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PHYTOCHEMICAL AND ANTIBACTERIAL ACTIVITY OF ETHANOL AND ETHYL ACETATE EXTRACTS OF *PARKIA BIGLOBOSA*

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ABSTRACT

Phytochemical and Antibacterial activity of ethanol and ethyl acetate extracts of *Parkia Biglobosa.* The antibacterial potential of *Parkia Biglobosa*, Bark, root and leaf extracts against bacteria isolated from food items. The test bacteria were isolated from food items and identified using standard microbiological methods; ethanol and ethyl acetate were used as solvents in the extraction of the extract from the Bark, root and leaf of *Parkia Biglobosa*. The phytochemical screening showed that the ethanol and ethyl acetate extracts contained tannins, Phenols, Steroids and saponins in varying proportions. The Antibacterial activity of the extract against the test bacteria was determined using cup agar plate method and diameter inhibition zones formed around wells on the agar plates were measured. The mean antibacterial activity of the extracts *in vitro* showed that the Ethyl acetate extract of leaves was most efficacious at 50mg amount of the concentration inhibiting *Salmonella* sp (11.02mm)**,** *E. coli* (14.10mm), *Klebsiella* sp (18.0mm), *Bacillus* sp (15.0mm) and *S. aureus* (13.0mm). The ethanol extract at 12.5mg amount of the concentration inhibited *Salmonella* sp (7.0mm), *E. coli* (8.0mm) and *Klebsiella* sp (10.0mm). The ethyl acetate extract of the Root of *P. biglobosa* inhibition zone varied from 7.0mm - 21.0 mm against the tested bacteria such as *Salmonella* sp, *E. coli, Klebsiella* sp, *Bacillus* sp and *S. aureus.* The ethyl acetate Bark extract of *P. biglobosa* extracts were found to be less efficient than ethanol extracts. This study has revealed that the extract of *P. biglobosa* contains antibacterial and phytochemical substances which can be harnessed.

KEYWORDS: P. biglobosa extract, phytochemical screening, inhibition zone.

INTRODUCTION

Plants have provided as sources of inspiration for drug compounds development, as plant derived medicines which have made large contribution to the well-being of human and health.

Although many synthetic drugs that are more potent have replaced drugs that generally come from trees, trees remain a source for some drug ingredients.[1] Medicinal plants have become important for the treatment of different disease conditions, such as diabetes, malaria and anemia for a long time now.[2] But the potential of higher plants as source for new drugs is still largely unexplored.[3] Systematic screening of them may result in the discovery of novel effective compounds.[4] *Parkia biglobosa* commonly called African locust bean tree is known in Yoruba as Igba or Irugba, in Hausa as Dorowa and in lgbo as Ogiri, it belongs to the family Fabaceae-Mimosoideae, it is the most versatile, multifarious trees of tropics, with immense potential. It possesses maximum useful non-wood products (leaves, bark, flowers, fruits, seed, and oil) than any other tree species.

Various parts of the *Parkia biglobosa* tree have been used as traditional medicine in African.

Parkia biglobosa oil and the bark and leaf extracts have been therapeutically used as folk medicine to control diabetes, intestinal helminthiasis, respiratory disorders, and constipation and also as a general health promoter. *Parkia biglobosa* Bark and leaf has been found useful to control various skin infections. Bark, leaf, root, flower and fruit together cure blood morbidity, biliary afflictions, itching, skin ulcers, burning sensations and pthisis.[5]

Parkia biglobosa tree has adaptability to a wide range of climatic, topographic and edaphic factors. It thrives well in dry, stony shallow soils and even on soils having hard calcareous or clay pan, at a shallow depth. *Parkia biglobosa* tree requires little water and plenty of sunlight.[6]

The tree grows naturally in areas where the rainfall is in the range of 450 to 1200 mm. However, it has been introduced successfully even in areas where the rainfall is as low as 150 to 250 mm. *Parkia biglobosa* grows on

altitudes up to 1500 m. It can grow well in wide temperature range of 0 °C to 49 °C. In Nigeria it grows in regions with hot weather like northern part of the country. Therefore, we used *Parkia biglobosa* samples from the regions, in order to determine the antimicrobial activity of *Parkia biglobosa* plant extracts against some specific bacteria isolated from food items.

MATERIAL AND METHODS

Collection of Samples and Preparation

Fresh and healthy leaves of *Parkia biglobosa* were obtained at locations within Nasarawa state, North-Central Nigeria and identified at the Plant Science and Biotechnology herbarium in Nasarawa State University, Keffi. The leaves were washed in distilled water and allowed to dry at room temperature for 7 days. The leaves were then pulverized into coarse powder using blending machine.

