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IN VITRO AMELIORATIVE POTENTIAL OF SOME ORGANIC ACID IDENTIFIED FROM *BOSWELLIA DALZIELII* STEM BARK EXTRACT AGAINST FREE RADICALS

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ABSTRACT

Due to their safety and availability, medicinal plants are natural alternatives to orthodox medicines in health management, particularly in developing countries. One such plant is *Boswellia dalzielii*, a renowned tree in northern Nigeria that is extensively used due to its ethnomedicinal history. This study aimed to evaluate the antioxidant of some organic acids identified in the stem bark extract of *B. dalzielii* by Liquid Chromatography Mass Spectrometer (LCMS). The stem bark was extracted with ethanol using the cold maceration method. Antioxidant assay using 2,2-Diphenyl 1-Picryl-Hydrazyl-hydrate (DPPH), Ferric Reducing Antioxidant Property (FRAP), and Hydrogen Peroxide (H_2O_2) methods were used, and the results showed the highest antioxidant activity with higher concentrations of the extract; 49.580% ± 0.214, 3.540 ± 0.022 and 45.145% ± 0.234 for DPPH, FRAP and H_2O_2 respectively. And significantly lower than the standard (Ascorbic acid) used, 95.01 ± 0.001, 3.540 ± 0.120, and 91.390%± 0.022 respectively. the LCMS profile revealed the presence of many organic acids and other metabolites; Malonamic acid, D-pyroglutamic acid, Maleamic acid, Benzohydroxamic acid, 2-bromo-1,10-phenanthroline, *p*-tropoquinone (Quinone derivatives) among others. Therefore, the stem bark of *B. dalzielii* extract possesses antioxidant potential and could be due to its organic acid presence, it can be concluded that the stem bark of this plant may perhaps be useful in health management.

Keywords: Antioxidant, Metabolite, Profile, Stem bark

INTRODUCTION

The majority of the world's population still uses plants to treat a variety of illnesses, a practice that dates back to the earliest human civilizations (Abubakar *et al*., 2024). Natural plants continue to be an alternative to conventional medications in the treatment of medical conditions because of the phytoconstituents found in medicinal plants. Medicinal plants are a valuable resource in drug development procedures because they include a variety of beneficial, physiologically active compounds (Wachtel-Galor and Benzie 2011). Due to these constituent, various plant have been investigated for potential medicinal benefits (Taylor *et al*., 2001; Abdulrahman *et al*., 2018).

The *Boswellia* genus, which includes trees in the Sapindales order, includes *Boswellia dalzielii*. *Boswellia* species are blooming plants of moderate size that grow into trees and shrubs. They are primarily found in tropical parts of Asia and Africa. *B. dalzielii* has fragrant white flowers and is more than 13 meters tall. It has been used for many ailment management in African traditional preparation (Owolabi *et al*., 2020; Dogara *et al.*, 2022).

Oxidative stress is triggered by a variety of cell metabolic processes that produce Reactive Oxygen Species (ROS), also referred to as free radicals, including hydrogen peroxide, hydroxyl radicals, and superoxide anion (Muhammad *et al*., 2022). Numerous plant phytochemicals are known to play a significant role in the treatment of human illness and are naturally occurring antioxidants (Muhammad *et al*., 2022). The *in vitro* potential of the metabolites found in the stem bark extract of *Boswellia dalzielii* was evaluated in this investigation.

MATERIALS AND METHODS

Sample collection and Identification

The Plant sample *Boswellia dalzielii* (stem bark) was collected from Gombe town with the help of practitioners and authenticated at the Herbarium unit, Department of Botany, Gombe State University, Gombe with the voucher number 900121.

Extraction of the Plant Materials

The stem bark was extracted according to Sofowora (2008) report. The sample (stem bark) was air-dried and mechanically ground to produce a fine powder. The weighed sample (200g) was extracted with ethanol exhaustively using the cold maceration technique for 48 hours. Then, filtered and evaporated to dryness using a vacuum rotary evaporator.

