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Phytochemical Investigation And *In Vitro* Antioxidant Activity of Leaf Extracts Of *Blumea Oxyodonta* DC

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Abstract
The current study was conducted to explore the phytochemical composition and in vitro antioxidant activity of *Blumea oxyodonta* leaves. Preliminary phytochemical analysis of methanol and ethanol extracts from the leaves showed the presence of alkaloids, catechins, coumarins, flavonoids, phenols, glycosides, saponins, steroids, terpenoids, sugars, and xanthoproteins. Extracts from petroleum ether, benzene, ethyl acetate, methanol, and ethanol demonstrated the ability to scavenge DPPH, hydroxyl, superoxide, and ABTS radical cations, as well as exhibit reducing activity in a concentration-dependent manner. The methanol extract exhibited the highest DPPH and hydroxyl scavenging activities, while the ethanol extract displayed greater superoxide and ABTS radical cation scavenging activity. The methanol extract also showed the highest reducing power. Overall, the study highlights that *Blumea oxyodonta* leaves possess significant antioxidant and free radical scavenging properties, supporting the plant's traditional use for treating various ailments.

Keywords: Blumea oxyodonta, phytochemical, antioxidant, reducing power.

Introduction

Antioxidants are substances that significantly delay or inhibit oxidation of an oxidizable substrate when present at low concentrations in comparison with those of the substrate. The activities of free radicals have been implicated in aging, destruction of DNA, obstruction of arteries, cancer, strokes, cardiac and central nervous system (CNS) disorders which have led to an increase in the investigation of substances that can protect against these reactive oxygen species and thus may play a role in disease prevention (Kapadiya *et al*., 2016; Adebiyi *et al*., 2017).

Free oxygen and nitrogen species are unstable molecules that are present in the environment (exogenous) and are also generated in the body (endogenous) during the normal aerobic metabolic processes in the body (Bhat *et al*., 2015). The growing need to complement these endogenous antioxidants has led to an increased supplementation by exogenous sources. At present, there are keen interests and widespread researches on exogenous antioxidants from natural sources perhaps, due to the fact that they are less expensive, readily available and believed to have lesser side effects when compared to their synthetic counter parts (Adebiyi *et al*., 2017).

Considering the available alternative and complementary strategies, medicinal plants stand a better chance of providing potent, safer, affordable and easily accessible therapies for oxidative stress – related maladies (Goyal and Suleria 2019). Medicinal plants contain various secondary metabolites, which have demonstrated a wide spectrum of pharmacological activities. Antioxidants properties of plants have been demonstrated to play a protective role in the body against diseases, since their consumption lowers the risk of cancer, heart disease, hypertension, dementia and stroke (Ojiewo *et al*., 2013). Keeping all these into account, the present study was undertaken to evaluate preliminary phytochemical screening and antioxidant activity of *Blumea oxyodonta* DC leaves. In the traditional system of medicine the decoction of the roots is used for treatments of impotency and spermatorrhea (Quattrocchi 2012).

Materials and Methods

Collection of leaf sample

Fresh leaves of *Blumea oxyodonta* DC (BOL) were collected from Karumathra village, near Thrissur, Kerala. The plant specimens were taxonomically identified with the assistance of local floras, and the botanical identity was verified and authenticated by the Botanical Survey of India, Coimbatore, Southern Circle, Tamil Nadu, India. The collected leaves were then chopped into small pieces and shade-dried. Once dried, the samples were ground into a fine powder using a blender and sieved to ensure a uniform consistency.

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Preliminary phytochemical screening
Qualitative tests were performed to identify various chemical components in the solvent extracts (petroleum ether, benzene, ethyl acetate, methanol, ethanol, and aqueous) of *B. oxyodonta* leaves, following the methods outlined by Brinda *et al.* (1981). The extracts were screened for the presence of alkaloids, anthraquinones, catechins, coumarins, flavonoids, phenols, quinones, saponins, steroids, tannins, terpenoids, glycosides, sugars, xanthoproteins, and fixed oils. The ethanol extract was then selected for the in vitro antioxidant activity study.

Antioxidant activity
Petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of *Blumea oxyodonta* were used to determine the *in vitro* antioxidant activity.

