Experimental Investigation on Antimicrobial Properties of *Elaeocarpus Ganitrus* (rudraksha) and *Cassia Occidentalis l.*

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Abstract

Objective: This study aims to evaluate the antimicrobial properties of Elaeocarpus ganitrus (Rudraksha) and Cassia occidentalis extracts against Staphylococcus aureus, determine the optimal concentrations, and investigate possible mechanisms of action.

Methods: The experimental design was conducted using response surface methodology (RSM), with statistical analysis performed in Design Expert software. Variables such as extract concentration, pH, and physiological temperature were manipulated to assess their effects on antimicrobial activity.

Results: Both Elaeocarpus ganitrus and Cassia occidentalis extracts exhibited significant antimicrobial activity against Staphylococcus aureus. The highest antimicrobial activity was observed with Elaeocarpus ganitrus extract at a concentration of 50%, pH 6.5, and at a physiological temperature of 37°C. These findings suggest that the extracts are effective under specific conditions.

Conclusions: The study concludes that Elaeocarpus ganitrus and Cassia occidentalis extracts have significant potential for developing skincare products to treat infections caused by antibiotic-resistant Staphylococcus aureus. The results are particularly relevant for combating bacterial infections related to acne, highlighting the importance of these traditional medicinal plants in modern healthcare.

Keywords: Acne; *Cassia occidentalis*; *Staphylococcus aureus*; Antimicrobial Properties; RSM

Introduction

The emergence of antibiotic-resistant strains, particularly Staphylococcus aureus, represents a major challenge in modern healthcare.[1] Conventional antibiotics are becoming increasingly ineffective, necessitating the search for alternative sources of antimicrobial agents. It is well established that the gut microbiota plays an important role in host health and is perturbed by several factors including antibiotics. Antibiotic-induced changes in microbial composition can have a negative impact on host health including reduced microbial diversity, changes in functional attributes of the microbiota.[2] The traditional medicinal plants *Elaeocarpus Ganitrus* (Rudraksha) and *Cassia Occidentalis* are promising due to their antimicrobial properties.[3-4] However, comprehensive studies examining the specific effects of these plant extracts on *Staphylococcus aureus* are lacking.[5] Staphylococcus aureus is a versatile gram-positive bacterium that colonizes human skin and mucous membranes.[4,6] Although it is a normal component of the human microbiota, it can cause a range of infections, from mild skin and soft tissue infections to serious and life-threatening diseases such as pneumonia, endocarditis and sepsis.[7] The ability of Staphylococcus aureus to develop resistance to multiple antibiotics, including methicillin-resistant Staphylococcus aureus (MRSA), has become a global health concern.[8]

The plant kingdom harbors a wealth of secondary metabolites, many of which exhibit significant antibacterial activities. *Elaeocarpus Ganitrus* (Rudraksha) and *Cassia Occidentalis* are two such plants known for their traditional medicinal uses, and recent studies have indicated their potential antimicrobial efficacy. Rudraksha, derived from *Elaeocarpus Ganitrus*, has been reported to possess various pharmacological properties, including antimicrobial activity.[9] *Cassia Occidentalis*, commonly known as "Coffee Senna," has demonstrated antimicrobial potential attributed to its phytochemical composition.[10] Despite the growing interest in the antimicrobial properties of these plant extracts, a comprehensive investigation into their specific effects on *Staphylococcus aureus* is lacking.[11]

The discovery rate of active novel chemical entities, however, is dropping, according to recent trends. Higher plant natural products could provide a fresh source of antibacterial compounds with potentially unique modes of action.[12] A huge number of researchers from all around the world have investigated how plant extracts affect bacteria. India has seen a lot of research on herbs. *Cassia occidentalis L*., is an annual Ayurvedic plant which is used in several traditional medicines to cure various diseases.[13] Antimicrobial activity of these Indian herbs against plant pathogen and treatment of different human disease were reported. *Elaeocarpus Ganitrus* (Rudraksha) and *Cassia occidentalis L*. are traditional medicinal plants that have been studied for various pharmacological activities, including antimicrobial properties. Rudraksha seeds have been reported to possess antibacterial effects against both Gram-positive and Gram-negative bacteria.[15] *Cassia occidentalis L*. has demonstrated antimicrobial potential against various pathogens, making it a subject of interest in the search for novel antimicrobial agents.[16]

