritsche, 2013) [21].

Plants are rich sources of natural antioxidants (Biswas *et al*., 2005) [4]. Antioxidants acts against the oxidative stress in the animal body, so the defence mechanism gets weakened and causes oxidative damages to lipids, proteins and DNA (Dosek *et al*., 2007) [9] and inhibits the initiation and propagation of Reactive Oxygen Species (ROS) (Velioglu *et al*., 1998) [20].

Hexadecanoic acid (fatty acid) reported to have antibacterial activity against *S. aureus* and *E. Coli* (Alamin *et al*., 2016) [2]. Linoleic acid (Fatty acid) possesses the antibacterial activity against gram positive (*B. cereus*, *B. pumilus*, *B. subtilis* and *S. aureus)* bacteria (Dilika *et al*., 2000) [8]. Octadecanoic acid (Fatty acid) also have antimicrobial activity against *S. aureus* and *S. pyogenes* (Zheng *et al*., 2005) [24]. Hexadecanoic acid, methyl ester belongs to the class of organic compounds known as fatty acid methyl esters, reported to have antibacterial and antifungal activities (Chandrasekaran *et al*., 2011) [5]. In another study, nematicidal and pesticidal activities of Hexadacanoic acid, methyl ester has been reported (Uraku, 2016) [19]. Hexadecanoic acid ethyl ester (fatty acid ester) acts as an antioxidant, hypocholesterolemic nematicide, pesticide, antiandrogenic flavour, haemolytic, and 5-alpha reductase inhibitor (Bihana *et al*., 2018).

1,2:5,6-Dianhydrogalactitol (DAG) is a bifunctional DNA-targeting agent causing N^7-guanine alkylation and inter-strand DNA crosslinks currently in clinical trial for treatment of different cancers (Zhai *et al*., 2018) [23].

Table 1: GC-MS analysis of methanol extract of *M. hortensis* root.

S. No.	R. Time	Area%	Compound name	Molecular formula	Molecular wt.
1.	4.570	0.59	1,2,3-Propanetriol	$C_3H_8O_3$	92
2.	4.808	0.41	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	$C_6H_8O_4$	144
3.	5.700	0.37	2,3-Dimethyl-1,4-dioxene	$C_6H_{10}O_2$	114
4.	5.942	0.29	2,5-Dimethyl-3(2H)furanone	$C_6H_8O_3$	128
5.	6.262	4.51	3-Diazo-2,4-Pentanedione	$C_5H_6N_2O_2$	126
6.	6.527	0.48	Oxalic acid, isobutyl pentyl ester	$C_{11}H_{20}O_4$	216
7.	7.169	6.87	Glyceraldehyde	$C_3H_6O_3$	90
8.	7.324	1.12	1,5-Anhydro-6-Deoxyhexo-2,3-Diulose	$C_6H_8O_4$	144
9.	8.540	5.07	5-hydroxymethylfurfural	$C_6H_6O_3$	126
10.	8.732	2.07	1,2,3-Propanetriol, monoacetate	$C_5H_{10}O_4$	134
11.	9.266	1.41	3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one	$C_6H_8O_4$	144
12.	9.354	0.18	Acetic acid, Propyl ester	$C_5H_{10}O_2$	102
13.	9.836	0.24	1-Methyl-3-chlorophospholene-1-oxide	C_5H_8ClOP	150
14.	10.185	1.31	3-Hydroxy-3methylvaleric acid	$C_6H_{12}O_3$	132
15.	10.460	0.86	2-methyl-3-nonanol	$C_{10}H_{22}O$	158
16.	11.000	1.32	1'-Hydroxy-4,3'-dimethyl-bicyclohexyl-3,3'-dien-2-one	$C_{14}H_{20}O_2$	220
17.	11.200	0.16	1-Hexanesulfonic acid, methyl ester	$C_7H_{16}O_3S$	180
18.	11.807	47.99	Guanosine	$C_{10}H_{13}N_5O_5$	283
19.	12.078	1.83	11-(3 Ethenylcyclopentyl)undec-10-enoic acid, ethyl ester	$C_{20}H_{34}O_2$	306
20.	12.342	0.75	Formic acid, octyl ester	$C_9H_{18}O_2$	158
21.	12.817	0.28	Isopentyl alcohol, Valerate	$C_{10}H_{20}O_2$	172
22.	13.066	0.33	3-Hydroxy-4-methoxybenzoic acid	$C_8H_8O_4$	168

23.	13.317	0.24	6-Methyl-1-heptanol	$C_8H_{18}O$	130
24.	13.461	1.50	1,2-Benzenedicarboxylic acid, Diethyl ester	$C_{12}H_{14}O_4$	222
25.	13.765	0.37	4-Ethylcyclohexanol	$C_8H_{16}O$	128
26.	13.922	0.52	3-Deoxy-d-mannonic acid	$C_6H_{12}O_6$	180
27.	14.513	0.69	Santolina triene	$C_{10}H_{16}$	136
28.	15.336	0.28	3-Amino-2(1H)-Pyridinone #	$C_5H_6N_2O$	110
29.	15.590	0.10	3,3-Difluoro-1-Tetradecen-4-Ol	$C_{14}H_{26}F_2O$	248
30.	17.324	4.06	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256
31.	18.297	0.08	Pentanoic acid, 2-Methylpropyl ester	$C_9H_{18}O_2$	158
32.	18.659	0.19	2-Octyne	C_8H_{14}	110
33.	19.015	8.33	Linoleic acid	$C_{18}H_{32}O_2$	280
34.	19.236	0.63	Octadecanoic acid	$C_{18}H_{36}O_2$	284
35.	22.596	1.39	1,2-Benzent dicarboxylic acid	$C_{24}H_{38}O_4$	390
36.	24.138	0.65	2-Amino-4-methylpyrrole-3-carbonitrile	$C_6H_7N_3$	121
37.	25.232	1.44	Dianhydrogalactitol	$C_6H_{10}O_4$	146

