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# Antioxidant, Cytotoxicity Studies and Isolation of Trans-Anonene and Lupeol from leaves and stem bark extracts of *Croton membranaceus*

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## Abstract
The antioxidant activity of the dichloromethane ($CH_2Cl_2$) and methanol (MeOH) extracts of the leaves and stem bark of *Croton membranaceus* was carried out using a 2,2-Diphenyl-1-picryl-hydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and metal chelation assay along with cytotoxicity analysis using the Brine shrimp lethality assay (BSLA). The silica gel-based chromatographic separation of the $CH_2Cl_2$ and MeOH extracts successfully isolated trans-annonene and lupeol. The structures of the isolated compounds were subsequently identified using Fourier-transform infrared (FTIR) spectroscopy and nuclear magnetic resonance (NMR) based on 1D and 2D data compared with the literature. The $CH_2Cl_2$ and MeOH extracts exhibited antioxidant activity in a concentration-dependent manner but were highly cytotoxic. In conclusion, the study showed that the leaves and stem bark of *C. membranaceous* are potentially viable sources of bioactive compounds with potential health and medicinal benefits.

**Keywords:** Antioxidant activity, Cytotoxicity, *C. membranaceus*, Trans-Anonene, Lupeol
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## Introduction
Plants with therapeutic properties have important roles in healing practices and the treatment of diseases (Toma *et al.,* 2009). The use of natural products in drug discovery is challenging because it requires the isolation of the bioactive compounds from natural sources and the pharmacological investigation of the isolated compounds (Sircar, 1982 and Tariq *et al*., 1985). *Croton membranaceus* Mull. Arg. (Euphorbiaceae) is an undershrub or herb that thrives in coastal areas of West Africa, particularly in Ghana, Ivory Coast, Nigeria, and Niger. The plant can grow up to 1-2 meters in height with slender and densely stellate hairy branches. It has simple, alternate, and green leaves with a characteristic membranous texture. It grows widely in Wuru near the confluence between Rivers Niger and Benue in Nigeria (Appiah *et al.,* 2013). It occurs at low altitudes in moist bush vegetation and savanna but has

limited distribution (Schelmeizer and Gurib-Fakin, 2008).

*Croton membranaceus* is a widely used medicinal plant in many parts of West Africa. Similarly, the root, leaves, and stem bark extracts are widely reported in the literature as remedies for various ailments. The root bark of the plant is ethnomedicinally used in Ghana to treat Benign Prostatic Hyperplasia (BPH), urinary retention caused by an enlarged prostate, and measles (Appiah *et al.,* 2013). In Nigeria, it is used to treat stomach pain or diarrhea (Adesogan, 1981). The leaves of *C. membranaceus* are used to aromatize tobacco and as an aromatic bitter and tonic, which improves digestion (Asare *et al.,* 2011). According to Bayor *et al.* (2009a), the essential oil of *C. membranaceus* bark is used to treat diarrhoea, cough, fever, flatulence, and nausea. *C. membranaceus* is also used in combination with other medicinal plants to effectively treat prostate cancer and uterine fibroid (Appiah *et al.,* 2013).

Over the years, numerous studies have been conducted to isolate and examine the nature, composition, and mode of action of the bioactive/medicinal compounds present in *C. membranaceus*. The phytoconstituents of *C. membranaceus* can inhibit the proliferation of HL-60 and PC-3 (prostate cancer) cells by inducing apoptosis (Appiah *et al.,* 2013). The methanol extract of *C. membranaceus* root exhibited cytotoxic activity against the DLD-1 and MCF-7 cells (Bayor *et al.,* 2009b). Compounds such as scopoletin and crotomembranafuran (a novel furano-clerodane diterpenoid), isolated from *C. membranaceus* exhibited modest activity against human prostate cancer (PC-3) cells, (IC50 = 4.1 ± 0.6 μg/ml; 10.6 μM) but was inactive against both DLD-1 and MCF-7 cells (IC50 > 5 μg/ml). Afryie *et al.* (2015) reported that the aqueous root extract of *Croton membranaceus* exhibited mitochondria-dependent apoptogenic activity on human BPH-1 cells. Asare *et al.* (2015) showed that ethanolic root extract *C. membranaceus* successfully shrunk of prostate of BPH patients potentially resulting in improved quality of life. Similarly, the team of Yeboah *et al*. (2023) observed that the hydroethanolic root extract of *C. membranaceus* exhibited antiproliferative, antimigratory and anticlonogenic effects on 22Rv1 castration-resistant prostate cancer cells in humans. Other studies in the literature have examined the phytochemistry of other Croton species such as *Croton mubango* (Isyaka *et al.,* 2020a), *Croton megalocarpus* (Langat *et al*., 2020), *Croton dictyophlebodes* (Munissi *et al.,* 2020), and *Croton haumanianus* (Isyaka *et al*., 2020b) among others for the isolation of bioactive compounds.

Recent acute and sub-chronic toxicity tests on the root extracts of *C. membranaceus* have confirmed its safety, anti-antherogenic potential and anti-ischaemic activity (Asare *et al.,* 2011 and Afryie *et al.,* 2013). The findings also suggest that *C. membranaceus* contains additional compounds with cytotoxic effects particularly in other plant parts (Bayor *et al*., 2008). Therefore, this current study examined the antioxidant and cytotoxicity properties of the leaves and stem bark extracts of *C. membranaceus*. To the best of the authors' knowledge, this is the first study on the isolation of bioactive compounds from the leaf and stem bark of *C. membranaceous*, which will complement knowledge already published in the significant studies reported on the roots in the literature. It is envisaged that the findings will provide further insights into the nature, composition, and mode of action of the bioactive/medicinal compounds in *C. membranaceus* future medicinal applications.

