**Scholars Academic Journal of Pharmacy**
Abbreviated Key Title: Sch Acad J Pharm
ISSN 2347-9531 (Print) | ISSN 2320-4206 (Online)
Journal homepage: http://saspublishers.com

OPEN ACCESS

**Medicine**

# Evaluation of Total Flavonoids, Total Phenolics Content and in Vitro Antioxidant Activities of Extract of *Platycerium bifurcatum* Leaf: A Potential Antihypertensive Medicinal Plant

Ikenna Elvis Nnaoma[1*], Chibuzor Okechukwu Okeke[2]

[1]Department of Pharmaceutical Technology, School of Industrial and Applied Sciences Federal Polytechnic Nekede, Owerri, Nigeria
[2]Department of Chemistry/Biochemistry, School of Industrial and Applied Sciences, Federal Polytechnic Nekede, Owerri, Nigeria

**DOI:** https://doi.org/10.36347/sajp.2024.v13i08.003 | **Received:** 19.09.2024 | **Accepted:** 24.10.2024 | **Published:** 30.10.2024

***Corresponding author:** Ikenna Elvis Nnaoma
Department of Pharmaceutical Technology, School of Industrial and Applied Sciences Federal Polytechnic Nekede, Owerri, Nigeria

**Abstract** **Original Research Article**

Plant bioactive compounds possess the potential to function as antioxidants, which makes interest in the use of ethnomedicine for treatment or prevention of diseases associated with oxidative stress to be growing. This study evaluated the flavonoid contents, total phenolics and in vitro antioxidant activity of *Platycerium bifurcatum* leaf extract. The free radical scavenging activity was determined using diphenyl-picrylhydrazyl (DPPH) and nitric oxide assay, and the reducing power by ferric reducing antioxidant power (FRAP) and the total antioxidant capacity assays. The total phenolics content of the extract was 4.7 ± 0.56 mg GAE/g extract and flavonoid contents of the extract was 46.73 ± 2.03 mg QE/g extract. The extarct exhibited a dose dependent nitric oxide and DPPH free radical scavenging capacity, which showed a strong correlation between the antioxidant activity and the flavonoid contents and total phenolics. The appreciable amount of flavonoid contents, total phenolics and its strong antioxidant properties suggested that *Platycerium bifurcatum* is a promising source of novel lead antioxidants and could be utilized in the treatment of oxidative stress mediated cardiovascular disease like hypertensive and obesity

**Keywords:** Bioactive compounds, antioxidants, *Platycerium bifurcatum*, free radical, oxidative stress.



## INTRODUCTION

Oxidative stress, which is commonly caused by an imbalance between reactive oxygen species (ROS) and the biological system's capacity to detoxify the reactive oxygen intermediates, is regarded as the major cause of several illnesses (Rajan *et al*., 2017) High levels of reactive oxygen species (ROS) such as superoxide anion (O2-), hydrogen peroxide, hydroxyl radicals (OH-), and peroxyl radicals (ROO-) has high tendency to cause oxidative stress in the body, leading to pathogenic and degenerative effects such DNA and cell damage. Cancer, ageing, atherosclerosis, ischemia injury, inflammation, and neurological diseases. Many compounds found in plants used in traditional medicine can be employed as medications to treat serious chronic non-communicable diseases as well as infectious disorders (Ayoka *et al*., 2022). To reduce oxidative cell damage, there is a lot of interest right now in discovering antioxidants from natural sources.

Plants not only provide essential nutrients for humans but also contain biologically active compounds that are beneficial for human health and the treatment of various diseases (Liu, 2003). They encompass a wide range of compounds, including phytochemicals, pharmaceutics, flavors, and fragrances, with antioxidant potential (Velmurugan & Anand, 2017).

While various plant species harbor a wealth of secondary metabolites, only a few have been extensively studied as substantial sources of bioactive compounds. The development of effective screening procedures is crucial for the discovery of novel chemicals and quality control (Yadav *et al.,* 2017). The extraction and characterization of these bioactive compounds have led to the development of specific medications with high activity profiles (Loza-Mejia *et al.,* 2018).

