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Determining the Phytochemical Constituents and the Antimicrobial Activity of Ethanolic Extract of Acassia Leaf *(Senna Siamea*) On Some *Enterobacteriaceae*

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ABSTRACT: *Microorganisms are known to cause different types of infection in both humans and animals. The use of medicinal plants to prevent and treat such infections is increasing. This study* was *aimed at investigating the phytochemical constituents of ethanolic extract of Senna siamea leaf and also to determine its antimicrobial activity on some Enterobacteriaceae which include Escherichia coli, Proteus mirabilis and Klebsiella pneumonia, using agar disc diffusion method. The result revealed that the ethanolic extract of Senna siamea leaf inhibited the growth of these microorganisms to varying proportions with zones of inhibition ranging from 16 to 20 mm as lowest and highest zones of inhibition. Furthermore, the phytochemical screening of the leaf extract indicated the presence of alkaloids, flavonoids, steroids and tannins, while saponins were absence. The presence of alkaloids, flavonoids and tannins in the extracts could be responsible for the observed antimicrobial activity. Findings therefore, recommend the use of Senna siamea leaf in the treatment of enteric fever in traditional medicine.*

KEYWORDS*: Antimicrobial, Phytochemical, Senna siamea and Enterobacteriaceae*

I. INTRODUCTION

MEDICINAL plants possess therapeutic properties or exert beneficial pharmacological effects on the human and animal body (Ghani, 2003). Plants are the source of about 25% of prescribed drugs in the world (Rate *et al*., 2001). In developing countries about 80% people rely on traditional plant based medicines for their primary health care needs (FAO, 2004). There is abundant number of medicinal plants and only small amounts of them were investigated for its biological and pharmacological activities.

The wide range of medicinal plant parts like flowers, leaves, barks, stems, fruits, roots extracts are used as powerful raw drug, possessing a variety of pharmacological activities. Discovery of new pharmaceutical agents from medicinal plants can combat the drastic increase in infectious diseases in many countries especially in rural areas and it has been used as an economic reason as well. Nowadays, there is widespread interest of drugs derived from plants which reflect its recognition of the validity of many traditional claims regarding the value of natural products in health care (Nair *et al*., 2005). Thus, in order to determine the potential use of medicinal plants, it is essential to intensify the study of medicinal plants that finds place in folklore. The application of herbs and medicinal plants in traditional medicine to diagnose, prevent or treat diseases dates back to many centuries among rural communities throughout the world (Conco *et al.,* 1999). The active Phytochemical produced by plants include, alkaloids, phenolic, anthraquinones, flavonoids, phenols, saponins, steroid, tannins, terpenes e.t.c. (Gahukar, 2010).

In recent years, advances have been made in the development of antimicrobial compounds in an effort to check the harmful effects of microorganisms (Bashir, 2012). Bacterial disease results when the harmful bacteria enter the organism then multiply and invade the body's defence mechanism. These pathogenic bacteria enter the body through inhalation, ingestion or damaged skin tissue. The inability of the immune system to stop the bacteria from reproducing and spreading consequently results in the symptoms of bacterial disease (Namukobea *et al.,* 2011). The antimicrobial resistance is the foremost problem all over the world with present antibiotic therapy in treating infectious diseases (Manikandan *et al*., 2011). The development of dug resistance by microorganisms reduces the effectiveness of modern drugs (WHO, 2000). Thus, resistance to antibacterial agents poses threat in many areas of the world especially in the developing countries (Shears, 2000). The integration of traditional and modern medicine is gaining increase recognition globally (Abebe, 1996; WHO, 2000).

Senna siamea belongs to the sub- family fabaceae (Caesalpinioideae) of family leguminosae (Fowler, 2006). Leaf of this plant has been used as vegetables in Thailand (Otimenyi *et al*., 2007). Furthermore, Aliyu (2006), revealed that *S. siamea* is ethno medicinally used as laxative, blood cleaning agent, cure for digestive system, urinogenitory disorders, herpes and rhinitis. A traditional claim have cited *Senna Siamea* Lam to be used for the treatment of typhoid fever, jaundice, abdominal pain, menstrual pain, in addition, it is claimed to be used for reducing sugar level in the blood. It has become necessary to further evaluate the pharmacological potential use of *S. siamea* leaves for the treatment of many other diseases. This research was aimed at investigating the phytochemical constituents of ethanolic extract of *Senna siamea* leaf and evaluating its antimicrobial activity on some *Enterobacteriaceae (Escherichia coli, Proteus mirabilis and Klebsiella pneumonia).*

II. MATERIALS AND METHODS

2.1 Collection and Identification of Plant Materials

Fresh leaves of *Senna siamea* Lam were collected from Biological Sciences Department Garden, of Gombe State University and were authenticated by a botanist in the Department of Biological Sciences.

2.2 Preparation of *Senna siamea* Plant Material

The *Senna siamea* leaves were neatly washed then shade dried for seven days before grounding into fine powder using motor and pestle. The powdered material was then stored in an appropriate container in a cool, dry and dark place until required for use.

