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Chemical composition and antibacterial activity of the essential oil from *Diphasia klaineana* fruits and its fractions obtained by fractional distillation

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Abstract
This study aimed to analyse the chemical composition of essential oils from *Diphasia klaineana* fruits, its fractions and evaluate their antibacterial activity. The GC-MS analysis and agar disc diffusion and micro-broth dilution methods were carried out. A total of 43 constituents were identified in the essential oil from fruits of *Diphasia klaineana* (F_R) and the major compounds were *β*-elemol (30.23%), sabinene (9.28%), guaiol (5.12%), *(E)-β*-ocimene (5.11%) and δ-elemene (3.53%). The F_{R1} and F_{R3} fractions collected after one hour and after three hours were rich in hydrocarbon monoterpenes and showed bactericidal and bacteriostatic activities, respectively. No activity was found with F_{R5}, F_{R7} and F_{R9} fractions. Therefore, fractional distillation may be an interesting process to upgrade the antibacterial activity of *D. klaineana* fruits essential oil againt *Staphylococcus aureus*.

Keywords: *Diphasia klaineana*; essential oil; GC-MS; chemical composition, antibacterial activity, *Staphylococcus aureus*

Introduction
Essential oils (EOs) are natural compounds extracted from different parts of a plant, such as flower, leaves, stems, fruits, seeds, roots, barks, or resin. It is an important part of traditional healing practices in the human diseases. It is used as raw materials in cosmetics, spices, foods, perfumes, and in treatment of several health disorders [1]. In recent years, there has been a resurgence of interest in researching plant products as antimicrobial instead of several antibiotics with their side effects. Throughout history, natural substances and their derivatives have been an all-important source of therapeutic agents. The *in vitro* antimicrobial assays have effectively served as valid methods to reveal secondary metabolites with antimicrobial activity [2]. Plants extracts have contained different metabolites which have strong antimicrobial activity against both biofilms and pathogens resistant to several drugs. It is more difficult for bacteria to develop resistance to the multi-component EOs than to the antibiotics that are often composed of only a single molecular entity [3]. Since the biological activities of EOs are composition-dependent, no particular resistance or adaptation to EOs has been described to date. In addition to antimicrobial activity, EOs can act in synergy with some antibiotics, enhancing their biological properties [4]. Essential oils consist mainly of monoterpenes, sesquiterpenes and their oxygenated derivatives. The qualitative and quantitative analysis of the chemical composition of essential oils is important, as it is responsible for their effectiveness. That is, not only which compounds are present, but their proportion and amounts are important for their activity [5].
Among the medicinal plant, there is the Rutaceae family, which has species of ecological, economic and therapeutic importance. It belongs to the order of Sapindales with about 150 genders and over 1600 species. They are hugely distributed throughout the tropical and temperate regions of the globe, being more abundant in tropical America, South Africa and Australia [2]. Among the representatives of this essential oil family producers, stand out *Diphasia klaineana* Pierre, popularly known as courou-la-lemourou in Malinké, lobo in Abbey, pahiri in Bété, grénian in Krumen, hugely used as a medicinal resource by local people throughout Côte d'Ivoire.
In folk medicine, there are several therapeutic properties described from *D. klaineana* P., which include the use of this plant in respiratory conditions, including sinusitis. Furthermore, few studies have investigated the chemical composition underlying the health benefits due to this medicinal plant.

Our previous work revealed the chemical composition of the essential oil from *D. klaineana* leaves, its fractions, their biological activities and the influence of flowering on the chemical composition and biological activity [6,7]. Therefore, the purpose of the present study was to determine the chemical composition and to evaluate the biological activities of *D. klaineana* fruits EO and its fractions as new potential source of natural antibiotic components.

Materials and methods

Collection and pre-treatment of plant material

Fruits of *Diphasia klaineana* were collected in June 2017, from the Denguélé region (north-west of Côte d'Ivoire). The samples were identified at the Floristic National Center of Felix Houphouët-Boigny University (Abidjan-Cocody, Côte d'Ivoire). They were deposited in our laboratory for future references. The fresh plant material was stored in an air-conditioned chamber at 18 °C for three days, protected from light, before extraction.

Essential oil distillation

The essential oils were extracted from the fruits of *D. klaineana* by continuous and fractional distillation hydro-distillation using an apparatus of Clevenger type. The essential oils were collected over water and dried over anhydrous sodium sulfate. The pure essential oils were stored in airtight glass containers in a refrigerator at 4 ºC until oils analysis and evaluation antibacterial activity test.

