RJC

RASĀYAN *J. Chem.*
Vol. 8 | No.4 |396 - 403 | October - December | 2015
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

ANTIBACTERIAL ACTIVITY OF ESSENTIAL OILS AND EXTRACTS FROM THE LEAVES OF *HYPTIS SUAVEOLENS* AND *LIPPIA MULTIFLORA* ON MULTI-RESISTANT BACTERIA

GOLY Kouassi Roselin Cyrille [1,2], SORO Yaya [1*], DADIE Adjehi [2], KASSI Amian Brise Benjamin [3]and DJE Marcellin [2]

[1]Laboratoire des Procédés Industriels de Synthèse, de l'Environnement et des Energies Nouvelles, Institut National Polytechnique Félix HOUPHOUËT-BOIGNY, BP 1093 Yamoussoukro (Côte d'Ivoire).

[2]Laboratoire de Biotechnologie et Microbiologie des aliments, Université Nangui Abrogoua d'Abobo, 02 BP 801 Abidjan 02 (Côte d'Ivoire).

[3]Laboratoire de Chimie Organique des Substances Naturelles, Université Félix HOUPHOUËT-BOIGNY de Cocody, 22 BP 582 Abidjan 22 (Côte d'Ivoire)

* E-mail : soro_y@yahoo.fr

ABSTRACT

The essential oils and 70% ethanol extracts from leaves of *Hyptis suaveolens* and *Lippia multiflora* were tested on two multi-resistant bacterial strains. The results of the tests in agar and liquid suspension show a higher antibacterial activity for essential oil of *Lippia multiflora* and for ethanol extract from *Hyptis suaveolens* after essential oil extraction. This extract inhibit the growth of *S. aureus* ATCC 25923 with inhibition diameter of 30 mm and the growth of *S. aureus* Meti-R and *P. aeruginosa* ceft/Imp-R with a Minimum Inhibitory Concentration value of 0,78 mg/mL. The essential oil obtained from *Lippia multiflora* inhibits the growth of *P. aeruginosa* Ceft/Imp-R with an inhibition diameter of 28 mm and a Minimum Inhibitory Concentration of 0.9 mg/mL for all bacterial strains. A synergic anti-bacterial activity has been observed between essential oils and antibiotics against several bacterial strains.

Keywords: *Hyptis suaveolens*, *Lippia multiflora*; antibacterial activity, Essential oil, synergic activity

INTRODUCTION

Nature is a source of medicinal agents and an impressive number of modern drugs have been isolated from natural sources. The presence of various life sustaining constituents in plants made scientists to investigate these plants for their uses in treating certain infectious diseases and management of chronic wounds[1,2]. *Lippia multiflora* (Moldenke) (Verbenaceae) and *Hyptis suaveolens* (L.) Poit (Lamiaceae*)* are two herbaceous plants among the plants of traditional pharmacopoeia of Côte d'Ivoire, which grow in the wild savannah.

Hyptis suaveolens (*H. suaveolens*) is traditionally used for treating respiratory infections, colds, pains, fevers and cramps[3]. The leaves of the plant have insecticide properties essentially against mosquitoes and *Callosobruchus maculates* and are also used as antiseptics in case of skin burns or wounds[4,5]. The leaves extracts have antimalaric, antibacterial, larvicide, nematophagic, antioxidant, anticonvulsive and fungitoxic activities[6,7]. The essential oil from the leaves has showed intense antibacterial and antifungal activities against *Mucor Sp* and *Fusarium moniliforme*[8,9,10].

