Chemical Composition, Cytotoxicity, Antimicrobial and Antioxidant Activities of the Leaf and Stem Essential Oils of *Agelaea Obliqua* (P.Beauv) Baill (Connaraceae)

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ABSTRACT

Agelaea obliqua is widely used in West African ethnomedicine for irritating throat, parasitic skin infections, bronchitis and as an aphrodisiac. Despite its extensive use, information on the chemical composition and biological activities of its essential oil (EO) remains limited. This study evaluated the chemical composition, cytotoxicity, antimicrobial and antioxidant activities of the leaf and stem EOs of *A. obliqua*. The plant (voucher FHI 113363) was collected from Agbegi-odofin secondary forest, Ikire, Osun State, Nigeria, and authenticated at the Forestry Research Institute of Nigeria (FRIN), Ibadan. EOs were obtained from air-dried leaves and stem by hydro-distillation using an all-glass Clevenger-type apparatus, and their constituents identified by Gas Chromatography-Mass Spectrometry (GC-MS). Cytotoxicity was assessed using the brine shrimp lethality test (BSLT); antimicrobial activity was evaluated against two Gram-positive and four Gram-negative bacteria and two fungi by the broth micro-dilution method, with Minimum Inhibitory Concentration (MIC) and Minimum Microbicidal Concentration (MMC) determined; and radical-scavenging activity was assessed by the DPPH assay against ascorbic acid and butylated hydroxyanisole (BHA) as standards. GC-MS identified 41 compounds (81.20%) in the leaf EO and 40 compounds (80.74%) in the stem EO, dominated by n-hexadecanoic acid (14.31% and 35.09%, respectively), phytol, oleic acid and 6,10,14-trimethyl-2-pentadecanone. Both EOs were cytotoxic to brine shrimp nauplii (LC50 15.69 and 109.51 µg/mL for leaf and stem, respectively). The stem EO showed strong antimicrobial activity against all tested bacteria and fungi, requiring as little as 0.78 µg/mL to eliminate *Staphylococcus aureus* and *Klebsiella pneumoniae*, while the leaf EO showed weak activity and no effect against *Pseudomonas aeruginosa* and *S. aureus*. Both oils showed moderate, concentration-dependent DPPH radical-scavenging activity relative to the reference standards. These findings support the ethnomedicinal use of *A. obliqua* and identify its essential oil, particularly from the stem, as a potential lead source for antimicrobial and antioxidant drug development.

Keywords: *Agelaea obliqua*; essential oil; GC-MS; antimicrobial activity; antioxidant activity

INTRODUCTION

Higher plants remain one of the richest sources of structurally diverse secondary metabolites, including terpenoids, alkaloids, flavonoids, tannins and saponins. Many of these compounds underpin the ethnomedicinal use of plants across West Africa (Sofowora *et al.*, 2013, Adedotun *et.al.*, 2026). Natural products and their derivatives have contributed a large share of the small-molecule drugs approved over the past four decades, particularly in the anti-infective and anticancer fields (Newman & Cragg, 2020). Advances in analytical chemistry, genomics and screening technologies have renewed interest in plant-derived chemistry as a source of new scaffolds (Atanasov *et al.*, 2021). Plants from biodiverse but under-investigated tropical floras are therefore a logical place to look for new leads.

Essential oils are volatile, lipophilic mixtures dominated by mono- and sesquiterpenoids, often with phenylpropanoids and other minor constituents. They are among the most pharmacologically active plant metabolites (Masyita *et al.,* 2022). Their biological effects are usually attributed to the combined and sometimes synergistic action of many constituents rather than a single compound, and their composition varies with plant organ, geographical origin, harvest time and extraction method (Dhifi *et al.,* 2016). Essential oils are widely screened for cytotoxic, antimicrobial and antioxidant activity, three properties that match pressing global health needs. Cancer caused an estimated 19.3 million new cases and almost 10.0 million deaths worldwide in 2020 (Sung *et al.,* 2021). Bacterial antimicrobial resistance was directly responsible for about 1.27 million deaths in 2019 and associated with nearly 5 million (Murray *et al.,* 2022). Oxidative stress is implicated in the pathogenesis of cardiovascular, neurodegenerative, metabolic and neoplastic diseases (Pizzino *et al.,* 2017). Essential oils and their terpenoid constituents have shown antiproliferative effects on diverse cancer cell lines (Blowman et al., 2018) and broad-spectrum antimicrobial activity, including against drug-resistant strains (Chouhan *et al.,* 2017). This has made them attractive as leads and as adjuncts to conventional therapy.