Isolation and Identification of Bacterial Species

Bacterial species were isolated from food item samples collected from Keffi Market, Nigeria. The food item samples were cultured by streaking on Eosin Methylene Blue (EMB), MacConkey Agar (MA) and Mannitol Salt Agar (MSA), and incubated at 37 °C for 24 hrs.

Identification of the bacterial isolates was based on the cultural and morphological and biochemical characteristics such as, Indole, Methyl Red/Vorges-Proskauer, Citrate utilization, Catalase, Coagulase, Nitrate reduction, Urease and Sugar fermentation tests following standard microbiological procedures as described by Cheesebrough.[7]

Extraction of *Parkia biglobosa Bark*, *root* and leaves

Extracts were prepared following the method described by Ekeleme *et al*.[8] Briefly, 100 g of dried pulverized *Parkia biglobosa Bark, root* and leaves were soaked in 500 mL of ethanolic and ethyl acetate for 24 hrs at room temperature, under occasional shaking. Extraction was repeated three times, and the extracts obtained were filtered using Whatman filter paper number 1. After that, the extracts were concentrated to dryness under reduced pressure using a rotary evaporator at 45 °C.

Phytochemical Analysis

Test for Alkaloids

The extracts (20 μL) was applied on TLC plate (Silica Gel 60G, 5 × 10 cm) and eluted using toluene-ethyl acetate-diethylamine (70: 20: 10) as solvent system. Alkaloid was detected after spraying Dragendorff's reagent as orange-brown spots on the TLC plate.[9]

2.4.2. Test for Quinones

Extracts suspended in ethanol (1 mL) were treated with 1mL of concentrated sulfuric acid. Formation of red colour showed the presence of quinones.[9]

Test for Glycosides

One ml of glacial acetic acid, 3 drops 5% W/V ferric chloride and concentrated sulphuric acid were added to test tubes containing 2 ml of extracts and observed. The disappearance of reddish brown colour at the junction of two layers and bluish green in upper layer indicates the presence of glycosides.[10]

Test for Tannins

Extracts were treated with 1mL of 5% ferric chloride. The presence of tannin was indicated by the formation of bluish black or greenish black precipitate.[8] Ekeleme *et al.* (2017)

Test for flavonoids

Few fragments of magnesium metal ribbon (3-4 pieces) was added to 1 mL of the extracts, followed by drop wise addition of concentrated hydrochloric acid. Formation of pink or red colour indicated the presence of flavonoids.[10]

Test for Saponin

The 2 mL of distilled water was added to extracts suspended in ethanol and was shaken vigorously. The formation of profuse foam layer indicated the presence of saponins.[8]

Test for Terpenoids

One mL of acetic anhydride and 5 drops of concentrated sulfuric acid (H2SO4) was added to the extracts.A colour change from violet to blue confirms the presence of steroids[8] and formation of blue-green ring indicates the presence of terpenoids.[11]

Determination of Antibacterial Activity of *Parkia biglobosa*

Antibacterial activity of crude extracts (ethanolic and ethyl acetate) of *Parkia biglobosa* bark, root, leaves was carried out using cup-plate agar diffusion bioassay.[12] As follows; 100 μL of fresh culture (Standardized to 0.5 McFarland) was spread uniformly on a sterile Mueller-Hinton agar (MHA) plates and allowed to air dry. After that, wells of 6 mm in diameter were made in the MHA plates using a sterilized cup-borer and the base was seeded with molten MHA and approximately 100 μL for each concentration (50 mg/L, 25 mg/L, 12.5 mg/L, 6.25 mg/L and 3.125) of the extract was dispensed into the wells and the plates were allowed to stand for 1 hr at room temperature for pre-diffusion and then incubated at 37 °C for 24 hrs and the diameter zone of inhibition against the test strain is measured and recorded. Ciprofloxacin (5μg) was used as control.

RESULTS AND DISCUSSION

Table 1 shows the qualitative screening of phytochemical properties of *Parkia biglobosa* bark, root and leaves extracts. it showed the presence of moderate quantities (+) and absence (-) of tannins, alkaloids, saponins, glycoside, terpenoids, and flavonoids in the different solvents used.

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

Sl. No	Phytochemicals	Bark		leaves		Root	
		Ethanol	Ethyl acetate	Ethanol	Ethyl acetate	Ethanol	Ethyl acetate
1	Flavonoids	-	-	-	+	-	-
2	Phenols	+	+	+	+	+	+
3	Alkaloids	-	-	-	-	-	-
4	Tannins	+	+	+	+	+	+
5	Steroids	+	+	-	-	+	+
6	Saponins	+	-	+	-	+	-

