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# Antibacterial efficacy and phytochemical screening of *Senna siamea* leaves extracts on some pathogenic bacteria

## Abstract

The study was aimed to investigate the phytochemical constituents and antibacterial activity of *Senna siamea* leaves extracts against some pathogen bacteria. Aqueous and ethanol extracts from *Senna siamea* leaves were prepared, screened for phytochemical analysis and tested for its antibacterial activity against 6 pathogenic bacteria (*Klebsiella pneumoneae, Salmonella typhi, Shigella spp, Escherichia coli,* and *Pseudomonas aeruginosa*) recovered from stool sample of patients attending Murtala Muhammad Specialist Hospital Kano. Phytochemical screening of the extracts showed that *Senna siamea* extract contain Alkaloid, Anthraquinone, saponin, tannin, phenol, steroid, flavonoid, terpenoid and glycosides. Statistical analysis of the result showed that highest antibacterial activity was recorded in ethanol extract with average zone of inhibition of 14.12±1.51 mm among the isolates while aqueous extracts recorded an average zone of inhibition of 12.00±1.06 mm. Based on the susceptibility of the organisms to the extracts, *E. coli* was found to be the highest susceptible organisms with average zone of inhibition of 14.29±1.12 mm, followed *S. aureus*(13.61±1.23mm), *Salmonella typhi*(13.56±1.89mm), *Shigella* (13.22±1.41mm), *Pseudomonas*(12.36±1.38mm) while least average zone of inhibition is shown by *Klebsiella*(11.33±0.80mm). The MIC and MBC of the extracts ranges from 3.125 to 50mg/ml There is no significant different on the susceptibility of the organisms against the extracts at $p<0.05$. The results of the present study have supported the therapeutic potential of *Senna siamea* and its use as medicinal plant.

**Keywords:** antibacterial activity, pathogenic bacteria, phytochemicals, *Senna siamea*

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## Introduction

Recently, there has been a lot of attention focused on producing medicines and products that are natural to complement the existing synthetic antimicrobial drugs that are gradually becoming less potent against pathogenic microorganisms. Several leaves and leaves extracts have been found to have antimicrobial activity against microorganisms. Several hundred genera of plants were utilized traditionally for medicinal purposes. The world health organization[1] reported that 80% of the world population relies chiefly on traditional medicine and a major part of the traditional therapies which involve the use of plant extract and their constituents.[2] The presence of phytochemical constituents in medicinal plants made them useful for healing as well as for curing of human diseases.[3] Phytochemicals are naturally occurring compounds in the medicinal plants such as terpenoid, flavonoids, steroid, alkaloids and phenolic compounds. The phytochemicals have impressive pharmaceutical properties such as analgesics, aesthetic, antibiotics, antiparasitic, anti-inflammatory, oral contraceptive, hormones and ulcer therapeutic laxative.

*Senna siamea* Lam. belongs to the family Fabaceae. It is commonly called kassod tree or yellow cassia,[4] or in Hausa as ''Malga'',[5] was introduced to Africa from tropical Asia. It is widely grown throughout tropical Africa. Different parts of *S. siamea* can be used for various medical purposes.[6–8] The leaves, stems, roots, flowers and seeds of *C. siamea* regardless the subspecies have been used for the treatment of several illnesses including mostly malaria, a tropical endemic disease with high morbidity and mortality.[9]. The leaves are the most used parts' the plant especially by African and Asian population in preparation of the herbal remedies. In Burkina Faso, fresh and dried leaves decoction (boiled for 20 min in 1L of water) is drunk with lemon juice or for body bath throughout the day to treat malaria and liver disorders.[10]

*S. siamea* has been reported to be used in the management of constipation, diabetes, insomnia, hypertension, asthma, typhoid fever, and dieresis.[11] Leaves and bark of medicinal plants were reported to be used locally as antimalarial medications.[12] Traditionally Senna siamea is used for the treatment of typhoid fever, jaundice, abdominal pain, menstrual pain and is also used to reduce sugar level in the blood. Ethno medicinally *S. siamea* is used as laxative, blood cleaning agent, cure for digestive system and genitourinary disorders, herpes and rhinitis.[13] In traditional medicine, the fruit is used to charm away intestinal worms and to prevent convulsion in children.[14]

