



ORIGINAL RESEARCH

Phytochemical and Antioxidant Activity of Water and Methanol Extracts of *Oldenlandia corymbosa*

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ABSTRACT

Background: *Oldenlandia corymbosa* is a small perennial herb used traditionally to clear toxins, activate blood circulation, and relieve urinary obstruction.

Objectives: This study investigated the phytochemical and antioxidant activity of water and methanol extracts of *Oldenlandia corymbosa*.

Materials and Methods: Total phenolic, flavonoids, and tannin contents of *Oldenlandia corymbosa* were determined using Folin-Ciocalteu reagent, Colorimetric AlCl₃ method, and spectrophotometry method respectively. The antioxidant activities were analyzed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, ferric reducing antioxidant power (FRAP), metal chelating activity (MCA), 2,2'-azino-bis-(3-ethyl) benzothiazoline-6-sulfonic acid (ABTS) radical scavenging, and phosphomolybdenum assay (PMA).

Results: The total phenolic, flavonoid and tannin of the methanolic extracts were found to be higher than that of the water extracts. The DPPH, FRAP, MCA, ABTS and PMA for the water extracts were found to be 56.05 ± 0.1 %, 0.018 ± 0.002 mg Fe(II) equivalent/g, 0.346 ± 0.04 mg EDTA equivalent/g, 63.67 ± 0.6 mg trolox equivalent/g, 0.82 ± 0.03 ascorbic acid equivalents/g and greater than that of the methanol extract 47.26 ± 0.09 %, 0.009 ± 0.0001 mg Fe(II) equivalent/g, 0.198 ± 0.002 mg EDTA equivalent/g, 62.68 ± 0.7 mg trolox equivalent/g and 0.365 ± 0.008 ascorbic acid equivalents/g respectively. These results show that, though the methanol extracts contain more polyphenolic compounds, the water extract exhibits more antioxidant activity.

Conclusion: The water extract of *Oldenlandia corymbosa* shows superior antioxidant activity due to the synergistic effects of its phytoconstituents than that of methanol extracts and can serve as a natural remedy for managing oxidative stress.

Keywords: *Oldenlandia corymbosa*; phytochemicals; antioxidant activity water extract, methanol extract

INTRODUCTION

Oldenlandia corymbosa Linn, is a plant of flowering plant group belonging to the Rubiaceae family has ascending stems that are angled and the leaves are 1-4 cm in length and 6 mm in width^{1,2}. Flowers tube consist of 4 petals and it is about 1.5-2 mm in length¹. *Oldenlandia corymbosa* is a plant species belonging to the Rubiaceae family². They are native to various regions, including Africa, Asia, and Australia. *Oldenlandia corymbosa*, also known as the "diamond flower," has a long history of traditional use in herbal medicine^{3,4}. In the traditional Chinese medicine systems, more than 20 species of *Oldenlandia* have been used as folk medicine with *H. corymbosa* as the most prominent plant and is the active ingredients of some Chinese herbal medicinal preparations such as Peh-huejuwa-chi-cai[®], Jiedu Yanggan Gao powder[®], Xiao Wei Yan Powder[®] and Feibao syrup[®] used in treating cancer and other related diseases⁵. The plant is also known to clear toxins, activate blood circulation, promote diuresis, and relieve urinary obstruction. It inhibits the tumor activity of lymphosarcoma and carcinoma and of the liver and larynx. This plant is also useful for sore throat, ulcers, and skin infections. There is not much literature about the antioxidant activity of this plant in Nigeria, therefore this study investigated the phytochemical and antioxidant activity of water and methanol extracts of *Oldenlandia corymbosa* to provide evidence for its usefulness in traditional remedies.

MATERIALS AND METHODS

Collection of plant material

Oldenlandia corymbosa was collected from the University of Ilorin, Kwara State, Nigeria and identified properly by a botanist at the herbarium, Department of Botany, University of Ilorin, Ilorin, Nigeria with voucher number UILH/001/034/2024. The whole plant without the roots was dried at 45 °C for 72 hours and powdered using an electric blender.

Extraction of plant materials

The pulverized plant (100 g) of *Oldenlandia corymbosa* was soaked in distilled water (20% w/v) at room temperature for 24 h with intermittent shaking after 12 h. Another 100 g pulverized plant was also soaked in methanol (20% w/v) and the same procedure as in distilled water followed. The extracts were filtered using a clean white muslin piece of cloth to remove debris after which they were each filtered through a Whatman No 1 filter paper. The resulting *Oldenlandia corymbosa* water extract (OCW) and *Oldenlandia corymbosa* methanol extract (OCM) were evaporated over a water bath at 40 °C⁸. The evaporated extracts were then stored in small-capped bottle containers at +4°C until required for analysis.

