Journal of Agriculture and Environment
Vol. 20 No. 1, 2024: 141-149

Antioxidants and vitamins content of methanol extract of *Piliostigma reticulatum* leaves

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ABSTRACT

Medicinal plants play an important role in treatment of various diseases or health conditions. *Piliostigma reticulatum* has many medicinal applications in Northern Nigeria. The leaves are used as antimalarial, antihypertensive, diuretic, anti-cancer, to name but few. Geographical location and soil nature can lead to variations in the phytochemical and nutritional contents of any given plant. The aim of this work was to determine the antioxidant property, phytochemical and vitamin contents of *P. reticulatum* leaves found in Gusau, Zamfara state. Antioxidant potentials were determined using the 2,2 diphenyl-1-picryl hydrazyl (DPPH), free radical scavenging assay and the ferric reducing antioxidant power (FRAP). Using the two antioxidants capacity tests, antioxidant power of the extract increased with increase in concentration of the extract. The IC_{50} for DPPH and FRAP assays were 5.22µg/ml and2.8 µg/ml respectively. The test revealed strong presence of saponins, and glycosides (+++). The extract was rich in vitamins C, E, and A at the concentrations of µg/ml 232.03, 80.71 µg/ml and 68.05 µg/ml respectively. The results of this study revealed methanolic leaves extract of the plant to be very rich in antioxidants. Further research to ascertain the toxicity index and quantify the phytochemicals for further in cooperation into modern pharmaceuticals is recommended.

Keywords: *Piliostigma reticulatum*; phytochemicals; antioxidant; vitamins

INTRODUCTION

Medicinal plants have played a significant role in the treatment of many ailments over the centuries. This is why there is renewed interest to study the antioxidant potential and nutritional benefits of the medicinal plants. *Piliostigma reticulatum* is one such plant that has been used over the centuries in Sub-Saharan Africa for many medicinal purposes. The plant is a shrub that can grow into a tree in many parts of Sub-Saharan Africa. Although, it is widely used in traditional medicine practice in Northwestern Nigeria, it is also used as food by man and animals (Zerbo *et al.*, 2010). The leaves of *P. reticulatum* known as *Kalgo* (in

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https://dx.doi.org/10.4314/jagrenv.v20i1.14

Hausa), *Abafin* (in Yoruba), and *Okpo atu* (in Igbo) are extensively used in the treatment of malaria, hypertension, cancer, gonorrhea, hookworm, and ascites. Decoctions of the leaves are also used as antidote against plant poisons and liver or gall complaints (Tira-Picos *et al*., 2010). It is important to mention the root decoctions are also used in vapor baths against cough, diarrhea, constipation, stomachache and muscular pains (Dieng *et al*., 2020). The bark extract is widely used on wounds, cuts, ulcers and sores as astringent and hemostat (Tira *et al*., 2010).

Plants are known to synthesize a wide range of chemical compounds like primary and secondary metabolites. While this is true, each medicinal plant species has different phytochemicals and nutritional composition some of which are essential for the physiological functions of human and animal body (Daniel, 2021). Large number of bioactive compounds with high natural antioxidant capacity but little or no side effects are contained in these medicinal plants. This explains why medicinal plants, could be major source of antioxidant compounds and nutrients (Xu *et al*., 2017).

Studies have reported amazing composition of medicinal plants such as phenolic acids, flavonoids, and tannins, that are known for their health benefits as antioxidants (Sharma *et al*., 2021). These compounds that possess antioxidant properties help to capture free radicals (Munteanu and Apetrei, 2021). Knowing the chemicals available in plants is important for the discovery of therapeutic agents and new resources for subsequent application to various uses (Alotaibi *et al*., 2021).

