

**Tropical Journal of Phytochemistry & Pharmaceutical Sciences**Available online at <https://www.tjpps.org>**Original Research Article****Phytochemistry and Bioactivity of Nigerian *Croton lobatus* Stem Bark**Sani M. Isyaka<sup>1,2,3,6</sup>, Khalifa B. Al-Adam<sup>2</sup>, Ahmed Umar<sup>2</sup>, Bilkis Adedoyin<sup>2</sup>, Bintu Muhammad Mofio<sup>2</sup>, Bemgba B. Nyakuma<sup>6</sup>, Zakari Abdu<sup>3</sup>, Saidu Jibril<sup>3</sup>, Yakubu Rufai<sup>4</sup>, John V. Anyam<sup>1,5,6\*</sup>, and John O. Igoli<sup>5</sup><sup>1</sup>Centre for African Medicinal Plants Research, North-Eastern University, Gombe (Formerly, Pen Resource University) – Gombe State, Nigeria<sup>2</sup>Department of Chemistry, Faculty of Science, University of Abuja, Gwagwalada, Nigeria<sup>3</sup>Department of Chemical Sciences, Faculty of Science, Federal University of Kashere, Gombe, Nigeria<sup>4</sup>Chemistry Department, Federal College of Education, Okene, Kogi State, Nigeria<sup>5</sup>Department of Chemistry, College of Physical Sciences, Joseph Sarwuan Tarka University, Makurdi, Benue State, Nigeria<sup>6</sup>Department of Chemical Sciences, North-Eastern University, P. M. B. 0198 Gombe, Gombe State, Nigeria.

## ABSTRACT

*Croton lobatus* L. (*Euphorbiaceae*) is an important medicinal plant commonly used in traditional medicine. In this study, the *C. lobatus* plant was subjected to detailed phytochemical investigation with the view to isolate and characterize compounds from its dichloromethane and methanol stem bark extracts. Using silica gel column chromatography, lupeol, lupenone, and octylferulate, were isolated and subsequently characterized by nuclear magnetic resonance (NMR) spectroscopy, and gas chromatography–mass spectroscopy (GC-MS). The evaluation of the antioxidant and cytotoxicity potentials of the crude extracts and isolated compounds showed remarkable antioxidant activity at variable concentrations (0.5 mg/mL, 1 mg/mL, and 1.5 mg/mL), highlighting the therapeutic prospects of *C. lobatus* L. Furthermore, the stem bark extracts exhibited high toxicity, as evidenced by an LC50 value of 58.012 µg/mL observed during the brine shrimp toxicity assay. The study findings not only add to the understanding of the phytochemical composition of *C. lobatus* L. but also highlights the plant's potential as a source of bioactive chemicals with strong cytotoxic and antioxidant effects. Lastly, the findings will facilitate more pharmacological research and therapeutic applications by providing insightful information about the plant's potential for medicinal use.

**Keywords:** Bioassay, *Croton lobatus*, *Euphorbiaceae*, Phytochemical Investigation, Medicinal Plants

Received 26 December 2024

Revised 10 January 2025

Accepted 23 January 2025

Published online 01 March 2025

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*Croton lobatus* L. (*Euphorbiaceae*) (synonym: *Astraea lobata* (L.) Klotzsch) is native to South America but has been introduced to tropical Africa where it is used as herbal medicine and vegetable.<sup>1</sup> In Nigeria, the plant is known as *Gaásàyaá* (Hausa), *Òkwè* (Igbo), and *Àjẹìofòlè* (Yoruba) in the three major languages.<sup>2</sup>

Multiple biological activities of *C. lobatus* L. have been documented on its aerial parts, encompassing antibacterial,<sup>2</sup> antidiabetic,<sup>2,3</sup> antiplasmodial, antioxidant,<sup>2</sup> antitrypanosomal,<sup>4</sup> leishmanicidal, cytotoxic, and toxic effects.<sup>4,5</sup> The aerial parts of the plant which have numerous applications in African ethnomedicine have also been subject to the most phytochemical investigations. Attioua *et al.*,<sup>6</sup> have isolated the alkaloids Palmitanoide, Onosmin B, and Onosmin A from aerial parts of *Croton lobatus*. Stuart and Woo-Ming,<sup>7</sup> have isolated the diterpene Vomifoliol, the phenolic acid (E)-3-(4-methoxy-phenyl)-2-phenyl-acrylic acid and the steroidal ketone, Cholestan-3-one from the leaves of the plant. Attioua *et al.*,<sup>6</sup> have isolated Cholesta-5,7-dien-3-ol, Ergosterol, 3-Hydroxycholest-5-en-7-one while Lagnika *et al.*,<sup>4</sup> isolated the flavonoids Tiliroside, Isovitexin, and Vitexin from the leaf of *Croton lobatus*.

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Research on Nigerian *Croton lobatus* and its specific plant parts and extracts remains scarce. This knowledge gap extends to its natural product composition, phytochemistry, and bioactivity. In this study we seek to isolate and characterize the bioactive constituents present in stem extracts of *C. lobatus* sourced from Nigeria.

