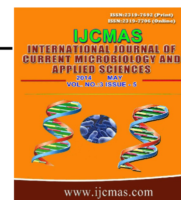

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Original Research Article

Evaluation of *in vitro* antibacterial activity in *Senna didymobotrya* roots methanolic-aqua extract and the selected fractions against selected pathogenic microorganisms

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A B S T R A C T

The study was done to evaluate antibacterial activity in *Senna didymobotrya* roots methanolic-aqua extract and the selected fractions against selected pathogenic bacterial organisms**.** The aqueous fraction of *S. didymobotrya* root inhibited *B.cereus* giving the best results followed by *Salmonella typhi*, *P.vulgaris*, *S. liquefaciens* and *E. coli* respectively. There was no inhibition for *E. aerogenes*. Analysis of variance (ANOVA) showed that the organisms were significantly different in the zones of inhibition ($p< 0.05$). The ethyl acetate fraction of *S. didymobotrya* root extract inhibited *B. cereus* the best followed by, *Salmonella typhi*, *P.vulgaris*, *S. liquefaciens* and *E. coli* respectively(Table 1). The extract fraction did not inhibit *E. aerogenes*. The crude extract of *S. didymobotrya* root inhibited all the organisms, with the best zone of inhibition been that of Bacillus cereus (29.67±0.882), followed by *P.vulgaris* (16.67±0.667), *Salmonella typhi* (15.67±0.667), *E. coli* (13.33± 0.667), *E. aerogenes* (12.00±0.000) and *Serratia liquefaciens* (11.33±0.667). These results have shown that *S. didymobotrya* extract show significant activity against all the organisms tested. The inhibition of the plant roots extract to the growth of all the organisms greatly depicts the plant roots to have great potency towards the treatment of diseases caused by the organisms. The antibacterial activity of the plant roots is due to the presence of important phytochemicals as observed in previous studies. Further research needs to be done in order to isolate the active compounds, their structural elucidation, mode of action and their effect in the in vivo environment.

Keywords

Senna, antibacterial, medicinal, plant, pharmaceutical, roots.

Introduction

Plants have been known since ancient times and therefore scientists have found them to be a better choice in such for bioactive compounds (Khan, et al., 2011;

Jeyaseelan, et al., 2010). In continuation with our research on medicinal plants , we have turned our attention to the Roots of *Senna didymobotrya* plant. It is mainly found along lakeshores, streams, rivers, deciduous, bush land and old plantations. The plant is hardly attacked by disease or pests. *Senna didymobotrya* is locally known as senetwet. It is used locally in the preparation and preservation of 'mursik' which is the local name for fermented milk, hence, the name mursik plant (Tabuti, J.R.S. 2007; Ngule, et al., 2013). Microbial resistance to the currently used antibiotics has greatly increased in the last four decades despite efforts by pharmaceutical industries to produce new antibiotics. Several measures have been put in place in various countries all over the world to control the spreading of drug resistant microorganisms, however, the microorganisms have continued to develop new ways to mutate and acquire resistance to drugs (Nasciment, et al., 2000. According to Montellia and Levy (1991), data collected on resistant microorganisms shows the period between 1980-1990 to have recorded the highest number of microbial drug resistance. The increase on the number of drug resistance microorganisms calls for quick action to control the situation.

Plants have been used since time immemorial to treat most of the diseases affecting human kind. The introduction of synthetic drugs, however, changed the trend and attracted many to turn to use them on the expense of botanical drugs, a trend which according to researchers is changing and many people are using medicinal herbs. According Ngule (2013), about 80% of the individuals from developing countries use traditionally known plants as medicine. The world health organization (WHO), recommends medicinal plants to be the best source of a variety of drugs (Santos Filho et al., 1990). Botanical medicine is the oldest known type of medicine. The use of plants as source of medicine is as old as the origin of man himself. Medicinal plants have been used widely over all the cultures as a source of drugs for treatment of various ailments affecting human beings and animals (Sigh, and Singh, 2010).

The medicinal values of plants are attributed to pharmacologically active compounds that have no direct impact on the plants main processes but research has proved these compounds to have great medicinal values. These compounds that the plant uses to protect itself against predators are called secondary metabolites or phytochemicals. Over the recent decades scientist have developed great interest on botanicals to isolate these compounds through various methods such as column chromatography and thin layer chromatography in order to purify them and study their structural elucidation. The studies already done have shown plants to have great potentials in the treatment against drug resistant microorganisms (Muroi, H., Kubo, I. 1996).

Medicinal plants have been tested extensively and found to have great pharmacological uses such as anti-inflammatory activity, antibacterial activity, anti- diabetic activity, anti-fungal activity, anticancer activity, antioxidant activity, hepatoprotective activity, haemolytic activity, larvicidal activity, anthelmintic activity, pain relief activity, central nervous system activity, sexual impotence and erectile dysfunction (Hosahally, et al., 2012; Farook, et al., 2011; Kisangau, et al., 2007; Kamatenesi-Mugisha, M. and Oryem-Origa, 2005; Adu, et al., 2011; Deepa, N. and

Rajendran, 2007; Joshi et al., 2011; Arivoli, S. and Tennyson, 2012). The plant *Senna didymobotrya* is used traditionally to treat against various diseases. The great potency which the plant has demonstrated traditionally therefore creates the need for scientific justification on the medicinal value of the plant. The plant is used traditionally in the treatment of enteric problems, as an anthelmintic, treatment against fungal infections and in the preservation of milk by the Nandi community in Kenya. The current study was done to analyse the antibacterial activity of the plant against selected pathogenic microorganisms.

Materials and Methods

Sample Collection and Preparation

The herb was randomly collected in the natural forest around University of Eastern Africa, Baraton and identified by a taxonomist in the University of Eastern Africa, Baraton. The samples were thoroughly mixed and spread to dry at room temperature in the chemistry laboratory for about three weeks. They were then ground into fine powder and put in transparent polythene bags.

