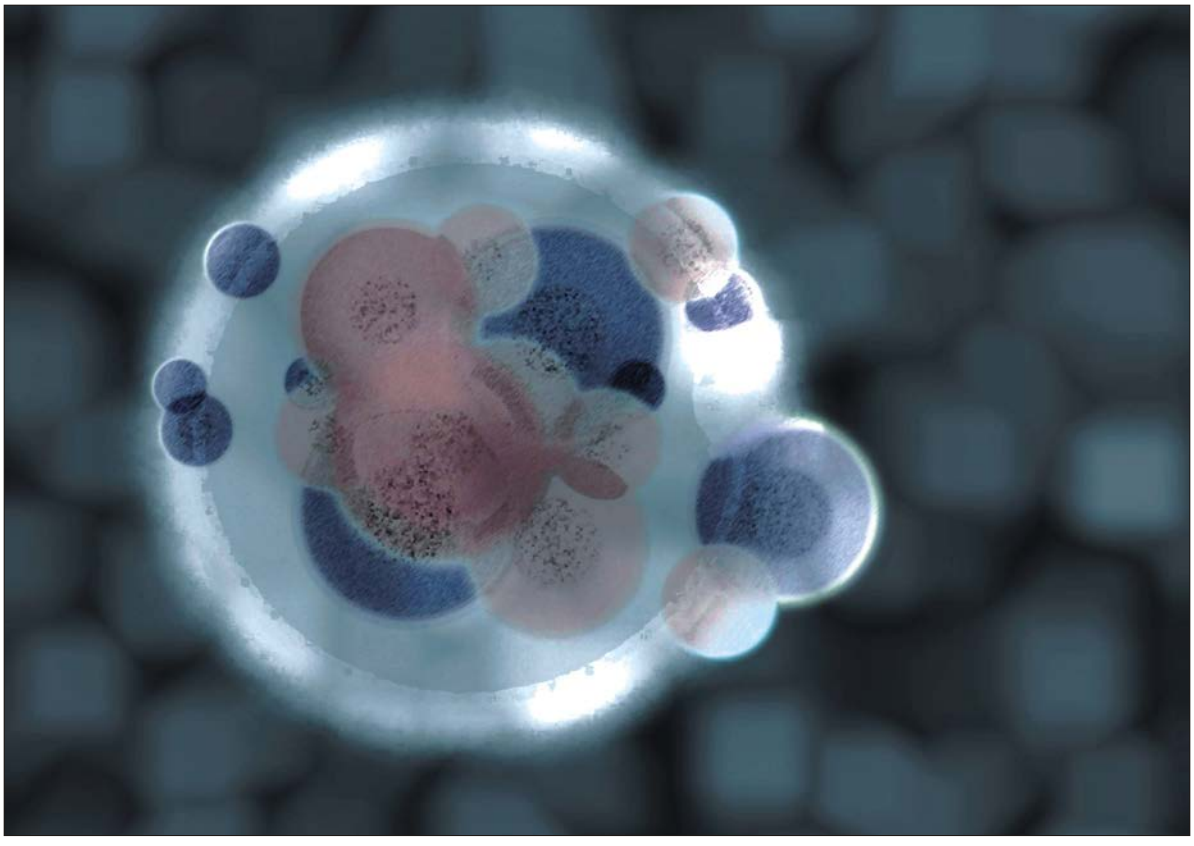

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Antioxidant Properties of the Nigerian Piliostigma Species

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Abstract: In this study a comparative antioxidant activity of the leaf extracts of *P. reticulatum* and *P. thonningii* have been evaluated using the DPPH free radical scavenging assay. The butanol fraction of *P. reticulatum* exhibited the highest activity (EC_{50} = 10.27±0.02 μg mL^{-1}), while crude extract of *P. thoninngii* had the least activity (EC_{50} = 50.94±0.27 μg mL^{-1}). These results suggested strong antioxidant activity potentials of the plants but less than ascorbic acid.

Key words: *P. reticulatum*, *P. thoninngii*, antioxidant activity, radical scavenging, DPPH

INTRODUCTION

Piliostigma reticulatum (DC.) Hochst and *Piliostigma thonningii* (Schum) Milne-Redhead belong to the family Caesalpinaceae. The two species are widely distributed in Nigeria but are often confused with each other 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[1,2]. Both plants are frequently used interchangeably in ethnomedicine in Africa to treat wounds, chronic ulcers, diarrhea, cough, respiratory disorders and toothache[1,2].

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[3]. Other reported bioactivity of *P. thonningii* includes larvicidal activity against common intestinal parasites of cattle[4] and antimicrobial activity against the yeast *S. cerevisiae*[5] and *S. luted*[6]. On the other hand, the alcoholic extract of the leaves, pod and root of *P. reticulatum* were found to exhibit no molluscicidal activity[7]. Recent study in our laboratory have shown that the aqueous ethanolic extract of the leaf of *P. reticulatum* also possess both antimicrobial and anti-inflammatory activities[8].

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*[9,10]. Previous investigation of the leaf extract of *P. reticulatum* has also yielded similar C-methylflavonols[11]. Earlier studies on both species have revealed the presence of tannin content of up to 20%[2].

