Phytochemical Analysis and Antimicrobial Activity of Parkia Biglobosa (Jacq.) Benth. Extracts Against Some Food – Borne Microrganisms

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ABSTRACT

Phytochemical analysis of aqueous and ethanol extracts of Parkia biglobosa leaf and pod was carried out using standard protocols. The antimicrobial activity of the same plant materials was carried out using the disc diffusion method. The extracts were tested against Staphylococcus aureus, Enterobacter aerogenes, Escherichia coli, Salmonella typhi, Salmonella typhimurium, Shigella spp and Pseudomonas aeruginosa (bacteria) and Mucor spp and Rhizopus spp (fungi) isolated and characterized from some selected food samples. Results of the phytochemical chemical analysis revealed variation in the type of phytochemicals detected in the extracts. Results of antimicrobial activity of P. biglobosa extracts confirmed a broad spectrum of activity on all the bacteria tested by the aqueous and ethanol pod extracts at concentrations between 500 μg and 4000 μg with Minimum inhibitory concentrations (MIC) ranging between 1.0 and 4.0 mg/ml. The antifungal activity results however, showed that Parkia leaf aqueous and ethanol extracts inhibited by 50% growth of Mucor and Rhizopus species at 1mg/ml concentration. The antimicrobial activity exhibited by these extracts on food – borne pathogens and spoilage causing organisms showcased the preservative potentials these extracts possess in controlling the growth of such organisms in foods.

Key words: Parkia biglobosa, Preservative, Food – borne pathogens, Spoilage causing organisms.

Introduction

Microorganisms are associated in a variety of ways with all the foods we eat. Food products serve not only as sources of nutrition for human and other animals, but also as substrates for the growth of microorganisms [1]. The uncontrolled growth of microorganisms in food causes spoilage, a serious problem accounting for sizeable losses of food products that are critically needed to meet global food requirements [2]. Growth in food of pathogenic, toxigenic and spoilage causing microorganisms such as Salmonella spp, Shigella spp, Escherichia coli, Pseudomonas spp, Enterobacter aerogenes, Mucor spp, Rhizopus spp, Aspergillus spp etc need to be controlled in food as they pose the risk of causing food – borne infections, food – borne intoxications and decay of the food. Control of pathogenic and spoilage causing microorganisms is achieved by sanitizing the food, to reduce its microbial load and ultimately extend its shelf life by application of chemical and physical methods such as use of chemical preservatives (lactic acid, sodium nitrite, benzoic acid, sodium benzoate etc) and irradiation (ultraviolet radiation). These methods have been reported to be detrimental to the health of consumers of such preserved foods [3]. Currently, there is growing pressure from consumers on food industries to replace use of synthetic chemicals with natural alternatives (plant – derived sanitizers/preservatives) in order to ensure safety [4]. As a result of this, a lot of researches are conducted

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to screen plant materials (leaves, stem-bark, roots etc) for bioactive compounds that could be used as sanitizers/preservatives in foods. A product of such researches is the Grape Seed Extract (GSE), which has been accepted and presently applied as a ‘safe plant derived preservative’ for use in foods [5]. A very important step in the screening of a plant material for sanitizing/preservative activity is to evaluate its antimicrobial activity against food – borne microorganisms. The determination of a plant’s antimicrobial profile against food – borne microorganisms may promote the plant to further tests geared towards its evaluation as a sanitizer or preservative in foods.

It is in view of this, that the present research was set up to evaluate the phytochemical constituents and antimicrobial activity of aqueous and ethanol extracts of Parkia biglobosa pod and leaf (claimed by local herbalists to treat diarrhoeal diseases in man) against some food – borne microorganisms. The objective is to further research on its sanitizing and preservative activity, if the test organisms are found to be susceptible.

Materials and methods

Collection, Authentication and Processing of Plant Materials:

The leaf and pod of Parkia biglobosa were collected from Old Campus, Bayero University, Kano, Nigeria. The plant material was identified and authenticated by a Botanist at the Biological Sciences Department, Bayero University, Kano, Nigeria. The leaf and the pod were air dried and ground into fine powder and kept for future use.

Extraction:

The powdered plant material (50g) each of pod and leaf were percolated in 500mL redistilled ethanol in 1L capacity conical flask and stoppered and kept for two weeks with intermittent shaking. The percolate was filtered with Whatman’s No 1 filter paper. The ethanol extract was concentrated at 40°C under reduced pressure using rotary evaporator (R110). The same quantity of plant material were again percolated with distilled water for one week and after filtration, the aqueous extract was concentrated in hot oven at 40°C [6]. The concentrated extracts were labeled PPW (aqueous pod extract), PPE (ethanol pod extract), PLE (leaf ethanol extract) and PLW (aqueous leaf extract) respectively. Each extract was screened for some important phytochemicals.

