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RESEARCH ARTICLE

COMPOSITION, ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF ESSENTIAL OIL FROM *ENANTIA POLYCARPA* AND *CLERODENDRUM POLYCEPHALUM*

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ABSTRACT

This reseach was conducted to contribute to the values of medicinal and aromatic plants of Cote d'Ivoire. As such, we carried out the phytochemical composition of essential oil from *Enantia polycarpa* and *Clerodendrum polycephalum* baker by gas chromatography coupled to mass spectroscopy (GC-MS), estimate the antioxidant activity per spectrophotometer and finally achieve some antimicrobial tests upon reference strain of the Swiss center in Cote d'Ivoire. Thus, we identified 96.61% of the total composition of essential oil from *Enantia polycarpa* leaves which is mainly made up of oxygenated compounds (45.45%) followed by sesquiterpenes (42.42%) and monoterpenes (12.12%). The main compounds are β-Elemene (27.14%), γ-Elemene (23.46%) and α-Pinene (6.43%). Concerning essential oil from *Clerodendrum polycephalum* leaves, 95.84% of the compostion was identified. It is mainly composed of sesquiterpenes (69.04%) followed by oxygenated compounds (23.80%) and monoterpenes (7.14%). The main compounds are Caryphyllene (30.27%) and Germacrene D (23.96%). The IC_{50} of essential oil from *Enantia policarpa* and that of *Clerodendrum polycephalum* are respectively 0.27 (±0.05) mg/mL and 0.73 (± 0.2) mg/mL. The vitamin C taken as a reference has an IC_{50} of 0.06 (±0.003) mg/mL. Moreover, the antimicrobial tests have revealed that the essential oil of *Clerodendrum polycephalum* leaves has got no effect upon the tested strains. The essential oil from *Enantia polycarpa* has got no effect on *Staphylococcus epidermidis, Pseudomonas aeruginosa, Escherichia coli* and *Candida tropicalis* but has got an inhibitory activity on *Bacillus subtulis*, *Staphylococcus aureus, Salmonella typhimirium, Klebsielle blse, Candida albicans* and *Candida glabrata*.

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INTRODUCTION

Using plants for the treatment of diseases in Cote d'Ivoire is systematic. Many aromatic plants that produce essential oil are part to species used in the ivorian traditonal therapy. *Enantia polycarpa* (*Annickia polycarpa*) is small to medium-size tree up to 20 m; bark smooth to slightly rough or fissured, green to blackish, inner bark fibrous and bright yellow. They are eight (8) species of *E. polycarpa* limited to West and Central Africa, from Sierra Leone to Nigeria and western Cameroon, but one endemic to Tanzania north-eastern. It is traditionally used to treat sores, ulcers, leprosy and ophthalmia. The Guéré use bark extract as a nerve poison of hunting arrows. Bark decoction is also used to treat fever and malaria (Anonymous, 2018).

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Several phytochemical and antimicrobial tests have been carried out on solvent extracts from the bark (Ambé, 2017; Ajali, 2000; Anonymous). However, the essential oil of the leaves of this plant has not yet been biologically investigated to our knowledge. As for *Clerodendrum polycephalum,* it's an erect or scandent shrub to 4 m high, of savanna and closed jungle, in Guinea to Southern Nigeria, and in Easten Cameroun. Leaf-sap is used in Côte d'Ivoire to wash the face of persons subject to fainting, giddiness and attacks of epilepsy. It is therefore used in traditional treatment as a painkiller, antidote and for paralysis (Anonymous). The essential oil extracted from *Clerodendrum polycephalum* of Côte d'Ivoire was not studied before. This work is to determine the chemical composition, antioxidant and antibacterial activities of essential oils extracted from the leaves of *Enantia polycarpa* and *Clerodendrum polycephalum*, two aromatic plants species from the flora of Côte d'Ivoire.

