ejpmr, 2019,6(12), 185-194

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

CHEMICAL COMPOSITION OF TEN ESSENTIAL OILS FROM *CALOPHYLLUM INOPHYLLUM* LINN AND THEIR TOXICITY AGAINST *ARTEMIA SALINA*

Emmanuel O. Ojah[1], Dorcas. O. Moronkola[1,2]*, Riccardo Petrelli[3], Franks Kamgang Nzekoue[3], Loredana Cappellacci,[3] Cristiano Giordani, [4,5] Marcel Jaspars[2] and Jioji N. Tabudravu[2,6]

[1]Department of Chemistry, University of Ibadan, Ibadan, Nigeria.
[2]Marine and Biodiversity Center, Chemistry Department, Meston Building, University of Aberdeen, Scotland, United Kingdom.
[3] School of Pharmacy, Medicinal Chemistry Unit, via S. Agostino 1, 62032 University of Camerino, Italy.
[4]Instituto de Física, Universidad de Antioquia, UdeA, Calle 70 No 52-21, Medellín-Colombia.
[5]Grupo Productos Naturales Marinos, Facultad de Ciencias Farmacéuticas y Alimentarias, Universidad de Antioquia, UdeA, Calle 70 No 52-21, Medellín-Colombia.
[6]School of Forensic and Applied Sciences, Faculty of Science and Technology, University of Central Lancashire, PR1 2HE, United Kingdom.

***Corresponding Author: Dorcas. O. Moronkola**

Marine and Biodiversity Center, Chemistry Department, Meston Building, University of Aberdeen, Scotland, United Kingdom.

Article Received on 14/10/2019 **Article Revised on 03/11/2019** **Article Accepted on 24/11/2019**

ABSTRACT

Essential oils from ten different parts of *Calophyllum inophyllum* were analyzed by Gas Chromatography-Mass Spectrometry (GC-MS) to study their chemical compositions. The yields were between 0.219 and 0.506 %. A total of 102 compounds were identified in the ten *C. inophyllum* essential oils, which are mostly monoterpenes, sesquiterpenes and their oxygenated derivatives. The numbers and percentages of identified compounds varied in the different parts of the plant: leaf (71, 54.94%), leaf stalk (22, 79.55%), flower (25, 51.24%), seed (25, 89.39%), seed-coat (69, 73.80%) fruit-pulp (15, 46.10%), stem wood (55, 59.40%), stem bark (9, 69.38%) root wood (51, 58.73%), and root bark (24, 74.66%). High content of cymene, terpinene, and limonene in the oils may be responsible for the vast ethno-medicinal applications of the plant. Toxicity experiments show that the oils were fairly toxic. Each part after 24 hours of exposure against *Artemia salina* gave the following LC_{50} values in µg/mL: leaf (68.8740 µg/mL), leaf-stalk (102.5692 µg/mL), flower (114.4410 µg/mL), seed (132.2324 µg/mL), seed coat (137.1206 µg/mL), fruit-pulp (135.0350 µg/mL) stem wood (126.1410 µg/mL), stem bark (149.7237 µg/mL), root wood (110.6539 µg/mL) and root bark (110.6539 µg/mL). The chemical compositions and toxicity levels of these ten *Calophyllum inophyllum* essential oils are reported for the first time in literature.

KEYWORDS: *Calophyllum inophyllum*, Guttiferae, Essential oils (EO), GC-MS, *Artemia salina*, Toxicity.

1. INTRODUCTION

The genus *Calophyllum* comprises of 180 to 200 species of which *Calophyllum inophyllum* is the most abundant species. It is widespread in tropical areas, which tolerates varied kinds of soil such as coastal sand, clay or even degraded soil.[1] The plant possesses a wide variety of uses ranging from traditional, medicinal and industrial applications; the wood has been used in general construction and boat building, as well as for flooring, furniture, musical instruments, handicrafts, and a variety of other purposes.[2] The stem bark has been identified as a potential anti-solar agent.[3] Several species of this genus are known to be used in folk medicine.[4] The extracted oil from the fruit is used as a remedy for sciatica, shingles, neuritis, rheumatism, ulcers, and skin diseases; while seed oil is reported to have medicinal and healing properties.[5] The dried leaf and its decoction are widely used in curing rheumatism, skin infections, cuts and sores.[6] Extracts from leaf and stem bark expressed antidiabetic, antihyperglycemic and antihyperlipidemic activities[7], while leaf extract was identified to inhibit oxidative stress.[8] Its fruits are effectively utilized in the treatment of dermatitis[9]; bark is locally utilized for treating vaginal disorders after childbirth, the passing of blood, gonorrhea, and internal haemorrhages.[10] Recently, *C. inophyllum* has been identified as the most suitable feedstock for the future generation of biodiesel.[11] Seed oil has been identified as highly potent for production of vegetable oil.[12]

The broad spectrum of *C. inophyllum* has been associated with the chemical composition of its different parts. The root is furnished with xanthones such as brasilixanthone, 1,3,5-trihydroxy-2- methoxy-xanthone, caloxanthone A, pyranojacareubin, caloxanthone B and tovopyrifolin[13,14] The genus *Calophyllum* has been

reported to be rich in coumarins,[15-17], triterpenoids[18,19] and flavonoids.[20] Several coumarins isolated from two *Calophyllum* species were found to inhibit HIV-1 replication and cytophaticity activities.[21,22] Xanthone derivative obtained from the root bark of *C. inophyllum* has been identified as antimicrobial and cytotoxic agent.[23] Five bioactive compounds isolated from *C. inophyllum L*. leaves namely mixture of calophyllic and isocalophyllic acids, 3-oxo-friedelin-28-oic acid, canophyllic acid, amentoflavone, and shikimic acid showed dose-dependent lipid-lowering activity in *in-vivo* experiments.[24] Calophyllolide a complex coumarin from *Calophyllum inophyllum L*. was reported as an anticoagulant and anti-inflammatory agent.[25,26] The plant has also been identified as a good anticancer agent.[27] Although the phytochemical constituents of some parts of the plant have been reported, no study has been performed to characterize essential oils composition from different parts of the plant, which this study is on.

Fruits and leaves are reported to be poisonous[28], hence it would be interesting to also assess the toxicity level of *C. inophyllum* essential oils. Brine shrimp (*Artemia salina*, the fairy shrimp or sea monkeys) lethality assay is an essential tool commonly employed in the preliminary assessments of the cytotoxic effect of plant extracts. *A. salina* has been used from immemorial time to establish the toxicity levels in substances.[29-33] The lethal concentration which is the concentration at 50% level (LC_{50}) expresses the extent of toxicity. LC_{50} above 1000 µg/mL implies a non-toxic property; LC_{50} between 500-1000 µg/mL signifies a less toxic property while LC_{50} between 100-500 µg/mL indicates a moderately toxic property. LC_{50} less than 100 µg/mL implies a high toxic property.[34] The toxicity level indicates the potency of using such natural products as larvicidal, insecticidal and many other broad-spectrum evaluations and assessments.

Therefore, this study aims to assess for the first time, the chemical compositions and toxicity levels of ten essential oils (EOs) from leaf, leaf-stalk, flower, seed, seed coat, fruit pulp, stem wood, stem-bark, root wood, and root bark of *C. inophyllum*.

