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Antibacterial Activity of the seed of *Dialium guineense* against Selected Enteric Bacteria

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Abstract: This study was aimed at evaluating the antibacterial activity and screening the phytochemical composition of the seed of *Dialium guineense*. The aqueous, methanolic and ethanolic extracts of the seed of *Dialium guineense* were analyzed against some clinical isolates. The phytochemical composition and antibacterial sensitivity testing were carried out using standard methods. The clinical isolates were *Escherichia coli*, *Proteus mirabilis, Klebsiella pneumoniae* and *Salmonella typhi*. Broad spectrum antibiotics were used as positive control for the antibiotic sensitivity test. Phytochemical screening of the extracts revealed the presence of saponin, tannins, alkaloid and glycoside while antimicrobial activity test at various concentrations for ethanolic and methanolic extracts showed significant results against the selected enteric bacteria with the exception of aqueous extracts which showed no antimicrobial activity against any of the isolates. The highest zone of inhibition (13.33mm) was obtained against *S. typhi* using methanolic extract while *E. coli* had a zone of inhibition of 10.67mm using ethanolic extract. *P. mirabilis* showed the lowest zone of inhibition (3.67mm) using ethanolic extract. Ciprofloxacin showed the highest sensitivity to the test organisms while Gentamicin showed the lowest

sensitivity. MIC results for the methanolic and ethanolic seed extract against the microbial isolates varied. However, a higher concentration above 225 mg/ml would be required for bactericidal activity, From these findings, the seed of *Dialium guineense* is a potential source of bioactive compounds and may have implications in the management of infectious diseases caused by some enteric bacteria.

Keywords*:* Dialium guineense, *phytochemicals, solvent extraction, enteric bacteria, antimicrobial activity.*

Introduction

Medicinal plants represent a rich source of antimicrobial agents. A wide range of medicinal plant parts is used for extract as raw drugs and they possess varied medicinal properties. Although, hundreds of plant species have been tested for antimicrobial properties, the vast majority of them have not been adequately evaluated, considering the vast potentiality of plants as sources of antimicrobial drugs with reference to antibacterial and antifungal agents. It's been reported globally about the antimicrobial properties of various medicinal plants and their use for medicinal purposes [1, 2, 3,]. Quite a number of plants have been used traditionally for medicinal purposes as they contain a variety of compounds of known therapeutic values[4, 5, 6].It is expedient that extracts from these medicinal plants showing antimicrobial activities at specific sites other than those used by synthetic drugs will be active against drug-resistant microbial pathogens. However, information the activity of such medicinal plants is inadequate [7]. Plants have ability to synthesize diverse aromatic secondary metabolites, and these groups of compounds show antimicrobial action and serve as plant defense mechanisms against pathogenic microorganisms [8, 9].*Dialium guineense* (Wild) belongs to the family of Fabaceae, commonly called black velvet or velvet tamarind. The fruit pulp is edible and sweet, with some quantities of tannins and ascorbic acid. It is quite a good source of protein and minerals as reported by Arogba *et al*. [10]. *D. guineense* is called "Awin" among the Yoruba speaking Nigerians. It is also known as "Icheku" among the Igbos in the Eastern part of Nigeria and as "Tsamiyarkurm" among the Hausa speaking Nigerians [9, 11]. Nwosu [11] reported that the bark and leaves of *D. guineense* have shown some medicinal properties and are used against several diseases. The usefulness of *D. guineense*as an anti-ulcer agent has been reported [12]. The antimicrobial activities of the fruit pulp extracts of *D. guineense* against some clinical isolates have been elucidated [13]. According to Bero *et al*. [14] the leaves and stem bark are used for the treatment of some infections such as diarrhoea, severe cough, bronchitis, stomach aches, malaria fever, jaundice and haemorrhoids.