MATERIAL AND METHODS

Plant material and hydrodistillation: The vege Table material is composed of leaves of *E. polycarpa* from Azaguié (5° 37' 59.999" N 4° 4' 59.999" W) (Anonymous, 2019) and *C. polycephalum* leaves from Prikro (7° 10' 59.999" N 4° 55' 0.001" W) (Anonymous, 2019). They have been identified thanks to herbarium of National Floristic Center of Cote d'Ivoire, respectively under the numbers UCJ001183 and UCJ017398. The material was dried in a room temperature. The essential oil extraction was realised in four (4) hours with a Clevenger type hydro-distiller.

GC-MS analysis: A GC (7890A, Agilent Technologies) instrument coupled with MS (5975C, Agilent Technologies). The liquid sample volume of 1 µl was injected to a liner with 250°C and a split ratio of 100:1. The capillary column HP-5MS was used. Oven temperature programming was as follows: 40°C (hold 5 min), then a rate of 2°C/min to 250°C; then a rate of 10°C/min to 300°C. The carrier gas helium flow was 1 mL/min. Solvent delay: 2 min. The source and transfer line of MS detector were at 230 and 280°C, respectively, while the detector voltage was 1.4 kV, and the scan range of mass-to-charge ratio of ion was 40-500.

In vitro radical scavenging test: 2.2-Diphenyl-1-picrylhydrazyl (DPPH, Cat.: D913-2, Lot : STBB0555), is solubilized in the absolute methanol to get concentrated solution of 0.03 mg/mL. 10 mg of essential oil are diluted within 5 mL of the same solvant. Different concentrated ranges comprised between 0.0625 to 2 mg/mL of each sample of essential oil are made by successive dilutions in the absolute methanol. In some dry and sterile tubes we introduce 2.5 mL of essential oil sample and 1 mL of methanolic solution of DPPH. After stirring, we put the tubes in dark for 30 min. the absorbance is then measured with a spectrophotometer (UV-visible WPA S800, $N^0$113648) at 517 nm againt a blank consisting of 2.5 mL of pure methanol and 1 mL of alcoholic solution of DPPH. The positive control vitamin C prepared under the same condition as the study samples. The inhibitory half-concentration (IC_{50}) of the sample was determined graphically.

In vitro antibacterial activities Evaluation: The strains used are reference strain from the laboratory of Swiss center in Cote d'Ivoire. Antibacterial activity of the different essential oil with different concentrations was determined against each bacterial strain by Berghe and Vlietinck methods (Berghe, 1991). Inoculum sowing of 1 mL takes 18 to 20 h (10^5-10^6 UFC/mL), on Mueller Hinton (MH) agar. After 15 min, some wells are cut, using pasteur pipettes. At the bottom of the wells are closed with a drop of MH agar to limit the diffusion of the oil under the agar. Then, 50 µL of the different dilutions of the oil is poured into each well. After diffusion, the cultures are incubated in incubators at 37 °C for 24 h. The inhibition rings are measured with caliper. 0.1 mL of 18 hours broth of *Escherichia coli, Salmonella, Bacillus, klebsiella, Candida* is transplanted into 10 mL of MH broth and 0.3 mL for *Staphylococcus aureus* and *epidermidis*. Incubated at 37 °C for 3 to 5 hours until the appearance of a slight opalescence about 5 10^7 bacteria/mL. Then 1 mL of these broths is added to 10 mL of MH broth warmed before at 37°C (inoculum). Afterward, 100 µL of the extracted solution of essential oil is put in the column n°12 of the microplate, 50 µL of MH broth from the column n° 11 to the n°2 and 100 µL of MH broth in the column n°1. A dilution from the column n°12 to the n°3 taking 50 µL each time, and 50µl of the inoculum is poured into each well of the columns n°12 to n°2.the reading is made with naked eye after incubation at 37°c during 18h.

