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IN SILICO MOLECULAR DOCKING OF ESSENTIAL OIL FOR ANTIBACETERIAL ACTIVITY

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ABSTRACT: Over the past few years, a great deal of scientific research has focused on the quest for naturally occurring antimicrobials found in plants. This study aimed to evaluate the Lemmon gras essential oil's in vitro antibacterial activity and in silico toxicity against strains of bacteria. First, the essential oil was dissolved in a solution of tween 80 and dimethyl sulfoxide (DMSO). The antibacterial action was evaluated using the minimum bactericidal concentration (MBC), which was determined by the depletion in nutrient agar (NA) technique with aliquots of 10μL of the MIC, MIC × 2, and MIC × 4. The minimum inhibitory concentration (MIC) was determined from the microdilution in double-concentrated brain heart infusion broth (BHI). In Only the Citronellol, Geraniol, and Citral fractions showed a hazardous potential in the in silico toxicology investigation. Nonetheless, there was no indication of toxicity in the Citral fraction. Consequently, in comparison to other substances frequently used in the treatment of bacterial infections, lemon grass essential oil exhibits adequate antibacterial activity and a low potential oral toxicity.

Keywords: Lemon grass Essential oil, Antibacterial activity, In-silico

INTRODUCTION: Since the rapid emergence of multiresistant infections is endangering the clinical efficacy of several currently available medications, infectious illnesses have gained international attention (1). Finding novel antibacterial compounds with suitable chemical structures and a fresh mode of action against pathogenic pathogens is an ongoing necessity (2). Natural products, particularly antimicrobials, offer a wealth of characteristics and versatility for the development of novel pharmaceuticals with potential therapeutic applications for the treatment of infectious disorders (3). The isolated compounds of essential oils (EOs), such as terpenes, terpenoids, and aromatic compounds, have demonstrated antimicrobial activity against a diverse range of pathogens with varying spectrums of activities, making them suitable as antimicrobials both individually and in combination (4). The primary sources of novel medications and potential substitutes for conventional medications are phytotherapeutic agents (5).

Because Cochin Port in India is the hub for 90% of the world's lemongrass exports, the plant is also known as Cochin grass (6). Given its abundance in vitamins A, C, E, folate, niacin, and riboflavin, protein, antioxidants, and mineral nutrients like N (0.74%), P (0.07%), K (2.12%), S (0.19%), Mg (0.15%), Ca (0.36%), Zn (35.51 ppm), Mn (155.82%), Fe (126.73%), and Cu (56.64 ppm), the lemongrass plant has a wide range of uses as food and fodder (7, 8).

Lemongrass oil and extract have antimicrobial properties against a broad range of pathogens. LEO has frequently been employed as an antiviral (12), antifungal (11), and antibacterial (9, 10). Similarly, at varying degrees of susceptibility, lemongrass extract prevented the growth of Escherichia coli, Bacillus cereus, Klebsiella pneumoniae, Candida albicans, and Staphylococcus aureus (13, 14). Different functional groupings in EO components show varying degrees of antibacterial activity; aldehydes and phenols have the highest activities, while hydrocarbons and esters have the lowest. (15) Nonetheless, the citral (aldehyde) found in lemongrass oil is widely responsible for the plant's antibacterial properties (16, 17, 18). However, when the entire EO and a combination of the main oil components were examined, the entire EO showed improved antibacterial activity.

MATERIALS AND METHODS:

Phytoconstituent and substances: Essential oil was procured of local market. The tween 80 and the DMSO were solubilized in a proportion which did not exceed 0.5% in the tests, subsequently was diluted in sterile distilled water with the EO of the lemon grass in order to obtain a doubly concentrated emulsion of 2048µg/mL.

Test organism: Microorganisms were obtained from the Department of Microbiology, Christian Medical College, Vellore, India and Institute of Basic Medical Sciences (IBMS), Chennai, India. Gram-negative bacteria [Escherichia coli (ATCC 25922), Klebsiella pneumoniae (ATCC 15380), Pseudomonas aeruginosa (ATCC 27853), Proteus vulgaris (MTCC 1771)] and two strains of gram-positive bacteria [Bacillus subtilis (MTCC 441) and Staphylococcus aureus(ATCC 25923)] were used. The cultures of bacteria were maintained in their appropriate agar slants at 4°C throughout the study and used as stock cultures.

