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GC-MS, FTIR AND NMR ANALYSIS OF METHANOL EXTRACT OF *EUCALYPTUS GLOBULUS*

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ABSTRACT

Eucalyptus globulus commonly known as blue gum tree is one of worlds mostly planted genera. It is well known because of its ethnomedicinal and therapeutic importance, as it possesses various pharmacological effects. The current research is aimed at identification of chemical constituents from the methanol extract of *Eucalyptus globulus* using GC-MS, FTIR and NMR analysis. The extraction of leaves was done by using Soxhlet's method. TLC-bio autography was carried out to separate and find the antibacterial fraction from *Eucalyptus globulus* methanol extract. The GC-MS analysis revealed presence of five main compounds Phthalic acid bis (7-methyloctyl) ester, Oxalic acid allyl hexadecyl ester, Pentadecanoic acid 14-methyl, methyl ester, 2-piperidinone N-(4-bromo-n-butyl) and Cyclopentane decanoic acid, methyl ester. The results of FTIR analysis showed presence of alcohol, carboxylate ion, C-H str, C=O str and C=C str functional groups. The ^{1}H NMR results showed presence of aliphatic OH, methyl, aromatic OH and aromatic proton, while ^{13}C results revealed presence of carbonyl, aromatic carbon, quaternary carbon, olefinic carbon and methyl group. The compounds identified by GC-MS analysis showed various pharmacological activities such as antimicrobial, antifungal, anti-inflammatory, anti malarial and antioxidant. *Eucalyptus globulus* plant contains high medicinal compounds and can be used further for production of antimicrobial drugs against plant pathogenic bacteria.

Keywords: Eucalyptus globulus, TLC-bio autography, GC-MS, FTIR, NMR.

Introduction

There are mainly two groups of phytochemicals based on their function in plant metabolism which are basically primary and secondary metabolites. The carbohydrates, aminoacids, proteins, and chlorophylls comprise as common primary metabolites while secondary metabolites are alkaloids, saponins, steroids, flavonoids, tannins and phenolic compounds. These phytochemicals are important in establishing several pharmaceutical industries and play a major role in identification of crude drugs (Mukherjee *et al.,* 2007). The interest in studying organic compounds of plant origin and their activities has increased in recent years (Singh *et al*. 2014). For the discovery of therapeutic agents, not only the knowledge of plant secondary metabolites is desirable, but it also helps in disclosing new sources of economic phyto compounds. For the synthesis of complex chemical constituents and for discovering the actual significance of folkloric remedies the knowledge regarding phyto chemicals is essential (Milne, 1993).

Nowadays, the researchers are focused on finding out new antimicrobials from alternate sources as there is increased resistance in bacteria due to extensive use of antibiotics which simultaneously led decrease in use of synthetic antimicrobial drugs (Amghalia *et al.,* 2009; Lakshmi *et al*., 2014). Plants are one of the most important sources of medicine and most of the modern drugs used today are derived plant products (Singh *et al*., 2012). In the practice of ethnomedicine, the plant and plant products are commonly used ingredients and thus is hinged on the fact that plant products are cheap, nutritive, and easily accessible (Toyang *et al*., 2007). These plant metabolites work through a diverse mechanism which may inhibit the microorganism like bacteria and may also provide values for treatment of

infection caused by plant bacteria (Stein *et al*., 2005). These plants notably produce secondary metabolites when they face abiotic stress or when they encounter herbivores. By various pathways secondary metabolites are produced from primary metabolites, either from amino acids or from acetyl co-A under specific conditions. Synthesis and bioassay guided isolation of bioactive compounds is currently an important approach for development of plant derived products. Moreover, the increasing use of plant extracts in the food, cosmetic and pharmaceutical industries suggests that, to find active compounds, a systematic study of medicinal plants is very important. The presence of rich source of biologically active secondary metabolites in plants possess antimicrobial properties (Jadhav *et al*., 2014; Sasikala and Mohan, 2014). The wide range of secondary metabolites are found in *Eucalyptus* species of which many are found to harbor a diverse range of biological activities (Cheng *et al.,* 2009). *Eucalyptus globulus* commonly known as blue gum of Myrtaceae family is one of world's most widely planted genera and its constituents are known to have antibacterial, antifungal, antioxidant, and repellent activities (Safaei-Ghomi and Ahd 2010, Amini *et al.,* 2012).

This plant is well known to possess biological and pharmacological propertiesas it bears different secondary metabolites like tannins, flavonoids, sterols, carotenoids, saponins, triterpene, alcohols, polysaccharides,alkaloids, fatty acid methyl esters, dicarboxylic acids, palmitic acids, and compounds of plasticizer nature (Ray *et al*., 2015). These group of compounds possess different pharmacological activities such as antioxidant, antimicrobial, antifungal (Elaiyaraja and Chandramohan, 2016), anti-inflammatory (Meenakshi *et al*., 2012), antitumour (Lee *et al.,* 2000), antiseptic,acaricide, CNS-paralytic, fatal, haemostatic, irritant, pesticidal, renotoxic and varroacidial (Zayed and Samling, 2016). As per the previous review, no clear report is yet found for controlling *Xanthomonas citri* causing canker disease using *Eucalyptus globules* plant. Therefore, this research was aimed at identification of different bioactive compounds from the methanol leaves extract of *Eucalyptus globulus* plant using GC-MS, FTIR and NMR analysis.

