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Research Article

Distribution, Phytochemistry and Antioxidant Properties of the Genus *Parkia* R.br. (Mimosaceae) in Nigeria

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ABSTARCT

The present study examined the ecological distribution range of the two occurring Nigeria species of *Parkia* (Allopatric taxa): *Parkia bicolor* and *Parkia biglobosa*. The phytochemical content and antioxidant activities of leaves and stem bark of both species were also studied using standard techniques. The antioxidant activities of the crude extract was carried out using the 2,2-diphenyl-1-picyrylhydrazyl (DPPH) radical scavenging method. Methanolic extracts of the leaf and stem bark of the two species were analyzed quantitatively by simple, sensitive and reproducible spectrophotometric methods for the following phytochemicals: alkaloid, saponin, phenols and flavonoids. Preliminary phytochemical screening showed that both plants had similar constituents namely cardiac glycoside, alkaloids, saponin, tannin and flavonoid. Anthraquinone was present only in the leaf of *P.bicolor* and stem bark of *P. biglobosa* respectively. Both plant species showed promising antioxidant activity considering their scavenging activity on DPPH. The DPPH method indicated that the antioxidant activity of the stem bark extract of both species showed a significant free radical activity in a concentration dependent manner. Its action was comparable to standard antioxidants like ascorbic acid (Vitamin C), rutin, butylated hydro-anisole (BHA) and alpha-tocoherol (Vitamin E).The leaf extracts of *Parkia bicolor* showed a high antioxidant activity in a concentration dependent manner when compared with the leaf extracts of *Parkia biglobosa.* The quantitative screening of phytoconstituents contained in the leaf and bark extract of the two species revealed that phenols and saponin are relatively high, while flavonoid is relatively moderate with the alkaloid content relatively low. This study justifies the therapeutic usage of *Parkia* species in traditional medicine.

Key Words: *Parkia*, Phytochemical constituents, Antioxidant activity, Ecology, Allopatric.

INTRODUCTION

The genus *Parkia* belongs to the tribe Parkieae. It consists of about 35 species with a pantropical distribution1.[1,2] Only 3 species all belonging to the section *Parkia*, occur in continental Africa, and a fourth one in Madagascar.[3] Two of these species are found in Nigeria. These are *P. bicolor* A. Chev. and *P. biglobosa* (Jacq.) R. Br. ex G. Don. The taxonomic characters separating both species are well documented.[4]

In the West African sub-region, the seed of *P. biglobosa* has been recognized as the most valuable product, topping the list of acceptable indigenous multipurpose trees in Nigeria[5] and ranked highest of a list of eighteen traded edible forest products in Mali.[6,7] The seed are fermented into daddawa, a strong smelling tasty seasoning, rich in protein and used in improving the taste of soups in most part of West Africa.[8] Traditional medicinal uses include: stem bark of *P. biglobosa* used as a mouth wash to steam and relieve toothache as well as a bath for fever.[9] Infusion of the stem-bark can also be used as tonic against diarrhea in Nigeria and Cote d' Ivoire.[10] Pulverized stem bark of *P. bicolor* is reported to be used in the treatment of wounds.[9]

Presently, there is a growing interest in phyto-medicinal research. Emphasis so far are directed towards a systematic search for useful bioactive compounds in medicinal plants as a rational approach in drug research. However, the genus *Parkia* have been relatively understudied in Nigeria[9], with respect to the phytochemistry of its component parts. Most importantly, the antioxidant properties is yet to be fully explored. Antioxidants are compounds that can delay or inhibit the oxidant of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions.[11] The oxidative effect in plants is mainly due to phenolic components, such as flavonoids[12], Phenolic acids and phenolic diterpenes.[13,14] This constitutes a diverse and ubiquitous group of phytochemicals in the plant kingdom.[15] Depending on their concentration, phenolic compounds have a dual bioactive role in plants, acting as both antioxidant and pro-oxidant agents at low and high concentrations, respectively.[15] The antioxidative capacity of phenolic compounds has investigated the search for natural antioxidants as alternatives to the available synthetic antioxidants, such as butylated hydroxytoluene (BHT)/ anisole (BHA), that are widely used in the food and pharmaceutical industries. The dietary intake of synthetic antioxidants, for example, BHT could be toxic at high concentration.[16,17] In contrast, natural antioxidants are often presumed to be safe for consumption, due to their plant origin[18], but this may vary depending on the

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Table 1: Qualitative phytochemical screening of leaf and bark extracts.

