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ESSENTIAL OILS AND ISOLATED COMPOUNDS WITH ANTIBACTERIAL EFFECT

ÓLEOS ESSENCIAIS E COMPOSTOS ISOLADOS COM EFEITO ANTIBACTERIANO

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Keywords: Myrocarpus fastigiatus, Cymbopogon winterianus, Escherichia coli, Staphylococcus aureus, Salmonella spp.

Resumo

It is remarkable that essential oils and isolated volatile compounds have biological activities with high pharmaceutical, biochemical, biotechnological and agricultural potential. This study aimed to evaluate essential oils and volatile compounds isolated on groups of Gram-positive and Gram-negative bacteria, verifying their bactericidal effect. The essential oils of Myrocarpus fastigiatus and Cymbopogon winterianus, and the isolated compounds aldehyde C:8, Cinnamaldehyde, α-ionone and hexyl cinnamic aldehyde were obtained and donated by the laboratory of Organic Chemistry and Natural Products of the Jataí Federal University, Goiás, Brazil . The antibacterial assay was performed using the paper disk diffusion method and the inhibition halo determined in millimeters (mm) for Escherichia coli, Staphylococcus aureus, Salmonella serovar Thyphymurium and serovar Enteritidis, and Enterococcus faecalis. The essential oils showed good antibacterial action, although the isolated compounds showed greater bactericidal aptitude on most tested bacteria. Salmonella serovar Thyphymurium and serovar Enteritidis demonstrate resistance to essential oils and isolated compounds. However, the other bacterial strains showed sensitivity to the complex groups of phytomolecules verified in this study. It is concluded that both the essential oils and the isolated volatile compounds tested, have potential pharmaceutical and biological use for the strains evaluated.

Abstract

It is remarkable that essential oils and isolated volatile compounds have biological activities with high pharmaceutical, biochemical, biotechnological and agricultural potential. This study aimed to evaluate essential oils and volatile compounds isolated on groups of Gram-positive and Gram-negative bacteria, verifying their bactericidal effect. The essential oils of Myrocarpus fastigiatus and Cymbopogon winterianus, and the isolated compounds aldehyde C:8, Cinnamaldehyde, α-ionone and hexyl cinnamic aldehyde were obtained and donated by the laboratory of Organic Chemistry and Natural Products of the Jataí Federal University, Goiás, Brazil . The antibacterial assay was performed using the paper disk diffusion method and the inhibition halo determined in millimeters (mm) for Escherichia coli, Staphylococcus aureus, Salmonella serovar Thyphymurium and serovar Enteritidis, and Enterococcus faecalis. The essential oils showed good antibacterial action, although the isolated compounds showed greater bactericidal aptitude on most tested bacteria. Salmonella serovar Thyphymurium and serovar Enteritidis demonstrate resistance to essential oils and isolated compounds. However, the other bacterial strains showed sensitivity to the complex groups of phytomolecules verified in this study. It is concluded that both the essential oils and the isolated volatile compounds tested, have potential pharmaceutical and biological use for the strains evaluated.

1. INTRODUCTION

It is notable that essential oils (EOs) and isolated volatile compounds (IVCs) have high potential on a numerous genus of pathological bacteria. EOs are a complex group produced from the special metabolism of plants, presenting a complex chemical constitution of monoterpenes, triterpenes and phenylpropanoids (Santos et al., 2012; Boz; Dunca, 2018; Gomes et al., 2018; Rabib et al., 2019).

Biological characterization studies with EOs and IVCs, in particular with bacterial action, are widely studied throughout the world. A wealth of results obtained in antibacterial assays brings optimism to researchers for the development of new volatile biomolecules capable of matching the synthetic antibacterials currently on the market (Li et al., 2019; Hu et al., 2019; Selmi et al., 2020).

However, when conducting an experiment with volatile compounds, one must pay attention to several potential points that present a variability of responses on microorganisms, including the circadian circle, collection time, collection site, solar irradiation, biotic and abiotic stress and chemotypes are some of the obstacles observed in numerous studies with bactericidal or antimicrobial activity (Ribeiro et al., 2018; Neves et al., 2021; Santana et al., 2022).

Another important point to be evaluated, is the resistance of these microorganisms to the main antibacterial agents in the medical market. The uncontrolled use of antibiotics and their incorrect disposal, in the environment have caused numerous bacterial and fungal strains to show resistance to the standard doses used, in the home and within the hospital environment (Costa; Júnior, 2017; Ntondini et al., 2021).

