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In Vitro Sensitivity of *Salmonella typhi* Strain to Essential Oils of *Syzygium aromaticum* and *Cymbopogon citratus*, Medicinal Plants Used in the Kisangani Region (DR Congo)

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Abstract

The growing resistance of *Salmonella typhi* to antibiotics is a major public health challenge, justifying the search for natural therapeutic alternatives. This study aims to evaluate in vitro the antibacterial activity of essential oils of *Syzygium aromaticum* and *Cymbopogon citratus* on a strain of *S. typhi* isolated in Kisangani in the Democratic Republic of Congo. The essential oils were extracted by hydrodistillation and characterized organoleptically and physicochemically. Antibacterial activity was evaluated using the agar diffusion method, supplemented by determination of the minimum inhibitory concentration (MIC), and then statistically analyzed by ANOVA. The results show a higher extraction yield for *S. aromaticum* (0.85%) than for *C. citratus* (0.31%). The essential oil of *S. aromaticum* showed more pronounced antibacterial activity (inhibition diameter: 10 mm) compared to that of *C. citratus* (3 mm) and the reference antibiotics. The MIC of *S. aromaticum* was estimated at 80%, indicating concentration-dependent activity. Thus, the essential oil of *S. aromaticum* is an effective source of natural antibacterial agents against *S. typhi*, although further studies (molecular and in vivo) are needed.

Keywords: *Syzygium Aromaticum*, *Cymbopogon Citratus*, Essential Oil, Antibacterial Activity, *Salmonella Typhi*.

I. INTRODUCTION

Salmonella typhi is a bacterium that infects the intestinal tract and blood. Known as typhoid fever, it is an infectious disease that can be fatal if left untreated [1]. Typhoid fever remains endemic mainly in developing countries, particularly in South Asia and sub-Saharan Africa. According to the World Health Organization (WHO), approximately 14 to 20 million cases are reported each year worldwide, resulting in nearly 135,000 deaths, mainly due to limited access to care and increasing antibiotic resistance [2].

This fever most often occurs in areas where hygiene is poor and mainly affects low- and middle-income countries. In France, 100 to 250 cases are reported each year among travelers or people from endemic areas (Africa, Asia, Latin America) [3, 4].

However, ultra-resistant strains exist that are resistant to all antibiotics except macrolides and carbapenems [2].

Antibiotic resistance leads to more severe infections, an increase in hospitalizations, and greater difficulty in the therapeutic management of typhoid fever [5].

Essential oils, as complex mixtures of natural compounds, have promising antimicrobial potential, offering a natural alternative to the growing resistance to conventional antibiotics [6].

This study aims to evaluate in vitro the antibacterial activity of essential oils from *Syzygium aromaticum* (clove) and *Cymbopogon citratus* (lemongrass) against a strain of *S. typhi* resistant to antibiotics commonly used in the Kisangani region (DR Congo). The results could contribute to proposing a natural therapeutic alternative to the growing resistance to conventional antibiotics.

II. MATERIALS AND METHODS

II.1. Study Environment

This study was conducted in the city of Kisangani, capital of Tshopo Province, in the Democratic Republic of Congo. The city is located at approximately 0°31' north latitude and 25°11' east longitude, about 57 km north of the equator, at an average altitude of 428 meters above sea level [7].

II.2. Materials

The essential oil was extracted by hydrodistillation. Dried leaves of *C. citratus* and flower buds of *Syzygium aromaticum* were used. The essential oils were collected and stored in airtight bottles, protected from light [6].

The microbial strain consisting of *S. typhi* was used as biotic material. As a result, vancomycin and erythromycin, two commonly used antibiotics, were selected as positive controls.

II.3. Methods

II.3.1. Isolation of the Strain

Germs were isolated using the swab technique. Sterile swabs were dipped into a test tube containing sterile peptone water. These swabs were then rubbed on the urinals at the Faculty of Natural Sciences and Biotechnology at the University of Kisangani and the Bar Beach BRALIMA. Finally, each swab was returned to its test tube, sealed tightly, and sent directly to the Microbiology and Phytopathology Laboratory at the same faculty for isolation of the strains on a selective medium [8].

