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(RESEARCH ARTICLE)

Comparative antimicrobial activity of *Cymbopogon citratus* essential oil and thiosemicarbazones derived from this oil

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Abstract

Introduction: The presence of microbes in our environment is always a permanent public health problem. In this context, research on natural treatment, less expensive and accessible to fight these microbial germs would be beneficial.

Methods: During this work, molecules of thiosemicarbazones due to their numerous biological activities were hemi-synthesized *in situ* in the essential oil of *Cymbopogon citratus* in order to evaluate their antimicrobial activities.

Results: Analysis of the essential oil extracted by hydrodistillation revealed the presence of 72.91% of citral. Citralthiosemicarbazone (CThio) and citral 4-phenyl-3-thiosemicarbazone (CPthio) were hemi-synthesized in this oil with interesting yields of 83% and 91%, respectively. After purification and confirmation of the structures of these molecules, the three substances were tested on eleven strains of microbes. Determination of the inhibition diameters showed that the activity of the essential oil is best in over 80% of strains. However, the largest diameter of inhibition (26 mm) was noted with CPthio against *Salmonella typhi* R 30951401. The determination of the minimum inhibitory concentrations showed that the oil remains more active with the smallest value of 0.3125 mg / ml against *Micrococcus luteus*. The lower value of minimum bactericidal concentrations was also obtained with the essential oil against *Enterococcus foecalis* ATCC 29212.

Conclusion: The essential oil of *C. citratus* remains more active in the majority cases. It could be a great alternative in the fight against bacteria, and the advantage is that it remains a natural substance.

Keywords: Thiosemicarbazones; Hemi-synthesized; *Cymbopogon citratus*; Antimicrobial activities; Minimum inhibitory concentrations; Minimum bactericidal concentrations.

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1. Introduction

Antibacterial drugs save countless lives since the beginning of the antibacterial era. But the problems of multi-drug resistant microorganisms have reached an alarming level in many countries around the world [1-3]. Microbial resistance to antimicrobial drugs has led to severe health and economic problems. The extensive use of antimicrobial drugs and their resistance against microbial infections is positively correlated with the use of antimicrobial agents in clinical practice. That is why it is very much essential to find out safe, more effective and inexpensive new chemical compounds or natural substances as antimicrobial agents.

During this work, we were interested in thiosemicarbazones molecules whose numerous biological activities have been widely described in the literature [4-8]. Among them, we can mention the antifungal [9,10], anti-HIV [11,12], anticancer [13,14], anti-inflammatory [15,16], tuberclostatic [17] activities. The antimicrobial properties of these molecules were also known [18-20]. The starting substrate which is the essential oil of *Cymbopogon citratus* according to the literature also had very interesting biological properties [21-23]. In our previous work, molecules of thiosemicarbazones hemi-synthesized *in situ* in essential oils rich in carbonyl compounds revealed very impressive antitrypanosomal activity [24-26]. Due to the spread of microbial diseases, we considered testing some of these molecules and the starting substrate on strains of microbes to assess their antimicrobial properties. So the aim of this work is therefore to hemi-synthesize *in situ* molecules of thiosemicarbazones in the essential oil of *Cymbopogon citratus* in order to evaluate their antimicrobial properties on strains of microbes.

This work is all the more interesting because the leaves of *C. citratus* are easily grown and are very available in Benin.

2. Material and methods

2.1. Extraction and analysis of essential oil

2.1.1. Extract Preparation

Extraction of volatile compounds from *Cymbopogon citratus:* That is the essential oil of the plant. The dry leaves of *Cymbopogon citratus* harvested in the morning at Abomey-Calavi (Republic of Benin) on the shores of Nokoué Lake are used as material plant. The essential oil is obtained by hydrodistillation using a Clevenger type apparatus [27].

