Gas Chromatography-Mass Spectrometry Analysis of the anti-MRSA fractions of *Chromolaena odorata* (L.) R.M. King & H. Rob. leaves

Maureen Uchechukwu Okwu[1]*, Tonye Grace Okorie[1], Matthias Ikenna Agba[2] , Omose Josephine Ofeimun[3]

[1]*Department of Biological Sciences, College of Natural and Applied Sciences, Igbinedion University, Okada. P.O. Box 0006, Edo State, Nigeria.*

[2]*Department of Medical Microbiology, College of Health Sciences, Igbinedion University Teaching Hospital, Okada, Edo State, Nigeria.*

[3]*Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Edo State, Nigeria.*

ABSTRACT

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a strain of the bacterium *Staphylococcus aureus* characterized by its multi-drug resistance to penicillins, clindamycin, tetracyclines, macrolides, aminoglycosides, fluoroquinolones, etc. *Chromolaena odorata* R.M. King & H. Rob is a potential and promising plant that should be explored for the management of diseases caused by MRSA because its fractions have anti-MRSA activities. **Objective:** The aim To screen and identify the chemical constituents of the anti-MRSA fractions of *C. odorata* leaves. **Materials and Methods:** Seven isolates of MRSA and methicillin-susceptible *S. aureus* (MSSA) and a control strain *S. aureus* NCIBB 8588 were used for this study. Fresh leaves of *C. odorata* were collected from Igbinedion University, Okada environs, Edo State, Nigeria. The fractions obtained were analyzed with a Gas Chromatography-Mass Spectrometry (GC-MS) analyzer to identify their chemical constituents. **Results:** A total of eight fractions (F1, F2, F3, F4, F5, F7, F8, F9) obtained from the leaves of *C. odorata* were analyzed for their chemical constituents. They contain essential oils which were: α-pinene, β-pinene, 1,8-cineole, σ-elemene, terpineol, camphene, cymene, linalool, terpinolene and α-phallandrene. Free fatty acids were also identified: namely, hexanoic acid (caproic acid), dodecanoic acid (lauric acid), decanoic acid (capric acid) and octanoic acid (caprylic acid). Constituents common in fractions F2 and F3 with the highest anti-MRSA activities were: α-pinene, camphene, octanoic acid and decanoic acid.

Conclusion: *C. odorata* is a promising anti-MRSA agent that can be explored for the synthesis of novel drugs for use in the treatment of MRSA infections.

Key words: *Chromolaena odorata*, Free fatty acids, Gas Chromatography-Mass Spectrometry, Methicillin-resistant, Monoterpenes, *Staphylococcus aureus*

Citation: Okwu M, Okorie TG, Agba MI, Ofeimun OJ. Gas Chromatography-Mass Spectrometry Analysis of the anti-MRSA fractions of Chromolaena odorata (L.) R.M. King & H. Rob. leaves. Int J Pharmacol and Clin Sci 2015; 4 (2): 16-22.

INTRODUCTION

Medicinal plants have been used by human being since ages in traditional medicine due to their therapeutic potential and the search on medicinal plants has led to the discovery of novel drug candidates used against diverse diseases. According to the World Health Organization (WHO) in 2008, more than 80% of the world's population relies on traditional medicine for their primary healthcare needs.[1] Globally, plant extracts are employed for their antibacterial, antifungal and antiviral activities.[2] *Chromolaena odorata* R. M. King and H. Rob is a member of the family Asteraceae. In traditional medicine, it is used as: antispasmodic, anti-protozoal, anti-trypanosomal, astringent, diuretic, hepatotropic, antifungal and antibacterial agents.[3,4] It also has anti-methicillin-resistant *Staphylococcus aureus* (anti-MRSA) activities and this suggests a need to isolate and evaluate the active constituents

