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# Antioxidant Effects of *Cocos nucifera* L. (Asteraceae) Stem Bark Extracts using Diphenyl-I-picrylhydrazyl; Hydrogen Peroxide and Nitric Oxide Scavenging Assay

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**ABSTRACT:** Coconut (*Cocos nucifera* L. (Arecaceae)) supplies nearly all of the basics of life, including food, oil and phyto-medicines. However, reports on the antioxidant effects of the stem bark of *C. nucifera* are scanty. Therefore, the objective of this study is to evaluate the antioxidant effects of coconut stem bark extracts using diphenyl-i-picrylhydrazyl (DPPH); hydrogen peroxide ($H_2O_2$) and nitric oxide (NO) scavenging assay using standard techniques The stem bark of the plant was pulverized and macerated with 70% ethanol. Antioxidant analysis was evaluated on the extract using diphenyl-I-picrylhydrazyl (DPPH), hydrogen peroxide ($H_2O_2$) and nitric oxide (NO) scavenging assay. Results indicated that the ethanol extract possesses dose-dependent antioxidant activity. DPPH scavenging activity assay showed an $IC_{50}$ of 7.65 g/mL, hydrogen peroxide scavenging activity assay revealed an $IC_{50}$ of 22.92 µg/mL and an $IC_{50}$ of 79.83 µg/mL for the nitric oxide scavenging activity. In conclusion, the ability of the extract to scavenge for free radicals, demonstrates its ability to neutralize numerous radicals in different systems, suggesting that it might be helpful as a cheap source of phyto-antioxidant for the management of pathologies associated with free radicals' damaging effects.

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The mechanisms of oxidation are necessary for cellular viability. Organisms with aerobic cellular respiration produce free radicals that harm cells during metabolism while also supplying energy from the metabolism of glucose (Gulcin and Alwasel, 2023). Exogenous sources of free radicals also impact the human system negatively in addition to the ones generated from the metabolic pathways (Akbari *et al.*, 2022). These oxidative species are commonly termed reactive oxygen species (ROS), which include both free radical and non-radical groups such as singlet oxygen ($1O_2$), superoxide anion radical ($O_2$•), hydrogen peroxide ($H_2O_2$) and hydroxyl radical (•OH) (Michalak, 2022). When the activity of ROS overwhelms the body's antioxidant defenses such as β-Nicotinamide adenine dinucleotide phosphate (NADPH), glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) the result is the development of chronic allergic diseases including asthma (Michaeloudes *et al.*, 2022), arthritis (Zamudio-Cuevas *et al.*, 2022) and Alzheimer's disease (Du *et al.*, 2022). Antioxidants from plant sources often strengthen the human defence system against free radicals. Compounds such as flavonoids, carotenoids, terpenes and vitamins (Dias *et al.*, 2021) expressed by plants are known to take part in the oxido-reduction pathways in the human system thus

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ameliorating the effect of oxidative stress and improving the prognosis of ailments associated with it.

*Cocos nucifera* L. (Arecaceae), commonly called coconut is widely distributed in the tropical region of the world. It is a large palm common to the tropical and subtropical regions and can grow up to 30 m tall (Lima and Block, 2019), where it forms an integral part of the local culture of the people. The coconut plant offers a variety of resources that are required for human survival. It is used to produce culinary items including copra, edible cooking oil, coconut chips, coconut honey, coconut jam, coconut candy, coconut milk, and coconut sweets (Naliapara *et al.*, 2022). Coconut oil is produced from the coconut fruit and is used as a functional food additive (Chan *et al.*, 2022). Furthermore, toothpaste can be substituted with coconut butter, which is produced from coconut fruit (Mardiati *et al.*, 2022).

Reports have shown that the stem bark of *C. nucifera* contains numerous bioactive constituents including flavonoids, alkaloids, saponins, tannins and vitamins (Omoboyowa, 2015). Several studies have investigated the antioxidant effects of coconut sap (Asghar *et al.*, 2020), water (Mantena *et al.*, 2003), root (Uy *et al.*, 2019) and oil (Mohammed *et al.*, 2021). However, literature reports are scanty on the antioxidant profile of the stem bark of *C. nucifera*. Therefore, the objective of this study is to evaluate the antioxidant effects of coconut stem bark extracts using diphenyl-i-picrylhydrazyl (DPPH); hydrogen peroxide ($H_2O_2$) and nitric oxide (NO) scavenging assay.

## MATERIALS AND METHODS

*Plant identification, collection and extraction: Cocos nucifera* stem bark was collected at Eyotong village in Oron Local Government Area of Akwa Ibom State, Nigeria. The plant was authenticated in the Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo Voucher specimen was deposited at the herbarium section of the Department with specimen number UUPH8b. The plant organ collected was dried in the oven at 40$^{o}$ C for 5 days and was pulverized. The powdered plant's part (200 g) was extracted with 70 % ethanol through the maceration method for 72 hours. The extract was concentrated at 60 $^{o}$C, transferred to labelled bottles and stored in the refrigerator at 4$^{o}$ C until required.