Extraction procedure

Using electric analytical beam balance 100 grams of the powdered roots of the *Senna didymobotrya* was placed in 1000 ml conical flask, methanol and water were then added in the ratio of 9:1 respectively until the roots were completely submerged in the solvent. The mixture was then agitated for thorough mixing and kept for 24 hours on a shaker for effective extraction of the plant components. The extract was filtered using Butchner funnel; Whatman no.1 filter paper and a vacuum and pressure pump. The filtrate was re-filtered again using the same apparatus. The solvent was evaporated using rotary vacuum evaporator (R-11) with a water bath at 40°C. The crude extract was then dissolved in different solvents according to polarity and the resulting extracts concentrated to remove the solvents. The solvents used were chloroform, ethyl acetate, butanol and water respectively. The residues were then obtained and used for the experiment.

Bioassay Study

Preparation of the Bacterial Suspension

The turbidity of each of the bacterial suspension was prepared to match to a 0.5 McFarland standard, a procedure similar to that used by *Biruhalem* (2007) and Donay *et al.,* (2011). The McFarland standard was prepared by dissolving 0.5 g of $BaCl_2$ in 50 ml of water to obtain a 1% solution of Barium chloride (w/v). This was mixed with 99.5 ml of 1% sulphuric acid solution. Three – five identical colonies of each bacterium was taken from a blood agar plate (Himedia) culture and dropped in Mueller Hinton broth (Himedia). The broth culture was incubated at 37^{0}C for 2 - 6 hours until it achieved turbidity similar to the 0.5 McFarland standards. The culture that exceeded the 0.5 McFarland standard were each adjusted with the aid of a UV spectrophotometer to 0.132A^{0} at a wavelength of 600 nm in order to obtain an approximate cell density of $1x10^{8}$ CFU/ml.

Preparation of the Extract Concentrations and Antibiotic

Extracts stoke solutions were prepared by dissolving 500 mg in 1 ml of dimethylsulfoxide (DMSO). An antibiotic

control was made by dissolving 500 mg of penicillin in 1 ml of sterile distilled water. DMSO served as a negative control.

Determination of bioactivity of the Extract

Mueller Hinton agar plates were prepared by the manufacturer's instruction. 0.1 ml of each of the prepared bacterial suspension for the test was transferred to 3 plates for each organism to give a triplicate for each concentration and organism. Five wells were drilled in each agar plate. Three of the wells were filled with the extract dilution and the other wells were filled with penicillin and DMSO control respectively. Three plates were made for each bacterial organism and extract giving a triplicate reading for each microorganism and extract. The plates were labeled on the underside and incubated at 37^{0}C for between 24 to 48 hours and the zones of inhibition measured in millimeters with the aid of a ruler.

Results and Discussion

The *S. didymobotrya* crude root extract inhibited all the organisms (table 1) with the best zone of inhibition been that of *Bacillus cereus* (29.67±0.882) as shown fig.6, followed by *P. vulgaris* (16.67±0.667) as shown in fig. 7, *Salmonella typhi* (15.67±0.667), *E. coli* (13.33± 0.667), *E. aerogenes* (12.00±0.000) and *Serratia liquefaciens* (11.33±0.667). These results have shown that *S. didymobotrya* root extract had significant activity against all the organisms tested. The control penicillin also inhibited the organisms and DMSO negative control showed no inhibition at all. The zones of inhibitions of the organisms were also represented in a bar graph in order to clearly show the variation among inhibition caused by the plant extract and that caused by the positive control (fig. 1).

The *S. didymobotrya* root showed significantly higher zones of inhibition against *B. cereus* compared to *E. coli* ($p<0.001$). The extract inhibited *P. vulgaris* significantly higher than *E. coli* ($p<0.05$). Inhibition for *Salmonella typhi* was significantly higher than that of *S. liquefaciens* ($p<0.001$), while that of *Salmonella typhi* was higher than that of *E. aerogenes* ($p<0.05$) and *Bacillus cereus* was significantly higher than that of *Salmonella typhi* ($p<0.001$). Inhibition against *B. cereus* was significantly higher than all the organisms (Table 2). Inhibition for *P. vulgaris* was significantly higher than *S. liquefaciens* ($p<0.001$) and that of *P. vulgaris* was significantly higher than that of *E. aerogenes* ($p<0.001$). All the organisms were inhibited by the penicillin control significantly higher than the extract. The DMSO negative control did not show any inhibition against any of the organisms.

The ethyl acetate fraction of *S. didymobotrya* root extract inhibited *B. cereus* the best followed by, *Salmonella typhi*, *P. vulgaris*, *S. liquefaciens* and *E. coli* respectively (Table 3). Extract fraction did not inhibit *E. aerogenes.* All the organisms were inhibited by the penicillin positive control but were not inhibited by the DMSO negative control. The difference in the zones of inhibition caused by the plant extract and those caused by the positive control are shown in fig. 2.

Multiple comparison showed that *S. liquefaciens* was inhibited significantly higher than *E. coli* ($p<0.001$), but *E. coli* was significantly higher than *E. aerogenes*

($p<0.001$). *E. coli* was however not significantly different from *P. vulgaris* and *Salmonella typhi* ($p>0.05$). *Salmonella typhi* was also not significantly different from that of *P. vulgaris* ($P>0.05$). *B. cereus* was significantly higher than all the organisms ($p<0.001$). All the organisms were inhibited significantly higher by the penicillin control compared to the extract ($p<0.001$). The DMSO negative control had no inhibitory effect on the organisms. Zones of inhibition for *Salmonella typhi* was significantly lower than that of *S. liquefaciens* ($p<0.001$) but higher than *E.aerogenes* ($p<0.001$). *S. liquefaciens* zone of inhibition by the extract was higher than *E.aerogenes* and *P.vulgaris* ($p<0.001$). *P.vulgaris* zone of inhibition by the extract was significantly higher than *E. aerogenes* ($p<0.001$). These results (Table 4) have proved that it is possible to limit the spread of the selected microorganisms using *S. didymobotrya* root extract and hence, the extract can be incorporated as a component of pharmaceutical formulations against the pathogenic organisms.

