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# ANTIOXIDANT AND PHYTOCHEMICAL ANALYSIS OF METHANOL EXTRACT OF PHYLLANTHUS AMARUS

**Ogochukwu Tracy Njideaka, *Onyeka Benjamin Onyeukwu and Dumebi Chibuzor Dibie**

Department of Chemical Sciences (Biochemistry Unit), University of Delta, Agbor, Nigeria.

*Corresponding authors' email: benjamin.onyeukwu@unidel.edu.ng

## ABSTRACT

*Phyllanthus amarus* is a widely recognized plant that has been traditionally utilized to cure a variety of ailments. The current study explored the phytochemical and antioxidant characteristics of *P. amarus* methanol extract. Standard procedures were used to analyze: 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) percentage (%) inhibition, lipid peroxidation % inhibition, total phenolic content (TPC) and total flavonoid content (TFC). A higher quantity of the plant extract resulted in a greater inhibition of DPPH and lipid peroxidation percentage. The highest DPPH % inhibition (74.4%) and lipid peroxidation % inhibition (83.44%) were at 0.05 mg/ml while the lowest DPPH % inhibition (25.23%) and lipid peroxidation % inhibition (39.11%) was at 0.03 mg/ml ($p < 0.05$). The most prevalent compound was flavonoid, with a TFC of 460.22 µg/ml while the TPC was 7.428 µg/ml. Owing to its antioxidant and phytochemical qualities, *P. amarus* methanol extract may potentially find application in medicine.

**Keywords**: *Phyllanthus amarus*, Phytochemicals, Antioxidants, Extract, Total phenolic content, Total flavonoid content

## INTRODUCTION

Since the beginning of human history, medicinal plants have been used in complementary and traditional medicine to cure, manage, or prevent a wide range of illnesses. (Ekweogu et al., 2019; Onyeukwu et al., 2024). The preference for using herbal medicines over conventional ones is on the rise, which is interesting. This could be explained by the effectiveness of the active ingredients in herbal medicine as natural healing agents, as well as the fact that they are readily available, accessible, affordable, and have less or no toxic effects. (Ijioma et al., 2021).

Natural antioxidants come in a variety of forms within the kingdom of plants. On the other hand, not much has been discovered about the majority of their actual utility. Numerous herbal drinks, commonly employed in traditional medicine, possess pharmacological and antioxidant qualities associated with an abundance of phenolic chemicals, particularly flavonoids. (Cabrera, Artacho et al, 2006; Pinto 2010). Different components of plants have been shown to provide nutritional and medicinal benefits by many investigators. (Onyeukwu et al., 2024; Achuba, 2018; Aganbi et al., 2017).

Antioxidant prevents other molecules from oxidising. The body uses antioxidants as part of its defense mechanisms to fight off diseases linked to free radical damage. Therefore, consuming antioxidants produced from plants helps prevent degenerative diseases including cancer, Parkinson's disease, Alzeheimer's disease, and atherosclerosis that are brought on by oxidative stress (Pisochi & Nagulescu 2011). It has been demonstrated that antioxidants can stop or slow down the oxidation of other substances. By eliminating radical precursors and oxidising themselves, they have the power to stop chain reactions and prevent oxidation processes (Awuchi and Okpala, 2022). Chemicals originating from plants are known as phytochemicals. Numerous fruits, vegetables, cereal grains, edible macrofungi, microalgae, and medicinal plants contain antioxidant phytochemicals (Breslin, 2017). *P. amarus* is a *Euphorbiaceae* family member. Common names for this plant include "carry me seeds," "stone breaker," "gala of wind," "bhumi amla," and "jangli amli." (Deora et al., 2021). *P. amarus* is an erect, glabrous, annual herb that can reach heights of 10 to 60 cm. The herb's primary stem can be simple or branching, and in its earlier stages, it can be smooth or scabridulous. (Okiki et al., 2015). African traditional medicine uses the plant for numerous applications, suggesting its hepatoprotective, anti-diabetic, anti-hypertensive, analgesic, anti-inflammatory, and antibacterial effects. (Ogunmoyole et al., 2020). Many conditions, including hepatitis B, jaundice, diarrhoea, dysentery, dropsy, sporadic fevers, Herpes Simplex virus, inflammation, oxidative stress, hypotension, urinary problems, etc., have been treated with *P. amarus* (Mao et al., 2016). The present study investigated the antioxidant and phytochemical properties of methanol extract of *P. amarus*.

