

Essential oil of *Alchornea laxiflora* (benth): phytochemical, antimicrobial and toxicity evaluations

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ABSTRACT

Alchornea laxiflora belonging to the Euphorbiaceae family has ethnomedicinal applications as antimalarial, anti-inflammatory, and antimicrobial agent. This present study investigated the spectroscopic, antibacterial, and toxicity profile of essential oil of *Alchornea laxiflora* (ALEO). The composition of ALEO was detected using Gas Chromatography-Mass Spectrometry (GC-MS) and Fourier Transform Infrared (FTIR) Spectroscopy. Using agar disc diffusion, the antibacterial activity of ALEO against five clinical isolates: *Bacillus subtilis* ATCC 6633, *Bacillus cereus* ATCC 10872, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 9027, and *Staphylococcus aureus* ATCC 25923 was evaluated. The toxicity profile of ALEO was obtained in studies involving Wistar rats. GC-MS analysis identified eight bioactive compounds, mostly fatty acids and their ester derivatives. The FTIR spectrum revealed peaks at 3500-3180 cm⁻¹ and 2950-2800 cm⁻¹ corresponding to O-H stretch band of alcohol and the C-H stretch of aliphatic alkane, respectively. The highest zone of inhibition diameter was recorded against *Bacillus cereus* ATCC 10872 and *Escherichia coli* ATCC 25922, while the least zone of inhibition was against *Pseudomonas aeruginosa* ATCC 9027. Oral administration of ALEO caused an elevation in Alanine aminotransferase activity. The essential oil of *Alchornea laxiflora* has potential as an antibacterial. However, the doses used in this study might be slightly hepatotoxic.

Keywords: *Alchornea laxiflora*; antimicrobial activity; essential oil; GC-MS; toxicity

1. INTRODUCTION

The Pharmaceutical industry has benefitted immensely from modern medicine based on naturally occurring molecules and their derivatives. Plants, chemically, and structurally heterogeneous have continued to play a vital role in drug development [1]. Medicinal plants are cheaply sourced, with little or no side effects; hence, they have continued to be valuable resources of natural antimicrobial compounds in the treatment of bacterial infections [2].

Essential oils are complex mixtures of organic compounds that give characteristic odor and flavour to the plants [3]. A myriad of biological activities has been attributed to essential oils extracted from the flowers, leaves, stems, and roots of aromatic plants. Specifically leaf-derived essential have been studied for antibacterial [4, 5], antioxidant [6, 7], antifungal [8], anti-diabetic [9], anti-cancer [10, 11]; anti-parasitic [12, 13], anti-aging [14], anti-inflammatory [15] and hepatoprotective [16, 17] effects. The components and functional groups of the essential oils contribute to their bio-activities. For example, essential oils rich in aldehydes, phenols, and terpenes have been reported to be bioactive [18, 19].

2. MATERIALS AND METHODS

Plant materials.

Fresh samples of *Alchornea laxiflora* leaves were collected from Okigwe in Imo State situated in South-Eastern Nigeria (5°28'58.80" N; 7°32' 60''.00 E). The plant was identified by Mr. Thomas Odewo of the University of Lagos Herbarium, Lagos,

The Euphorbiaceae plant family is widely distributed all around the world, and several plants in this family have therapeutic application. *Jatropha mutabilis* leaves, a source of essential oil, has been shown to have a high content of phytol (41.38 %), which is notable for its antinociceptive and antioxidant activities [20, 21]. In the same vein, the pharmacologically active sesquiterpenes were the main constituent of essential oil derived from *Croton matourensis* leaves [22]. *Alchornea laxiflora* is another notable member of the Euphorbiaceae family. An ethnobotanical survey in South West Nigeria revealed that the leaves, root and bark parts of *A. laxiflora* were highly medicinal, although the leaf parts were the most versatile in the treatment of different ailments [23]. The anti-anaemic [24], antimalarial [25], antibacterial [26], anxiolytic [27] and hepatoprotective [28] effects of the various solvent extracts of *A. laxiflora* leaves have been reported. This study, therefore, aims to evaluate the spectroscopic, antibacterial, and toxicity characteristics of essential oil extracted from *Alchornea laxiflora*.

Nigeria. The essential oil was obtained from the fresh leaves *Alchornea laxiflora* using the hydro-distillation method, as earlier described by Oloyede et al. [29]. Briefly, 120 g of sliced fresh leaves, 550 mL of distilled water and 1 mL of n-hexane measured into a flask and introduced to the Clavenger apparatus. After 3

hours of boiling and condensation, the distillate and essential oil were collected in a tightly sealed MacCartney bottle to prevent the essential oil from evaporating.

Gas chromatography and Fourier Transform Infrared Spectroscopic Studies.

