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PHYTOCHEMICAL SCREENING AND ANTIOXIDANT PROFILE OF STEMBARK Extracts *Piliostigma thonningii* (Schum.)

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Abstract
This study was conducted to examine the phytochemical screening and antioxidant activity of Piliostigma thonningii (Schum.) stembark. Extraction of the stembark was carried out using serial extraction starting with less polar to most polar solvent. The plant extracts (n-hexane, dichloromethane, ethyl acetate and methanol) were screened for the presence of phytochemicals using standard methods and their effect on 2,2-diphenyl-1-picryl-hydrazyl radical (DPPH) were used to determine their antioxidant activity. The results of the study showed that the ethyl acetate extract had highest antioxidant activity with IC_{50} of 0.58 mg/cm^3 which is greater than vitamin C with IC_{50} of 0.65 mg/cm^3. Dichloromethane, methanol and hexane extracts showed lesser antioxidant values compared with vitamin C. The findings of this study suggest that the stembark extract of P. thonningii is rich in antioxidants and thus could be used as a potential source of natural antioxidants and therapeutic agents. Further studies should be carried out to investigate the bioactive compounds of this species and their possible characterization
***Keywords**: antioxidant activity, extract, P. thonningii, phytochemical and stembark*

Introduction

Natural products are chemical compounds or substances isolated from plants or animals, which historically played a key role in discovering new drugs especially for cancer and infectious diseases treatment (Atanas *et al*., 2021). The World Health Organization (WHO, 1993) had reported about 80 % of the people living on earth depends mainly on traditional medicine for the treatment of diseases and the traditional treatment involves mainly the use of plant extracts.

Phytochemicals are biologically active, plant-based bioactive compounds produced by plantsfor their protection against predators. They are natural bioactive components which are rich in foods like whole grain products, fruits, vegetables, legumes, nuts, seeds and dark chocolates among others (Jianho and Weibin, 2019).

Piliostigma thonningii (Schum.) commonly referred to as monkey bread belongs to *Fabaceae* family and is used in the tropics for nutritional as well as medicinal purposes. It is also called *Kalur*, *Kalgo*, *Abafe* and *Okpachu* in Kanuri, Hausa, Yoruba and Igbo respectively. *Piliostigma thonningii* is utilized for treatment of human diseases among them are: malaria, rheumatism, snake bites, fever, dysentery among others (Farida and Mathew, 2023). It is widely believed that plant pharmacological actions are driven by their secondary metabolites. Identification of the secondary metabolites have led to discovery of many useful drugs and leads for designing of novel compounds to enhance human health.

Plants with therapeutic properties have been utilized in health care delivery since the birth of human civilization (Moni *et al*., 2021). Reza *et al*. (2021) also reported that the enormous health benefits associated with herbal medicines is accredited to the phytochemicals they contain. Plants contain phytochemicals in them which include alkaloids, flavonoids, phenolic acids, terpenoids, sterols and triterpenes among others (Reza *et al.*, 2021). Phenolic compounds are known to possess various pharmacological activities including anti-aging, anti-inflammation, anti-oxidant, anti-atherosclerosis, anti-malarial, and others, (Shrestha *et al.*,

2015). Saponins are reported to possess anti-inflammatory and anti-bacterial potency (Chang *et al.*, 2023).

There are so many challenges for drug discovery, such as technical barriers to screening, isolation, characterization and optimization, which had contributed to a decline in the pursuit by the pharmaceutical industries from early 90s to date (Atanas *et al*., 2021). Caffeine was reported to be isolated from the ethyl acetate root bark extract and its toxicity tested using Drosophila assay and was found to be safe for oral consumption (Babagana *et al*., 2022). (Babagana *et al*., 2019) reported the presence of alkaloids, flavonoids and steroids, saponins and glycosides and tannins were found to be present in various solvent extracts of root bark of *P*. *thonningii*. Sodium, potassium, iron, zinc and magnesium were also reported in the root bark of the plant (Babagana *et al*., 2019).In another research carried out to comparatively study the phytochemical and the antioxidant activity of P. thonningii leave and its mistletoe (*Tapinanthus globiferus*) leaves grown on the host plant showed both the plant and the mistletoe have good antioxidant activities (Halilu *et al*., 2017).

Total polyphenols, flavonoids, condensed tannins, and vitamin C were also found to be present in *P*. *thonningii*root bark extract which is believed to have contributed to the antioxidant and anti-inflammatory activities of the plant (Zango *et al*., 2023).In a related research, the GC MS analysis of the Dichloromethane stem bark extract to investigate the phytochemicals present, it was found that flavonoids, phenolics, phytosterols, fatty acids,oleic acid, squalene, valencene and α-cedrene were present in the plant which is believed to contribute to the therapeutic potential of the plant (Okioga and Ngugi, 2023).