Materials and Method

Extraction of leaves:

The fresh leaves of *Eucalyptus globules* were collected washed under tap water to remove impurities and were dried under shade for one to two weeks. The dried leaves were grinded into fine powder using grinder and stored it in airtight container. Then extraction was done using Soxhlet's apparatus as given by Ayeploa and Adeniyi, 2008 using ethyl acetate, chloroform, methanol, and distilled water as solvents. The supernatant obtained in flask was then filtered separately through Whatman No.1 filter paper and evaporated at room temperature. Air dried extracts were weighed separately and transferred into small vials and kept in refrigerator at 5˚C until further use.

Thin Layer Chromatography

Thin layer chromatography was performed by the method of Gende *et al*. 2010 for identification of active antibacterial component present in the leaves of *Eucalyptus globulus* using toluene: ethyl acetate (93:7) solvent system.The *Eucalyptus globulus* methanol extract was diluted in DMSO from which 10 µl samples was applied on TLC plates at equal distance with the help of capillary tubes. The TLC plates were kept in toluene: ethyl acetate (93:7) solvent system and were allowed to run until it reached to 3/4th position. The developed chromatogram on TLC plates was allowed to air dry and observed under visible and UV light. The bands with their Rf value (Relative front) were calculated by measuring the distance travelled by solute and the solvent front.

Bioautography

The bioactive compounds were isolated using bio autography method as given by Ishnava *et al*. 2013. Bioautography of the TLC plate was used to confirm the position of compound showing antibacterial activity. The area of inhibition zone on TLC plates appeared transparent against a red background. Later, the compound was eluted from the developed plate by scrapping off the silica gel and was dissolved well in methanol. Then it was centrifuged at 10,000 rpm for 10 min. By using the vacuum concentrator, the supernatant was evaporated at 60°C for 50 min for complete evaporation of solvent. The remaining purified compound was stored in refrigerator for further analysis.

FT-IR, GC-MS, ^{1}H and ^{13}C NMR analysis

Further analysis was carried out of purified compound by Fourier Transform Infrared Spectroscopy (FT-IR), Gas Chromatography–Mass Spectroscopy (GC-MS) and Nuclear Magnetic Resonance (NMR) techniques. For this analysis approximately 70mg purified active compound was separately stored in small, sterilized glass bottles. Samples were outsourced for chemical analysis like FT-IR, GC-MS and ^{1}H and ^{13}C NMR at Sophisticated Analytical Facility, IIT, Pawai, Mumbai. Results are interpreted and represented in the form of Table and Figures.

Results and Discussion

Extraction yield of *Eucalyptus globulus*

Extraction yield of *Eucalyptus globulus* leaves in different solvents are presented in Table 1 and Fig 1.

Table 1: Effect of different solvents on percent extraction yield from dry weight of leaves

Plant	Solvent	Yield in%
Eucalyptus globulus	Chloroform	7.64
	Ethylacetate	7.1
	Methanol	9.54
	Distilled water	7.2

Extraction yield of *Eucalyptus globulus*

Methanol exhibited (9.54%) maximum extraction from *Eucalyptus globulus* leaves whereas minimum extraction yield was observed in ethyl acetate (7.1%).

The results of extraction yield obtained from *Eucalyptus globulus* leaves by using Soxhlet method is given in Table 1. The results revealed highest extraction in methanol solvent, which principally depends on polarity and capability to extract substances that can be dissolved in the used solvent. Thus, methanol was most capable to extract substances from *Eucalyptus globulus* plant. Madouri *et al*. (2015) extracted the essential oil from leaves of *Eucalyptus globulus* using methanol solvent. The yield obtained from methanol extract of *Eucalyptus globulus* leaves was (2.53%). Also, the present results were in confirmation with findings of Badrunnisa and Pai (2017) who recorded highest extraction yield (41.6%) in methanol extract.

Thin Layer Chromatography

Thin layer chromatography was used for separation of different chemical constituents present in methanol extract of *Eucalyptus globulus.*

Standardization of solvent system

Various solvent systems were screened for efficient separation of bands according to polarity. Total 9 solvent systems were used in present investigation to know most suitable solvent system for separation of compounds in methanol extract of *Eucalyptus globulus*. The Rf values and colour of separated bands in different solvent systems under UV transilluminator are summarised in Table 2 Plate 1.

Table 2 : Standardization of solvent system for methanol extract of *Eucalyptus globulus*

Sr. No.	Solvent system	Proportion	Methanol extract of *Eucalyptus globulus*	
			Rf	Color
1	Chloroform: methanol	3:2	0.18	Light orange
			0.50	Violet
			0.81	Light violet
			0.91	Pink
2	Ethylacetate: methanol: water	81:11:8	0.78	Pink
			0.94	Violet
3	Hexane: ethylacetate	9:1	0.22	Pink
			0.31	Light violet
			0.50	Light pink
			0.55	Violet
			0.66	Light pink
			0.72	Black
			0.97	Light violet
4	Chloroform: methanol	8:2	0.17	Light orange
			0.47	Violet
			0.70	Light violet
			0.94	Pink
5	Petroleum ether: toluene: ethyl acetate	3:1:1	0.05	Light pink
			0.21	Blue
			0.42	Light pink
			0.61	Pink
			0.71	Light pink
			0.89	Pink
			0.94	Light violet
6	Hexane: ethyl acetate	3:1	0.16	Light pink
			0.66	Pink
			0.72	Black
			0.93	Light violet
7	Toluene: ethyl acetate: acetone	7:2:1	0.11	Light green
			0.45	Light pink
			0.68	Light orange
			0.73	Light orange
			0.88	Pink
			0.96	Blue
8	Petroleum ether: ethyl acetate: acetone	7.8:2.2:0.2	0.05	Light green
			0.21	Light pink
			0.44	Pink
			0.66	Light blue
			0.72	Black
			0.88	Light pink
			0.94	Black
9	Toluene: ethyl acetate	93:7	0.03	Green
			0.07	Orange
			0.12	Pink
			0.25	Light pink
			0.37	Blue
			0.43	Pink
			0.56	Light violet