Received : 30-05-2015; **Revised** : 12-06-2015; **Accepted** : 14-06-2015

***Correspondence** : **mokwus@gmail.com**

Conflict of interest: Nil ; **Source of support** : Nil

DOI : 10.5530/ijpcs.4.2.2

of the plant which can be used for the development of novel chemotherapeutic agents for the effective treatment of MRSA infections[5] MRSA is a strain of the bacterium *Staphylococcus aureus* characterized by its multi-drug resistance to penicillins, clindamycin, tetracyclines, macrolides, aminoglycosides, fluoroquinolones etc. It can cause the same types of infections as *S. aureus* isolates such as: skin and soft tissue infections including impetigo, folliculitis, furunculosis, cellulitis, abscesses and wound infections. MRSA can also cause invasive infections such as: pneumonia, endocarditis, septic arthritis, meningitis, osteomyelitis, septicemia, toxic shock and staphylococcal scalded skin syndromes in infants and adults. Patients with compromised immune systems are at greater risk of symptomatic secondary infections. MRSA infections have now become a major public health concern and its prevalence is increasing globally.[6-7] *C. odorata* is a potential and promising plant that should be explored for the management of diseases caused by MRSA (and perhaps some other drug-resistant microorganisms) because its fractions have more anti-MRSA activity than its crude extracts and partitions. However, further research is necessary to determine the chemical composition of the anti-MRSA fractions (compounds) and to isolate the anti-MRSA compound(s) from this plant.[8] Mass spectrometry coupled with gas chromatography (GC-MS) is normally used for direct analysis of components existing in traditional medicines and medicinal plants. In recent years, GC-MS studies have been increasingly applied for the analysis of medicinal plants as this technique has proved to be a valuable method for the analysis of non-polar components and volatile essential oils, fatty acids, lipids and alkaloids.[1] The aim of this work was to screen and identify the chemical composition of the anti-MRSA fractions of *C. odorata* R.M. King & H. Rob leaves.

MATERIALS AND METHODS

Study setting

Eleven clinical isolates (7 MRSA and 4 MSSA) of *S. aureus* and a control strain *S. aureus* NCIB 8588 were used for this work. The isolates were identified using standard microbiological methods which included colonial morphology, Gram's staining, biochemical and oxacill in screen agar tests.[9] Fresh leaves of *C. odorata* were collected from their natural habitats in Igbinedion University, Okada environs, Edo State, Nigeria in the month of September, 2012. The samples were identified and authenticated in the Department of Botany, University of Benin, Edo State, Nigeria and Forestry Research Institute of Nigeria (FRIN). The voucher specimen (Fhi no. 109890) was deposited in the herbarium.[8]

Preparation of *C. odorata* fractions

The crude aqueous leaf extract of *C. odorata* was prepared using the methods described by earlier workers.[10,11] The concentrated extract (300 g) was dissolved in 800 ml of distilled water. The solution was partitioned with chloroform to obtain two partitions, the aqueous (polar) and chloroform (non-polar) partitions. The partitions were evaporated to dryness using a rotary vacuum evaporator and weighed. They were screened for anti-MRSA activities[8] The chloroform partition (CP) with higher activity was selected and subjected to the fractionation process. It was fractionated by column chromatography (CC) using silica gel 60 (Qualikems)[8] The fractions obtained were subjected to analytical thin layer chromatography (TLC) using commercially pre-coated silica gel 60 F254 plates (Merck, Germany). The solvent system used for the TLC analysis was chloroform-methanol in 4:1 ratio.[8] The fractions obtained were screened for anti-MRSA activity and then analyzed using gas chromatography-mass spectrometry to identify the chemical constituents.

Identification of anti-MRSA components of *C. odorata* fractions

Quantitative and qualitative analyses of the *C. odorata* fractions derived from TLC were carried out using a gas chromatography/mass spectrometry (GC-MS) analyzer (Agilent 7890 A). The analyzer was equipped with a HP-5 fused silica column (30 m x 0.25 mm, film thickness 0.25 μm) and interfaced with a detector (Model 5975C). The column temperature was programmed from 70–240°C at the rate of 12°C/min. The holding time for the initial temperature was 2 minutes and the average velocity was 0.22 mm/sec in the split less mode, the pressure applied was 11 psi. An aliquot of 1 μl of each fraction dissolved in dimethyl sulfoxide (DMSO, Kermel) was injected into the column with injector (auto injector 7683 B series) temperature at 250°C. The mass spectrum of compounds in the fractions was obtained by electron ionization at 70 eV, the total running time was 28 minutes.[1,12] The identification of the constituents was assigned on the basis of the comparison of their retention indices and mass spectra using the database of National Institute Standard and Technology (NIST) library software.[1] The relative amount (in percentage) of each component was calculated by comparing its average peak area to the total areas.