For the continuous distillation, the extraction was carried out for 9 h to mix 500 g of plants in 1500 mL of distilled water. The extract obtained was encoded F_R.

For fractional distillation, the essential oils of plant material (500 g) were obtained by hydrodistillation for 9 h. The essential oil fractions were captured at regular time frames after 1 h: 0-1, 1-2, 2-3, 3-4, 4-5, 5-6, 6-7, 7-8, 8-9 h nonstop control. Thus, the F_{R1}, F_{R2}, F_{R3}, F_{R4}, F_{R5}, F_{R6}, F_{R7}, F_{R8} and F_{R9} fractions were collected without interrupting the hydro distillation process. But we only used odd fractions for this study (F_{R1}, F_{R3}, F_{R5}, F_{R7} and F_{R9}).

GC and GC-MS analysis

The identification of the major constituents was conducted by GC-MS analyses. GC/MS analyses were carried out on Perkin Elmer auto system XL Gas equipped with a Rtx-1 Column nonpolar phase (60 m × 0.22 mm, coating thickness 0.25 µm) and a Perkin Elmer TurboMass mass detector. Analytical conditions were as follows: 0,2 µL of sample was injected using flow splitting 1:50; as carrier gas was helium with flow velocity of 1 mL/min; carrier gas helium at a regular pressure of 25 psi; oven temperature was programmed from 60 to 230 °C at 2 °C/min, with injector temperature at 250 °C and detector temperature at 280 °C. All mass spectra were acquired over the mass range 35-350 Da in-electron impact (EI) mode with ionization voltage 70 eV. The assignment of peaks in the chromatogram was based on the comparison of retention times with those of authentic samples, comparing their linear retention indices with respect to the series of n-hydrocarbons, and the computer matching with the mass spectra of libraries comprise pure substances and components of known oil and MS literature [8, 9].

Bacterial strain and growth conditions

The essential oils were tested against a common pathogenic bacterial strain: *Staphyloccocus aureus* ATCC 25923, a Gram-positive bacteria obtained from the Swiss Center for Scientific Research of Côte d'Ivoire. The strain was streaked on to an agar plate to obtain single colonies, and then freshly grown on agar-based nutrient medium in the dark. All incubation accomplished aerobically for 24 h at 37 °C.

Determination of antimicrobial activity by the agar-well diffusion method

The antibacterial activity was carried out with the agar-well diffusion method [10,11]. Mueller-Hinton Agar medium was poured into a sterile petri dish and allowed to solidify. Colonies of *S. aureus* ATCC 25923 were directly suspended in 0.85% saline to obtain turbidity comparable to that of the 0.5 McFarland standard. Aliquots (0.1 mL) of the inoculum were spread over the surface of pre-dried agar plates with a sterile spreader. Six wells were drilled into the inoculated medium using a sterile cork borer (6 mm). Wells, every 6 mm, were cut through the agar using a sterile cork borer and the agar was removed leaving empty wells which were filled with 50 µL of each essential oil, the positive control (gentamycin) or the negative control / solvent. For about 30 minutes, it was allowed to diffuse and incubated for 18 to 24 hours at room temperature. The zone of inhibition was observed and measured in mm. The assay was carried out in triplicate.

Determination of minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) in liquid medium

The determination of the MIC of the essential oils was performed by microdilution assay following the standard protocol from the Clinical and Laboratory Standards Institute, with some modifications [12,13]. For the assay, the standard inoculum was prepared in sterile saline (0.85% w/v) from living colonies of *S. aureus* contained in plates of agar (final inoculum). The essential oil stock solution was prepared with MH broth using Tween 80 as emulsifier. From the stock solution, two-fold serial dilutions were made in a range from 50 to 0.39 mg/mL. 10 µL from the final inoculum were added to each well containing 50 µL of several essential oil concentrations, being the final volume in each well of 60 µL. The following controls were used: culture medium control (60 µL of MH broth); growth control (50 µL of MH broth + 10 µL of inoculum); Tween 80 emulsifier control (60 µL of MH broth with Tween 80) and growth control containing the emulsifier (50 µL of MH broth with Tween 80 + 10 µL of inoculum). Finally, microplates were incubated for 18-24 hours at 37 °C. The MIC was considered as the lowest essential oil concentration that inhibited visible bacterial growth.

The determination of MBC was performed from wells containing essential oil concentrations where there was no visible bacterial growth. So, an aliquot of 100 µL was taken from each well and seeded in MH agar. Plates were incubated at 37 °C for 24 h. The MBC was defined as the lowest concentration of essential oil able to cause total bacterial death, represented by the visible absence of colonies of *S. aureus* on the agar plates.