Quantitative Phytochemical Analysis

Total Phenolic content

The amount of phenol in the methanol and aqueous extract of *Oldenlandia corymbosa* were determined using the Folin-Ciocalteu reagent method^{9,10}. 2 mL of 10 % Folin-Ciocalteu reagent and 1.6 mL of 2 % solution of sodium bicarbonate was added to 2 mL of the plant extract. Different concentrations (0.1 - 0.7 mg/mL) of gallic acid (standard) was prepared the same way as the extracts. The resulting mixture was incubated for 30 minutes at room temperature and the absorbance of the standard and samples was measured at 765 nm. A graph of the absorbance of standard versus concentration was plotted as the standard curve. All tests were performed in triplicates. The results were determined from the standard curve and were expressed as gallic acid equivalent (mg/g of extracted compound).

Total Flavonoid content

Colorimetric method using aluminium chloride was used to determine the flavonoid content⁹. The methanol and aqueous extracts solution (0.3 mL each) were each mixed with 1.5 mL of methanol and 1.5 mL of 10 % aluminium chloride and allowed to stand for 1 hour at room temperature. Different concentrations (10 µg to 1000 µg) of quercetin

(standard) were prepared the same way as the extracts. The absorbance was measured at 420 nm. Extract samples were evaluated at a final concentration of 0.1 mg/mL. Quercetin at different concentrations of 0.06 to 0.18 mg/mL was prepared (as standard) in the same way as the extracts. All tests were performed in triplicates. Total flavonoid contents were calculated as quercetin equivalents (mg/mL).

Determination of total tannin content (TTC)

The vanillin-HCl method¹¹ with minor modifications was used to determine total tannin content. Briefly, the extracts (methanol and aqueous extracts of *Oldenlandia corymbosa*) or standard catechin (100 µL) and 1 mL reagent (4% vanillin and 8% conc. HCl; 1:1 in methanol) were mixed and incubated at room temperature for 20 min and absorbance were taken at 500 nm. All the results were expressed as mg catechin equivalent (CE)/g extract.

Antioxidant Assay

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging

The DPPH radical-scavenging activity¹² of the test extracts were examined. Different concentrations (0.6 and 0.4 mg/mL) of each extract (methanol and aqueous extracts of *Oldenlandia corymbosa*) were prepared. 1.5 mL of the different concentrations was added to 1.5 mL of the methanolic solution of DPPH (100 µM). The mixture was allowed to react to room temperature in the dark for 30 minutes. Vitamin C was used as standard control while a mixture without the extract was taken as blank. Three replicates were made for each test sample. After 30 minutes, the absorbance (A) was measured at 518 nm and converted into percentage antioxidant activity using the following equation:

$$\% \text{ DPPH antioxidant activity} = \frac{\text{Absorbance (DPPH)} - \text{Absorbance (Extract)}}{\text{Absorbance (DPPH)}} \times 100$$

Ferric reducing antioxidant property assay (FRAP)

FRAP assay was performed according Patel *et al.*¹⁰. Briefly, FRAP reagent was prepared by

mixing acetate buffer (pH 3.6), 10 µM TPTZ in 40 µM HCl, and 20 µM FeCl₃ at ratio of 10:1:1 (v/v/v). Methanol extract of each of the samples (methanol and aqueous extracts of *Oldenlandia corymbosa*) (2 mL from 2 mg/mL stock) and 2 mL reagent was mixed, incubated at 37 °C in water bath for 30 min, and absorbance was read at 593 nm. Standard FeSO₄·7H₂O (20–120 mg/mL) was used to plot curve. Results were expressed in mg Fe (II) equivalent/g extract.

Metal chelating activity (MCA)

To determine total antioxidant capacity of the extracts (methanol and aqueous extracts of *Oldenlandia corymbosa*), metal chelating assay was performed¹³. In brief, 5 mL of 2 mM FeCl₂ was added to 4 mL (2 mg/mL stock) extract or standard EDTA (20–120 µg/mL). The reaction was started by the addition of 10 mL of 5 mM ferrozine solution and 650 µL distilled water was added to make up the volume. The mixture was vigorously shaken and left to stand at room temperature for 10 min. The absorbance of the solution was thereafter measured at 562 nm. Na₂-EDTA was used as a positive control. The results were expressed as mg EDTA equivalent (EE)/g extract.

