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Research Article

Antibacterial Activities of *Cassia Fistula* Leaf Bark and Root Extracts

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Abstract: The medicinal value of Cassia fistula which is commonly referred to as the golden shower is captured in traditional medicine as the "disease killer". The aim of this study is to determine the antibacterial activities of leaf, bark and root of extracts *C. fistula*. Aqueous extraction of leaf, bark and roots of the plant was carried out and the extracts were diluted to 500mg/ml, then doubling dilution was carried out for 250mg/ml, 125mg/ml, 62.5mg/ml and 31.25mg/ml. Four test Bacteria used in this work were *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtillis*, *Klebsiella pneumoniae*. The antibacterial activities of the aqueous extracts of the leaf, bark and root *C. fistula* was determined using modified Kirby Bauer well-diffusion sensitivity testing method. The three extracts had bactericidal effect on the Gram Positive test bacteria than the Gram Negative test bacteria. The boost in medicinal herb development and sustenance should be encouraged by policy makers and philanthropists.
Keywords: Traditional Medicine, *C. fistula*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtillis*, *Klebsiella pneumoniae*.

INTRODUCTION

Medicinal plants are the main resource for trado-medicine; from these plants, numerous modern medicines were produced (Mahomoodally, 2013). Medicinal plants have been used for a wide variety of different purposes for thousands of years all over the world. A good number of plants including trees and shrubs have been reported by ethnoveterinary practitioners, ethnomedical practitioners and other rural dwellers to have medicinal advantages against communicable and non-communicable diseases of man and his animal. Examples of these diseases in man include malaria, blood pressure, wounds, menstrual issues (Mahomoodally and Chintamunnee, 2012).

Medicinal plants have numerous advantages as antibacterial agents when processed to treat some bacterial infection or diseases. Among many medicinal plants are Papaya, Chili, bitter leaf, *Acacia albida*, *Adansonia digitata* (Baobab), *Vangueria madascariensis (V. acutiloba)*, *Warburgia ugandensis (W. salutaris)*,and *Cassia fistula* (Mbuya *et al.*, 1994). Research articles on phytochemical analyses have reported that plants with antibacterial properties contain tannins, flavonoids, alkaloids and saponins as bioactive constituents. (Dall_Agnol *et al.*, 2003; Ogunleye and Ibitoye, 2003; Edeoga *et al.*, 2005; Latha and Kannabiran, 2006; Awoyinka *et al.*, 2007).

C. fistula is one of the plants reported to have antibacterial characteristics (Seyyednejad et.al, 2014). *C. fistula* is an herbaceous plants belonging to Cassia PiniaCea family which are largely used in traditional medicine in so many countries. Aside having antibacterial properties, *C. fistula* root, bark, leaves and flowers have laxative properties but at a lesser extent. Bacterial infectious diseases are endemic in various regions of Nigeria in both human and veterinary medicine. Several studies world over have reported the emergence and re-emergence of antibiotic resistant pathogens and World health Organization has declared it a public health problem. The aim of this study is to determine the antibacterial activities of leaf, bark and root of extracts *C. fistula*.

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MATERIALS AND METHODS

Plant Collection

Samples were collected from randomly selected species of *C. fistula* (leaf, bark and root), thoroughly washed and then cleaned with 45% ethanol to reduce the microbial load on the back of the fruits. Thereafter they were sliced separately and dried at 40^{o}C in a hot air oven.

Extraction Process

Aqueous extraction (Hot water) was carried out for all the extracts. Dried *C. fistula* parts were finely pounded and homogenized in hot water for one hour, filtered using What Man No. 1 filter paper and steam dried. Extracts were weighed and labelled. The extracts were diluted to 500mg/ml then doubling dilution was carried out for 250mg/ml, 125mg/ml, 62.5mg/ml and 31.25mg/ml.

Test Bacteria

Four test Bacteria used in this work were *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtillis*, *Klebsiella pneumoniae*. They were bacterial isolates of livestock preserved in agar slants gotten from the Microbiology Laboratory of Federal College of Animal Health and Production Technology Vom.

Antibacterial Screening by Zone of Inhibition

The antibacterial activities of the aqueous extracts of the leaf, bark and root *C. fistula* was determined using modified Kirby Bauer well-diffusion sensitivity testing method. Overnight broth culture of each of the test bacteria was adjusted to 0.5 McFarland's turbidity standard using sterile normal saline. Thereafter, 0.2ml of the adjusted broth culture were added to 20mls of molten Nutrient Agar before aseptically pouring into a petri dish. After solidifying, seven holes with diameters of 6mm were made on the media using a sterile cork-borer and labeled appropriately. Five holes had the concentrations of the respective extracts, while the other two had the positive and negative controls respectively. Ampiclox of 50mg/ml concentration was used as the positive control while sterile distilled water was used as the negative control. Incubation was done at 37°C for 24 hours. The diameter zone of inhibition was measured using a transparent plastic ruler in millimetre.

