Evaluation of the antimicrobial activity of the extracts from cashew nutshell and castor oil plant

Avaliação da atividade antimicrobiana dos extratos da casca de caju e do óleo ricinoleico

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ABSTRACT
Essential oils are natural extracts rich in bioactive compounds that possess antioxidant, antimicrobial, antiviral, and insecticidal properties. For this reason, they are investigated as a possible alternative to the use of antibiotics. Micellar and aqueous solutions from extracts derived from the cashew nut shell liquid and the ricinoleic acid extracted from the castor oil plant were screened to determine their minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against common bacteria, such as *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, Methicillin-Resistant *Staphylococcus aureus*, and *Staphylococcus epidermidis*. The cashew nut shell oil showed activity against Gram-positive bacteria with the micellar suspension having the best results. Neither the micellar nor the aqueous solutions of the castor bean extract showed antimicrobial activity in this study.

Keywords: *Anacardium occidentale*, *Ricinus communis*, microbial resistance, essential oil, antibacterial activity, medicinal plants.

RESUMO
Os óleos essenciais são extratos naturais ricos em compostos bioativos que possuem propriedades antioxidantes, antimicrobianas, antivirais, e insecticidas. Por esta razão, são investigados como uma possível alternativa ao uso de antibióticos. As soluções micelar e aquosa de extratos derivados do líquido da casca do caju e do ácido ricinoleico extraído da mamona foram testados para determinar a sua concentração inibitória mínima (MIC) e concentração bactericida mínima (MBC) contra bactérias comuns, tais como *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus* resistente à meticilina, e *Staphylococcus epidermidis*. O óleo de castanha de caju mostrou atividade contra bactérias Gram-positivas, tendo a suspensão micelar os melhores resultados. Nem a micelar nem a solução aquosa do extrato de mamona mostraram actividade antimicrobiana neste estudo.

Keywords: *Anacardium occidentale*, *Ricinus communis*, resistência microbiana, óleos essenciais, atividade antibacteriana, plantas medicinais.

1 INTRODUCTION

Essential oils (EOs) are concentrated natural compounds extracted from plants, which have been used for their medicinal and antimicrobial properties since the Middle Ages [1-3]. Formed from secondary plant metabolites, they are biologically active, volatile complexes with characteristic colors and odors, which can be obtained through various extraction methods, such as hydrodistillation - first developed in the Middle Ages by the Arabs for extraction and commercialization of these compounds -, extraction by organic solvents, extraction with Clevenger apparatus, cold extraction and others [1-4]. EOs, in general, are composed of terpenes, mainly monoterpenes, and sesquiterpenes, terpenoids, such as alcohols, aldehydes, ethers, ketones, phenols, and aromatic compounds [1-4].

Long recognized for their microbial properties, changes in the population's consumption profile have revived scientific interest in EOs. As the use of known antibiotics has been restricted in recent decades, mainly due to the selection of agents resistant to their action potentials, antibiotics used to treat humans and animals act as an important selective pressure for the emergence and persistence of resistant strains. The search for alternatives to chemical additives used in food products, to reduce environmental impact, and other factors drive related research in various industrial sectors [2,4].

According to the literature, EOs or their components can present, in addition to antimicrobial activity, antiviral, antimycotic, antiparasitic, and insecticidal activity [5-8] These characteristics may be related to their function in nature, since their role is to protect the plant from predators, such as insects and microorganisms, and to attract pollinating organisms [2,3,5,6].

The antibacterial activity of essential oils has been a topic of interest in the last decade. Their mechanism of action on bacteria occurs by cell wall degradation, which ultimately compromises the metabolism of the microorganism. The effect of EOs has been shown to be effective even on multidrug-resistant strains [1,2,9,10]. The market demand for more natural products and the increase in bacterial resistance to conventional antibiotics contribute to the search for alternative ways to control and eliminate bacteria, such as the use of EOs. [2,5,6,9,10]

This study aims to evaluate the antimicrobial action of oil extracted from cashew nutshell and ricinoleic acid extracted from the castor seed, *Anacardium occidentale*, and *Ricinus communi*, respectively, on common bacterial strains: *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*.

2 METHODOLOGY

2.1 BACTERIAL STRAINS AND INOCULUM PREPARATION

The chosen strains are representative of the pathogenic bacteria most commonly involved in human infections, including strains with high antibiotic resistance or high environmental adaptability. Ten registered microorganisms were tested for their susceptibility to ricinoleic acid (Table 1). For susceptibility to the liquid extracted from the shell of the cashew nut, the tests were made on three Gram-positive and two Gram-negative bacteria (Table 2).

Bacterial strains were revitalized on blood agar and checked for purity with biochemical identification tests. In sterile saline, from an isolated culture, was prepared a standard inoculum of 0.5 McFarland units (2 × 108 Colony Forming Units/ µL). Then ten microliters of inoculum were transferred in 9990 µL of 2x Mueller-Hinton.

