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Phytochemical Analysis and Anti-Bacterial Activities of Siannasiamea (Yellow Cassia) Leaf Extract against Staphylococcus aureus and Escherichia coli

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Abstract

In recent decades, there is a progressive increase in antibiotic resistant strains of clinically important pathogens. Despite the advancement in science and technology on the discovery of many natural and synthetic drugs, infectious diseases are still the leading cause of morbidity and death, especially in developing countries. This study therefore aimed at evaluating the phytochchemicals and antibacterial potentials of ethanol and Acetone extracts of Siannasiamea leaves on Escherichia coli and Staphylococcus aureus. The phytochemical test was carried out using standard methods and investigation the presence of saponin, glycoside, steroid phenol and reducing sugar, while alkaloid and flavonoid were absent. The antibacterial activity of the leaf extract was bio-assayed using the agar well diffusion method and broth (tube) dilution method. Both extracts were found to be inactive on the test organisms at the concentration of 125mg/ml, 100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml, but inhibit the growth of both Staphylococcus aureus and Escherichia coli at the concentration of 1000mg/ml, 500mg/ml and 250mg/ml. It was also subjected to obtain minimum inhibitory concentration and minimum bactericidal concentration. The mean zone diameter of inhibitions of 13.17mm, 11.27mm and 8.17mm on E. coli and 14.77mm, 12.4mm, 8.50mm on S. aureusforethanolic extract and 13.83mm, 12.17mm, 8.13mm on E. coli and 14.77mm, 12.67mm, 9.17mm on S. aureus for acetone extract at the concentration of 1000mg/ml, 500mg/ml and 250mg/ml respectively. The minimum bactericidal concentration was found to be bactericidal at concentration of 250mg/ml for both Staphylacoccus aureus and Esherichia coli. The leaf extract of Siannasiamea possess different phytochemicals including glycoside, steroid, saponin phenol and reducing sugar and has antibacterial activity against some bacterial species and therefore has the potential of being used as ethenomedical

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drug and required further studies to identify the specific active chemical that can be used to formulate specific antibacterial drug. The best inhibitory concentration of the extarct was found to be effective at 250mg/ml for both ethanolic and Acetonic extract on Escherichia coli and Staphylococcus aureus respectively. Further studies is recommended for isolation and identification of several bioactive constituent which are not been tested and biological assay methods ought to be carried out for the standard drug preparation.

Keywords: Anti-Bacterial Activities, Leaf Extract, Phytochemical Analyses, *Siannasiamea*

INTRODUCTION

Various medicinal properties have been attributed to natural herbs. Medicinal plants constitute the main source of new pharmaceuticals and health care products (Ivanova *et al.,* 2005). The history of plants being used for medicinal purpose is probably as old as the history of mankind. The use of medicinal plants in the industrialized societies has been traced to the extraction and development of several drugs from these plants as well as from traditionally used folk medicine (Ivanova *et al.,* 2005). Extraction and characterization of several active phytocompounds from these green factories have given birth to some high activity profile drugs (Mandal *et al.,* 2007). The use of traditional medicine is widespread in India (Jeyachendra and Mahesh, 2007). A growing body of evidence indicates that secondary plant metabolites play critical roles in human health and may be nutritionally important (Hertog *et al.,* 1993). It is believed that crude extract from medicinal plants are more biologically active than isolated compounds due to their synergistic effects (Jana and Shekawat, 2010). Phytochemical screening of plants has revealed the presence of numerous chemicals including *alkaloids*, tannins, flavonoids, steroids, glycosides and saponins etc. Secondary metabolites of plants serve as defense mechanisms against predation by many microorganisms, insects and herbivores (Jeyachendra and Mahesh, 2007). Herbal medicines have become more popular in the treatment of many diseases due to popular belief that green medicine is safe, easily available and with less side effects. Indeed, the market and public demand has been so great that there is a great risk that many medicinal plants today face is either extinction or loss of genetic diversity. Plant products have been part of Phytomedicines since time immemorial. These can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, etc. I.e. any part of the plant may contain active components. Knowledge of the chemical constituents of plants is desirable because such information will be of value for the synthesis of complex chemical substances. Such phytochemical screening of various plants is reported by many workers. The medicinal plants are useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents (Nostro *et al.,* 2000). Phytochemicals are naturally occurring in the medicinal plants, leaves, vegetables and roots that have defense mechanism and protect from various diseases. Phytochemicals are primary and secondary compounds. Chlorophyll, proteins and common sugars are included in primary constituents and secondary compounds have *terpenoid, alkaloids* and *phenolic* compounds (Krishnaiah *et al.,* 2007). Terpenoids exhibit various important pharmacological activities i.e. anti-inflammatory, anticancer, anti-malarial, inhibition of cholesterol synthesis, anti-viral and anti-bacterial activities (Mahato and Sen, 1997). *Cassia siamea is* medicinal plant that is widely used to treat various diseases in Nigeria and other West African countries. The leaves of *Cassia siamea* are locally used in Indonesia for treating malaria (Krishnaiah *et al.,* 2007). An *alkaloid* in the pods and leaves has been reported to be fatal to pigs (Krishnaiah *et al.,* 2007). The main objective of this research work is to analyze the presence or absence of different phytochemicals and to test the antibacterial effect of the leaf extract of S*iannasiamea*.

