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RESEARCH ARTICLE

CHEMICAL COMPOSITION AND ANTIBACTERIAL PROPERTIES OF *EUCALYPTUS CAMALDULENSIS* DEHN ESSENTIAL OIL ON PATHOGENIC BACTERIA: EXPLORING THE MECHANISM OF ACTION

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ABSTRACT

The uncontrolled use of antibiotics has led to the emergence of multi-resistant bacteria affecting human health. Many solutions have been sought in the hope of developing alternative andeffective molecules, includingmedicinalplants. The aim of the present study is to evaluate the membrane destabilization potential of the essential oil extracted from fresh leaves of *Eucalyptus camaldulensis* harvested in Benin. For this purpose, the essential oil was extracted by steam stripping. It was separated and identified by gas chromatography coupled to a mass spectrophotometer. This oil was subjected to physicochemical characterisation and sensitivity tests on reference and clinical bacterial strains. Subsequently, the minimum inhibitory concentrations, minimum bactericidal concentrations and antibiotic power were determined. The mode of action of the antibacterial effect of *Eucalyptus camaldulensis* essential oil was explored using the outer membrane destabilization test of bacterial strains. The average extraction yield was 0.57%, and its major compound was Terpinolene. All strains tested were sensitive to the essential oil except *Klebsiella oxytoca NCTC 13442*. The inhibition diameters of the essential oil ranged from 7 to 16 mm. The minimum inhibitory concentrations ranged from 3.106 to 12.5 mg/ml. In addition, the essential oil affected the bacterial strains tested by destabilizing their outer membrane. The percentage of outer membrane destabilization of *Salmonella typhi* and *Klebsiella pneumoniae* by the essential oil was significantly better than that of Imipenem used as a reference. The present study revealed that the essential oil of *Eucalyptus camaldulensis* has a remarkable inhibitory activity on Gram negative bacteria with a better percentage of membrane destabilization than the reference molecule. This essential oil is therefore a good alternative to counteract antibiotic resistance.

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INTRODUCTION

The discovery of antibiotics was a major breakthrough in medicine, as their use allowed the cure and prevention of many previously life-threatening infectious diseases of bacterial origin (el boujnouni, *2020*). Indeed, antibiotics have been essential for the treatment of bacterial infections in humans and animals since their discovery *(Khaoula et al., 2020*). However, the inappropriate use of these antibiotics in human and animal health has accelerated the emergence of antibiotic resistance in microbes (el boujnouni, *2020*). Antibiotic resistance is a real public health problem, causing the deaths of 700,000 people worldwide each year according to a British study (Konaté, *2021*). Faced with this resistance to antibiotics, many studies have been carried out to develop alternative and effective molecules, particularly of plant origin, to combat infections. Medicinal and aromatic plants are a promising source of potentially exploitable bioactive substances (Khaoula *et al., 2020*). Experimental evidence suggests that essential oils have significant potential, not only in neutralising drug-resistant bacteria, but also in reversing resistance to conventional antibiotics (Tisserand *and Young, 2014)***.** The applications of essential oils for different purposes are varied and often related to their therapeutic potential (antibacterial, antifungal, antiviral, antioxidant and anti- inflammatory).The most demanded essential oils on the world market are: lemongrass, peppermint, *Eucalyptus* oil, orange, *Eucalyptus* (*Camaldulensis* type), clove leaf, exotic verbena and spearmint (Bessah and Benyoussef, *2015*).*Eucalyptus* essential oil is used as a natural remedy for coughs, flu and especially as an anti-inflammatory. Numerous studies have highlighted the anti-oxidant, anti- cancer, anti-bacterial, anti-inflammatory, anti-infectious, anti-spasmodic, insecticidal and acaricidal properties of *Eucalyptus* essential oil (Aleksic Sabo and Knezevic, 2019; Allanto et al., 2022; Baba-Moussa et al., 2012; Ghasemian et al., 2019; Malti, 2019; Taheri et al., 2020).

However, no laboratory study has addressed the evaluation of the mode of action of *Eucalyptus camaldulensis Dehn* essential oil on pathogenic bacteria. The present study is therefore timely to explore the mode of action of *Eucalyptus camaldulensis* Dehn essential oil on pathogenic bacteria by studying cytoplasmic membrane destabilization.

MATERIALS AND METHODS

Plant material

Figure1. Leaves of *Ecamaldulensis* Dehn (PantList, James Harvey 2021)

We used fresh leaves of *Eucalyptus camaldulensis* Dehn collected in Abomey- Calavi and identified in the national herbarium under the number YH 681/HBN.

Biological materials: The microbial strains used in this study were obtained at the Research Unit for Applied Microbiology and Pharmacology of Natural Substances (U.R.M.A.Pha). The characteristics of these strains are summarised in Table I.

