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Test of the Reduction of Pathogenic Microorganisms in Wastewater from the Agro-food and Domestic Industries by the Essential Oil of *Eucalyptus citriodora*

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Abstract

The study evaluates *Eucalyptus citriodora* essential oil (EO) as an eco-friendly alternative to chemical disinfectants for treating wastewater from agro-food and domestic sources. It demonstrates the oil's antimicrobial effectiveness against Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa, achieving bacterial growth inhibition at low concentrations. While less potent than chlorine in total microbial elimination, the EO presents fewer environmental and health risks. The study concludes that EO is a viable option for sustainable wastewater disinfection and encourages further research into its applications.

Keywords: *Eucalyptus citriodora*; Essential Oils; Wastewater; Agro-Food Industries; Pathogenic Microorganisms.

INTRODUCTION

Water is an essential component for all living beings (humans, animals, plants); it is necessary for life and constitutes a means of cleaning. According to OMS (2015), 1.6 million children die each year due to unsafe water, lack of basic sanitation services, and poor hygiene. Thus, wastewater management must be a major concern for the authorities but also for the population. In 2017, according to the UN, in the metropolises of emerging countries with high demographics, poor wastewater treatment is still a source of serious health risks. 70% of wastewater is treated on average in high-income countries, but only 8% in developing countries.

In the world and particularly in the DRC, several practices is observed that threaten the balance of aquatic ecosystems: among others, the direct discharge of wastewater of all types into the receiving environment, without prior treatment, from spontaneous housing districts; dry latrines for traditional districts; septic tanks followed by cesspools in residential districts Kerspern and Carré (2008). This represents a high risk of spreading waterborne diseases and pollution of receiving environments. However, the rise of urbanization and population growth are the basis of the ever-increasing demand for water and, moreover, the production of wastewater. Humans have become familiar with pathogenic microorganisms under ignorance, if not negligence, in the sanitation and lack of hygiene in their indoor and outdoor environments. Pathogenic bacteria, fungi, and viruses contaminate and affect the health, much more, of children and the elderl OMS (2016).

In fact, in the DRC, in the city province of Kinshasa, the agro-food industries, in particular the slaughterhouses, discharge wastewater loaded with organic matter into receiving environments. The latter drain the city, such as the Masina slaughterhouse in the commune of Masina, discharging its effluents into the N'djili River Kakundika et al (2009). Furthermore, mainly in the communes along the coast of the N'djili River in Kinshasa, wastewater from septic tank emptying, households, and agricultural activities is channeled directly into the river, where it is discharged in disregard of environmental protection provisions. This wastewater not only affects the quality of the N'djili River and the living conditions of the organisms living there, but also hinders subsequent uses of the river's water (Helga-Jane 2017).

So to overcome this problem, it is obvious that the wastewater from this IAA and the agglomerations listed above undergo treatment before being discharged into the receiving ecosystems, in particular the N'djili River.

In addition, several collective sanitation techniques are used to treat water before its discharge into the environment. In the city of Kinshasa, exceptionally, the BIAMBA MUTOMBO hospital, in particular, uses the biodisc system coupled with chlorination. It is then important to emphasize that chlorine is the most commonly used disinfectant; its reaction with organic molecules can generate carcinogenic by-products and also cause chlorine to lose its disinfectant power in its residual action (Tangou, 2016).

Much work has been done by many researchers on the properties of essential oils for their uses. Indeed, mixtures of essential oils have proven to be very bactericidal and fungicidal (Gbenou 1994). On the other hand, their antiviral properties have been little studied. Interesting therapeutic effects have been obtained by inhalation on mice infected with *Pneumococus*. Some authors recommended *Cochlearia armoracia* oil for the treatment of urinary tract infections by Gram-positive and Gram-negative bacteria. In 1962, it was noted that most often, plant essences have a greater germicidal power than their vapors. Other authors have suggested their practical applications in cosmetics, the manufacture of disinfectants, and in the cure of microbial diseases (Gbenou 1994). Also, the essential oils of rosemary, savory, thyme, and lavender have antibacterial and antifungal properties (Pellecuer and CoU 1976). Furthermore, essential oils, particularly those extracted from garlic, onion, oregano, and thyme, have inhibitory effects on thirteen types of yeast (Conne and Boucha 1984). Most of the essential oils of forty Egyptian aromatic plants are fungicidal (Gbenou 1994).

