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Phytochemical Analysis and Antibacterial Activity of Stem Bark Extracts of *Detarium Microcarpum* Against Bacteria Causing Gastrointestinal Tract Infections in Humans

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Abstract
Detarium microcarpum is used by different ethnic groups for treatment of various diseases in Nigeria and several parts of West African. The phytochemical constituents of the stembark extract of D. microcarpum were analyzed using qualitative methods. The antibacterial activity of the stembark extracts against Escherichia coli and Staphylococcus aureus were tested using the agar well diffusion method. The phytochemical investigation revealed that presence of tannins, saponin, steroids, flavonoids, glycosides, phenols and terpenoids. The plant extracts exhibited antibacterial potential against the tested organisms at different concentrations (100 mg/mL, 50 mg/mL, 25 mg/mL and 12.5 mg/mL), with S. aureus having the highest zone of inhibition of (21 mm at 100 mg/mL) with ethanolic extract. Therefore, this study suggests that D. microcarpum stembark has phytochemical constituents. The antibacterial activity exhibited by the extracts could be as a result of the phytochemicals presents.

Keywords: Antibacterial activity, ***Detarium microcarpum*,** Phytochemicals, *Staphylococcus aureus*, *Escherichia coli*

INTRODUCTION

Gastrointestinal tract infections are among the major cause of high morbidity rate in the world, though its highest incidence occurs in the unindustrialized countries (Mengelle *et al.,* 2013). A wide group of bacteria including *E. coli*, *Salmonella*, *Shigella*, *S. aureus*, etc. are said to be responsible for gastrointestinal tract infection presenting similar clinical manifestations such as vomiting, diarrhea, abdominal pain, nausea, fever and dehydration at different levels of severity (Rintala *et al.,* 2016).

Antibiotics have had vital impacts, including the decline of mortality and morbidity rate caused by bacterial infections in humans, particularly in the developing countries with limited public health infrastructure (Rossolini *et al.,* 2014). However, these antibiotics have been abused in the general population, resulting in an increase in their resistance rates among numerous microorganisms (Aslam *et al.,* 2018). Hence, antibiotic resistance has been recently recognized as a major threat to global health (Anand *et al.,* 2019). Therefore, efforts have been made to thwart the antibiotic resistance menace, while exploring alternative sources of antimicrobial agents, like medicinal plants.

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Traditional medicinal plants are natural plant resources which are used for the treatment of various diseases at either local or regional scale (Alyaa, 2015). Because they are natural with relatively less complications, these plants have been used in developing and developed countries for thousands of years. It has been estimated that around three-quarter of the global populace depends on either the plants or theirs extracts for health care (Bhawani *et al.,* 2013). Most of the medicinal plants are used fresh so as to obtain the extract from the whole plant or part of it (leaves, roots, flowers or fruit). The woody parts, including, the bark and the roots are also used (Kurhekar, 2017). With the consistent increase in antimicrobial resistance among bacterial populations, medicinal plants remain the potential source of antimicrobial agents (Rouamba *et al.,* 2017).

Detarium microcarpum from the family of Fabaceae is commonly called tallow tree in English and the Hausa ethnic group from the Northern Nigeria refers to it as "Taura". The tree is usually found in savannah area, growing up to 9.114 m long, with reddish-brown scaly stembark. Different parts of *D. microcarpum* were reported to possess medicinal properties (Moghimipour, 2015). The anti-viral property of *D. microcarpum,* cytotoxicity, antibacterial properties, and hypoglycemic activity were reported by Nitbani *et al.* (2016).

Wadood *et al.* (2013) reported that different parts (bark, leaves and roots) of *D. microcarpum* were extensively employed due to their diuretic astringent properties. Likewise, different parts of *D. microcarpum* were prepared as either decoction or infusions to cure rheumatism, urogenital infections, vomiting, intestinal worms and diarrhea including dysentery (Reuben and Jada, 2013). The Hausa ethnic group from the Northern Nigeria claimed that the infusion of stem bark of *D. microcarpum* treats gastrointestinal discomfort. However, there is no scientific evidence to verify the claim, thus the basis for this study. The aim of the study is to analyzed the phytochemical constituents and antibacterial potential of *D. microcarpum* stem bark extracts against *E. coli* and *S. aureus* causing Gastrointestinal tract infections in humans.

