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In vitro Evaluation of the Antibacterial Activity of *Drypetes gilgianna Essential Oil* on Escherichia Coli and *Staphylococcus aureus* Strains in the Kisangani Region (DR Congo)

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Abstract

Resistant bacterial infections are a major public health problem, requiring the search for new therapeutic alternatives. This study aims to evaluate in vitro the antibacterial activity of essential oil extracted from *Drypetes gilgiana* leaves on strains of *Escherichia coli* and *Staphylococcus aureus* isolated in Kisangani (DR Congo). The essential oil was obtained by hydrodistillation and then characterized organoleptically and physicochemically. Its antibacterial activity was analyzed using the agar diffusion method and by determining the minimum inhibitory concentration (MIC). The extraction yield was low (0.086%), but the essential oil had physicochemical and organoleptic characteristics favorable to its biological activity. The oil showed very strong antibacterial activity against *E. coli* (inhibition diameter 78 mm) and moderate activity against *S. aureus* (9 mm). The minimum inhibitory concentration (MIC) confirmed a higher sensitivity for *E. coli* (40%) than for *S. aureus* (80%). These results suggest that *D. gilgiana* essential oil could be a promising source of antimicrobial agents, warranting future studies to isolate the active compounds and evaluate their mechanism of action.

Keywords: *Drypetes Gilgiana*, Essential Oil, Antibacterial Activity, *Escherichia Coli*, *Staphylococcus Aureus*.

I. INTRODUCTION

Bacterial infections remain a major public health problem worldwide. Their impact is particularly pronounced in sub-Saharan Africa, where poor hygiene, limited access to healthcare, and inadequate surveillance contribute to the morbidity and mortality associated with these infections [1, 2]. Recent estimates attribute more than one million deaths directly to bacterial resistance.

Among the pathogens responsible for common infections in humans, Escherichia coli and *Staphylococcus aureus* occupy a prominent place. *E. coli* is frequently involved in gastrointestinal and urinary tract infections, while *S. aureus* causes skin, osteoarticular, respiratory, and serious systemic infections [3, 4]. The effectiveness of conventional antibiotics is threatened by the emergence and spread of resistant strains, making it necessary to explore new sources of antimicrobials [5].

Medicinal plants are a promising avenue for the discovery of alternative antimicrobial agents [6]. Essential oils (EOs) in particular are rich in volatile compounds (phenols, terpenes, alcohols, aldehydes) that have demonstrated in vitro antibacterial activity against a wide spectrum of bacteria, including *E. coli*, *Salmonella spp*. and *S. aureus*; however, sensitivity varies depending on the bacterial species and the chemical composition of the oils [7].

The genus Drypetes (family Putranjivaceae) includes several species traditionally used in Africa to treat various infectious and gastrointestinal conditions.

Pharmacognostic and phytochemical studies on this genus reveal the presence of triterpenes, alkaloids, and other metabolites with biological activity, and some species have shown moderate and promising antimicrobial activity in preliminary studies [8].

Drypetes gilgiana in particular is cited in ethnobotanical inventories and studies as a medicinal plant used locally. However, published data specific to the essential oil of this species and its antibacterial activity on clinical strains of *E. coli* and *S. aureus* remain limited. This gap warrants rigorous investigation to characterize the essential oil and evaluate its in vitro efficacy on locally isolated strains [9].

The aim of this study is therefore to evaluate in vitro the antibacterial activity of essential oil extracted from *D. gilgiana* leaves on strains of *Escherichia coli* and *Staphylococcus aureus* isolated in the Kisangani region (DR Congo) in order to contribute to the promotion of local phytotherapeutic resources on the one hand and to guide further studies on the identification of active compounds and the evaluation of their mechanism of action on the other.

II. MATERIALS AND METHODS

II.1. Study Setting

This research was conducted in the city of Kisangani, capital of Tshopo Province, in the Democratic Republic of Congo (DRC). Kisangani is located at approximately 0°31' north latitude and 25°11' east longitude, about 57 km north of the equator. It lies at an average altitude of 428 meters above sea level [10].