The study would reveal more bioactive compounds, which corroborates the bioactivities of *C. inophyllum*, responsible for its wide ethno-medicinal uses.

2. MATERIALS AND METHODS

2.1 Plant materials

Fresh samples of *C. inophyllum* were collected from the trees growing in Botany Department, University of Ibadan, Ibadan, Oyo State, Nigeria. The samples were authenticated in the Herbarium, Department of Botany, University of Ibadan, Nigeria, where voucher samples were deposited with specimen voucher number UIH - 22659. The collection of the samples was done during the daytime. The plant was sorted into ten parts: leaf, stalk, flower, seed, seed coat, fruit pulp, stem wood, stem bark, root wood, and root bark.

2.2 Extraction of essential oils

Each separated part (leaf, stalk, flower, seed, seed coat, fruit-pulp, stem wood, stem bark, root wood, and root bark) of *C. inophyllum* was air-dried, pulverized and hydro-distilled for 3 hours in an all-glass Clevenger-type apparatus designed to British Pharmacopeia (BP) specifications. EOs were procured in 0.219 to 0.560% yields (Table 1). Each of the oils had a distinct characteristic pleasant smell. The EOs were refrigerated until further analyses were carried out.

2.3. Identification of Essential oils by Gas Chromatography-Mass Spectrometry (GC-MS) Analyses

GC-MS analyses were carried out by using an Agilent 7890B-5977B GC-MS (Santa Clara, CA, USA) system operating in the EI mode at 70 eV, using an HP-5MS capillary column (5% phenylmethyl polysiloxane, 30 m, 0.25 mm i.d., 0.1 µm film thickness) (J & W Scientific, Folsom), which was programmed with the following conditions: 60 °C for 4 min, then up to 4 °C/min to 160 °C, then 11 °C/min up to 280 °C, held for 15 min, finally 15 °C/min up to 300 °C. The carrier gas was helium at a flow rate of 1.2 ml/min; the injector temperature was 280 °C, while the transfer line temperature was 300 °C; injection volume: 1 µl; split ratio: 1:100; run time: 57 min; acquisition mass range: 29–400 amu. Identification of the essential oil components were based on their retention indices (experimentally determined using homologous series of C8–C30 alkanes), and by comparison of their mass spectral fragmentation patterns in computer matching against library Linear retention index and mass spectra taken from Adams and NIST 17[25] FFNSC2 and MAGGI libraries (Adams 2007; NIST 17 2017; FFNSC2 2012). Relative peak area percentages were obtained by peak area normalization without using correction factors and were the mean of three determinations with a RSD% in all cases below 10%.

2.4 Toxicity Assay (Brine shrimp Lethality test)

1g of Brine shrimp (*Artemia salina*) eggs (Sanders Great Salt Lake, Brine Shrimp Company L.C., U.S.A.) was aerated in 1L shallow rectangular dish seawater at room temperature (29-31 °C). Brine shrimp eggs were hatched in a shallow rectangular dish (150 mm × 5 mm), filled with sea water. A plastic divider with several holes was made at the middle of the dish to make two unequal compartments. The shrimp eggs were sprinkled into the larger compartment which was darkened, while the smaller compartment was illuminated. After 48 hours free swimming phototropic larvae (nauplii) were collected using Pasteur pipette from the illuminated compartment. The toxicity of the oils were tested at various concentrations viz. 10, 100, and 1000 µg/mL in sea water containing 2% DMSO (v/v). Ten nauplii were used in each test. Three replications were used for each concentration. A parallel series of tests with the standard potassium dichromate solution were tested and the blank control was always included. After 24 hours, survivors

were counted using a dissection microscope and the percentage of the mortality (%M) of each dose was calculated as compared with control.

Positive and negative control groups were used to validate the test method and ensure that the results obtained were only due to the activity of the test agent. Pure 2% DMSO and seawater were used as negative control while 2% DMSO, sea water and the Essential oil were used as positive control. After 24 hours, the total number of dead shrimps was counted and the lethal concentration at 50% level (LC_{50}) was determined by the Finney probit computer program.

3. RESULTS AND DISCUSSION

3.1. Percentage Yield

EOs obtained from the ten (10) parts of *C. inophyllum* gave characteristic odours (Herbal, Floral, woody). The oils were procured in 0.219 to 0.506 % yields (Table 1), with the highest yield from fruit pulp, which gave 0.560 %, while the root had the lowest yield (0.219%), which may be due to its high fiber content.

Table 1: Percentage (%) yield of essential oils of *Calophyllum inophyllum LINN*.

S/N	Plant parts	Weight of sample (g)	Weight of EO (g)	% yield of essential oils	Physical examination
1.	Leaf	800	2.66	0.333	Leafy
2.	Leaf-stalk	900	2.82	0.313	Herbal
3.	Flower	700	2.02	0.288	Floral
4.	Seed	950	2.90	0.305	Pleasant
5.	Seed coat	600	3.04	0.506	Nut-like
6.	Fruit pulp	550	3.08	0.560	Fruity
7.	Stem wood	850	2.90	0.341	Woody
8.	Stem Bark	900	2.76	0.307	Slightly choking
9.	Root wood	950	2.08	0.219	Woody
10.	Root Bark	900	2.51	0.279	Nut-like

3.2. Essential Oils composition

102 compounds were identified in the ten essential oils (EOs), which are mostly monoterpenes, sesquiterpenes, and their oxygenated derivatives. 71 compounds were characterized in leaf oil, which corresponded to 54.94% of the identified peaks, while 22 were identified in leaf-stalk (79.55%); 25 compounds were identified in flower oil (51.24%); 25 in seed coat (89.39%); 69 compounds in seed coat oil (73.80%); 15 compounds were characterized in fruit-pulp (46.10%); 55 in stem wood oil (59.40%); 9 in stem bark oil (69.38%); 51 compounds in root wood essential oil (58.73%); and 24 in root bark oil (74.66%). Compounds identified are presented in Table 2.

The predominant compounds in percentages (%) for each EO are: cis-cadina-1(6),4-diene (6.50), hexadecanal (6.16), and cis-calamenene (5.41) for leaf; limonene (23.79), γ-terpinene (13.06), and *p*-cymene (9.28) for leaf-stalk; cis-cadina-1(6),4-diene (15.42), β-Alaskene (9.63), and γ-Bisabolene (7.20) for flower; limonene (25.40), γ-terpinene (14.00), and *p*-cymene (10.03) for seed; limonene (16.85) γ-terpinene (9.82), and p-cymene (6.70) for seed-coat; cis-cadina-1(6)-4-diene (15.6%), β-alaskene (8.4%), and β-acoradiene for fruit-pulp; hexadecanal (6.87%), E-nerolidol, (5.86%) and 1,8-Cineole (5.63) for stem wood; hexadecanal (46.80), E-anethole (6.12), and limonene (3.24) for stem bark; n-hexadecanoic acid (9.86), E-nerolidol, (5.83) and α-Bisabolol (4.36) for root heartwood; and Cembrene-A-3Z (15.05) limonene (13.93), and hexadecanal (10.61) for root bark.