The aqueous fraction of *S. didymobotrya* root as shown in table 5, inhibited *B.cereus* giving the best results followed by *Salmonella typhi, P.vulgaris, S. liquefaciens* and *E.coli* respectively. There was no inhibition for *E.aerogenes*. Analysis of variance (ANOVA) showed that the organisms were significantly different in the zones of inhibition ($p< 0.05$). Fig 3 shows the difference in the zones of inhibition caused by the plant extract and that caused by the penicillin control.

Tukey's multiple comparison, however, showed (table 6) that the zone of inhibition for *E. coli* was significantly higher than that of *E. aerogenes* ($p< 0.001$) but significantly lower than those of *B. cereus*, *Salmonella typhi, P. vulgaris* ($p<0.001$) and *S. liquefaciens* ($p< 0.05$). Zones of inhibition of *Salmonella typhi* compared to those of *S. liquefaciens* and *P. vulgaris* were significantly higher but significantly lower than those of *B. cereus* ($P<0.01$). There was no significance difference between the zones of inhibition of *Salmonella typhi* and those of *E. aerogenes* ($p>0.05$). The zones of inhibition of *S. liquefaciens* were found to be significantly higher than those of *E. aerogenes* but significantly lower than those of *B. cereus* ($p<0.001$). There was no significance difference in the zones inhibition between *S. liquefaciens* and those of *P. vulgaris* ($p> 0.05$). The zones of inhibition of *E. aerogenes* were significantly lower than those of *B. cereus* and those of *P. vulgaris*, while those of *B. cereus* were found to be significantly higher than those of *P. vulgaris* ($p<0.001$). The zones of inhibition of penicillin against all the tested microorganisms were found to be significantly higher than those on the organisms against the microorganisms ($p<0.05$).

The butanol extract (table 7) was found to inhibit the growth *Enterobacter aerogenes* (14.000±1.528), and *Bacillus cereus* (24.667±0.333). The extract did not inhibit the growth of all the other organisms. The positive control inhibited all the organisms while DMSO showed no zone of inhibition. Analysis of variance (ANOVA) showed that the organisms were significantly different in the zones of inhibition ($p< 0.05$). Fig. 4 shows the difference in the zones of inhibition caused by the butanol fraction and those caused by the penicillin positive control.

Tukey's pair wise comparison (Table 8) showed that the zones of inhibition of

E.coli were significantly lower than those of *E. aerogenes* and *B. cereus* ($p < 0.05$), however, the organism showed no significance difference against all the other organisms ($p > 0.05$). The zones of inhibition of *S. liquefaciens* were significantly lower than those of *E. aerogenes* and *B. cereus,* however the organisms zones of inhibition were not significantly different as compared to those of *P. vulgaris* ($p>0.05$).The zones of inhibition of *Salmonella typhi* were significantly lower than those of *E. aerogenes* and *B. cereus* ($p< 0.05$), the organism inhibitions were not significantly different in comparison to the other organisms ($p>0.05$). The zones of inhibition of *E. aerogenes* were significantly higher than all the organisms but significantly lower than those of *B. cereus* ($p<0.05$). The zones of inhibition of *B. cereus* were significantly high than all the other organisms. The zones of inhibition of penicillin were significantly higher than all the inhibitions caused by the plant extract. The data obtained shows the butanol fraction can be used to treat against *Bacillus cereus* and *E. aerogenes* bacteria.

The chloroform fraction (Table 9) was found to inhibit the growth of *Bacillus cereus* (20.667±0.333), *S. liquefaciens* (10.000±0.000) and Proteus vulgaris (12.667±0.333); however, the plant did not inhibit the growth of all the other organisms it was tested against. Penicillin inhibited the growth of all the organisms while DMSO did not show any inhibition zones against all the organisms it was tested against. The analysis of variance showed that the organisms were significantly different in their zones of inhibition ($p < 0.05$). The bar graph (fig.5) shows the difference in the zones of inhibition caused by the chloroform fraction extract and those caused by the positive control against the microorganisms.

Tukey's pair wise comparison (table 10) showed the zones of inhibition of *E. coli* were significantly lower than those of *S. liquefaciens, B. cereus and P. vulgaris* ($p<0.05$), however, here was no significant difference in the zones inhibition between the *E. coli* all the other remaining organisms ($p>0.05$). The zones of inhibition of *Salmonella typhi* was significantly lower than those of *B. cereus, S. liquefaciens and P. vulgaris,* however, there was no significant difference in the zones of inhibition of *Salmonella typhi and E. aerogenes* ($p>0.05$). The zones of inhibition of *S. liquefaciens* were significantly higher than those of *E. aerogenes* but significantly lower than those of *B. cereus;* however, the zones of inhibition of *S. liquefaciens* had no significance difference as compared to those of *P. vulgaris*. The zones of inhibition of *E. aerogenes* were significantly lower than those of *B. cereus and P. vulgaris.* The zone of inhibition of *B. cereus* were significantly higher than those of *P. vulgaris.* The zones of inhibition of penicillin against all the tested bacteria were significantly higher than those of the crude and the selected fractions.

The results obtained in this research are inconformity with those obtained by Ngule (2013), in which the plant leaves were found to inhibit the growth *Salmonella typhi* with 12.50±0.563, *Klebsiella sp*., 14.33±0.211, *Bacillus cereus* 19.00±0.258, *Streptococcus pyogenes* 11.67±0.494, *Escherichia coli* 12.17±0.477, *Proteus vulgaris* 10.83±0.477, *Enterobacter aerogenes* 10.33±0.615. The roots of the plant contain the important phytochemicals such as, saponins,

Table.1 Antimicrobial activity of *Senna didymobotrya* roots Crude extract (Mean Zone of Inhibition ± S.E.)

