Phytochemical Screening and Antimicrobial Property of *Cassia arereh*

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Abstract: The phytochemical screening of the root and stem bark of *Cassia arereh* revealed that the root possesses alkaloids, flavonoids, phlobatanins, saponins, steroids and while the stem back possesses alkaloids, anthraquinones, saponins, steroids and tannins. The antimicrobial activity on *Shigella* spp, *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Salmonella typhi* were determined at the concentration of 200mg/ml using the disc diffusion technique. The ethanolic extract of the stem back produced the highest antimicrobial effect against *E. coli, S. aureus* and *Pseudomonas aeruginosa* with the zones of inhibition of 13mm respectively and 12mm against *S. typhi* compared with the other extracts. Equally, the stem back extracts were observed to be more potent against the microbial isolates than the root extracts as *E. coli, S. aureus* and *Pseudomonas aeruginosa* were observed to be resistant to stem back extracts. Minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) values were in the range of 12.5mg/ml-200mg/ml concentrations. Analysis of active extracts against the test organisms showed that the activity of the extract was dependent on the solvent used and the type of target bacteria and the difference observed were significant ($P<0.05$).
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1. Introduction

Traditional medicine is undoubtedly the oldest form of medicine and probably evolved simultaneously with the evolution of human beings or even much earlier (Wynn, 2001; Wanzala *et al.,* 2005). The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. In plants, these components are mostly secondary metabolites such as alkaloids, steroids, resins, fatty acids, gums flavonoids, tannins and phenol compounds which are capable of producing definite physiological action on the human body (Erdogrul, 2002). The development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substance from other sources including plants (Erdogrul, 2002). Microbial diseases that remain most challenging in today's health care system tend to be complex involving multiple mechanisms, targets and drugs of effective disease management. In contrast to current combination therapies, however, plant based drugs contain a mixture of components thereby saving considerable time and expense (Karnath, 2002).

Cassia arereh is used for the treatment of diarrhea, dysentery, dermatitis, malaria and skin infections (De *et al.,* 2009). Studies by De *et al.* (2009) and investigations Ngulde *et al.* (2010) have shown antimicrobial effects of the extracts although with somewhat conflicting results with regards to some isolates. In this locality, the plant (north central Nigeria), the plant is also used by the indigenous people for essentially the same ailments. This research was warranted by the need to investigate further the antimicrobial effect of the plant with the view of resolving some contradictions and possibly taking it to the next step which is isolation of active components.

Materials and Methods

The root and stem bark of *C. arereh* plant were collected from Gwarinpa in Abuja Municipal Area Council of the Federal Capital Territory Abuja – Nigeria. After which it was identified Prof. O. O. Olorode of the Department of Biological Sciences, University of Abuja for identification. After which the voucher specimens were preserved in the Herbarium.

Preparation of The Plant Extracts

The root and stem bark of *C. arereh* were cut into small pieces using a clean sharp knife and then air direct in the herbarium under room temperature for 7 days. The dried samples were then crushed using mortar and pestle; later the crushed materials were further grinded to powder form with an electric blender. This was done to enhance the penetration of the extracting solvents into the cells, thus facilitating the release of active principles (Jigna and Charida, 2006). The ground samples were then used for extraction purposes.

Ethanol, Methanol and Water were employed as solvents for the extraction of both samples (root and stem bark) of *C. arereh.*

Aqueous Extracts

About 200ml of distilled water was added to 50g each of the two powdered samples (root and stem bark) of *C. arereh* in a 250ml conical flask and was then soaked for 2 hours. After that period, the flask was agitated manually, after every 30 minutes for 3 hours. The mixtures was then allowed to settle down and left overnight in the refrigerator. In each case, the extract was filtered using a clean Whatman's No. 1 filter paper. The filtrates were concentrated using an air – circulating oven at 42^0C until total dryness. The dried extracts obtained were stored in the refrigerator at 4^0C for further use.

Ethanol Extracts

About 50g each of the powdered samples was dispensed into a 250ml conical flask and 200ml of 97% ethanol was added to the sample. The procedure was similar to that of aqueous extract except that ethanol was used instead of water. The recovered extracts were kept in the refrigerator for further use.

