

Parkia biglobosa Fruit Husks: Phytochemistry, Antibacterial, and Free Radical Scavenging Activities

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Abstract

Known for their role in the manufacture of eco-materials in buildings, *Parkia biglobosa* fruit husks are also used in folk medicine. The present study focuses on the metabolites content and antiradical, antibacterial activities of the hydroethanolic extract of *P. biglobosa* husks. Secondary metabolites were identified using staining and/or precipitation tests. The mineral content is determined according to the standard NF EN 14082. The antioxidant activity performed by 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging capacity, and antibacterial activity strain against three Gram-negative and two Gram-positive strains by microdilution for Minimal Inhibitory Concentration. Fruit husks contain tannins, anthocyanins, leuco-anthocyanins, anthraquinones, saponins, reducing compounds, sterols, terpenes, and 1.225% of potassium. The hydroethanolic extract of *P. biglobosa* fruit husks scavenges the DPPH radical with an EC₅₀ = 64 µg/ml. The extract is more active in one Gram (*Pseudomonas aeruginosa* and *Escherichia coli*) with a minimum inhibitory concentration of 1.25 mg/mL than the three Gram + studied. This study showed that *P. biglobosa* fruit husks extract could be used for its antioxidant and antibacterial activities.

Keywords

Parkia biglobosa, Husks, Constituent, Therapeutic Potential

1. Introduction

Parkia biglobosa (Jacq.) Benth or *Parkiaclappertoniana* (Keay) is a perennial tropical plant legume of the family Fabaceae found in gallery forests in Benin republic, Burkina Faso, Cote d'Ivoire, Cameroun, Nigeria, and Mali. It is a tree that can go up to 30 m in height, with red globular, red globose inflorescence; pod variable, flat, and seed-bearing [1] [2]. The fruit is a slightly bent, brown indehiscent pod, 30 to 40 cm long and 2 to 3 cm wide producing up to 20 seeds [3]. The different parts (leaves, bark, and roots) of this plant are known for several of their biological properties: anti-inflammatory, antibacterial, diarrhea, antidiabetic, abdominal pains, Gastric and duodenal ulcer, antihypertensive activity, and hepatic deficiency [2] [3]. The seeds were known for: food condiments obtained by fermentation, such as *afitin* and *sonru* in the republic of Benin [4]; *iru* and *dawadawa* in Nigeria [5]; *soumbala* in Burkina Faso [6], and for their oil termiticidal properties [7]. The fruit pulp is known for its great nutritional value due to its carbohydrates, proteins, and mineral content [8] [9]. The fruit husks extract has been used as a bonding agent between locally manufactured clay tiles and the soil beneath [10]. The decoction has been used on floors, walls of rooms, and in soil constructions in West Africa to improve their durability, [11] [12] to the production of laterite blocks for buildings to prove their durable protection and waterproofing [13] [14]. Scientific works had shown that they can be used as biopesticides in soils [15] [16]. Traditionally the husks are used in Burkina Faso, as an anti-poison [17], and in Benin, they are used as an anthelmintic in small ruminants [18]. In terms of scientific research, only: Abagale *et al.* [19] have done chemical analyses of the aqueous extract of *Parkia biglobosa* fruit husks collected from Northern Ghana, and Salit *et al.* [20] have evaluated the phytochemical, antimicrobial, toxicity, and antioxidant characteristics of seeds husks from Nigeria.

A medicinal plant is any plant that, in one or more of its organs, contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs [21]. This study aims to explore the potential of hydroethanolic extract of *P. biglobosa* fruit husks, through its: mineral content, secondary metabolite profile, free radical scavenging, and antimicrobial potential on some bacterial strains.

2. Materials and Methods

2.1. Chemicals and Reagents

2,2-Diphenyl-1-picrylhydrazyl (DPPH) and Iodonitrotetrazolium chloride (INT) were purchased from Sigma Aldrich Chemie GmbH, Steinheim, Germany, Mueller Hinton Broth and Agar provide from Oxoid, Basingstoke, United Kingdom.

2.2. Plants Materials

Plant material consisted of *P. biglobosa* fruits (Figure 1) and was collected in



Figure 1. *Parkia biglobosa* fruits.

Parakou in the department of Borgou, northern Benin in April 2018. After the fruits were separated from their husks; the husks (**Figure 2**) were dried at room temperature in the laboratory and reduced to powder.

2.3. Bacterial Strains

Two (2) Gram-negative *Escherichia coli* (ATCC25922) and *Pseudomonas aeruginosa* (CIP82118), and three (3) Gram-positive: *Enterococcus faecalis* (ATCC25212), *Staphylococcus aureus* (ATCC25923), Methicillin-resistant *Staphylococcus aureus* were used for the antibacterial test. They were obtained from the Laboratory of Biochemistry and Bioactives, Natural Substances, Faculty of Science and Technology, University of Abomey-Calavi of Benin republic.

