Research article Available online www.ijrpsonline.com ISSN: 2249–3522

International Journal of Research in Pharmacy and Science

Gas Chromatography - Mass Spectroscopy Analysis of Ethyl Acetate and Ethanolic Extracts of *Cordia obliqua* Willd. Leaves

Jeyaraman Amutha Iswarya Devi, Velayutham Mani Mala* and Narayanan Venkateshan

Department of Pharmaceutical Chemistry, Arulmigu Kalasalingam College of Pharmacy, Anand Nagar, Krishnankoil-626126, Srivilliputtur (via) Tamil Nadu, India

ABSTRACT

To qualitative analysed the chemical composition in ethyl acetate and ethanolic extract from the plant leaves of *Cordia obliqua* Willd, by gas chromatography-mass spectrometry (GC-MS). The shade dried leaves plant powder was extracted with pet. ether, ethyl acetate and ethanol by using Soxhlet extractor was obtained. GC-MS analysis of ethyl acetate and ethanolic extract of leaf showed several active components. Among the identified compounds of ethyl acetate contains 16 phytochemical compounds and ethanol contains 4 phytochemical compounds.GC-MS analysis showed the existence of ethyl acetate extract with different phytochemical compounds are Didodecyl phthalate, 2-Heptadecenal, 9,12-Octadecadienoic acid (Z,Z)-, Cyclobarbital, Trans-2,3-methylenedioxy-b-methyl-b-nitrostyrene, 1,1,1,3,5,5,5-Heptamethyltrisiloxane, 1H-indole, 1-methyl-2-phenyl-, Hexahydropyridine, 1-methyl-4-[4,5-dihydroxyphenyl]-, Benzo [h] quinolone, 2,4-dimethyl-, 2-methyl-7-phenylindole, 2-Ethylacridine, Suprane, 1,4-Benzenediol, 2,5-bis(1,1-dimethylethyl)-, 1-methyl-3-phenylindole, 9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester, 6-Octadecenoic acid. GC-MS analysis showed the existence of ethanolic extract with different phytochemical compounds are 2-(2-(2-(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)ethoxy)ethoxy) acetic acid, Phytol, Benzene, 2-[(tert-butyldimethylsilyl) oxy]-1-isopropyl-4-methyl-, Acetamide, 2-chloro-N-[(5-chloro-8-hydroxy-7-quinolinyl)methyl]-. The studies performed on various parts of this plantconstituents are medicinally important and many have reported biological activities. Thus GC-MS analysis revealed the existence of various types of constituents in *Cordia obliqua* leaf ethyl acetate and ethanol extract fraction which confirm the application of this plant for a number of medicinal activities.

KEYWORDS: *Cordia obliqua*, Ethanolic extract, Ethyl acetate extract, GC-MS analysis, Phytocomponents.

***Corresponding author**

Velayutham Mani Mala

Department of Pharmaceutical Chemistry

Arulmigu Kalasalingam College of Pharmacy

Srivilliputtur (via) Tamil Nadu, India

E-mail id: rammala824@gmail.com

1. INTRODUCTION

Cordia obliqua Willd, Plant belongs to genus *Cordia* and family *Boraginaceae.* Commonly it is known as "Clammy Cherry" and "Lasora" in hindi. It is a medium sized deciduous tree and very vigorous in growth. The ripe fruits are traditionally eat by local tribes and raw fruits are used as pickle. *Cordia obliqua* is otherwise called as "Narivili" in tamil. It is one of the most traditional system of medicine in Ayurvedic and Siddha.

The leaves are useful in ulcers and in headache. The juice of the bark along with coconut oil is given in gripes. The barks and also the unripe fruit are used as a mild tonic. Fruits are used as anthelmintics, astringent, demulcent, diuretic, expectorant in bronchial affections and irritation of urinary passages. The kernals are a good remedy in ringworm. The santals use the powder of the bark as an external application in prurigo. The Japanese use the bark in fever.

The leaf is dorsiventral, xeromorphic and hypostomatic. The midrib is thick and wide and plano-convex in sectional view; the adaxial side is flat and the abaxial part is semicircular. It is 900µm thick and 850µm wide. The epidermal layer is thin and the cells are spindle shaped, thick walled and darkly stained. The ground tissue around the vascular strand is parenchymatous; the cells are circular, thick walled and compact. The trichome is 140µm long. The stomata are 20 × 30µm in sizes. The lamina is 200µm thick. The palisade cells are 80µm in height.

The *Boraginaceae* family consists of about 2,700 species, which are distributed in tropical, sub-tropical and warmer regions around the world. About 300 species of genus *Cordia* have been identified worldwide. There are 13 species of this genus found in India. The *Cordia obliqua* Willd., is a medium-sized deciduous tree, found scattered throughout the mid-Himalayas up to elevations of 1,470 meters, this plant having small fruits is commonly found.

Medicinal plants are the nature's gift to human beings to help them pursue a disease-free healthy life. The world's cultures have an extensive knowledge of herbal medicine. Plants are a valuable source of a wide range of secondary metabolites, which are used as pharmaceuticals, agrochemicals, flavors, fragrances, colors, bio pesticides and food additives. Traditionally the fruit has been used for the treatment of coughs, chronic fever, to remove joint pains and spleen diseases. Natural polysaccharides and their derivatives are widely used in pharmaceutical and food industry as biodegradable and biocompatible polymers for a large number of applications such as binding, thickening, emulsifying and gelling agents, etc.

Gum *Cordia*, an anionic gum obtained from fruits of *Cordia obliqua* Willd (Family: *Boraginaceae*), is one such polysaccharide with potential applications. *C. obliqua*, commonly known as **lassora**, is the medium-size deciduous tree native to Indian subcontinent. The tribal

population traditionally eats the ripe fruits of the plant, while the raw fruits are used as vegetable and for making pickles The seeds of the plant contain anti-inflammatory constituents.

2. MATERIALS AND METHODS

2.1 Collection and preparation of plant material

The leaves plant of *Cordia obliqua* was collected from Tenkasi, Thirunelveli District of Tamil Nadu, India. Taxonomic distinguishing proof was produced using The American College, Madurai, Madurai District, Tamil Nadu. The plant of *Cordia obliqua* and were dried under shadow, segregated, crushed by a mechanical processor and went through a 40 lattice sifter. The plant powdered materials were put away in a hermetically sealed holder. The leaves were shade dried and ground into fine powder. The powdered materials were stored in air tight polythene bags until use.

