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Evaluation of antioxidant, anti-inflammatory, and antimicrobial activities of the leaf extracts of *Luffa cylindrica*

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ABSTRACT

Introduction: *Luffa cylindrica* has been used traditionally in the treatment and management of several disease conditions. This study was aimed at evaluating the antioxidant, anti-inflammatory, and antimicrobial properties of leaf extracts of the plant, and identifying some of its phytoconstituents.

Methods: The crude ethanol and ethyl acetate extracts were evaluated for antioxidant and anti-inflammatory activities using the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) and paw-fluid displacement methods, respectively. The extracts were tested for antimicrobial activity using the agar well diffusion and agar dilution methods. The ethyl acetate leaf extract of the plant was further subjected to high-performance liquid chromatography-diode-array detection (HPLC-DAD) analysis for the identification of the bioactive compounds.

Results: The ethanol and ethyl acetate extracts of *L. cylindrica* showed average antioxidant properties at 100 µg/mL, with inhibitions of 53.31% and 54.73%, respectively. The ethanol extract displayed significant anti-inflammatory activity at 50 mg/Kg with an inhibition of 31.1% compared to 39.7% recorded for the control (diclofenac). The ethyl acetate extract produced an inhibition of 15%. In the antimicrobial evaluation, the ethanol and ethyl acetate extracts showed moderate antibacterial activity against *Staphylococcus aureus, Salmonella typhi,* and *Bacillus subtilis*. The ethyl acetate extract exhibited considerable antimicrobial activity against the test isolates compared to the ethanol extract. HPLC-DAD analysis of the ethyl acetate extract suggested the presence of two flavonoid compounds - luteolin and apigenin as key components of the leaf extract of *L. cylindrica*. These compounds are known to possess antioxidant, anti-inflammatory, and antimicrobial activities.

Conclusions: The results of this study showed that the leaf extracts of *L. cylindrica* possess antioxidant, anti-inflammatory, and antimicrobial properties.

Key words: *Luffa cylindrica*; antioxidant; anti-inflammation; antimicrobial; high-performance liquid chromatography-diode-array detection analysis

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INTRODUCTION

Plants have been used in the treatment of various infectious and non-infectious diseases. The use of plants and plant products play an essential role in primary health care in developing countries such as

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Nigeria. *Luffa cylindrica*, commonly called sponge gourds, belongs to the Cucurbitaceae family (1). It is reported to have originated from India (2) but can be found growing like a weed in most parts of Nigeria. *L. cylindrica* has been reported to possess both medicinal and nutritional properties. Its seeds have been used in the treatment of asthma, sinusitis, and fever (3). According to Muthumani et al. (4), the plant seeds possess hepatoprotective, anesthetic activity, anti-inflammatory, anti-helminthic, antimicrobial, anticancer, and enzyme inhibitor effects.

Several bioactive compounds have been isolated from various parts of the *L. cylindrica*. Apigenin, rho-coumaric acid, luteolin, kaempferol, quercetin, and apigenin-7-O-beta-D-glucuronopyranoside were isolated from the leaves (5,6). Shim and Park (7) identified some bioactive components in the sprouts of *L. cylindrica.* These include myricetin, luteolin, quercetin, and some apigenin metabolites, such as apigenin-glucuronic acid and apigenin-acetyl glucuronic acid.

Eight compounds with antioxidant activities were isolated by Du et al. (8) from the fruits of *L. cylindrica*. The compounds are *p*-coumaric acid, 1-O-feruloyl-beta-D-glucose, 1-O-p-coumaroyl-beta-D-glucose, 1-O-caffeoyl-beta-D-glucose, 1-O-(4-hydroxybenzoyl) glucose, diosmetin-7-O-beta-D-glucuronide methyl ester, apigenin-7-O-beta-D-glucuronide methyl ester, and luteolin-7-O-beta-D-glucuronide methyl ester. Ismail et al. (9) reported the isolation of 3-hydroxy-1-methylene-2,3,4,4-tetrahydroxynapthalene-2-carbaldehyde and 22,23-dihydroxy spinasterol from the fruits of the plants. Also from the fruit of *L. cylindrica*, Xiong et al. (10) reported the isolation of six compounds. They were identified as lucyosides C, E, F, and H, a mixture of alpha-spinasterol and stigmasta-7,22,25-trien-3 beta-OH and a mixture of alpha-spinasteryl glucoside and delta 7,22,25-stigmasteryl-beta-D-glucoside.

