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Research Article

Evaluation of the Antibacterial Activity of Eucalyptus Globulus 3C and 6C against Uropathogens

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A B S T R A C T

Introduction: Infections of the urinary tract are one of the most frequently encountered problems reported in outpatient settings. In the majority of the instances, clinicians initiate empirical antimicrobial therapy before the laboratory results of urine culture are available. This happens to be one of the most prominent causes of resistance to antibiotics in diverse uropathogens. These microbes can cause a new form of infectious disease because of this drug resistance and may become more resistant because of mutation after some time.

Methods: The purpose of this study was to assess the antimicrobial properties of Eucalyptus globulus 3C and 6C directed at typical bacterial strains of *Pseudomonas aeruginosa, Escherichia coli* and *Proteus mirabilis*. The broth culture of the above organisms was prepared and colonies were allowed to be formed, following which, the zones of inhibition and minimum inhibitory concentrations were determined across each of the bacterial strains.

Results: In this study, the medicine Eucalyptus globulus 3C and 6C demonstrated significant antimicrobial properties against *Pseudomonas aeruginosa* and *Escherichia coli*.

Conclusion: In this in vitro study, Eucalyptus globulus 3C and 6C showed a maximum zone of inhibition of about 9 mm and 12 mm against *Escherichia coli*.

Keywords: Eucalyptus Globulus, Zone Of Inhibition, Drug Resistance, Empirical Treatment

Introduction

Due to antibiotic resistance, problems arising from gram-negative bacterial colonies (GNB) pose one of the world's most serious public health issues. These germs are of tremendous clinical relevance in hospitals because they put patients in the intensive care unit (ICU) at risk and cause significant complications and fatalities.[1,2] Among these organisms, *Escherichia coli* is a well-known microbe that causes infections of the urinary tract ranging from renal infection to infection of the bladder and urethra. Similarly, *Klebsiella pneumoniae* (K. pneumoniae) and *Proteus mirabilis* are also responsible for urinary tract infections. The former is considered to be the cause of infections involving hospitalised individuals who have had many bouts of infections of the urinary tract and have taken earlier antibiotics and Proteus species is responsible for urinary tract infections in the age group of 2–12 years, though it is uncommon.[3]

Antimicrobial resistance (AMR) is a severe hazard to public health because it diminishes the beneficial effects of antimicrobial therapy, resulting in higher death rates, and expenditures on healthcare.[4] The prevalence of antibiotic resistance is often greater in the healthcare industry and mostly affects immunocompromised, elderly, and even young individuals who frequently need medical treatment.[5] Antimicrobial resistance continuously poses a major challenge to the available procedures and solutions for dealing with them.[6]

Homoeopathic medicines act via an appropriate host response through the body's innate self-regulatory mechanism to restore the normal physiological activities of health. No microbial resistance is known to develop against the administration of homoeopathic drugs. The purpose of this study was to assess the antimicrobial activity of Eucalyptus globulus 3C and 6C against uropathogens.[7,8]

Materials and Methods

Bacterial Strains

Typical bacterial strains of *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *Escherichia coli* were used for this study. The cultures were purchased from a National Accreditation Board for Testing and Calibration Laboratories (NABL)-accredited laboratory - Vivek Laboratories, Nagercoil, Kanyakumari District, Tamil Nadu.

Type of Study

It was an in vitro study carried out on *Escherichia coli*, *Pseudomonas aeruginosa*, and *Proteus mirabilis*. The total duration of the study was three weeks from October 1, 2022 to October 20, 2022.

Homoeopathic Medicine

In this study, homoeopathic medicine Eucalyptus globulus 3C and 6C was purchased from Tata 1mg company. The control used was dispensing alcohol.

Antibacterial Screening

In this study, Mueller–Hinton agar plates were used for antibacterial screening.

Preparation of Disc

A plain sterile disc was acquired from HiMedia and immersed with every dosage of samples before being air-dried for 6 hours at the ambient temperature. Then, the disc paper was labelled and used for the antibacterial study.

Mueller-Hinton Agar (MHA)

The culture of *Escherichia coli*, *Pseudomonas aeruginosa*, and *Proteus mirabilis* was prepared in nutrient broth. After that, using Mueller-Hinton agar media, a bacterial lawn was prepared in a Petri dish. The colonies were allowed to be formed and the bacterial growth was assessed. The colonies were then subjected to the administration of homoeopathic medicines Eucalyptus globulus 3C and 6C.

The Petri dish with bacterial colonies and the medicated disc of different potencies with alcoholic control were incubated at 370 °C for 24 hours. The inhibitory zone was calculated in millimetres. The lack of an inhibitory zone was perceived as a lack of activity. The results were categorised as resistant if the inhibitory zone was below 7 mm, moderate if it was 8–10 mm, and reactive if the calculated inhibitory zone went above 11 mm.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC was assessed through broth dilution assay. Here 0.2 ml of the pathogens were cultured in various volumes (0.2, 0.4, 0.6, 0.8 and 1 ml) of Eucalyptus globulus 3C and 6C. The culture was then incubated for 24 hours. After 24 hours, the optical density was measured at 630 nm using a UV-Vis spectrophotometer.

