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In vitro antibacterial activity of *Dacryodes edulis* and *Dacryodes buettneri* essential oil as well as their synergies with antibiotics commonly prescribed to treat bacterial gastroenteritis in children

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Abstract

The emergence of antibiotic-resistant bacteria has revived interest in a large group of plant antimicrobial compounds, such as essential oils, as alternative antimicrobial agents to combat AMR. The essential oils of *Dacryodes buettneri* and *Dacryodes edulis* were obtained by hydrodistillation of the resins, then tested against the bacteria responsible for childhood diarrhea by the disc diffusion method. The MIC was determined by the microdilution method (CLSI). Temporal killing of bacteria was obtained using the standardized CLSI M26-A test. The checkerboard test was used to evaluate the in vitro synergy of essential oils with the antibiotics prescribed to combat bacterial gastroenteritis in Gabon. Essential oils have shown antibacterial activity against high-level penicillinase and ESBL strains. Additionally, *D. edulis* demonstrated antibiofilm activity against all strains studied. Similarly, *E. coli* ATCC 25922 and *Shigella* spp were sensitive to *D. buettneri* essential oil. The (CFM)-*D. edulis* combination showed synergy on penicillinase strains, and the (CRO)-*D. edulis* combination against ESBLs of *E. coli*. Overall, our results showed that both essential oils can be considered as alternatives to antibiotics in the fight against AMR. However, their simultaneous use with antibiotics should be avoided.

Keywords: Antibacterial activity, Antibiofilm, Essential oils, *Dacryodes buettneri*, *Dacryodes edulis*

.Introduction

The first decade of the twentieth century saw the emergence of the first artificial antibacterial, arsphenamine (Salvarsan) synthesised in 1910 by the chemist Alfred Bertheim, marking the beginning of the golden age of antibiotics, notably with the discovery in 1928 of penicillin by the physician biologist and pharmacologist Alexander Fleming (Gould, 2016; Nicolaou and Rigol, 2017; Hutchings *et al.*, 2019). These discoveries had a considerable impact on increasing human life expectancy (Abdallah *et al*., 2023). Unfortunately, the success of antibiotics has had a snowball effect, leading to their overuse in healthcare as well as in other industries such as animal husbandry, resulting in the emergence of bacterial resistance to antibiotics. Diarrheal diseases remain a major cause of illness in children under 5 years of age, causing over 500,000 deaths worldwide each year, mainly in Africa and South-East Asia (Murray *et al.*, 2022).

The antibacterials of first choice for treating diarrhoea caused by bacteria are beta-lactam antibiotics, sulphonamides and quinolones, although we are now seeing the emergence of resistance to these antibiotics by certain microbial agents (Shaikh *et al.*, 2015). According to the WHO, antimicrobial resistance (AMR) is one of the top ten global threats to public health. Despite efforts to combat AMR, drug-resistant infections are estimated to have contributed to 4.95 million deaths globally in 2019, with most of the burden attributed to low- and middle-income countries (LMICs), particularly sub-Saharan Africa. However, in the absence of a global policy to combat AMR, it is estimated that the number of deaths attributable to AMR worldwide could reach ten million per year by 2050 (Murray *et al.*, 2022; Walsh *et al.*, 2023), rekindling interest in an important group of plant antimicrobial compounds, such as essential oils, as alternative or complementary antimicrobial agents to combat pathogenic microorganisms (Magi *et al., 2015*; Chávez-González *et al.*, 2016).

Essential oils are liquid, aromatic and volatile extracts obtained by distillation from plant materials such as leaves, rhizomes, flowers, roots, bark, seeds, resin and whole plants (Morsy and Morsy, 2017). They can be defined as products or mixtures of fragrant or odourless substances and vary considerably depending on genetics, climate and geographical origin. They are composed of more than 200 chemical constituents (Ramsey *et al.*, 2020), which give them various properties such as viscosity, colour and antimicrobial activities (Aghoutane *et al.*, 2020; Sadgrove *et al.*, 2022). In addition to antimicrobial activities, essential oils and their constituents can act synergistically with certain antibiotics, enhancing their antimicrobial activity (Magi *et al.*, 2015).

Therefore, the aim of this study was to assess the antimicrobial activity of essential oils from two Gabonese plants and to evaluate their synergy with common antibiotics prescribed to children under five suffering from bacterial gastroenteritis.

1. Materials and methods

1.1. Plant materials

The essential oils used in this study were extracted from the resins of two plants, Ozigo (*Dacryodes buettneri*) and safoutier (*Dacryodes edulis)* collected respectively at three different sites: Akanda, Ntoum and Ayem in one of the Gabon's nine regions called Estuaire.

1.2. Biological material

Four micro-organisms including a reference strain *Escherichia coli* ATCC25922, an extended-spectrum betalactamine (ESBL)-producing strain (*Escherichia coli* spp) and two highly penicillinase-producing strains (*Shigella* spp and *Salmonella* spp) were used to assess the antimicrobial activity and synergy of the different essential oils against antibiotics commonly prescribed to children under five suffering from bacterial gastroenteritis. All organisms, with the exception of *E. coli* ATCC25922 (Microbiologics, USA) were isolated from stool samples taken from children under five years of age suffering from gastroenteritis in the microbiology laboratory of the Owendo University Hospital Center, Gabon.

1.3. Antibiotics commonly prescribed as probabilistic treatment for childhood diarrhoea

The standard antibiotics used in this study were selected on the basis of a questionnaire submitted to prescribers on antibiotics commonly prescribed as probabilistic treatments for childhood diarrhoea.

1.4. Extraction of essential oils

The essential oils of *Dacryodes buettneri* and *Dacryodes edulis* were obtained from their resins using the lambic hydrodistillation method at the N'kira cosmetics laboratory (Libreville/Gabon) (De Almeida-Couto*et al.*, 2022). The essential oil was then stored at -20°C (Boonyanugomol *et al.*, 2017).

1.5. Yield

The essential oil yield is the ratio of the quantity of oil collected after hydrodistillation to the quantity of plant treated, expressed as a percentage (AFNOR, 1996).

$$R = \frac{PB}{PA} X\ 100$$

R: yield of essential oil in % ;PB: quantity of essential oil in g ;PA: quantity of plant in g.

1.6. Antimicrobial activity of essential oils

The antibacterial activity of essential oils was screened using the disc diffusion method, which was used to select essential oils with antibacterial activity**.** Stock solutions of the essential oils were prepared by diluting the different oils in 5% (v/v) DMSO (Fischer, USA) to facilitate their diffusion onto Muller hinton agar (Balouiri*et*

al., 2016; Arsene *et al.*, 2022). Under aseptic conditions, a microbial suspension with a turbidity of 0.5 McFarland units was spread on a Petri dish containing Mueller-Hinton agar, empty sterilised discs (Whatman No. 5, 6 mm in diameter) were impregnated with 50 µl of oils and deposited on the agar surface, as well as a disc soaked in aqueous DMSO (Fischer, USA) serving as a negative control. Inhibition diameters were interpreted as follows: non-sensitive = diameter < 8 mm; sensitive = diameter from 8 to 14 mm; very sensitive = diameter from 15 to 19 mm; extremely sensitive = total diameter > 20 mm (Boonyanugomol *et al.*, 2017).