Connaraceae is a pantropical family of about 25 genera of trees, shrubs and twining climbers. Agelaea (about 50 species), Connarus, Rourea, Cnestis and Byrsocarpus are the largest genera (Vickery & Vickery, 1974; Plants of the World Online, 2024). Species of Agelaea are used across tropical Africa as folk remedies, as an emetic, for dysentery and gonorrhoea, and to treat irritating throat and skin infections. Chemical work on the genus is nonetheless sparse. Essential oil constituents have been reported for only two species. *Agelaea borneensis* yielded the monoterpenes limonene and α-pinene and the sesquiterpenes β-caryophyllene and D-germacrene (Sardans *et al.,* 2015). Coumarins and the flavonoid tricin have been isolated from *Agelaea pentagyna* (Kuwabara *et al.,* 2003). Because the volatile fraction of most *Agelaea* species is unexplored, the genus is a largely untapped source of potentially bioactive terpenoids.

Agelaea obliqua (P.Beauv.) Baill. is a scrambling shrub or forest and secondary-jungle climber up to 6 m high, widespread from Guinea-Bissau to West Cameroon. It bears white fragrant flowers and scarlet fruits (Zhang et al., 2013; Vickery & Vickery, 1974). In Ivory Coast, a root-bark decoction of *A. obliqua* combined with Costus is chewed as an aphrodisiac, the leaf sap is taken to treat illness in nursing infants, and the ash of the root, mixed with salt, is used for irritating throat (Obiang *et al.,* 2015). These traditional uses suggest that the plant contains pharmacologically relevant metabolites. Despite them, information on the essential oil composition and biological activities of *A. obliqua* is limited, and no comparative account of the oils from different organs of the plant is available. Such a comparison is important because the leaves and stem differ in metabolic function and tissue type, which may give oils of distinct chemical profiles and biological potency.

Given this gap, the present study evaluated the chemical composition, cytotoxicity, antimicrobial and antioxidant activities of the essential oils obtained from the leaves and stem of *A. obliqua*. The aim was to provide a chemical and pharmacological basis for its ethnomedicinal applications and to assess its potential as a source of bioactive leads.

MATERIALS AND METHODS

Plant Collection and Identification

The whole plant of *A. obliqua* was collected from Agbegi-odofin secondary forest, Ikire, Osun State, Nigeria, in April 2021. Upon collection, the plant was identified and authenticated at the Forestry Research Institute of Nigeria (FRIN), Ibadan, where a voucher specimen was deposited and assigned herbarium number FHI 113363.

Chemicals and Reagents

Methanol, ethyl acetate, n-hexane and acetone (General Purpose Reagent grade) were obtained from chemical distributors and further distilled and purified before use. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), butylated hydroxyanisole (BHA) and ascorbic acid (Vitamin C) were obtained from the Department of Chemistry store, University of Ibadan.

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Preparation of Plant Material

The leaves and stem of the plant were separated and air-dried under shade for about a month to reduce moisture content while minimising loss of volatile constituents. Afterwards, they were pulverised at the Wood Extraction Laboratory, Department of Chemistry, University of Ibadan, to increase surface area, and stored in air-tight containers prior to extraction.

