International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 19-08-2021, Accepted: 05-09-2021, Published: 20-09-2021
Volume 6, Issue 5, 2021, Page No. 1550-1555

GC-MS analysis of phytocomponents in the ethanolic extract of *Azadirachta indica* A. Juss (Neem) flower

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Abstract
Neem is a medicinal plant traditionally used for the cure of diabetes, leprosy, fever and respiratory ailments. The present study was undertaken to explore the possible bioactive compounds found in *Azadirachta indica* A. Juss which have been analysed using Gas Chromatography–Mass Spectrometry analysis. The mass spectra of the compounds discovered were matched to the National Institute of Standards and Technology (NIST) library. The results revealed the presence of twenty major compounds in the ethanolic flower extract which are compound are dihydroxy-Propanedioic acid (3.6257%), Glyceraldehyde (3.5567%), Erythritol (0.8183%), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (0.3838%), 2 Pentadecanone, 6,10,14-trimethyl- (0.1734%), n-Hexadecanoic acid (2.1955%), Hexadecanoic acid, ethyl ester (0.5283%), Linoleic acid ethyl ester (0.1385%), Ethyl Oleate (0.2051%), Tetracosane (0.1311%), Tetracontane (0.6984%), Eicosane (0.2829%), 2-Methyltetracosane (0.6592%), Hexatriacontane (2.5669%), n- Hexadecanoic acid (2.1955%), 2-hydroxy-1-(hydroxymethyl)ethyl ester (0.6974%), beta.-Sitosterol (3.3581%), Nonacosane (0.1475%), Tetrapentacontane (11.5463%), Dotriacontane (1.7905%).

Keywords: GC-MS, *Azadirachta indica* A. Juss., flower, phytocomponents

Introduction
Neem (*Azadirachta indica* A. Juss) is a native plant of India, belongs to the family Meliaceae. It is found all over the world and can be grown in tropical and subtropical countries (Sharma and lokeshwar, 2011) [29]. *Azadirachta indica* usually, known as margosa or neem (Parrotta and Chaturvedi, 1994) [24]. The genus including two species of *Azadirachta*. *Azadirachta excelsa and Azadirachta indica. Azadirachta indica* is native to India, Bangladesh, Thailand, Nepal and Pakistan (Prashanth and Krishnaiah, 2014) [26]. It has alternate, usually pinnate leaves, without stipules and syncarpous, bisexual flowers (Subapriya and Nagini, 2005) [31]. The flowers are pentamerous, small, delicate, whitish-pink blooms and produced on axillary cymose panicles. Flower buds bloom throughout the afternoon and evening leaving a rich aroma in the evening. The nectar-producing flowers, which are around 5 mm long, have a fragrant jasmine-like aroma and generate ample quantities of nectar (Loke *et al*., 1992) [18].

In India, Neem is recognized as “the village pharmacy” because of its therapeutic usefulness. Because of its therapeutic characteristics, the tree has been employed in Ayurvedic medicine. In Sanskrit, neem is known as 'arista,' which means 'perfect, complete, and imperishable. Bitter substances may be found in the fruit, seeds, oil, leaves, roots, bark, and almost every other part of the tree (Koul *et al*., 1990) [14]. The many components of this tree have several functions, give Neem the Sanskrit name "sarva roga nivarini," which means as "curer of all ailments." Each part of the neem tree has medicinal properties and may thus be exploited economically (Biswas *et al*., 2002) [7].

Siddha and Ayurvedic practitioners used neem products as an anthelmintic, antidiabetic, contraceptive and sedative. In ayurvedic herb, neem was also used for baths (Ogbuewu and Odoemenam 2011) [23]. The leaves, flowers, and bark, seeds of *Azardirachta indica* A. Juss have a wide range of pharmacological action and are utilised as raw materials for pesticides, medicines, and other goods (Aromdee and Sriubolmas, 2006) [2]. Both the bark and the flower of the neem tree have hypoglycemic properties, however the floral extract has a greater hypoglycemic agent than the bark (Purohit and Dixit, 1991) [27]. The flowers and seed oil of the *Azardirachta indica* A. Juss were used for antioxidant properties (Nahak and Sahu, 2011) [21].

