Original Research Article

BIOCHEMICAL CONSTITUENTS AND ANTICANCER ACTIVITIES OF THE ESSENTIAL OILS OF *Lannea egregia* Engl. & K. Krause

ABSTRACT

Aims: To analyze the chemical constituents *and determine the anticancer properties of the essential oils obtained from Lannea egregia* Engl. & K. Krause Leaf, twig and stem-bark.

Study design: This study was designed to assay the chemical constituents of *Lannea egregia* and based on the ethnobotanical claims, the anticancer activities of this plant was studied.

Place and Duration of study: Department of Chemistry, Dipartmento di Farmacia, Via Bonanno 6, Universita di Pisa and University of Ibadan and Department of Virology, University of Ibadan, Ibadan, Nigeria. July 2018- April 2021

Methodology: Essential oils (EOs) from leaf, twig and stem-bark of *Lannea egregia* were extracted by hydro-distillation. Chemical compositions were characterized using Gas Chromatography–Mass Spectrometry (GC–MS). Cytotoxicity screenings of EOs were determined on human rhabdomyosarcoma (RD) and breast adenocarcinoma (MCF-7), using MTT [3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] cell viability assay.

Results: Leaf, twig and stem-bark EOs gave good yields of 0.12%, 0.43% and 0.33%, respectively.
Fifty-five, thirty-three and thirty-eight compounds were identified in leaf, twig and stem-bark EOs, accounting for 99.1%, 97.8% and 95.8% of them. Most abundant compound in leaf, stem-bark and twig EOs was β-caryophellene (28.2, 23.4 and 13.2%, respectively).
Leaf EO displayed strong cytotoxicity against RD and MCF-7, with 50% cytotoxicity (CC_{50}) of 1.69 and 15.81 µg/mL, respectively. Twig EO exhibited good selectivity with selectivity index of 24.8 and 10.8 for RD and MCF-7, respectively.

Conclusion: Results showed that EOs have good anticancer properties.

Keywords: *Lannea egregia*, Essential oils, GC-MS, Sesquiterpenes, Anticancer activity.

1. INTRODUCTION

A projection made in 2011 suggested that cancer deaths will continue to rise with an estimate that 11.4 million people might die of cancer in 2030 **(1)**. In Nigeria, the statistics of cancer deaths was 15% of 681,000 cases in Africa **(2)**. Rhabdomyosarcoma (RD or RMS) and Michigan Cancer Foundation-7 adenocarcinoma (MCF-7) are tumors derived from the muscle and breast respectively **(3, 4)**. RD is the most common cancer in children and adolescents, and it accounts for about 5% of tumors in the pediatric **(5)** while breast cancer is the most common type of cancer affecting women and a progressive increase in patients has been noticed **(6)**. The observed resistance, severe side effects and deficiencies in the present anti-cancer drugs have given rise to more

research being conducted on possible natural products which would solve the existing chemotherapy problems **(7)**. Natural sources produce a diverse array of bioactive molecules, thus making them a rich source of diverse type of medicines, such as antimicrobial, anticancer, antiviral, antioxidant, hepatoprotective agents among others **(8)**.

Essential oils are mixtures of volatile lipophilic constituents, consisting mainly of terpenes **(9)**. The varied therapeutic potential of essential oils has attracted the attention of researchers in recent years for their potential activity against cancer. These essential oils are used to target the discovery of new anticancer natural products **(10).**

Some *Lannea* species (Anacardiaceae), such as *L. acida* A. Rich., *L. discolor* (Sond.) Engl., *L. edulis* (Sond.) Engl. and *L. microcarpa* Engl. & K. Krause have been reported to have antibacterial, antipyretic, antiviral and antifungal effects **(11).** Leaf of *L. coromandelica* has been used in traditional medicine for the treatment of tumor, scurvy, ulcers, bruises, cancer, skin diseases and dysentery **(12).** Compounds such as (2*R*,3*S*)-(+)-3′,5-dihydroxy-4′,7-dimethoxydihydroflavonol, (2*R*,3*R*)-(+)-4′,5,7-trimethoxydihydroflavonol, (2*R*,3*R*)-(+)-4′,7-di-*O*-methyldihydroquercetin, (2*R*,3*R*)-(+)-4′,7-di-*O*-methyldihydrokaempferol, **(13),** 4'-methoxy-myricetin 3-O-α-L-rhamnopyranoside and myricetin 3-O-α-L-rhamnopyranoside, were among compounds isolated from the *Lannea* genus **(14).** *L. egregia* is a tree up to 13 m high, usually growing in the savanna of Guinea, Ivory coast, Dahomey and Nigeria. It is locally known as Ekudan in Nigeria, Fula-Pulaar in Guinea and Moore in Ivory Coast. Ethno-botanically, the bark of *L. egregia* and the seeds of *Capsicum frutescens, Afromamomum melegueta, Aframomum melegueta, Capsicum frutescens Pterocarpus osun, Sorghum bicolour, Daniellia oliveri, Anogeissus leiocarpus, Piliostigma thonningii* soaked in local gin was reported as a remedy for stomach pain **(15)**. A documentation on medicinal plants suggested that the bark of *L. egregia* can be used as blood tonic **(16),** while an ethnobotanical survey on anti-cancer plants reported the leaf as an anticancer plant **(17)**. The stem-bark possesses tannins, terpenoids, flavonoids, anthraquinones, saponins and alkaloids; and exhibited antimicrobial and antioxidant properties **(18)**. There is little or no scientific information on its essential oil constituents and anticancer activities. Therefore, this paper reports the essential oil compounds and anticancer properties of *L. egregia.*