2.1.2. Chemical analysis equipment of essential oil

The analysis is performed on a FOCUS GC with a capillary column CP Wax 52 CB (J & W Scientific from Agilent Technologies Column, No. US1670726A, USA) of dimension 15 x 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 (ThermoQuest, Rodano, Italy), equipped with an AS2000 autosampler (GC System ThermoQuest coupled to a mass spectrometer type TheroQuest Trace MS) operating in electron impact mode. The compounds are identified by comparing their retention time and mass spectra with those of reference compounds [28]. Identification of compounds

2.2. Synthesis and identification of compounds

The melting points were taken on a fusionometer type *electrothermal 1A 9000* and are uncorrected. The IR spectra were recorded on a Perkin-Elmer FTIR 286. The frequencies of absorption bands are expressed in cm-1. The NMR spectra were registered on a Brucker 500 in CDCl3 (chloroform-d6) or DMSO-d6 (dimethylsulfoxide-d6) which frequencies for 1H and 13C are 400 MHz and 100 MHz respectively. Chemical shifts are given in parts per million (ppm) relative to tetra-methyl silane as a benchmark. Multiplicity is designated as singlet (s), triplet (t), doublet (d) and multiplet (m).MS spectrometrical data of compounds were reported in APCI mode.

2.2.1. Hemi-synthesis of Citralthiosemicarbazone and citral-4-phényl-3-thiosemicarbazone

To a stirring mixture of 0.001 mol of essential oil of *Cymbopogon citratus* (152 mg) dissolved in 1.5 ml of ethanol at 95 ° was added 0.001 mol of thiosemicarbazide or substituted thiosemicarbazide dissolved in 2 ml of 1N hydrochloric acid. This mixture was stirred until thiosemicarbazone or substituted thiosemicarbazone crystals were observed after three minutes. Stirring continued for one hour. The precipitate is filtered, washed until neutral, dried, weighed and then recrystallized in ethanol [24-26].

2.3. 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, *Streptococcus oralis*, *Enterococcus foecalis* ATCC 29212, *Salmonella typhi* R 30951401 and *Escherichia coli* O157 were used.

2.3.1. Preparation of essential oil emulsion

In a test tube, 3920 µl of Mueller Hinton broth was mixed with 80 µl of essential oil. To this mixture was added 5% of different concentrations of tweens 80. Indeed, the tween 80 has surfactants which 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 [29].

2.3.2. Sensitivity test

It was done according to the disc method inspired from the one described by [30]. Briefly, 1 ml of pre-culture of 18-24 h (10^6 UFC/ml) enabled planting a box of Petri dishes containing agar Mueller Hinton by 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 compound (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 hours using a ruler graduated [31]. For each compound, the experiment was performed induplicate.

2.3.3. Determination of the Minimum Inhibitory Concentration (MIC)

The MIC has been determined by macrodilution method with Visual assessment of the growth of microorganisms [32]. Briefly, nine concentrations (10 000, 5 000, 2 500, 1 250, 625, 312.5, 156.25, 78.12 and 39.06 µg/ml) was performed in screw tube. To 1 ml of the above concentrations was added 1 ml of the bacteria inoculum (10^6 UFC/ml). After 24 h of incubation turbidity tubes was examined relative to the control tube containing distilled water and the inoculum (10^6 UFC/ml).

2.3.4. Determination of the Minimum Bactericidal Concentration (MBC)

The MBC was determined by 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 [33].

2.3.5. Data treatment and analysis

The spreadsheet Microsoft Excel version 2013 has been used for the capture and encoding the data.

3. Results and discussion

3.1. Extraction and chemical composition of the essential oil of *Cymbopogon citratus*

From 500 g of plant material, 11.4 g of essential oil was obtained, that represented a yield of 2.28%. The leaves were dried at 18 ° C before extraction, which explained the high yield of extraction. Usually the essential oil yield of *C. citratus* does not exceed 3% [34]. The yield of essential oil depends on the fresh or dry material plant. The fresh material containing more water, its mass is higher and thus leads to a lower yield. Some authors have reported yields of 1.02 to 1.5% from the dry material by location. 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 [35]. 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). This achievement of the essential oil of *Cymbopogon citratus* is made possible thanks to its availability in large quantities in Benin and its essential oil yield substantial. In addition to reducing the cost of synthesis, this work will develop a new line of research in the field of essential oils.

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
iso neral	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
neric acide	1317	0.27
citronellyl formate	1335	0.62
geranic acide	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

3.2. Synthesis of molecules

We performed the hemi-synthesis of two thiosemicarbazones which have been prepared by this method for the first time. The spectroscopic analysis showed the presence of the thiosemicarbazones of the two isomers (neral and geranial) of citral.