Siannasiamea is a plant that claimed to have a lot of medicinal values. These claims have not been clearly justified. This research and experiment is therefore centered on investigation analyzing and justifying the claims made on this plant. And also to know the chemical composition responsible for the medicinal value in the plant. The study of this research was aimed to determine the phytochemicals constituents and antibacterial effect of *Siannasiamea* leaves extract on *E. coli* and *S. aureus*.

MATERIALS AND METHODS

Collection and Authentication of Plant Sample

The fresh leaves of *Siannasiamea* was carefully collected from their natural habitat in the school farm of Federal University Dutse, Jigawa State. The sample of the plant leaves in a polyethene bag were conveyed to herbarium section of Bayero University Kano for taxonomic identification and authentication. A voucher specimen number BUKHAN70 was given to the specimen. The plant material was brought to the laboratory rinsed with water to remove dirt and dried at room temperature before examination. The leaves were removed from the dried leaves and crushed in to powder form using pestle and mortal.

Collection of the isolates

Clinical isolate of *Escherichia coli* and *Staphylococcus aureus* collected from Rasheeed Shekoni Specialist Hospital Federal University Dutse, and was transported to the Biological sciences laboratory Federal University Dutse immediately and kept in the refrigerator for used. The isolates were confimed by Mr Nura Haris.

Isolate of *E. coli* and *Staphylococcus aureus* colony on MacConkey Agar and Nutrient Agar were stain using the Gram staining method as described below:

The Gram staining smear was examined microscopically using 100 oil immersion objective lens.

Preparation of plant extract

50g of air dried and grounded *Siannasiamea* leaves powder was extracted by percolation with 200 ml (1:4 ratio) each of Ethanol and Acetone at room temperature for one week the content were filtered using whatman no 1 filter paper and the residue was discarded and later the extract were obtained following evaporation to dryness using rotary evaporator.

Phytochemical Analysis of Plant Extract

Phytochemical analysis were conducted to qualitatively determine the presence or absent of the following secondary metabolites that is alkaloid, glycoside, phenol, steroid, saponin, flavonoid, and carbohydrate. Using method outline by (Sofowora 1993; Evans and Trease 1999; Joseph *et al.*,2013).

Alkaloids

Using pipette, 3ml of dragendoff reagent was added to the extract, forming creamy precipitate indicated the presence of alkaloid as reported by Evans and Trease (1999).

Tannins

Few drops of $FeCl_2$ solution was added to 3 ml of the extracts in a test tube followed by shaking. A result of dirty green coloration confirmed the presence of tannins as demostrated by Evans and Trease (1999).

Flavnoids
One ml of the extract was treated with 1ml of dilute NaOH. The presence of a cloudy precipitate confirm the presence of flavonoid as described Evans and Trease (1999).

Saponin
Five mililiters (5ml) of distilled water was added to the 2ml of the extract in a test tube and shaken vigorously. The formation of foams or stable thing following the shaking indicated the presence of saponin as demostarted by Evans and Trease (1999).

