Antimicrobial activity and phytochemical screening of *Senna didymobotry* used to treat bacterial and fungal infections in Kenya

Authors Names:

a) Catherine Kadogo Kitonde[1] (Corresponding Author);
E-mail address: ckitonde@uonbi.ac.ke or ckitonde@yahoo.com; phone number: +254-708 962 471

b) DossajiSaifudin Fidahusein[2]; E-mail address: saifuddin.dossaji@uonbi.ac.ke; Phone number: +254-20-4449004; ext: 2477.

c) Catherine Wanjiru Lukhoba[3], E-mail address: clukhoba@uonbi.ac.ke; Phone number: +254-20-4449004; 4447865; ext: 2470.

d) Miriam Musamia Jumba[4]; E-mail address: Miriam.jumba@uonbi.ac.ke; Phone number: +254-20-2180089.

Address for All Authors: University of Nairobi, School of Biological Sciences. P.O. Box 30197-00100. Nairobi- KENYA.

Abstract

Infectious diseases are prevalent and life threatening in Kenya. The majority of the sick are seeking herbal remedies in search of effective, safe, and affordable treatments. This study aimed to investigate the antimicrobial activity and presence of chemical compounds in different parts of *Senna didymobotrya*. Results showed that, organic extracts of *root* with Mean inhibition zone (MIZ) of 1.58 cm, recorded the highest activity against *S. aureus* than the standard antibiotic (Streptomycin MIZ of 1.30 cm. Flavonoids were the chemical compoundshighly present. The results of this study suggest that *S. didymobotrya* has significant antimicrobial properties and justify its use in traditional herbal medicine for the management of microbial based diseases. Cytotoxicity assays are highly recommended for *S. didymobotrya* in order to verify, validate and document its safety in medicine.

Key words: Prevalent, Effective, Herbal, *Senna didymobotrya*.

1.0 INTRODUCTION

Infectious diseases are the leading cause of death in children and young adults accounting for one in every two deaths in developing countries, according to WHO (1999, 2000), as cited in Smolinski and Hamburg(2003). The issue of population health and socioeconomic development is particularly acute in Sub-Saharan Africa with a high burden of bacterial, fungal, and viral infections, such as: tuberculosis, skin rushes, mouth rush, diarrhoea, gonorrhoea, syphilis, and ringworms among others (Bloom & Canning, 2008). It has been estimated that every hour, 1,500 people die from an infectious disease. Over half of them are children under 5 years of age.

Adenisa, Idowu, Ogundaini, Oladimeji, Olugbade and Pais (2000) in their studies found that, microbial infections are prevalent in Kenya and have contributed to unsustainable socio-economic development due to high mortality rate of the infected patients, emergence of antibiotic resistance in pathogens which threatens to overwhelm modern healthcare systems, side-effects of these antibiotics to the hosts, unaffordable medicine to the poor, and eventually the suffering people result to use of alternative medicine/or herbal remedies in such of effective and safe cure. Natural products of higher plants may provide a new source of antimicrobial agents with possibly novel mechanisms of action.

Throughout history, microbial infections have been a major threat to human and animal health and a prominent cause of morbidity and mortality which lead to reduced work productivity and long-term poverty, according to WHO/FAO/OIE (2003).World Health Organization(2012) reported that, in our increasingly interconnected world, new diseases are emerging at unprecedented rates, often with the ability to cross borders rapidly and spread.

Mariita, Mariita, Ogol, Oguge, and Okemo(2010) reported increased antibiotic resistance which has become a global concern, coupled with the problem of microbial persistence, thus highlighting the need to develop novel microbial drugs that are not only active against drug resistant microbes, but more importantly, kill persistent micro-organisms and shorten the length of treatment. Apart from toxicity, lengthy therapy also creates poor patient compliance

The high cost of important conventional drugs and / or inaccessibility to modern health care facilities has led to overreliance on traditional medicine since it is affordable and available to rural people. On the other hand, even when conventional health facilities are available, traditional medicine is viewed as an efficient and an acceptable system from a cultural perspective, according to Munguti, (1997). WHO (2007), posited that today's universal vulnerability to these threats, better security calls for global solidarity in search for safe, effective, and affordable drugs .

