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Phytochemical Trial and Bioactivity of the Essential Oil from Coriander Leaves (*Coriandrum sativum*) on Pathogenic Microorganisms

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Coriandrum sativum (Apiaceae), known popularly as coriander, has nutraceutical and functional properties and presents bioactive antimicrobial potential. The essential oil of this herb has been widely investigated and demonstrated efficiency in the inhibition of pathogenic microorganisms. Knowing this, we aimed to perform a phytochemical screening and to verify the bioactivity of the essential oil of the coriander leaves cultivated in Roraimense Savannah, Brazilian Amazon, on bacteria and yeasts. The leaves were collected in a cultivated area, duly selected, washed and processed, and subsequently destined to a hydro distillation process using Clevenger. Identification and quantification of the volatile constituents were provided by Gas Chromatography of Coupled Mass Spectrometry (GC-MS) and High Resolution Gas Chromatography (GC-FID), respectively. Five microorganisms were used in the bioassays for inhibition and IC_{50} tests. The oil had an inhibitory potential against *Staphylococcus aureus* and *Candida albicans*, greater than 80%. There was less than 40% inhibition for *Salmonella typhimurium* and there was no activity for *Bacillus cereus* and *Escherichia coli*. Seven constituents were identified, of which three were: tetradecenal (22.9%); 2-dodecanal (21.6%); and palmitic acid (10.7%). It is believed that the synergy of the secondary constituents of the oil, can positively aid the antimicrobial action.
Keywords: *Coriandrum sativum*, *Staphylococcus aureus*, *Candida albicans*, GC-MS, Tetradecenal.

1. Introduction

Coriandrum sativum, popularly known as coriander, from the eastern Mediterranean and western Asia, belongs to Apiaceae family, is among the most consumed and cultivated olive groves in tropical and subtropical regions of the world, mainly in the North and Northeast of Brazil (Brazil, 2018). Its citric exotic flavor makes coriander a very characteristic herb. Coriander is a highly known seasoning herb, many times related as part of traditional food, as for it is used for flavoring many dishes such as curries, sauces and soups in India (Priyadarshi et al., 2018). This vegetable has important nutraceutical (Wei et al., 2019) and functional properties (Nishio et al., 2019) and is already known to be rich in proteins, vitamins and minerals. Its inhibitory activity and other negative effects on the life cycle of several biological and pathogenic organisms has also been described (Ashraf et al., 2018) and are extensively investigated in different areas of scientific knowledge (Salamon et al., 2018; Huzar et al., 2018; Ildız et al., 2018; Mansouri et al., 2018; Jabir et al., 2019).

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In addition, the antimicrobial properties of different types of essential oils, extracted from different parts of the vegetal structure of different botanical species has been extensively investigated (Begnami et al., 2018; Abdelkader et al., 2018, Abdella et al., 2018), mainly its activity on Gram-positive and Gram-negative bacteria, as well as its effect on pathogenic yeasts of interest to public and collective health. Many of these oils have been showing important biological activities. For example, essential oil from *Hyptis dilatata* (Lamiaceae) flowers and from *Pouteria caimito* seed showed to be active for acetylcholinesterase inhibition, making them interesting targets to develop new medicines or food supplements for patients with neurodegenerative diseases such as Alzheimer's disease (Almeida et al., 2018; Melo Filho et al., 2018).
In this context, the bioactivity of essential oil of coriander leaves and its efficiency has been widely reported, including: antioxidant, antimicrobial, analgesic, anti-inflammatory, anticonvulsive and anticancer effects, emphasizing its inhibitory action on bacteria and yeasts harmful to human health (Beyzi et al., 2017; Almeida Freires et al., 2014).
It is proposed in many studies that the action of natural products on biological organisms, in general, but especially on harmful microorganisms, is linked to the synergy among the secondary chemical constituents. Recent studies in this line of research indicate that the synergistic effect of the secondary metabolites in different chemotypes presents superior control or suppression action compared to the effect of each component isolated. This is also proposed and correlated with the efficiency of allopathic synthetic products recommended for inhibition of pathogenic microorganisms (Trinh et al., 2018; Ben; Djenane, 2018).
In this context, many studies indicated that biological activity of plant species varies according to seasonal factors, plant age (Msaada et al., 2007) and, when essential oils are taken into consideration, the extraction method is also important. Investigations with chemotypes of this botanical species cultivated under Amazon Savannah conditions were not identified in the literature in this line of research. Thus, the objective of the present study was to carry out a phytochemical screening of volatile components and to verify the bioactivity, from the perspective of the synergism among secondary constituents, of the essential oil of coriander leaves cultivated under Savana conditions in the Brazilian Amazon, on Gram-positive and Gram-negative bacteria and pathogenic yeast *Candida albicans*.