2.4. Hydroethanolic Extraction

Ten grams (10 g) of *Parkia biglobosa* fruit husks powder was extracted by maceration with 100 mL of hydroethanolic solvent (50/50 v/v) for 24 hours under stirring. After filtration on Buchner, the filtrate was concentrated to dryness using a rotary evaporator and then stored at 4 °C until further use.

2.5. Determination of Mineral Content

The mineral content was determined on the ashes of the husks. The ash was digested for 30 min in a mixture of 1 M nitric acid and 3 N hydrochloric acid (ISO 15587-2). The filtrates obtained were used to determine the mineral content according to the standard NF EN 14082, using atomic absorption spectroscopy (VARIANT with spectra A110 software).

2.6. Phytochemical Screening

Phytochemical constituents of *Parkia biglobosa* fruit husks were determined by qualitative tests such as tannins (Ferric chloride test and Stiasny reaction), an



Figure 2. *Parkia biglobosa* fruits husks.

alkaloid (Dragendorff's), anthraquinones, flavonoids (Magnesium and hydrochloric acid reduction), saponins (Foam index), terpenes and sterols (Liebermann-burchard's test), mucilages (Alcohol 95% test), coumarins (UV-Lamp at 366 nm) and reducing compounds (Fehling's test) using the methods variously described by Bothon *et al.* [22] and Aswathi *et al.* [23].

2.7. Free Radical Scavenging Assay

Free radical scavenging activity of the alcoholic extract of *Parkia biglobosa* fruit husks was evaluated using 2, 2-diphenyl-1-picrylhydrazil (DPPH), as described by Bothon *et al.* [22] with slight modifications. 200 μL of the different concentrations (0 - 250 mg/mL) of the extract was added to 2.8 mL of DPPH solution at 120 μM . Ascorbic acid was used as a positive control. Absorbance (Abs.) at 517 nm was determined after 1 hour, and IC₅₀ (Inhibitory concentration 50%) was determined. IC₅₀ value denotes the concentration of sample required to scavenge 50% of the DPPH free radicals. The percent inhibition was calculated from Equation (1):

$$\% \text{ Inhibition} = (\text{Abs. of control} - \text{Abs. of the sample}) / (\text{Abs. of control}) \times 100 \quad (1)$$

2.8. Antimicrobial Activity

The antimicrobial test was performed using the method described by Atindehou *et al.* [24] Bacteria were cultured aerobically at 37°C in a Mueller Hinton Broth (MHB) for 18 h. The fruit huskshydroethanolic extract was suspended in acetone/water (10:90 v:v, 1 mL) and diluted to a concentration range from 10; 5; 2.5; 1.25; 0.625... mg/mL in MHB in 96 wells microplates in 100 μL . 100 μL of a midlogarithmic phase culture of bacteria with a concentration of 10⁶ CFU/mL at 620 nm was added. Each assay was performed in triplicate. Acetone/water control and negative control which consists of the mixture of MHB without bacteria

were realized. After 18 h of incubation at 37°C under agitation, the Minimal Inhibitory Concentration (MIC) was determined by the addition of 40 µL of Iodonitrotetrazolium chloride at 0.2 mg/mL in each well. Bacterial growth was determined by a reddish-pink color in the well after 1 hour of plate incubation at 37°C. The Minimal Bactericidal Concentration (MBC) was determined by sub-culturing the extract on agar from wells that showed no growth during the MIC determination.

3. Results and Discussion

3.1. Mineral Content

With moisture of 11.263% and an ash content of 5.745%, the most minerals present in *P. biglobosa* fruit husks were: potassium (1.225%), nitrogen (0.650%), and calcium (0.234%) (Figure 3).

Aqueous extract of Ghana husks [12], contains potassium (0.096%), calcium (0.0531%), magnesium (0.0245%), and iron (0.0107%). Although low in minerals compared to the results of this work; in both cases, the most important mineral is Potassium. *P. biglobosa* husks could be a good source. Potassium plays important role in muscle contraction - heart function - carbohydrate and protein metabolisms - acid-base balance [25]. A diet rich in potassium, magnesium, and calcium reduces the risk of hypertension [26]. Nitrogen makes an indispensable contribution to protein synthesis [27].

3.2. Phytochemical Screening

The phytochemical screening of the fruit husks of *P. biglobosa* showed the presence of tannins, coumarin, saponins, anthocyanin, reducing compounds, sterols, and terpenes (Table 1).

