Research Article

Chemical Composition and Antibacterial Activities of Essential Oils Extracted from the Leaves of *Harungana madagascariensis* Lam. Ex Poiret (Hypericaceae) and *Allanblackia parviflora* a. Chev (Clusiaceae)

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ABSTRACT

Harungana madagascariensis and *Allanblackia parviflora* essential oils have been analyzed by Gas Chromatography coupled with Mass Spectrometry. Thus that of *Harungana madagascariensis*, obtained by hydrodistillation contains a total of 44 compounds representing (99.79%) of the total composition. The extraction yield is 0.05%. The oil, on one hand consists essentially of hydrocarbon monoterpenes (13.36%), oxygenated monoterpenes (0.44%), hydrocarbon sesquiterpenes (67.50%), oxygenated sesquiterpenes (11.99%) and other compounds (6, 50%). The major compounds are: β-caryophyllene (27.46%), δ-amorphene (12.82%), 3-carene (12.62%), bicyclosesquiphellandrene (8.88%), khusimone (4.47%), cadineol T (4.10%). On the other hand, we have identified in the essential oil of *Allanblackia parviflora* obtained by hydrodistillation 27 compounds representing (99.90%) of the total composition. The extraction yield is 0.02%. The oil mainly consists of hydrocarbon sesquiterpenes (73.87%), oxygenated sesquiterpenes (23.27%), diterpenes (0.61%) and other compounds (2.15%). The main compounds are α - caryophyllene (27.06%), copaene (21.62%), caryophyllene (17.89%), hedycaryol (7.74%). The study of the antibacterial activity has been carried out on reference strains from the laboratory of the Institut Pasteur in Côte d'Ivoire. The results have shown that *Harungana madagascariensis* essential oil (id = 8.64±0.08; 9.45±0; 9.49±0.01; 12±0.05; 14±0) was effective against *Enterobacter cloaceae* 543T/20CNRa, *Escherichia coli* 466 TR/20 CNRa, *Klebsiella pneumoniae* 471UB/20CNRa and very effective against *S. aureus* ATCC 25923 (id= 15.74±0.05), *Acinetobacter baumanii* 531UB/20 CNRa (id = 15.94±0.01). Then that of *Allanblackia parviflora* (id= 11.8 ±0; 12.84±0; 14±0) has been effective against *S. aureus* ATCC 25923, *S. aureus* 211UB/20CNRa, *S. aureus* 483UB/20CNRa and very effective against *Acinetobacter baumanii* 531UB/20 CNRa (id= 15.65 ±0). Thus, the oils of *Harungana madagascariensis* and *Allanblackia parviflora* has had a bactericidal effect on the strains *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* 483UB/20CNRa, *Staphylococcus aureus* 211UB/20CNRa, *K. pneumoniae* 471UB/20CNRa, *Acinetobacter baumanii* 531UB /20 CNRa. This study therefore has shown a good antibacterial potential of the extracted essential oils.

Keywords: *Harungana madagascariensis*, *Allanblackia parviflora*, Essential oil, Chemical composition, Yield.

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INTRODUCTION

Essential oils have always occupied a place of choice both in the perfume industry and in the pharmaceutical, cosmetic and food fields.[1] The popularity that these essential oils and aromatic plants in general have enjoyed for a long time remains linked to their medicinal properties.[2] For example, we note the anti-inflammatory, antiseptic, antiviral, antifungal, bactericidal, antitoxic properties, etc..[2] Exploration of the active ingredients of these plants for therapeutic use has seen renewed interest in recent years.[3, 4] This is why the valorization of these natural plant resources constitutes today a necessary and important concern for the research of new drug molecules.[4,5] The strengthening of research in order to have a better knowledge of the chemical composition and physico-chemical properties of the essential oils of these aromatic plants is necessary to take advantage of their benefits.[6] It is in this context that the study of *Harungana madagascariensis* [7-10] and *Allanblackia parviflora*[11,13] two species of the Ivorian flora falls within. *Harungana madagascariensis* is a shrub or tree with much branching foliage to a small tree reaching 12 m in height.[7] It is native to Madagascar, tropical Africa and grows in humid forests.[7] It is a vigorous colonizer also called weed.[7] The wood is used as fuel in the local metallurgy.[7]