**Materials and Methods***Sample collection*

The plant material was collected from Chikaji village, Zaria, Kaduna State on 22nd April 2021 and authenticated at the Department of Botany, Ahmadu Bello University, Zaria, by Dr U. S. Gallah. The stem bark and roots of *C. lobatus* were collected in plastic bags and transported to the Chemistry laboratory of the University of Abuja. The plant material was weighed after collection and air-dried at room temperature. The dried stem and root bark was further size reduced into small pieces, pulverized to powder, weighed, and stored in a tight polythene bag.

*Extraction of plant material*

The pulverized air-dried plant material (1 Kg) was successively extracted with dichloromethane (2.5 L) exhaustively. The marc was air dried and further extracted methanol (2.5 L) for 72 hours and then filtered. The extracts were evaporated to dryness using a rotary evaporator at 50 °C to yield the crude extracts. Before evaporation to dryness, thin layer chromatography (TLC) was run using a silica gel pre-coated TLC plate with different solvent systems. The weight, state, and colour of dry extracts were noted (Figure 9).

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**Citation:** Isyaka MS, Al-Adam KB, Umar A, Adedoyin B, Mofio BM, Bemgba BN, Abdu Z, Jibril S, Rufai Y, Anyam JV, Igoli JO. Phytochemistry and Bioactivity of Nigerian *Croton lobatus* Stem Bark. Trop J Phytochem Pharm. Sci. 2024; 4(2): 79 – 84  
<http://www.doi.org/10.26538/tjpps/v4i3.7>

Official Journal of Natural Product Research Group, Faculty of Pharmacy,

### Thin-layer chromatography (TLC)

Thin-layer chromatography spotting was performed manually using capillary tubes and developed in an air-tight saturated chromatographic tank at room temperature using combinations of n-hexane and ethyl acetate as solvent systems. The developed chromatograms were air dried and visualized under normal day light, and ultraviolet light (254 nm and 366 nm) before spraying. In this study, the spraying agents used were *p*-anisaldehyde and 10% sulfuric acid followed by heating with an air dryer for 5 – 10 minutes (Figure 9).

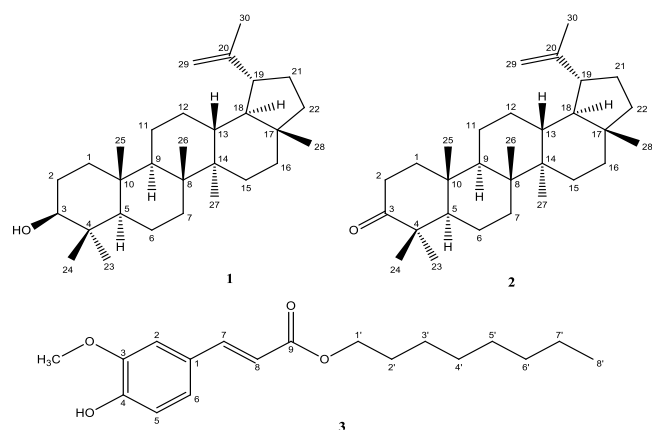
### Column chromatography of dichloromethane (CH<sub>2</sub>Cl<sub>2</sub>) crude extract

The root extract (5 g) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (30 mL) solvent, mixed with silica gel (20 g), and crushed. The sample was dried and ground using a porcelain mortar and pestle. It was then loaded onto a silica gel column (5 × 75 cm) using wet packing and dry sample loading methods. CH<sub>2</sub>Cl<sub>2</sub> (100%) was used as the solvent to obtain 22 fractions (10 mL each). Afterward, the column was washed with MeOH. Fractions 1, 3, 8, and 9, from the dichloromethane elution, with a single spot on TLC were combined. These combined fractions were further analyzed by TLC using a hexane: ethyl acetate (8:2) solvent system. Furthermore, column chromatography using a gradient solvent system was used to elute the combined CH<sub>2</sub>Cl<sub>2</sub> fraction of the stem bark, starting with n-hexane (100%) and gradually increasing the polarity with CH<sub>2</sub>Cl<sub>2</sub> (5% stepwise), followed by CH<sub>2</sub>Cl<sub>2</sub>/EtOAc (90:10) to 100% ethyl acetate. Methanol was used for final washing. A total of 227 10 mL aliquots were collected. Fractions with similar TLC profiles were combined to obtain major fractions. Fractions 158-162 and 177-178 showed single spots, which were further confirmed using other solvent systems (hexane: ethyl acetate (8:2,1:1); hexane: dichloromethane (8:2,1:1); dichloromethane: methanol (9:1)), resulting in the isolation of two white crystalline compounds, named CL-1 and CL-2. These compounds underwent 1D and 2D-NMR analysis (Figure 9).

### Characterization Procedure

#### Solubility test

The isolated compounds (Figure 1) were subjected to solubility tests using different solvents (*n*-hexane, chloroform, ethyl acetate and methanol).