*Antioxidant Evaluation of Extract:* Diphenyl-I-picrylhydrazyl (DPPH) Radical Scavenging Activity
The solution of DPPH (0.1 mM) in methanol was prepared and 1 mL of this solution was added to 3 mL of various concentrations (20-100 µg/mL) of the extract dissolved in methanol in different test tubes. The same procedure was repeated for different concentrations (2-10 µg/mL) of the standard drug (Ascorbic acid). The mixture was vortexed and then incubated in a dark chamber for 30 minutes after which the absorbance was measured at 517 nm against a blank containing only 3 mL of methanol in place of extract. All tests were performed in triplicates (Blois, 1958). The 50 % inhibitory concentration ($IC_{50}$) was calculated from the dose-response curve obtained by plotting percentage inhibition versus concentrations.

*Nitric Oxide Radical Scavenging (NO) Assay:* Three millilitres of 10 mM sodium nitroprusside in phosphate buffer is added to 2.0 mL of extract and the reference compound in different concentrations (20-100 µg/mL). The resulting solutions are then incubated at 250C for 60 mins. A similar procedure is repeated with methanol as blank, which serves as a control. To 5.0 mL of the incubated sample, 5.0 mL of Gries reagents (1 % sulphanilamide, 0.1 % naphthyl ethylene diamine dihydrochlorides in $H_3PO_3$) is added and absorbance of the chromophore formed is measured at 540 nm per cent inhibition of the nitrite oxide generated is measured by comparing the absorbance values of control and test preparations. All tests were done in triplicates (Blois, 1958). The 50% inhibitory concentration ($IC_{50}$) was calculated from the dose-response curve obtained by plotting percentage inhibition versus concentrations (Green *et al.*, 1982).

*Hydrogen Peroxide ($H_2O_2$) Scavenging Activity*: Plant extract (4 mL) prepared in distilled water at various concentrations (20-100 µg/mL) was mixed with 0.6 mL of 4 mM $H_2O_2$ solution prepared in phosphate (0.1 m, pH 7.4) and incubated for 10 minutes. The absorbance of the solution was taken at 230 nm against a blank solution containing the plant extract without $H_2O_2$. All tests were conducted in triplicates (Blois, 1958). The 50% inhibitory concentration ($IC_{50}$) was calculated from the dose-response curve obtained by plotting percentage inhibition versus concentrations (Ruch *et al.*, 1989).

## RESULTS AND DISCUSSION

The ethanol extract of the stem bark of *C. nucifera* was compared to ascorbic acid in terms of the DPPH radical scavenging effect based on their dose-response curve. *C. nucifera*'s ability to scavenge DPPH was observed to rise in a concentration-dependent manner, with an $IC_{50}$ of 7.65 g/mL, while that of the standard was observed to be 3.70 g/mL (Figure 1). Antioxidants are known to impact DPPH because of their capacity to donate hydrogen. Although *C. nucifera*'s power to scavenge DPPH radicals was about 50% lower than that of ascorbic acid, the result of the study has revealed that the extract is a potential antioxidant.

