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Research Article

Antibacterial Activity of *Ocimum basilicum* Essential Oil and Linalool on Bacterial Isolates of Clinical Importance

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ABSTRACT
Ocimum basilicum, popularly known as Basil, is a Lamiaceae family species widely known to treat different diseases. This species has as its main compound the monoterpene linalool. This study aimed to determine the antibacterial activity of *O.basilicum* essential oil and linalool against *S. aureus* and *P. aeruginosa* strains, as well as times to bacterial death facing each substance. The extraction of the *O. basilicum* leaves components was made by steam distillation. The Minimum Inhibitory Concentration (MIC) was calculated using microdilution technique and assessment of bacterial kinetics was performed with time-to-kill methodology. The results showed that *O. basilicum* essential oil and linalool display antibacterial activity against both *S. aureus* and *P. aeruginosa*, with certain strains of *P. aeruginosa* being resistant to the oil. Bacterial kinetics testing showed bacteriostatic activity against the strains in almost all concentrations, while only the MIC x 4 concentration of either essential oil or linalool against *S. aureus* displayed bactericidal activity. We conclude that the *O. basilicum* essential oil has antibacterial activity characterized as bacteriostatic or bactericidal against clinical isolates, and this activity is likely associated with linalool, its major compound.

Key words: Basil, bacterial resistance, medicinal plants, linalool.

INTRODUCTION

The appearance of antibiotics was a milestone in the history of health because it brought forward the possibility of effective combat and treatment of the numerous diseases caused by microorganisms. Unfortunately, what looked like a problem solved became a worldwide public health problem due to the emergence of resistant bacteria, the beginning of the era of bacterial resistance to existing antibiotics had begun. The current situation of drug resistance has its origin in many factors, including selection of resistant mutants through exposure to antimicrobial agents; genetic transfer of resistance determinants among bacterial strains; and clonal spread of resistant strains between both hospitalized patients and hospitals. The consequences are increased patient morbidity and mortality, a reduced number of usable drugs for future generations, and the economic impacts brought by the cost of infections[1].

The species *Pseudomonas aeruginosa* is responsible for a variety of infections; affecting the skin, urinary tract, the eyes, and the ears. A wide distribution of *Pseudomonas* in the environment is ensured by its non-fastidious growth requirements and *Pseudomonas* possesses many structural factors, enzymes and toxins that enhance virulence. This also makes them resistant to most common antibiotics[2].

Staphylococcus aureus is often found colonizing the natural microbiota, especially the skin. With the breakdown of skin barriers or immunity *S. aureus* can become pathogenic. It causes a variety of skin and subcutaneous infections, post-surgical infections, osteomyelitis, pneumonia, abscesses, endocarditis and bacteremia[3].

Plants used in traditional health care with therapeutic properties are an important source of new biologically active compounds. They have been part of traditional health care in many parts of the world for decades, and have aroused the interest of many researchers[4].

Ocimum basilicum (Lamiaceae) is widely distributed in tropical and warm temperate regions. It is a multi-purpose medicinal herb commonly used in folk medicines to treat different diseases like upper respiratory tract infections, diarrhea, headaches, eye problems, skin disease, pneumonia, coughs, fevers, and conjunctivitis[5].

Linalool, 3,7-Dimethyl-1,6-octadien-3-ol, is a monoterpene found in most aromatic plant essential oils. It is the major constituent of *Ocimum basilicum* oil. It has been widely used as starting compound for several

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Table 1: Chromatography of essential oil of *Ocimun basillicum*

RI	Compounds	%
928	α-pinene	0.4
972	β-pinene	1.1
987	Myrcene	0.7
1034	1,8-Cineole	8.8
1041	*trans*-β-Ocimene	0.6
1099	Linalool	55.2
1182	Terpinen-4-ol	0.9
1356	Eugenol	3.2
1421	B-Caryophyllene	0.4
1439	α-*trans*-Bergamotene	7.0
1489	Germacrene D	2.2
1515	γ-Cadineno	2.9
1638	Muurolol	2.9

RI: Retention index

important syntheses, such as ethyl linalyl acetate, and is a certified acaricide, bactericide and fungicide. In medicine it has been applied successfully as a sedative and is currently being analyzed for its anticonvulsant properties. Thus, linalool enjoys wide application in various areas of human knowledge, necessitating its production in ever greater quantities[6].

