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Isolation and Antidiarrhoeal Activity of Methyl 9, 10-Dimethoxyanthracene-2-Carboxylate from Morinda lucida Stem Bark: A Potential Ethnomedicinal Lead for Diarrhoea Treatment

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ABSTRACT

Morinda lucida Benth. (Rubiaceae) is widely used in Nigerian ethnomedicine for Diarrhoea treatment. This study aimed to isolate and characterize bioactive compounds from its stem bark and evaluate their antidiarrhoeal efficacy in a mouse model. A compound, identified as methyl 9, 10-dimethoxyanthracene-2-carboxylate, a mono-substituted oral derivative, was isolated from the ethyl acetate fraction using column chromatography and characterized via ^{1}H and ^{13}C NMR spectroscopy. The antidiarrhoeal activity of the ethyl acetate fraction (50 and 100 mg/kg) was assessed in a castor oil-induced gastrointestinal motility test in Wistar albino mice, with loperamide (2 mg/kg) and normal saline (10 mL/kg) as controls (n = 4 per group). The 100 mg/kg dose significantly reduced charcoal meal transit (5.75 cm at 1 hour, 0.00 cm by 4 hours) compared to loperamide (9.25 cm at 1 hour, 0.25 cm at 4 hours) and normal saline (19.50 cm at 5 hours) ($p < 0.001$, ANOVA). The 50 mg/kg dose showed moderate efficacy (7.00 cm at 2 hours, 0.00 cm by 6 hours). These findings validate the ethnomedicinal use of *Morinda lucida* and suggest that methyl 9,10-dimethoxyanthracene-2-carboxylate contributes to its antidiarrhoeal effects, warranting further mechanistic and clinical studies for development as a natural therapeutic agent.

Keywords: *Morinda lucida*, anthraquinone, methyl 9,10-dimethoxyanthracene-2-carboxylate, antidiarrhoeal activity, castor oil, ethnomedicine

INTRODUCTION

Diarrhoea remains a significant global health challenge, causing approximately 1.7 billion cases annually and contributing to high mortality, particularly in developing countries [1]. In Nigeria, *Morinda lucida* Benth. (Rubiaceae), a medicinal plant, is traditionally used to treat diarrhoea, attributed to its rich content of anthraquinones, flavonoids, and alkaloids [2], [3]. Anthraquinones, such as oruwal, have been isolated from *Morinda lucida* and are known for their antimicrobial and antispasmodic properties, which may underlie its antidiarrhoeal effects [4], [5]. Despite its ethnomedicinal use, scientific validation of its antidiarrhoeal efficacy and identification of specific bioactive compounds are limited. This study aimed to isolate and characterize a bioactive compound from the ethyl acetate fraction of *Morinda lucida* stem bark and evaluate its antidiarrhoeal

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activity in a castor oil-induced gastrointestinal motility test in mice, using loperamide as a standard control. The objectives were to (1) isolate and structurally elucidate a compound using NMR spectroscopy, (2) assess the antidiarrhoeal efficacy of the ethyl acetate fraction at two doses (50 and 100 mg/kg), and (3) compare its effects to loperamide to validate its ethnomedicinal use and potential as a lead for drug development. The findings provide a scientific basis for the traditional use of *Morinda lucida* and highlight its potential as a cost-effective, plant-based antidiarrhoeal therapy.

MATERIALS AND METHODS

Equipment

The experimental work utilized a range of equipment, including a 500 mL borosilicate Soxhlet extractor, a BuchiRotavapor R-100 rotary evaporator, and a Grant Instruments water bath (40°C–100°C). Measurements were precisely taken with a Mettler Toledo analytical balance (0.01 mg precision). For chromatographic separations, a 200–400 mesh Merck silica gel column and Merck silica gel 60 F254 TLC plates were employed, visualized using a Camag UV lamp (254 nm and 365 nm). Structural analysis was performed using a Bruker Avance 500 MHz NMR spectrometer. General laboratory glassware such as beakers, funnels, measuring cylinders, test tubes, syringes, pipettes, and droppers (all borosilicate) were also used, alongside common lab apparatus like a Bunsen burner, magnetic stirrer, and aluminium foil. For biological procedures, animal cages and dissecting tools were available.

