Original Research Article

Chemical composition, antioxidant and antibacterial activity of the essential oil of *Harungana madagascariensis* Lam. Ex Poiret (Hypericaceae).

Abstract

Essential oils derived from aromatic plants are highly sought after due to their many biological properties. This is the case with *Harungana madagascariensis*, a plant widely used in traditional African medicine for various indications such as malaria, dysentery, and anemia. The aim of this study is to promote this Ivorian species. The essential oil was extracted by steam distillation. Gas chromatography coupled with mass spectrometry (GC/MS) was used to identify and quantify the volatile constituents. For antioxidant activity, the DPPH test was performed on the extract. The antibacterial activity was tested on 13 reference strains using the liquid dilution method at the Pasteur Institute laboratory in Ivory Coast. The chemical composition showed that this oil is rich in hydrocarbon sesquiterpenes (74.31%). It is dominated by β-caryophyllene (18.82%). The yield is 0.01%±0. The study reveals that this oil has strong anti-radical activity (CR50 = 0.051±0.01 mg/mL; vit C: CR50 = 0.016±0.089 mg/mL). The antibacterial test shows that *Acinetobacter baumanii, Staphylococcus aureus, Enterobacter cloaceae, Klebsiella pneumoniae, and Escherichia coli* 466 were sensitive to the extract. Bactericidal activity (CMB/CMI = 1) was demonstrated against these bacterial strains.

Keywords: Essential oil, antioxidant activity, antibacterial activity, *Harungana madagascariensis*

1-Introduction

Essential oils from plants are highly sought after because they generally have interesting biological properties **[1].** Some have recognized pharmaceutical properties, while others are used as perfume bases or food additives **[1].** The quality of these essential oils depends on a large number of parameters of different origins, such as botanical origin, vegetative cycle, production site, geographical and climatic conditions, etc. **[1].** This is the case with *Harungana madagascariensis*, a shrub or tree with highly branched foliage reaching 12 m in height, with a fairly straight trunk bearing evergreen branches **[2,3].** The trunk is covered with bark that can peel off in short strips **[4].** All young parts of the plant are covered with reddish hairs, such as the buds, stems, and inflorescence. The plant produces a yellow or orange sap commonly known as “dintin-karongana” or harongana resin. It is found particularly in Madagascar, Nigeria, Zimbabwe, Senegal, the Democratic Republic of Congo, Burundi, Rwanda, Equatorial Guinea, Sierra Leone, Kenya, Mozambique, and Cameroon **[2].** *Harungana madagascariensis* has traditionally been used for many years in African herbal medicine to treat a wide range of human diseases **[2].** In Ivory Coast, for example, a decoction of the bark is used in enemas or baths to treat malaria **[5].** In Cameroon, leaf decoctions are used to treat dysentery, diarrhea, anemia, and typhoid **[6, 7].** To contribute to the promotion of this Ivorian species, the aim of

this study is to determine the chemical composition and evaluate the antioxidant and antibacterial activity of the essential oil of *Harungana madagascariensis*.

2- Materials and methods

2.1- Plant material

The plant material consists of fresh leaves of *Harungana madagascariensis* harvested in a forest in Djorobité (5° 25' 58.393“ N 4° 2' 20.011” W) in the municipality of Cocody, part of the Autonomous District of Abidjan in Ivory Coast. The plant was identified using the herbariums of the National Center for Floristics of Ivory Coast (CNF) at Félix Houphouët-Boigny University (Abidjan/Cocody) under the numbers *UCJ008606.*

2.2 - Methods

2.2.1- Extraction of essential oil

A steam distillation technique using a stainless steel device with four compartments was used to extract the essential oil from the plant material. The boiler (60 l capacity) is connected to a large tank by a stainless steel pipe. The large tank (height: 100 cm, internal diameter: 51 cm, volume: 0.2 m³) contained four grids attached to a removable rod. 1.9 kg of plant material for each species was placed separately on the grids. From this tank, the water vapor carries the volatile compounds to a third tank (height: 100 cm, internal diameter: 41 cm, volume: 0.13 m3) which serves as a condenser. The essential oil is obtained in a fourth compartment which serves as a collection system. recovery. It is then dried on anhydrous MgSO4 for approximately 10 minutes and stored in a pill box in a refrigerator at 4°C.