			0.62	Pink
			0.75	Light violet
			0.81	Pink
			0.87	Light violet
			0.88	Light pink
			0.97	Pink

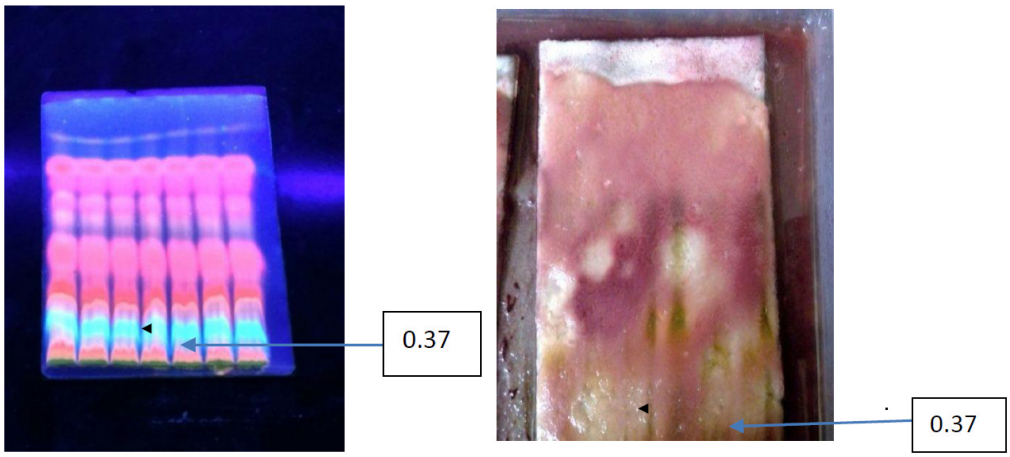
It is observed from data presented in Table 2, different solvent systems showed differences in number of bands and their Rf values in methanol extract of *Eucalyptus globulus*. Among all the tested solvent systems most promising solvent system produced good results on TLC plates was toluene: ethyl acetate (93:7) for methanol extract of *Eucalyptus globulus*.The Rf values of methanol extract of *Eucalyptus globulus* run under Toluene: ethyl acetate (93:7) solvent system was0.03, 0.07, 0.12, 0.25, 0.37, 0.43, 0.56, 0.62, 0.75, 0.81, 0.87, 0.88 and 0.94.

TLC was done to separate the different antibacterial fractions of *Eucalyptus globulus* plant. Total nine different solvent systems were screened for separation of bands as per polarity. The Rf value and color of separated bands in different solvent systems under UV transilluminator are summarized in Table 2. Most prominent solvent system found among all was Toluene: ethyl acetate (93:7) separating thirteen bands (Fig 2). Similar findings were reported by Obiorah *et al*. (2012) who showed three Rf values 0.15, 0.83 and 0.83 of methanol extracts of *Eucalyptus globulus* eluted with chloroform: petroleum ether: diethyl ether (10:7:3) solvent system. More *et al*. (2016) identified ten active fractions of *Aegle marmelos* leaves extract on thin layer chromatography technique eluted with petroleum: ethyl acetate (2:1) solvent system.

Bioautography

TLC plates run in Toluene: ethyl acetate (93:7) was used for bioautography technique to determine antibacterial activity of separated compounds against tested bacterium. The TLC plate after spraying with 2,3,5-tri phenyl tetrazolium chloride, showed a transparent zone of inhibition against red background around band which contain active principle responsible for antibacterial activity. The one compound from *Eucalyptus globulus* methanol extract showed well resolved inhibition of *Xanthomonas axonopodis* pv.*citri* at Rf-0.37 showing blue colour under UV transilluminator (Plate 1 and 2).

Bioautography was done to identify the antibacterial fraction of *Eucalyptus globulus* plant on TLC plates eluted in toluene: ethyl acetate (93:7) solvent system. The TLC plate after spraying with 2, 3, 5-triphenyl tetrazolium chloride showed a whitish or transparent zone of inhibition against red background at Rf value 0.37. Guleria *et al*. (2011) evaluated the essential oil from *Eucalyptus teretecornis* leaves using TLC-bioautography and analysed it by gas chromatography/mass spectrometry (GC/MS) and revealed presence of two main bioactive components, β-fenchol (oxygenated monoterpene) and α-eudesmol (oxygenated sesquiterpene) of Rf 0.27 and Rf 0.33. The essential oils from different parts of *Eucalyptus lanceolatus* plant were detected by using Gas Chromatography/Mass Spectrometry and TLC-bioautography and were named as, alpha-pinene and limonene with Rf 0.95 and 0.73 by Baghat *et al*. (2016).