According to Sandiga, Chacha, and Kanunah (1995), it is estimated that about 75% of the population in Kenya seeks health care among traditional healers. In certain instances people utilize both traditional and modern medicine simultaneously. As has been shown in studies ofethnomedical surveys ofMiaron, Kassim, and Ekaya (2004) and Kareru, Kenji, Gachanja, Keriko, and Mungai (2007), traditional medicine is widely practiced in Kenya.Infections associated with bacterial and fungal pathogens are among some of the indications treated using traditional remedies in Kenya , as reported by Njoroge and Bussmann(2007).

Therefore, the aim of this study was to determine antimicrobial activity (efficacy) in different parts of*Senna didymobotrya*, claimed to treat microbial infections in Kenya, against *Escherichiacoli*, *Staphylococcus aureus*, *Candida albicans* and *Aspergillus niger*, and thereafter, find out the chemical profiles of the active crude extracts, which would be a prospective open for future search to identify the active compounds.

2.0 Material and Methods

2.1 Collection of Plant Material

The *Senna didymobotrya* was selected based on ethno-medicinal information from literature and collected from Kibuye in Kisumu County, Kenya in January 2010. The specimen was authenticated

by a plant taxonomist in University of Nairobi and a voucher specimen (CK 2010/02) deposited at the University of Nairobi Herbarium.

2.2 Crude plant Extracts preparation

The seeds, seedpods, leaves, stem, and roots were air-dried under the shade at room temperature, ground into powder and extracted using Dichloromethane/Methanol in the ratio 1:1, and water, according to standard extraction methods (Harborne, 1998).

2.3 Sources of Micro-organisms and Preparation of Standard inoculums

Pure cultures of bacteria; *Staphylococcus aureus* ATCC 259213, *Escherichia coli* NC 35218 (from School of Pharmacy, University of Nairobi) were maintained on nutrient broth slants at 4^{o}C. Two fungi; *Candida albicans* SC 5314 (Provided by Ted White, from Seattle Biomedical Research Institution U.S.A), and *Aspergillus niger*ATCC 16404 (a collection of the late Professor George M. Siboe, School of Biological Sciences, University of Nairobi) were maintained on Sabourauds' Dextrose agar slants at 4^{O}C. The standard inoculums suspensions were adjusted to turbidity equivalent to 0.5 McFarland standards to give a density of $1x10^{8}$ cells or spores/ml, according to Nostro, Germano, Marino and Cannatelli (2000).

2.4 Antimicrobial Activity

Disc diffusion technique was used as the standard method for antimicrobial activity and minimum inhibitory concentrations (MICs) for active extracts against *Staphylococcus aureus*, *Escherichia coli*,*Aspergillus niger*, and *Candida albicans*. Stock solution at concentration 2000 mg/2 ml (1000 mg/ ml) was prepared for each plant part and entire plant used. Two fold serial dilutions were prepared from each stock solution. Readily manufactured sterile paper discs were used for each concentration prepared by pipetting 100µl onto individual paper discs (0.6cm) drop by drop using a micropipette. Paper discs with crude extracts were transferred onto plates inoculated with 1ml of standard inoculum for each test organism. The plates were labelled, and incubated at 37^{o}C for bacteria and *Candida albicans*, and 25^{o}C for *A. niger*. Streptomycin (for bacteria) and Nystatin (for fungus) were used as standards, while discs with extraction solvents only were used as controls. These were done in duplicates under sterile conditions and results recorded after 24, 48, 72 and 96 hours. The antimicrobial activity was determined by measuring clear inhibition zones diameters (including diameter of paper discs) formed using a transparent ruler (cm). Minimum inhibitory concentrations were determined by recording the lowest concentration of the active extracts that inhibited growth of the micro-organisms, Ochei and Kolhatkar, (2000).