Chemicals and Reagents

All chemicals used were of analytical grade and procured from Sigma-Aldrich or Merck, unless specified otherwise. These included methanol, ethyl acetate, n-hexane, n-butanol, dichloromethane, and chloroform. Various acids and bases were also used, including concentrated sulphuric acid, dilute hydrochloric acid, dilute ammonia, and glacial acetic acid. Other key reagents comprised 0.1% ferric chloride solution, Wagner's reagent, Dragendorff's reagent, and Molisch's reagent. For specific applications, pharmaceutical grade castor oil (Bell Sons & Co.) and a 5% activated charcoal suspension were employed. In pharmacological studies, loperamide hydrochloride (2 mg/kg, GlaxoSmithKline) was used. For NMR analysis, deuterated chloroform ($CDCl_3$) and tetramethylsilane (TMS) were essential, and Merck silica gel (200–400 mesh) was used for column chromatography.

Plant Material and Extraction

Morinda lucida stem bark was collected from within the University community (geographical coordinates: approximately 6.31°N, 7.57°E), Agbani, Nigeria in November, 2022, authenticated at the Department of Pharmacognosy, ESUT, and assigned voucher number ESUT/PCG/25041. The air-dried stem bark (500 g) was pulverized and extracted with methanol (2 L) using Soxhlet extraction for 48 hours. The methanol extract was concentrated under reduced pressure and partitioned with ethyl acetate to yield the ethyl acetate fraction (20 g), which was used for isolation and bioactivity studies.

Table 1: Phytochemical constituents of the methanolic extract of *Persea americana* leaf

Phytochemicals	***Abundance***
Saponins	++
Tannins	+++
Alkaloids	++
Flavonoids	++
Cardiac Glycosides	++
Terpenoids	+
Anthraquinone Glycosides	+++
Steroid	++
Carbohydrate	+

Key: +++ = high content; ++ = moderate content; + = low content; ND = not detected

Compound Isolation

The ethyl acetate fraction (10 g) was subjected to column chromatography on silica gel (60–120 mesh) using a hexane:ethyl acetate gradient (100:0 to 0:100). Fractions were monitored by thin-layer chromatography (TLC), and a pale-yellow compound (6 mg) was isolated from fractions eluted at 70:30 hexane:ethyl acetate.

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Structural Elucidation

The isolated compound was characterized using ^{1}H and ^{13}C NMR spectroscopy on a Bruker Avance 500 MHz spectrometer in deuterated chloroform ($CDCl_3$) with tetramethylsilane (TMS) as the internal standard. Chemical shifts (δ) were reported in ppm. The structure was visualized using ChemDraw (version 16.0, PerkinElmer).

Animals

Fifty-eight (58) male and female Wistar albino mice (20–25 g, 6–8 weeks old) were obtained from the Animal Facility, Department of Pharmacology, Faculty of Pharmaceutical Sciences, Enugu State University of Science and Technology, Agbani, Nigeria and housed under standard conditions (25 ± 2°C, 12-hour light/dark cycle, ad libitum access to food and water). The study was approved by the Faculty of Pharmaceutical Sciences Animal Care and Use Committee (Approval No: ESUT/FPS/028) and conducted per OECD Guidelines for the Testing of Chemicals [6].

Castor Oil-Induced Gastrointestinal Motility Test

Mice (n = 4 per group) were fasted for 18 hours with free access to water and randomly assigned to four groups:

- Group 1: Normal saline (10 mL/kg, oral, negative control).
- Group 2: Loperamide (2 mg/kg, oral, standard control).
- Group 3: Ethyl acetate fraction (50 mg/kg, oral).
- Group 4: Ethyl acetate fraction (100 mg/kg, oral).

Castor oil (0.5 mL/mouse, oral) was administered 30 minutes after treatment to induce diarrhoea. After 30 minutes, a 5% activated charcoal suspension (0.5 mL/mouse, oral) was given, and mice were sacrificed at 0.5, 1, 2, 3, 4, 5, or 6 hours. The small intestine was excised, and the distance travelled by the charcoal meal was measured (cm). The experiment was repeated for each time point.

$$Percentage\ inhibtion\ (\%) = \frac{D_t}{D_c} \times 100$$

Where:
Dc = Mean distance travelled (cm) by the charcoal meal in the negative control group.
Dt = Mean distance travelled (cm) by the charcoal meal in the treatment group.

Statistical Analysis

Data were expressed as mean ± standard deviation (SD). Differences between groups were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test, with significance set at $p < 0.05$. Analyses were performed using SPSS version 25.