The main objective of this study is to evaluate the inhibitory effect of *Elaeocarpus Ganitrus* and *Cassia occidentalis L*. extracts on the growth of *Staphylococcus aureus*. The research investigates the reactions of these plant extracts corresponding to concentration, pH value and physiological temperature against *Staphylococcus aureus* with the aim of determining optimal

inhibitory concentrations. In addition, the study aims to elucidate the possible mechanisms of action behind the observed antimicrobial effects, including the identification of specific bioactive compounds.[2]

Material and Method

Collection of sample materials

Figure 1 presents images of seeds from *Elaeocarpus Ganitrus* and *Cassia occidentalis L*. The seeds were systematically procured from both a local market and an adjacent garden situated in Bareilly, Uttar Pradesh. Figure 1 (a) corresponds to *Elaeocarpus Ganitrus* seeds, while Figure 1 (b) signifies *Cassia occidentalis L*. seeds. The thorough selection of samples ensures a comprehensive and representative analysis of *Elaeocarpus Ganitrus* and *Cassia occidentalis L*. seeds within the specified geographic context, contributing valuable insights into the diversity of local plant species.

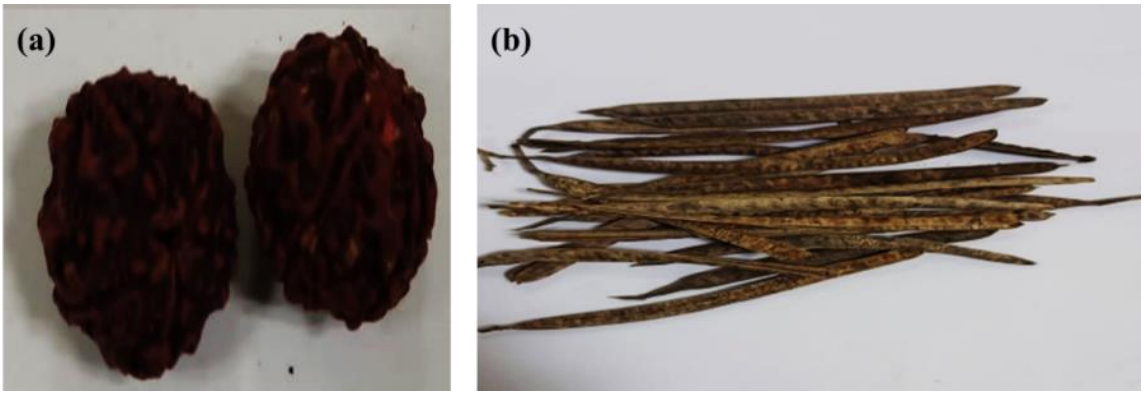

Figure 1 Sample materials (a) *Elaeocarpus Ganitrus* (b) *Cassia occidentalis L*. seed

Extracts preparation of Rudraksha and Cassia occidentalis L.

First, the Rudraksha seed was thoroughly washed from distilled water until it was completely clean and air dried at room temperature. Thereafter, a thin powder from the inner part of Rudraksha and weigh it into five different tubes was prepared. The tubes contained different concentrations: 100 mg, 200 mg, 300 mg, 400 mg, 500 mg of Rudraksha powder.