Test	***Parkia bicolor***		***Parkia biglobosa***	
Phytoconstituents	Leaf Extract	Stem bark Extract	Leaf Extract	Stem bark Extract
Alkaloid	-	+	+	+
Saponin	+	+++	++	++
Tannin	++	++	+++	+++
Cardiac glycosides	+	+	++	++
Anthraquinone	+	-	-	+
Flavonoid	+	++	++	+
Terpenoid	-	++	-	+++

+++ Appreciable amount; ++ Moderate amount; + Trace; - Complete absence

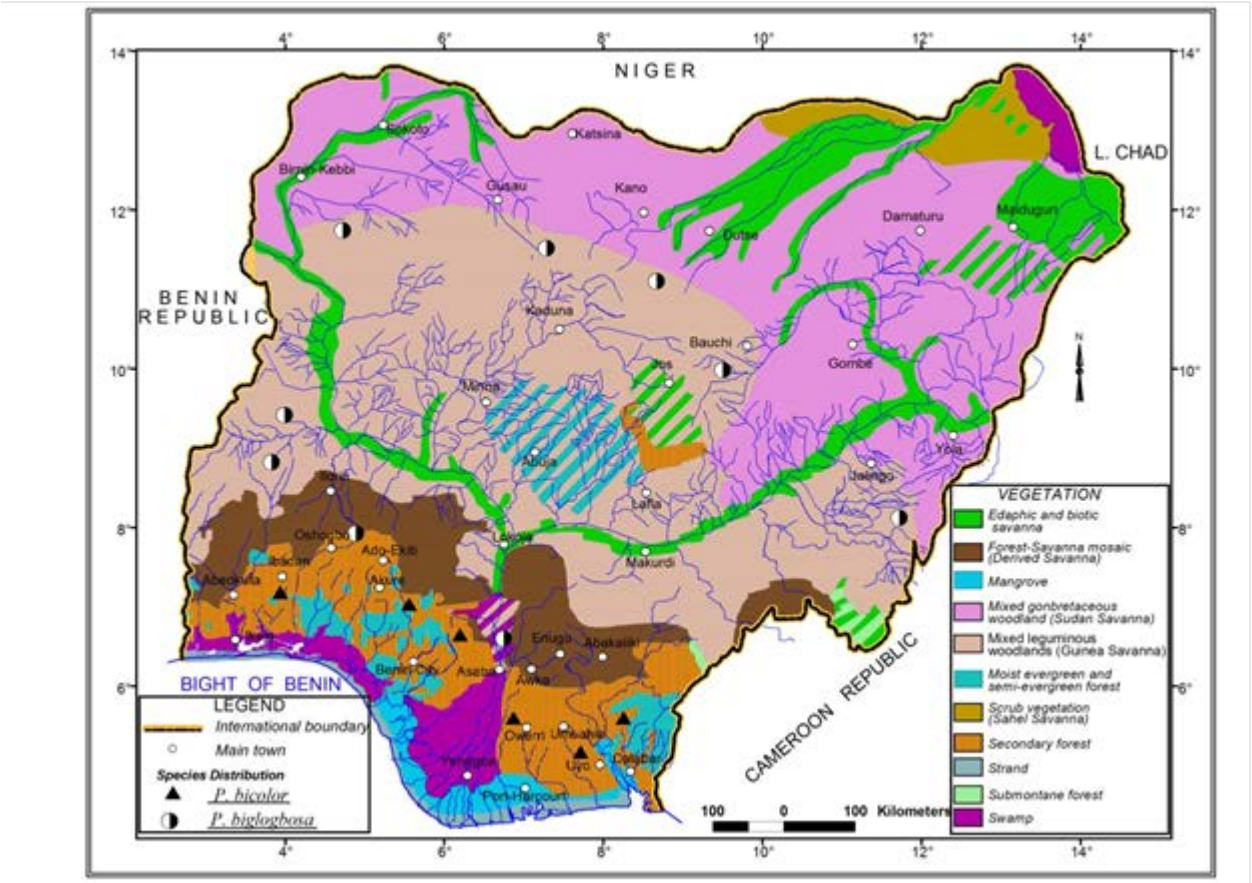

Table 2: Quantitative phytochemical screening of leaf and bark extracts.