Results and discussion

Chemical composition of essential oil from continuous hydro-distillation

The continuous hydro-distillation of the fruits of *Diphasia klaineana Pierre* (Rutaceae**)** from Côte d'Ivoire was performed in a Clevenger-type apparatus, which yielded a yellowish color oil and specific aroma. Based on the fresh

weight of the plant material, the essential oil of the fruits (F_R) of *Diphasia klaineana* Pierre (Rutaceae) was obtained in yields of 0.14% w/w; in contrast, the essential oils of the same plant were found to be 1.65% and 1.53% w/w from the leaves before flowering and during flowering, respectively [6]. However, the yield of essential oil was 0.12% from the fruits of *Hortia oreadica* (Rutaceae) after 2 h. In general, plants belonging to the Rutaceae family are highly aromatic and have significant importance as a source of citrus fruits and as ornamental plants. Many essential oils of species of this family are used in the pharmaceutical and cosmetic industries, nutritional supplements and aromatherapy [14].

The chemical composition of essential oil from the fruits of *Diphasia klaineana* was analyzed by GC and GC-MS and the result was presented in Table 1. In total, 38 components were identified, representing 81.28% of the total amount. *β*-elemol (30.23%), a sesquiterpenoid that is isopropanol which is substituted at position 2 by a (3*S*,4*S*)-3-isopropenyl-4-methyl-4-vinylcyclohexyl group was found as the main component. Sabinene (9.28%) was the second major compound detected in F_R coded essential oil, followed by guaiol (5.12%), *(E)-β*-ocimene (5.11%), γ-eudesmol (3.54%), terpinen-4-ol (3.37%), germacrene D (3.22%), limonene (2.92%), myrcene (2.52%), *β*-elemene (2.20%), (Z)- *β*-ocimene (1.74%), E-caryophyllene (1.46%), γ-terpinene (1.40%), linalool (1.08%), *α*-pinene (1.03%) and others were found to be the minor components in the essential oil from the fruits of *Diphasia klaineana*. Furthermore, the oxygenated sesquiterpenes (39.11%) and monoterpene hydrocarbons (27.52%) were the main chemical groups in the essential oil from the fruits of *D. klaineana*, and small amount of sesquiterpenes hydrocarbons (8.85%) and oxygenated monoterpenes (5.80%) were found (Fig. 1).

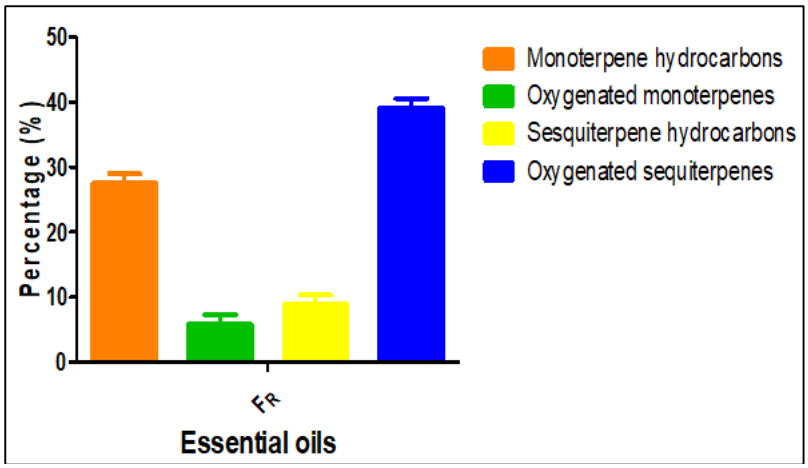

Fig 1: Monoterpene and sesquiterpene content of total essential oil (F_R)

Data from literature showed that essential oils contain a large variety of substances with great potential as valuable source of bioactive molecules. Some of the identified compounds have been reported to have many biological activities. Many studies carried out with essential oils had reported that they exhibited many biological effects such as antibacterial, antifungal, anti-inflammatory, hepatoprotective, antiviral, anti-leishmanial, antioxidant and anti-proliferative properties [15, 16].

