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Comparative analysis of GC-MS of *Isolona* Engl. (*Annonaceae*) in Nigeria and the Cameroons

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Nijerya ve Kamerun'daki *Isolona* Engl. (*Annonaceae*) bitkisinin karşılaştırmalı GC-MS analizi

Abstract: The comparative assessment of six (6) *Isolona* species occurring in Nigeria and the Cameroons was undertaking using GC-MS analysis. The analysis was carried out with methanol extract and one hundred and seventy-six (176) phyto-constituents identified and scored at different retention times ranges from 3.22min in *I. hexaloba* (Pierre) Engl. & Diels. to 34.56min in *I. campanulata* Engl. & Diels.. The prominent compounds were scored in carboxylic and its derivates while the least compound was identified in alkyne. Highest M.wt. was 741.5 in *I. congolana* (De Wild.&T.Durand) Engl. & Diels. at retention time of 34.52 min and the lowest M.wt. was 84.15 in *I. thonneri* (De Wild.&T.Durand) Engl. & Diels. at retention time 3.44 min. Hexadecanoic acid, methyl ester formula $C_{17}H_{34}O_2$ and M.wt of 270.4 was scored in all the six species suggested a diagnostic characters for the genus *Isolona*. Quantitatively, the amount of the analytes isolated in all the species ranges from 0.1% in *I. hexaloba* to 59.36% in *I. thonneri.* Two UPGMA distant trees were constructed with RMSD and Euclidean index to illustrate the relationships among the *Isolona* species based on the difference chemical constituents. The distance tree revealed the separation of *I. thonneri* from all other species, also a sub-cluster tree of *I. hexaloba, I. campanulata* and *I. zenkeri* (Engl.) Dyer. was also constructed. The highest distance value level is 9.576 scored between *I. thonneri* and *I. congolana* while the highest similarity level was 2.911 scored in *I. hexaloba* and *I. campanulata*. The overlapping phyto-constituents characters revealed the closeness of the taxa studied, this invariably will update the existing data in the genus and ultimately in the family *Annonaceae*.

Key words: Comparative analysis, GC-MS, *Isolona*, Phyto-constituents

Özet: Nijerya ve Kamerun'da bulunan altı (6) *Isolona* türünün GC-MS analizi kullanılarak karşılaştırmalı değerlendirmeleri yapılmıştır. Analiz metanol özütü ile gerçekleştirildi ve yüz yetmiş altı (176) fito-bileşen tanımlandı ve *I heksaloba*'da 3.22 dakika ile *I. campanulata*'da 34.56 dakika arasında değişen farklı alıkonma sürelerinde tanımlanmaları gerçekleştirildi. Öne çıkan bileşikler karboksilik ve türevleri olur iken, en az tepit edilen bileşikler alkinlerde tanımlandı. En yüksek M.wt. 34.52 dakikalık alıkonma zamanında *I. congolana*'da 741.5 olarak ölçülürken, düşük M.wt. değeri 84.15 ile 3.44 dk alıkonma süresinde *I. thonneri'*de tespit edildi. Heksadekanoik asit, metil ester formülü $C_{17}H_{34}O_2$ ve 270.4 M.wt olarak değerlendirilen bileşik, altı türün tamamında tespit edildi ve *Isolona* cinsi için bir tanısal karakter önerildi. Kantitatif olarak, tüm türlerde izole edilen analit miktarı, *I. hexaloba*'da %0.1 ile *I. thonneri'*de %59.36'ya kadar değişim gösterdi. Farklı kimyasal bileşenlere dayalı olarak *Isolona* türleri arasındaki ilişkileri göstermek için RMSD ve Öklid indeksi ile iki UPGMA ağacı oluşturuldu. Oluşturulan ağaç, *I. thonneri*'nin diğer tüm türlerden ayrıldığını ortaya çıkarmış, ayrıca *I. hexaloba, I. campanulata* ve *I. zenkeri*'nin bir alt ağaç oluşturduğu görüldü. En yüksek uzaklık değeri *I. thonneri* ve *I. congolana* arasında 9.576 olarak puanır iken, en yüksek benzerlik düzeyi *I. hexaloba* ve *I. campanulata*'da 2.911 puan almıştır. Örtüşen fito-bileşen karakterleri, incelenen taksonların yakınlığını ortaya çıkardı, bu durum her cinsteki ve nihayetinde Annonaceae familyasındaki mevcut verileri güncellenmesine neden olacaktır.

Anahtar Kelimeler: Karşılaştırmalı analiz, GC-MS, *Isolona*, Fito-bileşenler

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1. Introduction

Annonaceae Juss. are a pantropical family of trees, shrubs, and lianas belonging to the order Magnoliales (APGII, 2003) with *c.* 130 genera and *c.* 2500 species (Chatrou et al., 2004). In Africa, the family comprises *ca.* 42 genera and 500 species (Bremer et al., 2009; Smith et al., 2010; Couvreur et al., 2012; Zeng et al., 2014). The African genera were treated as a whole for the last time over 100 years ago by Engler and Diels (1901). Since then, regional accounts have been published in the second half of the last century such as those for Flore du Gabon (Le Thomas, 1969), Flora of West Tropical Africa (Hutchinson et al., 1954) and Flora of Tropical East Africa (Verdcourt, 1971). African *Annonaceae* have been largely understudied in recent years when compared to Neotropical and South-East Asian taxa (Maas et al., 2003; Couvreur et al., 2006; Couvreur et al., 2008). One of these genera is *Isolona* Engl., a sizeable genus with *ca.* 21 species distributed across the tropical zone. *Isolona* Engl. flowers are hermaphroditic and are unusual as all six petals are basally connate, forming a single whorl (Couvreur, 2009). Relatively little is known about the reproductive biology, although the flowers of *I. campanulata* Engl. & Diels have been shown to be protogynous, with the reproductive phases extending over a 2-day (or possibly 3-day) period, with diurnal receptivity (Gottsberger et al., 2011). The species are mainly trees and there are accounts on exomorphology (Maas et al., 2003; Couvreur, 2009).

According to Panichpol and Waterman (1978), the Annonaceae is perhaps one of the least chemically known families'. A comprehensive review article published by" Leboeuf et al. (1982) updates all the important phytochemical research on Annonaceae members. Moreover, many earlier studies are only fragmentary and systematic reinvestigations are necessary in many cases. In order to draw valid conclusions on chemotaxonomic features of the Annonaceae, more chemical investigations are necessary. However, there is paucity of information on the structures of these chemicals and it has not been entrenched into taxonomic studies. Therefore, in search for additional identification criteria and pharmacological properties of the genus, the phyto-constituents characters of 6 African *Isolona* species occurring in Nigeria and Cameroons were investigated with a view to update the existing data within the family.

2. Materials and Methods

Dried herbarium samples of the six (6) species of *Isolona* from West and Central African obtained from the National Herbarium Yaounde, Cameroon YA(IH) were used for the study. The herbarium abbreviations follow Holmgren and Holmgren (2003) while the specific samples of the studied species and their herbarium information were *Isolona campanulata*: P.T. Francis, Dec. 1945; *I. congolana*: Westphal, 15/5/78; *I. dewevrei*: R. Letouvzey, 8/7/75; *I. hexaloba*: R. Letouvzey, 16/5/63; *I. thonneri*: R. Letouvzey, 23/3/70 and *I. zenkeri*: Endengle Elais, 1955-1956; showing species name, collector and date of collection respectively.