Materials and Methods

Sample collection and identification

Stem bark of *Detarium microcarpum* was collected at Birnin Gwari L.G.A Kaduna state. It was then identified and confirmed by the department of Biological Science in Kaduna State University.

Preparation and extraction of the plant materials

The stem bark collected was washed and air dried in the laboratory then ground into powder form with mortar and pestle. Fifty grams of the powdered material was macerated in 500 mL of ethanol while another 50 g in 500 mL of distilled water. It was then allowed to stand at room temperature for three days with intermittent shaking. The macerated solution was strained using Whatman (No 1) filter paper and the residue was discarded. The filtrate of ethanol was allowed to dry at ambient temperature and that of water was left to evaporate for three days at room temperature, which was later stored in a clean dry form as described by Bashir *et al.* (2013).

Phytochemical screening of plant extract

The aqueous and the ethanolic extract were investigated for the presence of bioactive phytochemical constituents as demonstrated by Abdu and Dimas (2016).

Collection of test isolates

Escherichia coli and *Staphylococcus aureus* isolated from GIT patients were obtained from NNPC Industrial Clinic, where *S. aureus* on Mannitol Salt Agar (MSA) and *E. coli* was sub-cultured on Eosin Methylene Blue Agar (EMB)

Preparations of concentration of the stem bark extract

Using sterile dilution technique, 0.2 g ethanolic plant extract was dissolved in 4mls of water to give the stock solution of 100 mg/mL. This was serially diluted with distilled water given different concentrations (50 mg/mL, 25 mg/mL, 12.5 mg/mL and 6.25 mg/mL). Aqueous dilution was also made using the same technique (Ekwe and Eleglam, 2005).

Preparation of McFarland Standard

The preparation involves the mixture of Sulphuric acid (H_2SO_4) (1%) and Barium Chloride (1.17%). 9.95 mL of H_2SO_4 was added to 0.05 mL of BaCl to form precipitate suspension. This serves as the 0.5 McFarland's standard which was set as the turbidity for the test organisms (Cheesbrough, 2006).

Standardization of Inoculum

The test isolates were sub-cultured on petri-dish containing nutrient agar and then incubated for overnight at 37°C. The few colonies were transferred into a test tube containing 5ml of normal saline (0.9%) and the volume was adjusted to achieve a turbidity which equaled that of 0.5 McFarland's standard (as prepared above) (Andrews, 2005).

Determination of antibacterial Activity

The agar well diffusion method previously demonstrated by Biradal *et al. (*2007) was adopted to test for the antibacterial activity of the stem bark extract of *D. microcarpum*. The adjusted bacterial suspensions were inoculated using a sterile swab onto the prepared and solidified Mueller-Hinton agar, and the allowed to stand for 15 minutes. Thereafter, six wells of 4 mm each were bored using a sterile cork borer. 0.2mls of the ethanol extract of varying concentration (100 mg/mL, 50 mg/mL, 25 mg/mL, 12.5 mg/mL and 6.25 mg/mL) were added to five wells. Ciprofloxacin was added as a positive control to the other well. The prepared plates were incubated overnight at 37°C. The effects of the crude extracts were evaluated by measuring the diameters of inhibition zones (mm). Same procedure was carried out for the aqueous concentrations.

Determination of Minimum Inhibitory Concentration (MIC)

To determine the minimum inhibitory concentrations (MIC) of the *D. microcarpum* stem bark extract, National Committee for Clinical Laboratory standards procedure as described by Lar *et al.* (2001) was followed. Briefly, seven tubes each containing 5 mLs of Muller-Hinton broth (labeled 1-7) were used for the MIC determination of the ethanolic extract. 1 mL of each of the crude extract concentrations (100 mg/mL, 50 mg/mL, 25 mg/mL, 12.5 mg/mL and 6.25 mg/mL) were introduced into the seven tubes (1-7), and were then mixed thoroughly. To six test tubes, 0.1ml of broth cultures of the test organism was added with the sixth serving as positive control (broth and culture) while the seventh as negative control (broth only). Thereafter, all the inoculated tubes were incubated overnight at 37°C after which they were observed for bacterial growth. The MIC of the test crude extract was defined as the lowest concentration of the extract capable of inhibiting the growth of the test organism. The same procedure was carried out for the aqueous extract concentrations.