Blood morbidity, biliary afflictions, itching, skin ulcers, burning sensations, and phthisis are all cured by utilizing the bark, leaf, root, flower, and fruit (Badam *et al*., 2004) [5]. Neem flowers are also effective in the fields of medicine, food, and pharmaceuticals (Aromdee and Sriubolmas, 2006) [2]. Neem flowers have recently been considered to have high chemopreventive potential in rats, inhibiting carcinogen-induced liver and mammary gland carcinogenesis (Tepsuwan *et al*., 2002) [33]. The extract of neem flower possesses Bile suppression, elimination of intestinal worms and phlegm properties (Biswas *et al*., 2002) [7]. The administration of neem flowers to rabbits resulted in hypolipidaemia, Glutathione S-transferasse activity in the liver was significantly increased when Neem flowers were consumed (Kusamran *et al*., 1998) [17]. Rat Liver Micronucleus Assay was used to assess the clastogenic and anticlastogenic potential of *Neem* Flower Extract (Kupradinun *et al*., 2013) [16].

The young flowers of the neem plant are a bitter tonic which are often consumed. The flowers are also used for treatment of fever (Kumara *et al*., 2021) [15]. The flowers are used in vitiated pitta (body heat balance) and kapha conditions (cough formation). Flowers are non-toxic and astringent. (Bhowmik *et al*., 2010) [6]. Recent studies show that neem flowers are also having the biological activities, they are hypocholesterolemic, antioxidant, Immunostimulatory (Kumara *et al*., 2021) [15]. Neem tree contains more than 100

bioactive ingredients (Nahak and Sahu, 2010) [20]. With this views, the present study deals with the GC-MS analysis of phytocomponents in the ethanolic extract of the flowers of *Azadirachta indica* A. Juss.

A B C

Fig 1: *Azadirachta indica* A. Juss. A. Habit B. flowering twig C. Flower

Materials and Methods

Collection of the plant material

The fresh *Azadirachta indica* A. Juss. flowers were collected from Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, in September 2018. The plant material was identified and authenticated by Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad.

Preparation of Flower powder and extract

The flowers of *Azadirachta indica* A. Juss. were washed thoroughly in running tap water to remove dust particles and adhered debris and finally washed with sterile distilled water and shade dried for 12 days. Sample of the material was ground separately into fine powder using an electric grinder. Plant material (Flower 10 Gms) was extracted with 250 mL of Ethanol at 60^0C for 8 hrs in soxhlet extractor. The ethanolic extracts were filtered through Whatmann No. 1 filter paper. The filtrate was evaporated to dryness at room temperature and stored until further analysis.

Preparation of stock solution

The extracts was reconstituted in Ethanol. Ethanolic extract (1 µl) was injected for GC-MS analysis

Gas Chromatography-Mass Spectrometry analysis

The ethanolic extract of the Flower of *Azadirachta indica* A. Juss. were subjected to GC-MS analysis on a GC- MS system (GC-2010 plus) Shimadzu, (Agilent Technologies Inc.,) equipped with an HP-5 MS capillary column (30 m × 0.25 mm, 0.25 mm, (Agilent Technologies Inc.,). The injection volume of sample was 1 µL. Helium (99.999%) was used as the carrier gas at a flow-rate of 1 mL/min. The temperature of the injection port was 250 ◦C, and the column temperature program was as follows: 50 ◦C for 2 min, followed by an increase to 180 ◦C at a rate of 5 ◦C/min, an increase to 270 ◦C at a rate of 20 ◦C/min, and maintenance at 270◦C for 5 min. The MS (QP-2020) conditions included an EI ion source temperature of 230 ◦C, an ionization energy of 70 eV, and a mass scan range of 40–500 amu. Mass spectra were taken at 70 eV; a 0.5 seconds of scan interval and fragments from 40 to 550 Da. Total GC running time was 40 minutes.