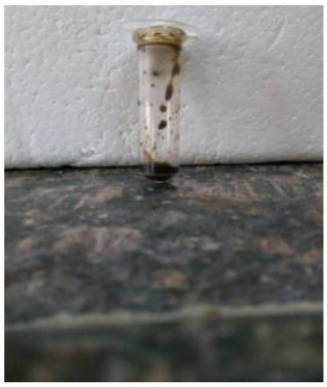


A) TLC of *E. globulus* B) Bioautography of *E. globulus*

Plate-1: T L C and Bioautography of methanolic extract of *Eucalyptus globulus*

A) Scrapped compound

B) Supernatant



C) Purified compound

Plate 2: Purification of scrapped compound

GCMS analysis of *Eucalyptus globulus*

The *Eucalyptus globulus* methanolic leaves extract showed presence of five main compounds with their retention time, peak area, and molecular weight. The five compounds mainly Pentadecanoic acid, 14-methyl, methyl ester at retention time 20.19, Mol. wt. 270 and peak area 520719.48, second was 2-Piperidinone, N-(4-bromo-n-butyl) at time 28.57, mol wt.233 and peak area 349497.62, third compound was Cyclopentanudecanoic acid, methyl ester at retention time 29.45, mol. wt. 268. While fourth and fifth compound present were Oxalic acid, allyl hexadecyl ester and Phthalic acid bis (7-methyloctyl) ester at retention times 31.51 and 32.04, mol. wt. 354 and 418 and peak areas were 5367778.52 and 8614812.74 respectively (Table 3 and Figure 1-5).

Table 3: Identification of compounds from methanol extract of *Eucalyptus globulus* leaves By GCMS

Sr. no.	Name of compound	Formula	MW	Retention time	Peak area
1	Pentadecanoic acid, 14-methyl,methylester	$C_{17}H_{34}O_2$	270	20.19	520719.48
2	2-Piperidinone, N-(4-bromo-n-butyl)	$C_9H_{16}BrN_0$	233	28.57	349497.62
3	Cyclopentanudecanoicacid, methylester	$C_{17}H_{32}O_2$	268	29.45	638324.60
4	Oxalic acid, allylhexadecylester	$C_{21}H_{38}O_4$	354	31.51	5367778.52
5	Phthalic acid bis (7- methyloctyl) ester	$C_{25}H_{42}O_4$	418	32.04	8614812.74

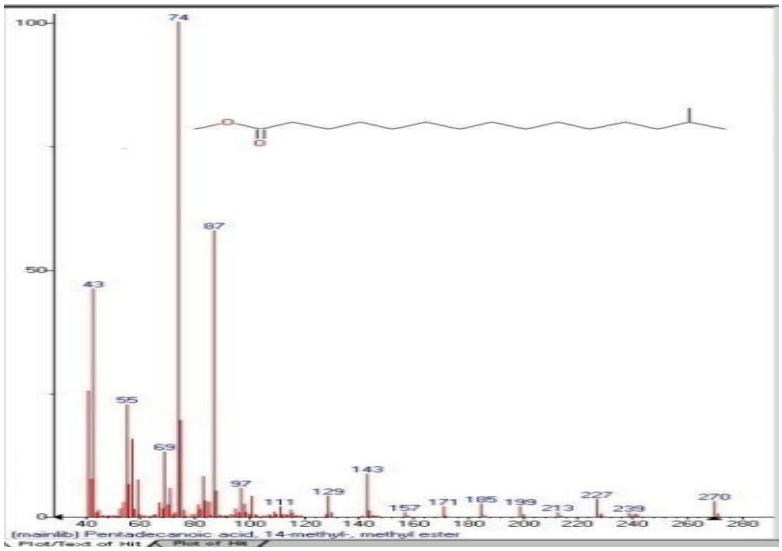


Fig. 1. : Mass spectra of compound Pentadecanoic acid 14- methyl, methyl ester from methanol extract of *Eucalyptus globulus* leaves

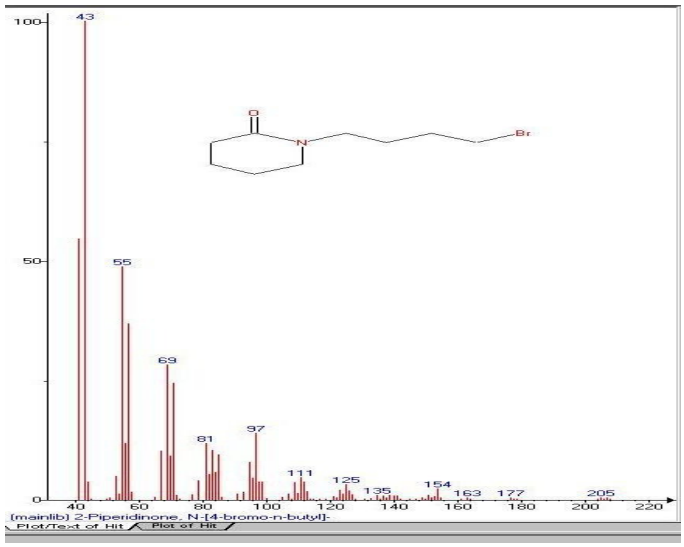


Fig. 2: Mass spectra of compound 2-piperidinone, N(4-bromo-n-butyl) from methanol extract of *Eucalyptus globules* leaves