Microorganisms	Mean ± S.E	Penicillin	DMSO
Escherichia coli	13.33± 0.667	40.00± 0.000	0.00±0.000
Salmonella typhi	15.67±0.667	35.33±0.333	0.00±0.000
Serratia liquefaciens	11.33±0.667	40.67±0.333	0.00±0.000
Enterobacter aerogenes	12.00±0.000	32.33±0.333	0.00±0.000
Bacillus cereus	29.67±0.882	39.00±0.000	0.00±0.000
Proteus vulgaris	16.67±0.667	34.67±0.333	0.00±0.000

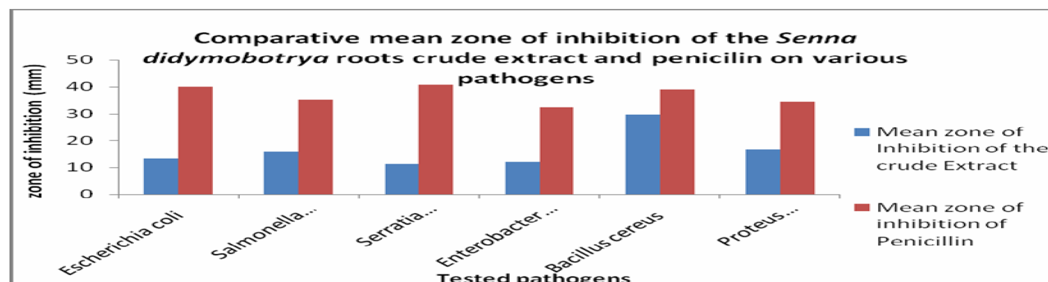

Fig. 1

Table.2 Tukey's honestly significant Difference among microorganisms using 500 mg/ml of *Senna didymobotrya* roots extract

Comparison	P-value	Significance
E. coli vs *S. typhi*	0.095	NS
E. coli vs *S. liquefaciens*	0.229	NS
E. coli vs *E. aerogenes*	0.757	NS
E. coli vs *B. cereus*	0.000	S
E. coli vs *P. vulgaris*	0.004	S
E. coli vs *E. coli control*	0.000	S
S. typhi vs *S. liquefaciens*	0.000	S
S. typhi vs *E. aerogenes*	0.001	S
S. typhi vs *B. cereus*	0.000	S
S. typhi vs *P. vulgaris*	0.949	NS
S. typhi vs *S. typhi control*	0.000	S
S. liquefaciens vs *E. aerogenes*	0.998	NS
S. liquefaciens vs *B. cereus*	0.000	S
S. liquefaciens vs *P. Vulgaris*	0.000	S
S. liquefaciens vs *S. liquefaciens control*	0.000	S
E. aerogenes vs *B. cereus*	0.000	S
E. aerogenes vs *P. vulgaris*	0.000	S
E. aerogenes vs *E. aerogenes control*	0.000	S
B. cereus vs *P. vulgaris*	0.000	S
B. cereus vs *B. cereus control*	0.000	S
B. cereus vs *B. cereus* control	0.000	S
P. vulgaris vs *P. vulgaris* control	0.000	S

Table.3 Antimicrobial activity (Zone of Inhibition ± S.E.) of the Ethyl acetate fraction of *Senna didymobotrya* roots

Microorganisms	Mean ±S.E	Penicillin	DMSO
Escherichia coli	11.67± 0.333	43.67± 0.882	0.00±0.000
Salmonella typhi	18.332±0.882	35.00±0.577	0.00±0.000
Serratia liquef aciens	15.33±0.882	44.00±0.577	0.00±0.000
Enterobacter aerogenes	00.00±0.00	36.67±0.333	0.00±0.000
Bacillus cereus	25.33±0.882	40.00±0.000	0.00±0.000
Proteus vulgaris	17.67±0.882	35.23±0.333	0.00±0.000

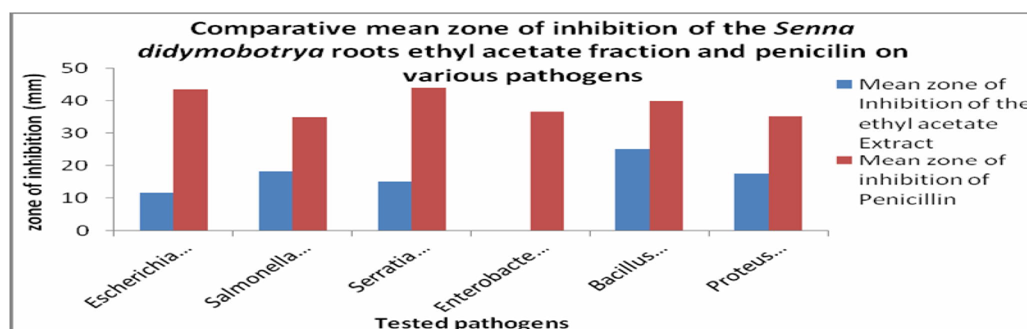

Fig. 2

Table.4 Tukey's honestly significant difference among microorganisms using 500mglml of *Senna didymobotrya* roots Ethyl acetate fraction

Comparison	P-value	Significance
E. coli vs *S. typhi*	1.000	NS
E. coli vs *S. liquefaciens*	0.018	S
E. coli vs *E. aerogenes*	0.000	S
E. coli vs *B. cereus*	0.000	S
E. coli vs *P. vulgaris*	0.998	NS
E. coli vs *E. coli control*	0.000	S
S. typhi vs *S. liquefaciens*	0.000	S
S. typhi vs *E. aerogenes*	0.000	S
S. typhi vs *B. cereus*	0.000	S
S. typhi vs *P. vulgaris*	1.000	NS
S. typhi vs *S. typhi control*	0.000	S
S. liquefaciens vs *E. aerogenes*	0.000	S
S. liquefaciens vs *B. cereus*	0.000	S
S. liquefaciens vs *P. vulgaris*	0.000	S
S. liquefaciens vs *S. liquefaciens control*	0.000	S
E. aerogenes vs *B. cereus*	0.000	S
E. aerogenes vs *P. vulgaris*	0.000	S
E. aerogenes vs *E. aerogenes control*	0.000	S
B. cereus vs *P. vulgaris*	0.000	S
B. cereus vs *B. cereus control*	0.000	S
B. cereus vs *B. cereus control*	0.000	S
P. vulgaris vs *P. vulgaris control*	0.000	S

Table.5 Antimicrobial Activity (Zones of Inhibition ± S.E.) of *Senna didymobotrya* aqueous fraction