2.2 Preparation of plant extraction

The leaves powder samples of *Cordia obliqua* were extracted with pet.ether, ethyl acetate and ethanol at temperature between 60-70ºC by using soxhlet extractor. The solvent was evaporated by rotavapor to obtained viscous semi solid masses. The semi dry ethyl acetate and ethanol crude extract from the leaves plant of *Cordia obliqua* analysed by GC-MS, it has led to the identification and characterization of different organic compounds.

3. GC-MS ANALYSIS

From GC-MS analysis 16 active components were detected from ethyl acetate extract and GC-MS analysis 4 active components were detected from ethanol extract. The identification of phytochemical compounds was based on retention time, molecular formula, peak area, molecular weight are presented in Table No: 1,3 and medicinal activity and nature of compound were presented in Table No: 2,4.

3.1. Ethyl acetate extract of Cordia obliqua willd.

Among the identified compounds, 1,4-Benzenediol, 2,5-bis(1,1-dimethylethyl)- was found to be the major compound attained the largest peak (19.03 %) with the retention time (17.622) minutes. Another major compound 2-Ethylacridine having peak area of (17.53 %) with retention time (17.253) minutes.

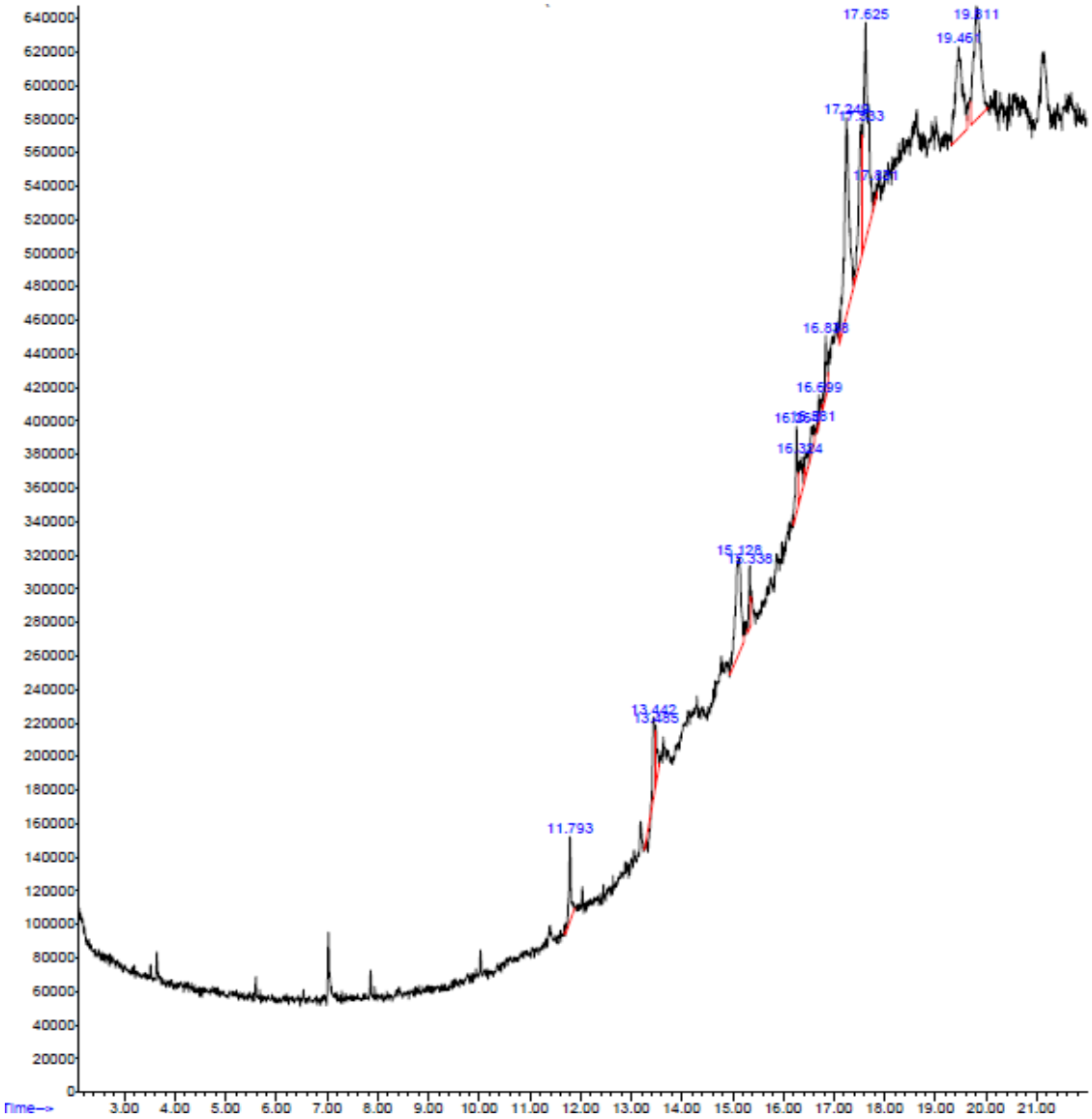

Fig. 1: GC-MS chromatogram of ethyl acetate extract of *Cordia obliqua* willd.