Reactive oxygen species (ROS) are produced in the human body as a result of metabolism, food consumed by humans, overproduction and imbalance of free radicals as a result of oxidative stress (Pizzino *et al*.,2017). Animal and human cells produce excess oxygenated free radicals that maintain inflammation and other stress situations. Under normal circumstances, the rate of oxidant production is balanced by the rate of their removal but when the level of free radicals exceeds the antioxidant power of living cells, they become a problem (Munteanu and Apetrei, 2021; Halliwell and Gutteridge 2015). The loss of balance between pro-oxidants and antioxidants then results to oxidative stress. High levels of reactive oxygen species (ROS) in biological cells have a large impact on cellular function, leading to impaired metabolism, aging or disease (Rodrigo *et al*., 2013). The free radicals are also involved in the pathogenesis of many diseases such as renal failure, cancer, diabetes, inflammation and hypertension (Nwozo *et al*., 2023). Overproduction of free oxygen radicals also lead to aging, cellular injury, chronic diseases such as cardio-vascular, cerebrospinal diseases and death (Sarkar *et al*., 2016).

Compounds with high antioxidants activities are of great interest to researchers so that they can be used in foods and pharmaceutical preparations to replace the synthetic ones (Zahran *et al* ., 2020). Antioxidants are known to prevent damage caused by the free radicals produced from biological processes or stress (Barhe and Tchouya 2016). The role of the antioxidants is to neutralize the free radicals in biological cells because they have a negative impact on living organisms. These free radicals are generated from degenerative diseases or excess physiologic stress in the human or animal body (Munteanu and Apetrei 2021).

Recently, there is a growing interest in substances exhibiting antioxidant and antimicrobial properties that are used by human as food or therapeutic options (Parham *et al.*, 2020). Importantly, the role of free radicals is becoming increasingly recognized in the pathogenesis of many human diseases, including inflammation, cancer, diabetes, renal failure, atherosclerosis and hypertension (Aliyu *et al*., 2013). Antioxidants are able to prevent the adverse effects of free radicals and sometimes used as additives in meat and poultry

products to prevent or slow oxidative rancidity of fats. Also to mention the fact that natural antioxidant compounds are receiving consideration due to their relatively fewer or absence of side effects compared to synthetic ones (Ito *et al*., 1983). It is known that plant extracts which contain phenolic and flavonoid compounds have antioxidant and antibacterial effects (Bruneton, 1999). People in rural communities or otherwise use the leaves in drinks, as food or feed them to animals despite lacking the knowledge of the composition of the leaves.

Despite the wide use of *Piliostigma reticulatum* in Sub-Saharan Africa, not much research has been documented about this plant. Research on the nutritional contents of the edible parts from this part of Nigeria is essential since environment, geographical location and climate can affect the phytochemical composition of plants (Kumar *et al*., en plant 2017). The present research aimed to assess the phytochemical contents, vitamins, and antioxidant reducing power of the methanol extract of the leaves of *Piliostigma reticulatum*.

MATERIALS AND METHODS

Specimen Collection and Preparation

Leaves samples of *Piliostigma reticulatum* were obtained from Wanke village of Gusau local government in Zamfara State (Latitude 12˚ 3’ 15” N; Longitude 6˚ 37’ 56” E). The samples were identified at the herbarium of Plant Science Department, Usmanu Danfodiyo University, Sokoto (Voucher number: UDUS/ANS/0137). The leaves were dried under shed and weighed until a constant weight was achieved and grounded into powder using pestle and mortar. After grinding about 250 grams of the powder was dissolved in 500 mL of methanol and left to soak overnight with constant agitation using a magnetic stirrer (BiBy sterline LTD, staford shire ST 15 OSA, UK) for 24 hours. The mixture was then filtered using Whatman filter paper (Grade 1) and filtrate was evaporated using a rotary evaporator (Fabricant and Farnsworth, 2001) and then reconstituted with 50% dimethyl sulfoxide after evaporation

