Chemical composition and in vitro antifungal activity of Cymbopogom citratus essential oil used in the Montepuez district/Mozambique

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ABSTRACT

The use of medicinal plants in the treatment of diseases is a practice widely used by the population of Montepuez. However, the present study aimed to evaluate the chemical composition and antifungal activity of the essential oil of Cymbopogom citratus. Plant leaves were harvested and dried and essential oils extracted by hydrodistillation in a Clevenger apparatus. Identification of the chemical composition was performed by gas chromatography coupled with mass spectrometry (GC-MS). The antifungal activity was assessed by the disc diffusion method and Minimum Inhibitory Concentration (MIC) at different dilutions with 99% DMSO with distilled water against Candida albicans ATCC 10231. Nystatin was used as a positive control for fungi at the concentration of 50 μg / disc. The essential oil of C. citratus revealed the presence of oxygenated monoterpenes, monoterpen and diterpene hydrocarbons as major compounds; of the activity to the micro-organism tested the MIC was 50 mg/ml with a percentage of 1.65%. The antifungal activity was greater in relation to the halos formed. In vitro results indicate that C. citratus may act as an antifungal agent against infections caused by fungi.

Key words: Essential oil, chemical composition, antifungal activity and C. citratus.

INTRODUCTION

The use of plants for the cure of illnesses has been in use since the existence of man because its use conditioned the advance to the beginning of medicine as a whole or as an area of interest with health, since man with the empirical knowledge possessed began to isolate active substances present in plants. The aforementioned therapeutic conduct dates back to the ancient people of China, Egypt, Asia and Rome, where scholars, on the basis of their knowledge classified numerous plant species with their respective indication of medicinal use. In Mozambique, this practice is not new, as historical accounts of the evolution of our society have told us that our ancestors used plants to cure their illnesses (Mozambique Government resolution 11/2004).

According to Mozambique Government resolution (No. 11/2004: 130), the epidemiological profile of the country is characterized by a predominance of communicable and parasitic diseases, with a greater focus on cholera, dysentery, meningitis, diarrheal diseases, malaria, tuberculosis and respiratory infections. Hence, Agra (1996) stated that "the use of plants in the treatment of diseases is quite common, especially in rural and urban areas and low-income populations, where cultural tradition and socio-economic problems make access to medicine difficult.

Mozambique is an important repository of biological diversity. This diversity is used by about 90% of the country's population mainly in rural areas to meet their housing, energy, food and health needs (Ribeiro et al., 2010: 1). According to Krog et al. (2006: 2) in Mozambique about 15% of total plant genetic resources (estimated at about 5,500 plant species) are used by rural communities for
medicinal purposes and play a key role in basic health care.

Work with medicinal plants begins with proper identification of the species, adequate collection, correct pre-treatment and storage, and ends with preparation and therapeutic use, which must also be adequate for satisfactory results. The aromatic plants of Mozambique are revealed in this and it confirmed the expectation that Mozambique can become a great world producer of aromatic oils. A strategy is proposed to reach this objective and will be impotent without plans of actions in relation to the potential of the aromatic oils in that country, if not instrumental, of the process that will become the strategy proposed for the reality (MBB, 2006).

However, scientific investigations aiming to determine the therapeutic potential of plants are limited, and there is a lack of experimental scientific studies confirming the possible antibiotic properties of a large number of these plants (Coutinho et al., 2004). According to the World Health Organization (2003) cited by Nascimento et al. (2000) and Caetano et al. (2002) "medicinal plants are the best sources for obtaining a variety of drugs to maintain human health and 80% of the world's population uses traditional medicine in the search for relief from some painful or unpleasant symptoms".

Essential oils are secondary metabolites. These are volatile, lipophilic, odoriferous and liquid substances extracted from leaves, flowers, fruits, stems and roots, with oily appearance at room temperature (Simões and Spitzer, 2000). Generally, they are presented as mixtures of terpenic chemical substances, including their alcoholic and aldehyde derivatives. Commercially, they are used in the pharmaceutical industry, comestible industry and as insecticides (Girard, 2005).

In order to contribute to the chemical and biological knowledge of the essential oils of these aromatic plants, the present work had as objective evaluated the in vitro antifungal activity and chemical composition of the dried leaves essential oil of C. citratus harvested in the district of Montepuez against Candida albicans.