Test	*Parkia bicolor*(mg/g)		*Parkia biglobosa*(mg/g)	
Phytoconstituents	Leaf Extract	Stem bark Extract	Leaf Extract	Stem bark Extract
Alkaloid	0.09±0.007	0.09±0.06	0.25±0.007	0.11±0.07
Saponin	1.35±0.07	4.35±0.07	3.55±0.24	3.43±0.08
Flavonoid	3.63±0.30	2.93±0.30	2.51±0.45	1.54±0.26
Phenols	8.82±0.02	5.24±0.30	9.25±0.8	8.92±0.02

Figure 1: Ecological distribution of P. biglobosa and P. bicolor

plant species and environmental factors that affect growth.

Hence, the present work was designed to examine the ecological distribution and compare the chemical constituents present in the leaf and bark extracts of *P. bicolor* and *P. biglobosa.* Also, a study on its antioxidant properties is presented.

MATERIALS AND METHODS

Ecological Data: The natural ecological distribution range of the respective species was obtained based on preserved plant specimens housed at the Forest herbarium Ibadan (FHI) listed in Holmgren *et al*[19]. Also, the ecological binders containing a list of occuring species from

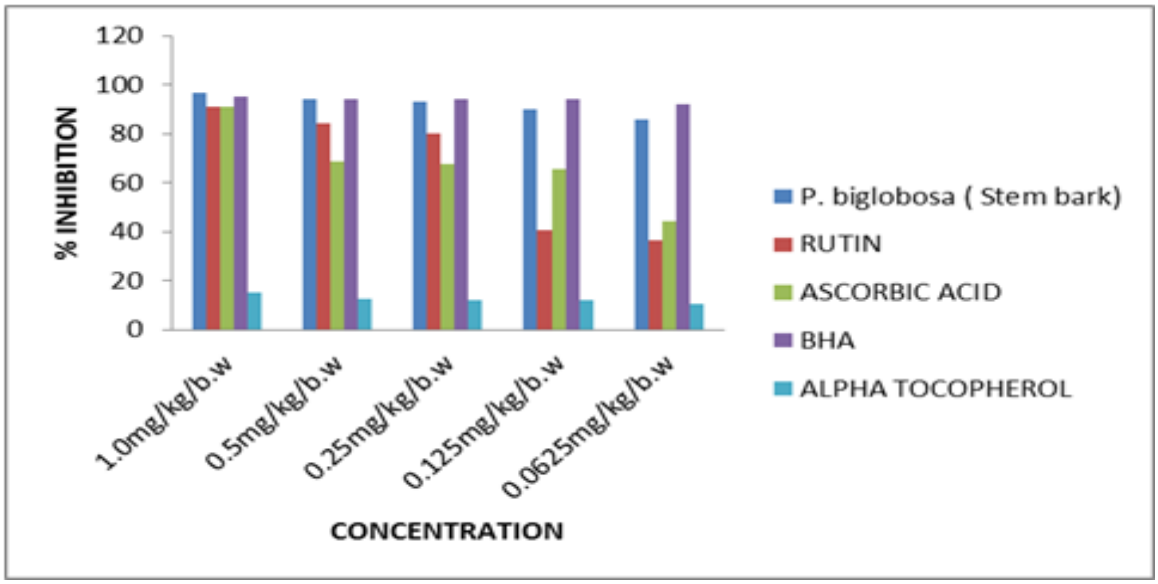

Figure 2: DPPH scavenging activity of P.biglobosa stem-bark extract

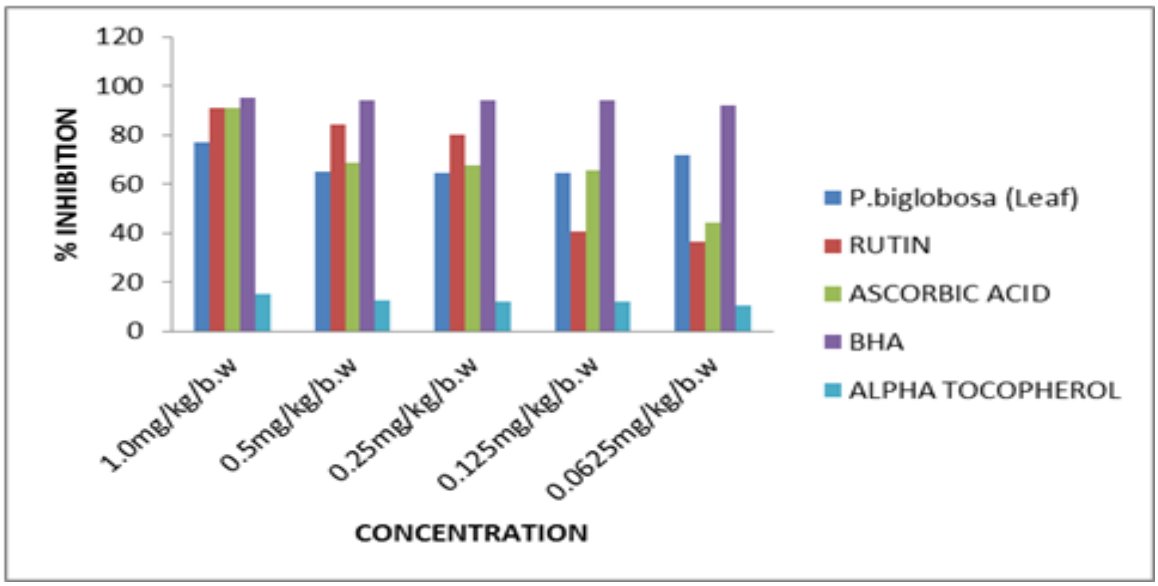

Figure 3: DPPH scavenging activity of P.biglobosa leaf extract

protected forest ecosystem in Nigeria were examined to enable a draw up of its ecological distribution range.