Table I. Origin of bacterial species

Strain	Origins
*Klebsiellapneumoniae*NCTC13443	Referencestrain
*Klebsiellaoxytoca*NCTC13442	Referencestrain
*SalmonellaTyphi*ATCC14028	Referencestrain
Shigella sonnei ATCC25931	Referencestrain
M2 *Klebsiella pneumoniae*	Clinical strain
42 *Klebsiella oxytoca*	Clinical strain
M10b *Salmonella typhi*	Clinical strain
M13 *Escherichia coli*	Clinical strain

Extraction and chemical analysis of the essential oil: The fresh leaves once in the laboratory were weighed and underwent steam extraction with an improved Clevenger type apparatus (BOUKHATEM *et al., 2019*). The essential oil, after decanting, was extracted from the leaves. The essential oil, after decanting and drying over magnesium sulphate (MgSO4), was collected in a brown bottle and stored at 4°C in a cold room. The yield of essential oil from fresh *Eucalyptus camaldulensis* leaves was calculated. GC/MS was performed on a TRACE GC 2000 series (Thermo Quest, Rodano, Italy) equipped with an AS2000 autosampler (Thermo Quest). The GC system was coupled to a Trace MS (Thermo Quest) operating in electron collision mode. The GC/MS was equipped with a CP- WAX52CB capillary column (Chrompack) measuring 25m x 0.25 mm with an internal diameter of 0.2 µm. The samples were injected in splitless mode (injection volume: 1 µl, inlet temperature: 230°C).

Table II. The various oven temperature settings.

Ramps	Rate (°C/min)	Temp (°C)	Hold time (min)
Initial	/	65	5
Ramp1	2.0	185	0
Ramp2	3.0	230	10

Helium was used as the carrier gas at a constant flow rate of 1.3ml/min.00. The coupling temperature of the GC is 260°C.
The electron energy is70 eV and the electron source is 250°C.The data was recorded and analysed using Xcalibur 1.1 software (Thermo Quest).

The mass spectra of the peaks obtained were analysed and compared with the reference compounds of the NIST/EPA/NIH 98 library. Following the GC/MS, physical and chemical characterization tests were carried out.

Bacteriological examination: The bacterial strains were plated on Mueller-Hinton (MH) agar and incubated in an oven at 37°C for 24 hours. The next day the colonies obtained on the agar were described. Two to three colonies obtained after culture were selected for Gram stainingaccordingtotheclassical Gram-Nicollemethod. Thentheidentificationof the strains was carried out with the Leminor Gallery and biochemical tests.

Sensitivity testing of bacterial strains to essential oil: The aromatogram technique was applied in order to evaluate the antimicrobial activity of essential oils (Andrianarison, 2021). This test was carried out by depositing a sterile 6mm diameter discimpregnated with a quantity of essential oil on an agar medium previously seeded with a microbial culture; then incubated in an oven at 37°C, under optimal culture conditions. After seeding the germs, different discs were placed on the petri dishes. In each seeded Petri dish, 04 discs were applied:

- The first will be a Control disc without essential oil and DMSO.
- The second one soaked with DMSO confirmed the inactivity of DMSO on the germs.
- The third was impregnated with pure essential oil.
- And the fourth disc was soaked with the mixture of DMSO and essential oil in equal volume.

After a lag time at 37.5°C, the diameter of the inhibition halo surrounding the discs was then measured in millimetres.

Table III. Standard used for reading the results of aromatogram tests (Andrianarison, 2021)

Inhibitor diameter (Δ)	Sensitivity to germs
Δ<7 mm	Resistant
7mm≤Δ<8mm	fairlysensitive
8mm≤Δ<9mm	Sensitive
Δ ≥ 9mm	Verysensitive

Determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the essential oil: The 96-well plate method was used in this study (Dougnon *et al., 2021a*). 100 µL of the initial essential oil solution at the concentration of 100 mg/mL was added to 100 µL of Mueller-Hinton broth. A series of successive well-to-well dilutions were made, and then 100 µL of various bacterial suspensions were added. Positive (100 µL ofMHbrothaddedto100 µL of bacterial suspension) and negative (100µLof MH broth added to 100 µL of essential oil) controls were prepared. The microplates were incubated at 37 °C for 24 h. All MIC wells at the highest concentrations were then inoculated onto Mueller Hinton agar and the Petri dish was placed at 37 °C for 24 h for MBC determination. The antibiotic potency (AP) of each extract was then calculated using the MBC/MIC formula. The antibacterial effect or potency is judged to be bactericidal or bacteriostatic on the basis of AP = MBC / MIC. If $1 \leq AP \leq 2$, the effect is bactericidal and if $4 \leq AP \geq 16$, the effect is bacteriostatic.

