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Nanoemulsions of *Canarium schweinfurthii* sap and *Dacryodes edulis* kernel essential oils: Chemical profile and in vitro efficacy against diarrheagenic bacteria

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Abstract

Background: The objective of this study was to evaluate the in vitro antibacterial activity of nanoemulsions based on the essential oils of *Canarium schweinfurthii* and *Dacryodes edulis* against enteric bacteria responsible for diarrhea.

Methods: The essential oils were extracted by the hydrodistillation method using a Clevenger-type apparatus, and the chemical composition was determined by gas chromatography with flame ionization detection (GC-FID) and gas chromatography (GC) coupled with mass spectrometry (GC/MS). Nanoemulsions were prepared, and their antibacterial activity was evaluated using the disc diffusion method and by the micro-dilution method in liquid medium, at the end of which the inhibitory diameters and minimum inhibitory concentrations (MICs) were obtained with respect to the different isolates.

Results: *C. schweinfurthii* showed 3.96%, and *D. edulis* showed 0.10% extraction yield with 19 and 20 compounds found, respectively. The chemical profile of *C. schweinfurthii* was composed mainly of *α* -pinene (40.92%), myrcene (16.02%), and *β*-phellandrene (12.73%); whereas that of *D. edulis* was majorly composed of limonene (35.52%), *α*-terpineol (30.72%), and *p*-cymene (8.82%). Both nanoemulsions showed a good antibacterial activity: *C. schweinfurthii* MICs were found to be between 312.5 µg/mL (*Salmonella typhi*) and 5000 µg/mL (*E. coli* and *E. aerogenes*), with a bactericidal effect on most strains and a bacteriostatic effect on *S. Typhi*. In the case of *D. edulis*, the MICs were 625 µg/mL to 5000 µg/mL with bactericidal activities against *E. aerogenes* and *E. coli* and bacteriostatic against *K. pneumoniae* and *S. typhi*.

Conclusion: Although these nanoemulsions were less active than the reference ciprofloxacin, they could retain the biological activities of the essential oils, making them promising plant-based substitutes to treat enteric infections by diarrheagenic bacteria.

Keywords: Antibacterial activities; *Canarium schweinfurthii*; Chemical composition; *Dacryodes edulis*; Essential oils; Nanoemulsions.

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Background

Infectious diseases, caused by pathogenic agents such as viruses, fungi, and bacteria, have constituted a major public health problem in recent decades [1]**.** The situation is further exacerbated by the rise of multidrug-(MDR) microbial strains and the emergence of atypical infections that compromise standard treatment efficacy. With an annual mortality rate of nearly 17 million [2]. Among these infectious diseases, approximately 43% of deaths are recorded in tropical countries. The case of Cameroon is a perfect illustration of this, with approximately 122 deaths of children under five years of age out of approximately 1000 live births, the main causes being malaria, pneumonia and diarrhea [3]. The most effective medicinal weapons used against these bacterial infections are antibiotics. However, despite advances in antibiotic therapy, there is a constantly increasing emergence of resistance in bacteria, thus hindering the control of bacterial infections. Furthermore, socioeconomic conditions such as poor hygiene, illiteracy, and limited access to healthcare and vaccines exacerbate this dramatic situation in developing nations [4]. The search for new treatments for infectious diseases remains a critical concern: the emergence and spread of resistance mechanisms to antibacterial drugs, combined with the limited pipeline of new antibiotics, constitute a genuine public health crisis. There is an urgent need to find and develop new therapeutic approaches for treating these infections. Numerous research avenues exist, but the exploration of natural resources appears to be one of the most particularly promising, as these resources, due to their diversity, constitute the largest reserve of active substances. Plants can be a valuable source of new antibiotic compounds. According to the WHO [5], nearly 80% of the population in developing countries primarily uses medicinal plants as medicine. These plants play a very important role in individual health due to the multitude of secondary metabolites they contain (alkaloids, tannins, phenols, saponins). The presence of these diverse compounds in plants makes them a reservoir of substances that combat pathogenic microorganisms [1]. Nowadays, in tropical countries like Cameroon, medicinal plants, such as aromatic plants rich in essential oils, offer an alternative for disease treatment due to their accessibility and lower toxicity. Numerous essential oils derived from Cameroonian flora have demonstrated significant efficacy. This was the case in the study of *Callistemon rigidus* (Myrtaceae), which highlighted the antioxidant and herbicidal activities [6]. Similarly, Jazet et al., [7] demonstrate the antifungal, antioxidant, free radical scavenging, and anti-inflammatory properties of the sap of *Canarium schweinfurthii* . Studies carried out on *Syzygium aromaticum* (Myrtaceae), known as the clove tree, by Chaieb et al. [8] revealed its antibacterial, anti-inflammatory, analgesic, antifungal and antioxidant properties. However, due to the complexity of the essential oil (EO) matrix, characterized by instability and volatility, as well as the dermo-caustic and photosensitivity issues observed during application, essential oil-based nanoemulsions (NEs) could constitute another potential source of antibacterial properties. Indeed, nanoemulsions protect bioactive substances, improve their intracellular penetration into biological tissues and skin, prolong the effect of these substances, and reduce tissue irritation. This study was undertaken to evaluate *in vitro* the antibacterial activity of nanoemulsions based on the essential oils of kernels of *Dacryodes edulis* and the sap of *Canarium schweinfurthii* against enterobacteria responsible for gastroenteritis.

