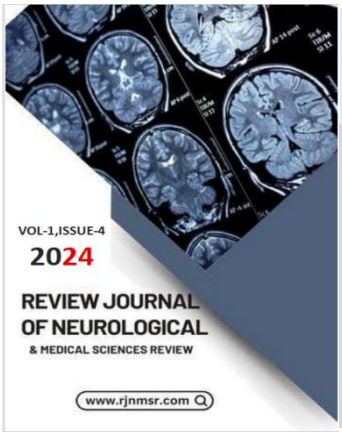


Vol. 2 No. 4 (2024): October - December
https://rjnmsr.com/index.php/rjnmsr/about

Review Journal of Neurological & Medical Sciences Review

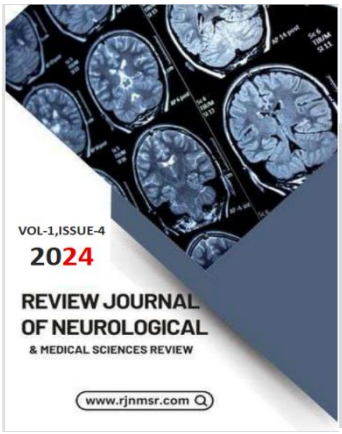
E(ISSN) : 3007-3073
P(ISSN) : 3007-3065

Antibiofilm Activity of Eucalyptus Camaldulensis for Combating Bacterial Infections

Ammara Bibi[1]
Department of Microbiology, the University of Haripur, Khyber Pakhtunkhwa, Pakistan
Syeda Asma Bano[2*]
Associate Professor, Department of Microbiology, the University of Haripur, Khyber Pakhtunkhwa, Pakistan. Coresponding Author Email: asma_baano@yahoo.com
Iqra Bibi[3]
Department of Microbiology the University of Haripur, Khyber Pakhtunkhwa, Pakistan

Abstract

Background: Traditional treatment of infectious diseases is based on compounds that kill or inhibit bacterial growth. Eucalyptus (river red gum) is considered an important medicinal plant worldwide. It is used for medicinal purposes, especially as a cough remedy, and has a significant role against most of the microbial pathogens that impose serious diseases. The main aim and objective of this study were to identify the antibiofilm activity of Eucalyptus camaldulensis against biofilm-forming bacterial pathogens affecting humans. Methodology; Samples were collected from Haripur. All samples (leaves of plants) were collected from different regions of Haripur. Agar well diffusion method was used for the initial antibacterial activity of plant from leaf extract sample (100g/ml). Muller Hinton agar was used for antibacterial activity of bacterial pathogens by using the temperature of 37°C for 1 to 2 days of incubation. Then antibiofilm activity was tested on 96 well plates. Cultured samples were stained using crystal violet stain. For confirmation of strains, Congo red method was performed. Blackish and red colonies with dry crystalline consistency indicated biofilm producers. Results: Plant extracts were rich in over 250 detected chemicals via GC-MS analyzer. The antimicrobial activity of some of the compounds was known e.g. eucalyptol(37%) and 10S,11S-HIMACHALA-3(12),4-DIEN(56%) which may be used against the antibiofilm activity of bacteria, while other compounds needs further research for which no record were found in literature



Vol. 2 No. 4 (2024): October - December
https://rjnmsr.com/index.php/rjnmsr/about

Review Journal of Neurological & Medical Sciences Review

E(ISSN) : 3007-3073
P(ISSN) : 3007-3065

review for antibacterial activity. Acetonic leaf extracts of Eucalyptus camaldulensis may be used in suitable formulation against bacterial diseases. Conclusion: These findings underscore the promising applications of Eucalyptus camaldulensis extracts in both antimicrobial and antibiofilm interventions. And can be effected against different diseases such as cough remedy, and disease caused by Escherichia coli and staphylococcus aureus. Further exploration of their specific mechanisms of action and potential therapeutic applications of different metabolites and chemical compounds are warranted to harness the benefits offered by these natural extracts.

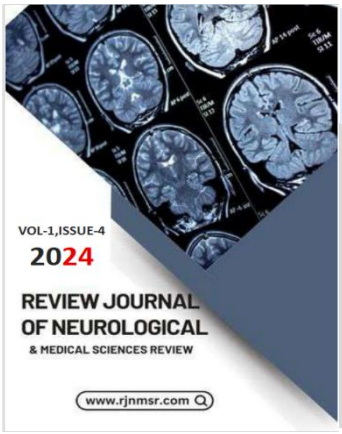
Keywords: Biofilm forming microorganism, Extracts, Antimicrobial and anti-biofilm activity

Introduction

The disaster of antimicrobial resistance increases day by day due to misuse of these agents that is why bacterial strains become more resistant(Adonizio et al., 2008). Pseudomonas aeruginosa which is resistant against many diseases while Staphylococcus aureus which is resistant against methicillin similarly enterococci is resistant against vancomycin antibiotic.(Nelson, 1997).Due to the emergence of multidrug-resistance, the efficacy of antibiotics against pathogenic bacteria is now reducing (Aslam et al., 2018).The development of biofilm is one of the most important pathway which is used in bacteria and emerging for developing such resistance(Tajkarimi et al., 2010) (El-Taweel, 2015) (Schmidt-Silva et al., 2011). Natural compounds has significant effect against multi-drug resistant microorganisms (Jadhav et al., 2013).These new-found antimicrobial agents could be unseen in medicinal plant extracts and essential oils. One of the significant medicinal plants is Eucalyptus camaldulensis (Dung et al., 2008).Eucalyptus camaldulensis common name is river red gum tree which belongs to Myrtaceous family and genus is Eucalyptus it is a common tree found in many countries and also called River Red Gum (Elaissi et al., 2012).

Eucalyptus camaldulensis a well-known medicinal plant. It is also considered one of the most widely planted trees in the world. Extracts are affecting against microorganism (El-Taweel, 2015).It is already identified that essential oils have antimicrobial activity due to presence of some bioactive compound, some specific compounds, mainly terpenoids and phenylpropenes with the most energetic being phenols like carvacrol, thymol and eugenol (Ventola, 2015).

Large quantity of bioactive compounds are obtained from plant Zhao et al., (2005). The important eucalyptus bioactive compounds are terpenoids and thymol). The vital component of plant is alpha pinene and aphellandrene



Vol. 2 No. 4 (2024): October - December
https://rjnmsr.com/index.php/rjnmsr/about

Review Journal of Neurological & Medical Sciences Review

E(ISSN) : 3007-3073
P(ISSN) : 3007-3065

(Robbins et al., 2017). Their percentage varies in different species of plants. aphellandrene (20.8%), 1,8-cineole(9.48%) c-terpinene are most commonly used. Their percentage varies in different species of plants.Many studies have been conducted to evaluate the repressive activity of essential oils of plants against microorganisms. There are few reports showing the action of the essential oil against Gram positive bacteria Ellwanger et al., (2015); Negreiros et al.,(2016).Dried leaves extract of Eucalyptus citriodora are traditionally used as anti-inflammatory remedies used as analgesic, respiratory infection, flu, cold and sinus congestion. (Gilling et al., 2014).

Eucalyptus camaldulensis are also known to contain bioactive products that showed antibacterial, antifungal, analgesic and anti-inflammatory effect, antioxidative and antiradical activities (Mabona et al., 2013)..The bacterium Pseudomonas aeruginosa causes very severe infection in immune-compromised patients (Sebei et al., 2015) and is responsible for about 57% of all nosocomial infections. Phytosterols from oil are sitosterol, stigmasterol and campesterol and they most commonly consumed. Their percentage varies in different portion in each specie kernel(Nasir et al., 2015).The presence of eucalyptol which is naturally occurring compound along with other medicines helps to improve the functioning of thyroid gland and immune system. (Nosratabadi et al., 2015) 1-8 cineole in essential oil has ability to control blood pressure by regulating blood cholesterol level (Reda et al., 2017).Biofilms are the large community of microorganisms. It is an extracellular membrane which is made up of proteins, lipids and extracellular DNA(Palomino et al., 2002). Pseudomonas aeruginosa is source of serious life threatening diseases may lead to AIDS, cancer, hospital required pneumonia and urinary tract infection in developing countries where there is limitation of disease control methods and ability of biofilm formation of Pseudomonas aeruginosa make it more susceptible (Thuille et al., 2003).

