



Phytochemical screening and antibacterial investigations of crude methanol extracts of *Senna didymobotrya* (Fresen.) H. S. Irwin & Barneby

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ABSTRACT

Objective: *Senna didymobotrya* (African senna, African wild sensitive plant, peanut butter cassia, peanut butter tree, popcorn cassia, popcorn senna or wild senna) is native to East Africa and is widely used as a medicinal plant among many communities in Kenya. The objective of this research was to evaluate the presence of phytochemicals present in the different plant parts and their antibacterial activity.

Methodology and results: Leaves, flowers, stem bark, immature pods and root barks were collected from Siaya, Nandi and Nakuru Counties. These were dried and ground. Methanolic crude extracts were in cooperated in nutrient media at 2.5 %, 5 %, 7.5 % and 10 %. Test organisms *Staphylococcus aureus* and *Escherichia coli* were inoculated on impregnated media, incubated and observed for colony development. Observation on growth of cultures was made at an interval of 2 days for 8 days. The area under disease progress stairs (AUDPS) was calculated using the derived colony surface areas. Results indicated that all plant parts contained terpenoids, phenols and steroids. The presence of alkaloids and flavonoids varied with the location the plant was collected and the plant part. Growth of *S. aureus* cultures grown on media impregnated with 2.5% root bark extract and that with 7.5% stem bark extract were completely inhibited (no growth). Media with 10% flower, pods and leaves extract had average reduction of colony sizes from AUDPS 10102 (control) to AUDPS 2475. Growth of *E. coli* was completely inhibited on media impregnated with 5% root bark extract and 7.5% stem bark extracts. At 10% concentration, the flowers, pods and leaves extract did not result in complete inhibition of colony growth.

Conclusions and applications: The present research suggests that *S. didymobotrya* extracts possessed antibacterial activity against bacterial pathogens thus supporting their folkloric usage, promising a future scope for its use against microbial populations. Methanolic extracts possessing high antibacterial effects should be further investigated for their therapeutic utility. This would be related to the presence of bioactive metabolites, which are soluble in methanol. There is need to explore further the quantities of phytochemicals in the root and

stem barks that make them more potent than the other plant parts. The structures of the bioactive metabolites should be examined in future.

Key words: Antibacterial activity, Methanolic extract, *Senna didymobotrya*, *Escherichia coli*, *Staphylococcus aureus*

INTRODUCTION

Herbal medicines have been used for many years in many parts of the world especially developing countries (Kareru *et al.*, 2007). Large body of evidence has accumulated to demonstrate the promising potential of medicinal plants used in various traditional, complementary and alternative systems of treatment of human diseases (Wong and Cuervo, 2010). In tropical countries, modern medicines are not affordable to most of the rural populations and WHO estimates that 80% of the world's population use botanical medicines for primary health care (Muregi *et al.*, 2004). The World Bank report further pointed out that in 2002, Kenya's Ministry of Health budget for medicine provided for only 30% of the population (WHO, 2002). This left 70% (21 million) of the population, which could not access the conventional medicines. The latter population group was therefore left to rely on traditional medicines for their healthcare needs (Sharma and Alagarsamy, 2012). The medicinal value of plants lies in some chemical substances that produce definite physiological actions to the human body. The most important of these compounds are alkaloids, flavonoids, tannins and phenolic compounds (Edeoga and Okwu, 2005). Biological and pharmacological activities of phytochemical compounds depend on factors such as the ecological factors, age of the plant, species and method of extraction. Thus, each plant has different chemical composition, toxicity and bioactivity (Rajakaruna *et al.*, 2002). The type and amount of phytochemical compounds present usually differ from one part of the plant to another part. This explains why in traditional medicine the same or different plant parts may be used in treating different diseases or same diseases (Addo-Fordjour *et al.*, 2008). The *Senna* genus formerly *Cassia* have been extensively studied for their phylogenetic diversity, phyto-constituents as well as for their biological activities. It is native to tropical Africa (Bett, 2010) and was introduced, naturalized and became an

