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Research Article

ANTIBACTERIAL ACTIVITY AND PHYTOCHEMICAL SCREENING OF LEAF AND STEM (BARK) EXTRACT OF *KHAYA SENEGALENSIS* AGAINST DIARREAL/STOOL ISOLATES

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ABSTRACT
Khaya senegalensis has the medicinal properties for the effective management of several ailments including; diarrhea. To establish the pharmacological rationale for its traditional use, the powdered leaf and stem (bark) were extracted with water and ethanol. All fractions were subjected to phytochemical screening and antibacterial activity against the gram –negative bacteria using well diffusion method. The extracts contained; Saponin, Flavonoids, alkaloid, and tannin. The stem (bark) and leaf of both aqueous and ethanolic extracts were active on the organisms (*E. coli*, *Shigella* and *Salmonella*). But statistically, there is no significant difference among the organisms themselves and the P- values for the test of independence of the organisms themselves are; (1.00 > 0.05), (1.00 > 0.05), (0.996 > 0.05) and lastly (1.00 > 0.05). The MIC of the isolates on the ethanolic stem (bark) extract ranged from (12.5mg/ml to 25.0 mg/ml), whereby, *E. coli* is more sensitive isolate with (12.5mg/ml). In contrast, the leaf ethanolic extract ranged from (25.0 to 50.0mg/ml) in which, *E. coli* and *Shigella* were more sensitive at (25mg/ml). While, both the aqueous leaf and stem (bark) extracts were less effective with MIC value of 50mg/ml. The MBC of the extracts shows the lowest concentration of (12.5mg/ml) on *E. coli* and *Shigella* in stem (bark) ethanolic extract and also in leaf ethanolic extract on the same *E. coli* and *Shigella* with the highest concentration of (25.0mg/ml), but in aqueous leaf and stem (bark) extracts MBC were not detected.

Keywords: *Antibacterial, Phytochemicals and Isolates*

INTRODUCTION
Diarrhea is the passage of loose or watery stools occurring three or more times in a 24-hours period. The three types of diarrhea are: acute, persistent, and dysentery diarrhea (WHO, 2006). Where diarrhea lasts less than 14 days, it is regarded as acute diarrhea. Acute watery diarrhea causes dehydration and contributes to malnutrition and may lead to death of a child. When diarrhea lasts 14 days or more, it is called persistent diarrhea.

Up to 20% of episodes of diarrhea become persistent**.** Persistent diarrhea often causes nutritional problems, creating the risk of malnutrition and serious non-intestinal infection and dehydration. Diarrhea with blood in the stool with or without mucus – is called dysentery. Dysentery pose a serious health problem because of its ability to lead to anorexia, rapid weight loss, and damage to the intestinal mucosa (WHO, 2006).

The plant product may contain recipient or inert ingredient, in addition to the active ingredient (Silva *et al*., 1996). Phytomedicines can also be naturally running substance, usually plant origin, used in prevention and treatment of disease. The medicinal flora in the tropical region has a preponderance of plant that provides raw material for addressing a range of medicine disorders and pharmaceutical requirement (Fatope *et al.*, 2001).

Khaya senegalensis, belongs to family Meliaceae. The plant is also known as the African dry zone mahogany, reaches height of 130 to 165 ft. The trunks bole is straight with branches generally occurring

approximately 32ft above the ground. The thick bark is reddish brown and coarse in texture. The pinnate leaf generally possesses four to seven pails of caplets that measure about 3 to 5.5 inches long. The flowers appear whitish with pyramid shape at the end of branches (Ijeome and Umar, 1996). The woody fruit is shaped similar to a capsule with five sections, or valves.

These valves contain winged seeds that measure about an inch diameter. *K. senegalensis* has been found to contain Anthracitic derivers and strained, which makes it a better Anti diabetic agent (Takin *et al.,* 2014).

In West Africa, Fulani herdsmen used the stem (bark) and leaf of *K. senegalensis* for the treatment of diarrhea, syphilis, pyrexia and malarial fever (Olayinka *et al.*, 1992). Similarly, in northern Nigeria the Hausa utilize *K. senegalensis* extract as a remedy for several human and animal ailments (Deen and Sadiq, 2002; Wurochekke, 2004).

