# PHYTOCHEMICAL AND ANTIMICROBIAL EVALUATION OF AQUEOUS AND METHANOL LEAF EXTRACTS OF *Sterculia setigera* ON SOME ABDOMINAL MICROFLORA

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Abstract: The aqueous and methanol extracts of *Sterculia setigera* was analyzed for some of its phytochemical components and evaluated forits antimicrobial properties on some gram-negative abdominal bacteria of humans, *in vitro*. Methanol and distilled water were used for the extraction and phytochemical analysis was performed using standard procedures. Strains of *Escherichia coli, Klebsiella spp* and *Salmonella spp* from stool specimen were inoculated in the appropriate agar and subcultured in Eosin Methylene Blue (EMB) agar, *Salmonella-shigella* gar (SSA) and Müller-Hinton agar, respectively. Disc diffusion method was used to test the antimicrobial effect of *S. setigera* extracts in triplicates at 37ºC for 24 h. The zones of inhibition were measured. Results of phytochemical analysis showed high levels of *tannins, alkaloids, saponins and flavonoids* in bott extracts. The methanol extract of *S. setigera* showed zero antimicrobial effect. The aqueous extract showed considerable effect with the medium and high doses on the gram-negative inoculum. This justifies the use of aqueous decoction of *S. setigera* by traditional medical practitioners for the treatment of abdominal ailments. There is need for further studies on the aqueous extract of *S. setigera* with a view of purifying and identifying the active principle responsible for this activity.

**Keywords:** Aqueous, extract, methanolic, *Sterculia setigera*, antimicrobial, microflora

## Introduction

A large proportion of the world population nowadays uses herbal based medicines for the treatment and management of diseases (Martson *et al*., 1993). According to World Health Organization (2003) statistical data, 80% of the African population relies primarily and sometimes exclusively on Traditional Medicine to solve their health problems. This medicine provides the basic information that help to develop out new drugs (Farns *et al*., 1995)

Rubiaceae are a family of plants widely used in Tanzania and other eastern African countries to treat various diseases including stomach ache, diarrhea, skin diseases, earache and venereal diseases (Louppe *et al*., 2008; Moris *et al*.,1996).

Nowadays, many people suffer from different forms of disease such as diarrhea, dysentery, snake bites, wounds, skin ache etc. In a bit to cure such diseases, many ignorantly engage in abuse of antibiotics thereby leading to antibiotic drug resistances resulting to more complicated health challenges. This research work aimed at finding the medicinal plant medical properties of *Sterculia setigera* which is traditionally available, easy and cheap to afford for the treatment. This will lessen the financial burden of treatment for the wellbeing of the communities. The knowledge of the phytochemical and antimicrobial activities of the *S. setigera* is important in developing different antimicrobial drugs.

There has been renewed interest currently being experienced by traditional medicine practitioners in the area of antimicrobial agents. This has led us to show great a great of interests in order to prepare effective extracts for the treatment of some infectious and parasitic diseases that affect humanity, especially people in remote areas who find it difficult to access orthodox medicines.

The proliferation of pathogenic microorganisms due to improper and inappropriate use of antibiotics currently poses a public health problem. Antibiotic resistance sometimes makes the therapeutic treatment expensive and ineffective (Cimanga *et al.*, 2002). Owing to diseases care requirements, less expensive traditional treatments based on medicinal plants are offered. Plants stand as a source of new molecules endowed with antimicrobial activity (Burt, 2004). This research therefore is one of the numerous efforts targeted at exploring local products as antimicrobial agents.

## Materials and Methods

### *Materials*

#### *Plant material*

The plant material used for the study was dried stem bark of *Sterculia setigera.* It was collected from Wumdio village, Askinal Uba Local Government in Borno State. Identification of the plant material was done at the Department of Plant Science and Biotechnology, Nasarawa State University, Keffi, Nigeria.

#### *Instruments/equipment*

The instruments/Equipment used for the study include; Mechanical blender, Electronic weighing balance, hot plate, incubator, rotary evaporator, spectrophotometer, incubator, water bath, autoclave (all from Jenway, U.K), Micropipette, Cuvette (quartz), strings and glasswares.