2.2 ESSENTIAL OILS AND WORK SOLUTIONS

The essential oils were purchased already in their commercial forms from the specialized company A. Azevedo Industria e Comercio LTDA®, manufactured on

01/15/2019, and were previously tested to research the quality of the composition and non-addition of other compounds.

Two microdilution methods were used, based on the Clinical & Laboratory Standards Institute (CLSI) 2018 methodology and similar previous studies. [1,11] The first method, solution 1, consisted of mixing equal parts of sterile water and essential oil in microcentrifuge tubes, the mixture was submitted to the Ultrasonic Sonicator (Bandelin Sonopuls) at 40kHz for 20 minutes at 25°C to obtain homogeneous micellar aggregates. The opalescent phase, which was deposited at the bottom of the tube, was recovered using a sterile pipette and used as a stock solution of essential oil micelle (MiEO).

For solution 2, equal amounts of pure EO and sterile water were mixed thoroughly overnight using an orbital plate mixer at a temperature of 25°C in sterile centrifuge tubes (15mL). The tubes were centrifuged for 15 minutes at 5000 rpm for separation of the aqueous and non-aqueous phases. After complete separation, the aqueous phase was recovered with a sterile pipette and used as the essential oil aqueous working solution (AqEO).

2.3 DETERMINATION OF THE MINIMUM INHIBITORY CONCENTRATION – MIC

The minimum inhibitory concentrations were determined using 96-well plates (8 rows, A-H, and 12 columns, 1-12). 200µL of each stock solution, AqEO, and MiEO, were dispensed into the first wells of each row, and 100µL of sterile distilled water in columns 2-12. Using a multichannel pipette, 100µL of AqEO / MiEO from the first column were mixed sequentially with water, achieving two-fold serial dilutions. The remaining 100µL in the last dilution mixture was discarded. The final concentrations of the EO solutions were 50%, 25%, 12.5%, 6.25%, 3.13%, 1.56%, 0.78%, 0.39%, 0.20%, 0.10%, 0.05%, and 0.025% v/v in the final volume. One plate was separated for each bacterial strain.

From the prepared inoculum, using a multichannel pipette and pipetting tray, 100µL were transferred to the wells containing the dilutions, resulting in a bacterial inoculum of approximately 2,104 CFU/well, in a final volume of 200µL. Negative (Müeller-Hinton broth and water) and positive (bacterial inoculum and water) controls were prepared in the last row of each plate. The plates were incubated at 35°C for 18 hours.

The MIC was interpreted at the last well where there was no visible bacterial growth and understood as a percentage v/v of the stock solution.

2.4 DETERMINATION OF THE MINIMUM BACTERICIDAL CONCENTRATION – MBC

The MBC was determined by inoculating the last three wells of each line, which showed no bacterial growth, onto blood agar plates, labeled with the coordinates of the corresponding wells. The plates were incubated at 35°C for 24 hours. The MBC was noted in the areas that showed no bacterial colony growth and interpreted as the percentage v/v of the stock solution.

3 RESULTS AND DISCUSSION

For the essential oil obtained from the cashew nutshell, the best activity was obtained with the essential oil micelle stock solution (Table 3). The MiEO extract was able to achieve bactericidal effect - at lower concentrations (0.025-0.1%) -, and bacteriostatic (0.78%) for Gram-positive bacteria (staphylococci and enterococci). The aqueous extract - AqEO - showed less bactericidal and bacteriostatic activity for the microorganisms tested and was not effective in controlling the strain of methicillin-resistant *Staphylococcus aureus* (Table 3).

For the ricinoleic acid, there was satisfactory bacterial growth in all the wells, with concentrations of 50%, 25%, 12.5%, 6.25%, 3.13%, 1.56%, 0.78%, 0.39%, 0.20%, 0.10%, 0.05% and 0.025%, showing the absence of antimicrobial activity of the oil, according to table 4.

Gram-negative bacteria maintained their viability even at concentrations higher than 50% for both oils tested.

Cashew nuts have been used in traditional medicine to treat thrush, ulcers, impinges, bronchitis, and arthritis, among others, due to their antiseptic and anti-inflammatory properties. The components of the extract derived from cashew nutshell are proven effective in controlling *Candida albicans* and *Candida utilis* and have antimicrobial action on Gram-positive cells, which corroborates the results obtained by this study [12-15].

Silva [14] describes the in vitro efficacy of the hydroalcoholic extract of *A. occidentale* against multidrug-resistant strains of *Staphylococcus aureus*, the results obtained by Gonçalves [15] demonstrate a lower susceptibility of Gram-negative bacteria to the compounds extracted from *A. occidentale*, the presence of the polysaccharide capsule may act as a barrier to hydrophobic compounds such as the EOs.

Castor oil has several applications in the industry, being used in products such as lubricant waxes, cosmetics, plastics, and biodiesel production. It has a hydroxyl (OH) attached to the carbon chain, which distinguishes it chemically from other oils since there is no other vegetable oil produced commercially with this property. Its medicinal characteristics have been researched in this context, just as other botanical elements have been explored by traditional health care over the centuries all over the planet [16-19].