Two fibrinolytic saponins (lucyoside N and P), two triterpenoids (sapogenins 1 and 2), and a plant-derived antifungal peptide (luffacylin) were isolated from the seeds of *L. cylindrica* (11-13). Furthermore, the flavonoid compound apigenin was identified as a key phytoconstituent in the plant seeds (7).

In our effort to authenticate the ethnomedicinal use of Nigerian plants, we investigated the antioxidant, anti-inflammatory, and antimicrobial properties of leaf extracts of *L. cylindrica.*

METHODS

Plant materials

Fresh, healthy leaves of *L. cylindrica* were harvested from a garden in Agulu, Anambra State-Nigeria in March 2014. The plant was identified by a plant taxonomist in the Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Anambra State-Nigeria. The herbarium specimen deposited with herbarium number PCG474/A/026.

Culture media, chemicals, and reagents

Culture media used in this study include Mueller-Hinton Agar (MHA) (Oxoid, UK) and Sabouraud Dextrose Agar (SDA) (Oxoid, UK). Chemicals/reagents used include ethanol and ethyl acetate (Sigma-Aldrich, Germany), high-performance liquid chromatography (HPLC) - grade methanol (Merck, Germany), 1, 1-diphenyl-2-picrylhydrazyl (DPPH), Sigma-Aldrich, Germany), and dimethyl sulfoxide (DMSO, Sigma-Aldrich, Germany).

Animals

Healthy Swiss albino mice of both sexes weighing about 20–28 g were obtained from animal house of the Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka-Nigeria. The animals were allowed unlimited access to food and water *ad libitum* and were placed under standard laboratory animal house condition. All authors hereby declare that "the principles of laboratory animal care" (NIH publication No. 85-23, revised 1985), were adopted, and experiments were examined and approved by the appropriate ethics committee.

Test microorganisms

Test microorganisms used in the study comprised four strains of both Gram-negative and Gram-positive bacteria (*Escherichia coli, Salmonella typhi, Staphylococcus aureus,* and *Bacillus subtilis)* and two fungal strains (*Aspergillus fumigatus* and *Candida albicans*). These were laboratory isolates obtained from the Department of Pharmaceutical Microbiology and

Biotechnology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka-Nigeria.

Plant extraction

The plant leaves were rinsed in clean water and dried under room temperature to a constant weight. The dried leaves were pulverized into a fine powder using a mechanical grinder. A weight of 400 g of the pulverized plant sample was macerated in 2 L of ethanol and ethyl acetate, respectively, for 48 h. These were filtered using No. 1 Whatman filter paper, and the filtrates were concentrated under vacuum using a rotary evaporator at 40°C.

Antioxidant assay

The free radical scavenging activity of the plant extracts was evaluated using the DPPH assay method described by Shen et al. (14). The plant extracts and DPPH solution in methanol were prepared. These were mixed bringing resulting to a final concentration of 0.1 mM for the DPPH solution and 100 µg/mL for the plant extracts. The mixtures were shaken vigorously and allowed to stand at room temperature for 30 min. The absorbance of the samples was measured at 517 nm using a ultraviolet (UV)-Visible spectrophotometer (JENWAY 6505, Bibby Scientific Ltd, UK). Ascorbic acid was used as the positive control and 0.1 mM DPPH solution was used as a blank. The capability of scavenging the DPPH radical was calculated using the following formula.

DPPH scavenging effect (% inhibition) = (Abs blank – Abs sample/abs blank × 100).