#### *Chemicals/reagents*

All the chemicals and reagents used for the work were of analytical grades and products of Sigma Aldrich Ltd. (USA). They include; Methanol, Hydrochloric acid, Lead acetate, Tetraoxosulphate (vi) while the reagents include; Wagner's reagent, sterilize saline, Eosin, methylene blue agar (EMB),

#### *Culture media for Salmonella shigella*

The culture media used include; *Salmonella shigella a*gar (SSA), Nutrient broth, Nutrient agar, Deoxycholate Citrate Agar (DCA) and MacConkey agar.

### *Methods*

#### *Preparation of the methanol extract*

250 g of sample powder was macerated by mixing with organic methanol and left for 48 h. Each resulting solution was filtered with Whaan filter paper No 1, the residual was soaked for the second time with 500 ml and filtered with filter paper for 24 h. Then, for the third term the residual was soaked with 250 ml for another 24 h. Concentration of the extract was done using rotary evaporator, following air circulation in an oven at 54ºC until total dryness.

***Preparation of aqueous extract***

The aqueous extract was prepared by suspending 114 g of sample powder in 250 ml of distilled water shaken and left for 48 h; solution was filtered with what man filter paper No. 1.

***Phytochemical analysis***

Phytochemical tests were carried with different solvent extracts using standard procedures to identify the constituents as described by Harbone (1984).

***Test for phenol***

**Ferric chloride test:** Extracts were treated with 3 – 4 drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenol. No bluish black color appeared.

***Test for flavonoids***

**a) Alkaline reagent test:** Extracts were treated with a few drops of sodium hydroxide solution. Formation of intense yellow color appeared, which becomes colorless on the addition of dilute acid, indicates the presence of flavonoids.

**b) Lead acetate test:** Extracts were treated with a few drops of lead acetate solution. Formation of yellow color precipitate appeared, indicates the presence of flavonoids.

***Test for alkaloids***

Extracts were dissolved individually in dilute hydrochloric acid and filtered.

**a) Mayer's test:** Filtrates were treated with Mayer's reagent (potassium mercuric iodide). Formation of a yellow colored precipitate appeared which indicates the presence of alkaloids.

**b) Wagner's test:** Filtrates were treated with Wagner's reagent (Iodine in potassium iodide). The formation of brown reddish precipitate appeared, indicates the presence of alkaloid.

**c) Dragendroff test:** Filtrates were treated with Dragendorff's reagent (solution of potassium bismuth iodide). Formation of a red precipitate observed indicates the presence of alkaloids.

**d) Hager's test:** Filtrates were treated with Hager's reagent (saturated picric acid solution). Formation of yellow color appeared which indicates the presence of alkaloid.

***Test for tannins (Ferric chloride test)***

To 0.2 g of extract was added 10 ml of 45% ethanol, boiled for 5 min and then filtered. To 1 ml filtrate, 200 µl of ferric chloride was added. An observation of brownish green precipitate indicates the presence of tannins.

***Test for saponin***

A quantity (0.2 g) of extract was dissolved with 10 ml distilled water, warmed for a minute and then filtered. To 1 ml filtrate was added 4 ml of distilled water, shaken thoroughly for 5 min and allowed to stand for 1 min. Persistence of foam indicates the presence of saponins.

***Collection of microorganism***

Human stool sample was collected from some volunteer donors at Nasarawa State University, Keffi. It was cultured in a prepared nutrient broth. It was then dissolved and sterilized in autoclave then cooled before stool samples were inoculated and incubated for 24 h. Standard microorganisms and clinical isolates were gotten from Microbiology Department laboratory, Nasarawa State University, Keffi where they are maintained lyophilized.

***Detection, identification and isolation of test***

Nutrient broth was prepared according to the manufacturers' direction; 0.9 ml of dissolved nutrient broth was measured into six different bottles and sterilized at 121ºC for 15 min in the autoclave. Then, little sample of stool was inoculated in a bottle and left for 24 h. Then, 1.15 g eosin methylene agar (EMB) was prepared and autoclave at 121ºC for 15 min. Then, poured in the petri dishes to solidify. Then, 1.2 g of *Salmonella shigella* Agar was prepared and poured in a Petri dishes to solidify, the petri dishes were then incubated for 24 h. Both *Escherichia coli* and *Klebsiella* were subcultured in EMB agar and *Salmonella* subcultured in SSA to confirmed, microorganism isolated and stored in nutrient agar as a slant for future use.