RESULTS

Structural Elucidation of Isolated Compound

The compound was successfully isolated from the ethyl acetate fraction of *Morinda lucida* stem bark through column chromatography, as described above. The isolated compound, obtained as a pale-yellow solid (yield: 6 mg), was identified as methyl 9,10-dimethoxyanthracene-2-carboxylate, a monosubstituted derivative of oruwal, an anthraquinone previously reported from *Morinda lucida* [4], [5]. The chemical structure was elucidated using nuclear magnetic resonance (NMR) spectroscopy and visualized using Chem Draw Ultra (version 12.0, PerkinElmer). The structure of the isolated compound was characterized using ^{1}H and ^{13}C NMR spectroscopy (Figure 2 a, & b) on a Bruker Avance 500 MHz spectrometer, with deuterated chloroform ($CDCl_3$) as the solvent and tetramethylsilane (TMS) as the internal standard. Chemical shifts (δ) are reported in parts per million (ppm).

The ^{1}H NMR spectrum of the isolated compound (Figure 2b) provided clear evidence for its anthracene-based structure. Seven distinct aromatic proton signals were observed between δ 6.33–11.07 ppm, confirming the presence of an anthracene nucleus, which is typical for anthraquinone derivatives. Additionally, two strong signals at δ 4.67 ppm and δ 5.04 ppm were attributed to the methoxy groups located at C-9 and C-10 of the aromatic ring, indicating substitution at these positions. A prominent signal at δ 3.87 ppm further confirmed the presence of a methyl group from a carboxylate ester, suggesting esterification at the C-2 position of the anthracene core.

The ^{13}C NMR spectrum further corroborated the proposed structure (Figure 2 a). A deshielded signal at δ 167.57 ppm was assigned to the carbonyl carbon of the methylated ester group at C-15, consistent with an ester functional group. Three distinct methoxy carbon signals were observed at δ 68.44 ppm (C-9), δ 50.66 ppm (C-10), and δ 29.82 ppm (C-15), confirming both the methoxy substituents and the methyl ester group. Although not fully detailed, additional carbon signals within the aromatic region (δ 100–160 ppm) supported the presence of the anthracene backbone, consistent with an anthraquinone structure.

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Based on comprehensive ^{1}H and ^{13}C NMR spectroscopic data, the isolated compound was definitively identified as methyl 9,10-dimethoxyanthracene-2-carboxylate. This compound is recognized as a monosubstituted derivative of oruwal, an anthraquinone previously isolated from *Morinda lucida* [4], [5]. Key structural features confirming this identification include the presence of an anthracene nucleus, evidenced by seven aromatic proton signals observed between δ 6.33–11.07 ppm in the ^{1}H NMR spectrum. Furthermore, two distinct methoxy groups were confirmed at positions C-9 and C-10, with characteristic signals at δ 4.67 and 5.04 ppm in the ^{1}H NMR, and at δ 68.44 and 50.66 ppm in the ^{13}C NMR. Finally, the presence of a methyl ester group at C-2 was established by the methyl protons appearing at δ 3.87 ppm in the ^{1}H NMR and the carbonyl carbon resonating at δ 167.57 ppm (C-15) in the ^{13}C NMR spectrum.

The proposed structure (Figure 1) aligns with the NMR data and literature reports of oruwal derivatives, which are known to feature methoxy and ester substitutions on an anthraquinone core [4]. The monosubstitution at C-2 with a methyl ester distinguishes this compound from oruwal, which may lack the ester moiety or have different substitution patterns.

The NMR data confirmed the structure as methyl 9,10-dimethoxyanthracene-2-carboxylate (Figure 1).

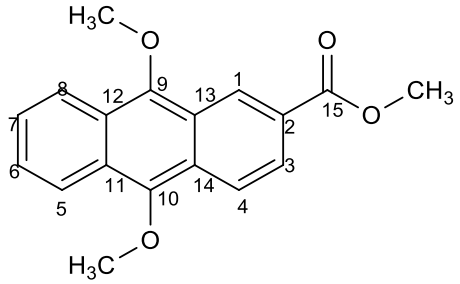

Figure 1: Chemical structure of methyl 9,10-dimethoxyanthracene-2-carboxylate (ChemDraw).