Extraction and Fractionation of Crude Extracts

A 400g portion each of dried leaves and stem was extracted by cold maceration in 5 L of methanol for 4 days. Each methanol extract was concentrated by distillation and dried in a vacuum desiccator, then partitioned sequentially with n-hexane and ethyl acetate using a separating funnel (Pavia *et al.,* 2007). The leaves methanol extract (19.43 g) yielded 3.78 g hexane and 4.02 g ethyl acetate fractions; the stem methanol extract (16.88 g) yielded 2.47 g hexane and 2.14 g ethyl acetate fractions.

Phytochemical Screening

The hexane, ethyl acetate and methanol extracts of the leaves and stem were screened qualitatively for alkaloids, flavonoids, anthocyanins, tannins, saponins, terpenoids/steroids, cardiac glycosides, terpenes, reducing sugars and anthraquinones using standard procedures (Sofowora, 1993; Harborne & Williams, 2000; Evans & Trease, 2002).

Extraction of Essential Oil

Essential oils were obtained from 300 g portions of air-dried leaves and stem by hydro-distillation for 3 h using an all-glass Clevenger-type apparatus, following Pavia et al. (2007). The oil, being less dense than water, was collected as an upper layer trapped in 2 mL of distilled hexane. This was subsequently decanted, weighed and stored at 4 °C. Percentage yield was calculated as: %Yield = (Weight of oil / Weight of plant sample) × 100.

GC-MS Analysis

The essential oils were analysed on an Agilent Technologies 7890 GC coupled with a 5975 mass spectrometer, fitted with an HP-5MS capillary column (30 m × 0.320 mm i.d., 0.25 µm film), using helium as carrier gas. The oven temperature was programmed from 80 °C (held 2 min) at 12 °C/min to 240 °C (held 6 min); the MS interface temperature was 250 °C, scan range m/z 50–550, and injection volume 1 µL. Constituents were identified by comparison of retention indices and mass spectral fragmentation patterns with the NIST 11.L database/Chemstation data system and with published spectra (Adams, 2007), and relative proportions were expressed as percentage of total peak area.

Brine Shrimp Lethality Assay

Cytotoxicity was assessed by the brine shrimp lethality test (Aboaba *et al.,* 2010). *Artemia salina* cysts were hatched in seawater under illumination for 48 h. Essential oils, pre-dissolved in DMSO, were tested at 1000, 100 and 10 µg/mL (in triplicate), with ten newly hatched nauplii per test tube and DMSO (≤0.05%) as negative control. Surviving nauplii were counted after 24 h and mortality calculated; LC50 values were determined using Probit Analysis on Statistical Package for Social Science (SPSS).

DPPH Radical-Scavenging Assay

Radical-scavenging activity was determined by the DPPH method (Onocha *et al.,* 2011). Stock solutions (10 mg/mL) of the essential oils and the standards (ascorbic acid, BHA) in methanol were serially diluted (10–0.625 mg/mL); 0.5 mL of each dilution was mixed with 3 mL of freshly prepared 0.1 mM methanolic DPPH and left in the dark for 30 min. Absorbance was read at 517 nm, and percentage inhibition calculated as: %Inhibition = [(Absorbance of control − Absorbance of sample) / Absorbance of control] × 100. IC50 values were obtained by plotting inhibition against concentration; all assays were run in triplicate and results reported as mean ± SD.

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Antimicrobial Assay

Antimicrobial activity was evaluated by the broth micro-dilution method (Mshelia *et al.*, 2008; Kalemba, 2003) against the Gram-negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Klebsiella pneumoniae*, the Gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis*, and the fungi *Candida albicans* and *Aspergillus niger*. Oil samples were dissolved in double-strength Tryptone Soya Broth (50 mg/mL) and serially diluted (25–0.396 mg/mL) in 96-well microplates; gentamicin (bacteria) and ketoconazole (fungi) served as reference standards. Plates were inoculated and incubated at 37 °C (bacteria, 12 h) or 25 °C (fungi, 48 h); the lowest concentration showing no visible growth was recorded as the MIC. For MMC, 10 µL of 0.2 mg/mL p-iodonitrotetrazolium violet (P-INT) was added to each well and re-incubated at 36 °C for 30 min; the lowest concentration showing no colour change (yellow to pink) was recorded as the MMC.