Microorganisms	Mean ±S.E	Penicillin	DMSO
Escherichia coli	11.67± 0.333	43.67± 0.882	0.00±0.000
Salmonella typhi	18.33±0.882	35.00±0.577	0.00±0.000
Serratia liquefaciens	15.33±0.882	44.00±0.577	0.00±0.000
Enterobacter aerogenes	0.00±0.00	36.67±0.333	0.00±0.000
Bacillus cereus	25.33±0.882	40.00±0.000	0.00±0.000
Proteus vulgaris	17.67±0.882	35.33±0.333	0.00±0.000

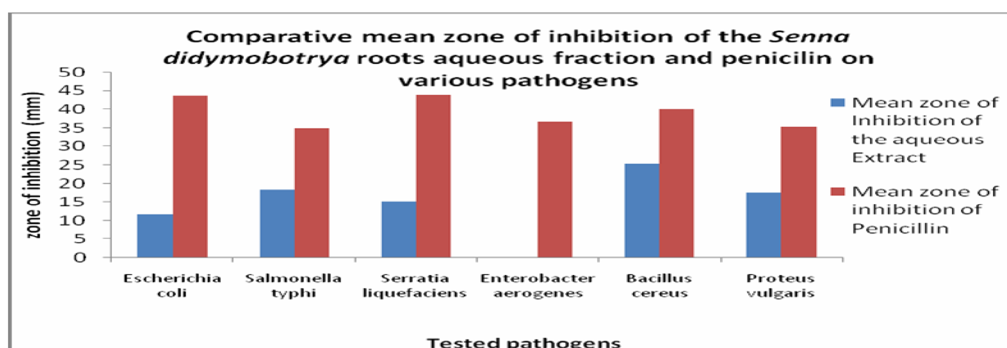

Fig. 3

Table.6 Tukey's honestly significant difference among microorganisms using 500mglml of *Senna didymobotrya* roots aqueous fraction extract

Comparison	P-value	Significance
E. coli vs *S. typhi*	0.000	S
E. coli vs *S. liquefaciens*	0.018	S
E. coli vs *E. aerogenes*	0.000	S
E. coli vs *B. cereus*	0.000	S
E. coli vs *P. vulgaris*	0.000	S
E. coli vs *E. coli* control	0.000	S
S. typhi vs *S. liquefaciens*	0.000	S
S. typhi vs *E. aerogenes*	0.090	NS
S. typhi vs *B. cereus*	0.000	S
S. typhi vs *P. vulgaris*	1.000	NS
S. typhi vs *S. typhi* control	0.000	S
S. liquefaciens vs *E. aerogenes*	0.000	S
S. liquefaciens vs *B. cereus*	0.000	S
S. liquefaciens vs *P. vulgaris*	0.340	NS
S. liquefaciens vs S. *liquefaciens* control	0.000	S
E. aerogenes vs *B. cereus*	0.000	S
E. aerogenes vs *P. vulgaris*	0.000	S
E. aerogenes vs *E. aerogenes* control	0.000	S
B. cereus vs P. vulgaris	0.000	S
B. cereus vs B. *cereus* control	0.000	S
B. cereus vs *B. cereus* control	0.000	S
P. vulgaris vs *P. vulgaris* control	0.000	S

Table.7 Zones of inhibition (mm ±S.E) of 500mg/ml of *Senna didymobotrya* roots butanol fraction

Microorganisms	Extract	Positive control	Negative control
Escherichia coli	0.000±0.000	45.333±0.882	0.000±0.000
Salmonella typhi	0.000±0.000	38.000±1.000	0.000±0.000
Serratia liquefaciens	0.000±0.000	41.667±1.202	0.000±0.000
Enterobacter aerogenes	14.000±1.528	30.333±0.333	0.000±0.000
Bacillus cereus	24.667±0.333	46.333±0.882	0.000±0.000
Proteus vulgaris	0.000±0.000	35.000±1.155	0.000±0.000

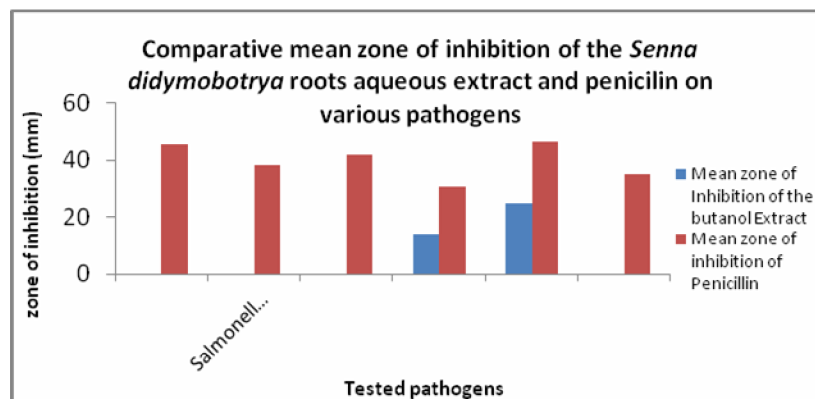

Fig. 4

Table.8 Tukey's multiple comparison of the zones of inhibition of bacteria isolates treated with *Senna didymobotrya* roots (butanol fraction) and penicillin antibiotic control

Pair wise comparison	p- value	Significance
E. coli vs *Salmonella typhi*	1.000	NS
E. coli vs *S. liquefaciens*	1.000	NS
E. coli vs *E. aerogenes*	0.000	S
E. Coli vs *B. cereus*	0.000	S
E. coli vs *P. vulgaris*	1.000	NS
E. coli vs *E. coli* control	0.000	S
S. typhi vs *S. liquefaciens*	1.000	NS
S. typhi vs *E. aerogenes*	0.000	S
S. typhi vs *B. cereus*	0.000	S
S. typhi vs *P. vulgaris*	1.000	NS
S. typhi vs *S. typhi* control	0.000	S
S. liquefaciens vs *E. aerogenes*	0.000	S
S. liquefaciens vs *B. cereus*	0.000	S
S. liquefaciens vs *P. vulgaris*	1.000	NS
S. liquefaciens vs *S. liquefaciens* control	0.000	S
E. aerogenes vs *B. cereus*	0.000	S
E. aerogenes vs *P. vulgaris*	0.000	S
E. aerogenes vs *E. aerogenes* control	0.000	S
B. cereus vs *P. vulgaris*	0.000	S
B. cereus vs *B. cereus* control	0.000	S
P. vulgaris vs *P. vulgaris* control	0.000	S