Cases of resistance to standard use antibiotics, are observed for the numerous strains of *Escherichia coli*, *Staphylococcus aureus*, *Salmonella serovar* Enteritidis and *serovar* Thyphymurium and *Enterococcus faecalis*. Countless cases of resistance and septicemia are registered annually around the world, with the highest percentage in under developed countries where the medical resource presents serious problems (Avancini; Both, 2017; Maciel et al., 2017; Silva et al., 2018; Lima et al., 2018; Lima et al., 2018; al., 2020; Nobre et al., 2021).

Although synthetic antibiotics are still the best option during an antibacterial treatment, studies need to be developed with the aim of finding, characterizing and synthesizing new molecules from EOs and IVCs in natural plants of a region, biome, domain or even species herbal medicines that already present some information on people who use these means to treat their human and animal pathologies (Owen; Laird, 2018; Atki et al., 2019; Ajilogba; Babalola, 2019).

This study aimed to evaluate the antibacterial activity on *E. coli*, *S. aureus*, *S. serovar* Thyphymurium and *serovar* Enteritidis and *E. faecalis* on essential oils of *Copaifera multijuga*, *Myrocarpus fastigiatus*, *Salvia officinalis* and *Cymbopogon winterianus*, and isolated compounds C:8 Aldehyde, Cinnamaldehyde, α-Ionone and Hexyl cinnamic aldehyde.

2. MATERIAL AND METHODS

2.1 Essential oils and compound isolates

Essential oils (*Myrocarpus fastigiatus* and *Cymbopogon winterianus*) and isolated volatile compounds (C:8 Aldehyde, Cinnamaldehyde, α-Ionone and Hexyl cinnamic aldehyde) were obtained from hydrodistillation in a Clevenger-type system. The material was donated by the Organic Chemistry and Natural Products laboratory of the Chemistry Departament at the Jataí Federal University, Goiás state, Brazil.

2.2 Antibacterial activity

The bacterial strains were obtained from the Technological Chemistry laboratory of the Goiano

Federal Institute, Campus Rio verde, GO, Brazil. The microbiological assay followed as described by Vieira et al. (2021) adapted, using the paper disc diffusion technique. Were used of strains from *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (LB 29212), *Salmonella serovar* Enteritidis (ATCC 13076) and *Salmonella serovar* Typhimurium (ATCC 14028).

The activation of microorganisms was carried out in a sterile solution of NaCl conc. 0.85% (Arboreto) until reaching the degree of 0.5 on the scale MacFarland conc. ($1x10^8$ CFU mL^{-1}) UV-*Vis* spectrometer (BELphotonics, Mod. M-51). *Petri* dishes (10 cm^2) were prepared with Plate Count Agar (PCA) after sterilization. The *Petri* dishes containing specific medium were inoculated using a sterile swab soaked with a microbial suspension, and spread across the plate.

Filter paper discs (Unifil) with a diameter of 7 mm were impregnated with 100 µL of the extract in different concentrations (100, 50, 25, 5 and 2.5 mg mL^{-1}), as a negative control, the saline solution used with 10% dimethylsulfoxide (DMSO) (*v*/*v*), and as positive control discs with antimicrobial agents, Azithromycin (15 µg), Cephalexin (30 µg) and Tigecycline (15 µg). The *Petri* dishes were incubated at 36 °C with an interval between 24-36 h, after that period, the halo of antibiosis when present was measured with a digital caliper. The minimum antibiosis halo was 5 mm. The test were carried out in quadruplicate.

2.3 Statistical Analysis

The treatment was carried out in quadruplicate and the experimental design was thoroughly randomized. Data were submitted to the analysis of variance (ANAVA), and the means of the treatments were evaluated by the *Scott-Knott* test at 5% significance level by the *ASSISTAT* software (free version).

3. RESULTS AND DISCUSSION

The results of bacterial growth inhibition halos observed for the EOs of *M. fastigiatus* and *C. winterianus* on *E. coli*, *S. aureus*, *E. faecalis*, *S. serovar* Enteritidis and *serovar* Thyphymurium are shown in (Table 1). As observed, the EO of *C. winterianus* showed antibacterial activity in all strains, including *S. serovar* Enteritidis and *serovar* Thyphymurium, which showed resistance to the EO of *M. fastigiatus* even at the highest concentration of 100 mg mL^{-1}.

Statistically, at the highest concentration of 100 mg mL^{-1} observed for *E. faecalis* in the EO of *M. fastigiatus*, and for *S. aureus* in the EO of *C. winterianus*, the results showed no significant difference by the *Scott-Knott* test with 5% probability, when compared to the reference antibacterials Azithromycin and Tigecycline, respectively. In this study, both EOs presented satisfactory results for most of the Gram-positive and Gram-negative strains tested, possibly this potential bactericidal effect occurs through the synergism of the complex chemical constitution of these EOs. Rocha et al. (2000) describe in a study of the chemical profile of *C. winterianus* EO the presence of Citronelal = 17.91%, β-citronelol = 16.68%, neral = 18.95% and elemol = 14.12%. Wanner et al. (2010) verified the presence of significant quantitative for (*E*)-nerolidol = 77% in the EO of *M. fastigiatus*.