Thus, in the context of the promotion and consumption of organic products as alternatives to chemicals that are dangerous for health and the environment, this research focused on the species *Eucalyptus citriodora* to solve multiple problems of contamination and water pollution. This choice is justified by the fact that this plant is rich in active ingredients, such as essential oils, and has diverse and important biological activities (Atmani-Merabet 2018).

MATERIALS AND METHODS

Plant Materials

The plant species *Eucalyptus citriodora* as well as effluents from the food industry and households constituted the main material for this study.

Eucalyptus Citriodora:

Is native to South America, Australia, China, Madagascar, and Vietnam. It grows particularly in tropical and temperate regions. It is distinguished by its lemony scent and its narrow, elongated leaves. This plant is widely cultivated for its medicinal, aromatic, and repellent properties. In terms of usage, *Eucalyptus citriodora* is often used in aromatherapy for its soothing and refreshing properties. Its essential oil is also known for its antiseptic, anti-inflammatory, antibacterial, and insect repellent properties. It is commonly used in the manufacture of cosmetics, cleaning products, and natural insect repellents Oliveira Pereira et al. (2015).

The plant material consists of the leaves of *Eucalyptus citriodora,* which were harvested in October 2023 on the site of the University of Kinshasa, in the commune of Lemba in the city province of Kinshasa. Its geographical coordinates are: 4°25'16.314" Latitude S; 15°18'38.628" Longitude E at 502 maltitude. The identification of this material was carried out at the INERA herbarium in the Department of Biology of the Faculty of Science and Technology of the University of Kinshasa.

Figure 1. *Eucalyptus citriodora leaves* found by author on the site of the University of Kinshasa

Effluents Sampling

The effluents from the collector of Quartier 8 of N'djili and those from the Masina slaughterhouse in the city province of Kinshasa, respectively, constituted the second experimental material.

The effluent samples were collected on December 22, 2023, at noon and then at 2 p.m., during the rainy season, at the outlet of the collector of district 8 and the abattoir, respectively. The water samples collected were placed in 750-ml plastic bottles containing newly purchased drinking water and rinsed with the effluents to be analyzed. The filled bottles were labeled and kept in a dark bag. Parameters such as pH, temperature, Total Dissolved Solids (TDS), and electrical conductivity were collected in situ using the HQ-006 multi-parameter probe before being sent to the Laboratory of Microbiology Applied to Natural Biological Resources (LaMarBn).

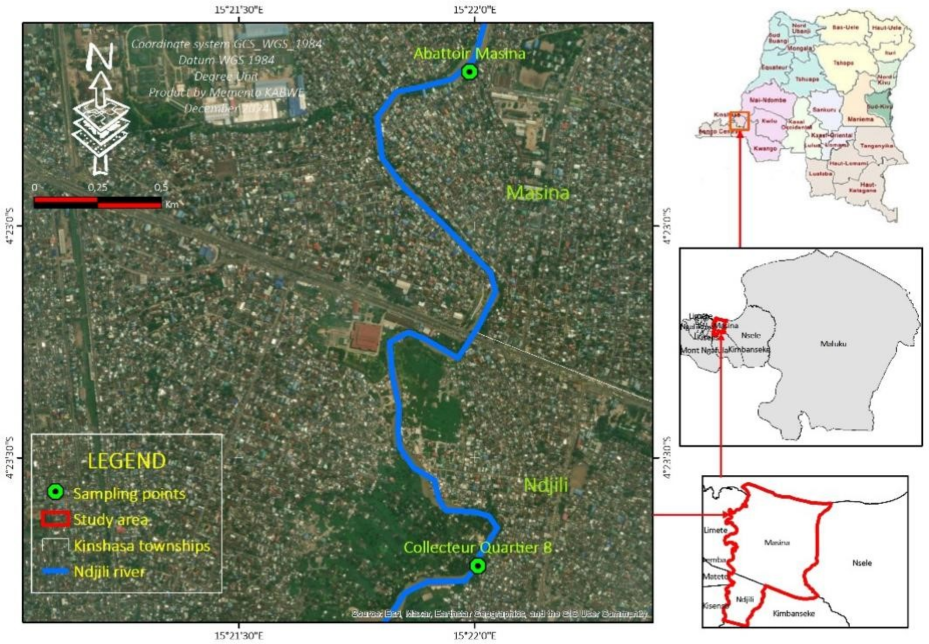

Figure 2. Illustration of the sampling area.