Outer membrane permeability test for Gram-negative bacteria: This test is based on the evaluation of the destabilising power of the membrane of bacteria by the essential oil of *Eucalyptus camaldulensis*. In a 96-well microplate, the MIC and 2CMI of the essential oil in triplicate were prepared by serial dilution.100 µL of the suspension of the tested bacteria ($1.5.10^6$CFU/mL) was added to all wells and the plate was incubated at 37˚C for 24 hours. Imipenem was used as a positive control.MuellerHintonbrothandbacterialsuspensionwereusedasnegativecontrol. Optical densities were read at 405 nm (the wavelength at which the complex form between lipopolysaccharide and membrane-stabilising divalent cations absorbs) (Djague*et al.,* 2020).

The percentage of destabilization was calculated using the formula below:

$$D = \frac{(A0 - AS)}{A0} \times 100$$

%D: Percentage of destabilization;
At_0: Absorbance of the negative control;
At_S: Absorbance of test samples.

Data analysis: The *in vitro* antibacterial tests were repeated three times and the results were analysed using Graph Pad 7 software. Analysis of variance (ANOVA) was used to compare the percentage of bacterial membrane destabilization between the different extracts and the reference Imipenem. In case of a significant difference, the data were compared using the Waller-Duncan test at a 95% confidence interval.

RESULTS

Extraction yield of the essential oil of *Eucalyptus camaldulensis:* According to the formula described above, the extraction yield of the essential oil of *Eucalyptus camaldulensis* was determined. Indeed, four extractions were carried out from the fresh leaves previously harvested.

Table IV. Yield after extraction of fresh leaves *of Eucalyptus camaldulensis*

Tests	Yields (%)
1er test	0,44
2eme test	0,7
3eme test	0,57
4eme test	0,58
Average	0,5725±0,11

The yield of fresh *Eucalyptus camaldulensis* leaves is an average of 0.57%.

Physico-chemical studies

Relative density: The relative density of the essential oil of *Eucalyptus camaldulensis* at room temperature: The average relative density of the essential oil of *Eucalyptus camaldulensis* is 0.89. Thisdensityof theEOobtainedislowerthanthatof waterwhichis equalto 0.9980,it is then lighter than water. The table below shows the different masses obtained which allowed us to calculate the relative density of the essential oil. The relative density of the essential oil of *Eucalyptus camaldulensis* is 0.89380.

Table V. Relative density

	1ertest
m0	1,0008
m1	2,0084
m2	1,9014
Density	0,89380

Acid number: The acid value of *Eucalyptus Camaldulensis* EO is 0.033

Table VI. Acid number

	Essential oil weight	KOH Volume	Acidnumber
1sttest	1,0154	0,6	0,033
2ndtest	1,0247	0,7	0,038
3rd test	1,0114	0,5	0,028
Average	0,033±0,005		

Miscibility with ethanol: *Eucalyptus camaldulensis* EO is miscible with ethanol. The gradual addition of 20 volumes of 98° ethanol to 0.5 mL of *Eucalyptus camaldulensis* EO did not cause turbidity. The mixture remained clear even after the addition of 20 volumes (10mL) of ethanol. Organoleptic characteristics of the essential oil of *Eucalyptus camaldulensis* leaves. The organoleptic characteristics of the EO were studied and their results are shownin Table XIV. *Eucalyptus Camaldulensis* essential oil is clear, light yellow oil with a characteristic odour of fresh *Eucalyptus Camaldulensis* leaves.

Table VII. Organoleptic characteristics of EO

Organoleptic characteristics	*Eucalyptus camaldulensis* essential oil
Aspect	Clear
Colour	Lightyellow
Smell	Characteristic odor of the *Eucalyptus camaldulensis* leaf

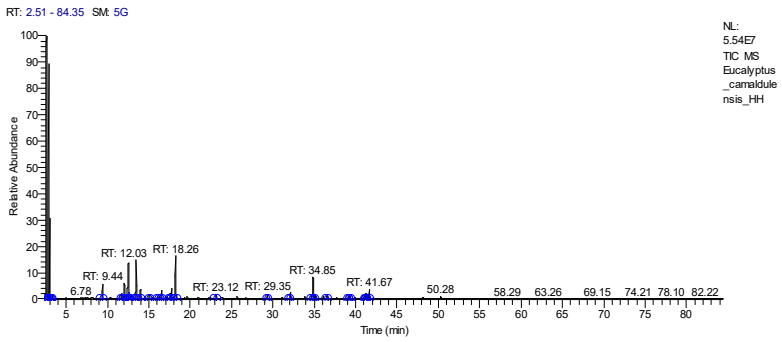


Figure 2. GC/MS chromatogram of *Eucalyptus camaldulensis*

Table VIII. The main chemical compounds of the essential oil of *Eucalyptus camaldulensis*

Name	RT	RSI	Area %	Molecular Weight
Terpinolène	3.04	935	32.21	136
M- cymène	2.93	877	26.35	134
Alloaromadendrene	12.53	944	6.03	204
alpha-Terpineol	18.26	905	6.06	154
Terpinène 4-acétate	13.46	89	4.63	154
α-terpinolene	3.04	935	4.56	136
Aromadendrène	34.85	922	3.02	204
(Z)-β-Caryophyllène	12.03	932	2.21	204
Borneol	41.67	785	1.11	152

The analysis of this table reveals that the majority compound of the essential oil of *Eucalyptus Camaldulensis* are: Terpinolene has 32.21% and M- cymene has 26.35%.