Two to 5 g of the plant sample was cut into small sizeable pieces and weighed then mixed with absolute methanol at a 1:20 ratio (100 g in 1 L solvent) and shaked thoroughly for 2-3 days. The extract was filtered by Whatman filter paper then the filtrate concentrated using the BUCHI Switzerland rotary evaporator to remove the methanol and obtain pure extract. 30 ml of the solvent was repeated twice in the process and the extract was concentrated using nitrogen concentration and later treated with silica gel and anhydrous sodium sulphate. The GC-MS used for analysis is Agilent technologies 7890GC system coupled with mass spectrometer of model Agilent technologies 5975. The mobile phase is helium gas and the stationery phase is the column with length 30 m, internal diameter 0.320 mm, thickness 0.25 µm. The oven temperature program, initial temperature is 80 °C to hold for 2 min at 10 °C per min to final temperature of 240 °C to hold for 6 min, the volume injected 1 µl. Method follows Cunha et al. (2004) and Phrompittayarat et al. (2008).

The phytocomponents of the extracts from the different species were identified based on direct comparison with the database of National Institute Standard and Technology (NIST) and Royal Society of Chemistry and National Library of Medicine.

2.1. Data analysis

The separated compounds by GC-MS analysis were scored in binary matrices, where 0 stands for the absence and 1 stands for the presence of a compound for all studied *Isolona* species; these codes were detailed in Table 1. Statistical analysis of the data of the compounds identified was carried out by using an online program Dendro UPGMA (A dendrogram Construction Utility) using RMSD coefficient and a software Paleontological Statistics software used for constructing Euclidean similarity index, scatter plot, and PCA using used for the constructing. This method follows the approaches of Gamal et al. (2017).

3. Results

The GC-MS comparative analysis of six (6) species of *Isolona* namely: *I. congolana, I. dewevrei, I. hexaloba, I. thonneri, I. zenkeri* and *I. campanulata* isolated and identified one hundred seventy six (176) compounds at different retention times between 3.26 min and 34.56 min (Table 1). The highest number of compounds separated and recorded was in *I. thonneri* at retention time between 3.44 and 33.86 min, while the lowest number of compounds was 29 at a retention time between 3.69 and 34.52 min in *I. congolona*. The highest M.wt. was 741.5 with a compound named Cyclodecasiloxane, eicosamethyl- and scored in *I. congolana* at retention time of 34.52 min and the lowest M.wt. was 78.13 (Mercaptoethanol, 2TMS derivatives) in *I. thonneri* at retention time 3.44 min. The first compound dissociated in all the species was between 3.26 min and 3.69 min retention time except *I. zenkeri* at about 8.24 min. The last compounds separated lies between retention time of 33.78 and 34.56 min but *I. dewevrei* last compound was dissociated at retention time of 20.39 min. The amount of analytes isolated in all the species ranges from 0.1% with decided name: 1H-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl)-3-methyl-4-oxo-4,5,6,7-tetrahydro-, isopropyl ester and hexasiloxane, 1,1,3,3,5,5,7,7,9,9, 11,11-dodecamethyl- in *I. hexaloba* to 59.36% with decided name: 1-Butanol, 2-methyl- ; cyclobutane, ethyl- and decane, 2,2,3-trimethyl- in *I. thonneri.* A compound named cyclononasiloxane, octadecamethyl was scored in *I. congolana, I. zenkeri* and *I. campanulata.* Also compounds with decided name octasiloxane, 1,1,3,3, 5,5,7,7,9,9,11,11,13,13, 15,15-hexadecamethyl- and heptasiloxane, 1,1,3,3, 5,5,7,7,9,9,11,11,13,13-tetradecamethyl- were scored in *I. hexaloba, I. thonneri, I. zenkeri* and *I. campanulata.* Likewise, hexadecanoic acid, methyl ester with the formula $C_{17}H_{34}O_2$ and molecular weight of 270.4 was scored in all the six species (Table 1).

3.1. *Isolona congolana*

There were twenty-nine phyto-compounds identified in *I. congolana* broadly grouped into alkanes, alcohol, organo-compound, alkaloid, carboxylic acids and its derivatives. A compound with decided name cyclodecasiloxane, eicosamethyl-, formula $C_{20}H_{60}O_{10}Si_{10}$ scored with the highest molecular weight of about 741.54 was found in *I. congolana*. Most prominent compounds in *I. congolana* were the carboxylic acids with fourteen (14) compounds and the least compound was an alkaloid with decided name benzene, nitroso- formula C_6H_5NO (Table 1). The retention time for the dissociation of the compounds was between 3.69 and 34.52min with M.wt ranged from 107.11 in Benzene, nitroso- to 741.53 in cyclodecasiloxane, eicosamethyl- (Fig. 1).

3.2. *Isolona dewevrei*

The M.wt of thirty-two (32) compounds separated between 3.36 and 20.39 min retention times was between 92.19 in a compound with decided name trimethylsilyl fluoride formula C_3H_9FSi and 270.45 in a compound with

Table 1. The GC-MS analysis of six (6) species of *Isolona* based on the phyto-chemical constituents