RESULTS AND DISCUSSION

Yield of extraction and chemical composition of the essential oils: essential oils extracted from *Enantia polycarpa* (annonaceae) and *Clerodendrum polycephalum Baker* (Verbenaceae) leaves are yellow with output of respectively 0.27±0.005% and 0.13±0.005%, releasing an aromatic odour. The identification of their constituants was realised through GC-MS. The retention indices were determined from retention times (Kovats, 1958; Iupac, 1997). Thirty-three (33) compounds have been identified in the essential oil of *Enantia polycarpa* leaves, that makes 96.61% of the global composition (table1). The oil composition shows 45.45% of oxygenated compound, 42.42% of sesquiterpene and 12.12% of monoterpenes. The main compounds are β-Elemene (27.14%), γ-Elemene (23.46%) and the α-Pinene (6.43%). Yapi and Al (Yapi, 2018), studied the chemical variability of the extracted essential oil from *Enantia polycarpa* leaves, and showed that its composition is dominated by β-Elemene, Germacrene B et D et β-Cubebene. Considering its global composition, the essential oil from *C. Polycephalum* brings out forty one (41) compound, that makes 95.84% (table2). It's mainly composed of sesquiterpenes (69.04%), of oxygenated compounds (23.80%) and of monoterpenes (7.14%). The main compounds are Caryophyllene (30.27%) and Germacrene D (23.96%). According to the bibliography consulted, a study realised by Akintayo and al., on the Nigeria species (Akintayo, 2016), showed that its composition is dominated by forty (40) compounds identified including sesquiterpenes (74.1%); the oxygenated sesquiterpenes (11.9%) and the monoterpenes (10.6%). The main constituents of the oil are β-Caryophyllene (28.9%), α-Muurolene (9.0%) and the β-Pinene (8.6%), representing 97.2% the total content of oil. According to this research, both ivoirian and nigerian species have some chemical similarities that seem to depend on pedoclimatic conditions.

Antioxydant activities of essential oil: essential oil possess an antioxydant activity. The essential oil of *E. polycarpa* leaves showed the good activity (IC_{50} = 0.27± 0.05 mg/mL). Concerning that of *C.Polycephalum*, the activity is comparatively low. (0.73± 0.2) mg/mL. Generally, the essential oil from *E. Polycarpa* activity is relatively high regarding that of *C. polycephalum* leaves for the same given concentration, (Picture 1). The vitamin C (**IC_{50}** = 0.06± 0.003 mg/mL) taken as a reference. The huge proportion of oxygenated compositions in the essential oil of *E. polycarpa* leaves could justify its high antioxydant activity ; that is not the case of that of *C.polycephalum* which is mainly composed of sesquiterpenes. In fact, according to the works of Kalemba and al in 2003, the activity of molecules depend on both the lipophilic character of their hydrocarbonate skeleton and the hydrophilic character of their functional group. The oxygenated molecules are generally more active than the hydrocarbon molecule (Oussou, 2009; Sipailiene et al., 2006; Kalemba, 2003).

Antibacterial Activities: The results of the antimicrobial tests have shown that the essential oil of *C.Polycephalum* leaves has got no effect on the bacterial strains and fungi.