Screening of Some Phytochemicals:

Phytochemical analysis for qualitative detection of alkaloids, flavonoids, tannins, saponins, reducing sugar and flavones was performed on the extracts as described by Trease and Evans [7], Sofowora [8].

Source of Microorganisms:

Staphylococcus aureus, Enterobacter aerogenes, Escherichia coli, Salmonella typhi, Salmonella typhimurium, Shigella spp and Pseudomonas aeruginosa were bacteria isolated, while Macor spp and Rhizopus spp were fungi isolated from food samples, which included Rice and Beans dish, Roselle calyx juice, Fresh tomato, Bread, lettuce, carrot, fried groundnut. Food samples were homogenized and streaked on appropriate media for isolation. Cultural and morphological identification was carried out and finally biochemical characterization of isolates using protocols described by Cheesbrough [9] was done. Pure cultures of the isolates were maintained in appropriate media for future use.

Antimicrobial Disc preparation:

Discs of about 6mm diameter were made from Whatman’s No.1 filter paper using a paper puncher. Batches of 100 discs were transferred into Bijou bottles and sterilized in the oven at 121°C for 15minutes. Stock solution (400mg/ml) of the plant extract was prepared by dissolving 0.8g of each fraction in 2ml Dimethylsulphoxide (DMSO). Serial doubling dilution was carried out by adding 1ml of DMSO at each serial dilution. Four concentrations were prepared from the stock solution such that each disc would absorb 0.01ml which is equivalent to 500 μg/disc, 1000 μg/disc, 2000 μg/disc and 4000 μg/disc respectively.

Standardization of Inoculum:

The inocula were prepared from the stock cultures which were maintained in nutrient agar slant at 4°C and subculture in nutrient broth using a sterilized wire loop. The density of suspension inoculated unto the media for susceptibility test was determined by comparison with 0.5 McFarland standard of Barium sulphate solution [9]. Spore suspension for fungal bioassay was prepared according to the procedure of Murugan et al. [10].

Susceptibility Testing:

Disc agar diffusion technique described by Bauer and Kirby [11] and demonstrated by Cakir et al. [12] was employed for antibacterial bioassay. For fungi susceptibility testing, the extracts were incorporated into appropriate medium and subsequently fungal
spore suspension inoculated. The preparation was incubated at appropriate temperatures. After incubation, zone of inhibition diameter formed in the medium was measured to determine antibacterial effectiveness of the different concentrations of the extracts, while sensitivity of the fungi to the test extract was recorded as described by Murugan et al. [10].

**Determination of Minimum Inhibitory Concentration (M.I.C.):**

The minimum inhibitory concentration for bacterial isolates was carried out using tube dilution as described by Akinseye et al. [13]. Stock solution of 80,000μg in 10ml sterilized distilled water was serially diluted to arrive at concentrations of 500 μg/ml, 1000 μg/ml, 2000 μg/ml and 4000 μg/ml respectively.

**Results and discussion**

The results of the phytochemical analysis are presented in Table 1. Only the pod possesses flavonoids, both pod and leaf possess reducing sugar and tannins. Alkaloids were only detected in the ethanol leaf extract. Saponins were not detected in both the leaf and pod. The result of the phytochemical analysis corroborate the work of Ajaiyeoba [14] who reported presence of tannins in two leaf extracts (water and ethanol), alkaloids only in leaf ethanol extract and saponins were absent in both leaf extracts. In terms of possession of phytochemicals, the result of the present research shows the pod extracts to possess more phytochemicals than the leaf extracts. Scrutiny of past works on *P. biglobosa* however, shows not much has been reported on the pod extracts.

Table 2 presents the results of antibacterial activity of the various extracts of *P. biglobosa*. The antibacterial activity of an extract is read from the zone of inhibition diameter produced by it around the disk, diameter >6mm indicates activity while <6mm showed failure by the extract [15]. It could be observed that *P. biglobosa* pod aqueous (PPW) extract had the broadest spectrum of activity on the test organisms. Seven (7) bacterial isolates were sensitive to the extract at different concentrations. *Enterobacter* spp (10mm), *S. aureus* (10mm), *E. coli* (10mm), *S. typhimurium* (10mm) were sensitive to the lowest concentration of 50mg/ml. *Shigella* spp (14mm), *P. aeruginosa* (07mm), and *S. typhi* (07) were only sensitive to concentration of 200mg/ml. The MIC of the extract is 1.0mg/ml for *Shigella* spp, *S. typhi* and Enterobacter spp, while MIC of 2mg/ml was observed for *E. coli*, *P. aeruginosa* and *S. aureus*. The MIC for *S. typhimurium* was found to be 4.0mg/ml (Table 2).

