International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
www.chemijournal.com
IJCS 2022; 10(6): 07-11

Received: 07-09-2022
Accepted: 12-10-2022

Kouame Bi KFP
a) LCOSN, UFR SSMT, 08 BP 582 Abidjan 08, Université F. H. B. Abidjan, Côte d'Ivoire
b) EBSI Group, CEISAM, LUNAM–CNRS UMR6230, BP 92208, 44322 Nantes, France
c) Département de Mathématiques, Physique, Chimie, UFR des Sciences Biologiques, Université Peleforo. GON COULIBALY. Korhogo, BP 1328 Korhogo, Côte d'Ivoire

Coulibaly Wacothon K
Département de Mathématiques, Physique, Chimie, UFR des Sciences Biologiques, Université Peleforo. GON COULIBALY. Korhogo, BP 1328 Korhogo, Côte d'Ivoire

Gooré Stéphane G
a) Laboratoire de Chimie BioOrganique et de Substances Naturelles, Université Nangui Abrogoua, Abidjan, Côte d'Ivoire de Chimie Organique Biologique
b) Université de Corse-Centre National de la Recherche Scientifique, Unité Mixte de Recherche 6134, Equipe Chimie et Biomasse, Ajaccio, France

Ouattara Zana
Laboratoire de Chimie BioOrganique et de Substances Naturelles, Université Nangui Abrogoua, Abidjan, Côte d'Ivoire de Chimie Organique Biologique

Bedi Gustave
LCOSN, UFR SSMT, 08 BP 582 Abidjan 08, Université F. H. B. Abidjan, Côte d'Ivoire

Tea Illa
EBSI Group, CEISAM, LUNAM–CNRS UMR6230, BP 92208, 44322 Nantes, France

Chalchat Jean C
Laboratoire de chimie des Hétérocycles et des Glucides Chimie des huiles essentielles, Les Cezeaux, 63177, Aubière France

Tonzibo Felix
LCOSN, UFR SSMT, 08 BP 582 Abidjan 08, Université F. H. B. Abidjan, Côte d'Ivoire

Corresponding Author:
Kouame Bi KFP
a) LCOSN, UFR SSMT, 08 BP 582 Abidjan 08, Université F. H. B. Abidjan, Côte d'Ivoire
b) EBSI Group, CEISAM, LUNAM–CNRS UMR6230, BP 92208, 44322 Nantes, France
c) Département de Mathématiques, Physique, Chimie, UFR des Sciences Biologiques, Université Peleforo. GON COULIBALY. Korhogo, BP 1328 Korhogo, Côte d'Ivoire

Comparative phytochemical analysis of essential oils from different biological parts of a species of *Crassocephalum* in Côte d'Ivoire

Kouame Bi KFP, Coulibaly Wacothon K, Gooré Stéphane G, Ouattara Zana, Bedi Gustave, Tea Illa, Chalchat Jean C and Tonzibo Felix

Abstract
Essential oils obtained by hydro-distillation of the leaves and stems of a species of *Crassocephalum* growing in Côte d'Ivoire have been studied for the first time. Essential oils were analyzed and characterized by GC and GC-MS. Analyses of the essential oils led to the identification and quantification of 39 and 84 constituents in the respective leaves oils and stems oil. Characterization of the oils revealed the predominance of the hydrocarbon monoterpene myrcene (leave: 87.48%, stem: 35.55%). Myrcene alone accounts for almost all of the leaf oil. The essential oils of the stems present a different chemotype from that of the leaves with the presence of other predominant compounds terpinolene (31.99%) and (*E*)-dec-2-enal (8.01%).

Keywords: Chemical composition, *Crassocephalum*, essential oils, myrcene, terpinolene