Plant Collection**:** Fresh leaves and stem-bark of *Parkia biglobosa* and *Parkia bicolor* were collected from the premises of the Forestry Research Institute of Nigeria (FRIN), Oyo state, Nigeria. The plant specimens was authenticated in the Herbarium section (FHI), where a voucher specimen was deposited.

Phytochemistry: The plant samples were air dried for five days and milled respectively to powder with the aid of a mechanical blender prior to extraction with the different solvents. About 120g of the powdered samples were first defatted with n-hexane for 6 hours with the aid of a soxhlet apparatus and the fat free plant material were air dried and stored for further analysis. Each stored fat free samples was then individually extracted with a suitable solvent for the various tests. The chemical tests were carried out in the methanol crude extracts for the qualitative determination of phytochemical constituents as described.[20,21,22]

Saponin determination: Twenty (20) grams of each powdered plant samples were dispersed in 200ml of 20% ethanol. The suspension was heated over a hot water bath for 4hrs with continous stiring at about 55^{O}C. The mixture was filtered and the rsidue re-extracted with another 200ml of 20% ethanol. The combined extracts were reduced to 40ml over water bath at about 90^{o}C. The concentrate was tranfered into a 250ml separator funnel and 20ml of diethy ether was added and shaken vigorously. The acqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60ml of n-butanol extracts were washed twice with 10ml of 5% acqueous sodium chloride. The

