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Research Article

Antibacterial Activity of Ethanolic Leaves Extracts of *Cassia Siamea* against Some Bacterial Isolates from Infantile Diarrhoeal Attending General Hospital Damaturu

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Abstract: The study was aimed to investigate the antibacterial activity of *Cassia siamea* leaves extracts against some bacterial isolates from infantile diarrhoeal samples. The ethanolic extracts of *Senna siamea* leaves were prepared, screened for phytochemical analysis and tested for its antibacterial activity against 3 pathogenic bacteria (*Shigella spp, Escherichia coli,* and *staphylococcus aurous*) recovered from infant diarrhoeal samples of patients attending General Sani Abacha Specialist Hospital Damaturu. Phytochemical constituents found in *Cassia siamea* leaves extract include; alkaloids, saponins, tannins, steroids and flavonoids as well as the Anthraquinones and phenols were absent. Based on the susceptibility of the organisms to the extracts, *staphylococcus aureous* was found to be the highest susceptible organisms with average zone of inhibition of 15. 00 (40gm/ml) followed *Escherichia coli 15.00* (50gm/ml), *and* Shigella14.00 (30gm/ml). The MIC of the extracts ranged from 4.125 to 50mg/ml. There was no significant difference on the susceptibility of the organisms against the extracts. The results of the present *study* have supported the therapeutic potential of *Senna siamea* and its use as medicinal plant.

Keywords: *Cassia siamea*, infantile diarrhoea, phytochemicals, *Staphylococcus aureus*, *Escherichia coli* and Minimum inhibitory concentrations.

INTRODUCTION

Plants have great potential uses, especially traditional medicine and pharmacopeia drugs. *Herbalism* is a folk medicine based on the use of their extract. Sometimes the scope of herbal medicine is extended to include fungi and bee al product as well as minerals, shell, and certain part of animal (Hudcub *et al*., 2008). However, the medicinal value of plants lies in some chemical substances present in them. The most important of these bioactive compounds of the plants are *alkaloids*, *tannins* and *phenolic* compounds of plants, the expanding bacterial resistance to antibiotics as become a growing concern worldwide. Increasing bacterial resistance is promoting resurgence in research of the antimicrobial role of herbs against resistance strains. A vast number of medicinal plants have been recognized as valuable resources of natural antimicrobial compound. Medicinal plants extracts offer considerable potential for the development of new agent effective against infection currently difficult to treat. A large proportion of the world population depend on traditional medicine because of the scarcity and high cost of orthodox medicines (El- mahmood and Doughari, 2008).

Infantile diarrhea is a leading cause of morbidity and mortality among children age more than five years in sub-Sahara Africa, in Nigeria, over 1500 death occurring yearly among children are attributed to diarrhea. Prevailing poor sanitary condition, scarce health services and limited access to these services in addition to immaturity of the immune system associated with this age group are contributory factors (Echemendia *etal*.,2004).Therefore Bacterial enteropathogen are important etiological agent acute diarrhea. Majority (64%) of the stool samples yielded bacterial isolates. The high prevalence of bacterial isolates in this study, when compared to studies of (Nweze, 2018).

Nevertheless, *Cassia Siamea* belongs to the family *leguminosae*: *caesalpinodeae*. The leaf, stem bark and root of the plant are used for medicinal purposes. A combination of the root, leaf and flower extract is taken for treating indigestion and as expectorant. The leaf root of the plant is used for treating conjunctivitis, the leaf is used for heart burn and as an antipyretic, leaf and flower extracts are combined to heal blotches on skin due to menstrual disorder. Micro-organisms such as *Escherichia coli*, staphylococcus species, *salmonella* species are reported to caused diseases like diarrhoea and typhoid etc. therefore, there is need to

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study plants which may have effect on these organisms and this will improve the effective use of the plant against diseases caused by pathogens. The acceptance of traditional medicine as an alternative form of health care and the development of microbial resistance to the available antibiotics have led researches to investigate the antimicrobial activity of herbal extracts (Hansel *et al*., 1972). The main aim of the study is to determine the activity of *Cassia siamea* leaves extract against the isolates causing infantile diarrhoea.