The isolated bacterial strains were transferred to a selective Xylose Lysine Deoxycholate (XLD) medium for the specific detection of Salmonella spp., in accordance with standardized microbiological recommendations [9].

For storage, the strains were transferred by deep inoculation into tubes containing soft agar [10].

II.3.2. Preculture and Characterization of the Strains to Be Tested

❖ **Preculture**

The strains to be tested were inoculated into tubes, each containing 3 ml of peptone water (1 g/100 mm) for revivification, then incubated at 37°C for 6 to 10 hours [11-13].

❖ **Characterization of the Strains to Be Tested**

a. Gram Staining

A clean slide is carefully cleaned with cotton wool soaked in 95% alcohol. In the case of a liquid culture, a drop of the culture is placed on the slide using a sterile Pasteur pipette or platinum loop [14, 15].

In the case of a culture obtained on a solid medium (tube or Petri dish), a drop of sterile distilled water or sterile physiological water is first placed on the slide, then a bacterial inoculum is taken and carefully dissociated [16]. The preparation is then covered with a coverslip and sealed with paraffin or petroleum jelly to prevent drying out.

Observation is carried out using an optical microscope, generally with a ×40 or ×60 objective. For better visualization of fine structures, particularly the cell wall, a ×100 immersion objective can be used [17].

b. Biochemical Characterization of *Salmonella Typhi* Strains

The biochemical characterization of *Salmonella typhi* strains is performed using pure colonies isolated and cultured in different selective and differentiating media incubated at 37°C for 24 to 48 hours. Tests include Kligler Iron Agar (KIA), MIU (Mobility, Indole, Urease) medium, Simmons citrate, the Vosges-Proskauer test, and methyl red [15].

In Kligler Iron Agar, the fermentation of lactose and glucose is observed by a yellow color change in the medium, the production of gas by air pockets or the lifting of the agar, and the production of hydrogen sulfide (H_2S) by a black precipitate at the interface between the slope and the base [15]. MIU medium is used to assess the motility of the bacteria, urease production (the phenol red indicator turns pink), and indole production (a pink ring forms after adding Kovacs reagent) [15]. Simmons citrate detects the use of citrate as the sole carbon source (change from green to blue) [18]. The Vosges-Proskauer test reveals the production of acetoin (light yellow color) [18], while methyl red indicates the production of acids by fermentation, resulting in the medium turning red [18].

II.3.3. Antibacterial Activity of Essential Oil

❖ **Impregnation of Discs**

Discs with a diameter of 7 mm were impregnated with 10µ of essential oil. They were then placed in Petri dishes to dry for at least 5 days.

❖ **Antibacterial Activity of Essential Oils**

The aromatogram method was used to evaluate the antibacterial activity of the essential oil under study. This technique is based on the diffusion of aromatic compounds in a gelatinous medium in a Petri dish, allowing the inhibition zone around each oil tested to be measured [19].

The results are read by measuring the diameter of the inhibition zone in millimeters [20].

❖ **Antibacterial Activity of Antibiotics**

The Mueller Hinton agar diffusion method was used to evaluate the activity of antibiotics [21].

The results are interpreted by measuring the diameter of the inhibition zone (Ø) in millimeters and comparing these values to the standard thresholds for each antibiotic [21].

II.3.4. Determination of the MIC

The minimum inhibitory concentration (MIC) was determined using the agar diffusion method. A 100% stock solution was prepared by mixing equal parts of essential oil and methanol. Successive dilutions were used to obtain concentrations ranging from 90% to 10%. Sterile discs soaked in 10 µL of each dilution were placed on Mueller Hinton agar previously spread with the strain to be tested, then incubated at 37°C for 24 hours. The MIC corresponds to the lowest concentration producing a visible inhibition zone [6-21].

II.4. Statistical Analyses

Statistical analyses were performed using R.4.5.1 software.

ANOVA was used to compare the inhibition diameters of essential oils with antibiotics on the *S. typhi* strain. Tukey's HSD (Honestly Significant Difference) post-hoc test was used to identify which pairs of group means differed significantly between essential oils and antibiotics.