Of note is that PPW shows the broadest activity on bacteria tested which range from enteropathogenic, toxigenic to spoilage causing organisms. Perhaps its broad spectrum of activity on food – borne bacteria coupled with its low MIC on most of the bacteria tested might give it an impetus as a potential preservative on foods.

*Parkia* pod ethanol (PLE) extract was active on six (6) bacteria with only *S. typhimurium* (07mm) sensitive to the lowest concentration of 50mg/ml. *Enterobacter* spp (10mm), *S. aureus* (10mm), *P. aeruginosa* (07mm) and *S. typhi* (07mm) were sensitive to concentration of 100mg/ml. *Shigella* spp (12mm) was only sensitive to concentration of 200mg/ml. The MIC of the extract is 1.0mg/ml for *S. typhimurium*, *Shigella* spp, *P. aeruginosa* and Enterobacter spp, while MIC for *S. typhi* (2mg/ml), *S. aureus* (4.0mg/ml) and >4.0mg/ml for *E. coli* (Table 2).

Literature related to phytochemical constituents of *P. biglobosa* pod extracts is scanty. This might be attributed to the fact that *P. biglobosa* is a tree indigenous to West Africa and therefore research on the plant is scanty and claims by traditional herbalists on the usefulness of the plant as medicinal mostly centered on the use of the stem bark, root and leaf. Personal discussion with traditional herbalists revealed the use of the pod by people in northern Nigeria to treat diarrhoeal diseases in humans.

The result of *P. biglobosa* leaf extracts is also presented in Table 2. *Parkia* leaf ethanol (PLE) extract was active on five (5) test bacteria; *Enterobacter* spp (09mm), *S. aureus* (07mm), *Shigella* spp (12mm), *P. aeruginosa* (07mm), *E. coli* (10mm), *S. typhimurium* (10mm) and *S. typhi* (07) were sensitive to concentration of 200mg/ml. The finding of the present study is corroborated by the report of Udobi and Onaolapo [16] who equally reported PLE as active on *S. aureus* at 200mg/ml. Ajaiyeoba [14] reported PLE as active on *S. aureus*, *E. coli* and *P. aeruginosa* with zone of inhibition diameters of 8mm, 7mm and 7mm respectively. Udobi and Onaolapo [16] also reported P. aeruginosa and *E. coli* to be sensitive to 200mg/ml concentration of PLE in disagreement with result of the present study.

*Parkia* leaf water (PLW) was active on 3 bacterial isolates; *Enterobacter* spp (08mm), *Shigella* spp (08mm), *S. typhi* (07mm) were sensitive to concentration of 200mg/ml. *S. aureus*, *S. aeruginosa*, *E. coli* and *S. typhimurium* were not sensitive to any of the concentrations tested (Table 2). This disagrees with the findings of Udobi and Onaolapo [16] who reported *S. aureus*, *P. aeruginosa* and *E. coli* to be sensitive to PLW at 200mg/ml. Ajaiyeoba [14] also reported PLE as active on *S. aureus*, *E. coli* and *P. aeruginosa*, which disagrees with the findings of the present study, which reported
the three bacteria not sensitive at 100mg/ml concentration. Variations in the sensitivity of the bacterial species tested on the extracts might be as a result of differences in the strains employed for the research as Udobi and Onaolapo [16] used clinical strains from patients, while Ajaiyeoba [14] used standard strains from the National Culture Type Collection (NCTC) which quite differ from the wild strains sourced from food samples used in this research. Wild strains of bacteria could possess genetic capabilities that could make them adapt well to the tough environments they dwell in as compared to stock cultures (ATCC, NCTC etc), which have been isolated and preserved.

The antimicrobial activity of plant extracts has been linked by many researchers to be due to the presence of phytochemicals in them [17, 18, 19]. The antimicrobial activity of the extracts tested in vitro could be higher than they are reported if active ingredients from the extracts are isolated and tested. Ebi and Ofoefule [20] reported that crude extracts may contain inactive substances which may also antagonize the antimicrobial actions of one other.

Table 3 shows the result of antifungal activities of *P. biglobosa* extracts on the test molds. PPW and PPE inhibited the growth of *Mucor* spp by 25% at 1000μg/ml concentration, while they were not active on *Rhizopus* spp at all concentrations. PLW and PLE inhibited the growth of *Mucor* spp by 50% at 1000μg/ml concentration, while PLW inhibited the growth of *Rhizopus* spp by 50% at 1000μg/ml concentration. On the other hand, PLE inhibited the growth of *Rhizopus* spp by 25% at the same concentration.

