International Journal of Innovative Research and Advanced Studies (IJIRAS)
Volume 10 Issue 8, August 2023

ISSN: 2394-4404

In-Vitro Antioxidant Potential Of The Methanolic Leaf Extract Of Piliostigma Thonningii

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Abstract: The aim of this study is to determine the In-vitro Antioxidant properties of the methanolic leaf extract of Piliostigma thonningii. The methanolic leaf extract of Piliostigma thonningii which is used in ethno-medicine for the treatment of wounds, ulcers and gingivitis was investigated for its in-vitro antioxidant potential in this present study. The antioxidant activity of the leaf extract of P. thonningii was determined using three methods: the 1,1-diphenyl-2-picryhydrazyl (DPPH) free radical scavenging assay, Ferric ion reducing antioxidant Assay, and hydrogen peroxide reducing assay. The result of the study of the plant extract showed marked antioxidant activity, as evidenced by the high percentage inhibition values as compared with that of the standards (α-tocopherol, Ascorbic and Butylated hydroxyanisole).This study therefore, suggest that the leaves of P. thonningii possess antioxidant potentials. Further studies is required to investigate the in-vivo antioxidant potential of the studied plant extracts, as well as isolation and characterization of bioactive antioxidant compounds that are potent against oxidative stress.

Keywords: Antioxidant, Piliostigma thonningii, Oxidative Stress

I. INTRODUCTION

Piliostigma thonningii is a legume belonging to Caesalpiniaceae subfamily under Fabaceae family. This plant is well distributed in many African countries ranging from Senegal to Zambia. Twigs and leaves of this plant are traditionally used to manage malaria fever, snake bites, and dysentery, among other conditions (Orwa *et al.,* 2009; PROTA 2020). Stem barks of *P. thonningii* are used for management of intestinal, respiratory, and inflammatory conditions (Afolayan *et al.,* 2018; Kwaji *et al.,* 2010). Besides the leaves, stem bark decotions and smoke are traditionally used to manage insanity and rheumatism among other conditions (Kaigongi *et al.,* 2015).

Piliostigma thonningii and other species in the genus have been reported to have a wide range of uses to mankind ranging from food for man and animals and also a wide range of medicinal uses (Ibewuike *et al.,* 1996). The medicinal uses include treating loose stool in teething children, wound dressing, ulcers treatment, worms' infestation, arrest bleeding, inflammations, bacterial infections, gonorrhea, stomach ache, headache, etc (Burkhil, 1995; Ozolua *et al.,* 2009).

The roots and twigs have been used locally in the treatment of dysentery, fever, respiratory ailments, snake bites, hookworm and skin infections and the leaf extracts has been used for the treatment of malaria all over Eastern Nigeria (Kwaji *et al.,* 2010). The plant is used in ropes making, making of dyestuff or tanning of leather, household utensils, roofing tiles, fencing, bridge building and farm implements,

because they are deep rooted they are used as erosion control measures, the woods are used as stakes to support plants of weak stems or creepers like yams.

Other Common names of *Piliostigma thonningii* include Camel's foot, monkey bread, kalgo(Hausa), Omukpakpaajalu (Igala), Mchekeche (Swahili), Kharub (Arabs), Abefe (Yoruba), Okpoatu (Ibo).

Oxidative stress is a phenomenon caused by an imbalance between the production and accumulation of free radicals and release of certain hormones and the ability of the biological system to eliminate them from the body (Gabriele *et al.,* 2017). These Free radicals contain unpaired electrons usually in the outer orbitals and as such are very reactive and if uncontrolled, can attack important molecules. Free radicals are of physiological importance when produced in adequate amount in the body. They are involved in normal cellular signaling, and in oxidative phosphorylation (Meda *et al.,* 2019). However, when produced in excess amount that can overwhelm the body's capacity to eliminate them by the way of the antioxidant systems, it usually results to oxidative stress, causing damages to important biological molecules such as proteins, DNA and lipids (Droge, 2002; Genestra, 2007; Pizzino *et al.,* 2017; Liu *et al.,* 2018). The free radicals of biological importance are those of the reactive oxygen species and that of the reactive nitrogen species including the superoxide radical, hydroxyl radical and peroxynitrite (Galley, 2011; Mantzarlis *et al.,* 2017). Oxidative stress is implicated in many diseases including diabetes, hypertension, cardiovascular diseases, cancer and even ageing (Taniyama and Griendling, 2003).

