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Investigating the Antioxidant Activity of Methanol Extract of *Zapoteca portoricensis* Root Using the 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) Radical Scavenging Method

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Abstract: Oxidative damage can be managed using various synthetic medications; however, these are often linked to undesirable side effects. A safer alternative to these challenges involves the consumption of natural antioxidants found in dietary supplements and traditional remedies. In vitro antioxidant assay of *Zapoteca portoricensis* root extract demonstrated antioxidant activity was identified. The inhibition rate in the antioxidant model (DPPH) indicated that the plant extract neutralized free radicals in a concentration-dependent fashion within the tested range. The scavenging activity percentage of the *Zapoteca portoricensis* root extract for DPPH showed the highest 83.51% DPPH inhibition at 1.25 mg/mol concentrations. From the above result, the % DPPH inhibition of this plant extract increases as the concentration of the extract increased. This demonstrates that the root extract of *Zapoteca portoricensis* possesses bioactive components capable of donating hydrogen atoms to free radicals, thereby neutralizing the unpaired electrons responsible for their reactivity. Based on the findings of the antioxidant evaluation using the DPPH model, it can be inferred that the extract likely contains significant amounts of phenolic and flavonoid compounds, exhibiting strong antioxidant properties and effective free radical scavenging capabilities.

Keywords: *Oxidative damage, Antioxidants, Zapoteca portoricensis, Antioxidant activity, DPPH, Free radicals, Scavenging activity, Phenolic compounds, Flavonoid compounds, Bioactive components.*

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I. INTRODUCTION

The process of oxidation is fundamental for energy production in living organisms, as it drives numerous biological functions. However, the continuous generation of oxygen-centered free radicals and other reactive oxygen species (ROS) within the body can lead to cellular damage and tissue destruction. Scientific findings indicate that during oxidative stress, oxygen radicals such as superoxide anions (O_2^-), hydroxyl radicals (OH), and peroxyl radicals (H2O2) are formed within biological systems [1]. These reactive molecules can inflict damage on DNA, causing genetic mutations and chromosomal abnormalities. They also oxidize cellular thiols and remove hydrogen atoms from unsaturated fatty acids, initiating lipid peroxidation of cell membranes [2].

Furthermore, the excessive production of free radicals triggers oxidative damage, which is linked to over a hundred health disorders, including atherosclerosis, coronary heart disease, neurodegenerative conditions, cancer, and the aging process. Antioxidants are crucial compounds that safeguard the body against damage caused by free radical-induced oxidative stress [3,1]. Due to concerns over the potential toxicity of synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), there has been a shift in focus toward natural alternatives. Interest has grown in the therapeutic value of plant-based antioxidants, which are believed to mitigate free radical-induced damage more safely and effectively than synthetic options [4].

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Efforts to discover plant-derived antioxidants have intensified, aiming to identify compounds with potent free radical-scavenging properties. Antioxidant substances such as phenolic acids, polyphenols, and flavonoids neutralize reactive molecules like peroxides and hydroperoxides, thereby inhibiting oxidative processes that contribute to degenerative diseases. Epidemiological studies suggest that diets rich in polyphenolic compounds may promote better health due to their antioxidant properties [5].

Medicinal plants have long served as sources of antioxidant compounds. Their effectiveness is attributed to the abundance of phenolic substances, including flavonoids, which are traditionally used to combat free radicals and prevent lipid peroxidation [6]. With rising interest in the therapeutic applications of plant-derived antioxidants, numerous vegetables, fruits, and plant species are either commercially utilized or actively studied for their antioxidant potential [7].