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2. MATERIALS & METHODS

2.1 Plant Collection and Identification

L. egregia (leaves, twig and stem-bark) were collected at Olokemeji forest reserve along Eruwa. Plant samples were identified at the Forest Research Institute of Nigeria (FRIN) and deposited at the herbarium unit of FRIN for future reference (FHI 112357). The collected plants were sorted into different parts and weighed.It was better to indicate the age of plant and the period and the condition of collection

2.2 Essential Oil Isolation and Analysis

The essential oil extraction of plant parts (leaves, twigs and stem-bark) were carried out separately. The weighed plant materials (896.47g of leaves, 300.11g of twig and 400.00g of stem-bark) were transferred into a 10 L round bottom flask and water was added to cover the plants. The extraction was done for a period of 3 h. The volatile oils were trapped using 1 mL of hexane, stored in glass sample vials and preserved in the refrigerator. Please add the reference of the methods used

2.3 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

Gas Chromatography–Electron Impact Mass Spectrometry (GC–EIMS) analyses were performed with an Agilent 7890B gas chromatograph (Agilent Technologies Inc., Santa Clara, CA, USA) equipped with an Agilent HP-5MS-5% Phenyl Methyl polysiloxane (Agilent Technologies Inc., Santa Clara, CA, USA) capillary column (30 m×0.25 mm; coating thickness 0.25µm) and an Agilent 5977B single quadrupole mass detector (Agilent Technologies Inc., Santa Clara, CA, USA). Analytical conditions were as follows: injector and transfer line temperatures 220 and 240 °C, respectively; oven temperature programmed from 60 to 240°C at 3°C/min; carrier gas helium at 1 ml/min; injection of 1 µL (0.5% HPLC grade *n*-hexane solution); split ratio 1:25. The acquisition parameters were as follows: full scan; scan range: 30–300 m/z; scan time: 1.0 sec. Identification of the constituents was based on a comparison of the retention times with those of the authentic samples, comparing their linear retention indices relative to the series of *n*-hydrocarbons. Computer matching was also used against commercial (NIST 14 and ADAMS) and laboratory-developed mass spectra library built up from pure substances and components of known oils and MS literature data (19).

2.4 Determination of effect of essential oils on cell proliferation by MTT assay

2.4.1 Cell culture

Cytotoxicity assay was carried out using human Rhabdomyosarcoma (RD) cells (CDC, Atlanta, USA). The cell lines were grown using Eagle's Minimum Essential Medium (MEM). The composition of the MEM was 10% FBS, 100 mg/mL of penicillin, 100 mg/mL of streptomycin, 2 mM L-glutamine, 0.07% $NaHCO_3$, 1% non-essential amino acids and vitamin solution. The cell cultures were placed in incubator with 5% CO_2 at 37°C.

2.4.2 Cytotoxicity assay

MTT [3-(4,5 dimethylthiazol-2-yl)-2,5- diphenyl tetrazolium bromide (Sigma, Chem, St. Louis, MO) was used to test for cell viability. The mechanism of action involves the cleavage of the tetrazolium salt by the mitochondrial enzyme succinate dehydrogenase (17).The essential oils were individually pre-solubilized in dimethylsulphoxide (DMSO) to give a stock solution of 1000 µg/mL. Ten-fold dilutions were carried out to give six concentration levels (1000–0.01 µg/mL). Plated Rhabdomyosarcoma (RD) was observed under the microscope to ascertain confluency of the cells in the 96 well-microlitre plates after 24 h. The six concentrations of the essential oils were incubated with RD cell lines. This was carried out in triplicate at 37 °C in a CO_2 environment for 72 h. The negative control was the maintenance medium alone, and the positive control was cyclophosphamide®. Cell viability and cytopathic effect (CPE) were examined under the microscope. The supernatant was pipetted out of each well after 72 h and 25 µL of the MTT solution (2 mg/mL in Phosphate Buffered Saline) was transferred into each well. This was incubated for 90 min at 37°C, and 125 µL of DMSO was added to each well to dissolve the formazan crystals. The plates were placed on a shaker for 15 min and the absorbance was determined at 540 nm on a multi-well spectrophotometer (Multiskan, Thermo Fisher Scientific, Waltham, MA). The 50% cytotoxic concentration (CC_{50}), which is the concentration required for the reduction of cell viability by half, was calculated using a non-linear regression curve in the Graph pad prism statistical software.

