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2, 2-Diphenyl-1-picrylhydrazyl Radical Effect and Phytochemical Constituents of *Combretum platypetalum* Welw. ex. M. A. Lawson subsp. oatesii (Rolfe) Exell Leaf

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Authors' contributions

This work was carried out in collaboration among all authors. Authors SDU and BBS designed the study, wrote the protocol, and the first draft of the manuscript. All authors conducted experimental work, managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To investigate the phytochemical constituents and antioxidant properties of *Combretum platypetalum* leaf.
Study Design: *In vitro* assessment of antioxidant properties and determination of the phytochemical constituents of *C. platypetalum* leaf extracts.
Site and Duration of Study: Department of Pharmaceutical Chemistry, Faculty of Pharmacy,

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University of Ibadan, Ibadan; Department of Biochemistry, University of Ibadan, and University of Agriculture, Makurdi (August 2013 –February 2015).
Methodology: Phytochemical constituents of the hexane, ethyl acetate, acetone and methanol extracts were determined following established methods. The extracts were investigated for their antioxidant properties using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay, total phenolic content and total flavonoid content assay models.
Results: Phytochemical screening showed the presence of alkaloids, terpenoids, saponins, flavonoids, and anthraquinones in the leaf extract. The methanol fraction contained the highest amount of polyphenols and flavonoids (1289.76 and 517.97 µg Catechin equivalent /g of extract respectively). The phenolics content of the extracts also correlated with the observed significant *in vitro* antioxidant effects. The highest DPPH free radical inhibition for catechin, *n*-hexane, ethyl acetate, acetone extracts and methanol extracts were observed at 90.41±0.05% at 2000 µg/mL, 20.60±12.38% at 750 µg/mL, 6.73±6.32% at 2000 µg/mL, 25.28±1.46% at 2000 µg/mL and 25.98±1.93% at 250 µg /mL respectively.
Conclusion: The results of this study substantiate the high phenolics content and free radical scavenging effect of *C. platypetalum* leaf extract and validate the claims for its traditional uses.

Keywords: Combretaceae; Combretum platypetalum; antioxidant activity; phytochemical constituents.

1. INTRODUCTION

Despite the intracellular defence mechanisms such as superoxide dismutase (SOD), glutathione peroxidise (GPX) catalase (CAT) and other endogenous antioxidants in the body which arrests the damaging effect of reactive oxygen species (ROS) [1-4], continuous exposure to the ROS beyond the capacity of the body can cause an irreversible oxidative damage [5] to the system. This had been reported to be associated with several health concerns including cancer, inflammation, atherosclerosis, coronary heart disease, diabetes, and cardiovascular disease [6-8]. Plants containing phenols, flavonoids, polypropanoids and other phytochemicals have been implicated to scavenge free radicals due to their hydrogen atom donating ability [9]. Thus, the need to investigate phytochemicals in plants cannot be overemphasised.

About 135 compounds belonging to the cycloratane, ursanes, oleananes and dammarane triterpenes and their glycosides structural groups; 51 flavonoids belonging to the flavonols, flavanone, flavonones and chalcone structural groups; four steroids; six stilbenes and eight dihydrostilbenes have been isolated and identified from the *Combretum* genus [10], with their unique biological relevance, mostly, as antibacterial [11,12], antioxidant [13,14] and anti-tumour [15,16], amongst several others. The chromosome number 2n= 39 has been reported for the dwarf shrub with annual stem, typically less than 30 cm, growing from a woody rootstock that usually flowers between August and October [17,18], locally known as the red wing with botanical name *C. platypetalum* from the *Combretum* genus of the Combretaceae family of plants that is widely distributed in approximately 21 genera [19,20] with over 599 species [21,22] of herbs, shrubs and trees. Traditionally, *C. platypetalum* has been used for the treatment of pneumonia, abdominal pains, diarrhea, antiemetic, dysmenorrhea, infertility in women, earache, pistaxis, and haemoptysis [23,24]. Ruvimbo et al. [23] investigated its effect on the growth and drug efflux system of *mycobacterium aurum* and *mycobacterium smegmatis* and established it *antimycobacterial* effect. Similarly, Fadzai et al. [25] established it`s nitric radical scavenging properties. This study investigates the phytochemical composition of the hexane, ethyl acetate, acetone and methanol leaf extracts of *C. platypetalum* and their *in vitro* antioxidant capacity using the DPPH radical model.