Present work reveals more secondary metabolites than those of Abagale *et al.* [19] from Ghana and Salit *et al.* [20] from Nigeria. Abagale *et al.* [19] revealed alkaloids, flavonoids, and saponins in the aqueous extract, and the same

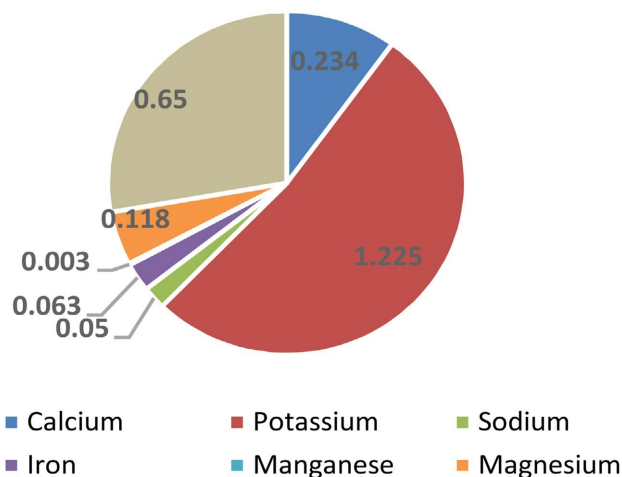


Figure 3. Minerals content of *Parkia biglobosa* fruit husks (%).

Table 1. Phytochemical screening of *Parkia biglobosa* fruit husks.

Secondary metabolites	Result
Alkaloids	–
Flavonoids	–
Tannins	+
Anthocyanins	+
Leuco-anthocyanins	+
Anthraquinone	+
Mucilage	–
Saponins	+
Coumarins	–
Quinones	–
Reducing compounds	+
Cyanogenic derivatives	–
Sterols and terpenes	+
Alkaloids	–

+ = present, – = not revealed.

metabolites were identified in ethanolic extract in addition to anthraquinones. Salit *et al.* [20] work showed the presence of flavonoids, saponins and steroids, and terpenes and no trace of tannins. This difference would be due to the ecological and edaphic factors diversity of the harvesting sites and to the fact that the screening was done on the powder of the whole sample while the other two authors did it on the aqueous, ethanolic, and methanolic extracts.

3.3. Free Radical Scavenging Capacity

Figure 4 shows the free radical scavenging ($EC_{50} = 64 \mu\text{g/mL}$) of the hydroethanolic extract of *P. biglobosa* fruit husks compare to ascorbic acid used as the positive control ($EC_{50} \approx 12 \mu\text{g/mL}$). Although it has an EC_{50} , five times higher than that of ascorbic acid, the hydroethanolic extract of *P. biglobosa* contains natural molecules that give it antiradical activity on DPPH. 250 $\mu\text{g/cm}^3$ of Nigeria husk's methanol extract, inhibited 80.54% DDPH radical [20]. The antioxidant activity observed with the hydroethanolic extract studied depends on its tannin content while, that observed with the Nigeria sample, depends on flavonoids because it does not contain tannins. Tannins and flavonoids possess strong antiradical properties due to the high number of hydroxy groups connected to the aromatic ring [28] [29] [30].

Free radicals in the living organism play a dual role. Though they have beneficial roles in the body to fight against certain pathogenic diseases; they are toxic by-products of aerobic metabolism causing oxidative damage and tissue dysfunction [31]. They may eventually lead to oxidative stress responsible for certain

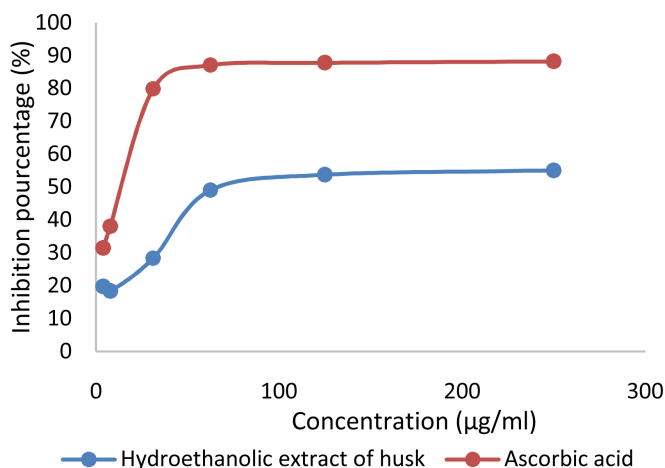


Figure 4. Free radical scavenging of hydroethanolic extract of *Parikia biglobosa* fruit husks.

metabolic diseases such as cancer, diabetes, atherosclerosis, hypertension, respiratory diseases, arthritis, cataract, cancer, and cardiovascular diseases. The search for new sources of anti-radical molecules will serve to the development of new drugs for the treatment of these various diseases.