**Figure 1:** Isolated compounds 1-3

#### Spectral analysis

The compounds' chemical structure and functional groups were determined using NMR and Fourier Transform Infrared (FTIR) spectroscopy at the Royal Botanic Gardens, Kew (United Kingdom). NMR experiments were conducted on the Bruker AVANCE III NMR spectrometer at 400.MHz CDCl<sub>3</sub> was referenced at  $\delta$ H 7.26 and  $\delta$ C 77.23 for <sup>1</sup>H and <sup>13</sup>C NMR spectra, respectively. NMR spectra were processed with academic Topspin software. GC-MS analysis was performed using an Agilent Technologies 6890N GC system coupled to an Agilent Technologies HP5973 mass electron detector, with samples dissolved in CH<sub>2</sub>Cl<sub>2</sub>.

### Antioxidant assay protocol

#### DPPH free-radical scavenging activity

DPPH free-radical scavenging was carried out as described by Isyaka *et al.*,<sup>8</sup> without modification. Crude extracts were reconstituted to concentrations of 0.25 mg/mL, 0.5 mg/mL, 1.0 mg/mL, and 1.5 mg/mL. The concentrations were arrived at by dissolving 0.49 mg, 0.98 mg, 1.47 mg, and 1.96 mg of each extract in 2 mL of solvent, respectively. A solution of DPPH (1 M) was prepared by dissolving of DPPH (39.4 mg) in methanol (MeOH) (100 mL). After a 10-minute incubation, absorbance at 517 nm was measured. A 0.5 mL aliquot of each test solution was mixed with 2 mL of the DPPH solution, incubated for 10 minutes, and the absorbance was measured against a control. The percentage inhibition was calculated (Equation 1). The same procedure was applied to butylated hydroxyanisole (BHA) as a standard, prepared in distilled water, with percentage inhibition calculated according to Equation 2.

$$\text{Inhibition} = \frac{(A_{DPPH} - A_S)}{A_{DPPH}} \times 100\% \quad (1)$$

A<sub>DPPH</sub> and A<sub>S</sub> represent the absorbance of the neat DPPH and test solutions, respectively.<sup>9</sup>

#### Trolox equivalent antioxidant capacity (TEAC) Assay

The TEAC assay evaluates the antioxidant capacity by quenching the ABTS radical cation, compared to Trolox, a water-soluble vitamin E analogue.<sup>10</sup> As reported previously,<sup>8</sup> a stable ABTS stock solution was prepared by reacting a 7 mM aqueous solution of ABTS with 2.45 mM potassium persulfate, incubated in the dark for 16 hours.. The assay involved mixing 1 mL of ABTS solution (156  $\mu$ M in 100 mM phosphate buffer, pH 7.4), 1 mL of NADH solution (468  $\mu$ M in 100 mM phosphate buffer, pH 7.4), and 1 mL of the sample solution (0.25, 0.5, 1.0, and 1.5 mg/mL) in their respective solvents. Absorbance at 734 nm was measured, and percentage inhibition was calculated using Equation 2.

$$\text{Inhibition} = \frac{(A_{\text{blank}} - A_{\text{sample}})}{A_{\text{blank}}} \times 100\% \quad (2)$$

The terms are defined as A<sub>blank</sub> is the absorbance of the blank in the absence of a sample, and A<sub>sample</sub> is an absorbance in the presence of the sample.

#### Metal chelating activity

Metal chelating activity was done as reported elsewhere.<sup>8,11-13</sup> The assay involved a reaction mixture containing 0.5 mL of the extract solutions (0.25, 0.5, 1.0, and 1.5 mg/mL), 1.5 mL of deionized water, and 0.5 mL of 1 M FeCl<sub>2</sub> solution. After 30 minutes, 1.0 mL of 5 M ferrozine solution was added, and the mixture was incubated for 10 minutes. Absorbance at 562 nm was measured, and percentage inhibition was calculated using Equation 3, with BHA as a positive control.

:

$$\% \text{ Activity} = \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \times 100\% \quad (3)$$

Th terms are defined as A<sub>control</sub> is the absorbance of the blank in the absence of the sample; and A<sub>sample</sub> is an absorbance in the presence of the sample.

#### Toxicity Procedure

##### Brine Shrimp Lethality Test (BSLT)

For the BSLT, brine shrimp eggs (70 g) were hatched in seawater (250 mL) for 48 hours. Extract solutions (1000, 100, and 10  $\mu$ g/mL) were prepared in triplicate, with DMSO as a solvent. Ten shrimp larvae were added to each vial, and survival was recorded after 24 hours. The LC50 was calculated using Finney probit analysis software. Procedure as previously elaborated by Isyaka *et al.*,<sup>8</sup> Adedoyin *et al.*,<sup>11</sup> and Chan *et al.*,<sup>14</sup> was followed without modification in the *Artemia salina* lethality assay.