Previous reports tells us that 10 to 20 % of hospital infections occurs due to Pseudomonas aeruginosa strain PAO1 (Aqil et al., 2014).There are certain compound which inhibit QS system of Pseudomonas aeruginosa can help to restrict it biofilm formation (Aqil et al., 2014). So the best system for inhibition of biofilm is quorum sensing. Numerous plants have been testified having antibacterial activity against various bacterial pathogens that causing disease in humans and animals. (Wang et al., 2021)

Plants also have phenolic compounds which bind with receptors to recognize the invading microorganisms and act as a medicine (Olawore and Ololade, 2017). Today natural compounds of plants have well developed to stop the attack of pathogen by inhibiting invasion of microorganism into the tissue of plants and triggering the wide range of defensive reactions against different types of pathogens(Selim, 2011). It is accepted that the extract of plant have anti-bacterial and antifungal properties (Jemâa et al., 2012).One of the most effective ways is biofilm inhibition and quorum sensing method (Nelson, 1997)

Vol. 2 No. 4 (2024): October - December
https://rjnmsr.com/index.php/rjnmsr/about

Review Journal of Neurological & Medical Sciences Review

E(ISSN) : 3007-3073
P(ISSN) : 3007-3065

Materials And Methods

The present study involves checking the antibiofilm activity of Eucalyptus camaldulensis plant leaf extract against pathogenic bacteria. Leaf extract prepared in ethanol, methanol, and acetone was used against biofilm-forming bacteria. Much of this study was carried out in the Microbiology Laboratory, The University of Haripur.

Study Pathogens

Six strains of bacteria Salmonella typhi. Bacillus subtilus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Staph. aureus and Escherichia coli Pathogens were collected from NESCOM Hospital Islamabad and transported to laboratory of microbiology university of Haripur.

Sample Collection

Sterilized polythene bags were used for sample collection. Samples were collected all over the fields Of Haripur district Pakistan. Collection of healthy leaves of Eucalyptus camaldulensis is started in summer season from university of Haripur. Plant were identified by taxonomist. Healthy leaves of Eucalyptus camaldulensis were collected and unhealthy leaves were detached rest were dried at room temperature. The collected samples were properly labelled and brought to Microbiology laboratory. The samples were stored at room temperature until process Samples were prepared from Haripur. Samples were washed under running tap water prior to surface sterilization. The surface sterilization was done by using 0.5% of sodium hypochlorite solution for 1-2 minutes and then washed in autoclaved distilled water. The sterilized samples were grind by using grinder. Samples were prepared for inoculation. Drying of samples at room temperature .The dried leaves were then ground to a fine powder using a grinder. The leaf powders 115g were weighed and stored in closed glass containers in the dark at room temperature.

Extraction

Acetone, methanol and ethanol solvents were used in extraction process. Powered leaf extract were dissolve in three solvents. Two gram of leaf sample were dissolved in 20ml of each solvent. Sonicater was used for 20 minutes, vigorously shaken the mixture and then discharged in centrifuge tube. Centrifuged these tubes for 10 min at 4000g.Next the supernatant was collect together and filtered through filter paper. For further drying rotatory apparatus were used. After drying leaf extract were weighted and concentration of 100mg/ml in acetone, methanol and ethanol were set for further assays.

Vol. 2 No. 4 (2024): October - December
https://rjnmsr.com/index.php/rjnmsr/about
Review Journal of Neurological & Medical Sciences Review
E(ISSN) : 3007-3073
P(ISSN) : 3007-3065

Figure 01: Plant Collection and Extraction Preparation

Well Diffusion Method

Antimicrobial activity was performed on muller Hinton agar .Then wells were makes with corn borer. Then inoculate bacterial strains such as Pseudomonas aeruginosa, Salmonella typhimurium, Escherichia coli ,Bacillus subtilis ,Klebsiella pneumonia and Staphylococcus aureus on surface of agar. Incubated these plates for 24 hour. Then the antimicrobial activity of the microbes is noticed by the appearance of zone of inhibition around the agar plug. ANOVA test was applied for antibacterial activities by means of SPSS software(Griffin et al., 2000)

Minimum Inhibitory Concentration

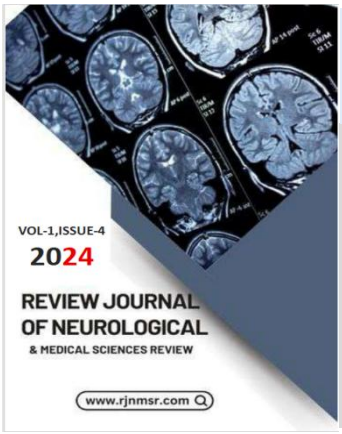
MIC was done by micro-dilution method. Test were performed in 96 well flat-bottom plates. Each cell containing 100ul of two fold dilution of plant extracts concentration was inoculated in wells. Two controls were included without plant extract one with broth and other with 100ul broth and bacteria. Plates were incubated at 37 °C for 24hr. After incubation reading of tested plates were was taken at 560nm.Next percentage was calculated. The lowest concentration that inhibited the bacterial growth i.e., no visible growth as compared to test control was considered as the MIC value of the tested plant extracts. For minimum bacterial concentration(MBC) for plant extracts 20ul from each concentration that showed no visible growth were inoculated on sterile nutrient agar media in plates. The cultured plates were incubated at 37°C for 24hr. After sub culturing the lowest concentration which showed no visible growth on nutrient agar was considered as MBC value.(Eloff, 1998).

Congo red agar(CRA)Method

0.8g of Congo red dye was dissolved in 36g of sucrose and 37g/L brain heart infusion agar. Mixed completely by dissolving the ingredients in distilled water. Media was autoclaved at 121°C.After autoclaving media was cooled for some time in laminar flow hood at room temperature. Media was poured in petri plates and left them for some time for the solidification of media. Bacteria were inoculated on media. Incubated plates for 24hr at 37°C.Black and red color growth indicate all strains are biofilm producers bacteria.

Biofilm Inhibition Assay

Fresh culture of bacteria was prepared in Muller Hinton broth. Incubated test tubes for 24hr at 37°C.Poured 10ul bacterial suspension with OD value 0.1 in



Vol. 2 No. 4 (2024): October - December
https://rjnmsr.com/index.php/rjnmsr/about

Review Journal of Neurological & Medical Sciences Review

E(ISSN) : 3007-3073
P(ISSN) : 3007-3065

which has OD value 0.1 in 96 well plate. Incubated well plate for 24hr at 37°C Next 50ul extract was added in each well. Again well plate was incubated for 24hr at 37°C.Applied 25ul crystal violet solution in each well and left for 15 minutes at room temperature. Removed crystal violet and washed well plate thrice with distilled water. Next 95% ethanol solution were added in each well. Absorbance was measured by spectrophotometer at 590nm.(Djordjevic et al., 2002).

Cytotoxicity

This test tells us about the safety of drug on target cells. Similarly, cytotoxicity of medicinal plant give gives the information about its safety. 600microliter blood was added with 400 microliter phosphate buffer saline solution. Source of blood was human. Centrifuged at 4000rpm speed for 10 minutes. Supernatant was removed and 400microliter phosphate buffer saline were added. Washing of blood was done with 400microliter phosphate buffer saline until the supernatant become colorless. Standard tube contained RBC, PBS and 1% Triton X-100.Control tube contained red blood cells and phosphate buffer saline only. Experimental tube contained 50microliter of plant extract containing both red blood cells and phosphate buffer saline. Incubated three tubes for 1 hour at 37 °C. Centrifuged these tubes at 40000rpm for 10 minutes. Then supernatant collected from pipette and pour in micro titer plate. Absorbance was measured at 540nm.(Dzoyem et al., 2016) .

GCMS Analysis

Crude leaf extract was used as a sample. Initial temperature was 50°C.Final temperature was 230°C. Peaks of various compounds eluted from the column of GC were recorded along with their retention time. Helium gas(1ml/m) worked as carrier. Initial temperature was 50°C but after 20 minutes run was directed then temperature reached at 250°C. 40-600m/z was the range of spectrum. The spectrum was noted in the range of 40-600m/z. Mass spectra were used for data analysis. Database of similar compounds was interrelated along with their retention time and molecular mass. GC-MS of methanol extract of leaves samples showed the presence of bioactive compound. Due to known biological activity of several bioactive compounds leaves extract of Eucalyptus camaldulensis and references are provided in in Table 2.ss

Results And Discussion

Antibacterial Activity Of Eucalapytus Camaldulensis

Methanol solvent showed maximum zones of inhibition against Escherichia coli and Pseudomonas aeruginosa as compared to other bacterial strains. Replicative data of observed zones of inhibition were calculated through excel tool. Observed zones of inhibition against bacterial pathogen on MHA plate shown in figure # 2.1.Measured values of zones of inhibition shown in table # 3.1

Vol. 2 No. 4 (2024): October - December
https://rjnmsr.com/index.php/rjnmsr/about
Review Journal of Neurological & Medical Sciences Review
E(ISSN) : 3007-3073
P(ISSN) : 3007-3065

Table-1: Zone of Inhibitions by Extracts

Pathogens	Eucalyptus camaldulensis		
	Methanolic	Ethanolic	Acetonic
Escherichia coli	24mm	23mm	27mm
Staph. aureus	14mm	12mm	11mm
Pseudomonas aeruginosa	20mm	21mm	15mm
Salmonella typhimurium	15mm	20mm	12mm
Klebsiella pneumoniae	Nil	20mm	20mm
Bacillus subtilis	Nil	Nil	15mm