The purpose of this study is to explore the phytochemical and antioxidant profile of *Piliostigma thonningii* stem bark extracts. Figure 1 showed *P*. *thonningii* tree in its natural habitat.



Figure 1:P. thonningii (Schum.) in its natural vegetation (Farida and Mathew, 2023)

Materials and Methods

Collection of Plant Material

The stembark of *Piliostigma thonningii* was collected from the plant farm of Lake Chad Research Institute, Maiduguri in the month of October, 2023. The plant was identified and authenticated by a Botanist at Department of Biological science, Borno State University, Maiduguri. The stembark sample was dried under shade for two weeks at 25°C and pulverized

using a wooden pestle and mortar and then the powdered sample was stored in separate clean, air-tight polythene bags until required for use (Sofowora *et al*., 2013).

Extraction of Plant Material

About 500 g of the powdered sample was extracted with 5000 cm^3 of n-hexane in a closed container by cold maceration for five days with occasional stirring. The extract obtained was filtered using Whatman No. 1 filter paper and then concentrated on water bath at 45 ^OC to obtain a semi-solid extract. The residue (marc) was allowed to dry and then extracted with solvent of higher polarity (i.e., ethyl acetate). The above procedure was repeated with dichloromethane and methanol. The percentage yields of the extracts were calculated and the extracts were stored in different containers until required for further analysis.

Qualitative Phytochemical Screening

Phytochemical screening was carried out on the four extracts (n-hexane dichloromethane, ethyl acetate and methanol) of the plant to validate the presence or absence of secondary metabolites such as alkaloids, anthraquinones, cardiac glycosides, flavonoid, quinine, saponins, steroids/terpenoids, and tannins using standard procedures as described by (Sofowora, 1993; Trease and Evans, 2002).

Antioxidant Activity

The free radical scavenging activities of the *P*. *thonningii* stembark extracts against 2,2-diphenyl-1-picrylhydrazine (DPPH) were determined by UV spectrophotometer at 517 nm. Radical scavenging activity was measured by a slightly modified method adapted by Ayoola *et al*, (2008). The following concentrations of the extracts were prepared: 2.5, 5, 10, 20 and 40 mg/cm^3 in methanol. Ascorbic acid was used as the antioxidant standard at concentration of 2.5, 5, 10, 20 and 40 mg/cm^3. One (1) cm^3 of the extract was placed in a test tube and 2 cm^3 of methanol was added and followed by 0.5 cm^3 of the DPPH solution. A blank solution was prepared containing the same amount of methanol and DPPH. The radical scavenging activity was calculated using the following

formula $\% \text{ Inhibition} = \frac{Xb - Xa}{Xb} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$

Where Xb is the absorption of the blank sample and Xa is the absorption of the extract. The IC_{50} of the various extracts were also calculated by plotting a graph of percentage inhibition against logarithm of concentration and then extrapolating from the 50 % inhibition to obtain the IC_{50} values.

Results and Discussion

Phytochemical Screening of Plant Materials

Table 1 showed the phytochemical screening of the plant extracts. Alkaloids and anthraquinones were detected in the ethyl acetate and methanol extracts while cardiac glycosides, flavonoids and quinine, saponins and tannins were present in all the four extracts. steroid/ terpenoids were detected in the n-hexane and dichloromethane extracts. Carbohydrates and fixed oils were not detected in all the extracts. This is in line with earlier report of Halilu *et al*. (2017) where these phytochemicals were detected in the leaves and mistletoes of the plant.

Table 1: Phytochemical Constituents

Test	n-Hex Extract	DCM Extract	EtOAc Extract	MeOH Extract
Alkaloids	-	-	+	+
Anthraquinones	-	-	+	+
Carbohydrates	-	-	-	-
Cardiac glycosides	+	+	+	+
Fixed oils	-	-	-	-
Flavonoids	+	+	+	+
Quinine	+	+	+	+
Saponins	+	+	+	+
Steroid/triterpenoid	+	+	-	-
Tannins	+	+	+	+

Key: + = detected, - = not detected, Hex= n-hexane, DCM = dichloromethane, EtOAc = ethyl acetate, MeOH = methanol

Antioxidant Activities of the Extracts

Figure 2 showed a plot of percentage inhibition of DPPH solution against concentrations of the extracts. The DPPH analysis provides insight on the reactivity of the test compounds with a stable free radical. DPPH gives a strong absorption band at 517 nm in visible region of a spectrum, as the colour changes from deep violet to light yellow (Ayoola *et al*. 2008). All the plant extracts exhibited potent antioxidant activity compared with standard vitamin C (Figure 2). The presence of flavonoids and tannins in all the extract is likely to be responsible for the free radical scavenging effects observed. Tannins and flavonoids are phenolic compounds and plant phenolic are a major group of compounds that act as free radical scavengers (Polterait, 1997).