The oils are dominated by cymene, terpinene, and limonene. Cymene, which is present in eight of the oils, is a good antioxidant, anti-inflammatory, anti-nociceptive, anxiolytic, anticancer and antimicrobial agent[35-37], which are also the ethno-medicinal applications of *C. inophyllum*. In a recent *in vivo* investigation on an experimental animal model, p-cymene was found to increase the activity of antioxidant enzymes, thereby reduced oxidative stress[38]; also, high antimicrobial potential of *Carum copticum* EO was attributed to the abundance of cymene and terpinene.[39,40]

The high content of γ-terpinene in leaf-stalk (13.06%), seed coat (6.77%) and root bark (7.75%) EOs is responsible for the anti-inflammatory and antioxidant effects, thus supporting the plant's anti-osteoarthritic activity. Terpinene in *Hyptis* species inhibited gastric lesions, reduced volume, and acidity of the gastric juice and increased gastric wall mucus.[41] Limonene, which is in an appreciable amount in stem heartwood (23.79%), stem bark (3.24%), and root bark (13.93%) essential oils of *C. inophyllum* is known to have sedative and stimulant effects in *Lippia alba*.[42,43]

Consumption of diets containing fruits and vegetables rich in monoterpenes, such as limonene, is known to reduce the risk of developing cancer of the colon, mammary gland, liver, pancreas, and lung. Limonene known to possess high anticancer properties.[44-45], is abundant in *C. inophyllum*: leaf-stalk (25.40%), seed (25.40%) and root bark (13.93%) EOs. The presence of non-ubiquitous compounds such as β-alaskene, β-

acoradiene, E-anethole is a unique feature of EOs from *C. inophyllum* (Table 2).

The mass spectrum of a compound eluting at a retention time of 23.66 minutes is in appreciable amount in fruit pulp (46.83%), flower (44.96%), leaf (19.16%) and seed coat (8.2%) EOs. The compound showed very similar fragmentation pattern with cis-thujopsene. (cyclopropane-*d*-naphthalene) (Figs.1a and 1b). This compound is likely the trans-isomer of cis-thujopsene which differs only in the stereo-centers, thus strengthening the broad spectrum of biological activity exhibited by *C. inophyllum*. The mass spectrum for another notable compound at retention time, 36.87 minutes, which was not identified, but in an appreciable amount in leaf oil (15.85%) is presented in Fig.2. With such a unique fragmentation pattern, we suggest that this compound is new.

Table 2: Chemical composition of ten Essential Oils from *Calophyllum inophyllum LINN*

S/N.	RI	Compound	Leaf	Stalk	Flower	Seed	Seed coat	Fruit pulp	Stem wood	Stem bark	Root wood	Root bark
1.	784	3-Hexanone	0.13	0.05	-	-	4.76	-	0.14	1.72	0.17	0.07
2.	789	2-Hexanone	0.50	0.07	-	-	3.21	-	1.00	1.85	0.82	0.12
3.	793	3-Hexanol	0.09	-	-	-	3.16	-	0.12	-	0.11	-
4.	800	Hexanal	0.92	0.42	0.06	0.67	3.82	-	0.84	-	1.24	0.16
5.	844	(E)-2-Hexenal	1.05	-	-	-	-	-	0.06	-	0.10	-
6.	846	3-Hexen-1-ol	0.23	-	-	-	-	-	-	-	-	-
7.	857	(E)-2-Hexen-1-ol,	0.99	-	-	-	-	-	-	-	-	-
8.	859	n-Hexanol	3.33	-	-	-	-	-	0.14	-	0.09	
9.	923	Acetonyl acetone	0.34	-	-	-	-	-	2.71		0.71	-
10.	926	α-Thujene	-	1.96	-	2.34	-	-	0.35	-	-	1.09
11.	932	α-Pinene	0.21	7.88	0.07	9.39	-	-	1.28	-	0.02	4.44
12.	945	Acetoxyhexane	-	-	-	-	4.80	-	-	-	-	-
13.	947	Camphene	-	0.62	-	0.70	-	-	-	-	-	0.39
14.	958	Benzaldehyde	0.09	-	-	-	-			-	-	0.5
15.	969	Sabinene	-	0.72	-	0.93	-	-	-	-	-	-
16.	975	β-Pinene	0.11	4.41	-	5.13			0.73			2.54
17.	978	1-Octen-3-ol	0.04	-	-	-	-	-	0.54	-	0.25	-
18.	987	6-methyl-5-Hepten-2-one,	0.10	-	-	-	-	-	0.11	-	0.17	-
19.	991	Myrcene	0.30	2.81	-	3.21	-	-	1.19	-	-	1.67
20.	1002	trans-2-(2-Pentenyl)furan	0.05	-	-	-	-	-	-	-	-	-
21.	1004	α-Phellandrene	-	0.27	-	0.32	-	-	0.26	-	-	-
22.	1010	δ-3-Carene	0.08	0.36	-	0.41	-	-	0.13	-	0.21	-
23.	1016	α-Terpinene	0.09	2.40	-	2.54	-	-	0.39	-	-	1.41
24.	1024	p-Cymene	0.34	9.28	0.06	10.03	8.50	-	1.42	2.29	-	5.39
25.	1028	Limonene	0.75	23.79	0.18	25.4	9.71	-	3.47	3.24	-	13.93
26.	1030	1,8-Cineole	0.30	5.33	-	5.69	-	-	5.63	-	0.39	3.54
27.	1034	2,2,6-trimethyl Cyclohexanone,	0.05	-	-			-	-	-	-	-
28.	1039	β-Ocimene	-	0.56	-	0.65	-	-	0.11	-	-	0.36
29.	1043	Benzeneacetaldehyde	0.08	-	-	-	-	-	-	-	-	-
30.	1049	(E)- β-Ocimene		0.84	-	0.90	-	-	0.15		-	0.51
31.	1058	γ-Terpinene	0.56	13.06	0.10	14.00	6.77	-	2.14	2.95	0.27	7.75
32.	1065	Acetophenone	0.61	-	-	-	-		0.43		0.21	-
33.	1071	1-Octanol	-	-	-	-	-	-	-		0.31	-
34.	1087	Terpinolene	-	0.70	-	0.84	-	-	-		-	0.53
35.	1100	Linalool	0.12	0.63	-	0.62	-	-	2.62		1.96	0.46
36.	1105	Nonanal	0.31	-	-	-	-	-	-		1.79	-
37.	1116	α-Cyclocitral	0.04	-	-	-	-	-	-		-	-
38.	1134	2,2,6-trimethyl-Cyclohexanone.	-	-	-	-	-	-	0.03		0.02	-
39.	1143	Camphor	-	-	-	0.29	-	-	0.07		-	-
40.	1160	(E)-2-Nonenal	0.08	-	-	-	-	-	0.33		0.69	-
41.	1176	Terpinen-4-ol	-	-	-	-	-	-	1.34		0.24	-
42.	1189	α-Terpineol	-	-	-	-	-	-	1.48		1.30	-
43.	1193	Methyl salicylate	0.08	-	-	-	-	-	-		-	-
44.	1195	Myrtenol	-	-	-	-	-	-	-		0.64	-
45.	1197	Methyl chavicol	-	0.59	-	0.73	-	-	0.09		-	-