1.6.1. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The MIC of essential oils was determined using the microdilution sensitivity test in accordance with Clinical Laboratory Standards (CLSI) recommendations (Boonyanugomol *et al.*, 2017; Ghavam, 2023). Using sterile Muller-Hinton broth, a twofold cascade dilution was performed with essential oil stock solutions to obtain final concentrations of 100; 50; 25; 12.5; 6.25; 3.125 µg/ml in microtiter plate wells. A 10 µl inoculum with a turbidity of 0.5 McFarland was introduced into microwells 1 to 11, while the essential oil was placed in microwells 1 to 10. Microwell 11 (broth and bacteria) was considered a positive control for microbial growth, while microwell 12 (broth alone) was considered a negative control (Al-Maharik *et al.*, 2023). The microtiter plate was then incubated at 37±2°C for 18 to 24 h. The MIC was obtained by determining the lowest concentration in the microwell with no microbial growth visible to the naked eye. The BMC was determined by plating the microwells without visible bacterial growth on Müller Hinton agar plates. The lowest concentration without bacterial growth on agar was taken as the BMC.

1.6.2. Synergy test for essential oils with antibiotics

The checkerboard test is one of the synergy tests used for essential oils. A two-dimensional array of serial concentrations of test compounds was used to evaluate antimicrobial combinations *in vitro*. Dilutions were based on the MIC of the essential oil and the antibiotic. The checkerboard test was used as the basis for calculating a fractional inhibitory concentration (FIC) index. Fractional Inhibitory Concentration (FIC) indices were determined as follows: CIF index = CIF A + CIF B where CIF A and CIF B correspond to EO and the antibiotic respectively. CIF A and CIF B were calculated as follows: CIF A = MIC AB (Minimal Inhibitory Concentration of A in the combination) / MIC A (Minimal Inhibitory Concentration of A alone) and CIF B = MIC BA (Minimal Inhibitory Concentration of B in the combination) / MIC B (Minimal Inhibitory Concentration of B alone) (Magi *et al.*, 2015; Siqueira *et al.*, 2021). Results were interpreted as follows: FIC synergy < 0.5; addition (0.5 ≤ FIC ≤ 1); indifference (1 <FIC ≤ 4); or antagonism (FIC> 4) (Huang *et al.*, 2019).

1.6.3. Time-killcurves of Bacteria

This test was standardised in accordance with CLSI M26-A (CLSI, 1999). It was performed in Muller-Hinton broth (Merck, Germany) using three tubes containing a bacterial suspension of 5×10^5 CFU/ml. The first tube contained the essential oil (EO) tested at a final concentration corresponding to the MIC, the second tube contained an antibiotic-EO combination for which synergy had previously been observed and the third tube was

considered the growth control. Incubation was carried out under appropriate conditions for different time intervals (0, 1, 2, 3, 4, 5 and 6h) (Balouiri *et al.*, 2016). Factor two serial dilutions were then performed on the first two tubes and inoculated onto Muller-Hinton agar (Merck, Germany) to obtain a plot of bacterial numbers against time (Boonyanugomol *et al.*, 2017). These tests were carried out in triplicate and the antimicrobials considered bactericidal were those with a percentage lethality of 90% for 6h, or 99.9% lethality for 24h (Balouiri *et al.*, 2016).

1.6.4. Assessment of antibiofilm activity

Many bacteria such as enteropathogenic bacteria like *E. coli* ESBL and *Salmonella* spp have the ability to produce biofilms (Tremblay *et al.*, 2014). These bacteria adhere to living or inert surfaces, forming amorphous mono- or polymicrobial structures called biofilms, which constitute one of their resistance strategies by preventing antimicrobial permeability.

The effect of essential oils on biofilm formation was studied using the crystal violet test. Two serial dilutions of the oils were prepared. Next, 100 µl of trypticase soy broth (TSB) (Merck, Germany) was adjusted to a final concentration of 5×10^5 CFU/ml and dispensed into each well of the sterile 96-well flat-bottom polystyrene microplate. After incubation at 37°C for 24h, the media were removed and the wells were washed three times with sterile distilled water. The biofilms were then air-dried and stained with 1% crystal violet dye for 20 minutes at room temperature. The excess dye was then removed and the microplate was washed three times with sterile distilled water. Next, 100 µl of 95% ethanol was introduced into each well and incubated for 15 minutes to solubilise the dye retained by the biofilm, and the mixture obtained was read with a microplatereader (Micro read 1000, Global diagnostic, USA) at an optical density of 630 nm (Fathi *et al.*, 2022; Ersanli *et al.*, 2023).

$$\text{Biofilm inhibition}\% = [(\text{positive Control} - \text{Experimental})/(\text{Positive Control})] \times 100$$

The lowest concentration of extract that showed at least 50% inhibition of biofilm formation was considered the Biofilm inhibition concentration (BIC).

1.7. Ethical considerations

The study and the protocol were approved by the scientific council of the doctoral school of fundamental and applied sciences (EDSFA) at the Université des Sciences and Techniques de Masuku (Gabon).

1.8. Statistical analysis

All experiments were performed in at least triplicate. Inhibition zones were calculated as means ± standard deviation using IMB SPSS 26 statistical software. Statistically significant differences were determined at the 5% level.

2. Results

2.1. Antibiotics commonly prescribed as probabilistic treatment for infantile diarrhoea

The antibiotics most commonly prescribed at home by the thirty doctors who took part in this study were cefixime (46.67%), followed by amoxicillin-clavulanic acid (26.67%), cotrimoxazole (16.66%) and ampicillin (10%). Ceftriaxone was the only antibiotic prescribed in hospital (100%) (**Figure 1**).