invasive species in parts of Kenya, Uganda and Tanzania (Sunarno, 1997). The flowers, roots and stems have both antibacterial and antifungal activity (Mazumder *et al.*, 2008). Njoroge *et al.* (2004) reported that that *S. didymobotrya* was utilized for back pains, antihelmintic, anaplasmosis, malaria, pimples, pneumonia (cattle), skin rashes, STD's, stomach ache, tonsils (human) and typhoid. In another study, Njoroge and Bussmann (2007) found that the leaves of *S. didymobotrya* were boiled and a patient bath in it for treatment of pimples, scabies, warts and measles in Muranga, Nyandarua and Kiambu Counties. An ethnobotanical survey carried out in Kiandutu and Kiang'ombe in Thika urban slums on herbal medicine found that *S. didymobotrya* was one of the 41 species identified which was used for management of diarrhoea. Its bark, sap, leaves and seeds were utilized (Njoroge and Kibunga, 2007). In Mozambique, the roots are used traditionally against infectious diseases like diarrhoea, fevers, abscesses of the skeletal muscles and venereal diseases (Madureira *et al.*, 2012). Pounded leaves and young stems are used in Kenya to treat skin diseases. The pulp is applied to the skin (Gachathi, 1989). The leaf sap is diluted in water and orally taken to treat diarrhoea, dysentery. It is also taken as a diuretic, laxative and emetic. Evidences are mounting concerning the resistance of microorganisms to antibiotics throughout the world (Akanwariwiak *et al.*, 2012). This development has awakened scientists to explore alternative approaches that target and block resistance by combining plant extracts with antibiotics to increase their activity (Akanwariwiak *et al.*, 2012). Therefore, there is a need to focus on the alternative sources of the antibiotics, as the pathogenic microbes are gaining resistance against standard antibiotics (Alo *et al.*, 2012). This study was therefore, aimed at determining the phytochemicals present in the different plant parts of *S. didymobotrya* and the

antibacterial activity of the methanolic extracts obtained from the plant parts.



Plate 1.1.1



Plate 1.1.2



Plate 1.1.3



Plate 1.1.4

Plate 1.1: *Senna didymobotrya* (1.1.1- Inflorescence, 1.1.2- Fruits {immature pods}, 1.1.3 –Roots and 1.1.4- Leaves).

MATERIALS AND METHODS

Collection and processing of plant materials: Surveys were conducted in Siaya, Nakuru and Nandi Counties in Kenya. The regions were purposively selected according to altitude, geographical location and environmental variability. During the surveys, one cluster of *S. didymobotrya* with at least 20 mature plants at flowering and fruiting stage was selected. Samples were selected from Nandi County at Ndurio (0.02 N and 35.01 E), Siaya County at Uyawi-Miandhe (0.11 S and 34.08 E) and Nakuru County at Rongai (0.23 S and 35.54 E). From these plants, (i) young leaves, (ii) flowers, (iii) stem bark,

(iv) immature pods and (v) root barks were obtained. The samples were collected ethically following standard procedures (WHO, 2003) and with the farmer's prior consent. Each sample was evenly spread on the laboratory bench and allowed to air dry at 28.7°C for 14 days (Sikuku *et al.* , 2010). Samples were milled and sieved to pass through the mesh of 1.0 millimetre (mm) and stored in airtight plastic bags awaiting extractions.

Extraction: Methanolic extraction was carried out according to methods as described by Kigundu *et al.* (2009). One thousand (1000) grams of powdered dried

plant part was weighed. At each time, 42.25 g was wrapped in a filter paper, placed in Soxhlet apparatus and distilled with 300 ml absolute methanol. This was repeated until all the 1000g sample was used up. The methanol extract was then placed in a rotary vacuum evaporator placed in a water bath at 40°C to recover the solvent (methanol) and concentrate the crude extract. The semi-solid crude extract was placed in sterile beaker and left in the laminar flow hood for 24 hours for complete evaporation of the solvent.