Anti-inflammatory assay

The anti-inflammatory assay was done using the paw-fluid displacement method described by Winter et al. (15). A total of 24 mice were used, and the animals were grouped into 8 groups of 3 animals per group. Group 1 received 0.5 mL of distilled water; Group 2 received 50 mg/kg of diclofenac; Groups 3, 4, and 5 received 50, 100, and 200 mg/kg of the ethanol extract of *L. cylindrica,* respectively; and Groups 6, 7, and 8 received 50, 100, and 200 mg/kg of the ethyl acetate extract of *L. cylindrica,* respectively. All the treatments were through the oral route. 1 h post-treatment inflammation was induced in all the animals by single sub-plantar injection of 0.05 mL of egg albumin at the right hind leg. The paw size was checked before and after inducing inflammation at 0.5, 1, 2, 3, and 4 h. The volume of liquid displaced by the inflamed paw was used as a measure of the edema.

% Inhibition = (a/b × 100) – 100

Where; a = mean paw volume of mice at 30 min; b = mean paw volume of mice at 4 h

Antimicrobial assay

Primary antimicrobial screening

Primary screening of the plant extracts for antibacterial and antifungal activities was carried out using the agar well diffusion method described by Onyegbule et al. (16). Dilutions of 250, 125, 62.5, 31.25, and 15.625 mg/mL in DMSO (100% v/v) were prepared for each of the plant extract in a 2-fold dilution process. 20 mL of molten MHA and SDA (for bacterial and fungal isolates, respectively) were poured into sterile Petri dishes (90 mm) and allowed to set. Standardized concentrations (McFarland 0.5) of overnight cultures of test isolates were swabbed aseptically on the agar plates, and holes (6 mm) were made in the agar plates using a sterile metal cork-borer. 20 µl of the various dilutions of the extract and controls were put in each hole under aseptic condition. This was kept at room temperature for 1 h to allow the agents to diffuse into the agar medium and incubated accordingly. Gentamicin (10 µg/mL) and fluconazole (50 µg/mL) were used as positive controls in the antibacterial and antifungal evaluations, respectively; while DMSO (100% v/v) was used as the negative control. The MHA plates were then incubated at 37°C for 24 h, and the SDA plates were incubated at 25–27°C for 2–3 days. The resulting inhibition zones diameters (IZDs) were measured and recorded. The size of the cork borer (6 mm) was deducted from the values recorded for the IZDs to obtain the actual zone diameters. This procedure was conducted in duplicate, and the mean IZDs calculated.

Determination of minimum inhibitory concentration (MICs)

The MICs of the plant extracts on the test isolates were determined by the agar dilution method as described by Onyegbule et al. (16). Dilutions of 1000, 500, 250, 125, 62.5, and 31.25 mg/mL in DMSO (100% v/v) were prepared for each extract.

Agar plates were prepared by pouring 9 mL of molten double strength MHA and SDA (for bacterial and fungal isolates, respectively) into sterile Petri plates containing 1 mL of the various dilutions of the extract to obtain final plate concentrations of 100, 50, 25, 12.5, 6.25, and 3.125 mg/mL. Standardized concentrations of overnight cultures of the test isolates were streaked onto the surface of the agar plates containing dilutions of the extract. The MHA plates were then incubated at 37°C for 24 h, and the SDA plates were incubated at 25–27°C for 2–3 days, after which all plates were observed for growth. The minimum dilution (concentration) of the extract completely inhibiting the growth of each organism was recorded as the MIC.

HPLC-diode-array detection (HPLC-DAD) analysis

The HPLC-DAD analysis of the ethyl acetate extract of the plant was carried out using a Dionex P580 HPLC system (Dionex Softron GmbH, Germany) coupled to a UVD 340S photodiode array detector (Dionex, Germany). The separation column (125 × 4 mm; length × internal diameter) was prefilled with Eurospher-10 C18 (Knauer, Germany). A weight of 2 mg of the plant extract was dissolved in 2 ml of HPLC grade methanol and the mixture sonicated for 10 min, followed by centrifugation at 3000 rpm for 5 min. A volume of 100 µL of the dissolved sample was then transferred into a vial containing 500 µL of the HPLC grade methanol. Auto-sampler injected 20 µL of the sample, and a linear gradient of nanopure water (adjusted to pH 2 by addition of formic acid) and methanol was used as eluent. Detection was at 254 nm, and the resulting chromatogram was analyzed for the retention time, UV visible spectra, as well as the peak distribution of the constituents from the extract. The absorption peaks for the plant extract were analyzed by comparing with those in the HPLC-UV/Visible library.