Some researchers have authenticated activities of the leaves and stem bark of *D. guineense* which include its antibacterial and analgesic activities [15, 16,], as well as antioxidant properties [17]. Globally, infectious diseases have been a main cause of death and diverse kinds of disability which accounts for about 23% of worldwide disease as opined by Murray and Lopez [18]. Lomovskaya

and Bostian [19] have suggested that improvement of the efficacy of available antibiotics might be a reasonable and sustainable option due to the challenge between the slow development of new drugs and the fast emergence of resistant strains. This may raise some hope rather than making the future management of infectious diseases look bleak. Although, various works have been done to investigate the antimicrobial and phytochemical screening of *D. guineense* plant using its leaves, bark, and roots but fewer or no researches have been recorded in investigating the potential antimicrobial effects of the plant seeds.

This work is therefore aimed at screening the phytochemicals and conducting antimicrobial activity tests on the seed of *Dialium guineense.*

Materials and Methods

Plant Collection

The fruits of *Dialium guineense* were purchased at Ketu in Lagos State, Nigeria. The seeds were separated from the fruit and were identified at the herbarium section of the Department of Plant Biology, University of Ilorin, Nigeria.

Collection of Microbial Isolates

The bacteriall isolates were obtained from the Department of Microbiology and Parasitology, University of Ilorin Teaching Hospital, Ilorin, Kwara State, Nigeria. The pure isolates collected were as follows; *Salmonella typhi*, *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus mirabilis*. The isolates were sub-cultured and the culture was maintained at 4 ^{o}C. However, further sub-culturing was done to keep the organisms viable.

Extraction of Plant Material

Forty grams of *D. guineense* powdered sample was soaked in 1000ml of sterile distilled water, 160 ml of 95 % ethanol and methanol respectively for 24 hours at room temperature on orbital shaker at 160 rpm. The content was filtered using muslin cloth and evaporated to dryness using water bath at 60 ^{o}C. The extracts were collected and stored at refrigerator temperature until when needed.

Antimicrobial Sensitivity Method

Antimicrobial activity study of the crude extracts of ethanol, methanol and aqueous extract of *Dialium guineense* seed was carried out on *Escherichia coli, Proteus mirabilis, Klebsiella pneumoniae* and *Salmonella typhi*. The agar well diffusion method of Collins *et al*. [20] with slight modification was adopted for this assay. The antibiotic discs of Ofloxacin, Gentamicin and Ciprofloxacin were placed aseptically on seeded plate of the isolates with the aid of a sterile pair of forceps.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC of the plant extracts were determined by using the broth dilution method [20]. One ml of 24 hours culture of test organisms (10^{7}cfu/ml) adjusted to McFarland turbidity standard was incubated in test tubes with varying concentrations of 125, 150, 175, 200 and 225 mg/ml of plant extracts in normal saline at 37 ^{o}C for 24 hours. The concentration with the lowest dilution and no detectable bacterial growth was considered as the minimum inhibitory concentration (MIC) [21].

Determination of Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentration was determined by first selecting plates that showed no growth during MIC determination and incubated for another 24 hours at 37 ^{o}C. The minimum bactericidal concentration was considered as the lowest concentration that could not produce a single bacterial colony [20, 21].

Qualitative Phytochemical Screening

The method described by Adebayo and Sofowora [22] was used to test the presence of saponins, tannins, phenolics, alkaloids, steroids and glycoside.

Statistical Analysis

Data obtained were expressed as mean and standard deviation of triplicates and were statistically analysed using SPSS statistical package of version 16.0. The results obtained were statistically analysed by ANOVA. Values were considered significant at $p<0.05$

Results

Antibacterial Activity of the Extracts.

Ethanolic and methanolic extracts showed significant result against the selected enteric bacteria with the exception of aqueous extracts which showed no zones of inhibition on any of the isolates.