Methods

Plant material

Canarium schweinfurthii resins were collected in August 2018 in Bana, located in the Haut-Nkam Department, West Region of Cameroon. The botanical identification and authentication of the plant were carried out at the National Herbarium of Cameroon in Yaoundé, registered under reference number 16929/SRF/Cam by the Forest Reserves Company of Cameroon. *Dacryodes edulis* kernels were collected in July 2018 in Makenene, Mbam-et-Inoubou Division, Center Region of Cameroon. Botanical identification and authentication of the plant were carried out at the National Herbarium of Cameroon in Yaoundé. registered under reference number18258/HNC.

Microorganisms

Microorganisms (*Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella* typhi and *Enterobacter aerogenes*) used in this study were Gram-negative bacterial isolates, all responsible for human infections (mainly gastroenteritis) from the University of Douala Cameroon with the characteristics summarized in the table 1.

Extraction of essential oils

The extraction of essential oils was carried out by hydrodistillation using Clevenger-type apparatus. For each plant material, 1 kg of *D. edulis* kernels or *C. schweinfurthii* resins was weighed and placed in a reactor with 2 liters of tap water, then the mixture was subjected to hydrodistillation for 4 hours until no more oil was collected. Heating causes the rupture of the secretory glands and steam entrainment of the essential oils. These oils, carried by steam, condense in the condenser where cold water circulates continuously. This results in the formation of a distillate consisting of two phases in the decanting column: the fragrant upper organic phase, consisting of essential oils was collected at the end of the distillation and then dried on an anhydrous sodium sulfate column to remove all traces of water contained in the essential oil. The essential oils were weighed and then stored in a refrigerator at a low temperature (**+4°C**) in dark bottles. Extraction yields were calculated relative to the mass of plant material after extraction using the following formula:

$$\textbf{Yield (\%)} = \frac{\textbf{Weight of essential oil}}{\textbf{Weight of the dry powder}} \times \mathbf{100}$$

Thus, the density of each oil was calculated relative to the density of water according to the formula shown opposite.

$$\text{Relative density } (d_{20}) = \frac{\text{Mass of 1 mL of EO at 20°C}}{\text{Mass of 1 mL of water at 20°C}}$$

The essential oils obtained were analyzed by Gas Chromatography (GC) and by Gas Chromatography coupled with Mass Spectrometry (GC/MS). This analysis was carried out at the Fine Chemistry Laboratory of the Max Mousseron Institute of Biomolecules at the University of Montpellier II.
Analysis by gas chromatography (GC)

The analysis of the essential oils was done through GC-FID (Varian CP-3380) and GC-MS (HP 5970). In GC-FID, a DB5 column (30 m, 0.25 mm, 0.25 µm) was employed that was ramped