*C. siamea* (leave) has been valued for its use in the treatment of infectious diseases. An aqueous extract of fresh or dried leaves of *S. siamea* has also been recommended for treatment of insomnia.[15] Aqueous leave extract is active against various bacteria gram at 500 and 1000µg/mL/disc, it inhibited *Pseudomonas aeruginosa* (iz. 16mm, respectively). At 0.1mL/disc/37°C for 24 hours, it showed inhibition on *Staphylococcus aureus* (11.7mm), *Bacillus cereus* (10mm) and *Escherichia coli* (10.2mm).[7] The ethanol leave extracts (ranged from 500-1000µg/disc) showed more activities than ciprofloxacin (30µg/disc) on *Staphylococcus aureus*.[16] Preliminary qualitative phytochemical screening of the leaves and stem bark of *S. siamea* revealed the presence of anthraquinones, alkaloids, tannins, polyphenols, glycosides, saponins and flavonoids in both the leaves

and stem bark.[17] The present study was aimed to determine the phytochemical constituents and antibacterial activity of *Senna siamea* leaves extract against some clinical isolate of bacteria recovered from stool samples of infected patients attending Murtala Muhammad General Hospital, Kano namely; *Klebsiella pneumoneae, Salmonella typhi, Shigella sp, Escherichia coli,* and *Pseudomonas aeruginosa*.

## Materials and methods

### Collection and authentication of plant materials

*Senna siamea* leaves were used in this study, which was collected from Bayero University, Kano old campus. Botanical Identification and Authentication of the plant material was done at Herbarium unit in the Department of Plant Biology, Bayero University Kano with the following Voucher specimen number: BUKHAN 0448. Voucher specimens were deposited there for future reference. The leaves were washed with water and removed dust and rinsed with distilled water, air dried for two-weeks and pulverized into powder form using sterile mortar and pestle under laboratory as described by Ali et al.[17] The powder sample was bagged in a black polythene bag and stored in air tight container for further work.

### Test organisms

Five(5) bacterial isolates recovered from stool sample of infected patients attending Murtala Muhammad General Hospital, Kano namely; *Klebsiella pneumoneae, Salmonella typhi, Shigella sp, Escherichia coli,* and *Pseudomonas aeruginosa* were obtained from Microbiology Laboratory of Kano University of Science and Technology Wudil, Kano. The bacteria were characterized to species level by using different laboratory procedures including; Gram's stain, cultural characterization and Biochemical tests include (Indole, Methyl red, Vougues Proskeaur, Catalase, Citrate utilization and coagulase tests) as described by Holt et al.;[18] Chessbrough[19] The isolates were maintained on Nutrient agar slants at 4°C.

### Indole test

Tryptophan broth was inoculated with an isolate of the test organism and incubated at 37°C for 24 hours. About 0.5ml of Kovack's reagents was added to the broth culture.

### Methyl red test

MR-VP broth was inoculated with an isolate of the test organism using sterile inoculating loop and incubated at $37^0$C for 24 hours. About 5 drops of Methl-red reagent was added to the broth culture.

### Voges proskauer

MR-VP broth was inoculated with an isolate of the test organism using sterile inoculating loop and incubated at 37°C for 24 hours. Six millilitre (6ml) of 5% alpha naphthol was added followed by 0.2ml of KOH. The tube was shaken gently and remained undisturbed for 5 minutes

### Citrate utilization test

Simmon's citrate agar was streaked back and forth with inoculums of the test organism and incubated aerobically at 37°C for 24 hours.