MATERIALS AND METHOD

Collection and Identification of Plant Materials

Cassia siamea (Kassod tree) was collected from the Botanical garden from the Department of Biological Sciences, Yobe state University , the leaves were identified and authenticated at the research laboratory Yobe state university, processing of plant materials The leaves of *Cassia siamea* were handpicked and washed thoroughly with distilled water. It was then air dried under shed at ambient room temperature. This was pounded into powder by using a clean mortar and pestle as described by (Abdallah *et al*., 2018). The powdered form was sieved to get a fine powder, and stored in a plastic container moreover 60grms of the powder leaves of *Cassia siamea* was weighed and it was suspended into 500ml of ethanolic and distilled water in separate between, however the suspended were kept in a room temperature for one day it was shake and filtered, the suspension is then filtered and the solvent was also removed (Abdallah *et al*., 2016).

Preliminary Phytochemical Screening

The phytochemical analysis has been divide into two: qualitative and quantitative method of plant extraction as highlighted by (Abdallah *et al.,* 2018*).* Tests for: tannin, steroids, phenols, antraqunones, flavonoids, alkaloids and saponins was carried out as described by (Abdallah *et al.,* 2018*).*

Culturing and Isolation of the Test Organisms

A sterile wire loop has been used to inoculate stool samples on eosin methylene blue that is differential media for *Escherichia coli*, Catalase test for *staphylococcus aureus* and Xylose lysine deoxy chocolate for *shigella*. The culture has been inoculated at 37°c for 24hours. *Escherichia coli* and *shigella* spp were both gram-negative bacteria and *Staphylococcus aureus* was gram-positive. However, during inoculation, the plates were dried because of easier growth and identification of the colonies. The wire loop was flamed and sterilized. The plates were placed invertedly overnight, to prevent falling of condensed water vapour on plate surface (Abdallah *et al*., 2016).

Gram Staining Technique

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 used to cover the smear for 60 second and after it was washed with distilled water .Secondly, lugol's iodine was also used to cover the surface for good 60 seconds. Acetone was used to decolourize the stain and lastly, the safranin solution was applied for counter staining 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 indicates gram negative bacteria (Cheesbrough, 2000).

Biochemical identification and Determination of Minimum Inhibitory Concentration

Bacterial isolates were identified using biochemical test technique as described by (Abdallah *et al*., 2016). As well as Minimum Inhibitory Concentration (MIC) was also determined using "Tube technique" as described in (Vollekova *et a.,l* 2001) and (Usman *et al*.,2004).

Statistical Analysis

The data of average zone of inhibition produced by the isolates against the extracts used were analysed using One-Way Completely Randomized ANOVA from statistical program 8.0. *Duncan's multiple range test (DMRT)*. The results were presented as the*** = Significant at 1%, * = Significant only at 5% and Ns = Not significant at 5%.*

RESULTS

Physical characteristics of aqueous extracts and ethanolic extracts of cassia siamea

S/N	EXTRACTS	WEIGHT CONC (g)	% YIELD	APPEARANCE	CHARACTERISTIC TEXTURES
1	Ethanolic extracts of *Cassia siamea* leaves	60	40	Dark green	powder
2	Aqueous extracts of *cassia siamea* leaves	60	50	Light green	powder

Formula for percentage yield = initial weight of sample /weight of extracts×100

Qualitative analysis, phytochemical constituent of the Ethanolic leaves extract of *Cassia siamea*

Phytochemicals	Status
Alkaloid	+ve
Flavonoid	+ve
Tannins	+ve
Steroid	+ve
Saponins	+ve
Phenols	-ve
Antraqunones	-ve

Key: + = present, - = absent.

Morphology and biochemical test for identification of the isolates

Biochemical test	*Escherichia coli*	*Shigella*	*Staphylococcus aureus*
Colony morphology			
Nutrient agar	Cream pinpoint clonies	Cream clonies	Cream cloured
Selective medium	EMB agar, shining green	XLD agar creamy white colonies	Catalyst test formation of bobbles indicate the presence of *staphylococcus* by using hydrogen peroxide
Grams nature	Gram negative	Gram negative	Gram positive
Cellular morphology	Rod	Cocci	Cocci
Motility	Motile	Motile	Non Motile
Indole	+ve	+ve	-ve
Methyl red	+ve	+ve	+ve
Voges proskauer	-ve	-ve	+ve

Citrate	-ve	-ve	+ve
Ureas	-ve	+ve	+ve
Catalyst	-ve	+ve	-ve

KEY:+ve= positive , -ve = positive

Zone of inhibition of various extracts against the organisms tested Ehanolic Leaves Extracts *Cassia Siamea*