46.	1198	Safranal	0.10	-	-	-	-	-	-		-	0.06
47.	1206	Decanal	0.09	-	-	-	-	-	0.46		0.73	-
48.	1220	β-Cyclocitral	0.26	-	-	-	-	-	-		-	-
49.	1238	Ascaridole	-	-	-	-	-	-	0.08		-	-
50.	1258	Edulan II	1.05	-	-	-	-	-	1.07		0.99	-
51.	1262	2-Decenal	0.11	-	-	-	-	-	0.09		0.08	-
52.	1271	α-Citral	0.04	-	-	-	-	-	0.12		0.14	-
53.	1285	E Anethole	0.13	2.80	-	3.66	25.45	-	0.87	6.12	-	2.04
54.	1293	(E,Z)-2,4-Decadienal	-	-	-	-	-	-	-		0.55	-
55.	1301	Carvacrol	-	-	-	0.42	-	-	0.12		-	-
56.	1314	Edulan I	1.69	-	-	-	-	-	-		-	-
57.	1316	(E,E)-2,4-Decadienal	-	-	-	-	-	-	-		1.34	-
58.	1350	α-Cubebene		-	0.07	-	-	-	-		-	-
59.	1352	1,2-dihydro-1,1,6-trimethyl Naphthalene.	0.14	-	-	-	-	-	-		-	-
60.	1363	2-Undecenal	0.07	-	-	-	-	-	-		-	-
61.	1367	Cyclosativene	0.04	-	-	-	-	-	-		-	-
62.	1376	Copaene	0.17	-	0.31	-	-	0.40	0.10		0.15	-
63.	1382	(3Z)-3-Hexenyl hexanoate	0.05	-	-	-	-	-	-		-	-
64.	1384	β-Bourbonene	0.06	-	0.27	-	-	-	-		-	-
65.	1387	n-Hexyl hexanoate	0.77	-	-							-
66.	1391	7-epi-Sesquithujene	0.47	-	0.58			0.40	-		-	-
67.	1399	Cyperene		-	-	-	-	-	-		-	1.14
68.	1400	Tetradecane	0.10	-	-	-	-	-	-		4.19	-
69.	1405	Methyl eugenol	0.03	-	-	-	-	-	0.05		0.10	-
70.	1413	β-Cedrene	0.21	-	0.69	-	-	0.80	-		-	-
71.	1424	β-Copaene	-	-	-	-	-	0.20	-		-	-
72.	1428	α-Ionone	0.44	-	-	-	-	-	-		-	-
73.	1454	6,10-dimethyl 5,9-Undecadien-2-one.	0.89	-	0.09	-	-	-	0.39		0.97	-
74.	1458	β-Farnesene	0.15	-	-	-	-	0.3	-		-	-
75.	1463	Cis-Cadina 1,6 4 diene	6.50	-	15.42	0.37	-	15.60	0.45		0.84	-
76.	1467	β-Acoradiene	2.54	-	5.72	0.15	-	5.60	0.25		-	-
77.	1477	γ-Muurolene	0.34	-	0.22	-	-	0.70	0.15		1.11	-
78.	1481	Germacrene	0.20	-	3.74	-	-	1.30	-		-	-
79.	1486	(E)-β-Ionone	1.94	-	-	-	-	-	0.07		1.31	
80.	1496	β-Alaskene	2.73	-	9.63	-	-	8.40	0.44		1.56	
81.	1509	β-Bisabolene	0.16	-	0.28	-	-	-	-		-	-
82.	1516	γ -Bisabolene	-	-	7.20	-	-	4.70	-		-	-
83.	1517	(Z)-γ-Bisabolene	2.00	-	-	-	-	-	-		1.21	-
84.	1524	δ-Cadinene	1.20	-	0.86			2.00	0.27		0.82	-
85.	1534	Cis-Calamenene	5.41	-	1.88	-	-	5.70	0.65		5.83	-
86.	1565	E-Nerolidol,							5.86		-	
87.	1580	(3E,7E)-4,8,12-Trimethyltrideca-1,3,7,11-tetraene	0.20	-	-	-	-	-	-		0.92	-
88.	1660	Neointermedeol	-	-	-	-	-	-	-		1.48	-
89.	1682	(Z)-3-Heptadecene,	0.77	-	-	-	-	-	-		-	-
90.	1689	α-Bisabolol	0.28	-	0.50	-	-	-	-		4.36	-
91.	1818	Hexadecanal	6.16		2.54	-	3.62	-	6.87	46.80		10.61
92.	1848	hexahydrofarnesyl acetone	0.77	-	-	-	-	-	0.18		0.72	-
93.	1973	Cembrene A 3Z	-	-	-	-	-	-	-		-	15.05
94.	1881	1-Hexadecanol	1.53	-	0.11	-	-	-	5.40	4.41	3.04	-
95.	1922	Farnesyl acetone	0.63	-	-	-	-	-	-		1.12	-
96.	1974	n-Hexadecanoic acid	-	-	-	-	-	-	-		9.86	-
97.	1997	9-Octadecenal	1.35	-	0.19	-	-	-	2.95		0.67	-
98.	2085	n-Octadecanol										0.90
99.	2086	2-Octadecen-1-ol	1.20	-	0.47	-	-	-	2.74		0.86	-
100.	2496	Pentacosane	-	-	-	-	-	-	-		1.08	-

101.	2599	Hexacosane	-	-	-	-	-	-	0.24		0.67	-
102.	2900	Nonacosane							0.23		0.32	
		% Identified	54.94	79.55	51.24	89.39	73.80	46.10	59.40	69.38	58.73	74.66

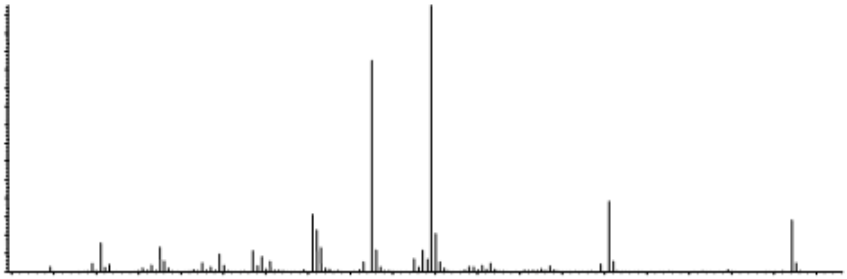


Figure 1a: Mass spectrum for compound with retention time 23.66 in Leaf (19.16%), Seed coat (8.02%) Fruit pulp (46.83%), and Flower (44.96%).

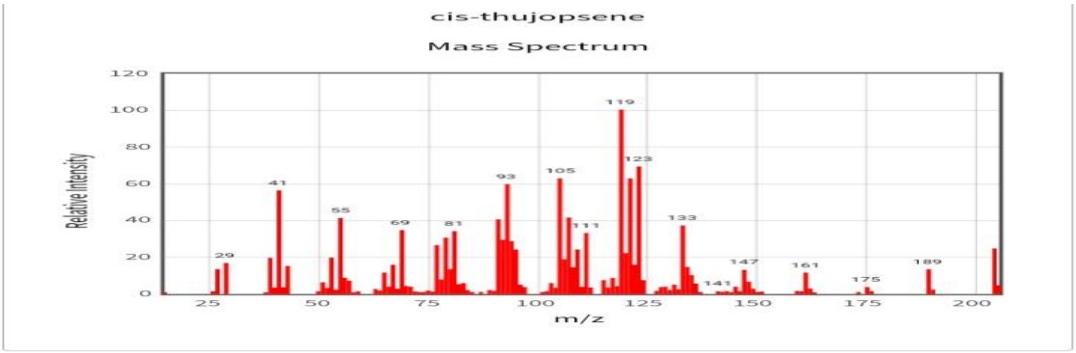


Figure 1b: Mass spectrum showing fragmentation patterns of cis-thujopsene.