### MATERIALS AND METHODS

The oil used in this work was obtained by the hydrodistillation process using a Clevenger type extractor. The extraction was carried out in the laboratory of the Center for Research and Development in Ethnobotany (CIDE) - Namaacha. The species used to obtain the essential oil was collected in the northern region of Mozambique in Cabo delgado, Montepuez district from March to June, 2017. The obtained oil was evaluated using the Kirby and Bauer method and the culture medium was Müller and Hinton supplemented with 2% glucose. A bacterial suspension was prepared in sterile saline and adjusted to 0.5 tube of the McFarland scale, which corresponds to approximately 1.5 x 106 CFU/ml of the strains of C. albicans ATCC 10231, owned by the Laboratory of Microbiology of the Faculty of Medicine, of EMU. 100 μl aliquots of each bacterial suspension were surface-seeded in Petri dishes containing about 15 ml of the Mueller-Hinton medium, with a thickness of approximately 4 mm (Kirby-Bauer, 1966). Six-millimeter diameter, sterilized filter paper disks containing volumes of 1, 3, 5 and 10 μl of pure oil without any dilution of each evaluated species were placed on the bacterial suspensions in the Petri dishes. The control test was performed with antibiotic Nystatin disks containing 50 μg per disc, commercially available.

Incubation was carried out in an oven at 36°C for 24 to 48 h. The tests were performed in triplicate and the results expressed in mm by the arithmetic mean of the diameter of the inhibition halos formed around the discs in the 3 replicates. The inhibition halo diameters were interpreted according to the interpretation criteria recommended by NCCLS (National Committee for Clinical Laboratory Standards, 1997, 2000a, b). Minimal Inhibitory Concentration (MIC) was determined by the disc diffusion method (NCCLS, 2003). Identification of the major compounds was performed using gas chromatography and gas chromatography coupled by mass spectrometry.

### RESULTS AND DISCUSSION

#### Yield of extracted essential oil

There appears to be no large fluctuations in yield in response to data obtained by other authors regarding oils from this plant. The first step in the process of preparing and obtaining the essential oil using the dried leaves is the drying of the plant material, which has the purpose of removing the water and, thus, preventing hydrolysis and microbial growth reactions (Bacchi, 1996). All plant materials were immediately dried after collection, since there are many enzyme systems in the plants that according to Calixto et al. (2001) can continue to act and degrade the active principles even after collecting them. Table 1 shows that the yield of the essential oil was

#### Table 1: Essential oils yield of Cymbopogom citratus.

<table>
<thead>
<tr>
<th>Name of plant</th>
<th>Dried leaves mass (g)</th>
<th>Essential oil mass (g)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cymbopogom citratus</em></td>
<td>390.7</td>
<td>3.0213</td>
<td>0.77</td>
</tr>
</tbody>
</table>

Source: Adapted by author (2017).
0.77% for the dry leaves of *C. citratus*. In this study we obtained 0.77% calculated on a dry basis. According to De Brito (2007), studying the essential oils of *C. citratus*, an essential oil yield calculated on a fresh basis of 0.4669% was obtained (Matasyoh et al., 2011). The average oil content in the green leaves of the plant ranges from 0.8 to 1.0% and if considered in the conditions of this study, the yield obtained has a reasonable percentage if compared to those presented in the literature. It was observed that in the aforementioned culture system and under the conditions of this experiment there was good yield.

According to Burt (2004), these yield variations of essential oils for the same plant species can be attributed to differences in harvest time, soil type, regional climate, drying time and relative humidity of the day of the harvest. Verma et al. (2013) verified that during the year the quantities of essential oil varied between 0.37 and 0.82%, corresponding to the months of May and September, respectively. Also, the concentrations of their constituents were not constant throughout the year. This study allowed us to relate the harvest season to the quantity and quality of the essential oil produced. Studies such as these are of great importance since they allow us to know the best harvesting time to obtain more of the components of interest resulting in a more profitable extraction.

The time of collection is one of the factors that most influence the pharmacological activity of plant drugs, since the nature of the active constituents is not constant during the year. Currently, seasonal variations in the content of virtually all secondary metabolite classes are reported with emphasis on essential oils (Giacomelli et al., 2014).