## MATERIALS AND METHODS

### Chemicals and reagents

1, 1-diphenyl-2-picryl-hydrazyl (DPPH), gallic acid and quercetin were from Sigma Aldrich Spruce Street, St. Louis, USA. All other chemicals and reagents were of analytical grade.

### Plant collection, identification, and preparation of extract

*P. amarus* fresh leaves were harvested from a nearby bush at the University of Delta, Agbor environment and identified with voucher number DELSUH:236 on 28th of November, 2023 in the Department of Botany, Delta State University, Abraka. After being allowed to dry naturally, the leaves were ground into powder and sieved. A soxhlet extractor was used to extract 20g of the plant with 50ml of 80% methanol for 24 hours and the resulting methanol extract was then concentrated using a rotary evaporator.

### Antioxidant activity

The extract's anti-lipid peroxidative and DPPH scavenging capacity were assessed using the methodology outlined by Idowu et al. (2016).

### Phytochemical capacity

TFC and TPC of plant extract were analyzed using the approach of Bello and Ibaba (2020).

**Statistical analysis**

The results were presented as the mean ± standard error of the mean (SEM) of the values obtained in triplicate. SPSS statistical software (version 21) was used to analyze the data and the least significant difference test was used to check for a significant difference at $P < 0.05$. Microsoft Excel was used to plot percentage inhibition against extract concentration and bar charts.

**RESULTS AND DISCUSSION**

Presented in Figures 1 and 2 are the DPPH % inhibition and lipid peroxidation % inhibition respectively. The DPPH % inhibition increased significantly ($p < 0.05$) with an increase in concentration. The highest % inhibition (74.4 %) was at 0.5 mg/ml concentration although insignificantly higher than the concentration of 0.25 mg/ml (73.43%) while the lowest DPPH % inhibition (25.23%) was at the concentration of 0.03 mg/ml. The lipid peroxidation % inhibition also increased significantly ($p < 0.05$) with increasing concentration with a concentration of 0.5 mg/ml (83.44%) having the highest lipid peroxidation % inhibition while the lowest lipid peroxidation % inhibition was at a concentration of 0.03 mg/ml.

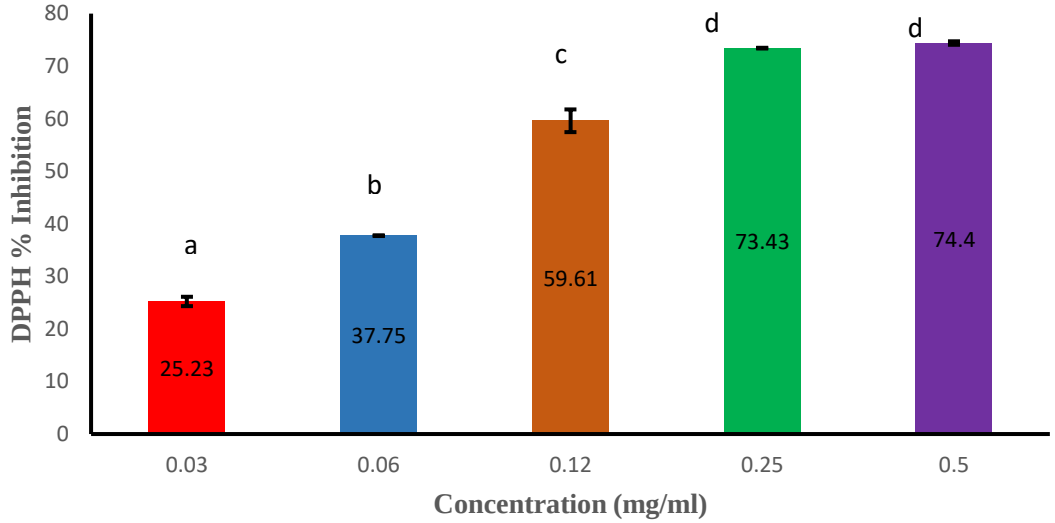


Figure 1: DPPH % inhibition of *P. amarus* methanol leaf extract

Results were expressed as mean ± SEM with a significant difference at $p < 0.05$. The same letter from a-d have no significant difference.