Phytochemical screening of crude extracts of *Senna didymobotrya*: The crude extracts obtained were subjected to phytochemical analysis to determine the presence or absence of various chemical constituents using thin-layer chromatography (TLC). The Thin layer separation was carried out using pre-coated potassium chloride (KCl) silica gel [60Å] TLC plates, size 20 × 20 cm, 1 mm thick. The chromatographic tank was allowed to equilibrate with the solvent system (Chloroform Methanol in the ratio of 98: 2, with 5 drops of glacial acetic acid), for at least 30 minutes prior to development of plates. The plates were sprayed with TLC visualization agents that gave specific reactions to specific group of compounds and visualized using standard methods i.e. UV- 254/364 nm (Harborne, 1984). The presence of different classes of compounds was determined by observing appropriate colour change on the TLC plate. Phytochemicals present in the plants were analysed according to the standard procedures described in previous studies (Harborne, 1984; 1998; Parekh and Chanda 2007).

Terpenoids: Vanillin spray test was used to determine the presence of terpenoids. One (1) g of vanillin was dissolved in 100 ml of concentrated sulphuric acid and this was used to spray the developed TLC plates and then placed in the oven for about 3 minutes at a temperature of 100 °C, colour change to purple was indicative of the presence of terpenoids (Harborne, 1984).

Alkaloids: Zero point five (0.5) grams of each extract was stirred with 5 ml of 1% aqueous hydrochloric acid on a steam bath. About 1 ml of the filtrate was treated with a few drops of Dragendorff's reagent. The formation of red precipitate or orange colour indicated the presence of alkaloids (Harborne, 1998).

Phenols (polyphenols): Zero point one (0.1) grams of ferric chloride and 0.1 g of potassium ferric cyanide was dissolved in 10 ml of water. Then the reagent was sprayed on TLC plate. Change of colour to blue (instant) indicated the presence of phenols (Harborne, 1984).

Flavonoids: Flavonoids presence was tested by dipping the developed TLC plates then exposed to fumes of

ammonia for five (5) minutes and any brightening yellow-brown spots of the present colours recorded indicated the presence of flavonoids.

Steroids: This was tested by the use of Kedde's reagent. Kedde's reagent was prepared by mixing 0.2 g of 3, 5 - Dinitrobenzoic acid in 10 ml methanol with 0.57 g sodium hydroxide in 10 ml distilled water. This was then used to spray the developed plates and any colour change to brown was indicative of the presence of steroids.

Antibacterial activity of *S. didymobotrya* extractson the selected bacterial isolates

Nutrient agar: Nutrient agar (28g) was dissolved in 1L of distilled water in a conical flask and then homogenized by boiling until boiling point using a hot plate. The medium was autoclaved at 121°C for 15 minutes. After sufficient cooling, 100 ml was measured and poured into 100 ml Erlenmeyer flasks. The medium was allowed to cool to 40°C.

Extract preparation: The constitutions of the extracts were carried out according to methods of Akanwariwiak et al. (2012) and Chad (2013). Using analytical balance, 2.5 g, 5 g, 7.5 g and 10 g of crude extract was weighed and placed in sterile beakers. Five millimetres of warm sterile water was added to the solid extract to reconstitute it. Each of the reconstituted extract was added into 100 ml of sterilized medium at 40 – 50 °C in a conical flask and stirred. The negative control consisted of media alone without addition of *S. didymobotrya* extracts. The positive control consisted of streptomycin sulphate (Str) incorporated at 1 g/L of nutrient agar. The mixtures were stirred and let to cool for 30 minutes with the flask mouths open under the laminar flow hood. Medium from each flask was poured in four disposable petri dishes (replicates).

Test strains: The test microorganisms were obtained from Kenya Medical Research Institute (KEMRI), Kisumu, Kenya and were chosen based on the ethno-botanical exploitation of the plant. The test strains consisted of:

(a) *Staphylococcus aureus* (ATCC 28923): Gram positive cocci and

(b) *Escherichia coli* (ATCC 25922): Gram-negative rod.