Statistical Analysis

Results are presented as mean ± standard deviation of triplicate determinations. Graphs were plotted in Microsoft Excel 2019.

RESULTS

Physical Properties of the Essential Oils

Hydro-distillation of *A. obliqua* leaves and stem gave colourless essential oils with a woody aroma (Table 1). The leaf oil gave a slightly higher yield (0.30%) compared to the stem oil (0.26%). Both oils showed low volatility, consistent with a high sesquiterpenoid content, given the higher molecular weight and carbon number of sesquiterpenoids relative to monoterpenoids.

Table 1: Physical properties of the leaf and stem essential oils of Agelaea obliqua

Essential oil	Weight of plant material (g)	Weight of essential oil (g)	% Yield	Colour
Leaves (AOL)	300	0.91	0.30	Colourless
Stem (AOS)	300	0.78	0.26	Colourless

AOL = *Agelaea obliqua* leaves; AOS = *Agelaea obliqua* stem.

Chemical Composition of the Essential Oils

GC-MS analysis identified forty-one compounds accounting for 81.20% of the leaf essential oil and forty compounds accounting for 80.74% of the stem essential oil (Table 2), which consisted of hydrocarbons, oxygenated mono-, sesqui- and diterpenes, fatty acids/fatty acid esters, and non-terpenoids.

The leaf oil was dominated by n-hexadecanoic acid (14.31%), phytol (8.90%), geranyl acetone (6.90%), 6,10,14-trimethyl-2-pentadecanone (5.76%), 5E,9E-farnesyl acetone (5.53%), oleic acid (4.59%) and (E)-α-ionone (3.25%), and was characterized by a high proportion of non-terpenoids (42.12%), oxygenated sesquiterpenes (12.61%), oxygenated monoterpenes (10.44%) and oxygenated diterpenes (9.16%).

The stem oil was dominated by n-hexadecanoic acid (35.09%), with oleic acid (4.78%), cetene (4.05%), zonarene (3.16%) and 6,10,14-trimethyl-2-pentadecanone (2.90%) as other major constituents, and was characterised by a high proportion of non-terpenoids (51.82%) and hydrocarbon sesquiterpenes (11.36%).

Both oils shared n-hexadecanoic acid, phytol, oleic acid, 6,10,14-trimethyl-2-pentadecanone and geranyl acetone, though at markedly different proportions. 3-Carene (2.11%) was the only monoterpene detected in the stem oil, while trans-calamenene (0.23%) was the only hydrocarbon sesquiterpene detected in the leaf oil.

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Table 2: Constituents of the leaf and stem essential oils of Agelaea obliqua

RT (min)	Identified Compound	% Area Leaves	% Area Stem
3.32	Hexanal	1.53	0.65
5.86	Salicylic aldehyde	0.76	0.14
6.34	6-Methyl-5-hepten-2-one	1.31	0.22
6.41	Pentyl furan	0.61	0.40
6.58	Octanal	-	0.22
7.47	1-Decene	0.20	-
7.68	n-Octanol	-	0.21
7.71	cis-Linalool oxide	-	2.18
8.14	Linalool	0.46	2.37
8.21	Nonanal	1.63	2.04
9.05	2-Decenal	0.19	-
9.16	1,3-Dimethoxybenzene	-	1.08
9.52	α-Terpineol	-	0.92
9.58	Methyl salicylate	0.38	0.85
9.66	Safranal	0.32	1.08
9.73	Decanal	0.31	-
9.82	Santene	0.63	-
9.67	β-Cyclocitral	0.62	0.11
10.06	3-Carene	-	2.11
10.53	Geranial	2.28	-
10.66	Neral	-	1.07
12.11	α-Copaene	-	2.09
12.22	(E)-β-Damascenone	0.65	-
12.77	α-(E)-Ionone	3.25	0.35
13.00	Cuminol	0.18	-
13.06	Geranyl acetone	6.90	0.52