Introduction
The plant species of our phytochemical study, *Crassocephalum* sp, is part of the genus *Crassocephalum*, which belongs to the large Asteraceae family comprising 1314 genera. This is the most important family, widespread all over the world, but mainly in temperate regions. There are nearly 24 species of *Crassocephalum* classified botanically (Wagner *et al.*, 1999) [1] of which only seven have been studied chemically. Phytochemical research on species of the genus *Crassocephalum* has led to the discovery of a variety of alkaloids, diterpenes and coumarins (Asada *et al.*, 1985, Kongsaeree *et al.*, 2003, Taïwe *et al.*, 2012) [2-4]. From the dried aerial parts of *Crassocephalum mannii* was isolated the diterpene labdane (de las Heras *et al.*, 2007, Taïwe *et al.*, 2012, Adjatin *et al.*, 2013) [5-7]. Other research work has identified leaves of *Crassocephalum crepidiodes*, saponins, tannins, flavonoids, steroids and polyphenols (Adjatin *et al.*, 2013, Lestari *et al.*, 2015) [5, 8]. Studies of the chemical composition of volatile secondary metabolites (essential oils) have been carried out for different species of *Crassocephalum*. In Vietnam, the main components of essential oils extracted from the leaves of *Crassocephalum crepidioides* are myrcene (59.3%), *β*-phellandrene (11.9%) and cryptone (6.4%) (Hung *et al.*, 2019) [9]. However, the chemical composition of the essential oils in the stems differs slightly from those in the leaves. Stem oils have as major compounds myrcene (26.1%), α-pinene (10.7%), *α*-humulene (5.9%) and (*E*)-*β*-farnesene (5.2%). On the other hand, the essential oils from the aerial parts (leaves + stems) of the plant *Crassocephalum crepidioides*, harvested in western India (Joshi, 2011) [10] have chemical compositions close to those of Vietnam. The essential oils extracted from the aerial parts contain 45.3% myrcene and 20.2% *β*-phellandrene (Joshi, 2011) [10]. Cryptone, which was relatively abundant in essential oils from Vietnam, was only observed in small proportions (0.1%) in oils from India. So much say that in Africa, The essential oils from leaves and stems of *Crassocephalum crepidioides* (Asteraceae) growing wild in southwestern Nigeria showed a different chemotype from those obtained in Vietnam and India. The essential oils of the leaves contained *α*-caryophyllene (10.29 %), *β*-cubebene (13.77 %) and *α*-farnesene (13.27%) as major constituents while the dominant constituents of the stems oil were thymol (43.93%), *α*-caryophyllene (15.16%) and 4-cyclohexybutyramide (20.94%) (Owokotomo *et al.*, 2012) [11]. In another report from Seven Cameroonian *Crassocephalum* Species, *Crassocephalum biafrae*, *Crassocephalum bougheyanum*, *Crassocephalum crepidioïdes*, *Crassocephalum mannii*, *Crassocephalum rubens*, *Crassocephalum vitellinum* and *Crassocephalum montuosum*, the principal constituents from leaves of seven *Crassocephalum* species were α-phellandrene, p-cymene, myrcene, limonene and (*E*)-*β*-ocimene (Zollo *et al.*, 2000) [12].

The chemical study of *Crassocephalum sp* is part of a project, the aim of which is to understand the biodiversity of the Gôh region, Center-West region of Côte d'Ivoire. The *Crassocephalum* sp species studied is a chemically unknown plant in Côte d'Ivoire and Africa. This work describes, for the first time in Côte d'Ivoire, the chemical composition of essential oils extracted from the leaves and stems of a species of the genus *Crassocephalum* using a combination of three GC and GC/MS techniques.

Materials and methods

Plant materials

Leaves and stems of *Crassocephalum sp* were collected at 8 am in the fallow lands of the village of Gabia in the Oumé sub-prefecture, Gôh region (Centre-West of Côte d'Ivoire). Plant materials were identified in the National Floristic Center of University of Felix Houphouët Boigny, Cocody-Abidjan, department of Botany, Côte d'Ivoire.

Isolation of essential oil

Extraction of essential oils was carried out by steam distillation using a Clevenger-type apparatus. Fresh leaves or stems (1000 g) were placed on a metal grid under which is distilled water (3000 mL) boiling for 4 hours. Oils are driven by steam. After condensation and liquefaction, the oil overcomes the water in the test tube. The obtained oil was dried over anhydrous sodium sulfate and after filtration, stored in a sealed sample tube at 0 °C until GC and GC-MS analysis.