Antibacterial assay: The disc diffusion method was utilised to screen essential oils for antibacterial activity. This method is typically utilised as a first step and to identify the most effective essential oils (19). In 10 millilitres of Mueller Hinton Broth, an 18-hour culture at 37°C was used to carry it out. The cultures were titrated with sterile saline solution to roughly 105 CFU/ml. Using a sterile cotton swab, 500 microliters of the solutions were applied to the Mueller-Hin- tonne agar plates, ensuring a consistent microbial growth on both the control and test plates. The essential oils were filtered through a 0.45 μm membrane filter after being diluted in 10% aqueous dimethylsulfoxide (DMSO) with Tween 80 (0.5% v/v for easy diffusion). Empty sterilised discs (Whatman no. 5, 6 mm dia) were impregnated with 50 µL of various essential oil concentrations under aseptic circumstances and then placed on the agar surface. (20) As a vehicle control, a paper disc dampened with aqueous DMSO was put on the seeded petriplate. As a reference control, a regular disc containing 25 µg/disc of streptomycin was utilised. To prevent the test samples from eventually evaporating, sterile laboratory parafilm was used to encapsulate each petridish. The plates were incubated at 37°C for 18 hours after being left at room temperature for 30 minutes to allow the oil to diffuse (18 h was established as the optimal as there was no change in the inhibition up to 24 h). After the incubation period, the zone of inhibition was measured with a calliper. Studies were per- formed in triplicate, and mean value was calculated. (21)

In silico analysis (Osiris): Based on a pre-computerized list of molecular fragments that, in the event that they are discovered in the currently planned chemical structure, give rise to the toxicity alerts, the Osiris software predicts the biological effects. Based on Osiris's toxicity predictions, molecular groupings have been identified that exhibit mutagenicity, tumorigenicity, irritability, potential reproductive system damages, cLogP, druglikeness, and drug-score. (22)

Lipinski's rule of five 23 is used to compare how similar two medications are. It ascertains whether a chemical compound with proven biological or pharmacological action possesses characteristics that would likely make it a potentially effective oral medication for use in humans. This rule explains the molecular characteristics that are crucial to understanding a chemical compound's pharmacokinetics—that is, how it is absorbed, distributed, metabolised, and excreted in the human body—in the context of ADME. (23) The number of hydrogen acceptors (nALH<10), number of hydrogen donors (nDLH ≤5), molecular weight (≤500 Da), and cLogP ≤ 5 are the accepted rules for most "drug-like" compounds. If a molecule deviates from any of these characteristics, their bioavailability could be seriously compromised (23, 24).

RESULTS AND DISCUSSION:

The results of the antibacterial activity of the EO of Lemon grass were found from the MIC determined by broth microdilution. (Table 1 and 2).

Table 1. Mic Values (µg/mL) Of Lemon Grass Essential Oil Against Bacterial Strains.

Conc.	S. aureus	P. vulgaris	P. aeruginosa	B. subtilis	K. pneumoniae	E. coli
32µg/mL	-	-	-	-	-	-
64µg/mL	-	-	-	-	+	+
128µg/mL	+	-	-	-	-	+
256µg/mL	+	+	-	-	+	+
512µg/mL	+	+	+	+	-	-
1024µg/mL	+	+	+	+	+	+
Negative control	-	-	-	-	-	-
Positive control	+	+	+	+	+	+

(+) inhibition (-) no inhibition

Table 2. MBC Values (µg/mL) OF Lemon Grass Essential Oil Against Bacterial Strains.

Conc.	S. aureus	P. vulgaris	P. aeruginosa	B. subtilis	K. pneumoniae	E. coli

64µg/mL	-	-	-	-	+	+
128µg/mL	+	-	-	-	-	+
256µg/mL	+	+	-	-	+	+
512µg/mL	+	+	+	+	-	-
1024µg/mL	+	+	+	+	+	+
Negative control	-	-	-	-	-	-
Positive control	+	+	+	+	+	+

(+) inhibition (-) no inhibition

In addition to determining the minimum inhibitory concentration (MIC), an in silico toxicological analysis was conducted on the constituents of lemon grass essential oil (EO). This analysis encompassed an assessment of the pharmacological properties of the oil, which allowed for an appraisal of its mutagenicity, tumorigenicity, irritability, and potential reproductive system harm in relation to conventional antibiotics.

Table 3. Osiris Calculations Of Toxicity Risks And Drug-Score Of Compounds citronellol, gerniol and citral Compared To The Standard Antibiotics Drugs.