Phenol
One ml (1ml) of the extract was added to 1ml of $FeCl_3$ and mix together. The presence of blue black precipitate confirmed the presence of phenols as described Evans and Trease (1999).

Glycoside
Approximately 2ml of glacial acetic acid were added to 5ml of the extract. Followed by one (1) drop of $FeCl_2$ and concentrated H_2S0_4. Brown ring precipitate indicated the presence of glycoside (Joseph *et al.,* 2013).

Test for reducing sugar:
Few miles of Fehling solution A and B were added to a few drops of the extract and boiled, a brick red colored indicate the present of reducing sugar (Joseph *et al.*, 2013).

Preparation of concentration
Eight bijou bottles were labeled as 1, 2, 3 and 4 each for methanol and acetone extract. Di - methyl sulfoxide (DMSO) was used as reagent for the preparation of ethanol and Acetone, four concentrations were made for each extract as follows; 100mg/mL, 50mg/mL, 25mg/mL and 12.5, mg/ml.

Biochemical Test
Indole test.
The organism was each grown in 5ml peptone water and incubated for 24hrs. After incubation; the reagents (Kovac's reagent) were added and shaken gently. A red color in the reagent layer above the broth within 1minute indicates a positive test while absence of the red layer indicate a negative test (Cheesbrough, 2000).

Catalase test
2-3 drops of hydrogen peroxide solution are place on a clean glass slide, and a sterile inoculating wire loop will use to pick some test colonies which was mixed with the solution.The presence of bubbles indicates positive result

Coagulase test
Drops of distilled water were place on a clean glass slide after which a colony of the test organism was emulsifying with loop full of citrated blood plasma are then added to the mixture and mixed gently for few seconds to observe for the presence of coagulation.

Different types of media were used which include nutrient agar (NA), MacConkey agar eucinemethalline blue (EMB) and MuellerHinton agar (NH). All the media used were prepared according to manufacturer's instruction.

Standardization of inoculum
The sub - culture colony of *E. coli* and *S aureus*(wire loop full) were placed in a test - tube containing 5ml of normal saline until a turbidity of 0.5 Macfarlands standard were reached (Cheesebrought, 2004).

Bioassay test
For the sensitivity test carried out, the antibacterial activity of the extracts was evaluated using agar well diffusion method on Mueller-Hinton agar (MH) as described by (Kirby-Baver, 1996). Two experiment were carried out using different concentration. For the first experiment, concentration of 100mg/mL, 50mg/mL, 25mg/mL and 12.5mg/mL were used, for the second experiment, concentrations of 1000, 500, 250 and 125mg/mL were used. During the experiment, agar plate were prepared and the surface of it was allowed to solidified at room temperature, using sterile swab stick, the Mueller-Hinton agar plate were aseptically inoculated with the test organism (*Escherichia coli* and *Staphylococcus aureus*) to ensure evenly distribution of the test organism on the plates. Sterilized 6mm in diameter cork borer was used to make holes on the agar, the prepared concentrated extracts aseptically pipetted in to each hole. Control were also incorporated on to the inoculated plates, two control were used, these are; positive control (Ciprofloxacin) and negative control (DMSO). Eight (8) diameter was consider as a positive result (Reagor*et al.,* 2002; Cakir *et al.,* 2004).
The experiment was performed in triplate under strict aseptic conditions to ensure consistency of all findings.

Determination of Minimum Inhibitory Concentrattion (MIC):
The minimum inhibitory concentration (MIC) is define as the lowest concentration of the compounds which inhibits the growth of microorganisms. The broth tube dilution method was used for the determination of minimum inhibitory concentration. I.e. the lowest concentration of the extract to completely prevent the growth of the test organisms.250 mg/ml, 125 mg/ml, and 62.5 mg/ml respectively using the dilution formula. (Ochei and Kolhatkar, 2008).

Determination of Minimum Bactericidal Concentration (MBC)
The minimum bactericidal concentration (MBC) is defined as the lowest concentration of the compounds that kills and show no growth of microorganism on an agar plate.For the determination of MBC, the fresh Mueller Hinton agar were inoculated with one loopfull of culture taken from each of the broth culture that show no growth in the MIC tubes. MBC assay plates were incubated for 24 hr. after the incubation period, the lowest concentration of the extract that did not produce any bacterial growth on the solid medium were regarded as MBC value for that particular extract (Ochei and Kolhatkar, 2008).