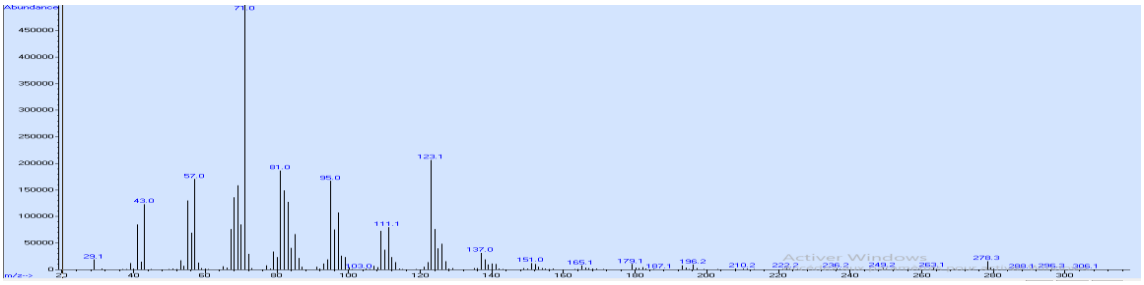


Figure 2: Mass spectrum for non-identified compound with retention time 36.87 in leaf oil (15.85%).

3.3. Brine Shrimp Lethality Assay

The brine shrimp lethality assay was used as a tool for the preliminary assessments of the toxicity levels of the EOs. The oils were analyzed for their toxicity levels after 24 hours of exposure against *Artemia salina* with the following LC_{50} values: leaf (68.8740 µg/mL), stalk (102.5692 µg/mL), flower (114.441 µg/mL), seed (132.2324 µg/mL), seed coat (137.1206 µg/mL), fruit-pulp (126.1410 µg/mL), stem wood (126.141 µg/mL), stem bark (149.7237 µg/mL), root wood (110.6539 µg/mL) and root bark (110.6539 µg/mL), respectively (Table 3). The LC_{50} of the standard cytotoxic agent $K_2Cr_2O_7$ was also evaluated as 110.6539 µg/mL. No mortality was recorded in both positive and negative controls. This is carried out to determine the lethal concentration at 50% level of toxicity (LC_{50}).

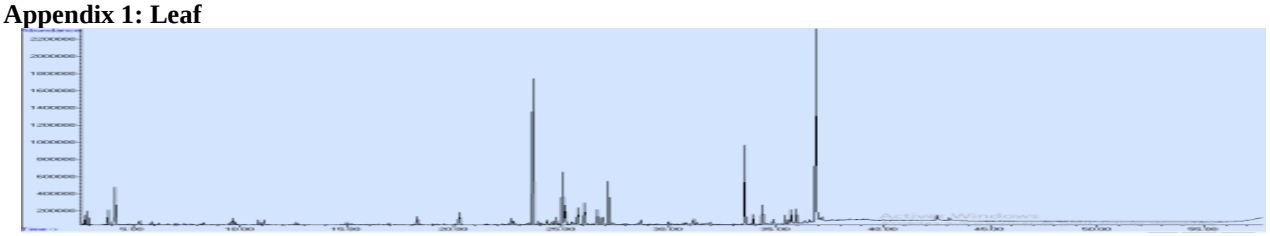
LC_{50} above 1000 µg/mL implies a non-toxic property; LC_{50} between 500-1000 µg/mL implies a less toxic property, while LC_{50} between 100-500 µg/mL implies a moderately toxic property. LC_{50} less than 100 µg/mL imply a high toxic property. Our results show that the leaf EO is the most toxic with LC_{50} value of 68.8740 µg/mL compared to the other nine oils, which are moderately toxic when compared with both positive and negative standards. Our findings further corroborate earlier reports in the literature.[28] It implies that the Leaf EO would possess higher larvicidal, insecticidal and other biological properties.

Table 3: The result of Brine shrimp lethality bioassay for essential oils from ten (10) parts of *C. inophyllum*.

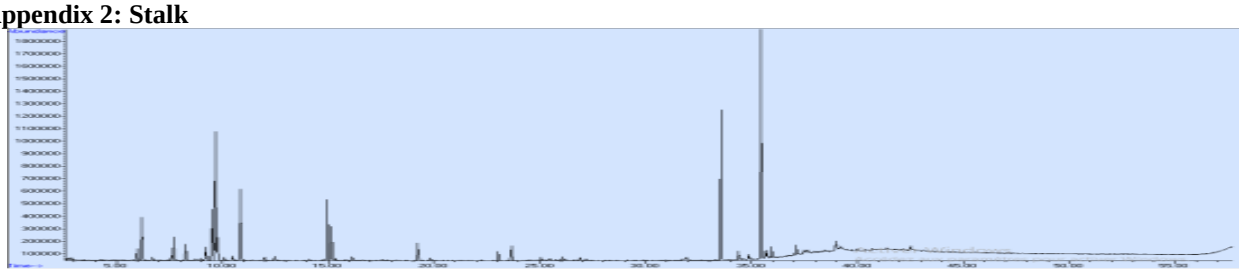
Part	1000 µg/mL	100 µg/mL	10 µg/mL	Lower limit µg/mL	Upper limit µg/mL	LC_{50} µg/mL	G
Leaf	100.00	40.00	23.33	35.4967	125.9052	68.8740	0.1225
Stalk	100.00	36.66	10.00	60.0776	171.2537	102.5692	0.1076
Flower	100.00	43.33	0.00	74.2648	178.4152	114.441	0.1671
Seed	96.67	40.00	0.00	83.1965	212.9240	132.2324	0.1459
Seed coat	100.00	33.33	10.00	82.8949	223.4220	137.1206	0.1137
Fruit pulp	100.00	30.00	10.00	79.8033	228.9448	135.035	0.1126
Stem wood	100.00	26.67	10.00	74.1654	212.6596	126.141	0.1110
Stem Bark	100.00	30.00	0.00	98.7794	238.9873	149.7237	0.1896
Root wood	100.00	43.33	0.00	64.2379	187.6848	110.6539	0.1042
Root Bark	96.66	36.66	10.00	64.2379	187.6848	110.6539	0.1042
$K_2Cr_2O_7$	100.00	43.33	0.00	64.2379	187.6848	110.6539	0.1042
Controls	0	0	0	0	0	0	

Values are the mean of triplicate studies (mean ± SEM), N = 10 (no. of shrimps). Score for LC50: Highly-toxic < 100 µg/mL, Moderately toxic- 100-500 µg/mL, Less toxic- 500-1000 µg/mL Non-toxic > 1000 µg/mL.

