

## Antimicrobial natural products

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Our ethnopharmacological studies, in several Angolan regions, showed that *Cymbopogon citratus* (DC) Stapf., it's applied in folk medicine to treat several diseases In order to validate antibacterial activity several studies were performed using 12 bacterial strains. The essential oil samples of *Cymbopogon citratus*, obtained by hydro-distillation, were analysed by GC and GC-MS. Constituents were identified [1] and tested for their antimicrobial activity by the disc agar diffusion technique and the dilution technique against *Staphylococcus aureus* (ATCC 25923) and *Staphylococcus epidermidis* (ATCC 12228) and other bacterial strains. Broad spectrum antibacterial activity was exhibited by the *Cymbopogon citratus* essential oil against both Gram-positive and Gram-negative bacteria as well as for MRSA that exhibited more sensitive towards the test substance compared to the non-MRSA. Our work was the first to show that *Cymbopogon citratus* essential oil has higher antibacterial activity against MRSA strains, than commercial antibiotics. These discover opens a new hope to fight against nosocomial infection.

**Keywords** *Cymbopogon citratus*, MRSA, nosocomial infections, antibacterial activity

### 1. Introduction

The indiscriminate use of antimicrobial agents resulted in the emergence of drug-resistant bacteria, fungi and viruses. Various populations in developing countries are using medicinal plants against infectious diseases by accidental discovery, and trust in the benefit of their use. To overcome the increased resistance of pathogenic microbes, researchers are using traditional knowledge as source of development of new drugs with high antimicrobiotic potential. The use of phytochemicals as natural antimicrobial agents, commonly called “biocides”, is gaining popularity. There is a growing interest among the medical proprietries of medicinal plants in terms of antibacterial activity. Several compounds in the essential oil of plants have been recognized for many years as having antibacterial proprietries [2] and their preparations have found applications as naturally antimicrobial agents in several fields.

In the ongoing search for better antibacterial compounds, plant-derived products are gaining ground [3,4]. Essential oils, plant extracts prepared by distillation, are composed by a huge diversity of small hydrophobic molecules, most of them accomplishing theoretical criteria of druglike prediction [5]. Such molecules easily diffuse across cell membranes, and consequently gain advantage in what concerns interactions with intracellular targets, this being a valuable research option in the search of anti-bacterial leads and drugs [6]. *C. citratus* (DC) Stapf, Family Poaceae, is a widely used herb in tropical countries viz. Southeast Asia, Africa and South America and is also known as a source of ethno medicines. *C. citratus* is commonly used in folk medicine in Angola for the treatment of gastrointestinal disturbances, and as an antispasmodic, anti-inflammatory, anti-pyretic, and diuretic treatment. Some studies have demonstrated its antimicrobial activity, specifically antibacterial, antifungal, and antiprotozoal properties; On the other hand, many aromatic plants are today considered as the most important sources for extraction of compounds with strong antioxidant activity. Numerous studies have focused on the various biological activities of the secondary metabolites of several aromatic species, indicating that several aromatic plants have antioxidant activity. In addition, aromatic plants are gaining the interest of pharmaceutical companies for their high potential as antioxidant, these being Rosemary, the first spice commercially available for the use as an antioxidant in Europe and USA, marked in an oil soluble form, in dry powder and also in water soluble formulations [7].

### 2. Antibacterial Activity of Angolan *Cymbopogon Citraus* essential oil

Infectious diseases are responsible for 14 million global deaths annually [8] amongst them bacterial infections being a major threat [9]. The only solution to the problem being the use of antibiotics or chemicals. However, the increasing failure of chemotherapy and antibiotic resistance exhibited by bacterial pathogens have prompted researchers to screen plants for their antimicrobial activity.