The cultures were obtained from the petri dishes using a sterilized loop and placed in 10 ml of distilled water in a sterilized tube. The inoculum was serially diluted from 10⁻¹ to 10⁻⁸. The original sample was stored in a refrigerator at 4°C. 1 ml each was obtained from the last two dilutions and plated on the Petridish. The dishes were incubated at 37°C for five days. The number of colonies developing in each plate was counted. Using this, the colony forming units (cfu) in the original sample was determined. The concentration of the original sample was adjusted using

sterile distilled water to obtain 10⁸ cfu/ml. Using a micropipette, 50 µl of the inoculums was obtained and inoculated in the middle of the Petri dish. The inoculum was allowed to dry for 2 hour. A line was drawn on top (on the petri dish cover) using a transparent ruler along the diameter of the dried inoculated position. The petri dishes were incubated at 37°C. The experiment was set in with four replicates in a completely randomized design (CRD). Colony growth was assessed at an interval of 2 days for 8 days. At each observation date, the diameter of the colony growth along the line was measured in millimetres.

Data analysis: The diameter (mm) of the colony size was used to calculate the actual surface area (mm²) covered by the pathogen. The disease areas for the different observations were summarized using the area under disease progress stairs (AUDPS) (Simko and Piepho, 2012):

$$AUDPS = AUDPC + \left[\frac{y_1 + y_n}{2} \times \frac{D}{n - 1} \right]$$

Where AUDPC is area under disease progress curve given with formula;

$$AUDPC = \left[\frac{1}{2} Obs_1t_1 + Obs_2t_2 + Obs_3t_3 + \frac{1}{2}Obs_4t_4 \right]$$

Where:

y_1 and y_n are assessments at the first and last observations respectively.

$D = t_n - t_1$ (when observations are performed at every time unit, then $D = (n - 1)$)

t_1, t_2, t_3 and t_4 are the time intervals of the first, second, third and the fourth observation.

Obs_1, Obs_2, Obs_3 and Obs_4 are first, second, third and fourth observation respectively.

n is the total number of observation.

The AUDPS was subjected to Analysis of Variance (ANOVA) and comparison of means was carried out on all data using SAS. Means were separated by the use of the least significant difference and compared at $P = 0.05$ for significance. SAS statistical package Release 8.02 copy 1999 - 2001 was used. The values are represented as means for AUDPS. Paired t - test was used for reporting the p value and significance with respect to the control.

RESULTS

Phytochemical screening of methanol extracts of *S. didymobotrya*: The methanolic extracts of *S. didymobotrya* contained a mixture of compounds namely, steroids, alkaloids, flavonoids, phenols and terpenoids (Table 1). Terpenoids, phenols and steroids were present in all plant parts extracts. Flavonoids were present in all

plant-parts extracts except in immature pods from Siaya and Flowers in Nandi. Alkaloids were not detected in all plant parts from Siaya. Alkaloids were present in immature pods, flowers, stem and root barks (Nandi) and in flowers, leaves and stem bark (Nakuru)

Table 1: Phytochemical compounds identified in the various parts of *S. didymobotrya* methanol extracts

County	Plant part	Phytochemical compound				
		Terpenoids	Alkaloids	Phenols	Flavonoids	Steroids
Siaya	Immature pods	+	-	+	-	+
	Flowers	+	-	+	+	+
	Leaves	+	-	+	+	+
	Stem bark	+	-	+	+	+
	Root bark	+	-	+	+	+
Nandi	Immature pods	+	+	+	+	+
	Flowers	+	+	+	+	+
	Leaves	+	-	+	-	+
	Stem bark	+	+	+	+	+
	Root bark	+	+	+	+	+
Nakuru	Immature pods	+	-	+	+	+
	Flowers	+	+	+	+	+
	Leaves	+	+	+	+	+
	Stem bark	+	+	+	+	+
	Root bark	+	-	+	+	+

Key: (+) = Presence (-) = Absence

Table 2: The area under disease progress stairs (AUDPS) of growth of *S. aureus* on culture media impregnated with different concentrations of *S. didymobotrya* obtained from different plant parts

Plant part	Concentration (%)					Strep (1g/L)
	0	2.5	5.0	7.5	10.0	
Flowers	11035 a	5993 a	4125 a	2648 b	2026 b	0
Pods	9584 bc	5146 b	3842 a	3309 a	2952 a	0
Leaves	9986 b	3920 c	3761 a	3674 a	2448 ab	0
Stem barks	11046 a	1485 d	572 b	0 c	0 c	0
Root barks	9252 c	0 e	0 b	0 c	0 c	0
Mean	10181	3309	246	1926	1485	0

LSD = 572, Means followed by the same letter in a column are not significantly different (P>0.05) using LSD.

