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RESEARCH ARTICLE

Structural Elucidation of Anti-*Pseudomonas* Component from *Eucalyptus Tereticornis*

S. Badrunnisa[1*] and Vinitha Ramanath Pai[2]

[1]Department of Microbiology, Krupanidhi Degree College, Bengaluru - 560035, India; badrunnisa.s@gmail.com

[2]Department of Biochemistry, Yenopoya Medical College, Yenepoya (Deemed to be University), Mangaluru - 575018, India

Abstract

Eucalyptus species is a well-known medicinal plant from ancient times with diverse biological functions, antimicrobial activity being one of them. This study attempts to isolate a secondary metabolite with antibacterial properties from ethanol extracts of *Eucalyptus tereticornis* leaf against *Pseudomonas aeruginosa* –using an activity-guided procedure. Ethanol extract of the leaf powder was obtained by soxhlation and subjected to liquid-liquid extraction with organic solvents: ethyl acetate, n-hexane, n-butane, and chloroform. The active n-hexane and chloroform extracts were purified by column chromatography, and the components of the active eluant fraction were separated by Thin-Layer Chromatography (TLC). The purity of the antibacterial compound was checked by High Performance Liquid Chromatography (HPLC), the molecular weight was determined by Liquid Chromatography-Mass Spectroscopy (LC-MS) and the structure was elucidated and identified by Nuclear Magnetic Resonance (NMR) and by Fourier Transform Infrared Spectrometer (FTIR). TLC of n-hexane extract from liquid-liquid extraction showed a single spot with antibacterial activity. A single major peak was observed on HPLC, and LC-MS revealed that the compound is a Formylated Phloroglucinol Component (FPC) with a molecular weight of 471.3. The NMR and FTIR analysis identified that the isolated, compound is Macrocarpal A. This study reveals that the isolated relatively pure anti-*Pseudomonas* compound from the leaf extracts of *E. tereticornis* is Macrocarpal A, a flavanoid from FPC.

Keywords: *Eucalyptus tereticornis,* Formylated Phloroglucinol Compounds (FPCs), High-Performance Liquid Chromatography (HPLC)

1. Introduction

An increase in the frequency of new, re-emerging strains of pathogens and their resistance to existing antibiotics creates an exigent necessity to ascertain new antibacterial combinations with assorted chemical structures and innovative appliances for action. In India, there are approximately 600 ancient plants with pharmaceutical importance[1]. *Eucalyptus* is one of the important species belonging to the family Myrtacea. The genus comprises *Eucalyptus alligtrix, Eucalyptus saligna, Eucalyptus andrewsii, Eucalyptus tereticornis, Eucalyptus tetragona, Eucalyptus amplifolia, Eucalyptus acies, Eucalyptus abdita, Eucalyptus apiculata, Eucalyptus albopurpurea, Eucalyptus ammophila,* etc[2,3]. This genus's species have higher vital oil content in their leaves than others, which have average or low oil content[4]. The commercial benefits of eucalyptus oils are due to their diverse bio-activities (antimicrobial, insecticidal, antiviral, and herbicidal). These properties are associated with their unique chemical composition[5]. In addition, Eucalyptus species proved to be foundations or raw materials of significance for various medicines, such as pain relievers (for overextended sore muscles), and it also acts as an antiseptic that helps in killing fungi and bacteria[6,7].

*Author for correspondence

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Coolant oil is the lubricant used in the steel industry, which helps extend the tool's life. However, the limitation of coolant oil is that it loses its properties due to bacterial and fungal contamination. Hence, it has to be discarded on soil or in water bodies, which leads to environmental pollution with bacteria and fungi and, more importantly, a health hazard for the workers[8-11]. There is an urgent need for new antimicrobials due to emerging resistance among microbes to existing antibiotics[1,12]. One of the alternatives to antibiotics is the use of secondary metabolites from plant sources, with antibacterial properties[13].

Our preliminary studies with the leaf extract of *E. tereticornis* show its antibacterial effect on *Bacillus cereus, Bacillus thuringiensis,* and *Pseudomonas aeruginosa*[8,9], isolated from used coolant oils of the steel industry. The ethanol extract has better antibacterial action than methanol extracts[14]. Therefore, the present study involves the isolation, purification, and structural elucidation of the secondary metabolite with antibacterial properties against *P. aeruginosa* from ethanol leaf extracts of *E. tereticornis*. Identifying the secondary metabolite could probably be a step toward finding an effective, alternate antibacterial that functions as a preventive measure for unused coolants or as an antidote for the microbial contaminants of in-use industrial coolants. *E. tereticornis* is a commonly available Eucalyptus species in the Bellary, Karnataka geographical area in south India.