2,2'-azino-bis-(3-ethyl) benzothiazoline-6-sulfonic acid (ABTS) radical scavenging activity

ABTS radical cation decolourization assay¹⁴ was performed on the methanol and water extracts of *Oldenlandia corymbosa*. ABTS radical cation (ABTS) was produced by reacting ABTS solution with 2.45 mM potassium persulfate (1:1 ratio) and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. ABTS solution was diluted in methanol (1:89 ratio) to obtain an absorbance 0.7 (±0.02) at 734 nm. In test tube, 1 mL ABTS solution and 2 mL of extracts (2 mg/mL stock) or standard was taken, incubated at 30 °C and absorbance taken at 730 nm exactly after 30 min using methanol as blank. All the solutions were used on the day of preparation. The results were

expressed as mg trolox equivalent (TE)/g extract.

Phosphomolybdate assay (PMA)

The method reported by Prieto and Ghane¹⁵ was adopted with some minor modifications. In this assay, 1.5 mg/mL plant extract (10 mg/mL stock) or standard ascorbic acid (0.2–2.0 mg/mL) was mixed with phosphomolybdate reagent (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The reaction mixture was then incubated in a water bath at 90 °C for 90 min, cooled to room temperature and absorbance was measured at 695 nm. The antioxidant capacity was expressed as mg of ascorbic acid equivalents (AAE)/g extract.

Data Analysis

The results are presented as mean $\pm$ standard error (SE) of at least three independent replicates. Statistical analysis was performed using Analysis of Variance (ANOVA) and the significances of the differences between sample mean were determined using Duncan's multiple range test using SPSS software ver. 16. Statistical significance was set at a level of $p < 0.05$.

RESULTS

Total phenolic content was expressed as mg/g Gallic acid equivalent using the following equation based on the calibration curve:

$$y = 1.6232x, R^2 = 0.6658$$

Where y was the absorbance and x was the concentration.

Total flavonoid contents were calculated as quercetin equivalents (mg/mL) using the following equation based on the calibration curve:

$$y = 0.23x, R^2 = 0.9424$$

Where y was the absorbance and x was the concentration.

The total phenolic, flavonoid and tannin contents in the methanol extract of *Oldenlandia corymbosa* were higher than those of the water extract as shown in Figure 1. The total phenolic, flavonoid and tannin of the methanol extracts were found to be (0.816 ± 0.03 mgGAE/g), (0.887 ± 0.01 mgCE/g) and (249.8 ± 0.5 mgCE/g) higher than that of the water extracts (0.542 ± 0.01 mgGAE/g), (0.411 ± 0.001 mgCE/g) and (141.8 ± 0.3 mgCE/g) respectively.

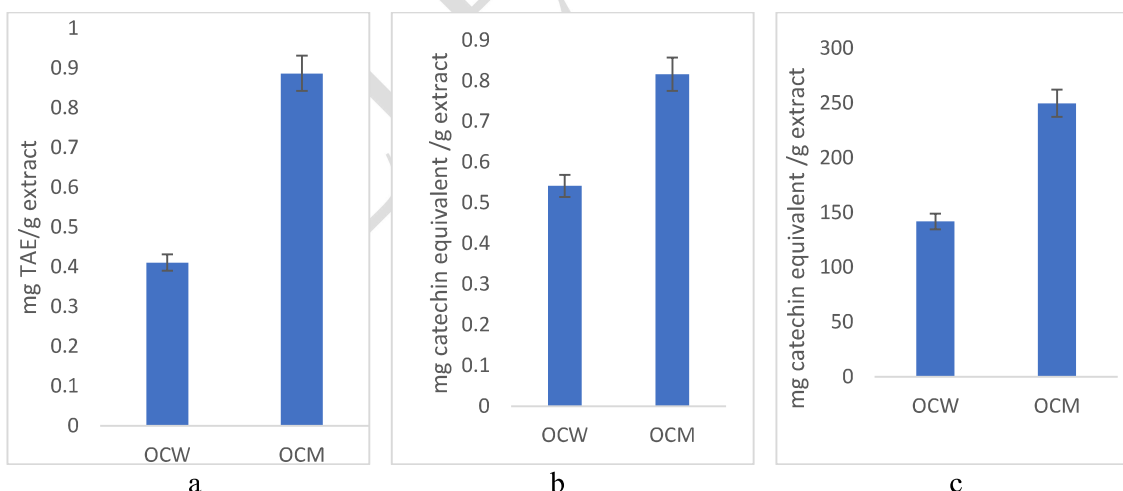


Figure 1. a: Total phenolic content, b: total flavonoids content, and c: total tannin contents of water extract (OCW) and methanol extract (OCM) of *Oldenlandia corymbosa*