2.2.2 - GC-MS analysis of the extracted essential oil

The analysis of the essential oil diluted in dichloromethane (1:100) was performed on a GC chromatograph (7890A, Agilent Technologies) coupled with 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 was set 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 was used as the carrier gas at a flow rate of 1 ml/min. The MS detector has a temperature of 280°C and a voltage of 1.4 kV. Only ions with a mass-to-charge ratio between 40 and 500 are detectable. Based on the retention times and mass spectra obtained with those from the National Institute of Standards and Technology (NIST) database and the literature.

We have $Ir = 100\left[n + \frac{t_R(C_i) - t_R(C_n)}{t_R(C_{n+1}) - t_R(C_n)}\right]$ **[8, 9]**.

n: number of carbon atoms in the linear alkane preceding the unknown compound;
t_R(ci): retention time of the unknown compound;
t_R (c_n): retention time of the linear alkane preceding the unknown compound;
t_R(c_{n+1}): retention time of the linear alkane following the unknown compound;
I_r : retention index of the unknown compound.

2.2.3- Evaluation of the antioxidant activity of essential oil

2,2-Diphenyl-1-picrylhydrazyl (DPPH) is solubilized in absolute methanol to obtain a solution with a concentration of 0.03 mg/mL. The concentrations of the various essential oil extracts in mg/mL prepared by successive dilution in absolute methanol are: 4; 2; 1; 0.5; 0.25; 0.125; 0.062; 0.031 and 0.015. In dry, sterile hemolysis tubes, 2.5 mL of the extract solution to be tested and 1 mL of the DPPH methanol solution are added. After shaking, the tubes are incubated for 30 min in the dark, then the absorbance of the mixture is measured at 517 nm.

The blank consists of 2.5 mL of absolute methanol and 1 mL of DPPH solution. The positive reference control is vitamin C prepared under the same conditions as the samples. The reduction percentages (%R) of the samples are calculated from the measured absorbances **[10,11].**

$$\%R = \left(1 - \frac{\text{Absorbance of the extract}}{\text{Absorbance of DPPH}}\right) \times 100$$

2.2.4- Evaluation of the antibacterial activity of the extracted essential oil

The strains used are reference strains from the Pasteur Institute laboratory in Ivory Coast. They were chosen because of their involvement in certain pathologies and their use in the treatment of several diseases. The prior completion of the antibiogram of the strains made it possible to assign different phenotypes to the inoculated bacterial strains, namely:

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

Gram-positive bacteria: *Staphylococcus aureus* 211 UB/20 CNRa, *Staphylococcus aureus* 483 UB/20 CNRa, *Staphylococcus aureus* ATCC 25923

3 - Results and discussion

3.1 - Extraction of *Harungana madagascariensis* essential oil

Harungana madagascariensis essential oil extracted by steam distillation has an orange-yellow color and an aromatic odor. The yield obtained is 0.01% ± 0. This value shows that the yield of Harungana madagascariensis essential oil is low. This low yield could depend on several factors, namely the species, genotype, environment, harvest period, geographical origin, or extraction technique **[12, 13,14].**