Ethanolic extract on organisms tested	Antibacterial activity of Ethanolic extract on	Antibacterial activity of Ethanolic extract on	Antibacterial activity of Ethanolic extract on
Extract	***Escherichia coli***	***Shigella***	***Staphylococcus***
10mg/ml	9.00	14.00	13.00
20mg/ml	0.00	0.00	0.00
30mg/ml	10.00	0.00	11.00
40mg/ml	0.00	12.00	15.00
50mg/ml	14.0	15.00	13.0

Showing the minimum inhibitory concentration (MIC) of ethanolic extracts of *Cassia siamea* on test organisms

Treatment	AL(eth)E
Test Organism	
ESC	6.60000[a]
SHG	8.2000[a]
ST	10.400a
S.E	4.2079
Sig.	NS
Conc. Levels (mg)	
10	12.0000[a]
20	0.00000[b]
30	7.0000[ab]
40	9.0000[a]
50	14.0000[a]
S.E	3.7947
Sig.	*

Means within a column followed by the same letters are statistically not significant at 5% level of probability using Duncan's multiple range test (DMRT)

*** = Significant at 1%, * = Significant only at 5% and Ns = Not significant at 5%. ESC = Escherichia coli, SHG = Shigella,ST = Staphylococcus, AL(eth)E =Antibacterial activity of ethanolic leave extract on C. siamea*

Aqueous Leaves Extract of *Cassia siamea*

Aqueous extract on organisms tested	Antibacterial activity of aqueous extract on	Antibacterial activity of aqueous extract on	Antibacterial activity of aqueous extract on
Extract	***Escherichia coli***	***Shigella***	***Staphylococcus***
10mg/ml	5.00	9.00	6.00
20mg/ml	0.00	0.00	0.00
30mg/ml	0.00	14.00	15.0
40mg/ml	10.0	0.00	12.00
50mg/ml	11.00	15.0	8.00

Showing the minimum inhibitory concentration (MIC) of Aqueous leave extracts of *Cassia siamea* on test organisms

Treatment	AL(AQ)E
Test Organism	
ESC	5,2000[a]
SHG	7.6000[a]
ST	8.2000a
S.E	3.9021
Sig.	NS
Conc. Levels (mg)	
10	6.6667[a]
20	0.00000[b]
30	9.6667[a]
40	7.3333[ab]
50	11.333[a]
S.E	4.1366
Sig.	NS

Means within a column followed by the same letters are statistically not significant at 5% level of probability using Duncan's multiple range test (DMRT)

*** = Significant at 1%, * = Significant only at 5% and Ns = Not significant at 5%. ESC = Escherichia coli, SHG = Shigella,ST = Staphylococcus, AL(AQ)E =Antibacterial activity of Aqueous leave extract on C. siamea*

Minimum inhibitory concentration (MIC) Ethanolic Leaves Extract of *Cassia Siamea*

Test organisms	Concentration of the extract	Concentration of the extract	Concentration of the extract	Concentration of the extract	Concentration of the extract

	10	20	30	40	50
Shigella	0.14	0.00	0.00	0.48	0.75
Staphylococcus	0.13	0.00	0.33	0.6	0.52
Escherichia coli	0.09	0.00	0.03	0.00	0.7

Showing the minimum inhibitory concentration (MIC) of Ethanolic Leave extracts of *Cassia siamea* on test organisms

Treatment	L(eth)E
Test Organism	
ESC	0.2740[a]
SHG	0.3160[a]
ST	0.1640a
S.E	0.1878
Sig.	NS
Conc. Levels (mg)	
10	0.1200[bc]
20	0.0000[c]
30	0.1200[bc]
40	0.3600[ab]
50	0.6567[a]
S.E	0.1412
Sig.	**

Means within a column followed by the same letters are statistically not significant at 5% level of probability using Duncan's multiple range test (DMRT)

*** = Significant at 1%, * = Significant only at 5% and Ns = Not significant at 5%. ESC = Escherichia coli, SHG = Shigella,ST = Staphylococcus, L(eth)E =Antibacterial activity of ethanolic leave extract on C. siamea*

Minimum inhibitory concentration (MIC) Aqueous Leaves Extract of *Cassia Siamea*

Test organisms	Concentration of the extract 10	Concentration of the extract 20	Concentration of the extract 30	Concentration of the extract 40	Concentration of the extract 50
shigella	0.09	0.00	0.42	0.0	0.75
Staphylococcus	0.18	0.00	0.45	0.48	0.4
Escherichia coli	0.05	0.00	0.00	0.4	0.55

