

ANTIBACTERIAL EFFECTS OF ETHANOIC ROOT EXTRACT OF *Parkiabiglobosa*

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ABSTRACT

This study examined the antibacterial effects of ethanoic root extract of Parkiabiglobosa. In Africa, decoctions of Parkiabiglobosa have been shown locally by traditional healers to treat various illnesses. The ethanoic extract of Parkiabiglobosa root is dark brown in colour and odourless. The result of this study showed that the ethanolic extract of the root of Parkiabiglobosa contain biochemical constituents that have antimicrobial effect against all the test organisms. These organisms includes: Proteus Vulgaris, Pseudomonas aeruginosa, Escherichia coli, Klebsiella Sp and Staphylococcus aureus. The result obtained in this study also showed that at high concentration (500mg/ml) the extracts were very active against the entire organism while at the lowest concentration (31.25mg/ml) most of the organisms were resistant, except the ethanolic extracts on staphylococcus aureus. staphylococcus aureus is the most susceptible of all the test organisms used. The lowest affect is in klebsiella while staphylococcus aureus showed the highest effect.

Keywords: Antibacterial, Effects, Ethanoic, Root Extract, Parkiabiglobosa

Introduction

Parkiabiglobosa is commonly known as the locust bean tree, African locust bean or nere. It is a perennial deciduous tree and a dicotyledonous angiosperm belonging to the family Fabaceae - Mimosoideae. It is categorized under spermatophytes, vascular plants. It grows to between 7 and 20 metres high, in some cases up to 30 metres. The tree is a fire-resistant heliophyte characterized by a thick dark gray-brown bark. It is found in a wide range of environments in Africa and is primarily grown for its pods that contain both a sweet pulp and valuable seeds. Various parts of the locust bean tree are used for medicinal purposes (Timmer, Kessler, & Slingerland, 1996, Sina & Traore 2012). As a standing tree, locust bean may have a positive effect on the yield of other nearby crops.

African locust bean is a multipurpose tree that is as highly valued as shea butter tree (Burkill 1995). Fermented seeds ('soubala', 'dawadawa', 'netetu') serve primarily as a condiment for seasoning sauces and soups. Roasted seeds are used as a coffee substitute known as 'Sudan coffee' or 'cafe negre'. Ground seeds are mixed with *Moringa oleifera* Lam. leaves to prepare a sauce, and are also used to make doughnuts. The mealy pulp from the fruits is eaten or is mixed with water to make a sweet and refreshing drink rich in carbohydrates. (Burkill 1995, Boffa 1999)

According to Sina & Traore (2012), boiled pods are used to dye pottery black; the ash is applied as a mordant. The bark is rich in tannins and may be used for tanning hides, but the resulting leather is often of moderate quality especially with regard to colour, which is often reddish, uneven, and darkens when exposed to light. The leaves are sometimes eaten as a vegetable, usually after boiling and then mixed with other foods such as cereal flour. Young flower buds are added to mixed salads. In West Africa the bark, roots, leaves, flowers, fruits and seeds are

commonly used in traditional medicine to treat a wide diversity of complaints, both internally and externally, sometimes in combination with other medicinal plants. The bark is most important for medicinal uses, followed by the leaves. Medicinal applications include the treatment of parasitic infections, circulatory system disorders, such as arterial hypertension, and disorders of the respiratory system, digestive system and skin. In veterinary medicine, a root decoction is used to treat coccidiosis in poultry. Green pods are crushed and added to rivers to kill fish. The nutritional value of the fish is not adversely affected so long as they are cooked or dried.(Nikiema 1993).

Materials and Methods

The apparatus and reagents used are pipettes, beakers, beam weighing balance, foil paper, cotton wool, white handkerchief, conical flasks, macartney bottles, petri plates, cork borer, spirit lamp, hot plate, spatula, measuring cylinder, autoclave, dryer (oven), pistle and mortar, ethanol, distilled water, masking tape incubator, inoculating loop, steam bath disposable gloves, refrigerator was bottles.

Sample preparation

The plant sample of *Parkiabiglobosawas* was dried at room temperature and then pulverized into powder using morta and pistle. This was done to allow for easy extraction of the plant extract.