3.2- Chemical composition of *Harungana madagascariensis* essential oil

Harungana madagascariensis essential oil, obtained by steam distillation, contains 37 compounds representing 99.84% of the total composition (Table 1). This oil consists of hydrocarbon monoterpenes (15.78%), oxygenated monoterpenes (0.48%), hydrocarbon sesquiterpenes (74.31%), oxygenated sesquiterpenes (6.15%), and other compounds (3.12%). The main compounds are: β-caryophyllene (18.82%), (z)-β-ocimene (14.29%), δ-amorphene (9.71%), α-selinene (9.35%), α-farnesene (7.55%), γ-cadinene (7.16%), and γ-selinene (5.77%). Those obtained by hydrodistillation are: β-caryophyllene (27.46%), δ-amorphene (12.82%), 3-carene (12.62%), khusimone (4.46%), bicyclosesquiphellandrene (8.88%), cadinol T (4.10%) according to N'dri et *al.,* (2023) **[12].** The results show that the chemical composition of the essential oil from *Harungana madagascariensis* leaves obtained by steam distillation differs from that reported in the study conducted by N'dri *et al.*, (2023) **[12].** This difference could be due to the extraction technique, certain ecological factors, the period of the vegetative cycle, or the origin of the plant **[13-17].**

Table 1: Chemical composition of *Harungana madagascariensis* essential oil obtained by steam distillation

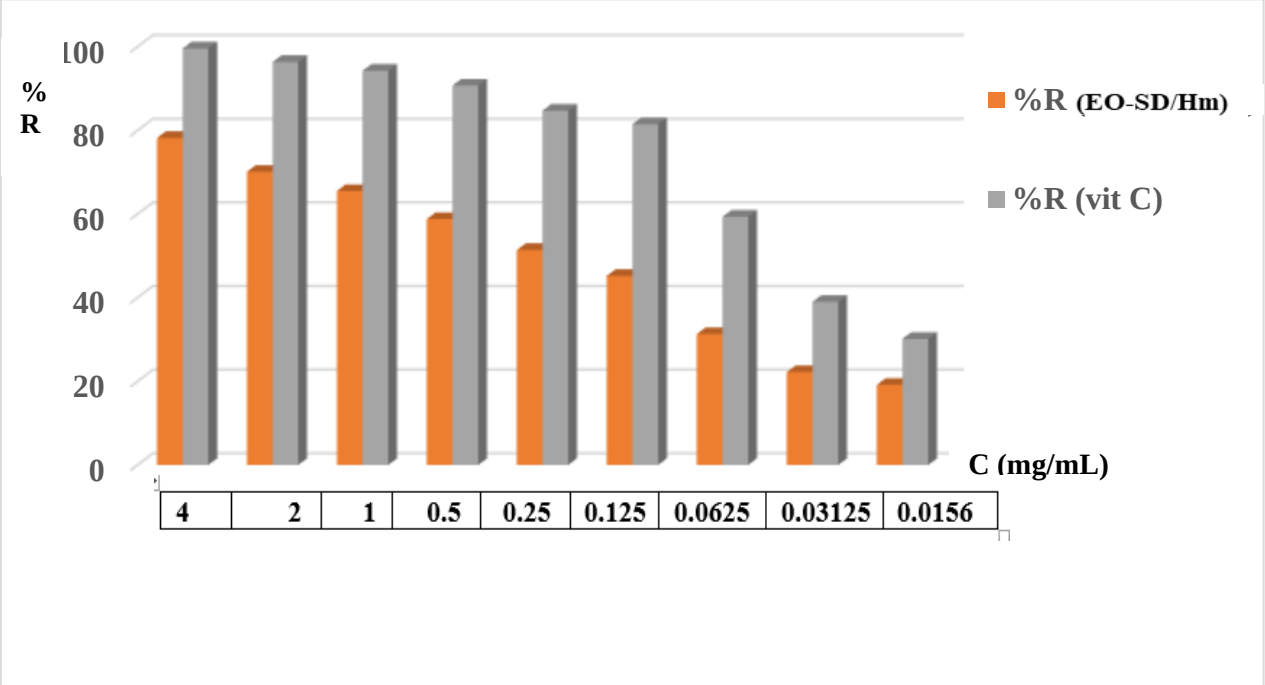
N°	Compounds	Tr	Ir	m/z	Content (%)
1	α -pinene	12.63	924	136	1.50
2	sulcatone	15.36	967	126	0.22
3	2,6-dimethylcyclohexan-1-one	16.76	989	126	0.50
4	salicylaldehyde	19.10	1023	122	0.20
5	acetophenone	20.08	1036	120	0.49
6	**(z) - β-ocimene**	**20.79**	**1046**	**136**	**14.29**
7	(3Z, 5Z)-2,6-dimethyl-3, 5,7-octatrien-2-ol	32.21	1208	152	0.15
8	2-undecenal	40.45	1331	168	0.12
9	cyclosativene	41.18	1342	204	0.18
10	cascarilladiene	42.17	1358	204	0.67
11	α-cubebene	42.80	1368	204	2.14
12	isoeugenol	43.32	1376	164	0.33
13	α-damascone	43.93	1385	192	1.47
14	isocomene	44.88	1400	204	0.18
15	**β- caryophyllene**	**45.44**	**1409**	**204**	**18.82**
16	calarene	46.08	1420	204	0.68
17	α-bulnesene	46.76	1431	204	1.82
18	α- caryophyllene	47.53	1443	204	3.07
19	aristolene	48.02	1451	204	0.97
20	**γ -cadinene**	**49.26**	**1472**	**204**	**7.16**
21	**α-selinene**	**49.51**	**1476**	**204**	**9.35**
22	**γ-selinene**	**50.09**	**1486**	**204**	**5.77**
23	α-amorphene	50.53	1493	204	2.66
24	**α-farnesene**	**51.27**	**1505**	**204**	**7.55**
25	**δ- amorphene**	**51.92**	**1516**	**204**	**9.71**
26	δ-selinene	52.38	1524	204	0.29
27	β-guaiene	52.68	1530	204	1.69
28	germacrene D	53.63	1546	204	1.59
29	epiglobulol	54.44	1560	222	0.13
30	caryolan-8-ol	55.10	1572	222	1.35
31	nérolidol	55.76	1583	222	0.27
32	γ-undecalactone	55.92	1586	184	0.12
33	eudesm-6-en-4- β-ol	57.04	1606	222	0.85
34	γ-eudesmol	57.78	1619	222	0.37
35	cadinol T	58.55	1633	222	1.64
36	α-cadinol	59.22	1645	222	1.42
37	α-bisabolol	61.08	1679	222	0.12
Hydrocarbon monoterpenes					**15.78%**
Oxygenated monoterpenes					**0.48%**
Hydrocarbon sesquiterpenes					**74.31%**