Showing the minimum inhibitory concentration (MIC) of Aqueous leave extracts of *Cassia siamea* on test organisms

Treatment	L(AQ)E
Test Organism	
ESC	0.2520[a]
SHG	0.3020[a]
ST	0.2000[a]
S.E	0.1697
Sig.	NS
Conc. Levels (mg)	
10	0.1067[b]
20	0.00000[b]
30	0.2900[ab]
40	0.2933[ab]
50	0.5667[a]
S.E	0.1482
Sig.	*

Means within a column followed by the same letters are statistically not significant at 5% level of probability using Duncan's multiple range test (DMRT)

*** = Significant at 1%, * = Significant only at 5% and Ns = Not significant at 5%. ESC = Escherichia coli, SHG = Shigella,ST = Staphylococcus, L(eth)E =Antibacterial activity of Aqueous leave extract on C. siamea*

DISCUSSION

In the present study, the preliminary phytochemical screening of the plant materials (leaves of *Cassia siamea*) revealed the presence of Tannin, Saponin, Steroid, Flavonoid, and Phenols, Anthraquenones were absent. The presence of various phytochemicals in *Cassia siamea* extracts has also been reported by many researchers.The result of phytochemical screening of this study was in Similar with that of (Abdallah et al., 2016), who reported the presence of flavonoids, tannins, polyphenols, saponins, and glycosides in *Cassia siamea* leaves extract. On the other hand, the result of the present study was contrary to that of (Bukar *et al*., 2009). Who reported the absence of flavonoids, saponins and alkaloids in ethanolic extract of S. siamea leaves.

Moreover, the active phytochemicals were known for their medicinal activity as well as physiological actions; as such they confer the therapeutic potentials of all medicinal plants. Alkaloids, saponins, and tannins have been reported to inhibit bacterial growth and protective to plants against fungal infections. Twenty four (24) Alkaloids comprising a large group of nitrogenous compounds are widely used as cancer chemotherapeutic agents, anaesthetics and Central Nervous Stimulants, the medicinal uses reported of *S. siamea* in managing constipation, its antimicrobial and antimalarial uses may be attributed to the presence of these phytochemical constituents. More so, results of antibacterial activity of *Cassia siamea* leaves extracts In this study indicated that different extracts of *Cassia siamea* leaves have broad spectrum antibacterial activity with variable degree of sensitivity against the tested bacterial species. The antibacterial activity of *S.*

siamea leaves extracts could be attributed to the chemical properties of *Cassia siamea* leaves as mentioned above. The antibacterial activity of *Cassia senna* has been previously reported by (Abo *et al*., 1999).

Moreover the dose–dependent antibacterial activity of *Cassia siamea* has been reported by (Ahmed-Alizaga *et al*., 2007) analysis of the result showes that ethanol extract demonstrated highest antibacterial activity with average zone of inhibition of 15.0mm (30mg/ml) among the isolates. This could be attributed to better solubility of the photochemical in ethanol when compared to water. Aqueous extracts exerted antibacterial activity against the tested isolates with average zone of inhibition of 15mm. The result of this study was inconformity with that of (Ahmed-Alizaga *et al*., 2007) who found *Cassia siamea* leaves extracts active against certain bacteria. The result of this study also supported that of(Bukar et al.,2009) who reported anti pseudomonal activity of *Cassia siamea* leaves extracts against pathogenic *streptococcus* The result of MIC of the extracts showed that dilutions of various concentrations of aqueous and ethanol extracts of *Cassia siamea* can inhibit and/or kill the isolates. Lower MIC (10mg/ ml) was shown by aqueous extract than ethanolic extract the antibacterial activities of the extracts are expected, perhaps due to the present of bioactive compounds like alkaloid, terpenoid, saponin, tannin, flavonoids and steroids which were dissolved in the solvents. The results of present study have provided the justification for therapeutic potential of *Cassia siamea* leaves and also used as medicinal plant. The result for statistical analysis of ethanolic extract of *Cassia Siamea* on test organisms showed significant difference, which implies that *staphylococcus* recorded the highest while *Escherichia coli* recorded the least. Similarly the concentration levels tested shows significant differences in the leaves extract of ethanolic while for the aqueous it shows no significant differences this shows that there are no any differences or changes as compared with that of (Sharma *et al*., 2010).

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