RESULTS

The result of the antioxidant assay of the leaf extracts of *L. cylindrica* is presented in Table 1. At 100 µg/mL, the ethanol and ethyl acetate leaf extracts of *L. cylindrica* showed inhibition of 53.31% and 54.73%, respectively. Furthermore, the result of the anti-inflammatory assay of the plant extracts is presented in Figure 1. The ethanol and ethyl acetate extracts exhibited anti-inflammatory activity at concentrations of 50, 100, and 200 mg/Kg. At 50 mg/Kg, the ethanol and ethyl acetate extracts showed inhibition of 31.1% and 15%, respectively, with the control (diclofenac) producing inhibition of 39.7%. At

TABLE 1. Result of antioxidant assay of *Luffa cylindrica* leaf extracts

Concentration (µg/mL)	Inhibition (%)		
	Ethanol extract	Ethyl acetate extract	Ascorbic acid (positive control)
100	53.31	54.73	62.78

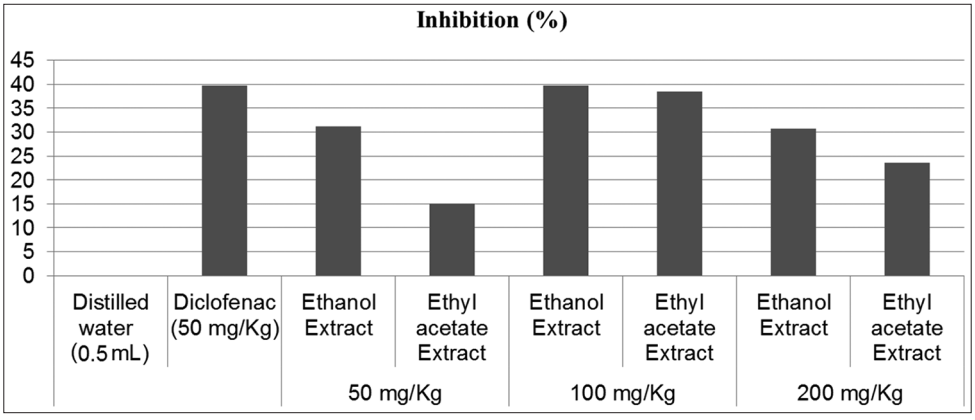


FIGURE 1. Anti-inflammatory activity of leaf extracts of *Luffa cylindrica* showing the percentage inhibition. Anti-inflammatory activity was recorded for the ethanol and ethyl acetate leaf extracts of *L. cylindrica* at 50, 100, and 200 mg/Kg, with the ethanol extract having better activity.

100 mg/Kg, a relatively similar anti-inflammatory activity was displayed by the ethanol and ethyl acetate extracts with inhibitions of 39.7% and 38.4%, respectively. At all concentrations analyzed, the ethanol extract showed better anti-inflammatory activity compared to the ethyl acetate extract.

Figures 2 and 3 present the results of the primary antimicrobial screening of the ethanol and ethyl acetate leaf extracts of *L. cylindrica,* respectively. It can be observed that the plant extracts displayed moderate antibacterial activity against *S. aureus*, *S. typhi,* and *B. subtilis;* with the best activity recorded against *S. typhi* followed by *S. aureus* and then *B. subtilis*. No antibacterial activity against *E. coli,* as well as antifungal activity against *A. fumigatus* and *C. albicans* was recorded for the plant extracts. Furthermore, the MICs of the plant extracts on the test isolates were determined, and the results are shown in Table 2. At the concentrations analyzed, the MICs of the plant extracts on test organisms ranged from 12.5 to