Chemical Analyses of the essential oil

The essential oil was investigated first by Gas Chromatography (GC) and then by GC coupled with Mass Spectrometry (GC-MS). Concerning GC analysis, the gas phase chromatography was carried out using a Delsi DI 200 instrument equipped with a flame ionization detector and a DB5 column (25 m x 0.25 mm, df: 0.25 µm) with a split flow rate of 60 mL/min. Nitrogen was used as carrier gas; temperature programming was 5 min at 50 °C and 30 °C/min up to 220 °C, injector and detector temperatures were respectively set to 220 °C and 250 °C. GC-MS analysis were performed using a Hewlett-Packard gas Chromatograph Model 6890 coupled to a Hewlett-Packard MS Model 6890 equipped with an HP5 column (30 m x 0.25 mm, df: 0.25 µm). The initial oven temperature was maintained at 50 °C for 5 min and then programmed at 50 °C/min to 300 °C (held 50 min). The carrier gas was helium (1.0 mL/min); a split injection with a split ratio of 1:10 was chosen. Injector and detector temperatures were respectively set to 250 °C and 320 °C. The electron multiplier was set at 2200 V with an applied electron ionization voltage of 70 eV, with the ion source temperature at 230 °C. Mass spectral data were acquired in the scan mode in the m/z range of 33-450. Identification of compounds was carried out by calculating Retention Indices (RI) or Kováts Indices (KI) and comparing mass spectra with those in data banks, *i.e*. Adams (Adams, 1995) [13] or Mc Lafferty and Stauffer (Mc Lafferty *et al.,* 1989) [14]. For quantification purposes, relative area percentages obtained by field ionization detection (FID) were used.

Results

The hydrodistillation of leaves and stems of *Crassocephalum* sp harvested in Côte d'Ivoire gave yellow essential oils. GC and GC-MS analysis of essential oils from *Crassocephalum* sp leaves led to the identification and quantification of a total of 39 major components representing 99.96% of the total components present. 84 constituents representing 99.23% were identified in the essential oils of the stems (Table 1).

Table 1: Chemical constituents of essential oil of leaves and stems of *Crassocephalum* sp.

				Leaf	Stem
N°	**KI**	**RI**	**Compounds**	**Contents%**	
1	836	835	Ethyl isovalerate	0.02	nd
2	885	886	Styrene	nd	0.18
3	909	900	2-Heptanol	nd	0.10
4	922	920	Tricyclene	0.04	0.05
5	926	929	*α*-Pinene	0.27	0.05
6	966	971	Sabinene	0.26	0.10
7	978	976	*β*-Pinene	2.72	0.79
8	983	982	Myrcene	87.48	35.55
9	996	996	2,2,4,6,6-Pentamethylheptane	nd	0.20
10	1007	1005	*α*-Phellandrene	0.04	nd
11	1018	1011	α-Terpinene	0.04	0.12
12	1025	1014	Paracymene	0.09	1.31
13	1022	1028	Limonene	4.11	2.23
14	1035	1027	(*Z*)-*β*-Ocimene	0.02	0.03
15	1047	1038	(*E*)-*β*-Ocimene	0.84	0.48
16	1060	1050	*γ*-Terpinene	0.21	1.15
17	1072	1060	1-Octanol	0.06	nd
18	1074	1073	*p*-Cymenene	0.02	0.22
19	1081	1083	Linalool	0.02	0.05
20	1083	1085	*trans*-Linalool oxide	0.06	0.08
21	1089	1089	Terpinolene	0.61	31.99
22	1095	1098	*α*-Pinene oxide	0.04	0.04
23	1113	1105	(*E*)-4,8-dimethyl-nona-1,3,7-triene	nd	0.04
24	1140	1136	Geigerene	nd	0.04
25	1163	1162	Terpinen-4-ol	0.06	0.22
26	1167	1169	1,4-Dimethoxybenzene	nd	0.06
27	1180	1173	*α*-Terpineol	0.06	0.03
28	1186	1185	Decanal	0.16	0.16