S/N	COMPOUNDS	R.T	Area%	M.F	M.W	A	B	C	D	E	F
	ALCOHOL										
1.	1,2-Benzenediol, 3,5-bis(1,1-dimethylethyl)-	32.86	4.13	$C_{14}H_{22}O_2$	222.32	0	0	0	0	0	1
2.	1-Butanol, 2-methyl-	3.44	59.36	$C_7H_{14}O_2$	130.19	0	0	0	1	0	0
3.	2-Naphthalenemethanol, decahydro-. alpha.,.alpha.,4a-trimethyl-8-methylene-, [2R-(2.alpha.,4a.alpha.,8a.beta.)]-	16.45	8.3	$C_{15}H_{26}O$	222.37	0	0	0	0	1	1
4.	Benzenemethanol, 4-methyl-	3.68	1	$C_8H_{10}O$	122.16	0	0	0	0	0	1
5.	Benzyl alcohol	7.384	7.81	C_7H_8O	108.14	0	1	0	0	0	0
6.	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, acetate, (1S-endo)-	11.258	5.88	$C_{12}H_{20}O_2$	196.29	1	0	0	0	0	0
7	Heptaethylene glycol, TBDMS	17.21	1.62	$C_{20}H_{44}O_8Si$	440.41	0	0	0	1	0	0
8.	Mercaptoethanol, 2TMS derivative	32.82	9.99	C_2H_6OS	78.13	0	0	0	0	1	0
9.	Pentaethylene glycol, TBDMS	17.21	1.62	$C_{16}H_{36}O_6Si$	352.54	0	0	0	1	0	0
10.	Phenol, 3,4-dimethyl-	3.693	2.55	$C_8H_{10}O$	122.16	1	0	0	0	0	0
11.	1H-1,2,3-benzotriazole, 5,6-dichloro	15.91	2.14	$C_6H_3Cl_2N_3$	188.01	0	1	0	0	0	0
	ALKALOID										
12.	Benzene, nitroso-	3.693	2.55	C_6H_5NO	107.11	1	0	0	0	0	0
13.	Benzo[h]quinoline, 2,4-dimethyl-	33.07	1.09	$C_{15}H_{13}N$	207.27	0	0	1	1	0	1
14.	Etiron	3.67	0.28	$C_3H_9BrN_2S$	184.9	0	1	0	0	0	0
15.	Hexestrol, 2TMS derivative	33.86	0.24	$C_{24}H_{38}O_2Si_2$	414.73	0	0	0	1	0	0
16.	Oxime-, methoxy-phenyl-_	5.376	1.29	$C_8H_9NO_2$	151.16	0	0	1	0	0	0
17.	1-Methyl-2-aminomethylimidazole	23.36	1.64	$C_5H_9N_3$	111.15	0	0	0	1	0	0
18.	4-Benzamido-4-dichloromethyl-2-phenyl-2-imidazolin-5-one	3.26	7.5	$C_9H_{10}N_2$	146.19	0	0	0	0	0	1
19.	Methenamine	10.69	1.03	$C_6H_{12}N_4$	140.19	0	0	1	0	0	0
20	5-Methyl-2-phenylindolizine	33.19	1.92	$C_{15}H_{13}$	207.27	0	0	1	0	0	0
21.	1H-Benzo[4,5]furo[3,2-f]indole	33.4	4.35	$C_{14}H_9NO$	207.23	0	0	0	1	0	0
22.	1H-Indole, 1-methyl-2-phenyl-	32.78	0.8	$C_{15}H_{13}N$	207.27	0	0	0	1	0	0
23.	1H-Indole, 5-methyl-2-phenyl-	33.51	1.04	$C_{15}H_{13}N$	207.27	0	0	0	1	0	0
24.	1H-Indole-2,3-dione, 5,7-dichloro-	15.91	2.14	$C_8H_3Cl_2NO_2$	216.02	0	1	0	0	0	0
25.	1H-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl)-3-methyl-4-oxo-4,5,6,7-tetrahydro-, isopropyl ester	32.58	0.1	$C_{21}H_{25}$	355.43	0	0	1	0	0	1
26.	3H-indole, 2-methyl-3-phenyl-	33.76	1.83	$C_{16}H_{15}N$	221.3	0	0	0	1	0	1
27.	3-Amino-6-oxo-7a-phenyl-4aH,5H,7H-pyrrolo[2,3-c]pyridazine-4,5-dicarbonitrile	3.68	1	$C_{12}H_{10}N_4O_2$	242.23	0	0	0	0	0	1
28.	2-Ethylacridine	31.83	1.3	$C_{15}H_{13}N$	207.27	0	0	1	1	0	1
29.	n(sup1),n(sup4)-dibenzoylpiperazin	3.362	19.97	$C_{18}H_{18}N_2O_2$	294.3	0	0	1	0	0	0
30.	N,N-Dimethyl-4-nitroso-3-(trimethylsilyl)aniline	34.05	1.2	$C_{11}H_{18}N_2OSi$	222.36	0	0	0	0	0	1
31.	Pyrido[2,3-d]pyrimidine, 4-phenyl-	33.55	0.34	$C_{13}H_9N_3$	207.23	0	0	1	0	0	0
32.	1-(4-Nitrophenyl)piperazine	4.81	1.08	$C_{10}H_{13}N_3O_2$	207.23	0	0	0	1	0	0
33.	.gamma.-Muurolene	13.92	1.52	$C_{15}H_{24}$	204.35	0	0	0	0	1	0
	ALKANE										
34.	2,3,5-Trioxabicyclo[2.1.0]pentane, 1,4-bis(phenylmethyl)-	11.67	0.35	$C_{16}H_{14}O_3$	254.28	0	1	0	0	0	0
35.	1-Bromoeicosane	8.359	2.29	$C_{20}H_{41}Br$	361.44	1	0	0	0	0	0
36.	2-Methylhexacosane	8.519	2.98	$C_{27}H_{56}$	380.7	1	0	0	0	0	0
37.	Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-, (1.alpha.,2.beta.,5.alpha.)	19.05	2.27	$C_{10}H_{18}$	138.25	0	0	1	0	0	0
38.	Cyclobutane, ethyl-	3.44	59.36	C_6H_{12}	84.16	0	0	0	1	0	0
39.	Decane, 2,2,3-trimethyl-	3.44	59.36	$C_{13}H_{28}$	184.36	0	0	0	1	0	0
40.	Decane, 2-methyl-	16.923	7.18	$C_{11}H_{24}$	156.31	1	0	0	0	0	0
41.	Dotriacontane	16.831	2.24	$C_{32}H_{66}$	450.87	1	0	0	0	0	0
42.	Eicosane	30.24	16.77	$C_{20}H_{42}$	282.55	0	0	0	0	1	0
43.	Eicosane, 9-octyl-	32.49	11.79	$C_{28}H_{58}$	394.76	0	0	0	0	1	0
44.	Hentriacontane	8.519	2.98	$C_{31}H_{64}$	436.84	1	0	0	0	0	0
45.	Heptacosane, 1-chloro-	30.24	16.77	$C_{27}H_{55}Cl$	415.18	0	0	0	0	1	0
46.	Hexadecane	15.395	2.24	$C_{16}H_{34}$	226.44	1	0	0	0	0	0
47.	Hexadecane, 2,6,11,15-tetramethyl-	16.923	7.18	$C_{20}H_{42}$	282.5	1	0	0	0	0	0
48.	Hexane, 2,2,3-trimethyl-	3.361	0.98	C_9H_{20}	128.25	0	1	0	0	0	0
49.	Methylene chloride	3.608	3.56	CH_2Cl_2	84.93	0	0	1	0	0	0
50.	Nonadecane	32.49	11.79	$C_{18}H_{38}$	254.49	0	0	0	0	1	0
51.	Octacosane	30.24	16.77	$C_{28}H_{58}$	394.77	0	0	0	0	1	0
52.	Pentane, 3-ethyl-2,2-dimethyl-	3.361	0.98	C_9H_{20}	128.25	0	1	0	0	0	0
53.	Tetracontane, 3,5,24-trimethyl-	15.395	2.24	$C_{43}H_{88}$	605.2	1	0	0	0	0	0
54.	Tridecane	16.093	2.87	$C_{13}H_{28}$	184.36	1	0	0	0	0	0
	ALKENE										
55.	5,7-Dimethylenebicyclo[2.2.2]oct-2 -ene	11.67	0.35	$C_{10}H_{12}$	132.2	0	1	0	0	0	0
56.	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-,[1R-(1R*,4 Z,9S*)]-	13.84	1.86	$C_{15}H_{24}$	204.35	0	0	0	0	1	0
57.	Neophytadiene	19.05	2.27	$C_{20}H_{38}$	278.51	0	0	1	0	0	0
58.	Tricyclo[4.2.1.0(2,5)]non-7-ene, 3,4-	32.69	0.64	$C_{27}H_{64}O_6Si_8$	709.48	0	0	1	0	0	1