The isolation of the principle was done by liquid-liquid extraction, purification by column chromatography, TLC (Thin Layer Chromatography), HPLC (High-Performance Liquid Chromatography), and LC-MS (Liquid Chromatography-Mass Spectroscopy). This investigation suggests that the component of *E. tereticornis,* which has antibacterial properties towards *P. aeruginosa,* belongs to the group of Formylated Phloroglucinol Compounds (FPCs). Further structure elucidation of the secondary antibacterial metabolite using analytical tools such as NMR and FT-IR (Nuclear Magnetic Resonance and Fourier Transform Infrared Spectroscopy) reveal that the compound is Macrocarpal A.

2. Materials and Methods

Isolation and purification were carried out using an activity-guided method; at every step, the extracts were tested for antibacterial potential against *P. aeruginosa* isolated from used steel industry coolant before deciding the next step of purification.

2.1 Antibacterial Assay

The antibacterial assay was done according to the diffusion method described earlier[8,9]. The Muller-Hinton method was used to prepare agar plates and inoculate with 10^6 (100 µL) cells per mL of *P. aeruginosa*, isolated from used steel industry coolant, by the spread plate culture method, which was incubated at 37°C in an incubator overnight. Wells (0.5 mm) were made in the agar plates using a borer. Two-fold diluted extracts (50 µL), with final concentrations ranging from 200 to 2500 µg/mL, were added into the wells. Ampicillin (30 µg/mL; volume 50 µL) was used as a positive control, and DMSO was the vehicle control[15]. The plates were incubated at 4°C for two h to allow the diffusion of the extract into the agar medium. The zone of inhibition was measured in the plates after incubation at 37°C for 24-48 h. The experiment was carried out in three duplicates, and the zone of inhibition was expressed as mean ± standard deviation. The extract/fraction showing the highest efficacy was used for further analysis.

2.2 Minimum Inhibitory Concentration (MIC) Test

The stock solution (10%) was diluted with DMSO to get concentrations ranging from 50 to 0.1 mg/ml. The final test concentration (50 µL) was used to determine MIC by agar diffusion. The mean zone of inhibition was calculated from the plates in triplicate, and the lowest concentration of extracts showing inhibition was expressed as the MIC (mg/mL).

2.3 Soxhlet Extraction

The leaves of *E. tereticornis* were washed in running water and dried in an oven at 60°C. Dried leaves were

powdered with the help of a blender. The dried powder (50 g) and ethanol (150 mL) were used for extraction in a soxhlet thimble. After 48 h, the solvent was evaporated under reduced pressure, and the extract was stored at room temperature until further study[16,17]. The extract was tested for antibacterial efficacy against *P. aeruginosa,* as described earlier.

2.4 Liquid/liquid Extraction

For liquid-liquid extraction, the dried ethanol extract (2 g) was dissolved in 50 mL of methanol: H_2O (MeOH-H_2O; 1:1 ratio) and mixed with 50 mL of organic solvents (ethyl acetate, n-hexane, n-butanol, and chloroform) stirred and allowed to stand for 2 h. The four extracts, each with one of the solvents, were dried with Na_2SO_4, and the solvent evaporated *in vacuo*[3,17]. The four extracts were checked for their antibacterial activity, as described earlier.

2.5 Column Chromatography

Dried n-hexane extract (2 g) was dissolved in 50 mL of methanol: H_2O (MeOH-H_2O; 1:1 ratio) and loaded on a silica gel column (7.5 × 30 cm) packed with 160 g silica-gel of mesh size 60–120, and eluted with 50 mL each of n-hexane-ethyl acetate solvent mixtures in six different ratios: 10:1, 9:1, 7:1, 5:1, 3:1 and 0:1. Six fractions (F_1 to F_6), of volume 50 mL each, were collected following the standard procedures described in previous reports[15,18,19]. The F_1 to F_6 fractions were dried with Na_2SO_4, and the solvent evaporated *in vacuo*[3,17]. As described earlier, the six fractions obtained from n-hexane were checked for their antibacterial activity. A similar procedure was done with the chloroform extract as well.