The two compounds were obtained with the good yields in this essential oil. There are: citralthiosemicarbazone **CThio** (83 %), and citral 4-phenyl-3-thiosemicarbazone **CPthio** (91%) (Table 2). The formulas of the various products are shown in Figure 1

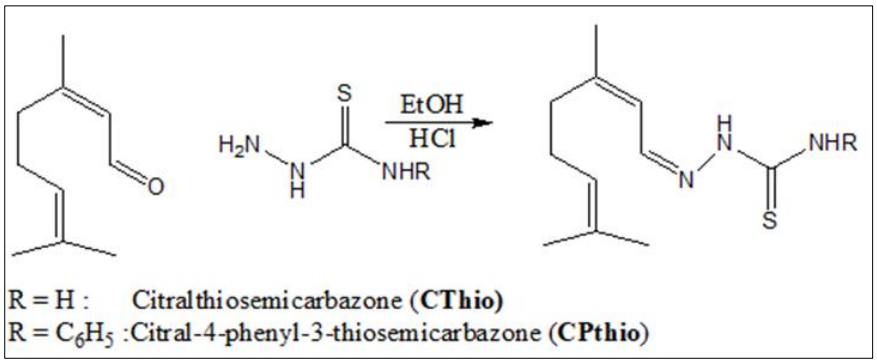

Figure 1 Chemical formulas of thisemicarbazones

3.3. Physical properties of thiosemicarbazones hemi-synthesized in *C. citratus* essential oil

Among hemi-synthesized molecules, only the synthesis of **CThio** had been reported in the literature from commercial citral [36]. To our knowledge, it was during this work that compounds **CPthio** had been synthesized for the first time by ours methods. **CPthio** yield was higher than that of **CThio** (Table 2). We deduce that the presence of phenyl in position 3 then promotes the reaction

Table 2 Physical properties of thiosemicarbazones

Names	Empirical formulas	M (g/mol)	Melting Points (°C)	Yield (%)
Citralthiosemicarbazone (CThio)	$C_{11}H_{19}SN_3$	225.10	105	83
Citral-4-phényl-3-thiosemicarbazone (CPThio)	$C_{17}H_{23}SN_3$	301.10	82	91

3.4. Confirmation of the formulas of the molecules

3.4.1. Citralthiosemicarbazone (CThio)

MS: [MH] + calculated: 226, 13 [MH] + found: 226, 10.

IR ν: (NaCl, cm-1): 3373 et 3271 (NH2); 3165 (NH); 1643 (C=N); 1609 (C=C) ; 836 (C=S). **NMR 13C** (CDCl3, 100MHz) δ(ppm): 178 (C=S); 142 (C=N) ; 139 et 121 (C=C) ; 31et 27 (CH2-CH2); 25; 22 et 19 (CH3).

NMR 1H (CDCl3, 400MHz), δ(ppm): 1,6 (d, 3H, -CHCH3); 1,7, et 1,8 (s, 6H, -C(CH3)2); 2,2 (m, 4H, -H2C-CH2); 5,1 (m, 1H, -CH=(CH3)2); 5,9 (d, 1H, =CH-CH=N-); 7,1 et 7,2 (s, 2H, -NH2); 7,9 (d, 1H,=CHCH=N); 10,2 (s, 1H, =NNH-).

3.4.2. Citral-4-phényl-3-thiosemicarbazone (CPthio)

MS: [MH] + calculated: 302, 16 [MH] + found: 302, 10.

IR ν (NaCl, cm-1): 3418 (-NHPh); 3280 (NH); 1644 (C=N); 1595 (C=C); 855 (C=S).

NMR 13C (CDCl3, 100MHz) δ (ppm): 181 (C=S); 140 (C=N); 135; 121(C=C); 121, 122, 124 (aromatiques); 40 ; 35 ; 23 ; 18 (CH3).