III. RESULTS AND DISCUSSION

III.1. Essential oil Extraction Yield

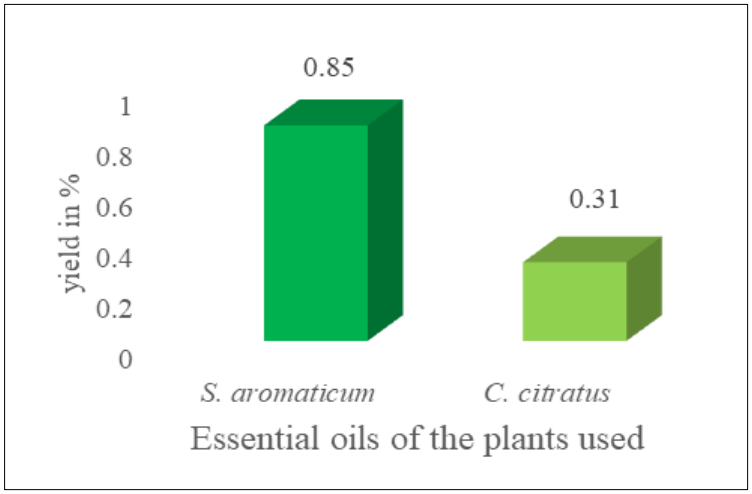

Figure 1: Essential oil extraction yield in %

The figure above shows the essential oil yields obtained from *S. aromaticum* and *C. citratus*. It shows that *S. aromaticum* has a significantly higher yield, reaching 0.85%, compared to only 0.31% for *C. citratus*.

This difference could be explained by the intrinsic richness of cloves (*S. aromaticum*) in volatile compounds, particularly eugenol, the main constituent of its oil [22]. On the other hand, *C. citratus* (lemongrass) is generally less concentrated in essential oils, which explains its low yield [23].

These results confirm the observations reported by Burt and Lawal [24, 25], which indicate that species rich in aromatic phenols often have higher yields, in line with their density of secretory glands.

III.2. Characterization of Essential Oils

III.2.1. Organoleptic Quality

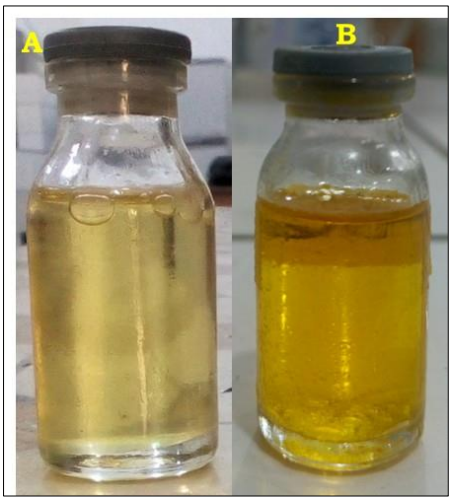

Figure 2: Images of essential oils from *Syzygium aromaticum* (A) and *Cymbopogon citratus* (B)

Organoleptic parameters showed a strong similarity between the essential oils of *S. aromaticum* and *C. citratus* in terms of appearance and odor. However, a notable difference was observed in color: the essential oil of *S. aromaticum* is pale yellow, while that of *C. citratus* is darker yellow. This variation in color can be attributed to the chemical nature of the major compounds present in each oil. Indeed, the essential oil of *S. aromaticum*, rich in eugenol, is generally characterized by a clear to pale yellow color, while *C. citratus*, dominated by citral, tends to have a more intense color ranging from yellow to light brown [22-26].

Such organoleptic differences are commonly reported in the literature and are often influenced by several factors, including geographical origin, the vegetative stage of the plant, climatic conditions, and the distillation technique used. Therefore, the darker color observed in the *C. citratus* oil obtained in this study could reflect a higher concentration of terpene aldehydes or distillation by-products [6-27].

Even though the oils are similar in appearance and smell, the difference in color is a relevant organoleptic indicator that may reflect qualitative and quantitative variations in their chemical composition.