Leaf decoctions are used to cure dysentery, diarrhoea, anemia, typhoid and certain heart conditions like tachycardia.[14] *Allanblackia parviflora* is a medium-sized tree reaching a height of about 40 m. It is found in Ghana, Nigeria, Cameroon and Tanzania in forest areas.[12] The leaves and bark of the genus *Allanblackia* are used for the treatment of dysentery, hypertension, cough, chest pain, wounds.[15] The vegetable oil extracted from the seed is used locally for cooking and soap making.[12]

MATERIALS AND METHODS

1. Plant material

The plant material consists of fresh leaves of *Harungana madagascariensis* and *Allanblackia parviflora* harvested in a wooded forest at Djorobité (5° 23' 10.423" N 3° 58' 57.866" W) in the commune of Cocody (5° 21' 22.792" N 3° 58' 3.706" W) belonging to the Autonomous District of Abidjan in Côte d'Ivoire. The plants have been identified thanks to the herbaria of the Center National de Floristique de Côte d'Ivoire (CNF) of the Félix Houphouët-Boigny University (Abidjan/Cocody) under the numbers UCJ008606 for *Harungana madagascariensis* and WAG0060197 for *Allanblackia parviflora*.

2. Methods

2.1 Extraction of essential oils

Extractions of essential oils have been carried out using a Clevenger-type hydrodistiller, associated with a pressure cooker of 10 L capacity containing 3 L of water. We lay on the grid 1000 g of plant material. The mixture (plant material and water) is brought to a boil on a hot plate. A glass column is connected above the cocote through which the vapors will condense in the cooler. The essential oil obtained is separated by gravity on the surface of the water after 4 hours of heating in a vase after decantation. It is then dried over anhydrous MgSO4 for approximately 10 minutes and then stored in a pill box in the refrigerator at 4°C.

2.2 GC-MS analysis of extracted essential oils

The analysis of essential oils diluted in dichloromethane (1:100) has been carried out on a GC chromatograph (7890A, Agilent Technologies) coupled to a mass spectrometer (5975C, Agilent Technologies). A sample of the essential oil (1µl) was injected into an HP-5MS capillary column at 250°C. The oven temperature has been programmed at 40°C for 5 min, then at 2°C/min for 15 min up to 250°C, with a flow rate of 10°C/min up to 300°C. Helium has been used as carrier gas with a flow rate of 1ml/min. The MS detector has had a temperature of 280°C and a voltage of 1.4 kV. Only ions whose mass/charge ratio is between 40 and 500, can be detected. The identification of the volatile compounds has been carried out by comparing the retention indices, calculated from the retention times and the mass spectra obtained with those National Institute of Standards and Technology (NIST) database and literature. We have

$$Ri = 100\left[n + \frac{t_r(C_i) - t_r(C_n)}{t_r(C_{n+1}) - t_R(C_n)}\right]$$ [16, 17] with

Ri: Retention index of the unknown compound;

n: carbon number of the linear alkane preceding the unknown compound;

tr (Ci): retention time of the unknown compound;

tr (Cn): retention time of the linear alkane preceding the unknown compound;

tr (Cn+1): retention time of the linear alkane according to the unknown compound.

2.3 Evaluation of the antibacterial activity of extracted essential oils

The strains used are reference strains from the laboratory of the Institut Pasteur in Côte d'Ivoire. They have been chosen thanks to their involvement in certain pathologies and their use in the treatment of several diseases. The prior realization of the antibiogram of the strains have made it possible to attribute different phenotypes to the subcultured bacterial strains, namely:

Gram (-) bacteria: *Escherichia coli* 466TR/20 CNRa *Escherichia coli* 470 UB/20 CNRa, *Salmonella* sp 109 UB/20 CNRa, *Acinetobacter baumanii* 531UB/20CNRa, *Klebsiella pneumoniae* 471UB/20CNRa, *Enterobacter cloacae* 543T/20CNRa, *Pseudomonas aeruginosa* 551UB/20CNRa, *Pseudomonas aeruginosa* ATCC 27853, *Pseudomonas aeruginosa* 469 U/20CNRa

Gram (+) bacteria: *Staphylococcus aureus* 211 UB/20 CNRa, *Staphylococcus aureus* 483 UB/20 CNRa, *Staphylococcus aureus* ATCC 25923

2.3.1 Efficacy test:

Blotting discs have been soaked in essential oil and placed on previously seeded agars. The reference antibiotics are Ceftriaxone (CRO), Imipenem (IPM) or Cefoxitin (FOX).