Also in Table 1, it is possible to observe that both strains of *Salmonella* were resistant to the EO of *M. fastigiatus*. Similar results were observed by Silva et al. (2018) evaluating the EOs of *Piper nigrum*, *Cymbopogon citratus* and *Rosmarinus officinalis* on *E. coli* and *Salmonella* spp. Wanner et al. (2010) did not observe bactericidal activity evaluating the EO of *M. fastigiatus* for *Bacillus cereus*, *Staphylococcus epidermidis*, *Citrobacter koseri*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens* and *Salmonella abony*, although in this study the researchers did not observe any inhibition activity for *S. aureus* and

E. coli, in the present study, a slight inhibition was observed at the highest concentration = 13 and 9 mm, respectively. However, Brito et al. (2020) observed important antibacterial activity on *Salmonella* Heidelberg, Enteritidis and Thyphymurium isolates using EO from *Origanum vulgare*.

Table 1. Antibacterial activity of the essential oils *Myrocarpus fastigiatus* and *Cymbopogon winterianus*.

	Inhibition zone (mm) *M. fastigiatus*				
	100 mg mL^{-1}	**50 mg mL^{-1}**	**25 mg mL^{-1}**	**5 mg mL^{-1}**	**2.5 mg mL^{-1}**
E. coli	9.11±0.51b	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c
S. aureus	13.54±0.98b	11.20±0.66b	8.07±0.99c	0.00±0.00d	0.00±0.00d
E. faecalis	23.48±0.31a	21.15±0.96a	18.17±1.03ba	15.03±0.68cb	11.77±0.67cb
S. Enteritidis	0.00±0.00b	0.00±0.00b	0.00±0.00b	0.00±0.00b	0.00±0.00b
S. Thyphymurium	0.00±0.00b	0.00±0.00b	0.00±0.00b	0.00±0.00b	0.00±0.00b
	Inhibition zone (mm) *C. winterianus*				
	100 mg mL^{-1}	**50 mg mL^{-1}**	**25 mg mL^{-1}**	**5 mg mL^{-1}**	**2.5 mg mL^{-1}**
E. coli	14.73±0.90b	13.09±1.07b	11.37±0.60cb	7.11±0.19d	5.16±0.32d
S. aureus	21.05±0.30a	17.66±0.14ba	12.08±0.95c	8.88±0.32dc	0.00±0.00e
E. faecalis	16.05±0.21b	12.68±1.07b	6.11±0.97c	0.00±0.00d	0.00±0.00d
S. Enteritidis	9.15±0.66b	7.08±0.09b	0.00±0.00c	0.00±0.00c	0.00±0.00c
S. Thyphymurium	6.54±0.37b	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c
Antibiotics	[a]23.52±0.40Aa	[b]28.07±0.19Ba	[a]28.01±0.08Ca	[a]27.53±0.84Da	[c]22.64±0.21Ea

Different lowercase letters on the same line differ statistically by the *Scott-Knott* test with 5% probability. [a]Azithromycin, [b]Cephalexin and [c]Tigecycline. A = *S. aureus*, B = *E. coli*, C = *S. serovar* Thyphymurium, D = *S. serovar* Enteritidis and E = *E. faecalis*.

Table 2 shows the results of growth inhibition activity in (mm) evaluating the isolated volatile compounds C:8 aldehyde, Cinnamaldehyde, α-ionone, and hexyl cinnamic aldehyde on *E. coli*, *S. aureus*, *E. faecalis* , *S. serovar* Enteritidis and *serovar* Thyphymurium.

All volatile compounds important inhibition activity against the bacterial strains tested, except for C:8 aldehyde, cinnamaldehyde and α-ionone which showed growth inhibition activity for *S. serovar* Enteritidis only at the highest concentration of 100 mg mL^{-1}. The hexyl cinnamic aldehyde compound, was the only volatile compound that presented at the highest concentration of 100 mg mL^{-1} a result similar to that observed by the reference antibacterial Azithromycin for *S. aureus* inhibition as evaluated by the *Scott-Knott* test with 5% probability. The highest allowances between 100 to 25 mg mL^{-1} concentrate the best inhibition results for most associated compounds. It is observed that both strains of *Salmonella* spp. have a certain resistance to these compounds, although they show a slight result and inhibition.

As observed in other studies, the *Salmonella* genus has high resistance even to the main reference antibiotics, which is a serious public health problem due to the constant bacterial outbreaks involving this genus (Cuevas et al., 2009; Toro et al. , 2014; Silva et al., 2018).