(50 200°C) in the presence of N_2 carrier gas (0.5 ml/min). The GC-MS used an HP1 column at 70 (ramp 10°C/min), He carrier gas (0.6 mL/min) and injected 0.1 uL of 10% oil in hexane. The identification of compounds was done through the comparison of the mass spectra and the Kovats retention indices (calculated using n-alkanes) with the literature values. Determinations were done using electronic integration, omitting factors of relative responses. The conditions were as followed: GC-FID: column of DB5; 50–200◦C (5◦C/min) with a flow rate of 1 mL/min of N_2 carrier gaz; GC-MS: column of HP1; 70–200°C (10°C/min) with a flow rate of 0.6 mL/min of the carrier gas.

Nano-emulsion (NE) formulation

The methodology described by Prinderre *et al., [9]* was used. Briefly, tween 80 was used as a surfactant at a 5% concentration. The NE was therefore prepared by mixing tween 80 (5%), essential oil (5%), and distilled water (90%). A mixture of 0.5 g of Tween 80 and 0.5 g of essential oil was stirred (mechanically at 400 rpm) for 15 minutes. Then, 9 mL of distilled water was added to the mixture at a rate of 2 mL/min while continuing to stir at the same speed. After adding the water, the water-oil-Tween mixture was stirred for two hours. At the end of the preparation, it was stored at a low temperature for two to three days, observed and physical characteristics recorded.

In vitro evaluation of the antibacterial activity of essential oil nanoemulsions

The antibacterial activity of the nanoemulsions (NE) of the different oils obtained was evaluated by the disk diffusion method on solid medium as described by Remmal *et al.,* [10] to determine the inhibition zone diameters and by the microdilution method in liquid medium as described by Satrani *et al.* [11] in order to determine the MIC (Minimum Inhibitory Concentration) and the MBC (Minimum Bactericidal Concentration) of our essences.

Preparation of the essential oil stock solution

The essential oil (EO) was mixed with Tween 80 and sterile distilled water in a 0.5/0.5/9 ratio. The resulting solution was poured into 15 ml glass bottles to obtain the desired concentration. Three trials were performed for each concentration. After pouring and solidifying the medium in Petri dishes, inoculation consisted of depleting an initial deposit of inoculum prepared beforehand by making successive spreads in different directions (this technique is known as the quadrant method of depletion). The dishes were then sealed with film and incubated upside down at room temperature for 24 hours. Four or five successive spreads were performed.

Diffusion method on agar medium (Disc method)

The culture medium Mueller Hinton Agar (MHA) was aseptically poured while supercooled into 90 mm Petri dishes at a rate of 15 ml per dish and allowed to cool and solidify on the benchtop. 1 ml of each bacterial culture suspension with a concentration of approximately (10^6 CFU/ml) was prepared from an 18-hour culture and then spread on the surface of the MHA agar medium. Using sterile forceps, 6 mm diameter Whatman No. 1 paper discs were soaked with the NE to be tested by only touching the tip of the disc. The disc gradually absorbed the NE until it was completely saturated, and then the discs were placed on the agar. The Petri dishes were then sealed and incubated at 37°C for 24 hours. After 18 to 24 hours of incubation, a clear zone or halo was present around a disc if the NE inhibited microbial growth. The larger the zone of inhibition, the more susceptible the organism. All tests were repeated three times for each bacterial strain and for each NE. In the control dishes, the discs were soaked in Tween 80 for negative controls and in the antibiotic for the positive control [12]. The reading was taken by measuring the diameter of the inhibition zone around each disc using a graduated ruler (in mm). The results were expressed as the diameter of the inhibition zone and symbolized by signs according to the sensitivity of the isolates to NEs, as follows: Non-sensitive (-) or resistant when diameter < 8mm; Sensitive (+) when diameter between 9 and 14 mm; very sensitive (++) when diameter between 15 and 19 mm; and extremely sensitive (+++) when diameter > 20 mm.

