



Exploring the antimicrobial efficacy of *Elaeocarpus ganitrus* extract against burn wound infection microorganisms

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Abstract:

In numerous countries, infections from burn wounds caused by multidrug-resistant (MDR) bacteria are recognized as significant and pervasive health threats. *Pseudomonas aeruginosa* and *Staphylococcus aureus* are the primary pathogens responsible for burn infections. The primary cases of infections worldwide are attributed to these bacterial pathogens. This data presents the utilization of natural plant leaf extracts as alternatives to combat multi-drug-resistant bacteria. The potential of the medicinal plant *Elaeocarpus ganitrus* for wound healing, traditionally employed for treating wounds and preventing bacterial infections, was assessed. Bacterial pathogens associated with fire burn wound infections were identified through conventional microbiological methods. The disc diffusion technique was employed for antimicrobial susceptibility testing, adhering to the Kirby-Bauer method. This study aimed to identify bioactive compounds in methanol extracts of *E. ganitrus* leaves through phytochemical screening and GC-MS methods. The methanolic extract of *E. ganitrus* revealed the presence of steroids, alkaloids, quinones, proteins, flavonoids, glycosides, tannins, and phenols. The methanol extract derived from *E. ganitrus* demonstrated significantly greater antibacterial activity than conventional antibiotics across all tested species. The study's findings indicate that *E. ganitrus* possesses the capability to neutralize *P. aeruginosa* and *S. aureus*, which have been isolated from infected wounds.

Keywords: *Elaeocarpus ganitrus*, Plant extract, Antibacterial activity; Burn wound infection

Introduction

Burns represent one of the most severe types of injuries that a person can endure. Burn injuries result in considerable psychological and physical repercussions. Infection remains a significant contributor to the elevated mortality rate observed in burn patients [1-4]. Burn patients exhibit a distinct susceptibility to various infections, attributed to compromised resistance resulting from the disruption of the skin's mechanical integrity and a generalized suppression of the immune system. The skin barrier is supplanted by a protein-rich, vascular environment that creates a favourable niche for the colonization and proliferation of microorganisms. This alteration allows for the potential invasion of one or more sepsis-causing agents, thereby enhancing their capacity to inflict damage on the host's sterile tissues. A wound can be infected by a variety of agents, including bacteria, fungi, and parasites, primarily consisting of gram-positive and gram-negative microorganisms [5-8]. Multidrug-resistant (MDR) bacteria present a significant threat in the context of burn wounds among hospitalized patients, particularly those suffering from full-thickness burns. The multidrug-resistant (MDR) organisms include Gram-negative *P. aeruginosa*, *K. pneumoniae*, *E. coli* and Gram-positive *S. aureus* [9]. *Pseudomonas aeruginosa* represents the primary etiological agent of infections associated with burns. *Staphylococcus aureus* represents the second most significant cause of infection in burn cases. A primary therapeutic challenge associated with these bacteria is their rapid development of resistance to antibiotics. Methicillin-resistant *S. aureus* (MRSA) represents a significant subset of *S. aureus*, recognized for its health risks and the challenges it poses in treatment [10].

Burn wound infections present growing management challenges due to the increasing antimicrobial resistance observed in various strains [11]. Pathogens are undergoing evolution and exhibiting a growing resistance. The prolonged misuse of antibiotics has contributed to the rise of microbial drug resistance, subsequently resulting in reduced efficacy of treatments. Therefore, it is crucial to formulate effective antimicrobials to decrease the morbidity and mortality rates in burn patients. In such instances, a viable alternative is the use of natural antimicrobial compounds, which include plant extracts [12-15]. Numerous plants and natural products exhibit antibacterial, antifungal, and antiprotozoal effects, which can be used either systemically or locally. The medicinal properties of plants are widely favoured globally, owing to their significant pharmacological activities, low toxicity, and economic viability in comparison to synthetic drugs. Medicinal plants contain a diverse array of bioactive secondary metabolites, including tannins, terpenoids, alkaloids, saponins, flavonoids, and phenolic compounds, which possess specific physiological effects on the human body [16-20].