29	1213	1215	Cumin aldehyde	nd	0.13
30	1237	1234	Chavicol	nd	0.02
31	1272	1269	(*E*)-dec-2-enal	1.28	8.01
32	1283	1278	carvacrol	0.12	0.29
33	1286	1285	Bornyl acetate	0.68	1.29
34	1290	1289	Thymol	nd	0.33
35	1294	1297	Nonyl acetate	nd	0.09
36	1302	1299	Methyl geranate	nd	0.06
37	1309	1312	Methyl decanoate	0.05	nd
38	1323	1326	*γ*-Nonalactone	nd	0.03
39	1348	1345	Silphin-1-ene	nd	0.46
40	1363	1362	(*E*)-*β*-Damascenone	nd	0.02
41	1368	1369	Cyclosativene	nd	0.05
42	1375	1375	*α*-Copaene	nd	0.09
43	1380	1381	Modhephene	nd	0.74
44	1383	1383	*β*-Cubebene	nd	0.02
45	1388	1387	*β*-Bourbonene	0.05	3.28
46	1393	1391	*β*-Elemene	nd	0.02
47	1399	1400	Cyperene	0.04	0.40
48	1405	1406	*α*-Barbatene	0.02	1.89
49	1419	1417	(*E*)-*β*-Caryophyllene	0.07	0.94
50	1430	1428	*β*-Copaene	nd	0.11
51	1435	1434	*trans*-*α*-Bergamotene	0.07	nd
52	1445	1446	Sesquisabinene	0.05	0.03
53	1449	1450	*α*-Humulene	0.02	0.43
54	1474	1475	*trans*-Cadina-1(6),4-diene	0.04	0.02
55	1480	1481	Germacrene-D	0.04	0.24
56	1491	1491	(*Z*)-*α*-Bisabolene	0.04	0.91
57	1494	1495	*(E)*,(*E*)-*α*-Farnesene	nd	0.32
58	1506	1507	*trans*-Calamenene	nd	1.33
59	1512	1510	7-epi-α-Selinene	nd	0.11
60	1514	1514	*δ*-Cadinene	nd	0.16
61	1535	1534	*trans*-cadina-1,4-diene	nd	0.16
62	1542	1542	Selina-3,7(11)-diene	nd	0.09
63	1548	1547	*β*-Elemol	nd	0.03
64	1551	1550	Elemol	nd	0.16
65	1563	1563	(*E*)-Nerolidol	nd	0.19
66	1568	1569	Germacrene-D-4-ol	0.05	0.32
67	1577	1577	Caryophyllene oxide	nd	0.03
68	1583	1582	Spathulenol	nd	0.06
69	1594	1593	Viridiflorol	nd	0.10
70	1601	1601	*α*-Humulene oxide	nd	0.02
71	1610	1607	Humulene-1,2-epoxide	nd	0.04
72	1615	1614	1,10-di-epi-Cubenol	nd	0.12
73	1625	1629	10-*epi*-*γ*-Eudesmol	nd	0.08
74	1630	1631	*β*-Eudesmol	nd	0.15
75	1635	1635	*α*-Cadinol	nd	0.14
76	1636	1637	*α*-Eudesmol	nd	0.08
77	1651	1653	*β*-Bisabolol	nd	0.04
78	1676	1677	Andro encecalinol	nd	0.07
79	1698	1695	*β*-Sinensal	nd	0.12
80	1715	1715	Mayurone	nd	0.02
81	1740	1745	(*E*,*E*)-*α*-Farnesene,	0.05	nd
82	1783	1786	*Cis*-Cadina-1(2),4-diene	nd	0.03
83	1834	1830	6,10,14-Trimethyl pentandecan-2-one	nd	0.06
84	1862	1866	1-Hexadecanol	nd	0.08
85	1899	1884	Nonadecane	nd	0.07
86	1955	1942	Isophytol	nd	0.38
87	1978	1976	Ethyl hexadecanoate	nd	0.02
88	2107	2106	(*E*)-hytol	0.06	0.84
			TOTAL	99.96	99.23

nd: no detected or percentage < 0.02%

The chemical composition of the essential oils extracted from the leaves and stems harvested in Côte d'Ivoire is dominated by the presence of monoterpenes (97.82% in the leaves, 74.62% in the stems) with the predominant compound being the hydrocarbon monoterpene, myrcene (87.48%) which alone represents almost all of the oil in the leaves. Two other hydrocarbon monoterpenes, limonene (4.11%) and *β*-pinene (2.72%) are in significant quantities compared to the rest of the constituents. The three predominant compounds alone represent 94.30% of the oil, which reflects a monoterpene hydrocarbon chemotype of the essential oils of the leaves of *Crassocephalum* sp. Figure 1 presents the main compounds of

the essential oils of the leaves and stems of *Crassocephalum* sp. The essential oils in the stems show a different chemotype than in the leaves. Indeed The main components were myrcene (35.55%), terpinolene (31.99%), (*E*)-dec-2-enal (8.01%), *β*-bourbonene (3.28%) and limonene (2.23%). The hydrocarbon monoterpene, terpinolene is high in the stems but low in the leaves.

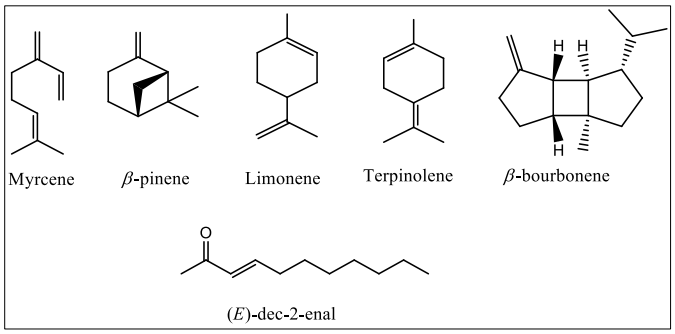

Fig 1: Main compounds of the essential oils of the leaves and stems of *Crassocephalum* sp.