NMR 1H (CDCl3, 400MHz), δ(ppm): 1,5 (d, 3H, -CHCH3); 1,7, et 1,8 (s, 6H, -C(CH3)2); 2,1 (m, 4H, -H2C-CH2) ; 5,1 (m, 1H, -CH=(CH3)2); 5,9 (d, 1H, =CH-CH=N-); 7,1 ; 7,2 ; 7,5 (aromatiques) 7,9 (d, 1H, =CHCH=N); 9,1 (s, 1H, -NHPh) ; 10,4 (s, 1H, =NNH-).

3.5. Antimicrobial activities of thiosemicarbazones and Essential Oil (EO)

3.5.1. Determination of substances inhibitory diameter zone against the reference strains

Figure 2 shows the diameters of inhibition of hemi-synthesized compounds and essential oil on the eleven strains of microbes presented above. This experiment was shown that the sensitivity of microbial strains varies from strain to

strain depending on the compounds and the essential oil. Of the three substances tested, the essential oil exhibited a pronounced antagonist effect by inhibiting the growth of more than 81% of the pathogenic strains used (Figure 2). The two compounds have practically the same power of inhibition on about 50% of strains. In all cases, the highest diameter of inhibition (26 mm) was obtained with CPThio against *Salmonella typhi* R 30951401. It would be important to emphasize that CPThio acted on fewer strains than the essential oil, but it presented the larger diameters of inhibition in case of activity. After the activity of this molecule, followed that of the essential oil. The smallest diameters of inhibition was noted with CThio. However, it should be noted that an absence of activity of each of the substances on certain germs was noted. The results obtained were consistent and confirm those obtained during our previous work on trypanosome parasites. CPThio had always been shown to be more active on parasites, followed by EO and finally Cthio [24-26]. These results obtained on inhibition diameters are also in agreement with those obtained by other researchers who have worked on the antimicrobial activities of else thiosemicarbazones [18-20]. We retain from this part that certain strains of microbes used during this work are sensitive to the three substances tested.