Table 1: Yields and antibacterial activities of TLC fractions of *Chromolaena odorata*

CC Fractions numbers	Mean R_f value	TLC fraction label	Yield (g)	Yield (%)	Diameter zone of inhibition (mm) at 2 mg/ml	
					Mean value	Standard error
1–5	0.86	F1	0.5	7.35	19.0[a]	± 2.00
6-12	0.66	F2	0.6	8.82	22.5[a]	± 0.50
13-35	0.56	F3	0.6	8.82	22.5[a]	± 2.50
36-65	0.59	F4	0.7	10.29	10.5[b]	± 6.06
66–67	0.85	F5	0.6	8.82	8.0[b]	± 4.62
68–88	0.82	F6	0.7	10.29	16.5[a]	± 0.50
89–100	0.74	F7	0.6	8.82	10.0[b]	± 5.77
101–108	0.43	F8	0.6	8.82	19.5[a]	± 4.5
109–120	0.49	F9	0.7	10.29	11.0[b]	± 6.35
121-142	0.93	F10	ND	ND	ND	ND

Key: R_f=Retention factor; CC=column chromatography, Negative control=Dimethyl sulphoxide; Positive control=Ciprofloxacin (1 mg/ml).[8] Fractions with mean values with the same letter (a or b) are not significantly different (P>0.05). Values with different letters are significantly different from each other (P<0.05).

Statistical analysis

Percentages were calculated for study variables and chi square was used to calculate significant difference between the fractions obtained using Statistical Package for Social Sciences (SPSS) 16.0 for Windows.

RESULTS

Table 1 shows the anti-MRSA activities of the *C. odorata* fractions, F1-F9.[8] All the fractions except F10 (not donebecause it was not distinctly isolated) showed anti-MRSA activities at 2.0 mg/ml. The least mean zone of inhibition against MRSA isolates was 8.0 mm for F5 and the highest was 22.5 mm for F2 and F3. There was significant difference in anti-MRSA activities between the fractions (P<0.05). Fractions with mean values with different letters were significantly different in activities from each other (P<0.05) while fractions with same letter were not significantly different from each other (P>0.05). Table 2 shows the GC-MS analysis of chemical contents of the fractions (except F6 which was not done as it was lost). F1 contained: camphene (20.5%), α-pinene (20.0%), cymene (18.0%), 1.8-cineole (10.0%), linalool (8.0%), hexanoic and octanoic acid (2.0% each). The total of chemical content identified was 80.5%. F2: camphene (50.0%), α-pinene (30.0%), cymene (10.0%), linalool (5.0%), undecanone, octanoic and decanoic acids were in traces. The total was above 95%. F3: decanoic acid (30.0%), dodecanone (20.0%), methyl dodecanoate (15.0%), hexanoic acid (10.0%), α-pinene (6.0%), octanoic acid and β-pinene (5.0% each). Camphene, terpineol and terpinolene were in traces and the total was above 91%. F4: decanoic acid (20.0%), hexanoic acid and α-phallandrene (15.0% each), octanoic acid (13.0%), β-pinene (8.0%), α-pinene (6.0%), camphene (3.0%), terpinol (2.0%). Terpinolene was in traces and the total was above 82%. F5: terpinolene (24.9%), camphene (23.8%), β-pinene (20.4%), α-pinene (20.0%), α-terpineol (5.0%), σ-elemene (1.0%). Do-decanoate ester and octanoic acid were in traces and the total was above 95.1%. F7: 10.0% of dodecanoate ester and a trace of octanoic acid. F8: camphene (18.0%), α-pinene (15.0%), β-pinene (12.0%), terpinolene (12.0%). Octanoic, decanoic and dodecanoic acid were in traces and the total was above 57%. F9: terpinolene and α-terpineol (5.0% each), methyl tetradecanoate, dodecanoate ester and dodecanoic acid (2.0% each), β-pinene (0.33%), α-pinene and camphene (0.22% each).