	di(tris(trimethylsilyloxy)silyl)-										
	ALKYNE										
59.	3-Octen-5-yne, (Z)-	9.078	7.55	C_8H_{12}	108.18	0	1	0	0	0	0
	BENZENE										
60.	1,3-Benzodioxole, 4-methoxy-6-(2-nitro-1-propenyl)-	14.89	1.1	$C_{11}H_{12}O_3$	192.21	0	0	0	1	0	0
61.	Benzene, 3-butenyl-	11.67	0.35	$C_{10}H_{12}$	132.20	0	1	0	0	0	0
	CARBOXYLIC AND ITS DERIVATIVES										
62.	2'-Hydroxy-5'-methylacetophenone, TMS derivative	33.57	0.4	$C_9H_{10}O_2$	150.17	0	0	1	0	0	0
63.	N-Benzenesulfonylazetidin-3-one	3.26	7.5	$C_9H_9NO_3S$	211.24	0	0	0	0	0	1
64.	Propenone, 3-(2-benzoxazolylthio)-1-phenyl-	12.67	0.93	$C_{16}H_{11}NO_2S$	280.99	0	0	0	1	0	0
65.	1,4-benzenedicarboxylic acid, mono(1-methylethyl) ester	29.28	2.74	$C_{11}H_{12}O_4$	208.21	0	0	0	0	1	0
66.	10-Octadecenoic acid, methyl ester	23.46	2.54	$C_{19}H_{36}O_2$	296.5	0	0	1	0	1	0
67.	12-Octadecenoic acid, methyl ester	23.35	7	$C_{19}H_{36}O_2$	294.47	0	0	0	0	1	0
68.	13-Octadecenoic acid, methyl ester	23.46	2.54	$C_{19}H_{36}O_2$	296.49	0	0	1	0	0	0
69.	14-Octadecenoic acid, methyl ester	23.35	7	$C_{19}H_{36}O_2$	296.5	0	0	0	0	1	0
70.	1-Benzazirene-1-carboxylic acid, 2,2,5a-trimethyl-1a-[3-oxo-1-butenyl] perhydro-, methyl ester	33.39	0.64	$C_{15}H_{23}NO_3$	265.35	0	0	1	1	0	0
71.	1-Butanol, 3-methyl-, acetate	4.7	0.93	$C_7H_{14}O_2$	130.19	0	1	0	0	0	0
72.	1H-Cyclopenta[1,3]cyclopropa[1,2]benzene, octahydro-7-methyl-3-methylene-4-(1-methylethyl)-, [3aS-(3a.alpha.,3b.beta.,4.beta.,7.alpha.,7aS*)]-	13.92	1.52	$C_{15}H_{24}$	204.35	0	0	0	0	1	0
73.	2-(5-Phenyl-2-oxazolyl)benzoicaci	3.68	1	$C_{16}H_{11}NO_3$	265.26	0	0	0	0	0	1
74.	2-(Acetoxymethyl)-3-(methoxycarbonyl)biphenylene	33.04	0.56	$C_{17}H_{14}O_4$	281.99	0	0	1	1	0	1
75.	2-(Isobutoxycarbonyl)benzoic acid	29.36	3.06	$C_{12}H_{14}O_4$	222.24	0	0	1	0	0	0
76.	2,4,6-Trimethylbenzoic acid, TMS derivatives	31.909	30.76	$C_{13}H_{20}O_2Si$	236.38	1	0	0	0	0	0
77.	2-Amino-5-methylbenzoic acid	5.376	1.29	$C_8H_9NO_2$	151.16	0	0	1	0	0	0
78.	2-Amino-6-methylbenzoic acid	5.376	1.29	$C_8H_9NO_2$	151.16	0	0	1	0	0	0
79.	3-(4-Hydroxyphenyl)propionitrile	3.693	2.55	C_9H_9NO	147.17	1	0	0	0	0	0
80.	3-Chloropropionic acid, benzyl ester	9.198	2.65	$C_{10}H_{11}ClO_2$	198.64	0	1	0	0	0	0
81.	3-Methyl-hexanoic acid	3.361	0.98	$C_7H_{14}O_2$	130.19	0	1	0	0	0	0
82.	3-Quinolinecarboxylic acid, 6,8-difluoro-4-hydroxy-, ethyl ester	32.74	0.19	$C_{12}H_9F_2NO_3$	253.2	0	0	1	1	0	1
83.	3-Trimethylsilyloxystearic acid, trimethylsilyl ester	30.03	10.04	$C_{24}H_{52}O_3Si_2$	444.8	0	0	0	0	1	0
84.	4-Benzyloxybenzoic acid	19.772	0.58	$C_{14}H_{12}O_3$	228.24	0	1	0	0	0	0
85.	4-pyridinecarboxylic acid, 3-cyano-2-hydroxy-6-methyl-5-nitro-, methyl ester	14.89	1.1	$C_8H_6N_2O_2$	162.15	0	0	0	1	0	0
86.	7-Hexadecenoic acid, methyl ester (Z)-	23.36	1.64	$C_{17}H_{32}O_2$	268.4	0	0	0	1	0	0
87.	9,12-Octadecadiynoic acid, methylester	13.84	1.86	$C_{19}H_{34}O_2$	294.47	0	0	0	0	1	0
88.	Acetic acid, [4-(1,1-dimethylethyl)phenoxy]-, methyl ester	32.59	2.11	$C_{13}H_{18}O_3$	221.99	0	0	0	0	0	1
89.	Acetic acid, 1,7,7-trimethyl-bicyclo[2.2.1]hept-2-yl ester	11.258	5.88	$C_{12}H_{20}O_2$	196.29	1	0	0	0	0	0
90.	acetic acid, 2-[bis(methylthio)methylene]-1-phenylhydrazide	32.74	0.19	$C_6H_6N_2S_2$	170.26	0	0	1	0	0	0
91.	Acetic acid, phenylmethyl ester	9.347	45.54	$C_9H_{10}O_2$	150.18	0	1	0	0	0	0
92.	Acridine-9-carbaldehyde	33.86	0.24	$C_{14}H_9NO$	207.23	0	0	0	1	0	0
93.	Allyl 2-ethyl butyrate	7.899	0.45	$C_9H_{16}O_2$	156.22	0	1	0	0	0	0
94.	Benzoic acid, 2-hydroxy-, phenylmethyl ester	19.772	0.58	$C_{17}H_{18}O_3$	270.32	0	1	0	0	0	0
95.	Benzoic acid, 2-methoxy-, methyl ester	4.81	1.08	$C_9H_{10}O_3$	166.17	0	0	0	1	0	0
96.	Benzoic acid, hydrazide	8.25	2.22	$C_7H_8N_2O$	136.15	0	0	0	0	0	1
97.	Benzoic acid, methyl ester	8.27	0.33	$C_8H_8O_2$	136.15	0	0	0	1	1	1
98.	Bis(2-ethylhexyl) phthalate	29.36	3.06	$C_{24}H_{38}O_4$	390.56	0	0	1	1	1	0
99.	Butanoic acid, ethyl ester	3.459	3.21	$C_6H_{12}O_2$	116.16	0	1	0	0	0	0
100.	Carbonic acid, prop-1-en-2-yl tridecyl ester	16.093	2.87	$C_{17}H_{32}O_3$	284.43	1	0	0	0	0	0
101.	Carbonic acid, undecyl vinyl ester	16.831	2.24	$C_{14}H_{26}O_3$	242.35	1	0	0	0	0	0
102.	cis-13-Octadecenoic acid	23.46	2.54	$C_{19}H_{36}O_2$	296.5	0	0	1	0	0	0
103.	Diethyl Phthalate	15.52	17.62	$C_{12}H_{14}O_4$	222.24	0	1	0	0	0	0
104.	Diisooctyl phthalate	29.28	2.74	$C_{24}H_{38}O_4$	390.6	0	0	0	0	1	0
105.	Dodecanoic acid, methyl ester	14.44	1.02	$C_{13}H_{26}O_2$	214.34	0	0	1	0	0	0
106.	Ethyl (2-hydroxyphenyl)acetate, TMS derivative	19.73	1.88	$C_{10}H_{12}O_3$	180.2	0	0	0	1	0	0
107.	Formic acid, 1-(4,7-dihydro-2-methyl-7-oxopyrazolo[1,5-a]pyrimidin-5-yl)-, methyl ester	2.33	34.33	$C_5H_{10}O_2$	102.13	0	0	0	0	0	1
108.	Heptadecanoic acid, 16-methyl-, methyl ester	23.77	4.87	$C_{19}H_{38}O_2$	298.50	0	0	0	0	1	0
109.	Heptanoic acid, methyl ester	20.39	1.92	$C_8H_{16}O_2$	144.21	0	0	0	1	0	0
110.	Hexadecanoic acid, 2-methyl-	20.42	8.23	$C_{20}H_{40}O_2$	312.53	0	0	0	0	0	1
111.	Hexadecanoic acid, methyl ester	20.39	0.38	$C_{17}H_{34}O_2$	270.45	1	1	1	1	1	1
112.	Hexanoic acid, 2-propenyl ester	7.899	0.45	$C_9H_{16}O_2$	156.22	0	1	0	0	0	0
113.	Isobornyl acetate	11.258	5.88	$C_{12}H_{20}O_2$	196.29	1	0	0	0	0	0
114.	Methoxyacetic acid, 2-pentadecyl ester	8.359	2.29	$C_{18}H_{36}O_3$	300.5	1	0	0	0	0	0
115.	Methoxyacetic acid, 2-tetradecyl ester	8.359	2.29	$C_{17}H_{34}O_3$	286.4	1	0	0	0	0	0
116.	Methoxyacetic acid, 3-tridecyl ester	16.093	2.87	$C_{16}H_{32}O_3$	272.42	1	0	0	0	0	0
117.	Methyl anthranilate	12.05	3.05	$C_8H_9NO_2$	151.16	0	1	0	0	0	0
118.	Methyl salicylate	9.827	3.61	$C_8H_8O_3$	152.15	0	1	0	0	0	0