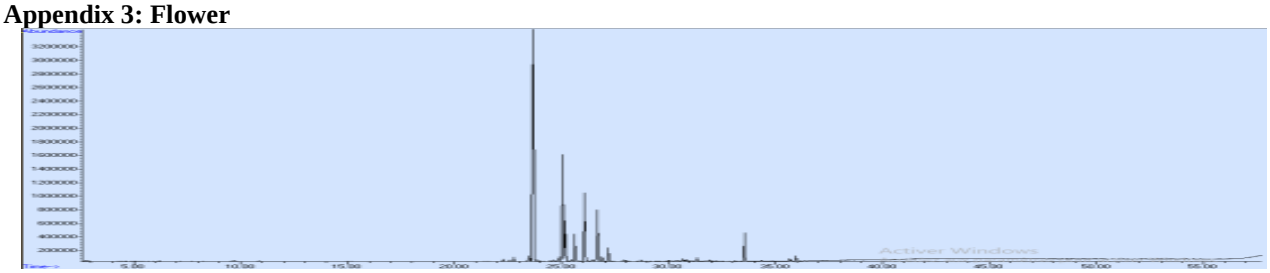
Appendix 1: Leaf



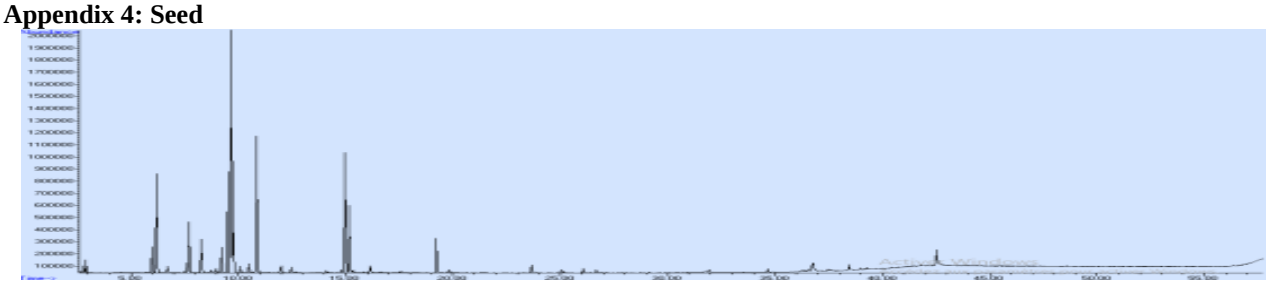
Appendix 2: Stalk



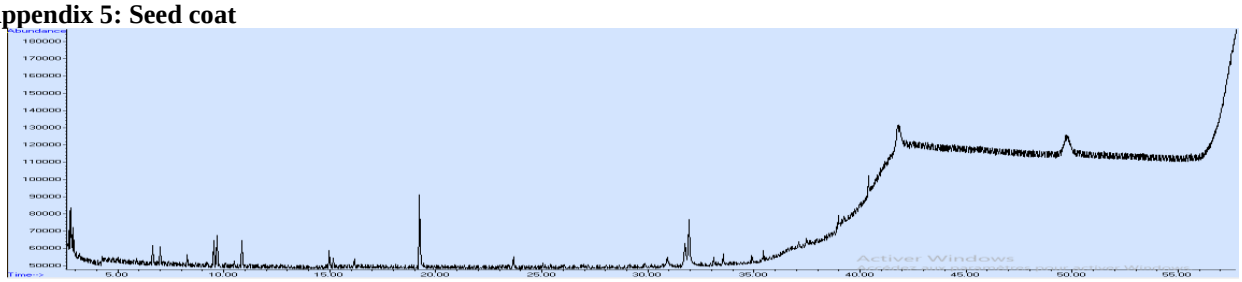
Appendix 3: Flower



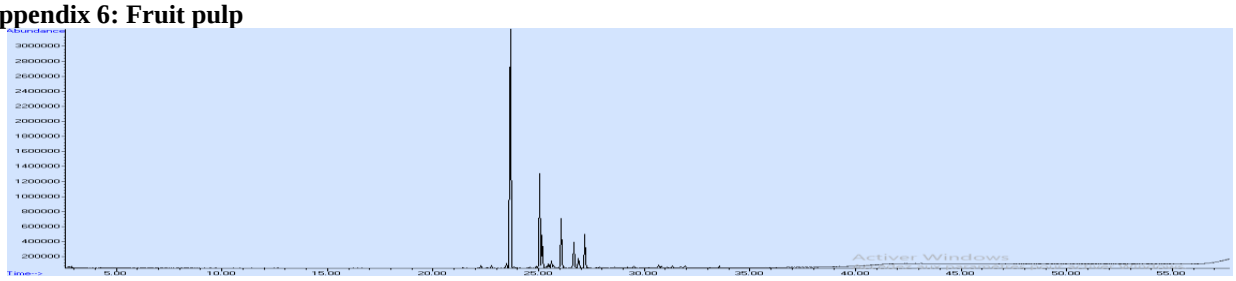
Appendix 4: Seed



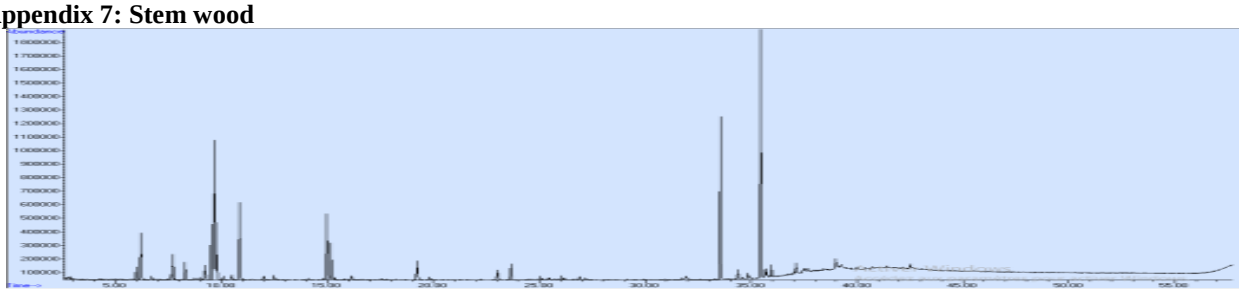
Appendix 5: Seed coat



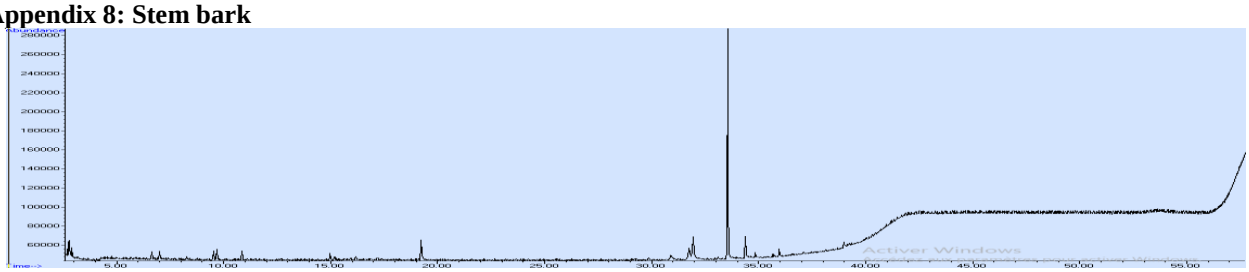
Appendix 6: Fruit pulp



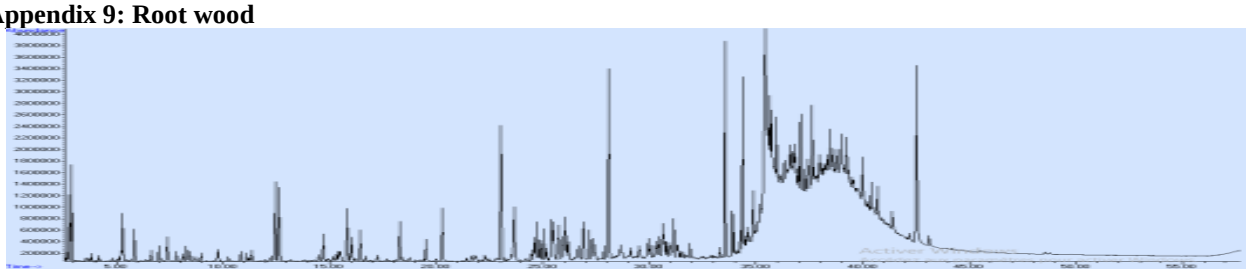
Appendix 7: Stem wood



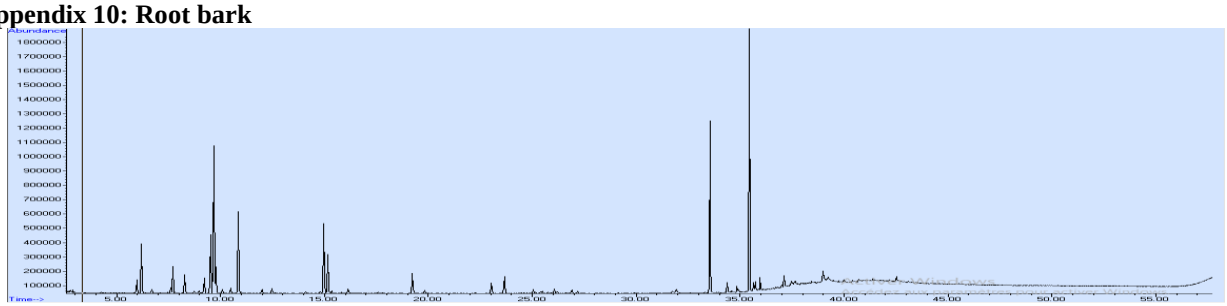
Appendix 8: Stem bark



Appendix 9: Root wood



Appendix 10: Root bark



4. CONCLUSION

We have been able to identify 102 compounds in the ten essential oils of *Calophyllum inophyllum* as well as assessing their toxicity levels, which is reported for the first time in literature. The essentials oils are rich in monoterpenes and sesquiterpenes. They have been identified as good sources of limonene, p-cymene, γ-terpinene, which, accounts for the vast ethno-medicinal applications of the plant. Toxicity assessment showed the oils are fairly toxic with LC_{50} mean values between 100-500 µg/mL. This is an indication that the oils have prospect to be applied as larvicidal or pesticidal and other related biological controls.

5. ACKNOWLEDGMENTS

We acknowledge the use of J laboratory facilities, Chemistry Department, University of Ibadan, Nigeria in essential oil extractions as well as postdoctoral fellowship ACTF DWS (honorary ID number: 42015006), utilized by DOMoronkola in Aberdeen UK, with Italian collaborations which were avenues for the use of state-of-the-art analytical equipment.

Conflict of Interest Disclosure

The authors declare no conflict of interest.

6. REFERENCES

1. Dweck AC, Meadowst T. Tamanu (Calophyllum inophyllum) – The African, Asian, Polynesian and Pacific Panacea. Int. J. Cosmetic Sci, 2002; 24: 1-8.
2. Shalan F, Locksley HD. Xanthones in the heartwood of *Calophyllum inophyllum*; A geographical survey. Phytochemistry, 1971; 10(3): 603-606.
3. Daveedu T, Prasad K. Phytochemical examination and anti-solar activity of *Calophyllum inophyllum* International Journal of Pharmacy and Pharmaceutical Science Research, 2014; 4(4): 76-77.
4. Dharmaratne HRW, and Wanigasekera WMAP. Xanthones from root bark of *Calophyllum thwaitesii*. Phytochemistry, 1996; 42: 249-250.
5 Burkhil HM. The useful plants of west tropical Africa. Edn 2, Families E-I. XX Royal Botanic Gardens Kew, 1994; 2: 522.
6 Uma SM, Murthy PN, Sudhir KS, Kanhu CS. Formulation and evaluation of herbal tablet containing methanolic extract of *Calophyllum inophyllum*. International Journal of Pharmacy, 2012; 2(1): 181-186.
7 Silpa S, Anees, Imran S. Phytochemical screening and evaluation of anti-hyperglycemic and anti-hyperlipidimic activity of methanolic extracts of *Calophyllum inophyllum* on Albino Wistar Rats International Journal of advanced research, 2014; 2(8): 743-752