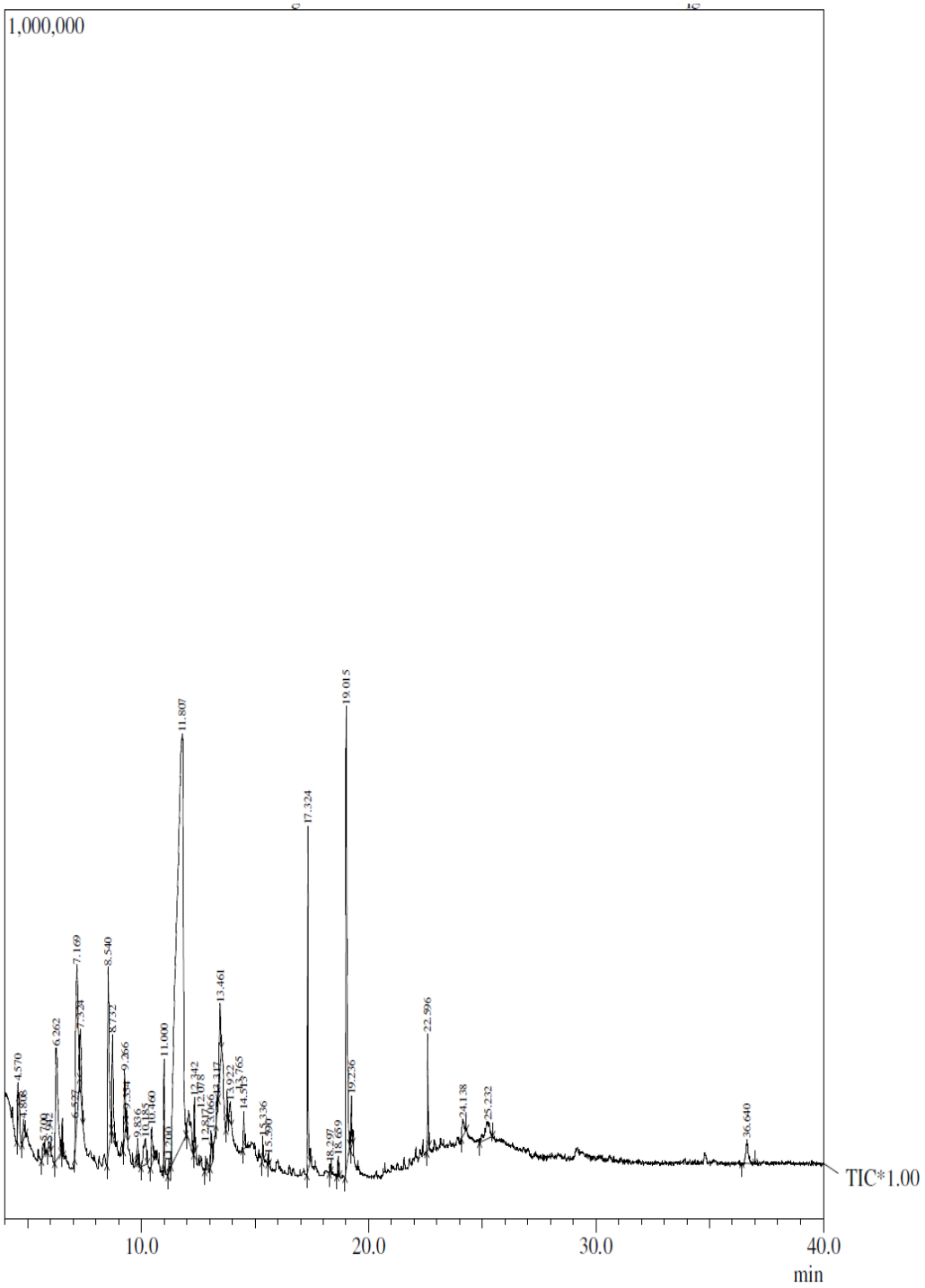

Fig 1: Chromatograph obtained by GC-MS analysis of methanolic extract of root of *M. hortensis*.

Conclusion

The present investigation concluded that methanolic extract of root of *M. hortensis* has many active constituents responsible for a lot of biological activities. So that those might be utilized for the development of traditional medicines and further investigation needs to elute novel active compounds from the medicinal plants which may be created a new way to treat many incurable diseases including cancer.

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Conflict of Interest

This statement is to declare that the author involved in this manuscript has no conflict of interest.

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