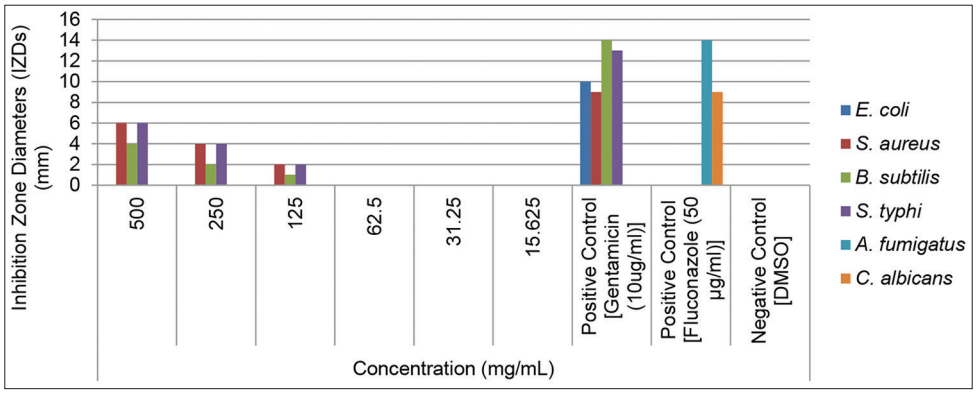


FIGURE 2. Mean inhibition zone diameters (mm) produced by the ethanol leaf extract of *Luffa cylindrica* on test isolates. The antimicrobial activity of the ethanol extract of the plant was observed at concentrations of 15.625–500 mg/ml. At these concentrations, the extract recorded antibacterial activity only against *S. typhi, S. aureus, and B. subtilis*. IZDs produced by the extract against the test isolates ranged from 0 to 6 mm with the best activity recorded equally against *S. typhi* and *S. aureus* followed by *B. subtilis*. No antimicrobial activity was recorded against *E. coli, A. fumigatus and C. albicans*.

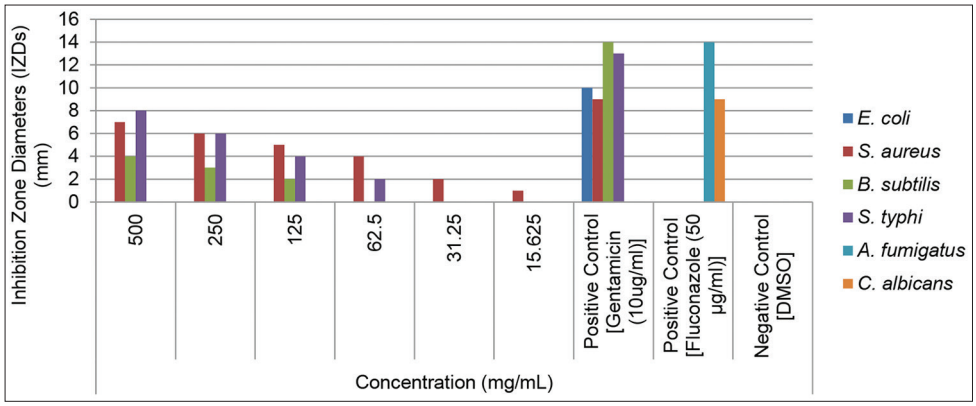


FIGURE 3. Mean inhibition zone diameters (mm) produced by the ethyl acetate leaf extract of *Luffa cylindrica* on test isolates. The antimicrobial activity of the ethyl acetate extract of the plant was observed at concentrations of 15.625–500 mg/ml. At these concentrations, the extract recorded antibacterial activity only against *S. typhi, S. aureus, and B. subtilis*. IZDs produced by the extract against the test isolates ranged from 0 to 8 mm with the best activity recorded against *S. tyhi*, followed by *S. aureus* and then *B. subtilis*. No antimicrobial activity was recorded against *E. coli, A. fumigatus and C. albicans*.

100 mg/mL. The ethyl acetate extract showed better antimicrobial activity against the test isolates compared to the ethanol extract.

The HPLC-DAD analysis of the ethyl acetate extract of the plant suggested the presence of two flavonoid compounds - luteolin and apigenin. The HPLC chromatogram, UV-spectra, and chemical structures of the two compounds detected in the ethyl acetate extract of *L. cylindrica* are presented in Figures 4, 5 and 6 respectively.

