IN VITRO EVALUATION OF THE ANTIMICROBIAL ACTIVITY OF BASIL (OCIMUM BASLICUM L.) AND CORIANDER (CORIANDRUM SATIRUM L.) OIL EXTRACTS ON STREPTOCOCUS MUTANS

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ABSTRACT

Plant extracts and essential oils show efficiency on growth control in a wide variation of microorganisms, including filamentous fungi, yeasts and bacteria. To evaluate antimicrobial activity of plant extracts, determine the lower quantity of substance to inhibit the microorganism test growth is necessary. This value is known as Minimum Inhibitory Concentration (MIC). This study had as aim to verify the antimicrobial action and the Minimum Inhibitory Concentration (MIC) of basil (Ocimum basilicum L.) and coriander (Coriandrum sativum L.) oil extract before S. mutans (ATCC 25175) strains. Antimicrobial activity determination was carried out by microdilution method and performed according to recommendations of CLSI (previously known as NCCLS), standard M7-A6 (NCCLS, 2003) for bacteria, and standard M27-A2 (NCCLS, 2002). All the experiments were carried out in triplicate. Results showed the Minimum Inhibitory Concentration (MIC) determination by microdilution method in broth showed Ocimum basilicum L. and Coriandrum sativum L. extract oils presented inhibitory activity before S. mutans strain. Basil in 1:4 concentration is bacteriostatic and in 1:3 concentration is bactericide. Coriander in 1:2 concentration is bacteriostatic and in 1:1 concentration is bactericide. We concluded that basil presented higher inhibitory activity regarding to the coriander. We also observed as bigger the extract dilution, lower their effectivity.o assess the attitude and practice of dental professionals towards using of advance Radiographic technique.

KEYWORDS: antimicrobial action, MIC, Ocimum basilicum, Coriandrum sativum, Coriandrum sativum L., Streptococus mutans.
http://dx.doi.org/doi: 10.19177/jrd.v5e2201740-45

INTRODUCTION

Emergence and dissemination of resistant microorganisms to antimicrobial available in the Market has been reported for decades, what encourages the search for new sources of substances with antimicrobial activity, like plants used in traditional medicine[22].

Data evidence that 66% of Brazilian population do not have access to medicines marketed. Then they use medicinal plants as the only alternative for treat their diseases.[4]

Essential oils from plants used as seasoning represent a large natural

antimicrobial group traditionally used in food to accentuate the flavor and scent. They showed efficiency in the growth control for a wide variety of microorganisms, including filamentous fungi, yeasts and bacteria. Essential oils are natural volatile composites, known by their scent and fungicide, antiviral and medicinal properties, and they can be used as antimicrobial, painkiller, sedative and anti-inflammatory[5,13,27].

According to Ostrosky et al., 2008[26] to evaluate the antimicrobial activity of plant extracts determine the lower amount of substance to inhibit the microorganism test growth is necessary, and this value is known as Minimum Inhibitory Concentration (MIC). A very significant aspect on determination of MIC of plant extracts is the concern regarding to the antimicrobial, toxicological and legal aspect to the usage of natural composites and their combination in human beings. For the same authors, MIC determination may suffer variations, depending on the microorganism and strain used in the test. Then, MIC test must be applied according to the primary etiologic agent and type of pathology in which the medicine will be proposed as therapy. Thereunto, this study had as aim to verify the antimicrobial action and Minimum Inhibitory Concentration (MIC) of basil (Ocimum basilicum L.) and coriander (Coriandrum sativum L.) oil extracts before S. mutans strains.

MATERIAL AND METHODS

PHARMACOLOGICAL AGENTS EVALUATED

Basil (Ocimum basilicum L.) and coriander (Coriandrum sativum L.) essential oils used were obtained from LASZLO company.

BACTERIAL AGENT TESTED

In this study, Streptococcus mutans (ATCC 25175) standard strains were used, which were refrigerated and conserved in glycerin nutrient broth 40%, and reactivated in the moment of usage.

MINIMUM INHIBITORY CONCENTRATION EVALUATION OF ANTIMICROBIAL AGENTS GROWTH

Determination of antimicrobial activity was performed through the microdilution method (Minimum Inhibitory Concentration -MIC) performed carried out to CLSI (previously known as NCCLS) recommendation, standard M7-A6 (NCCLS, 2003) for bacteria and standard M27-A2 (NCCLS, 2002).

In test tubes, a pure essential oil aliquot 200µl for each agent was reserved for posterior dilution. From the second test tube, 900µl of TSB (tryptic soy broth) were distributed with glycose 0.25%.

Serial dilutions were performed by transference of 100 µl from the first tube (pure essential oil) for the second tube (900 µl TSB), successively until obtain the dilution -5. At the end of dilutions, 100µl of bacterial suspension (1,5x10^8 UFC/ml of S. mutans) was added in each tube (Figures 1 and 2). Tubes were incubated at 37°C for 24 hours with no agitation.