Table 1. Constituents identified in the essential oil of *E. Polycarpa* leaves

N°	Compounds	RT (min)	RI	m/z	%
1	α-Pinene	12.74	926.52	136.12	6.43
2	β-Pinene	15.49	969.23	136.12	1.60
3	Limonene	19.25	1024.86	136.12	1.75
4	γ-Terpinene	27.50	1140.86	134.11	0.61
5	Myrtenal	31.08	1191.58	149.80	0.20
6	Copaene	42.95	1370.17	204.10	0.51
7	β-Elemene	44.10	1388.06	204.20	27.14
8	Caryophyllene	45.59	1411.87	204.20	2.87
9	Germacrene D	46.25	1422.71	204.10	0.29
10	Elixene	46.65	1429.28	204.20	2.90
11	Humulene	47.68	1446.14	204.20	0.51
12	β-Guaiene	49.05	1468.67	204.10	0.40
13	β-Selinene	49.66	1478.72	204.20	2.26
14	α-Selinene	50.23	1488.01	204.20	1.00
15	aromadendrene	50.80	1498.35	204.20	3.81
16	Cadina-1(10),4-diene	52.23	1522.08	204.10	0.51
17	Eudesma-4(14),7(11)-diene	52.54	1527.48	204.20	0.36
18	α-Bergamotene	52.89	1533.56	204.20	1.03
19	γ-Elemene	53.79	1549.23	204.20	23.46
20	Caryophyllene Oxide	55.26	1574.69	220.20	4.55
21	HumulèneEpoxide	56.21	1591.29	220.20	0.60
22	HumuleneEpoxide II	56.76	1600.78	220.20	0.58
23	γ-Eudesmol	57.09	1606.79	220.20	1.64
24	Epi-Cubenol	58.02	1623.83	222.20	1.00
25	α-Eudesmol	59.03	1642.03	222.20	0.70
26	Cadinol	59.34	1647.92	222.20	0.95
27	Cadina-1,4-diene-3-ol	59.95	1658.98	220.20	0.66
28	Santalol	60.37	1666.75	222.20	1.55
29	Eudesm-7(11)-en-4-ol	60.88	1676.08	220.0	1.25
30	(E)-Eudesma-4(15),7-dien-12-ol	62.32	1702.45	220.20	1.67
31	14-Hydroxy-alpha-Humulene	62.84	1712.36	220.00	3.22
32	α-Cyperone	63.88	1732.30	218.20	0.46
33	β -Vetivone	68.86	1829.18	202.17	0.16

RT: RetentionTime; RI : Retention Indice

Table 2. Constituents identified in the essential oil of *C. Polycephalum* leaves

N°	Compounds	RT (min)	RI	m/z	%
1	α -Pinene	13.10	932.14	136.10	0.93
2	Oct-1-en-3-ol	16.54	985.52	110.10	0.31
3	3-Octanol	17.67	1002.81	112.10	0.10
4	Limonene	19.64	1030.36	136.10	0.08
5	β-Myrcene	25.03	1105.76	136.00	0.17
6	Elemene d	41.08	1340.96	204.10	0.27
7	α -Cubebene	41.84	1352.84	204.20	0.85
8	α –Copaene	43.43	1377.65	204.20	2.55
9	β-Bourbonene	43.96	1385.89	204.20	3.04
10	β-Cubebene	44.41	1392.90	204.20	2.90
11	β-Elemene	44.57	1395.30	204.00	0.28
12	α -Gurjunene	45.54	1410.95	204.20	4.47
13	Caryophyllene	46.16	1421.21	204.20	30.27
14	β-Gurjunene	46.72	1430.42	204.20	0.92
15	γ -Elemene	47.15	1437.47	204.10	0.13
16	α -Bergamotene	47.31	1440.08	204.20	0.18
17	α-Muurolene	47.66	1445.82	204.20	0.19
18	α-Caryophyllene	48.18	1454.33	204.20	3.98
19	Aromadendrene allo(-)	48.61	1461.47	204.20	2.15
20	2-isopropyl-5-methyl-9-methylene-bicyclo(4.4.0)dec-1-ene	48.79	1464.33	204.20	0.26
21	γ -Cadinene	48.96	1467.20	204.20	0.23
22	Germacrene D	49.95	1483.46	204.20	23.96
23	α-Guaiene	50.30	1489.21	204.20	1.42
24	Bicyclosesquiphellandrene	50.51	1492.56	204.2	0.46
25	Bicyclogermacrene	50.83	1497.89	204.20	2.26
26	γ-Muurolene	51.16	1503.49	204.20	0.94
27	Valencene	51.30	1505.97	204.10	0.20
28	Cadina-1(10),4-diene	51,55	1510,20	204,20	0,15
29	Cadina-1,4-diene	51.91	1516.45	204.20	1.29
30	β-Cadinene	52.54	1527.50	204.20	4.06
31	Eudesma-3,7(11)-diene	52.98	1535.12	204.20	0.20
32	Cadina-3,9-diene	53.29	1540.46	204.20	0.24
33	α-Cadinene	54.04	1553.52	204.20	0.31
34	Germacrene B	54.28	1557.67	204.20	1.65
35	Palustrol	54.90	1568.43	222.10	0.29
36	Nerolidol	55.06	1571.28	222.20	1.00
37	Caryophyllene Oxide	56.92	1603.76	222.1	0.67
38	Cubenol	58.43	1631.29	222.20	0.07
39	α-Cadinol	59.19	1645.11	222.10	0.92
40	Guai-1(10)-en-11-ol	59.88	1657.77	222.20	1.17
41	3-(Trifluoromethyl) phenylacetic acid	61.56	1688.50	220.10	0.22
42	2-Tridecyloxirane	63.41	1723.25	226.23	0.10