## Results and Discussion

### Yield of extracts

The crude CH<sub>2</sub>Cl<sub>2</sub> and MeOH extracts of the stem bark of *C. lobatus* weighed 36.5 g, and 45 g respectively. The percentage yield was calculated thus:

$$\text{Percentage Yield} = \frac{\text{Weight of Extract}}{\text{Weight of Plant Material}} \times 100$$

For the CH<sub>2</sub>Cl<sub>2</sub> extract:

$$\text{Percentage Yield} = \frac{36.5 \text{ g}}{1000 \text{ g}} \times 100 = 3.65\%$$

For the MeOH extract:

$$\text{Percentage Yield} = \frac{45 \text{ g}}{1000 \text{ g}} \times 100 = 4.5\%$$

### Isolated compounds

**Lupeol (1)** (CL-1): White solid.  $[\alpha]_D^{19} = -22.8$  (c 0.50, CH<sub>2</sub>Cl<sub>2</sub>). LREIMS:  $m/z$  426.2 [M]<sup>+</sup> (C<sub>30</sub>H<sub>50</sub>O; 6 degrees of unsaturation). FTIR:  $\nu_{\text{max}}$  3416 (O-H), 3055 (=C-H), 2940, 2871 (C-H aliphatic). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$ H 4.68 (1H, *d*, *J* = 2.2 Hz, H-29A), 4.57 (1H, *d*, *J* = 2.2 Hz, H-29B), 3.17 (1H, *dd*, *J* = 5.7, 11.0 Hz, H-3), 2.38 (1H, *sext*, *J* = 5.7 Hz, H-18), 1.90–0.67 (m, CH<sub>2</sub>/CH<sub>3</sub>). Methyl resonances:  $\delta$ H 0.97 (H-23), 0.76 (H-24), 0.83 (H-25), 1.03 (H-26), 0.94 (H-27), 0.79 (H-28), 1.68 (H-30). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$ C 79.2 (C-3), 151.2 (C-20), 109.5 (C-29), 19.5 (C-30), 55.5 (C-5), 50.7 (C-9), 48.2 (C-18), 48.5 (C-19), 39.1 (C-4), 43.2 (C-17), 43.0 (C-14), 41.0 (C-8), 38.9 (C-1), 38.3 (C-13), 37.4 (C-10), 34.5 (C-7), 35.8 (C-16), 30.1 (C-21), 28.4 (C-23), 27.6 (C-15), 27.7 (C-2), 25.4 (C-12), 21.1 (C-11), 18.5 (C-6), 18.2 (C-28), 16.3 (C-25), 16.2 (C-26), 15.6 (C-24), 14.8 (C-27). The molecular formula, C<sub>30</sub>H<sub>50</sub>O ( $m/z$  426.2 [M]<sup>+</sup>; 6 degrees of unsaturation), and FTIR absorptions ( $\nu_{\text{max}}$  3416 cm<sup>-1</sup> for O-H, 3055 cm<sup>-1</sup> for =C-H) corroborate a pentacyclic structure with one double bond and five rings. The <sup>1</sup>H NMR spectrum revealed diagnostic signals for the  $\Delta$ 20(29) exocyclic methylene [ $\delta$ H 4.68 (H-29a) and 4.57 (H-29b), each *d*, *J* = 2.2 Hz], a  $\beta$ 3-oxymethine proton [ $\delta$ H 3.17 (*dd*, *J* = 5.7, 11.0 Hz, H-3)], and seven angular methyl groups ( $\delta$ H 0.67–1.68), while the <sup>13</sup>C NMR spectrum confirmed the oxygenated C-3 ( $\delta$ C 79.2), the  $\Delta$ 20(29) olefin ( $\delta$ C 151.2 and 109.5), and characteristic methyl carbons ( $\delta$ C 14.8–19.5). These spectral signatures, aligning with literature data for lupane triterpenoids, unequivocally established the structure as lupeol.<sup>8,10,13,15,16</sup>

