

Phytochemical Composition and In vitro Antioxidant Potential of Different Solvent Extracts of *Piliostigma thonningii* Schum RedH (Fabaceae)

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ABSTRACT

Traditionally, *Piliostigma thonningii* have been used in the management and treatment of diverse pharmacological conditions. This claimed potency could be attributed to its rich source of important phytochemicals. This study aimed at evaluating and comparing extracts from the different solvents of the plant leaves for its qualitative phytochemical composition, polyphenolic composition and *in vitro* antioxidant properties using different models. The plant leaves were collected, prepared and Soxhlet extracted using distilled water, methanol, ethanol, acetone, dichloromethane and ethyl acetate. While the phytochemical screening was performed using standard procedures, the antioxidant screening were performed using DPPH free radical scavenging and ferric reducing assay methods respectively. The result revealed the percentage extraction of the investigated solvents to be in the order MET (31.30) > ETH (28.60) > DW (19.70) > ACT (11.50) > DCM (9.40) > EA (5.30). MET was found to exhibit the highest (91.10 and 88.80%) and ACT have the least (27.30 and 31.30%) antioxidant activity among the investigated solvents at the highest investigated concentration of 100 µg/mL. AA however showed a 97.80% activity in the ferric reducing power and DPPH assay respectively. Methanol is a better extracting solvent if maximum extraction of phytochemicals with diverse pharmacological activity is desired from the leaves of *P. thonningii* when compared with the other solvents investigated.

Keywords: Antioxidant, Extraction, Phytochemicals, *Piliostigma thonningii*

Introduction

Africa is endowed with medicinal plants which has been reported to serve as a promising link in modern medicine development utilized globally for the management and treatment of diverse disease conditions (Toma *et al.*, 2009). The

increasing desire to scientifically screen medicinal plants for antioxidant potential is as a result of their health benefits recognition (Elajalde, 2001; Halliwell and Whitman, 2004; Armijos *et al.*, 2018). Medicinal plants have been scientifically proven to possess constituents capable of reversing oxidative stress caused by

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species like reactive oxygen species (ROS) and reactive nitrogen species (RNS) among others implicated to be responsible for atherosclerosis, cancer, diabetes, cataracts, asthma, cardiovascular problems, and ageing among others (Armijos *et al.*, 2018).

The West African plant *Piliostigma thonningii*, Schum (Milne-Redhead) belongs to the subfamily Caesalpinioideae with about 133 genera (Ogbiko *et al.*, 2020). It belongs to the legume family, Leguminosae/Fabaceae comprising of trees, shrubs or very rarely scramblers. The plant is mainly found in open and moist woodlands in the savannah regions as well as wooded grasslands in low to medium altitudes (Simpson, 1999; Jimoh and Oladiji, 2005). The plant is perennial in nature, its white or pink-colored flowers are produced around November and December. The twigs are hairy while the bark is rough, longitudinally fissured as well as creamy brown in color. It has a leathery leaf of up to 15 x 17 cm, bilobed one eight to one third the way down with a small bristle notch, glossy above and heavily veined and somewhat rusty hair below. It bears hairy flat-pod fruits that turn nasty-brown and woody on maturity and usually persist on the plant until around June to September (Jimoh and Oladiji, 2005). It is commonly known as monkey's bread or camel's foot tree. Locally, it is known as “kalgo” (Hausa), “kalur” (Kanuri) and “inpilataki” (Higgi). It has been used for the treatment of a variety of diseases in traditional medicine. The root and twig of the plant are used for the trado-medical treatment of dysentery, fever, respiratory ailments, snake bites, hookworm and skin diseases, gastrointestinal tract (GIT) problems (Iwalewa *et al.*, 1990; Asuzu and Onu, 1994). The stem is used in the management of dysentery, pile and male erectile dysfunction (Halilu *et al.*, 2017) while the leaves are used for the treatment of inflammation, bilharzia, eye diseases, catarrh (Kwaji *et al.*, 2010), malaria fever (Rabo and Sanusi, 2001), wounds, chronic ulcers, diarrhea, toothache, gingivitis, cough and bronchitis (Thagriki and Daniel, 2018). The decoction of the leaves and bark has been used for the treatment of heart pain, arthritis, pyrexia, leprosy, sore throat, toothache, gingivitis, cough and bronchitis among others (Ibewuiké *et al.*, 1997;

Ighodaro and Omole, 2012). This study is therefore designed to evaluate and compare the phytochemical composition as well as the *in vitro* free radical scavenging activities using DPPH and ferric reducing power models of the methanol, ethanol, water, acetone, dichloromethane and ethyl acetate solvent extracts of the *P. thonningii* leaves found in Sokoto State North - West Nigeria.