2. Materials and methods

2.1 Collection, processing and obtaining of essential oil

The coriander plants were collected in the area of Savana (Lavrado) in the locality of Roraima (Brazil). The climate of this region is characterized as Tropical Rainy (Aw), according to the types of climatic classification of Köppen. The access was duly registered in the National System of Management of Genetic Heritage and Associated Traditional Knowledge - SisGen linked to the Ministry of the Environment - MMA, according to specific Brazilian legislation, under number A5FD8B7.
For the processing of the vegetal material and obtaining the essential oil, the samples were destined to the Laboratory of Environmental Chemistry of the Federal University of Roraima - UFRR and the leaves were selected, washed, and while they were still fresh, submitted to a hydrodistillation process using Clevenger apparatus with double Spell condenser for an uninterrupted two-hour distillation period.

2.2 Phytochemical Screening

For identification and quantification of the volatile constituents, the oils were analysed by Gas Chromatography Coupled to Mass Spectrometry (GC-MS) and High Resolution Gas Chromatography (GC-FID), respectively, both procedures being performed in Chromatography Laboratory of the Federal University of Minas Gerais.
For identification of the phytochemical constituents present in the essential oil, a GCMS-QP2010 ULTRA (Shimadzu) equipment, equipped with a column Rxi-1MS 30 m x 0.25 mm x 0.25 μm (Restek) was used. Column temperature was 50 °C (3 min), 3 °C min^{-1}, to 220 °C. The injector worked with initial temperature of 220 °C, with split mode ratio (1: 50), GC-MS interface at 220 °C. Detector MS (Electronic Impact at 70 eV) at 220 °C. Flow of entrainment gas (Helium) was 3.0 mL min^{-1}. The mass spectrum of each essential oil component of *C. sativum* seeds was carefully analyzed in comparison to spectra of the library spectra (NIST11), using GC-MS Solution (Shimadzu) software and also by comparison with the indices of Kovats.
The quantification of the essential oil components of *C. sativum* seeds was performed using gas chromatography on a HP 7820A Gas Chromatograph (Agilent) equipped with a capillary column with dimensions 30 m x 0.32 mm x 0.25 μm (Agilent), with a temperature of 50 °C (0 min), 3 °C min^{-1}, up to 220 °C. Samples containing 1 μL of essential oil diluted (1%) in chloroform with an initial temperature of 200 °C in the split ratio (1:50) was injected. The GC-FID worked with a temperature of 220 °C. Drag gas, Helium at 3 mL min^{-1}. Data acquisition software was Ezchrom Elite Compact (Agilent).

2.3 Bioassays of antimicrobial activity

After preparation of the working solution and solubilization in dimethylsulfoxide (DMSO) at a concentration of 12.5 mg mL^{-1}, the bioassays of antimicrobial activity were carried out for bacteria and yeast pathogens by means of the Elisa plate reader with a reading capacity of up to 96 wells, in triplicate, as described by Dos Santos et al. (2015).

The microorganisms used in the activity bioassays were: i) *Staphylococcus aureus*: ATCC 29212 (Gram-positive bacterium); ii) *Bacillus cereus*: ATCC 11778 (Gram-positive bacterium); iii) *Escherichia coli*: ATCC 25922 (Gram-negative bacterium); iv) *Salmonella typhimurium*: ATCC 14028 (Gram-negative bacterium); and, v) *Candida albicans*: ATCC 18804 (yeast). Ampicillin and nystatin were used as a positive control for bacteria and yeast, respectively.

