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A comparative study of antibacterial potentials of leaf extracts of some selected trees from Benue State University Campus on *Staphylococcus aureus AND Escherichia coli*

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Abstract

Introduction: Medicinal Plant with antimicrobial properties are of great significance in therapeutic treatments of disease caused by bacteria. Their antibacterial properties are due to compounds synthesized in their cell sap during secondary metabolism, their antibacterial activity has shown that plants represent a potential source of novel antibiotic prototypes. This study aims to investigate the antibacterial potential of three plant extracts on S*. aureus and E. coli*.

Method: A comparative study of the antibacterial potentials of water and ethanolic leaf extracts of three plants, *Parkia biglobosa, Khaya senegalensis* and *Daniellia oliveri*, was carried out. Extracts at various regimes of concentrations, 0.2 g/ml, 0.4 g/ml, 0.6 g/ml, 0.8 g/ml and 1.0 g/ml were tested against Staphylococcus aureus and Escherichia coli. The disc diffusion method of Kirby Bauer with slight modification was adapted in determining zones of inhibition. Statistical significance was considered at ($p<0.05$).

Results: Ethanolic leaf extract of *P. biglobosa* recorded the highest zone of inhibition (20.0 mm) at 1.0 g/ml on S. aureus, while ethanolic leaf extract of *D. oliveri* had the least zone of inhibition (6.33 mm) at 0.2 g/ml *on E. coli. K. senegalensis* was most effective on *E. coli* with grand mean inhibitory effect of (13.33 mm and 12.13 mm) on ethanol and water extract respectively, while *P. biglobosa* was most effective on S. aureus with grand mean inhibitory effect of (13.00 mm and 12.47 mm) on ethanol and water extracts respectively. No significant difference in the grand mean inhibitory effect of the three plants on test bacterial or the type of solvent used.

Conclusion: The three plants are potential useful antibacterial agents, inhibiting bacterial growth at all concentrations of **the** study.

Keyword: Extracts; *E. coli*; *S. aureus*; *Parkia biglobosa*; *Daniellia oliveri*; *Khaya senegalensis*

1. Introduction

Medicinal Plant with antimicrobial properties is of great significance in therapeutic treatments of disease caused by bacteria, their antibacterial properties are due to compounds synthesized in their cell sap during secondary metabolism,

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their antibacterial activity has shown that plants represents a potential source of novel antibiotic prototypes[1]. Plant extracts and their active compounds have been used for antibacterial activities and have significant remedial properties, in recent years a wide range of investigation have been carried out throughout the world to confirm antibacterial properties of different medicinally important plants. A number of plants showed efficient antibacterial activities, which were comparable to that of synthetic standard antibacterial drugs[2]. An increase in the incidence of impending transferable diseases is hazard, isolation of different extracts and many other chemical compounds from plants with efficient antibacterial activities can be of immense impact in the health care. Medicinal action of these plants is linked to some important chemical compounds contained in them which pass on a definite physiological action on the human body[3]. In concern to negative aspect of conventional medicine, the utilization of natural product as an alternative way to the conventional drugs in healing of different ailments has been increased in the previous decades[4]. According to recent estimates by the WHO in 2001, more than 3.5 billion people in developing countries rely on plants as source of medicine for various ailments[5]. Over 20,000 plants have medicinal values and many plants are yet to be explored for their potentials[5]. In addition, many of the existing synthetic drugs cause various side effects. Hence drug development from plant-based compounds could be useful in meeting this demand for newer drugs with minimal side effects[6]. Bacteria such as *Escherichia coli* and *Staphylococcus aureus* are of human importance and cause diseases like Mastitis, Skin infections such as (boils), Upper respiratory complications and urinary tract infection[7]. This study aims to investigate the antibacterial potential of three plant on *S. aureus* and *E. coli.*

2. Materials and methods

2.1 Collection of plant material

Fresh leaves of three selected plant species, locust bean tree (*Parkia biglobosa*), Mahogany (*Khaya senegalensis*) and African Copaiba Balsam (*Daniellia oliveri*). Were collected in polytene bags and transported to the Botany laboratory of Benue state university Makurdi for identification and authentication.

2.2 Preparation of extracts

Fresh leaves of Water and ethanol extract of each selected plant species were prepared, 10 g each of fresh leaves were weighed for both water and ethanol extractions respectively, leaves were washed twice with tap water and rinsed with distilled water to reduce probable microbial load from the field. The measured leaves were pounded using mortal and pastel. The macerates were transferred into a 500 ml beaker each and soaked in 100 ml of distilled water and 100 ml (90%) ethanol respectively (for water and ethanol extraction). The set up was tied with foil paper and left for 12 hours, the macerates then squeezed and filtered through a muslin cloth into separate beaker and a fine filtration using a filter paper, the set up was left for 24 hours.

