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Phytochemical Screening and in Vitro Antioxidant Activity of Parkia biglobosa Extract

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Abstract
Parkia biglobosa leaves have popular folkloric ethnomedicinal use in the treatment of many diseases especially among the South-Western people of Nigeria. The present study was undertaken to find the antioxidant value of aqeous-methanolic extract of *Parkia biglobosa* leaf by investigating its phytochemicals and invitro antioxidant potentials. Antioxidant activity of extract was screened for by measuring its total flavonoid and total phenol content, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and ferric reducing property. Phytochemical screening was carried out on extract by standard method. Phytochemical analyses revealed the presence of saponins, flavonoids, tannins and cardiac glycosides. The total phenol and flavonoid contents are 144.18 mg gallic acid equivalent/g extract and 256.858mg Quercetin equivalent/g extract respectively. The extract showed considerably high and dose-dependent DPPH radical scavenging and ferric reducing property comparable with the respective standards, Quercetin and Ascorbic acid. The results of this study reveal that *Parkia biglobosa* leaf extract possess significant antioxidant properties and could be exploited as source of antioxidant additives.
Keywords: Parkia biglobosa, phytochemicals, Antioxidant activity

Introduction

Medicinal plants have been used for centuries before the advent of orthodox medicine. Leaves, flowers, stems, roots, seeds, fruit, and bark can all be constituents of herbal medicines. The medicinal values of these plants lie in their component phytochemicals, which produce definite physiological actions on the human body (Akinmoladun *et al*., 2007). The phytochemicals include alkaloids, saponins, tannins, phlobatannins, anthraquinones, glycosides, flavonoids, steroids, terpenoids e.t.c. The presence of these phytochemicals is suggestive of potent bioactivity. Animal studies have shown that dietary phytochemical antioxidants are capable of removing free radicals. Phenolic compounds are a class of antioxidant compounds which act as free radical terminators (Shahidi and Wanasundara, 1992). The antioxidant activity of phenolics is due to their redox properties which allow them to act as reducing agents, metal chelators and free radical scavengers (Rice-Evans *et al*., 1996; Oboh and Rocha, 2007). It has also been recognized that flavonoids show antioxidant activity and their effects on human nutrition and health are considerable. Antioxidants are the antidotes to the voracious electron apetite of free radicals and reactive oxygen species. Plant phytochemicals may be effective in combating or preventing disease due to their antioxidant effect (Halliwell and Gutteridge, 1992; Farombi et al., 1998).

Parkia biglobosa (Jacq.) Benth., a widespread savana tree was largely prescribed in traditional medicine for its multiple medicinal virtues in South western Nigeria. The bark and seeds were prescribed for the treatment of arterial hypertension, and the leaves against piles, amoebiasis, bronchitis, cough, burn, zoster, and abcess (Millogo-Kone et al., 2008)..

The present study was therefore designed to investigate the phytochemical constituents of *Parkia biglobosa* and its in vitro antioxidant properties.

Materials and Methods

Plant Materials

Fresh leaves of *Parkia biglobosa* were collected from a private farm in Ado Ekiti, Ekiti State, Nigeria, in the month of June, 2013. Botanical Identification and authentication was carried out at the herbarium (FHI) Forestry Research Institute of Nigeria, Ibadan, Oyo state Nigeria.

Chemicals

DPPH (2,2-Diphenyl-1-Picryl Hydrazyl), a product of Sigma Pharmaceuticals, China, was obtained from Rovet Chemicals, Benin City, Nigeria. All other chemicals were of analytical grade and were obtained from British Drug Houses, (Poole, UK). The water used was glass distilled.

Preparation of Aqeous-methanolic extract of *Parkia biglobosa leaf*

The leaves were air-dried for 28 days at room temperature. The air-dried samples were ground to fine powder using a blender. A 500 g sample of the powdered material was soaked in 1200 ml of a mixture of methanol and water (4:1) for 74 hours. This was filtered and concentrated to a small volume to remove the entire methanol using rotary evaporator. The small volume was later freeze-dried to obtain the extract powder. The extract was kept in the freezer at 4 ^{0}C for further studies.

Phytochemical Screening

Phytochemical screening of the extract was carried out to identify the constituents, using standard phytochemical methods as described by Trease and Evans (1989) as well as Sofowora (1993).. The screening involves detection of alkaloids, flavonoids, terpenoids, saponins, tannins, anthraquinones, and cardiac glycosides.

In vitro antioxidant assay

Total phenolic content

The total phenolic content was determined using the Folin–Colcalteu method, described by Singleton *et al*., (1999) and slightly modified according to Liu (2002).