Several members of the family enterobacteriacea are responsible for causing several infections (Fabio *et al.,* 2007). *Klebsiella pneumonia* and pseudomonas spp, are emerging as an important cause of neonatal nosocomial infections.

Escherichia coli causes septicemias diarrhea and can infect the gall bladder, meninges, skin lesions and the lungs especially in debilitates and immunodeficient patients.

However, *Proteus mirabilis* cause wound infections following catheterization or cystoscopy and it is a secondary invader of ulcers, pressure sores, etc**.** *Staphylococcus* and *Streptococcus* cause nosocomial infections, food poisoning upper respiratory infection and other type of infection (Pandey, 2007; Mishra *et al.,* 2011).

The objectives of this study are to collect, identify and determine the phytochemical constituents found in the leaf and stem (bark) extract of *Khaya senegalensis.* And also to determine the activity of the extracts against the diarrheal isolates.

MATERIALS AND METHODS

Collection and Identification of Plant Materials

The leaves and stem (Bark) of *K. senegalensis* were obtained from Wudil town (K.U.S.T) and identified at herbarium faculty of Agriculture, Ahmadu Bello University Zaria. The plant material was deposited at the herbarium with a voucher number 900181.

Preparation of Plant Material

The leaves and stems (bark) were air dried and grounded with the aid of pestle and mortar into a coarse powder, sieved with a 1 mm 2 and stored in a plastic container as described by Fatope *et al*., (1993). Moreover, 40 grams of the powder from both leaf and stem (bark) of *K. senegalensis* was weighted**,** which has then mixed with 400 ml of the required diluents (water and ethanol) for some days. The mixtures were filtered and the filtrat**e** was collected separately in a clean and label beaker (Fatope *et al*., 1993).

Phytochemical Screening

The phytochemical screening of the extracts was carried out for the possible detection and characterization of some secondary metabolites in the extracts such as, alkaloids, Tannins, Saponins, Flavonoids, Cardiac glycosides, Steroids, Anthraquinone, Terpenoids and lastly free sugar content. The phytochemical screening has been divided into: Qualitative and Quantitative method of plants extraction (Evans, 2000).

Test Organisms

The clinical isolates were obtained from diarrheal/stool samples collected from Muhammad Abdullahi Wase specialist hospital Kano, Kano state. The organisms include; *E. coli, Shigella* spp, *Salmonella* spp, etc.

The isolates were identified using the schemes of Cheesbrough (2006) and then sub-cultured into Mac conkey agar, Eosine methylene blue and Salmonella – Shigella agar for further confirmation Cheesbrough (2006).

Gram Staining Technique

A thin smear of about 200mm in diameter was made on grease free slide which was also fixed over a burning flame. A crystal violet solution was applied to cover the smear for 30 seconds and it was then washed with distilled water. Secondly lugol's iodine was applied to the surface for good 30 seconds**.** Acetone was used to decolorize the stain and lastly, the safranin solution was covered on the surface for a minute, which has been washed and allowed to dry at room temperature. Then the stains have been observed under microscope with oil immersion. Consequently, red stains indicate gram- negative bacteria (Cheesbrough, 2000).

Extraction of Khaya Senegalensis Leaves and Stem (Bark)

About 40g of the powdered form of both leaf and stem (bark) was suspended in 400ml ethanol and distilled water in a separate between and also, the suspension was shaken regularly for some days. More so, the suspension has been filtered and solvent or the residue was removed by evaporation to dryness at same room temperature (Fatope *et al*., 1993).

Minimum inhibitory concentration (MIC) of the extract on the bacterial test organisms: The MIC of the extract was determined by broth dilution method. Test tubes were labeled and 5ml of nutrient broth was introduced into each test tube, 0.5ml of bacterial suspension was inoculated. This was followed by the addition of different concentrations (25mg/ml, 50mg/ml, 100mg/ml, 150mg/ml, and 200 mg/ml). The mixtures in all test tubes were mixed properly before incubation at 37oC for 24hrs. Observation for turbidity was carried out.

The turbidity shows bacterial growth. The MIC was determined by the lowest concentration of the extract that prevented visible growth (Andrews, 2001).