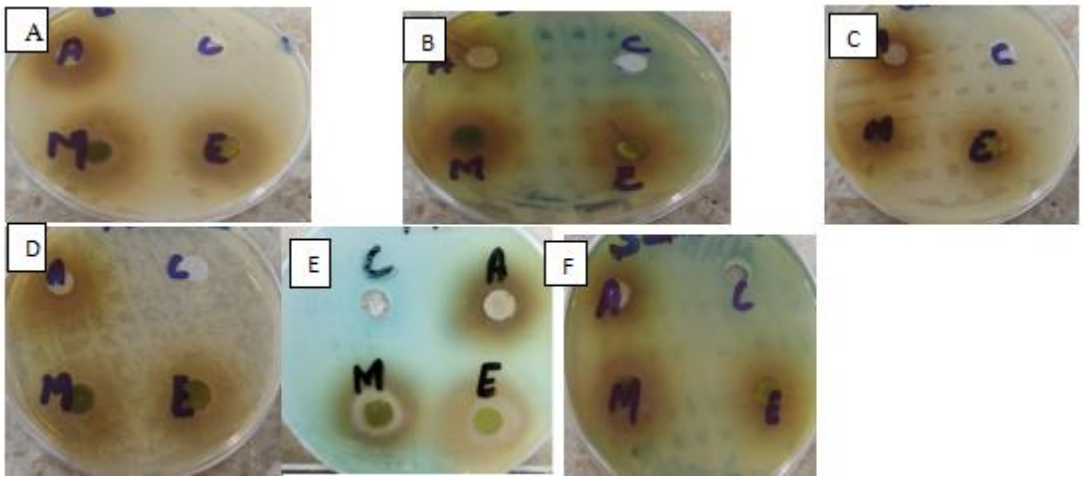


Fig-02: Zone of inhibition by different extracts against selected Pathogens

A= Zones of inhibition by Eucalyptus camaldulensis against Escherichia coli
B= Zones of inhibition by Eucalyptus camaldulensis against stap aureus
C= Zones of inhibition by Eucalyptus camaldulensis against Bacillus subtilus
D= Zones of inhibition by Eucalyptus camaldulensis against klebseilla pneumonae
E= Zones of inhibition by Eucalyptus camaldulensis against Pseudomonas aeruginosa

Vol. 2 No. 4 (2024): October - December
https://rjnmsr.com/index.php/rjnmsr/about
Review Journal of Neurological & Medical Sciences Review
E(ISSN) : 3007-3073
P(ISSN) : 3007-3065

F= Zones of inhibition by Eucalyptus camaldulensis against Salmonella typhimurium

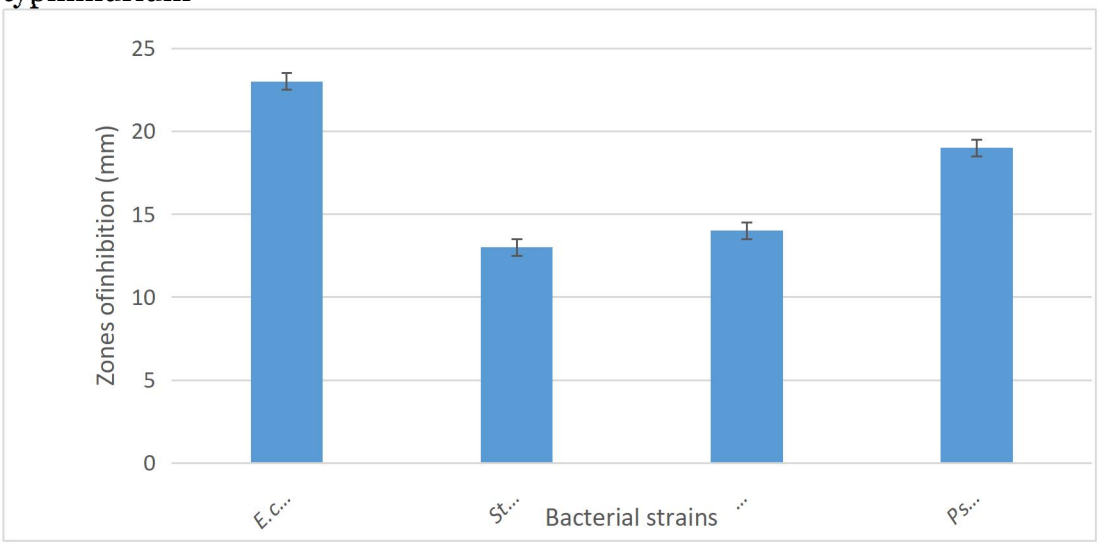


Fig- 3: Zones of inhibition showed by Eucalyptus camaldulensis leaves extract against selected pathogens in methanol solvent

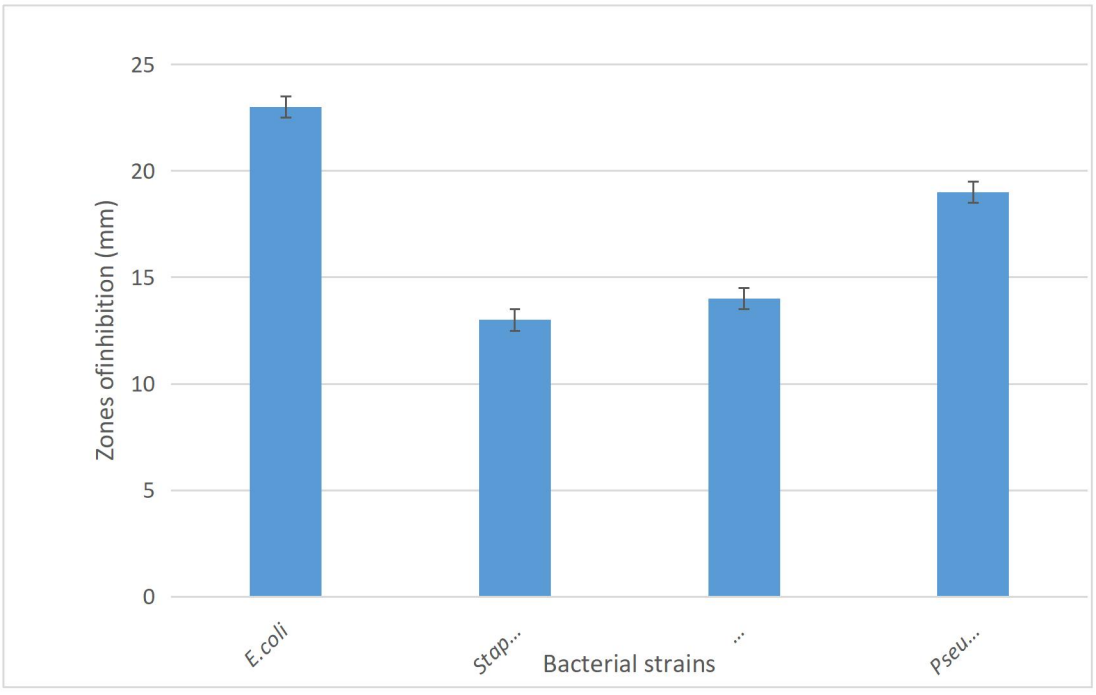


Fig-04: Zones of inhibition shown by Eucalyptus camaldulensis leaves extract against selected pathogens in acetone solvent

Vol. 2 No. 4 (2024): October - December
https://rjnmsr.com/index.php/rjnmsr/about
Review Journal of Neurological & Medical Sciences Review
E(ISSN) : 3007-3073
P(ISSN) : 3007-3065

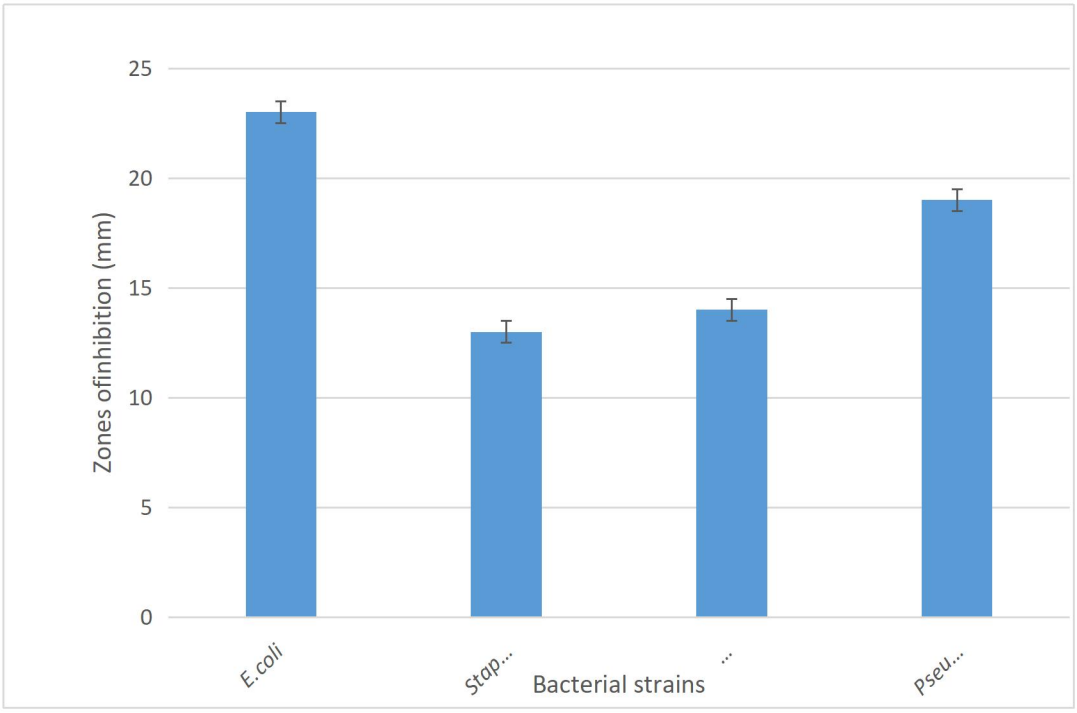


Fig-05: Zones of inhibition shown by Eucalyptus camaldulensis leaves extract against selected pathogens in ethanol solvent.