Identification of Compounds

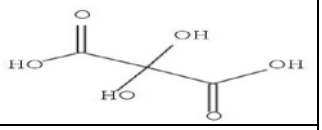
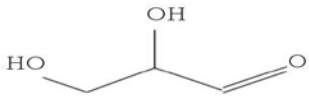
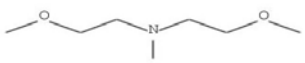
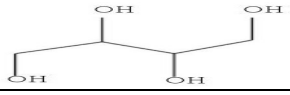
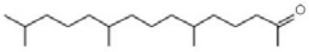
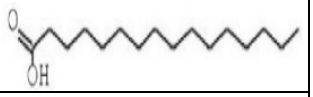
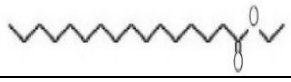
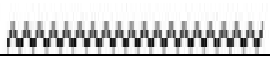
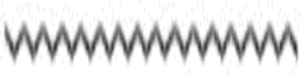
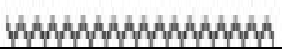
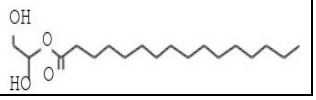
Analysis of the mass spectrum the GC-MS was carried out with the use of the National Institute of Standards and Technology's database, which has over 62,000 patterns. The NIST library contains a spectrum of known components. The components of the test materials were identified by their name, molecular weight, and structure.

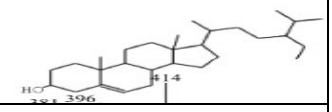
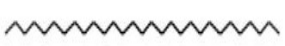
Results and discussion

The results pertaining to GC-MS analysis led to the identification of a number of compounds from the GC fractionations of the ethanolic extract of *Azadirachta indica* A. Juss flowers. These compounds were identified by mass spectrometry attached with GC. The results of the present study were tabulated in Table 1. The compound prediction is based on National Institute Standard and Technology Database. Table 2 Listed the major phytocomponents and its biological activities obtained through GC-MS study of *Azadirachta indica* A. Juss flowers. The results revealed that the presence of Propanedioic acid, dihydroxy- (3.6257%), 2,2'-Bioxirane (4.0598%), Glyceraldehyde (3.5567 %), Ethanamine, 2-methoxy-N-(2-methoxyethyl)-N-methyl- (3.5567%), Erythritol (0.8183%), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (0.3838%), 2-Pentadecanone, 6,10,14-trimethyl- (0.1734%), n-Hexadecanoic acid (2.1955%), Hexadecanoic acid, ethyl ester (0.5283%), Linoleic acid ethyl ester (0.1385%), Ethyl Oleate (0.2051%), Tetracosane (0.1311%), Tetracontane (0.6984%), Eicosane (0.2829%), 2-Methyltetracosane (0.6592%), Hexatriacontane (2.5669%), n- Hexadecanoic acid (2.1955%), 2-hydroxy-1-(hydroxymethyl)ethyl ester (0.6974%), beta.-Sitosterol (3.3581%), Nonacosane (0.1475%), Tetrapentacontane (11.5463%), Dotriacontane (1.7905%). The spectrum profile of GC-MS confirmed the presence of 22 major components with the retention time 3.6257, 4.0598, 1.829, 3.5567, 0.8183, 0.3838, 0.1734, 2.1955, 0.5283, 0.1385, 0.2051, 0.1311, 0.6984, 0.2829, 0.6592, 2.5669, 0.6974, 3.3581, 0.7297, 0.1475, 11.5463, 1.7905 respectively **(**Figure 2**)**.

These phytochemicals are responsible for various pharmacological actions like antimicrobial, *anti-helicobacter pylori* activity and *anti-candida* activity, antioxidant, antifertility, antimutagenic, anti-inflammation, anticancer, hepato-protective, diuretic, antiasthma activities.

Table 1: Phytocomponents identification in ethanolic extract of *Azadirachta indica* A. juss. flower by GC-MS.