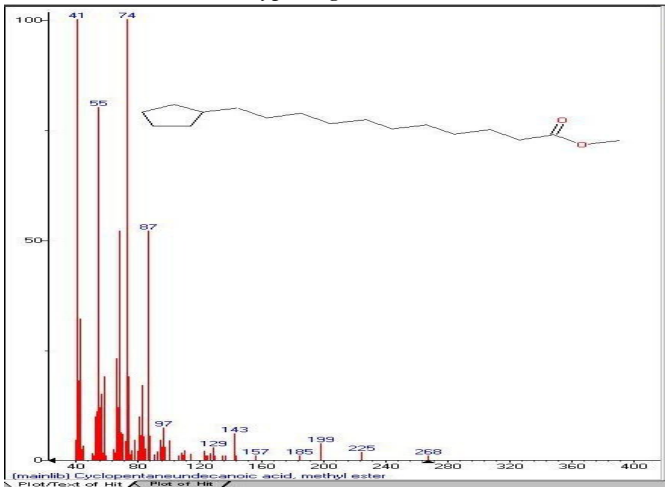


Fig. 3 : Mass spectra of compound Cyclopentanudecanoic acid, methyl ester from methanol extract of *Eucalyptus globules* leaves

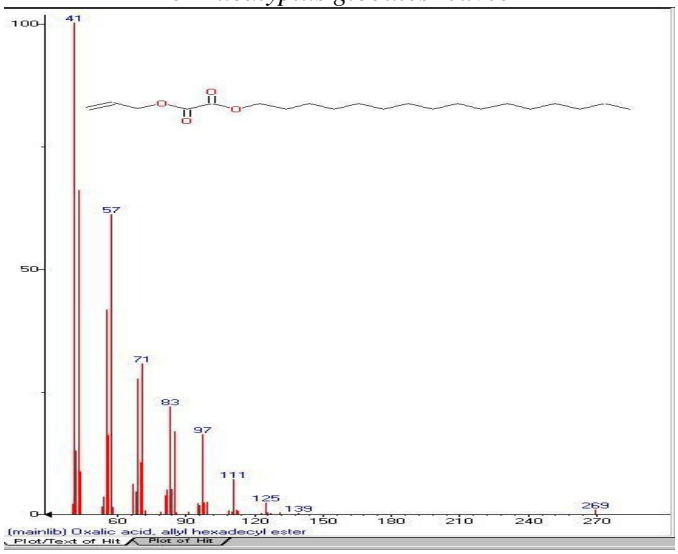


Fig. 4: Mass spectra of compound Oxalic acid, allyl hexadecyl ester from methanol extract of *Eucalyptus globulus* leaves

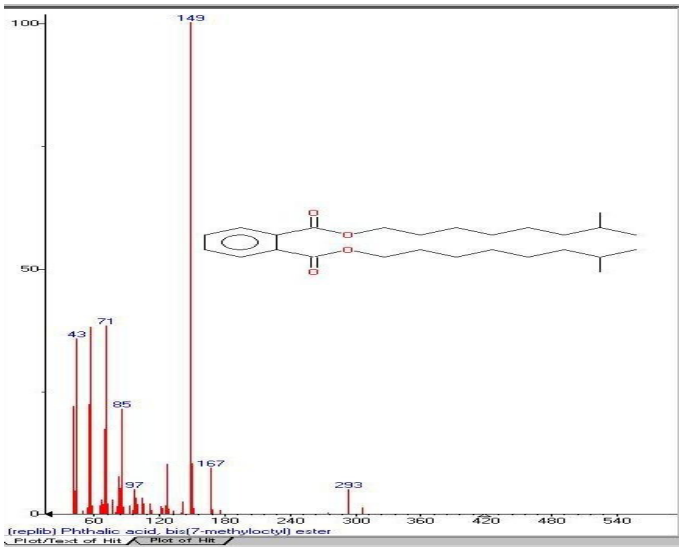


Fig. 5 : Mass spectra of compound Phthalicacidbis(7-methyloctyl) ester from methanol extract of *Eucalyptus globulus* leaves

The technique for reliable identification of bioactive compounds existing in medicinal plants including volatile matter, long and branched hydrocarbons, alcohol, acids and esters is done by Gas Chromatography Mass Spectroscopy (GCMS).

The compounds identified in methanol extract of *Eucalyptus globulus* extract with their retention time, molecular formula, molecular weight, and peak area are given in Table 3. The GCMS results showed presence of five compounds of alkaloid, plasticizer, dicarboxylic acid, fatty acid methyl ester and palmitic acid nature. In that, Phthalic acid bis (7-methyloctyl) ester showed highest 8614812.74 peak area with 32.04 retention time while 2-piperidinone, N-(4-bromo-n-butyl) showed lowest 349497.62 peak area with retention time 28.57. The compounds obtained are biologically active and play major role in different pharmaceutical industries.