The ethanolic extracts of medicinal plants were prepared by separately combining dried powder with 5 ml of ethanol. The mixture was then kept in a separatory funnel for seven days, shaking continuously this process continues after 24 hours for 7 days. After seven days, the extract was filtered and the ethanol was evaporated in an evaporator while the sediment fell to the bottom. After that we mix 1 ml of DMSO in each tube and shake it well. Figures 2 (a) shows the Rudraksha powder (b) extract mix with DMSO (c) Cassia occidentalis L. seeds (d) Cassia occidentalis L. extract

Figure 2 (a) Rudraksha powder **(b)** Rudraksha extract mix with DMSO **(c)** Cassia occidentalis L. seeds **(d)** Cassia occidentalis L. extract

Cassia occidentalis L. seed was properly washed in water to make it sterile and free from impurities and then dried at room temperature. Then made a thin powder using a mixer grinder and weighed the powder into five different tubes of 100 mg, 200 mg, 300 mg, 400 mg, 500 mg. Now the extract was dissolved in ethanol, which was used as solvent in each tube and shaken thoroughly by cyclomixture and brought to RT for shaking continuously this process continues for 7 days. After seven days, the extract was filtered and the ethanol was evaporated in an evaporator. The extract then desolved in 1ml DMSO. The sequential actions provided desired extracts of Rudraksha and *Cassia occidentalis L*.

Isolation of bacteria

Taking nutrient broth for bacterial cultivation. Here we take 3.25 g of nutrient broth to make 250 ml of media in the flask. Now we sterilize the media using an autoclave. We put media and other instruments in the autoclave for 30 minutes for sterilization at 15 psi. After that, we wiped the LAF with an alcohol cotton swab and started UV light for 15 minutes for sterilization. We collect the bacteria from culture collection centre of a laboratory of an academic institution in Uttar Pradesh and confirm their presence through a screening process. After observation, we identify the bacteria Staphylococcus aureus.

Experiment design

The experiments are designed through response surface methodology (RSM) in Design Expert 2013 software. RSM helps to develop the models with least standard error. The related former research explored to identify the significant process parameters for selected responses. Three controllable process parameters such as concentration of extract (Conc.), pH value and physiological temperature (Temp.) are selected based on their significance for responses. The range of parametric values is also selected based on previous research studies and the limiting conditions. The parameters varied at three levels i.e., low, medium and high as shown in Table 1 with 20 experimental runs.

Results and Discussion

Figure 3(a) shows the isolated *Staphylococcus bacteria*. Utilizing an inoculating loop, we grew the bacteria in LAF and incubated the flask at 37°C for 48 hours. After sterilizing it in an autoclave, prepare the NAM (nutrient agar media) 250ml media for this experiment. Spread the *staphylococcus* bacteria from the culture into a petri dish with NAM. Create six wells in a petri dish for the extract and control. Here, we utilised ethanol as the control and the remaining five wells for the extraction of 100 mg/ml, 200 mg/ml, 300 mg/ml, 400 mg/ml, and 500 mg/ml. Then, kept the petric plate into the incubator to provide a specific environment for the growth of the bacteria.

DMSO were used as a control in both experiments performed for Rudraksha and *Cassia occidentalis L*. antimicrobial activity against *Staphylococcus Aureus*. Result was observed after the 24 hours of culturing. we found easily visible zone of inhibition by extract to prevent growth of bacteria near wells as shown in (Figure 3b and c). The outcomes of the agar well diffusion method, which was employed to assess the rudraksha's antibacterial activity, are shown in Figure 3 (b). All extract concentrations showed effect, despite the fact that the clear zone widths differed between extract concentrations. The zones around the well were clearly defined by the different *Cassia occidentalis L*. extract concentrations, as shown in Figure 3 (c). This demonstrate that rudraksha and *Cassia occidentalis L*. have antimicrobial property against *Staphylococcus aureus* and help to make more product by using these herbal extract for resolving the acne problem.