II.2. MATERIALS

II.2.1. Essential Oil

The essential oil was extracted by steam distillation, a classic method for extracting volatile compounds. The dried leaves of *Drypetes gilgiana* were crushed and then immersed in a flask containing water. This flask, heated using a flask heater, allows the mixture to reach boiling point [11]. The water vapors carrying the volatile compounds are then directed to a coil condenser where they are condensed. The distillate obtained is collected in a separating funnel, allowing the essential oil to be separated from the hydrosol by difference in density. The essential oil is then recovered and stored in airtight bottles, protected from light [11].

The antibacterial activity of the extracted essential oil was tested on strains of *Escherichia coli* and *Staphylococcus aureus*. As a result, Ciprofloxacin and Ceftriaxone, two commonly used antibiotics, were used as positive controls.

The essential oil was then collected and stored in airtight bottles, protected from light [11].

II.2.2. Obtaining and Storing Strains

Germs were isolated using the swab technique. Sterile swabs were dipped into test tubes containing sterile peptone water. These swabs were then rubbed on the urinals of the Faculty of Sciences and the Bar Beach BRALIMA headquarters in Kisangani. Finally, each swab was returned to its test tube, sealed, and sent directly to the Microbiology and Phytopathology Laboratory of the Faculty of Sciences at the University of Kisangani for isolation of strains on two selective media: Eosin methylene blue (EMB) for *E. coli* strains and Staphylococcus agar for Staphylococci [12-14].

The strains were inoculated by deep puncture into tubes containing soft agar for preservation [13].

II.3. METHODS

II..3.1. Preculture and Characterization of Strains to Be Tested

a. Preculture

The strains to be tested were inoculated into tubes, each containing 3 ml of peptone water (1 g/100 mm) for revival, then incubated at 37°C in an incubator for 6 to 10 hours [14-16].

b. Characterization of Strains to Be Tested

❖ **Gram Staining**

Gram staining is an essential step in the characterization of isolated bacterial strains, as it allows Gram-positive bacteria to be distinguished from Gram-negative bacteria [17]. To do this, 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 [18].

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 [19]. 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 [20].

❖ **Biochemical Characterization of *Escherichia Coli* Strains**

The biochemical characterization of Escherichia coli 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 [21].

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 [21]. 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) [21]. Simmons citrate detects the use of citrate as the sole carbon source (change from green to blue) [22]. The Vosges-Proskauer test reveals the production of acetoin (light yellow color) [22], while methyl red indicates the production of acids by fermentation, resulting in the medium turning red [22].

❖ **Biochemical Characterization of the *Staphylococcus Aureus* Strain**

The biochemical characterization of the *S. aureus* strains was performed using the coagulase test with 0.5 ml of citrated human blood plasma mixed with 0.5 ml of inoculum, and the mixture was incubated at 37°C for 24 hours. Plasma coagulation indicates a positive result [23]. However, the catalase test uses a bacterial suspension brought into contact with 3% hydrogen peroxide; the formation of air bubbles indicates a positive reaction [24].

Mannitol fermentation is assessed by observing a color change in the medium from red to yellow using phenol red indicator. This test distinguishes certain coagulase-positive strains from coagulase-negative strains [23]. Finally, the DNase test consisted of inoculating the strains on DNA agar and then revealing the enzymatic activity by adding hydrochloric acid; the appearance of a clear halo around the inoculation indicates a positive reaction [23]. These tests thus confirm the biochemical identity of the isolated strains and evaluate their specific metabolic characteristics.

II.3.2. Antibacterial Activity of Essential Oil

Discs measuring 7 mm in diameter 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. The aromatogram, obtained by diffusing the aromatic compounds into a gelatinous medium in a Petri dish, made it possible to measure the inhibition zone around the disc (mm) [20].

The results were read by measuring the diameter of the inhibition zone in millimeters, which allows the sensitivity or resistance of the bacterial strains tested to be determined [20].

❖ **Antibacterial Activity of Antibiotics**

The Mueller Hinton agar diffusion method was used to evaluate the activity of antibiotics. This method involves placing antibiotic discs on agar. These discs diffuse according to a concentration gradient. This creates an inhibition zone around the disc, which varies in size depending on the sensitivity of the strain and the diffusion power of the antibiotic [26].