Results in Table 2 indicated that the negative control (0%) had a mean range colony growth AUDPS of 9252 – 11035. There were no colony growth of *S. aureus* (Table 2) observed on plates inoculated with streptomycin sulphate. When media were impregnated with 2.5% concentration of the extract, there was no culture growth observed on the medium containing root bark extract. This was also observed on medium impregnated with 5%, 7.5% and 10% of the root bark extracts. Media

impregnated with 2.5% stem bark extract had mean colony AUDPS of 1485. Media treated with immature pods, leaves and flowers had lower colony growths as compared to the negative control. The same trend was observed at 5% where root bark extract had no culture growth on medium whilst stem bark had mean culture growth AUDPS of 572. When media was impregnated with 7.5% and 10% of stem bark and root bark extracts there was no growth of bacteria observed.

Table 3: The area under disease progress stairs (AUDPS) of growth of *E. coli* on culture media impregnated with different concentrations of *S. didymobotrya* obtained from different plant parts.

Plant part	Concentration (%)					Strep (1g/L)
	0	2.5	5.0	7.5	10.0	
Flowers	7478 a	6658 a	6218 a	4001 a	3421 a	0
Pods	6422 b	5927 a	4880 b	4769 a	3004 a	0
Leaves	5785 b	4208 b	4141 b	3817 b	3541 a	0
Stem barks	5824 b	1309 c	173 c	0 c	0 b	0
Root barks	5620 b	929 c	0 c	0 c	0 b	0
Mean	6226	3806	3082	2517	1993	0

LSD = 871, Results are means of three replicate values. Means followed by the same letter in a column are not significantly different (P > 0.05) using LSD

Table 3 shows the negative control treatments had AUDPS range of 5619.7-7478.2. When medium was impregnated with 2.5% extract, root bark and stem bark had AUDPS of 929.1 and 1308.8 respectively. These were not significantly different (p > 0.05). Flowers, immature pods and leaf extracts had AUDPS range of 4208.3-6658.3. At concentration of 5%, the AUDPS for root bark was 0 whilst that of stem bark was 172.7. They

were significantly different (P < 0.05). When medium was impregnated with 7.5 and 10% of stem bark and root bark, there was no culture growth. At 7.5% and 10%, the flowers, immature pods and leaves had lower culture growth compared to the negative control. There were no colony growth of *E. coli* (Table 3) observed on plates inoculated with streptomycin sulphate

DISCUSSION

Phytochemical screening of *S. didymobotrya*: The presence of a good number of phytochemicals in the plant extracts screened can be a good source of bioactive components with antimicrobial potency, as they can be responsible together with the unscreened ones for the

antimicrobial activity of the extracts. This study showed that all plant parts of *S. didymobotrya* extracts contained terpenoids, phenols and steroids. The presence of alkaloids varied greatly depending on the location and plant part samples (Table 1). This may be attributed to the