Determination of minimum inhibitory concentrations (MICs): Liquid microdilution method

The bacterial growth inhibitory potential of each essential oil was determined by two liquid microdilution methods following the guidelines of CLSI [13]. The technique used is that of microdilution in liquid medium carried out according to a two-fold serial dilution. The tests were carried out in triplicate for 4 isolates of microorganisms. First, 100 µl of culture medium (MHB) was added to each well of a 96-well microplate. Next, 100 µl of stock solution (NE) was added to the upper wells, followed by a series of successive second-order geometric dilutions. Finally, 100 µl of bacterial inoculum was added to each well to obtain final NE concentrations ranging from 5000 to 39.06 µg/mL. The plates were covered with Parafilm and incubated at 37°C for 24 hours. The reference antibiotic (ciprofloxacin) was tested at the same concentrations. At the end of the incubation period, bacterial growth was detected using a 2% *p* -Iodonitrotetrazolium (INT) solution. Viable bacteria change the color of INT from colorless to pink. To achieve this, 40 µl of an INT solution were introduced into each well. The plates were incubated at 37°C for 30 min. Minimum inhibitory concentrations (MICs) were defined as the lowest concentrations of substances at which no color change in INT was observed.

Determination of minimum bactericidal concentrations (MBC)

For the determination of minimum bactericidal concentrations (MBCs), 150 µl of culture broth (from the wells of the microplates used for MIC determination) was taken and introduced into the wells of a new 96-well plate. 40 µL of the contents of wells with concentrations greater than or equal to the MIC was taken and introduced into the wells of the new plate. These plates were then incubated for 48 h at 37°C followed by INT detection as before. The MBC of each extract was recorded as the lowest dilution (concentration) at which no growth was observed on Mueller-Hinton agar.

MBC/MIC Report

Calculation of the MBC/MIC ratio allowed the determination of the bactericidal effect (MBC/MIC ≤ 4), bacteriostatic effect (MBC/MIC > 4) or "absolute bactericidal" effect if MBC/MIC =1 [14].

Statistical analysis

The data were entered into an Excel spreadsheet (Microsoft, 2010) and then analyzed using Statview version 5.0 software (SAS Institute Inc., USA). The results were presented in tables and graphs. One-way analysis of variance (ANOVA) was used to study the effect of the extract dose and incubation time on bacterial growth. The post-hoc test was used to compare inhibition

diameters. The significance level was set at a probability value less than 0.05.

Results

Extraction of essential oils from C. schweinfurthii and D. edulis

Hydrodistillation extraction allowed us to obtain the essential oils whose characteristics are listed in Table 2.

Essential oil complex - tween 80

Nanoemulsions were prepared using two essential oils: *Canarium schweinfurthii* and *Dacryodes edulis*. An emulsifier was used: Tween 80 (polyethylene sorbitan monooleate). These nanoemulsions were obtained from a ratio of 0.05/0.95/9 (v/v/v surfactant and distilled water) for a concentration of 5000 µg/mL for the essential oil. The *Dacryodes edulis* nanoemulsion was light, slightly milky white, while the *Canarium schweinfurthii* nanoemulsion was white and milky, exhibiting a bluish sheen on the glass.
According to Fernandes et al. [15], a good nanoemulsion should adhere to the surface and leave a bluish tint, as it was the case with our essential oils obtained in this way. This is thought to be due to the very small size of the droplets; upon adhering to the surface, the nanoemulsion leaves a very thin film that allows light to pass through, which is then reflected off the liquid's surface.

