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COMPARATIVE ANTIMICROBIAL ACTIVITY OF VOLATILE AND NON-VOLATILE EXTRACTS OF CYMBOPOGON CITRATUS LEAVES

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ABSTRACT: The present study, carried out on *Cymbopogon citratus* was interested in volatile and no-volatile extracts of the plant. It focused on the effect of two types of extracts on eleven strains of bacteria. Chemical analysis of the volatile extract obtained with a yield of 2.28% revealed the presence of 72.91% of citral and other minority compounds. The extraction yield of the ethanolic extract (no-volatile) is 10.8%. Chemical groups such as reducing compounds, alkaloids, flavonoids, phenolic compounds, leuco-anthocyanins, saponosides, coumarins, and finally, terpenoids have been identified in this extract. The Minimum Inhibitory Concentration (MIC) and Bactericidal (CMB) were determined by macro dilution method. The volatile extract showed the best inhibitory activities on more than 90% of the strains of bacteria studied. The lowest minimum inhibitory concentrations were obtained against Micrococcus luteus (0.3125 mg/ml), Staphylococcus epidermidis T22695 (0.625 mg/ml) and Proteus vulgaris A25015 (0.625 mg/ml). This extract also had the best inhibition diameters against most bacteria. The bactericidal activity of this extract was only obtained against Enterococcus faecalis (1.25 mg/ml). The no-volatile extract inhibited approximately 50% of the strains tested with values slightly higher than those noted in the case of the volatile extract. There was no bactericidal effect in all cases with this extract. In sum, the volatile extract of C. citraus is significantly more active than the non-volatile extract. Formulations of phyto-drugs based on the essential oil of *C. citratus* would therefore be more effective than those based on its non-volatile extract.

INTRODUCTION: The challenge of drug resistance, emerging and re-emerging diseases, is a serious concern to the field of phytomedicine, pharmacognosy and pharmaceutical microbiology and chemistry ¹.



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The extensive use of antibacterial drugs and their resistance against bacterial infections is positively correlated with the use of antibacterial agents in clinical practice.

That is why, it is very much essential to find out safe, more effective, and inexpensive new chemical com-pounds or plant extracts as antibacterial agents. Bacterial resistance to antibacterial drugs has led to severe health and economic problems ¹. Furthermore, plants have emerged as credible sources of new antimicrobials ^{2, 3}. As a result, research on the efficacy of plants has increased.

Several Plants have been widely reported to be effective against various disease conditions, including those caused by microorganisms. Studies have indicated that a significant number of the global population use herbal medicine for the treatment of several diseases, especially individuals residing in rural areas in many developing countries^{4, 5, 6}. According to some authors, phytomedicine or herbal medicine is a major component in all indigenous peoples' tradition, a common element in Ayurvedic, homeopathic, naturopathic etc. 6. During this study, we were particularly interested in Cymbopogon citratus whose numerous biological activities were known. Cymbopogon citrates, which is commonly known as lemongrass, belongs to the grass family of Poaceae ^{7, 8}. Lemongrass is a fast-growing, perennial aromatic grass native to South India and Sri Lanka, and now it's commonly cultivated in the tropical areas of America, Asia 9, 10, Africa, including the Republic of Benin. Typically, Cymbopogon represents an important genus of about 120 species that grow in tropical and subtropical regions around the world ². Several studies have been conducted with regard to the antimicrobial potentials of lemongrass 8, 10, 11, 12, 13,

Usually, the biological activities of volatile and novolatile extracts of this plant are studied separately. But the simultaneous comparative study of these two fractions remains unexplored. The aim of this work is to make a comparative study of the antimicrobial activity of volatile and no-volatile extracts of *Cymbopogon citratus* in order to deduce the most effective fraction.

MATERIALS AND METHOD:

Extract Preparation: Extraction of volatile compounds from *Cymbopogon citratus*. That is the essential oil of the plant. The dry leaves of *Cymbopogon citrates* harvested in the morning at Abomey-Calavi (Republic of Benin) on the shores of Nokoué Lake are used as a material plant. The essential oil is obtained by hydrodistillation using a Clevenger type apparatus ¹⁵.

Chemical Analysis Equipment of Essential Oil: The analysis is performed on a Focus GC with a capillary column CP Wa × 52 CB (J & W Scientific from agilent technologies column, No.