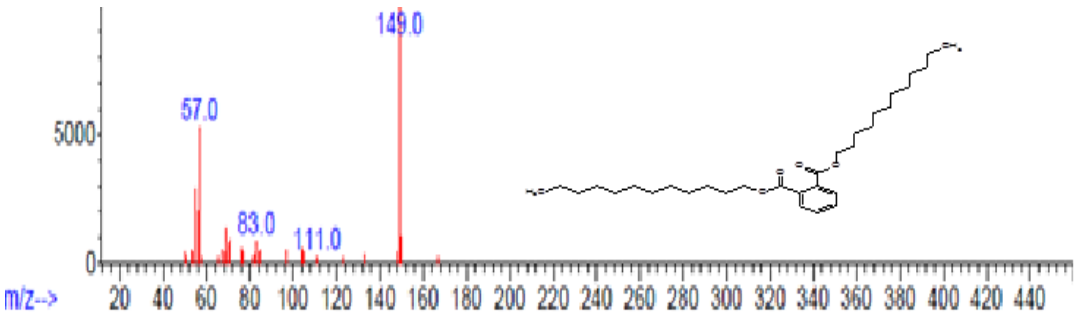

Fig. 1.1: Didodecyl phthalate

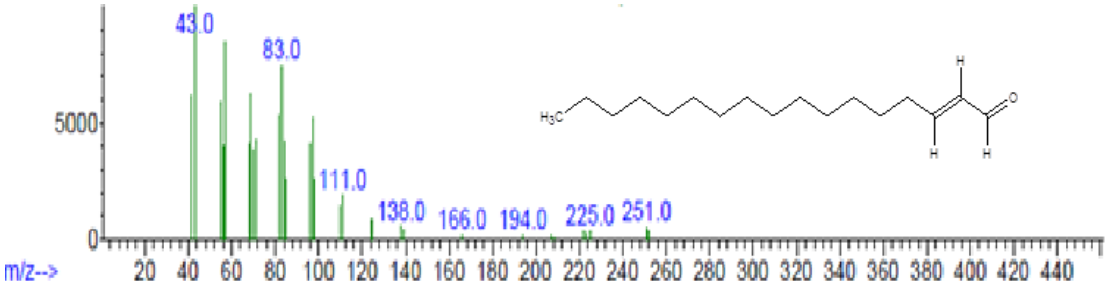

Fig. 1.2: 2-Heptadecenal

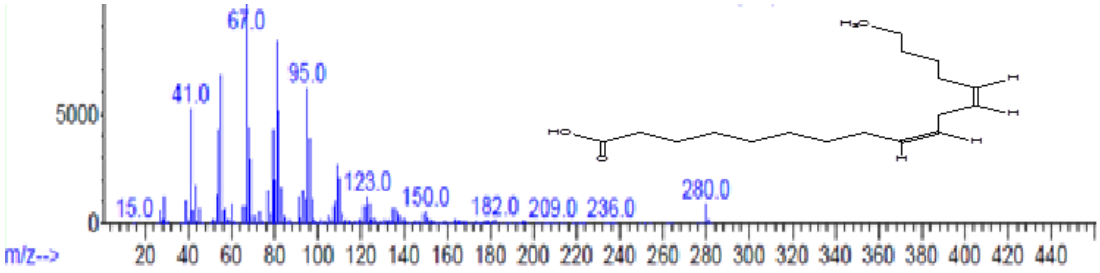

Fig. 1.3: 9, 12-Octadecadienoic acid (Z,Z)-

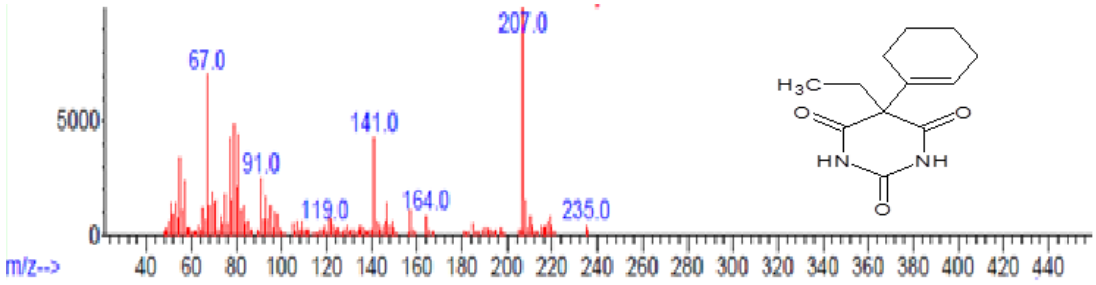

Fig. 1.4: Cyclobarbital

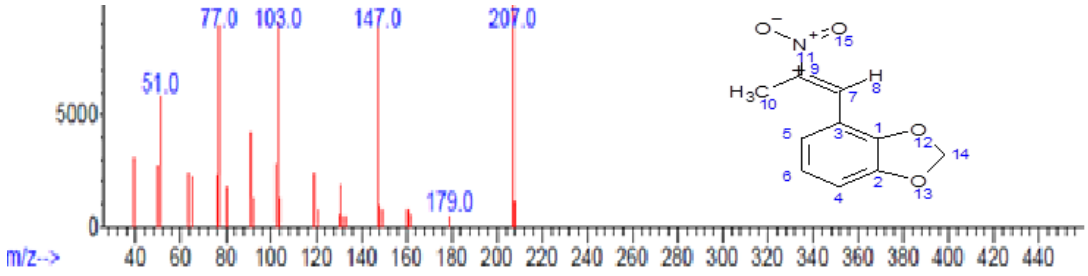

Fig. 1.5: Trans-2,3-methylenedioxy-b-methyl-b- nitrostyrene

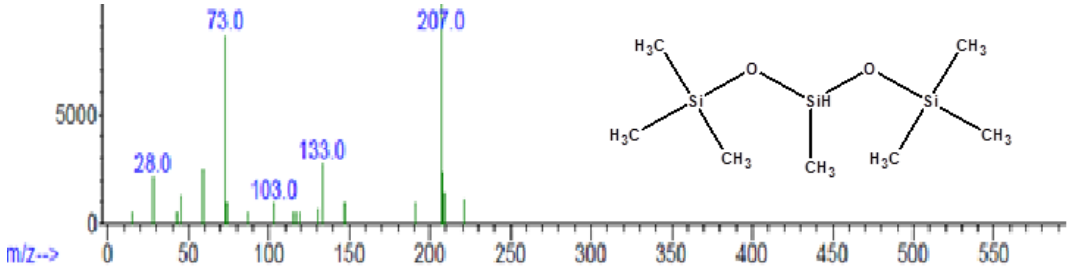

Fig. 1.6: 1,1,1,3,5,5,5-Heptamethyltrisiloxane

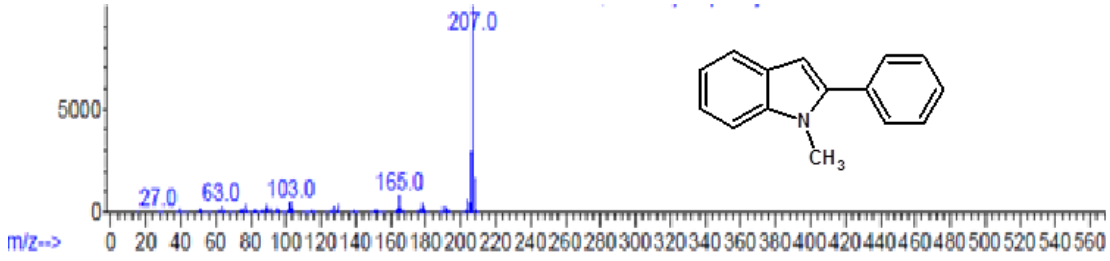

Fig. 1.7: 1H-indole, 1-methyl-2-phenyl-

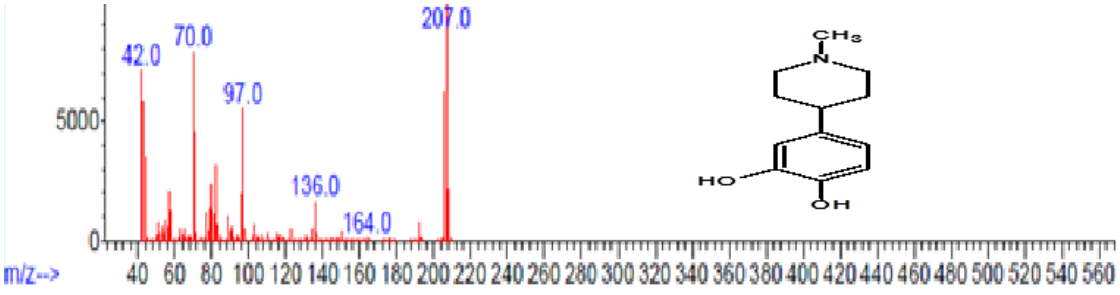

Fig. 1.8: Hexahydropyridine, 1-methyl-4-[4,5-dihydroxyphenyl]-

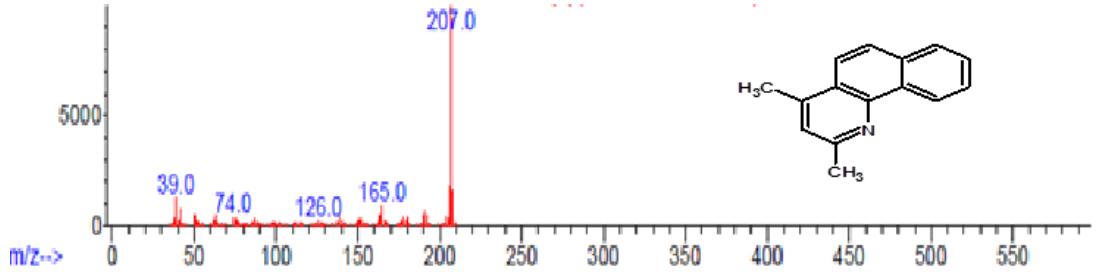

Fig. 1.9: Benzo [h] quinolone, 2,4-dimethyl-

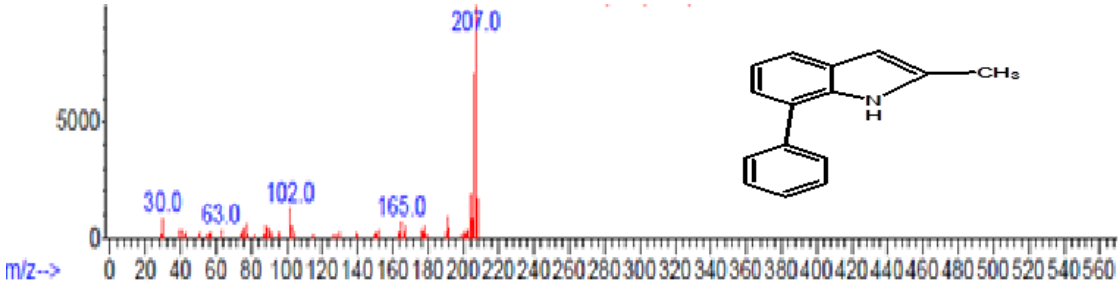

Fig. 1.10: 2-methyl-7-phenylindole

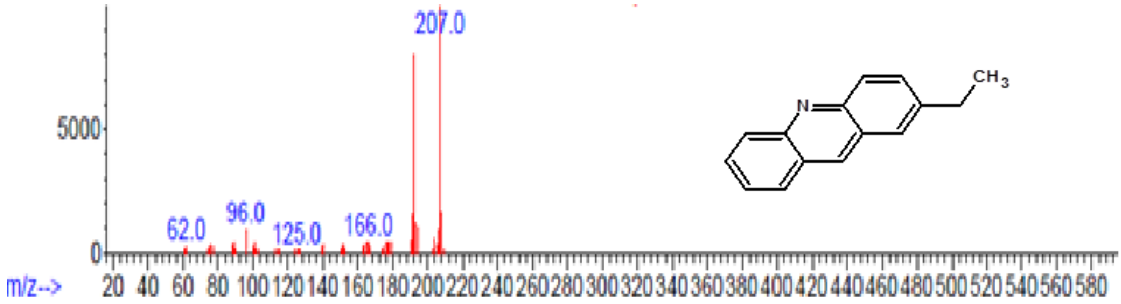