Minimum Bactericidal Concentration (MBC) of the Extracts on the Test Organisms:

The MBC was defined as the lowest concentration at which 99.9% of the bacterial inoculums were killed after 18 or 24 hrs of incubation at 370_C. The MBC of the extracts were determined by sub culturing the contents of the tubes that showed inhibition of growth onto extract Free- medium. The tubes that showed no turbidity were plated out on Mueller hint on agar plates which had neither antibiotics nor extract extracted for 24hrs (French, 2006).

RESULTS AND DISCUSSION

Results

Table 1: Revealed the Qualitative and Quantitative Analysis of Phytochemical Screening of *Khaya Senegalensis* Leaf Extract

Phytoconstituents	Status	Quantity (g)
1. Tannin	+	0.044
2. Anthraquinone	-	ND
3. Steroids	-	ND
4. Terpenoids	-	ND
5. Saponins	+	0.22
6. Flavonoids	+	15.49
7. Alkaloids	+	1.5
8. Cardiac Glycosides	-	ND

Key: ND= not detected,
+ = present and
- = absent

The phytoconstituents found in the leaf extract of *K. senegalensis* include; tannin with a 0.044g, saponin having 0.22g, flavonoids (15.49g) and alkaloid with a gram of 1.5g. While Anthraquinone, steroid, Terpenoids and cardiac glycoside were not detected.

Table 2: Shows the Qualitative and Quantitative Analysis of Phytochemical Screening of *Khaya Senegalensis* Stem (Bark) Extract

Phytoconstituents	Status	Quantity (g)
1. Tannin	+	0.166
2. Anthraquinone	-	ND
3. Steroids	-	ND
4. Terpenoids	-	ND
5. Saponins	+	1.27
6. Flavonoids	+	2.3
7. Alkaloids	+	1.16
8. Cardiac glycosides	-	ND

Key: ND= not detected,
+ = present and
- = absent

The phytoconstituents found in the stem (bark) extract of *K. senegalensis* include; tannin with a 0.166g, saponin having 1.27g, flavonoids (2.3g) and alkaloid with a gram of 1.16g. While Anthraquinone, steroid, Terpenoids and cardiac glycoside were not detected.

Table 3: Zone of Inhibition of Aqueous Stem (Bark) Extract *(SBAQ)* Against the Isolates

Exract Concentration (mg/ml)	Organisms/ Zone of Inhibition (mm)		
	E. Coli	***Shigella***	***Salmonella***
25	6.0	6.0	6.0
50	7.0	7.0	7.0
100	8.0	8.0	8.5
150	10.0	9.5	11.0
200	12.0	11.0	14.0
Control	18.0	14.0	16.0

Key: SBAQ = Stem (bark) aqueous extract

It shows that the mean zone of inhibition of aqueous stem (bark) extract (SBAQ) at concentrations; 25mg/ml - 200mg/ml. The effect of the extracts is more pronounced in *Salmonella* with zone of inhibition of 14 mm at 200 mg/ml. The lowest zone of inhibition is recorded in *Shigella* (6.0mm).

The P- value of Pearson chi – square, shows a P- value of 1.00 which signifies, that there is no significant difference in stem (bark) aqueous extract against the isolates.

Table 4: Zone of Inhibition of Ethanolic Stem (Bark) Extract *(SBETH)* Against the Isolates

Extract Concentration (mg/ml)	Organisms/Zone of Inhibition (mm)		
	E .coli	***Shigella***	***Salmonella***
25	8.5	7.0	6.5
50	11.5	10.0	9.5
100	14.0	12.5	13.0
150	16.5	16.0	16.0
200	20.0	21.0	20.5
Control	19.0	16.0	13.0

Key: SBETH= Stem (bark) ethanolic extract

Revealed the mean zone of inhibition of ethanolic stem (bark) extract (SBETH) at concentrations; 25-200mg/ml. The effect of the extracts is more pronounced in *Shigella* with zone of inhibition of 21mm at 200mg/ml. The lowest zone of inhibition is recorded in *Salmonella* (6.5mm).