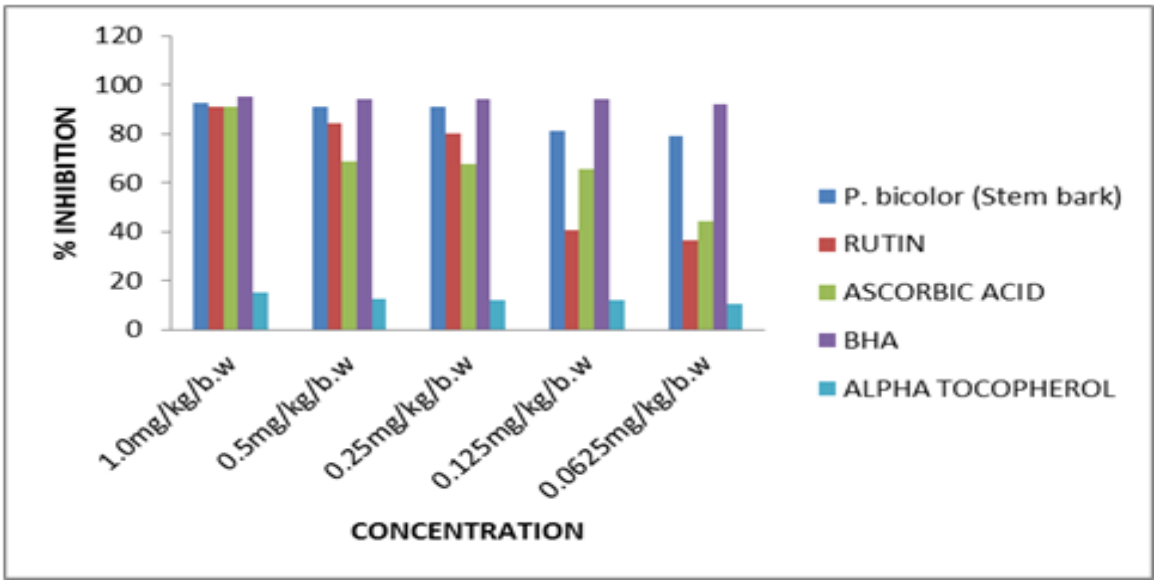

Figure 4: DPPH scavenging activity of P.bicolor stem-bark extract

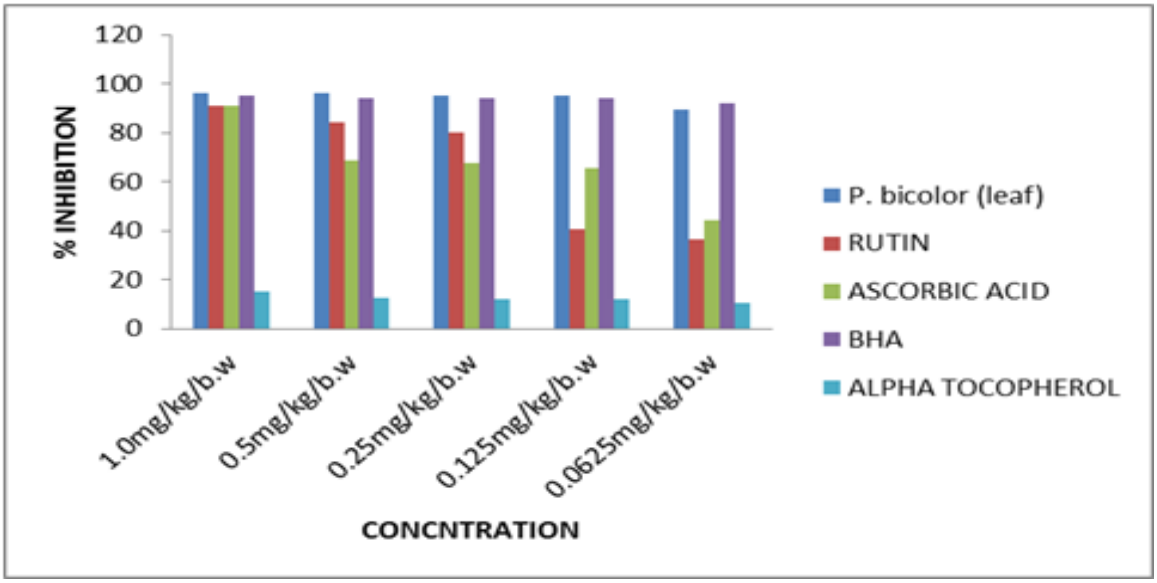

Figure 5: DPPH scavenging activity of P.bicolor leaf extract

remaining solution was heated in a water bath. After evaporation the sample were dried in the oven into a contant weight. The saponin content was calculated in percentage.[23]

Alkaloid determination: 5g of the sample were weighed into 250ml beaker and 200ml of 20% acetic acid in ethanol was added and covered to stand for 4h. This was filtered and the extract was concentrated using a water bath to one-quater of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitation was collected by filtration and weighed.[20,24]

Flavonoid determination: 10g of the plant samples were extracted repeatedly with 100ml of 80% acqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No. 42 (125mm). The filterate was later transffered into a crucible and evaporated to dryness over a water bath and weighed.[25]