Elaeocarpus ganitrus, also referred to by its synonym *E. sphaericus*, is a large evergreen tree that is commonly known as the Rudraksha tree in India. *E. ganitrus* is a significant medicinal plant recognized for its various applications in traditional medicine systems. It has been utilized to address various health issues across different regions of the world. Leaves and seeds possess a range of medicinal properties and have been traditionally utilized to address conditions such as stress, anxiety, depression, palpitations, nerve pain, epilepsy, migraines, lack of concentration, asthma, hypertension, arthritis, and liver diseases [21]. *E. ganitrus* is known to contain significant phytochemicals, including triterpenes, tannins, indolizidine alkaloids such as grandisine and elaecarpiline, as well as gallic acid, ellagic acid, and the flavonoid quercetin. These compounds exhibit a range of therapeutic activities, including antioxidant, hypoglycemic, antidepressant, antianxiety, antihypertensive, antimicrobial, and nephroprotective effects [22]. This study aims to assess the antibacterial properties of methanol extracts derived from the leaves of *E. ganitrus* against the bacteria *P. aeruginosa* and *S. aureus*, which were isolated from samples of burn wounds (FW).

2. Materials and Methods

2.1. Collection of samples and identification of isolates

Microorganisms were obtained from burn patients of different age groups and genders, who were admitted to the burn unit of a private hospital situated in Tirupur, Tamil Nadu, India. Wound swabs were collected from every patient. When there was direct contact with patients, a protective gown and disposable gloves were utilized. Swab samples were collected and inoculated onto MacConkey agar (Hi-media, Bangalore), mannitol salt agar, and pseudomonas agar medium (cetrimide). The plates were then incubated at a temperature of 37°C for a period of 18 to 24 hours. The blood agar plate (BAP) from Oxoid, Ltd. and the chocolate agar plate (CAP) were incubated in a humid atmosphere with 5% CO₂ at a temperature of 35°C–37°C for 18–22 hours. Each plate underwent a 24-hour aerobic incubation period. The plates that showed no growth were allowed an extra 48 hours for cultivation. The physiological and biochemical characteristics of the isolated organisms *P. aeruginosa* and *S. aureus* were assessed following the guidelines outlined in Bergey's manual of systematic bacteriology [23].

2.2. Antibiotics sensitivity test

The bacterial organism's susceptibility to the antibiotics was assessed through the Bauer disc-diffusion method, following the protocol outlined by Bauer et al. (1996). Commercially available antibiotic discs with different concentrations (µg) were employed. A total of 5 antibiotics were utilized in the study. The isolated microorganisms were uniformly distributed over the surface of a prepared Mueller Hilton agar plate, and a sterilized forceps was used to place the antibiotic disc onto the plate. After incubation, zones of inhibition were observed, and their diameters were measured with a caliper. The classification of the measurement into sensitive, moderate, or resistant categories was established according to the interpretative guidelines for standard zone size outlined by Cheesbrough [24].

2.3. Detection of Biofilm production by plate method

The isolates were inoculated in brain heart infusion broth containing 2% sucrose and incubated overnight at 37°C. They were then diluted with a 1 in 100 ratio of fresh BHI broth. 0.2 ml of the diluted cultures was carefully transferred to individual wells of sterile, polystyrene, 96 well-flat bottom microtitre plates. A control of uninoculated broth was utilized to verify the sterility of the media and to assess any non-specific binding. The microtitre plates were incubated at 37°C for 24 hours. The following day, the contents of each well were carefully removed by gently tapping the plates. The wells were rinsed four times with 0.2 mL of saline solution to eliminate any bacteria that were not attached. The plate's biofilms were fixed using a solution of 2% sodium acetate and then stained with 0.1% crystal violet. The optical density value (OD) of stained adherent bacteria was measured using an ELISA reader at a wavelength of 570 nm [25]. The experiments were conducted in triplicate and repeated three times. The data was averaged and the standard deviation was calculated. In order to account for background absorbance, the OD readings of the sterile medium, fixative, and dye were averaged and then subtracted from all the test values. All test OD values were adjusted by subtracting the mean OD value obtained from the media control well (Table 1).