**Materials and Methods**

General

The FTIR spectra were recorded in the range 4000 cm-1 – 600 cm-1 using a Perkin Elmer Spectrum-2 FT-IR Spectrophotometer, while NMR experiments were carried out in CDCl3 on a 400 MHz Bruker AVANCE III NMR spectrophotometer at the Department of Chemistry, Faculty of Engineering and Physical Sciences, University of Surrey, United Kingdom. The spectra were processed using Bruker NMR Topspin software

(TopSpin 3.5pl7). HRMS spectra were recorded using an Agilent 12 60 Infinity II coupled to an Agilent 6550 Quadrupole Time-of-Flight mass spectrometer using electrospray ionization. The LC conditions maintained viz inj vol. 1.00 µL, column Agilent Extend-C18, flow rate 1.0 mL/min, mobile phase (positive mode) Solvent A: MeCN (0.1% formic acid), Solvent B: water (0.1% formic acid), mobile phase (negative mode) Solvent A: MeCN, Solvent B: water; gradient: 0 to 3 min, 5% A; 3 to 3.5 min, 100% A; 3.5 to 4 min, 5% A.

**Sample Collection**

Fresh samples of *C. membranaceus* were collected and identified by Mr. Umar. M. Gallah of the Department of Botany, Kaduna State University Zaria, Kaduna State, Nigeria. The sample was deposited with the voucher specimen number KASU/BSH/677.

**Extraction**

The samples were cut into smaller sizes and air dried for 21 days for the leaves and 28 days for the stem bark before pulverization into powder. The ground leaves (200 g) were extracted with dichloromethane ($CH_2Cl_2$) and then methanol (MeOH) for one week using a Soxhlet extractor. This process was followed by filtration and evaporation to remove the solvents using a rotary evaporator at 40 °C to obtain the ($CH_2Cl_2$) and MeOH leaf extracts. The stem bark powder (150 g) was extracted with $CH_2Cl_2$) nd the extract was evaporated to remove the solvent using a rotary evaporator to yield the stem bark MeOH extract.

**Antioxidant Assay**

For the DPPH free-radical scavenging activity the solutions of the extracts were prepared according to the method (Mohan and Kakkar, 2020). The procedure involved dissolving 0.49, 0.98, 1.47 and 1.96 mg of extracts in 2 $cm^3$ of their respective solvents of extraction to give an approximate concentration of 0.25, 0.5, 1.0 and 1.5 $mg/cm^3$, respectively. DPPH (39.4 mg) was dissolved in 100 $cm^3$ methanol to give a 1M solution. The solution was allowed to stand for 10 minutes, and the absorbance was determined at 517 nm. The DPPH solution (2 $cm^3$) was added to 0.5 $cm^3$ of each of the test solutions and the mixture was shaken and left to stand for 10 minutes thereafter, their absorbance at 517 nm was also determined. The same procedure was applied to Butyrate Hydroxyl Anisole (BHA), Ascorbic acid and α-tocopherol which were used as standards. The standard was prepared in distilled water.

**Brine shrimp lethality Assay (BSLA)**

The cytotoxicity analysis was performed using the BSLA method. About 70.0 g of Brine shrimp (*Artemia salina*) eggs were added to 250 cm3 of seawater in a beaker and allowed to stand for 48 hours for the eggs to hatch into shrimp larvae. A 0.20 g portion of each extract was dissolved in 2 cm3 of its solvent of extraction. Using a pipette, 50, 5 and 1µ/L of each solution was transferred into vials and allowed to evaporate. Two drops of DMSO were added and made up to 2 cm3 with distilled water corresponding to concentrations of 1000, 100 and 10 µg/ $cm^3$ respectively. Each dosage was prepared in triplicates including the control (not containing the extract). Ten shrimp larvae were added to each vial. The number of surviving shrimp larvae at each dosage and the control was recorded after 24 h and the $LC_{50}$ was calculated using Finney probit analysis software (Gurib-Fakin, 2008 and Meyer *et al*., 1982) as shown in Equation 1.

$$\% \, mortality = \frac{Total\ larvae\ mortlity}{Total\ larvae} \times 100 \qquad (1)$$

**Column Chromatography**

About 6.0 g of the $CH_2Cl_2$ stem bark extract was loaded on top of a glass column of size 250 ml and diameter of 2.5 cm packed with silica gel of mesh size 200 – 400 mm dissolved in 100% $CH_2Cl_2$. The column was eluted using $CH_2Cl_2$ to obtain 100 fractions in glass vials. Similar fractions were combined based on their TLC profiles. Fraction CM 79-100 was further purified using a silica gel column and eluted starting with 100% n-hexane and then mixtures of n-hexane with $CH_2Cl_2$ in ratios 9:1, 8:2, 7:3, 6:4, 5:5, 4:6,3:7,2:8, 1:9 and finally, 100% $CH_2Cl_2$ to obtain 15 fractions for each of the solvent systems. A total of 165 fractions were

collected. Based on the TLC profile, fraction CM-91-104 were combined and on standing gave compound 1.

A similar procedure was followed for the isolation and purification of compound 2 from the MeOH leaf extract using the appropriate solvent mixtures as mobile phases. However, in this case, the column was packed with 100% $CH_2Cl_2$ and 15 fractions were collected. This was followed by increasing the polarity of the mobile phase using Ethyl acetate in the order; Dichloromethane: Ethyl acetate 9:1, 8:2, 7:3, 6:4, 1:1, 4:6, 3:7, 2:8, 9:1 and then 100% ethyl acetate. Similar fractions were combined based on their TLC profiles and a total of 150 fractions were collected. Fractions CM-78-88 which were combined based on the similarity of the TLC profile were purified using a mixture of $CH_2Cl_2$ and ethyl acetate. About 165 fractions were collected and fractions from 61 to 70 were combined to yield compound 2 as a white precipitate.