Gas Chromatography-Mass Spectrometer (GC-MS; Agilent Technologies 7890A GC chromatograph system; 7683 series injector) equipment was deployed for the analysis of ALEO. Attached to the GC is HP-5 column (30 m x 0.32 x 0.5 μ m), with helium as the carrier gas. The following conditions were observed; injector and oven temperature (250°C and 60-270°C, respectively), mass spectra (70 eV). Natural compounds in ALEO were identified based on retention times and fragmentation patterns of their mass spectra in comparison with reference compounds from National Institute Standard and Technology (NIST) database. The functional groups in ALEO were determined using FTIR (Shimadzu, 8400s). Samples were pelleted with KBr.

Antimicrobial analysis.

Five bacterial strains, obtained from the Federal Institute of Industrial Research (FIRO), Oshodi Lagos, Nigeria were used. They are *Bacillus cereus* ATCC 10872, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 9027, and *Escherichia coli* ATCC 25922. Susceptibility tests were performed using a modified protocol of the agar disk diffusion method, as previously reported by Osho et al. [30]. Briefly, a stock concentration of 100 mg/mL was prepared by measuring 1 g of ALEO into 10 mL of hexane. 7.5 mL of the stock was taken to prepare 75% of the sample's concentration, and this serial dilution was continued until 25% concentration level. Mueller-Hinton agar (MHA) served as the culture medium. Bacterial culture (18 hours old), was later transferred into a normal saline solution to obtain turbidity comparable to 0.5 McFarland standards (1.5×10^8 cfu/mL). The turbidity of the bacterial suspension was further readjusted to about 10^6 organisms by the addition of 100 μ L of saline solution. Ampicillin was used as the positive control, whereas hexane served as the negative control 24 h. Inhibition zones were measured in millimeters.

Experimental animals and study design.

3. RESULTS

GC-MS and FTIR analyses of ALEO.

The constituents of the oil revealed 8 compounds, composed of fatty acids (84.45%) and fatty acid esters (15.54%) as shown in Table 1. The percentage yield of the oil is as follows: n-Hexadecanoic acid (35.07%), 6-Octadecenoic acid (28.26%), Oleic acid (17.90%), Ethyl 9-tetradecenoate (9.35%), Octadecanoic acid, ethyl ester (3.99%), Pentadecanoic acid (3.22%), Ethyl cyclohexane propionate (1.31%), and Undecanoic acid ethyl ester (0.89%).

The FTIR spectrum of ALEO is shown in Figure 1. The peak at 3178.87 cm^{-1} corresponds to the O-H stretch band of alcohol while the peaks between 2800-2950 cm^{-1} indicate the C-H stretching vibration of aliphatic alkane. The peaks at 2731.29 and 2667.84 cm^{-1} correspond to the C-H aldehyde stretch.

Antimicrobial activity of ALEO.

Of the five strains used in this study, three of them showed clear zones of inhibition (a minimum of 8 mm in diameter). The zone

of inhibition of the other strains ranged from 4 to 7 mm (Table 2). ALEO exhibited the highest inhibitory activity against *Bacillus cereus* ATCC 10872 and *Escherichia coli* ATCC 25922, followed by *Bacillus subtilis* ATCC 6633. The least diameter was recorded for *Pseudomonas aeruginosa* ATCC 9027). Interestingly, ALEO showed a better inhibitory effect against *Bacillus cereus* and *Escherichia coli* than the positive control, Ampicillin. ALEO significantly inhibited microbial growth at 100 μ L/mL.

Growth performance indices, absolute weight and organo-somatic index (OSI).

The effects of ALEO on absolute organ weight and organo-somatic-index (OSI) of the liver are presented in Table 3. The absolute weight of the liver significantly ($p < 0.05$) increased at the highest dose (ALEO 3) when compared to the control. The OSI of the organs were not significantly changed across treatment groups when compared to the control. There were non-significant

Biochemical Estimations.

Liver function enzymes [Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP)], and Albumin (ALB) levels were determined in plasma using diagnostic kits (Randox, Crumlin, UK). Total protein was determined in liver post-mitochondrial fraction according to the method of Gornal et al. [31].

Statistical Analysis.

Values are reported as mean \pm SEM. One-way analysis of variance (ANOVA), followed by Duncan's post-hoc test (Graph Pad Prism 8). *P* - values less than 0.05 were considered to indicate statistical significance

increases in food and water consumption in the treatment groups compared to the control (Table 3).