Antioxidant potential from the water and methanol extract of *Oldenlandia corymbosa* was estimated by measuring DPPH, FRAP, MCA, ABTS, and PMA activities. The

DPPH, FRAP, MCA, ABTS and PMA for the water extract were found to be $56.05 \pm 0.1\%$, 0.018 ± 0.002 mg Fe(II) equivalent/g, 0.346 ± 0.04 mg EDTA equivalent/g,

63.67±0.6 mg trolox equivalent/g, 0.82±0.03 ascorbic acid equivalents/g and greater than that of the methanol extract 47.26±0.09%, 0.009±0.0001 mg Fe(II) equivalent/g,

0.198±0.002 mg EDTA equivalent/g, 62.68±0.7 mg trolox equivalent/g and 0.365±0.008 ascorbic acid equivalents/g respectively (Figure 2).

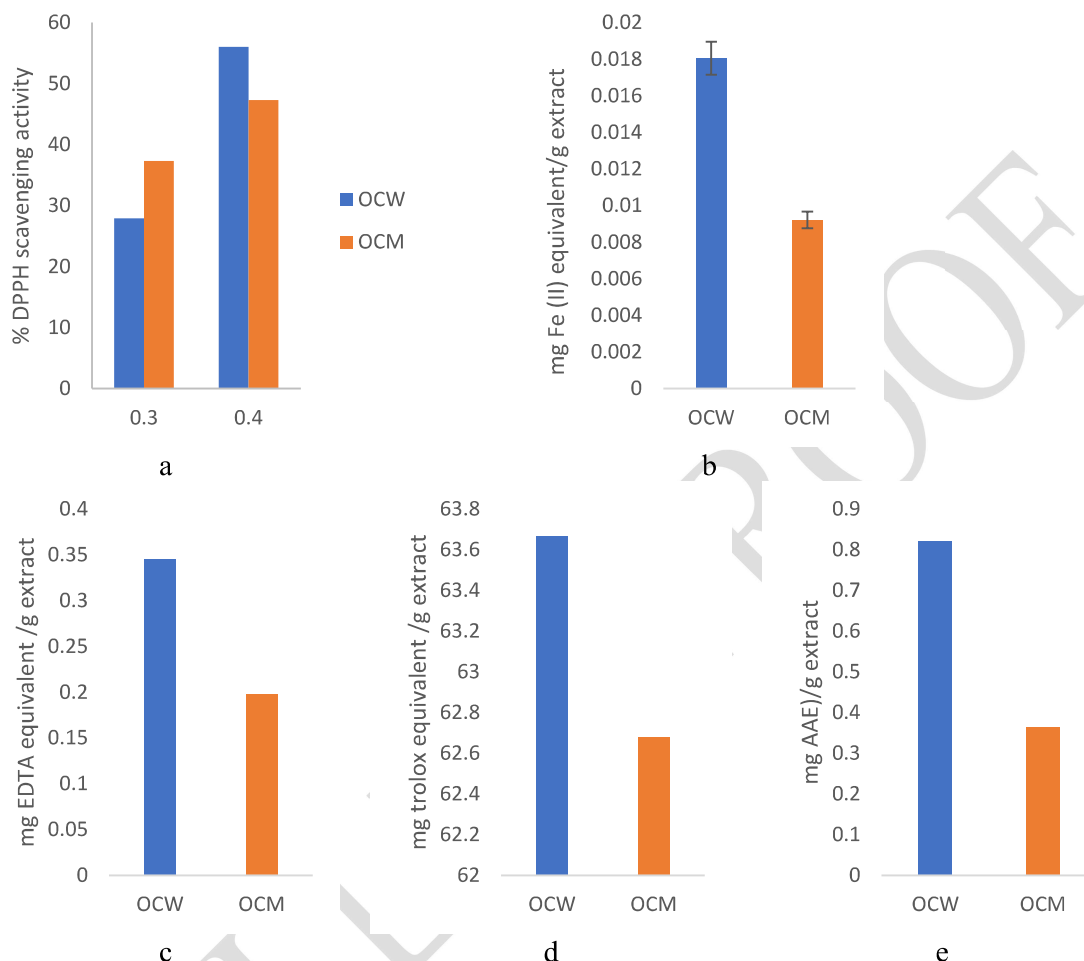


Figure 2. a: 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, b: Ferric reducing antioxidant power assay, c: Metal chelating activity, d: 2,20-azino-bis-(3-ethyl) benzothiazoline-6-sulfonic acid radical scavenging activity, e: Phosphomolybdenum assay of water extract (OCW) and methanol extract (OCM) of *Oldenlandia corymbosa*

DISCUSSION

Studies have proposed that the different polarities of solvents exert an important effect on the recovery of polyphenols from different sources¹⁶. It has also been shown that the amounts of components extracted by solvents increased as the polarity of the solvent used increased^{17,18}. The total phenol, flavonoid and tannin contents in the methanol extract of *Oldenlandia corymbosa* is higher than that of the aqueous extract. This result is in

agreement with previous literature¹⁹. The total phenol in this study was lower than that reported by Aziz and colleagues²⁰ and the flavonoid also lower than that reported by Al Bashera and colleagues²¹. Those reported in literature^{20,21} were harvested in India while ours was in Ilorin, Nigeria, thus, the difference in phytoconstituents concentrations can be due to differences in geographical location, climate and soil.

Phenolics, flavonoids, and tannins continue to be studied for their health benefits, and recent

research has further expanded their potential effects. Phenolic compounds have been reported to have antioxidant, anti-inflammatory, and anticancer properties. Recent studies have explored phenolics' role in gut microbiota modulation. Phenolic compounds can positively influence the gut microbiota composition, which is crucial for maintaining overall health and preventing various diseases²². Flavonoids, in addition to their antioxidant and anti-inflammatory effects, recent research has focused on flavonoids' impact on brain health. Some flavonoids have been found to cross the blood-brain barrier and exhibit neuroprotective properties, which may help prevent neurodegenerative diseases such as Alzheimer's and Parkinson's²³. Tannins have been studied for their potential role in weight management. Recent research suggests that tannins may help reduce body weight and fat mass by affecting gut microbiota and metabolic pathways²⁴. Therefore *Oldenlandia corymbosa* may possess all these health benefits including antioxidant activity, which helps protect cells from oxidative stress and damage caused by free radicals.