119.	Methyl stearate	23.86	2.53	$C_{19}H_{38}O_2$	298.50	0	0	1	0	1	1
120.	Methyl tetradecanoate	17.29	1.3	$C_{15}H_{30}O_2$	242.39	0	0	1	0	0	0
121.	Methyl tropate, (.alpha.)-, TMS derivative	19.73	1.88	$C_{10}H_{12}O_3$	180.20	0	0	0	1	0	0
122.	N-Methyl-1-adamantaneacetamide	32.54	0.45	$C_{13}H_{21}NO$	207.31	0	0	1	1	1	1
123.	Octacosylheptafluorobutyrate	16.831	2.24	$C_{32}H_{57}F_7O_2$	606.78	1	0	0	0	0	0
124.	Pentadecanoic acid, 14-methyl-, methyl ester	20.39	14.1	$C_{17}H_{34}O_2$	270.5	0	0	0	0	1	1
125.	Pentadecanoic acid, methyl ester	20.39	1.92	$C_{16}H_{32}O_2$	256.42	0	0	0	1	0	0
126.	Perfluoropropionic acid, TBDMS derivate	3.67	0.28	$C_3HF_5O_2$	164.03	0	1	0	0	0	1
127.	Phthalic acid, heptyl oct-3-yl ester	29.36	3.06	$C_{23}H_{36}O_4$	376.53	0	0	1	0	0	0
128.	Prop-2-enoic acid, 2-cyano-3-(3-methyl-2-thienyl)-, methyl ester	29.28	2.06	$C_{10}H_9NO_2S$	206.98	0	0	0	1	0	0
129.	Silicic acid, diethyl bis(trimethylsilyl) ester	33.1	1.91	$C_{10}H_{28}O_4Si_3$	296.58	0	0	0	1	0	1
130.	Sulfurous acid, butyl heptadecyl ester	8.519	2.98	$C_{21}H_{44}O_3S$	376.6	1	0	0	0	0	0
131.	Swep	15.91	2.14	$C_8H_7Cl_2NO_2$	219.98	0	1	0	0	0	0
132.	Tetradecanoic acid, 12-methyl-, methyl ester	20.436	4.71	$C_{16}H_{32}O_2$	256.42	1	0	0	0	0	1
133.	Tridecanoic acid, 12-methyl-, methyl ester	17.29	1.3	$C_{15}H_{30}O_2$	242.39	0	0	1	0	0	0
134.	Trimethylsilyl 2-(trimethylsilyloxy)propaneperoxoate	12.67	0.93	$C_{13}H_{33}NO_3Si_3$	335.66	0	0	0	1	0	0
135.	Undecanoic acid, 10-methyl-, methyl ester	14.44	1.02	$C_{13}H_{26}O_2$	214.34	0	0	1	0	0	0
136.	Benzylcarbamate	9.347	45.54	$C_8H_9NO_2$	151.16	0	1	0	0	0	0
137.	benzeneacetaldehyde, .alpha.-(methoxymethylene)-4-nitro-	32.78	0.8	$C_{10}H_9NO_4$	207.18	0	0	0	1	0	0
138.	Octanal, 2-(phenylmethylene)-	17.74	0.92	$C_{15}H_{20}O$	216.32	0	1	0	0	0	0
138.	(2E,4E)-N-Isobutyltetradeca-2,4-dienamide	33.24	0.32	$C_{18}H_{33}NO$	279.46	0	0	1	0	0	0
139.	Benzamide, N-[6-(2-furyl)-2-oxo-2H-pyran-3-yl]-	8.22	0.41	$C_{16}H_{11}NO_4$	256.99	0	0	0	1	0	0
140.	Salicylamide	9.827	3.61	$C_7H_7NO_2$	137.14	0	1	0	0	0	0
141.	Silane, ethenyldiethylmethyl-	23.67	1.79	$C_7H_{16}Si$	128.29	0	0	1	0	0	0
142.	1-(2-Thienyl)ethanonesemicarbazoneditms	17.21	1.62	C_6H_6OS	126.18	0	0	0	1	0	0
143.	1-(4-Methoxy-phenyl)-5,5-dioxo-hexahydro-5.lambda.(6)-thieno[3,4-b]pyrrol-2-one	29.28	2.06	$C_{13}H_{15}NO_4S$	281.33	0	0	0	1	0	0
144.	1,2,4-Triazine-3,5(2H,4H)-dione, 6-benzoylthio-	8.22	0.41	$C_7H_{11}N_3O_2$	169.18	0	0	0	1	0	0
145.	2-(Ethyl)oxybenzylideneacetophenone	33.61	0.58	$C_{17}H_{16}O_2$	252.31	0	0	0	0	0	1
146.	2-(n-Propyl)oxybenzylideneacetophenone	33.55	0.34	$C_5H_{12}O_2$	104.15	0	0	1	1	0	1
147.	1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene-	13.84	1.86	$C_{15}H_{24}$	204.35	0	0	0	0	1	0
148.	Cryptomeridiol	16.48	2.1	$C_{15}H_{28}O_2$	240.38	0	0	0	0	0	1
149.	Phytol	23.67	1.79	$C_{20}H_{40}O$	296.53	0	0	1	0	0	0
150.	2(3H)-Furanone, 5-hexyldihydro-	15.18	1.3	$C_{11}H_{20}O_2$	184.28	0	1	0	0	0	0
151.	.delta.-Dodecalactone	7.899	0.45	$C_{10}H_{18}O_2$	170.25	0	1	0	0	0	0
	NAPHTALENE										
152.	2-[4-Acetamidophenylsulfonyl]-1,4-naphthoquinone	19.73	1.88	$C_{18}H_{13}NO_5S$	355.4	0	0	0	1	0	0
153.	Anthracene, 9-ethyl-9,10-dihydro-9,10-dimethyl-	33.46	0.84	$C_{18}H_{20}$	236.4	0	0	0	1	0	0
154.	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-m ethylethyl)-, (1.alpha.,4a.beta.,8a.alpha.)-	13.92	1.52	$C_{15}H_{24}$	204.35	0	0	0	0	1	0
155.	Anthracene, 9,10-dihydro-9,9,10-trimethyl-	32.73	0.29	$C_{17}H_{18}$	222.32	0	0	1	0	0	0
	ORGANO-COMPOUNDS										
156.	1,1,1,3,5,5,5-Heptamethyltrisiloxane	32.39	3.89	$C_7H_{22}O_2Si_3$	222.51	0	0	0	0	0	1
157.	1,1,1,5,7,7,7-Heptamethyl-3,3-bis(trimethylsiloxy)tetrasiloxane	34.523	36.3	$C_{13}H_{39}O_5Si_6$	443.96	1	0	0	0	0	0
158.	3,3-Diisopropoxy-1,1,1,5,5,5-hexamethyltrisiloxane	32.73	0.29	$C_{12}H_{32}O_4Si_3$	324.63	0	0	1	1	0	1
159.	Cyclodecasiloxane, eicosamethyl-	34.523	36.3	$C_{20}H_{60}O_{10}Si_{10}$	741.54	1	0	0	0	0	0
160.	Cyclononasiloxane, octadecamethyl-	32.82	9.99	$C_{18}H_{54}O_9Si_9$	667.39	1	0	0	0	1	1
161.	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-	33.86	0.91	$C_{14}H_{42}O_6Si_7$	503.07	0	0	1	1	1	1
162.	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-	32.58	0.1	$C_{12}H_{38}O_5Si_6$	430.94	0	0	1	1	0	0
163.	Hexasiloxane, tetradecamethyl-	31.909	30.76	$C_{14}H_{42}O_5Si_6$	458.99	1	0	0	0	0	1
164.	Methyltris(trimethylsiloxy)silane	32.11	3.32	$C_{10}H_{30}O_3Si_4$	310.69	0	0	1	0	0	1
165.	Octasiloxane, 1,1,3,3,5,5,7,7,9,9, 11,11,13,13,15,15-hexadecamethyl-	32.54	0.72	$C_{16}H_{48}O_7Si_8$	577.2	0	0	1	1	1	1
166.	Silane, [[5,5-dimethyl-4-methylene-2-(trimethylsilyl)-1-cyclopenten-1-yl]methoxy]trimethyl-	33.76	1.83	$C_{15}H_{30}OSi_2$	282.57	0	0	0	1	0	0
167.	tert-Butyldimethylfluorosilane	3.362	19.97	$C_6H_{15}FSi$	134.27	0	0	1	0	0	0
168.	Tetrasiloxane, decamethyl-	33.03	1.49	$C_{10}H_{30}O_3Si_4$	310.69	0	0	0	1	0	0
169.	Trimethylsilyl fluoride	3.67	0.28	C_3H_9FSi	92.187	0	1	0	0	0	0
170.	Trimethylsilyl-di(timethylsiloxy)-silane	32.54	0.72	$C_9H_{27}O_2Si_4$	279.65	0	0	0	1	0	0
171.	Tris(tert-butyldimethylsilyloxy)arsane	33.27	0.68	$C_{18}H_{45}AsO_3Si_3$	468.7	0	0	1	0	0	0
172	1-methyl-4-phenyl-5-thioxo-1,2,4-triazolidin-3-one	34.27	2.7	$C_9H_9N_3OS$	207.25	0	0	0	0	0	1
173.	4-Methylphenol, n-propyl ether	9.198	2.65	$C_4H_{10}OC_{10}H_{14}O$	150.22	0	1	0	0	0	0
174.	4-n-Hexylthiane, S,S-dioxide	23.36	1.64	$C_{11}H_{22}O_2S$	218.36	0	0	0	1	0	0
175.	Benzenecarbothioic acid	8.22	0.41	C_7H_6OS	138.19	0	0	0	1	0	0
176.	Benzoyl bromide	8.24	3.89	C_7H_5BrO	185.02	0	0	0	0	1	0

