# PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF STEM BARK OF *Parkia biglobosa* (Jacq) Benth

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***Abstract***
*Parkia biglobosa is a wild medicinal plant used against ailments by the local people. Antimicrobial investigation of the ethanolic extracts of the stem bark of Parkia biglobosa was carried out against pathogenic bacteria (Staphylococcus aureus, Shegella dysentriae, Pseudomonas aeruginosa, Klebsiella spp and Escherichia coli). At the concentration used in the study, it exhibited (inhibited) activity against Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli but not against Shegella dysentriae and Klebsiella spp. The stem bark is more potent with the highest stem bark extracts concentration (200mg/ml) resulting in inhibition zone diameter of 14, 13 and 12mm respectively. Phytochemical screening revealed the presence of Terpenoids, Saponins, Tannins, Alkaloids, Phlobatannins, Steriods, Quinins and free anthraquinones in stem bark extracts. Carbohydrates was not detected. Result obtained explains why parts of this plant are used traditionally for the treatment of various infections.*

**Keywords:** *Parkia biglobosa*, ethanol, phytochemical, antimicrobial, Phlobatannins.

## Introduction

*Parkia biglobosa* commonly called African Locust Bean has the following local names; Dorowa (Hausa), Nune (Tiv), Ahinyo (Jukun), of North-Central Nigeria.

Phytochemical analysis study the constituents of plant materials with emphasis on the active constituents preparation. The phytochemicals are chemicals derived from plant; the term is often used to describe large number of secondary metabolic compounds found in plants. Many of these are known to provide protection against insect attack and plant diseases and they also exhibit a number of protective functions for human consumer (Skene and Philip, 2006).

Based on the growing knowledge of potency of traditional medicinal plants and coupled with the fact that numerous infectious agents are becoming resistant to synthetic drugs, researchers all over the world have intensified the screening of these acclaimed medicinal plants in order to provide a documented scientific backing and ultimately, recommend them as novel sources of future antimicrobial agent (WHO 2008). Therefore, the continuous screening of these acclaimed medicinal plants by scientists cannot be over-emphasised (Stucke 1993, Udobi, 2009).

Traditionally, various preparations of the leaves, stem and roots of the plants are used to treat a range of illness. The stem bark of *Parkia biglobosa* has been successfully used for the treatment of many infectious diseases (Mayer, 2006).

Despite the arrays of traditional application to which the leaves, stem bark and roots of *Parkia biglobosa* are subjected to, available literature revealed little information on the scientific elucidation of this plant as remedy for the acclaimed ailments.

The aim of this work is to increase to the available information already existing in literature concerning this plant.

## Experimental

### Sample collection and preparation

The stem bark of *Parkia biglobosa* was collected from Taraba State University

Farm land, Jalingo on 26th June, 2013. The plant was identified and authenticated by a Botanist, Mal. Kareem of the Department of Horticulture and Landscape, Federal College of Forestry, Jos. The specimen was deposited in Chemistry Department, Taraba State University, Jalingo. The plant sample was dried under shade and pulverized using mortar and pestle into powder, labeled and stored in a dry container.

**Solvent Purification**

The organic solvent used for the extraction was ethanol. It was purified by Soxhlet Xxtractor before use.

**Extraction Procedure**

The pulverized plant sample (150g) was introduced into 250ml of ethanol and allowed for 72 hours extraction. The extracts were concentrated in vacuum, using a Rotary Evaporator.

**Phytochemical Analysis**

**Tannins:** A small quantity of the extract was stirred with 10ml of distilled water, filtered and ferric chloride reagent added to the filtrate (Trease and Evans, 1978). Where a blue-black precipitation was observed, it indicated the presence of tannins.

**Saponins:** 2.0ml filtrate + 5ml distilled water, shaken for 40 seconds. Persistence frothing or foaming indicated the presence of saponins (Evans, 1996).

**Terpenoid/Steroids:** 200mg plants material in 10ml chloroform, filtered; 2ml filtrate + 2ml acetic anhydride + 1ml of concentrated $H_2SO_4$. A blue-green ring indicates the presence of terpenoids/steroids (Parekh and Chanda, 2007).