### Preparation of the leaves extracts

Aqueous and ethanol extracts of *Senna siamea* leaves were prepared separately. Fifty grams (50g) powder of the plant material was soaked in 500ml each of distilled water and ethanol respectively. The flasks were kept at room temperature for 3 days with intermittent shaking after which filtration was done using Whatman filter paper. The ethanol extracts was evaporated at 50°C using rotary evaporator while the aqueous extract was evaporated at 40°C in water bath until dried extract samples were obtained. All the dried extract samples were dissolved in 10% DMSO separately to the final concentration of 200mg/ml as a stock concentration. The extract solutions were stored at 4°C before use.[17]

### Qualitative phytochemical screening

The phytochemical screening of the plant materials for various phytochemical constituents such as terpenoids, flavonoids, alkaloids, reducing sugars, steroid, glycoside, phenol, Anthraquinones, saponin and tannin was conducted using standard methods as described by Sofowora[20] & Trease.[21]

### Antibacterial activity of the extracts

The sensitivity of each extracts was determined using the agar well diffusion method as described by Ahmed[22] with modifications. The prepared bacterial suspension equivalent to 0.5McFarland Standard ($1.5x10^6$CFU) was inoculated into sterile Mueller- Hinton agar medium in a sterile Petri-dish. A sterile 6mm diameter sterile cork borer was used to bore 5 wells into the agar medium. The wells were then filled up with approximately 0.1ml of the extract solution at a concentration of 25,50,75 and 100mg/ml taking care to prevent spillage onto the surface of the agar medium. The plates were allowed to stand on the laboratory bench for 1 hour to allow proper diffusion of the extract into the medium after which the plates were incubated at 37°C for 24hours, and thereafter the plates were observed for zones of inhibition and measured. The experiment was conducted in triplicate and the average values were recorded. Ciprofloxacin 50mg/ml (Micro Lab limited) was served as a control (positive) for the experiment.

### Minimum inhibitory concentration (MIC) of the extracts

The minimum inhibitory concentration MIC of the extracts was determined using broth dilution technique. Double fold serial dilutions of the extracts were prepared by adding 2ml of 100mg/ml of the extract into a test tube containing 2ml of Nutrient broth, thus producing solution containing 50mg/ml of the extract. The process continue serially up to test tube No.5, hence producing the following concentrations; 50,25,12.5,6.25,3.125mg/ml. Test tube No. 6 do not contain extracts and serve as negative control. Exactly 0.5ml of 0.5McFarland equivalent standards of test organisms were introduced into the test tubes and incubated at 37°C for 24hours. After incubation the test tubes were observed for growth by checking for turbidity.[22]

### Minimum bactericidal concentration (mbc) of the extracts

From each tube that did not show visible growth in the MIC, Briefly, 0.1ml bacterial culture was pipetted from the mixture obtained in the determination of MIC tubes which did not show any growth and sub cultured onto the surface of Mueller Hilton agar plates and incubated at 37°C for 24h. After incubation the concentration at which there was no single colony of bacteria was taken as MBC.[22]

### Statistical analysis

The data of average zone of inhibition produced by the isolates against the extracts used were analyzed using One-Way ANOVAs from statistical program SPSS 21.0(Statistical Package for the

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Social Sciences). The results were presented as the means±standard deviation. Significance level for the differences was set at $p<0.05$.

# Result

## Phytochemical constituents of the extracts

Phytochemical screening of *Senna siamea* leaves extracts in Table 1 indicates the presence of alkaloid, tannin, saponin, glycoside, steroid and anthraquinone flavonoid, terpenoid, and phenols while reducing sugar was absent.