Sensitivity testing of bacterial strains to essential oil: The tests were carried out in triplicate and the average of the results was used. The graph above represents the results of the sensitivity test of the essentialoil to the different bacterial strains and the results of the sensitivity to the strains ofthe volume versus volume mixture of the essential oil and DMSO. From the analysis of this graph it appears that the essential oil of *Eucalyptus Camaldulensis* had an activity on the majority of the strains with the exception of *Klebsiella oxytoca* on which the essential oil had no activity.

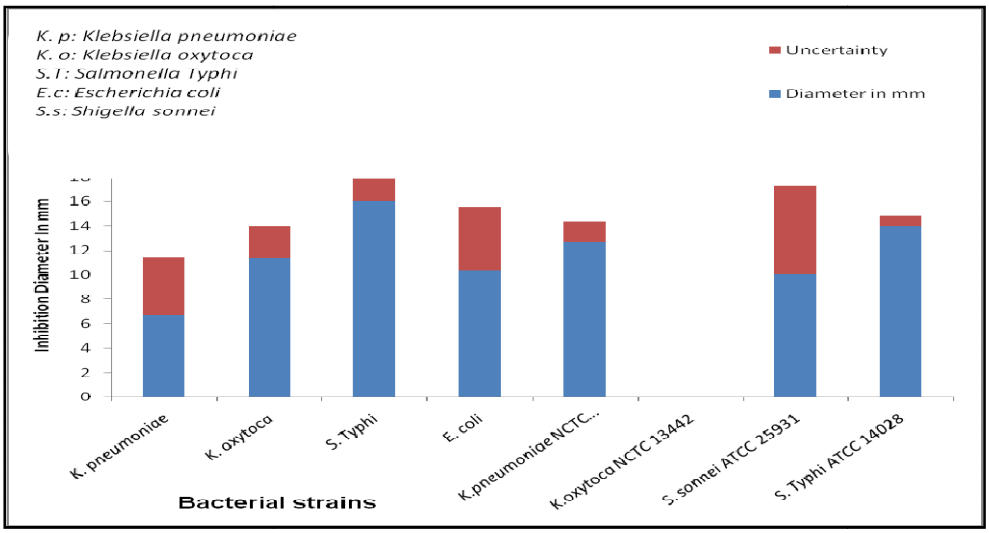


Figure 3. Results of the sensitivity test of the essential oil to the different strains

Determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the essential oil

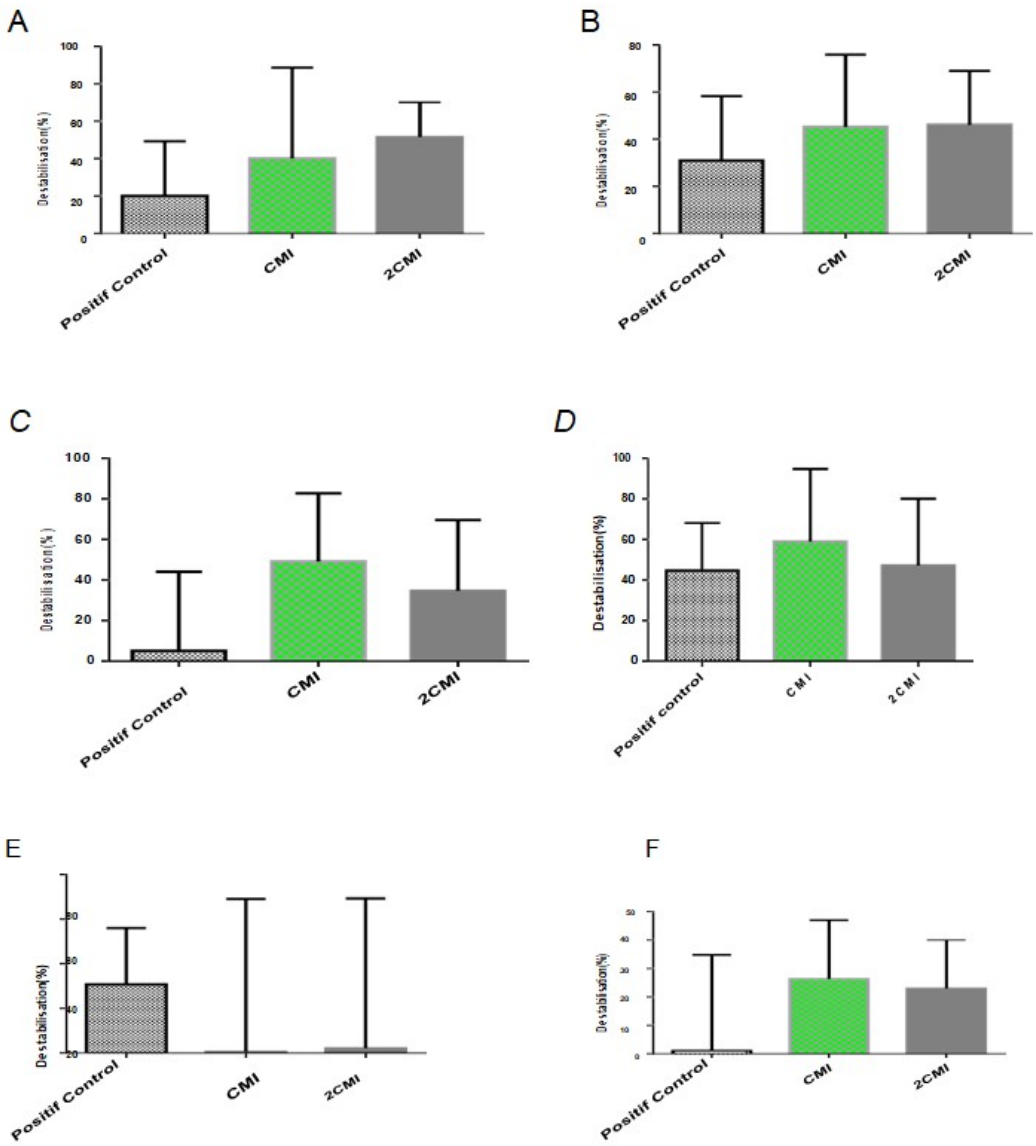
The table above provides information on the minimum inhibitory concentration, minimum bactericidal concentration and antibiotic potency of the essential oil on each strains. From the analysis of the table it appears that for the strains of *M13 Escherichia coli, Klebsiella pneumoniae, M10b Salmonella typhi*, the MIC is equal to the MBC. The essential oil of *Eucalyptus camaldulensis* has a bactericidal power on all the tested strains.

Table IX. MIC, BMC and AP results for different bacterial strains

Species	CMI	CMB	PA
M2 Klebsiella pneumoniae	9,375	18,75	2,005 Bactericide
42 Klebsiella oxytoca	78,125	9,375	1,2 Bactericide
M10b Salmonella typhi	46,875	46,875	1 Bactericide
M13 Escherichia coli	6,25	6,25	1 Bactericide
Klebsiella pneumonia NCTC13443	6,25	6,25	1 Bactericide
Salmonella typhi ATCC 14028	12,5	15,625	1,25 Bactericide
*Shigella sonnei ATCC*14028	12,5	18,75	1,5 Bactericide

Outer membrane permeability test for Gram-negative bacteria: To evaluate the mode of action of the essential oil on the different strains tested the percentage of membrane destabilization was used. A statistical difference between the different values was noted if $P<0.05$. From the analysis of these different graphs, it appears that a statistically significant difference ($P<0.05$) was obtained at the level of the strains:

- Of *M10 Salmonella typhi*, between the average of the values of the percentage of membrane destabilization by Imipenem (5.125%) and the average of the values of the percentage of MIC destabilization of the *M10 Salmonella typhi* strain (49.17%)
- *And for M2 K pneumoniae,* between the average of the values of the percentage of membrane destabilization by Imipenem (20.13%) and the average of the values of the percentage of destabilization of the 2CMI of the strain *M2 K pneumoniae* (51.50%). For the other strains there was no significant difference between the percentage of membrane destabilization by Imipenem and the different inhibitory concentrations of *Eucalyptus camaldulensis* essential oil. However, it is important to note that for the strains of :
- *ATCC14028 Salmonella typhi,* the percentage of membrane destabilization by Imipenem (30.75%) is higher that the percentage of membrane destabilization at MIC (0.5833%) and at 2CMI (2.083%).
- *ATCC 25931 Shigella sonnei,* the percentage of membrane destabilization by Imipenem (1.125%) is lower than the percentage of membrane destabilization at MIC (26.42%) and at 2CMI (24.67%).
- *M13 E coli,* the percentage of membrane destabilization byImipenem (44.63%) is almost the same as the percentage of membrane destabilization at MIC (59.00%) and at 2MIC (47.17%).
- *42 Klebsiella oxytoca,* the percentage of membrane destabilization by Imipenem (30.88%) is lower than the percentage of membrane destabilization at MIC (45.25%) and 2MIC (46.08%).