The parameters evaluated in relation to the pathogenic microorganisms were: a) Percentage (%) of inhibition at 250 μg mL^{-1}; and b) IC_{50} values (Concentration of the sample which inhibits 50% of the microorganisms present) in μg mL^{-1}.

2.4 Data analysis

For the determination of the secondary constituents of the essential oil of *C. sativum* leaves, Kovats index was used. In relation to the investigation of antimicrobial activity, the results were submitted to descriptive statistical analysis by means of percentage from the reading of bioassay plates; and, for IC_{50} values calculation, the log of the tested concentrations was used, through the regression study with the respective determination coefficients (R^2). The program used for data processing was OriginPro 8®.

3. Results and discussion

3.1 Phytochemical Screening

Gas Chromatography analysis of essential oil of coriander leaves (EOF), Table 1, showed a profile constituted mainly by medium chain aldehydes like decanal (6.7%), 2-decenal (6.8%), 2-dodecanal (21.6%), tetradecenal (22.9%), and palmitic acid (10.7%).

Table 1: Phytochemical composition of the essential oil of coriander leaves (EOF) grown in the Savana area of the state of Roraima, Brazil, in the dry period, by Gas Chromatography Coupled to Mass Spectrometry (GC-MS) and by Chromatography High Gas Chromatography Resolution (GC-FID).

Constituents	%	KI*
Decanal	6.7	1199
2-decenal	6.8	1256
2-dodecanal	21.6	1471
tetradecenal or 7-hexadecenal	22.9	1672
palmitic acid	10.7	1935
Total	68.6	

*Kovats Index

3.2 Bioassays of antimicrobial activity

EOF showed an interesting pattern of antimicrobial activity since, Table 2, among the bacteria assayed, it was selectively active mostly against *Staphylococcus aureus (*83.46 ± 2.3%), a Gram-positive bacterium which is responsible for many serious infections since its resistant strains are a major and clinical concern over the last decades, afflicting mainly hospitalized patients. The essential oil of coriander leaves was moderately active against *Salmonella typhimurium* (39.83 ± 3.01%). This is an important finding since Gram-negative bacteria are usually less susceptible to antimicrobial agents than the Gram-positive ones. Under the conditions of conduction of this experiment, there was no activity for *Bacillus cereus* and *Escherichia coli.* On the other side, coriander essential oil was highly active against *Candida albicans* (91.94 ± 1.61%). This demonstrates the bioactive potential of EOF on the respective pathogenic microorganisms evaluated *in vitro*.

Table 2: Percentage (%) inhibition of the essential oil of coriander leaves (EOF), chemotype grown in the Savana area of the state of Roraima, Brazil, in the dry period, against pathogenic microorganisms.

Sample (μg mL^{-1})	*S. aureus*	*B. cereus*	*S. typhimurium*	*E. coli*	*C. albicans*
EOF (%)	83.46 ± 2.3	-	39.83 ± 3.01	-	91.94 ± 1.61
Ampicillin (%)	95.76 ± 0.26	92.48 ± 0.52	90.39 ± 0.30	96.64 ± 2.52	-
Nystatin (%)	-	-	-	-	90.18 ± 1.15

It was also found that the concentration of the sample that inhibited 50% of the microorganisms present at the dose of 250 μg mL^{-1}, Table 3, was, respectively, 46.73 μg mL^{-1} and 68.12 μg mL^{-1} (dose-response), for *S. aureus* and *C. albicans*, having as control ampicillin (1.66 μg mL^{-1}) and nystatin (7.53 μg mL^{-1}), in due order.