III.2.2. Physicochemical Profile

a. Density

The density values of the essential oils of the plants studied are shown in Figure 3 below:

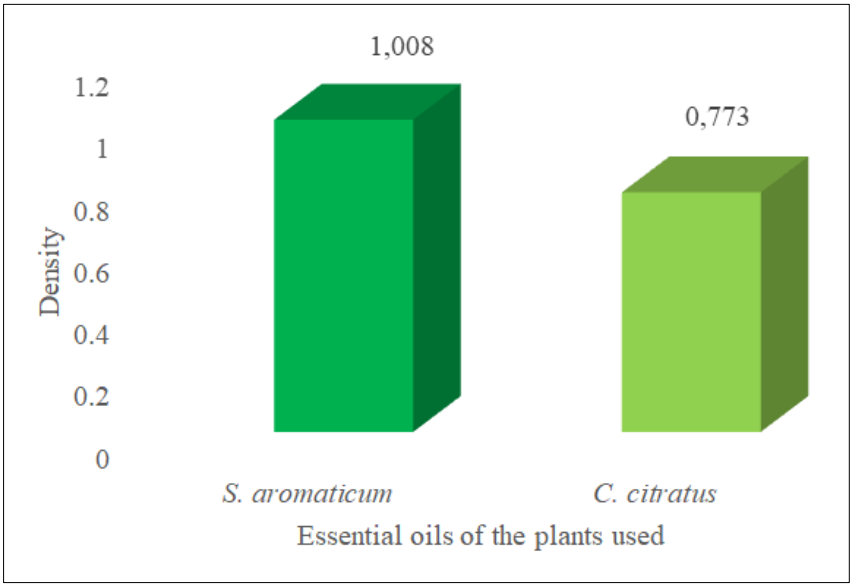

Figure 3: Density values

Figure 3 shows that the essential oil of *S. aromaticum* is denser, at 1.0081, while that of *C. citratus* is less dense, at 0.7734 (at 28.5°C). These results show a significant difference between the two oils, probably related to their chemical composition [22-28].

The high density observed in *S. aromaticum* can be explained by the oil's high eugenol content, an aromatic phenol with a relatively high molecular weight, which contributes to the oil's heaviness [22]. Conversely, *C. citratus* is characterized by a high content of citral, a lighter monoterpene aldehyde, which explains the lower density observed [26].

These observations are consistent with data in the literature, which generally report densities close to 1 for clove oil and between 0.70 and 0.80 for lemongrass oil [29]. However, it should be noted that the density of essential oils can vary depending on botanical origin, harvesting conditions, plant development stage, and extraction process [6].

We believe that the difference in density observed between *S. aromaticum* and *C. citratus* not only reflected their distinct phytochemical profiles, but also constitutes a relevant physicochemical criterion for their identification and quality control.

b. Hydrogen Potential (pH)

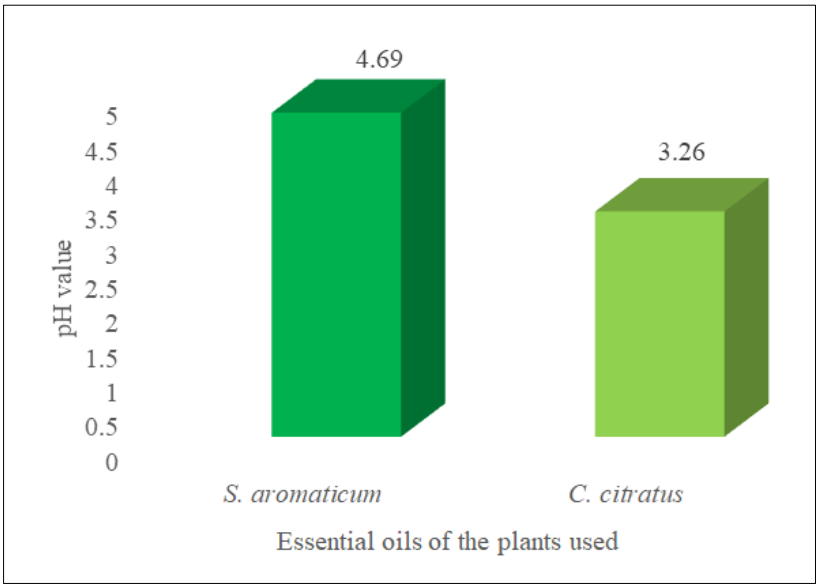

Figure 4: pH values of essential oils

This figure shows that the essential oil of *S. aromaticum* has a higher pH value (4.69) compared to that of *C. citratus* (3.26). This difference suggests that *S. aromaticum* oil is less acidic than *C. citratus* oil.