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13.52	trans-β-Ionone	2.79	0.37
13.76	Tridecanal	0.24	-
14.12	3-Methyl-bicyclo[4.1.0]heptane	1.89	-
14.21	β-Calacorene	-	2.10
14.42	Nerolidol	0.79	-
14.71	Cetene	0.93	4.05
15.18	Zonarene	-	3.16
15.37	β-Guaiene	-	2.28
15.46	Ylangenol	-	1.60
15.74	Cadelene	-	0.26
16.05	Longifolene	-	0.07
16.10	Pentadecanal	0.76	-
16.21	Allo-aromadendrene	-	0.14
16.22	α-Curcumene	-	1.28
16.43	2E,6E-Farnesal	0.25	-
16.65	Tetradecanoic acid	0.69	-
16.74	Aciphyllene	-	0.12
16.99	Octadecane	0.48	-
17.51	6,10,14-Trimethyl-2-pentadecanone	5.76	2.90
17.92	Linoleic acid	0.73	-
18.00	5E,9E-Farnesyl acetone	5.53	-
18.50	Isophytol	0.26	-
18.85	n-Hexadecanoic acid	14.31	35.09
19.00	Eicosane	1.48	-
19.83	trans-Calamenene	0.23	-
19.94	Heneicosane	1.29	0.59
20.13	Phytol	8.90	0.91
20.35	Oleic acid	4.59	4.78

20.83	Docosane	1.50	0.64
21.69	Nonadecane	1.42	0.69
22.50	Tetracosane	3.48	0.72
23.20	Nopinone	-	0.25
25.73	Squalene	0.64	0.18
	Total %	**81.20**	**80.74**
	Classification		
	Hydrocarbon monoterpenes	0.63	2.11
	Oxygenated monoterpenes	10.44	7.17
	Hydrocarbon sesquiterpenes	0.28	11.36
	Oxygenated sesquiterpenes	12.61	2.46
	Hydrocarbon diterpenes	0.64	0.18
	Oxygenated diterpenes	9.16	0.91
	Fatty acids and fatty acid esters	5.32	4.78
	Non-terpenes/terpenoids	42.12	51.82

RT = Retention time; – = not detected.

Percentage Yield of Crude Extracts

Cold maceration of the leaves and stem in hexane, ethyl acetate and methanol gave the highest yield with methanol (4.85% for leaves, 4.22% for stem), followed by ethyl acetate and hexane (Table 3). The leaves extracts were greenish while the stem extracts had a woody colour.

Table 3: Percentage yield of *Agelaea obliqua* leaf and stem extracts in different solvent systems

	Leaves Hexane	**Leaves Ethyl acetate**	**Leaves Methanol**	**Stem Hexane**	**Stem Ethyl acetate**	**Stem Methanol**
Weight of sample (g)	400	400	400	400	400	400
Weight of extract (g)	3.78	4.02	19.43	2.47	2.14	16.88
% Yield	0.95	1.01	4.85	0.61	0.53	4.22

Phytochemical Screening

The hexane, ethyl acetate and methanol extracts of the leaves and stem of *A. obliqua* tested positive for tannins and saponins in all solvent systems (Table 4). Alkaloids were detected in the ethyl acetate and methanol extracts of both leaves and stem, but not in the hexane extracts. Steroids, flavonoids and terpenoids were

present in most extracts, while cardiac glycosides were confined to the methanol extracts. Anthraquinones and reducing sugars were variably present across the extracts.