Antioxidants are the opposite of oxidants. They function to counterbalance the effects of oxidants (free radicals) in the body. Antioxidants can either be enzymatic or non-enzymatic. They exhibit various mechanisms of action including inhibiting lipid peroxidation (by inactivating lipoxygenase), scavenging free radicals and reactive oxygen species, preventing the decomposition of hydrogen peroxides into free radicals and chelating metal ions (Ghimeray *et al.,* 2009; Veeru *et al.,* 2009). Inadequacy of antioxidants within the biological system usually poses a medical challenge as free radicals would normally increase. This can be prevented through the ingestion of plant products that are rich in antioxidants such as fruits and vegetables.

II. MATERIALS AND METHODS

A. CHEMICAL

Methanol, 1,1 diphenyl-2-Picryhydrazyl acid, Hydrogen Peroxide (H_2O_2), Phosphate buffer saline, Butylated hydroxyanisole (BHA), Ascorbic (Vitamin C), α-tocopherol (Vitamin E).

B. COLLECTION AND IDENTIFICATION OF PLANT SAMPLE

Fresh leaves of *Piliostigma thonningii* were used for this study. They were collected from the Forest in Federal university of Agriculture, Makurdi, Benue State. They were authenticated by a plant taxonomist at the Department of Botany, Federal University of Agriculture, Makurdi.

C. PREPARATION OF PLANT EXTRACT

Upon collection of the leaves of *P. thonningii,* they were washed and kept under shade until they were completely dried. The leaves were then milled into powder using ceramic mortar and pestle.100g of the milled sample was extracted by cold maceration techniques for 24hrs in 300ml of methanol, this was filtered using a sterilized Whatman filter paper No.1 and the filtrates was concentrated in a water bath at 50^0C until a solid residue of constant weight was obtained. The concentrated extract was stored in the refrigerator until use to prevent microbial growth (Gülçin *et al.,* 2006). The percentage yield of the extract was calculated as follows:

$$\text{The percentage yield of the extract} = \frac{\text{Weight of concentrated extract}}{\text{Weight of original sample}} \times \frac{100}{1}$$

D. IN-VITRO ANTIOXIDANT ASSAY OF METHANOLIC LEAF EXTACT OF PILIOSTIGMA THONNINGII

a. PREPARATION OF SAMPLE EXTRACT

2g of extract was dissolved in 20ml of methanol and the resultant solution was filtered with whatman filter paper to get the filtrate.

b. DETERMINATION OF IN-VITRO 1,1-DIPHENYL-2-PICRYHYDRAZYL (DPPH) RADICAL SCAVENGING ACTIVITIES OF METHANOLIC LEAF EXTRACT OF PILIOSTIGMA THONNINGII

Experimental Procedure

The ability to scavenge the "stable" free radical DPPH or antioxidant activity was determined using the DPPH free – radical scavenging method as described by Gulcin *et al.* (2002) with slight modification:

3.92 mg of 1, 1-diphenyl-2-picryhydrazyl radical (DPPH), was weighed and dissolved in 100 ml of methanol to give a 2mM solution. 3.0 ml of the methanolic solutions of DPPH was added to each of the extracts at varying concentrations of 0.5ml, 0.25ml and 0.13ml with doses ranging from 50 mg/ml to 13 mg/ml. The reaction mixture was then allowed to stand at room temperature in a dark chamber for 10 minutes. The change in color from deep violet to light yellow was then measured and the decrease in absorption at 517 nm of DPPH was measured 10 minutes later. The actual decrease in absorption was measured against that of the control and the percentage inhibition was also calculated. The same experiment was carried out on butylated hydroxyanisole (BHA), α-tocopherol and ascorbic acid which are known antioxidants.

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Calculation Of DPPH Value

The radical scavenging activity (RSA) was calculated as the percentage inhibition of DPPH discoloration using the equation below:

$$\%RSA = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times \frac{100}{1}$$

c. *DETERMINATION OF HYDROGEN PEROXIDE REDUCING EFFECT OF THE METHANOLIC LEAF EXTRACT OF P. THONNINGII*

Procedure

The hydrogen peroxide reducing effect was determined using the method described by (Chang *et al.,* 2012; Oloyede and Farombi, 2010).