8 Varsha G, Uma MB, Ramasamy M, Karunanithi M. Effect of Ethanolic Extract of *Calophyllum inophyllum* Leaves on Oxidative stress Complications in Mouse Model. Asian Journal of Pharmaceutical and Clinical Research, 2016; 9(3): 250-252.
9 Yu LU, Young WJ. Inhibitory effects of *Calophyllum inophyllum* extract on atopic dermatitis induced by DNCB in mouse. AJPCT, 2016; 4(6): 165-173.
10 Burkhil HM. The useful plants of west tropical Africa. Edn 2, Families E-I. XX Royal Botanic Gardens Kew, 1994; 2: 522.
11 Hathurusingha HMSD, Ashwath N. Beauty leaf (*Calophyllum inophyllum* L.) tree: A tree with great economic potential. In: Proceedings of 12th International Forestry and Environment Symposium, Kalutara, SriLanka, 2007; 20.
12 Muhammed F, Sri NB, Elvi R. The potential of nyamplung (*Calophyllum inophyllum* L.) seed oil as biodiesel feedstock: effect of seed moisture content and particle size on oil yield. 2nd International Conference on Sustainable Energy Engineering and Application, ICSEEA. Elsevier Energy procedia, 2015; 68: 177-185.
13 Kijjoa A, Jos M, Gonzalez TG, Pinto MM, Damas AM, Mondranondra IO, Silva AMS. and Herz W. Xanthones from *Calophyllum teysimannii*. Phytochemistry, 2000; 55: 833-836.
14 Ee GCL, Jong VYM, Sukari MA, Rahmani M and Kua ASM. Xanthones from *Calophyllum inophyllum* Journal of science and Technology, 2009; 17(2): 307-312.
15 Kashman YK, Gustafson R, Fuller RW, Cardellina JH, McMahon JB, Currens MJ, Buckheit RW, Hughes SH, Cragg GM. and Boyd MR. The Calanolide, a novel HIV-Inhibitory class of coumarin derivatives from the tropical rainforest tree. *Calophyllum lanigerum*. Journal of Medicinal Chemistry, 1992; 35: 2735-2743.
16 McKee TC, Richard WF, Conni DC, John HC, Robert JG, Benjamin LK, James BM and Michael R. New pyranocoumarins isolated from *Calophyllum lanigerum* and *Calophyllum teysmannii*. Journal of Natural Product, 1996; 59: 754-758.
17 Pengsuparp T, Serit M, Hughes SH, Soejarto DD, and Pezzuto, JM. Specific inhibition of human immunodeficiency virus type 1 reverse transcriptase mediated by soulattrolide, a coumarin isolated from the latex of *Calophyllum tesmannii*. Journal of Natural Products, 1996; 59: 839-842.
18 Govindachari TR, Vishwanathan N, Pai BR, Ramadas RU, Srinivasan M. Triterpenes of *C. inophyllum* Linn. Tetrahedron Lett, 1967; 23: 1901-1910.
19 Hang NTM, Chien NQ, Hung NV. Triterpenes from the leaves of Vietnamese plant *C. inophyllum* L. J Chem, 2006; 44: 115-118.
20 Li YZ, Li LZ, Hua HM, Li ZG, Liu MS. Studies on flavonoids from stems and leaves of *C. inophyllum*. Journal of Chinese Materia Medica, 2007; 32: 692-694.
21 Patil AD, Freyer AJ, Eggleston DS, Haltiwanger RC, Bean MF. The inophyllum's novel inhibitors of