DISCUSSION

Comparative anti-MRSA activities of seven selected Nigerian medicinal plants have been previously determined by 'a in earlier study'[5] The solvents used for the plants extraction were: hexane, ethanol and water. The researchers reported that *Ageratum conyzoides, Bryophyllum pinnatum, Peperomia pellucida* and *Ocimum gratissimum* showed no anti-MRSA activities while *Chromolaena odorata*, *Piper guineense* and *Curculigo pilosa* showed activities. In their study, they concluded that the crude aqueous and ethanolic extracts

Table 2: GC-MS analysis of *Chromolaena odorata* fractions with anti-MRSA activities

Fraction Label	Retention index	Component	Percentage (%) content
F1	939	α – pinene	20
	955	Camphene	20.5
	1026	Cymene	18.0
	1033	1, 8-Cineole	10.0
	1086	Linalool	8.0
	-	Hexanoic acid	2.0
	-	Octanoic acid	2.0
	-	Total identified	80.5
F2	939	α-pinene	30.0
	955	camphene	50.0
	1026	cymene	10.0
	1086	linalool	5.0
	1209	octanoic acid	Traces
	1283	undecanone	Traces
	1365	decanoic acid	Traces
	-	Total identified	95 + traces
F3	939	α-pinene	6.0
	981	β-pinene	5.0
	955	camphene	Traces
	-	terpineol	Traces
	1086	terpinolene	Traces
	-	octanoic acid	5.0
	-	hexanoic acid	10.0
	1365	decanoic acid	30.0
	-	methyl dodecanoate	15.0
	-	dodecanone	20.0
	-	Total identified	91 + traces
F4	939	α-pinene	6.0
	981	β-pinene	8.0
	955	camphene	3.0
	1190	terpinol	2.0
	1086	terpinolene	Traces
	-	α-phallandrene	15.0
	-	octanoic acid	13.0
	-	hexanoic acid	15.0
	1365	decanoic acid	20.0
	-	Total identified	82 + traces
F5	939	α–pinene	20.0
	981	β-pinene	20.4
	955	camphene	23.8
	1086	terpinolene	24.9
	1190	α-terpineol	5.0
	1340	σ-elemene	1.0
	-	do-decanoate ester	Traces
	-	octanoic acid	Traces
	-	Total identified	95.1 + traces

F7	-	Octanoic acid	Trace
	-	Dodecanoate ester	10.0
	-	Total identified	10.0 + trace
F8	939	α–pinene	15.0
	981	β-pinene	12.0
	955	Camphene	18.0
	1086	terpinolene	12.0
	-	octanoic acid	Traces
	-	Methyl dodecanoate	Traces
	1365	decanoic acid	Traces
	1205	dodecanoic acid	Traces
	-	Total identified	57 + traces
F9	939	α–pinene	0.22
	981	β-pinene	0.33
	955	Camphene	0.22
	1086	terpinolene	5.0
	1190	α-terpinol	5.0
	1002	methyl tetradecanoate	2.0
	1101	dodecanoate ester	2.0
	1205	dodecanoic acid	2.0
	-	Total identified	16.77