Statistical Analysis

The statistical analysis of the study was done using one-way ANOVA test. The multiple comparisons of selected microorganisms were statistically analysed using Honestly Significant Difference (HSD).

Results

It was seen that Eucalyptus globulus 6C and 3C produced zones of inhibition of about 12 mm and 9 mm, respectively against *Escherichia coli* as shown in Figure 1. Against *Pseudomonas aeruginosa*, a zone of inhibition of 8 mm

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was formed with Eucalyptus Globulus 6C as shown in Figure 2. No zone was formed against *Proteus mirabilis* as shown in Figure 3. The details of the observed zones of inhibition have been documented in Table 1.

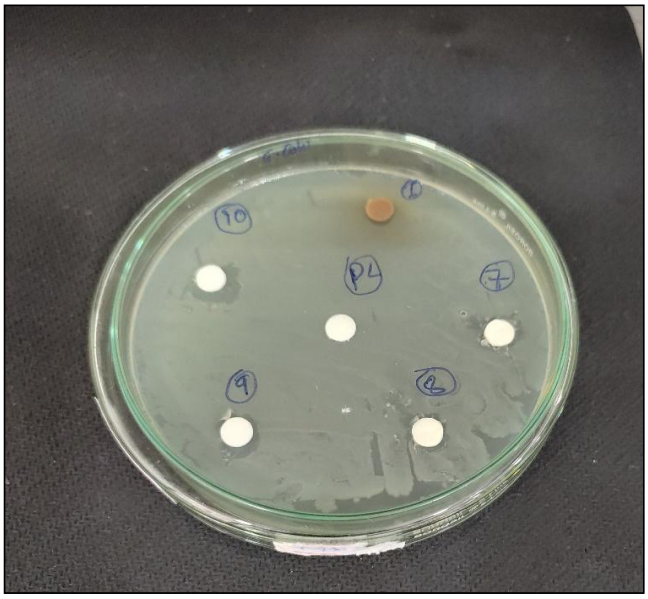

Figure 1.Antibacterial Assay of Homoeopathic Medicine and Control by Kirby–Bauer Method in *Escherichia coli*

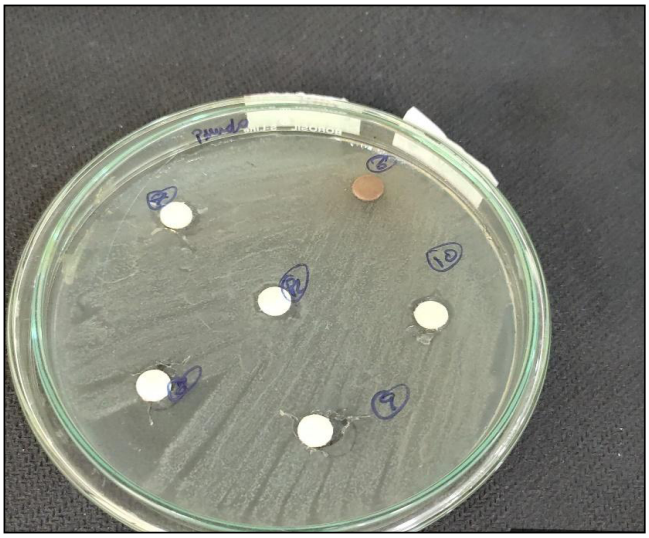

Figure 2.Antibacterial Assay of Homoeopathic Medicine and Control by Kirby–Bauer Method in *Pseudomonas aeruginosa*

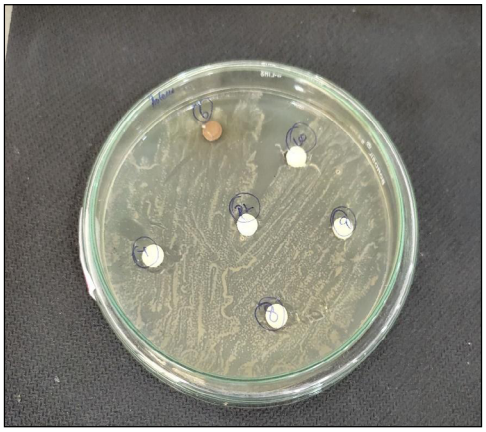

Figure 3.Antibacterial Assay of Homoeopathic Medicine and Control by Kirby–Bauer Method in *Proteus mirabilis*

Table 1.Zones of Inhibition of Eucalyptus Globulus and Control by Kirby–Bauer Method

Bacterial Strain	Potency	Inhibition Zone (mm)
Escherichia coli	3C	9
	6C	12
	PL (dispensing alcohol)	6
Pseudomonas aeruginosa	3C	7
	6C	8
	PL (dispensing alcohol)	7
Proteus mirabilis	3C	6
	6C	6
	PL (dispensing alcohol)	6

PL: Placebo

Minimum Inhibitory Concentration

Eucalyptus globulus 3C and 6C were able to produce mild to moderate minimum inhibitory changes in broth dilution assay. The value below 0.1 indicates the MIC of Eucalyptus globulus, which was more significant in 6C potency than in 3C potency, especially against *Escherichia coli* and *Pseudomonas aeruginosa* as shown in Tables 2 and 3. No significant changes were observed against *Proteus mirabilis* as shown in Table 4.