2.6 Separation of Components by TLC

The F_3 eluant fractions obtained from column chromatography of both n-hexane and chloroform extracts were spotted on the TLC plate. The plate was prepared using silica gel of 60 F_{254} as per the procedure[3,19-22]. A hot air oven was used to activate the plates at 110°C for 20 min. The line of application was 1.5 cm from the bottom of the plate, and the points of application were 1.5 cm from the edge and 1.5 cm from each other. Samples were applied to the absorbent surface on the application line using a capillary tube. The glass chambers (6 × 25 cm) were previously saturated with the solvent vapour system. Seven dissimilar solvent systems, such as chloroform: methanol: water (9:0.5:0.5), v/ v/ v, chloroform: methanol: acetic acid (9:0.5:0.5), butanol: acetic acid: water (4:1:5), chloroform: acetone (7:1), chloroform: acetic acid (7:1), chloroform: methanol (9:1) and chloroform: ethanol (9:1), were used for the selection of the most appropriate solvent system. All TLC separations were done at room temperature (37°C). When the solvent travelled four-fifths of the length of the absorbent, the plates were removed and dried. UV light visualization was performed (366-254 nm), and the plates were exposed to iodine vapors. Three replicates of each sample were used for the calculation of the mean R_f (retention factor) values using the following formula:

$$R_f = \frac{Disteance\ travelled\ by\ the\ compound}{Distance\ travelled\ by\ the\ solvent\ front}$$

2.7 Purification of the Isolate using HPLC

The compound, extracted with n-hexane and separated by TLC (Thin layer Chromatography) was dissolved uniformly in 100% DMSO. The sample (1 mL), containing 10 µL of an isolated compound and solvent mixture (Methanol: Water (50:50 v/v), was injected into the High-Performance Liquid Chromatography System, which is equipped with an LC8A pump and photo array detector (SPD-M 10 A vp) combined with Agilent software (LC 10 A). The experimental procedure carried out[23,24] . The required chromatographic conditions for the analysis used were:

- mobile phase containing acetonitrile: 0.1% and 10:90 v/v formic acid,
- column specifications are: ODS (Octadecylsilane) Zorbax SB C18, length and breadth of the column is 4.6 × 150 mm,
- the configuration of the 3.5 µm detector was a photo array detector (SPD-M 10 A vp),
- 254 nm wave length with flow rate: 1.0 mL/min,
- 5 µL of sample were injected in duplicate, and (6) the time of retention observed was 6.4 min. The absorbance of the eluant at 254 nm was documented by DAD[23,24].

2.8 Purification of Compound by Liquid Chromatography- Mass Spectroscopy (LC-MS)

The peak obtained from HPLC was dissolved in 100% DMSO and subjected to further analysis by the LC-MS. Solution (5 µL) containing isolated sample and solvent mixture (Methanol: Water (1:1 v/v) was used for the LC-MS. The experiment was performed on a Triple Quad-6410 from Agilent Technologies (Time of flight mass spectrometer), Connected to a 1000 amu, HPLC – Detector – DAD. Separation of the fraction was done using C18 column as per the procedure[24,25] with the column composition Zorbax SB and dimensions 4.6 × 150 mm. Solvent mixture of acetonitrile (0.1%) and formic acid (10:90 ratio) was used as the mobile phase. The spectrometer was operated in a positive mode with an elution profile of 0 to 21 min, and 0.5 mL/min constant flow rate. The interpretation of the data was analyzed using software. The calibration of the system was done, at the end, using sodium formate[24,25].

2.9 Nuclear Magnetic Resonance (NMR) C^{13} and H^{1}

2.9.1. Preparation of the Sample

Sample was prepared for NMR as reported earlier[26,27], by dissolving the extract in D_2O (600 µL). The evaporated extract was suspended in 1.5 mM TSP as the final concentration**,** and the same was transferred to a NMR tube (5 mm) for sample analysis. However, for media analysis, an aliquot of media (400 µL) under sterile condition was mixed with D_2O (200 µL) containing TSP (4.5 mM). NMR spectra (^{13}C and ^{1}H) of the sample, and media were acquired on a 700 MHz ^{1}H, Varian Instruments (16.4T Varian INOVA) equipped with an indirect cold probe (5 mm)[26,27].

2.9.2. Spectral Processing

The processing of the data was carried out by setting the base line of the FID by zero filling to 64,000 points, and the correction of the baseline was done by setting the TSP peaks to 0.00 ppm. Further 20 Hz broadening of exponential line done for analyzing ^{13}C NMR spectra. Advanced Chemistry Development, Inc., Toronto, version 12.0, Canada, was used for NMR analysis.