Lippia multiflora (*L. multiflora*) is highly valued for its numerous nutritional and medicinal properties, and it is especially used to treat hypertension[11,12]. It has bacteriostatic properties on *Staphyloccocus aureus* and on other species like *Enterococcus* as well as bactericidal and larvicide activities on *Aedes*

aegypti[13,14]. We have recently reported the in vitro antifungal activity of this oil on *Apergillus flavus*, *Asperguillus niger* and some species of *Fusarium sp*[15].
In Côte d'Ivoire, research carried out on *H. suaveolens* and *L. multiflora* mainly concern chemical compounds, agronomic and economic aspects[16,17,18]. A better knowledge of the activities of their extracts could however support their valorization and popularization. This study aims to evaluate in vitro the antibacterial potential of the essential oils and 70% ethanol extracts from the leaves of these two plants (before and after the extraction of the essential oil) on the growth of multi-resistant bacteria.

EXPERIMENTAL

Plants materials

Plants materials were constituted by Fresh leaves of *H. suaveolens* and *L. multiflora*, which were collected from July to September 2013 around the National Polytechnic Institute Félix HOUPHOUËT-BOIGNY of Yamoussoukro (Côte d'Ivoire). The leaves were identified by the botanist of the Institute, and a sample was deposited in the Herbarium. They were dried for ten days out of direct sun light at room temperature (27 ± 2°C) before being used.

Bacterial strains

The bacterial strains used for biological tests were provided by the antibiotics unit of natural substances and Survey of Resistance of Micro-organisms for anti-infectious (ASSURMI) Department of Bacteriology at Pasteur Institute of Côte d'Ivoire (IPCI). The strains used were *Staphylococcus aureus* resistant to methicillin (*S. aureus Meti-R*), *Pseudomonas aeruginosa* resistant to ceftazidime and imipenem (*P. aeruginosa* Cefta/Imp-R), Referenced strains of *S. aureus* ATCC 25923 and *P. aeruginosa* ATCC 27853. In order to get young colonies for the tests, the different bacterial strains were subcultured by streaking method and incubated in an oven at 37°C for 18 to 24 hours[19].

Essential oils extraction

Essential oils were obtained by hydrodistillation for 3h at normal pressure using a Clevenger apparatus[20]. The collected oils were dried on anhydrous magnesium sulfate then conserved at 4°C in a hermetically closed bottle.

Hydro alcoholic extraction procedure

The air-dried powdered sample before the extraction of the essential oil (100 g) was exhaustively extracted with 1L of a mixture of ethanol/water (70/30) (v/v) at room temperature (28°C) by constant stirring during 24 hours. This operation was repeated three times. After filtration on cotton then Watmann paper n°2, the extract was concentrated at 2/3 under reduced pressure at 40°C using Rotary evaporator Buchi and then lyophilized. The residual powder was the hydro alcoholic extract or 70% ethanol extract ($E_{eth.}$ I). The obtained power was conserved at 4°C in a hermetically closed bottle. After the extraction of the essential oil, the air-dried powdered sample was extracted under the same conditions as previously to afford the hydro alcoholic extract or 70% ethanol extract ($E_{eth.}$ II). The obtained power was conserved at 4°C in a hermetically closed bottle.

Preparation of the samples and inoculums

The essential oil (EO) was mixed in Tween 80 (Merck-Schuchardt) in the ration Tween/EO (1/9) as described by Opalchenova and Obreshkova and by Oussou et al. [21,22]. The crude extracts and their fractions (200 mg) were mixed in 1 mL of a mixture of DMSO/distilled water in the ratio 1/13 (v/v). Two referenced antibiotics which are Oxacilline (Medicef, Tunisia) and Ofloxacine (Sanofi aventis, France) (200 mg) were also tested under the same conditions in combination with 1 mL of the mixture essential oil/Tween 80.