Phytochemical Analysis

Analysis of some major phytochemicals was done using various methods as described by Harborne 1984. Alkaloids and saponins were assessed using the Harborne test, while tannins were assessed using gelatin test. For flavonoids, cardiac glycosides, steroids, the Shinoda’s test, Lieberman’s, Salkowski tests were done, respectively. To test for alkaloids 1% of hydrochloric acid was added to 0.2 grams of the plant extract and a few drops of Dragendorff’s reagent added. The appearance of a white precipitate confirms alkaloids. For saponins, 0.2 grams of the same extract is dissolved in distilled water and about 2 mL of the solution dispensed in several test tubes and shaken forcefully for a while. Presence of froth that persists even after mild heating indicates saponins. About 1% gelatin solution mixed with sodium chloride was added to the plant extract to observe the presence of a white precipitate which indicates the presence of tannins. To test for flavonoids, the plant extract was dissolved in about 2 mL of sodium hydroxide (NaOH). Formation of yellow solution that turns pale on adding HCL acid indicates flavonoids presence. Acetic acid anhydride is mixed with 5 g of the leaves extract and cooled using ice after which H_2SO4 is added. A change in colour from violet to blue and finally green indicates the presence of cardiac

glycoside. To test for steroids 0.5 g of the extract is mixed with about 2 mL of chloroform and 0.2 mL H_2SO4. Production of a red/brown ring confirms the presence of steroids.

Antioxidant Screening

The antioxidant property was assessed using the diphenyl 2,2 1-1 picryl hydrazyl (DPPH). The free radical scavenging activity was assessed using the methods of Gyamfi *et al*. (1999); Ayoola *et al*., (2008). The ability of different concentrations of the leaves extract to reduce the DPPH free radical from its purple color to yellow diphenyl picryl hydrazyl is measured using a spectrometer at 517 nm. The absorbance was compared to that of a blank sample. The procedure was repeated, and average reading taken. The percentage scavenging activity of the plant extract was calculated using the formula:

$$\% SA = \frac{Absorbance\ of\ control - absorbance\ of\ sample}{Absorbance\ of\ control}$$

Ferric Reducing Antioxidant Power (FRAP)

Ferric reducing activity power of the extract was also evaluated using the ferric chloride method. Plant extract (150 µL) was allowed to react with 2850 µL of the FRAP solution for 30 min in darkness. The formation of a coloured product complex was measured at 593 nm as described by Daniel and Temikotan (2021); Benzie and Szeto (1999).

$\frac{AS\ 100}{AC}$ *where AS = absorbance of sample and AC = absorbance of control*

Vitamin Content Evaluation

The presence of vitamin A was analyzed according to the methods of Babarinde and Fabunmi (2009) for vitamin C and E the methods used were that of Arlai 2009; Haijun & Bozhong 2007. To test for vitamin A presence, the principle is to add ethanol to the extract to break up the complex and permit vitamin A to partition into the heptanes. Formation of a colourless retain was measured using a spectrometer at 450 nm. For vitamin E the test is based on the reduction of ferrous ions to ferrous which forms red colour with α:α' dipiperidyl and the absorbance is measured using a spectrometer at 539 nm. To test for vitamin C, the method is based on reaction of the extract with phosphotungstate to form a green colour that is measured at 700 nm.

RESULTS

Phytochemical analysis yielded the presence of alkaloids, glycosides, steroids, tannins and saponins. Only Flavonoids, saponins, tannins were quantified and their concentrations in the sample using methanol extraction were 3.70 µg /ml, 5.29 µg /ml and 2.90 µg /ml, respectively (Table 1).

Table 1: Phytochemical and vitamins contents of methanol extract of leaves of *Piliostigma reticulatum*

Phytochemical	Presence	µg /ml	Vitamins	µg %
Flavonoids	++	3.70	A	68.05
Tannins	++	2.90	C	232.03
Saponins	+++	5.29	E	80.71
Alkaloids	++			
Cardiac glycosides	+++			
Steroids	++			

+ Trace amount, ++Moderate, +++Large amount

Table 2: Percentage scavenging activity on DPPH and Ferric reducing antioxidant power of extract

Conc.µg/mL	Absorbance at 517nm (DPPH)	% SA	Absorbance at 700nm (FRAP)	% SA
20	1.878	11.037	1.713	14.521
40	1.433	32.117	1.622	19.062
60	0.957	54.666	1.573	21.507
80	0.521	73.319	1.39	30.589
100	0.390	81.525	1.112	44.511

2,2 diphenyl-1-picryl hydrazyl (DPPH); Ferric reducing antioxidant power (FRAP)