DPPH radical scavenging assay
The 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging ability of different solvent extracts of *B. oxyodonta* leaf was determined by the method of Shen *et al*. (2010). DPPH solution of 0.1 mM was prepared using methanol. 1 mL of this prepared solution was added to 3 mL of different concentration (50, 100, 200, 400 and 800 μg/mL) of extracts. Then it was shaken vigorously and allowed to stand. After 30 min, absorbance was measured at 517 nm using a UV-VIS spectrophotometer (Genesys 10S UV: Thermo electron corporation) using Ascorbic acid as standard. Solution with lower absorbance values indicates more free radical scavenging activity. It was calculated by using the following formula.
DPPH scavenging effect (% inhibition) = $\{(A_0 – A_1) /A_0) * 100]$
where, A_0 is the absorbance of the control reaction, and A_1 is the absorbance in presence of all of the extract samples and reference.

Hydroxyl radical scavenging assay
The Hydroxyl radical scavenging assay was measured using the modified method of Halliwell *et al*. (1987). Various stock solutions used in this method are EDTA (1 mM), $FeCl_3$ (10 mM), Ascorbic Acid (1 mM), H_2O_2 (10 mM) and Deoxyribose (10 mM). All the solutions were prepared in distilled deionized water. Hydroxyl radical scavenging activity was carried out by adding 0.1 mL EDTA, 0.01 mL of $FeCl_3$, 0.1 mL H_2O_2, 0.36 ml of deoxyribose, 1.0 mL of the extract of different concentration, 0.33 ml of phosphate buffer (50 mM, pH 7.9), 1.0 ml of ascorbic acid in sequence. This mixture was kept at 37 °C for 1 h. After incubation period, 1.0 mL from the mixture was added to 1.0 mL of 10% TCA and 1.0 mL of 0.5% TBA (in 0.025 M NaOH containing 0.025% BHA) to produce the pink chromogen. It was measured at 532 nm. The percentage inhibition was calculated by comparing the results of the test with those of the control using the above formula.

Superoxide radical scavenging assay
Superoxide anion scavenging activity was measured by the method of Srinivasan *et al*. (2007). The superoxide anion radicals were generated in 3.0 mL of Tris-HCL buffer (16 mM, pH 8.0), containing 0.5 mL of NBT (0.3 mM), 0.5 ml NADH (0.936 mM) solution, 1.0 mL extract of different concentration (50, 100, 200, 400 and 800 μg/mL), and 0.5 mL Tris-HCl buffer (16 mM, pH 8.0). An addition of 0.5 mL PMS solution (0.12 mM) was carried to start the reaction. This mixture is incubated at 25°C for 5 min and the absorbance was measured at 560 nm against a blank sample, ascorbic acid. The percentage inhibition was calculated by comparing the results of the test with those of the control using the above formula.

Antioxidant activity by radical cation (ABTS+)
It was carried out by using slightly modified method of Huang *et al*. (2011). In this method, 7 mM ABTS solution and 2.45 mM potassium per sulphate were used to produce ABTS radical cation (ABTS+). This reaction mixture is kept in dark for 12-16 h at room temperature. Then this solution was diluted with ethanol to get an absorbance value of 0.70 + 0.02 at 734 nm. Then 3.9 ml of diluted ABTS+ solution is added with sample extract and is used for measuring absorbance at 734 nm by Genesys 10S UV-VIS (Thermo scientific) exactly after 6 minutes. Trolox used a standard. Resultant data were expressed as trolox equivalent antioxidant capacity (TEAC).

Reducing power
Reducing power was determined by the method of Kumar and Hemalatha (2011). In this sodium phosphate buffer (5.0 mL, 0.2 M, pH 6.6) and potassium ferricyanide (5.0 ml, 1.0%) is mixed with 1.0 mL of the five studied concentration of extract respectively. This mixture was incubated at 50 °C. After 20 min, 5 ml of 10% trichloroacetic acid was added and centrifuged at 5000 rpm (10 min at 5°C) in a refrigerator centrifuge. After centrifugation, the upper layer (5.0 mL) was diluted with 5.0 mL of distilled water and ferric chloride and absorbance read at 700 nm.

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Statistical analysis
All the experiments were estimated in triplicate determinations. The statistical analysis system SPSS was used to analyze the data.