DISCUSSION

Plants are potential candidates in search of biologically relevant compounds, and the results of our study reveal the pharmacological properties possessed by *L. cylindrica*. The ethanol and ethyl acetate leaf extracts of *L. cylindrica* showed antioxidant, anti-inflammatory, and antimicrobial activities.

The ethyl acetate extract of the plant was further subjected to HPLC-DAD analysis since it displayed slightly better bioactivities compared to the ethanol extract. The HPLC-DAD analysis suggested the presence of two flavonoid compounds - luteolin and apigenin as crucial components of the leaf extract of *L. cylindrica.* The compounds are both known to possess antioxidant, anti-inflammatory, and antimicrobial activities (17,19-34) and may have contributed considerably to the pharmacological activities recorded by the plant extracts.

TABLE 2. MICs of *Luffa cylindrica* leaf extracts of on test organisms

Test Isolates	MICs (mg/mL)	
	Ethanol	Ethyl acetate
Escherichia coli	-	-
Staphylococcus aureus	100	12.5
Bacillus subtilis	100	100
Salmonella typhi	100	50
Aspergillus fumigatus	-	-
Candida albicans	-	-

MICs: Minimum inhibitory concentrations

Flavonoids are widely distributed throughout the plant kingdom and are commonly found in fruits, vegetables, and certain beverages. Luteolin (3',4',5,7-tetrahydroxyflavone) and apigenin (4',5,7-trihydroxyflavone) are naturally occurring plant flavones abundant in various fruits, vegetables, and medicinal plants. These biologically essential flavonoids are found to be useful as pharmaceutical agents (17-19).

Luteolin has been reported to exhibit a variety of pharmacological activities including antioxidant (17,20-22), anti-inflammatory (17,23-26), antimicrobial (17,27), antiparasitic (28,29), antidiabetic (30,31), and anticancer activities (17,32,33).

Apigenin is reported to possess anti-inflammatory (19), antioxidant (19,22), antimicrobial (27,34), and anticancer properties (35-37). Apigenin was identified as a major component in the seeds and leaves of *L. cylindrica* (5-7).

The detection of the abundance of luteolin and apigenin in the leaf extracts of *L. cylindrica* confirms the findings of Du et al. (8) and Liang et al. (5). They reported the isolation of a luteolin derivative (luteolin-7-O-beta-D-glucuronide methyl ester) from fruits of *L. cylindrica* (8) and apigenin from *L. cylindrica* leaves (5).

The medicinal properties exhibited by the plant may be attributed to the presence of the flavonoid compounds - apigenin and luteolin, as well as other

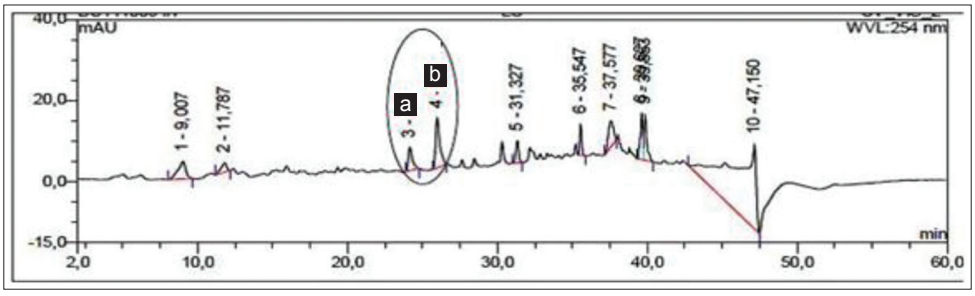


FIGURE 4. HPLC of ethyl acetate extract of *Luffa cylindrica* at showing the detection of Luteolin (a) and Apigenin (b) at a wavelength of 254 nm.