DISCUSSION

Preliminary phytochemical screening showed that, the leaves of *Piliostigma reticulatum* possess several phytochemicals as shown in Table 1. The amount of tannins was 2.9 µg/ml which is in agreement with the findings of Boualam *et al.* (2021) from Dakar, Senegal who also reported moderate presence of tannins in methanol extract of *P. reticulatum*. The presence of tannins, flavonoids, and alkaloids in the plant has also been acknowledged in a study carried out by Boualam *et al.* (2021). The antioxidant power of the tannins found in *P. reticulatum* was also documented by Dieng *et al*. (2020); Boualam *et al.* (2021). Tannins are known to have significant medicinal importance also due to their astringent properties (Fraga-Corral *et al*., 2021). They promote rapid healing and formation of new tissues on wounds and inflamed mucosa. Tannins are used in the treatment of varicose ulcers, hemorrhoids, minor burns, as well as topical inflammations (Chung *et al*., 1998). Tannins have also been reported to exert other physiological effects, such as acceleration of blood clotting, lowering blood pressure, decreasing serum lipids, and modulation of host immune responses (Chung *et al*., 1998).

In this study, saponins were found to have the highest concentration in the extract (5.9 µg/ml). This finding is similar to the work of Ajayi *et al*. (2019) who also reported the methanol leaves extract of *P. reticulatum* had high saponins presence. Traditionally, saponins are subdivided into triterpenoid and steroid glycosides, or into triterpenoid, spirostanol, and furostanol saponins (Vincken *et al*., 2007). Saponins decrease blood lipids, lower cancer risks, and lower blood glucose response. A high saponin diet can be used in the inhibition of dental caries and platelet aggregation, in the treatment of hypercalciuria in humans, and as an antidote against acute lead poisoning (Shi *et al*., 2004). Saponins are also widely used in

the pharmaceutical industry as adjuvants to enhance absorption of other drugs by increasing solubility or interfering in the mechanisms of absorption.

The flavonoids concentration was 3.7 µg/ml. Flavonoids are powerful antioxidants which can protect animal or human body from free radicals and reactive oxygen species. Flavonoids possess a number of medicinal benefits, including anticancer, antioxidant, anti-inflammatory, and antiviral properties. They also have neuroprotective and cardio-protective effects. These biological activities depend upon the type of flavonoid, its mode of action, and bioavailability (Ullah *et al*., 2020). Flavonoids are said to have a good effect on human health and nutrition due to their strong antioxidant capacity (Cook and Samman, 1996). They are known to have anti-inflammatory, antiviral, anticancer, and many other uses (Cook and Samman, 1996). They are able to scavenge and chelate free oxygen species (Cao *et al*., 1997). Flavones are one of the important subgroups of flavonoids. Flavones are widely present in leaves, flowers and fruits as glucosides. Celery, parsley, red peppers, chamomile and mint are among the major sources of flavones. Luteolin, apigenin and tangeritin belong to this subclass of flavonoids (Panche *et al*., 2016). Many studies have shown the benefits of these plant chemicals (phytonutrients). Researchers suggest that a diet rich in flavonoids could reduce the risk for cardiovascular disease, diabetes, and some types of cancer. It was observed that, the free radical scavenging capacity increased in multiple folds as the extract concentration was increased. However, at the concentration of 0.8 mg/ml the percentage radical scavenging only steadily increased. The FRAP method also showed an increase with higher concentrations. This is similar to the findings of Boualam *et al*. (2021).

Vitamins A, C and E have antioxidant properties. This means they can override harmful molecules, known as free radicals, which are produced within the cells and which may cause tissue damage or disease. Vitamin C is an antioxidant that helps to maintain healthy status. Vitamin E helps the body to form red blood cells and use Vitamin K (Traber and Stevens, 2011). The extract showed the presence of vitamins A, C and E, which is similar to the findings of Daniel and Temikotan (2021).

CONCLUSION

The methanol extract of *P. reticulatum* leaves has shown ability to scavenge free radicals and the potentials of being powerful antioxidant and a cheap source of vitamins C, E and A. Based on these findings, the leaves can be used as vitamin or dietary supplement and a rich source of antioxidants.

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