Fig. 1.11: 2-Ethylacridine

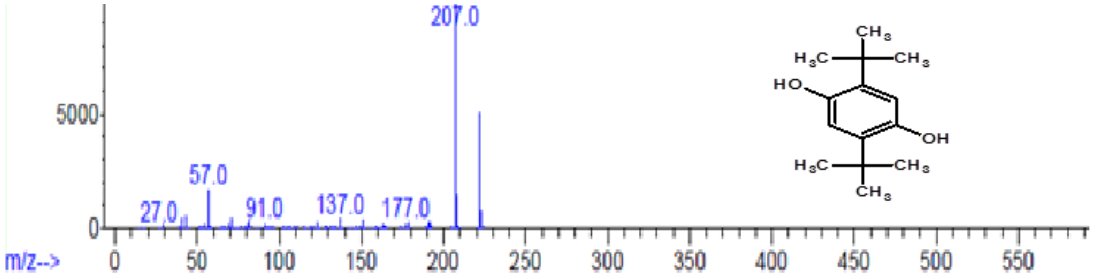

Fig. 1.12: 1,4-Benzenediol, 2,5-bis(1,1-dimethylethyl)-

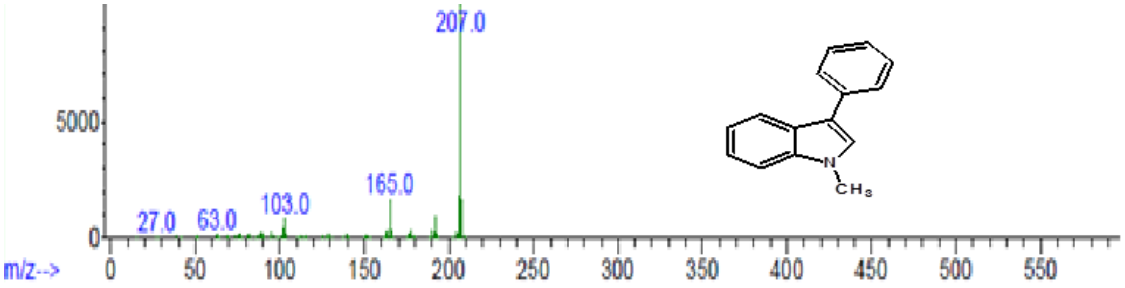

Fig. 1.13: 1-methyl-3-phenylindole

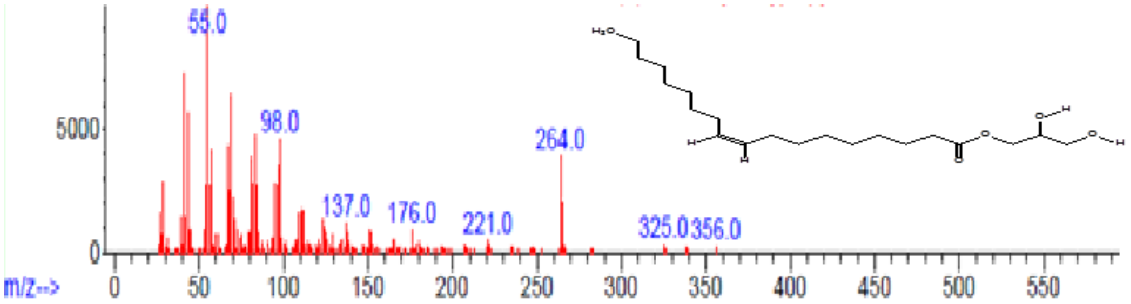

Fig. 1.14: 9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester

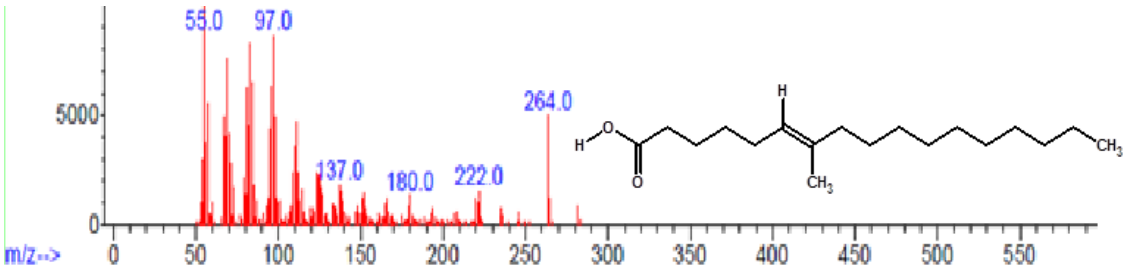

Fig. 1.15: 6-Octadecenoic acid

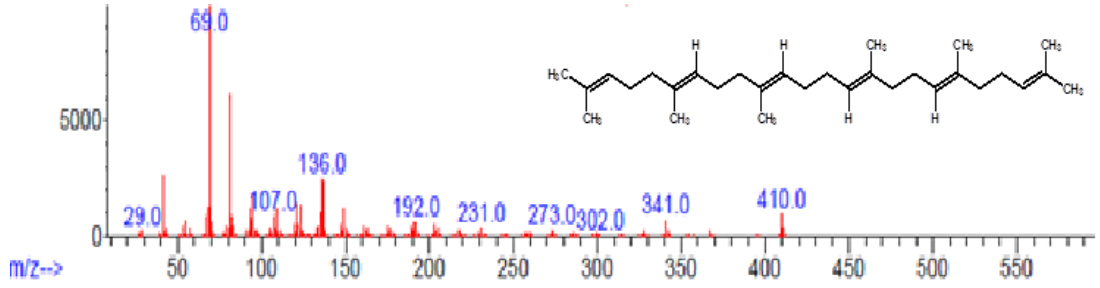

Fig. 1.16: Suprane

Table 1: Chemical composition of ethyl acetate extract of *Cordia obliqua* willd.

S. No.	RT	NAME OF THE COMPOUND	MOLECULAR FORMULA	MW	PEAK AREA %
1.	11.797	Didodecyl phthalate	$C_{32}H_{54}O_4$	502	3.06
2.	13.443	2-Heptadecenal	$C_{17}H_{32}O$	252	2.38
3.	13.490	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	1.79
4.	15.126	Cyclobarbital	$C_{12}H_{16}O_3N_2$	236	10.13
5.	15.334	Trans-2,3-methylenedioxy-b-methyl-b- nitrostyrene	$C_{10}H_9NO_4$	207	1.49