Table 1: Compounds identified in the essential oils of *D. klaineana*

No.	Compounds	I_R^1	I_R^2	% Occurrence					
				F_R	F_{R1}	F_{R3}	F_{R5}	F_{R7}	F_{R9}
1	*α*-Thujene	922	1023	0.23	0.44	0.43	0.34	0.31	0.19
2	*α*-Pinene	930	1020	1.03	2.53	1.84	1.38	1.17	0.76
3	Camphene	943	1069	0.19	0.42	0.30	0.21	0.16	0.09
4	Sabinene	965	1126	9.28	21.96	11.84	5.31	2.49	0.82
5	*β*-Pinene	970	1114	0.46	0.85	0.64	0.45	0.35	0.20
6	Myrcene	980	1164	2.52	3.43	2.84	2.07	1.79	1.16
7	*α*-Phellandrene	996	1163	0.05	0.05	0.04	-	0.03	0.03
8	*α*-Terpinene	1008	1184	0.77	0.55	0.64	0.14	0.50	0.44
9	*p*-Cymene	1011	1269	0.91	1.46	1.72	2.53	1.52	0.98
10	Limonene	1021	1204	2.92	4.69	3.53	2.53	2.01	1.31
11	*(Z)-β*-Ocimene	1024	1236	1.74	1.57	1.51	0.86	0.91	0.60
12	*(E)-β*-Ocimene	1035	1253	5.11	5.09	4.56	2.21	2.56	1.72
13	*γ*-Terpinene	1048	1248	1.40	1.00	1.27	0.48	1.06	0.89
14	trans-Sabinene hydrate	1053	1464	0.12	0.81	0.22	0.10	0.04	0.01
15	Terpinolene	1078	1286	0.91	0.44	0.84	0.31	0.57	0.44
16	Linalool	1083	1548	1.08	3.38	0.87	0.43	0.27	0.18
17	Allo-ocimene	1116	1370	0.05	0.02	-	-	-	0.02
18	Terpinen-4-ol	1161	1603	3.37	3.35	4.68	3.89	2.58	1.63
19	*α*-Terpineol	1171	1697	0.52	0.89	0.78	0.64	0.46	0.36
20	Bornyl acetate	1267	1576	0.16	0.23	0.17	0.13	0.09	0.06
21	*δ*-Elemene	1331	1461	0.10	-	0.08	0.12	0.14	0.17
22	Neryl acetate	1340	1723	0.08	0.07	0.08	0.07	0.06	0.04
23	Geranyl acetate	1357	1753	0.14	0.10	0.13	0.13	0.10	0.07

24	Methyl eugenol	1366	2010	0.06	0.10	0.06	0.05	0.05	0.05
25	*α*-Copaene	1372	1486	0.25	0.36	0.14	0.15	0.17	0.21
26	*β*-Elemene	1386	1581	2.20	1.53	2.21	2.68	2.77	2.94
27	*(E)*-Caryophyllene	1415	1590	1.46	1.10	1.06	1.18	1.45	1.71
28	*α*-Humulène	1448	1668	0.72	0.65	0.45	0.57	0.72	0.91
29	*(E)*-Methyl isoeugenol	1457	2168	0.12	0.09	0.09	0.10	0.13	0.18
30	*γ*-Muurolene	1466	1681	0.16	0.10	0.09	0.10	0.14	0.20
31	Germacrene D	1474	1707	3.22	2.64	2.72	1.97	3.31	3.93
32	*β*-Selinene	1479	1717	0.21	0.16	0.20	0.24	0.24	0.27
33	*α*-Murolene	1488	1716	0.11	0.08	0.33	0.33	0.38	0.43
34	γ-Cadinene	1504	1758	-	0.51	0.34	0.19	0.20	-
35	δ-Cadinene	1513	1756	0.52	0.33	0.38	0.39	0.50	0.70
36	*β*-Elemol	1535	2081	30.23	17.92	27.17	36.01	36.03	38.09
37	Caryopyllene oxide	1568	1980	-	1.03	0.89	1.07	0.92	0.92
38	Guaiol	1584	2089	5.12	2.94	4.07	4.99	5.64	6.16
39	epi-*γ*-Eudesmol	1606	2101	0.22	0,10	0.20	0.26	042	0.45
40	*γ*-Eudesmol	1616	2167	3.54	1,71	3.10	4.22	4.68	5.54
41	β-Eudesmol	1633	2220	-	2,59	3.61	4.55	4.97	5.13
42	α-Eudesmol	1638	2221	-	2.01	3.30	4.29	5.07	5.61
43	Bulnesol	1650	2208	-	0,41	0.87	1.10	1.35	1.47

I_R^1 : Retention indices on non-polar column ; I_R^2 : Retention indices on polar column

Antibacterial activity of the essential oil against *S. aureus*

Based on previous research, diameters of inhibition zone were appreciated as follows: Not sensitive (diameter ≤ 8.0 mm), moderately sensitive (8.0 < diameter < 14.0 mm), sensitive (14.0 < diameter < 20.0 mm), and extremely sensitive (diameter ≥ 20.0 mm) [17]. The results showed the F_R-encoded essential oil had certain antibacterial activity on *Staphylococcus aureus* (*S. aureus* ATCC 25923, growth inhibition zone of 9 mm). The MIC and MBC determinations were made using F_R-encoded essential oil from *D. klaineana*. The MIC for *D. klaineana* was 6.25 mg/mL and the MBC was 50 mg/mL. Several studies, which investigated the action of essential oil against pathogenic microorganisms, agreed that essential oils are more effective against Gram-positive bacteria than against Gram-negative [2].