Based on the above, this study aimed to evaluate the antibacterial activity of *O. basilicum* essential oil against isolated *S. aureus* and *P. aeruginosa*, and to determine the time of bacterial death for sensitive bacteria.

MATERIALS AND METHODS

Compounds

The essential oil of *O. basilicum* was acquired commercially from Quinarí® (Ponta Grossa-PR), and linalool from Sigma-Aldrich.

Antibiotics

The antibiotics used in this work were Imipenem and Ciprofloxacin acquired commercially from Sigma-Aldrich, based on the sensitivity profile of the strains.

Bacterial Strains

16 bacterial strains were used as follows: two strains of *Staphylococcus aureus* ATCC (25926 e 6538), 2 strains of *Pseudomonas aeruginosa* ATCC (25853 e 9027) and 12 clinical isolates, 6 of each species. The strains of clinical origin used were provided by the clinical analysis Laboratory of Hematology in João Pessoa- PB-Brazil. All other microorganism strains were obtained from the Laboratory of Mycology collection. Bacteria were kept on Nutrient Agar (NA) slants at 4 °C. Inoculum was obtained from overnight cultures grown on NA slants at 37 °C, and diluted in sterile saline solution (NaCl 0.85% w/v) to provide a final concentration of approximately 10^6 colony forming units per mL (CFU mL^{-1}), and adjusted according to turbidity at 0.5 McFarland tube scale.

Determination of Minimum Inhibitory Concentration (MIC)

The microplate bioassay was used to determine the minimum inhibitory concentrations (MIC) for (Imipenem, Ciprofloxacin, and the linalool). For this purpose, 96-well plates were prepared by dispensing 100 µL of double strength Nutrient Broth (NB) inoculated with the bacteria into each well prior to the assay. Aliquots (100 µL) of each compound (at its respective concentrations) were transferred into consecutive wells. The highest substance concentration (1024 µg/mL) solution was added to the first well with the smallest concentration (2 µg/mL) in the antepenultimate well. The penultimate and the last wells containing 200 µL of the NB were respectively inoculated with the microorganism suspension, and Imipenem (100 µg/mL), being the negative control and positive controls. The microplate was aseptically sealed and incubated at 37 °C for 24 h. The antibacterial activity was detected using colorimetric method adding 20 µL of resazurin (0.1 g/100 mL) aqueous staining solution to each well at the end of the incubation period. The MIC was defined as the lowest sample concentration able to inhibit the bacterial growth as indicated by resazurin staining. All experiments were carried out at least twice with consistent results[7].

Determination of Minimum bactericidal concentration (MBC)

After reading the MIC results, the determination of minimum bactericidal concentration (MBC) was performed; three 10 µL dilutions from the MIC were inoculated in Mueller-Hinton broth (100 µl/well) medium in sterile microdilution plates, and then were incubated at 35-37 ° C for 24-48 hours. Then, 20 µL of resazurin was added. The plates were incubated for 24 hours at 35-37 °C and then to confirm the concentration capable of inhibiting the overall growth of bacterial species, checked by no-change in the indicator dye staining[8,9].

Table 2: Phenotypic sensitivity profile of the species of *S. aureus*

S.aureus	Amoxicillin	Amoxicillin/ Acid clavulonic	Ampicillin	Azithromycin	Cefalexina	Cefalotina	Ciprofloxacin	Clarithromycin	Clyndamicin	Erythromycin	Oxacillin	Penicillin	Teicoplanin
72-1	R	S	R	S	S	S	S	S	S	S	S	R	S
M-289	R	S	R	R	S	S	S	R	R	R	S	R	S
A-197	R	S	R	R	S	S	S	R	R	R	S	R	S
M-177	R	S	R	R	S	S	S	R	R	R	S	R	S
M-137	R	S	R	S	S	S	S	S	S	S	S	R	S
M-117	R	S	R	S	S	S	S	S	S	S	S	R	S