S. tyhpi showed the highest inhibition to the crude methanolic extract of *D. guineense* seed with 13.33mm zone of inhibition followed by *E.coli* with 11.33mm zone inhibition. *P. mirabilis* showed the lowest zone of inhibition to the crude methanolic extract with 3.67mm zone of inhibition. The methanolic extract however showed the highest zones of inhibition on the enteric bacteria. Also, the ethanolic crude extract showed a different range of inhibition on the isolates with *E. coli* being the most sensitive with 10.67mm diameter zone of inhibition followed by *S. typhi* with 10.00 mm diameter zone while *K. pneumoniae* showed the lowest zone of inhibition with 2.67mm (Figure 1).

Minimum Inhibitory Concentration

About five-fold dilutions was done from the crude methanolic and ethanolic extracts with the concentration of 225 mg/ml, 200 mg/ml, 175 mg/ml, 150 mg/ml and 125 mg/ml for each extract respectively. The MIC (minimum inhibitory concentration) of the extracts was also determined. All the organisms showed different MIC concentrations of each extract. *P. mirabilis* showed low MIC range at 150 mg/ml using methanolic extract and 200 mg/ml using ethanolic extract. However, the MIC of both the methanolic and ethanolic extract against *E. coli* and *S. typhi* was 225 mg/ml. The MIC value for *K. pneumoniae* was 200 and 225 mg/ml for ethanolic and methanolic seed extract respectively. Using ANOVA to analyse the data obtained, there was no significant difference at $P < 0.05$.

Minimum Bactericidal Concentration (MBC)

All the plates that showed MIC after 18-24hours of incubation were plated out for MBC. Minimum bactericidal concentration was evaluated in order to ascertain the bactericidal effect of the methanolic and ethanolic extracts on the enteric bacteria. Both methanolic and ethanolic extracts at the various concentrations used did not show any bactericidal activity. This

implies that a higher concentration well above the MIC would be required for bactericidal activity. For all concentrations plated out there was growth of organisms after 18-24hours of incubation.

Antibiotics Sensitivity Testing

Using some broad antibiotics as reference drugs, the sensitivity of the antibiotics, Ofloxacin (5 µg), Ciprofloxacin (30 µg) and Gentamicin (10 µg), were investigated on Muller-Hinton agar plates (MHA). Reading was taken after incubation for 18-24hours at 37 ^{o}C. Among all, Ciprofloxacin showed the highest zones of inhibition to isolate *K. pneumoniae*. Gentamicin showed the lowest range of inhibition zones to the organisms with zero effect i.e. no zones of inhibition on *E .coli* and *P. mirabilis* but about 4.0mm and 0.67mm on *K. pneumoniae* and *S. typhi* respectively. *K. pneumoniae* showed high sensitivity to Ofloxacin having a zone of 14.00mm. Zones of inhibition of 11.67mm and 11.00 mm was obtained for *E. coli* and *S. typhi* respectively. *P. mirabilis* expressed very low sensitivity with a zone of 4.33 mm (Figure 2).

Qualitative Phytochemical Screening of *D. guineense* extracts.

The ethanolic, methanolic and aqueous extracts of the seed of *D. guineense* showed positive test for all the screened phytochemicals except for steroid which showed a negative result for all extracts. Table 3 showed the phytochemical screening result for each extract.