Table 4: Phytochemical screening of the crude extracts of the leaves and stem of *Agelaea obliqua*

	Leaves			Stem		
	Hexane	**Ethyl Acetate**	**Methanol**	**Hexane**	**Ethyl Acetate**	**Methanol**
Alkaloids	-	+	+	-	+	+
Steroids	+	+	-	+	+	+
Flavonoids	+	+	-	+	+	-
Terpenoids	+	+	+	+	+	+
Cardiac glycosides	-	-	+	-	-	+
Tannins	-	+	+	+	+	+
Saponins	+	+	+	+	+	+
Anthraquinone	+	-	-	+	-	-
Reducing sugar	-	+	+	-	+	+

+ = present; – = absent.

Brine Shrimp Lethality Assay

Both essential oils were cytotoxic to Artemia salina nauplii, with LC50 values below 1000 µg/mL, the threshold generally taken to indicate biological activity (Table 5). The leaf oil (LC50 = 15.69 µg/mL) was substantially more lethal than the stem oil (LC50 = 109.51 µg/mL), causing 96.6% mortality at 1000 ppm compared with 83.3% for the stem oil.

Table 5: Brine shrimp lethality test result of the leaf and stem essential oils of Agelaea obliqua

Essential oil	**Total no. of shrimps**	**Dead @1000ppm**	**% mortality**	**Dead @100ppm**	**% mortality**	**Dead @10ppm**	**% mortality**	**LC50 (µg/mL)**
AOL	30	29	96.6	23	76.6	18	60	15.69
AOS	30	25	83.3	15	50	4	13.3	109.51

AOL = *Agelaea obliqua* leaves; AOS = *Agelaea obliqua* stem.

Antimicrobial Activity

The stem essential oil showed strong activity against all eight test organisms, with MIC/MMC values as low as 0.78 µg/mL against *S. aureus* and *K. pneumoniae*, whereas the leaf essential oil showed weak, inconsistent activity and no measurable activity against *P. aeruginosa* and *S. aureus* at the concentrations tested (Table 6).

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Table 6: Minimum Inhibitory Concentration (MIC) and Minimum Microbicidal Concentration (MMC) (µg/mL or mg/mL as indicated) of the leaf and stem essential oils of Agelaea obliqua

Organism	**AOL MIC**	**AOL MMC**	**AOS MIC**	**AOS MMC**	**Gentamicin MIC/MMC (mg/mL)**	**Ketoconazole MIC/MMC (%)**
E. coli	50	–	0.78	1.56	5 / 10	NA
P. aeruginosa	–	–	1.56	6.25	>10 / >10	NA
S. aureus	–	–	0.78	0.78	1.25 / 5	NA
K. pneumoniae	–	–	0.78	0.78	10 / 10	NA
S. typhi	50	50	1.56	1.56	>10 / >10	NA
B. subtilis	25	50	1.56	1.56	5 / 5	NA
C. albicans	25	50	1.56	1.56	NA	0.5 / 0.5
A. niger	50	50	1.56	6.25	NA	0.5 / 1.0

AOL = *Agelaea obliqua* leaves; AOS = *Agelaea obliqua* stem; – = no activity; NA = not applicable. Inhibitions for AOL and AOS are expressed in µg/mL unless otherwise stated.

Antioxidant Activity (DPPH Assay)

Both essential oils exhibited concentration-dependent DPPH radical-scavenging activity (Tables 7 and 8). At the highest concentration tested (10 mg/mL), the leaf oil showed 50.97% inhibition and the stem oil 48.56% inhibition, comparable to BHA (45.08%) but lower than ascorbic acid (58.09%). IC50 values were 2.31 mg/mL for the leaf oil and 7.94 mg/mL for the stem oil, against 5.22 mg/mL for ascorbic acid and 10.58 mg/mL for BHA. The moderate antioxidant activity of the essential oils relative to the standards may reflect the comparatively low abundance of phenolic/hydroxyl-bearing constituents identified by GC-MS, since such moieties are generally associated with stronger radical-scavenging activity (Ogunlana *et al.*, 2006).