2.3.2 Determination of the minimum inhibition concentration (MIC) by dilution in liquid medium:

Concentrations of 0.03 mg/mL and 0.07 mg/mL of the essential oils to be tested have been distributed in the microplate wells from the highest concentration to the lowest. To these quantities are added respectively 0.07mg/mL and 0.03mg/mL of the bacterial inoculum. For the growth control, 1mg/mL of bacterial inoculum is placed in the well of the microplate. The microplate was incubated at 37°C for 24 hours. The MIC therefore corresponds to the concentration of the first experimental well above which no disturbance is observed with the naked eye. This operation was repeated 3 times in succession. The dilutions were made according to the technique obtained from the literature. Four successive tenth dilutions from 10-1 to 10-4 were made from the starting inoculum. These dilutions and the inoculum were inoculated in 5 cm streaks on the different MH agars, then incubated at 37°C for 24 hours. These Petri dishes constituted boxes A.[18-20].

2.3.3 Determination of the minimum bactericidal concentration (MBC):

The wells in which no disorder has been observed with the naked eye were inoculated in 5 cm streaks on the MH agars starting with the CMI well. The wells have then been incubated at 37°C for 24 hours. These boxes constituted boxes B. The Minimum Bactericidal Concentration (MBC) has been determined by comparing the densities of the bacterial colonies of the streaks in strips of boxes A and B.

The concentration of the essential oil seeded in strips (box B) having the same colony density as a band in box A is considered the Minimum Bactericidal Concentration CMB.[18-20].

RESULTS AND DISCUSSION

1 Extraction of essential oils

The results relating to the color, odor and yield **(Table 1)** of the oils extracted from the two study plants by hydrodistillation are shown below.

Table 1: Physical properties of extracted essential oils

Organs used	*Harungana madagascariensis*	*Allanblackia parviflora*
Color of essential oil	yelow	yelow
Odor of essential oil	Aromatic	Aromatic
Yield of Essential oil	0.05%	0.02%

The yield of essential oil extracted from the leaves of *Harungana madagascariensis* is twice as high as that of *Allanblackia parviflora*. It is lower than that of the study conducted by Moronkola et al, (2015) whose yield varies from 0.08% to 0.13%.[7] These low extraction yields observed could be due to the methods of processing the plant material, the species, the nature of the soil, the distillation technique and the harvest period.

2 Chemical composition of the essential oils extracted.

2.1 Harungana madagascariensis essential oil.

The essential oil of the Ivorian species has been analyzed by GC/MS. A total of 44 compounds representing 99.79% of the total composition have been identified **(Table 2)**. The oil therefore consists of hydrocarbon monoterpenes (13.36%), oxygenated monoterpenes (0.44%), hydrocarbon sesquiterpenes (67.50%), oxygenated sesquiterpenes (11.99%) and other compounds (6.50%). This composition is dominated by hydrocarbon sesquiterpenes (67.50%). The major compounds are: β-caryophyllene (27.46%), δ-amorphene (12.82%), 3-carene (12.62%), bicyclosesquiphellandrene (8.88%), khusimone (4.46%), cadineol T (4.10%). The Nigerian species is rich in α-caryophyllene (33.41%) while that of the Ivorian species is rich in β-caryophyllene (27.46%).[7]

So the results show that the chemical composition of the essential oil of the leaves of *Harungana madagascariensis* is different from that of the study conducted by Moronkola et al, (2015).[7] Indeed, this difference observed in the chemical composition of the essential oil of the Ivorian species and that identified by previous work carried out on the plant could be due to certain ecological factors, to the period of the vegetative cycle, to the origin of the plant and the extraction technique.[21-28]