Minimum Inhibitory Concentration

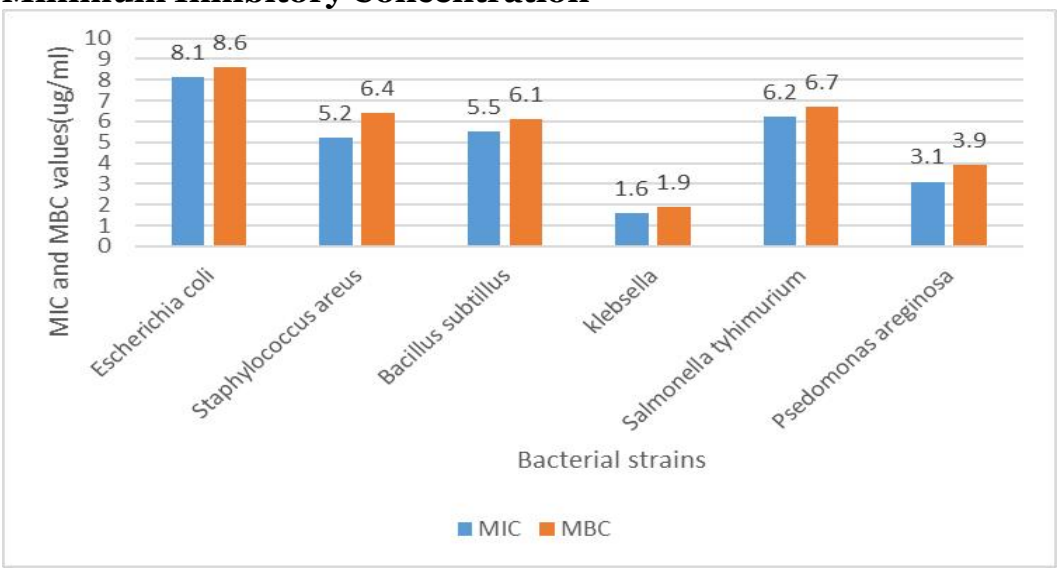


Fig-06: MIC and MBC determination of extracts in Ethanol solvent

Vol. 2 No. 4 (2024): October - December
https://rjnmsr.com/index.php/rjnmsr/about
Review Journal of Neurological & Medical Sciences Review
E(ISSN) : 3007-3073
P(ISSN) : 3007-3065

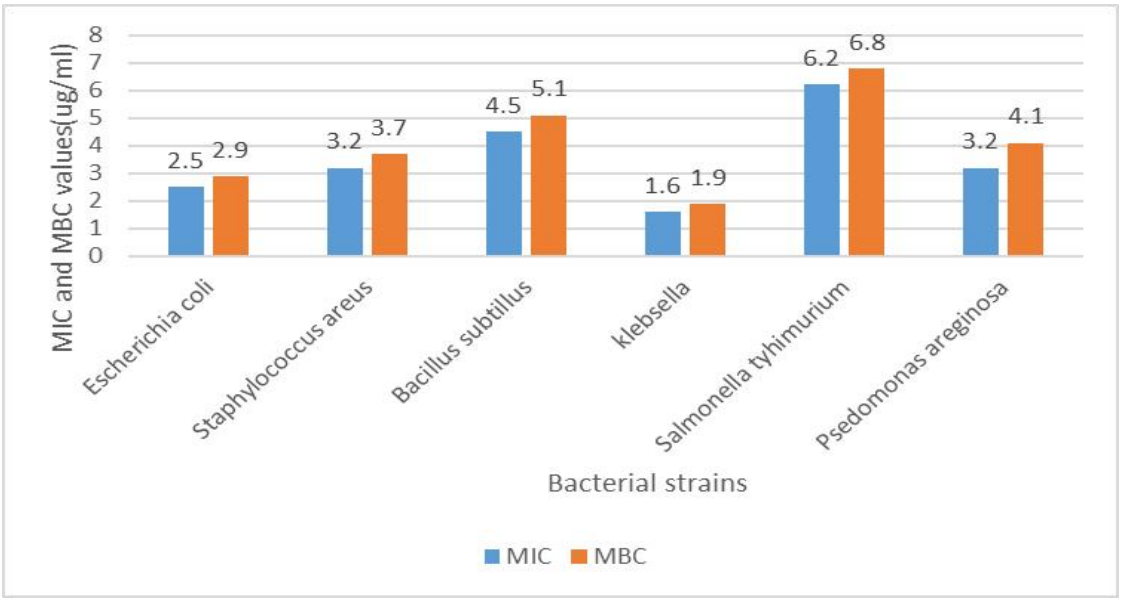


Fig-07 MIC and MBC determination of plant extracts in Methanol solvent

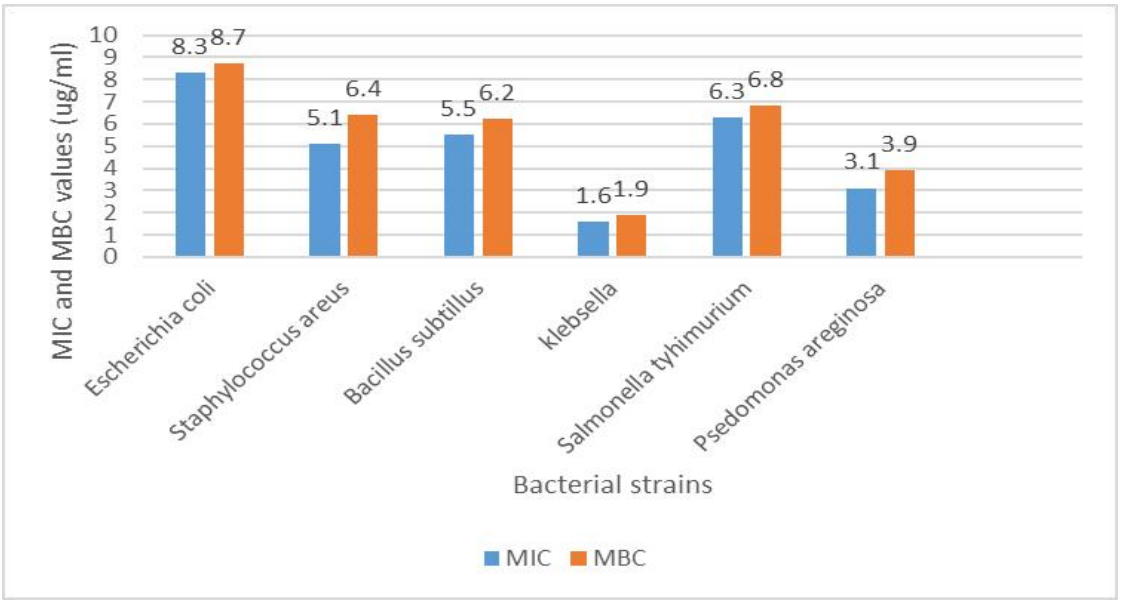


Fig-08 MIC and MBC determination of plant extracts in Acetone solvent

Vol. 2 No. 4 (2024): October - December
https://rjnmsr.com/index.php/rjnmsr/about