It is well known that antioxidant activity in higher plants has often been associated with phenolic compounds[12], 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[13]. 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: taxol and artermisinin[14]. In this study, a comparative antioxidant activity potentials of the leaf extracts of the two species was evaluated using the DPPH free radical scavenging assay. This was with a 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 chemika.

Plant material: Leaves of *P. reticulatum* and *P. thonningii* were collected at Ikire, Osun State and the Teaching and Research Farm, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria, respectively.

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Mr. O.A. Oladele of the Herbarium section, Faculty of Pharmacy, O.A.U. Ile-Ife, authenticated the plants. Where voucher specimens were deposited.

Preparation of the extracts: The plant materials were air dried for two weeks and then powdered. A known weight of each of the powdered leaves of *P. reticulatum* and *P. thonningii* was then percolated with ten volumes of 50% aqueous ethanol at room temperature for 24 h and filtered. The extracts obtained were concentrated *in vacuo* at 40°C using rotary evaporator (Buchi, Switzerland) to give the crude extracts of both plants.

Solvent partitioning of the crude extract of *P. reticulatum* and *P. thonningii*: A known weight of each of the crude extracts was suspended in the distilled water and extracted with two volumes of ethyl acetate three times. The combined organic layers of each sample was evaporated to dryness *in vacuo* using rotary evaporator to afford the ethyl acetate fractions as dark brown solids. The resultant aqueous portions was subsequently extracted separately with two volumes of butanol three times. The combined butanol fraction for each plant was then concentrated to dryness *in vacuo* at 40°C using rotary evaporator, to afford butanol fractions.

Evaluation of antioxidant activity: The determination of the radical scavenging activity of each of the crude extracts and solvent fractions was carried using the DPPH (1,1-diphenyl-2-picrylhydrazyl) assay as described by Mensor *et al.* 2001[15], with a slight modification. To 1.0 mL of DPPH (0.25 mM) in methanol was added 2.0 mL of the varying concentrations of the test samples (250, 125, 50, 25, 10 and 5 μg mL^{-1}). The reaction mixture was then allowed to stand at room temperature in a dark chamber for 30 min. 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:

$$AA\% = 100 - \{[(Abs_{sample} - Abs_{blank}) \times 100] / Abs_{control}\}$$

Blank = Methanol (1.0 mL) plus sample solution (2.0 mL), Negative control = DPPH solution (1.0 mL, 0.25 mM) plus methanol (2.0 mL), Ascorbic acid was used as positive control. 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.

Statistical analysis: The results are express as mean±SEM (Standard error of mean) and mean EC_{50} values of test samples were compared using the student's t test (Statistica® software version 5, Statsoft Inc). A probability of 0.05 or less was considered significant. The EC_{50} values obtained from the regression plots (SigmaPlot® 2001, SPSS Science) showed a good coefficient of determination ($r^2 \geq 0.937$).

RESULTS AND DISCUSSION

Table 1 that shows the ethyl acetate and butanol fractions from *P. reticulatum* and *P. thonningii* exhibited substantial inhibition of DPPH activity, with a 50% inhibition (EC_{50}) values ranging between 10.27±0.22 to 19.70±0.10 μg mL^{-1}. The crude extracts on the other hand exhibited moderate antioxidant activity in both species with EC_{50} values of 40.10±0.27 and 50.94±0.27 μg mL^{-1} for *P. reticulatum* and *P. thoninngii* respectively. These values were significantly ($p < 0.05$) higher than those obtained for the solvent fractions as well as that of the ascorbic acid standard. There were also significant differences ($p<0.05$) between the EC_{50} values of the corresponding test samples from both species.

Table 1: EC_{50} values (concentration to give 50% activity) in μg mL^{-1} for scavenging of the DPPH radicals

Samples	*P. reticulatum*	*P. thonningii*
Crude extract	40.01±0.27	50.94±0.27
Ethyl acetate fraction	19.70±0.10	18.51±0.29
Butanol fraction	10.27±0.22	14.70±0.05

Ascorbic acid as Standard, EC_{50} = 3.94±0.01 μg mL^{-1}

The results from the present investigation seem to suggest that the crude extracts and solvent fractions from both Piliostigma species 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 agent, with an EC_{50} value of 40.72 μg mL^{-1} [15]. Thus, implying that both plant extracts contain compounds with strong radical scavenging and antiradical generating effects.

The comparable antioxidant activity exhibited by the ethyl acetate fraction of both species 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.*[10] has 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.*[11] 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 ethyl

acetate fraction. The nature of the antioxidants present in butanol fractions of both piliostigma species are yet to be established, therefore there is need for the investigation of this fraction from both plants to ascertain the active constituents responsible for the observed strong activity.

Many flavonoids have shown strong antioxidant properties[16] and quercetin as been established as a strong antioxidant principle and had been used as standard in antioxidant experiments[12]. The observed significant difference ($p<0.05$) in the activity exhibited in the crude as well as the solvent fractions tested for *P. reticulatum* when compared to that of *P. thoninngii* may imply that *P. reticulatum* may probably contain some additional antioxidant principles in the butanol fraction. Thus further fractionation study of the butanol fraction from both plants is necessary in order to isolate, characterize and evaluate the antioxidant principles.

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