R.T- Retention time, P.A- Peak Area %, M.F- Molecular formula, M.W- Molecular weight, A- *Isolona congolana*, B- *I. dewevrei*, C- *I. hexaloba*, D- *I. thonneri*, E- *I. zenkeri*, F- *I.campanulata*

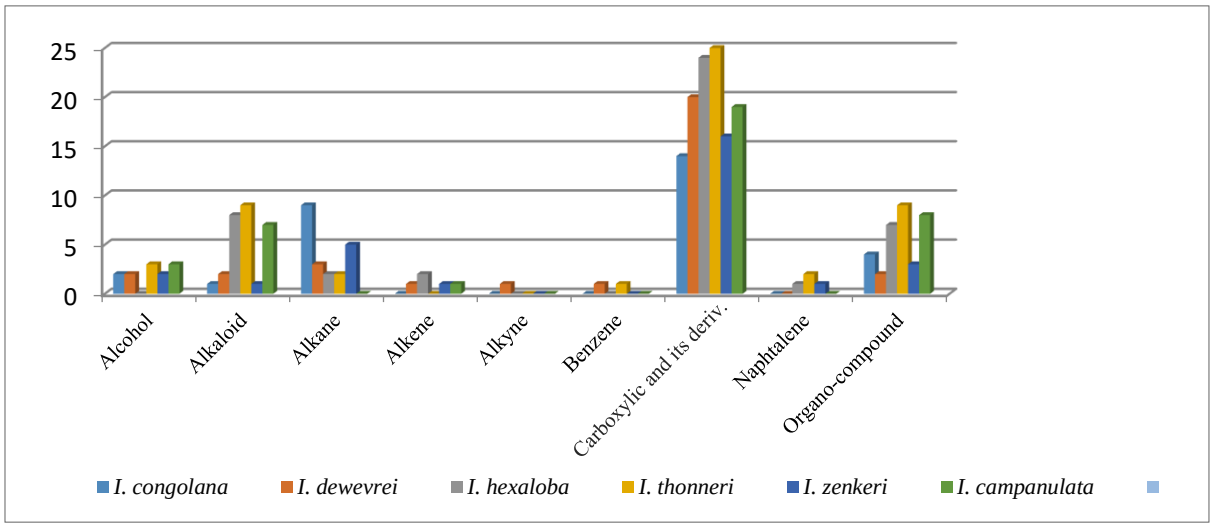

Figure 1: Showing the distribution of phyto-chemical compounds present in the Six (6) *Isolona* species

decided name hexadecanoic acid, methyl ester formula $C_{17}H_{34}O_2$. The prominent group of compounds in these species were scored in carboxylic and its derivatives with about twenty (20) while the least compounds were scored in alkene, alkyne and benzen with the compounds named: 5,7-dimethylenebicyclo [2.2.2]oct-2 –ene; 3-octen-5-yne, (Z)- and benzene, 3-butenyl- respectively (Table 1). In this species, the amount of the analytes isolated ranges from 2.28% with etiron; perfluoropropionic acid, TBDMS derivates and trimethylsilyl fluoride to 45.54% with acetic acid, phenylmethyl ester and decided name: 1-butanol, 2-methyl-; cyclobutane, ethyl- and benzylcarbamate (Table 1, Fig. 2).

3.3. *Isolona hexaloba*

In *I. hexaloba*, the retention time was between 3.36 and 33.78 min separated forty-four (44) compounds grouped into alkaloid, alkanes, alkenes, naphtalene, organo-compounds, carboxylic acid and its derivatives with M.wt ranging from 84.9 in methylene chloride formula CH_2Cl_2 to 709.48 in Tricyclo[4.2.1.0 (2,5)]non-7-ene,3, 4-di(tris(trimethylsilyloxy)silyl)- formula $C_{27}H_{64}O_6Si_8$. The following compounds: N-Methyl-1-adamantane-acetamide; Heptasiloxane,1,1, 3,3,5,5,7,7,9,9,11,11,13, 13-tetradecamethyl- and Octasiloxane,1,1,3,3,5,5,7,7,9, 9,11,11,13,13,15,15- hexadecamethyl- were replicated in *I. thonneri, I. zenkeri* and *I. Campanulata.*

A compound with a decided named Methyl stearate formula $C_{19}H_{38}O_2$ and M.wt of 298.50 was replicated in *I. zenkeri and I. campanulata* while Bis(2-ethylhexyl) phthalate formula $C_{24}H_{38}O_4$ with M.wt 390.56 was scored in *I. thonneri* and *I . zenkeri.* The most prominent were Carboxylic and its derivatives with about twenty-four (24) compounds (Table 1, Fig. 1).

