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Phytochemical Screening and Anti-Microbial Activity of *Parkia biglobosa* Seed' Oil Extract

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ABSTRACT

Parkia biglobosa is a deciduous tree also known as African Locust Bean. It is a forestry types well known and exploited by Africans. The tree pods are refer to as the fruits of the plant and has colour ranges from pink brown to dark brown when fully matured. Phytochemicals screening and antimicrobial activity of the seed' oil against some clinical isolates was carried out to ascertain suitability of the it as for the control of epidermal, urinary tract and other infectious diseases. Soxhlet extractor was used for the extraction of oil from the seeds, which later subjected to qualitative phytochemical analysis using standard methods. Antibacterial activity of the oil extract was perfomed using Agar Disc Diffusion Method. The clinical isolates used were *Escherichia coli* (*E. coli*), *Pseudomonas aeurigonasa* (*P. aeurigonasa*) and *Staphylococcus aureus* (*S. aureus*). The percentage yield of the oil is 30ml/120g x 100 % = 25%. The phytochemical analyses revealed the presence of saponins, flavonoids, steroids and glycosides. The oil possess significant antimicrobial activity against the isolates, particularly at 100% and 50% concentration. Zone of inhibition increases as the concentration of the oil increases. Positive correlation was observed based on the oil concentration (increase). This study revealed the medicinal value of the seed' oil, in addition to it's the traditional uses.

Keywords: *Parkia biglobosa*, Seed' oil, Phytochemical, Clinical Isolates.

INTRODUCTION

Medicinal plants are the major sources of obtaining antimicrobial agents (Ahmad & Wajid, 2013). This is because plants produce diverse types of bioactive molecules, making them a rich source of different types of medicines. Such plants have been using since ancient times in virtually all cultures as a source of drugs, and are of great importance to the health care of individuals and communities (Garg & Roy, 2020). Many plants have been confirm to have a variety of antimicrobial effects and antidiabetic properties. In fact, many researches have been directed towards the provision of empirical proofs to back up the use of plant species in trade and medicinal practices. Some have examined the effects of plants used traditionally by indigenous healers to support treatment of various diseases. Moreover, determining the antimicrobial activity of many plant species has become the subject of many intensive investigations (Aziz et al., 2018).

According to Ajaiyeoba (2002), majority of Nigerian homes, maintain some sort of private family traditional medicine practitioner. Existing data and contemporary researchers seem to authenticate the assumption for general health improvement of the masses by traditional healers. Ethnobotany is a preliminary method of research, suitable for gathering information on the use of plants

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(Bothon et al., 2023). It has been prove that the medical knowledge handed down by the common people constitutes sources of information useful for scientific research and that many plants utilized exclusively in popular tradition, but when exposed under scientific investigation, some were found to be useful and effective in curing illness. *Parkia biglobosa* (*P. biglobosa*) tree as one of the medicinal plant species as tested by many researchers have been used especially in African countries to cure certain diseases. Especially, the *P. biglobosa* leaves and pod husks were used for anti-diarrheal activities, analgesic and anti-inflammatory activities because they contain glycine flavonoids. Apart, they equally have spasmolytic activity on smooth muscles, as well vasodilatory and antiseptic effects (Bothon et al., 2023). The bark is used as a mouthwash, vapour inhalant for toothache, or for ear complaints (Ofosu, 2022; Famojuro, 2023; (Bothon et al., 2023). The leaves can macerated in baths for leprosy and bronchitis, pneumonia, skin infection, fever, malaria, burns and vomiting. So also, an alcoholic extract of crude seeds showed anti-hypertensive activity and contractile effect on smooth muscles of intestine and increased the tonus and mobility of the uterus (Al-Snafi 2022). In addition, plant is crucial toward overcoming food-borne diseases; which have remained a major concern for public health and an important economic problem in many countries over the years. Therefore, one can point out that *P. biglobosa* is a very important plant in our society, Northeastern Nigeria. The aim of the study is to extract oil from the seed of the *P. biglobosa*, determine the phytochemicals and antimicrobial effect against some clinical isolates.

MATERIALS AND METHODS

Sample Collection

The part of the plant used for the study was the seeds; dry *P. biglobosa* seeds was purchased from Gombe main market, Gombe LGA of Gombe State, Nigeria.