R=resistance; S= sensible; NT= not tested

Table 3: Phenotypic sensitivity profile of the species of *P.aeruginosa*

P.aeruginosa strains	Ciprofloxacin	Levofloxacin	Polymyxin B	Gentamicin	Amikacin	Ceftazidime	Cefepime	Piperacillin-tazobactam	Imipenem	Meropenem	Ceftriaxone
M 116-1	R	R	S	S	S	S	S	S	S	S	NT
166.22.260	R	NT	S	NT	S	R	R	NT	S	S	R
166.23.39	S	NT	S	NT	NT	R	S	NT	S	S	R
M-163	S	S	S	S	S	S	S	S	S	S	NT
LAC-21-1	S	S	S	S	S	S	S	S	S	S	NT
LM-07	S	S	S	S	S	S	S	S	S	S	NT

Table 4: Minimum Inhibitory Concentration of *O.basilicum* essential oil on *Staphylococcus aureus* and *Pseudomonas aeruginosa* strains.

	Microrganism	Control (Mo)	OB (µg/mL)		LIN (µg/mL)		IMP (µg/mL)	CPF (µg/mL)
			MIC	MBC	MIC	MBC		
S.aureus	ATCC 25923	+	1024	>1024	32	>1024	8	2
	ATCC 6538	+	1024	>1024	1024	>1024	4	2
	72-1	+	512	>1024	64	>1024	2	2
	M-289	+	1024	>1024	1024	>1024	4	2
	A-197	+	512	>1024	128	>1024	4	4
	M-177	+	1024	>1024	1024	>1024	4	2
	M-137-2	+	512	>1024	512	>1024	2	2
	M-117	+	512	>1024	512	>1024	2	2
P.aeruginosa	ATCC 25853	+	1024	>1024	1024	>1024	4	2
	ATCC 9027	+	1024	>1024	1024	>1024	2	2
	M 116-1	+	R	>1024	1024	>1024	16	4
	166.22.260	+	1024	>1024	1024	>1024	4	R
	166.23.39	+	1024	>1024	1024	>1024	4	2
	M-163	+	R	>1024	1024	>1024	2	2
	LAC-21-1	+	R	>1024	32	>1024	2	2
	LM-07	+	R	>1024	1024	>1024	2	2

Mo= microrganism; MIC= Minimum Inhibitory Concentration; MBC= Minimum bactericidal concentration; (+) Bacterial Growth; OB= O*cimum basilicum* essential oil; LIN =linalool; IMP= imipenem; CPF= ciprofloxacin; R= resistance.
R=resistance; S= sensible; NT= not tested

Determination of bacterial kill time

The essential oil of basil, and linalool were tested against bacterial strain viability by the colony counting method. From the MIC results obtained using the microdilution technique, the tests were prepared at the following concentrations: MIC/2, MIC, MIC x 2, and MIC x 4, control, and standard antibiotic. A bacterial suspension in saline 0.9%, equivalent to 0.5 McFarland tube scale, containing approximately 10^6 CFU/mL was prepared. To the 150 x 15 mm test tubes was added 9 ml of sterile BHI broth, the test product in the defined concentration, and 1 mL of the bacterial suspension. The test solutions were incubated at 37 °C, and during scheduled times (0, 1, 2, 4, 6, 8, 12 and 24 hours) an aliquot of 10µL was inoculated into a Mueller Hinton agar plate and incubated at 37 °C for 24 hours. Then, colony counting was done, where the average numbers of colonies (log10 CFU/mL) were labeled versus time for each strain, and used to compare the mean, and the extent of antibacterial activity at various concentrations. The analyses results for the test product were considered bactericidal if causing microbial death ≥ 99.9% (≥ 3 log10), and bacteriostatic if ≤ 99.9% (≤ 3 $\log_{10}$) taking into account the initial inoculum. The assay was performed in triplicate[10,11,12,13].

RESULTS AND DISCUSSION

The indiscriminate use of antibiotics has fomented the emergence of bacterial resistance to commonly used drugs and, consequently, the need (and search) for new products that can replace those which are no longer effective [14].