Table.9 Zones of inhibition (mm ±S.E) of 500mg/ml of *Senna didymobotrya* roots chloroform fraction

Microorganisms	Extract	Positive control	Negative control
Escherichia coli	0.000±0.000	41.667±1.202	0.000±0.000
Salmonella typhi	0.000±0.000	36.667±0.333	0.000±0.000
Serratia liquefaciens	10.000±0.000	43.667±0.667	0.000±0.000
Enterobacter aerogenes	0.000±0.000	44.333±1.202	0.000±0.000
Bacillus cereus	20.667±0.333	35.333±1.202	0.000±0.000
Proteus vulgaris	12.667±0.333	23.806±2.990	0.000±0.000

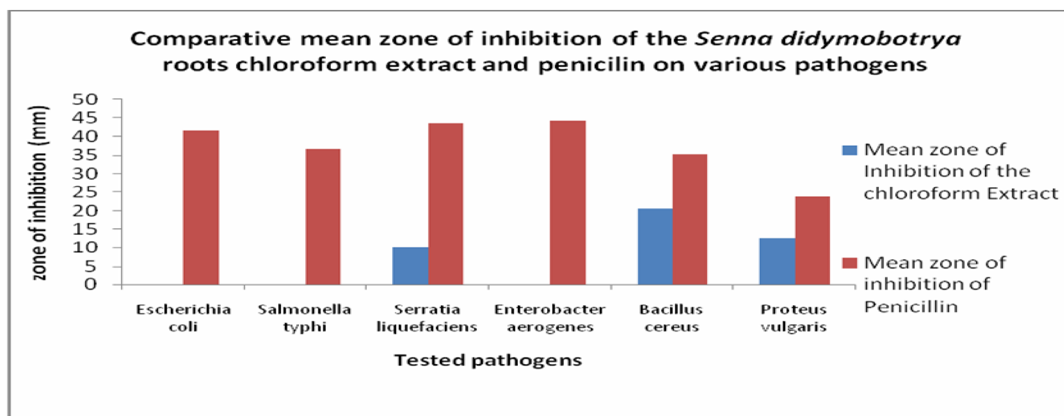

Fig. 5

Table.10 Tukey's multiple comparison of the zones of inhibition of bacteria isolates treated with Senna didymobotrya roots (chloroform fraction) and penicillin antibiotic control.

Comparison	p- value	Significance
E. coli vs *S. typhi*	1.000	NS
E. coli vs *S. liquefaciens*	0.000	S
E. coli vs *E. aerogenes*	1.000	NS
E. coli vs *B. cereus*	0.000	S
E. coli vs *P. vulgaris*	0.000	S
E. coli vs *E. coli* control	0.000	S
S. typhi vs *S. liquefaciens*	0.000	S
Sa S. typhi vs *E. aerogenes*	1.000	NS
S. typhi vs *B. cereus*	0.000	S
S. typhi vs *P. vulgaris*	0.000	S
S. typhi vs *S. typhi* control	0.000	S
S. liquefaciens vs *E. aerogenes*	0.000	S
S. liquefaciens vs *B. cereus*	0.000	S
S. liquefaciens vs *P. vulgaris*	0.357	NS
S. liquefaciens vs *S. liquefaciens* control	0.000	S
E. aerogenes vs *B. cereus*	0.000	S
E. aerogenes vs *P. vulgaris*	0.000	S
E. aerogenes vs *E. aerogenes* control	0.000	S
B. cereus vs *P. vulgaris*	0.000	S
B. cereus vs *B. cereus* control	0.000	S
P. vulgaris vs *P. vulgaris* control	0.000	S

flavonoids, tannins, phenols, steroids and cardiac glycosides (Anthoney , 2013) The antibacterial activity of the plant was attributed to these compounds. Comparing the zones of inhibition of the plant leaves as recorded by Ngule (2013); the plant roots have a better antibacterial activity. The data recorded is also in conformity with that recorded by Nyaberi [34], in which the stem charcoal of the plant inhibited the growth of E.coli (15.3±0.6) and *P.auroginosa* (13.6±0.5). The antibacterial activity of the plant could be attributed to presence of important phytochemicals as reported by Anthoney (Anthoney 2013; Nyaberi, et al., 2013), in which the extract of ethyl acetate was found to contain tannins, saponins, terpenoids, flavonoids, and steroidal rings. The chloroform extract was found to contain only saponins and terpenoids the low amount of phytochemicals in this extract could also be attributed to its weak antibacterial activity against most of the bacteria it was tested against. The aqueous extract was found to contain the highest percentage of the phytochemicals in which tannins; saponins terpenoids, flavonoids alkaloids and steroidal rings were detected. The butanol extract phytochemical analysis indicated the presence of tannins, saponins, terpenoids, flavonoids, steroidal rings.

From the results obtained in the study it is clear the number of phytochemicals present in the extract directly influences the pharmacological activity of the plant. According to Jeyaseelan (2010), plant extracts may act by interfering with peptidoglycan bacterial cell wall synthesis in the effect Organisms. They may also inhibit protein synthesis, interfere with nucleic acid synthesis, breaking the peptide bonds, preventing the utilization of available nutrients, lysis of microbial cells and acting as chelating agents inhibiting metabolic pathway (Gobalakrishnan, et al., 2013).

The result from this study shows that the plant roots have great pharmacological value against all the organisms it was tested against. The data shows that the water and ethyl acetate extracts to have highest number of inhibited organisms. This shows the two extracts to have great potency in extraction of the active compounds of the plant roots. The butanol and chloroform extracts inhibited at least two of the organisms' they were tested against. The plants crude methanol-water extract showed the greatest inhibition zones. The results shows that the compounds from the plant can be extracted with water hence eliminating the use of chemical solvents and in the end solving the problem of pollution associated with these solvents. The plants antibacterial activity is attributed to the presence of important pharmacological compounds in the plant.