of *C. odorata* were considered the most efficacious of the seven selected medicinal plants with minimum inhibition concentration (MIC) of 12.5 mg/ml each.[5] The crude aqueous extract of *C. odorata* was partitioned with chloroform to yield two partitions, the aqueous partition (AP) and the chloroform partition (CP). The anti-MRSA activities of AP and CP were 12.5 mg/ml and 3.13 mg/ml respectively.[8] CP was fractionated (via column and thin layer chromatographic techniques) by the researchers into fractions to further determine the anti-MRSA activity of the plant. These fractions, F1-F9 of *C. odorata* at 2.0 mg/ml have been observed to have more anti-MRSA activities than the crude aqueous (MIC of 12.5 mg/ml) and chloroform (MIC of 3.13 mg/ml) extracts of the plant.[8] The chemical constituents of fractions, F1-F9 (F6 was lost and therefore, not analyzed) of *C. odorata* obtained in this study were identified using Gas Chromatography-Mass Spectrometry (GC-MS) technique. The chemical constituents identified in this study were essential oils which were: α-pinene, β-pinene, 1, 8-cineole, σ-elemene, terpineol, camphene, cymene, linalool, terpinolene and α-phallandrene. Free fatty acids also identified were: hexanoic acid (caproic acid), dodecanoic acid (lauric acid), decanoic acid (capric acid) and octanoic acid (caprylic acid). Chemical compounds common in fractions F2 and F3which had the highest zones of anti-MRSA activities (Table 1) were: α-pinene, camphene, octanoic acid (also identified in the eight fractions in this study) and decanoic acid (identified in five fractions) (Table 2). In this study, it appears that the major chemical compounds responsible for the anti-MRSA activity of *C. odorata* leaves could be: α-pinene, camphene, octanoic acid and decanoic acid. This observation is in agreement with the reports of Bamba, *et al.,*[13] and Owolabi, *et al.,*[14] who isolated similar essential oils. The chemical compounds, camphene and linalool identified in the leaves of *C. odorata* in this study have also been isolated from *P. guineense* by Tchoumbougnang, *et al.*[15] The essential oils identified in *C. odorata* in this study were mostly monoterpenes which are well known for their antimicrobial activities. Monoterpenes, α- and β-pinenes show microbicidal activity against fungi and bacteria including MRSA.[16] The modes of action of monoterpenes have been reported to include interference of membrane structure and function. This could lead to: membrane expansion, increase in membrane fluidity and permeability, disruption of membrane-embedded proteins, inhibition of respiration, alteration of ion transport and other enzymatic reactions.[17] The anti-MRSA activity of *C. odorata* leaves in this study could have been due to the contribution of the membrane disruption activities of the monoterpenes on the cells of MRSA.

The presence of free fatty acids identified in this study agrees with the previous report which stated that the

proximate analysis of the crude fat content of *C. odorata* leaves was 23.10%.[18] Also, Hanh, *et al.,*[19] reported the isolation of six fatty acids from the leaves of *C. odorata*. On the contrary, Reotutar and Gemma,[20] reported that the leaves of *C. odorata* do not contain free fatty acids. A variety of free fatty acids and their monoglycerides have been reported to exert antimicrobial activity against a wide range of microorganisms. The fatty acids: caprylic acid, capric acid, lauric acid and caproic acid identified in the leaves of *C. odorata* in the present study have been reported by Ruzin and Novick,[21] to be active against gram-positive bacteria such as: *S. aureus*, *S. epidermidis, Corynebacterium diphtheriae, Bacillus cereus* and *Streptococcus pyogenes*. The prime target of free fatty acids is the cell membrane where they disrupt the electron transport chain and oxidative phosphorylation. The modes of action also include: inhibition of enzyme activity, impairment of nutrient uptake, generation of toxic peroxidation and auto-oxidation degradation products or direct lysis of bacterial cells.[22-24] Sun, *et al.,*[25] and Kenny, *et al.*[26] reported that fatty acids broad spectrum of activity, non-specific modes of action and safety make them attractive as antibacterial agents for various applications in medicine, agriculture and food preservation. This is particularly attractive where the use of conventional antibiotics is undesirable or prohibited. Moreover, the evolution of inducible free fatty-acid resistant phenotypes is less problematic than with conventional antibiotics. Free fatty acids have a longstanding safety record. When combined with antibiotics, these acids might prove useful in the prevention and treatment of several bacterial infections.[25-26] Methicillin-resistant *S. aureus* (MRSA) has been proven to be one of the most globally spread nosocomial and community-acquired pathogen of the twentieth century. It is highly problematic among hospitalized patients with significant morbidity and mortality.[6,27] This scourge of MRSA could be minimized by treatment with purified aqueous extracts of *C. odorata* which is more cost effective compared with its chemical extracts counterpart; for example, alcoholic extracts.

The combination of the two cell membrane disrupting group of compounds namely, the mono terpenes and fatty acids observed in the chloroform fractions (with higher anti-MRSA activities) in this study probably contribute to the great potential of *C. odorata* as a promising medicinal plant that could be explored as an anti-MRSA agent to treat or synthesize novel drugs against MRSA infections in the future.The present study has therefore, contributed greatly to solving drug resistance problem especially in MRSA isolates.

