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GC-MS Study of Stem Bark Extract of *Juglans regia* L.

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ABSTRACT

Natural products have been a major source of drugs for centuries. In the last few decades there has been an exponential growth in the field of herbal medicines. Medicinal plants and herbs contain substances known to modern and ancient civilizations for their healing properties. Plants produce chemical compounds as part of their normal metabolic activities. These metabolites are the source of active principles capable of curing health ailments. *Juglans regia* L. is a medicinally important plant of family Juglandaeceae. All parts of the plant have medicinal properties and are used in folk medicines since long. Taking into consideration the medicinal importance of the species hexane extract from the bark of this plant was analyzed using GC-MS and the structures were confirmed by genesis. The major constituents were n-octedecane, n- hexadecanoic acid (palmitic acid), 9-E-Hexadecenoic acid, Tetra, tetracotane, 4,8,12,16 tetramethylheptadecane-4-olide, n-heptadecanoic acid, 1-iodohexadecane, stearic acid, oleic acid, erucic acid and Di-n-octyl phthalate.

Keywords: *Juglans regia* L., GC-MS, Di-n-octyl phthalate, palmitic acid, erucic acid.

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INTRODUCTION

Natural products are an unending source of chemical compounds which are often useful in pharmaceutics, cosmetics and agro ecosystem developments [1]. Herbal remedies have proved successful in folk medicines and Ayurvedic system of medicine and it is now accepted worldwide. Increasing resistance of synthetic antibiotics towards existing as well as newly born species of pathogens and deadly infectious diseases prompts the researchers to investigate the traditional medicinal plants as a source of active principles capable of treating various diseases. *Juglans regia* L. is an important medicinal plant belonging to family Juglandaeceae. All parts of the plant have significant medicinal applications. This species, from Himalayan regions in India, is used in folk medicines to treat various diseases including cancer [2]. Different extracts of the leaves show anticancer activity [3]. Decoction of the stem bark is useful in dental complaints [4]. The species has been used to treat ailments like 'tisis' and 'scrofula', which are synonyms of tuberculosis, and tuberculosis of the cervical glands [5].The stem bark extracts are promising source of compounds with antibacterial activity [5]. GC-MS is one of the best techniques to identify the constituents like long chain, branched chain hydrocarbons, alcohols acids, esters etc. Literature survey reveals that twenty volatile components in the essential oil extracted by hydro distillation from the leaves of Juglans spp. were identified by GC-MS. The main components were terpenoids (84.89%), aromas (3.9%), and esters (1.34%) [6]. Flavonols were isolated and identified [7]. Seeds exhibit the presence of major five fatty acids as hexadecylic, stearic, oleic, linoleic and linolenic acid by gas chromatography [8]. With reference to the above facts, GC-MS analysis of hexane extract of the stem bark of J. regia L. has been executed. Results predict the presence of n-octedecane, n- hexadecanoic acid (palmitic acid), 9-E-Hexadecenoic acid, Tetra tetracotane, 4,8,12,16 tetramethylheptadecane-4-olide, n-heptadecanoic acid , 1-iodohexadecane, stearic, oleic, erucic acids along with Di-n-octyl phthalate were noted as the major constituents.

MATERIALS AND METHODS

Plant material used in this study was collected from local market Pune, India. It was authenticated at Agharkar Research Institute, Pune, India. Its authentication number is 14319. Air shade dried powdered bark material (50 g) was extracted with hexane (200 ml) using soxhlet extractor for 12 hours. The solvent was collected under reduced pressure to yield the crude sticky oily semisolid mass.

GC-MS Analysis

The extract thus obtained was analyzed using GC-MS technique. Gas chromatography analysis was performed by Agilent 6890N with FID using HP-5 capillary column. GC-MS analysis was performed using a Shimadzu QP 5050A mass spectrometer coupled with a Shimadzu 17A gas chromatograph fitted with a split-split less injector and a DB-5 fused silica capillary column (30m X 0.25 mm i. d., 0.25 µm film thickness). Helium was used as a carrier gas at a flow rate of 1.0 ml/min. The injection port was maintained at 250^{o}C, and the split ratio was 40:1.

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Oven temperature programming was done from 50 to 280°C, at10°C/min, and it was kept at 280°C for 5 min. Interface temperature was kept at 250°C. Ionization mode was electron impact ionization and the scanning range was from 40 amu to 400 amu. Mass spectra were obtained at 0.5 sec. interval. The spectra of the compounds were matched with NIST and Wiley's library. Their structures were defined by the % similarity index. The presence of compounds was confirmed by conventional genesis.

RESULTS AND DISCUSSION

Organic materials are products of the secondary metabolism of plants, and are generally consisting of complex mixtures of mono-, sesqui-, di-, tri-terpene hydrocarbons, and oxygenated materials biogenically. Solvent extraction of bark sample using hexane yielded 1.3% of organic matter. Use of GC-MS enabled identification of chemical constituents present in it. Some of the compounds identified are listed (**Table: 1)**.