*Platycerium bifurcatum* (Cav.), commonly known as the 'staghorn fern,' is an epiphyte naturally found on branches and trunks of trees in tropical and

subtropical jungles and rainforests. *Platycerium bifurcatum* is a lower plant that lacks roots and reproduces through spores rather than flowers. It belongs to the Polypodiaceae family and is propagated from its spores. Besides its ornamental uses, it has been reported to have various medicinal applications. In Nigeria, young leaves of *Platycerium bifurcatum* are used as a common antiulcer remedy (Pemberton, 2003). The leaf extract of *Platycerium bifurcatum* is known to have diverse uses, such as preventing miscarriages in women when taken two months after conception, and for treating edema, coughs, and hypertension. This study evaluated the total phenol, total flavonoids and antioxidant potential of leaf extract of *Platycerium bifurcatum.*

## MATERIALS AND METHODS

### Plant Collection and Identification

The leaves of *Platycerium bifurcatum* was collected from Nekede, Owerri West Local Government of Imo State. The leaves was identified by a plant taxonomist, Dr. C. Duru, in the Department of Science laboratory Technology, Environmental Biology Option, Federal Polytechnic Nekede Owerri.

### Preparation of Extract

The leaves of *Platycerium bifurcatum* were washed with clean tap water and dried at room temperature for 4 weeks. After drying, the leaves were pulverized into fine powder using a pulverizing machine and stored in an air tight container for further analysis.

Three Hundred and sixty gram (360g) of the powder was extracted with 1.5L of 80% Methanol using maceration process for 72 hours. The methanol was evaporated using rotary evaporator at 45$^{o}$c and the extract was refrigerated at 4$^{o}$c.

### Determination of the total phenolic content (TPC)

The method of Harbone 1984 using Gallic acid was as internal standard with a small modification, was employed. In a volumetric flask (20 mL), the extract (1.0 mg/mL) was combined with distilled water (9.0 mL). A 2.5 mL addition of 10-fold diluted Folin-Ciocalteau phenol reagent (FCPR, 1:10) was made. After waiting for five minutes, 10 mL of 7.5% Na2CO3 solution was added to the mixture and adjusted with distilled water to the proper concentration. The mixture was incubated at room temperature for 90 minutes in the dark. The same method used to prepare the extracts was used to prepare a set of standard solutions of gallic acid (20, 40, 60, 80, and 100 mg/L). Using a UV/Visible spectrophotometer (UV-1800, Shimadzu, Japan), the absorbance of the extract and standard solutions were measured against the reagent blank at 760 nm. The total phenolic content of the extracts was calculated in triplicate. The calibration curve was used to calculate the total phenolic content, which was then reported as milligrams of gallic acid equivalent (GAE) per gram of the extract 15.

### Determination of total flavonoid content (TFC)

Same method of Harbone 1984 was used. The total flavonoids was assayed using an aluminium-chloride colourimetric test. In a 20 mL volumetric flask, the extract (1.0 mg/mL) was combined with distilled water (4.0 mL). The flask received 0.30 mL of 5% sodium nitrite. 10% AlCl3.6H2O solution (0.30 mL) was added to the mixture after 5 minutes, and after another 5 minutes, 1.0 M NaOH (2.0 mL) was added. The mixture was then diluted to the desired strength using distilled water. The same procedure used to make the extracts was used to create a set of standard solutions of quercetin (20, 40, 60, 80, and 100 mg/L). Using a UV/Visible spectrophotometer Model 721G from Yoke Instruments Co., China. The absorbance of the extract and standard solutions was measured against the reagent blank at 510 nm. In triplicates, the total flavonoids in the extract and standards were determined.

### Quantitative antioxidant activity

### Total antioxidant capacity (TAC)

The total antioxidant capacity was assayed following a method described by Prieto *et al*., (1999). It is determined by the extracts reduction of Molybdenum (VI) to Molybdenum (V). At an acidic pH, a green phosphate/molybdenum (V) complex is formed.

### Assessment of DPPH free scavenging ability

The radical scavenging ability of the extracts was determined as described by Brand-Williams *et al.*, (1995).

### Inhibition of nitric oxide radical

The extracts was evaluated for nitric oxide radical inhibition activity according to the method of Green *et al*., (1982) as described by Marcocci *et al*., (1994).