Chemical composition of essential oils

Analysis of the chemical composition of the EO of *Dacryodes edulis* and *Canarium schweinfurthii* by GC and GC/MS allowed the obtaining of the chromatograms in supplementary data 1, which allowed the identification of the compounds they contain by comparing the Kovats indices obtained with those in the laboratory database [16] and the literature. The chromatograms presented 20 peaks corresponding to 20 chemical compounds for the essential oil of *Dacryodes edulis;* and 19 peaks corresponding to 19 chemical compounds for the HE of *Canarium schweinfurthii*. After calculation of their Kovats Index (KI), the identified chemical compounds were subsequently grouped into two main subclasses, namely: hydrogenated monoterpenes (MTH), oxygenated monoterpenes (MTO) and trace of hydrogenated sesquiterpenes. Table 3 shows that the essential oils of *Dacryodes edulis* and *Canarium schweinfurthii* are very rich in monoterpenes, with respective proportions of 99.95 % and 99.92% for these two plants. Hydrogenated monoterpenes (MTH) account for 66.16% and 98.71% of the composition. Oxygenated monoterpenes (MTO) follow, with percentages of 33.79% and 1.21%. Among the thirty-nine (39) compounds identified from the essences of *Canarium schweinfurthii* and *Dacryodes edulis* studied, three (03) compounds were identified as major compounds respectively for the essential oils of *Dacryodes edulis* : Limonene (35.52%), α-Terpineol (30.72%), Para-Cymene (8.82%), and of *Canarium schweinfurthii* : α-pinene (40.92%), Myrcene (16.02%), β-phellandrene (12.73%) .

In vitro antibacterial activity of essential oil nanoemulsions

The antibacterial potential of the NE of the essences of *C. Schweinfurthii* and *D. edulis* was evaluated *in vitro* against four bacterial isolates.

Antibacterial activity according to the disc method

After 24 hours in the incubator, the observation of the halos and the inhibition of the isolates in contact with the NE of *Dacryodes edulis and Canarium schweinfurthii* at different concentrations was done by measuring inhibition diameters which were compared to reference standards. The essential oils from both plants possess antibacterial activity against all four bacterial isolates. The inhibition zones increased with the EO dose. In other words, we observe a dose-concentration effect regardless of the isolate on which the essential oil-based NE was tested. Ciprofloxacin was more active than the EOs against all bacterial isolates. Table 4 shows that the NE of the sap of *C. schweinfurthii* is more active on all isolates than the NE of *D. edulis kernels. The NE of C. schweinfurthii* essential oil exhibited inhibition zones with diameters ranging from 17.33 to 32.33 mm. NE of *D. edulis kernels* exhibited inhibition zones with diameters varying from 10.77 to 24.82 mm. NE based on *Canarium schweinfurthii essential oil* showed strong activity against bacterial isolates with the following inhibition zones: 32.33 mm (*E. coli)*, 25.56 mm (*K. pneumoniae*) , 29.38 mm *(E. aerogenes)*, and 27.06 mm *(S. typhi)*. NE based on the HE of *Dacryodes edulis* showed strong activity on bacterial isolates of *E. aerogenes* (27.87 mm). *K. pneumoniae* **(**24.82 mm), *E. coli* (24.56 mm) and *S. typhi* (23.97 mm). Statistical analyses using post-hoc testing showed that at 5000 µg/mL and 1250 µg/mL there were no significant difference between the two NEs based on the essential oils of *C. schweinfurthii* and *D. edulis* compared to *K. pneumoniae* and *E. aerogenes* respectively a ($P < 0.05$).

Antibacterial activity evaluated through the microdilution method

In vitro antibacterial activity of the NEs of the essential oil derived from the sap of *Canarium schweinfurthii* and the kernels of *Dacryodes edulis* against *E. coli, K. pneumoniae, S. typhi, and E. aerogenes* isolated from the strains at the following concentrations (5000, 2500, 1250, 625, 312.5, 156.25, 78.125, and 39.06 µg/mL) allowed the determination of the MIC and MBC values. A summary of these MIC and MBC values is presented in Table 5.
Based on the results obtained, as with the agar test, both NEs possess antibacterial activity against all four bacterial isolates used. The NE of *C. schweinfurthii* exhibited MICs ranging from 312.5 to 5000 µg/mL, while the NE of *D. edulis* exhibited MICs ranging from 625 to 5000 µg/mL. Both NEs possess similar *in vitro* antimicrobial activities, *as* they showed similar MICs for *E. coli and E. aerogenes isolates* (MIC = 5000 µg/mL), except for *S. typhi* and *K. pneumoniae* (MIC = 625 and 1250 µg/mL) for the *C. schweinfurthii NE,* which showed better antimicrobial performance compared to the D. *edulis NE.* (MIC = 625 µg/mL). According to the MBC/MIC reports, the NE of *Canarium schweinfurthii* was 75% bactericidal on the isolates. of *E. aerogenes, K. pneumoniae* and *E. coli* and 25% bacteriostatic against *S. typhi*. On the other hand, 50% bactericidal effects are noted against *E. coli and E. aerogenes,* 50% bacteriostatic on *K. pneumoniae* and *S. typhi* for the NE of *Dacryodes edulis.*