- A : Effect of destabilization of *M2 Klebsiella pneumoniae* membrane by EC essential oil
- B : Effect of destabilization of the membrane of *42 Klebsiella oxytoca* bythe essential oil of *EC*
- C: Effect of destabilization of the membrane of *M10 Salmonella typhi* by *EC* essential oil
- D: Effect of destabilization of the *M13 E coli* membrane by *EC* essential oil
- E: Effect of destabilization of the membrane of *ATCC 14028 Salmonella typhi* by the essential oil of *EC*
- F: Effect of membrane destabilization of *ATCC 25931 Shigella sonnei* by *EC* essential oil

Figure 4. Percentage of membrane destabilization of the different strains by the essential oil and that of the positive control

DISCUSSION

The yield of essential oil extracted by steam stripping from fresh leaves of *Eucalyptus Camaldulensis* harvested in the vicinity of Abomey-Calavi precisely at Gbetagbo in Benin is an average of 0.57%. The latter is close to that previously obtained in Benin on the same plant with values ranging from 0.6 to 1.4 % (Aleksic Sabo and Knezevic, 2019). The work of Chalchat and colleagues (2000) also gave a similar yield of 0.5% in Jerusalem. However, this yield is lower than the one quotedby Siramon et al (2008) in their work in Thailand, which was 2.23%. Considerable variation in the yield of essential oil from *E. camaldulensis* leaves has been reported depending on multiple bio topical factors, as well as genetic and/or epi genetic characteristics of the plant and the method of extraction of the essential oil (Aleksic Sabo and Knezevic, 2019). The highest yields were obtained during the hot summer seasons with values above 2.7% and the lowest yields were recorded during the months of February with an average value of more than 1.2% (Dembélé *et al.*, 2020). A similar yield of essential oils (0.77-2.53%) was reported for *E. globulus*, as one of the economically important plants for essential oil production (Harkat-Madouri *et al., 2015;* Joshi*, 2012;* Selvakumar*,2012)*.The essential oil yield reported for other *Eucalyptus* species is slightly higher, ranging from 1.2 to 3%: the highest yield was obtained from *E. cinerea*F. Muell. Ex Benth and *E. sideroxylon* A. Cunn.exWoolls (3.0%) *(*Sebei*et al., 2015).* The analysis of the chemical composition of the essential oil extracted from EC by gas chromatography coupled with mass spectrophotometry (GC/MS) revealed that the majority compounds contained in the latter are: Terpinolene (32.21%) and M- cymene (26.35%). These results are not in line with those obtained during studies conducted in Benin which reported that the main component of *Eucalyptus camaldulensis* essential oils originatingfromBenin was 1,8-cineolerangingfrom31.0 to 72.5% (Aleksic Sabo and Knezevic, 2019; Baba-Mousse*et al.*, 2012).They are rather close to those of Allanto*and* collaborators (2022) who obtained a Terpinolene composition of 35.6% and M- cymene of 21.2% for fresh leaves collected in the vicinity of the University of Abomey-Calavi at around 1pm. Even if the composition is variable according to the time of harvest, these results are close to our results at the same time

of harvest. The majority of the compounds extracted from the essential oil of *Eucalyptus camaldulensis* Dehn are only monoterpenes and sesquiterpenes. Monoterpenes are the majority compounds while sesquiterpenes are the minority. It is important to point out here that according to the chemical composition, *Eucalyptus camaldulensis* Dehn can generally be divided into two different types. Type I is a cineol-rich essential oil containing 80-90% 1,8-cineole plus pinene, and type II is an essential oil containing much less cineole ranging from 0 to 5%. (Hamilton *et al., 2011*)**.**The one studied is poor in cineol but rich in Terpinolene and M-cymene. Variations in the chemical composition of *Eucalyptus* essential oils in relation to the seasons have also been reported (Tsiri *et al.,* 2003). This difference in the chemical composition of the essential oils of *Eucalyptus camaldulensis is of interest* and we can already put forward two hypotheses according to which: Environmental factors suchas the composition of the soil, theharvestingtimeandthematurityof the leaves can influence the chemical composition of the essential oil on the one hand (Aleksic Sabo and Knezevic, 2019) and on the other hand, the plant genotype also contributes (Djilani and Dicko*,* 2012*).* The physico-chemical properties of the essential oil extracted from the leaves of *Eucalyptus camaldulensis* have revealed that the acidity index of the latter doesnot contain a significant level of free acids that could be harmful to humans when used externally or internally (AFNOR, 2009 EN ISO 660). Its relative density is 1.005. It is an essential oil with a relative density close to that of water. It is a slight as water. The progressive addition of 98° ethanol to 0.5 mL of *Eucalyptus camaldulensis* EO didnotcauseany turbidity.The mixtureretaineditsclarityevenafter the addition of 20 volumes (10 mL) of ethanol. The essential oil of *Eucalyptus camaldulensis* is therefore highly miscible with ethanol. The good miscibility of *Eucalyptus camaldulensis* essential oil with 90°C ethanol would be due to its composition of polar compounds such as alcohols, terpene oxides and sesquiterpenes. Asfar as the organoleptic qualities of the oil are concerned, it is a clear yellow oil with a characteristic odour of the *Eucalyptus camaldulensis* leaf. The organoleptic parameters of the essential oil are in accordance with those listed in the Standards (AFNOR, 2000). The antimicrobial activity of essential oil extracted by steam entrainment using Clevenger's device on pathogenic strains was tested by agar disc diffusion method and well dilution technique. Eight (08) different strains were tested including: *Klebsiella pneumoniae* (reference as clinical), *Shigella sonnei, Escherichia coli, Salmonella typhi* (reference as clinical)*, Klebsiella oxytoca* (reference as clinical); all of them enterobacteria and therefore Gram-negative bacteria (B-).These strains have been chosen because of their wide implications in infectious diseases but also because of their ever increasing resistance to antibiotics. In the majority of publications, Gram positive bacteria are more sensitive to essential oils than Gram negative bacteria *(*Jeyaseelan and Jashothan*, 2012;* Lu *et al., 2007;* Mishra and Mishra*, 2011*)**.** The evaluation of the antibacterial properties of the essential oil extracted from the leaves of *Eucalyptus camaldulensis* allowed us to determine whether it would be more effective against Gram-negative bacteria than most essential oils.