From the study it is also worthy to mention that the antibacterial activity of the plant could be due to synergistic effect of two or more compounds in the plant. The data obtained in this research is a scientific justification of the plant roots use in the treatment of various diseases affecting human beings. It is, therefore, worthy to recommend the plant for the treatment of all diseases caused by all the organisms the plant was tested against. The plant extract can be used to treat infections caused by *Bacillus cereus* viz posttraumatic wounds, self-limited gastroenteritis, burns, surgical wounds infections, ocular infections such as endophthalmitis, corneal abscess and panophthalmitis (Garcia-Arribas et al., 1988; Sankararaman, S. and Velayuthan,

S. 2013). The plant extracts can be used to treat immunologically compromised patients including AIDS and malignant disease victims (Cotton, et al., 1987; Tuazon, et al., 1979). The plant's ability to inhibit the growth of *E. coli* is a scientific justification that the plant can be used treat against enteric infections caused by the bacteria. The plants extract can also be used to treat against gastro-intestinal diseases, ear infections, urinary tract infections and wounds infections caused by *Proteus vulgaris* (Goodwin, et al., 1971; Neter, R.E. and Farrar. H.R. 1943).Further research needs to be done to isolate the active compounds and analyse their structural composition, their mode action and their effect in the in vivo environment

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References

Adu, F., Gbedema, S.Y., Akanwariwiak, W.G., Annan, K. and Boamah, V.E. The effects of *Acanthospermum hispidum* extract on the antibacterial activity of amoxillin and ciprofloxacin, 2011; 31: 58-63.

Anthoney S.T., Ngule, C. M. and Obey, J. 2013. *In Vitro* control of selected Pathogenic organisms by *Vernonia adoensis* roots. *International Journal of Pharmacy and Life Science.* Vol.4 8 pp 2855-2859. ISSN: 0976–7126.

Anthoney S.T., Obey, J. K. and Ngule, C. M. 2014. *In vitro* Antibacterial Activity of Methanolic – Aqua Extract of *Plectranthus agentatus* Leaves. *World Journal of Pharmaceutical Research WJPR*, 2014, Vol.3 1, 339-349. .ISSN: 2277 – 7105.

Anthoney T.S., Ngule, C. M, and Machoka, R. 2014 Histopathological Effects of *Vernonia adoensis* Roots Extract on the Larvae of the Mosquito *Culex quinguefasciatus. Journal of Pharmaceutical Biology, 41: 11-13.*

Anthoney, S.T, Ngule, C. M., Ramesh, F. and Ngule, M. E. 2013. Preliminary Phytochemical Screening of *Plectranthus argentatus* Plant in Kenya. *International Journal of Medicinal Chemistry & Analysis*. Vol 3 2: 83-88. e ISSN 2249 – 7587; Print ISSN 2249 – 7595.

Anthoney, S.T., and Ngule, C. M. 2013. Chemical Constituents of Infused *Plectranthus argentatus* leaves. *World Journal of Science* wjp. 2013; 13:151-160. ISSN: 2320-1835.

Anthoney, S.T., Ngule, C .M. and Obey, J. 2013. Phytochemical Analysis of *Vernonia adoensis* Leaves and Roots Used as a Traditional Medicinal Plant in Kenya. *International Journal of Pharmacy and Biological Sciences*, Vol.3 3 pp 46-52. ISSN: 2230-7605.

Anthoney, S.T., Ngule, C. M. and Obey, J. 2013. *In Vitro* control of selected Pathogenic microorganisms by *Vernonia adoensis* leaves. *Int. J. Bioassays*. Vol. 02 08, pp 1113-1117. ISSN: 2278-778X

Anthoney, S.T., Ngule, C. M. and Obey, J. 2014. Phytopharmacological Analyis of Chemical Constituents of Infused *Vernonia adoensis* Roots. *International Journal of Pharmacology and Toxicology IJPT, 2014, 41, pp: 28-33.* ISSN- 2249-7668; Print ISSN- 2249-7676.

Anthoney, S.T., Ngule, C. M. and Obey, J.K. 2013. Phytopharmacological Analysis of Methanolic-Aqua Extract Fractions of *Senna didymobotrya* Roots. *Int. J. Bioassays*, 2013, 02 11, 1473-1479. ISSN: 2278-778X.

Anthoney, S.T., Ngule, C. M. and Obey, J.K. 2014. *In Vitro* Antibacterial activity of

Methanolic-aqua extract of *Tragia brevipes* Leaves. *Int. J. of Pharm. Life Sci. 2014*, Vol. 5 2, 3289-3294. ISSN: 0976-7126

Anthoney, T. S., Ngule, C. M. and Obey, J. 2014. Qualitative Assessment of Phytochemicals of Infused *Senna didymobotrya* leaves. *International Journal of Pharmacology IJP,* 2014, 4 1, pp: 40-45. e-ISSN: 2249-7684; Print ISSN- 2249-7692.

Anthoney, T. S., Ngule, C. M., Ngule, M. E. and Ramesh, F. 2013. Qualitative Analysis of Phytoconstituents in *Targia brevipes* Plant. *International Journal of Pharmaceutical Research & Analysis.* Vol.3 2: 93-98. e-ISSN: 2249 – 7781;Print ISSN: 2249 – 79X.

Arivoli, S. and Tennyson, S. Larvicidal efficacy of *Strychnos nuxvomica* Linn.Loganiaceae leaf extracts against the filarial vector *Culex quinquefanciatus* Say Diptera : Culicidae. *World Journal of Zoology*, 2012; 71: 06-11.

Biruhalem T., Giday M., Animut A., Seid J. Antibacterial activities of selected medicinal plants in traditional treatment of human wounds in Ethiopia. *Asian Pacific Journal of Tropical Biomedicine,* 2011; 370-375.

Cotton, D.J., Gill, V.J., Marshall, D.J., Gress, M., Thaler, M. and Pizzo, P. 1987. Clinical features and therapeutic interventions in 17 cases of *Bacillus bacteremia* in an immunosupressed patient population *.J. Clin. Microbiol*, 25: 672-674.

Deepa, N. and Rajendran, N.N. Antibacterial and anti-fungal activities of various extracts of *Acanthospermum hispidum DC. Journal of Natural Remedies*, 2007; 72: 225-228.