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 [26].

II.3.3. 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 [11-26].

II.4. Statistical Analyses

Statistical analyses were performed using R.4.5.1 software.

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

III. RESULTS AND DISCUSSION

III.1. Essential Oil Extraction Yield (RHE = $\frac{M'}{M}$×100)

The yield obtained is 0.086%. This value is very low compared to other medicinal plants often used for essential oil extraction, such as *Syzygium aromaticum*, which can exceed 1% [27], *Cymbopogon citratus* is around 0.3 to 0.5% [28].

The low yield observed could be explained by several factors, including the botanical nature of *D. gilgiana*, the low concentration of volatile metabolites in its leaves, and the harvesting and drying conditions [11]. This result corroborates previous studies showing that species of the genus Drypetes generally produce low yields of essential oil but contain powerful bioactive compounds such as alkaloids, flavonoids, and terpenoids [29].

III.2. Characterization of Essential Oil

III.2.1. Organoleptic Quality

Fig. 1: Organoleptic profile of the essential oil

The essential oil of *D. gilgiana* was reddish in color and had a pungent odor. These characteristics reflect the presence of volatile aromatic compounds typical of essential oils, including phenols, aldehydes, and oxygenated terpenes, which are often responsible for colorful hues and strong odors [30]. The reddish color is thought to be due to the presence of natural oxidizable pigments or certain phenolic compounds derived from plant material. Such colors have already been observed in oils rich in oxidized aromatic compounds [31].

The pungent odor, on the other hand, is indicative of a high concentration of active volatile compounds, such as eugenols or aromatic aldehydes, known for their antimicrobial and antiseptic properties. These results confirm the interest of *D. gilgiana* as a potential source of bioactive molecules for pharmaceutical or cosmetic use.

III.2.2. Physicochemistry

a. Density

The density of *D. gilgiana* essential oil is 0.946. This value is within the expected range for essential oils (0.850 to 1.050), indicating a composition characteristic of volatile essential oils [32].

The measured density (0.946) is close to that reported for several plant-derived essential oils such as *Eucalyptus globulus* (0.905–0.925) and *Mentha spicata* (0.920–0.940) [33, 34]. This variability depends on the specific chemical composition of the oils, particularly the proportion of monoterpenes, sesquiterpenes, and phenolic compounds. The value obtained for *D. gilgiana* suggests an intermediate composition, probably dominated by terpenic and flavonoid metabolites, as has been reported for other species of the genus Drypetes [29].

In addition to serving as a characterization parameter, density is also a quality control criterion. It confirms that the oil obtained complies with reference standards and can be used as study material for biological evaluation [35].

b. pH

The pH measurement of *D. gilgiana* essential oil revealed a value of 4.87. This acidic value is within the range generally observed for essential oils, between 3.5 and 6.0 [30].

The pH of 4.87 obtained in our study indicates the presence of acidic or phenolic compounds that give the essential oil its acidic character. Similar results have been reported for essential oils from *E. globulus* (pH 4.6–5.1) and *Melaleuca alternifolia* (Tea tree, pH 4.7–5.4) [36, 37]. The acidity of essential oils is considered a factor that promotes their antimicrobial activity because it can enhance the inhibitory effect by creating an environment that is unfavorable to bacterial growth [11].

In addition, pH is also a physicochemical quality parameter that can be used to monitor the stability of essential oils. A value close to neutrality would indicate a low content of active compounds, while a value that is too acidic could indicate degradation or oxidation [35]. The pH of 4.87 observed here is therefore consistent with a good quality essential oil, capable of retaining its biological properties.

c. Refractive Index

The refractive index measured for *D. gilgiana* essential oil is 1.5233, a relatively high value that reflects a high optical density. Essential oils generally have refractive indices between 1.450 and 1.570, depending on their richness in aromatic, phenolic, or terpenic compounds [38]. The value obtained places *D. gilgiana* among oils with a complex composition, probably rich in sesquiterpenes, diterpenes, or phenolic compounds, which are known to increase the refractive index [11].