Table 3: IC_{50} values of the essential oil of coriander leaves (EOF) and the respective controls effects on *pathogenic microorganisms.*

Sample (μg mL^{-1})	*S. aureus*	*S. typhimurium*	*C. albicans*	*B. cereus*
EOF (%)	46.73	-	68.2	-
Ampicillin (%)	1.66	4.20	-	1.90
Nystatin (%)	-	-	7.53	-

It was also observed that the effect of EOF on yeast was superior to the control treatment, Nystatin (90.18%), when correlated with the respective inhibition treatment against *C. albicans* (91.94%). C. albicans is a yeast of relevant pathogenicity due its high virulence. Candidiasis and other C. albicans-associated pathologies, including invasive infections are usually of difficult treatment (Borel et al., 2017). This result indicates the possibility of using coriander oil for the amplification of systematic studies of *C. albicans* inhibition, in other experimental conditions, both *in vitro* and *in vivo*.

Although the effect of essential oil of coriander leaves was less than 40% against *S. typhimurium*, it could still be considered as promising for the inhibitory action of this bacterium when compared to other studies. However, it is worth noting that the efficiency of the antibacterial effect of natural products also depends on their concentration. Priyadarshi et al. (2018) reported the *in vitro* activity of hydroalcoholic extract of *C. sativum* leaves and of a pure compound (Heneicos-1-ene) dissolved in chloroform. Growth of *Salmonella typhi* was only 11%. The same authors did not find significant antibacterial activity against three other bacterial pathogens tested, among them, *Staphylococcus aureus*, differing from the results found in this investigation, where the inhibition was superior to 80% against this bacterium. However, *Litsea cubeba* essential oil (Laureceae) showed superior effects on bacterial inhibition in relation to the effect of EOF towards *Staphylococcus aureus*, as reported by Hu et al. (2019), with 99.999% inhibitory action on the *S. aureus* strain analyzed. Borah et al. (2019) as well as Mandal and Mandal (2015) also reported antibacterial properties of the essential oils of *Curcuma caesia* (Zingiberaceae) leaves and coriander seeds, respectively, against *Staphylococcus aureus* and *Salmonella typhimurium* and antifungal effect against *Candida albicans*.

Therefore, the deleterious effect on the Gram-positive bacteria and yeast detected in the present study is associated with synergism of the secondary constituents identified in the phytochemical screening of the specific chemotype evaluated in this investigation. When investigating the antifungal activity and mode of action of EOF on *Candida* spp. associated to the profile of secondary metabolites detected by CG-MS, De Almeida Freires et al. (2014) found similar results in relation to part of the phytochemical constitution - with the presence of volatile compounds, from which three major ones were identified: decanal (19.9%); 2-decenal (17.54%); and 2-dodecanal (10.72%). These compounds were also found in the present study, however in different proportions and not all of them as the very major constituents. The same group also verified by Scanning Electron Microscopy (SEM) that of the five yeast species evaluated in their studies, the EOF effect was also responsible for lesions more representative on *C. albicans.* Decrease in their activity proteolytic - blocking cell wall biosynthesis and/or increasing in the ionic permeability of the membrane of the yeasts investigated was also noticed by them. The authors also state that these properties are probably due to the synergistic effect of mono- and sesquiterpene hydrocarbons and demonstrated that the EO represented a statistically significant inhibition between 42-85% towards the different strains evaluated, which were lower than those found in the present study, probably due different quail and quantitative EO composition.

In this sense, it is assumed that the synergism of the most prominent compounds identified in this study, are directly related to the inhibition of the harmful microorganisms *S. aureus* and *C. albicans*. The use of EOF confers a possible suppression of the microorganisms growth. Other studies also suggest the synergistic effect of natural products and their antimicrobial potentials, as well as their effectiveness in combating multidrug resistance (Elhidara et al., 2018, Sadiki et al., 2019, Hu et al., 2019).

Therefore, associated to other biological functions relevant to human health, as the hepatoprotective effect of its essential oil (Iqbal et al., 2018) and nutraceutical and functional properties, the chemotype of coriander evaluated in this investigation presented antimicrobial potential against *S. aureus* and *C. albicans*.

4. Conclusions

The synergism of the constituents present in the OEF of this chemotype, under the *in vitro* conditions of this investigation may be responsible for the inhibition superior to 80% of *Staphylococcus aureus* and *Candida albicans*. Despite the promising effects on the bacterium and pathogenic yeast studied, new research must still be done to obtain more information on the bioactivity profile of EOF and its possible actions in other experimental conditions.

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