After the incubation period, the Minimum Inhibitory Concentration was considered the lower extract concentration able to inhibit the bacterial growth (naked eye visualization).

The positive and negative controls were: for the first one, S. mutans suspension + TSB / glucose culture medium; and the second one, only 0.9 ml TSB broth with 0.25% de glucose. They were not exposed to any type of bacterial agent. All the experiments were performed in triplicate.

RESULTS

Ocimum basilicum L. (basil) and Coriandrum sativum L. (coriander) essential oils antimicrobial activity on S. mutans strains was performed by evaluation of Minimum Inhibitory Concentration determination of crude extract and diluted over S. mutans strains.

Minimum Inhibitory Concentration (MIC) determination by microdilution method in broth showed Ocimum basilicum L. and Coriandrum sativum L. essential oils presented inhibitory activity before S. mutans strains.

After 24 hours incubation of Ocimum basilicum L. (basil) essential oil, bacterial growth was inhibited until dilution -4 (Figure 1, Table 1). In a second Reading with 48 hours incubation, growth inhibition was reduced at dilution -3 (Figure 2, Table 1).

Figure 1. Ocimum basilicum L. (basil) antibacterial activity after 24 hours incubation at dilution -4, observing turbidity absence.

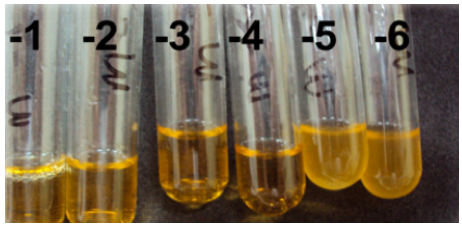
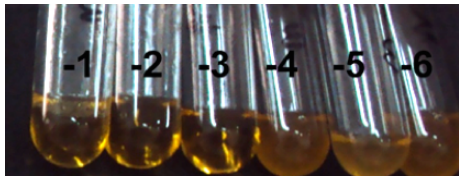


Figure 2. Ocimum basilicum L. (basil) antibacterial activity after 48 hours incubation at 37°C, dilution -3 with turbidity absence.



After 24 hours incubation, Coriandrum sativum L. (coriander) essential oil inhibited bacterial growth until dilution -2 (Figure 3, table 1). In a second reading with 48 hours incubation, growth inhibition was reduced at

dilution -1 (Figure 4, Table 1).

Figure 5. Coriandrum sativum L. (coriander) antibacterial activity after 24 hours incubation at dilution -2, observing turbidity absence.

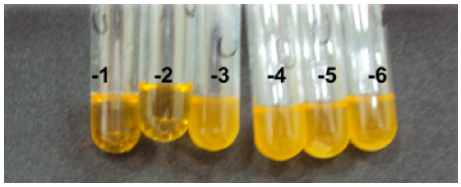
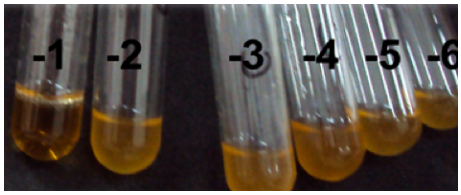


Figure 6. Coriandrum sativum L. (coriander) antibacterial activity after 24 hours incubation at dilution -1, observing turbidity absence.



DISCUSSION

Natural products usage in dentistry clinical has been justified by its low cost, minimum side effects and by antimicrobial and anti-inflammatory actions[7,8,12,36]. Before this, the literature has showed several works with medicine plants that verified these substances actions on bacterial growth [1,2,3,8,9,10,17,18,23,25,30,32,33,37]. Secondary metabolism products, like essential oils may act as antibacterial activity enhancer, or as attenuating virulence, fitting the host immune system answer to the infection[16].

It is important highlight the ideal antimicrobial should have the following characteristics: low toxicity, known interaction with buccal epithelium, low permeability, do not provoke unbalances which lead to other recurrent diseases and show good substantivity. Ocimum basilicum L. (basil) and Coriandrum sativum L. (coriander), our study objects are researched because they present antimicrobial, antifungal and antibacterial actions[3,5,9,14,15,21,25,27,31,32,33]. However, it is known that the extraction method (extract or oil), the seasonal harvest season and the species of plant used derivate different results. Regarding to the extraction method, the hydro alcoholic presents lower antimicrobial activity, when compared to the essential oil, which maintains more the medicinal properties from the plant[6]. Concerning the harvest season, higher antimicrobial potential was proved in the winter and the fall, when compared to the summer and spring. However, this data is hardly provided by handling laboratories[18]. Sedenho et al. (2014)[31] found antibacterial Ocimum basilicum L. (basil) extract in S. mutans biofilm.

Silva et al. (2011)[34] observed in studies that (Coriandrum sativum L.) coriander essential oil exercised effects on respiratory processes, flow pump and potential of Gram positive and negative bacterial membranes; it is bacterial for most lineages tested.