According to the results of the previous researches, the major constituents of essential oils including monoterpene or sesquiterpene hydrocarbons, and their oxygenated derivatives demonstrate antibacterial activity [10]. This activity against *Staphylococcus aureus* may be due to the presence of the major compounds in the essential oil or linked to a synergy of action of all the compounds. After determining the components and antibacterial activity of the essential oil of fruits of *D. klaineana*, an analysis of main components responsible for the antibacterial activity was performed.

Fractionation of the essential oil and the effect of various fractions on *S. aureus*

The essential oil extracted from the fruits of *D klaineana* was subjected to fractional distillation. Fractionation had given five main fractions (F_{R1}, F_{R3}, F_{R5}, F_{R7}, F_{R9}) and their extraction yields are shown in Fig. 2.

The chemical composition of these fractions obtained by fractional was different in each of them. GC and GC-MS analysis revealed the presence of 42 chemical constituents in F_{R1} fraction, F_{R3}, F_{R7} and F_{R9} fraction, and 41 chemical constituents in F_{R5} fraction (Table 1). Sabinene (21.96%), β-elemol (17.92%) and (E)-β-ocimene (5.09%) were the three most abundant chemical compounds in F_{R1} fraction. F_{R3} fraction was dominated by *β*-elemol (27.17%), sabinene (11.84%) and terpinen-4-ol (4.68%) while F_{R5} fraction was mostly composed of β-elemol (36.01%), sabinene (5.31%) and guaiol (4.99%). The three most abundant compounds in F_{R7} fraction were *β*-elemol (36.03%), guaiol (5.64%) and α-eudesmol (5.07%), while the five most abundant chemical compounds in F_{R9} fraction were β-elemol (38.09%), guaiol (6.16%), *α*-eudesmol (5.61%), *γ*-eudesmol (5.54%) and *β*-eudesmol (5.13%) (Fig. 3).

