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Comparative Evaluation Antioxidant Potentials and Phenolic Composition of Ethanolic Extracts from *C. odorata*, *A. occidentale*, and *P. amarus* in Calabar

Chibuzor Onyinye Okonkwo[1,*], Gregory Elayeche Oko[1], Nelson Bikom Oyong[1], Sunday Nnamdi Okonkwo[2], Chizuruoke Oluomachi Chukwu[3], David-Oku Essien[1]

[1] Department of Biochemistry, University of Calabar, Nigeria
[2] Department of Ophthalmology, University of Calabar, Teaching Hospital Calabar, Nigeria
[3] Department of Pharmaceutical Microbiology, Abia State University, Uturu, Abia State.
*Corresponding E-mail: oko210@unical.edu.ng

ABSTRACT

Oxidative stress contributes to the development of degenerative and metabolic diseases, and plants rich in phenolic compounds serve as natural antioxidants capable of mitigating its effects. This study comparatively evaluated the antioxidant potentials and phenolic composition of ethanolic leaf extracts of *Chromolaena odorata*, *Anacardium occidentale*, and *Phyllanthus amarus* harvested in Calabar, Cross River State, Nigeria. Standard in vitro assays were employed, including DPPH radical scavenging, ferric reducing antioxidant power (FRAP), hydrogen peroxide scavenging, catalase activity, and total phenolic content (TPC). The results revealed that *A. occidentale* exhibited the highest DPPH radical scavenging ability followed by *C. odorata* and *P. amarus*. However, *P. amarus* demonstrated the highest reducing and enzymatic antioxidant capacities, with FRAP (0.41 ± 0.01 µmol Fe^{2+}/g), catalase (4.77 ± 0.12 µmol H_2O_2/min/mg), hydrogen peroxide scavenging (156.60 ± 1.42 µmol/mL), and TPC (2.97 ± 0.50 mg GAE/g) rather than the other sample. The overall results indicate that *P. amarus* possesses superior total antioxidant capacity, suggesting its potential as a promising natural source of antioxidant compounds for pharmaceutical and nutraceutical applications.

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1. INTRODUCTION

Medicinal plants have long been recognized as invaluable sources of therapeutic agents, providing bioactive compounds with profound pharmacological effects. The global resurgence of interest in plant-derived antioxidants is largely due to the increasing incidence of oxidative stress-related diseases such as cancer, diabetes, and cardiovascular disorders. These conditions are linked to the excessive generation of reactive oxygen species (ROS), which damage biomolecules and disrupt cellular functions (Das Sarma, *et al.*, 2010; Prochazkova, *et al.,* 2011). Plants synthesize a wide range of secondary metabolites, including phenolics, flavonoids, tannins, alkaloids, and terpenoids, which function as antioxidants by neutralizing free radicals and protecting against oxidative damage (Fabricant & Farnsworth, 2001; Dey, *et al.,* 2016). Tropical countries with significant biodiversity, such as Nigeria, have abundant but underexplored medicinal flora with antioxidant potential. Among these, *Chromolaena odorata*, *Anacardium occidentale*, and *Phyllanthus amarus* are three ethnomedicinal plants traditionally used in managing infections, inflammation, diabetes, and liver disorders. Their bioactive compositions and antioxidant profiles, however, vary considerably with species and extraction methods, necessitating comparative evaluation using standardized assays (Do, *et al.,* 2020; Nguyen, *et al.,* 2021).

Chromolaena odorata which is perennial shrub belongs to the family Asteraceae and native to Central and South America. Studies suggest that ethanolic extracts of *C. odorata* display strong DPPH and ABTS radical scavenging capacities, positioning the plant as a valuable natural antioxidant source for pharmaceutical and nutraceutical applications (Gorawade, *et al.,* 2021). *Anacardium occidentale* L., or the cashew tree, belongs to the family Anacardiaceae and is cultivated widely across tropical and subtropical regions for its nutritional and medicinal value (Ojewole, 2003; Keshinro & Ketiku, 2009). Phytochemical investigations have identified phenolic acids, flavonoids, tannins, and cardanols as the principal bioactive constituents contributing to its pharmacological effects (Konan & Bacchi, 2007; Leite, *et al.,* 2016). Compounds such as quercetin, catechin, and myricetin have been shown to exhibit potent antioxidant and anti-inflammatory activities (Ahn, *et al.,* 2004; Gupta, *et al.,* 2005). Recent chromatographic studies have reported phenolic content values up to 8.5 mg GAE/g and flavonoid concentrations near 0.9 mg QE/g, confirming *A. occidentale* as a rich source of phenolic antioxidants (Sassi, *et al.,* 2023). *Phyllanthus amarus* Schum. & Thonn., a member of the family Phyllanthaceae, is an annual herb widely distributed in tropical and subtropical regions, including Nigeria, India, and the Philippines. Known locally as "ngwu" or "eyin Olobe," this species has been used for centuries to treat a wide range of ailments such as liver disorders, diabetes, hypertension, viral infections, and gastrointestinal diseases (Adeneye, 2006). Its therapeutic potential is attributed to a spectrum of secondary metabolites, notably phenolics, alkaloids, flavonoids, and terpenoids (Kumar, *et al.,* 2015). The ethanolic extracts of *P. amarus* have demonstrated significant free radical scavenging activities in DPPH and ABTS assays and inhibit α-glucosidase and α-amylase enzymes relevant to diabetes management (Hashim, *et al.,* 2013; Sulaiman & Ooi, 2014).