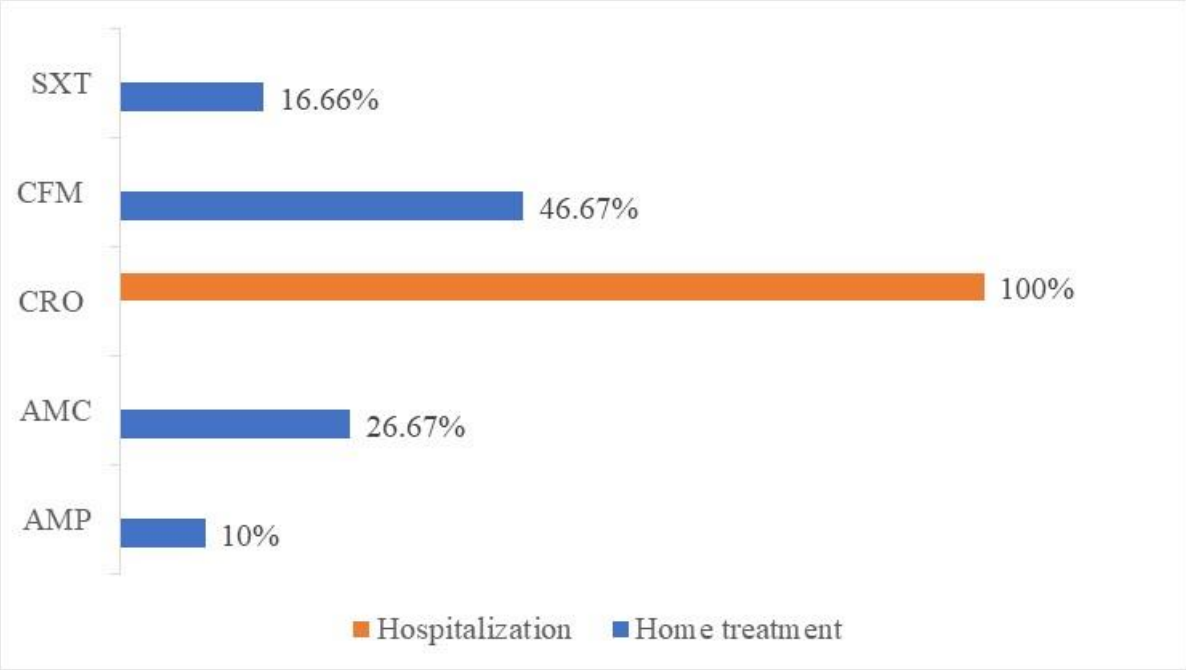

Figure 1: *Antibiotics prescribed as probabilistic treatment for infantile diarrhoea*

Cotrimoxazole (SXT), cefixime (CFM), ceftriaxone (CRO), amoxicillin-clavulanic acid (AMC), ampicillin (AMP).

2.2. Yield

A higher yield of essential oil was obtained with the resin of *Dacryodes buettneri*, at 7.80%, characterised by a pale amber colour, while the essential oil of *Dacryodes edulis*, with its limpid colour, was obtained with an extraction yield of 7.40%.

2.3. Antimicrobial activity of essential oils

The present study showed very good sensitivity of *Dacryodes edulis* essential oil against all the strains tested, as well as a larger inhibition diameter (varying from 14.33 to 18.67 mm), compared with the *Dacryodes buettneri* essential oil, whose inhibition diameter (ID) varied from 11.33 to 16.67 mm, with the exception of *Salmonella* spp (ID of 17 mm). The results of the antibiotic susceptibility tests confirmed the phenotypes of the strains studied (**Table 1**).

Table1: Inhibition diameter of essential oils and commonly prescribed antibiotics for gastroenteritis

Bacteria	*E. coli* ATCC 25922		*E. coli* ESBL		*Salmonella* spp		*Shigella* spp	
Antibacterial	DI (mm)	Int.	DI (mm)	Int.	DI (mm)	Int.	DI (mm)	Int.
Dacryodes buettneri	16.67± 0.57 Sd	Very sensitive	11.33 ± 0.57 Sd	Sensitive	17 ± 1 Sd	Very sensitive	11.33± 0.57 Sd	Sensitive
Dacryodes edulis	18.67 ± 0.57 Sd	Very sensitive	16 ± 0 Sd	Very sensitive	16± 1 Sd	Very sensitive	14.33 ± 0.57 Sd	Very sensitive
AMC	24± 0 Sd	Sensitive	20.67 ± 1.15 Sd	Sensitive	12± 1 Sd	Resistant	14 ± 0 Sd	Resistant
CFM	25.33± 0.57 Sd	Sensitive	12 ± 0 Sd	Resistant	23.33 ± 1.15 Sd	Sensitive	26± 0 Sd	Sensitive
CRO	35 ± 0 Sd	Sensitive	13± 1 Sd	Resistant	33 ± 1 Sd	Sensitive	30,33 ± 0.57 Sd	Sensitive

ID: Inhibition diameter (mm); Int: Interpretation; AMC: Amoxicillin-clavulanic acid, CFM: Cefixime, CRO: Ceftriaxone.

2.4. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

For *Escherichia coli* ATCC 25922, the essential oil of *Dacryodes edulis* had a lower MIC (20 µg/ml) than that of *Dacryodes buettneri* (50 µg/ml). The trend was the same for *Escherichia coli* ESBL, where the essential oil of *Dacryodes edulis* had a MIC of 50 µg/ml compared with 100 µg/ml for *Dacryodes buettneri* (**Table 2**).

For *Salmonella* spp, the essential oil of *Dacryodes edulis* (40 µg/ml) had a better MIC than that of *Dacryodes buettneri* (50 µg/ml). It was also observed that for the *Shigella* spp strain, the essential oil of *Dacryodes edulis* had a higher activity with an MIC of 20 µg/ml than that of *Dacryodes buettneri* (50 µg/ml) (**Table 2**).

For *Dacryodes buettneri*, the BMCs for the different strains in the study were identical to the MIC, but for *Dacryodes edulis*, the BMC was twice the MIC for *Escherichia coli* ATCC 25922 and *Shigella* spp, identical to the MIC for *Escherichia coli* ESBL and 10 units higher than the MIC for *Salmonella* spp (**Table 2**).

Table 2: MIC and MBC of the essential oils in the study

	Dacryodes buettneri		*Dacryodes edulis*	
Strain	MIC (µg/ml)	CMB (µg/ml)	MIC (µg/ml)	CMB (µg/ml)
E. coli ATCC 25922	50	50	20	40
E. coli ESBL	100	100	50	50
Salmonella spp	50	50	40	50
Shigella spp	50	50	20	40

2.5. Synergy test for essential oils

In this study it was observed that out of 24 combinations, only seven synergies were identified, followed by two antagonisms, seven additions and finally eight indifferences (**Table 3**).

In the case of the highly penicillinase bacteria in the study, namely *Salmonella* spp and *Shigella* spp (both resistant to AMC), no synergy was observed between AMC and the essential oils, with only the cefixime (CFM)-*D. edulis* combination showing synergy for both strains. As for the *E. coli* ESBL strain, synergy was observed between ceftriaxone (CRO), a third-generation cephalosporin, and *D. edulis* essential oil. Similarly, two combinations and an antagonistic effect were observed in *E. coli* ESBL against the AMC-*D. buettneri* and AMC- *D. edulis* combinations. For *E. coli* ATCC 25922, no antagonistic effect was observed with the different combinations, and this strain also showed the most synergistic effects, i.e. 4/6 of the combinations (**Table 3**).