Peak no.	R.T.	Name of Compound	MW	MF	Peak area (%)	Compound Structure
1.	1.550	dihydroxy-Propanedioic acid,	136	$C_3H_4O_6$	3.6257	
2.	1.670	2,2'-Bioxirane	86	$C_4H_6O_2$	4.0598	
3. 1	1.830	Glyceraldehyde	90	$C_3H_6O_3$	3.5567	
4. 2	1.940	Ethanamine, 2-methoxy-N-(2-methoxyethyl)-N-methyl-	147	$C_7H_{17}NO_2$	3.5567	
5. 5	4.000	Erythritol	122	$C_4H_{10}O_4$	0.8183	
6. 6	4.090	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	144	$C_6H_8O_4$	0.3838	
7. 7	12.940	2-Pentadecanone, 6,10,14-trimethyl-	268	$C_{18}H_{36}O$	0.1734	
8. 8	14.545	n-Hexadecanoic acid	256	$C_{16}H_{32}O_2$	2.1955	
9. 9	14.985	Hexadecanoic acid, ethyl ester	284	$C_{18}H_{36}O_2$	0.5283	
10. 10	17.450	Linoleic acid ethyl ester	308	$C_{20}H_{36}O_2$	0.1385	
11. 11	17.550	Ethyl Oleate	310	$C_{20}H_{38}O_2$	0.2051	
12. 12	19.035	Tetracosane	338	$C_{24}H_{50}$	0.1311	
13. 13	19.605	Tetracontane	562	$C_{40}H_{82}$	0.6984	
14. 14	21.150	Eicosane	282	$C_{20}H_{42}$	0.2829	
15. 15	22.115	2-Methyltetracosane	352	$C_{25}H_{52}$	0.6592	
16. 16	22.680	Hexatriacontane	506	$C_{36}H_{74}$	2.5669	
17. 17	22.845	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	330	$C_{19}H_{38}O_4$	0.6974	

18. 18	22.975	beta.-Sitosterol	414	$C_{29}H_{50}O$	3.3581	
19. 19	25.125	Nonacosane	408	$C_{29}H_{60}$	0.1475	
20. 20	25.675	Tetrapentacontane	758	$C_{54}H_{110}$	11.5463	
21. 21	27.125	Dotriacontane	450	$C_{32}H_{66}$	1.7905	

Abbreviation R.T. - Retention time, M.W. - Molecular weight, M. F. - Molecular formula

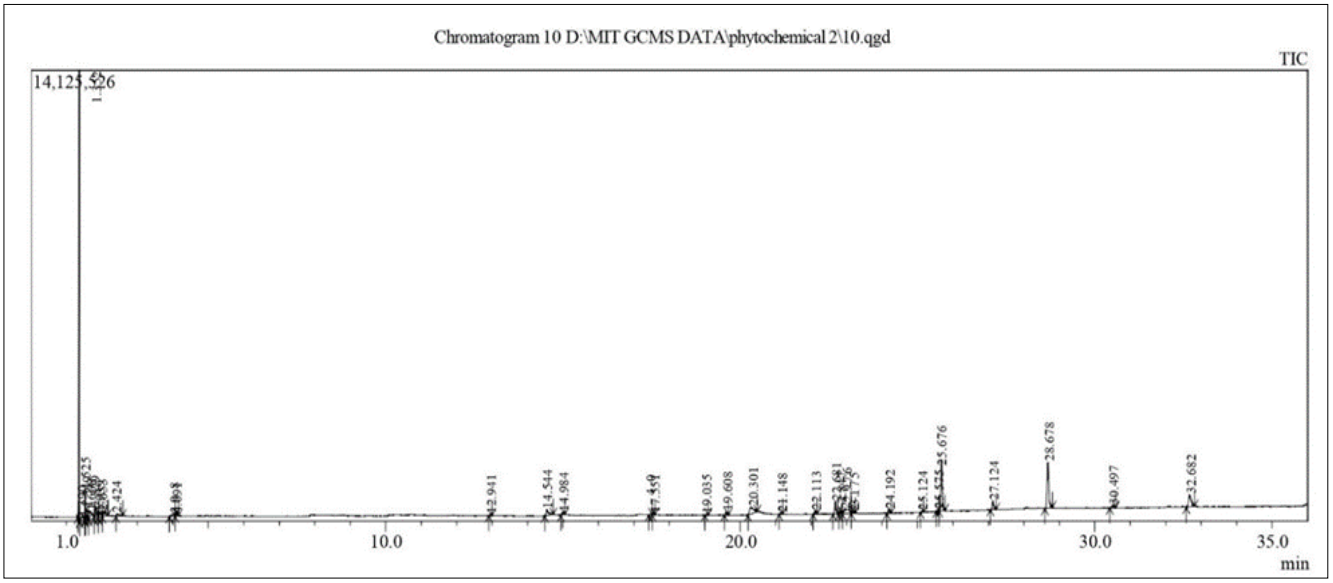


Fig 2: GC-MS Chromatogram of ethanolic extract of the *Azadirachta indica* A. Juss. Flower

Table 2: Activity of Phyto-components identified in the ethanolic extracts of *Azadirachta indica* A. Juss. Flower.