**Alkaloids:** 200mg plant material boiled. 20ml of 1% $H_2SO_4$in 50% ethanol, filtered, filtrate + 5 drops concentrated $NH_4OH$ + 20ml chloroform and the two layer separated, chloroform layer was extracted with 20ml dilute $H_2SO_4$extract + 5 drops of Mayer's/Wagner's/Dagen-dorff's reagents, a creamy/brownish red/orange-red precipitate indicate the presence of alkaloid (Evans and Trease, 1989).

**Carbohydrates:** 2ml of a samples solution is placed in a test tube, 2 drops of iodine/potassium iodide solution and 1ml of water are added, a positive test is indicated by the formation of a blue-black complex.

**Phlobatannins:** A small portion of the extract was boiled with 1% aqueous hydrochloric acid (Trease and Evans, 1978). A deposition of a red precipitate indicated the presence of phlobatannins.

**Free anthraquinones:** Equal volumes of dilute ammonia solution and chloroform were mixed with the extract and shaken (Trease and Evans, 1978). Where a pinkish precipitate were observed, it indicated the presence of free anthraquinones.

**Preparation of media**

Mueler-Hinton Nutrient and Sabouraud Dextrose Agar were the media used as the growth media for the microbes. The media were prepared according to the manufacturer's instruction, boiled to dissolve and sterilized at 121°c for 15 minutes. The media was allowed to cool to 45°c and 20ml of the sterile media was then poured into sterile petri-dishes, the plates were covered and allowed to cool and solidified. The Mueler-Hinton Agar seeded with 0.1ml of standard inoculums of the test bacteria, micro-organism and the Sabouraud Dextrose Agar was seeded with the bacteria each inoculums was spread evenly over the surface of the medium by the use of sterile swab. The seeded plates were dried at 37°c for 30 minutes. By the use of a standard cork borer of each 6mm in diameter as well is cut at centre of each inoculums plates, 0.1ml of solution of the

extract was then introduced into each cut well on the surface of the medium. The inoculated media were incubated at 37°c for 72 hours, for the bacteria which plates were observed for the zone of inhibition of growth. The zones were measured with a transparent ruler and result recorded in millimeter, prepared according to the manufacturers instruction.

**Determination of Minimum Inhibitory Concentration (MIC) of the Extracts**

Minimum Inhibitory Concentration of ethanol and extracts was determined using broth dilution method. Nutrient Broth and Sabouraud Dextrose Broth were prepared according to the manufacturer instruction; 10ml of each broth was dispensed into test tubes and were sterilized at 121°c for 15 minutes. The broth were allowed to cool. Mac. Farland's Turbidity Scale Number 0.5 was prepared to give turbid solution. Normal saline was prepared. 10ml was dispensed into test tubes and the microorganism was inoculated into the normal saline. Incubation was made at 37°c for 4 hours, dilution of test microorganism in the normal saline was performed until the turbidity that of the Mac. Farland's scale of visual compares at this point the microorganism has a concentration of about $1.5x10^8$cfu/ml. Two fold serial dilution of the extraction in the sterilized broth was performed to obtain concentration of 200mg/ml, 100mg/ml dissolving 0.4g of extracts in 10ml of the sterile broth having obtained the different concentration of the extract in the sterilized broth. 0.1ml of the standard inoculums of the test microbes in the normal saline was then inoculated in the concentration of the extract in the broth, the inoculated broths were observed for turbidity (growth). The lowest concentration of the extract in the broth which shows no turbidity was the MIC.

**Determination of Minimum Bactericidal Concentration (MBC) of Extracts.**

Minimum Bactericidal Concentration (MBC) of the extract was also determined to check whether the test organisms were killed or only their growth was inhibited. Mueller Hinton and Sabouraud Dextrose Agar were prepared, sterilized and were poured into sterilized petri-dish; Media were allowed to cool and solidified. The contents of the MIC in the serial dilution were then sub-cultured into the prepared media. (The Mueller-Hinton gar for bacteria and Sabouraud Dextrose Agar for bacteria). Incubation was made at 37°c for 24 hours for the bacteria and 30°c for 48 hours after which each medium was observed for colony growth. The MBC was then made.