Table 3 : Results of the checkerboard test

Strain	Antimicrobial combination	CIF A/B	CIF	Interpretation
E. coli ATCC 25922	AMC / *D. buettneri*	0.12 / 0.02	0.14	Synergy
	AMC / *D. edulis*	0.5 / 0.07	0.57	Additional
	CFM / *D. buettneri*	0.06 / 0.05	0.11	Synergy
	CFM / *D. edulis*	0.01 / 0.50	0.51	Additional
	CRO / *D. buettneri*	0.06 / 0.10	0.16	Synergy

	CRO / *D. edulis*	0.06 / 0.25	0.31	Synergy
Salmonella spp	AMC / *D. buettneri*	0.01 / 1	1.01	Indifference
	AMC / *D. edulis*	0.01 / 1.25	1.26	Indifference
	CFM / *D. buettneri*	0.01 /0.50	0.51	Additional
	CFM / *D. edulis*	0.06 / 0.44	0.50	Synergy
	CRO / *D. buettneri*	1 / 1.60	2.60	Indifference
	CRO / *D. edulis*	0.5 / 0.01	0.51	Additional
Shigella spp	AMC / *D. buettneri*	0.03 / 2.50	2.53	Indifference
	AMC / *D. edulis*	0.01 / 0.50	0.51	Additional
	CFM / *D. buettneri*	1 / 0.87	1.87	Indifference
	CFM / *D. edulis*	0.12 / 0.25	0.37	Synergy
	CRO / *D. buettneri*	2 / 0.87	2.87	Indifference
	CRO / *D. edulis*	2 / 0.62	2.62	Indifference
E. coli ESBL	AMC / *D. buettneri*	4 / 0.45	4.45	Antagonism
	AMC / *D. edulis*	8 / 0.40	8.40	Antagonism
	CFM / *D. buettneri*	0.5 / 0.50	1	Additional
	CFM / *D. edulis*	0.5 / 1	1.50	Indifference
	CRO / *D. buettneri*	0.5 / 0.40	0.90	Additional
	CRO / *D. edulis*	0.06 / 0.25	0.31	Synergy

2.6. Time-killcurves of Bacteria

The results of the tests on the elimination of bacterial strains under the effect of essential oils of *D. bettneri* and *D. edulis* and synergistic combinations are presented in **Figures 2 to 5**.

Combinations tested: Amoxicillin/clavulanic acid-*D. buettneri* (AMC-*D. buettneri),* Cefixime-*D. buettneri* (CFM-*D. buettneri),* Cefixime-*D. edulis* (CFM-*D. edulis*), Ceftriaxone-*D. buettneri* (CRO-*D. buettneri*), Ceftriaxone-*D. edulis* (CRO-*D. edulis*).

After incubation of the strains in the presence of essential oil (EO) MICs and synergistic combinations (antibiotic-essential oils) (**Table 3**), *E. coli* ATCC 25922 was completely suppressed by the EO MICs after 6h. Of the synergistic combinations, only one (ceftriaxone-*D. edulis* synergy) was unable to completely eliminate this germ (**Figure 2**). *Salmonella* spp was also completely suppressed by the EO MICs after 6h of incubation, but in its case, neither of the two synergistic combinations (cefixime-*D. buettneri* and cefixime-*D. edulis*) were able to eliminate it completely (**Figure 3**). For *Shigella* spp, an almost identical elimination curve was observed between the MICs of the two EO and the two combinations tested (AMC-*D. edulis* and CFM-*D. edulis*) (**Figure 4**). However, in the case of *E. coli* ESBL, the MICs of the EO and the only synergistic combination tested, ceftriaxone-*D. edulis*, had all destroyed this bacterium after 6h of incubation (**Figure 5**).