2.3 Extraction of *Senna siamea* Plant Material

Twenty five (25) gram of the powdered leaf materials was extracted with 250 mL of ethanol using a soxhlet extractor for one hour and the filtrate was evaporated at 40 ^{0}C as previously described by Fatope *et al*., (1993)

2.4 Phytochemical Screening

2.4.1 Test for Tannin

0.5 g of the extract was mixed thoroughly with 10 mL distilled water and then filtered; 5 mL of the filtrate was then added to 1 mL of 5% Ferric chloride solution. The appearance of blue black, greenish or blue green precipitate indicates the presence of tannins (Ciulci, 1994).

2.4.2 Test for Flavonoid

A few drop of concentrated hydrochloric acid was added to a small amount of an alcoholic extract of the plant material. The immediate development of a red colour indicates the presence of flavonoid (Sofowora, 1993).

2.4.3 Test for Saponin

0.1 g of the powdered plant material was boiled with 10 mL of water for 5 minutes and then filtered. After cooling, 5 mL of filtrate was then diluted with water and shaken vigorously. The formation of persistent foam indicated presence of saponin (Sofowora, 1993).

2.4.4 Test for Steroid

1 mL solution of the extract was added to 1 mL sulphuric acid. The appearance of red colour indicates the presence of steroid (Sofowora, 2006).

2.4.5 Test for Alkaloid

0.5 g of the extract was stirred with 5 mL of 1% hydrochloric acid on a steam bath and filtered. 1 mL of the filtrate was then treated with few drops of Mayer's reagent, the development of a white or creamy white precipitate indicates the presence of alkaloids (Ciulci, 1994).

2.5 Preparation of Sensitivity Disc

Sensitivity discs of about 6 mm in diameter were punched from Whatman's no. 1 filter paper using a file punch, then put onto Bijou bottle. The sensitivity discs were then sterilized in an autoclave at 121 ^{0}C for 15 minutes. Various concentration of sensitivity discs were prepared by measuring 0.2 mL, 0.5 mL and 0.75 mL of the ethanolic leaf extract in different test tubes, and diluted with Dimethyl-Sulphoxide (DMSO) up to 1 mL to produce three different concentrations of 25 % (v/v), 50 % (v/v), and 75 % (v/v) respectively. While 1 ml of the undiluted extract served as 100 % (v/v) concentration. This was followed by placing the improvised paper discs in each concentration. The disc were then allowed to absorb the solution and kept in the refrigerator at 4 ^{O}C before use (Bukar *et al.*, 2010).

2.6 Test Organisms

Clinical bacteria isolates of *Klebsiella pneumonia*, *Escherichia coli* and *Proteus mirabilis* were collected from Gombe State Specialist Hospital and subjected to sub-culturing process and purification by streaking plating methods. Appropriate confirmatory biochemical tests such as gram staining, urease, citrate, indole and KIA were carried out on each of the isolates. Pure culture of various bacteria isolates were maintained in agar slants in refrigerator (4^0C) prior to use.

2.7 Inoculums' Standardization

A loopful of each of the test bacteria isolates were picked using sterile wire loop and emulsified onto 3-4 mL of sterile physiological saline. The turbidity of the suspension was then matched with that of 0.5 Mc farlands standard (Cheesebrough, 2004).

2.8 Sensitivity Testing

Using sterile swab stick, standardized inocular of each isolate was swabbed onto the surface of Mueller Hinton agar contain in separate Petri dishes. Discs of different concentration of the ethanolic extract of *Senna siamea* were then placed on the surface of each of the inoculated plates, then the plates were inverted and allowed to stay for 30 minutes for extract to diffuse into the agar, afterwards, the plates were incubated aerobically at 37 ^{o}C for 24 hours. Zones of inhibition formed around each of the discs were then measured using meter rule (Cheesebrough, 2004).

III. RESULTS AND DISCUSSION

Table I: Shows the Phytochemical screening of the ethanolic extract of *Senna siemea* leaf. The result revealed the presence of alkaloids, flavonoids, tannin and steroids, while saponins were not detected.

Table I. Phytochemical components of ethanolic extracts of *Senna siemea* Leaf

Extract	Alkaloid	Flavanoid	Saponins	Steroid	Tannin
ESE	+	+	-	+	+

ESE= Ethanoloc soxhlet extract, + = Present, - =absence

Table II: Shows the antibacterial activity of the ethanolic extract of *Senna siemea* leaf. The result revealed that *Ethanolic* extract of *Senna siemea* leaf was active against all the tested microorganisms. The result further revealed that the higher the concentration of the leaf extract, the higher its efficacy. Highest activity was observed at 100 % (v/v) and the least at 25 % (v/v) concentration.