Ethanoic extract

50g of the powdered sample was weighed using a weighing balance and pour into 300mls beaker containing 200mls ethanol. It was stirred using a stirrer to allow even mixture. It was then kept in the fridge for 5 days and then the extraction was done using white handkerchief. The chaff was air dried and weighed again. The percentage yield obtain was 9.70%

Preparation of Extract Concentration by Serial Dilution

Five grams (5g) of the ethanoic extract was dissolved in 10ml of distilled water to produce the stock was solution of 500mg/ml. 2mls of sterile distilled water was measured into four sterile test tubes. 1ml of the stock solution was taken and diluted into the first test tube to produce a concentration of 250mg/ml and the process was repeated for all the test tube. Thus we have 500mg/ml, 250mg/ml, 125mg/ml, 62.5mg/ml and 31.25mg/ml.

After this, nutrient agar was prepared on 20 petri plates and allow to cool to a temperature of 45°C (not solidify) for easy inoculation. Nutrient broth (peptone water) was prepared using six grams nutrient broth powder, balance, conical flask, distilled water, pipette and mackatney bottles which were firmly capped.

Preparation of sample bacteria

The samples of bacteria used are pseudomonas aeruginosa, proteus vulgaris, Escherichia coli, staphylococcus aureus and klebsiella sp. These were subcultured in peptone water. Suspensions of the sampled bacteria were made in peptone water and stored in a refrigerator. A bacterial sample were prepare from the samples in peptone water. This was done by using a sterile syringe to dispense 1ml of the organism into a sterile peptri plate and then, nutrient agar was poured and shaken to allow for even distribution of the organism.

Antibacterial activities test

Thiswas donethrough the following methods;

Agar diffusion method

The nutrient agar with the bacteria in the petri plate was allowed to solidify. A cork borer of 10mm in diameter was used to bore five holes in each plates to make for the five different concentrations. The plates were labeled according to the differnte concentration for the different sampled bacteria. A sterile syringe was used to drop 1ml of the different concentrations into the different wells (holes). The plates were kept in the incubator at 37°C for 18 hours and monitored growth and zones of inhibited were measured using a calibrated ruler ad the readings recorded appropriately.

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) was determined. This was done to be able ascertain the lowest concentrations of the plant extracts that allow for growth or inhibition of each test organisms.

Doubling serial dilution was done for both the ethanolic extracts in a test tube to give a concentration of 500mg/ml, 125mg/ml 62.50mg/ml and 31.25mg/ml respectively. These dilutions were shaken vigorously and labeled appropriate and kept till required.

A nutrient broth that has been inoculated with the test organism taken from different zones of inhibitions were incubated for 24 at 37°C for 24 hours. The test tubes containing the different concentration ad test organisms were checked for turbidity after incubating for 24 hours at 37°C

The lowest concentration that prevented visible growth of the test organism of that extract on that particular organism was noted and taken as the minimum inhibitory concentration (MIC)

Minimum bacteriocidal /bacteriostatic concentration (MBC)

Tubes with no turbidty from the MIC (Minimum Inhibitory Concentrations)was taken for each organism and each organism and each concentrations that showed no turbidity. One loopful from each was taken and streaked on petri plates with nutrient agar and this was incubated for 24 hours at 37°C in an incubator and label according to concentrations and organisms.

After 24 hours the petri plats were observed and anyone that showed growth of the organism is said to be bacteriocidal at the concentration of the plant extract and any particular one that do not show any growth means that the plant extract killed the bacteria at that concentration ad is said to be bacteriostatic.

Results

The ethanoic extract of *Parkiabiglobosais* dark brown in colourand odourless. The extract showed antimicrobial activities againstall test organisms. The lowest affect is in klebsiella while staphylococcus aureus showed the highest effect.

Table 2: Reaction of test organisms to ethanolic extracts of Parkiabiglobosaroot

Test organisms	Mean diameter zone of inhibition (mm) at different Gentamysin concentration (mg/ml) of extract					
	500	250	125	62.50	31.25	40
	mg/ml	mg/ml	mg/ml	mg/ml	mg/ml	ug/ml
Proteus Vulgaris	25	22	18	17	15	26

<i>Pseudomonas aeruginosa</i>	32	32	31	27	19	25
<i>Escherichia coli</i>	21	18	15	12	0.0	22
<i>Klebsiella Sp.</i>	12	9	5	0.0	0.0	20
<i>Staphylococcus aureus</i>	32	32	31	27	19	30

From the result shown in table 1, *staphylococcus aureus* is the most susceptible to the ethanolic extracts. *Klebsiella sp* is the least susceptible and is resistant to 62.50mg/ml and 31.25mg/ml of the ethanolic extract. *E.coli* is resistant to 31.25mg/ml of the ethanolic extracts concentrations while *pseudomonas aeruginosa* is also resistant to the 31.25mg/ml of ethanolic extract concentration. *Staphylococcus* is not resistance to any concentrations of the extracts.