For more than 50 years, natural products have served us well in combating infectious bacteria and fungi. Microbial and secondary plant metabolites have helped to: double our life span during the 20th century, reduce pain and suffering, and revolutionized medicine. Essential oils are involved in many important processes related to plant survival, playing a prominent role in defense against microorganisms [15].

Among the various species of the genus Ocimum, *O. basilicum* L. is the most widely commercialized, due to its

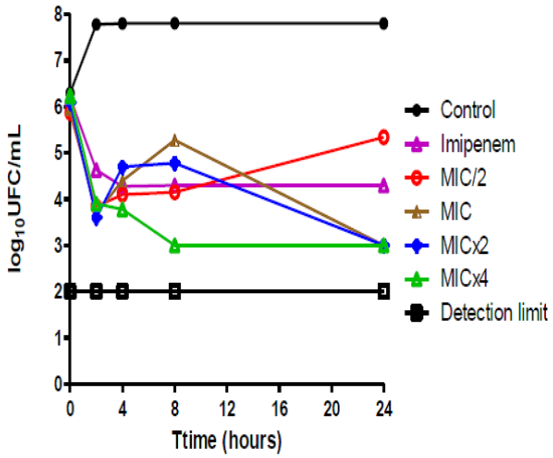

Figure 1: Curve of bacterial kill time, *Staphylococcus aureus* strain 72-1 by *O. basilicum* essential oil.

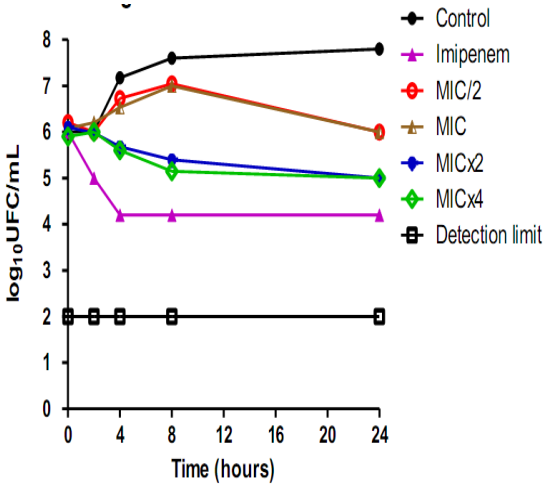

Figure 2: Curve of bacterial kill time, *Staphylococcus aureus* strain 72-1 by linalool

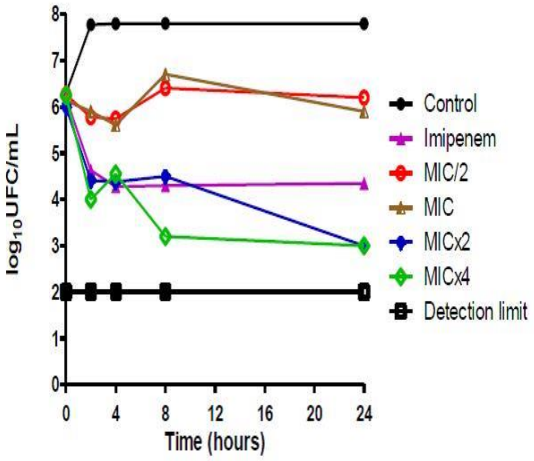

Figure 3: Curve of bacterial kill time, *P.aeruginosa* 166.23.39 strain by *O. basilicum* essential oil.

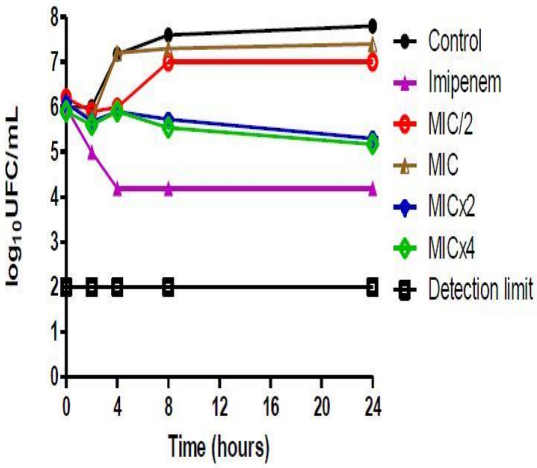

Figure 4: Curve of bacterial kill time, *P.aeruginosa* 166.23.39 strain by linalool.