RESULT
The of result phytochemical analysis showed that steroid, saponin, glycoside and reducing sugar were present in all the extract phenol is present only in Acetone extract while Alkaloid and flavonoid was absent in all the extract (Table 1).

Table 1: phytochemical analysis of leaf extract of *Cassia siamea*

Solvents		
Phytochemical	Ethanol	Acetone
Alkaloid	–	–
Steroid	+	+
Flavonoid	-	-
Saponin	+	+
Phenol	-	+
Glycoside	+	+
Reducing sugar	+	+

Keys: + = present - = absent

The result of susceptibility test showed that the ethanolic extract of *Siannasiamea* has no effect on the test organisms at the above concentration (Table 2)

Table 2: Antibacterial susceptibility of ethanolic extract on *E. coli* and *S. aureus*

Isolate	zone of inhibition at different concentration Of the extract				positive control
	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	
E. coli	00	00	00	00	39
Staph.	00	00	00	00	49

The result of susceptibility test showed that the Acetone extract of *Siannasiamea* has no effect on the test organisms at the above concentration (Table 3)

Table 3: Antibacterial susceptibility of Acetone extract on *E. coli* and *S. aureus*

Isolate	zone of inhibition at different concentration Of the extract				positive control
	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	
E. coli	00	00	00	00	39
Staph.	00	00	00	00	50

The results of antibacterial susceptibility showed that all the extract has antibacterial effects at the concentration of 1000 mg/ml, 500 mg/ml and 250 mg/ml but there is no effect at the concentration of 125 mg/ml (Table 4 and 5)

Table 4: Antibacterial susceptibility of ethanolic extract on *E. coli* and *S. aureus*

Isolate	zone of inhibition at different concentration of the extract				positive control
	1000 mg/ml	500 mg/ml	250 mg/ml	125 mg/ml	
E. coli	13.17±0.08	11.27±0.06	8.17±0.08	00	39.33±1.33
Staph.	14.33±0.33	12.4±0.13	8.50±0.25	00	49.66±2.33

Table 5: Antibacterial susceptibility of Acetonic extract on *E. coli* and *S. aureus*

Isolate	zone of inhibition at different concentration Of the extract			positive control
	1000 mg/ml	500 mg/ml	250 mg/ml	125 mg/ml
E. coli	13.83±0.08	12.17±0.08	8.13±0.17 00	39.33±1.33
Staph.	14.77±0.16	12.67±0.33	9.17±0.08 00	50.00±1.00

The result of minimum inhibitory concentration showed that the minimum inhibitory concentration of ethanolic and Acetone extracts was 250 mg/ml. in all the test organisms (Table 6 and 7).

Table 6: Minimum inhibitory concentration of ethanolic leaf extract of *Siannasiamea* on *Escherichia coli* and *Staphylococcus aureus*

	Concentrations (mg/ml)		
Isolate	250	125	62.5
Eschericia coli	-	+	+
Staphylococcus aureus	-	+	+

Key - = Non turbid i.e. inhibit the growth + = Turbid i.e. do not inhibit the growth

Table 7: minimum inhibitory concentration of Acetonic leaf extract of *Siannasiamea* on *Eschericia coli* and *Staphylococcus aureus*

	Concentrations (mg/ml)		
Isolate	250	125	62.5
Eschericia coli	-	+	+
Staphylococcus aureus	-	+	+

Key - = Non turbid i.e. inhibit the growth + = Turbid i.e. do not inhibit the growth.

The result of minimum bactericidal concentration showed that the minimum bactericidal concentration of ethanolic and acetone leaf extract was 250 mg/ml for all the test organisms (Table 8).