Review Journal of Neurological & Medical Sciences Review

E(ISSN) : 3007-3073
P(ISSN) : 3007-3065

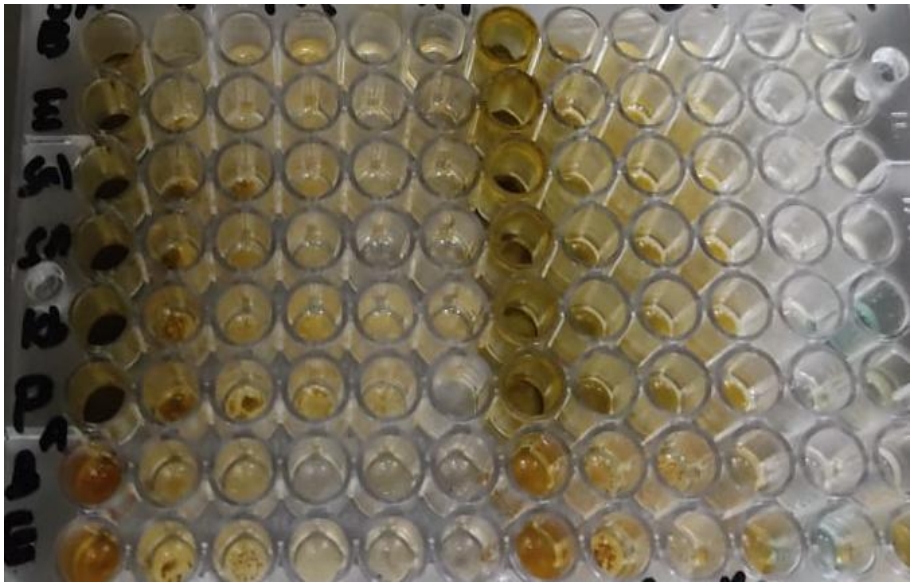


Fig-09: MIC and MBC determination of plant extracts of selected pathogens

Congo Red Agar Method

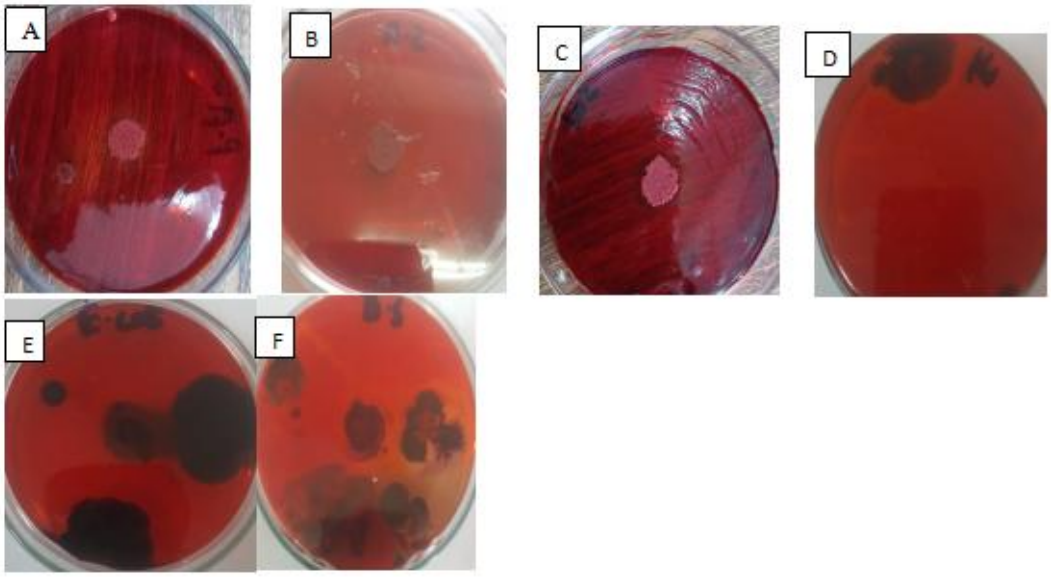
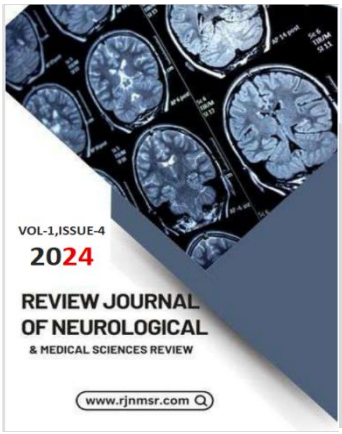


Fig:10: Congo red agar method against (A) Pseudomonas aeruginosa (B)Staphylococcus aureus, (C) (Salmonella typhimurium), (D) Klebsiella pneumoniae,, (E) Escherichia coli, (F) Bacillus subtills



Vol. 2 No. 4 (2024): October - December
https://rjnmsr.com/index.php/rjnmsr/about

Review Journal of Neurological & Medical Sciences Review

E(ISSN) : 3007-3073
P(ISSN) : 3007-3065

Biofilm Inhibition Assay

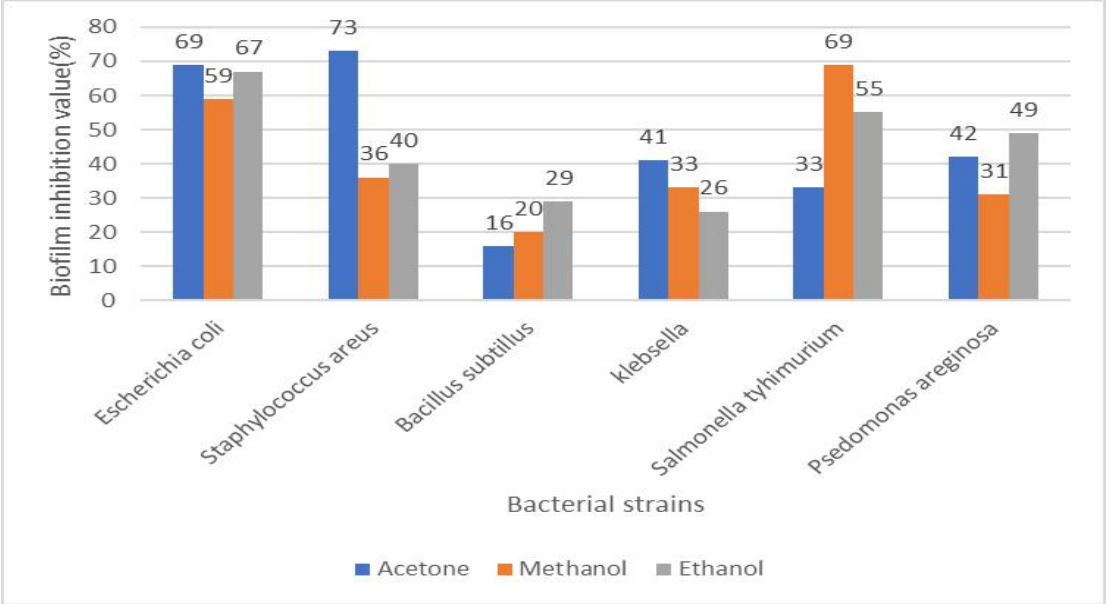


Fig-11: Biofilm inhibition by (Eucalyptus camaldulensis) plant extracts against bacterial strains. Acetone was the best solvent in biofilm inhibition assay against Escherichia coli and Staphylococcus aureus while methanolic solvent showed maximum inhibition against Salmonella typhimurium as compared to other solvents

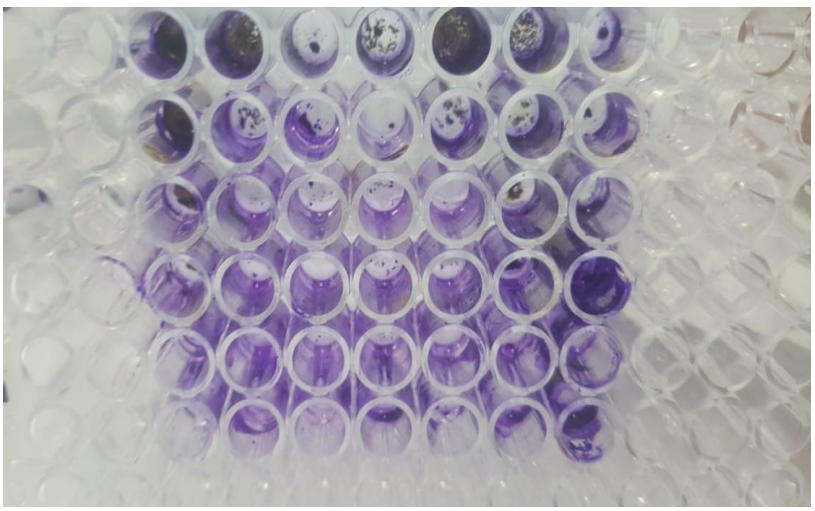
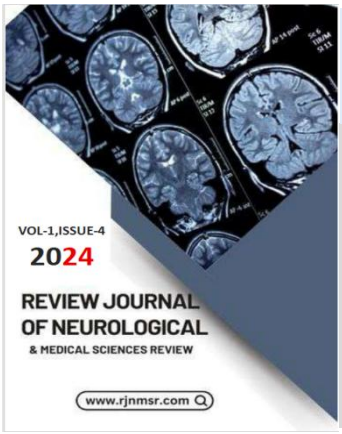


Fig-12: Biofilm inhibition by(Eucalyptus camaldulensis) plant extracts against bacterial strains



Vol. 2 No. 4 (2024): October - December
https://rjnmsr.com/index.php/rjnmsr/about

Review Journal of Neurological & Medical Sciences Review

E(ISSN) : 3007-3073
P(ISSN) : 3007-3065

Cytotoxicity

Table 02: Cytotoxicity value of plant extract in different solvents.