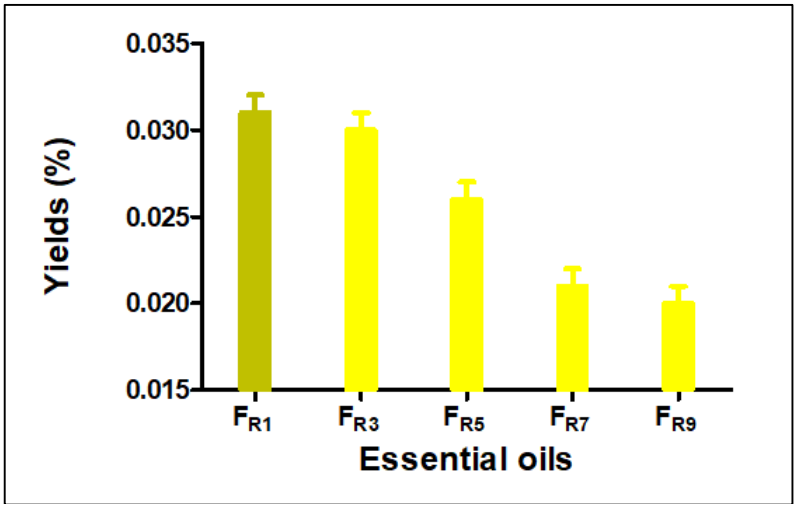

Fig 2: Yields of fractions of total essential oil

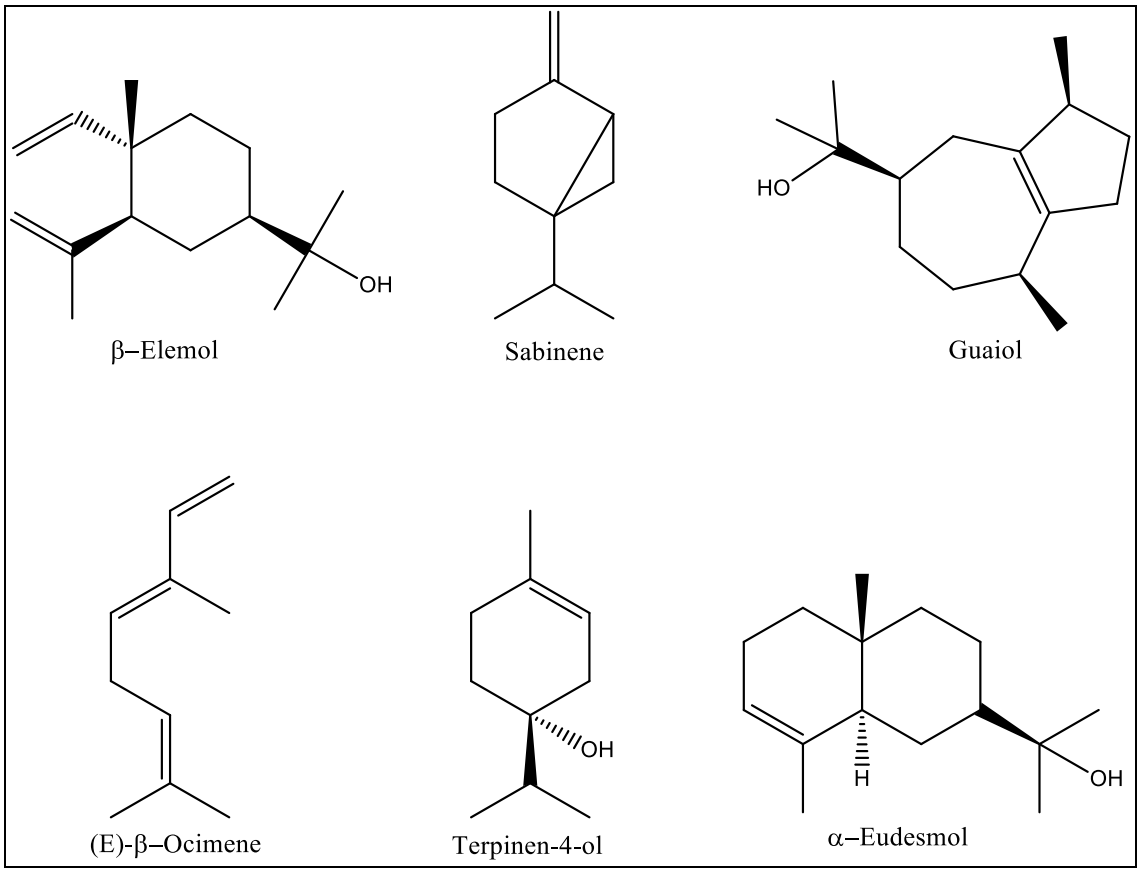

Fig 3: Some molecules in essential oils of *D klaineana*

Sabinene, α-thujene, *α*-pinene, camphene, β-pinene, myrcene, limonene, trans-sabinene hydrate, linalol, *α*-terpineol bornyle acetate and *γ*-cadinene were found in greater proportion in F_{R1} and F_{R3} fractions, this means that are considered more volatile**.** This coincides with the report by Rostro-Alanis et al [5]. Semerdjieva et al demonstrated that a high sabinene essential oil fraction (80% sabinene) could be obtained in the first 0-3 min hydro distillation time, following a grinding of plant material in water [18].

On the other hand, *β*-elemol, *β*-elemene, *δ*-elemene, *(E)*-methyl isoeugenol, *β*-selinene, *δ*-cadinene, guaiol, γ-epi eudesmol, *γ*-eudesmol, *β*-eudesmol, *α*-eudesmol and bulnesol were present in a larger proportion in F_{R7} and F_{R9} fractions. These components were further classified into three groups: high volatile monoterpene hydrocarbons; medium volatile oxygenated monoterpenes and sesquiterpene hydrocarbons; and low volatile oxygenated sesquiterpenes [19] (Fig. 4).