PLW was observed to show the highest activity as compared to the other extracts as it inhibited growth of both *Mucor* spp and *Rhizopus* spp by 50%. Ketoconazole, which is the standard drug, inhibited both *Mucor* spp and *Rhizopus* spp by 100% at 500μg/ml concentration (Table 3).

Not much have been reported on the antifungal activity of *P. biglobosa* extracts, except report by Udobi and Onaolapo [16] who reported on the antifungida activity of PLE and PLW.

### Table 1: Phytochemicals present in various extracts of *P. biglobosa*

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>PPW</th>
<th>PPE</th>
<th>PLW</th>
<th>PLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
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<tr>
<td>Reducing sugar</td>
<td>+</td>
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<td>-</td>
<td>+</td>
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<tr>
<td>Saponins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Flavones</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Key: PPW = *Parkia* pod water extract, PPE = *Parkia* pod ethanol extract, PLW = *Parkia* leaf water extract, PLE = *Parkia* leaf ethanol extract

### Table 2: Antibacterial activity of *Parkia biglobosa* extracts on food-borne bacterial isolates

<table>
<thead>
<tr>
<th>Extract</th>
<th>Conc. (mg/ml)</th>
<th>Zone of Inhibition (mm)</th>
<th>MIC (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPW</td>
<td></td>
<td>ENT STA SHI PA EC STM ST</td>
<td>1.0</td>
</tr>
<tr>
<td>PPE</td>
<td>50</td>
<td>06 06 06 06 07 09 06</td>
<td></td>
</tr>
<tr>
<td>PLE</td>
<td>50</td>
<td>07 06 06 06 06 06</td>
<td></td>
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<tr>
<td>PLW</td>
<td>50</td>
<td>06 06 06 06 06 06</td>
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</table>

### Table 3: Antifungal activities of *Parkia biglobosa* extracts on food molds

<table>
<thead>
<tr>
<th>Extract</th>
<th>Conc. (mg/ml)</th>
<th>Zone of Inhibition (mm)</th>
<th>MIC (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPW</td>
<td>50</td>
<td>06 06 06 06 07</td>
<td>1.0</td>
</tr>
<tr>
<td>PPE</td>
<td>50</td>
<td>07 06 06 06 06</td>
<td>1.0</td>
</tr>
<tr>
<td>PLW</td>
<td>50</td>
<td>06 06 06 06 06</td>
<td>2.0</td>
</tr>
</tbody>
</table>

Key: PPE = *Parkia* pod ethanol, PPW = *Parkia* pod water, PLE = *Parkia* leaf ethanol, PLW = *Parkia* leaf water, 06mm = size of disc μg = microgramme, Control = Streptomycin, ENT = Enterobacter aerogenes, STA = *S. aureus*, SHI = Shigella spp, PA = *P. aeruginosa*, EC = *E. coli*, STM = *S. typhimurium*, ST = *S. typhi*
Table 3: Antifungal activities of various extracts of *P. biglobosa*

<table>
<thead>
<tr>
<th>Extract</th>
<th>Conc. (μg/ml)</th>
<th><em>Mucor</em> spp</th>
<th><em>Rhizopus</em> spp</th>
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<tr>
<td>PPW</td>
<td>100</td>
<td>+++</td>
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<tr>
<td></td>
<td>500</td>
<td>+++</td>
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<td></td>
<td>1000</td>
<td>++</td>
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<tr>
<td>PPE</td>
<td>100</td>
<td>+++</td>
<td>+++</td>
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<tr>
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<td>500</td>
<td>+++</td>
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<td>++</td>
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<td></td>
<td>500</td>
<td>+++</td>
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</tr>
<tr>
<td></td>
<td>1000</td>
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<td>+</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>500</td>
<td>+</td>
<td>-</td>
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</table>

Key: PPE = *Parkia* pod ethanol, PPW = *Parkia* pod water, PLE = *Parkia* leaf ethanol, PLW = *Parkia* leaf water

++++ = normal growth, +++ = 25% inhibition, ++ = 50% inhibition, + = 75% inhibition
- = 100% inhibition

Conclusion and recommendation:

The result of the present study has shown the potential *Parkia biglobosa* aqueous and ethanol extracts possess as preservatives. This is due to the fact that they were found to possess antimicrobial activities against food – borne microorganism often implicated in the spoilage of foods and also food – borne illnesses. Further research should be conducted to test the preservative effect of the extracts on some foods.

Acknowledgement

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References