***Preparation the of cultured medium and inoculated of test microorganism***

A ten-fold serial dilution was made to reduce the concentration of the cultured microorganisms by a factor of ten that is to one-tenth of the original concentration by the use of a micropipette to transfer 1 ml of the test solution into 9 ml of the diluent in a glass tube. The tube was mixed by shaking and then 1 ml was micropipette from the first bottle into another glass tube containing 9 ml of the diluent and from second to third and from third to fourth bottle respectively.

***Determination of susceptibility of bacterial isolates to plant extract.***

***Preparation of Muller Hinton agar***

Muller Hinton agar was prepared according to manufacturer's directions. The test microorganisms were streaked on three different Mueller Hinton agar plates and incubated for 24 h (Igoli *et al*., 2005).

***Preparation of dosages***

Three different concentrations of each extract were obtained by suspending 1.083 g of extract in 30 ml of absolute methanol and another 1.083 in 30 ml distilled water until it dissolved which is equivalent to 36 g/ml served as low dosage. 2.17 g extract dissolved in 30 ml of methanol and another 30 ml of distilled water until it dissolved equivalent to 72 g/ml served as mid dosage. Lastly, 4.33 g of extract dissolved in 30 ml of methanol and as well as 30 ml of distilled water equivalent to 144 g/ml served as high dosage as outlined in Zaruwa *et al*. (2015).

**Table 1: Preparation of dosages**

| Dosage | Weight of extract (g) | Methanol solvent (ml) | Distilled water (ml) | Conc. (mg/ml) |
|---|---|---|---|---|
| Low | 1.08 | 30 | 30 | 36 |
| Medium | 2.17 | 30 | 30 | 72 |
| High | 4.33 | 30 | 30 | 144 |

Then, a perforated paper was used whose diameter was 6mm made from whatman No. 4 filter paper and sterilized about 151 pressure for 15 min at 121ºC temperature soaked inside different concentrated extract, impregnated over the surface of plate inoculated with microorganism and allow to incubate for 37ºC the 24 h.

***The disc diffusion method***

Antibacterial activity was determined using the agar disk diffusion method on plant extracts immediately after this preparation (Akpauka *et al*., 2003). Nutrient broth was used to culture bacteria, fresh overnight cultures of inoculum (0.1 ml) of each culture, was spread on agar plate or Muller Hinton agar. The plates were then kept for 10 min, and then the prepared sterilized discs were impregnated over the surface of the plate inoculated with the microorganism and allowed to incubate at 37ºC for the 24 h. The zone of inhibition in millimeter was determined after incubation period, the microbes were plated in triplicated and average zone of inhibition diameter were noted.

**Results and Discussion**

***Phytochemical composition of methanol leaf extract of Sterculia setigera plant***

Phytochemical analysis of *Sterculia setigera* aqueous and methanol extracts showed the presence of tannins, alkaloids, saponins and flavonoids (Table 2).

**Table 2: Phytochemical composition of aqueous and methanol leaf extract of *Sterculia setigera***

| Phytochemical | Inferences |
|---|---|
| Tannins | + |
| Alkaloids | + |
| Saponins | + |
| Flavonoids | + |

+ = present

**Table 3: Inhibition zones of aqueous leaf extract of *Sterculia setigera***

| Extract | Conc. (mg/ml) | Microorganisms (mm) | | |
|---|---|---|---|---|
| | | *Salmonella spp* | *Klebsiella spp* | *E. coli* |
| AQE | 36 | – | – | – |
| AQE | 72 | 10 | 7 | 8 |
| AQE | 144 | 12 | 11 | 11 |

– = No zone of inhibition, AQE = Aqueous extract

***Inhibition zones of aqueous leaf extract of Sterculia setigera***

The aqueous extract of *Sterculia setigera* at concentration of 36 mg/ml showed no inhibition for any of the innoculum (*Salmonella spp, Klebsiella spp* and *E. coli*). At 72 mg/ml, *Salmonella spp* showed inhibition of 10 mm, *Klebsiella spp* (7 mm) and *E. coli* (8 mm) and at 144 mg/ml, *Salmonella* showed inhibition of 12 mm, *Klebsiella spp* (11 mm) and *E. coli* (11 mm) (Table 3).