**Lupenone (2)**: white solid. LREIMS: [M]<sup>+</sup> at  $m/z$  424.4 (C<sub>30</sub>H<sub>48</sub>O, seven degrees of unsaturation). FTIR (cm<sup>-1</sup>): 2940, 2869 (C-H aliphatic stretches), 1705 (C=O stretch, ketone). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$ H: 1.43 (*m*, H-1 $\alpha$ ), 1.38 (*m*, H-1 $\beta$ ), 2.48 (*m*, H-2 $\alpha$ ), 2.40 (*m*, H-2 $\beta$ ), 1.32 (*m*, H-5), 1.68 (*m*, H-6 $\alpha$ ), 1.46 (*m*, H-6 $\beta$ ), 1.44 (*m*, H-7 $\alpha$ ), 1.44 (*m*, H-7 $\beta$ ), 1.38 (*m*, H-9), 1.44 (*m*, H-11 $\alpha$ ), 1.40 (*m*, H-11 $\beta$ ), 1.75 (*s*, H-12 $\alpha$ ), 1.66 (*m*, H-12 $\beta$ ), 1.68 (*s*, H-13), 1.67 (*m*, H-15 $\alpha$ ), 1.01 (*m*, H-15 $\beta$ ), 1.49 (*m*, H-16 $\alpha$ ), 1.39 (*m*, H-16 $\beta$ ), 1.38 (*m*, H-18), 2.40 (*m*, H-19), 1.90 (*m*, H-21 $\alpha$ ), 1.25 (*m*, H-21 $\beta$ ), 1.19 (*m*, H-22 $\alpha$ ,  $\beta$ ), 1.07 (*s*, H-23), 1.02 (*s*, H-24), 0.93 (*s*, H-25), 1.07 (*s*, H-26), 0.95 (*s*, H-27), 0.80 (*s*, H-28), 4.69 (*d*, *J* = 2.2 Hz, H-29A), 4.57 (*d*, *J* = 2.2 Hz, H-29B), 1.68 (*s*, H-30). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$ C: 39.8 (C-1), 34.4 (C-2), 218.4 (C-3), 47.6 (C-4), 55.1 (C-5), 19.9 (C-6), 33.8 (C-7), 41.0 (C-8), 50.0 (C-9), 37.1 (C-10), 21.7 (C-11), 25.4 (C-12), 38.4 (C-13), 43.2 (C-14), 27.6 (C-15), 35.7 (C-16), 43.1 (C-17), 48.5 (C-18), 48.2 (C-19), 151.1 (C-20), 30.1 (C-21), 40.2 (C-22), 26.9 (C-23), 21.3 (C-24), 16.2 (C-25), 16.0 (C-26), 14.7 (C-27), 18.2 (C-28), 109.6 (C-29), 19.5 (C-30). Specific rotation:  $[\alpha]_D^{19} = -56.0$  (c 0.80, CH<sub>2</sub>Cl<sub>2</sub>). The spectroscopic data is consistent with a lupane-type triterpenoid bearing a 3-keto group. The molecular formula C<sub>30</sub>H<sub>48</sub>O (LREIMS:  $m/z$  424.4 [M]<sup>+</sup>; seven degrees of

unsaturation) and FTIR absorption at 1705 cm<sup>-1</sup> (C=O stretch) confirm the replacement of the  $\beta$ 3-hydroxyl group in lupeol with a ketone, introducing an additional double bond equivalent. The <sup>1</sup>H NMR spectrum retains diagnostic signals for the  $\Delta$ 20(29) exocyclic methylene [ $\delta$ H 4.69 (H-29a) and 4.57 (H-29b), each *d*, *J* = 2.2 Hz], characteristic of the lupane skeleton, while the absence of the oxymethine proton (H-3) and the presence of a deshielded <sup>13</sup>C NMR resonance at  $\delta$ C 218.4 (C-3) unequivocally establish the ketone functionality. Angular methyl groups ( $\delta$ H 0.80–1.75;  $\delta$ C 14.7–26.9) and carbons associated with the pentacyclic framework (e.g.,  $\delta$ C 151.1 for C-20,  $\delta$ C 109.6 for C-29) align with lupeol's core structure, albeit with a reduced proton count (C<sub>30</sub>H<sub>48</sub>O vs. C<sub>30</sub>H<sub>50</sub>O) due to oxidation at C-3. The pronounced laevorotatory optical rotation ( $[\alpha]_D^{19} = -56.0$ , CH<sub>2</sub>Cl<sub>2</sub>) reflects stereochemical rigidity imparted by the ketone. These data, corroborated by literature, confirm lupenone as the 3-oxo derivative of lupeol, distinguished by its carbonyl group and retained  $\Delta$ 20(29) exocyclic olefin, hallmarks of lupane triterpenoids.<sup>12</sup>

**Octyl trans-Ferulate (3)** (CL-3): Oil <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$ H 7.59 (1H, *d*, *J* = 16.0 Hz, H-7), 6.28 (1H, *d*, *J* = 16.0 Hz, H-8), 7.04 (1H, *d*, *J* = 1.9 Hz, H-2), 6.91 (1H, *d*, *J* = 8.2 Hz, H-5), 7.06 (1H, *dd*, *J* = 1.9, 8.2 Hz, H-6), 3.92 (3H, *s*, OCH<sub>3</sub>), 4.19 (2H, *t*, *J* = 7.0 Hz, H-1' $\alpha$ ), 4.12 (2H, *t*, *J* = 6.7 Hz, H-1' $\beta$ ), 1.26–1.68 (10H, *m*, H-2'–H-7'), 0.88 (3H, *t*, *J* = 6.7 Hz, H-8'). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$ C 127.3 (C-1), 109.5 (CH, C-2), 147.0 (C-3), 148.1 (C-4), 114.9 (CH, C-5), 123.3 (CH, C-6), 144.8 (CH, C-7), 115.9 (CH, C-8), 167.6 (C=O, C-9), 56.2 (OCH<sub>3</sub>, C-3'), 64.8 (CH<sub>2</sub>, C-1'), 22.9–33.2 (CH<sub>2</sub>, C-2'–C-7'), 14.3 (CH<sub>3</sub>, C-8'). Trans olefinic protons were observed at  $\delta$ H 7.59 (H-7) and  $\delta$ H 6.28 (H-8), methoxy group at  $\delta$ H 3.92 (OCH<sub>3</sub>), an aromatic ABX system:  $\delta$ H 7.04 (H-2), 6.91 (H-5), 7.06 (H-6), a carbonyl carbon at  $\delta$ C 167.6 and trans olefinic carbons at  $\delta$ C 144.8 (C-7) and 115.9 (C-8). Literature comparison led to unambiguous confirmation of the structure as octyl trans-ferulate.<sup>17</sup>

