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Author(s): R. Habib
A. Mohyuddin
Z. Khan
T. Mahmood

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Analysis of Non-polar Chemical Profile of *Melia Azedarach* L.

Habib, R.[1], Mohyuddin, A[1]., Khan, Z[2]. and Mahmood, T[3].

[1]Department of Chemistry, University of Management and Technology, Lahore

[2] Department of Botany, GC University, Lahore

[3]Central Research Lab, Lahore College for Women University, Lahore

[*]rabiraho@gmail.com,

Abstract

Medicinal plants are conventionally used for the treatment of various diseases due to their world-wide occurrence and least side effects. Melia azedarach L. belongs to the family Meliaceae, is a highly significant medicinal plant. Extracts of M. azedarach obtained from its different parts such as seed, fruit, flower, leaf, and young branches are reported to exhibit antifungal, antihelmintic, nematicidal, diuretic, cytotoxic, antiproliferative, insecticidal and antioxidant activities. Thus the aim of this study was to explore the chemical profile of non-polar extract of M. azedarach leaves through GC-MS analysis. The identification of phytochemical compounds is based on molecular ion peak, base peak, and fragmentation pattern. GC-MS analysis of hexane extract of M. azedarach showed a highly complex profile, containing ketones, ethers, fatty acid derivatives, methyl esters, 1,3-dipalmitate, 7,8-dihydrocarpesterol, and 2-Undecanol. This study will be useful to explore the active components of medicinal plants and *can validate their medicinal value*.

Keywords: *Melia azedarach L., Meliaceae, GC-MS method, Phytochemical compound.*

Introduction

There is an increasing demand for the herbal medicine to cure variety of diseases as these medicines are efficient and safe without any side effects as compared to synthetic drugs [1]. For this purpose, scientist, botanists, chemists, and pharmacists all over the world are working on herbal medicines [2]. *Melia azedarach* Linn. *(M. azedarach)* commonly known as chinaberry or Persian lilac tree is a plant species of the family *Meliaceae* that contain 45 genus and 750 species. It is deciduous tree of medium sized that grows to a height of five to fifteen meter tall and thirty to sixty cm in diameter. It can be grown successfully in a wide variety of situations even in alkaline soil where other trees might fail to grow. It is native to Indochina, Pakistan, India, Australia, and Southeast Asia. It is cultivated in most of the countries located in tropics and subtropical region. This plant has long been known as an insecticidal and medicinal plant all over the world due to its world-wide availability and fewer side effects. Extracts of *M.*

azedarach obtained from its different parts such as seed, fruit, flower, leaf, and young branches have been used for the treatment of diabetes, malaria, intestinal worms, cough, nausea, vomiting and paroxysmal fever, and skin disease [3-5].

GC-MS has been known as a powerful technique for providing metabolic profiling of plants [6-9]. Hexane extracts of many species of *Meliaceae* family are analyzed by this technique but there has been no report on GC-MS of hexane extract of *M. azedarach* or any species of genus Melia. *Non polar profile of M. azedarach* has not been explored yet. Therefore, the aim of the study was to explore the chemical profile of non-polar extract of *M. azedarach* leaves through GC-MS analysis.

2. Material and Methods

2.1. Preparation of plant extract: The fresh leaves of plant *M. azedarach* was collected from the Botanical Garden of Government College University, Lahore and washed individually to remove impurities and dried under shade. Dried leaves were crushed into fine powder using a grinder.

The dried plant powder were weighed and dipped in a hexane and left for seven days. The hexane extract was then filtered. The hexane extract was concentrated under reduced pressure and low temperature in a Rotary Evaporator. The semi solid extract were obtained.

2.2. Phytochemical analysis of plant extract: Phytochemical analysis were carried out by employing standard procedures to sort out the presence of flavonoids, alkaloids, tannins, saponins and steroids in hexane extract of *Melia azedarach* [10, 11].

2.3. Preparation of sample for GC/MS analysis: A measured amount of semi solid was re-dissolved in *n*-hexane of GC grade and micro-filtered to prepare the samples of 5.0 mg /10 mL.