Wanner et al. (2010) evaluated several isolated volatile compounds such as α-bisabolol, cedrol, (*E,E*)-farnesol, (*Z*)-nerolidol, sabinene and thujopsene, where they found antibacterial activity for *B. cereus*, *S. aureus*, *S. epidermidis*, *C. diversus* and *E. coli*. QU et al. (2008) evaluated two compounds isolated forsythiaside and forsythin from *Forsythia suspensa* where they observed potential bactericidal action on *E. coli*, *P. aeruginosa* and *S. aureus* only for forsythiaside. Forsythin, did not show any activity against the three selected bacterial strains.

Table 2. Antibacterial activity of isolated volatile compounds, C:8 aldehyde, Cinnamaldehyde, α-ionone and hexyl cinnamic aldehyde.

Strains	**Inhibition zone (mm) C:8 aldehyde**				
	100 mg mL^{-1}	**50 mg mL^{-1}**	**25 mg mL^{-1}**	**5 mg mL^{-1}**	**2.5 mg mL^{-1}**
E. coli	9.96±1.05b	7.30±0.17b	0.00±0.00c	0.00±0.00c	0.00±0.00c
S. aureus	14.16±0.66b	13.55±0.91b	7.77±0.68c	0.00±0.00d	0.00±0.00d
E. faecalis	10.00±0.44b	7.98±0.90cb	0.00±0.00d	0.00±0.00d	0.00±0.00d
S. Enteritidis	7.31±0.00b	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c
S. Thyphymurium	18.07±0.83b	15.19±1.03bc	11.03±0.51d	9.14±1.15d	5.01±0.94e
	Inhibition zone (mm) cinnamaldehyde				
	100 mg mL^{-1}	**50 mg mL^{-1}**	**25 mg mL^{-1}**	**5 mg mL^{-1}**	**2.5 mg mL^{-1}**
E. coli	8.08±0.64b	5.00±0.37c	0.00±0.00d	0.00±0.00d	0.00±0.00d
S. aureus	14.14±0.94b	10.06±0.01cb	6.55±0.11dc	0.00±0.00e	0.00±0.00e
E. faecalis	17.01±0.15b	10.11±0.25c	0.00±0.00d	0.00±0.00d	0.00±0.00d
S. Enteritidis	11.02±0.38b	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c
S. Thyphymurium	10.04±0.09b	7.90±1.08b	0.00±0.00c	0.00±0.00c	0.00±0.00c
	Inhibition zone (mm) α-ionone				
	100 mg mL^{-1}	**50 mg mL^{-1}**	**25 mg mL^{-1}**	**5 mg mL^{-1}**	**2.5 mg mL^{-1}**
E. coli	21.01±0.99b	20.18±1.03b	17.56±0,90c	11.05±0.80dc	7.77±0.09e
S. aureus	14.14±0.37b	12.45±1.07b	9.68±0.14c	0.00±0.00d	0.00±0.00d
E. faecalis	18.16±0.41b	15.07±0.94cb	11.13±0.08d	0.00±0.00e	0.00±0.00e
S. Enteritidis	9.33±1.17b	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c
S. Thyphymurium	11.16±0.90b	10.82±0.50b	6.96±1.09c	0.00±0.00d	0.00±0.00d
	Inhibition zone (mm) hexyl cinnamic aldehyde				
	100 mg mL^{-1}	**50 mg mL^{-1}**	**25 mg mL^{-1}**	**5 mg mL^{-1}**	**2.5 mg mL^{-1}**
E. coli	7.33±0.68b	5.55±0.77b	0.00±0.00c	0.00±0.00c	0.00±0.00c
S. aureus	25.16±0.34a	24.12±0.08a	21.69±0.72ba	18.19±0.73cb	13.04±1.66cd
E. faecalis	10.11±0.65b	7.45±0.90bc	0.00±0.00d	0.00±0.00d	0.00±0.00d
S. Enteritidis	11.58±0.22b	8.17±0.93bc	5.05±0.98d	0.00±0.00e	0.00±0.00e
S. Thyphymurium	14.06±0.29b	11.95±1.09b	7.36±1.05c	0.00±0.00d	0.00±0.00d
Antibiotics	[a]23.52±0.40Aa	[b]28.07±0.19Ba	[a]28.01±0.08Ca	[a]27.53±0.84Da	[c]22.64±0.21Ea

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4. CONCLUSIONS

The isolated compounds showed high antibacterial potential for all strains tested, being superior to those observed for essential oils. Isolated compounds tend to have greater antibacterial action due to the high concentration of a particular volatile compound. Future studies should be carried out evaluating other physicochemical and biological characteristics, thus evaluating other desirable effects both for essential oils and for isolated volatile compounds.

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