Invitro antioxidant activity DPPH (2,2-Diphenyl 1-Picryl-Hydrazyl-hydrate) Assay

The DDPH assay is a widely used method for assessing antioxidants' ability to scavenge free radicals. This test involves mixing the plant extract with a stable free radical (DPPH) and measuring the amount of color change. Higher antioxidant activity is indicated by more discoloration. After transferring 500 microliters (500 µl) of the plant extract and standard (ascorbic acid) serial concentrations into separate Eppendorf tubes, each of the mixtures received 500 µl of DPPH solution (0.06 mM). The mixtures were then shaken and allowed to sit at room temperature for 30 minutes. Using DPPH solution as a blank, the absorbance was measured at 520 nm.

$$\% \text{ inhibition} = \frac{(Blank\ DDPH\ solution\ absorbance - actual\ absornace)}{Blank\ DPPH\ solution\ absorbance} X\ 100\ \%$$

***Invitro* antioxidant activity FRAP (Ferric Reducing Antioxidant Power) Assay**

The FRAP assay is based on the rapid reduction of ferric-tripyridyltriazine in samples by antioxidants resulting in ferrous-tripyridyltriazine (a blue-colored product). By adding the FRAP reagent to a range of known concentrations of Fe^{2+} solutions, a standard curve was produced allowing the Fe concentration of the sample to be determined and thereby indicating antioxidant capacity. The plant extract was serially prepared into different test tubes (Concentrations). 2,5 ml of phosphate buffer (pH) was added to all the tubes and thoroughly mixed, followed by the addition of 2.5 ml of 1% potassium ferricyanite to each tube, the reaction mixtures were vortex for 5 seconds and incubated at 50ºC for 20 minutes. After incubation 2.5 ml of 10% trichloroacetic acid was added and centrifuged at 3000 rpm for 10 minutes. The supernatant was collected and measured at 700 nm.

***Invitro* antioxidant activity H_2O_2 (Hydrogen Peroxide) free Radical scavenging activity assay**

The H_2O_2 assay assesses a plant extract's capacity to scavenge hydrogen peroxide, a dangerous reactive oxygen species. Cells and tissues may sustain oxidative damage as a result of hydrogen peroxide. The assay gauges the plant extract's antioxidant potential by measuring the drop in hydrogen peroxide concentration following exposure. In separate test tubes, 100 microliters (100 µl) of the serially prepared plant extract (Concentration) and the standard (ascorbic acid) were put. 600 µl of hydrogen peroxide (H_2O_2) was added to each tube, and the final volume was adjusted to 4 ml using phosphate buffer (pH). The tubes were then allowed to sit at room temperature for 10 minutes. At 300 nm, the absorbance was measured.

Scavenging activity % =

$$\frac{(Absorbance\ of\ control - Absorbance\ of\ sample)}{Absorbance\ of\ control}\ X\ 100$$

***Invitro* antioxidant activity DPPH (2,2-Diphenyl 1-Picryl-Hydrazyl-hydrate) Assay**

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% inhibition= =

$$\frac{(Blank\ DDPH\ solution\ absorbance - actual\ absornace)}{Blank\ DPPH\ solution\ absorbance}\ X\ 100\ \%$$

LCMS Analysis of the stem bark extract of *B. dalzielii*

The Sample extract was reconstituted in methanol and filtered using a membrane filter (0.45 µm). The filtered samples were injected into LC system (Water e2695), and separations were done in C18 column (Sunfire C18 5.0µm 4.6mm x 150 mm). The analysis was conducted at a flow rate of 1.0 ml /min, sample, and column temperature at 25ºC. Solvent A (0.1% formic acid in water) and B (0.1% formic acid in Acetonitrile) were used as mobile phase with the following gradient: A ratio of A/B 95:5 was used and maintained for 1 min, then followed by A/B 5:95 for 13 min, to 15 min, A/B 95:5 to 17min, 19min and finally 20min. the PDA detector was set at 210-400nm with a resolution of 1.2nm and a sampling rate at 10 points/sec. The mass spectra were acquired with a scan range from m/z 100 – 1250 after ensuring the following settings: ESI source in positive and negative ion modes; capillary voltage 0.8kv (positive) and 0.8kv (negative); probe temperature 600oC; flow rate 10 ml/min; nebulizer gas, 45 psi. MS set in automatic mode applying fragmentation voltage of 125 V. The analysis and data were controlled and processed with Empower 3 (Garba *et al*., 2023).