Pentaneudecanoic acid, methyl ester compound identified was known of palmitic acid methyl ester nature which showed antimicrobial activity in *Indoneesiella echioides* plant (Elaiyaaraja and Chandramohan, 2016). The compound also showed antioxidant activity in *Lagenaria breviflora* plant (Adeyemi *et al*., 2017) in *Leucaena leucocephala* plant (Zayed and Samling, 2016), antifungal activity in *Azadirachta indica* (Sandanasamy *et al*., 2014). The second compound 2-piperidinone, N (4-bomo-n-butyl) of alkaloid nature was found in different plants *Purpura persica* (Santhi *et al*., 2016), *Wattakaka volubilis* (Vishnusithan and Kamaraj 2012), *Amaranthus caudatus* (Pranthaman *et al*., 2012) and *Seidenfia rheedii* (Ruthisa *et al.,* 2017). The compound showed antioxidant and antimicrobial activity (Leena, 2015). The third compound Oxalic acid, allyl hexadecyl ester is dicarboxylic acid in nature (Zayed and Samling, 2016). This compound has also been reported elsewhere in other studies on other plant species such as in seaweed *Laurencia brandenii* (Manilal *et al.,* 2010), *Aloe vera* (Arunkumar *et al.,* 2009), *Leucaena leucocephala* (Zayed and Samling, 2016) and in *Capsicum annum* (Sathya *et al*., 2016). It is known to possess antimicrobial activity (Sathya *et al.,* 2016), acaricide, antiseptic, fatal, hemostatic, irritant, pesticidal, renotoxic and varroacide activities (Zayed and Samling, 2016). The fourth compound Phthalic acid, bis (7-methyloctyl) ester of plasticizer nature was found to be present in different plants *Purpura persica* (Santhi *et al*., 2016), *Calotropis gigantea* (Singh and Javed, 2015), *Centratherum punctatum* (Sivasubramanian and Brindha, 2013). The compound showed different activities like antitumor against mice sarcoma 180 cell lines (Lee *et al*., 2000), also antimicrobial and antifouling activity (Vennila and Udayakumar, 2015). The results of fifth compound Cyclopentane decanoic acid, methyl ester was in line with the findings of Vimalavady and Kadavul 2013 who showed presence of Cyclopentane decanoic acid, methyl ester compound in the *Hugonia mystax* plant. Also, same compound was identified in the essential oil of *Citrus sinensis* plant (Egharevba *et al*., 2016). The above compounds possessing antibacterial activities are responsible for the controlling the

Xanthomonas citri causing citrus canker disease plant pathogenic bacteria.

FTIR analysis of *Eucalyptus globulus*

FT-IR analysis provided following peaks with their corresponding functional groups. Peak observed at 3441 suggest the presence of R-CH_2OH, R_2-CHOH and R_3-C-OH. While C-H stretching was found at 2927 and 1728 peak represents C=O stretching as in Carboxylic acids. Similarly, C=C stretching was observed at 1464 and carboxylate ion was found at 1268 peak (Table 4 and Figure 6).

Table 4 : Identification of functional group from methanol extract of *Eucalyptus globulus* leaves by FTIR Analysis

Sr.no.	Peak	Functional group	Average range
1	3441	R-CH_2OH, R_2-CHOH,R_3-C-OH	3400-3600
2	2927	C-H str. Hydrocarbons aliphaticaromatic	2850-3000
3	1728	C=Ostr. Carbonylgroup	1650-1800
4	1464	C=Cstr. Aromatic compounds	1450-1600
5	1286	Carboxylateion–C00-	1200-1300

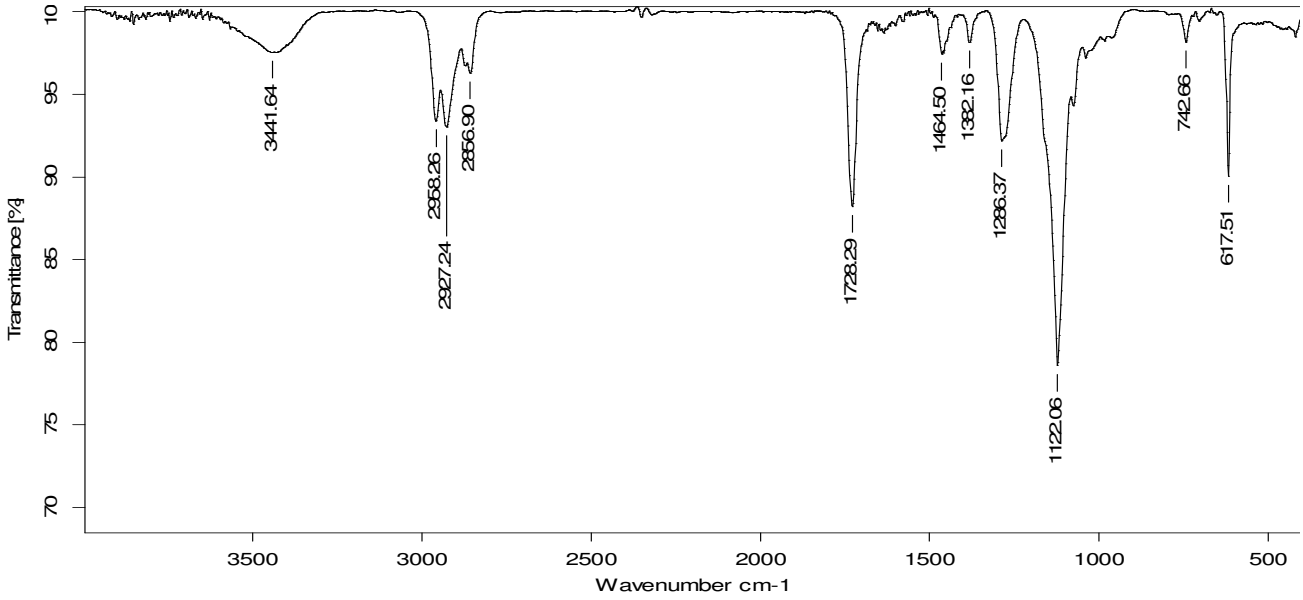


Fig. 6 : FTIR spectra of compound isolated from methanol extract of *Eucalyptus globules* leaves