6.	16.260	1,1,1,3,5,5,5-Heptamethyltrisiloxane	$C_{16}H_{28}OSi$	264	2.98
7.	16.327	1H-indole, 1-methyl-2-phenyl-	$C_{15}H_{13}N$	207	2.48
8.	16.582	Hexahydropyridine, 1-methyl-4-[4,5-dihydroxyphenyl]-	$C_{10}H_{28}O_4Si_3$	296	2.55
9.	16.695	Benzo [h] quinolone, 2,4-dimethyl-	$C_{15}H_{13}O_2Cl_2N_2$	285	0.40
10.	16.837	2-methyl-7-phenylindole	$C_{12}H_{17}NO_2$	207	2.11
11.	17.253	2-Ethylacridine	$C_{15}H_{13}N$	207	17.53
12.	17.537	Suprane	$C_{30}H_{50}$	410	7.81
13.	17.622	1,4-Benzenediol, 2,5-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O_2$	222	19.03
14.	17.830	1-methyl-3-phenylindole	$C_{15}H_{13}N$	207	0.29
15.	19.456	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	$C_{21}H_{40}O_4$	356	11.59
16.	19.806	6-Octadecenoic acid	$C_{18}H_{34}O_2$	282	14.36

Table2: Activity of phyto-components identified in ethyl acetate extract of *Cordia obliqua* willd by GC-MS.