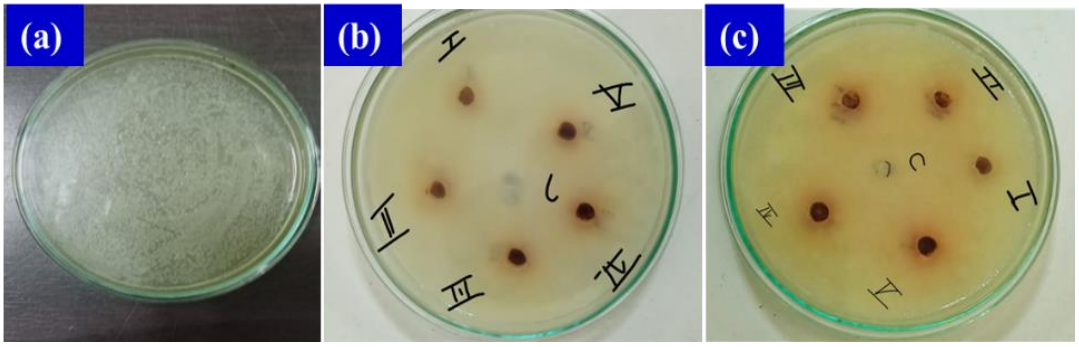

Figure 3 (a) Isolated *Staphylococcus bacteria* **(b)** *Elaeocarpus Ganitrus* zone **(c)** *Cassia occidentalis L*. zone of inhibition

The responses corresponding to designed experiments represented in Table 1. The results show that the variations in the selected parameters affects the zone of inhibition for both rudraksha and cassia. These results are further considered for the statistical analysis.

Table 1 Experimental results

Exp. Run	Conc. (%)	pH	Temp. (℃)	ZOI [Rudraksh] (mm)	ZOI [Cassia] (mm)
1	30	5.5	35	0.700±0.01	0.595±0.006
2	30	5.5	35	0.680±0.008	0.578±0.007
3	10	6.5	30	0.420±0.007	0.270±0.005
4	10	5.5	35	0.590±0.008	0.430±0.005
5	50	6.5	30	0.620±0.010	0.527±0.006
6	30	6.5	35	0.720±0.011	0.650±0.004
7	50	4.5	30	0.560±0.010	0.476±0.006

8	30	4.5	35	0.600±0.009	0.510±0.008
9	10	6.5	40	0.480±0.006	0.350±0.007
10	30	5.5	35	0.780±0.012	0.6030.009
11	30	5.5	30	0.620±0.008	0.527±0.006
12	30	5.5	35	0.690±0.011	0.581±0.007
13	50	6.5	40	0.720±0.011	0.612±0.010
14	30	5.5	35	0.770±0.012	0.644±0.010
15	50	5.5	35	0.760±0.012	0.710±0.011
16	30	5.5	35	0.760±0.010	0.646±0.010
17	30	5.5	40	0.700±0.012	0.560±0.009
18	10	4.5	40	0.400±0.006	0.330±0.007
19	50	4.5	40	0.630±0.008	0.523±0.006
20	10	4.5	30	0.440±0.006	0.280±0.004

The results of the analysis of variance (ANOVA) for ZOI (Rudraksha) are obtained Model F-value of 21.78 signifies the significance of the model. The likelihood of such a large F-value occurring due to random variability is only 0.01%. Model terms with p-values less than 0.0500 are considered significant, and in this instance, terms A, B, C, A^2, B^2, and C^2 are found to be significant. Conversely, model terms with values exceeding 0.1000 are deemed not significant. The Lack of Fit F-value of 0.32 indicates that the Lack of Fit is not statistically significant compared to pure error. The probability of observing a Lack of Fit F-value of this magnitude due to random fluctuations is 88.40%. The Predicted R^2 of 0.8268 demonstrates reasonable concordance with the Adjusted R^2 of 0.9078, with a difference of less than 0.2. Consequently, this model proves suitable for navigating the design space. The mathematical model for ZOI (*Elaeocarpus ganitrus*) is expressed in Eq. 1.

ZOI (*Elaeocarpus ganitrus*) = +0.7302 + 0.0960A +0.0330B + 0.0270C + 0.0112AB + 0.0187AC + 0.0162BC - 0.0555A^2 - 0.0705B^2 - 0.0705C^2 [1]

The ANOVA for ZOI (*Cassia occidentalis L.*) shows the significant results. The Model F-value of 28.19 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. In this case A, B, C, A^2, B^2, C^2 are significant model terms. The Lack of Fit F-value of 1.57 implies the Lack of Fit is not significant relative to the pure error. The Predicted R^2 of 0.8171 is in reasonable agreement with the Adjusted R^2 of 0.9279; i.e. the difference is less than 0.2. Adeq Precision measures the signal to noise ratio. This model can be used to navigate the design space. The mathematical representation of the Zero-One Integer (ZOI) for *Cassia occidentalis L.* is expressed in Eq. 2.