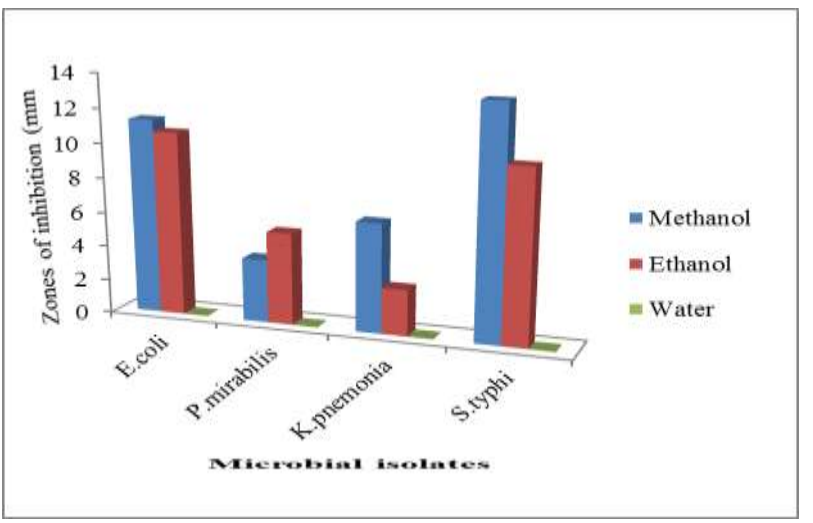

Figure 1: Zones of inhibition of the crude extracts of *D. guineense* on the bacterial isolates

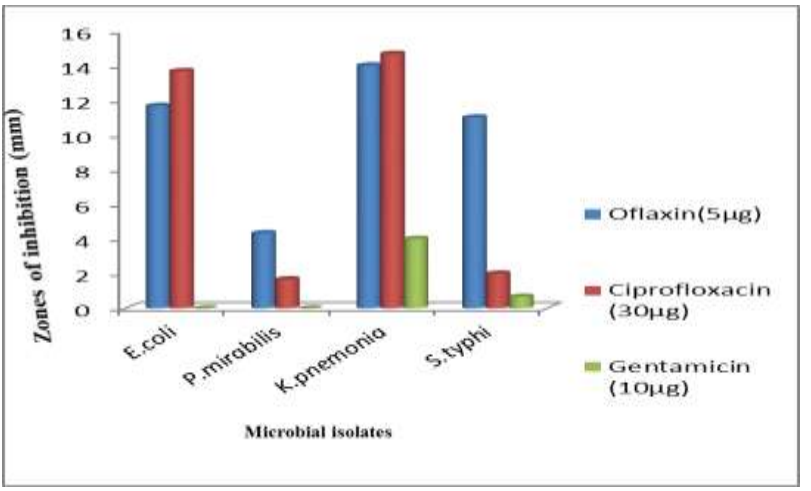

Figure 2: The effect of antibiotics on the bacterial Isolates.

Table 1: MIC and MBC of the ethanolic and methanolic seed extract of *D. guineense*

Enteric Bacteria	Ethanolic extract MIC	Conc. mg/ml MBC	Methanolic extract MIC	Conc. mg/ml MBC
Escherichia coli	225	-	225	-
Proteus mirabilis	200	-	150	-
Klebsiella pneumoniae	200	-	225	-
Salmonella typhi	225	-	225	-

Table 2: Qualitative phytochemical screening of the ethanolic, methanolic and aqueous seed extracts of *D. guineense*

Phytochemicals	Ethanolic extract	Methanol extract	Aqueous extract
Saponin	+++	+	++
Tannin	+++	+++	+
Alkaloid	++	++	+++
Steroid	-	-	-
Glycoside	+++	++	++

Key Notes:
+ → Positive, ++ → More positive, +++ → Most positive, - → Negative

Discussion

The seed of *Dialium guineense* had a significant effect on all test bacteria. However, the crude aqueous extract did not have any antimicrobial effect on enteric bacteria such as *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumoniae* and *Salmonella typhi*. Orji *et al*. [16] explains that the active ingredient in the plant bark and leaf are more soluble in ethanol than water. This may be the reason why aqueous extract had no effect on the enteric isolates. However, the evaluated phytochemicals of the aqueous extract of the seed of *Dialium guineense* revealed the presence of saponins, tannins, alkaloids and glycosides, although, there is a relatively low presence of tannin and absence of steroids. This supports the trends of Gideon *et al*. [17] who said flavonoids, alkaloids, tannins and saponins are present in the leaf and bark of *D. guineense* of ethanolic and aqueous extract. Akinpelu *et al*. [9] also supported the fact that there are active components present in *D. guineense*. He reported that the phenolic compounds from medicinal herbs and dietary plants play important role in health; in addition to enhancing antimicrobial activities in these plants. Udoh *et al*. [23] reported that the sensitivity of aqueous extract of *Lasianthera africana* possess antimicrobial effect against *Salmonella typhi*, *Escherichia coli* and *Proteus vulgaris*.