Comparative evaluation of the antioxidant and phenolic profiles of *C. odorata*, *A. occidentale*, and *P. amarus* is of scientific importance, particularly within the ecological context of Calabar, Cross River State, Nigeria, where environmental and climatic factors influence phytochemical accumulation. Variations in soil nutrients, sunlight exposure, and harvest season are known to affect the biosynthesis of phenolic compounds and flavonoids (Do, *et al.,* 2020; Nguyen, *et al.,* 2021). This study aims to elucidate the phenolic composition and antioxidant capacities of these three species. The findings will enhance understanding of their therapeutic potential, validate traditional medicinal uses, and contribute to the development of natural antioxidant agents for health applications. Moreover, establishing comparative data from plants harvested under uniform environmental conditions in Calabar provides a valuable baseline for regional pharmacognostic research and sustainable bioprospecting initiatives.

2. METHODS

2.1. Plant Collection and Identification

Fresh leaves of *Chromolaena odorata*, *Anacardium occidentale*, and *Phyllanthus amarus* were collected from the premises of the University of Calabar, Cross River State, Nigeria. The plant specimens were taxonomically identified and authenticated by experts at the Herbarium Unit, Department of Botany, University of Calabar. Voucher specimens were prepared and deposited in the herbarium for future reference. The collected leaves were rinsed thoroughly with distilled water to remove surface contaminants, then air-dried at ambient temperature until a constant weight was achieved. The dried materials were pulverized into fine powders using a mechanical grinder and stored in airtight containers until required for extraction.

2.2 Preparation of Plant Extracts

A hundred grams (100 g) of each powdered plant sample was separately macerated in 2000 mL of 90% ethanol. The mixtures were left to stand for 48 hours with intermittent agitation to facilitate efficient extraction of bioactive constituents. The extracts were first filtered through muslin cloth, followed by filtration with Whatman No. 1 filter paper. The resulting filtrates were concentrated using a rotary evaporator under reduced pressure at 40°C to yield semi-solid crude extracts. These extracts were transferred into labeled vials and preserved at 4°C until further analyses. Extraction yield (%) was determined as the ratio of the weight of dried extract to the initial weight of the plant material.

2.3 Determination of Total Phenolic Content (TPC)

The total phenolic content of each extract was quantified using the Folin–Ciocalteu colorimetric method (Wolfe, *et al.,* 2005) with minor modifications. Briefly, 1 mL of extract (1 mg/mL) was mixed with 5 mL of 10% Folin–Ciocalteu reagent and 4 mL of 7.5% sodium carbonate solution. The reaction mixture was incubated at room temperature in the dark for 30 minutes, after which absorbance was recorded at 765 nm using a UV–visible spectrophotometer. Gallic acid served as the calibration standard, and results were expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g).

2.4 DPPH Radical Scavenging Assay

The antioxidant activity of the extracts was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method, as described by Desmarchelier, *et al.* (2000). One milliliter (1 mL) of each extract at various concentrations (25–200 µg/mL) was added to 1 mL of 0.1 mM DPPH solution prepared in methanol. The mixtures were vigorously shaken and allowed to stand in the dark for 30 minutes. Absorbance was measured at 517 nm against a methanol blank. Ascorbic acid was used as the reference antioxidant.

2.5 Ferric Reducing Antioxidant Power (FRAP) Assay

The ferric ion reducing potential of the extracts was determined using the FRAP method (Benzie & Strain, 2000). The FRAP reagent was freshly prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) solution, and 20 mM $FeCl_3 \cdot 6H_2O$ in a 10:1:1 ratio. A 0.1 mL aliquot of extract was added to 3 mL of the FRAP reagent and incubated at 37°C for 30 minutes. Absorbance was measured at 593 nm, and results were expressed as micromoles of Fe^{2+} equivalents per gram of extract using a standard ferrous sulfate calibration curve.