Table 1: Classification of bacterial adherence

Organism	OD value (570nm)	Adherence	Biofilm production
Blank	<0.006	Non	Non
<i>Pseudomonas aeruginosa</i> (FW – 1)	>0.329	Strong	High
<i>Staphylococcus aureus</i> (FW – 2)	>0.418	Strong	High

2.4. Detection of Biofilm production by Tube Method

This method represents a qualitative approach for biofilm detection, as described by Christensen et al. A loopful of the organism was inoculated into 5 ml of Trypticase soy broth and incubated at 37° C for 24 hours. The tubes were subsequently poured off and rinsed with phosphate-buffered saline (pH 7.2) before being allowed to dry naturally. The tubes were subsequently stained with a 0.1% crystal violet solution for 10 minutes. Subsequent to the staining procedure, the tubes underwent a careful rinsing with deionized water. The tubes were subsequently allowed to dry naturally in an inverted position. The assessment of biofilm formation was performed by taking into account the control strains employed. When a discernible layer develops on the walls and base of the tube, the organisms are classified as biofilm producers. If a ring forms at the interface of the liquid medium, it indicates that the organism does not produce biofilm. The experiment was performed in triplicate [26].

2.5. Plant collection and identification

The *Elaeocarpus ganitrus* plant was sourced from a remote location in Wayanad, Kerala, India. The plant was identified and authenticated by the Botanical Survey of India, Southern Regional Centre, TNAU Campus, Coimbatore, Tamil Nadu, India.

2.6. Preparation of extracts

The aerial components of the *E. ganitrus* plant were thoroughly rinsed with running tap water to remove any contaminants. They were then washed with distilled water and allowed to dry in the shade. Following the drying process, the leaves were ground into a fine powder utilizing an electric blender. Twenty grams of powdered plant material were wrapped in thick filter paper to form a thimble. The thimble was afterwards positioned within the extractor tube and linked to a condenser. A round-bottom flask contained 100 ml of methanol, which was heated to 60°C for a duration of 20 minutes. After the solvent lost its colour, it was collected in the round-bottom flask and concentrated with a rotary vacuum evaporator. The extract was refrigerated for subsequent use [27].

2.7. Phytochemical Screening

The plant extracts were subjected to qualitative phytochemical screening in order to determine the key compound classes. The compounds identified were tannins, saponins, flavonoids, alkaloids, phenols, glycosides, steroids, and terpenoids. The screening adhered to standard protocols established by Dubale et al. (2023) [28].

2.8. Gas Chromatography Mass Spectrometry (GC -MS) analysis

The analysis performed a GC-MS study of the extract using the Agilent 7890A (GC) and Agilent 5975C (inert) MSDs, both made by Agilent Technologies and equipped with triple-axis detectors. A 30 m long and 0.25 mm thick J&W CP-Sil-8 GC column was utilized for the GC separation. With a carrier gas of methanol (5 mg/ml), helium was injected at a rate of 1 ml/min into an injection volume of 1 µl. They employed a split-mode approach (1:20) to inject the samples. The GC oven was set to heat up to 280 °C at 5 °C/min after reaching 120 °C at 10 °C/min after reaching 50 °C for one minute (held for two minutes). The performance lasted for sixty-five minutes. To identify the compounds, spectral configurations were obtained and compared with the mass spectral database NIST -08 SPECTRAL DATA [29].

2.9. Antimicrobial activity of leaf extracts using the Kirby–Bauer method

The study evaluated the antimicrobial properties of methanol extracts of *E. ganitrus*, emphasizing on their effectiveness against pathogens associated with fire burn wounds, namely *P. aeruginosa* and *S. aureus*. The screening was performed utilizing the Agar well diffusion technique, employing a concentration of 50 mg/ml of the plant extract. Streptomycin and Tetracycline, both at a concentration of 10 µg/ml, were employed as standard antibacterial agents. Following incubation, the zone of inhibition was measured [30].