Briefly, 0.5 ml of deionised water and 125 µl of the Folin–Colcalteu reagent was added to 125 µl of the suitably diluted sample extract. The mixture was then allowed to stand for 6 min and then 1.25 ml of a 7% aqueous Na_2CO_3 solution was added. The final volume was adjusted to 3 ml. The mixture was allowed to stand for 90 min and the absorption measured at 760 nm against water as a blank. The amount of total phenolics was expressed as gallic acid equivalents (GAE, mg gallic acid/g sample) through the calibration curve of gallic acid.

Total flavonoid content

The total flavonoid content was determined using a colorimetric method described by Dewanto *et al*. (2002).

To 0.25 ml of the suitably diluted sample, 75 µl of a 5% NaNO2 solution, 0.150 ml of a freshly prepared 10% $AlCl_3$ solution, and 0.5 ml of 1 M NaOH solution was added. The final volume was then adjusted to 2.5 ml with deionised water. The mixture was allowed to stand for 5 min and the absorption measured at 510 nm against the same mixture, without the sample, as a blank. The amount of total flavonoids was expressed as mg Quercetin equivalent/g extract.

Reducing power assay

This assay was determined according to the method reported by Oyaizu (1986) with slight modifications. Briefly, 1 ml of reaction mixture, containing varying concentrations (10-250µg/ml)of the test extracts or compounds in 500 µl of phosphate buffer (0.2 M, pH 6.6), was incubated with 500 µl of potassium ferricyanide (1%, w/v) at 50^0C for 20 min. The reaction was terminated by adding trichloroacetic acid (10%, w/v), then the mixture was centrifuged at 3000 rpm for 10 min. The supernatant solution (500 µl) was mixed with distilled water (500 µl) and 100 µl of ferric chloride (0.1%, w/v) solution, before measuring the optical density (OD at 700nm.

Free radical scavenging

Free radical scavenging activity was evaluated with the DPPH (1,1-diphenyl-2-picrylhydrazil radical) assay. The antiradical capacity of the sample extracts was estimated according to the procedure reported by Brand-Williams and Cuvelier (1995) and slightly modified.

Briefly, 2ml of the sample solution, suitably diluted with ethanol, was added to 2 ml of an ethanol solution of DPPH (0.0025 g/100 ml) and the mixture then allowed to stand for 20 min, before taking the absorption at 517nm against blank.

Statistical Analysis

All values are expressed as mean ± SD of triplicate results.

RESULTS

Phytochemical screening

Table 1 shows the phytochemical constituents of Parkia biglobosa leaf extract which revealed the presence of saponins, tannins, terpenoids, flavonois and cardiac glycosides in the extract.

Invitro Antioxidant properties

Table 2 gives the antioxidant property results of the aqeous-methanolic extract of Parkia biglobosa leaf. Total phenolic content was 144.18mg gallic acid equivalent/g extract while the total flavonoid was 256.858mg Quercetin equivalent/g extract.

Table 1*: The phytochemical constituents of *Parkia biglobosa* leaf extract

Phytochemical Constituents	Results
Alkaloid	Absent
Saponins	Present
Tannins	Present
Phlobotannins	Absent
Anthraquinones	Absent
Cardiac glycosides	Present
Flavonoids	Present
Terpenoids	Present

Table 2: In vitro antioxidant indices of *Parkia biglobosa* leaf extract

Property	Level
Total Phenol	148.18mg gallic acid equivalent/g extract
Total flavonoid	256.858mg Quercetin equivalent/g extract

Fig 1 shows the ferric reducing property of *Parkia biglobosa* leaf extract. The results showed that *Parkia biglobosa* leaf extracts exihibited considerably high reducing potential when compared with vitamin C. The observed effect was dose dependent with the highest activity occurring at the 250µg/ml concentration of the extract.

Fig 2 shows the DPPH radical scavenging activity of *Parkia biglobosa* leaf extract. The results revealed that *Parkia biglobosa* leaf extracts exihibited considerably strong radical scavenging activity against DPPH radical. The observed effect was dose dependent with the highest activity occurring at the 250µg/ml concentration of the extract.