### Antioxidant assay

The results (Figure 2-7) obtained show that the CH<sub>2</sub>Cl<sub>2</sub> stem bark extract in DPPH (Figure 2), ABTS (Figure 6) and Metal Chelation (Table 7) at 0.25 mg/mL is 42.3 - 50.3%, 0.5 mg/mL is 54.8 - 58.2%, 1.0 mg/mL is 67.1 - 71.1% and 1.5 mg/mL is 70.4 - 83.3% compared to BHA (Figure 3), Ascorbic acid (Figure 2) and  $\alpha$ -tocopherol (Figure 4) at 0.25 mg/mL is 45.7 - 50.7%, 0.5 mg/mL is 58.5 - 62.3%, 1.0 mg/mL is 63.3 - 78.4% and 1.5 mg/mL is 69.7 - 81.2%. The stem bark extracts show good antioxidant activity at 0.5 mg/mL, 1 mg/mL and 1.5 mg/mL for extracts and drugs used (Figure 5). Antioxidant activity (86%) has been reported from the ethanol extract of *C. gratissimus*.<sup>18</sup> A 3.4 to 4.9% variation in the antioxidant activity of *C. lobatus* leaves extract has been reported.<sup>18</sup> The antioxidant activity of *Croton caudatum* was 83.29% with a concentration of 40 mg/mL.<sup>19</sup> *Croton bonplandianum* species, at 0.5 mg/mL exhibited 59.62% activity.<sup>20</sup> The compounds responsible for antioxidant activity are reported to be phenolic, condensed tannins and flavonoid compounds.<sup>21-23</sup> Table 1 presents the values for the effective concentrations (EC<sub>50</sub>) of *C. lobatus* deduced in this study.

**Table 1:** Effective Concentration (EC<sub>50</sub>) of *C. lobatus*

Sample	EC <sub>50</sub> (µg/mL)		
	DPPH assay	ABTS assay	Metal Chelation assay
CH <sub>2</sub> Cl <sub>2</sub> Extract	36.4	75.2	195
BHA	6.9	2.8	15.0
Ascorbic acid	2.81	15.0	75.0
$\alpha$ -tocopherol	35.3	125.0	250

$$R^2 = 0.912$$

$$EC_{50} = 2.81 \mu\text{g}/\text{cm}^3$$

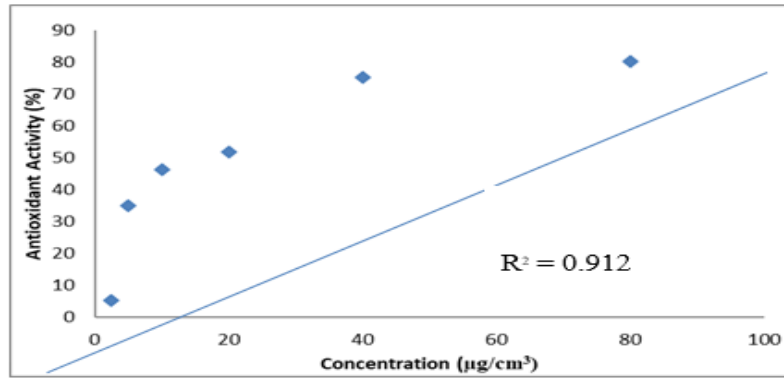


Figure 2: Effective Concentration (EC<sub>50</sub>) of Ascorbic acid in DPPH Assay