2.3 Concentration of crude extracts

Serial Dilution of water and ethanol crude extracts was prepared to yield five different concentration 0.2 g/ml, 0.4 g/ml, 0.6 g/ml, 0.8 g/ml and 1.0 g/ml respectively.

2.4 Collection of test organisms

Isolates of *Staphylococcus aureus* and *Escherichia coli* (from human source) in nutrient broth was obtained from Benue State University Teaching Hospital (BSUTH) Makurdi.

2.5 Identification isolation of microbes

The bacterial isolates were differentiated first on the basis of colonial morphology by microscopic examination after Gram staining techniques, positive for *S. aureus* and negative for *E. coli*, colonies were further identified by their biochemical properties, hydrogen peroxide test for *S. aureus* and sorbitol MacConkey agar culture for *E. coli,* and stored as stock culture.

2.6 Antibacterial susceptibility test

The activity of the plant extracts on microorganisms was determined by paper disc diffusion techniques according to Kirby-Bauer[8]. with slight modification. The paper disc (5 mm) were prepared with whatmann filter paper using a perforator, the paper disc was impregnated into the different concentrations of the water and 90% ethanol extracts and were placed on the surface of the inoculated media on Petri-dishes. After proper diffusion of extracts into the media, the plates were incubated for 24 hours at 37^{oc}, the diameter of the resultant zone of inhibition was measured in millimeters using meter rule. The test was carried out in triplicate for each concentration of both water and ethanol extracts of each

plant species to ensure precision, test organisms were treated with ordinary distilled water and 90% ethanol to serve as control.

2.7 Data Analysis

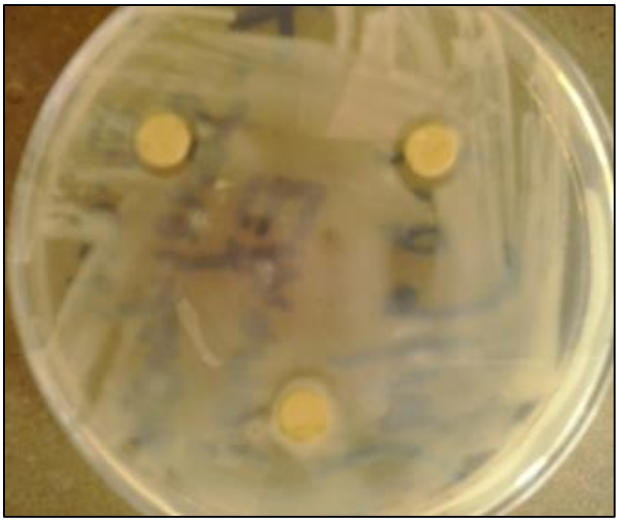

Figure 1 Zones of inhibition on *E. coli*

Table 1 Mean Inhibitory effect of ethanolic extract of leaves of *Parkia biglobosa* on test organisms

Concentration (g/ml)	SA (mm)	EC (mm)
0.2	10.67±3.5[a]	9.67±3.2[a]
0.4	11.00±3.6[a]	11.33±3.7[a]
0.6	11.333.7[a]	12.67±4.2[a]
0.8	12.00±4.0[a]	12.33±4.1[a]
1.0	20.00±6.6[b]	19.67±6.5[b]
F	23.971	17.208
p-value	0.002	0.001

Result is significant where p<0.05; Means tagged with different letter alphabets are significant; Values are Mean ± SD; **SA**=*Staphylococcus aureus,* **EC**=*Escherichia coli,*

Table 2 Mean inhibitory effect of ethanolic extract of *Daniellia oliveri* on test organisms

Concentration (g/ml)	SA (mm)	EC (mm)
0.2	9.33±3.1[a]	6.33±2.1[a]
0.4	11.00±3.6[a]	9.33±3.1[a]
0.6	11.67±3.8[a]	10.673.5[a]
0.8	12.67±4.2[b]	12.33±4.1[b]
1.0	14.33±4.7[c]	13.67±4.5[c]
F	8.371	22.091
p-value	0.031	0.002

Result is significant where p<0.05; Means tagged with different letter alphabets are significant; Values are Mean ± SD; **SA**=*Staphylococcus aureus,* **EC**=*Escherichia coli,*

Analysis of Variance (ANOVA) was used to test significant difference in the effect of different plant extracts on test organisms. Post Hoc test (LSD) was used for mean separation. All test was carried out at 95% confidence interval using SPSS version 27.0. Results were presented using tables.