Discussion

The chemical compositions of the essential oils of the leaves and stems of *Crassocephalum sp* from Côte d'Ivoire are different from those obtained for the leaves and stems of the species *Crassocephalum* crepidioides harvested in Vietnam (Hung *et al.*, 2019) [9], India (Joshi, 2011) [10] and in Nigeria (Owokotomo *et al.*, 2012) [11]. The leaves and aerial parts respectively from Vietnam and India which contain myrcene (59.3%), *β*-phellandrene (11.9%) and cryptone (6.4%) as predominant compounds, have the same major compound, myrcene, with different proportions. The other major compounds are different from those of the plant studied. Moreover, *β*-phelladrene and cryptone, which are also major compounds of *Crassocephalum crepidioides* oils, were not identified in our studied plant. In West Africa, Nigeria, the chemotypes reported for the oils extracted from the leaves and stems of *Crassocephalum crepidoides* are different from the chemotypes obtained in our work on *Crassocephalum* sp. The essential oils from the leaves of *Crassocephalum crepidoides* from Nigeria contained α-caryophyllene (10.29%), *β*-cubeben (13.77%) and *α*-farnesene (13.27%) as major constituents and the dominant stem oil constituents were thymol (43.93%), α-caryophyllene (15.16%) and 4-cyclohexybutyramide (20.94%). These major compounds in the oils of the leaves and stems of *Crassocephalum. crepidoides* from Nigeria (Owokotomo *et al.*, 2012) [11] are in the minority or absent in the oils of the species studied, *Crassocephalum* sp.

The myrcene chemotype obtained in the essential oils of the leaves and the myrcene, terpinolene, (*E*)-dec-2-enal chemotype identified in the essential oils of the stems of the species *Crassocephalum* sp studied are also different from the chemotypes reported in α-phellandrene, p-cymene, myrcene, limonene and (*E*)-*β*-ocimene for seven *Crassocephalum* species from Cameroon, *Crassocephalum biafrae*, *Crassocephalum bougheyanum*, *Crassocephalum crepidioïdes*, *Crassocephalum mannii*, *Crassocephalum rubens*, *Crassocephalum vitellinum and Crassocephalum montuosum* (Zollo *et al.*, 2000) [12]. *Crassocephalum crepidioides* essential oils showed excellent larvicidal activity against three species of mosquitoes in a comparative study of the larvicidal activity of many essential oils. This activity of essential oils is probably due to the high amount of myrcene (59.3%), which is also known for its larvicidal properties (Hung *et al.*, 2019) [9]. The essential oils of the leaves of *Crassocephalum* sp from Côte d'Ivoire containing more myrcene (87.46%) could have a higher larvicidal activity than those of the leaves of *Crassocephalum crepidioides* from Vietnam. Our study suggests that *Crassocephalum* sp from Côte d'Ivoire may be a potential source of myrcene source for larvicidal properties in the prevention of malaria. In addition, myrcene, the major constituent of essential oils from the leaves of *Crassocephalum* sp, could transmit these different biological properties to the oils from the leaves of this plant. Indeed, in studies of the biological activities of several monoterpenes, myrcene showed a significant antibacterial effect (Rasoul *et al.*, 2012) [15]. Inoue *et al*., (2004) also showed that myrcene contributed to the antibacterial activity of essential oils of *Melaleuca alternifolia* (Inoue *et al*., 2004) [16].

Conclusion

This study is a contribution to the promotion of tropical medicinal plants and very few known species of the genus *Crassocephalum*. This research work presents for the first time the phytochemical study of essential oils extracted from the leaves and stems of a species of *Crassocephalum* harvested in Côte d'Ivoire. Our study showed a monoterpene myrcene, limonene and *β*-pinene chemotype for the oils extracted from the leaves and a myrcene, terpinolene and (*E*)-dec-2-enal chemotype for the oils extracted from the stems. Myrcene, which represents almost all of the volatile matter of the oils in the leaves of the plant studied, is also the major compound of the species *Crassocephalum crepidioides* with a lower percentage. The large amount of myrcene, an antibacterial and larvicidal, in the oils extracted from the leaves of *Crassocephalum sp* could indicate important larvicidal and antibacterial activities of the oils.