The P- value of Pearson chi – square, shows a value of 1.00 which signifies, that there is no significant difference in stem (bark) ethanolic extract against the isolates

Tables 5: Zone of Inhibition of Aqueous Leaf Extract (LAQ) Against the Isolates

Extract Concentration (mg/ml)	Organisms/Zone of Inhibition (mm)		
	E .Coli	***Salmonella***	***Shigella***
25	6.0	6.0	6.0
50	8.0	7.0	8.0
100	10.0	8.0	9.5
150	12.5	10.0	13.0
200	15.0	12.5	20.5
Control	18.0	15.0	14.0

Key: LAQ= Leaf aqueous extract

It shows that the mean zone of inhibition of aqueous leaf extract (LAQ) at concentrations; 25 - 200mg/ml with *Shigella* having the highest zone of inhibition 20.5mm at 200mg/ml. The lowest zone of inhibition is recorded in *Salmonella* (6.0mm).
The P- value of Pearson chi – square, shows a P- value of 0.996 which signifies, that there is no significant difference in leaf aqueous extract against the isolates.

Table 6: Zone of Inhibition of Ethanolic Leaf Extract *(LETH)* Against the Isolates

Extract Concentration (mg/ml)	Organisms/Zone of Inhibition (mm)		
	E .coli	***Shigella***	***Salmonella***
25	7.5	6.5	6.0
50	9.5	9.0	7.5
100	13.0	12.0	9.0
150	15.0	16.0	14.0
200	20.0	17.0	17.0
Control	18.0	16.0	14.0

Key: LETH= leaf ethanolic extract

The mean zone of inhibition of ethanolic leaf extract (LETH) at concentrations; 25- 200mg/ml. Shows that the effect of the extract is more pronounced in *E. coli* with zone of inhibition of 20.0 mm at 200mg/ml. The lowest zone of inhibition is recorded in *Salmonella* (6.0).
The P- value of Pearson chi – square, shows a P- value of 1.00 which signifies, that there is no significant difference in leaf ethanolic extract against the isolates.

Table 7: Minimum Inhibitory Concentration (MIC) of the extract of *Khaya Senegalensis* against the Isolates

Organism	SBAQ (mg/ml)	SBETH (mg/ml)	LAQ (mg/ml)	LETH (mg/ml)
E. coli	50.0	12.5	50.0	25.0
Salmonella	50.0	25.0	50.0	50.0
Shigella	50 .0	25.0	50.0	25.0

Key: SBAQ = Stem (bark) aqueous extract, SBETH= Stem (bark) ethanolic extract, LAQ =leaf aqueous extract and LETH = Leaf ethanolic extract *Khaya senegalensis*

Shows the MIC of the isolates in which the ethanolic stem (bark) extract ranged from 12.5 to 25.0 mg/ml, the *E. coli* is more sensitive isolate at (12.5mg/ml) than *Salmonella* and *Shigella* (25mg/ml) each. Whereas, ethanolic leaf extract ranged from (25.0 to 50mg/ml). Whereby, *E. coli* and *Shigella* are more sensitive at (25mg/ml) than *Salmonella* which is resistance at 50mg/ml. Moreover, stem (bark) aqueous extract and leaf aqueous extract are less effective with MIC of 50mg/ml.

Table 8: Minimum Bactericidal Concentration (MBC) of the Extract of *Khaya Senegalensis*

Organism	SBAQ (mg/ml)	SBETH (mg/ml)	LAQ (mg/ml)	LETH (mg/ml)
E. coli	ND	12.5	ND	25.0
Salmonella	ND	25.0	ND	12.5
Shigella	ND	12.5	ND	25.0

Key: ND= not detected, SBAQ = Stem (bark) aqueous extract, SBETH= Stem (bark) ethanolic extract, LAQ =leaf aqueous extract and LETH = Leaf ethanolic extract *Khaya senegalensis*

Shows the Minimum Bactericidal Concentration (MBC) of the extract of *Khaya senegalensis* where the *E. coli* and *Shigella* has the lowest concentration of (12.5mg/ml) in stem (bark) ethanolic extract and *Salmonella* has the highest concentration of (25mg/ml) in stem (bark) ethanolic extract. While in leaf ethanolic extract, *E. coli* and *Shigella* has the highest concentration of (25.0mg/ml), but in aqueous stem (bark) and leaf extracts are not detected.