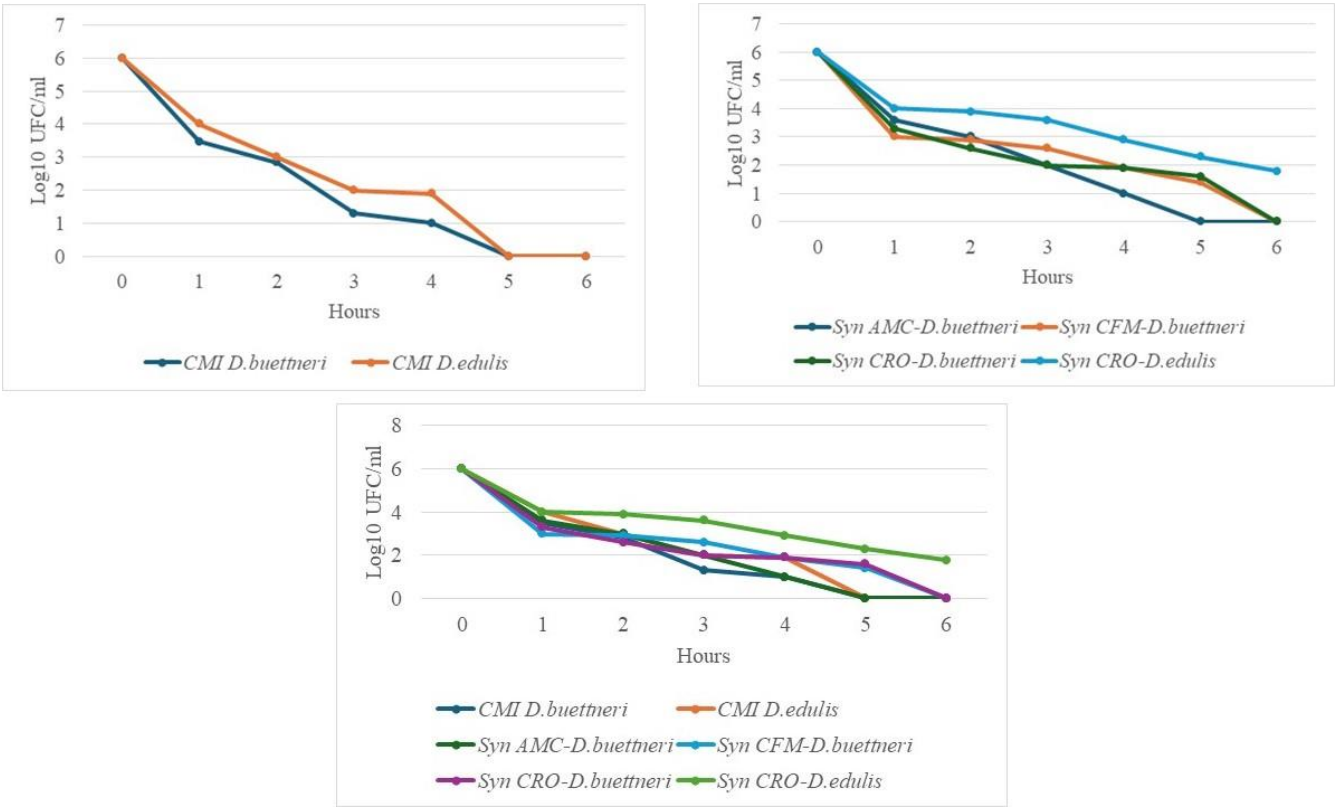

Figure 2: *Time-kill of E. coli* ATCC 25922

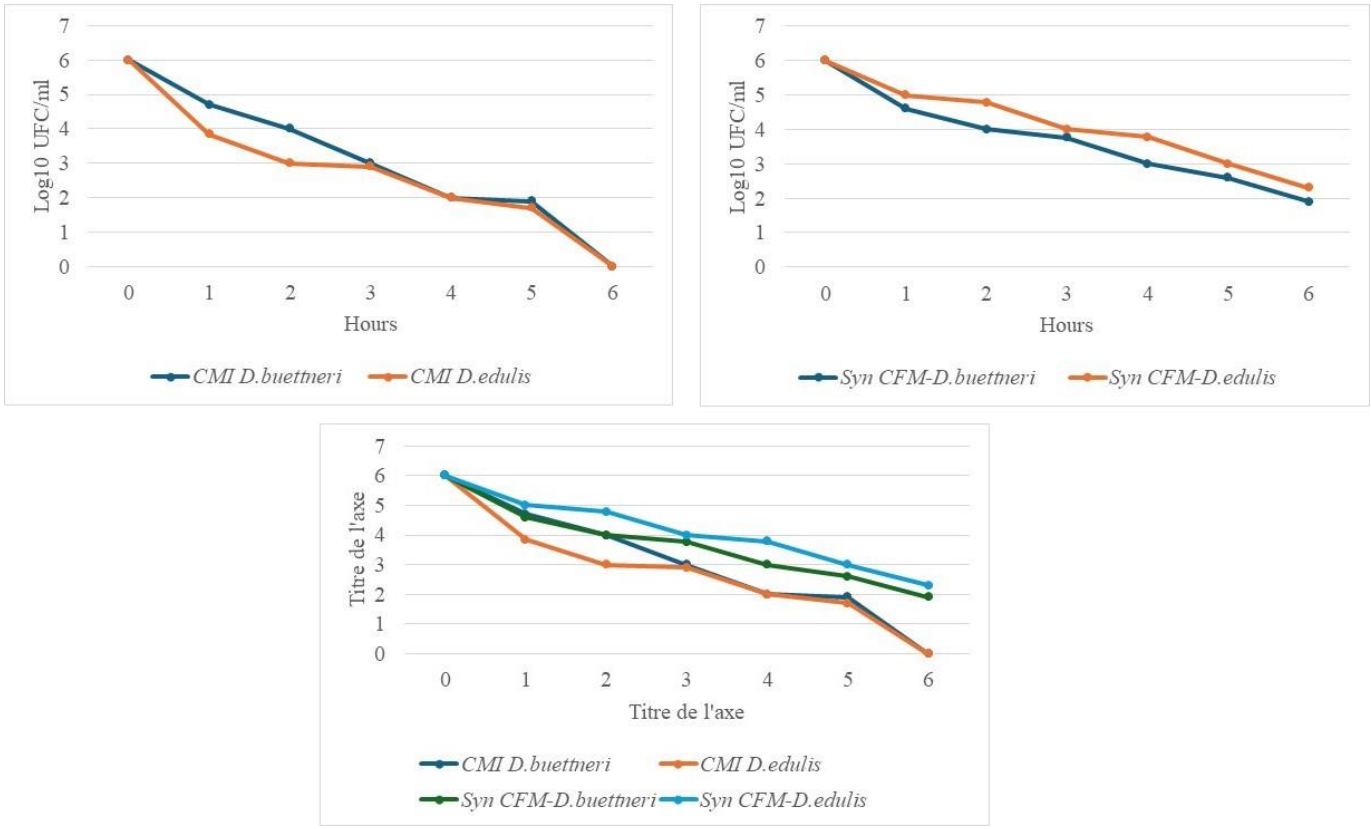

Figure 3: *Time-kill of Salmonella* spp

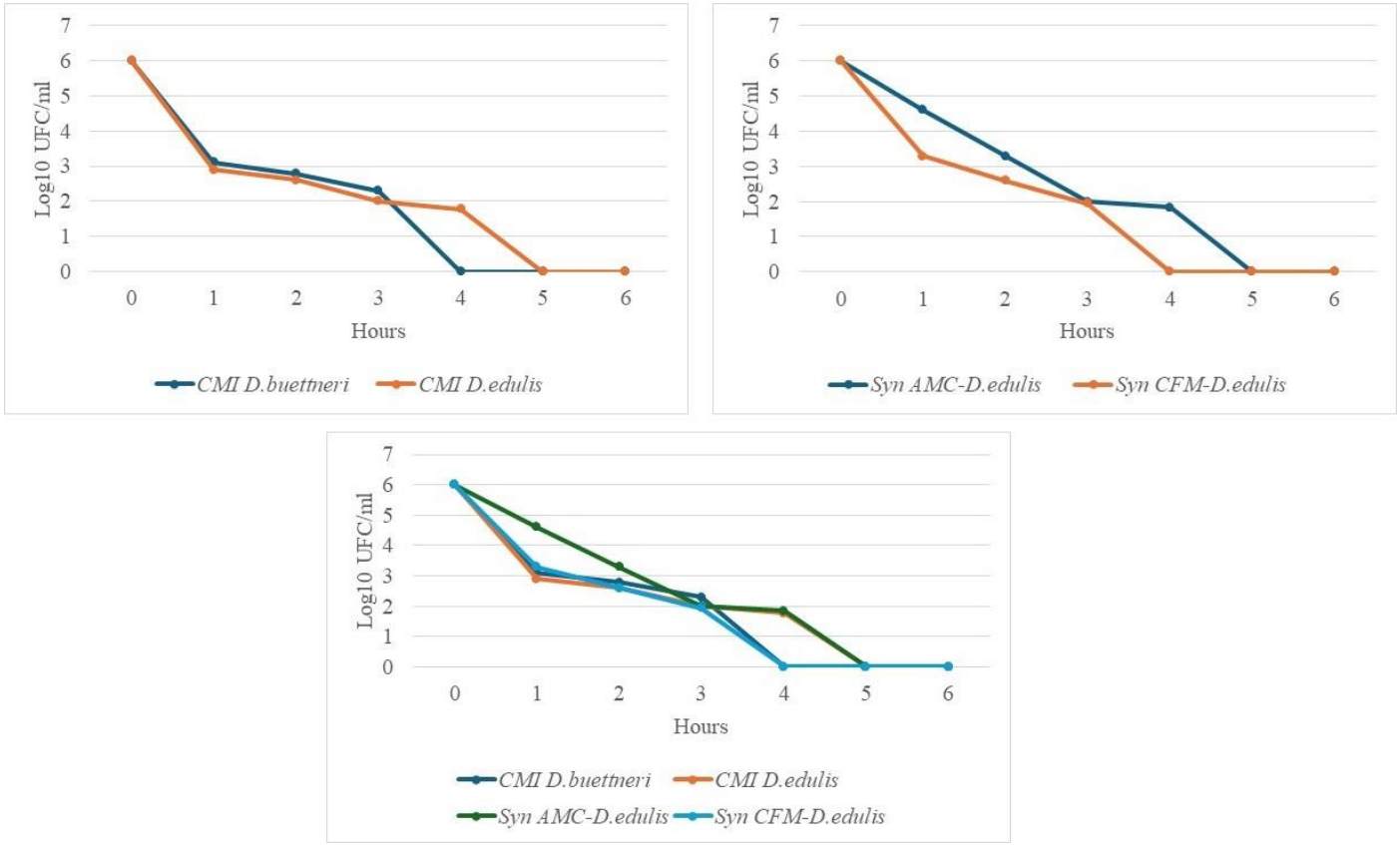

Figure 4: *Time-kill of Shigella* spp

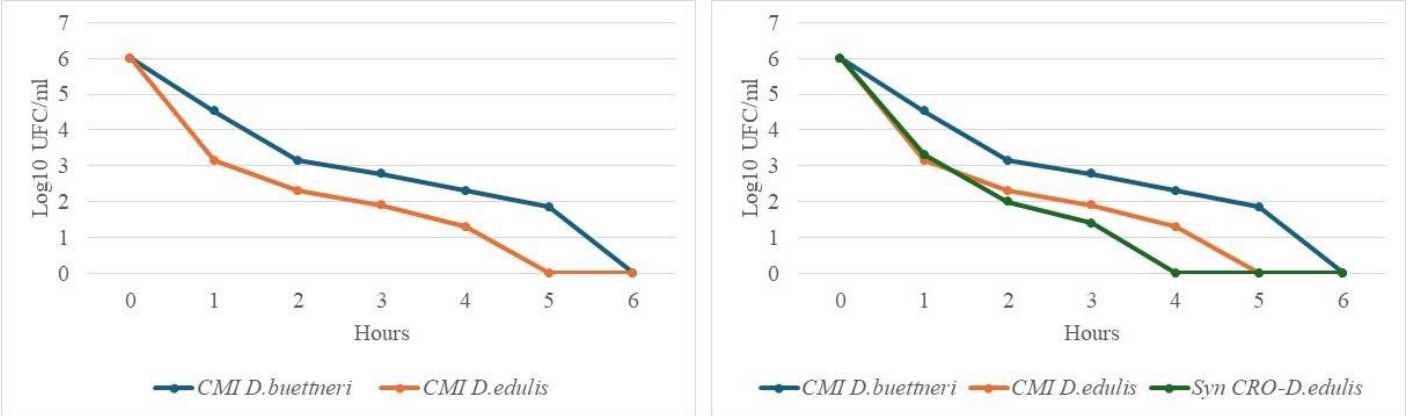

Figure 5: *Time-kil of E. coli* ESBL

2.7. Assessment of antibiofilm activity

The antibiofilm activity of *D. buettneri* and *D. edulis* essential oils was evaluated against *E. coli* ATCC 25922, *E. coli* ESBL and *Salmonella* spp.

Anti-biofilm activity was demonstrated for both EO on *E. coli* ATCC 25922 and *Salmonella* spp strains, while for *E. coli* ESBL, only *D. edulis* was shown to have antibiofilm activity with a minimum antibiofilm inhibitory concentration (MIBIC) of 50 µg/ml and a lethality rate of 57.25%. For *E. coli* ATCC 25922, the MIBC of *D. buettenri* (1.56 µg/ml) was lower than that of *D. edulis* (100 µg/ml) with respective case-fatality rates of 61.03% and 58.82%. On the other hand, for *Salmonella* spp, EO *D. edulis* had a lower MIBC (50 µg/ml) than *D. buettenri* (100 µg/ml) with a case fatality rate of 52% and 55% respectively (**Table 4**).

Table 4. Minimum antibiofilm inhibitory concentration (MIBIC) values

	Escherichia coli ATCC 25922		*Escherichia coli* ESBL		*Salmonella* spp	
Essential oil	CMIB (µg/ml)	Lethality (%)	CMIB (µg/ml)	Case fatality (%)	CMIB (µg/ml)	Lethality (%)
D. buettneri	1.56	61.03	-	-	100	55
D. edulis	100	58.82	50	57.25	50	52

CMIB: Minimum concentration of essential oil with a biofilm lethality rate of at least 50%.

3. Discussion

This study aimed to identify potential phyto-medicinal antibacterial agents and essential oils, with reference to traditional medicine, in order to find alternatives to antibiotics in the fight against the emergence of multi-resistant bacteria.

Previous studies have described how several parts of the plants of *Dacryodes* species have long been used in pharmacopoeia by indigenous peoples to treat various illnesses. The bark, leaves, resin and edible fruits of *D.*

edulis are used to treat headaches, fever and malaria while the resin of *D. buettneri* is used as an antimicrobial, disinfectant and astringent agent (Tee *et al.*, 2014).