Solvents	Hemolysis (%)
Acetone	2.4%
Methanol	9%
Ethanol	3%

GCMS Analysis

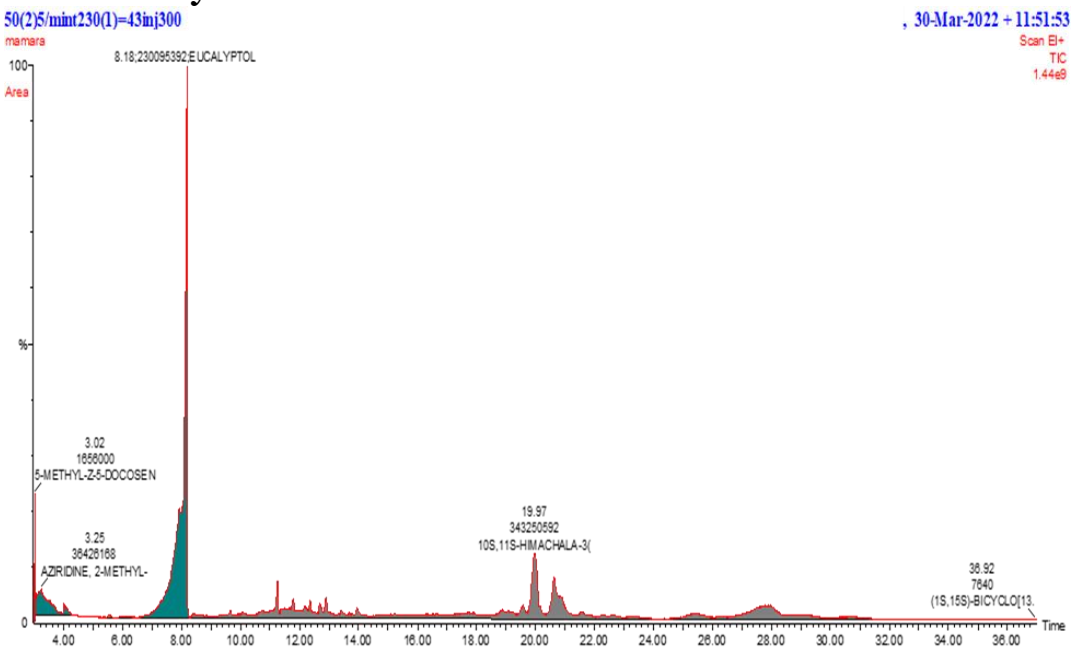
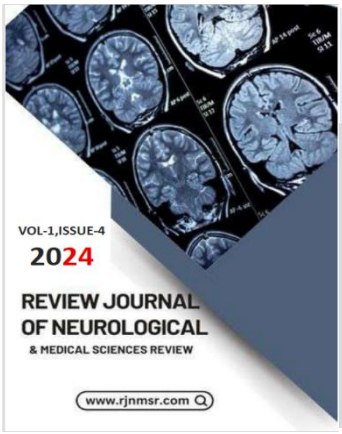


Fig-13:Compound detection, area and retention time in Eucalyptus camaldulensis leaf extracts by GCMS

Gcms Studies of Eucalyptus Camaldulensis

In figure 13 identified compounds EUCALAPTOL at retention time 8.1 with molecular formula C10H18O has antimicrobial activity (Aslam et al., 2018).Another identified compound in figure 13 10S,11S-HIMACHALA-3(12),4-DIEN at retention time 19.9 with molecular formula C15H24 has antimicrobial activity (Ashour, 2008).

In figure 13 identified compounds METHYL-Z-5-DOCOSENEat retention time 3.01 with molecular formula C23H46 has antioxidant activity (Khan et al., 2005). Another identified compound in figure13 BACCHOTRICUNEATIN C at retention time 36.1 with molecular formula C25H23O has antibacterial activity (Knezevic et al., 2016)



Vol. 2 No. 4 (2024): October - December
https://rjnmsr.com/index.php/rjnmsr/about

Review Journal of Neurological & Medical Sciences Review

E(ISSN) : 3007-3073
P(ISSN) : 3007-3065

Table-03: Compound detection in Eucalyptus camaldulensis leaf extracts by GCMS

Name	Formula	RT	Area	%comp
5-METHYL-Z-5-DOCOSENE	$C_{23}H_{46}$	3.018	1656000	0.270237
AZIRIDINE, 2-METHYL-2-(2,2,4,4	$C_{12}H_{25}$	3.249	36426168	5.94
Z,Z-6,28-HEPTATRIACTONTADIEN-2	$C_{25}H_{29}$	5.314	67095.92	0.01
CYCLOHEXANEMETHANOL, 4-METHYLE	C_6H_{12}	5.559	889331.4	0.145
CYCLOPROPANEOCTANAL, 2-OCTYL-	$C_{19}H_{36}O$	5.775	76287.89	0.012
CYCLOPROPANEOCTANAL, 2-OCTYL-	$C_{19}H_{36}O$	5.82	14240.13	0.0023
EUCALYPTOL	$C_{10}H_{18}O$	8.181	2.3E+08	37
10S,11S-HIMACHALA-3(12),4-DIEN	$C_{15}H_{24}$	19.971	3.43E+08	56
2-METHYL-3-(3-METHYL-BUT-2-ENY	$C_{25}H_{26}O$	35.8	154218.5	0.025
BACCHOTRICUNEATIN C	$C_{25}H_{23}O$	36.1	5735.9	0.0009
(1S,15S)-BICYCLO[13.1.0]HEXADE	C_6H_8	36.1	868.1	0.0014
2-METHYL-3-(3-METHYL-BUT-2-ENY	$C_{25}H_{26}O$	36.1	8963.6	0.004
2-METHYL-3-(3-METHYL-BUT-2-ENY	$C_{25}H_{26}O$	36.2	30488.6	0.004
TETRACONTANE-1,40-DIOL	$C_{40}H_{82}$	36.3	7591.4	0.01
(1S,15S)-BICYCLO[13.1.0]HEXADE	C_6H_{10}	36.4	18032	0.002
(1S,15S)-BICYCLO[13.1.0]HEXADE	C_6H_{10}	36.5	17436.7	0.002
(1S,15S)-BICYCLO[13.1.0]HEXADE	C_6H_{10}	36.5	5296.7	0.0008
(1S,15S)-BICYCLO[13.1.0]HEXADE	C_6H_{10}	36.6	11160.2	0.001
(1S,15S)-BICYCLO[13.1.0]HEXADE	C_6H_{10}	36.6	4760.8	0.0007
(1S,15S)-	C_6H_{10}	36.6	7978.6	0.001

Vol. 2 No. 4 (2024): October - December
https://rjnmsr.com/index.php/rjnmsr/about

Review Journal of Neurological & Medical Sciences Review

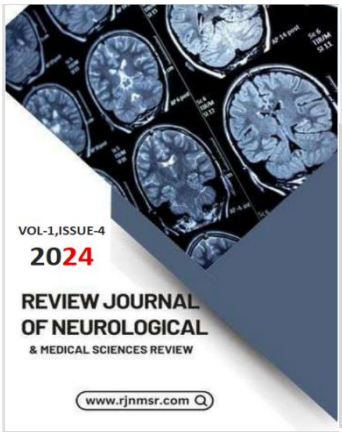
E(ISSN) : 3007-3073
P(ISSN) : 3007-3065

BICYCLO[13.1.0]HEXADE				
TRANS,CIS-1,8-DIMETHYLSPIRO[4.	$C_{12}H_{22}$	36.6	10963.7	0.001
NAPHTHALENE, DECAHYDRO-1,4A-DI	$C_{10}H_{18}$	36.7	7039.9	0.001
(1S,15S)-BICYCLO[13.1.0]HEXADE	C_6H_{10}	36.7	1598.0	0.002
TETRACONTANE-1,40-DIOL	$C_{40}H_{82}$	36.8	1598.0	0.002
(1S,15S)-BICYCLO[13.1.0]HEXADE	C_6H_{10}	36.9	7639.9	0.001
(1S,15S)-BICYCLO[13.1.0]HEXADE	C_6H_{10}	36.9	2053.5	0.001
(1S,15S)-BICYCLO[13.1.0]HEXADE	C_6H_{10}	36.9	2612.4	0.0004

Discussion

Eucalyptus plant is a medicinal plant to many bacterial pathogens that causes infectious diseases in humans and animals. Present research study showed the antibiofilm activity of Eucalyptus camaldulensis for combating bacterial infections. Leaf extract of Eucalyptus camaldulensis is effective against Pseudomonas aeruginosa, Salmonella typhimurium, Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli, Bacillus subtilis. Acetonic leaf extract samples showed significant inhibition against Staphylococcus aureus and Escherichia coli , while methanolic solvent show maximum inhibition against Salmonella typhimurium. Similar research was performed Jedidi et al., (2018) who reported that Pseudomonas aeruginosa inhibition against leaf extract. The bacterial strain were isolated from both fresh and stored samples.