Significance of the data

Statistical analyses post-hoc testing shows that at MICs of 625 and 1250 µg/mL for the NE of *C. schweinfurthii* and 312.5 and 625 µg/mL for *D. edulis*, both NE are significantly different at ($P < 0.05$) with respect to *K. pneumoniae* and *S. typhi.* However, at MIC 5000 µg/mL, there is no significant difference between the results of our two NE with respect to *E. coli* and *E. aerogenes* at ($P < 0.05$).

Discussion

The results showed that the essential oil extraction yields from the kernels of *Dacryodes edulis* and the sap *of Canarium schweinfurthii* are 0.10% and 3.96%, respectively. Based on these yields obtained, we can conclude that the sap *of C. schweinfurthii* is rich in essential oils. Thus, *C. schweinfurthii* could be the subject of further research with a view to its industrial exploitation with this yield. These results also show that within the same family, the essential oil yield differs from one species to another. The essential oil yield from the kernels of *Dacryodes edulis* (0.10%) is close to that obtained by Onayade et al. [17]. on fruits in Nigeria which had obtained a yield of 0.15%. However, this is lower than that obtained by Ajibesin et al., [18] in Cameroon which obtained a yield of 1.5% , much lower than that obtained by Obame et al., [19] in Ouagadougou, Burkina Faso, with a yield of 6.78%. The sap yield of *Canarium schweinfurthii* (3.96%) is higher to that obtained by Obame et al., [19] (2009) which had obtained a yield of 0.2%. But this yield is higher than that obtained by Jazet et al., [20] in Cameroon with (3.6%), and sabinene (1.4%). Another study by Onocha *et al.* [21] in Nigeria on the fruits, leaves, roots and bark of *Dacryodes edulis* showed that the EO of the fruits is composed predominantly of Myrcene (45.3%), caryophyllene (26.4%) for the essential oils of the leaves and bark is made up mainly of terpinen-4-ol (25.6%) and a mixture of thujiene and pinene (25.2%), phellandrene is the major compound for the oils of the roots [21]. The results obtained with the essential oil of *Canarium schweinfurthii* differ from those obtained by Obame et al. [19] in Ouagadougou , who identified 18 compounds, representing 95.3% of its chemical composition, with the major constituents being octyl acetate (60.0%), (E)-nerolidol (14.0%), and *n* -octanol (9.5%) . Similarly, our results differ from those obtained by Jazet et al . [20] in Mbouda and Lolodorf, Cameroon. which had identified 23 compounds representing 73.4% of the composition.

Our results differ from those obtained by Obame et al [19], whose inhibition zone diameters ranged from 9 to 25 mm for the NE of *C. schweinfurthii essential oil* against bacterial isolates. However, they subsequently showed that *Canarium schweinfurthii* has strong activity against bacterial isolates such as *Salmonella enterica* CIP105150 (27 mm), *Shigella dysenteria* CIP 5451, and *Escherichia coli CIP NCTC11602 (22 mm). Regarding D. edulis* resin oil , Obame et al. [19] showed that this oil has strong antibacterial activity with inhibition zone diameters ranging from 23 to 60 mm against bacterial isolates. NE showed the strongest activity on *Shigella dysenteria* CIP 5451 (60 mm), on *Salmonella enterica* CIP 105150 (28 mm) which corroborates the results of our tests.