2. MATERIALS AND METHODS

2.1 Identification and Preparation of Plant Material

Fresh leaf samples of *C. platypetalum* were collected during the rainy season (September, 2013), in the vicinity of the Faculty of Science, University of Ibadan. The plant was identified and authenticated at the Forestry Research Institute of Nigeria (FRIN) and herbarium sample was deposited with FHI Number 109750. The leaves were air dried and pulverised.

2.2 Extraction

The plant materials were extracted using Soxhlet apparatus by successive method. This extraction method was also monitored by cold maceration to validate the thermal integrity of the plant

components. The extraction process proceeded at a ratio of 1:10 plant material to solvent ratio.

2.3 Phytochemical Screening

The phytochemical evaluation of the n-hexane, ethyl acetate, acetone and methanol extracts of *C. platypetalum* leaf was performed using standard procedures [26,27].

2.6 Evaluation of DPPH radical Scavenging Activity

The DPPH radical scavenging activity of the extracts was determined according to the method of Mensor et al. [28] with slight modifications. Different concentrations of the extracts (100 to 1500 µg/mL) were prepared. Catechin was used as standard antioxidant at the same concentrations. 1 mL of extract was placed in a test tube and 2.5 mL of methanol was added followed by 0.5 mL of 1 mM DPPH in methanol. A blank solution (control) was prepared; the mixtures were allowed to react at room temperature. The absorbance was read after 30 minutes at 517 nm and converted to percentage antioxidant activity (inhibition) using the formula below:

Percentage inhibition of radical by sample = (Control – Test sample)/Control X 100

2.7 Total Phenolic Content

The total phenolic content was evaluated by adopting a modified colorimetric method described by Singleton and Rossi [29] with slight modifications. Different concentrations of the extract of (10 - 1000 µg/mL) were prepared and 0.5 mL of the extract was added to the test tube followed by 0.5 mL of 1:10 dilution of Folin C reagent. After few minutes, 0.5 mL of 15% w/v Na_2CO_3 solution was added and the solution was made up to 4 mL with water. The reaction mixture was kept in water bath for 20 minutes at 40ºC. The absorbance of the mixture was read at 760 nm and catechin was used as standard.

2.8 Total Flavonoid Content

This was determined colorimetrically using the method described by Jia et al. [30] with some modifications. Different concentration of the extract (10 – 1000 µg/mL) in 1 mL of distilled water was prepared, and 75 µL of 5% $NaNO_3$ was added. After five minutes, 150 µL of 10% $AlCl_3.H_2O$ was added followed by 500 µL of 1 M NaOH and 275 µL of H_2O after six minutes. The mixture was read using the UV spectrometer at 510 nm. Catechin was used as standard.

3. RESULTS AND DISCUSSION

The percentage yields of the hexane, ethyl acetate, acetone and methanol extracts of *C. platypetalum* were 48.71, 27.01, 14.91 and 9.37% respectively. Phytochemical screening showed the presence of alkaloids in the methanol extract; anthraquinones in ethyl acetate extract; saponins and flavonoids in ethyl acetate and acetone extracts and terpenoids in all leaf extracts (Table 1). This correlates with some of the identified phytochemicals in some members of the C*ombretum* as reported by Rodrigues et al. [31].

The hexane and ethyl acetate extracts were more of non-polar and intermediate polarity respectively. The acetone extract showed mainly two spots from TLC analysis (in various mobile phase systems) which was well resolved. The green coloured spot (of the acetone extract) observed in the normal phase which was suspected to be non-polar was left at the base line in the impregnated silica gel TLC plate in the mobile phase system of methanol: water and the polar component had R_f value of 0.62. The polar component was resolved by an artificial reverse phase; both components were better separated by solvent-solvent partitioning. The hexane and ethyl acetate extracts showed the presence of multiple components from thin layer chromatography.