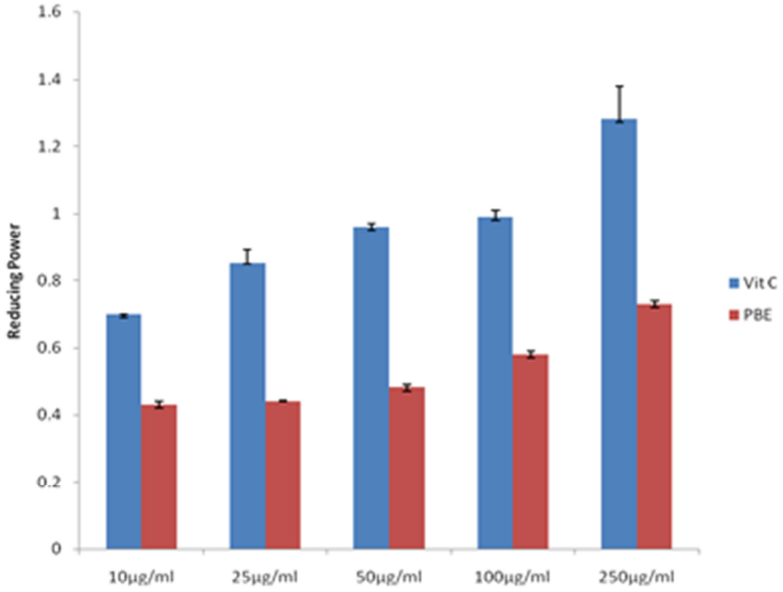

Fig 1: Ferric Reducing Property of *Parkia biglobosa* leaf extract
*Values are expressed as mean±SD (n=3).

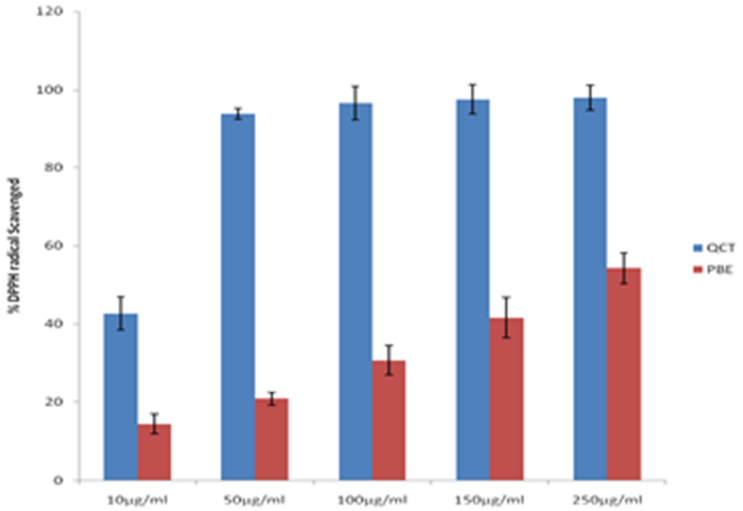

Fig 2: DPPH radical scavenging activity of *Parkia biglobosa* leaf extract
*Values are expressed as mean±SD (n=3).

Discussion

In recent years dietary plants with antioxidative property have been the center of focus. It is believed that these plants can prevent or protect tissues against damaging effect of free radicals (Osawa and Kato, 2005). Free

radicals and ROS have been implicated in a large number of human diseases (Wegener and Fintelmann, 1999). The beneficial medicinal effects of plant materials typically result from the combinations of secondary metabolites present in the plant, through additive or synergistic action of several chemical compounds acting at single or multiple target sites associated with a physiological process (Briskin, 2000).

The considerably high content of phenols and flavonoids (144.18mg gallic acid equivalent/g extract and 256.858mg Quercetin equivalent/g extract respectively) may be suggestive of considerable antioxidant potentials. Phenolic compounds are a class of antioxidant compounds which act as free radical terminators (Shahidi and Wanasundara, 1992). The major active nutraceutical ingredients in plants are flavonoids. A nutraceutical is any nontoxic food extract supplement that has scientifically proven health benefits for both the treatment and prevention of disease (Dillard and German, 2000). The best-described property of almost every group of flavonoids is their capacity to acts as antioxidants. As is typical for phenolic compounds, they can act as potent antioxidants and metal chelators. It has been recognized that flavonoids show antioxidant activity and their effects on human nutrition and health are considerable. They also have long been recognized to possess antiinflammatory, antiallergic, hepatoprotective, antithrombotic, antiviral, and anticarcinogenic activities. The mechanisms of action of flavonoids are through scavenging or chelating process (Kessler et al., 2003; Cook and Samman, 1996). They contain hydroxyl functional groups which are responsible for their antioxidant effects (Das and Pereira, 1990).

The presence of tannins, terpenoids and cardiac glycosides in Parkia biglobosa leaf is suggestive of its potent bioactivity. Presence of tannins suggests the ability of this plant to play a major role as antidiarrhoec and antihaemorrhagic agent (Asguith and Butler, 1986). The cardiac glycosides have been used for over two centuries as stimulants in cases of cardiac failure (Trease and Evans, 1989) hence; this plant could be investigated for this purpose.

The model of scavenging the stable DPPH radical and evaluation of reducing power is a widely used method to evaluate the free radical scavenging ability of various samples (Lee *et al.,* 2003). It was found that the radical- scavenging activities and hence antioxidant activity of all the extracts increased with increasing concentration.

Conclusion

The present study showed that Parkia biglobosa extract exhibited significant total flavonoid and phenolic content and also showed high radical scavenging activity. The plant could be exploited as source of antioxidant additives.

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