### Ferric reducing antioxidant power (FRAP)

The FRAP assay was carried out in this study as described by Benzie and Strain (1999). It uses antioxidants as reductants in a redox-linked colorimetric method with absorbance measured with a spectrophotometer.

### Statistical analysis

Statistical analysis of the data was carried out with SPSS version 22.0 using One Way Analysis of Variance (ANOVA). The statistically analysed data was reported as Mean+SEM. Significant difference will be accepted at 95% confidence level of probability ($P < 0.05$).

## RESULTS AND DISCUSSION

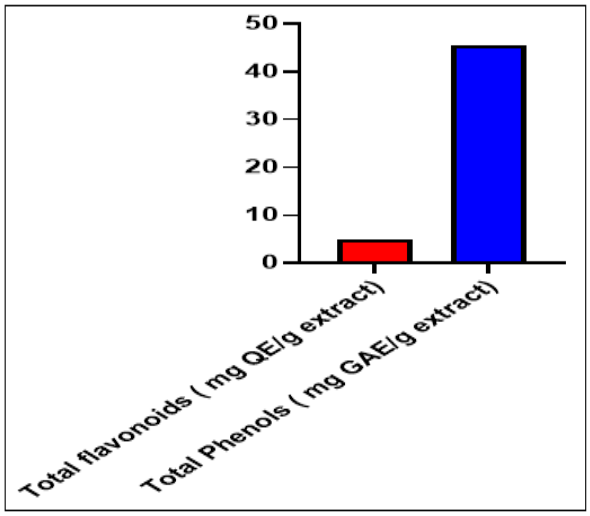


**Figure 1: Total phenols and Total flavonoids contents of *Platycerium bifurcatum* leaf**

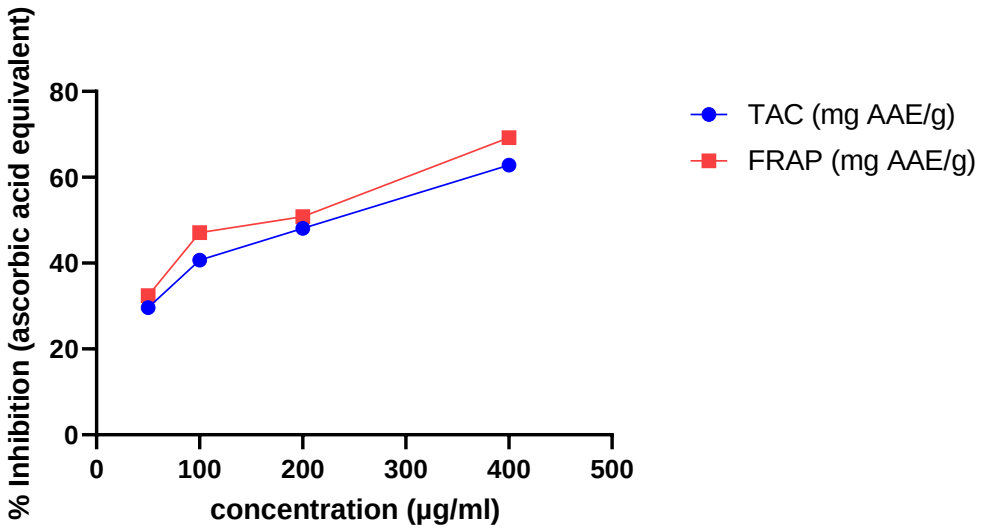


**Figure 2: Total antioxidant capacity and FRAP of *Platycerium bifurcatum* leaf**

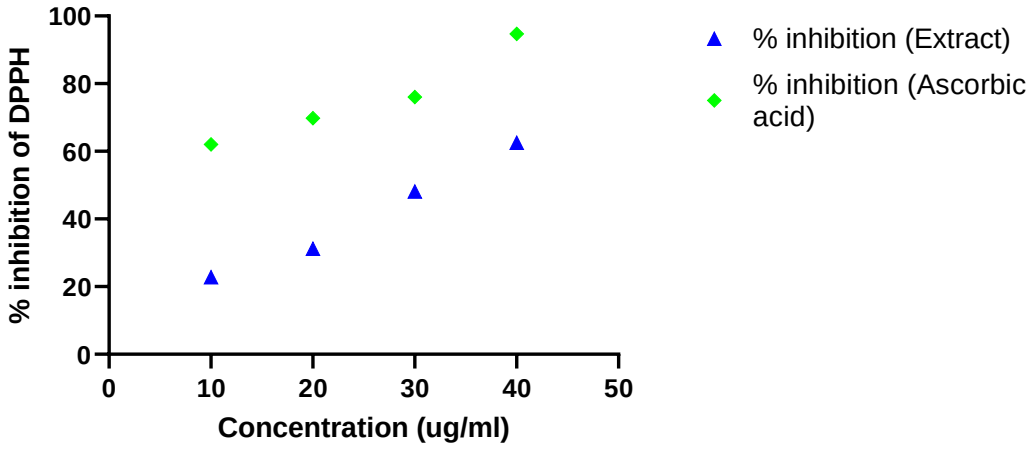


**Figure 3: Inhibition of DPPH potential of *Platycerium bifurcatum* leaf**

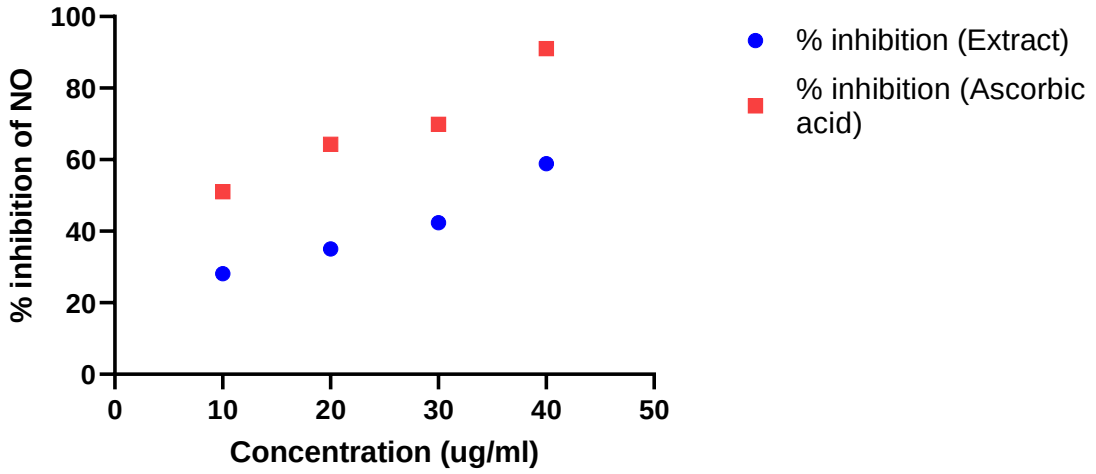


**Figure 4: Inhibition of nitric oxide (NO) potential of *Platycerium bifurcatum* leaf**

## DISCUSSION

The antioxidant potential of *Platycerium bifurcatum* leaf was evaluated using some quantitative antioxidant activities such as total antioxidant capacity (TAC), total phenolic contents, total flavonoids contents (TFC), DPPH free radical scavenging, nitric oxide inhibition, ferric and reducing antioxidant power (FRAP). The results of the assays are as shown in Figure 1-4.

The result showed a dose dependent antioxidant potential. The observation in this study indicates the effective capacity of *Platycerium bifurcatum* for scavenging radicals and associated with high total flavonoid content, showing its potential as an antioxidant. The inherent properties of phenolics may be connected to the capacity of extract in radical scavenging.

The result of TAC reveal that *Platycerium bifurcatum* can be an effective anti-oxidative stress agent. There was also concentration dependent antioxidant capacity of sample. The use of TAC model for antioxidant assay is well documented. TAC is known to derive its effect via the reduction of Mo (VI) to Mo (V) by various radicals which are released in the system. The strong antioxidant activity of *Platycerium bifurcatum* which compared well with ascorbic acid designates strong antioxidants in extract and could be credited to the presence of phenolic compounds and flavonoids (Obonga *et al*., 2019). The extract maintained higher concentration-dependent in the FRAP assay.