**Table 1** Phytochemical constituents of the extracts

| S/N | Phytochemical | Status |
|---|---|---|
| 1 | Alkaloids | + |
| 2 | Flavonoid | + |
| 3 | Glycosides | + |
| 4 | Reducing sugar | - |
| 5 | Saponin | + |
| 6 | Steroids | + |
| 7 | Phenols | + |
| 8 | Terpenoid | + |
| 9 | Anthraquinones | + |
| 10 | Tannin | + |

Key: +, presence of phytochemical; -, absent of phytochemical

## Antibacterial activity of aqueous extract

The antibacterial activity of aqueous *Senna siamea* extract is presented in Table 2. The results showed that zones of inhibition recorded by the isolates depend on the type of bacterial isolates and concentration of the extracts. Highest zone of inhibition is demonstrated by *E. coli* (18.23±0.36mm) at 100mg/ml. The zone of inhibition of the control (Ciprofloxacin 50mg/ml) ranges from to 19-22mm

## Antibacterial activity of ethanol extract

The antibacterial activity of ethanol extract is presented in Table 3. The results showed that zones of inhibition recorded by the isolates depend on the type of bacterial isolates and concentration of the extracts. Highest zone of inhibition is demonstrated by *Shigella sp* (18.87±0.37) at 100mg/ml. The zone of inhibition of the control (Ciprofloxacin 50mg/ml) ranges from to 19-22mm

## MIC and MBC of the extract

Minimum inhibitory concentration of aqueous and ethanol extract of *Senna siamea* is represented in Table 4. The result showed dilutions of various concentrations of aqueous and ethanol extracts can inhibit and/or kill the isolates. Lower MIC (3.125mg/ml) was shown by ethanol extract than aqueous extract. MBC of ethanol extract ranges between 12.5-50mg/ml.

**Table 2** Antibacterial activity of *Senna siamea* aqueous extract

| Concentration (mg/ml)/zone of inhibition(mm) | | | | | |
|---|---|---|---|---|---|
| **Isolates** | **25** | **50** | **75** | **100** | **Control** |
| *Klebsiella pneumoneae* | 8.00±0.00$^a$ | 8.84±0.00$^a$ | 10.56±0.11$^a$ | 12.17±0.13$^a$ | 22 |
| *Salmonella typhi* | 8.73±0.15$^a$ | 11.46±0.13$^a$ | 13.70±0.22$^a$ | 15.45±0.26$^b$ | 21 |
| *Shigella sp* | 9.40±0.17$^a$ | 10.28±0.20$^a$ | 13.82±0.09$^b$ | 14.71±0.31$^b$ | 22 |
| *Pseudomonas aeruginosa* | 9.55±0.00$^a$ | 10.77±0.26$^a$ | 12.49±0.14$^a$ | 12.65±0.21$^a$ | 20 |
| *Escherichia coli* | 10.33±0.20$^a$ | 12.59±0.12$^a$ | 14.88±0.17$^b$ | 18.23±0.36$^b$ | 22 |

Key:Values having different superscript on the same row are considered significantly different at $p<0.05$

**Table 3** Antibacterial activity of *Senna siamea* ethanol extract

| Concentration (mg/ml)/zone of inhibition(mm) | | | | | |
|---|---|---|---|---|---|
| **Isolates** | **25** | **50** | **75** | **100** | **Control** |
| *Klebsiella pneumoneae* | 10.28±0.20$^a$ | 12.64±0.12$^a$ | 13.62±0.17$^a$ | 14.54±0.17$^b$ | 22 |
| *Salmonella typhi* | 12.58±0.12$^a$ | 13.98±0.17$^b$ | 15.44±0.25$^b$ | 17.20±0.20$^b$ | 21 |
| *Shigella sp* | 10.76±0.32$^a$ | 12.85±0.25$^a$ | 15.10±0.32$^b$ | 18.87±0.37$^b$ | 22 |
| *Pseudomonas aeruginosa* | 11.60±0.12$^a$ | 12.82±0.36$^a$ | 14.29±0.15$^b$ | 15.54±0.23$^b$ | 20 |
| *Escherichia coli* | 12.38±0.32$^a$ | 13.92±0.20$^b$ | 15.18±0.12$^b$ | 16.87±0.32$^b$ | 22 |

Key:Values having different superscript on the same row are considered significantly different at $p<0.05$