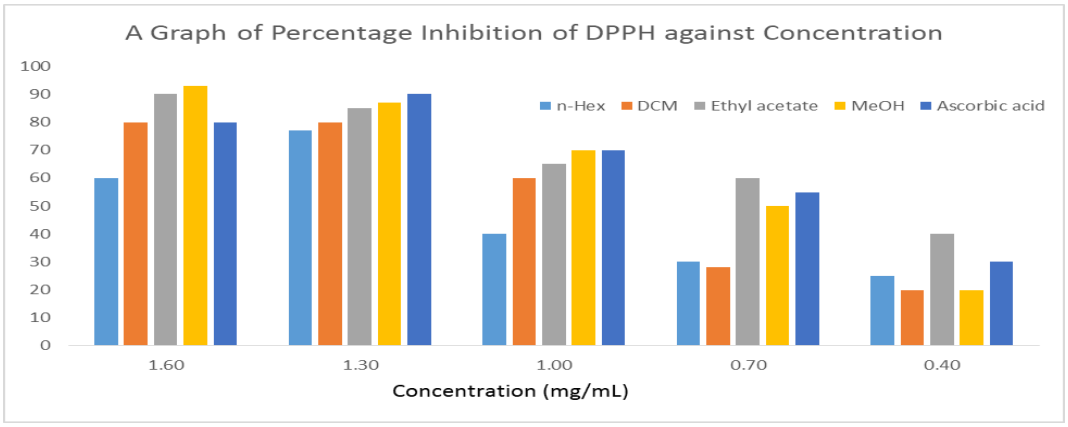

Figure 2: A plot showing %inhibition of DPPH against concentration
Key: n-Hex = n-hexane, DCM = dichloromethane, MeOH = methanol

Figure 3 is a plot of the percentage inhibition logarithm of the concentration of the extracts where IC_{50} values was extrapolated and obtained. The IC_{50} values of 1.08,0.90, 0.58, 0.70 and 0.65 mg/cm^3 for the n-hexane extract, dichloromethane extract, ethyl acetate extract, methanol extract and ascorbic acid respectively, were obtained. Based on this findings, the ethyl acetate extract has the highest antioxidant activity. This result is in line with finding of Halilu *et al*. (2017) where an IC_{50} of 0.60, 0.74, 0.40, 0.62 and 0.58 mg/cm^3 for n-hexane extract, dichloromethane extract, ethyl acetate extract, methanol extract and ascorbic acid respectively from the leaves of *P. thonningii*.

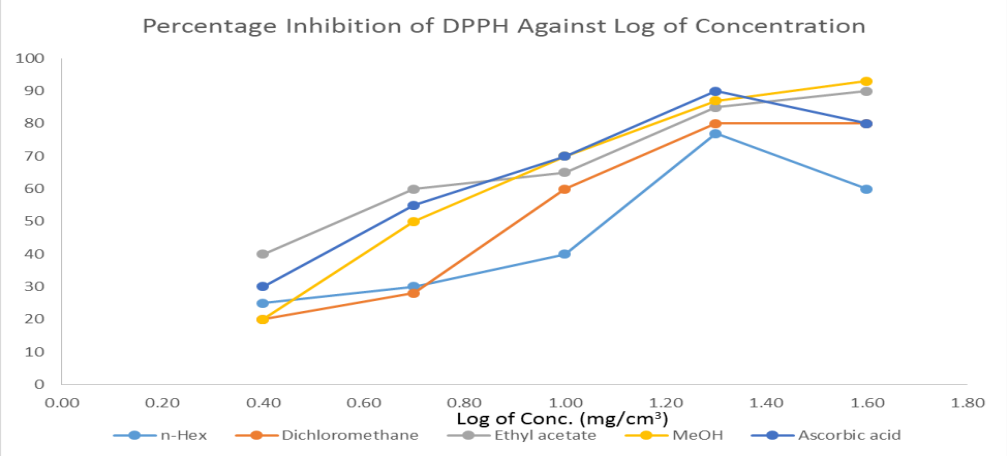

Figure 3: A plot showing IC_{50} values of the Extracts
Key: n-Hex = n-hexane, MeOH = methanol

Conclusion

Extracts from *Piliostigma thonningii* stembark showed varying antioxidant (free radical scavenging) activities when compared to vitamin C in the following order: ethyl acetate extract > vitamin C > methanol > dichloromethane > n-hexane. The results suggest that the antioxidant activity of these plant justifies the healing properties of many ailments.

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Conflicts of Interest: The authors declare no conflict of interest.

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