Methanol Extracts

The procedure was similar to ethanol except that methanol was used instead. The recovered extracts obtained from both the root and stem bark of *C. arereh* were stored in the refrigerator for further use.

Test Microorganisms

The test microorganisms used for the antibacterial activity were *Escherichia coli, Shigella* sp*, Salmonella typhi, Staphylococcus aureus and Pseudomonas aeruginosa.* The stock cultures used were obtained from University of Abuja Teaching Hospital. The isolates were cultured separately on nutrient agar plate and incubated for 24 hours.

Disc Preparation

Using a cork borer and a surgical blade 6mm diameter disc were cut out of clean sterile Whatman's No 1 absorbent filter paper. The discs were sterilized at 80^0C for 2 hours in the hot air oven. The 6mm diameter sterile discs of absorbent paper were placed into sterile Petri dishes – 12 in each and 0.02ml (a drop from a standard dropper) of an appropriate concentration of extracts and standard antibiotic drug (as control) was used to impregnate the discs.

The impregnated discs were put into the oven at 60^0C for about 15 minutes taking note of the concentration and antibiotic concerned after which the disc were ready for the susceptibility test (Isu and Onyeagba, 1998).

Phytochemical Screening

This was done on the extracts to ascertain the presence of bioactive components in *Cassia arereh* root and stem bark. The phytochemical analyses were carried out according to standard methods described by Trease and Evans (1997) and Sofowora (2006).

Antibacterial Assay

Each dried methanol, ethanol and aqueous extracts of the root and stem bark of *Cassia arereh* was reconstituted with sterile distilled water to get a final concentration of 200mg/ml.

The disc diffusion method as described by National Committee for Clinical Laboratory Standard (NCCLS, 1993) was used to determine the growth inhibition of bacteria by the plant extracts. Discs containing 200mg/ml concentration of dissolved extracts were prepared using sterile whatman's No. 1 filter paper (6mm in diameter). The discs were dried at 60^0C. Overnight cultures of each bacterial isolates was diluted with sterile distilled water to give inoculum size matched with 0.5 Mac Farland standard and sterile swab stick was used to uniformly seed sterile Mueller Hinton agar plates.

The discs were then aseptically placed evenly on the surface of the inoculation and gently pressed down to ensure contact using a pair of forceps. The plates were finally incubated at 37^0C for 24 hours. Chloramphenicol (200mg/ml) was used as positive control in each plate. Discs prepared using the same procedures without extracts or antibiotic were equally set as negative control. The plates were examined after 24 hours for clear zone of inhibition. The antibacterial activity by the extracts was measured using a transparent ruler.

Determination of Minimum Inhibitory Concentration

The method of Andrews (2001) was used to determine the MIC of the potent extract. Five sterile test tubes were arranged in a test tube rack and 0.5ml of sterile nutrient broth was pipette into each test tube. Half ml of the crude extracts containing 200mg/ml was pipette into each of the five tubes containing the broth. Thereafter, there was a serial dilution of the extract to obtain concentrations of 100, 50, 25 and 12.5 mg/ml respectively. The test tube organism (0.5ml) was pipetted into each of the test tube containing the mixture of the broth and extract and then finally incubated at 37^0C for 24 hours. The MIC was recorded as the least concentration of plants extract that completely inhibited the growth of the organism.

Determination Minimum Bactericidal Concentration

MBC were determined by first selecting the tubes that showed no growth during the MIC determination. A loopful from each of tube was sub cultured on the sterile nutrient agar and incubated for 24 hours at 37^0C. The bactericidal effect was demonstrated when no growth occurred on the sub cultured medium.

Statistical Analysis

The mean of the two reading measured for each zone in the sensitivity test was taken to be the zone of inhibition of the bacterial isolates. Two way ANOVA was used to compare means and differences were considered significant if $P < 0.05$.

Results

Table 1: Phytochemical Screening of the root and stem bark extracts of *Cassia arereh* plant.