Fourier Transform Infrared Spectroscopyis used for identification of functional groups present in bioactive compounds of different plant extract. The results of FTIR analysis showed presence of different functional groups with their peak and average range is summarized in Table 4. The above results of FTIR were in line with the findings of Zein *et al*., 2018 who showed presence of C-H stretching and C=O stretching i.e., carbonyl group at 2927 and 1728 peaks. Also, similar findings showed presence of O-H group and C-H stretching at 3406 and 2929.7 peaks (Egwaikhide *et al*., 2008), while the presence of O-H stretching at 3436 and 3423 peaks, C-H stretching at 2956, 2924 and 2927, C=O stretching in 1735 and 1727 peaks were given by Moein *et al*., 2012. This different functional groups found in *Eucalyptus globulus* plant are responsible for possessing different pharmacological activities shown by above compounds.

^{1}H NMR analysis of *Eucalyptus globulus*

^{1}H NMR analysis, showed presence of different types of protons at different signals. The first signal at 7.76 to 7.80 showed presence of aromatic proton. Whereas 4.22 to 3.7 signals showed presence of methylene group attached to electronegative atom or a hydroxyl group. The third signal from 2.52-2.82 showed presence of aliphatic hydroxyl group. While presence of aromatic methyl and methylene group was shown by 1.23 to 1.38 and 0.88 to 0.92 signals respectively (Table 5 and Fig. 7).

Table 5: Identification of type of proton from methanol of *Eucalyptus globules* leaves by Proton NMR analysis

Sr. no.	(δ) Chemical shift	Type of proton
1	7.76-7.70	Aromatic proton
2	4.22-4.30 and 3.6-3.7	CH or CH_2 attached to electronegative atom or aromatic OH
3	2.52-2.82	Ar-CH_3 or CO-CH_3 or aliphatic OH
4	1.23-1.38	CH_2 or CH_3
5	0.88-0.92	Ar-CH_3

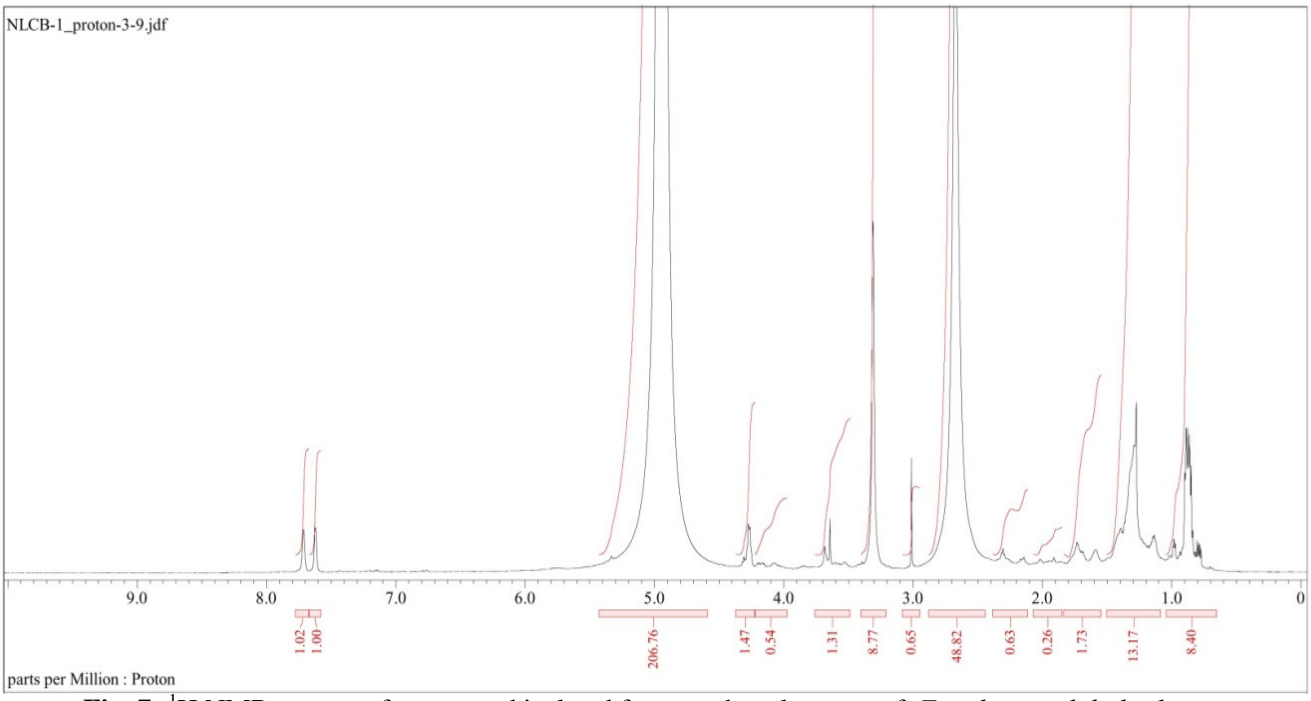


Fig. 7: ^{1}H NMR spectra of compound isolated from methanol extract of *Eucalyptus globulus* leaves

^{13}C NMR analysis of *Eucalyptus globulus*

^{13}C NMR analysis showed presence of carbonyl group at 169.41, 67.1 and 67.3 signals. The aromatic carbons were present at 129.96 and quaternary carbons at 132.47 and 133.54 signals. Similarly signals at 120.5 showed presence of olefinic or aromatic carbons and methyl group was present at 14.9 signal (Table 5a and Figure 7a).