Results and Discussion
Preliminary Phytochemical analysis
The qualitative distribution of phytochemical constituents in the petroleum ether, benzene, ethyl acetate, methanol, ethanol, and aqueous extracts of *Blumea oxyodonta* leaves (BOL) was evaluated, with the results provided in Table 1. Alkaloids, catechins, coumarins, flavonoids, glycosides, phenols, saponins, steroids, tannins, terpenoids, sugars, and xanthoproteins were found in the ethyl acetate, methanol, and ethanol extracts of *B. oxyodonta* leaves. This initial phytochemical analysis helps identify the chemical classes present in the extracts, contributing to their quantitative assessment and aiding in the identification of potential sources of pharmacologically active compounds. The presence of these phytochemicals in *B. oxyodonta* leaves suggests the plant's potential to address various ailments, as they may have notable therapeutic properties.

Table 1: Preliminary phytochemical screening of different solvent extracts of *B. oxyodonta* leaf

	Nature of extract					
Bioactive components	**Petroleum ether**	**Benzene**	**Ethyl acetate**	**Methanol**	**Ethanol**	**Aqueous**
Alkaloids	-	-	+	+	+	-
Anthraquinones	-	+	-	-	-	-
Catechins	+	+	+	+	+	-
Coumarins	-	-	+	+	+	-
Flavonoids	-	+	+	+	+	+
Glycosides	-	+	+	+	+	-
Phenols	+	+	+	+	+	+
Quinones	-	-	-	-	+	-
Saponins	-	-	+	+	+	+
Steroids	+	+	+	+	+	-
Tannins	-	+	+	+	+	+
Terpenoids	+	+	+	+	+	-
Sugars	+	+	+	+	+	+
Xanthoproteins	+	-	-	-	-	-
Fixed oil	+	-	+	+	+	+

+ Present, - absent

Antioxidant activity
Figure 1 shows the DPPH radical scavenging activity of the diverse solvent extracts of *B. oxyodonta* leaves and standard ascorbic acid. Among the solvent tested, methanol extract of *B. oxyodonta* leaves exhibited the highest DPPH radical scavenging activity. At a concentration of 800 µg/mL, the DPPH radical scavenging activity of methanol extract of *B. oxyodonta* leaves was 116.14% whereas at the same concentration, the ascorbic acid showed 110.36% DPPH radical scavenging activity. IC_{50} value of methanol extract of *B. oxyodonta* leaves and ascorbic acid showed 92.52 µg/mL and 76.32 µg/mL respectively. Previous report on ethanol extracts of *Grewia carpinifolia* leaf and stem exhibited 72.60% and 62.12% DPPH radical scavenging activity at the concentration of 1000 µg/mL respectively (Adebiyi *et al*., 2017).

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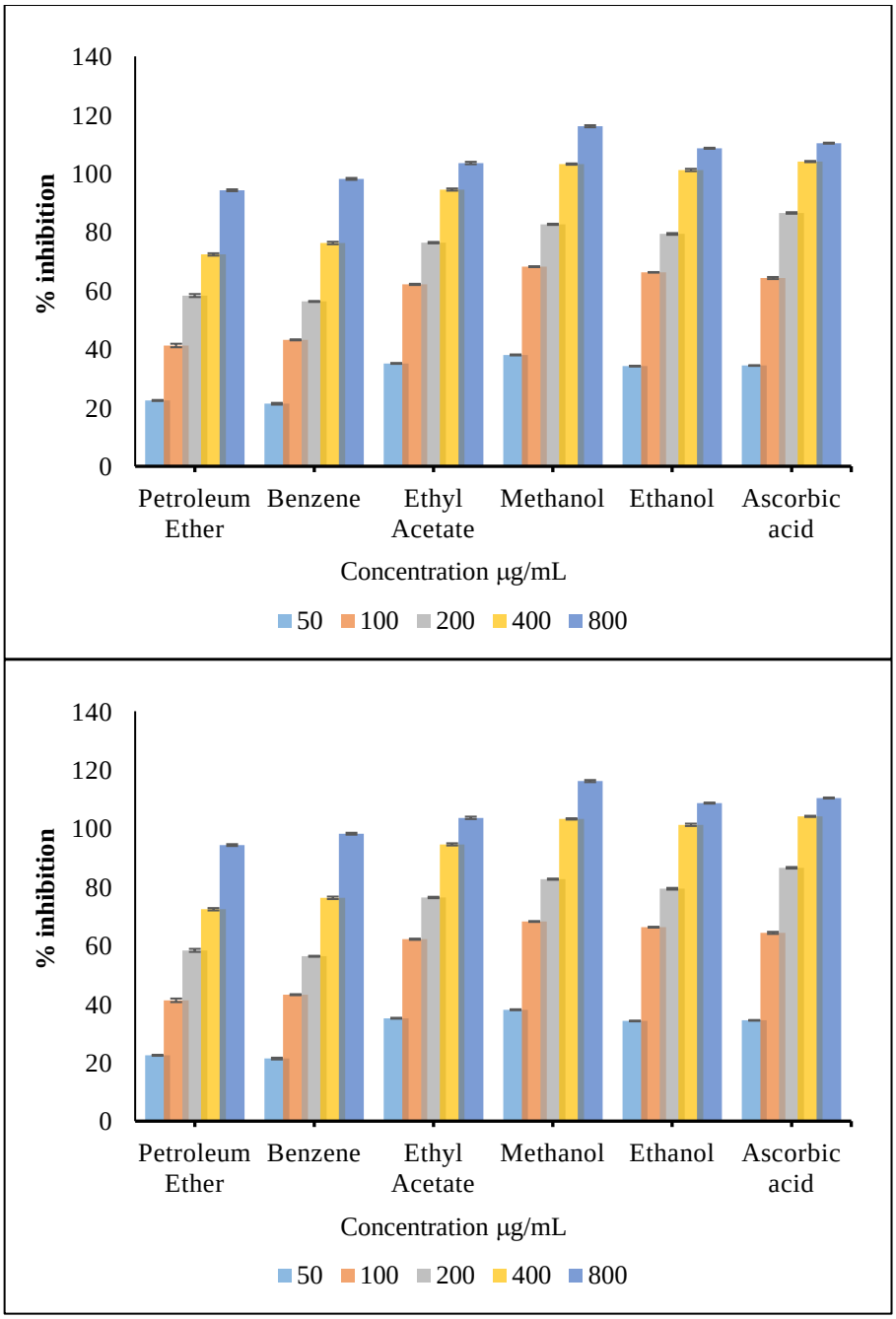