Sr.No.	Compound	Nature of Compound	Biological activity	Literature cited
1	dihydroxy-Propanedioic acid,	Dicarboxylic acids	Anti-Helicobacter pylori activity and Anti-Candida activity	Mohammed *et al*., 2016 [19].
2	Glyceraldehyde	Polyol compound	Preservative antimicrobial	Arockia, 2012 [1]
3	Erythritol	Organic compound	Antibacterial activity	Kadhim *et al*., 2016 [12]
4	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	Ketone	Anti-diabetic and anti-oxidant activity	Hameed *et al*., 2015 [9]
5	2-Pentadecanone, 6,10,14-trimethyl-	Ketone	Antibacterial activity against Gram +ve and Gram-ve bacteria	Nurettin *et al*., 2006 [22]
6	n-Hexadecanoic acid	Fatty acid	Anti-oxidant, Hypocholesterolemic, Nematicide, Anti-androgenic,	Tyagi and Agarwal, 2017 [34]
7	Hexadecanoic acid, ethyl ester	Organic compound	Antioxidant, hypocholesterolemic, flavour, nematicide, pesticide	Parthipan *et al*., 2015 [25]
8	Linoleic acid ethyl ester	Polyunsaturated fatty acid	Antiinflammatory, Cancer preventive Hepatoprotective Nematicide, Insectifuge	Vadivel and Gopalakrishnan, 2011 [35]
9	Ethyl Oleate	Fatty acid ethyl ester	It is used for vehicle for intramuscular drug delivery, Progesterone	Elaiyaraja and Chandramohan, 2016 [8]
10	Tetracosane	Alkane	Antioxiodant and Antibacterial	Huihua *et al*., 2013 [11]
11	Tetracontane	Alkane	Antioxidant and antimicrobial activities	Swamy *et al*, 2017 [32]
12	Eicosane	Fatty acid	cytotoxic properties, Antifungal, Antitumor, Antibacterial,	Arora *et al*., 2017 [3]
13	2-Methyltetracosane	Alkane	Free radical scavenging	Arora *et al*., 2017 [3]
14	Hexatriacontane	Alkane	Free radical scavenging,	Atolani *et al*., 2009 [4]
15	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	Fatty acid	Antioxidant	Arora *et al*., 2017 [3]
16	beta.-Sitosterol	Phytosterols	Antioxidant and antimicrobial	Hidayathulla *et al*., 2018 [10]
17	Nonacosane	Alkane	Antibacterial activity	Rani and Agrawal, 2006

				[28]
18	Tetrapentacontane	Alkane	Hair growth promoter, uric acid production, and arachidonic acid inhibitor	Shunmugapriya *et al*., 2017 [30]
19	Dotriacontane	Alkane	Antimicrobial agent	Kawuri and Darmayasa, 2019 [13]

Summary and Conclusion

The presence of various bioactive compounds justifies the uses of the neem flower for various ailments by local population. However, Individual phytochemical constituents separated from the plant and subjected to pharmacological action, on the other hand, will almost certainly provide positive results. The GC-MS analysis of ethanolic extract showed a number of components. n- Hexadecanoic acid, Tetracosane, Hexadecanoic acid, ethyl ester, Tetrapentacontane Dotriacontane and 2,3-dihydroxypropyl ester are commonly present in all plant parts with higher amount in root followed by stem, leaves and flowers. The n-Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester shows hemolytic, pesticide, antioxidant activity and used for flavor. 2, 3-dihydroxypropyl ester possess antimicrobial and anticancer properties. Ethyl Oleate and Eicosane both are fatty acid in nature, from this study it can be concluded that *Azadirictha indica* A. Juss. may serve as a new potential source of therapeutic drugs due to the presence of numerous important phytochemical bioactive compounds.

Acknowledgements

Junior author are thankful to UGC for awarding the Rajiv Gandhi National fellowship.

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