Compounds	**Toxicity risk[a]**				**Drug score[b]**						
	MUT	**TUMO**	**IRRI**	**REP**	**CLP**	**S**	**D-L**	**D-S**	**nALH**	**nDLH**	**Da**
Citronellol					3.19	-2.83	-2.52	0.21	0.00	0.00	134.22
Geraniol					3.45	-2.33	-2.89	0.41	0.00	0.00	136.24
Citral					2.72	-2.51	-3.11	0.17	0.00	0.00	136.24
Ciprofloxacin					-6.75	-9.42	1.82	0.23	33.0	19.0	1449.27
Streptomycin					-0.42	-2.36	-2.36	0.06	7.00	3.00	323.13

Nontoxic:,Slighlytoxic:, Highlytoxic:.[a]MUT: Mutagenic; TUMO: Tumorigenic; IRRI:Irritant;REP: Reproductive effective. [b]CLP: cLogP; S: Solubility; DL: Drug-likeness; DS: Drug-Score; nALH: number of acceptors hydrogen bonding; nDLH: number of hydrogen bond donor groups; Da: Molecular Weight.

Over time, the derived essential oils have demonstrated potential for bactericidal effects. Some plant species with documented bactericidal potential include lemon grass. In studies pertaining to the antibacterial activity of essential oils, Naveed et al. demonstrated that lemon grass exhibited remarkable efficacy against the strains of bacteria under investigation, which included Salmonella typhi G7 and Pseudomonas fluorescens. (25) The antimicrobial activity of lemon grass extract (EO) was examined against six bacterial strains, and it shown activity against every strain that was tested. The exceptional bactericidal power of lemon grass is confirmed by the low MIC and MBC values, particularly against strains of S. aureus, P. vulgaris, P. aeruginosa, B. subtilis, K. pneumoniae, and E. coli (Tables 1 and 2). These outcomes closely resemble those of Unlu et al., who found that lemon grass was a highly effective antibacterial agent by preventing the growth of both gram-positive and gram-negative bacteria. (26)

On the other hand, the compound's MBC adhered to the Hafidh et al. technique, taking into account the fact that a phytoconstituent exhibits bactericidal properties when the coefficient between the MBC/MIC is between 1 and 2, an effect that has the potential to ultimately result in the death of the bacterium. Because of this, lemon grass has a bactericidal effect and works well against most of the strains tested in this study. "In silico toxicity" describes scientific data analysis of chemical substance data by mathematical computations, computer experiments, or scientific data analysis using computational methods that anticipate potential toxicological activity (28). Osiris programme produces warnings on reproductive system damage, irritability, tumorigenicity, and mutagenicity. A colour code that denotes the strength of the effects is used to record the forecasts. Red denotes the possibility of unfavourable consequences, yellow represents a moderate risk, and green denotes no danger at all. Regarding the in silico analysis, Morales et al.'s concepts served as the foundation for the toxicological effects that the elements of lemon grass created. To determine the phytoconstituent's pharmacokinetic profile, the methodology suggested by Lipinski et al. is followed, meaning that the molecule must meet at least three of the four conditions (nDLH $\leq$ 5, nALH $\leq$10, DA $\leq$ 500 Da, and cLogP< 5). (29, 30)

Analysis was done on the three main EO fractions: geraniol, Citral, and citronellol. Comparing the phytoconstituents to other phytochemicals and conventional antibiotics, the results show that they are less harmful; they exhibit Drug-likeness values of -2.52, -2.89, and -3.11) and Drug-Score values of 0.21, 0.41, and 0.17, respectively. Only the monoterpene citral—which makes up the essential oil—showed signs of having a clearly hazardous potential, acting as an irritant and antibacterial agent. Geraniol did not exhibit any hazardous potential, however citral did exhibit a minor risk for tumorigenesis and irritation. On the other hand, the broad-spectrum antibiotic chloramphenicol, which is commonly used to treat severe bacterial infections, has been shown to be very toxic and to have irritating, tumorigenic properties. (31)

CONCLUSION: In summary, the antibacterial activity of lemon grass essential oil is excellent. proving once more that phytoconstituents are a useful substitute in medical treatments. Lemon grass showed promise against bacterial strains in this investigation. The in silico analysis also turned out to be intriguing, since every fraction of the EO complied with Linpinski's pharmacokinetics requirements, with the exception of two molecules' potentially harmful properties. As a result, both this essential oil and its main ingredients show promise as bactericides. Further research is required to fully examine the pharmacological and toxicological characteristics of these compounds both in vivo and in vitro.

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