The relatively high pH value observed in *S. aromaticum* could be attributed to its high content of eugenol, a phenol with slightly acidic properties, which tends to raise the pH of the solution [22]. In contrast, *C. citratus* oil, which is dominated by citral (a monoterpene aldehyde), is more acidic, which explains the lower pH measured in this study [26].

These results are consistent with the literature, where oils rich in aldehydes generally have a more pronounced acidity, while those with a high phenol content show more moderate pH value [29]. However, it should be remembered that the pH of essential oils can vary depending on the geographical origin of the plant, the stage of harvest, and storage conditions [24].

The difference observed between *S. aromaticum* and *C. citratus* in terms of pH reflects the diversity of their chemical composition and constitutes a complementary physicochemical parameter useful in their characterization and quality control.

c. Refractive Index

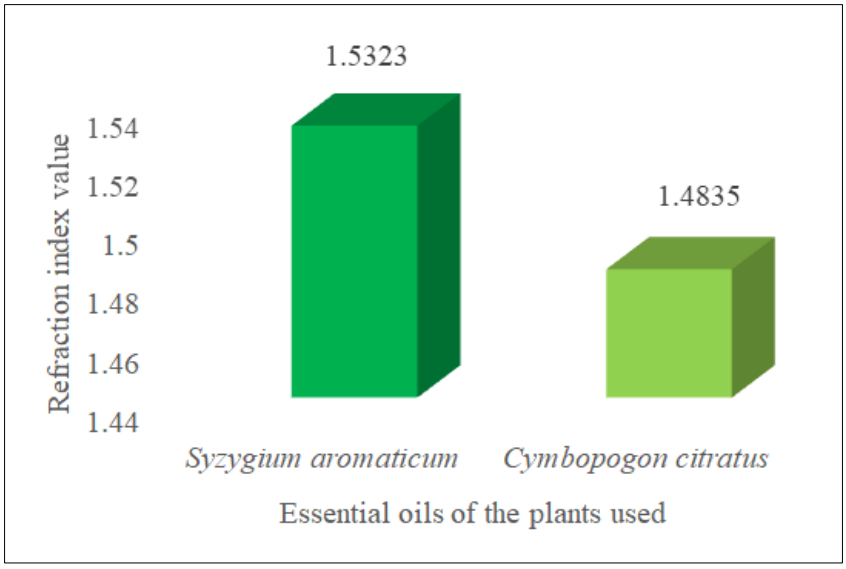

Figure 5: Refractive index of essential oils

The refractive index of *S. aromaticum* essential oil is 1.5323, while that of *C. citratus* is 1.4835. These values fall within the range generally reported for essential oils, between 1.450 and 1.600, depending on the richness in volatile and aromatic compounds [6]. They therefore reflect the characteristic optical nature of these oils.

The refractive index measured for *S. aromaticum* (1.5323) corresponds to the values reported by several authors (1.530–1.540), attributed to its high eugenol content, an aromatic phenol known to raise the optical index of essential oils [22]. Conversely, the refractive index of *C. citratus* (1.4835) is consistent with the ranges described in the literature (1.480–1.490), associated with oils rich in terpene aldehydes such as citral [30, 31].

These observations suggest that *S. aromaticum* oil is chemically denser in complex aromatic compounds, while *Cymbopogon citratus* contains lighter terpene molecules, consistent with the work of [24-32], on the chemical profiles of aromatic essential oils.