3.4. *Isolona thonneri*

There are fifty-one (51) compounds identified in *I. thonneri* ranges from alcohol, alkaloid, alkanes, benzene, naphtalene, organo-compounds, carboxylic acids and its derivatives. The retention time for the dissociation of the compounds was between 3.44 min with 1-Butanol, 2-methyl-; cyclobutane, ethyl-; and decane, 2,2,3-trimethyl- to 33.86 min with hexestrol, 2TMS derivative; acridine-9-carbaldehyde and heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-. Most prominent compounds were the carboxylic acid and its derivatives with twenty-five and least compound was scored in benzene with decided name 1,3-benzodioxole, 4-methoxy-6-(2-nitro-1-propenyl)- formula $C_{11}H_{12}O_3$ and W.mt 192.21. The following compounds namely: 2-ethylacridine ($C_{15}H_{13}N$); benzo[h]quinoline, 2,4-dimethyl- ($C_{15}H_{13}N$); 2-(Acetoxymethyl)-3-(methoxycarbonyl) biphenylene ($C_{17}H_{14}O_4$); 3-quinoline carboxylic acid, 6,8-difluoro-4-hydroxy-, ethyl ester ($C_{12}H_9F_2NO_3$); 1,2,4-Triazine-3,5(2H,4H)-dione, 6-benzoylthio- ($C_7H_{11}N_3O_2$) and 3,3-Diisopropoxy-1,1,1,5,5,5-hexamethyl trisiloxane ($C_{12}H_{32}O_4Si_3$) were scored and duplicated in *I. hexaloba* and *I. campanulata* (Table 1, Fig. 1).

3.5. *Isolona zenkeri*

At the retention time 34.14min, thirty different (30) compounds were separated with major compounds in carboxylic and its derivative. The M.wt for these compounds ranged from 78.13 to 667.39 having sixteen (16) compounds of carboxylic and its derivatives, five (5) alkanes, two (2) alcohols and a compound each of alkaloid and alkene. A compound with M.wt136.15, formula $C_8H_8O_2$ and decided name benzoic acid, methyl ester was scored in *I. campanulata* and *I. thonneri*. Also in this species, a compound with M.wt 667.39, formula $C_{18}H_{54}O_9Si_9$ and decided name cyclononasiloxane, octadecamethyl- was scored only in *I. congolana* and *I. campanulata* (Table 1, Fig. 1).

3.6. *Isolona campanualata*

At a retention time of 34.56min thirty-eight (38) different compounds were separated and about 27 duplicate compound with decided name octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-, formula $C_{16}H_{48}O_7Si_8$ and W.mt 577.2 at different quantity and retention time. The molecular weights for these compounds ranged from 104.15to 709.48 including fourteen (14) unique compounds (Table 1). Compound with M.wt667.39, formula $C_{18}H_{54}O_9Si_9$ and decided name cyclononasiloxane, octadecamethyl-was scored in *I. congolana* and *I. zenkeri.* A compound with decided name Methyl stearate, formula $C_{19}H_{38}O_2$ and M.wt

298.50 was scored with *I. hexaloba* and *I. zenkeri*. Two compounds name pentadecanoic acid, 14-methyl-, methyl ester and 2-naphthalenemethanol, decahydro- alpha., alpha., 4a-trimethyl-8-methylene-, [2R-(2.alpha., 4a.alpha., 8a.beta.)]- were both scored in *I. zenkeri* (Table 1, Fig. 2).

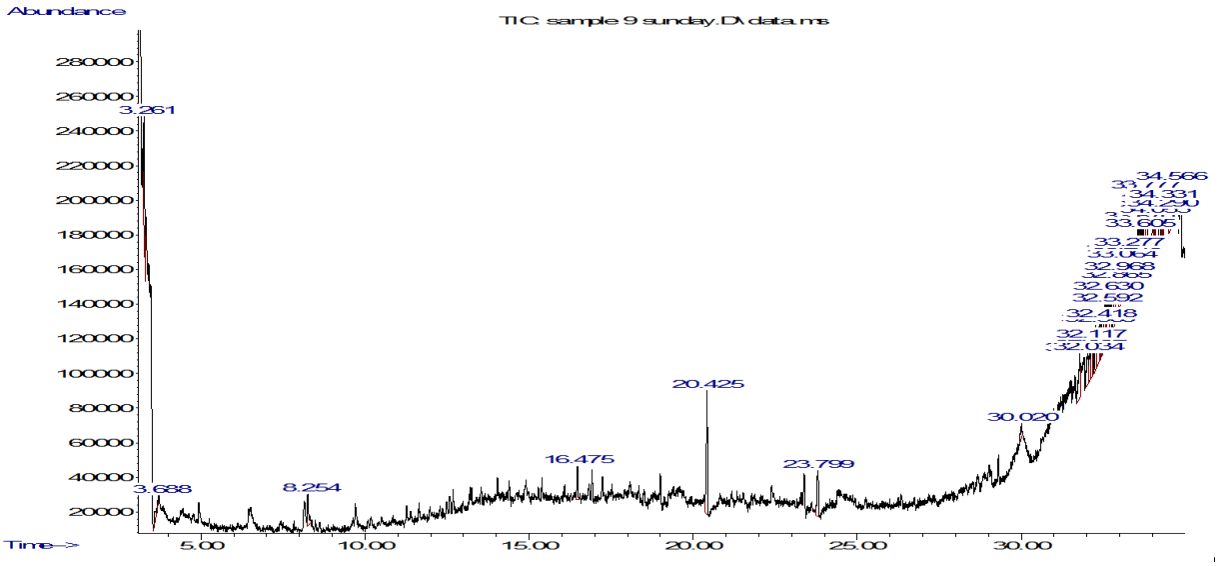

Figure 2. GC-MS Chromatogram of *I. campanualata*

3.7. Systematic implications of GC-MS analysis on *Isolona* species relationship

The distance values among the six (6) examined species showed evidence of similarity and differences using both the quantitative and qualitative phytochemical data. In general, the species varies from higher distance values to higher similarity (lower distance) values characterized by the species of the same genus indicated by the matrices (Figs. 4,5).

In a UPGMA distance tree based on the chemical constituents revealed by GC-MS between the six (6) *Isolona* species was presented. The distance matrix computed with Euclidean similarity index based on the quantitative characters of the constituents in each species (Fig. 4). The tree matrix produces clusters species and identified them to the specific. *I. thonneri* was well separated from all other species while the others were delineated within the sub-clusters. In figure 3, it was also clear in the scatter plot that *I. thonneri* has the highest distance from the centre. To corroborate the tree, the highest distance value level (9.576) was scored between *I. thonneri* and *I. congolana*. This was represented in the clustering tree. On the other hands, the highest similarity level was 2.911 scored in *I. hexaloba* and *I. campanulata.* The sub-cluster of *I. zenkeri*, *I. hexaloba* and *I. campanulata* was clearly grouped together in the scatter plot and closer to the centre. The distance between *I. zenkeri* and *I. hexaloba* was 4.168, whereas the similarity level scored in *I. campanulata* and *I. zenkeri* was 3.615 (Fig. 3).

The relationship between the examined species based on the analysis qualitative characters of chemical constituents as indicated by the RMSD coefficient tree constructed was illustrated in Figure 4. As observed in the quantitative analysis (Fig. 4). *I. thonneri* was also efficiently separated from the other *Isolona* species (Figure 5). A sub-cluster of the tree produced three species namely *I. hexaloba*, *I. campanulata* and *I. zenkeri* from the remaining taxa. Likewise in a cluster of *I. dewevrei* and I. *congolana* with genetic distance 5.367. The distance value between *I. zenkeri* and *I. thonneri* was 8.418 (Table 2).