+ = present; - = absent

The antimicrobial activity of crude extract of ethyl acetate leave of *P. biglobosa* against bacteria isolated from some food items (Table 2) showed that the ethyl acetate extracts has antimicrobial activity at concentrations 50 mg and 25mg against all tested bacteria with the exception of *Salmonella* sp which was inhibited only at 50 mg concentration. Table 3 showed the antimicrobial activity of crude ethanol leave extract of *P. biglobosa* against different bacterial isolates. There was highest inhibitory activity as observed against *K. pneumoniae* with 20.0mg, 16.0mg, 10.0mg and 6.0mg mean zone of inhibition was observed at 50mg, 25mg, 12.5mg and 6.25mg concentration. The most resistant to this extract were *S. aureus* and *Bacillus* which showed 8.0 mm and 9.0mm mean zone of inhibition at 25mg concentration. The antibacterial activity of crude ethyl acetate and ethanol leaves extract of *P. biglobosa* against bacterial isolated from food items (Table 2 and 3) showed significant level of activity against all test bacterial isolates at 50 mg concentration with decreased activity at lower concentrations.

This study on the antimicrobial and phytochemical properties of *P. biglobosa* leaves extracts on bacterial isolated from food items using ethyl acetate and ethanol as solvents revealed that the ethyl acetate extracts inhibits *E. coli*, and *K. pneumoniae* at 12.5 mg concentration whereas *Salmonella* sp was inhibited at 50 mg concentration of the ethyl acetate extracts. However, the ethanol extracts was potent at 6.25 mg concentration against *Klebsiella* sp.[13] has reported that the alcoholic and Ethyl acetate extract of *P. biglobosa* leaves have antimicrobial activity against the clinical bacterial and fungus *Tricophyton metagrophytes*.[14] Has also demonstrated the antimicrobial properties of *P. biglobosa* leaves extracts. In line with this research report also,[15] has reported that *P. biglobosa* has Gram-negative antibacterial characteristics.

Table 2: Antibacterial Activity of Ethyl acetate leave of *P. biglobosa* against bacteria isolated from some food items.

	Diameter of the Zone of inhibition (mm)(mean ± SD) of isolates at various amount of the extract				
Sl. No	Isolates	50.00mg	25.00mg	12.5mg	6.25mg	3.125mg
1	*Salmonella* sp	11.02±2.00	0.00	0.00	0.00	0.00
2	*E. coli*	14.1±1.00	11.0±0.21	9.0±1.10	0.00	0.00
3	*Klebsiella*	18.0±1.00	13.0±1.0	10.0±1.18	0.00	0.00
4	*Bacillus*	15.0±0.57	8.0±1.11	0.00	0.00	0.00
5	*S. aureus*	13.0±2.00	7.0±2.0	0.00	0.00	0.00

Table 3: Antibacterial activity of Ethanol leave extract of *P. biglobosa* against bacteria isolated from some food items.

	Diameter of the Zone of inhibition (mm)(mean ± SD) of isolates at various amount of the extract				
Sl. No	Isolates	50.0mg	25.0mg	12.5mg	6.25mg	3.125mg
1	*Salmonella* sp	12.0 ± 1.0	10.0 ± 0.48	7.0 ± 0.00	0.00	0.00
2	*S. aureus*	13.0 ± 1.00	8.0 ± 1.00	0.00	0.00	0.00
3	*E. coli*	16.0 ± 2.01	11.0 ± 0.27	8.0 ± 0.12	0.00	0.00
4	*Klebsiella* sp	20.0 ± 1.82	16.0 ± 2.00	10.0 ± 1.11	6.0 ± 1.27	0.00
5	*Bacillus* sp	12.0 ± 2.00	9.0 ± 1.42	0.00	0.00	0.00

Table 4 showed that the ethyl acetate root extract *of P. biglobosa* against bacteria isolated from some food items has antibacterial activity at concentrations 12.5 mg against all tested bacteria with the exception of *Bacillus* sp which was inhibited at 50 mg and 25.0mg concentration and was highly active against *S. aureus* and *Klebsiella* sp which had 7.0mm and 9.0mm mean zone of inhibition. Table 5 showed the antibacterial activity of crude ethanol root extract of *P. biglobosa* against bacterial isolates. In this result, the highest inhibitory activity was observed against *E. coli* with 22.0 mm mean zone of inhibition was observed at 50mg concentration. *E. coli* was mostly susceptibility to this extract and showed activity to all concentration. The

antibacterial activity of crude ethyl acetate and ethanol bark extract of *P. biglobosa* against bacterial isolates (Table 6 and 7) showed significant level of activity against all test bacterial isolates at 50 mg concentration with decreased activity at lower concentrations.

It was also observed in this research the presence of alkaloids, flavonoids, tannins, saponins, glycosides and terpenoids in the leaves, root and bark extracts of *P. biglobosa*. Also, these phytochemicals has been shown to have inhibitory activity against some bacterial isolated from food items *in vitro*. Reports have been made on the use of plants for therapeutic purposes dating back to centuries before the advent of antibiotics. The cinchona plant has been reportedly used to treat malaria.[16] Since microorganisms have become increasingly resistant to available antibiotics, scientists have been exploring various sources, including plants, to remediate the menace of antimicrobial resistance.