Antibacterial activity in agar medium

The antibacterial activity of various extracts was evaluated by the method of diffusion in agar medium as suggested by Woodman[23]. Wells of 6 mm in diameter were realized in a Mueller-Hinton (MH) (Bio-Rad,

france) agar previously prepared and poured into Petri dishes of 90 mm in diameter. From young bacterial colonies (12 to 24 hours), a suspension was prepared in Mueller Hinton Broth (MHB) (Bio-Rad, France) by introduction of this colony in 10mL of MHB followed by homogenization of the medium. A volume of 0.1 mL of the suspension obtained was introduced in a tube containing 10 mL of MHB. The suspension, thus obtained, corresponds to bacterial inoculum of approximately 5.10^6 CFU/mL. The inoculum was subcultured by flood on Mueller Hinton agar. After the culture, the wells were filed with 80 µL of each mixture of plant extract and DMSO or essential oil and Tween 80. The incubation of the cultures was carried out in an oven at 37°C for 18 to 24 hours.
The antibacterial activities of the extracts and essential oils were evaluated by Inhibition zone diameters around the wells. The tests were conducted in three replicates and average values were calculated.

Determination of the Minimum Inhibitory Concentration
The Minimum Inhibitory Concentration (MIC) corresponds to the weakest concentration of the extracts or essential oils from which no visible microbial growth is observed[24]. For each extract or essential oil, a concentration range was prepared in distilled water by the method of double dilution with concentrations ranging from 500 to 31.25 mg/mL. An inoculum resulting from bacterial suspension in Mueller Hinton Broth (MHB) was prepared as previously. A mixture of 50 µL of the concentrations ranges and 0.95 mL of the bacterial inoculums was prepared in hemolysis tubes. The concentration range of each extract was thus diluted twenty times to afford new concentrations ranges of 25, 12.5, 6.25, 3.12, 1.56 and 0.78 mg/mL. At the same time, control tubes containing 50 µL/mL of sterilized distilled water and 0.95 mL of bacterial inoculums, then 50 µL/mL of sterilized distilled water and 0.95 mL of sterilized Broth were also prepared.

Determination of the Minimum Bactericidal Concentration
For essential oils, 100 µL were introduced in 1.9 mL of Mueller Hinton Broth (MHB). A serial of double dilution afford concentration ranges of 86, 43, 21.5, 10.75, 5.37, 2.68, 1.34 and 0.67 mg/mL. The incubation was carried out during 24 hours at 37°C and turbidity of the medium was examined in each tube looking through at daylight using human eye[25]. The transparency of the tubes indicated the antimicrobial effect of the tested extract or essential oil, while its turbidity shows its ineffectiveness (a sign of bacterial growth).
After the MIC, the Minimum Bactericidal Concentration (MBC) was determined by a subculture in Mueller Hinton Agar medium of the tubes in which no visible growth was observed. At the same time, controls tubes were prepared by culture in agar medium from dilutions of $10°$, 10^{-1}, 10^{-2}, $10^{-3}$and 10^{-4} of the starting inoculum corresponding respectively to 100%, 10%, 1%, 0.1% and 0.01% of survival bacteria in culture. The incubation was carried out during 24 hours at 37°C. The MBC was determined in comparison of the control tube with experimental tubes. The first experimental tube in which the number of determined germs is less than or equal to the dilution concentration (10^{-4}) corresponds to the CMB.

Statistical analysis
The results were analyzed by the variance method (ANOVA) using the STATISTICA software version 6.0 (treatment by ANOVA 1 factor). Comparison of the means was performed by the Tukey's test at 5%.

RESULTS AND DISCUSSION

Yields of extractions
The average yields of the essential oils (EO) and 70% ethanol extracts ($E_{eth.}$ I) were respectively 0.34 ± 0.20% and 13.01 ± 1.30% for *H. suaveolens* and 1.30 ± 0.40% and 14.30 ± 1.30% for *L. multiflora* (table 1). After the extraction of essential oils, the 70% ethanol extracts ($E_{eth.}$ II) were obtained with yields of 14.40 ± 0.50% and 13.80 ± 1.70% respectively for *H. suaveolens* and *L. multiflora*.
The yields of 70% ethanol extracts were higher than those of essential oils for the two plants ($p < 0.01$). There was no significant difference between the yields of ethanolic extracts ($p > 0.01$). The yield of the essential oil of *L. multiflora* was higher than those of *H. suaveolens*.