The limitation of this study which therefore suggests areas of future research include: (1) *in vivo* studies of *C. odorata* to determine the toxicity of the bioactive constituents, their side effects, serum-attainable level, pharmaco dynamic actions, pharmacokinetic properties and diffusion in different body sites; (2) Determination of the synergistic/antagonistic tendency of the bioactive constituents of *C. odorata* when isolated and combined in different ratios and tested against MRSA isolates. In conclusion, the essential oils (monoterpenes) and free fatty acids identified in the leaves of *C. odorata* could be responsible for the significant anti-MRSA activity of the plant on clinical MRSA isolates. Therefore, the plant is a promising anti-MRSA agent which should be explored for the synthesis of novel drugs for use in the treatment of MRSA infections.

ACKNOWLEDGEMENTS

The authors thank Mr. Osakue Ohenhen Emmanuel (Nigerian Institute of Science Laboratory, Technology, Samonda, Ibadan, Nigeria) for the GC-MS analysis of the *C. odorata* fractions.

CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

ABBREVIATION

MRSA : Methicillin resistant *Staphylococcus aureus*

CC : Column chromatography

GC-MS : Gas chromatography-mass spectrometry

MIC : Minimum inhibitory concentration.

REFERENCES

1. Sermakkani M, Thangapandian V. Gas chromatography–Mass Spectrometry analysis of *Cassia italic* leaf methanol extract. Asian J of Pharmaceu Clin Res. 2012; 5: 90-4.
2. Aibinu I. Medicinal plants as antimicrobials. In: Odugbemi T, editor. Outlines and Pictures of Medicinal plants from Nigeria. Nigeria: University of Lagos Press; 2006. p. 53-6. ISBN: 978-38235-9-0.
3. Phan TT, Wang L, See P, Grayer RJ, Chan SY, See ST. Phenolic compounds of *Chromolaena odorata* protect cultured skin cells from oxidative damage: implication for cutaneous wound healing.