Minimum Inhibitory Concentration (MIC)

The MIC of each of the extracts was determined using the tube serial dilution method. Extracts concentrations of 500, 250, 125, 62.5, 31.25, mg/ml were used. An overnight broth culture of the test bacteria was adjusted to 0.5 McFarland turbidity standards (10^{6} CFUs/ml) and 0.2 ml of the cell suspension were added to each of the tubes containing the extracts. The tubes were incubated aerobically at 37°C for 24 hours. The MIC was defined as the lowest extract concentration that inhibited the growth of the test organism as indicated by absence of visible turbidity in the tube compared with the control tubes.

RESULT

The aqueous Extracts of *C. fistula* leaf extract exhibited antibacterial activity against Gram Positive and Gram Negative bacteria species. The aqueous Extracts of Cassia Fistula leaf extract efficiently inhibited the two gram positive test bacteria viz., *S. aureus* and *B. subtilis*, and the two gram negative bacteria viz., *E. coli* and *K. pneumoniae* as shown in Table 1.

Aqueous extracts of *C. fistula* roots exhibited antibacterial activity against only one gram positive bacteria which was *S. aureus* as in Table 2. While aqueous extracts of *Cassia Fistula* bark exhibited antibacterial activity against the four test bacteria used as shown in Table 3.

Table 1. Antibacterial activity of *C. fistula* leaf extract

Bacteria	Zone of inhibition (mm)						
	500 mg/ml	250 mg/ml	125 mg/ml	62.5 mg/ml	31.25 mg/ml	Positive control	Negative control
E.coli	15	12	10	6	6	40	0
K. pneumoniae	12	8	7	0	6	14	0
S. aureus	20	18	14	12	9	54	0
B. subtilis	25	15	10	6	0	42	0

Table 2. Antibacterial activity of *C. fistula* root extract

Bacteria	Zone of inhibition (mm)						
	500 mg/ml	250 mg/ml	125 mg/ml	62.5 mg/ml	31.25 mg/ml	Positive control	Negative control
E.coli	7	6	6	0	0	55	0
K. pneumoniae	8	6	0	0	0	10	0
S. aureus	12	10	10	7	7	44	0
B. subtilis	7	6	6	6	0	25	0

Table 3. Antibacterial activity of *C. fistula* bark extract

Bacteria	Zone of inhibition (mm)						
	500 mg/ml	250 mg/ml	125 mg/ml	62.5 mg/ml	31.25 mg/ml	Positive control	Negative control
E. coli	21	7	6	0	0	52	0
K. pneumoniae	15	9	7	6	0	21	0
S. aureus	23	17	8	7	6	52	0
B. subtilis	12	10	10	6	6	29	0

The mean result of MIC for all the extracts was 250 mg/ml.

DISCUSSION

In this study, *C. fistula* leaf extract exhibited high antibacterial activity against *B. subtilis* with the zone of inhibition of 25mm, and less against *K. pneumoniae* with the zone of inhibition of 12mm. The bark of *C. fistula* is more susceptible against *S. aureus* with the zone of inhibition of 23mm and less against *B. subtilis* with the zone of inhibition of 12mm.The root shows a low susceptible only in *S. aureus* with the zone of inhibition of 12mm and the remaining organisms viz., *E. coli*, *B. subtilis* and *K. pneumonia* showed resistance against the root of *C. fistula*. As was found in this study that the leaf extract had more activity against test bacteria, was discovered by Abo *et al*, 1999.

The three extracts had bactericidal effect on the Gram Positive test bacteria than the Gram Negative test bacteria. This is in direct contradiction with Seyyednejad *et al*, 2014 where the Gram Negative test bacteria were more sensitive than the Gram Positive bacteria to extracts of *C. fistula*. The contradiction of Seyyednejad *et al* could be as a result the ethanolic and methanolic extracts used, which may have had a stronger effect on the thing cell wall of the Gram Negative bacteria. The outer lipid membrane of the Gram Negative bacteria may have been affected by the alcoholic solvents that were used. The use of only aqueous method of extraction may have reduced the potency of these extracts. However, Rizvi *et al* 2009 and Vasudevan *et al* 2009 reported more activity of *C. fistula* against Gram positive microorganisms. This agrees with the result of this study. Rizvi *et* al 2009 claimed it was as a result of constituents like flavonoid and polysaccharides.

Phytochemical studies showed that this plant containing components like saponin, triterpnoids, glycosides, anthraquinore, steroids and flavonoids that inhibit the growth of the tested bacterial strains.

CONCLUSION

Due to indiscriminate use and misuse of antibiotics in Nigeria, antimicrobial resistance (AMR) is growing rapidly. AMR is becoming a global health threat which needs to be tackled from all fronts. One of the ways is the use of medicinal plants like *C. fistula*; scientific studies have proven that these herbs are good and affordable alternative to orthodox antibiotics.

Therefore, it is recommended that a boost in medicinal herb development and sustenance should be encouraged by the governments, pharmaceutical organizations, research-funding organizations, and Non-governmental organizations (NGOs).

Conflicts of Interest

The authors declare that they have no conflicts of interest in publishing this manuscript.

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