3. Results And Discussion

3.1. Bacteria isolation from fire burn wound

Wound from burns Bacteria from the private hospital situated in Tirupur were subjected to serial dilution. The swabs were inoculated onto several culture media (Blood agar, MacConkey agar, Mannitol salt agar, *Pseudomonas* agar) for cultivation, isolation, and preliminary identification. A significant number of isolates were cultivated on blood agar, exhibiting various morphologies according to their respective taxa. The suspected *Pseudomonas* and *Staphylococcus* isolates were grown on mannitol salt agar to identify *P. aeruginosa* and *S. aureus*. Certain isolates were cultivated on

MacConkey agar, exhibiting pale or pink colouration. Subsequently, each unique morphological characteristic was identified as either *P. aeruginosa* and *S. aureus* and subjected to the streak plate method for pure colony isolation, as shown in Figure 1 and 2. Forson et al. (2017) [18] indicated that *P. aeruginosa* constituted the highest percentage of bacterial species isolated from burn wounds. In a similar vein, Church et al. (2006) [31] reported that *P. aeruginosa* was the predominant bacterial species in infected burn wounds, followed by *E. coli*, while *Acinetobacter* spp. and *Bacteroides* spp. were found in the lowest percentages. Hateet, (2021) [32] recently reported that among bacterial isolations, *Pseudomonas aeruginosa* was identified as the most prevalent pathogen, accounting for 20%. This was followed by *Staphylococcus aureus* at 17.14%, *Enterobacter* spp. at 16.19%, *Proteus vulgaris* at 13.33%, *Proteus mirabilis* at 10.47%, *Escherichia coli* at 7.6%, *Klebsiella pneumoniae* at 6.6%, and finally, *Staphylococcus lentus* and *Aeromonas sobria*. Nevertheless, various studies indicate that *S. aureus* is the predominant organism found in burn wound colonisation [33]. Several studies have demonstrated that *Staphylococcus aureus* is a predominant etiological agent in burn wound infections [34].

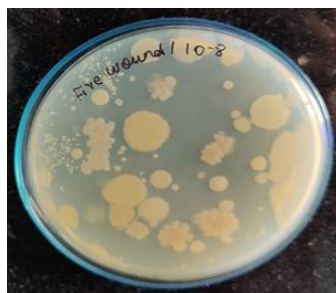


Figure 1: Bacterial Isolation by spread plate technique

3.2. Antibiotic sensitivity test

The antibiotics employed varied based on the bacterial species group, as the routinely used clinical antibiotics were different according to the specific clinical cases and species addressed in the study [35]. The assessment of the antibiotic susceptibility pattern indicated the existence of both resistance and sensitivity in the fire burn wound samples. The antibiotic resistance patterns of the common isolates *P. aeruginosa* and *S. aureus* are shown in Table 2 and Figure 3. The results illustrate the antibiotic sensitivity pattern of the predominant bacteria isolated from burn wound swabs. The isolated *P. aeruginosa* species exhibited high resistance to the majority of the antibiotics tested, including ampicillin (0 mm) and tetracycline (0 mm), with additional resistance observed against streptomycin (Figure. 3). Figure 3 demonstrates that the *S. aureus* isolates exhibited a high level of resistance to ampicillin, with a zone of inhibition measuring 0 mm, while demonstrating moderate resistance to tetracycline, with a zone of inhibition of 16 mm. Ciprofloxacin and Cefmetazole were identified as the most effective antibiotics against *P. aeruginosa* and *S. aureus*.

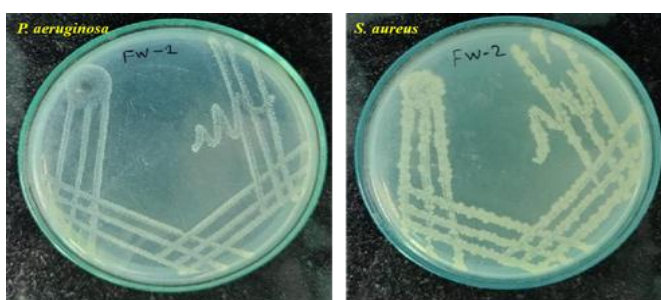


Figure 2: Single colony isolation by streak plate technique (*P. aeruginosa* and *S. aureus*)