Eleven compounds from n-hexane extract are identified by GC-MS for the first time from the stem bark of *Juglans regia* L. The spectra of the compounds are matched with NIST and Wiley libraries. Their structures are identified by the percentage similarity values (**Table**--). They are confirmed by the classical fragmentation pattern, base peaks, some intense peaks and molecular ion peaks of the compounds.

The GC pattern of the n-hexane extract shows a complex nature. The GC-MS study of thirteen major peaks reveal the presence of hydrocarbons(**1**&**4**), aliphatic -- saturated (**2**, **6**, **8**) and unsaturated acids (**3**, **9**, **10**), alkyl halide (**7**), cyclic ester-lactone (**5**) and aromatic ester (**11**). (**Table: 1**).

The detected compounds are n-octedecane ($C_{18}H_{36}$-**1**), n- hexadecanoic acid – palmitic acid ($C_{16}H_{32}O_2$-**2**),9-E-Hexadecenoic acid ($C_{16}H_{32}O_2$-**3**), Tetratetracotane (C44H90-**4**),4,8,12,16 tetramethylheptadecane-4-olide (C21H40O2-**5**), n-heptadecaqnoic acid, (C17H34O2-**6**), 1-iodohexadecane (C16H33I-**7**), stearic acid (C18H36O2-**7**), oleic acid (C18H34O2-**8**), erucic acid (C22H42O2-**9**), Di-n-octyl phthalate (C24H38O4-**10**).

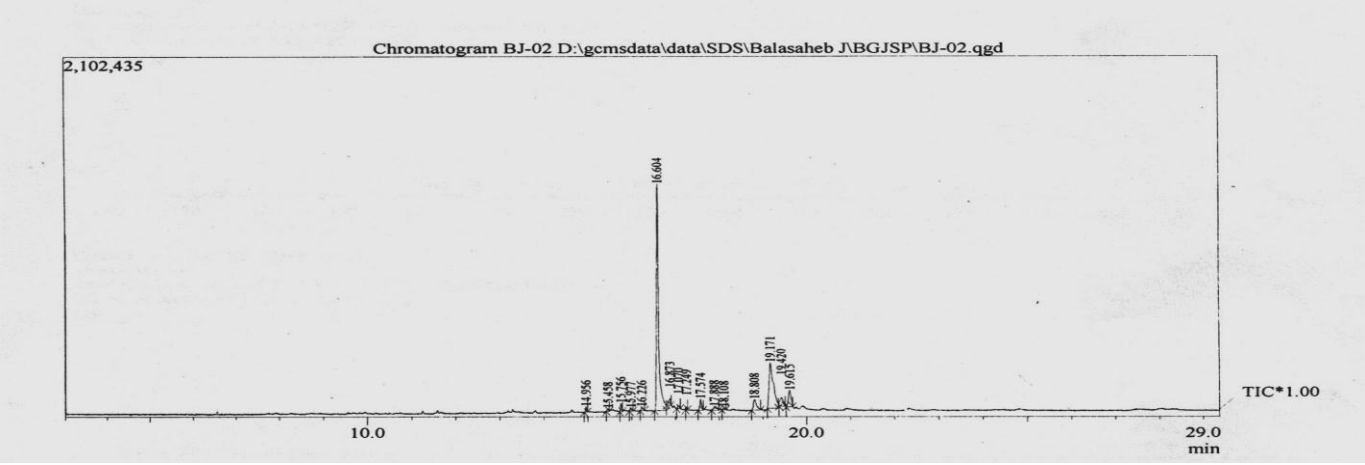

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Table 1: GC-MS Analysis of Hexane Extract

Sr No.	Molecular formula	Retention Time(min)	Base peak (amu)	$[M]^+$ peak (amu)	Name of compound	% S. I.
1	$C_{18}H_{38}$	16.23	57	254	n-octadecane	91
2	$C_{16}H_{32}O_2$	16.60	43	256	Palmitic acid	95
3	$C_{16}H_{30}O_2$	16.88	55	254	9-E-hexadecanoic acid	91
4	$C_{44}H_90$	17.07	57	618	Tetra-tetracontane	88
5	$C_{21}H_{40}O_2$	17.25	99	324	4,8,12,16----olide	83
6	$C_{17}H_{34}O_2$	17.58	43, 73	270	n-heptadecanoic acid	93
7	$C_{16}H_{33}I$	18.10	57	352	1-iodohexadecane	83
8	$C_{16}H_{34}O_2$	18.81	43	284	Stearic acid	95
9	$C_{18}H_{34}O_2$	19.17	55	282	Oleic acid	95
10	$C_{22}H_{42}O_2$	19.42	55	338	Erucic acid	92
11	$C_{24}H_{38}O_4$	19.62	149	390	Di-n-octyl Phthalate	94

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