(Pesci et al., 1997)screened antibacterial activity for pathogenic bacteria they reported 18 strains of bacteria including Pseudomonas aeruginosa, Salmonella typhimurium, Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli, Bacillus subtilis and enterococcus faecalis. These results are comparable to present study that showed among two compounds were effective against bacterial and fungal strains. A study undertaken by (Chaves et al., 2018) reported the presence of antibacterial activity of Eucalyptus camaldulensis and their essential oils. Samples were cultured on nutrient agar medium. Anti-bacterial activity was performed by first amplifying leaf extract and then essential oils. The isolated strain was identified as Salmonella spp. Similarly current study showed the methanolic leaf extract give significant result against Salmonella spp from leaf extract Eucalyptus camaldulensis samples of Haripur. The antibacterial activity result showed maximum inhibition against biofilm producing bacteria and no antibacterial activity were shown against Bacillus subtilis.



Vol. 2 No. 4 (2024): October - December
https://rjnmsr.com/index.php/rjnmsr/about

Review Journal of Neurological & Medical Sciences Review

E(ISSN) : 3007-3073
P(ISSN) : 3007-3065

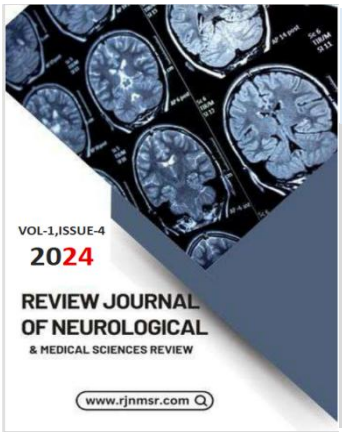
Current study also resulted no antibacterial activity of leaf extract against Bacillus subtilis. Only samples showed the antibacterial activity against, Salmonella typhimurium, Staphylococcus aureus, and Enterococcus faecalis. Present study is in accordance to Peng et al., (2015) who reported bacterial disease of humans. The caustic agent of this disease is Pseudomonas aeruginosa and their subspecies were also reported by (Chaves et al., 2018) from infected samples. The research aimed to investigate the antimicrobial compounds produced by leaf extract of plant

Two types of compounds were detected in leaf extract, EUCALAPTOL and 10S, 11S-HIMACHALA-3(12), 4-DIEN. Current research study also reported the detection of bioactive compounds against biofilm producing bacteria from the leaf extract samples of Eucalyptus camaldulensis from Haripur. Many different bacterial species were used which are biofilm producer including Pseudomonas aeruginosa, Salmonella typhimurium, Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli, Bacillus subtilis. Present research work also demonstrates the presence of antibiofilm activity of Eucalyptus camaldulensis against Staphylococcus aureus and Escherichia coli in the leaf extract sample of plant. Hence leaf extract of river red gum is effective against Staphylococcus aureus and Escherichia coli (Stepanović et al., 2007).

Present study detected antibacterial compounds s from leaf extract samples. GCMS results were comparable to study conducted by (Azwanida, 2015) identified EUCALAPTOL is used as a oral medicine in bacterial diseases, it has significant antibacterial activity and used as a drug against multi drug resistant bacteria. Bacterial strains was previously identified through morphological appearance and molecular basis. Strains used in the study were biofilm producers. Eucalyptol is one of the antimicrobial drug that limits the bacterial growth by degrading their enzymes, which is the major structural component of bacterial cell structure.

Present research showed the presence of 10S,11S-HIMACHALA-3(12),4-DIEN in leaf extract of river red gum plant.(Azwanida, 2015) reported from 10S, 11S-HIMACHALA-3(12),4-DIEN from Syzgium Grande showed maximum antibacterial activity , while fungal species were resistant to this compound. These two antibacterial compounds were also found in river red gum which may be utilize as antibiofilm compounds against bacteria. Different species of Eucalyptus are present in Pakistan, some of these do not contain these bioactive compounds.

Present study detected bioactive compounds from the leaf extract samples from Haripur. These results were comparable to study conducted by (Ashour, 2008) detected medicinal compounds which may effective against biofilm producing strain of bacteria. Acetonic leaf extract showed significant inhibition against Staphylococcus aureus and Escherichia col.



Vol. 2 No. 4 (2024): October - December
https://rjnmsr.com/index.php/rjnmsr/about

Review Journal of Neurological & Medical Sciences Review

E(ISSN) : 3007-3073
P(ISSN) : 3007-3065

Conclusions

The acetonic extracts of Eucalyptus camaldulensis shower maximum antimicrobial activity against biofilm producing strains two compound were detected against biofilm producing strains (Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium, Staphylococcus aureus, Klebsiella pneumoniae and Bacillus subtilis) as compared to methanolic and ethanolic leaf extract. Most effective solvent was acetone which inhibit Escherichia coli and Staphylococcus aureus. Some of the compounds detected by GCMS were also found as antibacterial agent by preview literature search e.g. 10S, 11S-HIMACHALA-3(12),4-DIEN and EUCAYPTOL. Antibacterial activity of other compound need to be tested, since more than 250 compounds were detected and they might have potential antibacterial activity against several bacterial strains. Hence eucalyptol and 10S, 11S-HIMACHALA-3(12),4-DIEN could be used against these biofilm forming bacteria.

Recommendations

Identification of other antibacterial and antifungal compounds is needed, to find out remedies to protect humans. Screening of other bioactive compounds in various plants which is resistant against viruses, bacterial and fungal is also needed. Hence transformation of pathogen resistant genes into nonresistant, usage of these bioactive compound will result in development of resistance in these microbes. This will certainly help to overcome the challenges of resistance in microbes which causes different infectious diseases in humans and animals.

Acknowledgements: I owe a debt of gratitude to the University of Haripur's Department of Microbiology for making my dissertation journey possible. Without the encouragement and assistance of my supervisor, associate professor Dr. Syeda Asma Bano, it would not have been feasible.

Availability of data and materials: Upon reasonable request, the corresponding author will make the datasets used and/or analyzed during the current investigation available.

Conflict of Interests: The authors declare that they have no conflicts of interests.

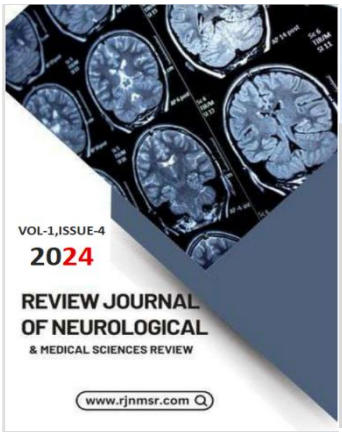
Funding: Hence it is an institutional study and it has not received any funding from any source.

Refrences

ADONIZIO, A., KONG, K.-F. & MATHEE, K. 2008. Inhibition of quorum sensing-controlled virulence factor production in Pseudomonas aeruginosa by South Florida plant extracts. Antimicrobial agents and chemotherapy, 52, 198-203.

AQIL, F., VADHANAM, M. V., JEYABALAN, J., CAI, J., SINGH, I. P. & GUPTA, R. C. 2014. Detection of anthocyanins/anthocyanidins in animal tissues. Journal of agricultural and food chemistry, 62, 3912-3918.

ASHOUR, H. M. 2008. Antibacterial, antifungal, and anticancer activities of volatile oils and extracts from stems, leaves, and flowers of Eucalyptus



Vol. 2 No. 4 (2024): October - December
https://rjnmsr.com/index.php/rjnmsr/about

Review Journal of Neurological & Medical Sciences Review

E(ISSN) : 3007-3073
P(ISSN) : 3007-3065

sideroxylon and Eucalyptus torquata. Cancer biology & therapy, 7, 399-403.

ASLAM, B., WANG, W., ARSHAD, M. I., KHURSHID, M., MUZAMMIL, S., RASOOL, M. H., NISAR, M. A., ALVI, R. F., ASLAM, M. A. & QAMAR, M. U. 2018. Antibiotic resistance: a rundown of a global crisis. Infection and drug resistance, 11, 1645.

AZWANIDA, N. 2015. A review on the extraction methods use in medicinal plants, principle, strength and limitation. Med Aromat Plants, 4, 2167-0412.

CHAVES, T. P., PINHEIRO, R. E. E., MELO, E. S., SOARES, M. J. D. S., SOUZA, J. S. N., DE ANDRADE, T. B., DE LEMOS, T. L. G. & COUTINHO, H. D. 2018. Essential oil of Eucalyptus camaldulensis Dehn potentiates β-lactam activity against Staphylococcus aureus and Escherichia coli resistant strains. Industrial Crops and Products, 112, 70-74.