Determination of total phenols: The fat free powdered sample wasboiled with 25ml of ether for 14min. 5ml of the extract was pipette into 50ml volumetric flask, then 5ml of distilled water was added. 1ml of ammonium hydroxide solution and 2.5ml of concentrated amyl alcohol were also added. The samples were made up to mark and left to react for 30min for colour development. A set of standard solution of tannic acid was prepared at concentrations ranging from 40mg/ml to 0.625mg/ml. The absorbance of the solution was read using a Model 752 Ultraviolet grating Spectrophotometer at 505nm wavelengths. The absorbances of the standard solutions were also read at the same wavelength.[20,24]

Antioxidant Activity by DPPH Radical- Scavenging Method: The radical-scavenging activity of the plant

extracts against 2,2-diphenyl-1-picryhydrazyl radical (DPPH) was determined using the method described by.[26] 394.33g of DPPH was dissolved in 1000ml methanol to give 0.1mM solution i.e. 3.9mg in 10mls of methanol. The stock solution was prepared by re-dissolving 10mg crude extract of the samples in 10mls of methanol to give 1mg/ml. To 1ml of the methanol solution of DPPH was added 2.5ml of the methanol plant extract solution taken from the stock solution. The mixture was shaken well and left to stand for 15min. Absorbance was measured at 517nm. The actual decrease in absorption induced by the test compound was calculated by subtracting that of the control. Other concentrations of methanol extract (1.0mg/ml, 0.5mg/ml, 0.25mg/ml, 0.125mg/ml, and 0.0625mg/ml) were prepared from the stock solution through the means of serial dilution and each of them was analyzed in the same way as the stock solution. The radical scavenging activity (RSA) was calculated as the percentage inhibition of DPPH discoloration using the equation below:

%inhibition = 1- (A1/A2) x 100

A_1= Absorbance of the test sample

A_2= Absorbance of control reaction

Ascorbic acid (vitamin C), Rutin, BHA and α- tocopherol (vitamin E) with known antioxidant activities were used as standards using the same concentrations as test solutions. The experiment was carried out in triplicate.

RESULTS

Ecological Distribution: The two *Parkia* species are Allopartric taxa. Being geographically isolated with *P. bicolor* occuring in the high forest zone while *P. biglobosa* occurs within the Savanna vegetation (Figure 1).

However, *P. biglobosa* (African locust bean) which is the most exploited, occur primarily within the Guinea savanna belt with pocket of presence within the derived Savanna zone (a transition between the forest in the south and the true Savanna in the north). The Guinea Savanna belt or wooded Savanna is the broadest vegetational zone in Nigeria. Annual Rainfall within the region is between 1000mm-1500mm, with a wet season of about 6-8months.[27]. *P. bicolor* occur mostly around water bodies or riparian vegetation within the high forest zone. Average annual rainfall within this region is above 2000mm with a wet season of about 8-10 months.

Qualitattive phytochemical analysis of crude extract Phytochemical screening of the *Parkia* species revealed that the leaf and stem bark extracts of the two species contained at least one class of secondary metabolite. Saponin, Tannin, Cardiac glycosides were present. Alkaloids were absent in the leaves of *Parkia bicolor* (Table 1) but a slight presence was indicated in the other samples of the two species. Terpenoids were absent in leaves of both species studied but a much more positive test was seen in their stem bark extract. However, there was an absence of Anthraquinone in the stem bark and leaves of *Parkia bicolor* and *Parkia biglobosa* respectively while Flavonoid was absent in the stem bark of *Parkia biglobosa* but was shown to be present in the other samples.

Quantitative Analysis Of Phytochemicals: Analysis of the leaves and stem bark methanol crude extract of *Parkia bicolor* and *Parkia biglobosa* in Table 2 shows that leaf extracts of both plant samples contains 0.09±0.007mg/g and 0.25±0.007mg/g of alkaloid, 1.35±0.07mg/g and 3.55±0.24 of saponin, 3.63±0.30mg/g and 2.51±0.45mg/g of flavonoid and 8.82±0.02mg/g and 9.25±0.8mg/g of phenols respectively while the stem bark of plant samples contains 0.09±0.06mg/g and 0.11±0.007mg/g of alkaloid, 4.35±0.07mg/g and 3.43±0.08mg/g of saponin, 2.93±0.30mg/g and 1.54±0.26mg/g of flavonoid and 5.24±0.30mg/g and 8.92±0.02mg/g of phenols respectively.