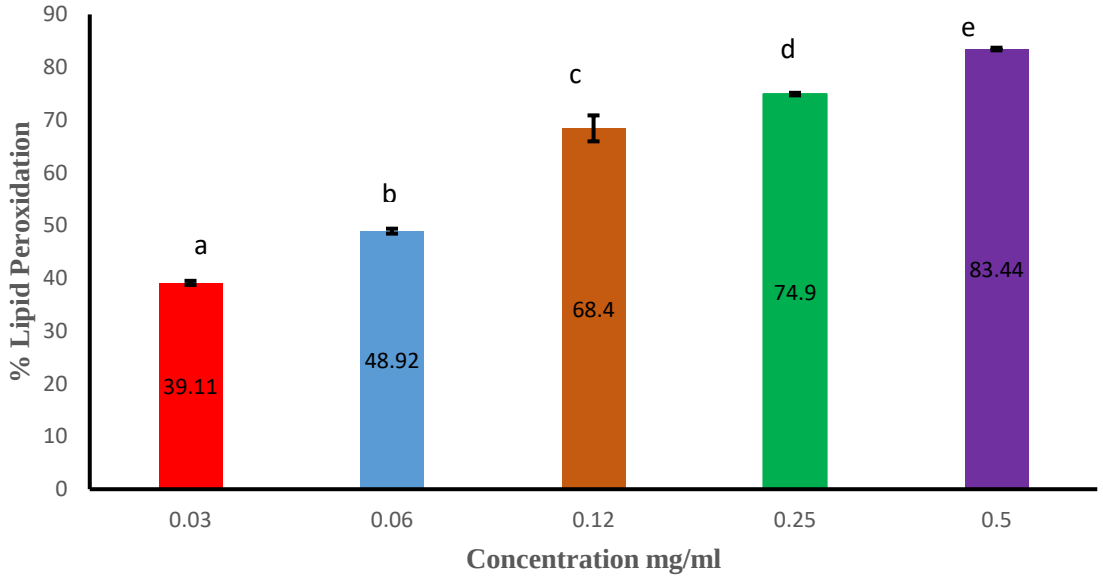


Figure 2: Lipid peroxidation % inhibition of *P. amarus* methanol leaf extract
Results were expressed as mean ± SEM with significant difference (a-e) at $p < 0.05$.

The total phenolic and total flavonoid content of *P. amarus* methanol leaf extract are presented in Table 1 while the standard curve of gallic acid and quercetin are presented in Figures 3 and 4 respectively. The total phenolic content was 7.428 µg/ml and the total flavonoid content was 460.22 µg/ml. This result showed that TFC was significantly higher than TPC ($p < 0.05$). *P. amarus* is thus shown to be rich in flavonoids.

**Table 1: Total phenolic and total flavonoid content of *P. amarus* methanol leaf extract**

| Extract (1.25 mg/ml) | Absorbance (750nm) | GAE (µg/ml) | Mean ±SEM |
|---|---|---|---|
| | 0.251 | 6.930 | |
| Total phenol content | 0.284 | 7.879 | 7.428 ±0.28 |
| | 0.270 | 7.476 | |
| | | QE (µg/ml) | |
| | 0.648 | 470.40 | |
| Total flavonoid content | 0.620 | 450.04 | 460.22 ±5.88 |
| | 0.634 | 460.22 | |