ZOI (Cassia) = +0.6161+0.1188A + 0.0290B + 0.0295C + 0.0162AB + 0.0002AC + 0.0085BC- 0.0585A^2 - 0.0485B^2 - 0.0850C^2 [2]

Effect of process parameters on ZOI (Elaeocarpus ganitrus)

The experimental results show the significant impact of process parameters on selected responses. The main effect plot Figure 4 shows that the ZOI (Rudraksha) increases by increasing the concentration percentage.[14] Though at maximum ZOI at 50% it stabilizes. Approximately the similar behavior is observed for the ZOI (*Cassia occidentalis L.*).

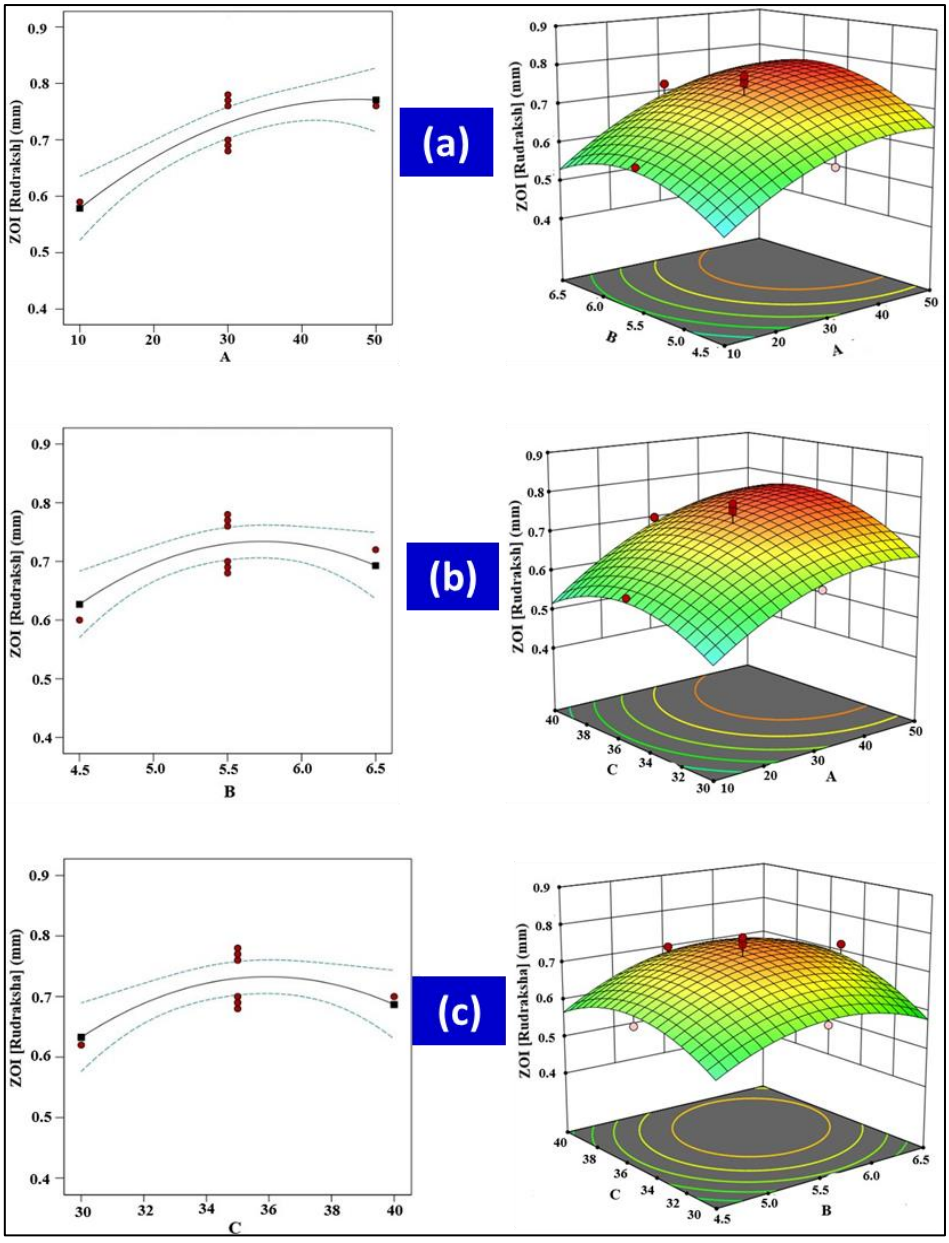

Figure 4 Parametric effects on ZOI (Rudraksha) [A- Concentration, B- pH, C-Physiological temp.]

Figure 4 (a) indicates that on increasing of the value of concentration, the ZOI is increasing. At the same time, Figure 4 (b) shows that the zone of inhibition is maximum at higher values of concentration and pH. The previous research also showed that the ZOI is maximum at a concentration of 50% Rudraksha extract. The antimicrobial activity at 50% concentration is higher due to the presence of a variety of secondary metabolites such as tannins, terpenoids,

alkaloids, flavonoids, antioxidants, glycosides, etc., which have antimicrobial properties in vitro.[17]

Effect of process parameters on ZOI (Cassia occidentalis L.)

The main effect plots and surface plots for ZOI (Cassia), corresponding to the concentration of *Cassia occidentalis L*. extract, are shown in Figure 5.