The model is based on the ability of tested phytochemicals to reduce ferric ions to ferrous ions which later formed a blue ferroustripyridyltriazine complex at 590 nm. Many scientific studies have validated the ethnomedicinal uses of *plant extract as natural* antioxidant, and this present study have also proven that *Platycerium bifurcatum* is an effective antioxidative agent

## CONCLUSIONS

This study concluded that *Platycerium bifurcatum* extracts had strong antioxidant activities. These agents could therefore be effective in the management of oxidative stress mediated diseases. These findings also justified the use of *Platycerium bifurcatum* extracts in folk medicine.

**Funding**

The research was funded by Tertiary Education Trust Fund (TETFund) Nigeria through the Institution Base Research (IBR) Project Grant.

**Acknowledgements**

The authors sincerely acknowledge Tertiary Education Trust Fund (TETFund) Nigeria for the financial support. We also acknowledge the Management of Federal Polytechnic Nekede Owerri for creating enabling environment for research.

## REFERENCES

- Aniya, Y. (2002). Antioxidants in traditional foods and medicinal plants from Okinawa. In Itokazu, D., Sho, H., & Nakahara, Y. Proceeding of Okinawa International Conference on Longevity. 50. Naha: OICL.
- Ayoka, T. O., Ezema, B. O., Eze, C. N., & Nnadi, C. O. (2022). Antioxidants for the Prevention and Treatment of Non-Communicable Diseases. *J Expl Res Pharmacol*, *7*(3), 178-188.
- Benzie, I. F. F., & Strain, J. J. (1999). Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity in biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Meth Enzymol*, 299, 15-27.

- Brand-Williams, M., Cuvelier, M. E., & Berset C. (1995). Use of free radical method to evaluate antioxidant activity. *LWT Food Sci Technol*, 28, 25-30.
- Green, L. C., Wagner, D. A., Glogowski, J., Skipper, P. L., Wishnok, J. S., & Tannenbaum, S. R. (1982). Analysis of nitrate, nitrite, and [15N] nitrate in biological fluids. *Analytical biochemistry*, *126*(1), 131-138.
- Harbone, J. B. (1984). Phytochemical methods: A guide to modern techniques of plant analysis, Chapman and Hall New York; 2nd edition: 288.
- Liu, R. H. (2003). Health benefits of fruits and vegetables are from additive and synergistic combinations of phytochemicals. *American Journal of Clinical Nutrition*, 78, 517S-520S.
- Loza-Mejia, M. A., Salazar, J. R., & Sanchez-Tejeda, J. R. (2018). In silico studies on compounds derived from Calceolaria: phenylethanoid glycosides as potential multi-target inhibitors for the development of pesticides. *Biomolecules*, 8, 121.
- Marcocci, L., Maguire, J. J., Droy-Lefaix, M. T., & Packer, L. (1994). The nitric oxide scavenging property of *Ginkgo biloba* extract EGb 761. *Biochem Biophys Res Commun*, 201, 748-755.
- Obonga, W. O., Nnadi, C. O., Chima, C. C., Okafor, S. N., & Omeje, E. O. (2019). In vitro antioxidant and In vivo Anti-inflammatory Potentials of Marantochloa Leucantha (Marantaceae) Extracts and Fractions. *Dhaka University Journal of Pharmaceutical Sciences*, *18*(2), 233-240.
- Prieto, P. Pineda, M., & Aguilar M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Anal Biochem*, 269, 337-341
- Rajan, V. K., & Muraleedharan, K. (2017). A computational investigation on the structure, global parameters and antioxidant capacity of a polyphenol, gallic acid. *Food Chem*, 220, 93–99.
- Velmurugan, G., & Anand, S. P. (2017). GC-MS Analysis of bioactive compounds on ethanolic leaf extract of Phyllodium pulchellum L. Desv. *International Journal of Pharmacognosy and Phytochemical Research*, 9, 114-118.
- Yadav, R., Khare, R. K., & Singhal, A. (2017). Qualitative phytochemical screening of some selected medicinal plants of Shivpuri District (MP). *International Journal of Life Science Research*, 3, 844-847.