Bioactive Constituents	Root	Stem Bark
Alkaloids	Present	Present
Anthraquinones	Absent	Present
Flavonoids	Present	Absent
Glycosides	Absent	Absent
Phlobatanins	Present	Absent
Sapronins	Present	Present
Steroids	Present	Present
Reducing Sugar	Absent	Absent
Tannins	Present	Present

Table 2 shows that the antimicrobial activity of 200mg/ml concentration of the aqueous, ethanolic and methanolic root extract of *Cassia arereh* against five Microbial isolates, which was carried out using the disc diffusion technique.

It was observed that *Shigella spp* and *Salmonella typhi* were susceptible to the aqueous , ethanolic, methanolic extracts and the chloramphenicol used as a standard antibiotic positive control while, *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa* were observed to be resistant extracts, while being susceptible to the chloramphenicol used as a standard antibiotic positive control.

However, it was observed that between the two organisms that were susceptible to the three root extracts *Shigella* spp has the highest zone of inhibition of 11mm while *Salmonella typhi* has the lowest of 10mm in methanolic root extracts and in ethanolic root extract *Salmonella typhi* has the highest zone of inhibition of 9mm while Shigella has the lowest with 7mm and in the aqueous root extract. *Shigella* has the highest zone of inhibition of 9mm while *Salmonella typhi* has the lowest with 8mm.

Table 2: Mean Zones of inhibition in (mm) of Aqueous, Ethanolic and Methanolic Extracts of *Cassia arereh* plant root.

Test Microorganisms	Aqueous Extract at 200mg/ml	Positive control at 200mg/ml	Ethanolic Extract at 200mg/ml	Positive control at 200mg/ml	Methanolic Extract at 200mg/ml	Positive control at 200mg/ml
Shigella spp	9	28	7	28	11	31
Escherichia coli	R	30	R	33	R	46
Staphylococcus aureus	R	28	R	40	R	35
Pseudomonas aeruginosa	R	39	R	40	R	40
Salmonella typhi	8	18	9	20	10	15

Key: R = Resistant, Positive Control = Chloramphenicol

Table 3 shows that antimicrobial activity of 200mg/ml concentration of the aqueous,ethanolic and methanolic stem bark extracts of *C. arereh* and a standard antibiotic control (Chloramphenicol) against five microbial isolates which was also carried out using the disc diffusion techniques.

It was observed that the entire microorganisms were susceptive to the aqueous, ethanolic and methanolic stem bark extractsand the chloramphenicol used as the standard positive antibiotic control. With *E. coli* and *Pseudomonas* having the highest zone of inhibition of 13mm, followed by *Shigella spp* which has 11mm and both *S. aureus* and *S. typhi* having the lowest zone of inhibition of 8mm in the Aqueous extract. In the ethanolic extract *E. coli, S. aureus* and *Pseudomonas* having the highest 13mm, followed by *S. typhi* which has 12mm and *Shigella* having the lowest of 8mm. further in the methanolic extract *shigella* and *E. coli* has the highest zone of inhibition of 12mm, followed by *Staphylococcus aureus and Pseudomonas aeruginosa* which has 10mm both and *S. typhi* having the lowest zone of inhibition of 8mm.

However, the stem bark were observed to be more active against the microorganisms than the root of *Cassia arereh* plant as all the microbial isolates showed susceptibility high zones of inhibitions measured in (mm) for the stem bark while only two organisms showed susceptibility for the root with lower zone of inhibition when compared with the stem bark while other three organisms showed resistant for the root extracts.

Table 3: Mean Zones of inhibition in (mm) of Aqueous Ethanolic and Methanolic extract of C. arereh stem bark.

Test Microorganisms	Aqueous Extract at 200mg/ml (mm)	Positive control at 200mg/ml (mm)	Ethanolic Extract at 200mg/ml (mm)	Positive control at 200mg/ml (mm)	Methanolic Extract at 200mg/ml (mm)	Positive control at 200mg/ml (mm)
Shigella spp	11	28	8	28	12	31
Escherichia coli	13	30	13	33	12	46
Staphylococcus aureus	8	28	13	40	10	35
Pseudomonas aeruginosa	13	39	13	40	10	40
Salmonella typhi	8.5	18	12	20	8	15

Key:R = Resistant, Chloramphenicol was used as positive control.