2.4. Gas Chromatography Mass Spectrometry analysis: A GC-MS analysis was carried out on a Shimadzu GCMS-QP2010A system in EI mode (70 eV) with DB-5MS capillary column (30 m × 0.25 mm i.d., film thickness: 0.25 μm, J and W scientific, Fulsom, CA, USA.1 μL of samples were injected at 250 ºC with a split ratio of 50/50 under electronic pressure to maintain a constant flow (0.67 mL/min) of the helium carrier gas. The oven temperature was programmed from 150 ºC for 4 min and heated to 300 ºC at a rate of 3 ºC/min and kept constant at this temperature for 2 min. The mass spectrometer was set to scan the mass range 40-600 amu with ion source temperature 200 ºC and interface temperature was 250 ºC. Analyses were performed in triplicate with a blank run after every analysis. The resulting data was interpreted by using Shimadzu Lab Solution, GC-MS Postrun analysis software. Compounds were identified by comparing the ions

fragmentation pattern of mass spectra with those of known compounds stored in NIST 147 and NIST 127 libraries.

3. Results and Discussion

The genus *Melia* is famous for the presence of liminoids and terpenoids that are water-insoluble plant components [12]. Previous researches have proved that these compounds have diverse effect against pathogens. Moreover, non polar profile of *M. azedarach* has not been explored yet. The waxy coating of leaves can only be dissolved by non polar solvent because waxes are non polar in nature. Therefore, the leaves of *M. azedarach* were extracted with n-hexane in order to isolate the wax.

3.1. Phytochemical analysis of plant extract: The phytochemical analysis was carried out and it was seen that alkaloids and flavonoids are absent in hexane extract of *M. azedarach*, whereas it contain tannins, terpenes, quinines, saponins and steroids as given in Table1.

Table1.
Phytochemical Analysis

Experiment	Observation	Inference
Test for Alkaloids		
Mayer's test	No yellow ppt.	Alkaloids absent
Wagner's test	No reddish brown ppt. No cloudy appearance	Alkaloids absent
Dragendorff test	Bluish green ppt.	Alkaloids absent
Test for Tannins		
5ml extract + 6 drops $FeCl_3$	Yellow floresence	Tannins present
Test for Coumarins		
plant extract+ 1N NaOH+ Pass through Uvlight	yellow florescence is not produced	Coumarins present
Test for Flavonoids		
Plant extract +2ml $AlCl_3$	Persistent froth	Flavonoids absent
Test for Saponins		
Shook the aqueous plant vigorously	Violet color	Saponins present
Test for Steroids		
Leibermann-Burchard test	Red color	Steroids present
Test for Anthraquinon		
Plant extract + few drops N,N-dimethylanaline		Anthraquinonses present

3.2. Gas Chromatography Mass Spectrometry analysis: Gas Chromatography and Mass spectroscopy analysis of compounds was carried out in n-hexane leaf extract of *M. azedarach* (Figure1).