A solution of 2 mM hydrogen peroxide was prepared in phosphate buffered-saline (PBS) pH 7.4 3mls of the H_2O_2 was placed into a test tube, the extract at the following concentrations; 50mg/ml, 25mg/ml and 13mg/ml was added to the H_2O_2 solution and incubated in the dark for 10min. Decrease in absorbance of H_2O_2 at 285nm was determined spectrophotometrically 10 minutes later against a Control containing the test extract in PBS without H_2O_2. The same experiment was carried out on Butylated hydroxyanisole (BHA), ascorbic acid and α-tocopherol which are known antioxidant standards.

Calculation Of Percentage Hydrogen Peroxide (H_2O_2) Reducing Effect

$$\text{Hydrogen Peroxide scavenged (\%)} = \frac{Acontrol - Atest}{Acontrol} X100$$

Where $A_{control}$ = absorbance of control sample and A_{test} = absorbance in the presence of the samples of extracts or standards.

E. REFERENCE STANDARDS

The standards used for comparison was α-tocopherol, Ascorbic and Butylated Hydroxyanisole

F. STATISTICAL ANALYSIS

Data was expressed as mean ± standard deviation and analyzed by One-way Analysis of Variance (ANOVA) using Statistical Package for social sciences version 22 (SPSS Inc., Chicago, USA). Statistical significance was accepted at $P < 0.05$.

III. RESULTS

Extract	Weight	%yield
Methanol	10.92g	10.92

Table 1: Percentage yields of methanol extracts of Piliostigma thonningii

The table above shows the weight to be 10.92g and the Percentage yield to be 10.92%.

A. IN-VITRO 1,1-DIPHENYL-2-PICRYLHYDRAZYL (DPPH) RADICAL SCAVENGING ACTIVITIES OF THE METHANOLIC LEAF EXTRACT OF P. THONNINGII

The *In-vitro* DPPH radical scavenging activities of the methanolic leaf extracts of *P. thonningii* was evaluated in this study. Generally, the obtained results depicted a positive dose-dependent increase in percentage DPPH radical scavenging activities of the extract and the standards except for a fluctuation at α-tocopherol (Table 2).The percentage radical scavenging activities of the studied plant extracts were compared among the tested concentrations in this study. The results showed that there is significant difference among the percentage radical scavenging activities at concentrations of 13mg/mL, 25mg/mL, and 50mg/mL of the Methanolic leaf extract of *P. thonningii* (P< 0.05).

The DPPH radical scavenging activities of the methanolic leaf extract of *P. thonningii* were compared to that of the standards at various concentrations. The results showed that at the concentration of 13mg/ml, there is significant (P< 0.05) decrease compared to α-tocopherol and Butylated hydroxyanisole. Also, the extract showed significantly (P< 0.05) higher DPPH radical scavenging effect compared to Ascorbic at the same concentration (Table 2).

At a concentration of 25mg/mL, the percentage DPPH radical scavenging activity of the methanolic leaf extract of *P. thonningii* as compared with α-tocopherol and Butylated hydroxyanisole were significantly different (P< 0.05; Table 2), but no significant difference with Ascorbic (P> 0.05; Table 2).

At a concentration of 50mg/mL, the percentage DPPH radical scavenging activity of the methanolic leaf extract of *P. thonningii* when compared with the standards (α-tocopherol, Ascorbic and Butylated hydroxyanisole) were significantly different (P< 0.05; Table 2) and showed a significant increase (Table 2).

Conc.(mg/ml)	MLEPT	α-tocopherol	Ascorbic	BHA
13	57.40 ± 0.87^{aB}	97.76 ± 0.77^{a}	40.61 ± 0.00^{aB}	89.10 ± 2.04^{aB}
25	73.50 ± 0.30^{aB}	53.30 ± 7.07^{aB}	75.35 ± 0.04^{B}	91.66 ± 0.23^{a}
50	76.77 ± 0.50^{aB}	97.40 ± 3.21^{a}	89.98 ± 0.53^{aB}	97.94 ± 0.37^{aB}

Table 2: Mean ± Standard Deviation of In-Vitro 1,1-diphenyl-2-picrylhydrazyl (DPPH) Radical Scavenging Activities of the Methanolic Leaf Extract of P. thonningii

Values with the same lowercase superscript letter across the rows compared to MLEPT and uppercase superscript letter along the columns compared at 13mg/ml concentration are significantly different (P< 0.05; one-way ANOVA by LSD).