RT: Retention Time; RI: Retention Indice

Table 3. Diameter of inhibition of essential oil (EO) on bacteria and fungi

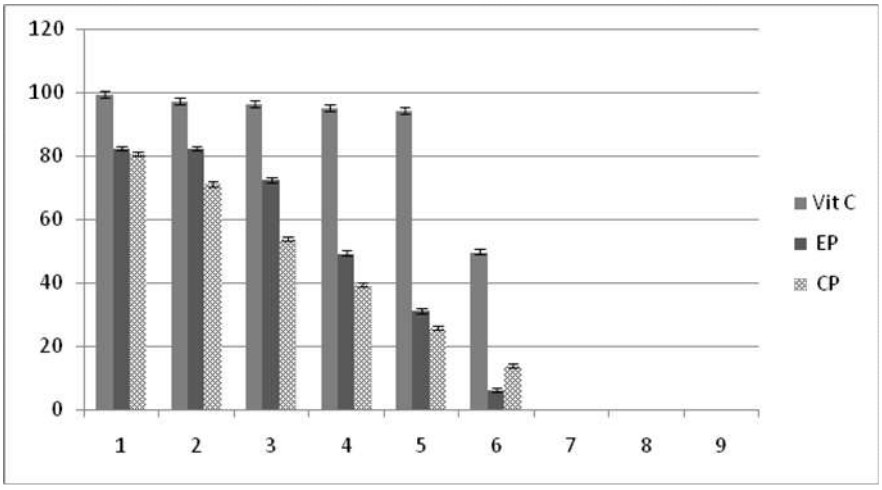
	d.i of EO CP (mm)	d.i of EP EO(mm)	d.i Oxy-tetracycline (reference)(mm)
Bacillus subtulis ATCC 6633	08	13	35
Staphylococcus aureus CIP 4.83	-	11	35
Staphylococcus epidermidis CIP.53124	-	-	35
Pseudomonas aeruginosa ATCC 27853	-	-	30
Salmonella typhimirium SO 66	08	15	25
Escherichia coli ATCC 25922	-	-	33
Klebsielle blse	09	11	35
Proteus mirabilis ATCC 14153	-	-	35
			Amphotericin B (reference)
Candida albicans ATCC 10231	08	18	20
Candida tropicalis ATCC 13803	08	08	20
Candida glabrata ATCC 66032	08	11	20

d.i : diameter of inhibition, CP : *Clerodendrum polycephalum*, EP : *Enantia polycarpa*

Table 4. Minimum inhibitory and bactericidal concentration of EO of the leaves of *E. Polycarpa*

	MIC mg/ml	MBC mg/ml	MBC/MIC	Effect of the EO
Candida Albicans ATCC 10231	12.5	50	4	bacteriostatic
Candida Glabrata ATCC 66032	6.25	50	8	bacteriostatic
Salmonella Typhimirium SO 66	12.5	50	4	bacteriostatic
Bacillus Subtilus ATCC 6633	6.25	50	8	bacteriostatic
Klebsiella Blse	6.25	50	8	bacteriostatic
Stahylococcus Aureus CIP 4.83	6.25	50	8	bacteriostatic