Figure 1: DPPH radical scavenging activity

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity of the different solvent extracts of *B. oxyodonta* leaves was dose dependent (Fig. 2). Among the solvent used, methanol extract of *B. oxyodonta* leaves had higher hydroxyl radical scavenging activity. At a concentration of 800 µg/mL, the hydroxyl radical scavenging activity of *B. oxyodonta* leaves exhibited 119.21% whereas at the same concentration, standard ascorbic acid was 118.30% hydroxyl radical scavenging activity. The IC_{50} value of methanol extract of *B. oxyodonta* leaves and ascorbic acid showed 91.24 µg/mL and 84.56 µg/mL respectively. Similar hydroxyl radical scavenging activity was reported by Guchu *et al*. (2020) in *Caesalpinia volkensis*, *Vernonia lasiopus* and *Acacia hockii*.

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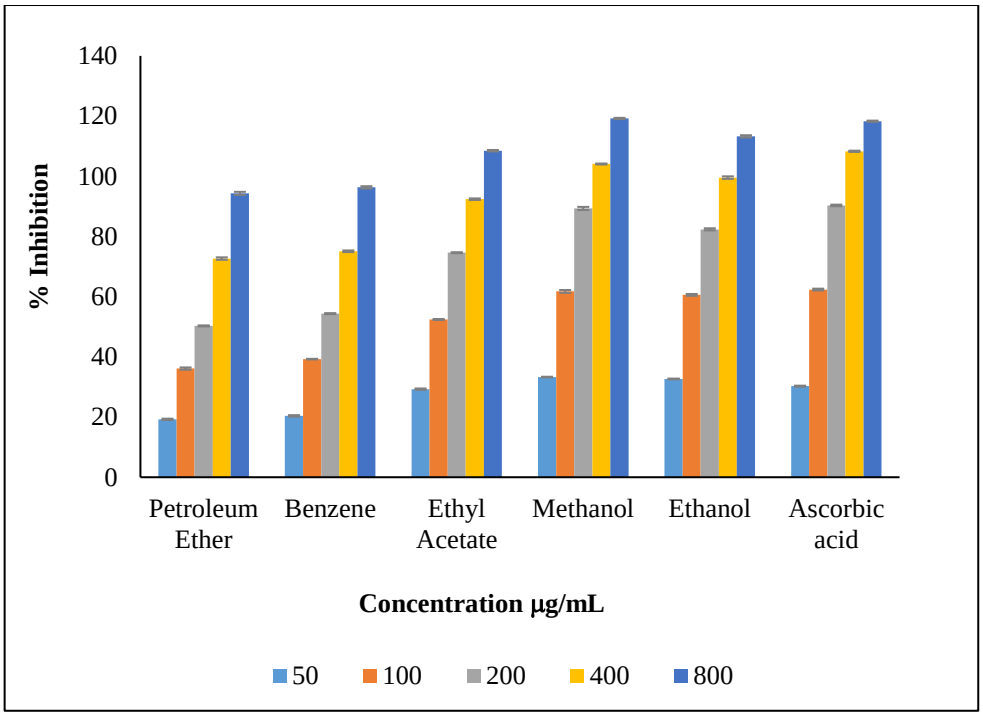