Table-1: Yields of extractions

Plants		$E_{eth.}$ I	EO	$E_{éth.}$ II
Hyptis suaveolens	Masses of power or leaves (g)	100	300	100
	Masses of extracts (g)	13.01	1.02	14.40
	Yields (%)	13.01 ± 1.30^{b}	0.34 ± 0.20^{a}	14.40 ± 0.50^{c}
Lippia multiflora	Masses of power or leaves (g)	100	300	100
	Masses of extracts (g)	14.30	4.00	13.80
	Yields (%)	14.30 ± 1.30^{c}	1.30 ± 0.40^{b}	13.80 ± 1.70^{c}

Alphabetical letters a, b and c indicates that the yields are significantly different ($P < 0.05$).

The essential oil has been extracted from the leaves of *H. suaveolens* with a yield of 0.34% similar to that obtained by Iwalokun et al. from a chemotype of the same plant[26]. However, weaker yields varying from 0.21% to 0.23% have been respectively obtained by Malele et al.[9] and by Adjou et al.[27]. The essential oil from the leaves of *L. multiflora* has been obtained with a yield of 1.32%. This yield is lower than the one obtained by Kunle et al. (1.57%)[28]. The differences in these yields could be explained by the temperature, the humidity rate, the components of the soil, the vegetative cycle of the plant and the method used for the extraction[29].

Antibacterial activities of extracts in agar medium

The results of the antibacterial activities of the extracts in agar medium were presented in table 2. The 70% ethanol extracts ($E_{eth.}$ I) inhibited the bacterial growth with diameters varying from 16 ± 0 to 24 ± 0 mm for *H. suaveoelens* and from 15 ± 0 to 24 ±0 mm for *L. multiflora*. The essential oils inhibited the bacterial growth with diameters varying from 0 ± 0 to 16 ± 0 mm for *H. suaveoelens* and from 20 ± 0 to 28 ± 0 mm for *L. multiflora*.

After extraction of the essential oils, the 70% ethanol extracts ($E_{eth.}$ II) inhibited the bacterial growth with diameters varying from 20 ± 0 mm et 30 ± 0 mm for *H. suaveoelens* and from 17 ± 0 et 21 ± 0 mm for *L. multiflora*.

The comparative analysis showed that 70% ethanol extracts ($E_{eth.}$ II) from *H. suaveoelens* inhibited more strongly the bacterial growth. The antibacterial activity of the essential oil from *L. multiflora* was better than those of the essential oil from *H. suaveoelens* ($p < 0.01$).

Table-2: Inhibition zone diameters (mm) of the bacterial growth of 70% ethanol extracts and essential oils of *Hyptis suaveolens* and *Lippia multiflora*

Inhibition zone diameters (mm)					
		Microorganisms			
Plants	Extracts	*S. aureus* Méti-R	*S. aureus* ATCC 25923	*P. aeruginosa* Ceft/Imp-R	*P. aeruginosa* ATCC 27853
Hyptis suaveolens	E_{eth}.I	24 ± 0^{a}	24 ± 0^{ad}	16 ± 0^{a}	20 ± 0^{a}
	EO	13 ± 0^{c}	16 ± 0^{b}	0 ± 0^{c}	0 ± 0^{c}
	E_{eth}.II	25 ± 0^{ad}	30 ± 0^{c}	20 ± 0^{d}	22 ± 1^{a}
Lippia multiflora	E_{eth}.I	24 ± 0^{a}	22 ± 0^{ad}	15 ± 0^{a}	18 ± 0^{a}
	EO	23 ± 0^{c}	25 ± 0^{b}	28 ± 0^{c}	20 ± 0^{c}
	E_{eth}.II	21 ± 0^{ad}	18 ± 0^{c}	17 ± 0^{d}	18 ± 1^{a}

The values with the same letter a, b, c or d doesn't present significant difference at 5%.