While the methanol extract of *Oldenlandia corymbosa* displayed significantly higher levels of total polyphenolic content compared to the aqueous extract of *Oldenlandia corymbosa*, it is noteworthy that the aqueous extract exhibited superior antioxidant activity. This disparity suggests that the aqueous extract of *Oldenlandia corymbosa* harbors specific polyphenolic compounds with remarkable antioxidant properties, emphasizing the importance of the quality rather than the quantity of bioactive compounds.

This phenomenon where methanol extracts generally contain higher levels of phenolics, flavonoids, and tannins compared to water extracts but the water extracts often exhibit more remarkable antioxidant activity has been reported in the literature²⁵⁻²⁷. This discrepancy can be attributed to the synergistic effects of different bioactive compounds present in the extracts²⁸, and also the different extraction

efficiencies of various bioactive compounds by different solvents¹⁶.

CONCLUSION

The methanol extracts of *Oldenlandia corymbosa* possess higher concentrations of phenolics, flavonoids, and tannins compared to the aqueous extract of *Oldenlandia corymbosa*, however, the aqueous extracts exhibit more remarkable antioxidant activity due to the synergistic effects of these compounds in the extracts which lead to a more potent overall antioxidant effect than that of methanol extracts. The superior antioxidant activity of the water extract explains its potential in traditional medicine, functional foods, and nutraceuticals

Further research is needed to explore the underlying mechanisms behind this phenomenon and to identify the specific compounds responsible for the synergistic antioxidant effects in water extracts. This knowledge could potentially lead to the development of more effective natural antioxidant sources for various applications in the food, pharmaceutical, and cosmetic industries.

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AUTHORS' CONTRIBUTIONS

NSN, FAUA, AOS, ODB, STA and OIE designed the study. SCC, SPO, PEI and CM determined phytochemistry and antioxidant activity. NSN, AOS and CM analyzed the data. The initial draft was written by MSD and AA. All authors approved the final article.

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