MLEPT = Methanolic Leaf Extract of *Piliostigma thonningii*

BHA =Butylated hydroxyanisole

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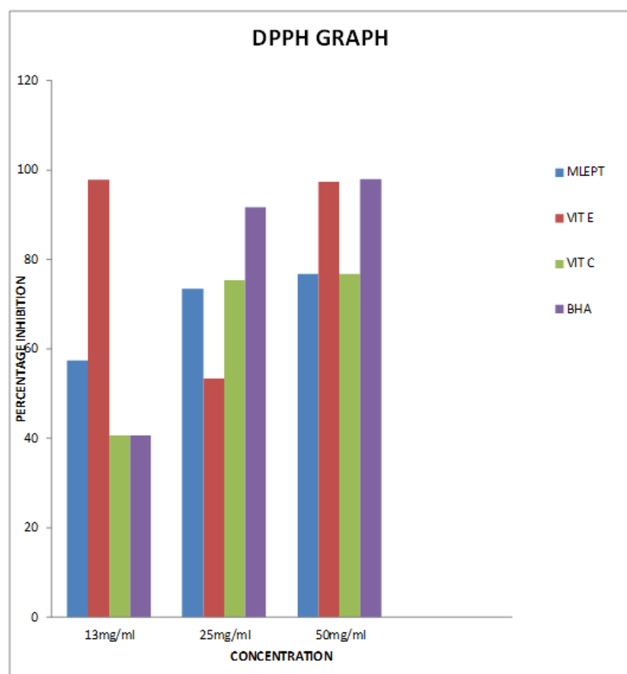

Figure 1: DPPH Assay GRAPH showing a comparison between MLEPT and Standards (α-tocopherol, Ascorbic and Butylated hydroxyanisole)

B. IN-VITRO HYDROGEN PEROXIDE REDUCING EFFECT OF THE METHANOLIC LEAF EXTRACT OF P. THONNINGII

The Hydrogen Peroxide reducing effect of the methanolic leaf extract of *P. thonningii* were investigated *in-vitro* in this study. A comparison among the percentage inhibitions caused by the studied plant extracts was done in this study. The results revealed that the methanolic leaf extract of *P. thonningii* showed a dose dependent decrease reducing effect which were significantly different ($P < 0.05$; Table 3) when compared at 13mg/mL as a baseline. The Hydrogen peroxide reducing effect of the methanolic leaf extract of *P. thonningii* was compared with that of the standards (α-tocopherol, Ascorbic and Butylated hydroxyanisole). The results showed that the effect of extract decreased compared to that of the standards at 13mg/ml but was only significantly ($P < 0.05$) different against BHA.

At 25mg/ml concentrations, the effect of the *P. thonningii* leaf extract significantly ($P < 0.05$) increased compared to α-tocopherol and BHA and significantly decreased against Ascorbic. On the other hand, the effect of the extract decreased significantly ($P < 0.05$) compared to all the standards at 50mg/ml concentrations ($P < 0.05$; Table 3).

Conc.(mg/ml)	MLEPT	α-tocopherol	Ascorbic	BHA
13	71.51 ± 7.77^a	82.33 ± 3.15	75.92 ± 1.37	92.87 ± 3.64^a
25	52.02 ± 0.45^B	36.41 ± 0.13^a	75.73 ± 4.67^B	32.38 ± 0.34^a
50	50.63 ± 0.07^B	81.46 ± 5.22	87.62 ± 1.58^a	75.99 ± 3.54^a

Table 3: Mean ± Standard Deviation of Hydrogen Peroxide Reducing effect of the Methanolic Leaf Extract of P. thonningii

Values with the same lowercase superscript letter across the rows compared to MLEPT and uppercase superscript letter along the columns compared at 13mg/ml concentration are significantly different ($P < 0.05$; one-way ANOVA by LSD).