Antibacterial parameters of ethanolic extracts and essential oils in liquid medium

The antibacterial parameters (MIC and MBC) of the 70% ethanol extracts and essential oil from the leaves of *Hyptis suaveolens* in liquid medium are presented in Tables-3 and 4.

Table-3: Antibacterial parameters of the 70% ethanol extracts and essential oil from the leaves of *Hyptis suaveolens*

Antibacterial parameters (mg/mL)												
	S.aureus Meti-R			*S.aureus* ATCC 25923			*P. aeruginosa* Ceft/Imp-R			*P.aeruginosa* ATCC 27853		
Extract	MIC	MBC	MBC/ MIC	MIC	MBC	MBC/ MIC	MIC	MBC	MBC/ MIC	MIC	MBC	MBC/ MIC
E_{eth}. I	3.12	6.25	2	3.12	12.50	4	12.50	12.50	1	3.12	12.50	4
EO	5.37	10.75	2	5.37	10.75	2	10.75	10.75	1	10.75	10.75	1
E_{eth}. II	0.78	1.56	2	3.12	3.12	1	0.78	1.56	2	3.12	3.12	1

MIC: Minimum Inhibitory Concentration; MBC: Minimum Bactericidal Concentration

Table-4: Antibacterial parameters of the 70% ethanol extracts and essential oil from the leaves of *Lippia multiflora*

Antibacterial parameters (mg/mL)												
	S.aureus Meti-R			*S.aureus* ATCC 25923			*P. aeruginosa* Ceft/Imp-R			*P.aeruginosa* ATCC 27853		
Extract	MIC	MBC	MBC/ MIC	MIC	MBC	MBC/ MIC	MIC	MBC	MBC/ MIC	MIC	MBC	MBC/ MIC
E_{eth}. I	3.12	12.50	4	6.12	12.50	2	3.12	25	8	1.56	25.00	16
EO	0.90	0.90	1	0.90	0.90	1	0.90	0.90	1	0.90	0.90	1
E_{eth}. II	12.50	12.50	1	3.12	12.50	4	12.50	>25	-	6.25	>25	-

MIC: Minimum Inhibitory Concentration; MBC: Minimum Bactericidal Concentration; (-): Not calculated

The bactericidal or bacteriostatic activity depends on the value of the ration MBC/MIC. According to Marmonier, when this ratio is less or equal to four (≤4), the tested substance is classified as bactericidal, otherwise it is a bacteriostatic[30]. All the extracts of *H. suaveolens* had a bactericidal effect (MBC/ MIC ≤4). However, the MICs of 70% ethanol extracts ($E_{eth.}$ II) were lower than or equal to those of the other extracts.

S. aureus Meti-R and *P. aeruginosa* Ceft/Imp-R were the most sensitive to this extract (MIC = 0.78 mg/ mL and MBC = 1.56 mg/mL). The essential oil from the leaves of *H. suaveolens* was more effective on *S. aureus* Meti-R and *S. aureus* ATCC 25923 with a MIC and MBC values of 5.37 mg/mL and 10.75 mg/mL respectively. From extracts of *L. multiflora*, the essential oil gave the best antibacterial activity on all bacterial strains with MIC = MBC = 0.9 mg/mL (table 4).

Ethanol is a solvent which facilitates extraction of antibacterial molecules. The obtained results in this study corroborate those of Renisheya et al., who showed the activity of ethanolic extracts of plant against human pathogens[31]. This extract is known to concentrate the active principles contained in the plant. Indeed, it is possible that the chemical components may be more soluble in ethanol[32]. The antibacterial activities of ethanolic extracts from the leaves of *H. suaveolens* were much stronger than those of the extracts from the leaves of *L. multiflora*. This difference could be explained by the difference in chemical compositions of the two plants.