2.5 Chemical Analysis of Selected Crude plant Extracts

The organic extracts that were active at low concentrations ($\leq$ 25 mg/100 µl) were analysed for presence or absence of alkaloids, Sapogenins, terpenoids, quinones, and flavonoids using Thin Layer Chromatography (TLC) technique, and the developed TLC plates were viewed under Ultra-Violet light and then sprayed with appropriate reagents for the detection of the chemical groups according to Harborne (1998).

2.6 Data Analysis

In order to analyse data, multiple way ANOVA was used to determine significant factors in production of inhibition zones, Tukey's Honest Significant Difference Test (THSDT) was used for means comparison within the significant factors (Brown, 2012).

3.0 Results

3.1 AntimicrobialActivity

In this study, mean inhibition zones were used as the results for figures 1, 2 and 3 below.Among the organic extracts of *S. didymobotrya* parts used, only the Stem, root, and seed extracts were active only against *S. aureus* (Figure 1).

The organic crude extract of root was more active with mean inhibition zone (MIZ) of 1.58 cm against *S. aureus*, with significant difference in activity from streptomycin (MIZ 1.30). Stem extract (MIZ of 1.10) was significantly different in activity from the root extract against *S. aureus*, but insignificantly different in activity from Streptomycin (MIZ of 1.30). The seed extract was less effective against *S. aureus* (MIZ of 0.18) with significant difference in activity from Streptomycin (MIZ of 1.30), root, and stem extracts. The seedpod, leaf, and whole plant extracts were not active against *S. aureus*, *E. coli*, *A. niger*, and *C. albicans* (Figure 1).

Only the stem and root aqueous extracts of *S. didymobotrya* were effective only against *S. aureus* (Figure 2).The stem extract was slightly more active (MIZ of 1.10) than the root extract (MIZ of 0.76) against *S. aureus*, with significant difference from each other. Streptomycin (MIZ of 1.30) was more active against *S. aureus* as compared to aqueous extracts of stem and root.

3.1.1 Susceptibility of test-organism

S. aureus was the only susceptible organism to organic extracts (MIZ of 0.11) and aqueous extracts (MIZ of 0.05) of *S. didymobotrya* with significant difference in susceptibility from *E. coli*, *A. niger*and *C. albicans* which were not affected by any of the extracts (Figure 3).

3.1.2 Extraction solvent

The organic extracts of *S. didymobotrya* were generally more active than the aqueous extracts most of which had lower activity or no activity against at least one of the four micro-organisms tested (Fig.3).

3.1.3Minimum Inhibitory Concentrations (MIC)

MIC of each extract and standards that were able to kill or inhibit the growth of at least one of the four micro-organisms tested in this study was exhibited by extracts that were active at concentration of 100mg/100µl to 0.02mg/100µl. The inverse of MICs was displayed on figure 4 below.

The organic extracts of stem and root against *S. aureus* recorded moderate activity (inverse MICs of 0.16 mg/100 µl each) as well as streptomycin, while the aqueous extracts of stem and root displayed low activity (inverse MIC of 0.04 and 0.02 mg/100 µl respectively).

3.1.4 Chemical Profile of five classes of Compounds identified in Different Parts of *S. didymobotrya*

The crude extractsof *S. didymobotrya* (stem and root) were screened for alkaloids, sapogenins, terpenoids, quinones, and flavonoids. The presence of classes of compounds was displayed by simple scoring (Table 1).

Terpenoids and flavonoids were present in all extracts in significant amounts. Sapogenins and quinones were moderately present, while alkaloids were least present because they were found only in trace amounts in stem extract and absent in root.