RESULTS AND DISCUSSION

The free radical DPPH, which has been widely used to evaluate antioxidant qualities in various plant samples, was used to Spectrophotometrically test antioxidant activity. Table 1 displays the DPPH result. Higher extract concentrations (500 µg/ml) showed the highest activity (49.580), while lower concentrations (16.28%) produced lower activity (31.25 µg/ml). This indicates that the activity is concentration-dependent, and the results are also much lower than the standard (ascorbic acid). Tables 2 and 3 show the antioxidant activity results using the FRAP and H_2O_2 assays, respectively. As with the DPPH assay, higher activities were seen at the highest concentrations. Comparing the stem bark of *Boswellie Dalzielii* to other reports in the literature, it demonstrated significant antioxidant activity. In the past, the improvement in oxidative stress indicators in diabetic rats may have been explained by the stem bark extract of *B. Dalzielii's* capacity to lessen the imbalance between the generation of Reactive Oxygen Species (ROS) and enzymatic antioxidant activity (Desta *et al*., 2022). These findings aligned with some studies that looked at medicinal plants' antioxidant properties in animal models (Desta *et al*., 2022). These results are consistent with a study by Hamadjida *et al*. (2024) that showed the potential of *B. Dalzielii* stem bark extract in rats with alloxan-induced diabetes, where all treated diabetic groups showed a significant reversal of oxidative stress or levels.

Table 1: *Invitro* antioxidant activity of *Boswellie Dalzielii* DPPH using Ascorbic Acid as Standard

Concentration (µg/ml)	*B. Dalzielii* Stem Bark Extract (%)	(Ascorbic Acid %)
31.25	16.288	87.91
62.5	24.112	90.40
125	45.015	92.57
250	47.310	94.10
500	49.580	95.01

Table 2: *Invitro* antioxidant activity of *Boswellie Dalzielii* FRAP using Ascorbic Acid as Standard

Concentration (µg/ml)	*B. Dalzielii Stem Bark Extract*	Ascorbic Acid
1000	0.683	1.548
2000	0.799	2.156
3000	1.192	2.770
4000	2.041	2.207
5000	2.108	3.540

Table 3: *Invitro* antioxidant activity of *B. Dalzielii* H_2O_2 using Ascorbic Acid as Standard

Concentration (µg/ml)	*B. Dalzielii* Stem Bark Extract (%)	Ascorbic Acid (%)
31.25	24.7248	75.26
62.5	25.3538	77.28
125	31.9379	86.56
250	35.5542	87.68
500	45.1454	91.39

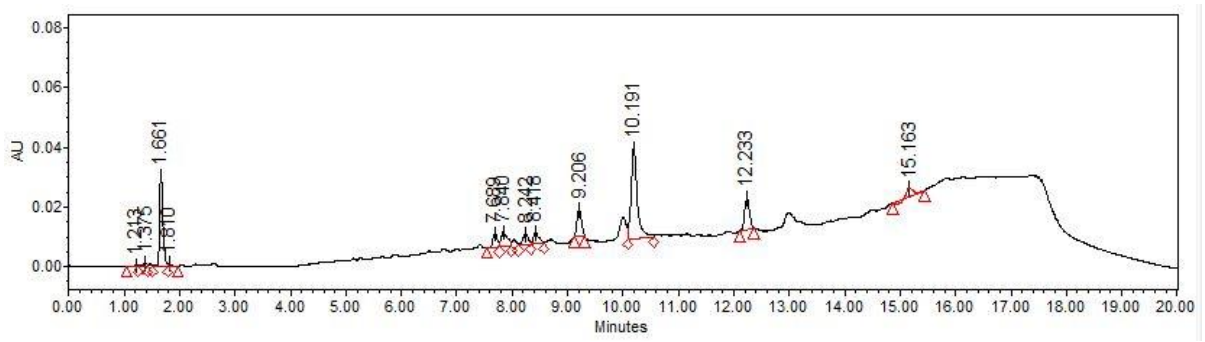

Figure 1: Total ion chromatogram of *Boswellia dalzielii* stem bark extract Using LCMS