DJORDJEVIC, D., WIEDMANN, M. & MCLANDSBOROUGH, L. 2002. Microtiter plate assay for assessment of Listeria monocytogenes biofilm formation. Applied and environmental microbiology, 68, 2950-2958.

DUNG, N. T., KIM, J. M. & KANG, S. C. 2008. Chemical composition, antimicrobial and antioxidant activities of the essential oil and the ethanol extract of Cleistocalyx operculatus (Roxb.) Merr and Perry buds. Food and chemical Toxicology, 46, 3632-3639.

DZOYEM, J. P., ARO, A. O., MCGAW, L. J. & ELOFF, J. N. 2016. Antimycobacterial activity against different pathogens and selectivity index of fourteen medicinal plants used in southern Africa to treat tuberculosis and respiratory ailments. South African Journal of Botany, 102, 70-74.

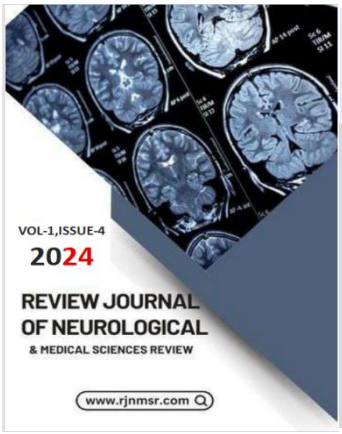
ELAISSI, A., ROUIS, Z., SALEM, N. A. B., MABROUK, S., BEN SALEM, Y., SALAH, K. B. H., AOUNI, M., FARHAT, F., CHEMLI, R. & HARZALLAH-SKHIRI, F. 2012. Chemical composition of 8 eucalyptus species' essential oils and the evaluation of their antibacterial, antifungal and antiviral activities. BMC complementary and alternative medicine, 12, 1-15.

ELOFF, J. N. 1998. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta medica, 64, 711-713.

EL-TAWEEL, H. A. 2015. Understanding drug resistance in human intestinal protozoa. Parasitology research, 114, 1647-1659.

GILLING, D. H., KITAJIMA, M., TORREY, J. & BRIGHT, K. 2014. Antiviral efficacy and mechanisms of action of oregano essential oil and its primary component carvacrol against murine norovirus. Journal of applied microbiology, 116, 1149-1163.

GRIFFIN, S. G., MARKHAM, J. L. & LEACH, D. N. 2000. An agar dilution method for the determination of the minimum inhibitory concentration of essential oils. Journal of Essential Oil Research, 12, 249-255.



Vol. 2 No. 4 (2024): October - December
https://rjnmsr.com/index.php/rjnmsr/about

Review Journal of Neurological & Medical Sciences Review

E(ISSN) : 3007-3073
P(ISSN) : 3007-3065

JADHAV, S., SHAH, R., BHAVE, M. & PALOMBO, E. A. 2013. Inhibitory activity of yarrow essential oil on Listeria planktonic cells and biofilms. Food Control, 29, 125-130.

JEMÂA, J. M. B., HAOUEL, S., BOUAZIZ, M. & KHOUJA, M. L. 2012. Seasonal variations in chemical composition and fumigant activity of five Eucalyptus essential oils against three moth pests of stored dates in Tunisia. Journal of Stored Products Research, 48, 61-67.

KHAN, M. T. H., ATHER, A., THOMPSON, K. D. & GAMBARI, R. 2005. Extracts and molecules from medicinal plants against herpes simplex viruses. Antiviral research, 67, 107-119.

KNEZEVIC, P., ALEKSIC, V., SIMIN, N., SVIRCEV, E., PETROVIC, A. & MIMICA-DUKIC, N. 2016. Antimicrobial activity of Eucalyptus camaldulensis essential oils and their interactions with conventional antimicrobial agents against multi-drug resistant Acinetobacter baumannii. Journal of ethnopharmacology, 178, 125-136.

MABONA, U., VILJOEN, A., SHIKANGA, E., MARSTON, A. & VAN VUUREN, S. 2013. Antimicrobial activity of southern African medicinal plants with dermatological relevance: from an ethnopharmacological screening approach, to combination studies and the isolation of a bioactive compound. Journal of Ethnopharmacology, 148, 45-55.

NASIR, M., TAFESS, K. & ABATE, D. 2015. Antimicrobial potential of the Ethiopian Thymus schimperi essential oil in comparison with others against certain fungal and bacterial species. BMC Complementary and Alternative Medicine, 15, 1-5.

NELSON, R. 1997. In-vitro activities of five plant essential oils against methicillin-resistant Staphylococcus aureus and vancomycin-resistant Enterococcus faecium. The Journal of antimicrobial chemotherapy, 40, 305-306.

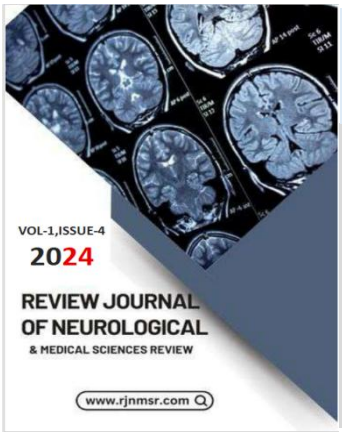
NOSRATABADI, S. J., SHARIFI, I., SHARIFIFAR, F., BAMOROVAT, M., DANESHVAR, H. & MIRZAIE, M. 2015. In vitro antileishmanial activity of methanolic and aqueous extracts of Eucalyptus camaldulensis against Leishmania major. Journal of parasitic diseases, 39, 18-21.

OLAWORE, N. & OLOLADE, Z. 2017. Eucalyptus camaldulensis var. Nancy.

PALOMINO, S. S., ABAD, M. J., BEDOYA, L. M., GARCÍA, J., GONZALES, E., CHIRIBOGA, X., BERMEJO, P. & ALCAMI, J. 2002. Screening of South American plants against human immunodeficiency virus: preliminary fractionation of aqueous extract from Baccharis trinervis. Biological and Pharmaceutical Bulletin, 25, 1147-1150.

PESCI, E. C., PEARSON, J. P., SEED, P. C. & IGLEWSKI, B. H. 1997. Regulation of las and rhl quorum sensing in Pseudomonas aeruginosa. Journal of bacteriology, 179, 3127-3132.

REDA, F. M., EL-ZAWAHRY, Y. & OMAR, A. 2017. Synergistic effect of combined antibiotic and methanol extract of Eucalyptus camaldulensis



Vol. 2 No. 4 (2024): October - December
https://rjnmsr.com/index.php/rjnmsr/about

Review Journal of Neurological & Medical Sciences Review

E(ISSN) : 3007-3073
P(ISSN) : 3007-3065

leaf against Staphylococcus aureus and Pseudomonas aeruginosa. International Journal of Applied Sciences and Biotechnology, 5, 486-497.

ROBBINS, N., CAPLAN, T. & COWEN, L. E. 2017. Molecular evolution of antifungal drug resistance. Annual review of microbiology, 71, 753-775.

SCHMIDT-SILVA, V., PAWLOWSKI, Â., DOS SANTOS, E. K., ZINI, C. A. & SOARES, G. L. 2011. Cytotoxicity of essential oils from two species of Heterothalamus (Asteraceae). Australian Journal of Botany, 59, 682-691.

SEBEI, K., SAKOUHI, F., HERCHI, W., KHOUJA, M. L. & BOUKHCHINA, S. 2015. Chemical composition and antibacterial activities of seven Eucalyptus species essential oils leaves. Biological research, 48, 1-5.

SELIM, S. 2011. Antimicrobial activity of essential oils against Vancomycin-Resistant enterococci (VRE) and Escherichia coli O157: H7 in feta soft cheese and minced beef meat. Brazilian journal of microbiology, 42, 187-196.

STEPANOVIĆ, S., VUKOVIĆ, D., HOLA, V., BONAVENTURA, G. D., DJUKIĆ, S., ĆIRKOVIĆ, I. & RUZICKA, F. 2007. Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci. Apmis, 115, 891-899.

TAJKARIMI, M., IBRAHIM, S. A. & CLIVER, D. 2010. Antimicrobial herb and spice compounds in food. Food control, 21, 1199-1218.

THUILLE, N., FILLE, M. & NAGL, M. 2003. Bactericidal activity of herbal extracts. International journal of hygiene and environmental health, 206, 217-221.

VENTOLA, C. L. 2015. The antibiotic resistance crisis: part 1: causes and threats. Pharmacy and therapeutics, 40, 277.

WANG, Q., PENG, K., LIU, Y., XIAO, X., WANG, Z. & LI, R. 2021. Characterization of TMexCD3-TOprJ3, an RND-type efflux system conferring resistance to tigecycline in Proteus mirabilis, and its associated integrative conjugative element. Antimicrobial agents and chemotherapy, 65, e02712-20.