Table 4: **Antibacterial activity of Ethyl acetate Root *of P. biglobosa* against bacteria isolated from some food items.**

	Diameter of the Zone of inhibition (mm)(mean ± SD) of isolates at various amount of the extract					
Sl. No	**Isolates**	**50.0mg**	**25.0mg**	**12.5mg**	**6.25mg**	**3.125mg**
1	*Salmonella* sp	14.0 ± 2.11	11.0 ± 0.47	8.0 ± 1.33	0.00	0.00
2	*S. aureus*	21.0 ± 2.00	17.10 ± 0.78	12.0 ± 2.00	7.0 ± 1.0	0.00
3	*E. coli*	19.0 ± 1.00	15.0 ± 0.88	11.0 ± 1.00	0.00	0.00
4	*Klebsiella* sp	18.0 ± 0.42	15.0 ± 2.00	13.0 ± 1.00	9.0 ± 1.0	0.00
5	*Bacillus* sp	12.0 ± 1.00	9.0 ± 2.00	0.00	0.00	0.00

Table 5: Antibacterial activity of Ethanol Root of *P. biglobosa* against bacteria isolated from some food items.

	Diameter of the Zone of inhibition (mm)(mean ± SD) of isolates at various amount of the extract					
Sl. No	**Isolates**	**50.0mg**	**25.0mg**	**12.5mg**	**6.25mg**	**3.125mg**
1	*Salmonella* sp	15.0 ± 0.78	12.0 ± 2.00	10.0 ± 1.00	6.0 ± 1.21	0.00
2	*S. aureus*	18.0 ± 1.00	16.0 ± 2.00	13.0 ± 0.78	9.0 ± 2.00	0.00
3	*E. coli*	22.0 ± 0.25	19.0 ± 2.01	17.0 ± 1.00	10.0 ± 1.10	6.0 ± 1.00
4	*Klebsiella* sp	15.0 ± 2.0	12.0 ± 1.41	8.0 ± 2.00	0.00	0.00
5	*Bacillus* sp	13.0 ± 1.0	9.0 ± 2.03	0.00	0.00	0.00

Table 6: Antibacterial activity of Ethyl acetate Bark of *P. biglobosa* against bacterial isolated from some food items.

	Diameter of the Zone of inhibition (mm)(mean ± SD) of isolates at various amount of the extract					
Sl. No	**Isolates**	**50.0mg**	**25.0mg**	**12.5mg**	**6.25mg**	**3.125mg**
1	*Salmonella* sp	11.0 ± 0.45	8.0 ± 1.00	0.00	0.00	0.00
2	*S. aureus*	8.0 ± 2.00	0.00	0.00	0.00	0.00
3	*E. coli*	14.0 ± 1.21	7.0 ± 1.00	0.00	0.00	0.00
4	*Klebsiella* sp	16.0 ± 2.00	12.0 ± 0.28	8.0 ± 1.11	0.00	0.00
5	*Bacillus* sp	0.00	0.00	0.00	0.00	0.00

Table 7: Antibacterial activity of Ethanol Bark of *P. biglobosa* against bacteria isolated from some food items.

	Diameter of the Zone of inhibition (mm)(mean ± SD) of isolates at various amount of the extract					
Sl. No	**Isolates**	**50.0mg**	**25.0mg**	**12.5mg**	**6.25mg**	**3.125mg**
1	*Salmonella* sp	13.0 ± 2.06	8.0 ± 0.33	6.0 ± 1.00	0.00	0.00
2	*S. aureus*	11.0 ± 0.68	9.0 ± 1.01	7.0 ± 0.16	0.00	0.00
3	*E. coli*	16.0 ± 1.00	11.0 ± 1.00	0.00	0.00	0.00
4	*Klebsiella* sp	18.0 ± 1.12	13.0 ± 1.00	8.0 ± 0.00	0.00	0.00
5	*Bacillus* sp	11.0 ± 10.2	7.0 ± 1.00	0.00	0.00	0.00

CONCLUSION

The phytochemicals and antimicrobial studies of *P. biglobosa* bark, root and leaf extracts provided scientific evidence for the rationale use of *P. biglobosa* bark, root and leaves in prevention of disorders due to presence of some useful phytochemicals, and in treatment of diseases caused by some bacterial pathogens such as *Salmonella* sp, *E. coli*, *S. aureus*, *Bacillus* sp and *K. pneumonia*e. Further research is necessary to reveal its detailed molecular mechanism behind these phytochemical and antibacterial activities.

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