Essential oils extraction

Essential oils were extracted from the leaves of *Eucalyptus citrodora*. The leaves, previously dried away from the sun for 1 week and then weighed, were subjected to hydrodistillation using a Clevenger-type apparatus for 1h, 1h30, 2h, and 2h30 at 97°C.

Method: 80 g of *Eucalyptus citriodora leaves* are placed in a Pyrex glass flask with the addition of 600 ml of distilled water; bring the whole to boiling. The water vapor is condensed by passing through a condenser. Then, the appearance of the first drop of distillate is observed at the outlet of the vapor condensation tube, which carries the essential oil fixed by a suitable support in a vertical position to facilitate the flow of the distillate. The time of this extraction is approximately three hours. The distillate obtained is collected in a separating funnel; the mixture is left to stand for a few minutes, and then two phases appear, one organic (essential oil) and the other aqueous. The essential oil of the leaves of *Eucalyptus Citriodora* is thus collected in a sterilized bottle.

The conservation of essential oil requires certain essential precautions Burt. (2004). For this reason, the essential oil of *Eucalyptus Citriodora leaves* was stored in a refrigerator at a temperature of around 4°C in a sterile glass bottle tightly closed and covered with aluminum foil to protect it from contact with air and light.

According to the AFNOR AFNOR (1986), the yield of essential oil is the ratio between the mass of the essential oil obtained after extraction and the mass of the plant material used, i.e.,

$$R\ (\%) = \frac{mass\ of\ extracted\ oil\ (g)}{mass\ of\ plant\ material\ subjected\ to\ extraction\ (g)} \times 100 \qquad (1)$$

The acid index evaluates the content of free fatty acids present in the essential oil. To determine it, 0.5 g of essential oil is introduced into a flask, 25 mL of ethanol and 5 drops of the phenolphthalein indicator (PPT) are added, and the solution is neutralized with 0.01N sodium hydroxide.

The acid index is calculated by: **AI=Mm of NaOH**

Compound search: To do this, the aqueous phase test was carried out. The preparation of the aqueous extracts consisted of a maceration of 20 g of *Eucalyptus citrodora* leaves in 100 mL of distilled water for 24 h of incubation at room temperature, then filtered using filter paper.

Polyphenols, steroids, flavonoids, anthocyanins, tannins, alkaloids, saponins, and leucoanthocyanins were investigated.

Antimicrobial Testing

- ***Strains used***

The microbial strains tested in this study were isolated from the effluents of the Masina slaughterhouse and from households in district 8 in the commune of N'djili at the Laboratory of Microbiology Applied to Natural Biological Resources (LaMarBn).

The evaluation of the antibacterial activity of *Eucalyptus essential* oil is carried out first by the disk diffusion method, due to its simplicity and effectiveness in testing the sensitivity of bacteria. The minimum inhibitory concentrations (MIC) are estimated by the microplate dilution method.

- ***Solid-state technique****:* we used two methods:

a. Aromatogram method

This method consists of highlighting a possible antimicrobial activity of the extract of the plants listed in the presence of the test germs. Sterile absorbent disks were impregnated with a quantity of extract and placed on an agar inoculated with the strains. The diffusion of the extract in the agar makes it possible to follow the inhibition and growth of the germs, which will result in a clear zone around the disk called the inhibition zone Mouna (2015).

b. Microplate dilution method

It allowed us to determine a range of values of the minimum inhibitory concentrations (MIC) of the strains. To do this, the supercooled agar culture medium is poured into Petri dishes. The inoculum of 1 ml is seeded on the surface (swab seeding) after solidification of the medium. One or more aromatogram discs are placed in the center of the dish. Each disc is then impregnated with a variable quantity (between 1000 and 125 µl) of the essential oil. The dish is then closed and placed in an incubator at 37° for 12 to 18 hours. When removed from the incubator, the absence of microbial growth is reflected by a translucent halo around the disc, identical to sterile agar, the diameter of which is measured and expressed either in cm or mm.