MLEPT = Methanolic Leaf Extract of *Piliostigma thonningii* BHA = Butylated hydroxyanisole

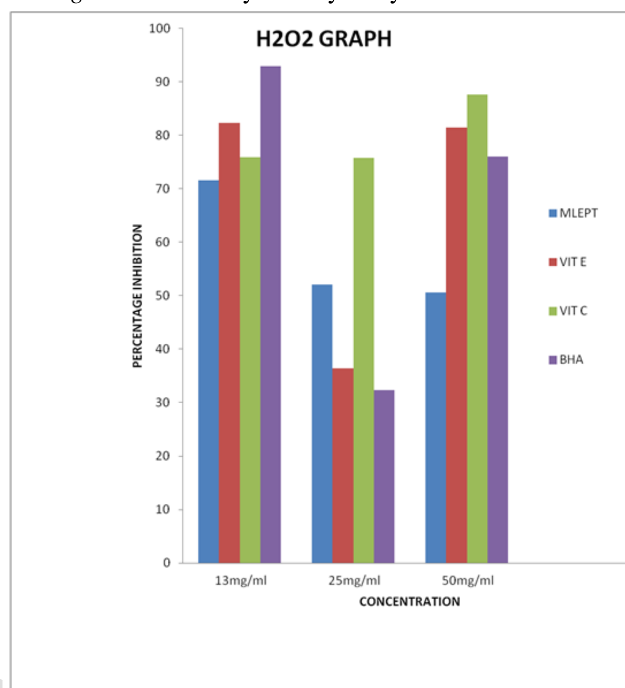

Figure 2: Hydrogen Peroxide (H_2O_2) Assay Graph showing a comparison between MLEPT and Standards (α-tocopherol, Ascorbic and Butylated hydroxyanisole)

IV. DISCUSSION

The present study investigated the *in-vitro* antioxidant potential of methanolic leaf extract of *P. thonningii.* 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and hydrogen Peroxide (H_2O_2) reduction assays were performed on the plant extract, while α-tocopherol, ascorbic acid and butylated hydroxyanisole served as standard antioxidants and the basis for comparison.

The results obtained for the *in-vitro* 1,1- diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of the methanolic leaf extract of *P. thonningii is* presented in Table 2. The results revealed that the methanolic leaf extract of *P. thonningii* has high DPPH radical scavenging activity as indicated by its dose- dependent increase in percentage inhibition value. However, this effect was less, compared to the effects produced by the α-tocopherol, ascorbic acid and BHA that served as the standards. The DPPH radical scavenging effect of the plant extract may have been due to the actions of the plant secondary metabolites that behaves like endogenous antioxidants (Sun and Ho, 2005).

The result of the hydrogen peroxide (H_2O_2) reducing effect of the methanolic leaf extract of *P. thonningii* revealed a positive effect. At 50mg/mLof the extract, percentage inhibition was 50.63 ± 0.07 while at 13mg/mL the highest percentage inhibition was recorded for the plant extract (71.51

± 7.77). At similar concentration with that of the standards (α-tocopherol, Ascorbic and BHA) the results obtained for the plant extracts, were lower i.e OH− free radical scavenging potential of the extracts was found to be lower than that of the reference compounds, except at the concentration of 25mg/mL. The hydrogen peroxide scavenging activities of the plant extract could be attributed to the presence of antioxidant phytochemicals as showed in this study and which agrees with previous reports (Oloyede and Farombi, 2010).

V. CONCLUSION

The methanolic leaf extract of *P. thonningii* have *in-vitro* DPPH radical scavenging effect and Hydrogen Peroxide reducing effect, because the results showed that the leaf extract of *Piliostigma thonningii* had considerable antioxidant activity with respect to their high percentage inhibition, this is due to the presence of Phytochemicals such as phenols, flavonoids, alkaloids and tannins. Therefore, the methanolic leaf extract of the studied plant may be a potential antioxidant compounds source and an alternatives for the management of oxidative stress associated conditions.

VI. RECOMMENDATION

This study recommends further research leading to isolation and characterization of the pure bioactive molecules of *P. thonningii* leaves. Furthermore, studies aimed at investigating the *in-vivo* antioxidant efficacy of the studied plant extracts are encouraged to ascertain if the *in-vitro* antioxidant results reported herein, can be replicated *in-vivo*.

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