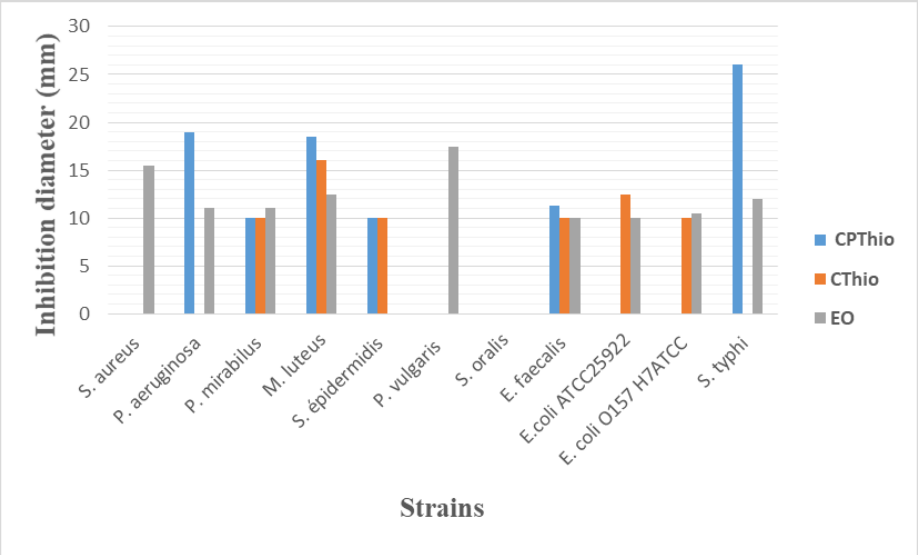

Figure 2 The substances inhibitory diameter zone with the reference strains

3.5.2. Minimum Inhibitory Concentrations of substances

On reading the table 3, we noted a correlation between the inhibition diameter values and the minimum inhibitory concentrations. The compounds and the essential oil in the proportions of approximately 50% and 81%, respectively, inhibit the proliferation of most pathogenic bacteria with varying minimum inhibitory concentrations. According to the results, the lowest MIC values were obtained with the essential oil on over 81% of the bacteria. The smallest MIC value (0.3125 mg / ml) was obtained against *Micrococcus luteus*. The values obtained with synthetic compounds remain higher than those obtained with essential oil. These compounds also inhibited less germs. The smallest MIC value (2.5 mg / ml) was obtained with CPThio against *Micrococcus luteus* ATCC10240. In all cases, between the two compounds CPThio remained more active than CThio. As in the case of our previous work, CPThio has been shown to be more active than CThio [24-26]. The presence of the phenyl group on this molecule therefore increased the activity of CPThio. This paragraph also told us that the essential oil remains more active than synthetic compounds. The activity of the oil was said to be due to the presence in large amounts of citral, but we also cannot overlook the synergistic effect of the various constituents of this oil [22]. This result is not surprising because it has been shown that microbes are very sensitive to essential oils and depending on their mechanism, EO particularly attack the membranes of germs during their destruction [22]. This probably explains the advantage of the oil over the compounds in this study. By comparing our previous work with current ones, we noted that trypanosome parasites are sensitive to synthetic compounds while the microbes studied during this work are rather sensitive to essential oil. The mechanism of action of the compounds on the parasites would be different from their mechanism on the microbes considered in the present study. Depending on the thiosemicarbazone molecules used, the results may differ because the literature reports on thiosemicarbazones molecules with very interesting antimicrobial activities [18-20].

Table 3 Minimum inhibitory concentrations (mg/ml) of substances on the studied reference strains

STRAINS	*S. aureus* ATCC 29213	*P. aeruginosa* ATCC 27853	*P. mirabilus* A24974	*M. luteus*	*S. épidermidis T22695*	*P. vulgaris A25015*	*S. oralis*	*E. faecalis ATCC29212*	*E. coli ATCC25922*	*E. coli O157 H7ATCC*	*S. typhi R 30951401*
CPThio	-	5	5	2.5	-	-	-	5	-	10	10
CThio	-	-	5	5	5	-	5	5	10	10	-
EO	0.625	2.5	2.5	0.3125	-	0.625	-	1.25	1.25	2.5	1.25

CPthio: Citral 4-phenyl-thiosemicarbazone; CThio: Citral thiosemicarbazone; EO: Essential oil of *Cymbopogon citratus*

3.5.3. Minimum Bactericidal Concentration (mg/ml) of substances

The values of the Minimum Bactericidal Concentration (MBC) are grouped together in Table 4. We noted a absence of bactericidal effect in most cases. The smallest value (1.25 mg / ml) of MBC was obtained with the essential oil against *Enterococcus foecalis* ATCC 29212. CPThio showed no bactericidal effect. On the other hand, CThio indicated at higher doses bactericidal effects on three germs. For the small Bactericidal Minimum Concentration noted in EO, we retained that the essential oil remains more active than the synthetic compounds. The presence of citral could always explain this activity. We insist on the fact that these noted bactericidal activities relate to the compounds obtained during this work, because thiosemicarbazone compounds according to the works of [36] have revealed very interesting bactericidal properties.

Table 4 Minimum Bactericidal Concentrations (mg/ml) of substances against reference strains

STRAINS	*S. aureus* ATCC 29213	*P. aeruginosa* ATCC 27853	*P. mirabilus* A24974	*M. luteus*	*S. épidermidis T22695*	*P. vulgaris A25015*	*S. oralis*	*E. faecalis ATCC29212*	*E. coli ATCC25922*	*E. coli O157 H7ATCC*	*S. typhi R 30951401*
CPThio	-	-	-	-	-	-	-	-	-	-	-
CThio	-	-	-	20	20	10	-	-	-	-	-
EO	-	-	-	-	-	-	-	1.25	-	-	-

CPthio: Citral 4-phenyl-thiosemicarbazone; **CThio**: Citral thiosemicarbazone; **EO**: Essential oil of *Cymbopogon citratus*

4. Conclusion

At the end of this work, we retain that the essential oil of *C. citratus* is very rich in citral which served as a substrate in the synthesis of thiosemicarbazones compounds. The resulting compounds as well as the oil exhibited a wide variety of interesting antimicrobial activities. In most cases, the oil has been shown to be more active than the hemi-synthesized molecules. In short, natural compounds such as essential oils can be more active than synthetic compounds and therefore can constitute a source of active ingredients against microbial diseases.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that they have no competing interests.

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