- ***Preparation of dilutions of Eucalyptus Citriodora essential oil***

The technique used to determine the antibacterial power of EOs has a great influence on the results. Practical difficulties arise from the insolubility of the constituents of EOs in water, their volatility and the need to test them at low concentrations. A range of four dilutions was prepared for each pure essential oil, ranging from 1/2, 1/4, 1/8, up to 1/16, by adding 0.5 ml of DMSO to the four tubes for each of the essential oils. 1 ml of the pure oil is put in a test tube, then 0.5 ml of each EO from the tube is taken and poured into the second tube containing the 0.5 ml of DMSO, and each time, 0.5 ml is taken from the previous tube and added to the next tube until the last tube, where the last 0.5 ml is thrown away, so each tube finally contains 0.5 ml.

- ***Disk Distribution and Reading***

Sterile discs of Wattman paper, 6 millimeters in diameter, are grasped with sterile forceps and impregnated with a small quantity of essential oils (EO of *Eucalyptus citriodora*) previously prepared at different concentrations. They are then applied to the surface of the previously seeded culture media. We placed 4 discs per 6-centimeter box, with a space of 20 millimeters between the discs, then incubated in an oven (28 C°) for 24 hours.

The reading was taken after 24 hours by measuring the diameter (in millimeters) of the possible inhibition zone around the discs using a metal caliper outside the closed boxes.

According to Satrani et al (2007), the inhibition diameters of the cultures are as follows:

- The germ is insensitive when the diameter of the halo in mm is < 6, we note –
- The germ is sensitive when the diameter of the halo is between 7 and 14, then we note +
- The germ is very sensitive when the halo diameter is between 15 and 19, we note ++
- The germ is extremely sensitive when the halo diameter is > 20, we note +++

Statistical analysis

MS Excel was used to perform ANOVA to compare the different means of the results obtained and to determine significant differences.

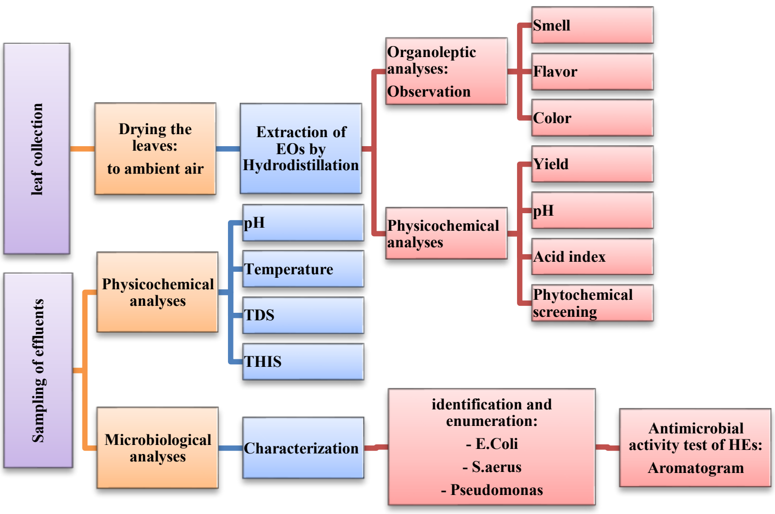

Figure 3. Summary diagram of the methodology.

RESULTS

Eucalyptus Citriodora Extracts gave the EO yields of 1.279±0.194%, 2.683±0.304%, 2.15±0.15%, and 11.69±0.15%, respectively, at 1h00, 1h30', 2h00, and 2h30'. All these different values are due to the fact that the yield of essential oil can be influenced by various factors, such as the genotype of the plant, its geographical origin, the harvest period, the climate, the physicochemical properties of the soil as well as the extraction method used, the plant parts used, and the drying conditions.