In this study, the essential oil of *Dacryodes edulis* showed good inhibition diameters on all the strains tested, *E. coli* ATCC 22922 (18.67 mm ± 0.57 Sd), *E. coli* ESBL (16mm ± 0 Sd), *Salmonella* spp *(*16 mm± 1 Sd) and *Shigella* spp (14, 33 mm ± 0.57 Sd). The essential oil of *Dacryodes buettneri* exhibited inhibition diameters as interesting as those of *D. edulis*, but smaller, except that observed with *Salmonella* spp where *D. buettneri* exhibited an inhibition diameter of 17 mm compared with 16 mm for *Dacryodes edulis*. Similar inhibition radii of *Dacryodes edulis* essential oil were observed on *E. coli* strains in studies conducted in Gabon and Cameroon respectively by Obame *et al.*, (2008) and Mordi *et al.*, (2019). However, the results differ from those of the study conducted by Riwonin Cameroon on the *in vitro* antibacterial activity of essential oils against strains associated with diarrhoea. In this study, *D. edulis* EO showed low inhibition diameters on *Salmonella* spp, *Shigella* spp and *E. coli* spp strains (Riwom*et al.*, 2015). This discrepancy between the two studies carried out in Cameroon could be due to the difference in the parts of the plants used to obtain the essential oils: the seeds in Mordi's study and the resin in Riwon's study (2015). On the other hand, the present study and that of Riwon (Cameroon) have in common the EO obtained from the resin and the strains isolated from the diarrhoea, but discordant results were observed, which could be explained by a genetic difference in the bacteria or by a different chemical composition of the essential oils which can vary considerably according to the climate or geographical origin (Ramsey *et al.*, 2020).

In the present study, MICs and BMCs ranged from 25 to 100 µg/ml for all bacterial strains tested (**Table 4**). In order to determine the antibacterial effect, the BMC/MIC ratios were calculated and it was observed that the essential oils of *D. buettneri* and *D. edulis* all had bactericidal activity with a BMC/MIC ratio less than or equal to 1, which was similar to the results found by Obame et al., (Obame Engonga *et al.*, 2007, 2008). Their antibacterial activities could be due to a chemical composition rich in limonene, terpinene, camphene, α-pinene, β-pinene, α-terpineole, which are molecules whose antimicrobial activities have already been reported (Obame Engonga *et al.*, 2007, 2008; Mahizan *et al.*, 2019; Fiacre Bédounguindzi, 2022).