**Antibacterial Screening**

*Staphylococcus aureus, Shegella dysentriae, Pseudomonas aeruginosa, Klebsiella spp* and *Escherichia coli* were collected from Federal Medical Centre, Jalingo, Taraba State and used for antibacterial screening.

**Results and Discussion**

The percentage yield value of the stem bark extract of *Parkia biglobosa* in this studies was 8.96%. Cyanogenic glycosides, saponins, tannins, alkaloids, free anthraquinone, steroids and quinone were detected in the stem bark while carbohydrates was not detected.

Antimicrobial activities of ethanolic stem bark of *P. biglobosa* are presented in tables 2 & 3 below. The stem bark extract inhibited the growth of *Staphylococcus aureus, Pseudomonas aeruginosa, E.coli* whereas it has no effect on the growth of *Shegella dysentriae*and *Klebsiella spp* at various test concentrations.

*P. biglobosa* stem bark extracts tested positive to some of the presence of these plants metabolites. Some of the metabolites were detected in the stem bark

extract. The higher inhibitory activity demonstrated by the stem bark extract corroborated with the report of Sukumar *et al.* 1991, that the activity of phytochemical compound on target specie varies with respect to plant part with among other factors.

The presence of alkaloids in the plant and the inhibition of the growth of S.aureus and others by the extract is an indication that indeed the result is in agreement with other works. This is because alkaloids are proven to be active against bacterial agents.

## Conclusion

The low yield value of extract used in this study may be due to the employed method of extraction (maceration). Maceration has been reported to result in low yield of extracts (as reported by Ubulom, *et al.* 2013). However, it was preferred to other methods because it does not require heating and as such serves to protect thermolopeutic phytoconstituents that may be present.

The antimicrobial screening of ethanolic extract had a remarkable effect on the test organism with minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of 200mg/ml and 100mg/ml for the test organism it inhibited.

This research has shown that the plant *P. biglobosa* has remarkable therapeutic effect on many diseases especially those that may be caused by the test organisms worked on.

## Recommendations

More precise chemical evaluation such as spectroscopic studies and chromatography be undertaken in order to isolate and characterize the activity of the constituents of plan, the active components could be used in drop preparation against ailments and diseases which are becoming resistant to existing drugs available in the market.

**Table 1: Phytochemical Screening of stem bark of *Parkia biglobosa***

| **Secondary metabolites in *P. biglobosa*** | **Indication/mode** |
|---|---|
| Cyanogenic glycosides | +++ |
| Saponins | +++ |
| Tannins | ++ |
| Alkaloids | +++ |
| Carbohydrates | - |
| Free anthraquinones | ++ |
| Steroids | +++ |
| Quinones | ++ |

Key: + = trace, ++ = moderate, +++ = abundant, - = not detected

**Table 2: Inhibitory zone diameter effect of ethanol extract of *P. biglobosa* stem bark on test bacterial species**

| **Test Organism** | **Inhibition zone diameter (mm)** | |
|---|---|---|
| | **200** | **100** |
| *Staphylococcus aureus* | *13* | *14* |
| *Shegella dysentriae* | *0* | *0* |
| *Pseudomonas aeruginosa* | *14* | *12* |
| *Klebsiella spp* | *0* | *0* |
| *Escherichia coli* | *13* | *14* |

*Zero values no inhibition*

**Table 3: The minimum inhibitory concentration (MIC) of methanol extract of *P. biglobosa* stem bark against test organisms**

| Test Organism | Concentration (mg/ml) | |
|---|---|---|
| | 200 | 100 |
| *Staphylococcus aureus* | - | - |
| *Shegella dysentriae* | + | + |
| *Pseudomonas aeruginosa* | - | - |
| *Klebsiella spp* | + | + |
| *Escherichia coli* | - | - |

Key: + = Scanty growth, - = No growth

**Table 4: The Minimum Bactericidal Concentration (MBC) of methanol extract of *P. biglobosa* stem bark against the test organism**

| Test Organism | Concentration (mg/ml) | |
|---|---|---|
| | 100 | 50 |
| *Staphylococcus aureus* | - | - |
| *Shegella dysentriae* | + | + |
| *Pseudomonas aeruginosa* | - | - |
| *Klebsiella spp* | + | + |
| *Escherichia coli* | - | - |

Key: + = Scanty growth, - = No growth

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