The extracted essential oil has a liquid, clear appearance, with a pale yellow color giving off a lemony odor. This corroborates with AFNOR AFNOR (2000), which describes the organoleptic aspects of the EO of *Eucalyptus citriodora* as being liquid, mobile and clear of colorless to pale yellow color and having a fresh odor, more or less eucalyptonic depending on the origin. The acid index and pH of the essential oil obtained are respectively 2.96±0.24 and 5.9±0.2. This would be due to the fact that eucalyptus essential oil is acidic in nature.

These different strains were found in the samples taken [Domestic effluent (Mabamaba) and Agro-food industry (Slaughterhouse)]: *E. coli, Proteus mirabilis, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella sp, Yersinia enterocolitica, Clostridium sp, Candida albicans*. The presence of these microorganisms is due to the lack of prior treatment of household and industrial wastewater constituting non-compliance with wastewater management standards.

Phytochemical screening of *Eucalyptus Citriodora* extracts revealed that its leaves contain seven metabolites out of the nine sought including: polyphenols, flavonoids, tannins, anthocyanins, alkaloids, leucoanthocyanins and steroids; and only Triterpenoids and Saponins were not present as was the case in the work of Erau P., who also reported that these compounds have antiviral and antimicrobial properties that could be used against pathogenic microbial strains.

Table 1. Physicochemical parameters of effluents.

Settings	Domestic effluent (Mabamaba)	Agro-food industry (Slaughterhouse)	NS 05-061 July 2001
pH	6.92±0.04	7.68±0.26	5.5-9.5
Temperature (°C)	25±0.0	26±0.0	≤ 30°C
TDS µg/L	0.062±0.0	59.26	1000mg/L
Conductivity µs/cm	77.41±0.0	75.31	<250µs/cm
MSS mg/L	210±0.0	482±222.7	
COD mgd'O_2/L	430±0.0	219.52±101.65	
BOD_5 mgd'O_2/L	190±0.1	181.34±136.52	
Phosphate mg/L	7±0.0	0.089±0.1	
Dissolved oxygen mg/L	-	1.8±1.0	

It is clear from this table that the wastewater is very loaded with organic matter in terms of COD, BOD_5 (181.34 mg/L). This is justified by the nature of the industry (agro-food industry), which discharges its effluents directly into the river without any treatment.

Table 2. Efficacy of the antimicrobial activity of *Eucalyptus Citriodora EO* and Chlorine (Cl) on *Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa*

Bacteria	Inhibition diameter in mm					
	Concentration in µg/ml of *Eucalyptus Citriodora* EO				P	Cl (50 µg/ml)
	250	125	65.5	31.25		
Staphylococcus aureus	9	9	9	-	3.5722E-06	15
Escherichia coli	10	10	10	10	1	17
Pseudomonas Aeruginosa	10	9	10	10	0.71889642	17

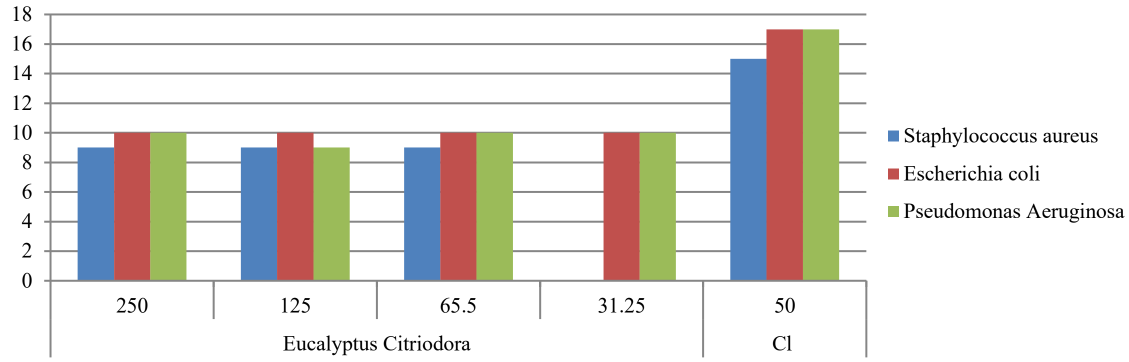

Figure 4. Efficacy of the antimicrobial activity of *Eucalyptus Citriodora EO* and Chlorine on *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa.*