Oxygenated sesquiterpenes	**6.15%**
Others	**3.12%**
Total	**99.84%**

Tr : Retention time; Ir : Retention index ; m/z : Mass to charge ratio ; % : Percentage

3.3 - Antioxidant activity of *Harungana madagascariensis* essential oil

The different percentages of inhibition of 2,2-diphenyl-1-picrylhydrazyl (DPPH) by essential oil extracted using the steam distillation technique are presented below in the form of histograms (Figure 1). Looking at this figure 1, the results show that *Harungana madagascariensis* essential oil exhibits better antioxidant activity.



% R (EO-SD/Hm): percentage reduction of *Harungana madagascariensis* essential oil obtained by steam distillation; % R (vit C): percentage reduction of vitamin C

Figure 1: Histogram of DPPH inhibition as a function of the concentration of *Harungana madagascariensis* essential oil

The CR_{50} value of *Harungana madagascariensis* essential oil obtained by steam distillation is 0.051±0.01 mg/mL, and that of vitamin C is 0.016±0.0089 mg/mL. This oil has shown good antioxidant activity. This activity could be attributed to β-caryophyllene (18.82%), α-farnesene (7.55%), and α-caryophyllene (3.07%). These major compounds all have very high antioxidant power according to Tepe *et al.*, (2005), Celik et al. (2014), and Ocete *et al.*, (1989) **[18-20].**

3.4 - Antibacterial activity of *Harungana madagascariensis* essential oil

The antibacterial activity of the essential oil obtained by steam distillation was evaluated. The diameter of the inhibition zones was measured to assess the effectiveness of this oil on the different bacteria tested. The results are summarized in **Table 2**.