Previous research has indicated that *Zapoteca portoricensis* possesses antioxidant properties. Therefore, this study aims to evaluate the plant's potential antioxidant activity using the DPPH free radical scavenging method [8]. The exploration of natural sources for potential antioxidant compounds has gained significant attention due to the increasing awareness of the adverse effects of oxidative stress on human health. *Zapoteca portoricensis*, a plant with diverse traditional uses, possesses a methanol extract that may harbor antioxidant properties [9,10]. However, a comprehensive assessment of the antioxidant potential of this extract is lacking. The problem to be addressed is the need to elucidate the antioxidant capabilities of the methanol extract of *Zapoteca portoricensis* through the DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging method [11,12,13]. This study aims to fill this research gap by evaluating the extract's efficacy in neutralizing free radicals, thereby contributing valuable insights to the potential use of *Zapoteca portoricensis* in combating oxidative stress-related diseases. This study is aimed at evaluating the methanol extract of *Zapoteca portoricensis* to assess their potential antioxidant activity, the study evaluated the plant using the DPPH radical scavenging assay.

II. MATERIALS AND METHODS

➢ *Reagents and Chemicals*

All the chemicals utilized in this research were of analytical quality and sourced from commercial suppliers located in Enugu and Onitsha, Nigeria. These reagents were selected to meet the required standards for scientific analysis such as absolute ethanol, methanol, NADPH, N-saline, phosphate buffer, starch solution, *P*-nitrophenyl-α-D-glucopyranoside and sodium chloride (NaCl).

➢ *Equipment, Instruments and Apparatus*

Equipment, instruments and apparatus used for this study were spectrophotometer (Spectronic 20D, England), electronic weighing balance (Gallenkanp, London), refrigerator (Thermocool, China), rotary evaporator, water bath (Gallenkamp), beaker, conical flask, micropipette (Perfect) and pH meter (Ecosan).

A. Methods

➢ *Sample Collection*

The freshly harvested roots of Zapoteca portoricensis were collected from a natural site in Udenu Local Government Area, Enugu State, Nigeria. The plant roots were authenticated by Mr. Alfred Ozioko, a taxonomist associated with the Bioresource Development and Conservation Program (BDCP) Research Center in Nsukka, Enugu State, Nigeria. The confirmation of the plant's identity was cross-checked against characteristics documented in plant databases, including The Plant List and IPNI.

➢ *Extraction Procedure*

The plant materials were cleaned of sand with portable water, and was drained of water followed by drying under shed for three weeks until crispy. Thereafter, the dried roots were ground into coarse powder. To prepare the crude methanol extract, 1000 g of the powdered root material was soaked completely in 5 L of absolute methanol for 48 hours. The mixture was filtered using a laboratory mesh, followed by filtration with Whatman paper. The remaining plant residue was re-extracted twice using fresh solvent to enhance the extraction yield. All the filtrates were combined and concentrated with the use of a rotary evaporator at 45^0C under reduced pressure to yield a darkish brown paste (methanol extract).

Assessment of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging potential of the methanol root extract of *Zapoteca portoricensis* was performed following a recently adapted method Agbo et al. [14] with minor adjustments. A methanolic DPPH solution at a concentration of 0.135 mM was prepared. One milliliter of this DPPH solution was combined with 1 ml of the extract solution (0.5 mg/ml) and varying concentrations (0.025 to 0.5 mg/ml) of the reference antioxidant, butylated hydroxytoluene (BHT), separately prepared in methanol. The mixtures were thoroughly mixed and left to stay for 30 minutes at room temperature in absence of light. The absorbance was measured at 517 nm using a spectrophotometer, and the scavenging efficiency of the sample was calculated accordingly.

$$\text{DPPH scavenging efficacy} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

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Where; Abs control represents the absorbance value obtained from the control test tube, while Abs sample corresponds to the absorbance reading from the test tube containing the extract.

B. Statistical Analysis

The results were expressed as mean values accompanied by their standard deviations (SD). The collected data underwent statistical evaluation for accuracy and significance using manual method of calculating mean and standard deviation.