RT	NAME OF THE COMPOUND	NATURE OF COMPOUND	ACTIVITY
11.797	Didodecyl phthalate	Esters	Non-central analgesic, antipyretic, anti-inflammatory, urinary system disorders.
13.443	2-Heptadecenal	Aldehyde	Antineoplastic agents, uses of additives.
13.490	9,12-Octadecadienoic acid (Z,Z)-	Monoterpenoid	Hepatoprotective, antiarthritic, hypocholesterolemic, cancer preventive.
15.126	Cyclobarbital	Aldehyde	Hypnotics and sedatives, nervous system.
15.334	Trans-2,3-methylenedioxy-b-methyl-b-nitrostyrene	Ethene	No activity reported.
16.260	1,1,1,3,5,5,5-Heptamethyltrisiloxane	Siloxane	Antimicrobial,antifungals, anti-infectives.
16.327	1H-indole, 1-methyl-2-phenyl-	Indole	Antiasthmatics, dermatological disorders, antipsoriatics.
16.582	Hexahydropyridine, 1-methyl-4-[4,5-dihydroxyphenyl]-	Pyridine	Non-specific cardiovascular stimulants.
16.695	Benzo [h] quinolone, 2,4-dimethyl-	Hydroquinone	Analgesia and anti-inflammatory
16.837	2-methyl-7-phenylindole	Indole	Non-central analgesic, antipyretic, anti-inflammatory, antineoplastic, allergic disorders, bone diseases.
17.253	2-Ethylacridine	Acridine	Antiseptic properties.
17.537	Suprane	Fluorinated Ether	Antineoplastic agents, dermatological disorders, treating wounds, ulcers, burns.
17.622	1,4-Benzenediol, 2,5-bis(1,1-dimethylethyl)-	Catechol	Antiasthmatics, dermatological disorders, joint disorders.
17.830	1-methyl-3-phenylindole	Indole	Non-central analgesic, antipyretic, anti-inflammatory, antineoplastic.
19.456	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	Long chain fatty acid	Antiasthmatics, bronchodilators, antitussive agents, urinary system disorders.
19.806	6-Octadecenoic acid	Long chain fatty acid	Urinary tract, kidneys, contrac-eptives, dermatological dis-orders, treating wounds, ulcers, burns.

3.2. Ethanolic extract of Cordia oblique willd.

Among the identified compounds, Acetamide, 2-chloro-N-[(5-chloro-8-hydroxy-7-quinolinyl)methyl]- was found to be the major compound attained the largest peak (47.14 %) with the retention time (16.459) minutes. Another major compound Phytol having peak area of (25.10 %) with retention time (13.188) minutes.

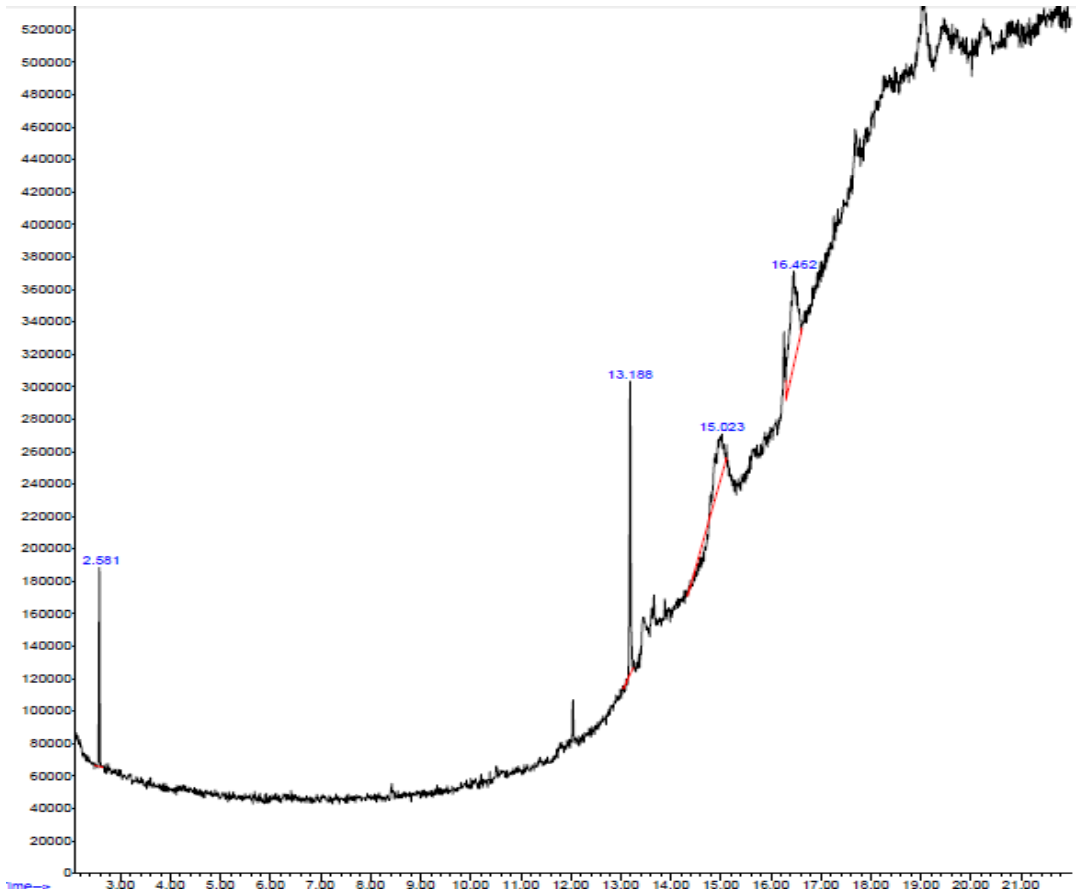

Fig. 2: GC-MS chromatogram of ethanolic extract of *Cordia obliqua* willd.

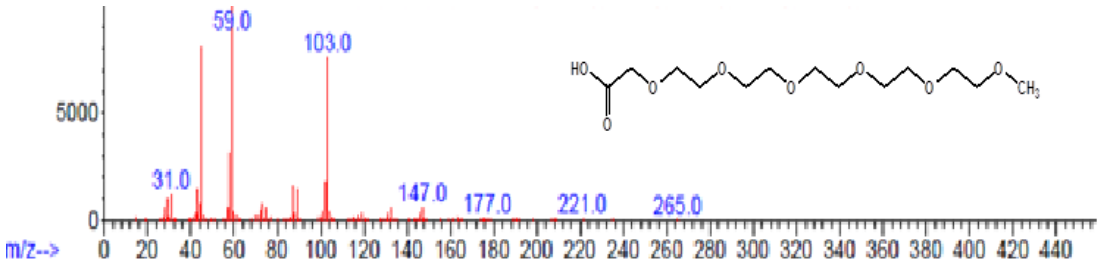

Fig. 2.1: 2-(2-(2-(2-(2-(2-methoxyethoxy)ethoxy)ethoxy) ethoxy)ethoxy) acetic acid