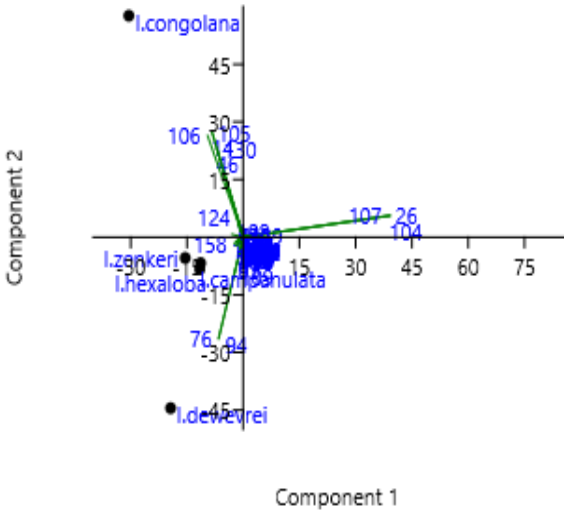

Figure 3. Scatter diagram based on the phyto-constituents of the six *Isolona* species

4. Discussion

Tetenyi (1987); Young et al. (1996); Akilan et al. (2014) and Gamal et al. (2017); have reported the systematics significance of phyto-constituents as an aid toward establishing the relationships among taxa below and above the species level. The GC-MS analysis separated all of the components in the examined samples and produced a specific spectral peak outputs. The total retention time to isolate the first compound and the last compound varies among the studied species. The differences in time taken for each chemical constituent to be dissociated are specific to the compounds and of great help in species identification.

Table 2. Matrix computed based on the analysis of variation in chemical constituents by GC-MS analysis for the *Isolona* species

	I. congolana	***I. dewevrei***	***I. hexaloba***	***I. thonneri***	***I. zenkeri***	***I. campanulata***
I. congolana	0	7.684	6.273	9.576	6.509	5.992
I. dewevrei		0	5.601	9.131	6.208	5.367
I. hexaloba			0	7.990	4.168	2.911
I.thonneri				0	8.418	7.815
I. zenkeri					0	3.615
I. campanulata						0

Cardoso et al. (2008) opined on the important of secondary metabolite in taxonomic questions. The size of the analytes determines the amount of the specific constituent isolated which can be a diagnostic character. The clear differences in chemical formula, Molecular weight, time taken to dissociate all the compounds, amount of compound produced at different time, number and shapes of the compounds in each species were all considered to delineate the six (6) species of *Isolona*. Faustino et al. (2017) profiled four species of *Calendula* L. by categorizing the phyto-chemicals into major compounds. In this study, the hundred and seventy-six (176) compounds were grouped into alcohol, alkaloid, alkenes, alkynes, benzenes, carboxylic and its derivatives, naphthalene and organo-compounds for additional identification. The absence or presence of these phyto-constituents among the studied *Isolona* species were profiled and used in grading and scoring each species, this established the interrelationship between them. The abundance of these compounds in number and amount of analytes were important in chemotaxonomy delimitation of *Isolona*. In all the species, carboxylic and its derivative were the most profound among the hundred and seventy-six compounds. It is noteworthy to mention that the present of Hexadecanoic acid, methyl ester was diagnostic for the genus *Isolona* having present in all the studied species.

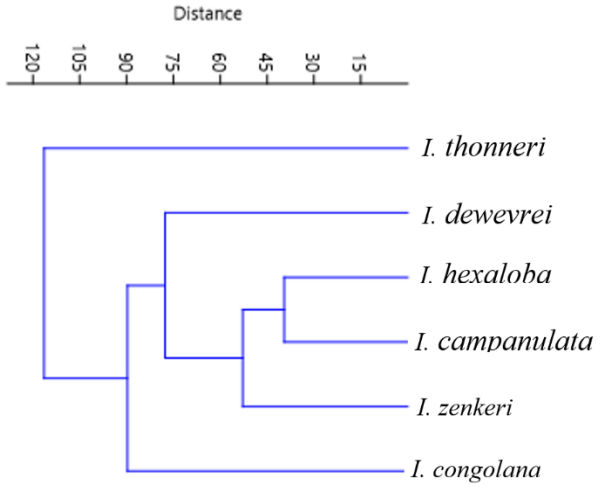

Figure 4. GC-MS analysis of UPGMA distant tree based on Euclidean similarity index showing the relationships among chemical constituents of *Isolona* species

Cardoso et al. (2008) and Mohy-UD-Din et al. (2010) investigated the phytoconstituents of the family Rubiaceae to analysis the taxonomic intricacies. The number of compounds produced was not the same in all the species studied. Graphical spectral peaks representation of each species revealed different phyto-compound at various retention times during dissociation. These were specific to the compound produced and scored. Gamal et al. (2017) categorized the compounds into six different retention times which was the basis for his work. In this 176 different compounds belonging to six (6) *Isolona* species were scored between 3.15 to 34.56 min. All the species except *I. zenkeri* has average of about 3.35 min to produce the first compound. The number of phytoconstituents in different species contributed to the determination of the rank of each species. Likewise, the profound majority of the compounds to be an extraction of carboxylic and its derivatives of the compounds suggest the important of such compounds in the chemotaxonomy of the genus.

In the cluster analysis of plant chemical using the RMSD and Euclidean index showed *I. thonneri* was efficiently separated from the remaining species. The response of scatter plot is in support of the trees where it clustered the trio of *I. zenkeri, I. hexaloba* and *I. campanulata.* This suggests the closeness of the three species because they shared more phyto-constituents than the rest of the species. The highest and lowest distance values by the matrix affirmed the relatedness among the six (6) species studied in this research.

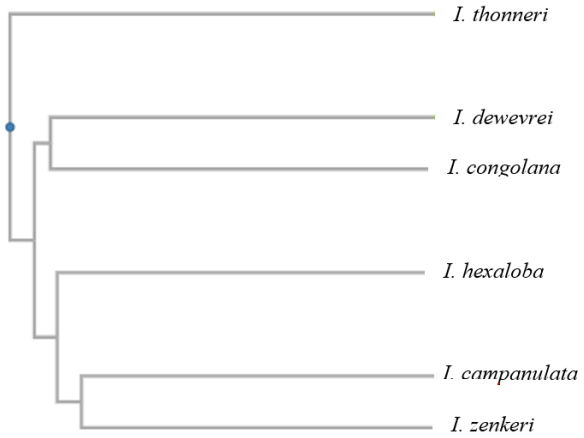

Figure 5. UPGMA distant tree constructed with RMSD coefficient showing the relationships among the *Isolona* species based on the qualitative analysis of chemical constituents

In conclusion, GC-MS analysis of methanol extract of six (6) *Isolona* species revealed one hundred and seventy-six (176) phytochemical constituents detected between 3.26 min to 34.56 min. Twenty-nine (29), thirty-two (32), forty-four (44), fifty-one (51), thirty (30) and thirty-eight (38) phyto-constituents were dissociated from *I. congolana, I. dewevrei, I. hexaloba, I. thonneri, I. zenkeri* and *I. campanulata*. The highest M.wt. was 741.5 scored in *I. congolana* at retention time of 34.52 min. and the lowest M.wt. was 84.15 in *I. thonneri* at retention 3.44

min. Hexadecanoic acid, methyl ester formula $C_{17}H_{34}O_2$ and M.wt of 270.4 was scored in all the six species studied. Similarity and distance matrices were calculated and two UPGMA distant trees constructed with RMSD and Euclidean similarity index to illustrate the relationships among the species based on the differences of chemical constituents. The overlapping phyto-constituents characters revealed the closeness of the taxa studied, this invariably will update the existing data in the genus and ultimately in the family.

Conflict of Interest

Authors have declared no conflict of interest.

Authors' Contribution

Kadiri AB and Olowokudejo JD contributed to manuscript development and literary work as supervisors of Adeniran SA who did data acquisition, writing and laboratory work.

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