Materials and Methods

Collection of sample

The fresh leaves of *P. thonningii* were collected from Jabo district in Tambuwal local government area of Sokoto, Sokoto State Nigeria. The leaves were taxonomically identified by Mallam Abdulaziz Salihu of the Botany unit, Department of Biological Sciences, Faculty of Sciences, Usmanu Danfodiyo University Sokoto. Sokoto State Nigeria. A herbarium specimen was prepared and deposited with voucher number UDUH/ANS/0137 issued.

Preparation of plant materials

The plant samples were washed severally using clean running tap water to remove the earthly impurities. The leaves were air-dried at room temperature for two weeks and pulverized into fine powder using an electric milling machine.

Extraction

One hundred gram (100 g) each of the pulverized plant sample was exhaustively extracted by Soxhlet extraction using 500 mL of distilled water, methanol, ethanol, acetone, dichloromethane, and ethyl acetate respectively for a 48 h duration at room temperature. The extract was filtered using Whatman filter paper No.1. Rotary evaporator (Polylab, India) was used to evaporate the filtrates, separately, and re-concentrated using water bath. The extracts obtained were stored in the freezer at - 4°C until needed for experiment. The extraction yield for each solvent was determined.

Qualitative phytochemical screening

The qualitative phytochemical analysis of *P. thonningii* leaves extracts were carried out, individually to screen for the presence of notable phytochemicals like alkaloids, flavonoids,

phenolics, saponins, tannins, cardiac glycoside, anthraquinone, and terpenoids using standard methods (Sofowra, 1993; Harborne, 1993).

Antioxidant Assay

DPPH free radical scavenging activity

The ability of the different solvent extracts of *P. thonningii* to scavenge 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radicals was estimated as previously described (McDonald *et al.*, 2001) with slight modifications. The extracts (3 mL) with five different concentrations (6.25, 12.50, 25.00, 50.00 and 100.00 µg/ml) were mixed with 1 ml of a 0.1 mM methanolic solution of DPPH. The absorbance was measured by a spectrophotometer at 517 nm at 30 minutes intervals against a blank (pure ethanol). The percentage of radical scavenging activity was calculated using the formula:

$$\% \text{ DPPH radical inhibition} = 1 - \frac{A_{\text{test}}}{A_{\text{control}}} \times 100$$

Ferric reducing or antioxidant power was determined as described earlier (Ebrahimzadeh *et al.*, 2008). Briefly, 100 µL of the extract (6.25 - 100 µg/mL) were mixed with 2.5 mL of 200 mmol/L phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide were incubated at 50°C for 20 min. Then, 2.5 mL of 10% trichloroacetic acid were added, and the tubes were centrifuged at 10,000 rpm for 10 min. 5 mL of the upper layer were mixed with 5.0 mL distilled water and 1 mL of 0.1% ferric chloride. The absorbance of the reaction mixtures was measured at 700 nm. Ascorbic acid was used as a positive control.

Statistical Analysis

Results were expressed as means ± standard deviations (SD). Statistical comparisons were made using the student t-test and one-way analysis of variance (ANOVA) where applicable.

Results

Extractive yield

The results of the extractive yield of the different solvent extracts is presented in

Table 1 using 100 g powdered leaf sample of *P. thonningii* after 48 h.

Table 1: Percentage yield of different solvent extracts of *P. thonningii*

Solvent	Percentage Yield
Distilled water (DW)	19.70
Methanol (MET)	31.30
Ethanol (ETH)	28.60
Acetone (ACT)	11.50
Dichloromethane (DCM)	9.40
Ethyl acetate (EA)	5.30

Qualitative Phytochemical Screening

The preliminary phytochemical analyses showed the presence of major classes of secondary metabolites as presented in Table 2.