This value is close to those reported for oils such as *Syzygium aromaticum* (1.530–1.535), whose high eugenol content explains its particularly high index [39]. However, it exceeds that of *Cymbopogon citratus* (1.480–1.491), which is characterized by a majority of citral, a less optically dense monoterpene [40]. The essential oils of *Thymus vulgaris*, *Cinnamomum zeylanicum*, and *Origanum compactum* also have indices above 1.50, often linked to a high concentration of phenols such as thymol or carvacrol [7]. The similarity between the value obtained for *D. gilgiana* and these oils suggests a composition with highly condensed molecules, consistent with the chemistry reported for other species of the genus Drypetes, known for their diversity in terpene and flavonoid metabolites [41].

The refractive index is also an excellent quality control parameter, allowing the chemical integrity of an essential oil to be assessed. A significant variation in this

value would indicate either oxidation or alteration due to prolonged storage or contamination. The result of 1.5233 therefore indicates a stable, non-oxidized oil, probably rich in bioactive compounds, justifying its interest for further biological analyses.

d. Miscibility of Essential Oils

The essential oil extracted from *D. gilgiana* was found to be immiscible in water but completely miscible in ethanol.

The immiscibility of this oil in water is consistent with the physicochemical properties generally observed for essential oils, which are mainly composed of hydrophobic compounds (terpenes, terpene alcohols, phenols, etc.) [11]. This hydrophobicity limits their dispersion in aqueous media, but explains their ability to interact with the lipid membranes of microorganisms, which contributes to their antimicrobial activity [7].

On the other hand, miscibility in ethanol is an expected characteristic, since ethanol is a polar organic solvent capable of effectively solubilizing the hydrophobic components of essential oils [27]. This property is often exploited to prepare stock solutions used in microbiological tests.

Our results are consistent with those reported for other essential oils, such as those of *Mentha piperita*, *E. globulus*, and *Lavandula angustifolia*, which are also immiscible in water but miscible in ethanol [38, 39].

III.3. Antibacterial Activity of Essential Oils and Antibiotics on *E. coli* and *S. Aureus* Strains Tested After 24 Hours of Incubation

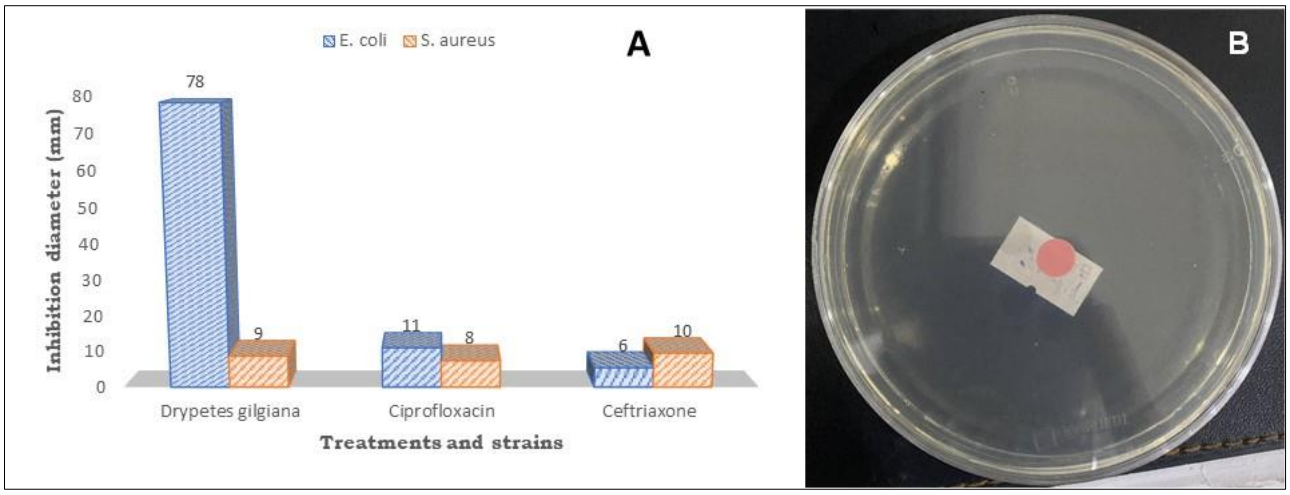

Fig. 1: (A) Sensitivity of *E. coli* and *S. aureus* strains to *D. gilgiana* essential oil and antibiotics, and (B) *D. gilgiana* disc on *E. coli* strain