For the development of this study the extraction was done with water by the hydrodistillation process using the Clevenger apparatus, at the boiling temperature of water, since many substances are unstable at high temperatures. This process was used in view of the fact that a good yield of the oils was observed since it allows the steam extraction until the exhaustion of the oils in the leaves; hydrodistillation is designated as the operation in which the extraction of the vegetal raw material is in a closed vessel, to a boiling temperature of water for an extended period (hours) under heating.

### Identification and quantification of constituents of essential oils

Essential oils of the species mostly terpenic compounds, such as monoterpenes, oxygenated monoterpenes, diterpenes and sesquiterpenes of this research were found. According to Simões and Spitzer (2003) "monoterpenes and sesquiterpenes are the most frequent terpenes in volatile oils". Table 2 shows the substances found in the essential oils of the dry leaves of this species. In studies of the chemical composition of essential oils, a major active compound is generally identified; however, because they are composed of complex mixtures of volatile substances, the oils include in their composition other chemical components, such as a series of terpene hydrocarbons, esters, organic, aldehydes, ketones and phenols among others in different concentrations (Burt, 2004).

The GC/MS analysis of the essential oil of the dried leaves allowed us to verify the percentage identification of the major components of the essential oil in this chromatographic analysis of *C. citratus* being 92.5%. The main compounds of the oils extracted from dry leaves were the monoterpenic hydrocarbon, oxygenated monoterpenes, sesquiterpenes and oxygenated sesquiterpenes and diterpene hydrocarbons whose contents varied between 0.7 and 77.9%, respectively. In relation to the majority of the species, 14 components were found in the majority of the essential oil and geranial (44.1%) was the majority. The data found are in line with those described in the literature, where it is stated that the essential oil of *C. citratus* (chabakate) presented geranial, neral and linalool, where the isomeric geranial was seen as a major compound that gives activity. Among these constituents already cataloged as antifungal agents, geranial, limonene and linalool deserve prominence, even by the high proportion with which they participate in the total essential oil of some aromatic species. Figure 1 shows the peak of the chromatographic profile of the major compounds (peak areas) characterized in dry leaves of *C. citratus*.

### Determination of antimicrobial activity by disk diffusion method

**Figure** 2 shows the formation of zones of inhibition halos of different sizes through diffusion technique (Bauer-Kirby, 1966). According to Black (2002), the antimicrobial activity in the disc diffusion technique occurs through the diffusion of the chemotherapeutic agent in all the directions of the agar during the incubation.

Although it was characterized as a qualitative technique,
the halos formed around the discs impregnated with the samples were used to compare these results with those obtained by the CIM, as was also done by Gonsalves (2010). Yeast was used for this diffusion test. According to Rios and Recio (2005), the disc diffusion method is especially good for determining the relative potential of extracts or essential oils and used to establish their antimicrobial spectrum, since it facilitates the use of different strains against the extract. According to the results of Table 3, it can be said that the species studied contains essential oil with activity in this micro-organism. According to Simões et al. (2007), this variation usually occurs as a function of the stage of the plant, as in full bloom or in the period before flowering. Hence, it can be inferred from the table that essential oil investigated had antifungal activity and significantly inhibited the test micro-organism C. albicans ATCC 10231.

The yeast, C. albicans ATCC 10231, was sensitive to the essential oil tested, since its antifungal growth was inhibited by 75% of the oil of the species. According to Fennell et al. (2004), the variations regarding the determination of the MIC of the essential oils of plants can be attributed to several factors. These include the methodology applied, the micro-organism evaluated, the origin of the plant and time of collection, whether the essential oils were prepared from dried plants and the
Table 3: Results of the mean minimum inhibitory concentrations (mg.mL-1) of the essential oil against the C. albicans strain.