Out of 24 combinations tested as part of the synergy research, seven synergies and two antagonisms were observed in this study. One interesting synergy was obtained, that of the *E. coli* ESBL strain in relation to the CRO-*Dacryodes edulis* combination, due to the fact that the EO of *D. edulis* lifted resistance to ceftriaxone (CRO). Furthermore, no synergy was observed between cefixime (CFM) and *D. edulis* EO on the same strain. This could be explained by the fact that *D. edulis* EO may have a mode of action other than beta-lactam inhibition. The observation of antagonisms showed that the simultaneous use of *D. buettneri* and *D. edulis* essential oils with antibiotics should be avoided, and that even the use of EOs to combat infections should be preceded by antimicrobial sensitivity testing.

In addition to the use of EOs as an alternative to antibiotics, this study showed that the essential oils of *D. bettenri* and *D. edulis* could also be used as surface disinfectants or detergents, thanks to their ability to

eliminate biofilm on inert surfaces. The antibiofilm capacity of these two essential oils could be explained by their chemical composition in p-cymene and γ-terpinene, compounds whose importance has already been demonstrated in inhibiting the growth and formation of biofilms of pathogenic bacteria (Obame Engonga *et al.*, 2007, 2008; Gadisa and Usman, 2021).

4. Conclusion

This study revealed that the essential oils of *D. buettneri* and *D. edulis* had broad bactericidal activity against enteropathogenic strains with high levels of ESBL and penicillinase, making them interesting alternatives for combating the emergence of multi-resistant bacteria. However, the simultaneous use of these essential oils with antibiotics should be avoided without prior antimicrobial resistance testing. In addition, the antibiofilm test on *D. buettneri* and *D. edulis* EOs has shown that they can be used as disinfectants on inert surfaces. The antimicrobial and antibiofilm activity of these two oils could therefore make it possible to develop new molecules, drugs or cleaning products to combat the emergence of drug-resistant bacteria (DRB).

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Conflicts of interest

The authors declare no conflicts of interest.

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Authors' contributions

Formal analysis**:** CIB, SMN and MLEN. Methodology**:** RLNMM, JFDS, JPO, LCOE. Editorial: CIB, RLNMM, JFDS and JPO. Review: CIB, and JPO.

References

Abdallah, E. M., Alhatlani, B. Y., de Paula Menezes, R., and Martins, C. H. G. (2023). Back to Nature: Medicinal plants as promising sources for antibacterial drugs in the post-antibiotic era. *Plants*, 12(17), 3077. https://doi.org/10.3390/PLANTS12173077/S1

AFNOR. (1996). *Huiles essentielles, recueil de normes françaises, AFNOR*. https://search.worldcat.org/title/25464123

Aghoutane, Y., Moufid, M., Motia, S., Padzys, G. S., Omouendze, L. P., Llobet, E., Bouchikhi, B., and Bari, N. El. (2020). characterization and analysis of okoume and aiele essential oils from Gabon by GC-MS, electronic nose, and their antibacterial activity assessment. *Sensors (Basel, Switzerland)*, 20(23), 1–17. https://doi.org/10.3390/S20236750

Al-Maharik, N., Jaradat, N., Al-Hajj, N., and Jaber, S. (2023). *Myrtus communis* L.: essential oil chemical composition, total phenols and flavonoids contents, antimicrobial, antioxidant, anticancer, and α-amylase inhibitory activity. *Chemical and Biological Technologies in Agriculture*, 10(1), 1–13. https://doi.org/10.1186/S40538-023-00417-4/FIGURES/4

Arsene, M. M. J., Viktorovna, P. I., Davares, A. K. L., Parfait, K., Andreevna, S. L., Mouafo, H. T., Rehailia, M., Vyacheslavovna, Y. N., Pavlovna, S. I., Manga, I. A. M., and Sergueïevna, D. M. (2022). Antimicrobial and antibiotic-resistance reversal activity of some medicinal plants from cameroon against selected resistant and non-resistant uropathogenic bacteria. *Frontiers in Bioscience - Elite*, 14(4), 25. https://doi.org/10.31083/J.FBE1404025/1945-0508-14-4-025/FIG15.JPG

Balouiri, M., Sadiki, M., and Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*, 6(2), 71–79. https://doi.org/10.1016/J.JPHA.2015.11.005

Boonyanugomol, W., Kraisriwattana, K., Rukseree, K., Boonsam, K., and Narachai, P. (2017). *In vitro* synergistic antibacterial activity of the essential oil from *Zingiber cassumunar* Roxb against extensively drug-resistant *Acinetobacter baumannii* strains. *Journal of Infection and Public Health*, 10(5), 586–592. https://doi.org/10.1016/J.JIPH.2017.01.008

Chávez-González, M. L., Rodríguez-Herrera, R., and Aguilar, C. N. (2016). Essential oils: a natural alternative to combat antibiotics resistance. *Antibiotic Resistance: Mechanisms and New Antimicrobial Approaches*, 227–237. https://doi.org/10.1016/B978-0-12-803642-6.00011-3

CLSI. (1999). *M26-A methods for determining bactericidal activity of antimicrobial agents; approved guideline this document provides procedures for determining the lethal activity of antimicrobial agents*. www.clsi.org.

De Almeida-Couto, J. M. F., Ressutte, J. B., Cardozo-Filho, L., and Cabral, V. F. (2022).Current extraction methods and potential use of essential oils for quality and safety assurance of foods. *Anais Da Academia Brasileira de Ciências*, 94(2), e20191270. https://doi.org/10.1590/0001-3765202220191270

Ersanli, C., Tzora, A., Skoufos, I., Fotou, K., Maloupa, E., Grigoriadou, K., Voidarou, C., and Zeugolis, D. I. (2023). The assessment of antimicrobial and anti-biofilm activity of essential oils against *Staphylococcus aureus*strains. *Antibiotics*, 12(2), 384. https://doi.org/10.3390/ANTIBIOTICS12020384

Fathi, M., Ghane, M., and Pishkar, L. (2022).Phytochemical composition, antibacterial, and antibiofilm activity of *Malva sylvestris* against human pathogenic bacteria. *Jundishapur Journal of Natural Pharmaceutical Products*, 17(1), 114164. https://doi.org/10.5812/JJNPP.114164

Fiacre Bédounguindzi, W. (2022). Caractérisation chimique et application à la préservation du bois des oléorésines *d'Aucoumea klaineana* (Okoumé), *Canarium schweinfurthii (Aiélé)*, *Dacryodes buettneri* (Ozigo) et *Dacryodes edulis* (Safoutier) du Gabon. http://www.culture.gouv.fr/culture/infos-pratiques/droits/protection.htm

Gadisa, E., and Usman, H. (2021). Evaluation of antibacterial activity of essential oils and their combination against multidrug-resistant bacteria isolated from skin ulcer. *International Journal of Microbiology*. https://doi.org/10.1155/2021/6680668

Ghavam, M. (2023). Antibacterial potential of biosynthesized silver nanoparticles using *Nepeta sessilifolia* Bunge and *Salvia hydrangea* DC. Ex Benth. extracts from the natural habitats of Iran's Rangelands. *BMC Complementary Medicine and Therapies*, 23(1), 1–17. https://doi.org/10.1186/S12906-023-04101-W/TABLES/3