Discussion

The study, established antibacterial activity of the aqueous and ethanolic extracts of *K. senegalensis*. The results of phytochemical analysis revealed the presence of Saponins, Flavonoids, Alkaloids and Tannins. Similar studies of Makut *et al.,* (2007), consistently reported phytochemical bioactive ingredients of *K. senegalensi*s to be alkaloids, tannins, saponins and Flavonoids. Therefore, the results of the phytochemical analysis of the extracts of *K. senegalensis* obtained in this study conform to the previous reports, with a difference in both qualitative and quantitative analysis of the confirmed constituents which is different from the previous results. The quantities of the constituents are of different values too. The antibacterial effects of the extracts of *K. senegalensis* were determined in comparison with the standard antibiotic (Tetracycline) against the test organisms. There was a significant difference between the zone of inhibitions by the extract and the antibiotic (control). The inhibitory effects of the extracts could be attributed to the phytochemical components of the extracts as reported in previous study by Kubmarawa *et al.,* (2008).

The aqueous stem (bark) extract had a higher activity in *E. coli*, *Salmonella* and also shows a lesser activity in *Shigella*. However, the aqueous leaf extracts in contrast, had a higher activity in *Shigella,* followed by *E. coli* and shows a lesser activity in *Salmonella.* Moreover, this finding is in conformity with the work of Kubmarawa *et al.,* (2008), that reported inhibitory effect of the aqueous stem and leaf extract of *K. senegalensis* on the isolates. The ethanolic stem (bark) extract shows more activity in *E. coli, Shigella* and *Salmonella*. While, the ethanolic leaf extract also shows more activity in all the isolates as in the ethanolic stem (bark) extract. Meanwhile, the ethanolic extract had better activity than the aqueous extracts. This shows that ethanol is a better extacting solvent than water in this study. The result of the present study agrees with that of Ahmed *et al.,* (1998), Parekh *et al.,* (2005) and Abalaka *et al.,* (2011). Generally, the antibacterial activity of the extracts of *K. senegalensis* was found to be more as the concentration of the extracts increases, which implies that the higher the concentration, the more the activity by the extracts on the organisms. This is also in line with the observations of Idu and Igeleke (2012).

But in contrast, no significant differences among the tested organisms, with the P- values test of independence; table 1: was (1.00 > 0.05), table 2: was (1.00 > 0.05), table 3: was (0.996 > 0.05) and lastly table 4: was (1.00 > 0.05). The minimum inhibitory concentration (MIC) is the smallest concentration that visibly inhibits the growth. The MIC is useful in determining the smallest effective dosage of a drug against bacteria (Prescott *et al.,* 2002). The MIC result obtained from this study revealed that different concentrations of the extract served as the MIC values against the organisms. Some of the organisms (*E. coli,* and *Shigella*) were more sensitive to the extracts even at a low MIC value compared to *Salmonella* with a high MIC value in most cases. The isolates (*E. coli* and *Shigella)* were more sensitive to the ethanolic extracts with an MIC value of 12.5 and 25 mg/ml with the exception of *Salmonella* which had the MIC value of 50mg/ml. The MIC value of the aqueous extracts of all the isolates was 50mg/ml, this is an indication of resistance but susceptible in the ethanolic extracts. This is also in line with the findings of

Ahmed *et al.*, (1998), Parekh *et al.*, (2005) and Abalaka *et al.*, (2011). The results of the minimum bacterial concentration (MBC) of the extracts yielded a lower MBC values ranging from 6.25 to 25 mg/ml in the ethanolic extracts while in the aqueous extracts no any results shown, which implies that a low concentration of the extracts is required to exert a bactericidal effect on the organisms. This is in contrary to the work of Abalaka *et al.*, (2011) which revealed a higher concentration, which also needed to exert a bactericidal effect on the organisms.

Conclusion

The ethno medicinal uses of the plant in Kano, was made from the information about the name from elders, that its parts such as; leaves and stem (bark) are used as medicines. Indeed, both the leaves and stem (bark) used to treat diarrhea. The results showed that the extracts were more effective against all the tests organisms with the least minimum inhibitory concentration (12.5mg/ml) and minimum bactericidal concentration of (25mg/ml). Moreover, the test organisms in both extracts were susceptible, especially at higher concentrations of 150- 200mg/ml of the extracts. Lastly, the phytochemical analysis revealed the presence of some bioactive ingredients such as; Alkaloids, Flavonoids, Tannins, and Saponins.

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