Table 2: Chemical composition of essential oil from *Harungana madagascariensis* leaves

N°	compounds	Rt	Ri	m/z	Content (%)
1	α -pinene	12.65	925	136	0.42
2	hept-2-en-1-ol	16.77	989	114	0.40
3	limonene	20.10	1036	136	0.11
4	(z) - β-ocimene	20.78	1046	136	0.24
5	**3-carene**	**24.47**	**1098**	**136**	**12.62**
6	décanal	30.65	1185	156	0.13
7	2-undecenal	40.46	1331	168	0.10
8	jasmone	42.79	1367	164	0.26
9	isoeugenol	43.36	1376	164	0.31
10	α-copaene	43.92	1385	204	1.61
11	β-damascone	44.91	1400	192	0.17
12	α-ionone	45.45	1409	192	0.56
13	(z)- β-farnesene	46.09	1420	204	0.14
14	**β – caryophyllene**	**46.76**	**1431**	**204**	**27.46**
15	α -bergamotene	47.52	1443	204	0.33
16	aristolene	47.97	1451	204	2.41
17	α- caryophyllene	49.23	1471	204	3.89
18	aromadendrene	49.58	1477	204	0.64
19	tridecanal	50.06	1485	198	0.14
20	**bicyclosesquiphellandrene**	**50.51**	**1492**	**204**	**8.88**
21	β-sesquiphellandrene	50.80	1497	204	1.74
22	γ-cadinene	51.33	1506	204	0.98
23	δ –selinene	51.91	1516	204	1.74
24	germacrene D	52.67	1529	204	0.61
25	eudesma-4(14) ,7(11)-diene	52.92	1534	204	1.72
26	**δ-amorphène**	**53.40**	**1542**	**204**	**12.82**
27	cadina-3,5-diene	53.61	1546	204	0.14
28	α-cadinene	54.44	1560	204	0.28
29	α -selinene	55.09	1572	204	1.76
30	γ-undecalactone	55.72	1582	184	0.09
31	**khusimone**	**56.14**	**1590**	**204**	**4.46**
32	Calarene	56.25	1592	204	0.34
33	tetradecanal	56.60	1598	212	0.16
34	eudesm-6-en-4- β-ol	57.04	1606	222	0.26
35	γ-eudesmol	57.80	1619	222	1.64
36	cubenol	58.54	1633	222	1.61
37	selin-11-en-4 α-ol	58.81	1638	222	2.86
38	7-epi- α-eudesmol	59.21	1645	204	0.83
39	**cadinol T**	**60.15**	**1662**	**220**	**4.10**
40	(z)- γ-atlantone	60.54	1669	218	0.36
41	β-sinensal	60.98	1677	218	0.11
42	α-bisabolol	61.12	1680	222	0.11
43	β-atlantone	65.16	1757	218	0.11
44	phytone	69.56	1843	250	0.14
Hydrocarbons monoterpenes					13.36
Oxygenated monoterpenes					0.44
Hydrocarbon sesquiterpenes					67.50
Oxygenated sesquiterpenes					11.99
Other					6.50
Total					99.79

Rt: Retention time; Ri : Retention index; m/z: mass on the load.

3.1.2 *Allanblackia parviflora* essential oil

The essential oil from the leaves of *Allanblackia parviflora* has been analyzed by GC/MS. The analysis of the chromatogram and the mass spectra have made it possible to identify 27 compounds representing (99.90%) of the total chemical composition **(Table 3)**. It consists of hydrocarbon sesquiterpenes (73.87%), oxygenated sesquiterpenes (23.27%), diterpenes (0.61%) and other compounds (2.15%). This oil is therefore rich in hydrocarbon sesquiterpenes (73.87%). The main compounds are: α-caryophyllene (27.06%), α-copaene (21.62%), caryophyllene (17.89%), hedycaryol (7.74%).