The results obtained with our NE are consistent with the work of Obame et al ., [19] who contributed to the study of antibacterial properties. They subsequently showed that the NE based on *C. schweinfurthii* demonstrates strong bactericidal activity on bacterial isolates of *E. coli* and *Salmonella enterica* and a bacteriostatic effect on *Shigella dysenteria* CIP5451. *In vitro* activity of NE based on *Dacryodes edulis* essential oil corroborates the results demonstrated by Ghaderi et *al.* who demonstrated antimicrobial activity *in vitro* of the NE of *Thymus daenensis* on Haemophilus influenza, Streptococcus pneumoniae, Pseudomonas aeruginosa [22].

Table 1. Summary of information on bacteria used.

Isolates	Code	Origin
Escherichia coli	EC 20	Laboratory isolate
Enterobacter aerogenes	ENT 95	Laboratory isolate
Salmonella typhi	*SAL120*	Laboratory isolate
Klebsiella pneumoniae	*KLP 50*	Laboratory isolate

Table 2. Physical characteristics of extracted essential oils.

Plants	Organ/part	Appearance	Color	Yield
C. schweinfurthii	Sap	liquid	Yellow	3.96%
D. edulis	kernels	liquid	Light yellow	0.10%

Table 3. Chemical composition of EO from *Dacryodes edulis* and *Canarium schweinfurthii*.

IK	Compounds	Plants	
		Dacryodes edulis	***Canarium schweinfurthii***
	Total identified (%)	**99.95**	**99.92**
	hydrogenated monoterpenes		
924	α-Thujene	0.33	3.2
931	α-Pinene	3.73	**40.92**
945	Camphene	0.12	0.35
971	Sabinene	6.38	2.54
974	**β-pinene**	3.36	**9.89**
990	6-Methylheptadi-3,5-en-2-one	0.29	-
991	**Myrcene**	-	**16.02**
999	Menthene	2.01	-
1004	α-Phellandrene	0.57	1.13
1009	Delta-3-Carene	-	1.30
1015	α-Terpinene	0.80	0.24
1020	Para-Menth-1-ene	0.16	-
1023	Para-Cymene	**8.82**	0.92
1027	**β-Phellandrene**	-	**12.73**
1029	Limonene	**35.52**	-
1148	Ortho-Cymene	0.11	-
1046	(E)-β-ocymene	-	0.85
1056	gamma-Terpinene	1.40	0.24
1064	cis hydrate sabine	0.95	-
1087	**Terpinolene**	-	**8.93**
1097	Cis-hydra-Sabinene	0.11	-
1459	(E)-Caryophyllene	-	0.15
	oxygenated monoterpenes		
1030	Eucalyptol	0.30	-
1144	Myrterol	-	0.35
1175	Terpinene-4-ol	2.66	0.36
1183	Para-Cymen-8-ol	-	0.37
1194	**α-Terpineol**	**30.72**	
1280	2-methyl-isoborneol	-	0.13

Table 4. Inhibition diameter (mm) of essential oil-based nanoemulsions against bacteria.