Finally, the refractive index is a crucial parameter for characterization and quality control: it allows the detection of alterations, adulterations, or the presence of non-volatile fractions. The values obtained confirm the purity and conformity of the oils analyzed, guaranteeing their reliability for biological testing [33].

d. Miscibility of Essential Oils

Table 2: Miscibility of essential oils in water and ethanol

Essential oils	Miscibility with water	Miscibility with ethanol
Cymbopogon citratus	Unmiscible	Miscible
Syzygium aromaticum	Immiscible	Miscible

This study showed that *S. aromaticum* essential oil is not miscible in water, while *C. citratus* essential oil has very low miscibility. However, both essential oils studied were found to be completely miscible in ethanol.

This behavior is consistent with the chemical nature of essential oils, which are mainly composed of hydrophobic compounds (terpenes, phenols, aldehydes). These molecules do not easily associate with the polar molecules of water, thus explaining the very low solubility observed [24]. On the other hand, their miscibility in ethanol is due to the amphiphilic nature of this solvent, which is capable of interacting with both the hydrophobic groups and the polar functions present in the oils [34].

Furthermore, the differences in miscibility observed between *S. aromaticum* and *C. citratus* can be attributed to their respective chemical compositions. *S. aromaticum* oil, rich in eugenol, a low-polar phenolic compound, shows very low affinity for water. Conversely, *C. citratus* oil, dominated by citral (monoterpene aldehyde), has slight aqueous solubility, although it remains limited [26].

These observations confirm the importance of miscibility as a parameter for the physicochemical characterization of essential oils, directly linked to their composition and potential for use in various pharmaceutical, food, and cosmetic formulations.

III.3. Antibacterial Activity of Essential Oils and Antibiotics on *Salmonella Typhi* Strains Tested After 24 Hours of Incubation

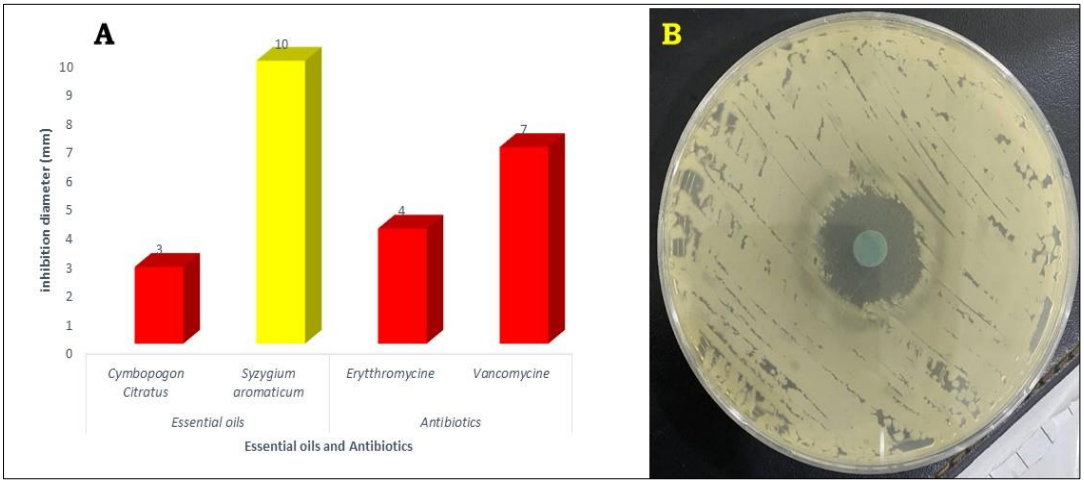

Figure 5: (A) Sensitivity of *Salmonella typhi* strain to essential oils and antibiotics and (B) *S. aromaticum* disc on *S. typhi* strain.