Determination of Minimum Bactericidal Concentration (MBC)

The MBC was evaluated by sub-culturing the contents of the tubes that showed no visible growth from the MIC test on prepared Mueller-Hinton agar. 0.1 mL of the inoculum was spread out using sterile wire loop. The inoculated plates were then incubated overnight at 37°C. MCB was defined as the lowest concentration that showed no visible growth on Mueller-Hinton agar after incubation. (Owuama, 2015).

RESULTS

The aqueous and ethanolic stem bark extracts revealed the presence of the following phytocontituents; Tannins, Alkaloids, Saponins, Glycosides, Phenols, Steroids, Flavonoids and Cardiac glycosides (Table 1).

Table 1: Phytochemical components of Aqueous and Ethanolic Extracts of *Detarium microcarpum* Stem bark

Phytochemical Compounds	Aqueous Extracts	Ethanolic Extract
Tannins	+	+
Saponins	+	+
Alkaloids	+	+
Glycosides	+	+
Quinones	-	-
Phenols	+	+
Terpenoids	-	-
Cardiac glycosides	+	+
Anthraquinones	-	-
Steroids	+	+
Phlobatannins	-	-
Flavonoids	+	+

Key
+ = Present
- = Absent

The result of the antibacterial screening of stem bark extracts of *D. microcarpum* at different concentrations (100 mg/mL, 50 mg/mL, 25 mg/mL, 12.5 mg/mL and 6.25 mg/mL) against *Staphylococcus aureus* and *Escherichia coli* isolated from gastrointestinal tract patients is presented in Table 2 and 3 respectively. The result of the aqueous extract showed that, *S. aureus* have the highest zones of inhibition of 14.5 mm at 100 mg/mL followed by 12 mm at 50 mg/mL, 9.5 mm at 25 mg/mL and 8 mm at 12.5 mg/mL compared to *E. coli* with lower zones of 5 mm at 100 mg/mL, 4.2 mm at 50 mg/mL and 2 mm at 25 mg/mL. Likewise, the ethanolic extract exhibited the highest inhibition against *S. aureus* with the inhibition zones of 21 mm at 100 mg/mL, 17.5 mm at 50mg/mL, 12.5mm at 25mg/mL and 11mm at 12.5mg/mL. On the other hand, *E. coli* showed zones of inhibition of 12 mm at 100 mg/mL, 9.5 mm at 50 mg/mL and 8 mm at 25 mg/mL. Ciprofloxacin was used as the control drug inhibiting *S. aureus* with 40mm and *E. coli* with 20mm.

Table 2: Diameter of inhibition zone of aqueous stem bark extract of *Detarium microcarpum* against the test isolates

Test	Zones of inhibition at different concentrations (in mm)					
Organisms	100 mg/mL	50 mg/mL	25 mg/mL	12.5 g/mL	6.2 mg/Ml	Ciprofloxacin
S. aureus	14.5mm	12mm	9.5mm	8mm	-	40mm
E. coli	5mm	4.2mm	2mm	-	-	20mm

Table 3: Diameter of inhibition zone of ethanolic stem bark extract of *Detarium microcarpum* against the test isolates

Test	Zones of inhibition at different concentrations (in mm)					
Organisms	100 /mL	50 mg/mL	25 mg/mL	12.5g/mL	6.25 g/Ml	Ciprofloxacin
S. aureus	21mm	17.5mm	12.5mm	11mm	-	40mm
E. coli	12mm	9.5mm	8mm	-	-	20mm

Key:
- = No Zone of Inhibition

The outcome of MIC of the aqueous and ethanolic stem bark extracts is presented in Table 4 and 5. The MIC of the aqueous extract was observed at 50mg/mL for *S. aureus* while *E. coli* showed no MIC on all the concentrations. The ethanolic extract also showed MIC for only *S. aureus* at 25mg/mL. This showed that both the extracts are only effective in inhibiting *S. aureus* at 50mg/mL in aqueous and at 25mg/mL in ethanol while *E. coli* is resistant to both extracts.

Table 4: Minimum Inhibitory Concentration for Aqueous Extract

Test Organism	Concentration					Positive Control	Negative Control
	100 mg/mL	50 mg/mL	25m g/mL	12.5 mg/mL	6.25 mg/mL		
S. aureus	-	-	+	+	+	+	-
E. coli	+	+	+	+	+	+	-

Table 5: Minimum Inhibitory Concentration for Ethanolic Extract

Test Organism	Concentration					Positive Control	Negative Control
	100 mg/mL	50 mg/mL	25 mg/mL	12.5 mg/mL	6.25 mg/mL		
S. aureus	-	-	-	+	+	+	-
E. coli	+	+	+	+	+	+	-

Key
+ = Presence of Turbidity (Negative result)
- = Absence of Turbidity (Positive result)

Both the aqueous and ethanol extracts showed no bactericidal effects on either of the test isolate (*S. aureus* and *E. coli*) at any of the concentrations (100mg/mL, 50mg/mL, 25mg/mL, 12.5mg/mL and 6.25mg/mL).