green and aromatic leaves which are used dried or fresh, as a condiment, or for obtaining essential oil. The composition of the essential oils extracted from the leaves and apices with basil inflorescence varies according to the species and the geographical location, being classified into four chemotypes, (according to the major components of the oil): linalool-methyl chavicol (European), methyl chavicol (Reunion), methyl cinnamate (Tropical), and eugenol (Java). The essential oil of the species contains at least five fatty acids: palmitic, stearic, oleic, linolic, and linoleic [16].

The results obtained for *O. basilicum* essential oil chromatography are shown in Table 1. It is observed that the oil has as its major compound, the monoterpene linalool.

This result corroborates studies by Veloso et al[17] which identified two major constituents present in the essential oils of the evaluated *O. basilicum* samples (from different regions): one monoterpene (linalool), the majority in both cultivars, and phenylpropanoid ((E) cinnamate methyl), the majority in wild accessions.

The sensitivity profile of the strains (table 2 and 3) is also revealed. The results for antibacterial activity of *O. basilicum* essential oil on *S. aureus* and *P. aeruginosa* strains can be seen in Table 4. The activity, in both cases, was measured in terms of presence of microorganism growth**.**

In Table 4, we observe that all *S. aureus* strains were sensitive to the *O. basilicum* essential oil and linalool with MICs in the ranges of 1024-512 µg/mL and 1024-32 µg/mL, respectively. According to the Sartoratto et al.[18] classification methods, antibacterial activity can be classified as moderate for the oil, and between moderate and strong for linalool. As for the *P. aeuruginosa* strains tested, it was observed that 50% of the strains were resistant to *O. basilicum* essential oil, and those which were sensitive had an MIC of 1024 µg/mL, which also characterizes moderate antibacterial activity.

The antimicrobial activity of basil essential oil has been linked in part to the presence of high amounts of the monoterpene linalool. Researchers have found that basil oil and linalool compounds display antimicrobial activity against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Aspergillus niger*[19]. According to the literature, the antimicrobial activity of the essential oil could be a result of the high percentage of oxygenated monoterpenes (94.47%), which are particularly active against microbial cells[20]. In this study, we observed that

linalool showed antibacterial activity for all of the tested *P. aeruginosa* strains, including those that were resistant to the essential oil, which supports the idea that linalool, is the substance primarily responsible for the antibacterial activity of *O. basilicum* oil.

Microbial death kinetic studies are commonly used in investigations of new antimicrobial agents because they are relatively easy to perform and economically viable[21]. They relate microorganism growth inhibition with exposure to various test drug concentrations over time, showing whether the same has bactericidal or bacteriostatic action.

It is observed that the bacterial kinetics (Figure 1 to 4) of the *S. aureus* and *P. aeruginosa* samples against the *O. basilicum* essential oil and linalool showed bacteriostatic activity at nearly all times and concentrations since there was a reduction lower than 3 $\log_{10}$CFU/mL (<99.9%) of the initial inoculum. Only at the concentration of MIC x 4 did *O. basilicum* essential oil and linalool show bactericidal activity against *S. aureus,* being a reduction in bacterial growth greater than 3 $\log_{10}$ CFU/mL (> 99.9%) of the initial inoculum after 8 hours of contact. The bacteriostatic action of a compound means that it prevents the growth of the bacteria, maintaining the same in the stationary phase, while bactericidal action kills the bacteria[22].

According to Greay & Hammer[23], monoterpenes such as linalool interfere with the integrity and function of the cell membrane; changing the membrane potential, causing loss of cytoplasmic material, and inhibiting the respiratory chain. Exposure to terpenes can interfere with the expression of virulence factor encoding genes, considered when producing strains of *S. aureus* enterotoxins[24], and the expression of cytoplasmic and membrane proteins in *Salmonella enterica*[25].

The results obtained in this study suggest that the compounds present considerable antibacterial effect against both Gram positive and negative bacterial species. Thus, further studies are necessary to explore this effect, investigate toxicities, and delineate mechanisms of action.

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