## Results

The $CH_2Cl_2$ and MeOH leaf extracts of *C. membranaceus* were obtained using the maceration method, while the $CH_2Cl_2$ stem bark extract was obtained using Soxhlet extraction. The yields of the extracts are given in Table 1.

### Antioxidant assay

The antioxidant activities of the MeOH and $CH_2Cl_2$ extracts for leaves and stem bark are shown in Fig. 1 to 3.

**Table 1: Percentage yield of *C. membranaceus* leaves and stem bark extracts.**

| Extract | Weight (g) | Yield (%) |
|---|---|---|
| $CH_2Cl_2$ leaves | 10.70 | 5.35 |
| MeOH leaves | 10.50 | 5.25 |
| $CH_2Cl_2$ stem bark | 9.11 | 6.07 |

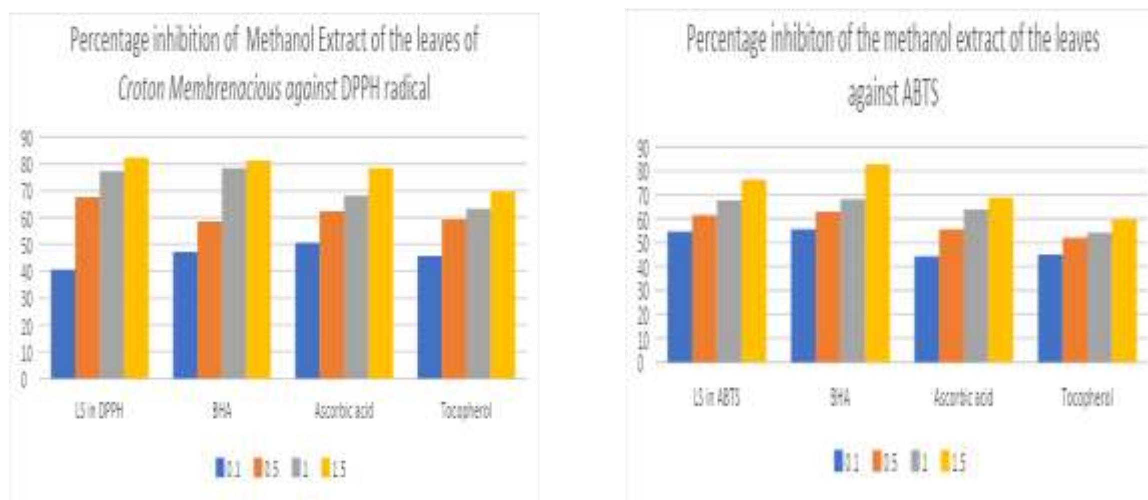


Figure 1: Percentage inhibition of MeOH leaves extract of *Croton membranaceus* against DPPH radical and ABTS free radicals.

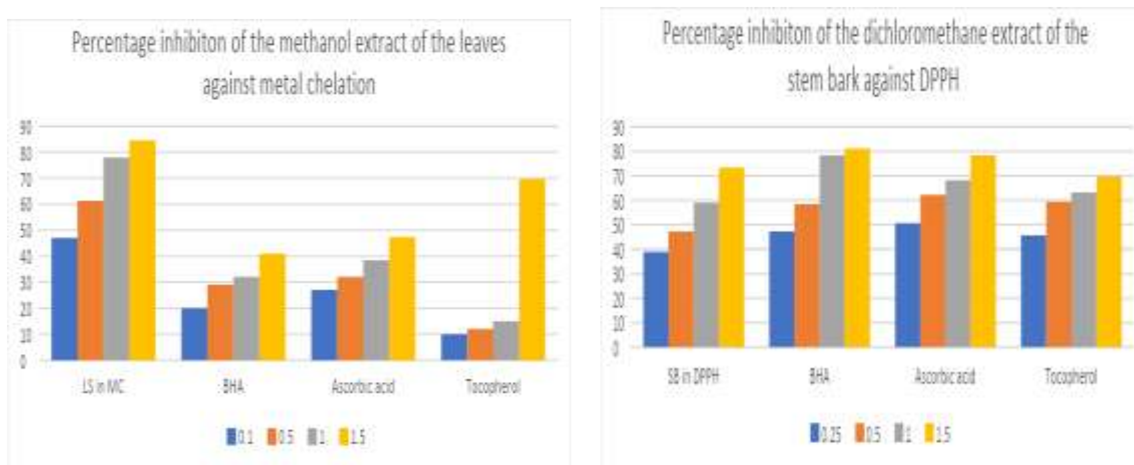


Figure 2: Percentage inhibition of the MeOH and $CH_2Cl_2$ extracts of the leaves and stem bark against DPPH and Metal Chelation free radicals