MIC and MBC values were obtained as described above for all isolates that were sensitive to a particular extract but for brevity we are reporting only values for *Shigella* sp which was sensitive to all extracts.

Table 4 shows minimum inhibitory concentration and minimum bactericidal concentration of different extracts of *cassia arereh* on *shigella* sp. The results showed that the MIC of aqueous root extract of *C. arereh* for *Shigella* is 100mg/ml, for ethanolic root extract, 12.5mg/ml while the value for methanolic root extract was 50mg/ml. The Minimum Inhibitory Concentration of aqueous stem bark extract of *Cassia arereh* against *Shigella* sp was 100mg/ml. However *S. aureus* and *Pseudomonas aeruginosa* had lower values of 50mg/ml (data not shown). For ethanolic stem bark extracts, The result showed that *Shigella* spp and *S. aureus* has MIC of 25mg/ml respectively while *Pseudomonas aeruginosa* and *Salmonella typhi* equally has MIC of 200mg/ml respectively and *E. coli* having the MIC 50mg/ml (data not shown). The MIC of the methanolic stem bark of *Cassia arereh* plant against were determined to be 50mg/ml for *Shigella* sp and *E. coli* and 100mg/ml for *S. aureus* and *Pseudomonas aeruginosa* while it was 200mg/ml for *S. typhi* (data not shown).

The MBC of the aqueous root extract against *Shigella* sp was 200mg/ml while for Salmonella typhi was 100mg/ml which was similar to ethanolic root extract mbc value for *Shigella* spp at 200mg/ml but different for *Salmonella typhi* at 50mg/ml (data not shown).

For ethanolic root extract of the MBC for *Shigella* sp was the 100mg/ml while while that of *Salmonella typhi* was 50mg/ml (data not shown)..

The MBC for aqueous stem bark extract was 100mg/ml for *Shigella* sp, *S. aureus, E. coli* and *Pseudomonas aeruginosa* respectively, while *Salmonella typhi* had a value of 50mg/ml (data not shown). For ethanolic stem bark extract of *Cassia arereh* , the MBC of *Shigella* sp*, S. aureus, E. coli* and *Pseudomonas aeruginosa* and *Salmonella typhi* was 200mg/ml respectively.

The MBC of Methanolic stem bark extract of *Cassia arereh against Shigella* spp and *Staphylococcus aureus* respectively was 100mg/ml while the MBC of *E. coli, Pseudomonas aeruginosa* and *Salmonella typhi* were 200mg/ml respectively.

Discussion

The preliminary study of the phytochemical compositions of the root extract of *Cassia arereh* plant showed that it composes of alkaloids, flavonoids, phlobatanins. Saponins, steroids and tannins which may accounts for its antimicrobial activity. However, the presence of a particular bioactive component like the anthraquinone in the stem bark extract together with alkaloids, saponins, steroids and tannins may account for the high antimicrobial activity of the stem bark extract against some selected microorganisms when compared with the root extract.

Table 4: Minimum Inhibition Concentration and Minimum Bactericidal Concentration of Different Extracts of *Cassia arereh* on *Shigella* sp

EXTRACT	MIC (**mg/ml**)	MBC (**mg/ml**)
ARE	100	200
ERE	12.5	200
MRE	50mg	100
ASE	100	100
ESE	25	100
MSE	50	100

Key: ARE= Aqueous root extract, ERE= Ethanolic root extract, MRE=Methanolic root extract, ASE= aqueous stem bark extract, ESE= Ethanolic stem extract, MSE= Methanolic stem extract

The aqueous, ethanolic and methanolic root extracts showed good antimicrobial activity against pathogenic bacterial strains like *Shigella* spp and *Salmonella typhi* with the methanolic extract having the highest zone of inhibition. However, they showed no antimicrobial activity against *E. coli, S. aureus* and *Pseudomonas aeruginosa*. The aqueous, ethanolic and methanolic stem bark extracts of *Cassia arereh* plant showed a very significant antimicrobial activity against all the pathogenic bacterial strains with the ethanolic extract having the highest zone of inhibition against *E. coli, S. aureus, Pseudomonas aeruginosa* and *S. typhi*.