Table 3: Constituents of the essential oil of *Allanblackia parviflora* obtained by hydrodistillation

N°	Compounds	Rt (min)	Ri	m/z	Content (%)
1	**α- copaene**	**42.81**	**1368**	**204**	**21.62**
2	β -damascenone	43.94	1385	190	1.12
3	**caryophyllene**	**45.45**	**1409**	**204**	**17.89**
4	α-damascone	46.19	1421	192	0.42
5	**α - caryophyllene**	**47.55**	**1444**	**204**	**27.06**
6	β - humulene	47.93	1450	204	0.62
7	β - guaiene	49.21	1471	204	1.29
8	α-muurolene	49.50	1476	204	0.58
9	β –selinene	50.08	1485	204	0.78
10	γ -cadinene	50.54	1493	204	0.72
11	α –farnesene	51.29	1505	204	0.67
12	δ -selinene	51.90	1516	204	2.64
13	β -elemol	52.94	1534	222	0.18
14	**hedycaryol**	**53.41**	**1542**	**222**	**7.74**
15	nerolidol	54.42	1560	222	1.52
16	Germacrene D-4-ol	54.72	1565	222	0.47
17	époxycaryophyllene	55.11	1572	220	1.76
18	guaiol	56.17	1590	222	3.25
19	humulene-1,2-epoxyde	56.61	1599	220	1.35
20	γ -eudesmol	57.01	1605	222	0.34
21	épi – cubenol	57.95	1622	222	2.02
22	cadinol T	58.55	1633	222	0.67
23	agarospirol	59.03	1642	222	3.57
24	valeranone	59.93	1658	222	0.40
25	isophytol	74.50	1944	296	0.20
26	acide palmitique	75.32	19,61	256	0.61
27	Phytol E	82.14	2110	296	0.41
Hydrocarbon sesquiterpenes			**73.87%**		
Oxygenated sesquiterpenes			**23.27%**		
Diterpenes			**0.61%**		
Other			**2.15%**		
Total			**99.90%**		

Rt: Retention time; Ri: Retention index; m/z: mass on the load.

3.3 Efficacy screening of extracted essential oils

According to Ponce et al. 2003[29], a bacterium is said to be resistant to an extract when its inhibition diameter around this extract is ≤ 8 mm and sensitive if this diameter is between 9 and 14 mm, very sensitive when it is between 15 and 19 mm and extremely sensitive for a diameter greater than 20 mm.

With regard to the diameters of the inhibition zones obtained **(Table 4)**. *Harungana madagascariensis* essential oil (id = 8.64±0.08; 9.45±0; 9.49±0.01; 12±0.05; 14±0) is effective against *Enterobacter cloaceae* 543T/20 CNRa *E. coli* 466 TR/20 CNRa, *K. pneumoniae* 471UB/20CNRa and very effective against *S. aureus* ATCC 25923 (id= 15.74±0.05), *Acinetobacter baumanii* 531UB/20 CNRa (id= 15.94± 0.01). Then that of *Allanblackia parviflora* (id= 11.8 ±0; 12.84±0; 14±0) is effective against *S. aureus* ATCC 25923, *S. aureus* 211UB/20CNRa, *S. aureus* 483UB/20CNRa and very effective against *Acinetobacter baumanii* 531UB/20 CNRa (id= 15.65 ±0). According to these zones of inhibition generated by the essential oils studied, we find that these oils have better activity on these strains tested.

Thus the activity of *Harungana madagascariensis* oil could be justified by the remarkable presence of compounds such as β-caryophyllene (27.46%) known to be active on *S. aureus*,[30] 3-carene (12.62%), bicyclosesquiphellandrene (8.88%), δ-amorphene (12.82%)[17] and certain monoterpenes such as α-pinene (0.42%), limonene (0.11 %) that possess antibacterial activities.[31] As for the activity of the essential oil of *Allanblackia parviflora*, it could also be justified by the presence of β-caryophyllene (17.89%), a very active compound,[30] α-caryophyllene (27.06%), α-copaene (21.62%).[17]