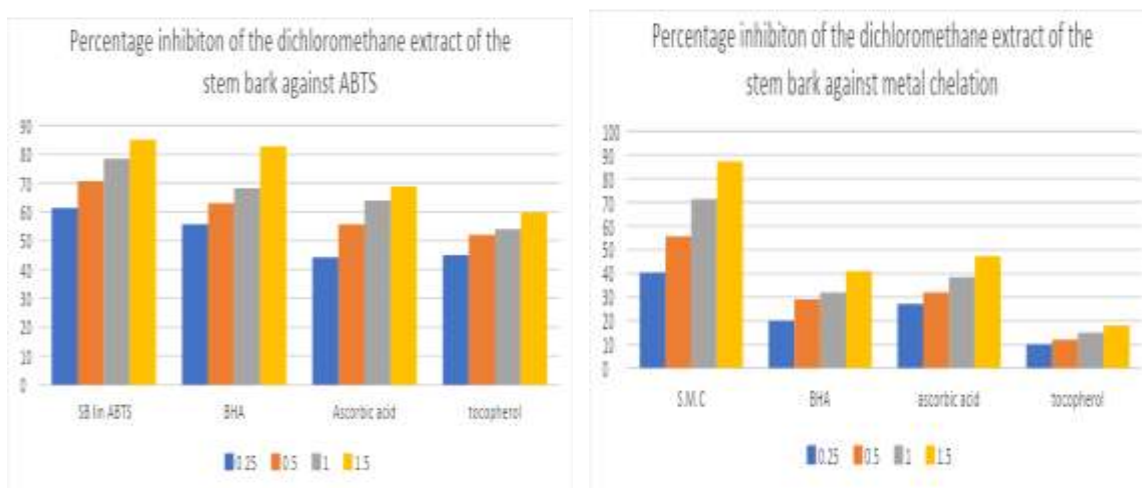


Figure 3: Percentage inhibition of the $CH_2Cl_2$ stem bark extract against ABTS and Metal Chelation free radicals

The median effective concentration of the two extracts MeOH and $CH_2Cl_2$ leaves, and stem bark are presented in Table 2.

**Cytotoxicity studies**

The cytotoxicity of the MeOH leaf and $CH_2Cl_2$ stem bark extracts using BSLA is presented in Table 3.

**Table 2: Median effective concentration ($EC_{50}$) of *C. membranaceous* extracts**

| Sample | EC50 (µg /cm3) | | |
|---|---|---|---|
| | DPPH assay | ABTS assay | Metal Chelation |
| MeOH leaf | 15.63 | 27.3 | 250 |
| $CH_2Cl_2$ stem bark | 20.0 | 125.0 | 220 |
| BHA | 6.9 | 2.8 | 15.0 |
| Ascorbic acid | 2.81 | 15.0 | 75.0 |
| α-tocopherol | 35.3 | 125.0 | 250 |

**Table 3: Cytotoxicity (BSLA test) results**

| Conc. | 1000 µg/ml | | 100 µg/ml | | 10 µg/ml | | Control | | LC50*U/L limit |
|---|---|---|---|---|---|---|---|---|---|
| **Methanol Leaf extract** | | | | | | | | | |
| | Survivor | Dead | Survivor | Dead | Survivor | Dead | Survivor | Dead | |
| 1st Trial | 0 | 30 | 5 | 25 | 16 | 14 | 10 | 0 | 65.22 (42.18/518.18) |
| 2nd Trial | 2 | 28 | 1 | 29 | 7 | 23 | 10 | 0 | 66.99 (47.06/515.14) |
| 3rd Trial | 2 | 28 | 12 | 18 | 9 | 21 | 10 | 0 | 70.05 (42.16/513.14) |
| **$CH_2Cl_2$ Stem bark extract** | | | | | | | | | |
| | Survivor | Dead | Survivor | Dead | Survivor | Dead | Survivor | Dead | |
| 1st Trial | 0 | 30 | 5 | 25 | 16 | 14 | 10 | 0 | 50.30 (47.06/377.31) |
| 2nd Trial | 2 | 28 | 1 | 29 | 7 | 23 | 10 | 0 | 52.28 (47.06/377.31) |
| 3rd Trial | 2 | 28 | 12 | 18 | 9 | 21 | 10 | 0 | 52.18 (42.36/379.34) |

**Characterization of compound 1 as trans-annonene**

Compound 1 CM-3 was obtained as a yellowish oil and the functional groups present were identified from its FTIR spectrum. The absorption band at 1655 cm-1 was attributed to the C=C double bond stretch, while the bands at 3016 cm-1, 2923 cm-1, and 2854 cm-1 are due to C-H stretches (Misra *et al.,* 1964). The 1H NMR showed three coupled aromatic protons consistent with a β- substituted furan ring at δH 6.27 (d, H-14), 7.35 (d, H-15) and 7.21 (s, H-16) (Urones 1990, 1989). An alkene proton signal was observed at 5.21 (m, H-3) and two methine protons at 1.57 (m, H-8) and 1.45 (m, H-10). It also showed resonances for four methyl groups at 0.84 (s, H-17), 1.61 (s, H-18), 1.03 (s, H-19) and 0.76 (s, H-20) and some methylene protons at 1.69 (m, H-11a) and 1.53 (m, H-11b), 2.32 (td, J = 4.3, 9.9 Hz, H-12a) and 2.24 (td, J = 4.3, 12.8 Hz, H-12b). The presence of twenty carbon atoms in its 13C NMR spectrum suggested that the compound could be a diterpene and the presence of a furan ring makes it possibly a furano-clerodane type diterpenoid. The corresponding carbon resonances were observed at δC 120.7 (C-3), 144.7 (C-4), 38.4 (C-5), 111.3 (C-14), 142.8 (C-15) and 138.6 (C-16). Other carbon signals were 38.9 (C-9), 37.1(C-6) and 46.7 (C-10). Using correlations in its 2D NMR spectra the compound was identified as follows: long-range correlations in its HMBC spectrum from H-18 identified C-3, C-4 and C-5, similarly the other methyl protons correlated to the carbon atoms in their vicinity. Correlations from H-14 and H-16 to C-12 confirmed the attachment of the Furan moiety to C-12 and the vicinal and adjacent protons were identified by correlations in its COSY spectrum and the spatial groups and protons by its NOESY spectrum. Finally, by comparison with literature reports (Silverstein 1962), compound 1 was characterized as trans-annonene (1) and it has been previously isolated from Solidago arguta Ait. (Urones 1990) and *Croton sonderianus* (Urones 1989). However, this is an initial report of the isolation of trans-annonene from *C. membranaceus*. Its full chemical shift assignments are given in Table 4.