US1670726A, USA) of dimension 15×0.25 mm with 0.25 µm internal diameter. In order to confirm the specificity and selectivity of the GC method, GC/MS analysis were performed on a TRACE GC 2000 series (Thermo Quest, Rodano, Italy), equipped with an AS 2000 auto sampler (GC System Thermo Quest. coupled to a mass spectrometer type There Quest Trace operating in electron impact mode. The compounds are identified by comparing their retention time and mass spectra with those of reference compounds ¹⁶. Extraction of no-volatile compounds Cymbopogon citratus: In some studies, the ethanolic fraction had been shown to be more active. In addition ethanol is a very little toxic solvent and according to some authors allows extracting the maximum of chemical compounds (Umar et al., 2016). That is what motivated us to take an interest in the ethanolic extract of their leaf. 50 g of powder of C. Citratus leaves were crushed and recovered in 500 ml of ethanol 96 °C. After agitation and homogenization, the mixture is filtered on Wathman paper and the filter is concentrated in a rotary evaporator at a temperature between 55 °C and 60 °C with help of vacuum pump to obtain the extract. The dry, watery triturated extract obtained was stored in a refrigerator at 4 °C.

Phytochemical Screening: The qualitative phytochemical screening is performed based on colouring or precipitation reactions. It is made directly on the ethanolic extract of *Cymbopogon citratus* leaves according to Houghton and Raman method ^{18, 19}.

Antimicrobial Activity Assessment Methods: Eleven references strains such as Escherichia coli ATCC 25922, Staphylococcus aureus ATCC 29213, Staphylococcus epidermidis T22695, Pseudomonas aeruginosa ATCC 27853, Proteus mirabilus A24974, Micrococcus luteus ATCC10240, Proteus vulgaris A25015, Streptococ cusoralis, Enterococcus foecalis ATCC 29212, Salmonella typhi R 30951401 and Escherichia coli O157 were used.

Preparation of Essential Oil Emulsion: In a test tube, 3920 μ l of Mueller Hinton broth was mixed with 80 μ l of essential oil. To this mixture were added 5% of different concentrations of tweens 80.

Indeed, the tween 80 has surfactants that make it possible to dissolve essential oils in water. The mixture (HD, oil, and tweens) was then homogenized to form the essential oil emulsion of 20 mg/ml concentration ²⁰.

Sensitivity Test: It was done according to the disc method inspired from the one described by ²¹. Brieflt, 1 ml of pre-culture of 18-24 h (10⁶ UFC/ml) enabled planting a box of Petri dishes containing agar Mueller Hinton by the flood. After seeding, the sterile Whatman paper discs (5 mm de diameter) were deposited with sterile pince. These discs have been carefully impregnated with 30 μl of plant extract (20 mg/ml. The dishes were kept for 15-30 min at room temperature before incubation at 37 °C. The inhibition zones diameters were measured after 24 to 48 h using a ruler graduated ²². For each extract, the experiment was performed induplicate.

Determination of the Minimum Inhibitory Concentration (MIC): The MIC has been determined by macro dilution method with Visual assessment of the growth of microorganisms²³. Briefly, nine concentrations (10 000, 5 000, 2 500, 1 250, 625, 312.5, 156.25, 78.12 and 39.06 μ g/ml) was performed in screw tube. To 1 ml of the above concentrations was added 1 ml of the bacteria inoculum (106 UFC/ml). After 24 h of incubation, turbidity tubes was examined relative to the control tube containing distilled water and the inoculum (10⁶ UFC/ml).

Determination of the Minimum Bactericidal Concentration (MBC): The MBC was determined by the solid medium culture of all of the tubes from the MIC to high concentrations. These dishes were incubated at 37 ° C for 24 h. The highest dilution that yielded no bacterial growth on solid medium was taken as MBC. ²⁴.

Data Treatment and Analysis: The spreadsheet Microsoft excel version 2013 has been used for the capture and encoding the data.