The mechanism of the antibacterial effect of ricinoleic acid is to denature and coagulate proteins through their activity on the bacterial cell wall structure. The alteration of plasma membrane permeability, more specifically, by hydrogen and potassium ions, causes the affected cells to lose control of chemical osmosis, leading to bacterial death [20].

A quick search on platforms of academic material shows that the terms "essential oil" produce increasing results, with publications that have been mainly concentrated in the last decade. This interest, in addition to being evident, is necessary given the increasing resistance to these drugs by microorganisms [21, 22]. In recent years, the significant increase in the occurrence of microorganisms that are increasingly resistant to antimicrobials highlights more and more the need for new products to enable proper treatment [23].

Essential oils have few adverse effects, low production cost, and may become a viable alternative for clinical therapy. For example, in situations where antibiotic therapy does not reach the site of colonization generating bacterial infections of difficult control, such as necrotic ulcers, or in situations of ischemia their topical use should also be considered [24].

Research for alternative methods of microbial control, especially with vegetable derivatives have shown promising results. However, the lack of standardization of methods for measuring antimicrobial activity hinders comparisons between studies, makes it impossible to reproduce pairs and the diversification of methodology ends up interfering in the results [1, 21]. Hood [25], for example, points out the inconsistency of the results obtained in studies using the disk diffusion, agar dilution, and well diffusion methods, accusing the dispersion instability of oils in a liquid medium. The absence of such standardization makes it difficult to obtain data about the true biological activity and therapeutic potential of these substances. Therefore, when characterizing the sensitivity of a microorganism to a specific agent, one must consider the technology used, the culture medium, the pathogen, and, obviously, the extract to be analyzed [21, 24].

4 FINAL CONSIDERATIONS

This study corroborates the data contained in the literature about the antimicrobial properties of cashew nut shell essential oil. However, the results differ for the ricinoleic acid, which despite being described as an agent with microbial potential, especially for Gram-positive microorganisms, was not effective in this evaluation. The lack of standardization of methods for measuring antimicrobial activity has proved to be a challenge, hindering comparative analysis and making it necessary to study new methods for identifying active antimicrobial compounds.

DISCLOSURE STATEMENT

No potential conflict of interest was reported by the author(s).

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Evaluation of the antimicrobial activity of the extracts from cashew nut shell and castor oil plant. (TABLES)

Table 1. Microorganisms tested for ricinoleic acid microbial activity.

Enterococcus faecalis	ATCC 29212
Escherichia coli	CCCD - E004
Klebsiella pneumoniae	CCCD - K001
Proteus mirabilis	CCCD - P001
Staphylococcus aureus	ATCC 13565
Staphylococcus aureus	ATCC 14453
Staphylococcus aureus	ATCC 27664
Staphylococcus aureus	MRSA USA 400
Staphylococcus aureus	WB 69
Staphylococcus epidermidis	CCCD - S010

Table 2. Microorganisms tested for microbial activity of cashew nut shell oil.

Enterococcus faecalis	ATCC 29212
Escherichia coli	CCCD - E004
Klebsiella pneumoniae	CCCD - K001
Staphylococcus aureus	MRSA USA 400
Staphylococcus epidermidis	CCCD - S010

Table 3. Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of micellar and aqueous extracts of cashew nut shell essential oil.

		Enterococcus faecalis	***Escherichia coli***	***Klebisiella pneumoniae***	***Staphylococcus aureus***	***Staphylococcus epidermidis***
MIC	AqEO	0,78%	>50%	>50%	>50%	0,78%
	MiEO	0,78%	>50%	>50%	0,78%	0,78%
MBC	AqEO	0,78%	>50%	>50%	>50%	0,20%
	MiEO	0,025%	>50%	>50%	0,10%	0,025%

Table 4. Microbial activity of ricinoleic acid on different microorganisms.

%	50	25	12,5	6,25	3,13	1,56	0,78	0,39	0,20	0,10	0,05	0,025
E. faecalis	+	+	+	+	+	+	+	+	+	+	+	+
E. coli	+	+	+	+	+	+	+	+	+	+	+	+
Klebisiella pneumoniae	+	+	+	+	+	+	+	+	+	+	+	+
P. mirabilis	+	+	+	+	+	+	+	+	+	+	+	+
S. aureus	+	+	+	+	+	+	+	+	+	+	+	+
S. aureus	+	+	+	+	+	+	+	+	+	+	+	+
S. aureus	+	+	+	+	+	+	+	+	+	+	+	+
S. aureus	+	+	+	+	+	+	+	+	+	+	+	+
S. aureus	+					+	+	+	+	+	+	+
S. epidermidis	+					+	+	+	+	+	+	+

O sinal + representa que houve crescimento bacteriano em ágar sangue nas concentrações indicadas.