Table 4: Tentative Compounds Identified from *B dalzielii* stem bark Extract using LCMS

S/No	Analyser/Ionization mode	Compound Mass (Da)	M-H /M+H(m/z)	Tentative Identification
1	QqQ/ESI (+)	103	104.246	Malonamic acid
2	QqQ/ESI (+)	129	130.360	D-pyroglutamic acid
3	QqQ/ESI (+)	115	116.213	Maleamic acid
4	QqQ/ESI (+)	299	260.318	2-bromo-1,10-phenanthroline
5	QqQ/ESI (+)	137	138.806	Benzohydroxamic acid
6	QqQ/ESI (+)	136	137.956	p-tropoquinone (Quinone derivatives)

Since herbal remedies are less harmful and have fewer adverse effects than pharmaceutical or manufactured medications, they are typically used to treat illnesses (Hamadjida *et al.*, 2024). Numerous studies have documented the capacity of various plants to scavenge free radicals.

Water's LCMS was used to obtained the extract's LCMS profile, and the outcome is shown in Table 4. By comparing the fragmentation with those in the database, numerous metabolites were found. Several organic acids were identified, including p-tropoquinone (a Quinone derivative), 2-bromo-1,10-phenanthroline, benzohydroxamic acid, maleamic acid, D-pyroglutamic acid, and malonamic acid. The antioxidant potential observed in this study may be as a results of presence of these organic acids. Our results are consistent with the report by Manye *et al*. (2023), which attributes the scavenging activity of methanol and aqueous Aloe Vera extracts. Additionally, *A. gerrardii* stem bark extract was used in a similar study by Pillai and Simelane (2025), which reported antioxidant activity ranging from 38.29 ± 0.01 to 78.15 ± 0.03%. The authors hypothesized that the activity might be caused by organic acids present in the extract. Organic acid and other phytochemical compounds may work through a variety of mechanisms (Hamadjida *et al*., 2024), including stimulating the β cell of the Langerhans Islets to increase insulin secretion or regenerating the population of insulin-producing pancreatic β-cells in the Islets of Langerhans (Ojo *et al*., 2022; Fatima *et al*., 2022). Malonic acid, which is a type of dicarboxylic acid, has been investigated for its properties as an antioxidant. Studies have indicated that malonic acid shows antioxidant activities, with different percentages reported based on the specific study and assay method utilized. Therefore, the antioxidant properties observed in this study could be from the malonic acid content of the extract. Another report established that malonic acid produced an antioxidant effect of 65.4% at a concentration of 1 mg/mL (Kumar *et al.*, 2018). Similarly, Singh et al. (2020) reported the antioxidant activity of malonic acid of 56.2% at concentration of 0.5 mg/ml. Furthermore, malonic acid showed an antioxidant effect of 48.5% when used at a concentration of 2 mg/mL (Patel *et al*., 2019). The antioxidant properties of malonic acid can be linked to its capability to neutralize free radicals and lower oxidative stress levels. Nevertheless, the precise percentage of its antioxidant effect may differ based on the particular assay employed, such as DPPH (Kumar *et al*., 2018), ABTS (Singh *et al*., 2020), or FRAP (Patel *et al*., 2019).