Donay J.L., Fernandes P., Lagrange P.H., Herrmann J.L. Evaluation of the inoculation procedure using a 0.25 McFarland Standard for the BD Phoenix Automated Microbiology System, *Journal of Clinical Microbiology,* 2007; 45 12, 4088-4089.

Farook, S.M. and Atlee, W.C. Antidiabetic and hypolipidemic potential of *Tragia involucrata* Linn. In streptozotocin-nicotinamide induced type II diabetic rats. *International Journal of Pharmacy and Pharmaceutical Sciences*, 2011; 34: 103-109.

Garcia-Arribas, M.L., Plaza, C.J., De La Rose, M.C. and Mosso, M.A. 1988. Characterisation of *Bacillus cereus* strains isolated from drugs and evaluation of their toxins. *J. Appl. Bacterial,* 64: 257-264.

Gobalakrishnan, R., Kulandaivelu, M., Bhuvaneswari, K., Kandavel, D. and Kannan, L. 2013. Screening of Wild plant species for antibacterial activity and phytochemical analysis of *Tragia involucrate* L. *Journal of Pharmaceutical Analysis,* 71:

Goodwin, C.S., Kliger, B.N., and Drewett, S.E. 1971. Colistin-sensitive Proteus organisms: Including indole-negative *Proteus vulgaris*, non-swarming on first isolation. *Br. J. exp. Path.*, 52: 138-141.

Hosahally, R.V., Sero, G., Sutar, P.S., Joshi, V.G., Sutar, K.P. and Karigar, A.A. Phytochemical and pharmacological evaluation of *Tragia cannabina* for anti-inflammatory activity. *International Current Pharmaceutical Journal*, 2012; 18:213-216.

Jeyaseelan, E.C., Pathmanathan, M.K., Jeyadevan, J.P. 2010. Inhibitory effect of different solvent extracts of *Vitex negundo* and *Allium sativum* L., on phytopathogenic bacteria. *Arch. Appl. Sci. Res*., 26: 325-331.

Joshi CG, Gopal M and Byregowda SM. Cytotoxic activity of *Tragia involucrata* Linn. extracts. *American-Eurasian Journal of Toxicology Sciences,* 2011; 32: 67-69.

Kamatenesi-Mugisha, M. and Oryem-Origa, H. Traditional herbal remedies used in the management of sexual impotence and erectile dysfunction in western Uganda. *African Health Sciences*, 2005; 51: 40-49.

Khan, A.V., Ahmed, Q.U., Mir, M.R., Shukla, I., Khan, A.A. 2011. Antibacterial efficacy of the seed extracts of Melia Azedarach agains some hospital isolated human pathogenic bacterial strains. *Asian Pac. J. Trop. Biomed*., 16: 452-455.

Kisangau, D., Hosea, K.M., Joseph, C.C. and Lyaruu, H.V.M. In vitro antibamicrobial assay of plants used in traditional medicine in Bukoba rural district, Tanzania. *Afr. J. Traditional, Complementary and Alternative Medicines,* 2007; 44: 510-523.

Montelli, A.C., Levy, C.E., Sistema, C.O.B.A. 1991. Aspectos reletivos aos dados dos laborato'rios de referência. *Rev. Microbiol*. 22: 197-205.

Muroi, H., Kubo, I. 1996. Antibacterial activity of anacardic acids and totarol, alone and in combination with methicillin, against methicillin- resistant *Staphylococcus aureus. J. Appl. Bacteriol*., 80: 387-394.

Nasciment, G.G.F., Locatelli, J., Freitas, P.C. and Silva, L.G. 2000. Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. *Brazilian Journal of Microbiology,* 31: 247-256.

Neter, R.E. and Farrar. H.R. 1943. *Proteus vulgaris* and *Proteus morganii* in diarrhea disease of infants. *The American Journal of Digestive Diseases,* 109: 344-347.

Ngule C. M and Anthoney, T. S. 2013. Phytochemistry of Infused *Tragia brevipes* Stem. *International Journal of Medical & Pharmaceutical Sciences Research & Review*. Vol. 1 3, 1 – 15. ISSN: *2321-7049*

Ngule, C. M., Anthoney, S.T., and Machoka, R. 2014. Evaluation of Larvicidal Activity of *Vernonia adoensis* Leaves Against *Culex quinguefasciatus. International Journal of Phytotherapy, 41:1-3.*

Ngule, C.M., Anthoney, S.T. and Jackie, O. 2013. Phytochemical and bioactivity evaluation of *Senna didymobotrya* Fresen Irwin used by the Nandi community in Kenya. *International Journal of Bioassays*, 207:1037-1043.

Nyaberi, M.O., Onyango, C.A., Mathoko, F.M., Maina, J.M., Makobe, M. and Mwaura, F. 2013. Bioactive fractions in the stem charcoal of *Senna didymobotrya* Fresen Irwin and Barney used by pastoral community of west Pokot to preserve milk. *Natural Resource Management*, pp980-985.

Sankararaman, S. and Velayuthan, S. 2013. *Bacillus cereus*.Peditrics in Review, 34: 196.

Santos Filho, D., Sarti, S.J., Bastos, J.K., Leitao Filho, H.F., Machado, J.O., et al. 1990. Atividade antibacteriana de extratos vegetais. *Rev. cien.Fam*, 12: 39-46.

Sigh, S.A. and Singh, R.N. 2010. Antibacterial activity of *Cassia didymobotrya* and *Phlogacanthus thyrsiflorus*. *Journal of Chemical and Pharmaceutical Research*, 2010, 24: 304-308.

Tabuti, J.R.S. 2007 *Senna didymobotrya* Fresen. H.S Irwin& barneby. In: Schmeltzer, G.H & Gurib. Fakim, A editors Prota 11i: medicinal plants 1. {CD-ROM}. PROTA, wageningen, Netherlands.

Tuazon, C.U., Murray, H.W., Levy, C., Solny, M.N., Curtin, J.A. and Shegren, J.N. 1979. Serious infections from Bacillus species. *JAMA*, 241:1137-1140.