**Table 4** Minimum inhibitory concentration (MIC) and MBC of the extracts

| | Aqueous extract | | Ethanol extract | |
|---|---|---|---|---|
| **Isolates** | **MIC(mg/ml)** | **MBC(mg/ml)** | **MIC (mg/ml)** | **MBC(mg/ml)** |
| *Klebsiella pneumoneae* | 25 | 50 | 6.25 | 25 |
| *Salmonella typhi* | 6.25 | 12.5 | 6.25 | 25 |
| *Shigella sp* | 12.5 | 50 | 6.25 | 50 |
| *Pseudomonas aeruginosa* | 12.5 | 50 | 12.5 | 25 |
| *Escherichia coli* | 6.25 | 25 | 3.125 | 12.5 |

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## Discussion

In the present study, the preliminary phytochemical screening of the plant material (leaves of *S. siamea*) revealed the presence of alkaloid, tannin, saponin, glycoside, steroid and anthraquinone Flavonoid, Terpenoid, and Phenols while reducing sugar was absent (Table 1). The presence of various phytochemicals in *S. siamea* extracts has also been reported by many researchers.[13,16,23] The result of phytochemical screening of this study was in conformity with that of Mohammad et al.,[16] who reported the presence of flavonoids, tannins, polyphenols, anthraquinones, saponins, and glycosides in *S. siamea* leaves extract. On the other hand, the result of the present study was contrary to that of Bukar et al.,[15] who reported the absence of flavonoids, saponins and alkaloids in ethanolic extract of *S. siamea* leaves.

These active phytochemicals are 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.[24] Alkaloids comprising a large group of nitrogenous compounds are widely used as cancer chemotherapeutic agents, anaesthetics and Central Nervous Stimulants.[25] Alkaloids are known to play some metabolic roles and control development in living system.[26] Anthraquinones were reported to be used as a laxative.[27] Flavonoids are also present in the extracts as a potent water-soluble antioxidant and free radical scavenger, which prevent oxidative cell damage and also have strong anticancer activity. Flavonoids were reported to suppress tumour growth and prevent blood clots.[28] Thus, 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.

The results of antibacterial activity of *S. siamea* leaves extracts In this study indicated that different extracts of *S. 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 *S. siamea* leaves as mentioned above. The antibacterial activity of *S. senna* has been previously reported by Abo et al.,[29,30] while the dose–dependent antibacterial activity of *S. siamea* has been reported by Ahmed-Alizaga et al.[23] Statistical analysis of the result showed that ethanol extract demonstrated highest antibacterial activity with average zone of inhibition of 13.77±2.16mm among the isolates. This could be attributed to better solubility of the phytochemicals in ethanol when compared to water. Aqueous extracts exerted antibacterial activity against the tested isolates with average zone of inhibition of 11.67±1.54mm. The result of this study was inconformity with that of Ahmed-Alizaga et al.,[23] who found *S. siamea* leaves extracts active against certain bacteria. The result of this study also supported that of Bukar et al.,[15] who reported antipseudomonal activity of *S. siamea* leaves extracts against pathogenic *Pseudomonas aeruginosa*. The result of MIC and MBC of the extracts showed that dilutions of various concentrations of aqueous and ethanol extracts of *Sienna siamea* can inhibit and/or kill the isolates. Lower MIC (3.125mg/ml) was shown by ethanol extract than aqueous extract. MBC of the extract ranges between 12.5-50mg/ml.

## Conclusion

In conclusion, this study revealed that *S. siamea* leaves extracts possess medicinal properties and antibacterial activity that inhibit bacterial growth. The results of the present study show that *S. siamea* leaves extracts are effective against all tested bacteria tested. The antibacterial activities of the extracts are expected perhaps due to the present of bioactive compounds like alkaloid, terpenoid, saponin, tannin, flavonoids and anthraquinones which were dissolved in the solvents. The results of present study have provided the justification for therapeutic potential of *S. siamea* leaves and also used as medicinal plant.

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## Conflict of interest

The author declares no conflict of interest.

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