2.10 Fourier Transform and Infrared Spectroscopy (FT-IR)

The IR spectrum of an isolated sample (hexane fraction) was recorded on an FTIR instrument combined with a Nic-Plan IR microscope (Nicolet MAGNA-IR 760 Spectrometer) using KBr pellets (400-4000 cm^{-1}) in the region[28].

3. Results and Discussion

3.1 Soxhlet Extraction

As already reported earlier by the researchers, the extractive yield of ethanol by soxhlet extraction is 41.65%[9] calculated using the formula[26]. Appreciable antibacterial activity towards *P. aeruginosa* isolated from used coolant oil, was observed with a zone of inhibition of 08.25 0.35 mm (Table 1), and the MIC was 2.0 mg/mL.

Table 1. Antibacterial activity: Soxhlet extraction

Test extracts	Zone of inhibition (in mm)*	MIC in mg/mL
Ethanol	08.25 0.35	2.00

Muller Hinton agar plates were inoculated with *P. aeruginosa* by spread plate method. 50 mm wells were made with the help of borer. 10µl of various extract was added to the wells and incubated the plates at 4°C and the zone of inhibition was recorded. * Mean zone of inhibition (n=3) ± Standard deviation.

3.2 Liquid-Liquid Extraction

The yield of each extract was as follows: ethyl acetate (1.8 gm), n-hexane (0.8 gm), chloroform (1.1 gm), and n-butanol (2.1 gm). n-Hexane and chloroform extracts showed anti-*Pseudomonas* activity. Although the yield of the n-hexane extract was less compared to the other three extracts, maximum anti-*Pseudomonas* activity was shown by n-hexane, with a zone of inhibition of 15.2 0.001 mm compared to the chloroform fraction of 11.14 0.12 mm. Butanol and ethyl acetate extracts did not show any anti-*Pseudomonas* activity (Figure 1 and Table 2).

Table 2. Antibacterial activity: Liquid-liquid extraction

Test extracts	Zone of inhibition (in mm)*	MIC in mg/mL
n-hexane	15.2 0.001	0.50
Chloroform	11.14 0.12	-
Butanol	Nil	-
Ethyl acetate	Nil	-

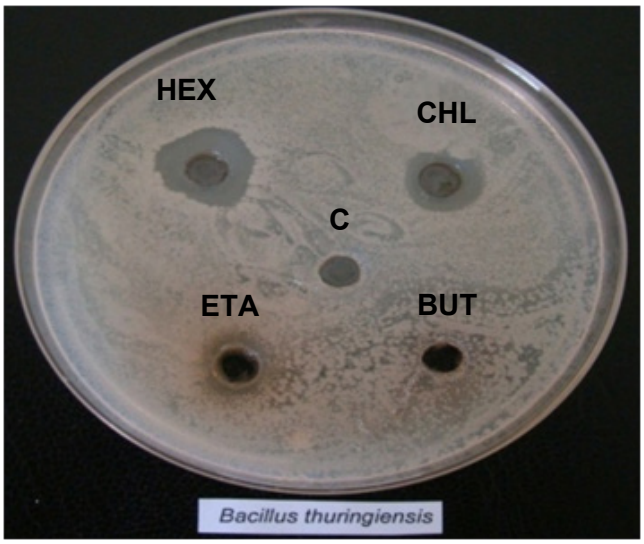

Figure 1. Antibacterial activity of the column fractions. HEX: n-hexane, CHL: chloroform, ETA: ethyl acetate, BUT: butanol, C: Control (DMSO).

3.3 Column Chromatography

The F_3 eluant fractions obtained from silica gel column chromatography of both n-hexane and chloroform extracts showed anti-*Pseudomonas* activity with a zone of inhibition of 11.8 0.03 mm and 08.54 0.12 mm (Table 3), respectively. All other fractions did not show any appreciable antibacterial activity. Hence these F_3 eluant fractions were used for further separation.

Table 3. Antibacterial activity: F_3 elution fraction from Column Chromatography of n-hexane and chloroform extraction

Test extracts	Zone of inhibition (in mm)*	MIC in mg/mL
n-hexane	11.8 0.03	Not done
Chloroform	08.54 0.12	

The six fraction effluents (F_1-F_6) were collected from silica gel column chromatography and tested for anti-*Pseudomonas* activity. As only F_3 Fraction showed the inhibition zone, hence only this fraction used for further study.