Table 2: Phytoconstituents present in the different solvent extracts of *P. thonningii*

Phytochemicals	DW	MET	ETH	ACT	DCM	EA
Alkaloids	+	+	+	-	-	+
Flavonoids	+	+	+	-	-	+
Phenolics	+	+	+	-	-	+
Saponins	+	+	+	+	+	+
Tannins	+	+	+	+	+	-
Cardiac glycosides	+	+	+	-	-	-
Anthraquinone	+	+	+	-	-	+
Terpenoids	+	+	+	+	+	+

Key: (+) = present; (-) = absent; DW = Distilled Water; MET = Methanol; ETH = Ethanol; ACT = Acetone; DCM = Dichloromethane; EA = Ethyl acetate

Antioxidant Assay

The radical scavenging property of the solvent extracts at varying concentrations of 6.25, 12.50, 25.00, 50.00 and 100 µg/mL is

represented in Figures 1 and 2. Statistically significant differences were observed in the

compared to the reference standard (ascorbic acid).

free radical scavenging activities of the extracts using the investigated models when

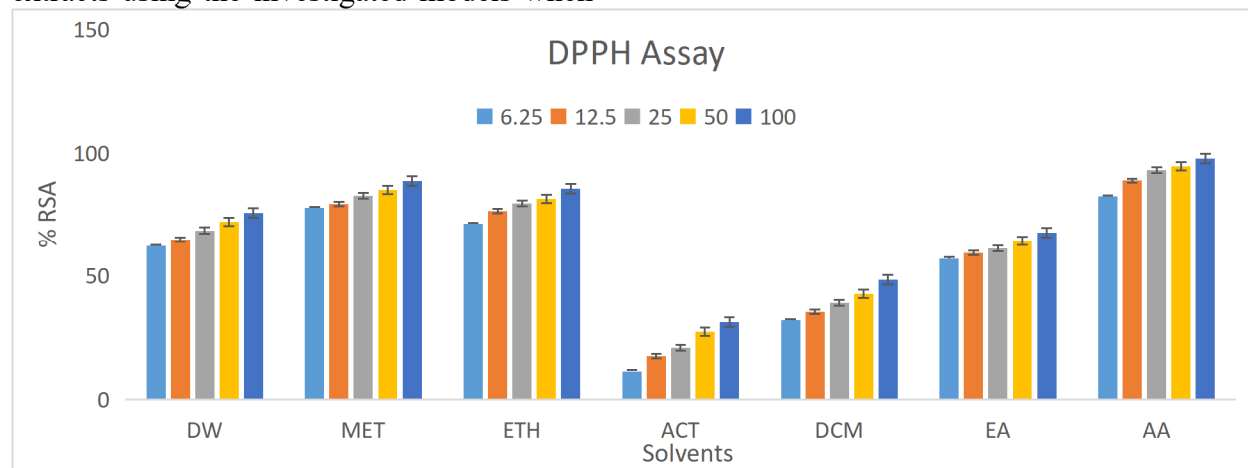


Figure 1: DPPH radical scavenging activity of different solvent extracts of *P. thonningii* and the synthetic antioxidant (ascorbic acid) at different concentrations (6.25, 12.50, 25.00, 50.00 and 100 µg/mL). Each point is a mean from triplicate measurements. RSA = Radical Scavenging Activity; DW = Distilled Water; MET = Methanol; ETH = Ethanol; ACT = Acetone; DCM = Dichloromethane; EA = Ethyl acetate