From the table, the zone of inhibition of *staphylococcus aureus* at 500mg/ml concentration of the ethanolic extracts was 32 mm. Also, the zones of inhibition of *E.coli* at 500mg/ml, 250mg/ml, 125mg/ml, 62.50mg/ml and 31.25mg/ml for the ethanolic extracts are 21mm, 18 mm, 15mm, 12mm, 10mm respectively and there was no inhibition at the 31.25mg/ml concentration.

TABLE 2: Minimum inhibitory concentration (MIC) of the extracts of *Parkia biglobosa* root on the test organisms

Test organisms	Broth clarity (inhibition concentrations using ethanol extract).				
	500 mg/ml	250 mg/ml	125 mg/ml	62.50 mg/ml	31.25 mg/ml
<i>Proteus Vulgaris</i>	+	+	+	+	-
<i>Pseudomonas aeruginosa</i>	+	+	+	-	-
<i>Escherichia coli</i>	+	+	+	-	-
<i>Klebsiella Sp.</i>	+	+	+	-	-
<i>Staphylococcus aureus</i>	+	+	+	+	-

Key:

+ =Inhibition (clear broth)

- = No inhibition (cloudy broth)

The result in table 2 shows that there was no clarity at 31.25mg/ml concentration of extracts. At 62.50mg/ml of the extracts, there was no inhibition of *Klebsiella Sp*, *Proteus vulgaris* and *E. coil*. For *pseudomonas aeruginosa* and *staphylococcus aureus* their minimum inhibitory concentration was 31.25mg/ml since at this concentration there was this inhibition.

Discussion

The result of this study showed that the ethanolic extract of the root of *Parkiabiglobosa* contain biochemical constituents that have antimicrobial effect against all the test organisms. These organisms includes: *Proteus Vulgaris*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella* Sp and *Staphylococcus aureus*. The findings agreed with the work of Ajaiyeoba (2002) and Burkill (1995) whose different works have reported the antimicrobial activities of *Parkiabiglobosa*.

The result obtained in this study showed that at high concentration (500mg/ml) the extracts were very active against the entire organism while at the lowest concentration (31.25mg/ml) most of the organisms were resistant, except the ethanolic extracts on *staphylococcus aureus*. *staphylococcus aureus* is the most susceptible of all the test organisms used.

Ajaiyeoba (2002) had tested the extracts of the stem (bark) at 12mg/ml and did not notice any antibacterial activity on *staphylococcus aureus* with ethanol and water extract display appreciable antimicrobial activities.

Conclusion

The findings of this study showed that the ethanolic extract of the root of *Parkiabiglobosa* have antimicrobial effect. It shows a broad spectrum of antimicrobial activities of the following organisms: *Proteus Vulgaris*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella* Sp and *Staphylococcus aureus*.

References

- Ajaiyeoba, A. (2002) antibacterial effect of *Parkiabiglobosa* stem using ethanolic extract. M.Sc research project, University of Ilorin.
- Boffia, J.M., (1999). Agroforestry Parklands in Sub-Saharan Africa. FAO conservation guide 34-230pp.
- Burkill, H.M., (1995). The useful plants of West tropical Africa. 2nd Edition. Volume 3, Families J-L. Royal Botanic Gardens, Kew United Kingdom 857 pp.
- Nikiema A., (1993). Regeneration of *Parkiabiglobosa* (Jacq) R.Br. ex Don in an agroforestry system. A pilot study in Burkina Faso. Thesis for MSc. Degree in tropical forestry. Wageningen agricultural University, Wageningen, the Netherlands. 42 pp.
- Sina, S. & Traore, S.A., (2002). *ParkiaBiglobosa* (Jacq)R.BR. ex G.Don. record from Protabase. Oyen, L.P.A & Lemmens, R.H.M.J. (Editors). PROTA (plant resources of tropical African Ressources Vegetales de l'Afrique tropicale), Wageningen, the Netherlands.
- Timmer, L.A., Kessler, J.J. & Slingerland, M., (1996) Pruning of nere trees (*Parkiabiglobosa* (Jacq.(Benth.) on the farmlands of Burkina Faso, West Africa. Agroforestry systems 33:87-98.