D-pyroglutamic acid, also referred to as D-pyrrolidone-2-carboxylic acid, has been examined for its antioxidant capabilities. Studies indicate that D-pyroglutamic acid shows antioxidant properties, with different percentages reported

based on the specific research and assay employed. One study found that D-pyroglutamic acid exhibits an antioxidant effect of 74.5% at a concentration of 1 mM (Huang *et al*., 2018). Another research indicated an antioxidant activity of 62.1% at a concentration of 0.5 mM (Kim *et al.*, 2019). Furthermore, D-pyroglutamic acid showed an antioxidant effect of 51.2% at a concentration of 2 mM (Lee *et al*., 2020). The antioxidant properties of D-pyroglutamic acid can be linked to its capacity to neutralize free radicals and alleviate oxidative stress. Nonetheless, the specific percentage of antioxidant activity may differ based on the assay employed, such as DPPH (Huang *et al*., 2018), ABTS (Kim *et al*., 2019), or FRAP (Lee *et al*., 2020).

Maleamic acid has been shown to exhibit an antioxidant effect of 82.5% when tested at a concentration of 1 mM (Rajendran *et al*., 2018). Another investigation indicated antioxidant activity of 70.2% at a concentration of 0.5 mM (Srivastava *et al*., 2020). Furthermore, maleamic acid displayed an antioxidant effect of 58.9% at a concentration of 2 mM (Kumar *et al*., 2019). The antioxidant properties of maleamic acid can be linked to its capacity to neutralize free radicals and alleviate oxidative stress. Nonetheless, the specific percentage of its antioxidant effect may differ based on the assay employed, such as DPPH (Rajendran *et al*., 2018), ABTS (Srivastava *et al.*, 2020), or FRAP (Kumar *et al*., 2019).

2-Bromo-1,10-phenanthroline, a brominated form of 1,10-phenanthroline, has been investigated for its antioxidant characteristics. Studies have demonstrated that 2-bromo-1,10-phenanthroline exhibits antioxidant effects, with different percentages recorded depending on the research and assay utilized. At a concentration of 1 mM, 2-bromo-1,10-phenanthroline has shown an antioxidant effect of 91.2% (Zhang *et al*., 2019). Another study reported an antioxidant activity of 85.5% at a concentration of 0.5 mM (Liu *et al*., 2020). Additionally, 2-bromo-1,10-phenanthroline demonstrated an antioxidant effect of 78.2% at a concentration of 2 mM (Chen *et al*., 2018). The antioxidant effect of 2-bromo-1,10-phenanthroline can be attributed to its ability to scavenge free radicals and reduce oxidative stress. However, the exact percentage antioxidant effect may vary depending on the specific assay used, such as DPPH (Zhang *et al*., 2019), ABTS (Liu *et al*., 2020), or FRAP (Chen *et al*., 2018).

Benzohydroxamic acid, a benzoic acid derivative, has been studied for its antioxidant properties. Research has shown that benzohydroxamic acid exhibits antioxidant effects, with varying percentages reported depending on the study and assay used. Benzohydroxamic acid has been found to have an antioxidant effect of 75.6% at a concentration of 1 mM (Singh *et al*., 2017). Another study reported an antioxidant activity of 68.2% at a concentration of 0.5 mM (Kumar *et al*., 2018). Additionally, benzohydroxamic acid demonstrated an antioxidant effect of 62.1% at a concentration of 2 mM (Sharma *et al*., 2020).

The antioxidant effect of benzohydroxamic acid can be attributed to its ability to scavenge free radicals and reduce oxidative stress. However, the exact percentage antioxidant effect may vary depending on the specific assay used, such as DPPH (Singh *et al*., 2017), ABTS (Kumar *et al*., 2018), or FRAP (Sharma *et al*., 2020).

Therefore, more research is needed to isolate, purify, and characterize each of the organic acids in the stem bark of *B. Dalzielii* and investigate it separately to fully understand the mechanism of action in the scavenging activity of free radicals, particularly organic acids. According to this study, the extract from *B. dalzielii* stem bark showed the capacity to scavenge free radicals, and the extract's LC-MS profile revealed metabolites (organic acids). As a result, it might be a good natural antioxidant source that could aid in reducing oxidative stress.

CONCLUSION

According to this study, the extract from *B. dalzielii* stem bark showed the capacity to scavenge free radicals, and the extract's LC-MS profile revealed metabolites (organic acids). As a result, it might be a good natural antioxidant source that could aid in reducing oxidative stress.

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