III. RESULTS

Table 1: Effects from the Methanol Extract of *Zapoteca portoricensis* roots DPPH Radical Scavenging Effects

Concentration of extract(mg/mol)	Inhibition of DPPH
0.25	18.49 ± 0.8^{a}
0.50	42.90 ± 0.11^{b}
0.75	64.72 ± 1.35^{c}
1.00	79.84 ± 0.25^{d}
1.25	83.51 ± 0.11^{e}

Data represent mean ± SD (n = 3). Average figures bearing distinct superscripts within a single column represent statistically different at $p < 0.05$

IV. DISCUSSION

As a result of metabolic processes and disease conditions, free radicals are continuously produced in the human body [21,22]. These radicals can inflict severe harm on tissues and biomolecules, contributing to different health issues, particularly degenerative disorders and also cell breakdown [23]. While oxidative damage can be mitigated using numerous synthetic drugs, their use often comes with undesirable side effects. A better alternative is the intake of natural antioxidants through dietary supplements and traditional herbal remedies [15]. To date, numerous natural antioxidants have been identified and documented for their effectiveness [24].

To counteract the harmful effects of free radicals, organisms are equipped with both internal (catalase, superoxide dismutase, glutathione peroxidase/reductase) and external (Vitamin C, Vitamin E, carotenoids, and uric acid) defense mechanisms. However, these protective systems may become insufficient under certain adverse conditions such as oxidative stress, environmental pollution, UV radiation, and microbial infections, where free radical production escalates significantly [16].

The *in vitro* antioxidant evaluation of *Zapoteca portoricensis* root extract demonstrated its antioxidant capabilities. The percentage inhibition observed in the antioxidant assay (DPPH) indicated that the plant extract scavenged free radicals in a concentration-dependent manner. At concentration of 1.25 mg/ml, the extract exhibited a peak DPPH inhibition of 83.51%. this result is comparable to findings from other researchers such as Ogbonna et al. [17], who reported approximately 85% inhibition for *Melissa officinalis* at 2 mg/ml, and Vaidyaratnam. [18], who observed around 80% inhibition in *Acacia nilotica* at 1 mg/ml, indicating that *Zapoteca portoricensis* possesses a potent free radical scavenging ability. Similarly, Agbafor et al. [19] found that *Zapoteca portoricensis* achieved about 74% inhibition at 1 mg/ml, and Joshua et al. [20] reported a lower inhibition of 60% for *Zapoteca portoricensis* at the same concentration, further supporting the superior antioxidant potential of *Zapoteca portoricensis.*

These findings suggest that the scavenging activity of the extract increased as its concentration rose. This indicates the availability of active components in the plant root extract capable of donating hydrogen atoms to neutralize free radicals by eliminating unpaired electrons responsible for their reactivity. The DPPH radical scavenging assay is highly regarded due to its reliability and independence from substrate polarity.

V. CONCLUSION

Based on the findings from the antioxidant assay conducted using the DPPH model, it can be inferred that the root extract of *Zapoteca portoricensis* likely contains substantial amounts of phenolic and flavonoid compounds, which contribute to its potent antioxidant and free radical scavenging properties. Additionally, the extract demonstrated notable reducing power. The findings of this antioxidant evaluation implores that the plant extract serves as a valuable natural source of antioxidants, potentially useful for mitigating various oxidative stress-related conditions. Consequently, more studies are essential in order to isolate, purify, and characterize the actual bioactive components causing the extract's antioxidant and protective effects.

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RECOMMENDATIONS

- Since this study have established *Zapoteca portoricensis* as a good antioxidant. Hence, it is recommended to be included in medicine in order obtain its therapeutic effect.
- In addition to its antioxidant properties. This plant has also been reported to be rich in several relevant therapeutic parameters such as phytochemicals. Therefore it should be used in the treatment of other complications aside oxidative stress
- Health service providers as well as food and nutrition experts should do well to sensitize the society on the invaluable health value in *Zapoteca portoricensis*
- The government should promote the cultivation and processing of *Zapoteca portoricensis* into a therapeutic product.

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