3.4 Thin-Layer Chromatography (TLC)

Out of the seven different solvent systems used for separation of the n-hexane F_3 eluant fraction, a mixture of chloroform: methanol: water (9: 0.5: 0.5) showed single spot R_f value of 0.38 and an appreciable anti-*Pseudomonas* activity with a zone of inhibition of 13.50 0.70 mm and a MIC found to be 0.5 mg/mL. The chloroform F_3 eluant fraction was separated into three spots with R_f values of 0.21, 0.32, and 0.45, which did not show any anti-*Pseudomonas* activity (Figure 2, Table 4). Hence F_3 fraction of n-hexane, which appeared to be relatively pure, was used for further purification.

Table 4. Antibacterial activity: Thin Layer Chromatography fraction

Test extracts	Zone of inhibition (in mm)*	MIC in mg/mL
n-hexane (spot 1)	13.50 0.70	0.5
Chloroform (spot 1)	Nil	nil
Chloroform (spot 2)	04.10 0.50	nil
Chloroform (spot 3)	02.50 0.30	nil

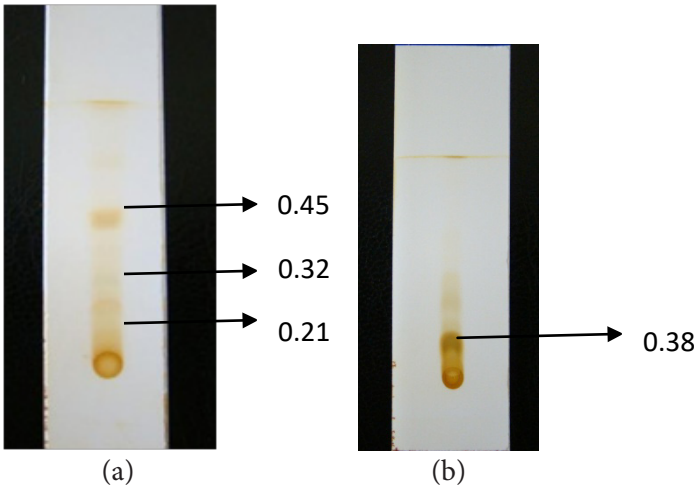

Figure 2. Fractionation of active extracts obtained from liquid-liquid extraction **(a)** Chloroform extract, **(b)** n-hexane extract.

Plate: silica gel 60 F_{254} , Merck , Developing system: Chloroform: Methanol/: water (9.0: 0.5: 0.5).

Indicator: Iodine, Detection: Visible light, UV254.

3.5 HPLC (High-Performance Liquid Chromatography)

The HPLC chromatogram of the F_3 eluant fraction of n-hexane extract showed a single highest peak at a retention time of 3.43 min (Figure 3, Table 5, 6), peak height = 156.56, area = 1391.27, and area % = 100. The tested anti-*Pseudomonas* activity was 15.25 0.00 in comparison with standard *Pseudomonas* (MTCC7903) with 12.05 0.00 (Table 6). Further, the MIC observed was 0.01 mg/mL for both the test and standard *Pseudomonas* strains.

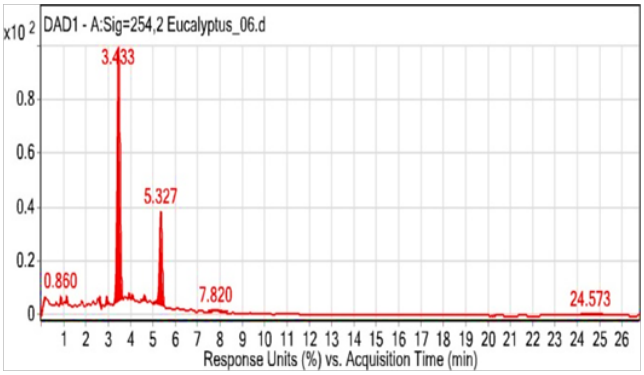

Figure 3. HPLC Chromotogram.

Table 5. Spectral analysis of n-hexane fraction: HPLC

Peak	Retention Time	Height	Area %
1	0.153	2.34	1.7
2	0.86	5.61	1.6
3	1.12	4.57	1.11
4	2.613	4.89	1.07
5	2.907	5.73	2.15
6	3.433	156.56	100
7	3.92	3.69	1.03
8	4.067	3.36	1.21
9	4.613	4.01	1.87
10	5.327	58.01	34.82

Table 6. Antibacterial activity: HPLC fraction

Test extracts	Zone of inhibition (in mm)*	MIC in mg/mL
Highest Peak	15.25 0.00	0.01
MTCC7903 (standard)	12.05 0.00	0.01

3.6 Liquid Chromatography-Mass Spectrometry

The mass analysis of the F_3 eluant fraction of the n-hexane extract gave a major peak with a ($M+H^+$) mass of 471.3 and a minor peak at 485.4 (Figure 4, Table 7).