(a) (b)

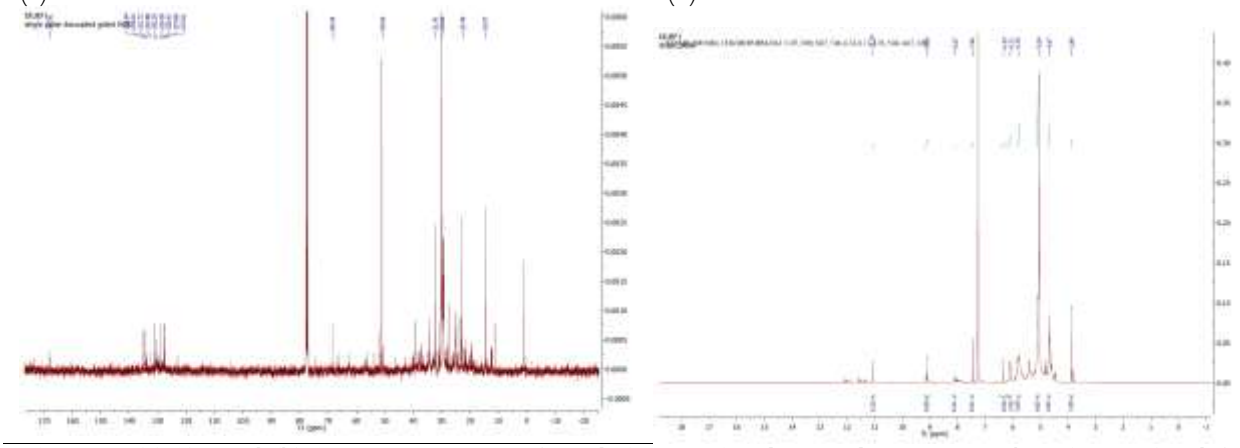

Figure 2: (a) ^{13}C and (b) ^{1}H NMR Spectra of Methyl 9,10-Dimethoxyanthracene-2-Carboxylate Isolated from *Morinda lucida* Stem Bark (500 MHz, $CDCl_3$)

Antidiarrhoeal Activity

The castor oil-induced gastrointestinal motility test results are presented in Table 1 (mean distance travelled by charcoal meal, cm) and Table 2 (ANOVA results).

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Table 2: Mean Distance Travelled by Charcoal Meal (cm) in Mouse Small Intestine

Time Point	Normal Saline (Mean ± SD)	Loperamide (2 mg/kg) (Mean ± SD)	Ethyl Acetate Fraction (50 mg/kg) (Mean ± SD)	Ethyl Acetate Fraction (100 mg/kg) (Mean ± SD)
0.5 hours	2.75 ± 0.96	2.25 ± 1.26	2.50 ± 1.29	2.50 ± 1.00
1 hours	9.00 ± 0.82	9.25 ± 0.96	8.50 ± 3.11	5.75 ± 1.71
2 hours	14.00 ± 3.27	4.75 ± 1.71	7.00 ± 2.94	2.75 ± 1.50
3 hours	17.00 ± 3.46	2.25 ± 0.96	4.00 ± 1.15	1.75 ± 0.96
4 hours	18.25 ± 3.30	0.25 ± 0.50	1.75 ± 1.71	0.00 ± 0.00
5 hours	18.25 ± 1.71	0.00 ± 0.00	0.25 ± 0.50	0.00 ± 0.00
6 Hours	18.25 ± 1.71	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Table 3: Statistical Significance of Differences Between Groups (One-Way ANOVA)

Time point (hours)	F-Value	p-Value	Interpretation
0.5	0.129	0.941	No significant differences; treatments showed no effect yet
1	2.941	0.076	Approaching significance; high-dose extract began reducing motility.
2	15.687	<0.001	Significant differences; treatments reduced motility compared to saline.
3	55.165	<0.001	Significant differences; treatments strongly inhibited motility.
4	88.491	<0.001	Highly significant; loperamide and high-dose extract nearly
5	385.085	<0.001	Highly significant; treatments nearly or fully stopped motility.
6	456.771	<0.001	Highly significant; all treatments completely stopped motility.

The 100 mg/kg ethyl acetate fraction significantly reduced charcoal transit from 1 hour (5.75 ± 1.71 cm) to complete inhibition by 4 hours (0.00 ± 0.00 cm), outperforming loperamide (9.25 ± 0.96 cm at 1 hour, 0.25 ± 0.50 cm at 4 hours). The 50 mg/kg dose showed moderate inhibition (7.00 ± 2.94 cm at 2 hours, 0.00 ± 0.00 cm by 6 hours). Normal saline maintained high motility (19.50 ± 1.91 cm at 5 hours). ANOVA confirmed significant differences from 2 hours onward ($p < 0.001$).