3. RESULTS & DISCUSSION

Percentage yields of the EOs extracted from leaf, twig and stem-bark were 0.12%, 0.43% and 0.33%, respectively. Chromatogram of each EO is presented ~~as~~ in Figures 1, 2 and 3, respectively. Fifty-five, thirty-three and thirty-eight compounds were identified in leaf, twig and stem-bark EOs, ~~representing~~ as levels of 99.1%, 97.8% and 95.8% of each EO, respectively (Tables 1, 2 and 3). β-caryophyllene, was the most abundant constituent present in the leaf (28.2%), twig (13.2%) and stem-bark (23.4%) EOs, respectively. However, the leaf essential oil composition reported by Ogundajo *et al*., (2021) **(20)** had indentified the α-panasinsen as the most abundant constituent. Though, these plants were collected from the same location, this could be due to the seasonal variation or different growth stages of the plant prior to extraction **(20)**. In the present study, some constituents such as β-caryophyllene (28.2, 13.2 and 23.4%), α-humulene (5.8, 5.6 and 10.1%), δ-cadinene (5.7, 4.0 and 4.1%) and germacrene D (5.4, 4.2 and 5.6%) were common to leaf, twig and stem-bark, EOs respectively. EOs were rich in sesquiterpenes with relative abundance of 80.9, 66.2% and 93.8% in leaf, twig and stem-bark EOs, respectively.

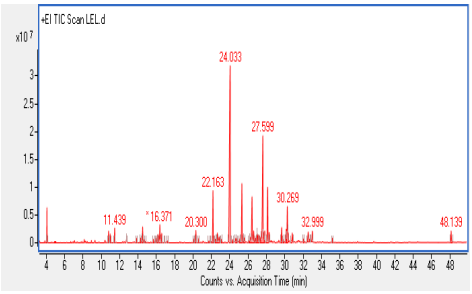

Figure 1: Gas Chromatogram of *Lannea egregia* leaf essential oil

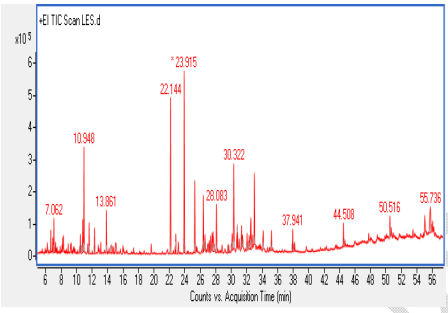

Figure 2: Gas Chromatogram of *Lannea egregia* twig essential oil

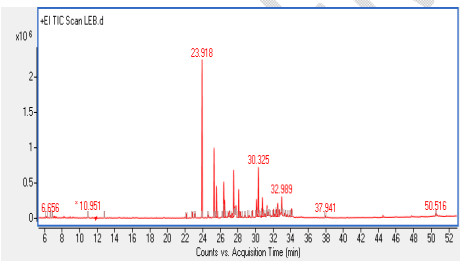

Figure 3: Gas Chromatogram of *Lannea egregia* stem-bark essential oil

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Table 1: Chemical Constituents of *L. egregia* leaf Essential Oil