DISCUSSION

The phytochemical analysis revealed the presence of Glycosides, Phenols, Tannins, Saponins, Alkaloids, Cardiac glycosides, Steroids and Flavonoids in both the ethanolic and aqueous extracts. The observed antimicrobial properties of the extracts could be due to the presence of phytochemical constituents present. This is in agreement with the findings of the study by Sani *et al.* (2014) where the same phyto-constituents were present in the extract of *D. microcarpum* stem bark. The antibacterial activity of aqueous and ethanol stem bark extracts of *D. microcarpum* against *E. coli* and *S. aureus* at different concentrations were observed.

Higher inhibition zones were observed in the ethanol extract than the aqueous extracts with the highest antibacterial activity on *S. aureus*. The highest activity of the ethanol extracts as compared to the aqueous extracts as reported by Tiwari *et al.* (2011) can be attributed to the differences in the polarity of the solvents used in extraction which could cause a wide variation in the level of the bioactive compounds in the extract. This means that extraction efficiency favors the highly polar solvents such as ethanol, methanol etc. According to Gera *et al*. (2016), the antibacterial activity displayed by *D. microcarpum* suggests the presence of growth inhibiting phytochemicals in the order of flavonoids and tannins which has shown to possess antibacterial potentials. Though, the traditional practitioners primarily use water for preparation but plants extracts from organic solvent have been found to display better antimicrobial activity compared to aqueous extracts.

Minimum inhibitory Concentration (MIC) is the lowest concentration of the antimicrobial agent required to inhibit microbial growth. Clinically MIC is not only used to determine the amount of antibiotics the patient will receive but also the type of antibiotics used which will lower the opportunity for microbial resistance to specific antimicrobial agent (Wiegand *et al.,* 2008). In this study the minimum inhibitory concentration was observed by *Staphylococcus aureus* and not *Escherichia coli* in both the ethanol and aqueous extracts. The MIC for *S. aureus* on aqueous extracts was 50 mg/mL while in ethanol extracts it was 25 mg/mL. This showed that both extracts are effective in inhibiting *S. aureus* at the above concentration and *E. coli* is resistant for both the two extracts. The resistance may occur due to the resistant mechanism the bacteria might possess and also due its thin peptidoglycan layer which is found in Gram-negative bacteria. This means that, even though Gram-positive bacteria are mechanically strong, but appears to proffer little resistance to the diffusion of small antimicrobial molecules. *E. coli* on the other hand, a Gram-negative bacterium is surrounded by a second membrane, the outer membrane which functions as an effective barrier (Nikolaidis *et al.,* 2014). It can also be that it needs a higher concentration which is above 100 mg/mL to be able to show any results which might be true as the findings of Chisom *et al.* (2018) where *E. coli* has shown minimum inhibitory concentration at a higher concentration of 150 mg/mL and 200 mg/mL. In this study the stem bark extracts of *D. microcarpum* showed no bactericidal effects against the clinical isolates of *E. coli* and *S. aureus*. This indicated that the stem bark extract can only inhibit the organisms but not kill them.

CONCLUSION

The phytochemical analysis of the stem bark extracts revealed the presence of bioactive components, including alkaloids, tannins, saponins, glycosides, phenols, cardiac glycosides, steroids and flavonoids. The ethanolic extract showed higher zones of inhibition against *S. aureus* than *E. coli* with 21 mm (100 mg/mL), 17.5 mm (50 mg/mL) and 12.5 mm (25 mg/mL). The higher values of the ethanol extract suggest that the use of polar solvents as the extraction solvent is a better choice for the secondary metabolites present in the plant. The observed antimicrobial properties could be due to the presence of phytochemical constituents present in the extracts. The result of this study supports the traditional use of *Deterium microcarpum* in the treatment of diarrhea, dysentery and other gastrointestinal ailments by the antimicrobial activity it has shown against the clinical isolates tested.

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