Angolan folk medicine is rich in plants with antibacterial activity. This activity the result of centuries of trials and error experiments. Angolan folk medicine also displays a remarkable potential of bioactive compounds. Various studies have revealed the activity of Angolan medical plants in human, animal and plant pathogens, however, very few

snapshots are available on their antibacterial activity. This chapter discusses the antibacterial activity of *Cymbopogon Citratus* leaves extracts in *S. aureus*, *S. epidermidis*, *E. coli*, *K. pneumoniae* and *P. mirabilis*, as well as multi-resistant drug strains.

Although, some studies have demonstrated that several constituents of essential oils have antibacterial activities [10], the antibacterial mechanism thereof is still unknown. We considered the possibility that permeabilization of bacterial cells' plasmatic membrane will be the major mechanism for bacterial death. In supported of this hypothesis, our results indicate that the composition of the essential oil of *Cymbopogon citratus* reveal the presence of several hydrophobic compounds. Permeabilization studies, using ethidium bromide as a fluorescent probe, were performed in order to validate these hypotheses, as described by Sato et al. [11].

## 2.1. Ethno Pharmacological Studies

The ethno pharmacological study performed in Angola reveal that shrubs are the plants more often used in folk medicine. These are followed by trees, the herbaceous and / or subshrubs and vines. Ten (10) of these are included in plant families such as: Anacardiaceae, Apocynaceae, Araceae, Capparaceae, Convolvulaceae, Cucurbitaceae, Menispermaceae, Nyctaginaceae, Polygalaceae, Rhamnaceae, Rubiaceae, Sapindaceae and Vitaceae. Although the leaves and roots are the organs most frequently used in the preparation of medicines, there are cases in which the stem bark is more important. In general, the medicines used results from decocting, soaking and spraying of these parts of the plants. Our results showed that usually the leaves are used fresh often than dried.

In addition, our results indicate that respiratory diseases, skin diseases, sexual diseases, malaria and diarrheal diseases are most often treated with ethno-pharmacs. Plants like Omundeo (*Diplorhynchos condilocarpon* (Müll. Arg.) Pichon), are used to treat malaria, while species from *Rhus* family, are indicated for antidiarrheal treatments, especially in cases of dysentery.

Of all the species identified, "Caxinde tea" (*Cymbopogon citratus*) is used in treatment of a greater range of diseases such as gastrointestinal, antispasmodic, anti-inflammatory, anti-pyretic, and diuretic disturbances. Some studies have demonstrated its antimicrobial activity, namely antibacterial, antifungal, and antiprotozoal properties [12-14].

## 2.2. Antibacterial studies

Analyses carried out by gas chromatography (GC) and by gas chromatography-mass spectroscopy (GC/MS) allowed the identification of compounds in the essential oil of *Cymbopogon citratus*, the retention time thereof, and its relative proportions. The main compounds from *C. citratus* is geranial (48.4%), neral (32.6%) and myrcene (6.4%) (Pires, 2012).

The results in Table I show that the essential oil concentrations between 15 and 80% inhibit the growth of both *S. aureus* and *S. epidermidis*, with diameters ranging from 8 to 37 mm in diameter, The inhibition of the positive controls ranging from 20.6 to 42.2 mm, corresponding to methacycline 5µg and Penicillin G 10mg.

The results of inhibition formed by disk diffusion method of essential oil of *Cymbopogon citratus* on *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus mirabilis* (Table I) demonstrate that concentrations of 5 and 20% has no effect on the three species tested, and that the maximum effect is obtained when using a figure of 60 to 100%. It should be noted that the halos obtained from the essential oil are always lower than those obtained for the positive controls. Similar studies were carried out, using the majority constituents present in the essential oil of *Cymbopogon citratus*. The results show that citral at low concentrations has no inhibitory activity on the growth of *S. aureus*. The dilutions between 20 and 45% *S. aureus* revealed to have only intermediate susceptibility, whereas at concentrations of 60 to 100% its activity is close to that obtained in positive controls. The same is observed for *S. epidermidis*, however above 45% its activity is higher than that obtained with the positive controls, although the difference was not statistically significant, being the same results obtained with the other major constituents of *cymbopogon citratos* essential oil.