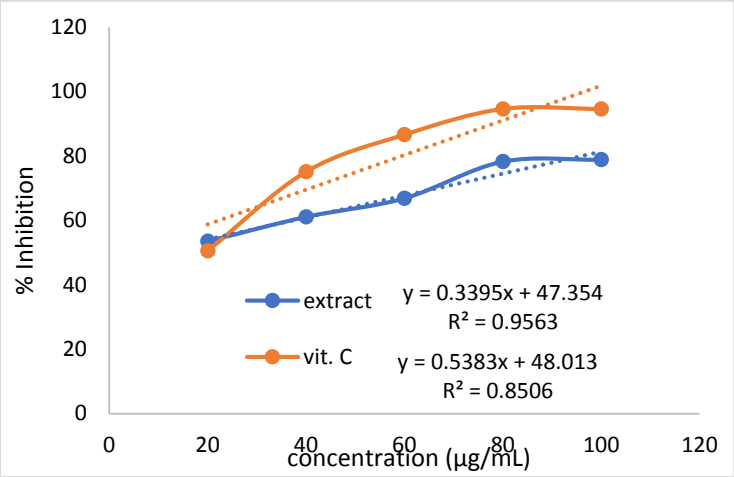


**Fig 1** DPPH radical scavenging activity of *C. nucifera* ethanol extract.

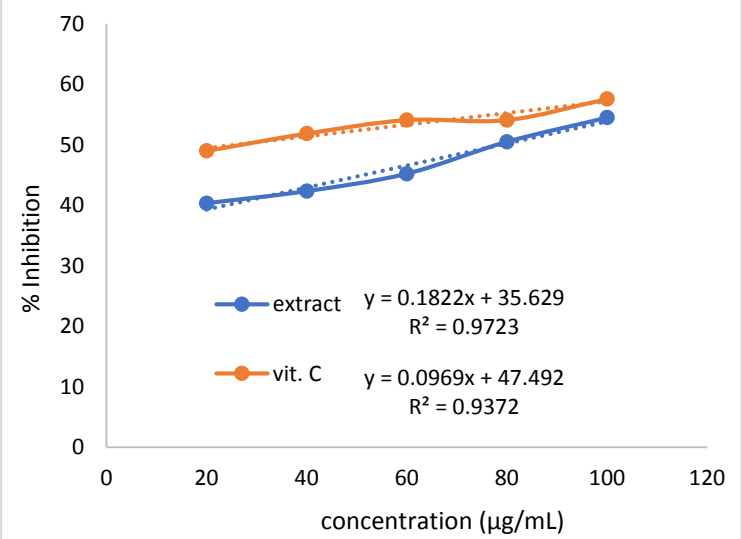


**Fig 2:** Nitric oxide radical scavenging activity of *C. nucifera* ethanol extract

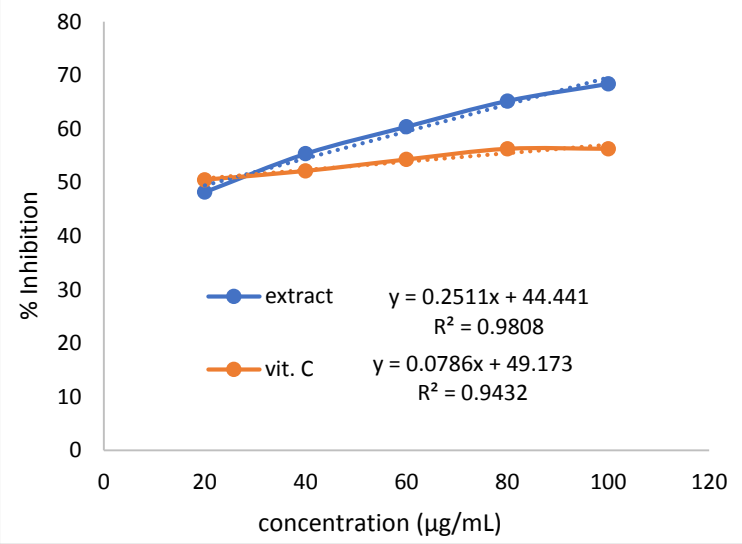


**Fig 3:** Hydrogen peroxide scavenging activity of *C. nucifera* ethanol extract

*C. nucifera* effectively reduced the generation of nitric oxide. The $IC_{50}$ was 79.83 µg/mL and 25.88 µg/mL for ascorbic acid (Figure 2). Nitric oxide is an intracellular and extracellular messenger that modulates a variety of signaling pathways in cells. It is responsible for many physiological processes, including neuronal signalling, immune response, phagocytic defence mechanism, penile erection and cardiovascular regulation, and its decompensation in atherogenesis. Immune system cells produce superoxide radicals during inflammation, and NADPH oxidase is crucial in vascular pathologies (Tuteja *et al.*, 2004). Oxygen ($O_2$) decomposes into singlet oxygen and hydroxyl radicals, which severely harm the mitochondria. In a dose-dependent manner, *C. nucifera* significantly reduces the production of NO and hydroxyl radicals. These findings show that the extract retains the potential to protect against the physiological harm that NO and $O_2$ radicals may inflict on a biological system.

The result of the hydrogen peroxide scavenging activity of *C. nucifera* extract revealed the $IC_{50}$ of 22.92 µg/mL and 9.22 µg/mL for the standard drug used in the study (Figure 3). This effect was observed to be concentration (20-100 µg/mL) dependent. Few enzymes may be directly inactivated by hydrogen peroxide. Hydrogen peroxide is a weak oxidizing agent that typically works by oxidizing thiol (-SH) groups. Usually, it crosses the cell membranes into the cell rapidly. It is the formation of the hydroxyl radical that is responsible for the harmful effects of $H_2O_2$ due to the combination of the hydroxyl radicals with Fe2+ and perhaps Cu2+ ions (Miller *et al.*, 1993). It is critical to monitor the amount of hydrogen peroxide that is permitted to build up in a biological system.

The food industry is becoming more interested in the crude extracts of antioxidant-rich foods, and other plant materials because they avert lipids from degrading via oxidation, enhancing food's flavour and nutritional content. Flavonoids and other plant phenolics such as phenolic acids, stilbenes, tannins, lignans, and lignin are prevalent in plant organs such as leaf, flower, and stem and bark (Kähkönen *et al.*, 1999). The ability of the GNEE to scavenge DPPH, $H_2O_2$ and NO radicals could be due to the presence of polyhydroxy phenolic compounds such as flavonoids and tannins in the extract. This further demonstrated the extract's potential to neutralize numerous free radicals in different systems, suggesting that they might be effective phototherapeutic agents for treating pathological damage caused by free radicals.

*Conclusion:* This study has authenticated the antioxidant effects of *Cocos nucifera* stem bark extract as a potential phytomedicine by highlighting its free radicals scavenging capabilities. The results add to the growing body of information regarding the antioxidant capacities of plant-based medicines and their prospective uses in the food, pharmaceutical, and cosmetic sectors.

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