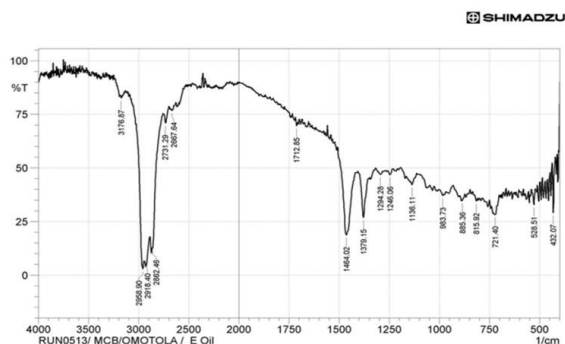


Figure 1 FTIR spectra of ALEO.

Table 1. GCMS characterization of ALEO.

SN	Name of compound	Retention time	Yield (%)
1	n-Hexadecanoic acid	20.97	35.07
2	Pentadecanoic acid	21.23	3.22
3	Oleic acid	23.05	17.90
4	Ethyl 9-tetradecenoate	23.18	9.35
5	6-Octadecenoic acid	23.26	28.26
6	Octadecanoic acid, ethyl ester	23.51	3.99
7	Ethyl cyclohexanepropionate	25.63	1.31
8	Undecanoic acid, ethyl ester	27.67	0.89

Table 2. : Effect of ALEO on diameters of zone of inhibition of five clinical isolates.

Microorganism	Zone of inhibition (mm)	
	ALEO	Ampicillin (positive control)
<i>Bacillus subtilis</i> ATCC 6633	8	8
<i>Bacillus cereus</i> ATCC 10872	9	7
<i>Escherichia coli</i> ATCC 25922	9	6
<i>Pseudomonas aeruginosa</i> ATCC 9027	4	8
<i>Staphylococcus aureus</i> ATCC 25922	7	8

Table 3. The growth performance indices, absolute organ weight and organo-somatic index (OSI) of control and ALEO-treated rats.

	Control	ALEO 1	ALEO 2	ALEO 3
Liver (g)	3.93 ± 0.45	4.74 ± 0.32	5.02 ± 0.43	5.30 ± 0.08*
OSI of the liver	3.69 ± 0.43	3.08 ± 0.20	3.62 ± 0.31	3.92 ± 0.06
Food intake (g)	59.60 ± 6.29	66.66 ± 6.98	61.24 ± 9.12	72.90 ± 7.07
Water intake (ml)	88.57 ± 12.99	84.29 ± 12.51	115.7 ± 19.01	132.90 ± 20.08

Values are expressed as the mean ± SEM (n=5); One-way ANOVA followed by Dunnet's multiple comparison test. * P<0.05.

Biochemical analyses.

Analysis of biochemical indices revealed a significant increase in the activities of the alanine aminotransferase at the 100 mg/kg, 200 mg/kg and 400 mg/kg doses of ALEO. Contrariwise, there was a significant depletion of aspartate aminotransferase activity at the dose of 100 mg/kg ALEO. At the medium and high doses of ALEO (200 and 400 mg/kg, respectively) bilirubin levels were significantly depleted. Administration of ALEO, at all doses, did not significantly alter the alkaline phosphatase and total tissue proteins (Figure 2).

Essential oils (EOs) are complex mixtures formed constituted of compounds with varying functional groups. Several therapeutic activities linked to EOs include anti-inflammatory, antimicrobial, analgesic, antimycotic, antidiabetic, antioxidant, anti-cancer [9,10, 32]. The GC-MS fatty acid analysis revealed eight fatty acids of which the primary constituents are n-Hexadecanoic acid, Octadecenoic acid, and Oleic acid with a percentage yield of

35.07%, 28.26%, and 17.90%, respectively. These results agreed with previous findings which identified hexadecanoic acid and oleic acid as the major fatty acid components of the essential oil from neem and *Tetrapleura tetraptera* [33, 34].

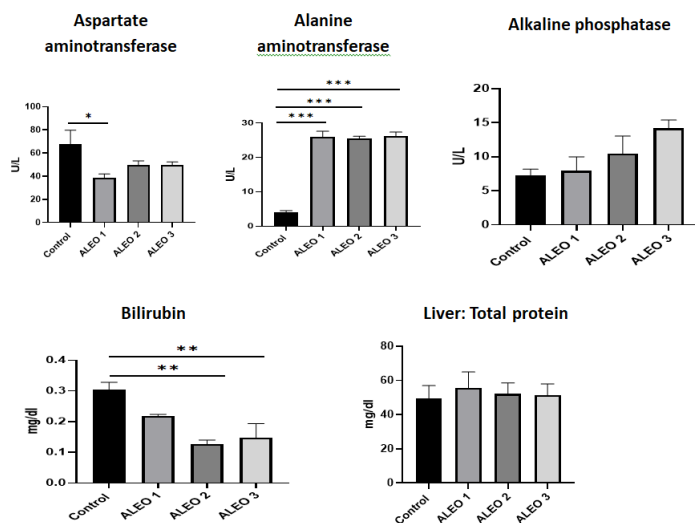


Figure 2. The effect of ALEO on biochemical parameters. Values mean ±SEM (n=5); One-way ANOVA followed by Dunnet's post-hoc test. * P<0.05, **P<0.01, ***P <0.001.