Table 8: Minimum bactericidal concentration of ethanolic and Acetone leaf extract of _*S. siamea* on *Escherichia coli* and *Staphylococcus aureus*
Concentration (250 mg/ml)

Isolate	Ethanol	Acetone
Escherichia coli	+	+
Staphylococcus aureus	+	+

Key: + = absent of growth - = present of growth

The result of Gram reaction and biochemical characterization of isolates showed that *E. coli*a gram negative bacteria, was positive for Indole and Catalyst tests, but negative for coagulase and oxidase tests, whereas *Staphylococcus aureus*a gram positive bacteria was positive coagulase and catalyst tests, but negative for indole and oxidase tests (Table 9)

Table 9: Gram Reaction and Biochemical Characterization of Isolates

Parameters	Isolate	
	E. coli	*Staphylococcus aureus*
Gram reaction	gram negative	gram positive
Indole test	+	_
Coagulase	_	+
Oxidase test	_	_
Catalyst test	+	+

Discussion

Photochemical compounds present in *Siannasiamea leaf* extracts include reducing sugars, steroid, glycoside and saponin. Phenol was found to be present in only the acetone extract, while flavonoids and alkaloids were not detected in all the extracts. The presence of various phytochemicals in *S. siamea*extract and fractions have also been reported by (Alli and Smith

2009; Ahmed and Olayanju, 2007). The variation in type of phytochemicals present in different solvents as shown in the result of phytochemical screening might be attributed to the ability of the solvents to dissolve into solution specific type of photochemical as reported by (Yusha'u*et al;*2008). The antibacterial activity of *S. siamea* leaves could be due to phytochemicals present such as saponin, alkaloids, and tanninsetc. which have been reported by (Dweck, 1994).

To act as plant protectants against pathogens in the wild. The protection is equally conferred on humans when plant parts are drunk as concoctions, decoctions in ethno medicine. The organisms were resistant to all the extracts at125mg/ml, 100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml concentrations, with antibacterial activity only exhibited at 1000mg/ml, 500mg/ml and 250mg/ml concentrations, for all the extracts. The mean zone diameter of inhibitions of 13.17mm, 11.27mm and 8.17mm on *E. coli* and 14.77mm, 12.4mm, 8.50mm on *S. aureus* for ethanolic extract and 13.83mm, 12.17mm, 8.13mm on *E. coli* and 14.77mm, 12.67mm, 9.17mm on *S. aureus* for acetone extract at the concentration of 1000mg/ml, 500mg/ml and 250mg/ml respectively. The antibacterial activity of *S. siamea* have been previously reported by (Abo *et al.,* 1999; Ayfer and Ozlem, 2003; Elujoba*et al.,* 1999; Kumar *et al.*, 2006: Ahmed and Olayanju,2007).While the dose – dependent antibacterial activity of *S. siamea* have been reported by (Ahmed and Olayanju, 2007).

The result also revealed acetone extract to possess higher antibacterial activity compared to ethanolic extract which exhibited the least activity. The high antibacterial activity of Acetone extracts over the ethanolic extracts could be attributed to its possession of more phytochemical constituents which include phenol which were absent in ethanol extract. The result obtain also shown that *Siannasiamea* leaf extract has low antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*as reported by (Nanasomba*et al.,* 2009).This result also shown that *Cassia siamea* leaves extract has antibacterial activity against *Escherichia coli* and *Staphylococcusaureus* only in higher concentration as such the plant can be used in the production of drugs. The minimum inhibitory concentration and minimum bactericidal concentration was found to be effective as 250mg/ml for both ethanolic and Acetone extract on *Escherichia coli* and *Staphylococcus aureus* respectively. However, further study is recommended to assess the presence of other bioactive compounds of chemotherapeutic potential and also to ascertain the efficacy, toxicity and suitability of using the extract in vivo.

Conclusion

The leaf extract of *Siannasiamea* possess different phytochemicals including glycoside, steroid, saponin phenol and reducing sugar and has antibacterial activity against some bacterial species and therefore has the potential of being used as ethenomedical drug and required further studies to identify the specific active chemical that can be used to formulate specific antibacterial drug. The best inhibitory concentration of the extarct was found to be effective at 250mg/ml for both ethanolic and Acetonic extract on *Escherichia coli* and *Staphylococcus aureus* respectively.

Recommendations

Further studies is recommended for isolation and identification of several bioactive constituent which are not been tested and biological assay methods ought to be carried out for the standard drug preparation.

It is also recommended that further studies should be carried out to identify the active ingredient present in *Siannasiamea* leaves which can be used in drugs development program for safe health care service.

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