When the antimicrobial activity of the root extracts was compared with the stem bark at the same concentration it was observed that the stem bark extracts were more potent than the root extracts against the first selected pathogenic bacterial strains used in the test study. And when both the root and stem bark extracts *C. arereh* was compared with the standard antibiotic (chloramphenicol) it was also observed that the standard antibiotic were more powerful than both plant extracts of *C. arereh.*

However, the very significant antimicrobial activity shown by the stem bark of *Cassia arereh* is beneficiary as it indicates probably the emergence of a new antibiotic with such a wide spectrum of activity.

The fact that treatment of infections caused by organisms such as *S. aureus, E.coli, S. typhi, Shigella* spp and *pseudomonas aeruginosa* are increasingly becoming difficult further strengthens the importance of these present findings and the need for a continuous search of chemotherapeutic agents.

The MIC of aqueous root extract of *C. arereh* indicates that the MIC of aqueous root extract of *C.*

arerah for both *Shigella* spp and *Salmonella typhi* is 100mg/ml respectively. While the MIC for ethanolic root extracts for *Shigella* spp is 12.5mg/ml and *S. typhi* are 50mg/ml. MIC of methanolic root extract of *C. arerah* for Shigella spp and *Salmonella typhi* is 50mg/ml respectively.

The MIC of aqueous stem bark extract of *C. arerah* indicated that *Shigella spp, E. coli* and *S. typhi* has individual MIC of 100 mg/ml respectively. While *S. aureus* and *Pseudomonas aeruginosa* also has MIC of 50mg/ml respectively. Also MIC of ethanolic stem bark shows that *Shigella* spp and *S. aureus* has the MIC of 25mg/ml respectively while *Pseudomonas aeruginosa* and *S. typhi* equally shared the same MIC of 200mg/ml and *E. coli* having the MIC of 50mg/ml. The MIC of methanolic stem bark indicates that *Shigella spp* and *E. coli* have the same MIC of 50mg/ml and *S. aureus* and *Pseudomonas aeruginosa* equally have the same MIC of 100mg/ml respectively while *S. typhi* has the MIC 200mg/ml.

The MBC of aqueous root extracts of *C. arereh* indicates that the MBC of *Shigella* is 200mg/ml while that of *S. typhi* is 100mg/ml. Also the MBC of ethanolic root extract shows that the MBC for *Shigella* spp is 200mg/ml while the MBC for *S. typhi* is 50mg/ml. While the MBC of methanolic root extract indicates that *Shigella* spp has the MBC of 100mg/ml while *S. typhi* has the MBC of 50mg/ml.

The MBC of aqueous stem bark of *C. arereh* plant showed that *Shigella* spp, *S. aureus*, *E. coli* and *Pseudomonas aerugenosa* had the MBC of 100mg/ml respectively while *S. typhi* has the MBC of 200mg/ml. Also the MBC of ethanolic stem bark extract shows that *Shigella* spp, *E. coli, S. aureus, Pseudomonas aureginosa* and *S. typhi* all has the MBC of 200mg/ml respectively. For *Shigella spp* and *S. aureus* is 100mg/ml respectively. The MBC of methanolic stem bark extract of *E. coli, Pseudomonas aeruginosa* and *S. typhi* are equally 200mg/ml respectively. This study demonstrated that the activity of the extracts depends on the solvent employed in the extraction from plant samples while the stem bark indicated strong antibacterial effect compared to the root which is in contrast to findings of of De *et al.,* (2009) which reported that the root extracts indicated more antibacterial effects compared to the stem bark. However, the variations could be due to geographical location of the plant and concentrations used.

In conclusion, this study has shown that extract from *C. arereh* possesses antimicrobial properties thus justifying its use in traditional medicine. From the experiment carried out, the stem bark of *C. arereh* was found to be highly active against *Shigella spp, E. coli, S. aureus, Pseudomonas aeruginosa and S. typhi* than the root extract. This supports the scientific basis for the use of the crude extract of *C. arereh* in the treatment boils, wound, scarlet fever, diarrhea and urinary tract infections.

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