great structural diversity of alkaloids (Fattorusso and Taglialatela- Scafati, 2008). Terpenoids have medicinal value such as antimicrobial, anti-carcinogenic, antimalarial and antidiuretics activity (Pichersky and Gershezon, 2002; Deganhart, 2003). They have shown great potentials in treatment against disease causing microorganisms. In addition, terpenoids have exhibited antibacterial activity against *E. coli*, *S. aureus* (Santos *et al.*, 2008; Piera *et al.*, 2011). The presence of alkaloids in the plant justifies its medicinal value since they have been identified for their functions, which include analgesic, antispasmodic, anti-hypertensive effects, cytotoxic, anti-inflammatory and antimalarial. Kokwaro (2009) indicated that most of the savannah and semi - arid plant contains large percentages of alkaloids. Phenolic compounds have excellent antioxidant properties (Narayana *et al.*, 2001). They, have biological and pharmacological properties especially their antimicrobial activity (Anpin Raja *et al.*, 2011), antiviral, anti-inflammatory and cytotoxic activity (Mungole *et al.*, 2010). Flavonoids are known to be antioxidant compounds, which protect human, animal and plant cells against the damaging effects of free radicals. Flavonoids and its derivatives have long been known to function as antimicrobial defense compounds (Kazmi *et al.*, 1994). Other authors have shown that flavonoids have effective antimicrobial activity against a wide range of microorganisms (Schewe and Sies 2003; Yadav and Agarwala, 2011). Plant steroids are known to be important for their cardiogenic activities, possess insecticidal and antimicrobial properties (Ngbede *et al.*, 2008; Anpin Raja *et al.*, 2011). These secondary metabolites play a defensive and attractive role in the interactions between plants and their environment with other plants, herbivores, pathogens and pollinators (Figueroa *et al.*, 2008). Secondary metabolites have been found to have a distribution, which is sometimes confined to a genus. Species frequently have different production and accumulation sites (Cowen, 2008). The presence of phytochemicals in different plant parts of *S. didymobotrya* have been observed in previous studies (Nyaberi *et al.*, 2013; Ngule *et al.*, 2013; Nyamwamu *et al.* 2015). Presence of phytochemicals has also been reported in different plant parts of *Bombax buonopozense* and *Monodora myristica* (Firempong *et al.*, 2016) and *Moringa oleifera* (Abubakar and Usman, 2016). The phytochemicals in the present study differed in their diversity with geographical areas or ecozones and the plant parts (Table 1). These results forms the scientific basis for the use of these plants by the herbal practitioners in the Nandi and Kipsigis communities community, since these secondary metabolites in plants

have been shown to possess biological activity (Jeruto *et al.*, 2008; Korir *et al.*, 2012). Generally, the composition of phytochemical compounds within the same plant species differed from one part to another. These observations clearly demonstrated the reason as to why traditional healers use more than one plant parts to make a concoction for the treatment of a given disease since they have some additive or synergistic activity among various phytochemicals against the pathogens of interest (Addo-Fordjour *et al.*, 2008; Ruttoh *et al.*, 2009). Bacterial bioassays: *Staphylococcus aureus* is a gram - positive bacterium that produces staphylococcal enterotoxin (SE) A which is responsible for almost all staphylococcal food poisoning, a form of gastroenteritis (Argudin *et al.*, 2010). It may cause non - food related health issues such as skin inflammations, mastitis, respiratory infections, wound sepsis and toxic shock syndrome (Stewart, 2003; Montville and Mathews, 2008; Musa *et al.*, 2011). Over time, *staphylococcus* bacteria have become difficult to treat with antibiotics related to penicillin. These resistant forms of *staphylococcus* are referred to as Methicillin - Resistant *S. aureus*, or MRSA. Other pharmaceuticals used include ampicillin and vancomycin, which has been the most reliable antibiotic against MRSA, though currently resistance strains have been, cited (Harris *et al.*, 2002). Attempts have been carried out by several authors in the world in managing *S. aureus* using alternative and herbal medicine. Results presented in Table 2 indicated that there was no growth of *S. aureus* on cultures impregnated with 2.5% root bark extract and 7.5% stem bark extracts. These were the only extracts that suppressed the growth of *S. aureus* within the range of concentrations studied. Even though the extracts from leaves, immature pods and flowers did not completely inhibit colony growth it was observed that there was significant suppression of the colony growth as the concentrations were increased. Thus, it was possible that the extracts from the other plant parts would completely suppress the growth of the organism at higher concentrations as the trend indicated (Table 2). In other studies, Anthoney *et al.* (2013b) reported that hot water extract of *S. didymobotrya* leaves inhibited the growth of all the gram positive and gram-negative bacteria that was tested against it. The extract inhibited the growth of *S. aureus* with a zone of inhibition of 11.00 ± 0.577 . Ngule *et al.* (2013) reported that the antibacterial activity of *S. didymobotrya* leaves was associated with the presence of phytochemicals, which included tannins and alkaloids. Observation that extracts from leaves, immature pods and flowers were not effective ($P \geq 0.05$) may be because they yielded less phytochemicals than in stem and root