3.7 ^{13}C- and ^{1}H-NMR (Nuclear Magnetic Resonance)

The ^{1}H-NMR (Nuclear Magnetic Resonance) spectra of isolated compounds exhibited de-shielded multiplets between δ 0.48-2.27 and at δ 3.79, 4.41, 4.65 and 5.27 for all O-H respectively. CHO proton appeared at δ 10.1 ppm. ^{13}C-NMR spectrum displayed all carbons from δ 15.-151.21 and at δ 180 for –CHO (Figure 5, 6, 7).

Table 7. Spectral analysis of n-hexane fraction: Mass Spectrometry

Peak	*Mass per Charge Ratio*	Abund
1	251.2	145788
2	385.3	65636
3	401.3	83857
4	471.4	525141
5	472.4	150751
6	485.4	293347
7	486.4	80374
8	487.4	161745
9	489.5	90897
10	499.4	94221

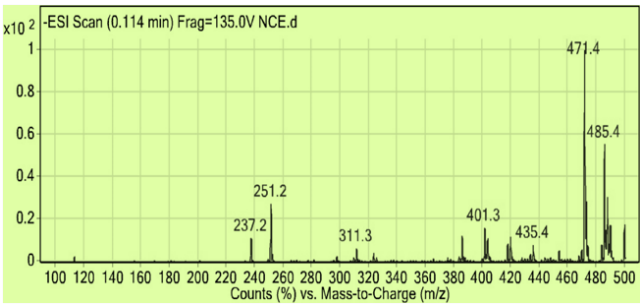

Figure 4. Liquid Chromatography-Mass Spectrometry.

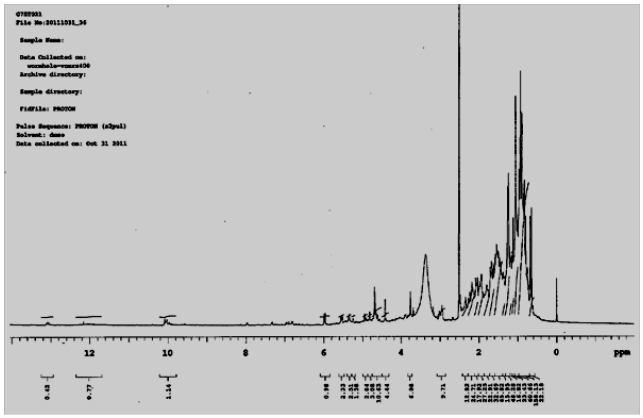

Figure 5. ^{13}C-NMR spectrum.

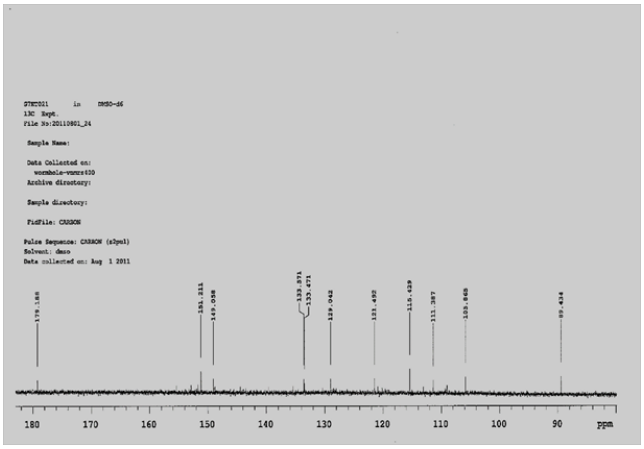

Figure 6. ^{13}C NMR.

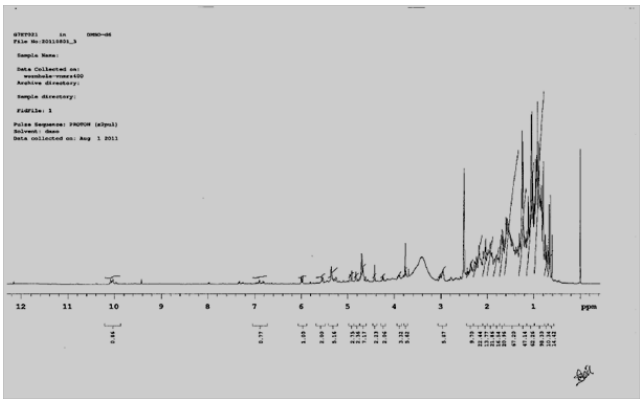

Figure 7. ^{1}H Proton NMR.