The 70% ethanol extract after the extraction of the essential oil (E_{eth} II) generated the most important inhibition zone diameters, which show a good antibacterial activity. This intense activity could be explained by the purification and the concentration of non volatile bioactive compounds, after the extraction of the essential oil. Indeed, the extracted essential oil may contain molecules capable of inhibiting any bactericide action of non volatile bioactive molecules. The achieved results might also

reveal the thermostable character of the antibacterial molecules of this plant or the positive effect of the temperature in the conversion of some molecules into more active ones, and might justify the choice of decoction in the traditional use[33].

The weak activity of the essential oil from the leaves of *H. suaveolens* could be explained by its bad diffusion in the agar. Indeed, the method used for the evaluation of the antibacterial activity has an effect on the results. Thus, Natarajan et al. and Fazeli et al. reported that the method of diffusion from wells on agar is the most suitable for the study of the activity of aqueous and organic extracts[34,35]. However, by the same method, the essential oil from *L. multiflora* leaves was active on all the studied strains. The intense antibacterial activity of this oil could come from the carvacrol and the thymol, the major compounds of this oil[26]. The *S. aureus* (positive GRAM) strain was the most sensitive. Several works, notably those of Derwich et al. and of Bari et al. have confirmed the great resistance of negative GRAM bacteria in comparison with positive GRAM bacteria[36,37]. This resistance of the negative GRAM could be related to the presence of the lipopolysaccharide (LPS) which would act as an efficient barrier against the bio-molecules[38].

Antibacterial activities of combinations essential oil-antibiotic

The antibacterial activities of combinations essential oil-antibiotic are presented in Table-5.

Table-5: Inhibition of bacterial growth by essential oils combinations of *Hyptis suaveolens* or *Lippia multiflora* and antibiotics

Inhibition zone diameters (mm)					
		Microorganisms			
Plants	Extracts	*S.aureus* Meti-R	*S.aureus* ATCC 25923	*P. aeruginosa* Ceft/Imp-R	*P.aeruginosa* ATCC 27853
Hyptis suaveolens	EO	13 ±0^{a}	16 ±0^{a}	0 ±0^{a}	0±0^{a}
	Ox	23 ±1^{b}	40 ±0^{b}	-	-
	Of	33 ±0^{b}	40 ±0^{b}	40 ±0^{b}	42 ±1^{b}
	Ox- EO	38 ±3^{b}	40 ±2^{b}	40 ±0^{b}	46 ±1^{c}
	Of- EO	46 ±0^{c}	58 ±1^{c}	54 ±0^{c}	44 ±0^{d}
Lippia Multiflora	EO	23 ±0^{a}	25 ±0^{a}	28 ±2^{a}	20 ± 0^{a}
	Ox	23 ±1^{b}	40 ±0^{b}	-	-
	Of	33 ±0^{b}	40 ±0^{b}	40 ±0^{b}	42 ±1^{b}
	Ox- EO	24 ±3^{b}	42 ±2^{b}	40 ±0^{b}	20 ±1^{c}
	Of- EO	50 ±0^{c}	44 ±1^{c}	60 ±0^{c}	54 ±0^{d}

The values with the same letter a, b, c or d don't present significant difference at 5%; (-): Not tested

The antibacterial activities of two used antibiotics were characterized by inhibition zone diameters varying from 23 to 40 mm and from 33 to 42 mm respectively for Oxacillin (Ox) and Ofloxicin (Of). The combination between the essential oils and Ofloxacin showed the better synergic antibacterial activity on all the tested bacterial strains. The best inhibition zone diameters produced by this combination were 58 mm on *S. aureus* with essential oil of *H. suaveolens* and 60 mm on *P. aeruginosa* with essential oil of *L. multiflora*.

The antibacterial activity of the essential oils of these plants has been amplified by the combination with antibiotic, and showed that there exists a synergic antibacterial activity on the bacterial strains between the two essential oils and antibiotics. The combination of these essential oils with ofloxacin afforded the best antibacterial activities.