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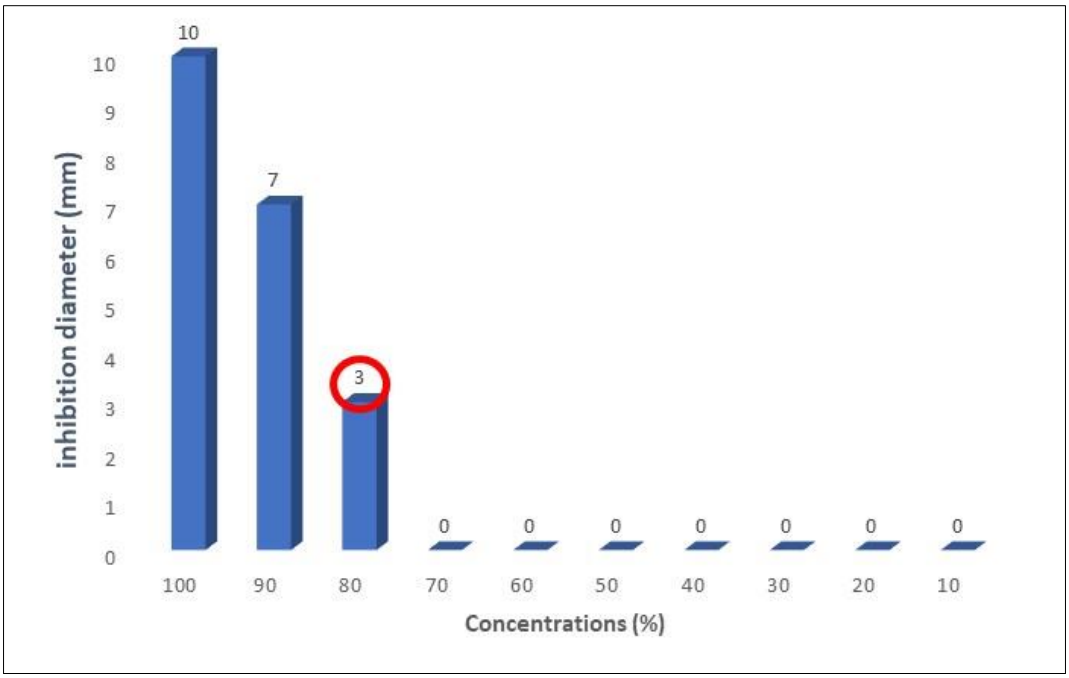

Figure 6: Minimum inhibitory concentration of *Syzygium aromaticum* essential oil on *Salmonella typhi strain*

The figure above highlights the antibacterial activity of *S. aromaticum* essential oil on *S. typhi*. It shows that this essential oil reaches the inhibition starting point (3 mm) at a concentration of 80%. This concentration (80%) is therefore considered to be the MIC of *S. aromaticum* essential oil on *S. typhi*.

At higher concentrations (90% and 100%), the inhibition zones reach 7 mm and 10 mm respectively, confirming increasing efficacy with concentration. These results confirm the known antimicrobial properties of *S. aromaticum*, which is rich in eugenol, a phenolic compound known for its bactericidal effects.

Our observations are consistent with those of Chaieb *et al*., [22], who demonstrated moderate activity of clove essential oil against enterobacteria. Similarly, Bouyahya *et al*., [29] report good efficacy of eugenol oils on Gram-negative bacteria such as Salmonella, although often less marked than on Gram-positive bacteria.

Thus, despite a relatively high MIC, *S. aromaticum* shows interesting potential as a natural antibacterial agent, particularly in the context of resistance to conventional antibiotics.

CONCLUSION

This study evaluated in vitro the antibacterial activity of *Syzygium aromaticum* and *Cymbopogon citratus* essential oils on a strain of *Salmonella typhi* isolated in Kisangani (DR Congo), in a context marked by increasing antibiotic resistance.

Extraction yields showed a clear superiority of *S. aromaticum* (0.85%) over *C. citratus* (0.31%). Antibacterial tests showed greater inhibitory activity of *S. aromaticum* essential oil against *S. typhi* (inhibition diameter: 10 mm) compared to *C. citratus* (3 mm) and reference antibiotics. The MIC of *Syzygium aromaticum* was estimated at 80%.

The essential oil of *S. aromaticum* has interesting antibacterial potential against *S. typhi* and could be a natural alternative to conventional antibiotic treatments. Further investigations, including in-depth phytochemical analyses and in vivo studies, are needed to confirm its efficacy, safety, and mode of action.

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