RESULTS AND DISCUSSION:

Composition of the Volatile Extract: From 500 g of plant material, 11.4 g of essential oil (volatile compounds) was obtained, which represented a yield of 2.28%. The leaves were dried at 18 °C before extraction, which explained the high yield of

volatile compounds extraction. Usually, the essential oil yield of *C. citratus* does not exceed 3% ²⁵. It emerges from the chemical analysis of the essential oil of *Cymbopogon citratus*, that citral (72.91%) is the majority compound. It is a mixture of two isomers: neral (31.26%) and geranial (41.65%) **Table 1**. The percentage of geranial is greater than that of neral ^{25, 26}. This observation can be explained by the fact that the geranial is the E-isomer generally more stable than the Z-isomer, which is the neral. We also noted the presence of other compounds such as myrcene (8.18%), geraniol (6.35%), geranyl acetate (2.56%), *etc.* in this oil **Table 1**.

TABLE 1: CHEMICAL COMPOSITION OF CYMBOPOGON CITRATUS ESSENTIAL OIL

Compounds	IK	Percentages (%)
6-methyl-hept-5-en-2-one	987,5	0,59
myrcene	991,3	8,18
δ-2 carene	998,7	0,28
(Z)-β-ocimene	1037	0,18
(E)-β-ocimene	1047	0,11
myrcene<6,7>epoxyde	1092	0,24
périllene	1098	0,07
linalol	1100	0,62
β-pinene-oxyde	1106	0,06
menth-3-en-9-ol	1150	0,22
citronellal	1153	0,30
isoneral	1162	1,02
iso geranial	1181	1,45
nerol	1225	0,29
citronellol	1229	0,20
neral	1242	31,26
geraniol	1253	6,35
geranial	1273	41,65
nericacide	1317	0,27
citronellylformate	1335	0,62
geranicacide	1352	0,96
geranyl acetate	1377	2,56
β-caryophyllene	1421	0,07
(E)-β-farnesene	1433	0,08
oxyde de caryophyllene	1584	0,10
Total		97,73

KI: Kowats index

Composition of the Non-volatile Extract: The ethanolic extract (non-volatile compounds) was obtained with a yield of 10.8%. This yield was very interesting compared to that of essential oil. The phytochemical screening of this extract had shown the presence of reducing compounds, alkaloids, flavonoids, phenolic compounds consisting of catechic and gallic tannins, leuco-anthocyanins, saponosides, coumarins, and finally terpenoids **Table 2**.

Previous work had shown that the ethanolic fraction allowed the maximum extraction of novolatile compounds compared to other solvents ¹⁷. Certain compounds that were sought after but not present were also mentioned in **Table 2**. These compounds could be found in the powder of the leaves and not in the extract. The objective being to identify the compounds responsible for the desired activity, the screening was therefore carried out on the ethanolic extract of the leaves.

TABLE 2: PHYTOCHEMICALS CONSTITUENTS OF CYMBOPOGON CITRATUS LEAVES' POWDER

Extract	Compounds		
+	Reducing compound		
+	Alkaloids		
+	Flavonoids		
+	Taninscatechic		
+	Taninscatechic		
+	Taninsgallic		
-	Anthocyanins		
	Leuco-anthocyanins		
-	Quinonics compounds		
	Saponin Coumarin		
+	Terpenoids		
-	Mucilages		
-	Cartenoids		
-	Free Anthracenics		

(+) = Presence; (-) = Absence

The Extracts Inhibitory Diameter Zone with the Reference Strains: Fig. 1 showed the diameters of inhibition of volatile and no-volatile compounds on the different strains of bacteria presented above. The sensitivity of bacteria depends on the type of extract. Between the two extracts tested, the essential oil (volatile extract), exhibited a pronounced antagonistic effect by inhibiting the growth of more than 90% of the pathogenic strains tested Fig. 1. The largest inhibition diameter (17.5 mm) was obtained with the volatile extract of C. Citratus against the strain of Proteus vulgaris, while the lowest inhibition diameter (5 mm) was obtained with the non-volatile extract against the pathogenic strains of Micrococcus luteus and Streptococcus oralis. In most cases, the diameters of inhibition of volatile compounds were much greater than the diameters of inhibition of novolatile compounds. The only exceptions where non-volatile compounds have an inhibitory diameter greater than the inhibitory diameter of volatile compounds were noted in Pseudomonas aeruginosa and Proteus mirabilus. From this observation, it appears that volatile compounds of

C. citratus have more effects on microbes than novolatile compounds.

In addition, this activity would be due to the presence of the two major isomers (citral) contained in this oil ²⁸.