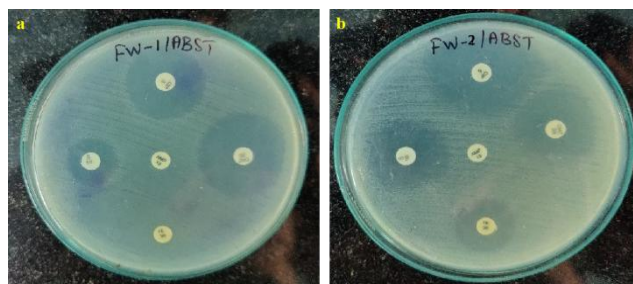


Figure 3: Antibiotic sensitivity pattern of Gram negative and positive bacterial isolates (a) *Pseudomonas aeruginosa* (AW – 1) and (b) *Staphylococcus aureus* (AW – 2)

Table 2: Antibiotic sensitivity pattern of Gram negative and positive bacterial isolates(mm)

Organism	Ciprofloxacin	Streptomycin	Ampicillin	Tetracycline	Cefmetazole
<i>Pseudomonas aeruginosa</i> (AW – 1)	28/S	17/R	-/R	-/R	31/S
<i>Staphylococcus aureus</i> (AW – 2)	34/S	21/S	-/R	16/R	24/S

indicate that these antibiotics may serve as effective alternatives for the treatment of these two pathogens. Both pathogens demonstrated resistance to Streptomycin, with *S. aureus* and *P. aeruginosa* exhibiting complete resistance to both Streptomycin and Tetracycline. The treatment of multidrug-resistant bacterial infections is often complicated by restricted susceptibility to antimicrobial agents and the emergence of antibiotic resistance throughout the course of therapy. In recent years, Morocco has emerged as a nation exhibiting one of the highest rates of antimicrobial resistance [36]. Additionally, *P. aeruginosa* strains possessing intrinsic resistance mechanisms to antibiotics have been observed. The findings indicated that *P. aeruginosa* demonstrated significant resistance levels to eight antibiotics, including ampicillin, ampicillin-sulbactam, cefazolin, ceftazidime, chloramphenicol, moxifloxacin, tetracycline, and trimethoprim - methoxybenzazole [37]. *S. aureus* is one of the pathogens exhibiting a rising resistance to antibiotics, therefore becoming a priority for antimicrobial resistance surveillance [38]. Over 25% of methicillin-resistant isolates exhibited resistance to ampicillin, ciprofloxacin, cotrimoxazole, erythromycin, clindamycin, azithromycin, and tetracycline [39].

3.3. Biofilm formation

Bacterial motility is crucial for the colonisation of surfaces by bacteria, leading to the development of resilient bacterial communities known as biofilms. The biofilm formation of *P. aeruginosa* and *S. aureus* was assessed using the crystal violet staining method. Microorganisms *P. aeruginosa* and *S. aureus* were allowed to form distinct biofilms in glass test tubes. Subsequently, the remaining biofilm was stained with crystal violet, followed by measurement and analysis. All the three bacterial strains successfully formed biofilms on the surface of the glass test tubes. In the absence of bacterial strains, biofilm formation was not observed. The data presented within highlight the significance of biofilm in wound healing, as bacterial biofilm has been identified as a critical contributor to delayed wound healing. Furthermore, elevated levels of biofilm production have consistently been documented in multidrug-resistant organisms [40]. Previous research conducted in Nepal and Egypt documented the prevalence of biofilm formers at 65.1% and 70.1%, respectively [41]. These findings underscore the significance of biofilm in the pathogenesis of microbial wound infections, as well as the difficulties associated with treatment regimens and the potential for persistent infections [42].

3.4. Phytochemical screening of methanol extract of *Elaeocarpus ganitrus*

The qualitative analysis of phytochemical components in the methanol extracts of *E. ganitrus* revealed the presence of steroids, alkaloids, quinones, proteins, flavonoids, glycosides, tannins, and phenols, as mentioned in Table 3. The phytochemical investigation demonstrated that the extracts are rich in essential phytochemicals, such as alkaloids, phenolics, tannins, flavonoids, and saponins. The test for saponin compounds in methanol extracts yielded a negative result. The ethanolic extract of *E. ganitrus* showed the presence of alkaloids, flavonoids, saponins, phenols, glycosides, steroids, terpenoids, and fixed oils [43]. Singh et al. [44] demonstrated that the ethanolic extract of *E. ganitrus* fruits exhibited notable anti-anxiety activity. A prior report indicates the presence of various phytochemicals, antioxidant activity, and alpha amylase inhibition properties in medicinal plants, derived from different parts such as leaves, bark, and roots [45].