CONCLUSION

This study confirmed the antibacterial activity of extracts from the leaves of *Hyptis suaveolens* and *Lippia multiflora*. The essential oil from the leaves of *Lippia multiflora* was more active than its 70% ethanol extract contrary to *Hyptis suaveolens* where the 70% ethanol extract was more active than its essential oil. After the extraction of the essential oil, the 70% ethanol extract had an even larger antibacterial activity.

The combination of the two essential oils with the ofloxacin showed a synergic antibacterial activity against some resistant strains. The studies are in progress to determine the structure of secondary bioactive metabolites after the extraction of the essential oils in order to compare them with those present before the extraction of the essential oils, and to understand the mechanisms of the formation of some potential new compounds resulting from the heating of the leaves.

ACKNOWLEDGEMENTS

The authors are grateful to M. N'GUESSAN Amany Antoine, taxonomist at the laboratory of botany of the department of Agriculture and Animal Resources of the National Polytechnic Institute Félix HOUPHOUET-BOIGNY of Yamoussoukro. His help was invaluable in the authentication of plants.

REFERENCES

1. S. Chitra, M. Patil and R. Kuma, *International Journal of PharmTech Research*, **1**, 737 (2009).
2. S. Yaya, K.A.B. Benjamin, B. Fanté, S. Sorho, T.S. Amadou and C. Jean-Marie, *RASĀYAN Journal of Chemistry*, **5 (3)**, 332(2012).
3. M. Iwu, Handbook of African Medicinal Plants, CRC Press Inc., Boca Radon. (1993).
4. S. Kéita, C. Vincent, J. Schmit, J. Arnason and A. Bélanger, *Journal of Stored Products Ressources*, **37(4),** 339(2001).
5. A. Chatterjee and S. Pakrashi, In: The Treatise on Indian Medicinal Plants. PID, New Delhi, p.15 (1997).
6. A. Amusan, A. Idowu and A. Arowolo, *Tanzanian Health Ressources Bulletin*, **7**, 174 (2005).
7. J. Chukwujekwu and J. Van Steden, *South Africa Journal of Botanic*, **71**, 316(2005).
8. O. Asekun, O. Ekundayo and B. Adeniyi, *Fitoterapia*, **7,** 440(1999).
9. R. Malele, C. Mutaya Barwa, J. Mwangi, G. Thoiti, A. Lopez, E. Lucini and J. Zigadlo, *Journal of Essential Oil Ressources,* **15**, 438(2003).
10. R. Malik, *Journal of Essential Oil Research*, **1-4** (2003).
11. D. Aly, M. Dah-Dovonon and A. Dansi, Deuxième rapport sur l'état des ressources phytogénétiques pour l'alimentation et l'agriculture au Benin. Ministère de l'Agriculture de l'Elévage et de la Pêche, p. 24,25 (2007).
12. A. Abena, M. Diatewa, G. Gakosso, M. Gbeassor, T. Hondi-Assah and J. Ouamba, *Fitotherapia,* **74(3),** 231(2003).
13. Y. Pelissier, D. Kone, Y. Loukou, C. Marion, Y. Nanga and J. Casadebaig, EPPOS. 735 (1998).
14. I. Bassolé, W. Guelbeogo, R. Nebié, C. Costantini, N. Sagnon, Z. Kaboré and S. Traoré, *Parasitology*, **45(1)**, 23(2003).
15. C. Goly, Y. Soro, B. Kassi, A. Dadié, S. Soro and M. Dje, *International Journal of Biological and Chemical Sciences*. **9(1),** 24(2015).
16. A. Ekissi, A. Konan, A. Yao-Kouamé, B. Bassirou, and S. Kati-Coulibaly*, Journal of Applied Bioscience*, **42**, 2854(2011).
17. A. Ekissi, A. Yao-Kouamé, A. Konan, K. Alui, N. Agbo and S. Kati-Coulibaly, *Asian Journal of Agricutural and Rural Development*, **3(8),** 597(2013).
18. M. Hien, A. Yao-Kouamé and N. Yao, *Journal of Applied Biosciences*, **56**, 4108(2012).
19. G. K. Julien, S. Sorho, S. Yaya, G. Nathalie, D. Mireille and D. A. Joseph, *British Microbiology Research Journal*, **9(1**), 1(2015).
20. E. Guenther, In: The Essential Oils, D. Van Nostrand, Princeton York, p 774 (1948).
21. G. Opalchenova and D. Obreshkova, *Journal of Microbiological Methodes,* **54**, 105(2003).
22. K. Oussou, C. Kanko, N. Guessend, S. Yolou, G. Koukoua, M. Dosso, Y. N'guessan, G. Figueredo and J. Chalchat, *Compte Rendu de Chimie*, **7**, 1081(2004).
23. K. E. Cooper and D. Woodman, *Journal of Pathology and Bacteriology*, 75(1946).
24. K. Oussou, S. Youlou, J. Boti, K. Guessend, C. Kanko, C. Ahibo and J. Cassanova*, European journal of science research,* **24(1),** 94(2008).