Table 2: Diameters of inhibition zones of *Harungana madagascariensis* essential oil obtained by steam distillation

Bacterial strains	dI (mm) of EO	
	EO-SD/Hm	ATB
***Staphylococcus aureus* ATCC 25923**	15.86 ±0.13	FOX : 20
***Staphylococcus aureus* 211UB/20 CNRa**	13±0	FOX : 27
***Staphylococcus aureus* 483UB/20 CNRa**	15±0	FOX : 30
Pseudomonas aeniginosa ATTCC 27853	0	IPM : 25
Pseudomonas aeniginosa 469UB/20 CNRa	0	IPM : 28
***Pseudomonas aeniginosa* 551UB/20 CNRa**	0	IPM : 33
***Escherichia coli* 466 TR/20 CNRa**	9.65 ±0.05	CRO : 0
***Escherichia coli* 470UB/20 CNRa**	6.73±0.87	CRO : 0
***Salmonella sp* 109 UB/20 CNRa**	7.09±0.08	CRO : 16
***Enterobacter cloaceae* 543T/20 CNRa**	10.78 ±0	CRO : 10
***Klebsiella pneumoniae* 471UB/20 CNRa**	9.92 ±0.02	CRO : 0
***Acinetobacter baumanii* 531UB/20 CNRa**	34.56 ±0	CRO : 0

di (mm): inhibition diameter; EO: essential oil; EO-SD/Hm: essential oil of *Harungana madagascariensis* obtained by steam distillation; ATB: reference antibiotic

In terms of the diameters of the inhibition zones **(Table 2),** the essential oil of *Harungana madagascariensis* obtained by steam distillation shows that *Acinetobacter baumanii* 531UB/20 CNRa (di= 34.56±0) is extremely sensitive to the extract. *Staphylococcus aureus* 483UB/20 CNRa (di= 15±0) and *Staphylococcus aureus* ATCC 25923 (di= 15.86±0.13) are also very sensitive to the extract. Next, *Staphylococcus aureus* 211UB/20 CNRa (di=13±0), *Enterobacter cloaceae* 543T/20 CNRa (di= 10.78±0), *Klebsiella pneumoniae* 471UB/20CNRa (di= 9.92±0.02) and *Escherichia coli* 466 TR/20 CNRa (di= 9.65±0.05) also show notable sensitivity. However, *Escherichia coli* 470UB/20 CNRa (di= 6.73±0.87) and *Salmonella* 109 UB/20 CNRa (di= 7.09±0.09) proved resistant to the extract. These oils showed strong activity against strains of *Staphylococcus aureus, Acinetobacter baumanii* 531UB/20 CNRa, *Escherichia coli* 466 TR/20 CNRa, *Klebsiella pneumoniae* 471UB/20 CNRa, and *Enterobacter cloaceae* 543T/20 CNRa. In addition, this essential oil has strong inhibitory power. Is due to these main constituents, namely: β-caryophyllene (18.82%), α-farnesene (7.55%), α-pinene (1.50%) (Celik et al., (2014), Sadou *et al*., (2015) **[19, 20].** According to Sadou *et al.,* (2015) and Celik *et al.,* (2014) **[19, 21],** these compounds have antibacterial properties. Thus, referring to the results in Table 3, the MBC/MIC activity ratio showed that this oil is bactericidal against the strains tested. These observed bactericidal activities could depend on antagonism between the different major and minor components of this essential oil, according to Noudjoub (2010) **[22].**

Table 3: MIC and MBC of *Harungana madagascariensis* essential oil obtained by steam distillation