Sample Preparation

The seed sample was screened to remove the unhealthy ones and cleaned thoroughly to ensure dirty free. The seeds were washed and sun dried before grinded using the laboratory blender as described by Ofosu, 2022 with little modification.

Oil Extraction

Extraction of oil from *P. biglobosa* seeds was carried out using Soxhlet extraction method. The amount of sample was placed in conical flask and added petroleum ether. The mixture was swirled sufficiently using incubator shake at 200rpm for two hours in the conical flask. Afterward, the mixture was filtered using filter paper and it was collected in a smaller beaker. Laboratory oven was set to 60-80^{0}C and the beaker containing the filtrate was left for 24 hours. The oil was removed from the obtained extract under reduced temperature and pressure, while the excess crude solvent was removed from extracted oil using rotary evaporator. The percentage yield was calculated using the formula (Olowokere, 2018):

$$\% \text{ yield} = \frac{\text{Amount of oil yielded in (ml)}}{\text{Amount of sample used in (g)}} \times 100$$

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Phytochemical Screening of the Seeds' Oil Extract

Phytochemicals analysis was conducted on the oil of *P. biglobosa* seeds to determine the bioactive constituents as described by Sani, (2014); Ajaiyeoba (2002).

***Detection of Alkaloids*:** 0.5ml of the extract was dissolved in chloroform (5ml of 1% aqueous hydrochloric acid) and filtered. It was steam on water bath, re-filtered and the filtrates was treated with three (3) drops of Dragendoff's reagent. Presence of alkaloids was confirmed by the formation of orange coloured precipitate.

***Detection of Saponins*:** 1ml of the oil was dissolved in chloroform and shaken with 2ml of water. Foam was produced and persists for ten (10) minutes, which confirmed the presence of saponins.

***Detection of Tannins*:** 1ml of extract was diluted with distilled after dissolving in chloroform and added 3-drops of 5% sodium chloride. White precipitate was formed which indicates the presence of tannins.

***Detection of Flavonoids*:** Extract was dissolved and were treated with few drops of sodium hydroxide solution and shaken until it is fully dissolved. Subsequently, few drops of H_2SO_4 was added and no colour solution indicate the absence of flavonoid.

***Detection of Steroids*:** 0.5ml of the extract was dissolved with petroleum ether and later treated with sufficient amount of chloroform, and then equal volume of H_2SO_4 was added. The solution divided into two layer; upper layer in red precipitate and yellow with green fluorescence in the sulphuric acid layer as lower layer that indicate the presence of steroids.

Media Preparation, Sub-Culturing and Purification of Test Organisms

The media used in the present study were all prepared according to manufacturer's instructions and autoclave at 121^0C for 15 minutes.

The test microbes were clinical isolates obtained from the Microbiology laboratory of Gombe State University (GSU) and reconfirmed by sub-cultured, identified using conventional approach. Stock cultures of the isolates; *Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa* were sub-cultured on nutrient agar by streaking on the plate, followed incubation at 37 ^{0}C for 16 hours.

Colonies of fresh cultures of the different test organisms were picked with sterile loop and pure cultures of the test organisms were obtained after second sub-culture. Distinct colony from the pure culture plates were sub-cultured on agar slants to obtain stock culture for each of the test organisms.

Preparation of Extracts for Anti-Microbial Analyses

Pure oil extract 2ml was diluted with 1ml of Dimethyl Sulphoxide (DMS) and used as stock solution. Afterward, 50% and 25% was prepared from the stock solution by adding 1ml of the stock and added DMS solution. Ampicillin and Tetracycline antbiotic was used as positive control.

Antibacterial Sensitivity Test

Different oil extract concentrations were used for the antibacterial susceptibility test as follows; 100%, 50% and 25% concentrations (Zaidan et al., 2005; Ilesanmi, 2024).

Disk Diffusion Method

The disk diffusion method is employ to determine microbial susceptibility to antibiotics of extract in which filter paper disk

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containing known concentrations of antibiotics or extracts is place on the agar surface that has been previously inoculated with the bacteria of interest. In the present, the plates were allowed to stay for 15minutes for proper diffusion of extract then incubated for 24 hours at 37 ^{0}C. Well along, microbial growth for some plates were determined by measuring the diameter of the inhibition zone (mm) using a transparent meter rule, while some were observed for the presence or absence of growth (Golus et al., 2016).