**Table 4: Correlation table for compound 1(Trans-annonene)**

| No. | 13C NMR (100 MHz) in $CDCl_3$ | 13C NMR (100 MHz) [25] | 1H NMR (400 MHz) $CDCl_3$ (J in Hz) |
|---|---|---|---|
| 1α | 18.4 $CH_2$ | 18.2 | 1.67* |
| 1β | | | 1.45* |
| 2α | 27.1 $CH_2$ | 26.7 | 2.06m* |
| 2β | | | 2.06m* |
| 3 | 120.7 CH | 120.6 | 5.21br s W1/2=9.34HZ |
| 4 | 144.7 C | 143.7 | - |
| 5 | 38.4 C | 37.9 | - |
| 6α | 37.1 $CH_2$ | 36.6 | 1.74m |
| 6β | | | 1.21* |
| 7α | 27.7 $CH_2$ | 27.4 | 1.45* |
| 7β | | | 1.23* |
| 8 | 36.5 CH | 36.2 | 1.57m |
| 9 | 38.9 C | 38.4 | - |
| 10 | 46.7 CH | 46.1 | 1.45* |
| 11A | 38.8 $CH_2$ | 38.5 | 1.69* |
| 11B | | | 1.53m |
| 12A | 18.5 $CH_2$ | 18.2 | 2.32td J=4.3,9.9HZ |
| 12B | | | 2.24td J=4.3,12.8HZ |
| 13 | 126.1 C | 125.2 | |
| 14 | 111.3 CH | 110.7 | 6.27 s |
| 15 | 142.8 CH | 142.3 | 7.35s |
| 16 | 138.6 CH | 138.0 | 7.21s |
| 17 | 16.3 $CH_3$ | 16.1 | 0.84d J=6.7HZ |
| 18 | 18.2 $CH_3$ | 17.9 | 1.61d J=1.5HZ |
| 19 | 20.2 $CH_3$ | 19.8 | 1.03s |
| 20 | 18.4 $CH_3$ | 18.2 | 0.76s |

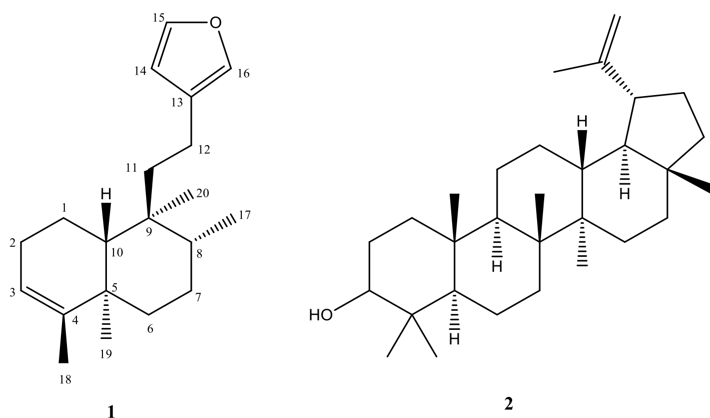


Figure 4: Structure of Trans-annonene (1) and Lupeol (2)

**Characterization of compound 2 as Lupeol**

Compound 2 was obtained as a white solid from the methanol extract of the leaves and was identified as Lupeol. This compound has been isolated from several sources, including *Croton haumanianus* (Tchissambou *et al*., 1990) and *Croton megalocarpoides* (Ndunda et al., 2016). The 1H NMR spectrum (Figure 8) for compound CM-5 showed characteristic resonances for an isopropenyl group typical of the lupane skeleton of pentacyclic triterpenes with the two methylene

protons observed at δH 4.66 (d, J = 2.2 Hz) and δH 4.59 (d, J = 2.2 Hz) for the H-29 protons. In addition, an oxymethine proton at δH 3.17 (dd, J = 5.7 Hz, 11.0 Hz, H-3) was observed and the corresponding C-3 carbon signal was at δC 79.2 ppm. By comparing the 13C-NMR data and its chemical shifts were confirmed by comparison with reports in the literature (Fortie *et al.,* 2006).

**Table 5: Correlation Table for compound 2 (Lupeol)**

| S/N | 13C NMR (100 MHz) in $CDCl_3$ | 13C NMR (100 MHz) in $CDCl_3$ (Lit.) | 1H NMR (400 MHz) $CDCl_3$ (J in Hz) |
|---|---|---|---|
| 1α | 38.9 $CH_2$ | 39.1 | 1.66 m |
| 1β | | | 0.98 m |
| 2α | 27.7 $CH_2$ | 27.8 | 1.68 m |
| 2β | | | 1.57 m |
| 3 | 79.2 CH | 79.3 | 3.17 dd J=5.7, 11.0 |
| 4 | 39.1 C | 39.2 | - |
| 5 | 55.5CH | 55.6 | 0.67 d J= |
| 6α | 18.5 $CH_2$ | 18.7 | 1.50 m |
| 6β | | | 1.38 |
| 7α | 34.5 $CH_2$ | 34.6 | 1.38 |
| 7β | | | |
| 8 | 41.0 C | 41.2 | - |
| 9 | 50.7 CH | 50.7 | 1.26 m |
| 10 | 37.4 C | 37.5 | - |
| 11α | 21.1 $CH_2$ | 21.3 | 1.39 m |
| 12α | 25.4 $CH_2$ | 25.5 | 1.66 m |
| 12β | | | 1.04 m |
| 13 | 38.3 CH | 38.4 | 1.64 m |
| 14 | 43.0 C | 43.2 | - |
| 15α | 27.6 $CH_2$ | 27.8 | 1.68 m |
| 15β | | | 1.57 m |
| 16α | 35.8 $CH_2$ | 35.9 | 1.46 m |
| 16β | | | 1.36 m |
| 17 | 43.2 C | 43.4 | - |
| 18 | 48.2 CH | 48.3 | 2.38 sext J = 5.7 |
| 19 | 48.5 CH | 48.6 | 1.34 m |
| 20 | 151.2 C | 151.1 | - |
| 21α | 30.1 $CH_2$ | 30.2 | 1.90 m |
| 21β | | | 1.19 m |
| 22α | 40.2 $CH_2$ | 40.4 | 1.37 m |
| 22β | | | 1.18 m |
| 23 | 28.4 $CH_3$ | 28.2 | 0.97 s |
| 24 | 15.6 $CH_3$ | 15.8 | 0.76 s |
| 25 | 16.3 $CH_3$ | 16.5 | 0.83 s |
| 26 | 16.2 $CH_3$ | 16.3 | 1.03 s |
| 27 | 14.8 $CH_3$ | 14.9 | 0.94 s |
| 28 | 18.2 $CH_3$ | 18.4 | 0.79 s |
| 29A | 109.5 $CH_2$ | 109.6 | 4.66 d J = 2.2 |
| 29B | | | 4.59 d J = 2.2 |
| 30 | 19.5 $CH_3$ | 19.7 | 1.68 s |