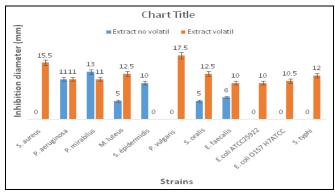


FIG. 1: DIAMETERS OF INHIBITION OF VOLATILE AND NON-VOLATILE EXTRACT OF C. CITRATUS

Minimum Inhibitory Concentrations of Volatile and Non-volatile extract of C. Citratus: The different extracts inhibited the proliferation of most pathogenic bacteria with variable minimum inhibitory concentrations in Table 3. According to these tests, the lowest concentrations (0.3125 mg/ml) were obtained with the volatile compounds of C. citratus against Micrococcus luteus (0.3125 Staphylococcus epidermidis T22695 (0.625 mg/ml) and *Proteus vulgaris* A25015 (0.625 The highest minimum inhibitory mg/ml). concentration for the volatile extract is 2.5 mg/ml. In terms of the non-volatile extract, which inhibited around 50% of microorganisms, the lowest concentration is 2.5 mg/ml obtained from three bacteria. The largest concentration is 5 mg/ml. These results confirm once again that volatile compounds were more active than no-volatile compounds. Microorganisms are, therefore, more sensitive to the volatile extract than to the nonvolatile extract. Some comparative studies have revealed the effectiveness of this oil compared to others ⁸. The citral, which constituted the majority compound of the volatile extract, would be responsible for this activity since it had been shown that the latter is active on certain strains of bacteria (Cronobactersakazakii) ²⁷. In addition, these authors have shown that citral attacks the cell membrane of these bacteria. However, we noted a slight inhibition with the non-volatile extract on Staphylococcus epidermidi, which did not exist at

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the level of the volatile extract. Flavonoids and tannins have been identified by some authors as being at the basis of the activity of the extract of the leaves of *C. citratus* ^{28, 29}. The volatile extract is lipophilic while the non-volatile extract is hydrophilic. *Staphylococcus epidermidi* would then be more sensitive to hydrophilic compounds and the others to lipophilic compounds.

TABLE 3: MINIMUM INHIBITORY CONCENTRATIONS (MG/ML) OF THE EXTRACTS ON THE STUDIED REFERENCE STRAINS

NVE	VE	STRAINS
0	0,625	S. aureus ATCC 29213
0	0,625	P. onasaeruginosa ATCC 27853
0	0,625	P. mirabilus A24974
0	0,625	M. luteus
0	0,625	S. épidermidis T22695
0	0,625	P. vulgaris A25015
0	0,625	S. oralis
0	0,625	E. faecalis ATCC29212
0	0,625	E. coli ATCC25922
0	0,625	E. coli O157 H7ATCC
0	0,625	S. typhi R 30951401

V E: volatile extract, N V E: non-volatile extract

Minimum Bactericidal Concentration (mg/ml) of the Extracts on the Reference Strains: In the case of the search for the Minimum Bactericidal Concentration (CMB), the only effect noticed was noted in the volatile extract against the strain *Enterococcus faecalis* (1.25 mg/ml) **Table 4**. The two extracts of *C. citratus* did not, therefore, have a major bactericidal effect on the microorganisms tested.

TABLE 4: MINIMUM BACTERICIDAL CONCENTRATIONS (mg/ml) OF EXTRACTS WITH REFERENCE STRAINS

NVE	VE	STRAINS
0	0	S. aureus ATCC 29213
0	0	P. onasaeruginosa ATCC 27853
0	0	P. mirabilus A24974
0	0	M. luteus
0	0	S. épidermidis T22695
0	0	P. vulgaris A25015
0	0	S. oralis
0	0	E. faecalis ATCC29212
0	0	E. coli ATCC25922
0	0	E. coli O157 H7ATCC
0	0	S. typhi R 30951401

V E: volatile extract. N V E: non-volatile extract

CONCLUSION: At the end of this work, it appears that the extraction yield of the non-volatile extract of *C. citratus*, obtained in ethanol, is better compared to the extraction yield of the volatile

fraction. The volatile extract consisting mainly of citral inhibited most of the strains of bacteria tested while the non-volatile extract inhibited less.

The volatile extract is the only one to show a bactericidal power against *Enterococcus faecalis*. This extract, despite its low yield, could serve as a very effective active ingredient in the formulation of bactericidal phyto-drugs.

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CONFLICTS OF INTEREST: The authors declare that they have no competing interests.

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