Bacterial strains	Essential oil extracts	MIC (mg/mL)	MBC (mg/mL)
***Staphylococcus aureus* ATCC 25923**	EO-SD/Hm	0.03	0.03
***Staphylococcus aureus* 483UB/20 CNRa**	EO-SD/Hm	0.03	0.03
***Staphylococcus aureus* 211UB/20 CNRa**	EO-SD/Hm	0.03	0.03
***Acinetobacter baumanii* 531UB/20 CNRa**	EO-SD/Hm	0.07	0.07
***Klebsiella pneumoniae* 471UB/20 CNRa**	EO-SD/Hm	0.07	0.07
***Acinetobacter baumanii* 531UB/20 CNRa**	EO-SD/Hm	0.07	0.07

EO: essential oil; essential oil of *Harungana madagascariensis* obtained by steam distillation; MIC: minimum inhibitory concentration; MBC: minimum bactericidal concentration

4 - Conclusion

This study determined the chemical composition and antioxidant and antibacterial properties of the essential oil extracted from Harungana madagascariensis. The oil was characterized through chemical analysis using gas chromatography coupled with mass spectrometry (GC-MS). The essential oil consists of hydrocarbon monoterpenes (15.78%), oxygenated monoterpenes (0.48%), hydrocarbon sesquiterpenes (74.31%), oxygenated sesquiterpenes (6.15%), and other compounds (3.12%). It revealed the presence of several compounds, the main ones being: β-caryophyllene (18.82%), (z)-β-ocimene (14.29%), δ-amorphene (9.71%), α-selinene (9.35%), α-farnesene (7.55%), γ-cadinene (7.16%), and γ-selinene (5.77%).This study also demonstrated that Harungana madagascariensis has an essential oil rich in hydrocarbon sesquiterpenes (74.31%), strong antioxidant activity, and promising antibacterial activity. These results justify the promotion of this plant in the search for new antioxidant or antibacterial agents from African plant resources.

6- DECLARATIONS

- **Conflict of interest**

The authors declare no competing interests.

- **Ethical approval**

This article does not contain any studies with human participants or animals performed by any of the authors.

- **Consent to participate**

Not applicable. This study did not involve human participants.

- **Consent to publish**

Not applicable.

6- DATA AVAILABILITY STATEMENT

All data generated or analysed during this study are included in this published article.

Bibliographic references

[1] : Salima M., Mounia H., Boufeldja T., (2009). Physicochemical study of the essential oil of *Ruta Chalepensis* L. from Tlemcen, Algeria. Department of Chemistry, Faculty of Sciences, Laboratory of Organic Chemistry, Natural Substances and Analysis, Abou Bekr Belkaid University, P.O. Box 119, Tlemcen 13000, Algeria. 05 (1). P. 67–81.

[2] : Moronkola D.O., Yeboah S.O., Majinda R.R.T., Sichilongo K. (2015). Compositions of *Harungana madagascariensis* Lam. Ex Poiret leaf and stem essential oils.-7 (5): p. 959-964.

[3]: Raharilina N. D. E. (2009). Valorization of medicinal plants: the case of *Harungana madagascariensis.* CAPEN thesis, CER Natural Sciences 51p.

[4]: Ramahandrimanana J.C.R. (1986). Inventory of plants active on the cardiovascular system, CAPEN thesis, CER Natural Sciences, 77p

[5]: Cooke B., Burren. C., Rakotoniaina M. (2009). Technical data sheets to promote tree planting, MYE Andohalo Antananarivo, 94p.

[6]: Berhaut J. (1975). Illustrated Flora of Senegal, Volume IV. Preface by Mr. Leopold Sendar Senghor, Dakar, Senegal, pp. 93–94.

[7] : Tona L., Kambu K., Ngimbi N., Mesia K., Penge O., Lusakibanza M., Cimanga K., De Bruyne T., Apers,S., Totte J., Pieters L., Vlietinck A.J.(2000). Antiamoebic and spasmolytic activities of extracts from some antidiarrhoeal traditional preparations used in Kinshasa. Congo. *Phytomedicine*, 7, p.31–38.