Eucalyptus Citriodora EO is capable of limiting the proliferation of Staphylococcus *aureus* to 9 mm, *Escherichia coli* to 10 mm, and *Pseudomonas aeruginosa* to 10 mm. The 31.25 µg/ml concentration did not affect s*taphylococcus aureus*, but it had the same effects as other concentrations on *Escherichia coli* and *Pseudomonas aeruginosa*.

ANOVA: The p-value (0.00) is less than that of α (0.05); hence, the null hypothesis is rejected, i.e., the concentrations have a significantly different impact on the strains of *Staphylococcus aureus*. The p-values for *Escherichia coli* and *Pseudomonas aeruginosa* are 1 and 0.719, respectively, higher than that of α(0.05); hence, we accept that there is no significant difference between the disk means and that the concentrations have an equivalent impact on the two strains.

Table 3. Comparison of antibacterial activities of *Eucalyptus citriodora EO* and chlorine before and after treatment.

	***Eucalyptus citriodora* EO**		**Chlorine**	
	Sample1 (10ml)	Sample2 (10ml)	Sample1 (10ml)	Sample2 (10ml)
Before treatment	Innumerable	Innumerable	Innumerable	Innumerable
After treatment	5 UFC	6 UFC	0 UFC	1 UFC

Treatment with *Eucalyptus citriodora* essential oil reduced the colonies to 5 CFU and 6 CFU respectively for the first and second samples while that of chlorine reduced to 1 CFU and 0 CFU respectively for the second and first samples. This would be due to the chemical composition of the essential oil rich in active compounds including citronellal, to the mechanism of action (the EO acts by several mechanisms, notably by disrupting the cell membranes of bacteria and interfering with their metabolic processes), to the resistance of microorganisms (chlorine can be less effective against certain strains of bacteria, notably those which develop resistance or which are protected by biofilms) and to the experimental conditions.

Table 4. Effects of disinfectants on the physicochemical parameters of the samples

Settings	***Eucalyptus citriodora* EO**		**Chlorine**		**Discharge standards**
	Before treatment	After treatment	Before treatment	After treatment	
pH	7.00 ± 0.50	6.50 ± 0.01	7.00 ± 0.40	6.09 ± 0.95	Between 6 and 9.5
TDS mg/l	56.87	62.60	56.99	62.72	<1000 mg/L
Conductivity µS/cm	74.12	77.41	89.04	98	<250µs/cm

The results obtained show that the treatment given to the effluents by both essential oil and chlorine has no remarkable effect on the physicochemical parameters. All the values before and after treatment meet the discharge standards according to the French Association for Standardization Gbenou (1994).

Discussion

The essential oil yields of *Eucalyptus citriodora* obtained in percentage were 1.279±0.194, 2.683±0.304, 2.15±0.15, and 11.69±0.15, respectively, at 1h00, 1h30', 2h00, and 2h30'. These yields are lower than those found by Gbenou (1994) in the Ifangni region, which is 4.5%.

Furthermore, the differences between these values may be due to the fact that the essential oil yield can be influenced by various factors, such as the genotype of the plant, its geographical origin, the harvest period, the climate, the physicochemical properties of the soil as well as the extraction method used, the plant parts used, and the drying conditions (Tolba 2017).

The organoleptic characteristics of the extracted *Eucalyptus citriodora* essential oil have a mobile liquid appearance, with a pale yellow color giving off a lemony odor, results that corroborate with those found by Tolba Saba et al. (2013) in the Pakistan region; moreover, Likibi et al. (2019) in the Congo-Brazzaville region found a liquid, lemony but colorless oil, which is due to the drying time of the leaves, the nature of the soil, etc.