3.4. Antibacterial Activity

The development of resistance to currently available antibiotics due to their prolonged use is a global concern and is an increasing global health crisis [32]. Many multidrug-resistant pathogens exist and are responsible for several diseases. *Pseudomonas aeruginosa* causes about 3% to 5% of nosocomial pneumonia. It is among the three top microorganisms causing healthcare respiratory infections [33]. *Escherichia coli* is the most common cause of bacteremia in high-income countries [34]. It is associated with many diseases like Crohn's disease, diarrhea in children hemorrhagic colitis, and Shigellosis-like, and is a probable source of food-borne disease [35]. Traditionally *Enterococcus faecalis* live safely in the intestines. However, if it spreads to other parts of your body and can cause a more serious infection. The bacteria can enter the blood, urine, and wound during surgery [36]. From there it can spread to different sites causing more serious infections including sepsis, endocarditis, and meningitis. *Staphylococcus aureus* is a leading causative agent in pneumonia and other respiratory tract infections surgical site prosthetic joint and cardiovascular infections as well as nosocomial bacteremia [37]. Methicillin-Resistant *Staphylococcus aureus* is a major public health problem worldwide and it is responsible for both hospital and community-associated infections and is a therapeutic challenge to treat [38] [39].

The use of natural antimicrobial compounds from plants; is important both for food preservation and also in the control of human infectious diseases. *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus aureus*, and Methicillin-Resistant *Staphylococcus aureus* are sensitive to certain

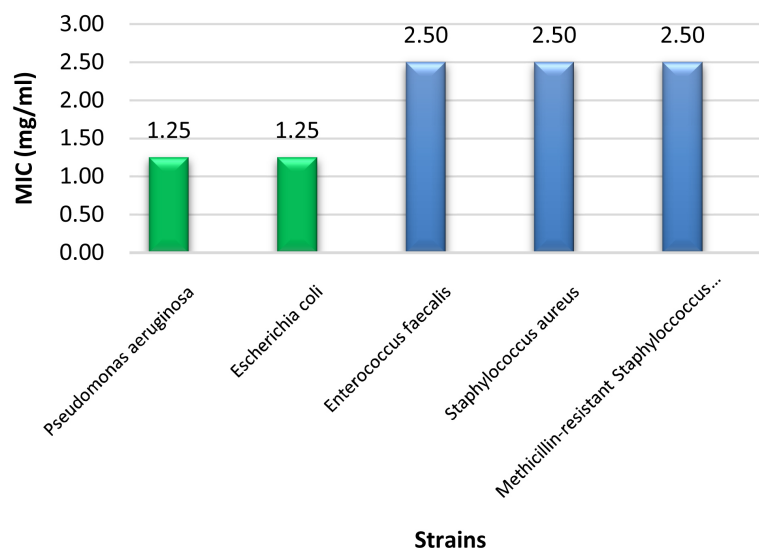


Figure 5. Free radical scavenging of hydroethanolic extract of *Parkia biglobosa* fruit husks.

compounds contained in the hydroethanolic extract of *Parkia biglobosa* fruit husks from Benin. **Figure 5** shows the MICs of the hydroethanolic extract on the three bacterial strains studied. The extract was more active on Gram-negative (1.25 mg/mL) than on Gram-positive (2.50 mg/mL).

The difference in the sensitivity of gram-positive and gram-negative bacteria to the extract may be explained by the fact that the gram+ wall consists of several layers of peptidoglycan while Gram-negative contains only one. The presence of water-soluble compounds like tannins, and saponins in the hydroethanolic extract could justify that they penetrate more easily the cell walls of Gram-negative bacteria than that of Gram-positive. Badmos *et al.* [40] show qualities of traditional West African soft cheese that may be preserved using honey in combination with ether extract of *Parkia biglobosa* fruits husks. Salit *et al.* [20] studied methanol extract of seed husks and showed that it has no inhibition activity on *Candida albicans*, *Escherichia coli*, *Bacillus subtilis* and *Pseudomonas species* because of its low secondary metabolite content mainly tannins, which is known for their antiseptic and astringent properties [41]. Saponins in general are reported to be significant antibacterial agents and several plants are claimed to be antibacterial [42] [43].

4. Conclusion

Secondary metabolites, mineral content, radical scavenging capacity, and antibacterial potential of hydroethanolic extract of *Parkia biglobosa* fruit husks were investigated. The results showed that the studied extract contains potassium as the main mineral; tannins, saponin, reducing sugars, sterol, and terpenes as secondary metabolites. Although the flavonoids and alkaloids were not revealed, the hydroethanolic extract of *Parkia biglobosa* fruit husks has a strong antiradical capacity compared to ascorbic acid and the growth of gram-negative bacteria is

inhibited at lower concentrations contrary to gram-negative bacteria. It would be interesting to study the structure of secondary metabolites contained in this part of *Parkia biglobosa* fruit and test their antibacterial power on a wide range of bacterial and fungal strains that have now become multi-resistant with antibiotics.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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