Peak No	Identified compound	% Relative Abundance	Retention Index
1	1-hexanol	1.7	871
2	Linalool	0.8	1101
3	Nonanal	0.7	1102
4	*(E)*-4,8-dimethylnona-1,3,7-triene	1.1	1116
5	4-terpineol	0.4	1178
6	α-terpineol	0.5	1189
7	Butyl hexanoate	1.3	1190
8	*(E)*-2-hexenyl butyrate	0.2	1193
9	β-cyclocitral	0.4	1217
10	*Cis*-3-hexenyl isovalerate	0.7	1234
11	Hexyl 2-methylbutyrate	2.1	1236
12	Hexyl 3-methylbutyrate	0.8	1244
13	β-cyclohomocitral	0.2	1256
14	*(E)*-2-methylbut-2-enoate	0.4	1319
15	Hexyl tiglate	1.0	1332
16	*(E)*-2-hexenyl tiglate	0.4	1339
17	Cyclosativene	0.3	1368
18	α-ylangene	0.1	1372
19	α-copaene	4.8	1376
20	*(Z)*-3-hexenyl hexanoate	0.3	1383
21	β-bourbonene	0.5	1384
22	Hexyl hexanoate	1.0	1385
23	β-elemene	0.9	1392
24	Cyperene	0.3	1398
25	β-caryophyllene	28.2	1420
26	β-copaene	0.4	1429
27	*Trans*-α-bergamotene	0.4	1438
28	α-humulene	5.8	1456
29	*(E)*-β-farnesene	0.7	1460
30	Alloaromadendrene	0.5	1461
31	*Trans*-cadina-1(6),4-diene	0.3	1470
32	γ-muurolene	0.7	1477
33	Germacrene D	5.4	1478
34	Bicyclosesquiphellandrene	0.4	1489
35	*(E)*-β-ionone	1.2	1490
36	Valencene	0.7	1492
37	Bicyclogermacrene	0.8	1495
38	α-muurolene	0.8	1498
39	α-bulnesene (=δ-guaiene)	0.7	1505
40	*(E,E)*-α-farnesene	12.7	1507
41	β-curcumene	1.2	1512
42	*(Z)*-γ-bisabolene	1.1	1515
43	δ-cadinene	5.7	1524
44	Selina-3,7(11)-diene	0.9	1542
45	*(E)*-nerolidol	1.4	1565
46	*(Z)*-3-hexenyl benzoate	0.3	1570
47	*n*-hexyl benzoate	1.2	1579

48	Caryophyllene oxide	0.9	1581
49	Guaiol	1.2	1595
50	1-*epi*-cubenol	0.4	1628
51	*Epi*-α-cadinol (=T-cadinol)	1.3	1640
52	*Epi*-α-muurolol (=T-muurolol)	0.3	1642
53	α-cadinol	1.3	1654
54	Pentadecanal	0.4	1712
55	Phytol	1.1	2114
	Total identified (%)	99.1	

Table 2: Chemical Constituents of L. *egregia* Twig Essential Oil

Peak No	Identified compound	% Relative Abundance	Retention Index
1	2-pentyl furan	1.9	993
2	2-nonanone	1.1	1094
3	Linalool	2.2	1101
4	Nonanal	5.7	1102
5	*Cis-p*-menth-2-en-1-ol	1.9	1124
6	*Trans-p*-menth-2-en-1-ol	1.6	1143
7	4-terpineol	2.7	1178
8	α-terpineol	1.2	1189
9	α-copaene	10.9	1376
10	β-elemene	1.4	1392
11	β-caryophyllene	13.2	1420
12	α-humulene	5.6	1456
13	Germacrene D	4.2	1478
14	*(E)*-β-ionone	1.1	1490
15	α-bulnesene (=δ-guaiene)	1.2	1505
16	*(E,E)*-α-farnesene	1.1	1507
17	δ-cadinene	4.0	1524
19	*(E)*-nerolidol	0.9	1576
20	Spathulenol	1.5	1581
21	Caryophyllene oxide	7.1	1590
22	Viridiflorol	1.1	1597
23	*(Z)*-7-tetradecenal	1.3	1607
24	Humulene epoxide II	1.7	1636
25	Caryophylla-4(14),8(15)-dien-5-ol	1.7	1640
26	Epi-α-cadinol (=T-cadinol)	3.3	1654
27	α-cadinol	5.3	1678
28	Aromadendrene oxide II	2.1	1712
29	Pentadecanal	1.7	1793
30	1-octadecene	1.6	1990
31	*n*-eicosene	1.6	2190
32	*n*-docosene	1.6	2200
33	*n*-tetracosane	4.5	2400