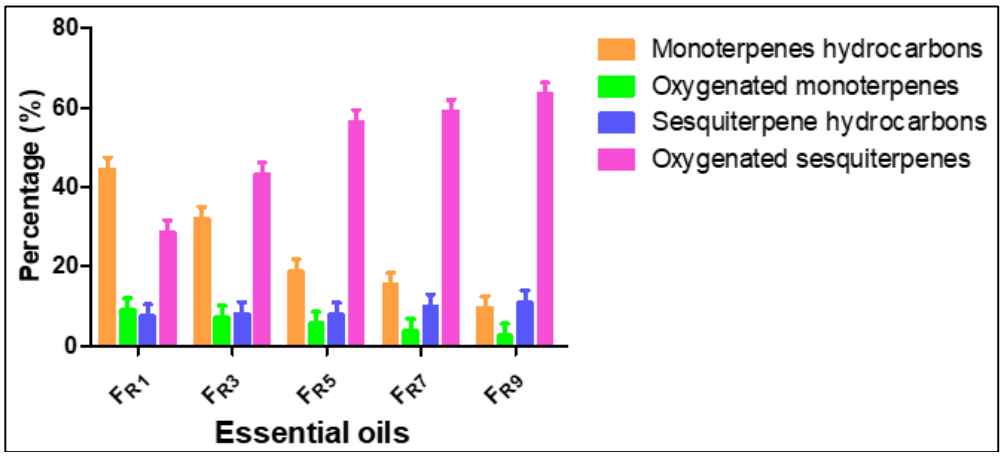

Fig 4: Change in monoterpenes and sesquiterpenes content during fractionation

The antibacterial activity of the fractions of essential oil from fruits of *D klaineana* was assessed *in vitro* against *S. aureus* ATCC 25923. Results are showed in Table 2.

It was found that best antibacterial results occurred with the F_{R1} fraction (minimal inhibitory concentration - MIC = 12.5 mg/ mL) and F_{R3} fraction (minimal inhibitory concentration - MIC = 12.5 mg/ mL). The F_{R5}, F_{R7} and F_{R9} fractions had no activity against *S. aureus* ATCC 25923. By referring to the chemical compositions of the fractions, the correlation between the antibacterial activities with the monoterpene hydrocarbons is evident. Other studies had confirmed that the monoterpene hydrocarbons confer antibacterial activity against *S. aureus* [20, 21].

Koné et al. (2015) obtained similar results when studying essential oils from the leaves of *D. klaineana*, finding that the fractions showed an increase in the antibacterial activity

against *S. aureus*, perhaps due to the presence of monoterpene hydrocarbons [6,7]. However, it was suggested that they may have synergy with minor oxygenated components [5].

Table 2: Determination of MIC and MBC of *Diphasia klaineana*

Bacterial strains	Fractions	Inhibition zones (mm)	MIC (mg/mL)	MBC (mg/mL)	MBC/MIC	Interpretation
Staphylococcus aureus ATCC 25923	FR_1	15	12,5	25	2	Bactericidal
	FR_3	12	12,5	50	4	Bacteriostatic
	FR_5	08	00	00	00	No effect
	FR_7	00	00	00	00	No effect
	FR_9	00	00	00	00	No effect

Conclusion

The essential oil from fruits of *Diphasia klaineana* and its fractions obtained by the fractional distillation process differ in terms of content and quantitative proportion of monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons and oxygenated sesquiterpenes, as well as in their antibacterial activity against *Staphylococcus aureus*.

Our results show the fractions collected after one hour and after three hours were rich in hydrocarbon monoterpenes and showed bactericidal and bacteriostatic activities respectively against *Staphylococcus aureus*. No activity was found against *Staphylococcus aureus* with F_{R5} et F_{R7} and F_{R9} fractions dominated by oxygenated sesquiterpenes. Even in low amounts, these compounds could work in synergy, and maintain biological activity.

These findings represent an important result for the rationalization of using essential oils of *Diphasia klaineana* in traditional medicine. The F_{R1} and F_{R3} fractions can be used for their antibacterial activity because they offer a greater antibacterial activity compared to the original oil. Therefore, fractional distillation may be used to upgrade the antibacterial activity of essential oil from *D. klaineana*.