<table>
<thead>
<tr>
<th>Micro-organism</th>
<th>Species</th>
<th>Concentration of essential oil (mg / ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C. citratus</td>
<td>50 (1.65%)</td>
</tr>
<tr>
<td>C. albicans</td>
<td></td>
<td>7</td>
</tr>
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</table>

Source: Adapted by the author (2017).

amount of extract tested. The results of antifungal activity against yeast were more effective in dry leaf oil of C. citratus against C. albicans. Several studies in the literature confirmed this data; however, the studies on the antimicrobial activity of C. citratus essential oil were evaluated by MIC values determined by the dilution in culture broth (Machado et al., 2012). Gutierrez et al. (2009) compared the MICs of several essential oils against different microbial species obtained by three test methods: broth dilution, agar dilution and agar diffusion. In this work, the results obtained with the methodology (disk diffusion) did not follow the same trend. However, there are often large differences in antibacterial activity reported on the oil from the same plant. The reasons for this variability may be due to geographic sources, harvesting times, genotype, climate and extraction method, factors influencing the chemical composition and relative proportions of each constituent in the essential oil of the plant (Burt, 2004). In the present article, these trends were observed both in relation to the concentration factor, whose inhibitory effects were positively associated to the oil concentration and species. These points were detected when analyzing the halos formed by this crude essential oil both on the micro-organism (Figure 2). The essential oil of C. citratus according to the data of the graph can be considered as effective antifungal.

**Determination of antimicrobial activity by minimum inhibitory concentration (MIC)**

The Minimum Inhibitory Concentration (MIC) assay is obtained through the macro or microdilution of compounds consisting of preparing successive dilutions of the antimicrobial being tested in solid culture media or liquid to sow the micro-organism and after the incubation verify the lowest concentration (greater dilution) of the antimicrobial that inhibited the growth of the micro-organism (CLSI, 2002, 2003). The advantages of this method are to provide more quantitative information and to be able to be applied to a wider variety of isolates than the diffusion tests (Koneman et al., 2001).

By the technique of determination of the MIC, the essential oil confirmed the activity observed by the disc diffusion technique and in addition, inhibited, in varied extensions the growth of the micro-organism. This variation can be justified by the presence of different substances in the samples, since more polar molecules or molecules of larger molecular mass may be more soluble and easier to disperse in liquid medium (Valgas et al., 2007). Thus, there is no standardized method for expressing the results of antimicrobial testing of natural products (Ostrosky et al., 2008).

The results obtained by each of these methods may differ due to factors such as variations between the tests, for example, microbial growth, exposure of micro-organisms to the extracts, solubility of the extract or its components and the use and amount of diluents. Therefore, some peculiarities such as the volatility, water insolubility and complexity of the essential oils must be taken into account since these characteristics can significantly interfere in the results (Nascimento et al., 2007).

The essential oil of the dry leaves of this aromatic species under study presented the lowest MIC for fungi (50 mg.ml-1), which constitute the following percentages, 1.65% for C. citratus (Chabalakate). The essential oils and extracts of this species present several biological activities, being able to emphasize antioxidant activity (Hakkim et al., 2008). Machado et al. (2012) evaluating the antimicrobial activity found that the minimum inhibitory concentration of C. citratus for the bacteria Escherichia coli and Salmonella aureus obtained mean values between 0.34 and 5.5 mg/ml, although it was not the same micro-organism you have to think about the value that differs significantly.

With these results, we verified that the studied plant which is a positive point presents for analyzed leaves, the antifungal activity and its essential oil that can be used because as it is known, most of the major compounds were the same for these species. As can be seen in the characterization of essential oils of plants with dry leaves, the levels of antifungal action showed to be promising, since they have activity for this micro-organism to the minimum values of the species of similar use.

**Conclusion**

In the experimental conditions used in this work we can conclude that the study of the essential oils of the dried leaves of the species C. citratus, allowed the identification of good yield with respect to the method used for extraction. The dried leaves of the herbs of the evaluated species (C. citratus) selected in this study have antifungal components which can be extracted by Clevenger hydrodistillation with yeast activity, as it has been shown to be a promising antifungal activity, showing satisfactory results for the
essential oil analyzed and proving the potential of these natural products as antifungal agents.

The essential oil tested showed activity against the microorganism tested using the minimal inhibitory concentration method per disc of 50 mg/ml, and its percentage was 1.65%, demonstrating a higher sensitivity of this technique. The results obtained in this study indicate the antifungal potential, but other studies should be carried out to complement these studies and applications with the isolation of the possible antifungal agents. The study of the chemical composition of the essential oils of the species under study suggests the presence of terpenes, oxygenated monoterpens, monoterpen and diterpene hydrocarbons with antifungal activity.

REFERENCES


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