It was noticed that the essential oil extracted from the leaves of *Eucalyptus camaldulensis* had moderate antibacterial activity against all strains except *Klebsiella oxytoca*, the reference strain (inhibition diameter 00 mm). Maximum inhibition of oilon bacteria was observed on both reference and clinical *Salmonella typhi* and a moderate effect was observed on *Escherichia coli* strains. These results are in agreement with those of Ostad Asiaei and collaborator who reported that *E. Camaldulensis* essential oil of Irish origin showed major activities against different pathogenic microorganisms and that the maximum effect was observed with *Klebsiella pneumoniae* (OstadAsiaei*et al.,* 2017*)*. However Khubeiz and colleagues in 2016 reported that the lowest inhibition diameters were observed in Gram-negative bacteria (*E. coli* and *Klebsiella pneumoniae)* (Khubeiz*et al., 2016*). In contrast, significant antibacterial activity against Gram-negative strains such as *E. coli* and *Salmonella typhi* has been reported in the literature (El-Baz et al., 2015; Lima et al., 2013). Furthermore Ghaffar and co-workers in 2015 had a very strong inhibition against *E. coli* (10-31 mm for inhibition diameter) for the essential oil of *Eucalyptus camaldulensis* at different concentrations **(**Ghaffar*et al., 2015***)**.** And more recently Kpadonou and collaborator obtained similar results stating that the oil was not effective against all gram positive bacteria **(**Kpadonou*et al., 2022***)**.** This difference between the results obtained in the literature can be explained by the variation in the chemical composition of the essential oil depending on the country but also on the time of harvest and the chemotype of the plant.Factors such as geography, temperature, day length, nutrients can influence the biosynthetic pathways of the plant with consequences in the relative proportion of the main compounds. This leads to the existence of differences in plant origins, as well as seasonal variations throughout the plant's vegetative cycle *(*Flaminie*t al., 2004)*. The lowest MIC was obtained on *Salmonella typhi* strains (4.6875mg/mL) and the highest MIC was obtained on *Shigella sonnei* strains (12.5 mg/mL). These different strains with the low and high MICs also have the high and low BMCs respectively. According to Okou's research team (Okou*et al., 2018*) the MBC/MIC ratio allows a better appreciation of the antibiotic power of the essential oil. When this MBC/MIC ratio ≤ 4, the extract is said to be bactericidal and when MBC/MIC > 4, the extract is described as bacteriostatic. The essential oil *of Eucalyptus camaldulensis* has a bactericidal antibiotic power on all the strains that were sensitive to it. These results are in line with those reported in the literature (Ghaffar*etal.,2015*; Khubeiz *et al., 2016*; Kpadonou *et al.* ,*2022*; OstadAsiaei *et al., 2017*). The study of the antimicrobial activity of EO is interesting to counter the ability of bacteria to develop bio-resistance against the classical antibiotics used. Indeed,the effectiveness of essential oils is found in a chemical compound giving them a broad spectrum of antimicrobial effect. That of the essential oil used in this study could be found in its composition of a mixture of monoterpenes (γ-terpinene, p- cymene**)** and oxygenated monoterpenes, but most of the antimicrobial activity in the oils was attributed to oxygenated monoterpenes. The antibacterial activity of the essential oil could not be truly elucidated without determining the mechanism of action of the latter on the different strains tested. Several models are known to study the mode of action of antibacterial agents on different bacterial targets (Pinto *et al.* , 2017). In this study, the bacterial outer membrane permeability test was used.