**Discussion**

The antioxidant activity varied between the extracts of the leaves and stem bark and demonstrated a concentration-dependent activity (Figure 1-3). The percentage inhibition of the methanol leaf extract at the highest concentration (1.5 mg/ml) against DPPH radical is 82.4 mg while the three standards, BHA, Ascorbic acid, and α-Tocopherol are 81.2 mg, 78.3 mg and 69.7 mg, respectively. It can be deduced that the percentage inhibition of the extract against DPPH is higher than the three standards used, hence the extract showed more antioxidant activity than the standards used. These findings agree with those obtained by Okoro *et al*., 2018. 94 he ABTS radical, the percentage in ı of the extract at the highest concentration is 67.7 mg/ml, while that of the three standards, BHA, Ascorbic acid, and α-Tocopherol are 82.7 mg/ml, 68.8 mg/ml, and 59.9 mg/ml respectively. The percentage inhibition of the extract against ABTS radical at the highest concentration is less than BHA and Ascorbic acid, although quite comparable to the results obtained for the two standards used but showed more activity than α-Tocopherol (Olowokudejo *et al*., 2008). For Metal chelation radical the percentage inhibition of the methanol extract at the highest concentration (see Figure 2) is 84.6 mg/ml, while the three standards, BHA, Ascorbic acid, and α-Tocopherol are 41 mg/ml, 47.3 mg/ml and 18 mg/ml respectively. The percentage inhibition of the extract against Metal chelation radical at the highest concentration is higher than that of BHA, Ascorbic acid, and α-Tocopherol. Hence the extract showed more antioxidant activities than the three standards. The metal chelating effects of the extracts are consistent with that of (Watt and Breyer-Brandwijk, 1962).

However, the stem bark chelating effect and three standards (BHA, Ascorbic acid, and α-Tocopherol) against DPPH at the highest concentration (1.5mg/ml) (Figure 2) is 73.3 mg/ml, 81.2 mg/ml, 78.3 mg/ml and 69.7 mg/ml respectively. The percentage inhibition of the $CH_2Cl_2$ extract and the drugs (BHA, Ascorbic acid, and α-Tocopherol) against ABTS radical at highest concentrations (see Figure 3) are BHA, Ascorbic acid and α-Tocopherol are 85.1 mg/ml, 82.7 mg/ml, 68.8 mg/ml and 59.9 mg/ml respectively. However, the percentage inhibition of the extracts and the drugs (BHA, Ascorbic acid, and α-Tocopherol) against metal chelation (see Figure 3) at the highest concentrations are 87.3 mg/ml, 41 mg/ml, 47.3 mg/ml and 18 mg/ml respectively. From the results, it is shown that the extracts have a lower percentage inhibition against DPPH value when compared to BHA, although the result is quite comparable and also has a higher percentage inhibition value against the other two standards. This pattern of inhibition is in strong agreement with the result of Bayor *et al*., 2009. Research has shown that *Croton argyratus* when evaluated for their antioxidant activity using DPPH radical scavenging activity, reducing power and total antioxidant capacity showed that the leaf extract exhibits the highest value of the antioxidant activity. Thus, the findings suggest the potential use of *C. argyratus* plant extracts as a natural source of antioxidants (Ali *et al.,* 2012).

The $EC_{50}$ value for the MeOH leaves extract of *C. membranaceus* against DPPH, ABTS, and metal chelation radical is shown in (Table 2). However, it should be noted that a lower $EC_{50}$ value indicates a stronger antioxidant potency of the test methanol extract. The results show that BHA and ascorbic acid had the best antioxidant potency than the methanol extract although the results are quite comparable with the findings of Zhang and Kim, 2015). The $EC_{50}$ value for the $CH_2Cl_2$ stem bark extract of *C. membranaceus* (Table 2) against DPPH, ABTS, and metal chelation radical. The results show that BHA and Ascorbic acid have more antioxidant activities than the extract although the extract showed a good antioxidant activity against the radicals. These findings are slightly similar to the results of Zhang and Kim, 2015). Therefore, the MeoH leaf extract has higher antioxidant activity than the $CH_2Cl_2$ stem bark extract of the same *C. membranaceus*.