Biol Pharm Bull. 2001; 24(12):1373-9.
4. Akinmoladun EO, Ibukun EO, Dan-Ologe IA. Phytochemcial constituents and antioxidant properties of extracts from the leaves of *Chromolaena odorata*. Sci. Res. Essay 2007; 2(6): 191-4.
5. Okwu M,Okorie T, Agba M, Ayinde B, Umumarongie H. Comparative anti-MRSA activities of seven selected Nigerian medicinal plants and phytochemical constituents of Piper guineense (*Schum* and *Thonn*.), *Curculigo pilosa* (*Schum* and *Thonn*.) and *Chromolaena odorata* (King and Robinson). IOSR J Pharm Biol Sci. 2014; 9: 7-13.
6. Peng Q, Hou B, Zhou S, Huang Y, Hua D, Yoa F, *et al*. Staphylococcal cassette chromosome mec (SCCmec) analysis and antimicrobial susceptibility profiles of methicillin resistant *S. aureus* (MRSA) isolates in a teaching hospital, Shontou, China. Afri J Microbiol Res. 2010; 4: 844-8.
7. Okwu M, Okorie T, Mitsan O, Osakue E. Prevalence and comparison of three methods for detection of methicillin-resistant *S. aureus* (MRSA) isolates in tertiary health institutions in Nigeria. Canadian Open Biol Sci J. 2014; 1: 1-12.
8. Okwu MU, Okorie TG, Agba MI. *In vitro* anti-MRSA (methicillin-resistant *S. aureus*) activities of the partitions and fractions of the crude aqueous leaf extract of *Chromolaena odorata* (King and Robinson). IOSR J Pharm Biol Sci. 2015; 10: 136-41.
9. Cheesbrough M. District Laboratory Practice in Tropical Countries. Vol. II. South Africa: Cambridge University Press; 2004. 2.
10. Okigbo R, Mbajaka C, Njoku C. Antimicrobial potential of (Uda) *Xylopia aethopica* and *Ocimum gratissimum* on some pathogens of man. Internat Mol Med Adv Sci Pakistan. 2005; 1(4): 392-4.
11. Nwinyi O, Chinedu N, Ajani O, Oguniran K. Antibacterial effects of extracts of *Ocimum gratissimum* and Piper guineense on *Escherichia coli* and *S. aureus*. African Journal of Food Science 2009; 3(1): 22-5.
12. Avlessi F, Alitonou GA, Djenontin TS, Tchobo F, Yehouenou B, Menut C, *et a*l. Chemical composition and biological activities of the essential oil extracted from the fresh leaves of *Chromolaena odorata* (L. *Robinson*) growing in Benin. J Biol Sci. 2012; 1(3): 7-13.
13. Bamba D, Bessiere JM, Marion C, Lelissier Y, Fouraste I. Essential oil of *Eupatorium odoratum*. Planta Med. 1993; 59: 184-5.
14. Owolabi MS, Ogundajo A, Yusuf KO, Lajide L, Villanueva HE, Tuten JA, *et al*. Chemical Composition and Bioactivity of the essential oil of *Chromolaena odorata* from Nigeria. Rec Nat Prod. 2010; 4(1): 72-8.
15. Tchoumbougnang F, Jazet DP, Sameza ML, Fombotioh N, Wouatsa NA, Amyam ZP, *et al*. Comparative essential oils composition and insecticidal effect of different tissues of *Piper capense* L., *Piper guineense* Shum et Thonn., *Piper nigrum* L. and *Piper umbellatum* L. grown in Cameroon. Afri J Biotechnol. 2009; 8: 424-31.
16. Rivas da Silva AG, Lopes PM, Barrosde MM, Costa DC, Alviano CS, Alviano DS. Biological activities of α-pinene and β-pinene enantiomers. Molecules 2012; 17(6): 6305-16.
17. Rasoul MA, Marei GI, Abdelgaleil SA. Evaluation of antibacterial properties and biochemical effects of monoterpenes on plant pathogenic bacteria. Afri J Microbiol Res. 2012; 6(15): 3667-72.
18. Danlami U, Adebisi FA, David BM, Lawal DR, Galadanchi KM. Proximate and phytochemical analyses of the hexane, ethyl acetate and ethanol extracts of *Chromolaena odorata* (Linn.) leaves. Asian J Pharmaceu Biol Res. 2013; 3(1): 34-5.
19. Hanh TT, Hang DT, Minh CV, Dat NT. Anti-inflammatory effects of fatty acids isolated from *Chromolaena odorata*. Asian Pacific J Trop Med. 2011; 4(10) :760-3.
20. Reotutar LP, Gemma AR. Phytochemical screening of Wellawel (*Chromolaena odorata*) leaves, its antimicrobial and coagulative properties. JPAIR. 2008; 1: 111-2. Retrieved, September, 2013 from: www.ejournals.ph/index.php?journal=JPAIRMJRpage=rtop.
21. Ruzin A, Novick R. Equivalence of lauric acid and glycerol monolaurate as inhibitors of signal transduction in *S. aureus*. J Bacteriol. 2000; 182(9): 2668-71.
22. Boyaval P, Corre C, Dupuis C, Roussel E. Effects of free fatty acids on propionic acid bacteria. Lait. 1995; 75(1): 17-29.
23. Wojtczak L, Wieckowski MR. The mechanism of fatty-acid-induced proton 1 permeability of the inner mitochondrial membrane. J Bioenerg Biomembr. 1999; 31(5): 447-55.
24. Shin SY, Ajpai VK, Kim HR, Kang SC. Antibacterial activity of 22 eicosapentaenoic acid (EPA) against food-borne and food spoilage microorganisms. LNT. 2007; 40:1515-9.
25. Sun CQ, O'Connor CJ, Roberton AM. Antibacterial actions of fatty acids and monoglycerides against *Helicobacter pylori*. FEMS Immunol Med Microbiol. 2003; 36(1-2): 9-17.
26. Kenny JG, Ward D, Josefsson E, Jonson I, Hinds J, Rees HH, *et al*. The *Staphylococcus aureus* response to unsaturated long chain free fatty acids. Survival mechanisms and virulence implication. PLOS ONE 2009; 4. Retrieved November, 2013 from: www.mcbinlm.nih.gov/pmc/articles/ pmc/2629846.
27. Obasuyi O. Molecular identification of methicillin-resistant *S. aureus* in Benin-City, Nigeria. Afri J Clin Experimen Microbiol. 2013; 14(1): 1-4.