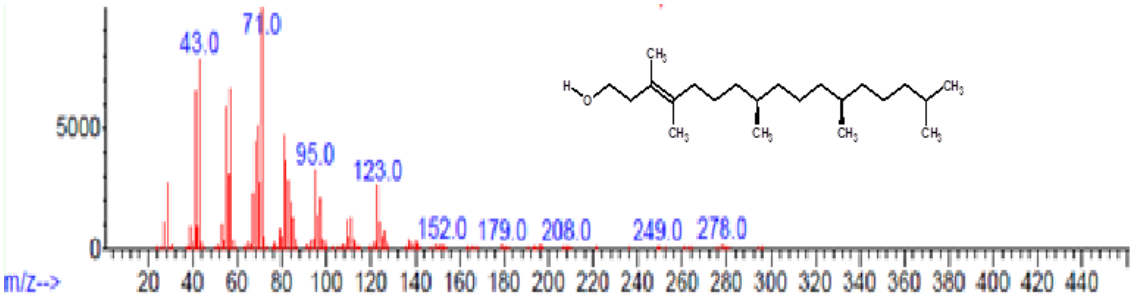

Fig. 2.2: Phytol

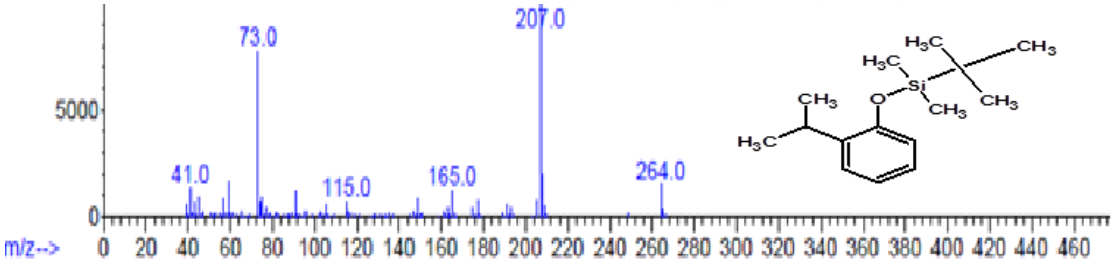

Fig. 2.3: Benzene, 2-[(tert-butyldimethylsilyl) oxy]-1-isopropyl-4-methyl-

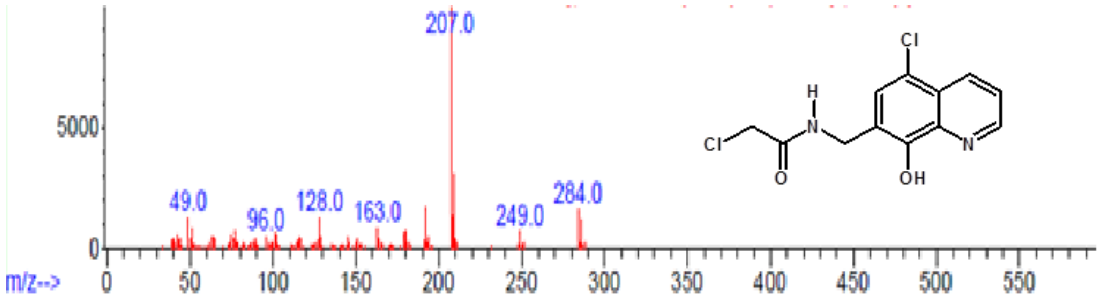

Fig. 2.4: Acetamide, 2-chloro-N-[(5-chloro-8-hydroxy-7-quinolinyl)methyl]-

Table 3: Chemical composition of ethanolic extract of *Cordia obliqua* willd.

S. NO.	RT	NAME OF THE COMPOUND	MOLECULAR FORMULA	MW	PEAK AREA %
1.	2.579	2-(2-(2-(2-(2-(2-methoxyethoxy)ethoxy)ethoxy) ethoxy)ethoxy)acetic acid	$C_{13}H_{40}O_2$	310	11.85
2.	13.188	Phytol	$C_{20}H_{40}O$	296	25.10
3.	15.022	Benzene, 2-[(tert-butyldimethylsilyl) oxy]-1-isopropyl-4-methyl-	$C_{16}H_{28}OSi$	264	15.91
4.	16.459	Acetamide, 2-chloro-N-[(5-chloro-8-hydroxy-7-quinolinyl)methyl]-	$C_{12}H_{10}O_2Cl_2N_2$	285	47.14

Table 4: Activity of phytocomponents identified in ethanolic extract of *Cordia obliqua* willd by GC-MS.

RT	NAME OF THE COMPOUND	NATURE OF COMPOUND	ACTIVITY
2.579	2-(2-(2-(2-(2-(2-methoxyethoxy)ethoxy)ethoxy) ethoxy)ethoxy)acetic acid	Ether	Centrally acting analgesics
13.188	Phytol	Diterpene	Antimicrobial, anticancer, cancer preventive, diuretic, anti-inflammatory
15.022	Benzene, 2-[(tert-butyldimethylsilyl) oxy]-1-isopropyl-4-methyl-	Benzene	Antibacterial
16.459	Acetamide, 2-chloro-N-[(5-chloro-8-hydroxy-7-quinolinyl)methyl]-	Ethanamide	Antimicrobial

RESULTS AND DISCUSSION

The results pertaining to GC-MS analysis of ethyl acetate and ethanolic extract from the plant leaves of *Cordia obliqua* Willd. These compounds were identified through mass spectrometry attached with GC. The results revealed that the presence of GC-MS analysis showed the existence of

ethyl acetate extract with different phytochemical compounds are Didodecyl phthalate having peak area (3.06%), 2-Heptadecenal having peak area (2.38%), 9,12-Octadecadienoic acid (Z,Z)- having peak area (1.79%), Cyclobarbital having peak area (10.13%), Trans-2,3-methylenedioxy-b-methyl-b-nitrostyrene having peak area (1.49%), 1,1,1,3,5,5,5-Heptamethyltrisiloxanehaving peak area (2.98%), 1H-indole, 1-methyl-2-phenyl-having peak area (2.48%), Hexahydropyridine, 1-methyl-4-[4,5-dihydroxyphenyl]-having peak area (2.55%), Benzo [h] quinolone, 2,4-dimethyl-having peak area (0.40%), 2-methyl-7-phenylindole having peak area (2.11%), 2-Ethylacridinehaving peak area (17.53%), Suprane having peak area (7.81%), 1,4-Benzenediol, 2,5-bis(1,1-dimethylethyl)-having peak area (19.03%), 1-methyl-3-phenylindole having peak area (0.29%), 9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester having peak area (11.59%), 6-Octadecenoic acid having peak area (14.36%). GC-MS analysis showed the existence of ethanolic extract with different phytochemical compounds are 2-(2-(2-(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)ethoxy)ethoxy)acetic acid having peak area (11.85%), Phytol having peak area (25.10%), Benzene, 2-[(tert-butyldimethylsilyl) oxy]-1-isopropyl-4-methyl- having peak area (15.91%), Acetamide, 2-chloro-N-[(5-chloro-8-hydroxy-7-quinolinyl)methyl]- having peak area (47.14%). The results from this research are quite promising for the use of *Cordia obliqua* as a multi-purpose medicinal agent, while *Cordia obliqua* has been used successfully in Siddha medicine for centuries, more clinical trials should be conducted to support its therapeutic use. Moreover, the therapeutic potential of the plant should also be checked when used in combination with other herbal drugs.