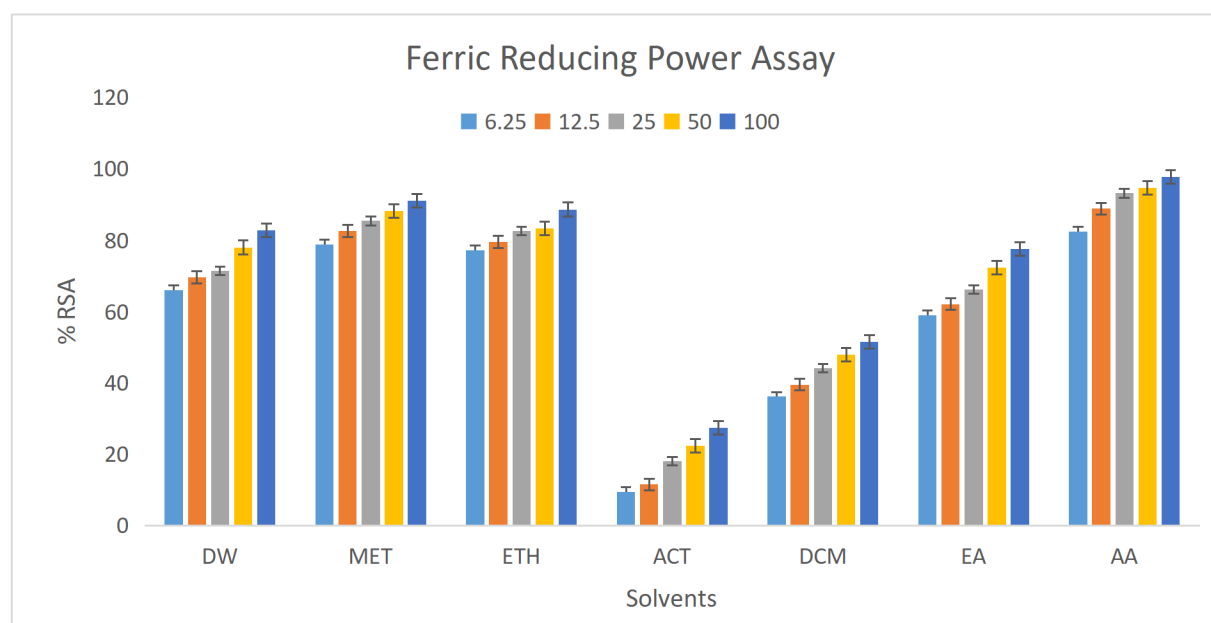


Figure 2: Ferric educing power activity of different solvent extracts of *P. thonningii* and the synthetic antioxidant (ascorbic acid) at different concentrations (6.25, 12.50, 25.00, 50.00 and 100 µg/mL). Each point is a mean from triplicate measurements. RSA = Radical Scavenging Activity; DW = Distilled Water; MET = Methanol; ETH = Ethanol; ACT = Acetone; DCM = Dichloromethane; EA = Ethyl acetate

Discussion

The variation in the percentage yield of the different extracts of the plant is expected as the solvents have varying degree of polarity. Methanol had the highest extractive value confirming it to be the best solvent for the extraction of the phytoconstituents present in the plant sample. Generally the polar solvents of DW, ETH and MET was found to have a higher extractive yield than that of the semi polar solvents of ACT, DCM and EA. Extraction methods and duration of extraction have been reported by Mehta *et al.* 2014 to affect the extractive yield of plant materials. Others related factors could be difference in ecotype, chemotype, phenophases, variations in environment conditions such as temperature, relative humidity, irradiance and photoperiod among others (Marotti *et al.*, 1994; Ngo *et al.*, 2017; Dieu-Hien *et al.*, 2019).

The results as presented in Table 2 revealed the presence of important secondary metabolites which have been established to possess diverse pharmacological activity. The role of medicinal plants in decreasing free radical-caused tissue injury reveals their antioxidant activity (Sylvie *et al.*, 2014). In both antioxidant assay, methanol and acetone extracts had the best and least free radical scavenging activity when compared to other solvent extracts. Their activities was not however statistically significant when compared to ascorbic acid standard used. The reported phytochemicals present in the solvent extracts (Table 2) have been established to be powerful protective agents that can prevent molecular cells from free radicals damages. Flavonoids and phenolics are important groups of secondary metabolites reported to enhance the protection of biological systems against the oxidative stress damages on the macromolecules, such as carbohydrates, proteins, lipids and DNA (Hamzah *et al.*, 2013).

In addition to attributing the observed antioxidant activity to the presence of the screened phytochemicals, several researchers have suggested that the antioxidant activity of plant extracts may also be partly due to some other polyphenolics such as anthocyanidins, carotenoids, and steroids among others which was

not screened for in this study. The superior antioxidant activity of the ascorbic acid standard compared to the solvent extracts may probably be due to its higher purity when compared to the solvent extracts which may possess inhibitory constituents to the investigated activity (Ogbiko *et al.*, 2018)

Conclusion

The promising *in vitro* antioxidant activity of the different solvent extracts of the plant might be due to the presence of supportive phytochemicals reported. This study lends scientific credence in support of the folkloric use of the leaves of *P. thonningii* plant for the investigated activities.

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