Essential oils, flavonoids, alkaloids and even tannins could induce a leakage of potassium ions at the level of the membrane and consequently irreversible lesions of this membrane *(*Bouhdid *et al., 2012)*. The results obtained from the present study show a better potential of the essential oil to destabilize the membrane of the bacterial strains with a better percentage compared to the positive control (Imipenem) which was used asreference molecule. Studies on other medicinal plants showed similar results compared to positive controls which were polymyxin B (Djague*et al.,* 2020*)* and Cefixime (Dougnon *et al*., 2021b). For all strains tested, the percentage of destabilization of the outer membraneof thestrains is almostthe sameforboth MICand 2MIC.This observation allows us to hypothesize that for *Eucalyptus camaldulensis* essential oil the percentage of membrane destabilization is not dependent on the concentration of the essential oil and that the higher the concentration, the more the percentage of membrane destabilization tends to decrease for some strains (*M10 Salmonella typhi, M13 E coli, Shigella sonnei)*. It is also important to underline that for M10 *Salmonella typhi* a statistically significant difference ($P<0.05$) was obtained between the average of the values of the percentage of membrane destabilization by Imipenem and the average of the values of the percentage of destabilization of the MIC of the strain. For the *M2 Klebsiella pneumoniae* strain, a statistically significant difference ($P<0.05$) was obtained between the average of the values of the percentage of membrane destabilization by Imipenem and the average of the values of the percentage of destabilization of the 2CMI of the strain. So for these two strains the essential oil is better than Imipenem at the respective MIC and 2 MIC concentrations. These different results confirm that even if the essential oil has a moderate bacterial activity on the strains involved in this study, it remains more effective than the last resort antibiotics used in the treatment of bacterial infections whose mode of action is the destabilization of the bacteria's membrane. In addition, previous work by Chimnoi and colleagues on determining the mechanism of action of *Ocimum gratissimum* essential oil on gastroenteritis pathogens allowed them to conclude that the best mechanism of action of the oil on Gram-negative bacteria is the destabilization of the bacterial wall by increasing the permeability of the microbial cell membrane, as evidenced by the LIVE/DEAD Bac Light Assay (Chimnoi

et *al.,* 2018). The potential of the essential oil to destabilize the membrane of the different strains can be attributed to its composition of monoterpenes (γ-terpinene, p-cymene) and monoterpene oxygens. Indeed, according to the work of Cristani and colleagues, the antimicrobial effect of thymol, carvacrol, p-cymene and γ-terpinene may result partly from a gross disruption of the lipid fraction of the microorganism's plasma membrane. In addition to being related to the physicochemical characteristics of the compounds (such as lipophilicity and water solubility), this effect seems todepend on the lipid composition and net surface charge of the membranes. In addition, the compounds can cross cell membranes, thus penetrating the cell interior by interacting with intracellular sites critical for antibacterial activity (Cristani et al., 2007). Furthermore, phenolic compounds are known to disrupt and weaken lipopolysaccharide interactions by complexing divalent cations that stabilize the outer membrane of bacteria (Dougnon *et al.* ,2021a). These data then explain the potential for membrane destabilization by *Eucalyptus camaldulensis* essential oil.

CONCLUSION

In the fight against the ever-increasing phenomenon of antibiotic resistance, plants remain the most promising alternative. The essential oil extracted from the fresh leaves of *Eucalyptus camaldulensis* Dehn by steam stripping and analysed by GC/MS revealed a high composition of chemical compounds including mainly oxygenated monoterpenes and sesquiterpenes. The physico-chemical tests revealed a good quality of the essential oil. It can therefore be used in cosmetics and pharmacology. This oil has also been tested on eight (08) different strains including : *Klebsiella pneumoniae* (reference as clinical), *Shigella sonnei*, Escherichia coli, *Salmonella typhi* (reference as clinical), *Klebsiella oxytoca* (reference as clinical). All these strains were susceptible to the test except *Klebsiella oxytoca*. Determination of the percentage of membrane destabilization revealed that the essential oil of *Eucalyptus camaldulensis* is more active on the strains than the reference molecule and that the effect does not vary with increasing concentration. In vivo experiments including other bacteria such as Gram-positive bacteria are planned, as well as another approach to highlight other mechanisms of action of the essential oil on the strains.

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AUTHORS' CONTRIBUTIONS

MEDEGAN FAGLA R. S.: Contributed to the study conception and analysis supervisor

GABA H. G. D.: Assistant in development of the technical protocols

AHOUANSOU C. A.: Elaboration of technical methods and contribution to the writing of this article

TOUKOUROU H.: Elaboration of technical methods

KASSEHIN C.U.: Elaboration of technical methods

DOUGNON T. V.: Performed the experiments

GBAGUIDI A. F.: Initiator and general supervisor

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