RESULTS

Phytochemical Screening

The percentage yield of the oil is 30ml/120g x 100% = 25% (Bothon et al.,2023; Ilesanmi et al., 2024). The presence or absent of the phyto-constituent is presented in Table 1.

Table 1: Qualitative phytochemical analysis of seeds oil of *P. biglobosa*.

Test	Results
Alkaloids	-
Saponins	+
Tannins	-
Flavonoids	+
Glycosides	+
Steroids	+

Key: + = presence, - = absent

Antimicrobial Analysis of Oil from *P. biglobosa* seeds

Table 2 indicate the antimicrobial potential of the *P. biglobosa* seeds extract at diverse concentration. The effect on the respective clinical isolates was similar at 100% concetration (Ilesanmi et al., 2024) Marpaung, L., & Sinaga, R. M. (2022). (Bukar et al., 2010), (Bothon et al.,2023).

Table 2: Antibacterial analysis of *P. biglobosa* seeds oil against three clinical bacterial isolates (mean in diametere).

		Concentrations of oil			
Bacteria	**Source of oil**	**100%**	**50%**	**25%**	**Positive Control**
E. coli	*P. biglobosa* seed	16	14	11	23
S. aureus	*P. biglobosa* seed	14	11	8	18
P. aeurigonasa	*P. biglobosa* seed	15	12	10	17

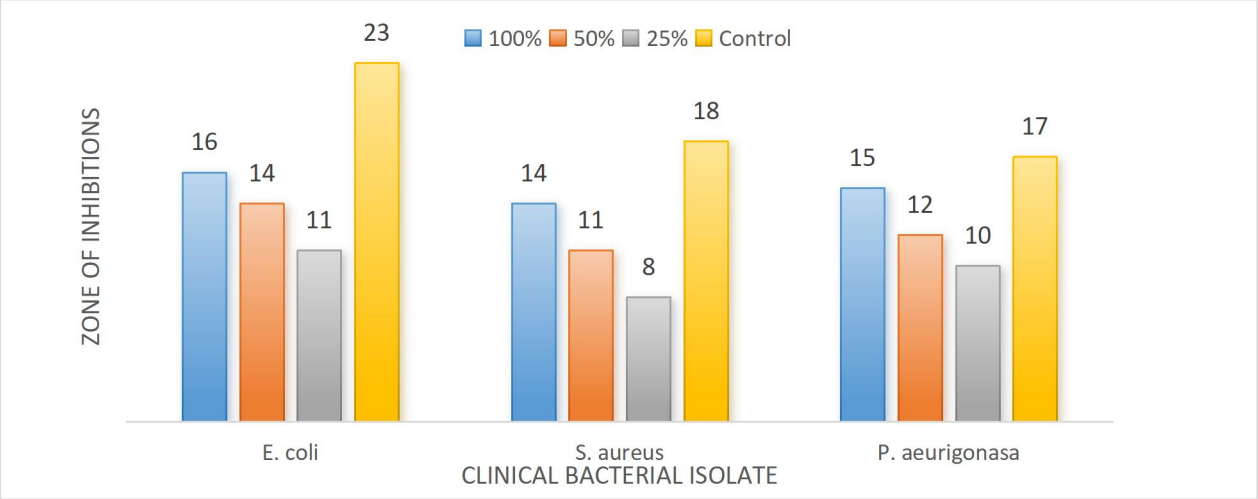

Figure 1: Antibacterial activities of *P. biglobosa* seeds' oil extract on *P. aeurigonasa, E. coli* and *S. aureus.*

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Table 3: Determination of MIC and MBC on *Escherichia coli, S. aureus* and *P. aeurigonasa*

Oil	MIC (*E. coli*)	MBC (*E. coli*)	MIC (*S. aureus*)	MBC (*S. aureus*)	MIC (*P. aerugonasa*)	MBC (*P. aeurigenosa*)
P. biglobosa	25%	100%	50%	50%	50%	100%