According to Meyer *et al.,* 1982 the criteria for toxicity of plant extract with the value of lethal concentration $LC_{50}$ >1000µg/cm$^3$ is not toxic, while $LC_{50}$ < 1000 µg/ml is toxic (Meyer *et al*., 1982). However, Clarkson *et al.,* 2004 criteria show that cytotoxicity on the plant extracts with $LC_{50}$ from 500 – 1000 µg/ml shows low toxicity, extracts with $LC_{50}$ from 100 – 500 µg/ml are toxic and extracts with $LC_{50}$ from 0-100 µg/ml shows that it is highly toxic. The results of the BSLA test conducted for the test samples, and $LC_{50}$ values obtained from each test extract in Table 3 for the methanol leaves and stem bark extracts, respectively. The MeoH leaves extract showed an $LC_{50}$ value of 65.22 µg/ml, 66.99 µg/ml, and 70.05 µg/ml with an average $LC_{50}$ value of 67.42 µg/ml. The average $LC_{50}$ of methanol extract shows that the leaves are toxic according to Meyer *et al.,* 1982 and however, very toxic according to Clarkson *et al.,* 2004. A similar effect was observed with $CH_2Cl_2$ stem bark extract, the LC50 values are 50.30 µg/ml, 52.28 µg/ml, and 52.18 µg/ml and the average $LC_{50}$ value of 51.57 µg/ml which shows that $CH_2Cl_2$ stem bark is also toxic according to Meyer *et al.,* 1982 and very toxic according to Clarkson *et al.,* 2004 criteria.

## Conclusions

The study successfully isolated two bioactive compounds, trans-anonene and lupeol, from the leaves and stem bark extracts of *Croton membranaceus*. The antioxidant and cytotoxicity analysis of the *Croton membranaceus* extracts was subsequently conducted to examine their suitability for future herbal or medicinal applications. Results demonstrated concentration-dependent activity against DPPH, ABTS, and metal chelation radicals. The leaf extract exhibited higher inhibition of DPPH and metal chelation radicals than standard antioxidants, aligning with previous research. The stem bark extract also showed antioxidant potential but slightly less than the leaf extract. Both extracts were found to be toxic based on LC50 values. Overall, the study suggests the potential use of C. species extracts as natural antioxidants, with the leaf extract displaying superior activity, but raises concerns about their toxicity based on established criteria. In conclusion, the study showed that the leaves and stem bark of *C. membranaceous* are potentially viable sources of bioactive compounds such as trans-annonene and lupeol with potential health and medicinal benefits.

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## References

Adesogan EK (1981). The structure of penduliflaworosin, a new furanoid diterpene from *Croton penduliflorus. Journal of the Chemical Society, Perkin Transactions* 1:1151-1153

Afriyie, D. K., Asare, G. A., Bugyei, K., Asiedu-Gyekye, I., Gyan, B. A., Adjei, S., Addo, P., Sittie, A. and Nyarko, A.K. (2013). Anti-atherogenic and anti-ischemic potentials of *Croton membranaceus* observed during sub-chronic toxicity studies. *Pharmacogn Res* 5(1): 10

Afriyie, D. K., Asare, G. A., Bugyei, K., Lin, J., Peng, J. and Hong, Z. (2015). Mitochondria-dependent apoptogenic activity of the aqueous root extract of *Croton membranaceus* against human BPH-1 cells. *Genet Mol Res* 14 (1):149-162. doi:10.4238/2015.January.15.18

Ali, N. M., Annegowda, H., Mansor, S., Ismail, S., Ramanathan, S. and Mordi M. (2012). Phytochemical screening, antioxidant and analgesic activities of *Croton argyratus* ethanolic extracts. *Journal of Medicinal Plants Research* 6(21): 3724-3731

Appiah A, Asomaning W, Oppong I, Duker-Eshun G, Clement J, Okine L, Achel G, Gyampo O, Adjei S and Nyarko A (2013). Prospects of *Croton membranaceus* for prostate health. African Natural Plant Products Volume II: Discoveries and Challenges in Chemistry, Health, and Nutrition. ACS Publications, pp 79-92

Asare, G. A., Afriyie, D., Ngala, R. A., Appiah, A. A., Anang, Y., Musah, I.,

Adjei, S., Bamfo-Quaicoe, K., Sule, D., Gyan, B. A., Arhin, P. and Edoh DA (2015). Shrinkage of prostate and improved quality of life: Management of BPH patients with *Croton membranaceus* ethanolic root extract. *Evid-Based Complement Altern Med*. doi:10.1155/2015/365205

Asare, G. A., Sittie, A., Bugyei, K., Gyan, B. A., Adjei, S., Addo, P., Wiredu, E. K., Nyarko, A. K., Otu-Nyarko. L. S. and Adjei, D. N. (2011). Acute toxicity studies of *Croton membranaceus* root extract. *Journal of Ethnopharmacology* 134(3): 938-943.

Bayor MT, Ayim JS, Marston G, Phillips RM, Shnyder SD, Wheelhouse RT and Wright CW (2008). A cytotoxic diterpenoid from *Croton membranaceus*, the major constituent of anticancer herbal formulations used in Ghana. *Natural Product Communications* 3(11): 1934578X0800301116

Bayor, M. T., Gbedema, S. Y. and Annan K (2009). The antimicrobial activity of *Croton membranaceus*, a species used in formulations for measles in Ghana. *Journal of Pharmacognosy and Phytotherapy* 1(4): 047-051

Clarkson, C., Maharaj, V. J., Crouch, N. R., Grace, O. M., Pillay, P., Matsabisa, M. G., Bhagwandin, N., Smith, P. J. and Folb, P. I. (2004). In vitro antiplasmodial activity of medicinal plants native to or naturalised in South Africa. *Journal of Ethnopharmacology* 92(2-3): 177-191

Fotie, J., Bohle, D. S., Leimanis, M. L., Georges, E., Rukunga, G. and Nkengfack, A. E. (2006). Lupeol long-chain fatty acid esters with antimalarial activity from *Holarrhena floribunda*. *Journal of Natural Products* 69 (1):62-67