Bacteria	Concentration (µg/mL)	Inhibitory diameters			Sensitivity test		
		NE-CS	NE-DE	CIP	NE-CS	NE-DE	CIP
S. typhi	5000	27.06 ± 1.01 ^{b}	23.97±0.58 ^{a}	47.43±0.51 ^{c}	ES	ES	ES
	2500	24.57±0.57 ^{b}	19.29±0.62 ^{a}	39.50±0.86 ^{c}	ES	VS	ES
	1250	22.47±0.50 ^{b}	13.93±0.29 ^{a}	31.67±0.58 ^{c}	ES	S	ES
	625	18.78±0.22 ^{b}	10.80±0.26 ^{a}	25.75±1.39 ^{c}	VS	S	ES
E. coli	5000	32.33±0.57 ^{b}	24.56±0.45 ^{a}	44.03±1.27 ^{c}	ES	ES	ES
	2500	26.89±1.02 ^{b}	19.77±0.22 ^{a}	42.53±0.50 ^{c}	ES	VS	ES
	1250	22.53±0.45 ^{b}	17.86±0.23 ^{a}	37.67±1.52 ^{c}	ES	VS	ES
	625	17.67±0.58 ^{b}	13.13±0.23 ^{a}	32.67±1.52 ^{c}	VS	S	ES
K. pneumoniae	5000	**25.56±0.58** ^{b}	**24.82±0.22** ^{b}	32.87±0.23 ^{c}	ES	ES	ES
	2500	23.17±0.50 ^{b}	18.33±1.15 ^{a}	29.13±0.77 ^{c}	ES	VS	ES
	1250	21.53±0.76 ^{b}	12.68±0.27 ^{a}	27.33±0.57 ^{c}	ES	S	ES
	625	17.33±0.58 ^{b}	10.77±0.28 ^{a}	23.53±1.28 ^{c}	VS	S	ES
E. aerogenes	5000	29.38±0.53 ^{b}	27.87±0.23 ^{a}	47.30±0.61 ^{c}	ES	ES	ES
	2500	27.73±0.46 ^{b}	23.26±0.64 ^{a}	43.67±0.33 ^{c}	ES	ES	ES
	1250	**23.20±1.70** ^{b}	**21.96±0.06** ^{b}	40.63±0.33 ^{c}	ES	ES	ES
	625	22.76±0.25 ^{b}	18.32±0.58 ^{a}	37.53±1.74 ^{c}	ES	VS	ES

Each value represents the mean ± standard deviation (n = 3). For the same row, values assigned different letters differ significantly at the 5% probability level. **ES** : Extremely sensitive; **VS** : Very sensitive; **S** : Sensitive

Table 5. Minimum inhibitory concentrations (MIC) and bactericidal concentrations (MBC) of NE against isolates.

Bacteria	NE *C. schweinfurthii*			NE *D. edulis*			CIP		
	MIC	MBC	R	MIC	MBC	R	MIC	MBC	R
E. coli	5000	10000	2	5000	10000	2	<1	-	-
S. typhi	312.5	2500	8	625	5000	8	<1	-	-
E. aerogenes	5000	10000	2	5000	10000	2	<1	-	-
K. pneumoniae	1250	5000	4	625	5000	8	<1	-	-

R = ratio MBC/MIC; (-): Not determined; NE: nanoemulsion

Conclusion

This paper has shown that the *C. schweinfurthii* and *D. edulis* essential oil nanoemulsions have a wide spectrum of action against bacteria that cause gastroenteritis. They showed promising inhibitory and bactericidal potential; hence, making them potential candidates for the development of natural antimicrobial therapies.

Abbreviations

ANOVA: Analysis of Variance
CFU/ml: Colony-Forming Units per milliliter
CIP: Ciprofloxacin
CLSI: Clinical and Laboratory Standards Institute
EO: Essential Oil
ES: Extremely Sensitive
GC: Gas Chromatography
GC-FID: Gas Chromatography with Flame Ionization Detection
GC/MS: Gas Chromatography coupled with Mass Spectrometry
INT: p-Iodonitrotetrazolium
KI: Kovats Index
MBC: Minimum Bactericidal Concentration
MDR: Multidrug-Resistant
MHA: Mueller Hinton Agar
MHB: Mueller-Hinton Broth
MIC: Minimum Inhibitory Concentration
MTH: Hydrogenated Monoterpenes
MTO: Oxygenated Monoterpenes
NE: Nanoemulsion
NE-CS: Nanoemulsion of *Canarium schweinfurthii*
NE-DE: Nanoemulsion of *Dacryodes edulis*
ORCID: Open Researcher and Contributor ID
S: Sensitive
UR-BIODEME: *Unité de Recherche Biomédicale et Développement des Médicaments*
VS: Very Sensitive
WHO: World Health Organization

Authors' Contribution

JAKN. and PMJD. conceived and designed the research. JAKN. and JNK. conducted laboratory experiments, including essential oil extraction, nanoemulsion formulation, and antibacterial testing. V.N and DED performed the data analysis and wrote the original draft of the manuscript. P.M.J.D. and J.N.K. supervised the project and critically revised the manuscript for important intellectual content. All authors read and approved the final version of the manuscript.

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Conflict of interest

The authors declare no conflict of interest

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