Table 3: Phytochemical screening of methanol extracts of *Elaeocarpus ganitrus*

Chemical constituent	Methanol leaf extract <i>Elaeocarpus ganitrus</i>
Tannins	+
Flavonoids	+
Saponins	-
Steroids	+
Glycosides	+
Phenols	+
Alkaloids	+
Quinones	+
Proteins	+

3.5. GC-MS analysis

The bioactive compounds found in the methanolic extract of the leaf of *E. ganitrus* were identified through GC-MS analysis (Figure. 4). GC-MS analysis revealed the identification of 12 distinct compounds, characterized by their unique retention times, peak areas, and mass spectra, as detailed in Table 4. The total running time for the analysis was 36.98

minutes. The spectra of the compounds were compared with the Wiley 9.0 and NIST libraries. In recent years, the application of GC-MS studies for the analysis of medicinal plants has seen a notable increase, as this technique has demonstrated its value in identifying non-polar components, volatile essential oils, fatty acids, lipids, and alkaloids [46]. The predominant compounds identified in the methanol extract of *E. ganitrus* were 9-Octadecenoic acid (Z)-methyl ester and 9,12-Octadecadienoic acid methyl ester. The compounds in question exhibit significant potential as antioxidants, as well as possessing anti-cancer and anti-inflammatory properties [47]. Furthermore, successfully isolated active compounds recognized as 2,3 Dihydroxypropyl elaidate. The compounds demonstrated significant antioxidant and anticancer activities [48].

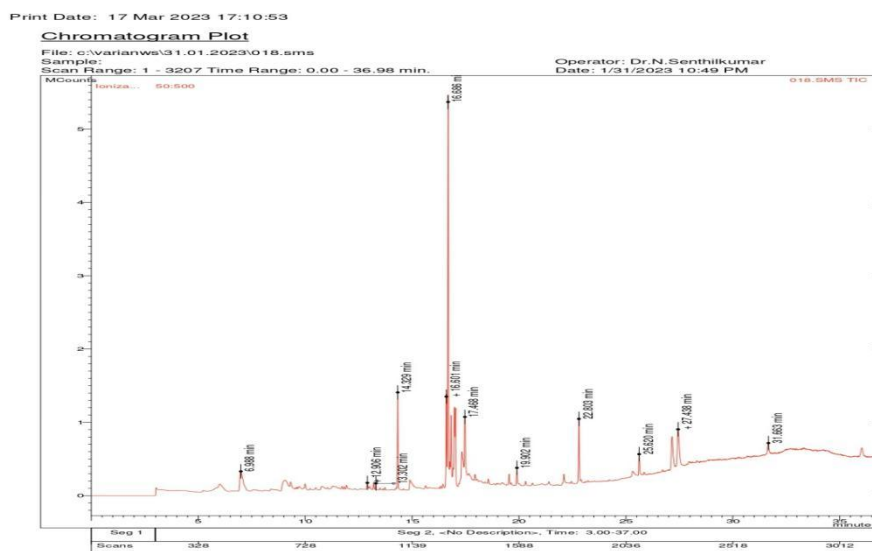


Figure 4: GC-MS spectra of methanol extract from the leaves of *Elaeocarpus ganitrus*

Table 4: Chemical profile of Methanol leaf extract *Elaeocarpus ganitrus*

Sno	Name of the Compound	Retention Time (RT)	Area
1	Pentandioic acid, (p-t-butyl	6.988	2.18E+06
2	7-Octadecyne, 2-methyl-	13.201	205330
3	2-Pentadecanone, 6,10,14-tri	13.302	181053
4	9,12-Octadecadienoic acid (Z	16.601	2.70E+06
5	9-Octadecenoic acid, methyl	16.686	1.36E+07
6	9-Octadecenoic acid, methyl	16.763	135556
7	Hexadecanoic acid, methyl es	14.329	2.68E+06
8	Heptadecanoic acid, 15-methy	17.025	1.76E+06
9	2,3-Dihydroxypropyl elaidate	17.328	1.20E+06
10	Eicosanoic acid, methyl este	19.902	582559
11	Docosanoic acid, methyl este	22.803	2.18E+06
12	Benzenamine, 4,4'-[sulfonylb	31.663	649175