References

1. Panda S, Sahoo S, Tripathy K, Singh YD, Sarma MK, Babu PJ et al. Essential oils and their pharmacotherapeutics applications in human diseases. Advances in Traditional Medicine 2020, 1-15.
2. França Orlanda JF, Nascimento AR. Chemical composition and antibacterial activity of *Ruta graveolens* L. (Rutaceae) volatile oils, from São Luís, Maranhão, Brazil. South African Journal of Botany 2015;99:103-106.
3. Değirmenci H, Erkurt H. Relationship between volatile components, antimicrobial and antioxidant properties of the essential oil, hydrosol and extracts of *Citrus aurantium* L. flowers. Journal of Infection and Public Health 2020;13(1):58-67.
4. Boudjedjou L, Ramdani M, Zeraib A, Benmeddour T, Fercha A. Chemical composition and biological activities of Algerian *Santolina africana* essential oil. Scientific African 2019;4:e00090.
5. de Rostro-Alanis MJ, Báez-González J, Torres-Alvarez C, Parra-Saldívar R, Rodriguez-Rodriguez J, Castillo S. Chemical composition and biological activities of oregano essential oil and its fractions obtained by vacuum distillation. Molecules 2019;24(10).
6. Kone Y, Boua Boua B, Ouattara Z, Mamyrbekova-Bekro JA, Bekro Y-A. Influence de la floraison sur la composition chimique et l'activité anti sinusite de l'huile essentielle des feuilles de *Diphasia klaineana* Pierre (Rutaceae) de Côte d'Ivoire. Journal of Applied Biosciences 2019;135(1):13742.
7. Kone Y, Boua BB, Mamyrbekova-Bekro J-A, Bekro Y.-A. Analyse CPG-MS et activité anti sinusite *in vitro* de l'huile essentielle fractionnée des feuilles de *Diphasia klaineana* Pierre (Rutaceae) de Côte d'Ivoire. Revue CAMES-Série Pharmacopée et Médecine Traditionelle Africaine 2018;19(1):10-16.
8. Bardaweel SK, Bakchiche B, ALSalamat HA, Rezzoug M, Gherib A, Flamini G. Chemical composition, antioxidant, antimicrobial and Antiproliferative activities of essential oil of *Mentha spicata* L. (Lamiaceae) from Algerian Saharan atlas. BMC Complementary and Alternative Medicine 2018;18(1):1-7.
9. Adams RP. Identification of essential oil components by gas chromatography/mass spectrometry, 4th ed. Allured Publ. Corp., Carol Stream, IL 2007.
10. Tanhaeian A, Sekhavati MH, Moghaddam M. Antimicrobial activity of some plant essential oils and an antimicrobial-peptide against some clinically isolated pathogens. Chemical and Biological Technologies in Agriculture 2020;7(1):1-11.
11. Berthold-Pluta A, Stasiak-Różańska L, Pluta A, Garbowska M. Antibacterial activities of plant-derived compounds and essential oils against Cronobacter strains. European Food Research and Technology 2019;245(5):1137-1147.
12. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Clinical and Laboratory Standards Institut (CLSI). www.clsi.org accessed on: 05.12.2020.
13. Ambrosio CMS, Ikeda NY, Miano AC, Saldaña E, Moreno AM, Stashenko E et al. Unraveling the selective antibacterial activity and chemical composition of citrus essential oils. Scientific Reports 2019;9(1):17719-17731.
14. Santos DL, Ferreira HD, Borges LL, Paula JR, Tresvenzol LMF, Santos PA et al. Chemical composition of essential oils of leaves, flowers and fruits of *hortia oreadica*. Revista Brasileira de Farmacognosia 2016;26(1):23-28.
15. Yagi S, Babiker R, Tzanova T, Schohn H. Chemical composition, antiproliferative, antioxidant and antibacterial activities of essential oils from aromatic plants growing in Sudan. Asian Pacific Journal of Tropical Medicine 2016;9(8):763-770.
16. León-Méndez G, Pájaro-Castro N, Pájaro-Castro E, Torrenegra- Alarcón M, Herrera-Barros A. Essential oils as a source of bioactive molecules. Revista Colombiana de Ciencias Químico-Farmacéuticas 2019;48(1):80-93.
17. Li Z-H, Cai M, Liu Y-S, Sun P-L, Luo S-L. Antibacterial activity and mechanisms of essential oil from *Citrus medica* L. var. sarcodactylis. Molecules 2019;24(8):1577.
18. Semerdjieva BI, Shiwakoti SL, Cantrell C, Zheljazkov VD, Astatkie T, Schlegel V et al. Hydrodistillation extraction kinetics regression models for essential oil

yield and composition in Juniperus virginiana, J. excelsa, and J. sabina. Molecules 2019;24(5):986.
19. Marković MS, Bošković-Vragolović NM, Ristić MS, Pavićevic VP, Veljković VB, Milojević S. Frakcionisanje eteričnog ulja kleke (*Juniperus communis* L.) Hidrodestilacijom i rektifikacijom. Hemijska Industrija 2017;71(6):471-477.
20. Borges CV, Amorim EP, Leonel M, Gomez HAG, Santos TPR dos, Ledo CA da S et al. Post-harvest physicochemical profile and bioactive compounds of 19 bananas and plantains genotypes. Bragantia 2019;78(2):284-296.
21. Mahizan NA, Yang S-K, Moo C-L et al. Terpene Derivatives as a Potential Agent against Antimicrobial Resistance (AMR) Pathogens. Molecules 2019;24(14):2631.