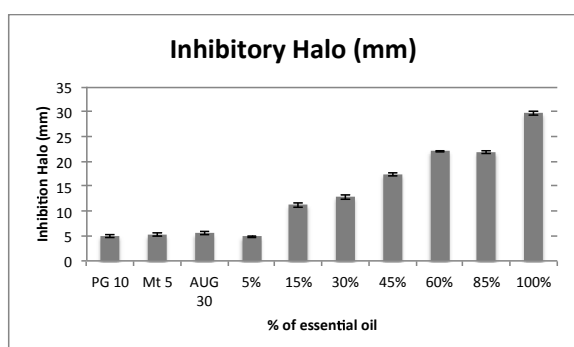
**Table 1** Inhibitory effect of *Cymbopogon citratus* essential oil on the growth of gram-positive bacteria, as well as on gram-negative bacteria.

Bacterial	<i>S. aureus</i> (ATCC 25923) (Halo diameter in mm)	<i>S. epidermidis</i> (ATCC 12228) (Halo diameter in mm)	<i>E. coli</i> (ATCC25922) (Halo diameter in mm)	<i>K. pneumoniae</i> (ATCC13883) (Halo diameter in mm)	<i>P. mirabilis</i> (ATCC25933) (Halo diameter in mm)
Concentration of essential oil (%)					
5	9,8 ±1,9	11,2±1,2	0	0	0
20	16,9 ±1,6	15,4±1,0	0	8,3±0,9	7,3±0,5
45	18,4±1,5	17,4±1,0	8,7±0,5	10,3±0,5	9,0±0,1
60	22,3±2,8	22,5±1,8	10,7±0,5	10,0±0,1	9,7±0,5
80	37,0±2,3	36,0±1,0	10,3±0,5	10,7±0,5	10,0±0,1
100	24,0±0,7	35,5±1,9	11,3±0,5	10,3±0,5	10,0±0,8
DMSO	0	0	0	0	0
Antibiotics					
Mt5	25,8±0,8	26,8±5,4			
PG10	42,2±1,2	20,6±5,7			
AUG30	34,0±0,5	32,5±3,6			
CIP5			28,7±1,2	33,7±0,5	34,0±0,8
NI300			22,3±1,2	17,7±1,2	10,3±1,2
CF30			25,3±1,2	19,3±1,2	20,3±1,2
GM10			18,3±1,2	24,3±1,2	31,0±1,6

ATCC - American Type Culture Collection; *S. aureus* - Staphylococcus aureus, *S. epidermidis* - Staphylococcus epidermidis; *E. coli* - Esherichia coli, *K. pneumoniae* - Klebsiella pneumoniae, *P. Mirabilis* - Proteus mirabilis; DMSO - dimethyl sulfoxide; CF 5 – Ceftazidima 30µg; 5 CIP-Ciprofloxacin 5µg, NI 300 - 300µg Nitrofurantoin, GM 10 - Gentamicin 10mg., Mt 5 - Metilicin 5µg, PG 10 - 10mg Penicillin G, Aug 30 - 30µg Augmentin. Sensitivity value: <8mm - Resistant; 9-14mm - Intermediate, 15-19mm - Sensitive;> 20mm - Very sensitive. # Values represent means ± standard deviation obtained from three determinations of inhibition zones.

### 2.3. Antibacterial studies on MRSA strains

The most important results were obtained when anti-bacterial activity of essential oil of *Cymbopogon citratus* where tested against Methicillin-resistant *Staphylococcus aureus* (MRSA).



**Fig. 1** Halos inhibition (mm) of different concentrations of essential oil of *Cymbopogon citratus* on methicillin resistant *S. aureus*, as compared to a positive control (augmentin AUG-30 PG-10 penicillin and methicillin-MT 5; NS - not significant. \*\*\* statistical significance.