We observed that essential oils that essential oils behave themselves differently before S. mutans strains, and when evaluated the exposition period of 24 and 48 hours, we found inhibitory activity, while in incubation time enlargement we could determine bacterial activity.

Thereunto, basil at dilution -4 and coriander at dilution -2 are bacterial growth inhibitors, and bactericidal at dilutions -3 and -1, both for basil and coriander, respectively.

Di Pasqua et al. (2006)[11] described that essential oils have cytotoxic characteristic because affects the cellular wall causing damages, because they are lipophilic and cross the membranes, making them more permeable, and this characteristic is associated to the protons pump collapse reduction on the membrane potential.

Most studies regarding to the basil antimicrobial activity which used Ocimum basilicum essential oil for analysis obtained positive antibacterial activity[3,5,9,15,25,27,32,33]. However, works performed by Pozzo et al. (2011)[27] and Freire et al. (2014)[15] did not found antibacterial activity. Silva et al. (2001)[33] compared basil essential oil and hydro alcoholic extract antimicrobial action, and verified that only the essential oil demonstrated antibacterial activity. However, bacteria used were Salmonella enteritidis and Staphylococcus. These results corroborated our findings, which achieved a positive answer to bacterial growth inhibition with basil and coriander oil extracts, but on S. mutans.

Activity evaluation of essential oils in crude aromatic species has becoming important for Discovery new drugs by oils biological effects with better therapeutic potential and lower side effects index. In this research, oils studied (basil and coriander) presented large amount of linalool in their composition.

Most common chemical contents of essential oils are formed by terpenes, phenols, aldehydes alcohols, esters, ketones, nitrogen and sulfur combinations[20]. These components are able to inhibit or retard the growth of bacteria, yeasts and molds, presenting activity against a variety of target, particularly the membrane and cytoplasm. In some cases, they change completely the cells morphology, depriving them and leading to the death for several target sites for action[24].

Terpenes are formed by isoprene unities, containing only hydrogen and carbon in their structure. By their diversity, they are largely studied and are able to produce several pharmacological and biological effects[19]. Umpteen essential oils and terpene contents present pharmacological actions already proved scientifically: antibacterial, pro-inflammatory, pro-nociceptive, anti-

inflammatory, anti-nociceptive, analgesic, antioxidant, anticonvulsant actions, and e Skeletal contractile modulator, cardiac and smooth muscles[28,29]. Linalool is a monoterpene composite, commonly found as major component of essential oils in aromatic species, like lavender (Lavandula officinalis), coriander (Coriandrum sativum), nolol (Citrus aurantium) and also in large amount in basil (Ocimum basilicum) essential oil, which has been used in researches because of its antibacterial activity[28].

Table 1. Essential oils MIC comparison regarding to the exposition time. (-) inhibition growth; + non inhibition growth.

Time	Essential oil	Dilutions					
		-1	-2	-3	-4	-5	-6
24 hours	Coriander	(-)	(-)	+	+	+	+
24 hours	Basil	(-)	(-)	(-)	(-)	+	+
48 hours	Coriander	(-)	+	+	+	+	+
48 hours	Basil	(-)	(-)	(-)	+	+	+

Gram positive and Gram negative bacteria wall cell allows that hydrophobic molecules penetrate easily for cells, and acts in both wall cells and cytoplasm. Phenolic compounds which are also present in essential oils generally show antimicrobial activity against Gram positive bacteria. The effect depends on the amount of the composite; in low concentrations they can interfere with enzymes involved in the energy production; in larger amount they can denature proteins, cellular metabolism, signaling effector pathways and destroy the cell wall [24,35]. In this research, a Gram positive bacteria was tested, Streptococcus mutans, and by the characteristics described previously we observe good action from the oils tested.

Regarding to the Linalool toxicity, its lethal dose is low, when compared to other contents of essential oils. For this reason, it becomes study target for therapeutic applying in the future. Researches on toxic effects of substances are related to the administration route, time, duration and frequency of doses, what Venâncio (2006)[37] demonstrated. Linalool presented low acute toxicity in several experimental models and different administration routes, what justifies the interest in this content in many areas of basic Science.

Basil and coriander essential oils demonstrated antimicrobial action, evidencing the potential for this plant usage as antibacterial agent. It is also important observe the plant species and characteristics of bacteria tested, because the oil action can be classified as bacteriostatic or bactericide, and like previously described, it can be associated to the structural characteristics of Gram positive and Gram negative bacteria, chemical composition and concentrations tested for each oil.

CONCLUSIONS

With the results we concluded Ocimum basilicum L. (basil) e do Coriandrum sativum L. (coriander) extracts presented antimicrobial actions over S. mutans strains. Basil oil extract presented higher activity regarding to the coriander. We also observed as greater the extracts' dilution, as lower effectivity for both.

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