Table 7: Mean absorbance of the leaf and stem essential oils of Agelaea obliqua and reference standards in the DPPH assay

Sample	**10 mg/mL**	**5 mg/mL**	**2.5 mg/mL**	**1.25 mg/mL**	**0.625 mg/mL**
AOL	0.493±0.002	0.525±0.004	0.560±0.001	0.590±0.001	0.658±0.001
AOS	0.517±0.002	0.530±0.000	0.551±0.003	0.588±0.002	0.669±0.001
Ascorbic acid	0.422±0.001	0.276±0.001	0.558±0.001	0.667±0.001	0.764±0.000
BHA	0.553±0.001	0.680±0.001	0.710±0.000	0.760±0.000	0.780±0.001

Values are mean ± SD of triplicate determinations. AOL = Agelaea obliqua leaves; AOS = Agelaea obliqua stem; BHA = butylated hydroxyanisole.

Table 8: Percentage inhibition and IC50 of the leaf and stem essential oils of Agelaea obliqua compared with reference standards

Sample	**10 mg/mL**	**5 mg/mL**	**2.5 mg/mL**	**1.25 mg/mL**	**0.625 mg/mL**	**IC50 (mg/mL)**
AOL	50.97	47.83	44.32	41.01	34.09	2.31

AOS	48.56	47.30	44.88	40.88	32.53	7.94
Ascorbic acid	58.09	72.55	44.55	33.73	24.13	5.22
BHA	45.08	32.47	28.83	24.52	22.54	10.58

AOL = Agelaea obliqua leaves; AOS = Agelaea obliqua stem; BHA = butylated hydroxyanisole; IC50 = inhibitory concentration causing 50% inhibition.

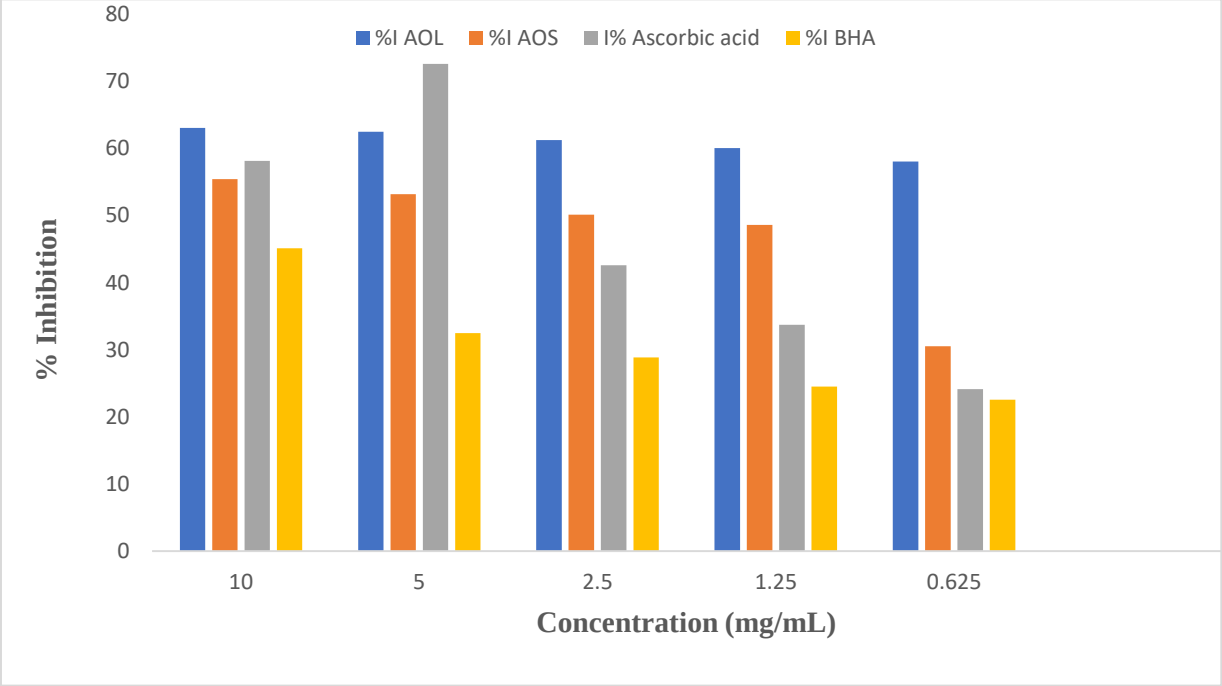


Fig 4.1: % Inhibition essential oil of leaves and stem of *Agelaea obliqua* antioxidant activity compared with the reference standard.