Table 5a : Identification of types of carbon from methanol extract of *Eucalyptus globulus* leaves by ^{13}C NMR analysis

Sr. No.	(δ) Chemical shift	Type of carbon
1	169.41	Carbonyl group
2	129.9	Aromatic carbon
3	132.47 and 133.54	Quaternary carbon
4	120.5	Aromatic or Olefinic carbon
5	67.1 and 67.3	Carbon atom attached to oxygen
6	14.9	CH_3

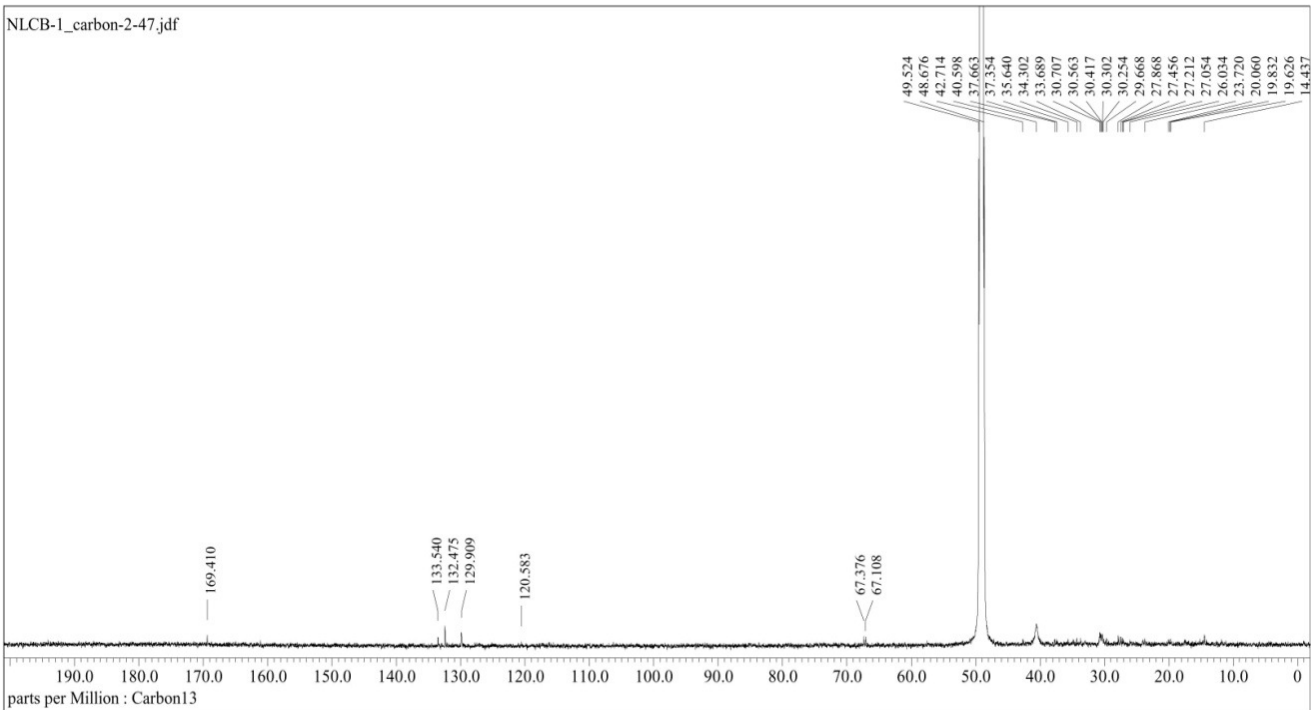


Fig. 7a: ^{13}C NMR spectra of compound isolated from methanol extract of *Eucalyptus globulus* leaves

The Nuclear Magnetic Resonance analysis is done to identify the types of protons and carbon of a bioactive compound. There are two types of NMR ^{1}H which identifies the type of proton and ^{13}C identifies the type of carbon**.** The results of ^{1}H and ^{13}C NMR with their chemical shift and types of protons and carbon are given in Table 5 and Table 5a.The several scientists showed presence of different groups from which Moein *et al.,* 2012 in ^{1}H NMR analysis showed presence of methyl group at 1.256 and 1.280, aromatic protons were observed at 7.260 and 7.264 peaks. Similarly, the presence of methyl group at 1.2-1.3 peak was observed in ^{1}H analysis by Karaki *et al*., 2016. Thus presence of the above groups plays a major role in possessing different activities shown by the compounds identified from *Eucalyptus globulus.*

Conclusion

This study demonstrates that TLC and direct bioautography are the best techniques for the identification of antibacterial compounds of various plant extracts. Direct bioautography is a relatively simple tool that ensures the rapid performance of the antibacterial efficacy of TLC-separated individual compounds of plant extracts. Also, FTIR, NMR and GC-MS techniques are most suitable spectroscopic techniques for identifying and studying the structure of partially purified antibacterial compounds. TLC and direct bioautography was successfully used for the identification of antibacterial compounds from *Eucalyptus globulus* extract.

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