3.8 FT-IR Spectroscopy (Fourier Transform-Infrared)

FT-IR spectra (Fourier Transform-Infrared) showed distinctive absorption band peaks for the hydroxyl group at 3411 cm^{-1} and aliphatic stretching C-H at 2937 and 2873 cm^{-1} (Figure 8). However, C=O stretching was observed at 1713 cm^{-1}.

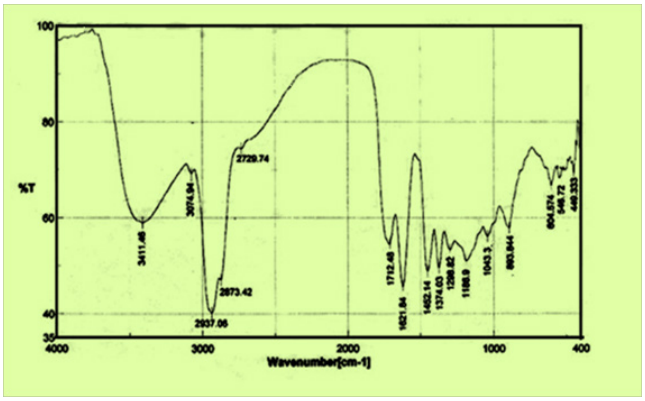

Figure 8. FTIR spectra.

3.9 Structure Elucidation

The spectral analysis of the compound suggested it is closely related to the structures of macrocarpal (Figure 9). The mass spectra displayed a molecular ion peak atm/z 471.3, which corresponds to the molecular formula of $C_{28}H_{40}O_6$. Its infrared spectrum revealed individual absorption bands for the hydroxyl group at 3411 cm^{-1} and C-H aliphatic stretching at 2937 and 2873 $cm^{-1}$and for C=O stretching at 1713 cm^{-1}. The proton NMR spectra of the compound exhibited de-shielded multiplets between δ 0.48-2.27 and at δ 3.79, 4.41, 4.65, and 5.27 for all O-H, respectively. CHO proton appeared at δ 10.1 ppm. ^{13}C-NMR spectra displayed all carbons from δ 15.-151.21 and at δ 180 for –CHO.

MF: Molecular formula: $C_{28}H_{40}O_6$

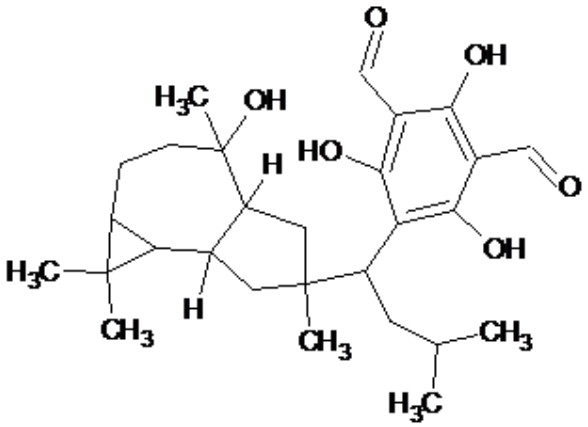

Figure 9. Macrocarpal A.

4. Discussion

Solvent extraction and liquid-liquid extraction of the extract are done to isolate the secondary metabolites

from plant sources, while the separation of the specific molecules is done by various chromatography techniques such as column chromatography and TLC. HPLC and LC-MS are used for checking the purity and determination of the molecular weight[22-24]. This study attempts to isolate, purify and identify secondary metabolites from ethanol extracts of *E. tereticornis* leaves using standard techniques, with anti-*Pseudomonas* activity against *P. aeruginosa* formerly isolated from used coolant oil[8,9].

A previous study[25] with methanolic extracts of *E. globules* showed antibacterial 'respond in contradiction' for *S. aureus, E. coli,* and *P. aeruginosa,* showing MIC of 5.0, 10.0, and 10.0 mg/mL[25]. In contrast, ethanol extract of *E. tereticornis* leaf showed antibacterial activity towards *P. aeruginosa, B. thuringiensis* and *B. cereus* with MIC of 2.0, 0.2, 0.5 mg/mL, respectively[9].