CONCLUSION

Ethno botanical and traditional uses of natural compounds, especially GC-MS analysis of plant leaves of *Cordia obliqua* Willd., in ethyl acetate and ethanol extracts. Traditionally, plants are used in the treatment of many infections and systemic disorders. More than hundreds of chemical compounds are derived from plants which have medicinal values due to their health-enhancing and therapeutic properties are referred as herbs. Through screening of literature available on *Cordia obliqua* depicted the fact that it is a popular remedy among the various ethnic groups Siddha and Ayurvedic properties.

ACKNOWLEDGEMENTS

The gratitude to Associate professor Dr. J. Amutha Iswarya Devi, M.Pharm., Ph.D., Department of Pharmaceutical Chemistry, Arulmigu Kalasalingam College of Pharmacy, Krishnakoil. Testing Laboratory and The Director, Science Instrumentation Centre of Ayya Nadar

Janaki Ammal College (ANJAC), Sivakasi, for providing the laboratory facilities (GC-MS) and support to carry out the work.

REFERENCE

1. Richa Gupta and Ghanshyam Das Gupta. A review on plant *Cordia obliqua* Willd. (Clammy cherry). *Pharmacogn Rev*. 2015 ; 9 (8): 127–131.
2. Richa Gupta, Ghanshyam Das Gupta. Formulation development and evaluation of anti-inflammatory potential of *Cordia obliqua* Topical Gel on Animal Model. *Pharmacogn J*. 2017; 9 (6): 93-98.
3. Bindu AR, NA Aleykutty, Jyoti Harindran. *Invitro* anti-inflammatory screening of stem bark of *Cordia obliqua* Willd. In RAW 264.7 cell lines. *Journal of Pharmacy Research*. 2016; 10 (6): 370-376.
4. Jalalpure SS, Ahirrao RA, Shaikh AF, Borse LB, Sayyed Hamid, and Patil JK. Anti-inflammatory activity of *Cordia obliqua* seed oil. *Pharmacology online.* 2009; 3 (5): 973-975.
5. Richa Gupta and Gupta GD. Pharmacognostic, phytochemical and antioxidant activity studies on *Cordia obliqua* willd. Leaf. *IJPSR.* 2016;7 (1): 469-477.
6. Sung Kwon Park, Chang Weon Choi, Yoo Kyung Lee, Park Jae Hyun and Selvaraj Arokiyaraj. Antimicrobial and free radical scavenging activity of *Cordia obliqua* W. *(Boraginaceae*) fruit extracts. *Journal of pure and applied microbiology.* 2014; 8 (1): 117-120.
7. Reenu Yadav, Govind Mohan, Ankur Choubey, Udit N. Soni, Jay Ram Patel. Isolation, spectroscopic characterization and screening of anti-microbial activity of isolated compounds from leaves and seeds of *Cordia obliqua* against some oral pathogens.*Indo American journal of pharmaceutical research.* 2015; 5(2): 125-143.
8. Munish Ahuja, Suresh Kumar, Ashok Kumar. Evaluation of mucoadhesive potential of gum *Cordia*, an anionic polysaccharide. *International Journal of Biological Macromolecules.* 2013; 5(9): 109– 112.
9. Monika Yadav, Munish Ahuja. Preparation and evaluation of nanoparticles of gum *Cordia*, an anionic polysaccharide for ophthalmic delivery. 2010; 8 (4): 871-877.
10. Richa Gupta, Ghanshyam Das Gupta. Toxicity assessment and evaluation of analgesic, antipyretic and anti-Inflammatory activities on *Cordia obliqua* leaf methanol extract. *Ph. cog. J*. 2017; 9 (6): 105-115.
11. Abou-Shaaban RR, Al-Angari AA, El-Tahir KE, Al-Khamis KI, Mirghani OM. Comparative hypotensive and respiratory stimulation effects of ripe and unripe fruit mucilage of *Cordia myxa* and *Cordia obliqua* in guinea pigs and rabbits. *Phytother Res*. 1989; 3 (7): 126–131.

12. Aswal BS, Goel AK, Kulshrestha DK, Mehrotra BN, Patnaik GK. Screening of indian plants for biological activity: Part XV. *Indian J. Exp Biol.* 1996;3 (4): 444–467.
13. Prakash Ramakrishnan, Dhivya Ramadoss, Priyadarshini Muthulingam, Ramya Nedunchezhian, Karthick Krishna moorthy. Anti-diabetic, anti-hyperlipidemic, antioxidant Property of *Cordia obliqua* on Streptozotocin Induced Diabetic Rats. *J. Young Pharm.* 2017; 9 (3): 321-326.
14. Gupta R and Gupta GD. Pharmacognostic, phytochemical and antioxidant activity studies on *Cordia obliqua* willd. Leaf. *IJPSR.* 2016; 71 (1): 4669-4677.
15. Reenu Yadav, Govind Mohan, Ankur Choubey, Udit N. Soni, Jay Ram Patel. Isolation, spectroscopic characterization and screening of antimicrobial activity of isolated compounds from leaves and seeds of Cordia obliqua against some oral pathogens. *Indo American Journal of Pharmaceutical Research.* 2015; 5 (2): 337-346.
16. Sivakrishnan S and Pradeepraj D. Gas chromatography- Mass spectroscopy analysis of ethanolic extract of leaves of *Cordia obliqua* willd. *Asian journal of pharmaceutical and clinical research.* 2019;12 (6): 441-445.
17. Tilak Raj and Punit Kumar. Preparation of hexane extract of *Cordia obliqua* and its analysis by TLC, FTIR and GCMS. *International research journal of pharmacy.* 2018; 95: 230-243.
18. Gupta R and Gupta GD. Determination of plant constituents in fractions of *Cordia obliqua* willd. Leaf methanolic extract using GC-MS analysis. *International journal of pharmacognosy and phytochemical research.* 2017; 9 (9): 1274-1279.
19. Amutha Iswarya Devi J and Kottaimuthu A.Gas chromatography-mass spectrometry analysis of bioactive constituents in the ethanolic extract of *Saccharum spontaneum* linn .*International Journal of Pharmacy and Pharmaceutical Sciences.* 2014; 6 (2): 975-983.