Gould, K. (2016). Antibiotics: from prehistory to the present day. *Journal of Antimicrobial Chemotherapy*, 71(3), 572–575. https://doi.org/10.1093/JAC/DKV484

Huang, R. Y., Pei, L., Liu, Q., Chen, S., Dou, H., Shu, G., Yuan, Z. X., Lin, J., Peng, G., Zhang, W., and Fu, H. (2019). Isobologram analysis: A comprehensive review of methodology and current research. *Frontiers in Pharmacology*, 10(10), 474554. https://doi.org/10.3389/FPHAR.2019.01222/BIBTEX

Hutchings, M., Truman, A., and Wilkinson, B. (2019). Antibiotics: past, present and future. *Current Opinion in Microbiology*, 51, 72–80. https://doi.org/10.1016/J.MIB.2019.10.008

Magi, G., Marini, E., and Facinelli, B. (2015). Antimicrobial activity of essential oils and carvacrol, and synergy of carvacrol and erythromycin, against clinical, erythromycin-resistant Group A *Streptococc*i. *Frontiers in Microbiology*, *6*(3), 130454. https://doi.org/10.3389/FMICB.2015.00165/ABSTRACT

Mahizan, N. A., Yang, S. K., Moo, C. L., Song, A. A. L., Chong, C. M., Chong, C. W., Abushelaibi, A., Erin Lim, S. H., and Lai, K. S. (2019). Terpene derivatives as a potential agent against antimicrobial resistance (amr) pathogens. *Molecules*, 24(14). https://doi.org/10.3390/MOLECULES24142631

Mordi, R. C., Olasehinde, G. I., Okedere, A. P., Elegwule, A. N., Ayo-Ajayi, J. I., Johnathan, H. O., Onibokun, A. E., Ajayi, A. A., and Uchenna, D. O. (2019). Antibacterial activity of moderately volatile components of the oil extracted from the seeds of *Dacryodes edulis* G. LAM. *Asian Journal of Pharmaceutical and Clinical Research*, 12, 246–249. https://doi.org/10.22159/AJPCR.2019.V12I3.30161

Morsy, N. F. S.,andMorsy, N. F. S. (2017). Chemical structure, quality indices and bioactivity of essential oil constituents.*Active Ingredients from Aromatic and Medicinal Plants*. https://doi.org/10.5772/66231

Murray, C. J., Ikuta, K. S., Sharara, F., Swetschinski, L., Robles Aguilar, G., Gray, A., Han, C., Bisignano, C., Rao, P., Wool, E., Johnson, S. C., Browne, A. J., Chipeta, M. G., Fell, F., Hackett, S., Haines-Woodhouse, G., KashefHamadani, B. H., Kumaran, E. A. P., McManigal, B., … Naghavi, M. (2022). Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *The Lancet*, 399(10325), 629–655. https://doi.org/10.1016/S0140-6736(21)02724-0

Nicolaou, K. C., and Rigol, S. (2017). A brief history of antibiotics and select advances in their synthesis. *The Journal of Antibiotics*, 71(2), 153–184. https://doi.org/10.1038/ja.2017.62

Obame Engonga, L.-C., Chalchat, J.-C., N Bassolé, I. H., Koudou, J., Bassolé, I., Edou, P., Ouattara, A. S., and Traore, A. S. (2007). Volatile components, antioxidant and antibacterial activities of *Dacryodes buettneri* H. J. Lam. essential oil from Gabon.*Scientific Research and Essay*, 2(11), 491–495. http://www.academicjournals.org/SRE

Obame Engonga, L.-C., Edou, P., Bassolé, N., Koudou, j, Agnaniet, H., Eba, F., and Traoré, A. S. (2008). Chemical composition antioxidant and antimicrobial properties of essential oil of *Dacryodes edulis* (G.Don) H.J. Lam from Gabon. *African Journal of Microbiology Research*, 2, 148–152.

Ramsey, J. T., Shropshire, B. C., Nagy, T. R., Chambers, K. D., Li, Y., and Korach, K. S. (2020). Focus: Plant-based medicine and pharmacology: essential oils and health. *The Yale Journal of Biology and Medicine*, 93(2), 291. /pmc/articles/PMC7309671/

Riwom, S. H., foe, F. M. C. N., Nyegue, M. A., Ambe Wanki, R., Voundi Olugu, S., and Etoa, F. X. (2015). Chemical composition and in vitro antibacterial activity of the essential oils of the leaves, resin and stem-barks of *Dacryodes edulis* (G. Don) H. J lam growing in Cameroon on diarrhea associated strains. *Journal of Applied Pharmaceutical Science*, *5*(10), 6–11. https://doi.org/10.7324/JAPS.2015.501002

Sadgrove, N. J., Padilla-González, G. F., and Phumthum, M. (2022). Fundamental chemistry of essential oils and volatile organic compounds, methods of analysis and authentification. *Plants*, 11 (6), 789. https://doi.org/10.3390/PLANTS11060789

Shaikh, S., Fatima, J., Shakil, S., Rizvi, S. M. D., & Kamal, M. A. (2015). Antibiotic resistance and extended spectrum beta-lactamases: Types, epidemiology and treatment. *Saudi Journal of Biological Sciences*, 22(1), 90–101. https://doi.org/10.1016/J.SJBS.2014.08.002

Siqueira, I. B., Teixeira Barbosa, A. A., Jain, S., Miranda Fernandes, R. P., Tavares Silva, A. R. S., Ferreira Barbosa, F. H., Schimieguel, D. M., Blank, A. F., Sacramento, A. G., de Castro Nizio, D. A., and Dolabella, S. S. (2021). *In vitro* antibacterial activity of essential oils of croton *Tetradenius bail*l. from the

Brazilian caatinga biome and its synergistic effect with ciprofloxacin and meropenem. *Journal of Essential Oil-Bearing Plants*, 24(1), 12–21. https://doi.org/10.1080/0972060X.2020.1871079

Tee, L. H., Yang, B., Nagendra, K. P., Ramanan, R. N., Sun, J., Chan, E. S., Tey, B. T., Azlan, A., Ismail, A., Lau, C. Y., and Jiang, Y. (2014). Nutritional compositions and bioactivities of *Dacryodes* species: A review. *Food Chemistry*, 165, 247–255. https://doi.org/10.1016/J.FOODCHEM.2014.05.084

Tremblay, Y. D. N., Hathroubi, S., and Jacques, M. (2014). Les biofilms bactériens : leur importance en santé animale et en santé publique. *Canadian Journal of Veterinary Research*, 78(2), 110. /pmc/articles/PMC3962273/

Walsh, T. R., Gales, A. C., Laxminarayan, R., and Dodd, P. C. (2023). Antimicrobial resistance: addressing a global threat to humanity. *PLOS Medicine*, 20(7), e1004264. https://doi.org/10.1371/JOURNAL.PMED.1004264