barks. Other researchers have demonstrated the antimicrobial activities of some plants against *S. aureus*. The leaf aqueous extracts of *Eucalyptus camaldulensis* Dehnh. using agar well diffusion technique was active against *S. aureus* (gram positive bacteria) even from a low concentration (12 mg / ml), the zones of inhibition increased in a dose dependent manner and it was bactericidal at 50 mg/ml (Musa *et al.* , 2011). The crude extracts showed activity that surpassed those of the standard antibiotics suggesting that they could be used in treating diseases caused by *S. aureus*. In their investigations on antibacterial investigations of moringa (*Moringa oleifera*) leaf extract on *S. aureus*, Abubakar and Usman (2016) reported that methanol extract had more antibacterial activity than the water extract, more so the extracts were discovered to be more active at higher concentration. They concluded that methanol extract of *M. oleifera* leaves have a potential antibacterial activity and to cure infections caused by bacteria such as *E. coli*, *S. aureus* and *S. pneumonia*. *Escherichia coli* is a gram-negative bacterium that lives in the digestive tracts of humans and warm-blooded animals. *E. coli* is a food borne pathogen that causes diarrhoea, hemorrhagic colitis and haemolytic uremic syndrome, which is a killer disease worldwide (Omori *et al.* , 2012). Young children, the elderly and immune-compromised individuals are particularly at risk (Zhao *et al.* , 2013). *E. coli* infection usually goes away on its own however the main treatment / prevention is to drink plenty of water since diarrhoea causes dehydration, which is especially dangerous for babies and older adults. With bloody diarrhoea, medicine or antibiotics can be used. Stem bark and root bark extracts completely inhibited colony growth of *E. coli* at concentrations of 5.0% and 7.5% respectively (Table 3). The other plant parts did not completely inhibit the colony growth at the highest concentration studied (10.0). However, a decrease in colony size was observed in

these extracts as the concentrations increased. The results obtained in this study are in conformity with those obtained by Anthoney *et al.* (2013 a), in which the plant roots were found to inhibit the growth of *E. coli* with significant zone of inhibition of 13.33 ± 0.667 . In other studies, Chandrabhan *et al.* (2011) found that with increased concentration of up to 15% methanol and aqueous extracts of *Terminalia chebula* inhibited growth of both *E. coli* and *S. aureus*. Using agar well diffusion method, methanolic extracts showed a greater antibacterial activity compared to aqueous extracts (Lakshmidivi *et al.* , 2011) with *Embllica officinalis* leaf extract giving a zone of inhibition of 20 mm on *E. coli*.

S. didymobotrya stem bark and root barks have shown effective antimicrobial activities against *S. aureus* and *E. coli* at low dosages/ concentrations. There is need to carry our research on these plant part extracts to identify the quantity of active phytochemicals that contribute to their high efficacies as compared to leaf, flower and immature pods extracts. On the conservation front, harvesting of these plant parts may lead to depletion of *S. didymobotrya* because they are potentially destructive unlike harvesting of the leaves, flowers and immature pods that regenerate every season hence reforestation of the plant is recommended for sustainability. *S. didymobotrya* extracts possessed antibacterial activity against bacterial pathogens thus supporting their folkloric usage, promising a future scope for its use against microbial populations. Methanolic extracts possessing high antibacterial effects should be further investigated for their therapeutic utility. This would be related to the presence of bioactive metabolites, which are soluble in methanol. There is need to explore further the quantities of phytochemicals in the root and stem barks that make them more potent than the other plant parts. The structures of the bioactive metabolites should be examined in future.

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