DISCUSSION

Scrutiny of past works on *P. biglobosa,* however, shows not much has been reported on the seeds oil extracts. Table 1 presents the results of phytochemical tests of the oil extract determine in this study that shows the presence of saponins, flavonoids, glycosides, steroids, while tannins and alkaloids was absence. The results of the phytochemical analysis are in correspondence with the research of Ajaiyeoba (2002) which reported on the medicinal value of pods and leaves of *P. biglobosa*. The pod possesses flavonoids, both pod and leaf possess reducing sugar and tannins (Kouamé et al., 2021; Bothon et al., 2023), although Alkaloids were only detected in the ethanol leaf extract and Saponins were not detected in both the leaf and pod. Ajaiyeoba (2002), 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. The absence of tanins in the extract of *P. biglobosa* seeds oil is in line with the study of Oluwaniyi and Bazambo (2014) and Ajiboye and Hammed (2020).

The above facts clarifying that the seeds oil has higher phytochemicals content as it served as the basis for the plant. The result further shows that the oil extracts possess more phytochemicals than the leaf and pods extracts. Phytochemical screening result of many research using different approaches for derived from various parts of *P. biglobosa* are ample in the scientific literature. Thus, there is less data concerning the phytochemical contents of it seeds oil. Alkaloids, polyphenols, cardiac glycosides, saponins, terpenoids, and steroids have all been determined in various extracts of the leaf, seed, root, and bark (Awuchi et al., 2020). In contrast to the work of Ajiboye and Hammed (2020) that make comparison on the fermented and unfermented (aqueous and acetone) crude oil seeds extracts of *P. biglobosa*, the fermented seeds oil extracts revealed the presence of all the following as follows Alkaloid, Flavonoid, Tannin, Saponins, Glycosides while the unfermented shows the absence of some of the phytochemicals. This information indicates the role some microorganism play ecologically during fermentation, to form a new substance.

Phytochemicals are secondary elements of plants that are accountable for biological activities, which have been reported to possess anti-oxidative potentials (Oluwaniyi and Bazambo, 2014). They are mainly associated in antimicrobial potentials of various medicinal plants as reported by different scholars. Antimicrobial analyses of the *P. biglobosa* seeds oil extract revealed that increasing concentration inclines to increase the mean zone of inhibition (Ilesanmi et al., 2024). Similarly, *S. aureus and E. coli* indicates increase in the mean zone of inhibition as the concentration increases, as well the same was observe in case of *P. aeruginosa*. The aqueous bioactive constituent's content may be responsible for this effect (Tijani *et al.,* 2009). The table 2*,* showed the results of the antibacterial activities of the oil extract based on the results obtained. The extract exhibited significant antimicrobial activity on *P. auerigonasa* was 15mm at 100% and 12mm at 50% concentration, the lowest zones of inhibition produced was 10mm at 25% concentration (Bukar et al., 2010).

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Eboma *et al*., (2020) reported on the antibacterial activity of the aqueous extract and oil of fermented locust bean seeds against 14 common pathogenic microorganisms. They and other researchers asserted that the oil had a higher efficiency, as indicated by a larger zone of inhibition (Ilesanmi et al., 2024). As in case of the aqueous extracts of *P. biglobosa* on *E. coli,* the highest zones of inhibition was 16mm at 100%, 14mm at 50% and lowest zone of inhibition was 11mm at 25% concentrations. Whereas, for *S. aureus* the highest zone of inhibition was 14mm at 100%, 11mm at 50% and the lowest was 8mm at 25% concentrations. Positive correlation was observed as increase in the concentration of the oil which showed increased zone of inhibition to the organism (figure1) (Bukar et al., 2010).

CONCLUSION

The research determined the antimicrobial properties of *P. biglobosa* seeds' oil extracts. The finding clarifying that the seeds oil has higher phytochemicals content as it served as the basis for the plant. The seeds' oil extract have demonstrated antimicrobial activity against some clinical isolates. *P. biglobosa* seeds' oil demonstrate optimal effect on *E. coli* with the highest zones of inhibition was 16mm at 100% and lowest zone of inhibition at 25% concentrations. This support the application of the seed oil as potential antimicrobial agent, which help in treatment of epidermal, and urinary tract infection caused by *E. coli and S. aureus*. *P. biglobosa*.

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