2.6 Hydrogen Peroxide (H_2O_2) Scavenging Assay

Hydrogen peroxide scavenging capacity was determined by using 40 mM hydrogen peroxide solution was prepared in phosphate buffer (pH 7.4). One milliliter (1 mL) of each extract was mixed with 2 mL of the hydrogen peroxide solution and incubated for 10 minutes. Absorbance was read at 230 nm against a blank solution containing phosphate buffer without hydrogen peroxide. Ascorbic acid served as the standard control (Ruth, *et al.,* 2000).

2.7 Catalase (CAT) Activity Assay

Catalase activity was measured by reaction mixture consisted of 1 mL phosphate buffer (pH 7.0), 0.5 mL hydrogen peroxide (0.2 M), and 0.1 mL of plant extract. The reaction was initiated by adding hydrogen peroxide, and the decrease in absorbance was recorded at 240 nm over 3 minutes. Catalase activity was expressed as micromoles of hydrogen peroxide decomposed per minute per milligram of extract (Ruth, *et al.,* 2000).

2.8 Data Analysis

All experiments were conducted in triplicate, and results were expressed as mean ± standard deviation (SD). Statistical significance among groups was assessed using one-way analysis of variance (ANOVA) and differences were considered significant at $p < 0.05$.

3. RESULTS AND DISCUSSION

Among the three species, *A. occidentale* showed the highest DPPH radical scavenging activity (30.93 ± 0.32%), indicating strong free radical neutralization. However, *P. amarus* demonstrated the highest ferric reducing antioxidant power (0.41 ± 0.01 µmol Fe^{2+}/g), hydrogen peroxide scavenging (156.60 ± 1.42 µmol/mL), catalase activity (4.77 ± 0.12 µmol H_2O_2/min/mg), and total phenolic content (2.97 ± 0.50 mg GAE/g extract), signifying a superior overall antioxidant capacity. *C. odorata* exhibited moderate antioxidant values across all parameters. The result of antioxidant activities and total phenolic content of ethanolic extracts of *Chromolema odorata, Anacardium occidentale*, and *Phyllanthus amarus* can be seen in **Table 1.**

The comparative evaluation of the antioxidant potentials and phenolic composition of *Chromolaena odorata*, *Anacardium occidentale*, and *Phyllanthus amarus* harvested in Calabar reveals significant interspecies variations in their biochemical profiles and antioxidant responses. The present study recorded higher DPPH radical scavenging activity and ferric reducing antioxidant power (FRAP) in *Phyllanthus amarus*, followed by *Chromolaena odorata* and *Anacardium occidentale*, respectively. The superior antioxidant activity of *P. amarus* (FRAP = 0.41 ± 0.01 µmol Fe^{2+}/g; catalase = 4.77 ± 0.12 µmol H_2O_2/min/mg extract) corresponds to its highest total phenolic content (2.97 ± 0.50 mg GAE/g extract), corroborating the established relationship between phenolic compounds and antioxidant capacity. These findings align with previous reports, which demonstrated that phenolic constituents of *P. amarus*—including gallic acid, ellagic acid, corilagin, and phyllanthusiin D—exhibit potent antioxidant activities both in vitro and in vivo (Londhe, *et al.,* 2008; Karuna, *et al.,* 2009; Maity, *et al.,* 2013). The remarkable hydrogen peroxide scavenging activity (156.6 ± 1.42 µmol/mL) further supports its role in mitigating oxidative stress, consistent with earlier evidence of the plant's hepatoprotective and anti-inflammatory potentials (Harish & Shivanandappa, 2006; Guha, *et al.,* 2010). The high phenolic and flavonoid contents of *P. amarus* identified through HPLC and GC-MS analyses (Kumar, *et al.,* 2015; Hajgude & Patil, 2025) reaffirm its dominance among the studied species, underscoring its pharmacological versatility.

Table 1. antioxidant activities and total phenolic content of ethanolic extracts of *Chromolema odorata, Anacardium occidentale*, and *Phyllanthus amarus*

Parameter	***C. odorata***	***A. occidentale***	***P. amarus***
DPPH radical scavenging activity (%)	18.10 ± 0.72	30.93 ± 0.32	9.48 ± 0.19
Ferric reducing antioxidant power (µmol Fe^{2+}/g)	0.28 ± 0.02	0.14 ± 0.002	0.41 ± 0.01
Hydrogen peroxide scavenging (µmol/mL)	116.07 ± 5.06	90.90 ± 1.37	156.60 ± 1.42
Catalase activity (µmol H_2O_2/min/mg extract)	4.30 ± 0.10	3.30 ± 0.20	4.77 ± 0.12
Total phenolic content (mg GAE/g extract)	0.86 ± 0.01	2.53 ± 0.56	2.97 ± 0.50