Table 1. Phytochemical screening of *C. platypetalum* leaf extracts

S/N	Test	n-hexane extract	Ethyl acetate extract	Acetone extract	Methanol extract
1	Alkaloid	-	-	-	+
2	Terpenoid	+	+	+	+
3	Saponin	-	+	+	-
4	Flavonoid	-	+	+	-
5	Anthraquinone	-	+	-	-

+ = Present; - = Absent

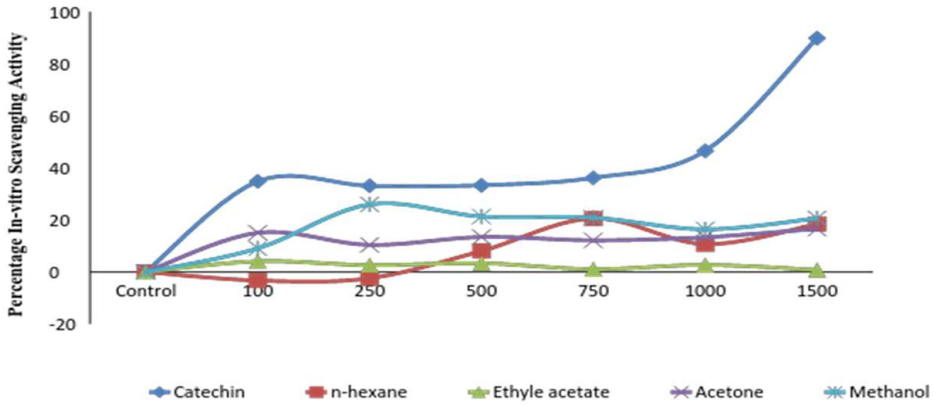

Fig. 1. In-vitro scavenging activity of *C. platypetalum* leaf extracts

Table 2. Total phenolics and flavonoids content of *C. platypetalum* leaf

Plant extract	Polyphenols (µg Catechin equivalent /g)	Flavonoids (µg Catechin equivalent /g)
Hexane	234.89	99.62
Ethyl acetate	429.33	369.84
Acetone	731.00	375.09
Methanol	1289.76	517.97

DPPH radical was used as a stable free radical to determine antioxidant activity. Fig. 1 illustrates the concentration of DPPH radical due to the scavenging ability of the leaf extracts of *C. platypetalum* and standard compound, catechin. The percentage inhibition of the free radical was dose dependent. Increase in concentration gave corresponding increased % inhibition. The percent inhibition of various extracts were found to be significantly different from the control at $P = 0.05$ except for the 750 and 1500 µg/mL concentration of the ethyl acetate extract with percentage inhibition of 1.09±1.42 and 0.79±0.41 respectively. The highest inhibitions for catechin, n-hexane, ethyl acetate, acetone extracts and methanol extracts were observed at 90.41±0.05% at 2000 µg/mL, 20.60± 12.38% at 750 µg/mL, 6.73±6.32% at 2000 µg/mL, 25.28±1.46% at 2000 µg/mL and 25.98± 1.93 at 250 µg/mL respectively.

The total phenolics and flavonoids content in *C. platypetalum* leaf are presented in Table 2. This accounts for the relatively high antioxidant effect of the extracts which strongly correlates with similar findings by Fadzai et al. [24]. The methanol fraction consisted the highest amount of polyphenols and flavonoids (1289.76 and 517.97 µg Catechin equivalent /g of extract respectively), while the hexane fraction showed relative low content of polyphenols and flavonoids (234.89 and 99.62 µg Catechin equivalent /g of extract respectively). The antioxidant activity of plant extracts containing polyphenol components is due to their capacity to be donors of hydrogen atoms or electrons and to capture the free radicals [9].

4. CONCLUSION

The results of this study substantiate the high phenolics content and free radical scavenging effect of *C. platypetalum* leaf extract and validate the claims for its traditional uses. *C. platypetalum* has shown the potential as a possible source of a new antioxidant agent.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

The authors have declared that no competing interest.

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