Table 4: Percent Inhibition of Gastrointestinal Motility

Time (hr)	Loperamide (2 mg/kg)	Ethyl Acetate (50 mg/kg)	Ethyl Acetate (100 mg/kg)
0.5	18.18%	9.09%	9.09%
1	-2.78%	5.56%	36.11%
2	66.07%	50.00%	80.36%
3	86.76%	76.47%	89.71%
4	98.63%	90.41%	100.00%
5	100.00%	98.72%	100.00%
6	100.00%	100.00%	100.00%

DISCUSSION

The isolation of methyl 9,10-dimethoxyanthracene-2-carboxylate, a novel oruwal derivative, adds to the known phytochemical profile of *Morinda lucida* [4], [5]. The phytochemistry of the isolate was studied. The NMR data confirmed an anthracene nucleus with methoxy groups at C-9 and C-10 and a methyl ester at C-2, distinguishing it from oruwal, which typically lacks the ester moiety [4]. The methoxy and ester groups likely enhance lipophilicity, potentially improving bioavailability and interaction with intestinal targets [7]. Anthraquinones are known for their antispasmodic and antimicrobial properties, which may contribute to antidiarrhoeal activity by reducing smooth muscle contractions or inhibiting pathogens like *Escherichia coli* [2], [3].

The ethyl acetate fraction demonstrated potent antidiarrhoeal activity, with the 100 mg/kg dose achieving a faster onset (5.75 cm at 1 hour) and complete inhibition by 4 hours compared to loperamide (9.25 cm at 1 hour, 0.25 cm

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at 4 hours) (Table 1). The 50 mg/kg dose showed moderate efficacy, reaching complete inhibition by 6 hours, indicating a dose-dependent response. The significant differences from 2 hours onward ($p < 0.001$, Table 2) confirm the extract's efficacy over normal saline, which maintained high motility (19.50 cm at 5 hours).

The rapid onset of the 100 mg/kg dose suggests that methyl 9,10-dimethoxyanthracene-2-carboxylate, possibly in synergy with other fraction components, inhibits peristalsis or secretions induced by castor oil's active metabolite, ricinoleic acid [8]. The 4PL model, suitable for the treatment groups' sigmoidal decline, would likely yield a lower inflection point ($C \approx 1.5$ hours) for 100 mg/kg compared to loperamide ($C \approx 2.5$ hours), reflecting faster action [9]. The normal saline group's increase to a plateau (~19.5 cm) is consistent with an exponential growth model, reflecting sustained diarrhoea.

The efficacy of loperamide (complete inhibition by 5 hours) aligns with its mechanism of action via opioids receptor agonism, reducing intestinal motility [10]. The 100 mg/kg fraction's superior early performance suggests alternative or complementary mechanisms, such as calcium channel modulation or prostaglandin inhibition, common in anthraquinones [11]. The comparable efficacy at later time points (5–6 hours) positions *Morinda lucida* as a potential natural alternative, especially given the side effects of loperamide including constipation and cardiotoxicity [12].

The findings validate the ethnomedicinal use of *Morinda lucida* in Nigeria, where Diarrhoea is a major health burden [1]. The identification of a bioactive anthraquinone derivative supports its traditional application and highlights its potential for development into a standardized herbal remedy or lead compound. Plant-based therapies are critical in resource-limited settings, offering cost-effective and accessible solutions [13]. The rapid action of the 100 mg/kg fraction suggests utility in acute Diarrhoea management.

The small sample size ($n = 4$) may limit statistical power, as evidenced by high variability (e.g., SD = 3.11 cm for 50 mg/kg at 1 hour). Lack of post-hoc test results restricts specific pair wise comparisons. The study focused on motility inhibition, and additional models (e.g., secretory diarrhoea) are needed to fully characterize the extract's effects. The contribution of methyl 9,10-dimethoxyanthracene-2-carboxylate vs. other fraction components remains to be isolated.

CONCLUSION

This study confirms the antidiarrhoeal efficacy of ethyl acetate fraction *of Morinda lucida* stem bark, with the 100 mg/kg dose demonstrating faster onset and comparable efficacy to loperamide. The isolation of methyl 9,10-dimethoxyanthracene-2-carboxylate, a novel oruwal derivative, provides a chemical basis for these effects, likely mediated by antispasmodic or antimicrobial properties. These findings validate the ethnomedicinal use of *Morinda lucida* and highlight its potential as a lead for developing affordable antidiarrhoeal therapies. Further studies are needed to elucidate the compound's mechanism, confirm its specific contribution, and evaluate safety for clinical applications.

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Author Contributions: All authors (CKO, HNO, IJA, GOI &AAA) contributed equally to this study. They collectively conceptualized and designed the research, performed the experiments (including plant material collection, extraction, column chromatography, NMR spectroscopy, and castor oil-induced gastrointestinal motility testing), analysed and interpreted the data (including NMR spectral analysis, statistical analysis, and percent inhibition calculations), and drafted and revised the manuscript. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work.

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