The results in Figure 1 show that the essential oil extracted from *D. gilgiana* has remarkable antibacterial activity, particularly against *E. coli*, with an inhibition diameter of 78 mm, significantly higher than that of the reference antibiotics used in this study (ciprofloxacin: 11 mm; ceftriaxone: 6 mm). On the other hand, the efficacy observed on *S. aureus* (9 mm) remains comparable to that of antibiotics (ciprofloxacin: 8 mm; ceftriaxone: 10 mm).

This observation is surprising in that, according to the literature, essential oils generally show better activity against Gram-positive bacteria (*S. aureus*) than against Gram-negative bacteria (*E. coli*), due to the complexity of the outer membrane of Gram-negative bacteria, which limits the penetration of hydrophobic molecules [7]. This strain of *S. aureus* is resistant to both the tested oil and reference antibiotics. This resistance is thought to be due to either a mutation or an acquired factor (plasmid) [40].

However, some studies have also reported high sensitivity of *E. coli* to oils rich in phenolic and terpenic compounds [30]. It is therefore likely that the specific chemical composition of *D. gilgiana* contains bioactive molecules capable of crossing this barrier.

The performance of this essential oil could be explained by the presence of secondary metabolites such as alkaloids, flavonoids, or terpenoids, which have already been identified in other species of the genus Drypetes [29]. These compounds are known to act on membrane integrity and cause leakage of cellular constituents, leading to inhibition of bacterial growth [41].

Our results suggest that *D. gilgiana* essential oil could be a promising alternative or potential adjuvant to antibiotics in the treatment of infections caused by *E. coli* and *S. aureus*. However, further studies, including chromatographic analysis and in vivo tests, are needed to

confirm this efficacy and identify the compounds responsible for this activity.

III.4. Minimum Inhibitory Concentration of *D. Gilgiana* Essential oil on *E. coli* and *S. Aureus* Strains

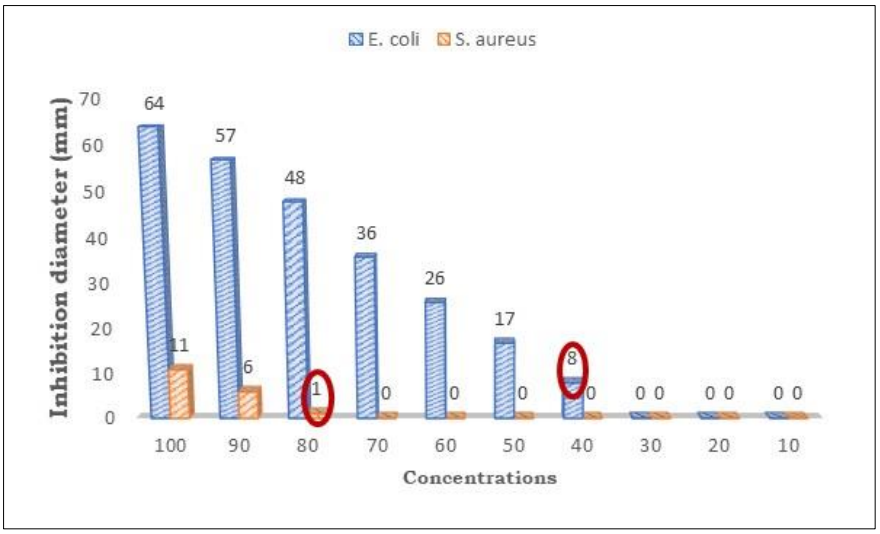

Fig. 2: Minimum inhibitory concentration of *D. gilgiana* essential oil on *Escherichia coli* and *Staphylococcus aureus* strains

The figure above shows that the minimum inhibitory concentration (MIC) of *D. gilgiana* essential oil is 40% for *Escherichia coli* and 80% for *Staphylococcus aureus*. This difference reflects a greater sensitivity of the *E. coli* strain tested compared to that of *S. aureus*. This observation contrasts with several previous studies, which generally report higher resistance in Gram-negative bacteria due to the complexity of their outer membrane, which is composed of lipopolysaccharides [7].