** Values are expressed as mean ± standard deviation (n = 3)*

Anacardium occidentale displayed moderate antioxidant activity (DPPH = 30.93 ± 0.32%; FRAP = 0.14 ± 0.002 µmol Fe^{2+}/g extract), supported by its total phenolic content (2.53 ± 0.56 mg GAE/g) and catalase activity (3.30 ± 0.20 µmol H_2O_2/min/mg). These results correspond with previous findings by Ajileye et al. (2015) and Pham et al. (2023), who demonstrated that ethyl acetate fractions of *A. occidentale* leaves possessed significant antioxidant and antimicrobial activities, largely attributed to quercetin glycosides, Agathisflavone, and tocopherol derivatives. The identification of γ-terpinene, 1,2,3-benzenetriol, and dl-α-tocopherol (Amarachukwu, *et al.,* 2024) further supports its redox capacity and potential anti-inflammatory effects consistent with previous research (Simic, 2000; Ahn, *et al.,* 2004). The antioxidant mechanism of *A. occidentale* likely involves hydrogen-donating and metal-chelating activities mediated by these phenolic and flavonoid structures. The moderate activity observed compared with *P. amarus* could be due to differences in phenolic concentration or structural diversity of the compounds. The presence of polyphenolic compounds with antioxidative roles complements its known pharmacological effects on metabolic disorders, inflammation, and microbial infections (Akinmoladun, *et al.,* 2010). Together, these findings validate *A. occidentale* as a potent natural antioxidant source, albeit less effective than *P. amarus* under identical extraction and testing conditions.

Conversely, *Chromolaena odorata* showed moderate antioxidant activity (DPPH = 18.10 ± 0.72%; FRAP = 0.28 ± 0.02 µmol Fe^{2+}/g extract) relative to the other species, although its phenolic content (0.86 ± 0.01 mg GAE/g) was lower. Nonetheless, its catalase activity (4.30 ± 0.10 µmol H_2O_2/min/mg) was comparable to *P. amarus*, suggesting an efficient enzymatic defense system. This finding corresponds with Magar, et al. (2023), who confirmed the strong antioxidant potential of *C. odorata* extracts, particularly from leaves rich in flavonoids and phenolic acids. Similarly, other research demonstrated immunomodulatory and antioxidant properties of *C. odorata* polysaccharides, complementing the free radical scavenging activities observed in this study (Boudjeko, *et al.,* 2015). The observed moderate activity compared to *P. amarus* can be attributed to compositional differences and environmental variations during growth, as suggested by recent researches, who reported phytochemical variability between geothermal and non-geothermal populations of *C. odorata* (Abubakar, *et al.,* 2023). The presence of flavanones and phenolic acids like 3,5-dihydroxy-7-methoxyflavanone (Putri & Fatmawati, 2019) explains its capacity to neutralize reactive oxygen species and inhibit oxidative degradation of cellular components. Thus, the current results reaffirm the importance of *C. odorata* as a moderate yet consistent antioxidant source among the three studied species.

Overall, the comparative analysis reveals that while all three species possess measurable antioxidant activity, *Phyllanthus amarus* exhibited the most potent effect across all assays, reflecting its high phenolic and flavonoid content, followed by *Anacardium occidentale* and *Chromolaena odorata*. The data demonstrate a positive correlation between phenolic content and antioxidant strength, reinforcing previous reports on the biochemical importance of phenolic compounds (Karuna, *et al.,* 2009). The consistency between the present findings and prior literature validates the role of polyphenols as primary determinants of antioxidant capacity in medicinal plants. The variation observed among species likely arises from genetic, environmental, and solvent-extraction differences influencing phenolic biosynthesis and stability (Nguyen, *et al.,* 2017; Pham, *et al.,* 2023). Importantly, the relatively high catalase and hydrogen peroxide scavenging activities across all extracts suggest that these plants, especially *P. amarus*, can mitigate oxidative stress by enzymatic and non-enzymatic mechanisms. The findings therefore establish that the studied plants represent valuable sources of natural antioxidants suitable for pharmaceutical, nutraceutical, and food applications.

4. CONCLUSION

The comparative analysis demonstrated that *Phyllanthus amarus* possessed the highest antioxidant potential and phenolic composition, followed by *Anacardium occidentale* and *Chromolaena odorata*. These findings confirm *P. amarus* as a superior natural antioxidant source capable of mitigating oxidative stress through enzymatic and non-enzymatic mechanisms. The study highlights the therapeutic and nutraceutical relevance of these species and supports further bioassay-guided isolation and characterization of their active phenolic constituents for potential pharmacological applications.

5. AUTHORS' NOTE

The authors declare that there is no conflict of interest regarding the publication of this article. Authors confirmed that the paper was free of plagiarism.

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