Table II Antibacterial activity of ethanolic extract of *Senna siemea* leaf on *E. coli*, *Klebsiella pneumoniae* and *Proteus mirabilis.*

	Zones of inhibition in different Conc. % (v/v)			
Isolates	25	50	75	100
Escherichia coli	14.0	14.5	15.5	16.0
Klebsiella pneumonia	12.5	13.0	14.5	15.0
Proteus mirabilis	12.0	13.5	14.5	14.5

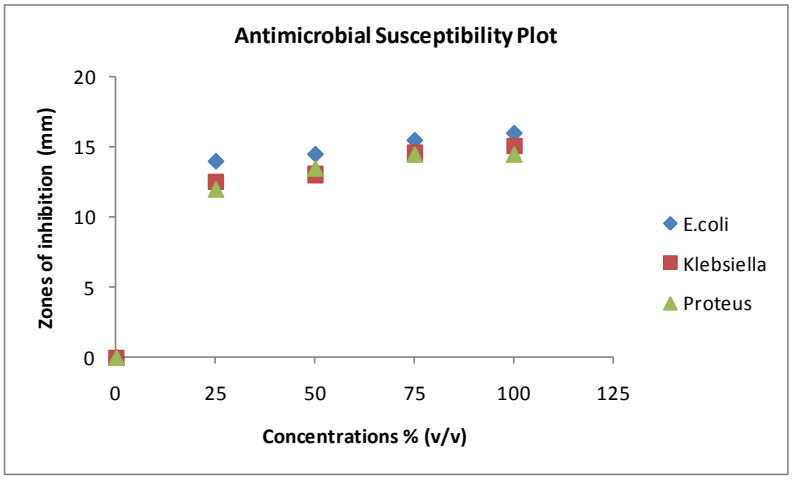

Fig.1: Shows the antibacterial activity of ethanolic extract of *Senna siemea* leaf on *E. coli, K. pneumonia* and *P. mirabilis*

There are abundant numbers of medicinal plants but only few have been investigated for their biological and pharmacological activities. The wide range of medicinal plant parts like flowers, leaves, barks, stems, fruits, roots extracts are used as powerful raw drug possessing a variety of pharmacological activities. This research was aimed at evaluating the antimicrobial activity of ethanolic extract of *Senna siamea* leaf on some *Enterobacteriaceae (Escherichia coli, Proteus mirabilis and Klebsiella pneumonia).*

Phytochemical screening of the ethanolic extract of *Senna siamea* leaf revealed the presence of steroid, alkaloid, flavanoid and tannins while saponins were absent. This finding is in accordance with the work done by Bukar *et al*. (2009), who also confirmed the presence of tannins and steroids in *Senna siamea* leaves. These active phytochemicals are known for their medicinal activity as well as physiological actions; as such they confer the therapeutic potentials of most medicinal plants. Alkaloids, saponins, and tannins have been reported to inhibit bacterial growth and confer protective to plants against fungal infections (Doughari and Okafor, 2008). Flavonoids were reported to suppress tumour growth and prevent blood clots (Seyfulla and Borisora, 1990). Thus, the reported medicinal uses of *S. siamea* in managing constipation, its antimicrobial and antimalarial uses may be attributed to the presence of these phytochemical constituents. Furthermore, this study also revealed that *Senna siemea* contain antimicrobial property, the result showed that the extract is active against *E. coli*, *K. pneumoniae* and *P. mirabilis.* It efficacy increases with an increased in concentration, the highest inhibition was observed in *E. coli* at 100 % (v/v) concentration with zones of inhibition of 16 mm, while the lowest inhibition was observed in *Proteus mirabilis* at 25 % (v/v) concentration, with zone of inhibition of 12 mm. Interestingly, this result is in accordance with the work of Daghouri *et al.*, (2008), which revealed that the ethanolic extract of *Senna siemea* leaf was active against *Salmonella*, and other *Enterobacteriaceae*. The result of this study was in contrast to the work conducted by Bukar *et al*., (2009), which revealed that the ethanolic extract of *Senna siamea* leaf was inactive to *E. coli* at the concentration levels of 100µg/disc and 200µg/disc; however the extracts were active at high concentration levels of 500µg/disc and 1000µ/disc revealing a dose-dependent antibacterial activity. The result of this study supports the fact that *S. Siamea* carries some active bio- components such as alkaloid, flavanoid, tannin and steroid that have therapeutic potentials, and as well support the local uses of this plant.

3.1 Conclusion

Conclusively, this work has confirmed the present of bioactive principles in the leaf of *Senna siamea* and the antimicrobial activity of the extract. The highest zone of inhibition observed on the bacterial isolates may justify the use of the leaf in traditional medicine practices especially for the treatment of infections.

3.2 Recommendation

The result of this study shown the medicinal potentials of leaf of *Senna siamea* as an important antimicrobial agents and can be used in the synthesis of modern drugs for the treatment of bacterial infection. Further research should be carried out with other extracting agents (e.g. methanol and water) to compare the antimicrobial effect of the leaf of with different extracting agents as well as other microorganisms will be a welcome idea.

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