The acid index of *Eucalyptus citriodora* essential oil found is 2.96±0.24 mg/g with a pH of 5.9±0.2; this would be due to the fact that Eucalyptus essential oil is acidic in nature. This low acidity index recorded suggests that the essential oils used contain low or even negligible amounts of free acids, which does not affect their qualities. These results are in accordance with the standards of the French Association for Standardization (AFNOR, 1986), Tolba, 2017)

This low index indicates that the essential oil can be well preserved, unlike a high acid index, which triggers the oxidation of the essential oil Erau (2019).

Furthermore, *Eucalyptus citriodora* leaves contain seven metabolites out of the nine sought, including polyphenols, flavonoids, tannins, anthocyanins, alkaloids, leucoanthocyanins, and steroids, and do not contain terpenoids and saponins, as was the case in the work of Erau (2019). Who also reported that these compounds have antiviral and antimicrobial properties that can be used against pathogenic microbial strains? It was observed that the discharged domestic effluents from Mabamaba have a potential of hydrogen (pH) of 6.92±0.04, a temperature of 25±0.0°C, and a total dissolved solids (TDS) value of 0.062±0.0 µg/L, lower than the world average (1000 mg/l). this is also proven by Falasi (2018), a conductivity of 77.41±0.0 µs/cm, a suspended solids concentration (SS) of 210±0.0 mg/L, a chemical oxygen demand (COD) of 430±0.0 mg/L, a biochemical oxygen demand for 5 days (BOD_5) of 190±0.1 mg/L, a phosphate concentration of 7±0.0 mg/L; those of the slaughterhouse (Agro-Food Industry) have a hydrogen potential (pH) of 7.68±0.26; a temperature of 26±0.0°C, a TDS value (total dissolved solids) of 59.26 µg/L, a conductivity of 75.31 µs/cm, 482±222.7 mg/L for suspended solids (SS), 1.8±1.0 mg/L of dissolved oxygen (O_2), a chemical oxygen demand COD of 219.52±101.65 mg/L, a biochemical oxygen demand for 5 days BOD_5 of 181.34±136.52 mg/L, and phosphate of 0.089±0.1 mg/l. These values corroborate the results of Driss et al. (2009), who state that the wastewater from the Kenitra slaughterhouse is very loaded with organic matter in terms of COD and BOD_5 (181.34 mg/L).

During the experiments, the concentrations of *Eucalyptus citriodora* essential oil, in particular that of 250 µg/ml, limited the multiplication of *Staphylococcus aureus* to 9 mm, *Escherichia coli* to 10 mm and *Pseudomonas Aeruginosa* to 10 mm; followed by 125 µg/ml of *Eucalyptus citriodora* essential oil which limited the proliferation of *Staphylococcus aureus* to 9 mm, *Escherichia coli* to 10 mm, *Pseudomonas Aeruginosa* to 9 mm; followed by 65.5 µg/ml of *Eucalyptus citriodora* essential oil which limited the activity of *Staphylococcus aureus* to 9 mm, *Escherichia coli* to 10 mm, *Pseudomonas aeruginosa* to 10 mm; then that of 31.25 µg/ml of *Eucalyptus citriodora* essential oil which limited the activity of *Escherichia coli* and that of *Pseudomonas aeruginosa* to 10 mm. It was observed that Eucalyptus EO is effective even at low concentrations (65.5 and 31.25 µg/ml), and the latter presented an antimicrobial effect, respectively, for *Escherichia coli* and *Pseudomonas aeruginosa*. Indeed, these results demonstrate the effectiveness of Eucalyptus essential oil and are similar to those of Erau P., who conducted a study on the evaluation of the larvicidal and pupicidal activity of Eucalyptus essential oils on the main vector of dengue and Zika viruses. In addition, the carpet formed by the strains, after treatment with *Eucalyptus citriodora* essential oil, counted 5 colony-forming units (CFU) for domestic wastewater (1st sample) and 6 CFU for industrial wastewater (2nd sample) when the concentration of *Eucalyptus citriodora* essential oil is equal to the minimum inhibitory concentration value (31.25 µg/ml) in 24 hours. On the other hand, with chlorine, 0 colony-forming units (CFU) were counted for the first sample and 1 colony-forming unit (CFU) for the second sample. Based on these results, *Eucalyptus citriodora* EO could be used in small artificial and temporary water bodies as an environmentally friendly vector control measure in the era of increasing resistance to chemicals (Falasi 2018).