Superoxide radical scavenging activity

Figure 3 shows the superoxide radical scavenging activity of different solvent extracts of *B. oxyodonta* leaves and standard ascorbic acid. The ethanol extract of *B. oxyodonta* leaves showed the maximum superoxide radical scavenging activity. At a concentration of 800 µg/mL, ethanol extract of *B. oxyodonta* leaves possessed 118.21% superoxide radical scavenging activity. IC_{50} value of ethanol extract of *B. oxyodonta* leaves showed 96.28 µg/mL. The percentage inhibition and IC_{50} value of standard ascorbic acid were 199.20% and 76.24 µg/mL respectively. This result aligns with that of *Suaeda monoica* (Sivagama Sundari *et al*., 2022) and *Enhalus acoroides* (Santhiya Selvam *et al*., 2022).

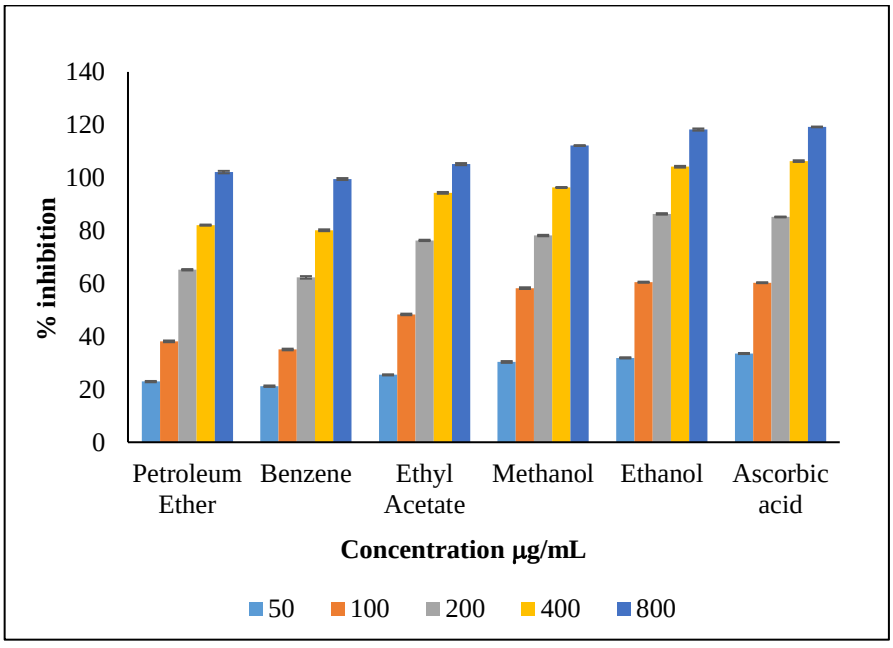

Figure 3: Superoxide radical scavenging activity

ABTS radical cation scavenging activity

Diverse extracts of *B. oxyodonta* leaves showed a concentration dependent rise in the scavenging of the ABTS radical (Fig. 4). Among the solvent tested, ethanol extract of *B. oxyodonta* leaves showed highest ABTS radical action scavenging activity. At a concentration of 800 µg/mL, the ABTS radical cation scavenging activity of ethanol extract of *B. oxyodonta*

leaves exhibited 118.10%, whereas at the same concentration, the standard trolox was 120.52% ABTS radical cation scavenging activity. IC_{50} value of ethanol extract of *B. oxyodonta* leaves and standard trolox showed 91.56 µg/mL and 82.66 µg/mL respectively. Recent study on methanol extract of *Vernonia amygdalina* leaf showed 61.98% ABTS radical cation scavenging activity at the concentration of 300 µg/mL (Hussen and Endalew 2023).