However, the ethanolic and methanolic crude extracts showed a greater range of inhibition to the microbial isolates with *E. coli* and *S. typhi* showing the highest sensitivity and *K. pneumoniae* and *P. mirabilis* being the least sensitive. The sensitivity of these extracts has been revealed from the result of the phytochemical screening of ethanolic and methanolic extracts. Ethanol and methanol extracts showed a positive result for the components tested except for steroid which showed a negative result for both extracts and also for the aqueous extracts. Fat and lipids are not present in the seed of *Dialium guineense*. This could be the reason why steriod was negative for all extracts. This is in line with Arogba *et al*. [24], who said that the edible part (pulp) of ripe *D. guineense* fruit is sweet but acidic and relatively poor in protein and oil with fairly low levels of ascorbic acid and tannin. However, the seed had also been reported to be mildly acidic, poor in oil but fairly good source of protein and minerals. This could also be evident from this study as ethanolic, methanolic and aqueous extract showed a negative composition of steroid. The findings of this study do not agree with Orji *et al*. [16] who reported the antimicrobial properties of the crude aqueous leaf extracts of *D. guineense* against *S. aureus* and *K. pneumoniae* but showed the presence of flavonoids, alkaloids, tannin and saponin in the aqueous extracts. This is in conformity with the findings from this present study as the aqueous extract of *D. guineense* seed had these phytochemicals. Akinpelu *et al*. [9] also reported the bioactivity of the methanolic crude leaf extract of *D. guineense* on fourteen environmental strains of *Vibro* species. Their findings are in conformity with this study in line with the antimicrobial activities of the methanolic and ethanolic extracts of the seed of *D. guineense* against *Escherichia coli*, *Salmonella typhi*, *Proteus mirabilis* and *Klebsiella pneumoniae*. Orji *et al*. [16] also reported the presence of alkaloids,

flavonoids, tannins and saponins in the ethanol extract of the stem bark of *D. guineense*. The fruit pulp of *D. guineense* has been reported by Arogba *et al*. [10] as antiulcer and as a vitamin supplement.

The range of MICs in this study is between 150-225mg/ml for all the isolates. Also, Orji *et al*. [16] reported MIC values for the aqueous and ethanolic extract of leaf and stem bark of *Dialium guineense* for *S. aureus* and *K. pneumoniae* to be 200mg/ml. This variation could be as a result of the variety of strains of microbial isolates used, varying phytochemical components of plant parts and the extraction methods used as well.

The present study has revealed evidence of the sensitivity of the microbial isolates to some of the broad-spectrum antibiotics while some were resistant. *E. coli* and *P. mirabilis* showed resistance to gentamicin while *K. pneumoniae* and *S. typhi* were susceptible. However, the isolates were susceptible to ofloxacin and ciprofloxacin, but *P. mirabilis* and *S. typhi* showed low zones of inhibition to ciprofloxacin. Ofloxacin is a broad-spectrum antibiotic of the class quinolones and due to its mode of action on the bacterial isolates, it was able to inhibit their growth.

In conclusion, the result of this study has revealed that the methanolic and ethanolic seed extract of *D. guineense* has antimicrobial activity against some microbial isolates which are enteric Also the seed extracts were shown to possess significant quantities of phytochemicals such as saponin, tannin, alkaloid, steroid and glycosides. This study also agrees with the findings of Ajiboye *et al*. [13] who also reported antimicrobial activity of the fruit pulp of *D. guineense* on some microbial isolates. Therefore, the seed of *D. guineense* can be employed as a potential antimicrobial agent. However, further research should be tailored towards investigating the different parts of *D. guineense* such as leaf, root, bark, stem and fruit pulp in relation to their phytochemical components and their effects on clinical isolates.

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