IN VITRO EVALUATION OF THE ANTIMICROBIAL ACTIVITY OF BASIL (OCIMUM BASILICUM L.) AND CORIANDER (CORIANDRUM SATIVUM L.) OIL EXTRACTS ON STREPTOCOCCUS 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.

KEYWORDS: antimicrobial action, MIC, Ocimum basilicum, Coriandrum sativum, Coriandrum sativum L., Streptococcus mutans.

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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. 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. 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.

According to Ostrosky et al., 2008, 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.) essential oils.

**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 (1,5x10⁸ 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.

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.](image1)

![Figure 2. Ocimum basilicum L. (basil) antibacterial activity after 48 hours incubation at 37ºC, dilution -3 with turbidity absence.](image2)

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...
(method (extract or oil), the seasonal

However, it is known that the extraction

antibacterial actions

antimicrobial, antifungal and

are researched because they present

sativum L. (coriander), our study objects

and show good substantivity. Ocimum

which lead to other recurrent diseases

permeability, do not provoke unbalances

characteristics: low toxicity, known

antimicrobial should have the following

It is important highlight the ideal

DISCUSSION

Natural products usage in dentistry

minimum side effects and by

antimicrobial and anti-inflammatory actions7,8,12,36. Before this, the literature

has showed several works with medicine

plants that verified these substances

actions on bacterial growth

Secondary metabolism products, like essential oils

may act as antibacterial activity

enhancer, or as attenuating virulence,

fitting the host immune system answer
to the infection16.

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 actions8,9,14,15,21,25,27,31,32,33. However, it is known that the extraction

method (extract or oil), the seasonal

harvest season and the species of plant

used derive 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 plant66.

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

laboratories38. Sedenho et al. (2014)33

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

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 basal

antimicrobial activity which used

Ocimum basilicum essential oil for

analysis obtained positive antibacterial

activity7,9,15,17,27,32,33. However, works

performed by Pozzo et al. (2011)37 and

Freire et al. (2014)35 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

combinations19. These components are

able to inhibit or retard the growth of

bacteria, yeasts and molds, presenting

activity against a variety of target,
p

particularly the membrane and
c
ytoplasm. In some cases, they change

completely the cells morphology,
depriving them and leading to the death

for several target sites for action24.

Terpenes are formed by isoprene

unities, containing only hydrogen and
c

carbon in their structure. By their

diversity, they are largely studied and are

able to produce several pharmacological

and biological effects19. Umpteen

essential oils and terpene contents

present pharmacological actions already

proved scientifically: antibacterial, pro-
inflammatory, pro-nociceptive, anti-
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.

<table>
<thead>
<tr>
<th>Time</th>
<th>Essential oil</th>
<th>Dilutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 hours</td>
<td>Coriander</td>
<td>(-) (-) + + + +</td>
</tr>
<tr>
<td>24 hours</td>
<td>Basil</td>
<td>(-) (-) (-) (-) + +</td>
</tr>
<tr>
<td>48 hours</td>
<td>Coriander</td>
<td>(-) + + + + +</td>
</tr>
<tr>
<td>48 hours</td>
<td>Basil</td>
<td>(-) (-) (-) (-) + +</td>
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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 effectiveness for both.

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