[8]: Tanoh S.K., N'gaman-Kouassi C.C., Boa D., Mamyrbekova-Békro A.J., Békro Y.A. (2019). Antioxidant activity of crude hydroethanolic and hydroacetonian extracts from the organs of four medicinal plants from Ivory Coast . *Nat. Tech*., 22: p.1-7

[9]: Paolini J. (2005). Characterization of essential oils by CPG/Ir, CPG/SM (IE and IC) and carbon-13 NMR of *Cistus albidus* and two Asteraceae endemic to Corsica: *Eupatorium cannabinum subsp. corsicum* and *Doronicum corsicum.* Chemistry. University of Corsica, 342p.

[10]: Daouda T. (2015). Chemical and biological studies of the essential oils of four aromatic medicinal plants from Ivory Coast . Organic chemistry. Felix Houphouet Boigny University, Ivory Coast , 154p.

[11] : Etekpo D.S., N'gamen K., Mamyrbekova-Békro A.J., Békro Y.A. (2018). Antioxant profiles of alcoholic tinctures from *Heterotis rotundifolia* (SM) Jacq. - Fel. (Melastomacaceae) by DPPH radical trapping. *European Journal of Biomedical and Pharmaceutical Sciences*, 5 (10), p.39-45

[12] : N'dri K., Atsain M., Konan N., Kouame B., Mamyrbekova-Békro J.A., Békro Y.A. (2023). 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), *International Journal of Pharmaceutical Sciences Review and Research*, 41, p.277-282.

[13]: Angioni A., Barra A., Coroneo V., Dessi S., Cabras P. (2006). Chemical composition, seasonal variability, and antifungal activity of *Lavandula stoecha*s L. ssp. stoechas essential oils from stem/leaves and flowers. *Journal of Agricultural and Food Chemistry*, 54, p. 4364-4370.

[14]: Baydar H. et Baydar N. G. (2005). The effects of harvest date, fermentation duration and Tween 20 treatment on essential oil content and composition of industrial oil rose (*Rosa damascena* Mill.). Industrial Crops and Products, 21(2): p.251-255.

[15]: Cole R., Haber W., Setzer W. (2007). Chemical composition of essential oils of seven species of *Eugenia* from Monteverde, Costa Rica. Biochemical Systematics and Ecology, *35*(12):p. 877-886.

[16]: Modzelewska A., Sur S., Kumar K., Khan S. (2005). Sesquiterpenes: Natural products that decease cancer growth. Current Medicinal Chemistry - Anti-Cancer Agents, 5:p. 477-499.

[17]: Svoboda K. *(*1999**).** Antibacterial, antioxidant, anti-inflammatory and other related pharmacological activities, Plant Biology department, Sac Auchincruve, ayr, Scotland, Uk, KA 65 HW, Bioactivity of essential oils of selected temperate aromatic plants, 17p.

[18] : Tepe B., Sokmen M., Sokmen A., Daferera D., Polissiou M. (2005). Antimicrobial and antioxidative activity of the essential oil and various extracts of *Cyclotrichium origanifolium* (Labill.) Manden. & Scheng. *Journal of Food Engineering*, 69: p. 335–342.

[19] : Celik K., Togar B., Turkez H., Taspinar N. (2014).In vitro cytotoxic, genotoxic, and oxidative effects of acyclic sesquiterpene farnesene. *Turk J Biol*, 38, p. 253 -259.

[20] : Ocete M.A., Risio S., Zarzuelo A., Jimenez J. (1989). Pharmacological activity of the essential oil of *Bupleurumg gibraltaricum* : Anti – inflammatory activity and effects on isolated rat uterius. *Journal of ethnopharmacology*, 25 (3), P.305-313.

[21]: Sadou N., Seridi R., Djahoudi A., Hadef Y. (2015). Chemical composition and antibacterial activity of essential oils from *Pinus halepensis* Mill. needles from northeastern Algeria, Rev. Sci. Technol., Synthèse 30: pp. 33-39.

[22]: Noudjoub M. (2010). Extraction of essential oils from *Thymus fontanesii* and application to the formulation of an antimicrobial medicinal product. Master's thesis, M'Hamed Bougara University, Boumerdes, 185p.