25. G. K. Julien, S. Sorho, G. Nathalie, S. Yaya, D. A. Josepha, D. Mireille, *Journal of Chemical and Pharmaceutical Research,* **4(5),** 2490(2012).
26. A. Iwalokun, A. Otunba and O. Oyenuga, *Current Ressources Journal of Biological Sciences*, **4(3)**, 265(2012).
27. E. Adjou and M. Soumanou, *Journal of Appl. Bioscience*, **70**, 5555(2013).
28. O. Kunle, J. Okogun, E. Egamana, E. Emojevwe and M. Shok, *Journal.of Phytomedecine*, **10,** 59(2003).
29. J. Bruneton, Pharmacognosie, Phytochimie, Edition Technique et documentation, 3ème Edition Lavoisier: Paris. (1999).
30. A. Marmonier, Introduction to antibiotics studies techniques. Medical Bacteriology, Common Techniques (In French), p.227, 236 (1990).
31. J. Renisheya, T. Jeba Malar, M. Johnson, M. Mary Uthith and A. Arthy, *Asian Pacific Journal of Tropical Biomedicine*, 76(2011).
32. R. Y. Stanier, J. L. Ingraham, M.L Wheelis and P.R Painter, The Microbial World. Englewood Cliffs, New Jersey: Prentice Hall (1986).
33. F. Magassouba, A. Diallo, M. Kouyaté, F. Mara, O. Mara, O. Bangoura, A. Camara, S. Traoré, A. Diallo, M. Zaoro, K. Lamah, S. Diallo, G. Camara, S. Traoré, A. Kéita, M. Camara, R. Barry, S. Kéita, S. Oularé and M. Barry, *Journal of Ethnopharmacology,* **114**, 44(2007)
34. D. Natarajan, J. Britto, S. Srinivasan, N. Nagamurugan, C. Mohanasundari and G. Perumal, *Journal of Ethnopharmacology*, **102**, 123(2005).
35. M. Fazeli, G. Amin, M. Ahmadian-Attari, H. Ashtiani, H.J amalifar and N. Samadi, *Food Control*, **18**, 646(2007).
36. E. Derwich, Z. Benziane, and A. Boukir, *Australian Journal of Basic and Applied Sciences*, **3(4),** 3818(2010).
37. M. Bari, W. Islam, A. Khan and A. Mandal, *International Journal of Agriculture and Biology*, **12**, 386(2010).
38. R. Upadhyay, P. Dwivedi and S. Ahmad, *Asian Journal of Medicine Sciences,* **2(3),** 152(2010).

[RJC-1345/2015]