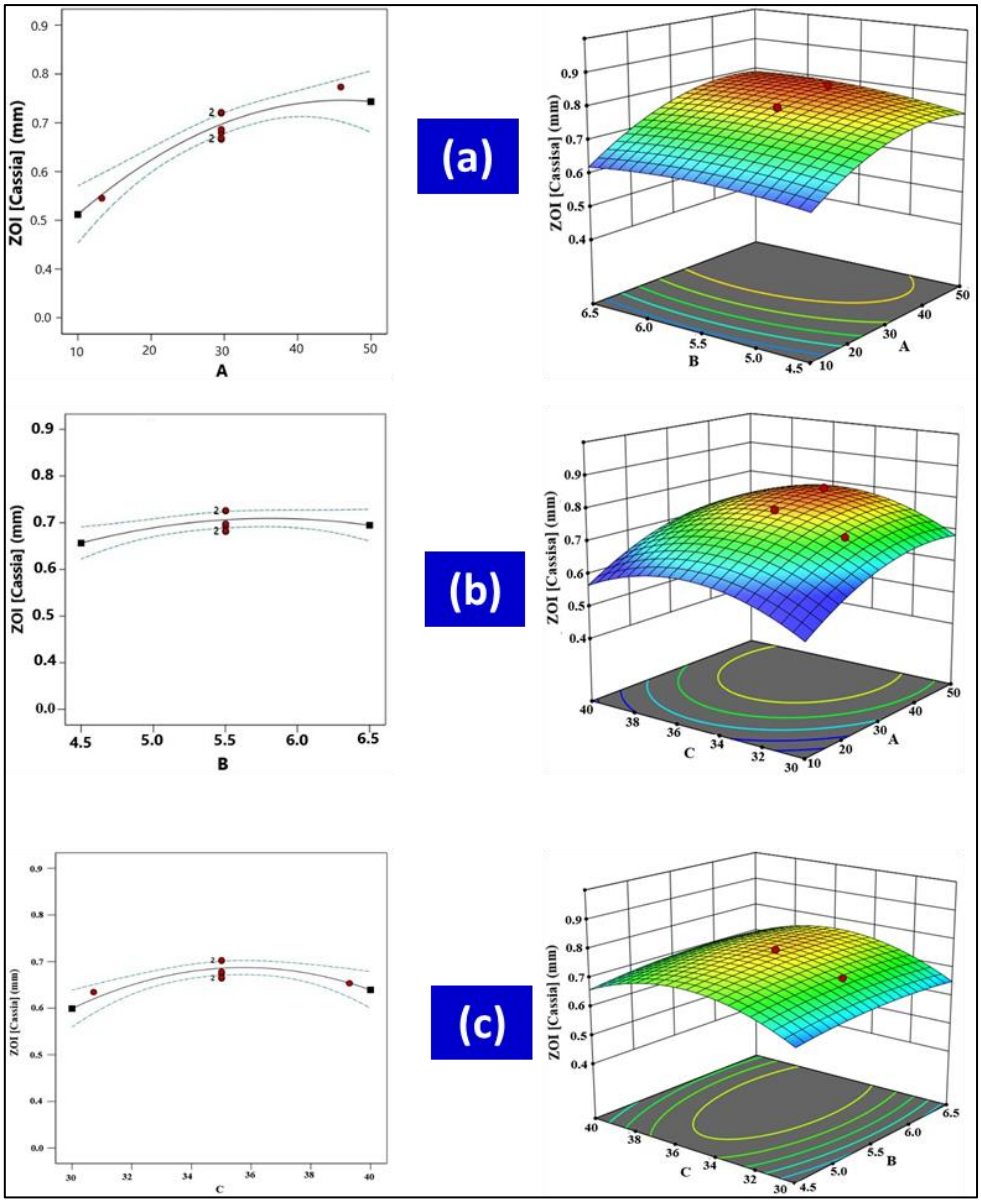

Figure 5 Parametric effects on ZOI (Cassia) [A- Concentration, B- pH, C-Physiological temp.]

Figure 5 (a) shows that the ZOI increases with increasing concentration value. At the same time, the ZOI is maximum at higher concentration and pH values but is lower compared to Rudraksha. Figure 5 (b) represent the main effect plot and surface plots represent the ZOI corresponding to the pH value. The ZOI does not have a major impact until the medium pH and then begins to decrease. The ZOI is maximum at a higher concentration value and around 37 °C at the medium temperature value. The former research also support the present results.[18]

Figure 5 (c) shows that the average values of pH and temperature are more suitable for the maximum ZOI.

The results of this study reveal promising antimicrobial activities of ethanolic extracts from *Elaeocarpus ganitrus* (Rudraksha) and *Cassia occidentalis L*. occidentalis against Staphylococcus aureus.[19] The investigation focused on evaluating the inhibitory effects of these plant extracts, determining concentration-dependent responses, exploring potential mechanisms of action, comparing the antimicrobial efficacy of the two plants, and offering insights for future therapeutic applications. The study demonstrated significant antimicrobial efficacy of both *Elaeocarpus ganitrus* and *Cassia occidentalis L*. extracts against Staphylococcus aureus. The observed zone diameters in the agar well diffusion method indicate the ability of these plant extracts to inhibit bacterial growth. Notably, the highest concentration of ethanolic extracts (50%) exhibited the maximum inhibitory effects, emphasizing the concentration-dependent nature of their antimicrobial activity. This indicates that Rudraksha extract can be safely considered for developing skin care products such as facial scrubs.

Conclusions

This study systematically investigated the antimicrobial potential of *Elaeocarpus Ganitrus* (Rudraksha) and *Cassia occidentalis L*. extracts against *Staphylococcus aureus*, addressing the pressing issue of antibiotic-resistant strains. The results showed a significant inhibitory effect, with both plant extracts showing responses according to concentration, pH and physiological temperature. It is noteworthy that the highest concentration (50%) of the DMSO extracts, pH 6.5 at 37 °C physiological temperature, showed maximum antimicrobial activity, highlighting the importance of dosage in their use. Comparative analysis showed that Rudraksha extract has superior potential in treating *Staphylococcus aureus* infections compared to *Cassia occidentalis L*. The research provides valuable insights into possible mechanisms of action underlying the observed antimicrobial effects. The results suggest that these plant extracts, particularly Rudraksha, hold promise as alternative or complementary agents in the development of antibacterial treatments. This research not only addresses the current healthcare challenge of antibiotic resistance, but also opens up opportunities for further exploration of these plant extracts in the development of skin care products such as facial peels to treat skin infections caused by *Staphylococcus aureus*.

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