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	Total identified (%)	97.8

Table 3: Chemical Constituents of *L. egregia* Stem-bark Essential Oil

Peak No	Identified compound	% Relative Abundance	Retention Index
1	Benzaldehyde	0.3	963
2	1-octen-3-ol	0.3	980
3	6-methyl-5-hepten-2-one	0.2	985
4	Nonanal	0.4	1102
5	2-ethylhexyl acetate	0.1	1159
6	α-copaene	0.6	1376
7	β-elemene	0.8	1392
8	Cyperene	0.7	1398
9	β-caryophyllene	23.4	1420
10	*Trans*-α-bergamotene	0.4	1438
11	α-humulene	10.1	1456
12	*(E)*-β-farnesene	0.4	1460
13	Alloaromadendrene	4.8	1461
14	γ-muurolene	0.4	1438
15	Germacrene D	5.6	1456
16	*Ar*-curcumene	2.5	1460
17	β-selinene	0.5	1461
18	Bicyclogermacrene	1.4	1495
19	α-muurolene	0.3	1498
20	α-bulnesene (=δ-guaiene)	1.1	1505
21	*(E,E)*-α-farnesene	6.3	1507
22	β-curcumene	0.9	1512
23	*(Z)*-γ-bisabolene	1.2	1515
24	δ-cadinene	4.1	1524
25	Selina-3,7(11)-diene	0.9	1542
26	α-calacorene	0.3	1546
27	*(E)*-nerolidol	1.0	1565
28	Spathulenol	2.5	1576
29	Caryophyllene oxide	8.4	1581
30	Viridiflorol	4.2	1590
31	Humulene epoxide II	1.6	1607
32	1,10-di-*epi*-cubenol	1.6	1614
33	*Epi*-α-cadinol (=T-cadinol)	3.0	1640
34	*Epi*-α-muurolol (=T-muurolol)	0.9	1642
35	α-cadinol	2.4	1654
36	Aromadendrene oxide II	1.5	1678
37	1-octadecene	0.3	1793
38	*n*-docosene	0.5	2200
	Total identified (%)	95.8	

Cytotoxic activity (Table 4) of the EOs was dose-dependent. Leaf EO showed the best inhibition against RD and MCF-7, having CC_{50} values of 1.69 and 15.81 µg/mL respectively. Twig EO gave good inhibition, with the CC_{50}

of 6.96 and 15.98 µg/mL respectively, whereas the stem-bark EO exhibited weak inhibition on RD and MCF-7 (170.9 and 16.39 µg/mL, respectively). Sesquiterpenes, such as β-caryophyllene and α-farnesene, have been found to possess strong selective cytotoxic properties against human colorectal cancer cells **(22).** The synergism of these compounds with other sesquiterpenes could be responsible for the observed activity of the EOs.

Table 4: Cytotoxicity Concentration (CC_{50}) of the Essential Oils of *L. egregia*

	CC_{50} (µg/mL)	
ESSENTIAL OILS	RD	MCF-7
Leaf	1.6	15.8
Twig	6.9	15.9
Stem-Bark	170.9	16.4
Vincristine® (Positive control)	0.8	0.9

The dDetermination of the selective indices (SI) for these EOs is important because these parameters demonstrate the differential activity on both normal and cancer cells. The greater the SI value, the more selective the EO is.p=Please complete this ~~.~~ ~~The~~sentences The selectivity index (SI) generally indicates the safety of an extract used for cytotoxic therapy. Unfortunately, most of the anticancer drugs currently available lack selectivity **(23).** EOs with good cancer cell growth inhibitory capacity were further tested on Vero, a normal cell line from African Green Monkey kidney to determine their selectivity for cancer cells. The selective index (SI) is usually obtained by taking the ratio of the normal cells (Vero) to the cancer cell lines (RD/MCF-7). Extracts with values lesser than 1 are tagged as toxic to the normal cells, between 1 and 10 are tagged as weakly toxic, while for 10 and above are considered non-toxic **(24).** Although the leaf EO showed the best inhibitory ability, the selectivity (Tab.5) of the twig EO for RD and MCF-7 was the highest, 24.8 and 10.8, which means they were approximately 25 and 11 times more toxic for RD and MCF-7 compared with normal cells. This selectivity is highly remarkable especially when compared with SI of vincristine, the standard drug (1.1 and 1.2 for RD and MCF-7, respectively).

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Table 5: Selective Index (SI) ~~of the Essential Oils~~ of *L. egregia* Essential Oil's

ESSENTIAL OILS	RD	MCF-7
Leaf	9.70	1.04
Twig	24.8	10.8
Stem-Bark	-	10.7
Vincristine® (Positive control)	1.1	1.2

A further improvement of this study would be to verify the pharmacokinetic and pharmacodynamic profile of twig EO to provide better information on the possible use of this EO as a potential cytotoxic agent. Currently, molecular docking of this research is been carried out.
Results were note well discussed please

4. CONCLUSION

Ethno-botanical claims on the leaf of *L. egregia* as an anticancer plant were confirmed by the cytotoxic results. In addition, the selectivity of twig essential oil was first demonstrated during this research. The synergism of sesquiterpenes such as β-caryophyllene, caryophyllene oxide and α-farnesene, whose cytotoxic properties were already known, could be responsible for the properties exhibited by these essential oils.

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