Table 3 Comparative grand mean inhibitory effect of water and ethanol extracts of the three plants on test organisms

	SA		**WA**	
Plant Extracts	**ET**	**WA**	**ET**	**WA**
P. biglobosa	13.00±4.3	12.47±4.0	13.13±4.3	10.20±3.4
K. Senegalensis	12.53±4.1	11.803.8	13.33±4.4	12.13±4.0
Daniellia oliveri	11.80±3.9	10.40±3.5	10.47±3.4	11.073.6
F	0.750	1.021	1.308	2.072
p-value	0.563	0.461	0.176	0.089

Result is significant where p<0.05; Values are Mean ± SD; **SA**=*Staphylococcus aureus,* **EC**=*Escherichia coli,* **ET=**Ethanol extract, **WT=**Water Extract

3. Results and discussion

The aqueous and ethanol leaf extract of all the three plants species, *P. biglobosa, K. senegalensis* and *D. oliveri* showed antibacterial activities against all test organisms at all concentrations. Susceptibility increased with increasing concentration of plant extracts on test organisms (tables 1-2). In general, the ethanol extract was observed to be more potent and consistent in activity than the aqueous extracts (table 1 and 2), these results confirm to earlier studies that observed plant extract in organic solvents to provide more consistent antimicrobial activity compared to water extracts[9,10]. From the study, ethanolic leaf extract of *P. biglobosa* recorded the highest zone of inhibition (20.0 mm) at 1.0 g/ml on *S. aureus*, while ethanolic leaf extract of *D. oliveri* had the least zone of inhibition (6.33 mm) at 0.2 g/ml on *E. coli* (table 1 and 2). The most sensitive test organism was *S. aureus* which had inhibitory diameter of 20.00 mm on ethanol extract of *P. biglobosa* (table 1), and least sensitive test organism was *E. coli* with inhibitory diameter of 6.33 mm on ethanol extract of *D. oliveri* (table 2). Comparatively *K. senegalensis* was most effective on *E. coli* with grand mean inhibitory effect of (13.33 mm and 12.13 mm) on ethanol and water extract respectively, while *P. biglobosa* was most effective on *S. aureus* with grand mean inhibitory effect of (13.00 mm and 12.47 mm) on ethanol and water extracts respectively (table 3). The higher effect of ethanol extract of *P. biglobosa* on *S. aureus* is consistent with the study of Ajaiyeoba[11], who reported similar higher effect of ethanol extract of *P. biglobosa* on *S. aureus*. The higher sensitivity of *S. aureus* to *P. biglobosa* is also in keeping with the sensitivity test carried out by two other studies who reported similar effect[12,13]. In a comparative sensitivity test, Adebayo *et al*. reported lower sensitivity of *E. coli* on *D. oliveri* among other plant extracts[14].

From this study *K. senegalensis* had a higher antibacterial potential on *E. coli,* while *P. biglobosa* higher effect on *S. aureus*. According to Bowersox *et al*[15]. *S. aureus* is the leading cause of skin and soft tissue infections such as abscesses (boils), furuncles and cellulitis including systemic infection like pneumonia, bone and joint infection like osteomyelitis, while *Escherichia coli* is the most common cause of urinary tract infection, neonatal meningitis and gastroenteritis[16], these indications agrees with another report elsewhere of *P. biglobosa* to treat sores, ulcers and pneumonia implicated by *S. aureus*[17]. It is also in agreement with two other reports of *K. senegalensis* to treat mucous diarrhea and urinary tract infection caused by *E. coli* [18,19].
The anti-bacteria potential of these plants extract can be explained by the phytochemicals present in them, according to three different studies[13,14,20], carried out on phytochemical screening of the leaves of these three plants revealed the presence of alkaloid, saponin, tannins, flavonoids, glycoside and phenol compounds, which have been found to form irreversible complexes with bacteria cell wall that results in the inhibition of cell wall synthesis of bacteria[21].

4. Conclusion

The three plants *Parkia biglobosa, Daniellia oliveri, Khaya senegalensis* are potential useful antibacterial agents, which had inhibitory effect on bacterial growth at all concentrations of the study, indicating their potential in the treatment of diseases caused by these bacterial.

Recommendations

More extensive studies especially involving phytochemical screening of the active metabolites of these plants found in the same study area, also an in vivo trial on their effects is recommended.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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