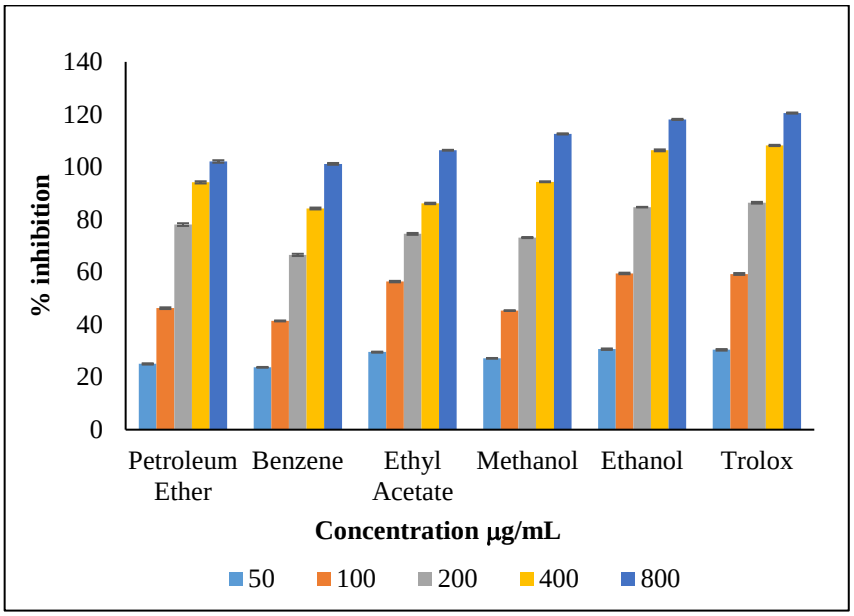

Figure 4: ABTS radical cation scavenging activity

Reducing power

Figure 5 shows the reducing activity of different solvent extracts of *B. oxyodonta* leaves. Absorbance of the solution was increased with the increasing concentration. A higher absorbance indicated a higher reducing power. Among the solvent tested, the methanol extract of *B. oxyodonta* leaves showed higher reducing power.

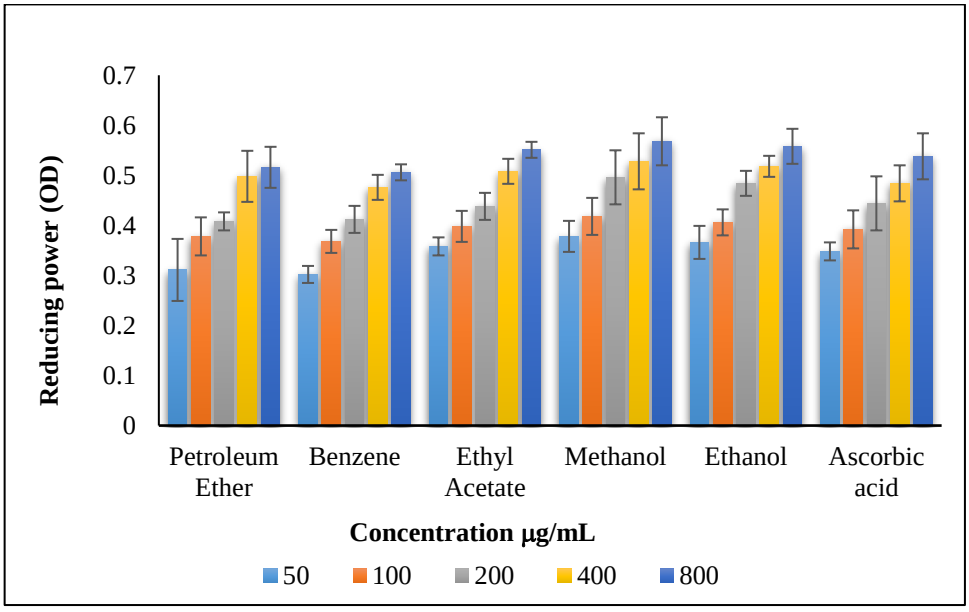

Figure 5: Reducing Power

From the obtained results, it was concluded that the methanol and ethanol extracts of *B. oxyodonta* leaves have appreciable antioxidant capacity and antioxidant associated phytochemicals. Further studies that aimed at isolating and characterizing the pure phytoactive principles for enhancement are recommended.

Conflicts of Interest
All authors declare that there is no conflicts of interest.

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