Thus, during the treatment of effluents based on *Eucalyptus citriodora* EO, the pH went from 7.00±0.50 to 6.50±0.01 after treatment. This drop in the pH of the treated effluents is justified by the fact that *Eucalyptus citriodora* EO is acidic in nature (pH-acid), the same result as Miloudi et al. (2020), who state that *Eucalyptus citriodora* EO are generally acidic. And after treatment with chlorine, the pH went from 7.00±0.40 to 6.09±0.95. This shows that chlorine influences the pH of the effluents more than *Eucalyptus citriodora* EO. In short, the essential oil tested showed some activity on the strains used. The greatest activity was noted on *Staphylococcus aureus*. It should be noted that the solvent used to prepare the essential oil at different concentrations does not influence the activity observed on bacteria. It should be noted that with *Eucalyptus citriodora* essential oil, the antimicrobial activity does not always increase with the increase in the amount of deposit.

The activity does not seem to be dose-dependent, unlike chlorine, whose total elimination of microbial germs from water depends on the dose of chlorine admitted (Christelle, 2018). This could be explained on the one hand by the more or less good dissolution, on the other hand by the diffusion or the load of the petri dishes (condition of absorption of germs by the agar) or even the composition of the essential oil Traoré et al (2010). The main limitation of *Eucalyptus citriodora* EO compared to chlorine is due to a lack of systematic increase in its antimicrobial activity with increasing applied dose. This means that, unlike agents such as chlorine, where the disinfection efficacy is directly related to the concentration used, the effect of the essential oil does not follow this trend. And there are other specific limitations such as microbial resistance (microorganisms can develop resistance to antimicrobial agents), different mechanisms of action (the essential oil acts through mechanisms that may not be directly related to concentration), complexity of components (essential oils are complex mixtures of chemical compounds; some of these components can interact synergistically or antagonistically), and environmental conditions (the effectiveness of an antimicrobial can also be influenced by environmental factors such as pH, temperature, and the presence of other substances in the water) Traoré et al (2010).

CONCLUSION AND RECOMMENDATIONS

This study aimed to test the effectiveness of the antibacterial activity of *Eucalyptus citriodora* EO as an alternative to chemical disinfectants of chlorinated compounds, on pathogenic microorganisms (*Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus*) found in the effluents of agri-food slaughterhouse and domestic activities.

The study had several interests, particularly on the environmental and health levels where it responds as a means of biological antimicrobial control that can be used on microbial strains contained in wastewater.

Initially, several hypotheses were put forward, including: the essential oil of *Eucalyptus Citriodora* would be effective in decontaminating effluents from food industry and households; the use of *Eucalyptus citriodora* essential oil as a bio-disinfectant would be a promising alternative to chemical disinfectants (chlorinated compounds) in the antimicrobial fight against *Escherichia coli, Pseudomonas aeruginosa* (Gram negative) and *Staphylococcus aureus* (Gram positive) species; these results can serve as a leitmotif for other microbial strains of the same categories studied, in particular Gram positive and negative microbial strains. After experimentation, all the hypotheses put forward are accepted; *Eucalyptus citriodora* essential oil has an influence on strains of *Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa*. In light of the above, the recommendations are addressed to:

- Industrialists: Pre-treat effluents before their discharge while respecting the discharge standards in force and invest in biological antimicrobial control methods, in particular the use of *Eucalyptus citriodora* EO;
- Researchers and economists: Further research on the antimicrobial power of *Eucalyptus citriodora*, to manufacture and make available to users finished products based on *Eucalyptus citriodora* in order to diversify the supply and reduce imports of antimicrobial products.
- Develop protocols based on the results of this study to determine the optimal concentrations of essential oil to be used, taking into account variations in the microbial load in wastewater.
- Conduct economic studies to compare the costs of applying essential oil with those of conventional chemical disinfectants, in order to justify its use in an industrial context.

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