Gurib-Fakim A (2006) Medicinal plants: traditions of yesterday and drugs of tomorrow. *Molecular aspects of Medicine* 27(1):1-93

Isyaka, S. M., Langat, M. K., Mas-Claret, E., Mbala, B. M., Mvingu, B. K., Mulholland, D. A. (2020a). Ent-abietane and ent-pimarane diterpenoids from *Croton mubango* (Euphorbiaceae). *Phytochemistry* 170:112217

Isyaka, S. M., Mas-Claret, E., Langat, M. K., Hodges, T., Selway, B., Mbala, B. M., Mvingu, B. K. and Mulholland, D. A. (2020b). Cytotoxic diterpenoids from the leaves and stem bark of *Croton haumanianus* (Euphorbiaceae). *Phytochemistry* 178:112455

Langat, M. K., Djuidje, E. F., Ndunda, B. M., Isyaka, S. M., Dolan, N. S., Ettridge, G. D., Whitmore, H., Lopez, I., Alqahtani, A. M. and Atiku, I. (2020). The phytochemical investigation of five African Croton species: *Croton oligandrus, Croton megalocarpus, Croton menyharthii, Croton rivularis* and *Croton megalobotrys. Phytochemistry Letters* 40:148-155

Meyer, B., Ferrigni, N., Putnam, J., Jacobsen, L., Nichols, D. and McLaughlin, J. L (1982). Brine shrimp: a convenient general bioassay for active plant constituents. *Planta medica* 45(05): 31-34

Misra, R., Pandey, R. and Dev, S. (1964). The chemistry of the oleo resin from *Hardwickia pinnata*: a series of new diterpenoids. *Tetrahedron letters* 5(49): 3751-3759

Mohan, B. and Kakkar, A. (2020). Evaluation of phenolic, flavonoid contents and antioxidant activity in leaves, stem and roots of *Solanum indicum* Linn. *Environment Conservation Journal* 21(1&2): 167-172

Munissi, J. J., Isyaka, S. M., Mas-Claret, E., Brabner, M., Langat, M. K., Nyandoro, S. S. and Mulholland, D. A. (2020) Ent-clerodane and ent-trachylobane diterpenoids from *Croton dictyophlebodes*. *Phytochemistry* 179:112487

Ndunda, B., Langat, M. K., Mulholland, D. A., Eastman, H., Jacob, M. R., Khan, S. I., Walker, L. A., Muhammad, I., Kerubo, L. O. and Midiwo, J. O. (2016). New ent-clerodane and abietane diterpenoids from the roots of Kenyan *Croton megalocarpoides* Friis & MG

Gilbert. *Planta Medica* 82(11/12): 1079-1086

Okoro, I., Tor-Anyiin, T., Igoli, J., Noundou, X. and Krause, R. (2018). Isolation and Characterisation of Stigmasterol and β–Sitosterol from *Anthocleista djalonensis* A. Chev. *Asian Journal of Chemical Sciences* 3(4): 1-5

Olowokudejo, J., Kadiri, A. and Travih, V. (2008). An ethnobotanical survey of herbal markets and medicinal plants in Lagos State of Nigeria. *Ethnobotanical Leaflets*, 12: 851- 65.

Schmelzer G and Gurib-Fakim A (2008). Plant resources of tropical Africa 11 (1). Medicinal plants 1. PROTA foundation. Backhuys Publishers, Leiden, Netherlands/CTA, Wageningen,

Silverstein, R. M. and Bassler, G. C. (1962). Spectrometric identification of organic compounds. *Journal of Chemical Education* 39(11): 546

Sircar, N. (1982). Medicinal plants. *The Eastern Pharmacist* 29(291): 49-52

Tariq, O. and Siddiqi, A. J. (1985). Vitamin C content of Indian medicinal plants-a literature review. *Indian Drugs* 23 (2):72-83

Tchissambou, L., Chiaroni, A., Riche, C. and Khuong-Huu, F. (1990). Crotocorylifuran and crotohaumanoxide, new diterpenes from *Croton haumanianus* J. Leonard. *Tetrahedron* 46(15): 5199-5202

Toma I, Karumi Y and Geidam M (2009) Phytochemical screening and toxicity studies of the aqueous extract of the pods pulp of Cassia sieberiana DC. (Cassia kotchiyana Oliv.). *African Journal of Pure and Applied Chemistry* 3(2):26-30

Urones, J., Marcos, I., Cubillo, I., Garrido, N. M. and Basabe, P. (1990). Terpenoid compounds from *Parentucellia latifolia*. *Phytochemistry* 29(7): 2223-2228.

Urones, J., Marcos, I., Cubillo, L., Monje, V., Hernández, J. and Basabe, P. (1989) Derivatives of malonic acid in *Parentucellia latifolia*. *Phytochemistry* 28(2): 651-653

Watt, J. M. and Breyer-Brandwijk, M. G. (1962). *The Medicinal and Poisonous Plants of Southern and Eastern Africa being an Account of their Medicinal and other Uses.* In Chemical Composition, Pharmacological Effects and Toxicology in Man and Animal. (Edn 2)

Yeboah, K. O., Emosivbe, M., Ntim, E. A., Sam, G. H. and Ainooson, G. (2023). The antiproliferative, antimigratory and anticlonogenic effects of *Croton membranaceus* Möll. Arg. (Euphorbiaceae) hydroethanolic root extract in human 22Rv1 castration-resistant prostate cancer cells. *Acta Pharm Sci* 61(2):121-140. doi:10.23893/1307-2080.APS6109

Zhang, Q. and Kim, H. Y. (2015) Antioxidant, anti-inflammatory and cytotoxicity on human lung epithelial A549 cells of Jerusalem artichoke (*Helianthus tuberosus* L.) tuber. *Korean Journal of Plant Resources* 28(3): 305-311