References

1. Wagner W, Herbs D, Sohmer S. Manual of the Flowering Plants of Hawaii, Revised Edition, Bernice P. Bishop Museum Special Publication. University of Hawaii Bishop. Vol 2, Museum Press, Honolulu; c1999. p. 1919.
2. Asada Y, Shiraishi M, Takeuchi T, Osawa Y, Furuya T. Pyrrolizidine Alkaloids from *Crassocephalum crepidioides*. Planta Med. 1985;51(6):539-40.
3. Kongsaeree P, Prabpai S, Sriubolmas N, Vongvein C, Wiyakrutta S. Antimalarial dihydroisocoumarins produced by *Geotrichum* sp., an endophytic fungus of *Crassocephalum crepidioides*. J Nat. Prod. 2003;66(5):709-711.
4. Taïwe GS, Bum EN, Talla E, Dimo T, Sidiki N, Dawe A, *et al*. Evaluation of antinociceptive effects of *Crassocephalum bauchiense* Hutch (Asteraceae) leaf extract in rodents. J Ethnopharmacol. 2012;141(1):234-41.
5. Adjatin A, Dansi A, Badoussi E, Loko YL, Dansi MPA, Gbaguidi F, *et al*. Phytochemical screening and toxicity studies of *Crassocephalum rubens* (Juss. ex Jacq.) S. Moore and *Crassocephalum crepidioides* (Benth.) S. Moore consumed as vegetable in Benin. Int. J Curr. Microbiol. Appl. Sci. 2013;2(8):1-13.
6. de las Heras B, Hortelano S, Girón N, Bermejo P, Rodríguez B, Boscá L. Kaurane diterpenes protect against apoptosis and inhibition of phagocytosis in activated macrophages. Br. J Pharmacol. 2007;152(2):249-255.

7. Taïwe G, Bum E, Talla E, Dimo T, Sidiki N, Dawe A, *et al*. Evaluation of antinociceptive effects of *Crassocephalum bauchiense* Hutch (Asteraceae) leaf extract in rodents. J Ethnopharmacol. 2012;141(1):234-241.
8. Lestari T, Nurmala A, Nurmalasari M. Penetapan kadar polifenol dan aktivitas antibakteri ekstrak etanol daun sintrong (*Crassocephalum crepidiodes* (Benth.) S. moore). Jurnal Kesehatan Bakti Tunas Husada. 2015;13(1):107-110.
9. Hung N, Satyal P, DN D, Tai T, Huong L, Chuong N, *et al*. Chemical Compositions of *Crassocephalum crepidioides* Essential Oils and Larvicidal Activities Against *Aedes aegypti*, *Aedes albopictus*, and *Culex quinquefasciatus*. Nat. Prod. Commun. 2019;14(6):1-5.
10. Joshi R. Terpene composition of *Crassocephalum crepidioides* from Western Ghats region of India. Int. J Nat. Prod. Res. 2011;1(2):19-22.
11. Owokotomo IA, Ekundayo O, Oladosu IA, Aboaba SA. Analysis of the Essential Oils of Leaves and Stems of *Crassocephalum crepidioides* growing in South Western Nigeria. Int. J Chem. 2012;4(2):34-37.
12. Zollo PHA, Kuiate JR, Menut C, Bessiere JM. Aromatic Plants of Tropical Central Africa. XXXVI. Chemical Composition of Essential Oils from Seven Cameroonian *Crassocephalum* Species. J Essent. Oil Res. 2000;12(5):533-536.
13. Adams RP. Identification of Essential oil Components by Gas Chromatography/Mass Spectroscopy. Illinois, USA: Allured Publishing Co; c1995.
14. Mc Lafferty FW, Stauffer DB. The Wiley NBS registry of mass spectral data. New York: 2ème Edition John Wiley and Sons; c1989.
15. Rasoul M, Marei GIK, Abdelgaleil S. Evaluation of antibacterial properties and biochemical effects of monoterpenes on plant pathogenic bacteria. Afr. J Microbiol. Res. 2012;6(15):3667-3672.
16. Inoue Y, Shiraishi A, Hada T, Hamashima H, Shimada J. The Antibacterial Effects of Myrcene on *Staphylococcus aureus* and Its Role in the Essential Oil of the Tea Tree (*Melaleuca alternifolia*). Nat. Med. 2004;58(1):10-14.