DISCUSSION

The colourless, low-yield essential oils obtained from *A. obliqua* leaves (0.30%) and stem (0.26%) reflect the generally low essential oil content typical of non-aromatic tropical shrubs. The dominance of n-hexadecanoic acid, phytol, oleic acid and 6,10,14-trimethyl-2-pentadecanone in both parts of the plant, alongside organ-specific differences like higher oxygenated mono-, sesqui- and diterpene content in the leaves versus a higher hydrocarbon sesquiterpene content in the stem is indicative of tissue-specific terpenoid biosynthesis within the plant. Only two other Agelaea species have been chemically characterised to date: *A. borneensis*, whose essential oil contains the monoterpenes limonene and α-pinene and the sesquiterpenes β-caryophyllene and D-germacrene (Sardans *et al.*, 2015), and *A. pentagyna*, from which coumarins and the flavonoid tricin have been isolated (Kuwabara *et al.*, 2003); the present findings extend the limited chemical record for the genus.

The markedly stronger cytotoxicity of the leaf oil (LC50 = 15.69 µg/mL) relative to the stem oil (LC50 = 109.51 µg/mL) in the brine shrimp lethality assay, and the reciprocal pattern in antimicrobial potency where the stem oil was active against all eight test organisms at concentrations as low as 0.78 µg/mL while the leaf oil was largely inactive together support the traditional use of *A. obliqua* for parasitic skin infections and bronchitis (Obiang *et al.*, 2015), and point to different bioactive constituents driving cytotoxic versus antimicrobial effects in the two organs. The absence of activity of both oils against *P. aeruginosa* and *S. aureus* suggests the essential oils are more effective against the other Gram-negative organisms and the fungal isolates tested.

The moderate DPPH radical-scavenging activity of both oils relative to ascorbic acid and BHA is consistent with the relatively low proportion of phenolic and hydroxyl-bearing constituents identified by GC-MS, since

such moieties are generally associated with stronger hydrogen/electron-donating capacity and hence stronger antioxidant activity (Ogunlana *et al.*, 2006).

Summarily, the presence of alkaloids, flavonoids, tannins, saponins, terpenoids/steroids and cardiac glycosides in the extracts and fractions of *A. obliqua*, together with the cytotoxic, antimicrobial and antioxidant activities of its essential oils, provides a pharmacological rationale for the ethnomedicinal uses reported for the plant.

CONCLUSION

The essential oils of *A. obliqua* leaves and stem differ both quantitatively and qualitatively: the leaf oil is richer in oxygenated mono-, sesqui- and diterpenes, while the stem oil contains a higher proportion of hydrocarbon sesquiterpenes. The leaf oil was more cytotoxic to brine shrimp nauplii, whereas the stem oil showed the stronger antimicrobial activity against the bacterial and fungal strains tested. Both oils showed measurable, though moderate, DPPH radical-scavenging activity. Phytochemical screening confirmed the presence of alkaloids, flavonoids, tannins, saponins, terpenoids/steroids and cardiac glycosides in the crude extracts and fractions. These findings support the ethnomedicinal use of *A. obliqua* for parasitic skin infections, bronchitis, irritating throat and as an aphrodisiac, and identify its essential oil, particularly from the stem, as a candidate lead for further antimicrobial drug development. Larger-scale collection and isolation of the essential oil, together with spectroscopic (UV, IR, NMR) elucidation of its major and minor constituents, is recommended for future work.

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