MIC: Minimal Inhibitory Concentration; MBC : Minimum Bactericidal Concentration



Picture 1. Histogram of inhibition % of the different extracts

However, the essential oil composition of *C. Polycephalum* having revealed a huge content in β-Caryophyllene could present good prospects. In fact, in 2014, Klauke and al. conducted a study examining analgesic effects of β-Caryophyllene on some mice that showed that when administrated orally, reduced both answers to inflammatory pains and neuro-inflammations spinals. They concluded that the β-Caryophyllene could be significantly efficaceous in the treatment of long, persistant and handicap pains, (Klauke, 2014). As for the essential oil of *E. polycarpa* leaves, it possesses a weak effect on *Bacillus subtulis*, *Staphylococcus aureus*, *Klebsielle blse* and *Candida glabrata*. It has a middle effect on *Salmonella typhimirium* and *Candida albicans* with a greater sensitivity on *Candida albicans.* In fact, for a diameter of inhibition (d.i.) inferior or equal to 8 mm, the activity is considered non- existent. Weak for a (d.i.) between 8 and 14 mm; average for (d.i.) between 14 and 20 mm ; high for (d.i.) superior or equal to 20 mm. The d.i. of oils upon bacteria and fungi are recoded on the Table 3. The MIC and MBC of the essential oil of *E.polycarpa* leaves were determined from the diameter inhibition superior or equalto 11mm (Table 4). The MBC/MIC reports, show that the essential oil of *E. polycarpa* leaves is bacteriostatic, with a greater sensitivity for *Salmonella typhimirium* SO 66 and *Candida albicans* ATCC 10231 thus, It inhibits the multiplication of bacteria without killing them. Inhibitting the bacteria, they could collaborate with the immune system to oust the bacteria from the body.

Conclusion

This study permitted to identify the composition through GC-MS, to evaluate the antioxydant and bacterial activities of essential oil of Enantia polycarpa and of Clerodendrum polycephalum leaves of Côte d'Ivoire. Thus, the essential oil of *E. polycarpa* leaves is made up of oxygenated compound

(45.45%), of sesquiterpenes (42.42%) and of monoterpenes (12.12%). As for the oil from *C. polycephalum* leaves, it is mainly composed of sesquiterpenes (69.04%), of oxygenated composition (23.80%) and of monoterpenes (7.14%). The main compounds are Caryophyllene (30.27%) and Germacrene D (23.96%). The essential oil of *E. polycarpa* leaves showed the good activity (IC_{50} = 0.27± 0.05 mg/ml). For that of *C.Polycephalum* leaves, the activity is relatively weak (0.73± 0.2) mg/ml. The results of the antimicrobial tests have shown that the essential oil of *C. Polycephalum* leaves has got no effect on the bacterial strains and fungi. However, the essential oil composition of *C. Polycephalum* having revealed a huge content in β-Caryophyllene could present good prospects. As for the essential oil of *E. polycarpa* leaves, it has got no effect on *Staphylococcus epidermidis, Pseudomonas aeruginosa, Escherichia coli* and *Candida tropicalis*, but possesses aninhibitory activity on *Bacillus subtulis*, *Staphylococcus aureus*, *Salmonella Typhimirium, Klebsielle blse, Candida albicans* and *Candida glabrata*. Thus, these activities would justify the use of extracted of *E.polycarpa* in the treatment of cutaneous infections and lesions by the populations.

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Keypoints

- Chemical composition of essential oil from *Clerodendrum polycephallum* leaves from Côte d'Ivoire ;
- Antioxydant activity of the essential oil of *Enantia polycarpa* and *Clerodendrum polycephallum* leaves from Côte d'Ivoire;
- The inhibitory activity of essential oil of *Enantia polycarpa*'s leaves on *Bacillus subtulis*, *Staphylococcus aureus*, *Salmonella Typhimirium, Klebsielle blse, Candida albicans* and *Candida glabrata*.

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