Thin Layer Chromatography (TLC) methods were reproducible in detecting the flavanoid, kaempferol-3-o-rectinoside, isolated in a sephadex column, using polar solvents followed by structural elucidation by spectroscopic technique[22,23]. Similarly, experiments were also conducted focusing on the extraction and TLC (Thin Layer Chromatography) for chlorophyll a and b extracted from spinach[29]. However, the present study's notable findings are the separation of formylated phloroglucinol compounds (FPCs) using TLC, and the standardization of the solvents used for the separation of FPCs. Among the seven different solvent systems used for separation of the n-hexane fraction, a mixture of chloroform: methanol: and water (9:0.5:0.5) showed good separation with a single spot (Figure 2b).

As previously reported[9], ethanol extracts of *E. tereticornis* leaves demonstrated significant antibacterial activity against *P. aeruginosa*, *B. cereus*, and *B. thuringiensis* isolated from used steel industry coolants. Earlier, the n-hexane extract of *E. globulus* was purified by HPLC with 45% methanol containing 1% TFA to give a tentative compound II[26,30,31]. In the current study, an n-hexane extract of *E. tereticornis* was purified by HPLC to yield a tentative compound III, which was compared to HPLC data reported[15] for detection of the Macrocarpal series from wood. *E. globulus* and *E. nitens* show single peaks, at a retention time of 30.0 min, for Macrocarpal A with a photodiode array detector[15,20,30]. While, the present study reports a peak retention time of 3.43 min with the DAD (Diode Array Detector) from the leaf extract of *E. tereticornis* (Figure 3).

Mass spectroscopy linked with gas chromatography or liquid chromatography is a powerful technique used by researchers for the separation of compounds based on mass to charge ratio. One such study using Gas Chromatography-Mass Spectrometry (GC-MS) analysis of stems, flowers, and roots of *Tripleurospernum collasum* (chloroform extracts) revealed the identification of 93 compounds[24,32]. While, LC-MS in the present investigation showed a single peak with the mass to charge ratio of 471.3 which supports the molecular formula $C_{28}H_{40}O_6$, matching the previously reported values of Formylated Phloroglucinol Compounds (FPCs) ranging from 454 to 472, isolated from other *Eucalyptus* species (Flower and Wood Extract)[28]. Further spectroscopic analysis using techniques such as nuclear magnetic resonance (NMR) and Fourier Transform - Infrared (FTIR) spectroscopy revealed structural data for macrocarpals[30-33] matched the data observed in the present investigation The ^{1}H-NMR spectrum of the compound exhibited de-shielded multiplets between δ 0.48-2.27 and at δ 3.79, 4.41, 4.65 and 5.27 for all O-H respectively. CHO proton appeared at δ 10.1 ppm. ^{13}C-NMR spectrum displayed all its carbons from δ 15.-151.21 and at δ 180 for –CHO matched the structure of macrocarpal A (Figure 3)[34-36].

There are significant variations among different formylated phloroglucinol compounds (FPCs), suggesting that they may have multiple roles depending on their composition, sites, synthesis and storage[30,37]. Fifteen compounds were obtained and identified from the fruit extract of *E. globulus*. To date, the chemical structures of some 50 specific macrocarpals and euglobals, which belong to the family of FPCs, have been published. But studies on its role in antibacterial defence are limited[30,31,33]. FPCs from the ethanolic extracts of the leaf of *E. globulus* are shown to have antibacterial activity against oral pathogenic organisms, *S. Ingbritt* and *P. gingivalis* ATCC 33277. While the residue of the methanolic extract of the same leaf showed antibacterial effects on different microorganisms such as *S. aureus, P. aeruginosa* and *E. coli*[22]. This is suggestive that there might be more than one secondary metabolite with antibacterial property, and with differences in their

solvent solubility[27,38,39]. However, in the present study, the Thin Layer Chromatography (TLC) profile of the n-hexane extract of *E. tereticornis* leaf showed a single spot (R_f = 0.38) and the component has inhibitory effects towards *P. aeuraginosa.* However, some of the macrocarpals isolated from *E. globulus* are shown to possess anti-HIV RTase inhibitory activity, with IC_{50} ranging from 5-12 mM as well[20,21,40].

5. Conclusion

On the basis of HPLC and LC-MS data, this study has tentatively identified a secondary metabolite as being related to Formylated Phloroglucinol Compounds (FPCs). FTIR and NMR data reveal it as Macrocarpal A with the molecular formula $C_{28}H_{40}O_6$, It shows antibacterial activity against *P. aeuraginosa* isolated from used steel industry coolant oil. Further standardization and scale up will probably be beneficial as a preservative for coolant oil.

6. References

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