HIV-1 reverse transcriptase isolated from the Malaysian tree, *C. inophyllum* Linn. J Med Chem, 1993; 36: 4131-38.
22 Spino C, Marco D, Subramaniam S. Anti-HIV coumarins from *Calophyllum* seed oil. Bioorg Med Chem Lett, 1998; 8: 3475-3478.
23 Marie C, Yimdjo, Anatole G, Azebaze AE, Nkengfack A. Michele Meyer, Bernard B, Zacharias T. Fomum a Antimicrobial and cytotoxic agents from *Calophyllum inophyllum* 2004 Phtochemisty, 2004; 65: 2789–2795
24 Janki P, Atul S, Khanna AK, Bhatia G, Awasthi SK, Narender T. Antidyslipidemic and antioxidant activity of the constituents isolated from the leaves of *Calophyllum inophyllum.* Phytomedicine, 2012; 19(14): 1245-1249.
25 Arora RB, Mathur CN, Seth SD. Calophyllolide, a complex coumarin anticoagulant from *C. inophyllum* Linn. J Pharm Pharmacol, 1962; 14: 534-535.
26 Bhalla TN, Saxena RC, Nigam SK, Misra G, Bhargava KP. Calophyllolide- A new nonsteroidal anti-inflammatory agent. Ind J Med Res, 1980; 72: 762-765.
27 Itoigawa M, Ito C, Tan HTW, Kuchide M, Tokuda H. Cancer chemo-preventive agents, 4-phenylcoumarins from *C. inophyllum*. Cancer Lett, 2001; 169: 15-19.
28 Austin DF. Poisonous plants of southern Florida, 9p. 1998. http://www.fau.edu/divdept/science/envsci/poison-pl.html.
29 Ghosh A, Banik S, Islam M. In vitro thrombolytic, anthelmintic, anti-oxidant and cytotoxic activity with phytochemical screening of methanolic extract of *Xanthium indicum* leaves. Bangladesh J Pharmacol, 2015; 10: 854-59.
30 Kibiti C, Afolayan A. Antifungal activity and brine shrimp toxicity assessment of *Bulbine abyssinica* used in the folk medicine in the Eastern Cape Province, South Africa. Bangladesh J Pharmacol. 2016; 11: 469-477.
31 Oberlies NH, Rogers LL, Martin JM, McLaughlin JH. Cytotoxic and insecticidal constituents of the unripe fruit of *Persea americana*. J Nat Prod, 1998; 61: 781-85.
32 Sufian M, Haque M. Cytotoxic, thrombolytic, membrane stabilizing and anti-oxidant activities of *Hygrophila schulli*. Bangladesh J Pharmacol, 2015; 10: 692-96.
33 Syahmi ARM, Vijayaratna S, Sasidharan S, Latha LY, Kwan YP, Lau YL, Shin LN, Chen Y. Acute oral toxicity and brine shrimp lethality of *Elaeis guinennsis* Jacq., (oil palm leaf) methanol extract. Molecules, 2010; 15: 8111-21.
34 Sherifat A., Akinsola A., and Guido F., Chemical constituents, toxicity and antimicrobial activities of the essential oil from the leaves of *tectona grandis* Elsivier Jounal of Biotechnology, 2013; 16: 16795-16798
35 DeOliveira TM, deCarvalho RBF, daCosta IHF, de Oliveira GAL, deSouza AA, deLima SG, deFreitas RM, Evaluation of p-cymene, a natural antioxidant. Pharm. Biol, 2015; 53: 423-428.
36 Quintans-Junior L, Moreira JC, Pasquali MA, Rabie SM, Pires AS, Schroder R, Rabelo TK, Santos JP, Lima PS, Cavalcanti SC. Antinociceptive Activity and Redox profile of the Monoterpenes(+-Camphene, p-Cymene, and Geranyl Acetate in Experimental Models ISRN 2013:459530.
37 DeOliveira TM, deCarvalho RBF, daCosta IHF, de Oliveira GAL, deSouza AA, deLima SG, deFreitas RM, Evaluation of p-cymene, a natural antioxidant. Pharm. Biol, 2015; 53: 423-428.
38 Quintans-Junior L, Moreira JC, Pasquali MA, Rabie SM, Pires AS, Schroder R, Rabelo TK, Santos JP, Lima PS, Cavalcanti SC, Antinociceptive Activity and Redox profile of the Monoterpenes(+-Camphene, p-Cymene, and Geranyl Acetate in Experimental Models ISRN 2013:459530.
39 Hassan W, Gul S, Noreen H, ShahZ, MI, and Zaman B. Chemical Composition, Essential oil Characterization and Antimicrobial Activity of *Carum copticum.* Vitam Miner, 2016; 5: 139.
40 Marino M, Bersani C, Comi G. Antimocrobial activity of the essential oils of *Thymus Vulgaris* L. measured using a biompedometric method. J Food prot, 1999; 62: 1017-1023.
41 Marcelo AP, Rafael MM, Lucio RLD. Gastroprotective effect of alpha-pinene and its correlation with antiulcerogenic activity of essential oils obtained from *Hyptis* species. Pharmacognosy Magazine, 2015; 11(41): 123-130.
42 Vale TG, Furtado EC, Santos-Jr JG, Viana GSB. Central effects of citral, myrcene and limonene constituents of essential oil chemotypes from *Lippia alba* (Mill.) N.E. Brown. Phytomedicine, 2002; 9: 709-714.
43 Viana GSB, Vale TG, Matos FJA. Anticonvulsant activity of essential oils and active principles from chemotypes of *Lippia alba* (Mill.) N.E. Brown. Biol. Pharm. Bull, 2000; 23: 1314-1317.
44 Chistani M, A rrigo MD, Mandalari G, Castelli F, Sarpietro MG. Interaction of four monoterpenes contained in essential oils with model membranes: implications for their antibacterial activity. Journal of agriculture and Food chemistry, 2007; 55: 6300-6308.
45 Marostica MR, Silva TAARE, Franchi GC, Nowill A, Pastore GM, Hyslop S, Antioxidant potential of aroma compounds obtained by limonene biotranformation of orange essential oil. Food chem, 2009; 116: 8-12.