3.6. Antibacterial effect of Methanol leaf extract *Elaeocarpus ganitrus*

This study investigates the antimicrobial activity of methanolic extracts derived from the medicinal plant *E. ganitrus* against pathogenic bacteria associated with fire burn wound infections. The bacterial strains employed in this study were *P. aeruginosa* and *S. aureus*. The plant extracts demonstrated significant antimicrobial properties against the tested microorganisms, as indicated by the disc diffusion method (Table 5). At a concentration of 50 mg/ml, the activity was notable when compared to antibiotics at 10 µg/ml. The methanol extracts of *E. ganitrus* demonstrated significant antibacterial activity against *P. aeruginosa*, exhibiting a zone of inhibition measuring 15 mm at a concentration of 50

mg/ml. The methanol extracts derived from *E. ganitrus* leaves demonstrated the most promising results against *S. aureus*, exhibiting a comparable zone of inhibition of 11 mm at a concentration of 50 mg/ml. It is important to note that it demonstrated greater antibacterial activity in comparison to the standard antibacterial drug (Figure. 5).

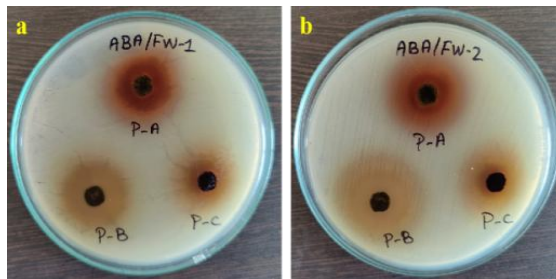


Figure 5: Activity of Methanol leaf extract *Elaeocarpus ganitrus* against the tested microbial species (a) *Pseudomonas aeruginosa* (FW – 1) and (b) *Staphylococcus aureus* (FW – 2)

Table 5: Inhibition zone of the Methanol leaf extract *Elaeocarpus ganitrus* activity against the tested pathogenic microbe

Organism	Zone of Inhibition (mm)		
	Methanol leaf extract <i>Elaeocarpus ganitrus</i> (P.A)	Streptomycin (P.B)	Tetracycline (P.C)
<i>Pseudomonas aeruginosa</i> (AW – 1)	15	16	7
<i>Staphylococcus aureus</i> (AW – 2)	11	16	5

According to Kumar et al. (2011) [49], the antimicrobial activity of the aqueous extract of *E. ganitrus* leaves was tested against clinical isolates of bacteria and fungi, with *E. coli* (12.3mm) showing the highest activity and *S. aureus* (11mm) showing the lowest activity. Petroleum ether, chloroform, ethanol, and aqueous extracts of *E. ganitrus* seeds showed potential broad-spectrum antifungal efficacy against *Aspergillus niger*, *Candidum geotrichum*, *Candida albicans*, *C. glabrata*, and *C. tropicalis* [50]. Previously, ethanolic extracts of *E. ganitrus* were most potent against *S. aureus* (16mm), with the lowest antibacterial activity against *E. coli* and *C. freundii* (9mm) at 200mg/ml concentrations [51]. This finding was corroborated by an additional study on other species in the *Elaeocarpus* genus, which found that the leaf extract of *E. ganitrus* was equally effective against gram-negative bacteria (e.g., *P. aeruginosa* and *K. pneumoniae*). This work successfully established the antibacterial activity of methanol extracts from *E. ganitrus* on burn sites and pathogenic microorganisms.

Conclusion

Our results show that *E. ganitrus* has considerable antibacterial action against bacterial strains isolated from burn wounds. The study found that a methanolic extract of *E. ganitrus* has superior antibacterial action against bacteria found in burn wound infections compared to selective antibiotics. The findings could help create cost-effective and accessible treatments for wounds infected with *P. aeruginosa* and *S. aureus*.

Consent for Publication

Not Applicable

Ethical Approval

Not Applicable

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Declaration of Competing Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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