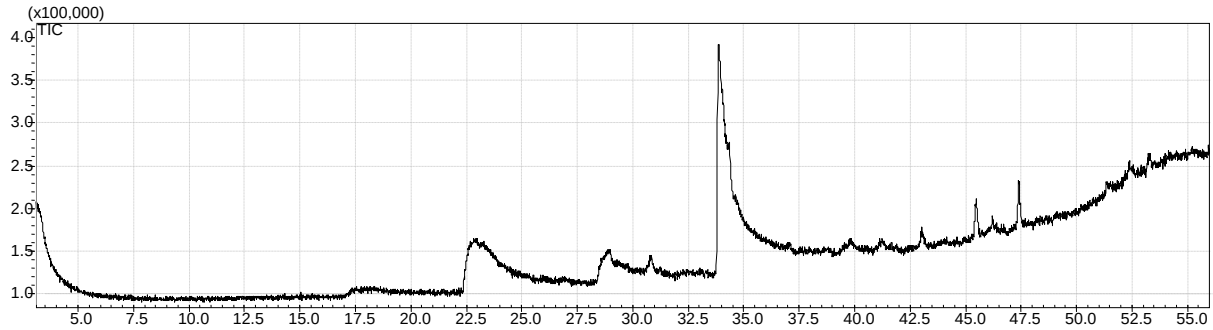

Figure1. GC chromatogram of *n*- hexane extract of *M. azedarach* leaves

Different compounds were identified in the hexane extract of *M. azedarach* on the basis of retention time, molecular ion peak, base peak, and mass fragmentation pattern. Eight peaks from non-polar plant extract were identified as: 2-Undecanol, Methyl 4, 6-decadienyl ether, 13-Docosenoic acid, 7, 8-Dihydrocarpesterol, Glutaric acid, dimethyl ester, Nonanoic acid, 1, 2, 3-propanetriyl ester, Glycerol 2-acetate 1, 3-dipalmitate and Docosenoic acid, 1 methyl-butyl ester as shown in Table2.

Table2.
Compounds from hexane extract of *M. azedarach*

Name of compound	Retention Time (mins)	Base Peak (m/z)
2-Undecanol	22.39	45
Methyl 4, 6-decadienyl ether	22.45	45
13-Docosenoic acid	33.88	55
7, 8-Dihydrocarpesterol	45.48	414
Glutaric acid, dimethyl ester	47.24	133
Nonanoic acid, 1, 2, 3-propanetriyl ester	52.09	151
Glycerol 2-acetate 1, 3-dipalmitate	52.18	40
Docosenoic acid, 1 methyl-butyl ester	54.90	44

2-Undecanol was eluted at 22.39 min. Base peak of the compound was observed at m/z 45. The fragment ions observed m/z 40, 60, 83, 97, 150. The fragment ion observed at m/z 83 was due to the loss of -CH2 group from molecular ion (M+). Methyl 4, 6-decadienyl ether was eluted at 22.45 min. Base peak of the compound was observed at m/z 45. The fragment ions observed m/z 41, 56, 70, 84, 150. The fragment ion observed at m/z 70 was due to the loss of -CH2 group from M+. 13-Docosenoic acid was eluted at 33.88 min. Base peak of the compound was observed at m/z 55. The fragment ions observed m/z 69, 83, 97, 185, 207, 320. The fragment ion observed at m/z 83 was due to the loss of -CH2 group from M+. 7, 8-Dihydrocarpesterol was eluted at 45.48 min. Base peak of the compound was observed at m/z 441. The fragment ions observed at m/z 91,

133, 147, 191, 208, 335, 441. The fragment ion observed at m/z 191 was due to the loss of -OH group from M+. Glutaric acid, dimenthyl ester was eluted at 47.24 min. Base peak of the compound was observed at m/z 133. The fragment ions observed m/z 40, 69, 83, 193, 209, 281.The fragment ion observed at m/z 40 was due to the loss of -C2H5 group from M+. Nonanoic acid, 1, 2, 3-propanetriyl ester was eluted at 52.09 min. Base peak of the compound was observed at m/z 151. The fragment ions observed m/z 44, 68, 85, 100, 169, 207, 281, 335. The fragment ion observed at m/z 68 was due to the loss of -OH group from M+. Glycerol 2-acetate 1, 3-dipalmitate was eluted at 52.18 min. Base peak of the compound was observed at m/z 40. The fragment ions observed m/z 68, 100, 169, 209, 267, 335, 441. Docosenoic acid, 1 methyl-butyl ester was eluted at 54.90 min. Base peak of the compound was observed at m/z 44. The fragment ions observed m/z 77, 101, 137, 150, 208, 341. The fragment ion observed at m/z 177 was due to the loss of -CH3 group from M+.

Conclusion

Traditionally, medicinal plants are largely used to cure many diseases owing to their least side effects. *M. azedarach* L. belongs to potent medicinal plant family *Meliaceae*. This was the first report on GC-MS of Hexane extract of *M. azedarach.* The results showed that non polar extract of the plant contained a variety of compounds such as ketones, ethers, fatty acid derivatives, methyl esters, 1,3-dipalmitate, 7,8-dihydrocarpesterol, and 2-Undecanol. This will contribute in completing the chemical profile of *M. azedarach.*

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