To further characterize the functional groups in ALEO, FTIR analysis was carried out. The spectrum revealed a sharp peak at 3178.87 cm⁻¹ corresponding to the O-H stretch band of alcohol. The peaks between 2800-2950 cm⁻¹ indicate the C-H stretching of alkane, while the peaks in the region of 1712cm⁻¹ expressed the C=O stretching of ketones. Observed spectra within 1500–1100 range corresponded to the C-O stretch common to flavonoids and terpenes [35, 36].

This present study also revealed the inhibitory effect of the ALEO against *Bacillus cereus* ATCC 10872, *Escherichia coli* ATCC 25922, and *Bacillus subtilis*. According to Sebel et al. [37] the essential oils of four *Eucalyptus* species (*E. maideni*, *E. astringent*, *E. cinerea*, *E. bicostata*) showed the highest antibacterial activity against *Listeria ivanovii* and *Bacillus cereus*. Similarly, the essential oil of *Coriandrum sativum* showed a strong inhibitory effect against *Bacillus cereus* and *E. coli* [38]. Several lines of scientific studies suggested that the essential oils exerted higher antimicrobial activity against gram-positive bacteria, compared to the gram-negative bacteria. However, our study showed that ALEO, at the concentration of 100 µl/mL, was active against both Gram-negative and -positive bacteria [5, 39].

From time immemorial, there has been an erroneous belief that herbal remedies or their semi-synthetic derivatives were free of any toxic effects [40]. Improper dosage regimen, indiscriminate, and prolonged use of herbal medicines have been implicated as causal effects of toxicity arising from their usage. In a study using five different medicinal plants *Cymbopogon*, *Artemisia*, *Cynanchum argel delile*, *Equisetum*, and *Vitex agnus-castus*, Brima [41] reported that these medicinal plants were sources of toxic elements including Aluminum, Lead, Arsenic, and Cadmium, that may be detrimental to human health. Studies have shown that some medicinal plants such as Ephedra species, *Aconitum* species, *Datura* species, and *Lobelia* species in long-term use have a strong toxic effect, particularly in the

children [42]. Recently, essential oil, produced from Myrrh, was found to be toxic to the liver, spleen, and kidney of mice treated at the higher doses of 20, 40, and 80 μL compared to animals treated with doses of 1, 5, and 10 μL for the same three-day duration [43]. Despite the salutary effects of essential oil as a natural remedy, there should also be an awareness of its potential toxicity. In this 7-day repeated toxicity study using graded doses of ALEO, there were no significant changes in the organo-somatic index (OSI) of the liver, water, and food intake in the test and control groups. Although the administration of ALEO at the dose of 400 mg/kg induced a significant increase in absolute liver weight, however, the OSI is a better toxicity indicator because it factors in organ-to-body weight ratio. Furthermore, there were no treatment-related changes in alkaline phosphatase and tissue total protein

4. CONCLUSIONS

The GC-MS analysis of ALEO showed hexadecanoic acid as the most prominent constituent followed by 6-Octadecenoic acid and oleic acid. The FTIR spectrum also revealed the presence of hydroxyl, amino, aldehyde, and ketone functional groups peculiar to flavonoids and terpenes. The antimicrobial effect of ALEO could be associated to the presence of these functional groups and bioactive principles. The antibacterial effect of ALEO

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levels. Despite these positive effects of ALEO on the liver, other sero-clinical indices of the treatment groups were altered. There was an opposing effect on the transaminases. While AST activity was significantly depleted at the 100 mg/kg dose, the activity of ALT was significantly elevated at all treatment doses. A similar study reported high levels of transaminases after an acute administration of neem oil to rats, suggestive of hepatocellular breakdown (34). Administration of ALEO also elicited a significant reduction in bilirubin levels, which may have clinical implications because depressed plasma bilirubin levels have been proposed as a potential biomarker of renal function [44]. Hence, further studies on the effect of ALEO on kidney function is warranted.

against gram-positive (*Bacillus cereus* ATCC 10872) and gram-negative (*Escherichia coli* ATCC 25922) bacteria is reported. Oral administration of ALEO, at the doses of 200 and 400 mg/kg, in rats elevated ALT activity and depleted bilirubin levels, indicating a possible derangement of hepatic function at high dose exposure. Based on these findings, ALEO may have practical applications in human health at lower doses of exposure.

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