However, some authors have also pointed out that bacterial sensitivity to essential oils depends largely on their chemical composition. Oils rich in phenols (such as eugenol, thymol, or carvacrol) and oxygenated monoterpenes have shown significant inhibitory activity against *E. coli* [30]. It is therefore likely that *D. gilgiana* oil contains specific compounds capable of altering the outer membrane of Gram-negative bacteria, thereby facilitating their inhibition.

In comparison, the weaker activity observed on *S. aureus* could be explained by a lower affinity of the oil's bioactive constituents with the peptidoglycan wall characteristic of Gram-positive bacteria [41]. In addition, some strains of *S. aureus* are known to produce enzymes (such as β-lactamases) and oxidative stress tolerance mechanisms that could reduce the effectiveness of essential oils [42].

These results suggest that *D. gilgiana* essential oil is a promising candidate against *E. coli*, but its effectiveness against *S. aureus* remains limited. This difference in sensitivity highlights the importance of conducting chromatographic analyses to identify the molecules responsible for this activity and possibly study synergies with other oils or antibiotics.

The ANOVA for the comparison of treatments on *E. coli* strains shows that the p-value (< 0.0001) is well below 0.05, which means that there is a highly significant difference between at least two of the treatments tested on E. coli.

Tukey's HSD post-hoc test showed that *D. gilgiana* has significantly higher antibacterial activity (78 mm) compared to Ciprofloxacin (11.1 mm) and Ceftriaxone (5.8 mm). The three treatments belong to distinct homogeneous groups (a, b, c), indicating real differences in efficacy. Thus, *D. gilgiana* is distinguished by its superior antibacterial power against E. coli.

D. gilgiana has significantly stronger activity, Ciprofloxacin has moderate antibacterial activity, and Ceftriaxone has the weakest activity.

The ANOVA test for comparing treatments on *S. aureus* strains showed that the p-value (< 0.001) is much lower than 0.05, confirming that not all treatments have the same antibacterial effect on this strain.

The Tukey HSD post-hoc test showed that Ceftriaxone has the highest activity, *D. gilgiana* has intermediate activity, and Ciprofloxacin has the lowest activity.

CONCLUSION

The aim of this study was to evaluate in vitro the antibacterial activity of essential oil extracted from *D. gilgiana* leaves on strains of *E. coli* and *S. aureus*

isolated in the Kisangani region of the Democratic Republic of Congo.

The essential oil was obtained by hydrodistillation and then characterized organoleptically and physicochemically. Its antibacterial activity was analyzed using the agar diffusion method and by determining the minimum inhibitory concentration (MIC).

This study shows that the essential oil tested has remarkable antibacterial properties, particularly against the *E. coli* strain, with an inhibition diameter (78 mm) significantly higher than that of the reference antibiotics, Ciprofloxacin (11 mm) and Ceftriaxone (6 mm). However, its activity against the *S. aureus* strain (9 mm) remains moderate and comparable to that of antibiotics, reflecting the relative resistance of this strain.

The extraction yield of *D. gilgiana* essential oil was low (0.086%), but its physicochemical and organoleptic characteristics (density of 0.946, acidic pH of 4.87, reddish color, and pungent odor) indicate the presence of bioactive compounds potentially responsible for the observed antibacterial activity. The oil's miscibility in ethanol and its insolubility in water confirm its hydrophobic nature, consistent with the properties of essential oils and their interaction with bacterial membranes.

The minimum inhibitory concentration (MIC) obtained shows a more marked sensitivity of *E. coli* (40%) compared to *S. aureus* (80%), highlighting that the effectiveness of the essential oil varies according to the strain and its chemical composition. Statistical analyses using ANOVA and Tukey's post-hoc test confirmed that *D. gilgiana* essential oil has significantly higher antibacterial activity against *E. coli* than the reference antibiotics, while its activity against *S. aureus* remains intermediate.

However, further research through in-depth phytochemical analyses and in vivo studies would be necessary to confirm the active ingredients responsible for this activity and to evaluate their toxicity, stability, and mode of action.

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