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ANTIOXIDANT POTENTIAL OF THE LEAF EXTRACT OF PILOSTIGMA THONNINGII (*CAESALPINIACEA*)

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ABSTRACT:

In this study, the antioxidant activity of the leaf extracts of *P.thonningii* was evaluated using the DPPH free radical scavenging assay. The butanol fraction of *P.thonningii* exhibited the higher activity (EC_{50}=15.39±0.01 µg ml^{-1}), while crude extract of *P.thonningii* had the activity (EC_{50}=10.75±0.10 µg ml^{-1}). These results suggested strong antioxidant activity potentials of the plant.

KEY WORDS: antioxidant, pilostigma thonningii, caesalpiniacae, free radicals

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INTRODUCTION:

Antioxidant agents are compounds that have the potentials to scavenge reactive oxygen species of free radicals. These free radicals play important roles in energy production, synthesis of some biomolecules, phagocytosis and cell growth. *Piliostigma thonningii* (Schum) Milne-Redhead belong to the family Caesalpinaceae. The species widely distributed in Nigeria but is often confused with Pilostigma reticulatum (DC) Hochst owing to their striking similarities in their morphology and common vernacular names *P.thonningii* is distinguishable for its larger leaves and on the undersurface of its leaf the inter-vein area is pubescent, while P. reticulatum leaf is glabrous (Burkill, 1995). Both plants are frequently used interchangeably in ethno medicine in Africa to treat wounds, chronic ulcers, diarrhea, cough, respiratory disorders and toothache (Assi and Guinko, 1991).

Pharmacological reports have shown that the ethanolic extract of the stem bark of *P. thonningii* bark induces persistent contractions of the isolated guinea pig ileum (Asusu and Ugwuja, 1991). Other reported bioactivity of *P. thoningii* includes larvicidal activity against common intestinal parasites of cattle (Asusu and Onu, 1993) and antimicrobial activity against the yeast. *S. cerevisiae* and *S. lutea* (Tanigushi et al, 1978). On the other hand, the alcoholic extract of the leaves, pod and root of *P. reticulatum* also possess both antimicrobial and antiflammatory activities (Adeyanju et al., 2011).

Phytochemical studies with this genus have demonstrated the presence of C-methylflavonols as the major principles responsible for both antimicrobial and anti-inflammatory activities in the leaf extract of *P. thonningii* (Ibewuike et al (1996); Ibewuike et al. (1997). Previous investigation of the leaf extract of *P. reticulatum* has also yielded similar C-methylflavonols (Aderogba et al., 2003). Earlier studies on both species have revealed the presence of taninin content of up to 20%.

It is well known that antioxidant activity in higher plants has often been associated with phenolic compounds (Thabrew, 1998), which have been demonstrated to be present in both *Piliostigma species*. Generation of free radicals in the body beyond its antioxidant capacity leads to oxidative stress which has been implicated in diseases like cancer, diabetes, hypertension, inflammation and AIDS (Burits and Bukar, 2005). Some of these diseases have no known remedy for now, many plant constituents have proven effective as -remedy for some diseases and accounted for about seven thousand pharmaceutical important compounds in Western Pharmacopoeia and a number of important drugs, for examples: taxoland artermisimin (Tashibangu et al., 2002). In this study, the antioxidant activity potentials of the leaf extracts of the *Pilostigma thonningi specie* was evaluated using the DPPH free radical scavenging assay. This was in view of our quest in finding novel antioxidant agent from natural 'sources.

MATERIALS AND METHODS

Chemicals: All chemicals used were of analytical grade obtained from BDH Chemicals Ltd, Poole England, Sigma chemical Co. USA and Fluka chemiika.

Plant material: Leaves of *P. thonningii* were collected at College of Forestry Jos, Nigeria.

Mr. Iliya I. Arabo of the Herbarium section, Plant Science Department, University of Jos authenticated the plants where voucher specimens were deposited.

Evaluation of antioxidant activity: The determination of the radical scavenging activity of each of the crude extracts and Butanol extract was carried using the DPPH (1,1--diphenyl-2-picryIhydrazyl) assay as described *by (*Mensor et al., 2001) with a slight modification. 1.0mL of DPPH (0.25 mM) in methanol was added 2.0 mLof the varying concentrations of the test samples (250, 125, 50, 25, 10 and 5 ug mL^{-1}). The reaction mixture was then allowed to stand at room temperature in a dark chamber for 30 mm. The changes in colour from deep violet to light yellow was then measured at 514 nm on a spectrophotometer (Pharmacie Biotech, Novaspec II). The decrease in absorbance was then converted to percentage antioxidant activity (AA%) using the formula:

$$\text{AA\%} = \frac{A_{blank} - A_{sample}}{A_{blank}} \; X\ 100$$

Where A_{blank} = blank absorbance

A_{sample} = sample absorbance.

The EC_{50} is defined as the concentration sufficient to elicit 50% of a maximum effect estimate in 100% Its values were calculated from the linear regression of plots of concentration of test samples against the mean percentage of antioxidant activity obtained from three replicate assays.

RESULTS AND DISCUSSION

Table 1 shows that crude extract and butanol extract from *P. thonningii* Exhibited substantial inhibition of DPPH activity, with a 50% inhibition (EC_{50}) values ranging between 10.75±0.10 to 15.39±0.01 ug mL"". The crude and butanol exhibited moderate antioxidant activity with EC_{50} values of 10.75±0.10 and 15.39±0.01 ug mL^{-1}respectively *P. thoninngii.* These values were significantly ($p< 0.05$) higher than that of the ascorbic acid which was used as standard.

Table 1: EC_{50} values in µg mL^{-1} for scavenging of the DPPH radicals

Samples	
Crude extract	10.75±0.10
Butanol extract	15.39±0.01

Ascorbic acid as Stand, EC_{50} = 13.94±0.01 µg mL^{-1}

The results from the present investigation seem to suggest that the crude extracts and Butanol extract from *Piliostigma thonningii* possess considerable antioxidant activity as demonstrated by the DPPH free radical assay. The EC_{50} of the test samples compare favourably with that of Ginkgo biloba (EGb 761), a standard antioxidant with an EC_{50} value of 40.72 ug mL^{-1} (Mensor *et al*., 2001). Thus, implying that both plant extracts contain compounds with strong radical scavenging and antiradical generating effects.

The antioxidant activity exhibited by the Butanol fraction could be due to the presence of flavonoid constituents contained in them Bioactivity guided fractionation study of the leaf extract of P. *thoninngii* by Ibewuike *et* al. (Ibewuike, et al., 1997) which led to the isolation of some 6-C-methyl and 6, 8-di-C-methylflavonols in addition to quercetin and quercetrin from the ethyl acetate fraction.. Similar studies by Aderogba *et* al (Aderogba et al., 2003); on the leaf extract of *P. reticulatum*have also indicated the presence of similar flavonoids. Thus, the antioxidant activity of this fraction is probably due to the presence of these flavonoids in the Butanol fraction.

Many flavonoids have shown strong antioxidant properties and quercetin as been established as a strong antioxidant principle and had been used as standard in antioxidant experiments. The observed significant difference ($p<0.05$) in the activity exhibited in the crude as well as the Butanol extract tested for may imply that *P.*thoninngii may probably contain some additional antioxidant principles in the butanol fraction. Thus further fractionation study of the butanol fraction from the plants is necessary in order to isolate, characterize and evaluate the antioxidant principles.

CONCLUSIION:

The result shows that the leaf extract of pilostigma thonningi had considrable antioxidant activity. This will eventually lead to the quest of finding novel antioxidant agent from natural sources.

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