***Antimicrobial activities of methanol leaf extract of Sterculia setigera***

Antimicribial activity of the methanol extract of *Sterculia setigera* showed no growth of the inoculated microorganisms (*Salmonella spp, Klebsiellar spp* and *E. coli*) nor turbidity of the solution (Table 4).

**Table 4: Antimicrobial activities of methanol plant extract of *Sterculia setigera***

| Extract | Conc. (mg/ml) | Microorganisms (mm) | | | | | |
|---|---|---|---|---|---|---|---|
| | | *Salmonella spp* | | *Klebsiella spp* | | *E. coli* | |
| | | T | G | T | G | T | G |
| MEE | 36 | T | G | – | – | – | – |
| MEE | 72 | T | G | – | – | – | – |
| MEE | 144 | T | G | – | – | – | – |

– = No zone of inhibition, G = Growth, T = Turbidity, MEE = Methanol extract

Many medicinal plants in traditional use are considered to be potential antimicrobial drugs as well as source for unique compounds with anti-microbial activities, with possibly new modes of action (Doughari *et al*., 2009). Phytochemical analysis conducted on the leaf extract of *Sterculia setigera* revealed the presence of constituents which are known to exhibit medicinal properties. The constituents include flavonoids, alkaloid, saponin and tannins. The antimicrobial properties of flavonoids have been shown to inhibit topoisomerase and to induce DNA mutations in the mixed-lineage leukemia (MLL) gene in *in vitro* studies (Essene *et al*., 2009). Tannins act by iron deprivation, hydrogen bonding or nonspecific interactions with vital proteins such as enzymes in some microorganisms. It also binds to proline rich proteins and interfere with the protein synthesis in bacteria (Scalbert *et al*., 1991). Antimicrobial properties of saponins may be due to its ability to cause leakage of proteins and certain enzymes from the organism's cell (Cowan *et al*., 1999). The antimicrobial effects of alkaloids may be through another mechanism, since the compound is known to be a DNA intercalator and an inhibitor of DNA synthesis through topoisomerase inhibition (Dassonneville *et al*., 2000). The antimicrobial potency of *Sterculia setigera* leaf extract have been further corroborated by this study with its observed inhibitory activities on *E. coli, Klebsiellar spp* and *Salmonella spp* in a concentration dependent manner as its inhibitory effects on the microbes increased with increased concentrations. The antimicrobial activity of aqueous extract was performed at different concentrations indicating that the activity of the extract decreased as concentration decreased. The non-inhibition activity of the aqueous extract on *Salmonella, Klebsiella spp* and *E. coli* may be attributed to the solvent or low amount of alkaloid and flavonoid present. Alkaloids have been implicated in the inhibition activities of many bacterial species (Nuhu *et al*., 2000; Hassan *et al*., 2004; and Alinnor *et al*., 2008).

Methanol extract showed no zone of inhibition in gram negative bacteria such as *salmonella spp, Klebsiella spp* and *E.coli.* Repeated test at the same dosage yielded no activity*;* this may be because there was no presence of phenols (Sun *et al*., 2005). According to certain studies acetone 80% is the best extraction solvent to extract secondary metabolite since it is able to extract more phenolic compounds than other solvent (Sun *et al*., 2005). On the whole, since traditional medicine practitioner attributes very high antimicrobial effects to this plant extracts, it is possible that some internal chemicals in the human gut (GIT) like amylases and sulfation enzymes may aid in making the extract active. It could possibly be that the bioactive compounds in the extract need to undergo biotransformations in order to become potent antimicrobials.

**Conclusion**

From the results, methanol and aqueous leaf extracts of *Sterculia setigera* contained some phytochemicals. While the aqueous extract showed growth inhibition of some gram negative bacteria *in vitro*, the methanol extract did not show significant antimicrobial activity against the isolated and tested microbes. Therefore, methanol leaf extract of *Sterculia setigera* used traditionally as medicine may have other factors yet to be elucidated scientifically for its speculated antimicrobial function while that of the antimicrobial activity of the aqueous extract may be justified by this findings.

**Conflict of Interest**

Authors have declared that there is no conflict of interest reported in this work.

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