DPPH Radical Scavenging Activity: The analysis of the antioxidant activities of the crude fraction of the plant sample as well as the antioxidant activities of standards are reported in the figures below. The radical-scavenging potential of the two species were dose-dependent (1.0-0.0625mg/ml). At absorbance of 517nm, *Parkia bicolor* sample showed a promising radical-scavenging activity. *Parkia biglobosa* bark also showed a promising radical-scavenging activity than its leaf sample which showed the lowest radical scavenging activity as compared to the other samples. The radical scavenging activity demonstrated by *Parkia biocolor* leaf and *Parkia biglobosa* bark were higher than that of the standards (rutin, ascorbic acid, Butylated hydro-anisole (BHA) and alpha-tocopherol) at all concentration (Fig.2-5). Report on the antioxidant properties of leaves and stem bark of *Parkia* species using DPPH are scarce. The presence of flavonoid could be responsible for the antioxidant activity of the plants. Recent studies have shown that many flavonoids and related polyphenols contribute significantly to the total antioxidant activity of many fruits and vegetables.[28,29.30] Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species such as singlet oxygen, super oxide, peroxyl radicals, hydroxyl radicals and peroxynitrile.[11] From our results, the two species of *Parkia* that were studied have shown appreciable level of antioxidant activity using the DPPH radical scavenging method.

DISCUSSION

In spite of the advances made in orthodox medicine, there has been an increasing interest in herbal medicine.[31] The analysis of the extracts of leaves and stem bark of the two *Parkia* species indicated the presence of tannins, cardiac glycosides, saponins, alkaloids, Anthraquinones, flavonoids and terpenoids. The presence of some of these metabolites in the plants could be linked to their therapeutic uses in traditional medicine. The result obtained in the phytochemical analysis seems to justify the use of the leaf of *Parkia biglobosa* for cardiac conditions[9] as an appreciable amount was present in the leaves. The appreciable amount found also in the stem bark of this plant species could possibly prove that the stem bark could also be used for this purpose. Cardiac glycosides of several plant species are currently being

investigated for anti-tumor properties and may augment cancer treatment strategies.[32] Our results confirmed the great potentials contained in *Parkia* species as been reported by researchers such as [8, 9, 33, 34] and goes further to reveal the antioxidants potential of the respective parts of the two species. Tannins which are phenolic compounds and that were found in the leaves and stem bark of the two species of *Parkia* are known to act by iron sequestration, hydrogen bonding or specific interactions with vital proteins such as enzymes.[35] In addition to its antimicrobial, anticancer activities, tannins are potent antioxidants.[22] Generally, plants containing tannins are astringents and used for treating intestinal disorder such as diarrhea and dysentery.[36] This further justifies the traditional medicinal uses of these plants in the treatment of different ailments. The appreciable amount of saponin in these plant species confirms it as an antimicrobial and antibacterial agents.[8,9] Saponins are known to produce inhibitory effect on inflammation.[37] The natural tendency of saponins to ward off microbes makes them good candidates for treating fungal and yeast infection. This compound serves as natural antibiotics which help the body to fight infections and microbial invasion.[38] The presence and high yield of flavonoid present in the leaves and stem bark of *Parkia bicolor* and the leaves of *Parkia biglobosa* justified their relatively high radical scavenging potential (antioxidant properties) of the two species. Recent studies have shown that many flavonoids and related polyphenols contribute significantly to the total antioxidant activity of many fruits and vegetables. [28, 39]

CONCLUSION

The two isolated species (allopatric taxa) of *Parkia* that were studied demonstrated a striking antioxidant activity in addition to the robust morphological and phytochemical characters. An appreciable amount of phytochemical was shown to be contained in its stem bark and leaf extracts. In addition, the quantitative phytochemical and antioxidant properties shown could also add to the description of the species thus indirectly providing information on the species. This result supports the traditional use of *Parkia* species. The reported activities are worthy of further pharmacological and phytochemical studies for possible isolation of active constituents responsible for the activities demonstrated.

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