Table 4: diameters of the zones of inhibition of essential oils

Bacterial strains	id (mm) of EO		
	EO-Hm	HE-Ap	ATB
S.aureus ATCC 25923	15.74±0.05	12.84±0	FOX : 20
S.aureus 211UB/20CNRa	12±0.05	11.8 ±0	FOX : 27
S.aureus 483UB/20CNRa	14±0	14±0	FOX : 30
P.aeniginosa ATTCC 27853	0	0	IPM : 25
P.aeniginosa 469UB/20 CNRa	0	0	IPM : 28
P.aeniginosa 551UB/20CNRa	0	0	IPM : 33
E.coli 466 TR/20 CNRa	9.45±0	0	CRO : 0
E.coli 470UB/20 CNRa	0	0	CRO : 0
Salmonella 109 UB/20CNRa	0	0	CRO : 16
Enterobacter cloaceae 543T/20CNRa	8.64±0.08	0	CRO : 10
K.pneumoniae 471UB/20CNRa	9.49±0.01	0	CRO : 0
Acinetobacter baumanii 531UB/20 CNRa	15.94±0.01	15.65 ±0	CRO : 0

id (mm): inhibition diameter; EO: essential oil; Hm: *Harungana madagascariensis*; Ap: *Allanblackia parviflora*

3.4 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Essential Oils

The determination of the antibacterial parameters (MIC and MBC) has made it possible to determine the activities, but also to characterize the nature of the effect revealed by the essential oil. Indeed, according to[32, 33].

The extract is said to be bactericidal, if the CMB = CMI. Then the results of **(Table 5)** show that the essential oil extracts of *Harungana madagascariensis* and *Allanblackia parviflora* have a bactericidal effect on the strains *S. aureus* ATCC 25923, *S. aureus* 483UB/20CNRa, *S. aureus* 211UB/20CNRa, *K. pneumoniae* 471UB/20CNRa, *Acinetobacter baumanii* 531UB/20CNRa.

Table 5: MIC, MBC of essential oils

Bacterial strain	Extracts of EO	MIC (mg/mL)	MBC (mg/mL)
S.aureus ATCC 25923	EO-Hm	0.03	0.03
	EO-Ap	0.07	0.07
S.aureus 483UB/20CNRa	EO-Hm	0.03	0.03
	EO-Ap	0.03	0.03
S.aureus 211UB/20CNRa	EO-Hm	0.03	0.03
	EO-Ap	0.03	0.03
K.pneumoniae 471UB/20CNRa	EO-Hm	0.03	0.03
Acinetobacter baumanii 531UB/20 CNRa	EO-Hm	0.07	0.07
	EO-Ap	0.07	0.07

EO: essential oil; Hm: *Harungana madagascariensis*; Ap: *Allanblackia parviflora*

MIC: Minimum Inhibitory Concentration; MBC: minimum bactericidal concentration

The bactericidal activities observed could be justified by the presence of sesquiterpenes with regard to their high content in the essential oils of *Harungana madagascariensis* and *Allanblackia parviflora* or the synergy of the compounds they contain. Indeed, sesquiterpenes have very wide applications. Most of them exhibit antiseptic and bactericidal properties.[34] Similarly, according to Hogg et al. 2005,[35] these bactericidal activities observed may also be due to the presence of alcohols identified on both sides in the essential oils of *Harungana madagascariensis* and *Allanblackia parviflora* because alcohols have bactericidal rather than bacteriostatic activity.

CONCLUSION

This study has determined the yield, chemical composition and antibacterial properties of essential oils extracted from *Harungana madagascariensis* and *Allanblackia parviflora*. The yields of essential oils of *Harungana madagascariensis* (RHm=0.05%) and *Allanblackia parviflora* (RAp=0.02%) extracted from fresh leaves are low. Chemical analyzes by GC/MS have identified 44 compounds in the essential oils of' *Harungana madagascariensis* and 27 compounds in that of '*Allanblackia parviflora*.

Harungana madagascariensis essential oil consists of monoterpenes (13.80%), sesquiterpenes (79.49%) and other compounds (6.5%). The majority compound is β-caryophyllene (27.46%). That of *Allanblackia parviflora* is essentially composed of sesquiterpenes (97.14%), diterpenes (0.61%) and other compounds (2.15%). The major constituents are α-caryophyllene (27.06%) and copaene (21.62%). The essential oils of the two species investigated have proved to be active against most of the bacteria tested. So this study shows that essential oils have good antibacterial activity. This activity would certainly be due to the presence of terpenes and also alcohols.

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