GAE- galic acid equivalent, QE- quercetin equivalent

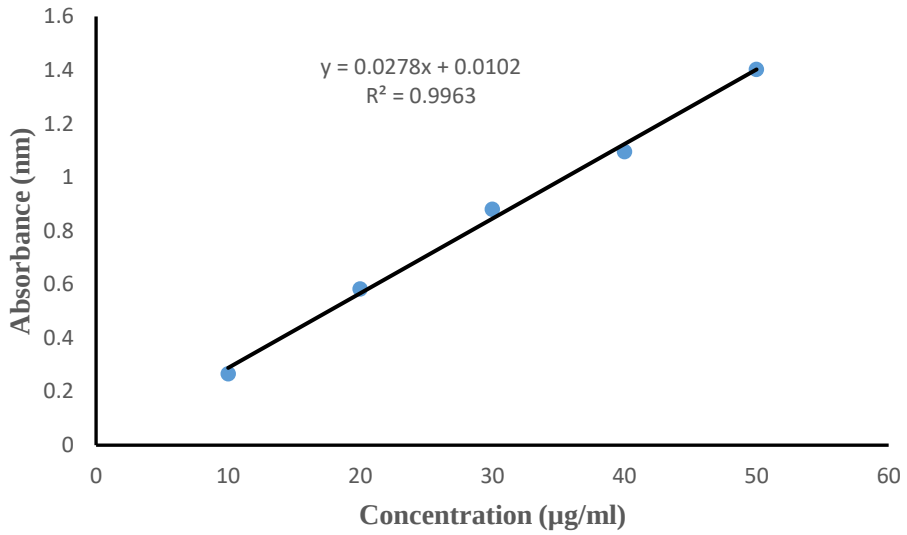


Figure 3: Standard curve of gallic acid with the linear equation

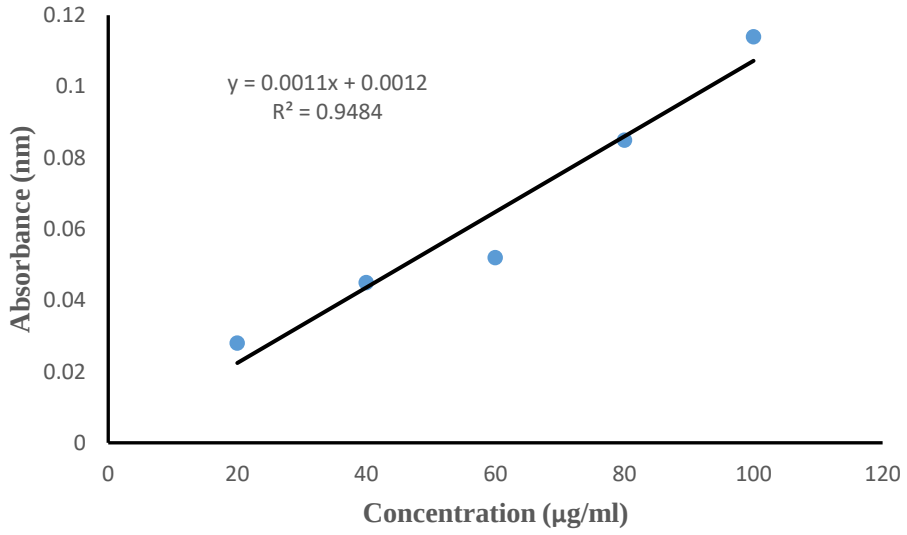


Figure 4: Standard curve of quercetin with linear equation

**Discussion**

According to Moukette et al. (2015), the most dependable and easily accessible technique for determining the antioxidant capacity of plants is to employ biochemical evaluations. As different antioxidants elicit different responses in different testing systems, it is crucial to employ multiple assay methods to fully comprehend the process underlying the action of the bioactive constituent (Bhakta and Siva, 2012). *P. amarus* exhibits strong antioxidant activity, according to the study's findings. This might be explained by the plant's phytochemical components. According to previous investigations, *P. amarus* leaf extract shows strong antioxidant activity (Verma et al., 2014; Oduola et al., 2018). Findings from the study demonstrate the mitigating effect of *P. amarus* leaf extract on lipid peroxidation. This supports the findings of Oyem et al. (2021), which demonstrated that plants have an inhibitory effect on lipid peroxidation. Researchers' interest in eliminating free radicals has grown over the past several years as it has become clear how important they are in treating infections and disorders. Their extensive involvement in biological systems has demonstrated their significance in mitigating the aetiology of

specific illnesses and the aging process (Vasthi and Devarajan, 2012). Numerous studies have demonstrated the effectiveness of taking antioxidant supplements in lowering the severity of oxidative stress and delaying or halting the emergence of disease-related consequences (Vasthi and Devarajan 2012).

This study found that the methanolic extract of *P. amarus* had phenolic content in addition to a high concentration of flavonoids. According to research by Umar et al. (2019), *P. amarus* has a high concentration of flavonoids and phenol, both of which are well-known for their array of therapeutic properties. As natural antibiotics, the majority of phytochemicals support the body's defenses against infections and microbial invasion (Lillehoj et al., 2018). For example, flavonoids have been demonstrated to have antioxidant properties and to prevent the development, growth, and spread of tumours (Batra and Sharma, 2013); they achieve this by either scavenging or quenching free radicals or by blocking the enzyme systems that produce free radicals (Deepak et al., 2015).

## CONCLUSION

*P. amarus* methanol leaf extract's strong inhibitory activity against DPPH radicals and inhibition of lipid peroxidation may be related to high concentrations of flavonoids and potentially phenols. As a result, *P. amarus* is a herbal remedy that may be utilized in developing drugs and for medical purposes.

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