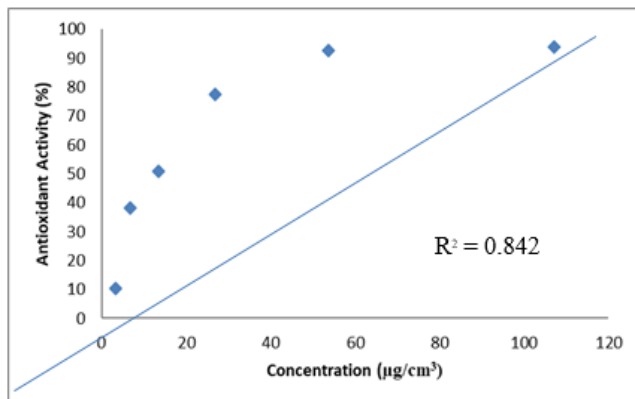


Figure 3: Effective Concentration (EC<sub>50</sub>) of BHA in DPPH Assay

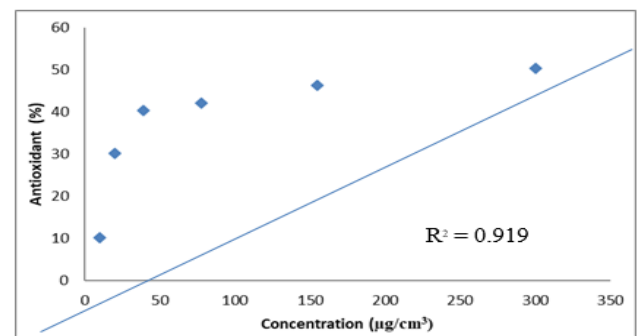


Figure 6: Effective Concentration (EC<sub>50</sub>) of CH<sub>2</sub>Cl<sub>2</sub> extract in ABTS Assay

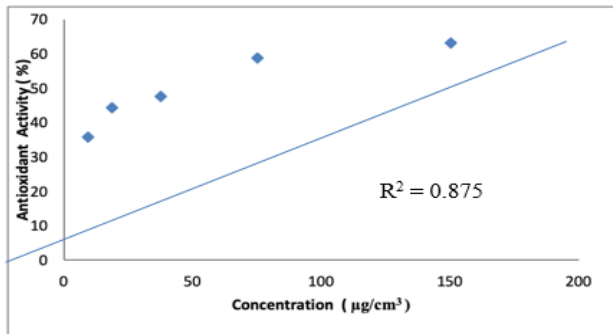


Figure 4: Effective Concentration (EC<sub>50</sub>) of α-tocopherol in DPPH assay

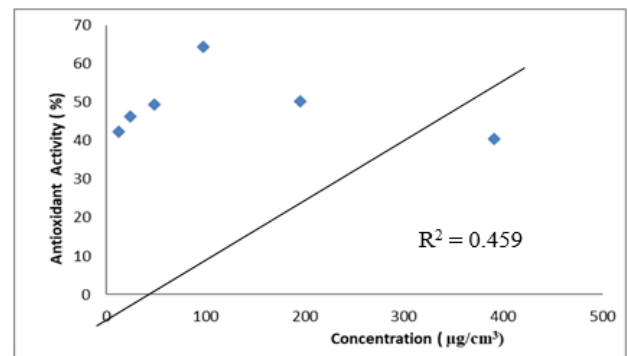


Figure 7: Effective concentration (EC<sub>50</sub>) of CH<sub>2</sub>Cl<sub>2</sub> extract in Metal Chelation Assay

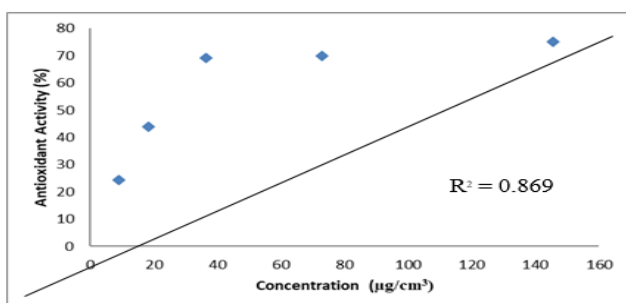


Figure 5: Effective concentration (EC<sub>50</sub>) of CH<sub>2</sub>Cl<sub>2</sub> Extract in DPPH Assay

#### Toxicity of *C. lobatus* stem bark CH<sub>2</sub>Cl<sub>2</sub> extract

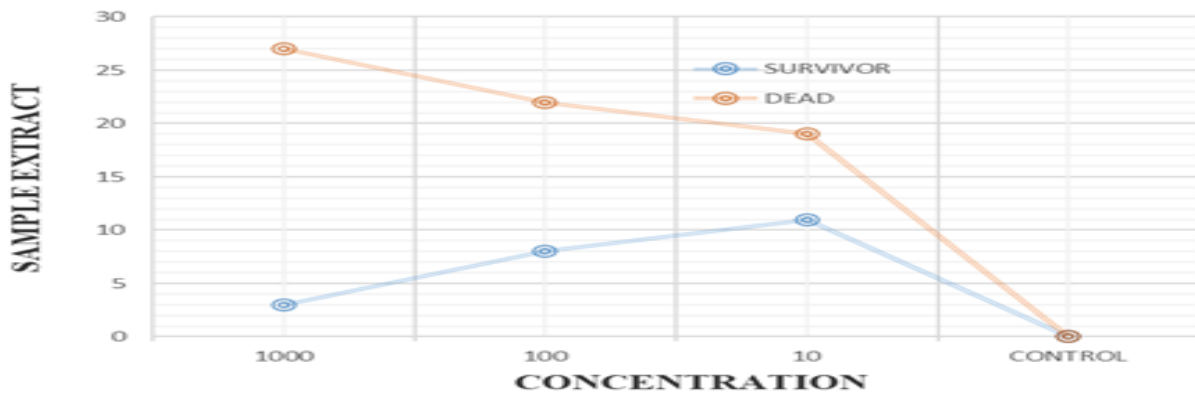
Toxicity activity was determined using the brine shrimp lethality assay.<sup>24</sup> *Artemia salina* is considered vulnerable to toxins at the early developmental stage. The survival rate rises with decreasing concentration, whereas the death rate falls with decreasing concentration.<sup>25-28</sup> Table 2 shows that the CH<sub>2</sub>Cl<sub>2</sub> extract of the stem bark LC<sub>50</sub> is 58.012 µg/mL.