As shown in figure 1, the hospital *S. aureus* is resistant to the combination amoxicillin / clavulamic acid (augmentin), penicillin G and methicillin, being classified as a MRSA strain.

Using the essential oil of *Cymbopogon citratus* at concentrations of 5%, we obtained an inhibitory halo similar to the one obtained when using methicillin, penicillin or Augmentin G, however, no statistically significant difference is observed ( $p < 0.001$ ). In all other concentrations used, the essential oil achieved a higher inhibitory effect (halo gave a major) with statistical significance ( $p < 0.001$ ) compared to the reference of the various antibiotics used in this study. Hospital strains of *S. epidermidis* demonstrate resistance to vancomycin. The results obtained after incubation of *S. epidermidis* showed that essential oil promotes a significantly bigger halo at the concentration of 15% ( $p < 0.001$ ) when compared with penicillin and methicillin.

These results were the first to demonstrate the antibacterial activity of *Cymbopogon citratus* essential oil against MRSA strains and nosocomial bacterial.

In recent years several screenings of medicinal plants used for the treatment of bacterial infections have been carried out, demonstrating the chromatographic fraction of the essential oil of *Cymbopogon citratus* in agar plate was active on *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus* [15-16] and *Salmonella paratyphi* and *Shigella flexneri* [17] confirming the importance of many plant species and essential oils as potential sources for the isolation of novel compounds with antibacterial effect. These activities are shown in two of the three main components of the oil identified through chromatographic and mass spectrometric methods. While the  $\alpha$ -citral (geranial) and  $\beta$ -citral (neral) compounds individually elicit an antibacterial action on gram-negative and gram-positive organisms, the third component, myrcene, did not show any observable antibacterial activity on its own [18]. The extract was also active when the volatile oil extract was oxidized via the active oxygen method [19–22].

A common feature of plant volatiles is their hydrophobic nature, and several studies addressing the mode of action of such compounds usually point at cell membranes as the primary target [23]. In our study we observed an increase in cell permeability measure by ethidium bromide entry to the cells. In this sense, we hypothesized that essential oil and citral may have a passive entry and may accumulate in bacterial cell membranes leading to an increased permeabilization, and pore formation. Other authors referred conclude similar to our work. The interaction affects membrane permeability, and this has been documented by loss of membrane potential, cellular uptake of ethidium bromide, and leakage of potassium ions, ATP, and carboxyfluorescein [24-27].

### 3. Conclusions

Briefly, it can be concluded that in this study, we found that the essential oil of *Cymbopogon citratus* has in its composition phenolic compounds, alcohols and terpenes. It was demonstrated that the antibacterial properties of the essential oil of *Cymbopogon citratus* may be attributed to citral (mixture of the two isomers geranial and neral).

The essential oil of *Cymbopogon citratus* revealed a strong antibacterial activity on *Staphylococcus aureus* and *Staphylococcus epidermidis* when compared to augmentin and antibiotics such as penicillin and methicillin. This has been observed in strains of reference, and enhanced effect in multidrug-resistant strains.

The antibacterial effect of the major compounds contributes to the final antibacterial effect of essential oil, since individually these compounds exhibit antibacterial effects smaller than essential oil. It was impossible to assign to a compound the antibacterial activity of the essential oil. All constituents tested were cytotoxic for various bacterial strains tested, but revealed less antibacterial effect than the essential oil, suggesting that other minor constituents contribute to overall antibacterial activity.

The determination of the minimum inhibitory concentration, which identified the bactericide effect, occurs at concentrations above 0.920 mM for strain *S. aureus* and 1.840 mM for strains of *E. Coli* and *S. epidermidis*.

This study proved the essential oil of *Cymbopogon citratus* has antibacterial activity, and as such being an option for obtaining natural antibiotics and the development of new pharmacologically active molecules against multi-resistant strains.

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