Table 2.Minimum Inhibitory Concentration in Different Concentrations of *Escherichia coli*

Eucalyptus Globulus	0.2 ml	0.4 ml	0.6 ml	0.8 ml	1.0 ml
3C	0.4752	0.378	0.2163	0.151	0.121
6C	0.3561	0.3212	0.2118	0.1855	0.01542

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Table 3.Minimum Inhibitory Concentration in Different Concentrations of *Pseudomonas aeruginosa*

Eucalyptus Globulus	0.2 ml	0.4 ml	0.6 ml	0.8 ml	1.0 ml
3C	0.4891	0.334	0.2021	0.150	0.110
6C	0.3580	0.3001	0.2005	0.1860	0.0162

Table 4.Minimum Inhibitory Concentration in Different Concentrations of *Proteus mirabilis*

Eucalyptus Globulus	0.2 ml	0.4 ml	0.6 ml	0.8 ml	1.0 ml
3C	0.6825	0.3458	0.2502	0.1687	0.1502
6C	0.3708	0.3501	0.2504	0.1864	0.1437

The results of one-way ANOVA are tabulated in Table 5.

Table 5.Statistical Analysis of Minimum Inhibitory Concentration

Minimum Inhibitory Concentration	Sum of squares	df	Mean square	F	Sig.
Between groups	0.018	2	0.009	0.414	0.005
Within groups	0.577	27	0.021	-	-
Total	0.595	29	-	-	-

The interpretations of multiple comparisons using HSD are tabulated in Table 6.

Table 6.Multiple Comparisons

Bacterial Strain (I)	Bacterial Strain (J)	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Escherichia coli	*Pseudomonas aeruginosa*	0.0085520	0.0653860	0.001	-0.153567	0.170671
	Proteus mirabilis	-0.0467280	0.0653860	0.007	-0.208847	0.115391
Pseudomonas aeruginosa	*Escherichia coli*	-0.0085520	0.0653860	0.001	-0.170671	0.153567
	Proteus mirabilis	-0.0552800	0.0653860	0.009	-0.217399	0.106839
Proteus mirabilis	*Escherichia coli*	0.0467280	0.0653860	0.007	-0.115391	0.208847
	Pesudomonas aeruginosa	0.0552800	0.0653860	0.009	-0.106839	0.217399

Tukey HSD, Dependent Variable: MIC

Bacterial strain can influence the MIC. The Tukey HSD test was conducted to compare the mean MIC values across different bacterial strains, namely *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus mirabilis*. For instance, comparing *Escherichia coli* to *Pseudomonas aeruginosa* revealed a statistically significant mean difference of 0.0085520 (p = 0.001, 95% CI [-0.153567, 0.170671]). This suggests that the MIC values for *Escherichia coli* and *Pseudomonas aeruginosa* are significantly different. Similar interpretations can be made for the other strain comparisons presented in Table 6, each indicating whether there are significant differences in MIC values between the respective bacterial strains.

Discussion

In the current study, Eucalyptus globulus 3C and 6C were evaluated for their antimicrobial properties. They were tested against the most prevalent microorganisms *Pseudomonas aeruginosa*, *Escherichia coli* and *Proteus mirabilis*. Eucalyptus globulus 3C was able to show a zone of inhibition of about 9 mm and 7 mm against *Escherichia coli* and *Pseudomonas aeruginosa*, respectively. When a higher potency of 6C was evaluated, zones of inhibition of about 12 mm and 8 mm were formed against *Escherichia coli* and *Pseudomonas aeruginosa*, respectively. Both Eucalyptus globulus 3C and 6C failed to demonstrate any sort of antibacterial activity against *Proteus mirabilis*.

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When compared to previous studies, the lower potencies of Eucalyptus globulus were able to form a mild to moderate zone of inhibition against selective uropathogens. It has been shown in previous studies that Eucalyptus globulus tincture showed very potent antibacterial properties against these microbes.[9,10] Several other studies were done so as to substantiate the role of Eucalyptus globulus against various microbes. It was found that the antibacterial activity of the medicine was due to its antioxidant properties. It has proven to be a very efficient diaphoretic.[11,12] Furthermore, the oil's hydrophobic properties promote enhanced cell permeability, which causes bacterial cell leakage.[13,14]

Conclusion

From this study, it was evident that Eucalyptus globulus 3C and 6C show moderate antibacterial properties. The potency 3C and 6C showed a maximum zone of inhibition of about 9 mm and 12 mm against *Escherichia coli*. Similarly, a zone of inhibition of about 8 mm was noticed against *Pseudomonas aeruginosa*.

There is no possibility of the development of drug resistance among homoeopathic medicines as they act via an effective host response by stimulating the body's innate self-regulatory mechanism. Hence, similar studies should be conducted in an effective manner to showcase the potential antibacterial activities of homoeopathic medicines.

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Conflict of Interest: None

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