Since it is reported that LC<sub>50</sub> that is less than 1000 µg/mL is toxic then it can be reported that the CH<sub>2</sub>Cl<sub>2</sub> extract of *Croton lobatus* stem bark is highly toxic. It has been suggested that some of the plant extracts with LC<sub>50</sub> value below 100 µg/mL which are categorized as toxic do not always indicate danger or out-right toxicity toward humans but may also suggest a potential antitumor or anticancer activity. The brine shrimp lethality test is reported to detect antitumor compounds in plant extracts.<sup>28-30</sup> Preliminary toxicity assay results suggest that the stem bark extract of *C. lobatus* contains bioactive compounds with cytotoxic properties (Figure 8, Table 2).

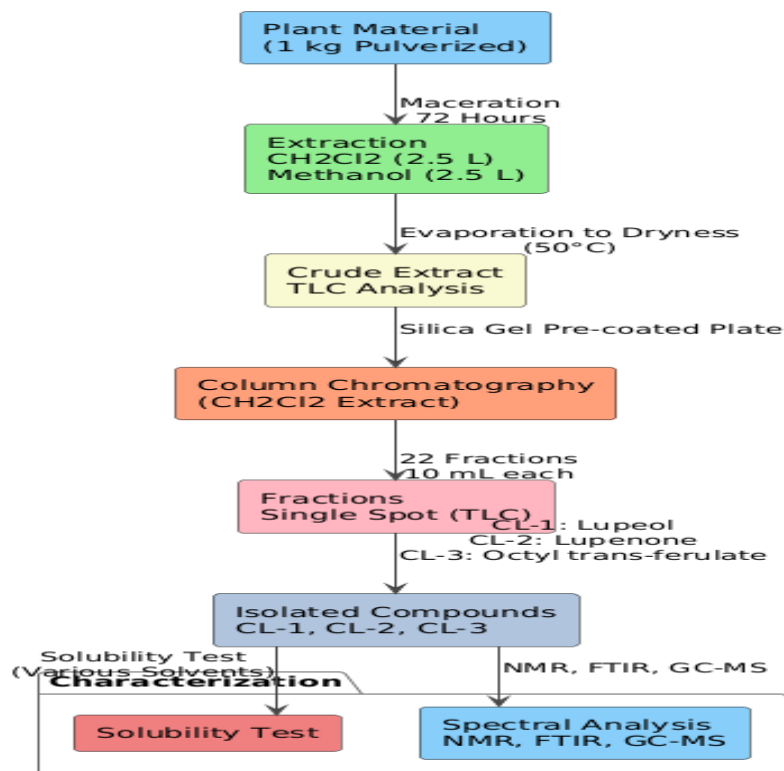
**Table 2:** Brine–shrimp lethality test result of *Croton lobatus* Crude extracts

Conc.	1000 µg/mL		100 µg/mL		10 µg/mL		Control		LC <sub>50</sub> U/L limit
	Survivor	Dead	Survivor	Dead	Survivor	Dead	Survivor	Dead	
Sample extract									
CH <sub>2</sub> Cl <sub>2</sub>	3	27	8	22	11	19	0	0	58.012 (32.36/203.34)

LC<sub>50</sub> 1000µg/mL = Toxic, LC<sub>50</sub> > 1000µg/mL = Not Toxic \*High/Low 95% Confidence interval



**Figure 8:** Average death of *Artemia salina* as a function of CH<sub>2</sub>Cl<sub>2</sub> extract concentration on stem bark of *Croton lobatus* was monitored after 24 hr exposure.



**Figure 9.** Extraction Chromatographic and Structure Elucidation Workflo

## Conclusion

Phytochemical investigation of *Croton lobatus* L. (Euphorbiaceae) has led to the isolation and characterization of lupeol, lupenone, and octylferulate from its dichloromethane and methanol stem bark extracts. Antioxidant assays demonstrated remarkable activity across variable concentrations, while brine shrimp lethality tests revealed high toxicity with an LC50 of 58.012 µg/mL. These findings highlight the therapeutic potential of *C. lobatus* L. as a source of bioactive compounds with significant antioxidant and cytotoxic properties.

## Conflict of interest

The authors declare no conflict of interest

## Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them

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