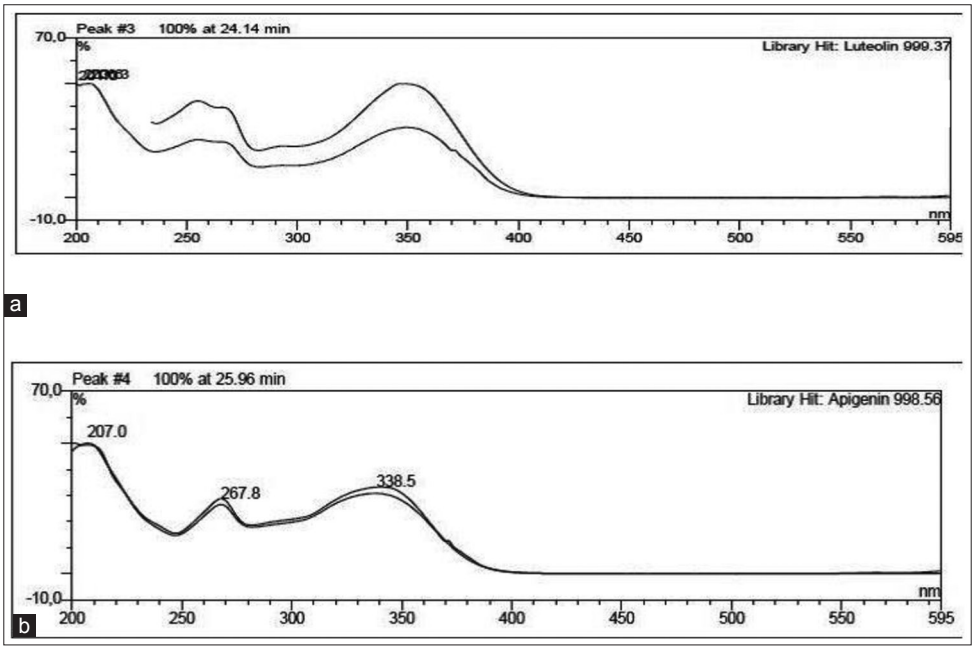
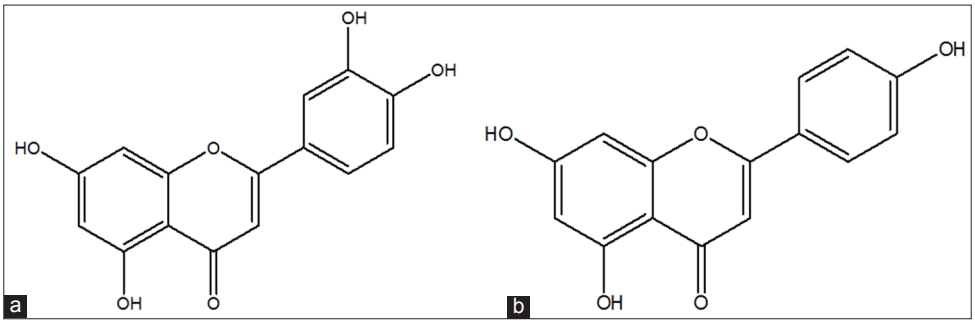


FIGURE 5. Ultraviolet spectra of detected compounds: Luteolin (a) and Apigenin (b).



Luteolin $C_{15}H_{10}O_6$. 286.24 g.mol^{-1} Apigenin $C_{15}H_{10}O_5$. 270.24 g.mol^{-1}

FIGURE 6. Chemical structures of detected compounds: Luteolin (a) and Apigenin (b).

flavonoids and phytocompounds not detected in this study.

According to Akpotu et al. (38,39), the HPLC-DAD analysis is still with limitations as only compounds with UV-spectra previously registered in the HPLC spectral library can be detected. The undetected compounds in the extract of *L. cylindrica* subjected to HPLC-DAD analysis in this study may constitute important or novel compounds with interesting biological properties. It is, therefore, necessary that further studies be carried out using more advanced and sensitive spectroscopic methods to validate the findings of this research.

CONCLUSIONS

The results of this study reveal that *L. cylindrica* possess antioxidant, anti-inflammatory, and antimicrobial properties. This provides a scientific basis for the use of this plant in ethnomedicine for the management of various disease conditions.

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COMPETING INTEREST

Authors declare that they have no conflicts of interest.

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