Discussion

The extent of the antimicrobial activity in this study was related to the extract of the plant part used. The organic extract of *S. didymobotrya* root elicited moderate mean inhibition zone of 1.58 cm, than the standard streptomycin (MIZ of 1.30 cm), followed by organicextract of stem (MIZ of 1.10 cm) against *S. aureus*. These results are significant in early stages of drug research as reported by Ochei and Kolhatkar, (2000 p. 811) that intermidiate susceptibility results indicate that the therapy with the drug can be successful only if it is administered in larger doses or when the drug is concentrated at a certain site such as urinary tract or dermal.

The organic extract of seed (MIZ of 0.18) and aqueous extract of root (MIZ of 0.76) were less active against*S. aureus*. Theextracts of leaf and seedpod were not active against any of the four micro-organisms used. The less activity and inactivity of these extracts could have been caused by the seasonal variations, in collection of plant material and the part of plant species at different stages of the plant growth and development. This could have affected the chemical composition of the plants and thus its antimicrobial activity. Therefore, the antimicrobial agents were either present in very little quantities or totally absent. This argument is in line with that of Birdi, Brijesh, and Daswan (no date), who argues that seasonal variations and part of plant species harvested at different stages of growth and development, can affect chemical composition of the plants, and thus its biological activity. In most cases, maximum accumulation of chemical constituents occur at the flowering season and declines at the beginning of the fruiting stage and this can lead to either presence of chemicals in high or very low quantities or totally absent in matured plants.

Results of this study did show some extracts of plant parts were more active when used singly, than those of the entire plant combined. Organic extract of *S. didymobotrya* entire plant was inactive compared to its single parts; stem and root extracts (MIZs of 1.10 and 1.58 respectively). This observation indicates that, the interactions of the active chemical substances present in crude extracts of plant parts used, formed strong synergistic effects to be able to inhibite the growth of test organisms, compared to those of the entire plant which had no effect against the four micro-organisms tested. It could be that *S. didymobotrya* parts combined, exhibited antagonistic effect that could have been caused by the interaction of the active compounds in the plants parts used, and therefore negating or lessening the activity of each other. These findings correlate with the results of Maryam,Aquil, Khan, and Ahmad (2010), who did research on different plant parts and mixtures (combinations) of plant extracts and found that, plant extracts or phytocompounds exhibiting strong antimicrobial activity may interact with each other and the interactions may be synergistic or

antagonistic. Therefore, the synergistic effects of these plant extracts in this study would be of significant importance in further search of novel compounds, with desirable synergistic effects, to kill persistent micro-organisms that are resistant to known antibiotics and delay emergency of microbial resistance.

The patterns of antimicrobial activity varied with the plant extract and the solvent used for extraction. The organic crude extracts showed more inhibition than the aqueous extracts on the average for all micro-organisms tested. This is seen in more active organic extracts of *S. didymobotrya* (MIZ of 0.11 cm) compared to its aqueous extracts (MIZ of 0.05 cm). These were compared with streptomycin (MIZ of 0.33) against *S. aureus*. Since traditional herbal remedy preparation use water as the extractant, it is a paradox that the aqueous extracts were inactive or less active in this study. It is possible that the aqueous crude extracts may contain antimicrobial constituents insufficient for efficacy in our study and which may explain why large amounts of the decoctions must be drunk by the patients. This observation is supported by Jigna and Chanda(2007), who also found out that, aqueous extracts showed little or no antimicrobial activity in contrast to those made using organic solvents. Yineger, Kelbessa, Bekele, and Lulekal(2008) in their studies reported that, the success in traditional medicines may be due to administration of the extracts in large quntities and over a long period of time.

Sensitivity of the test organisms to the plant extracts varied depending on the micro-organism. Most of the extracts were antibacterial especially against *S. aureus*, which was the most susceptible bacterium to the extracts compared to less affected *E. coli*. Differential sensitivity of bacteria to plant extracts, may be explained by the cell wall composition of Gram-positive and Gram-negative bacteria. According to (Nester, Roberts, Pearsall, and Nester (2004),the cell wall of gram-negativeve bacterium (*E. coli*), contains an outer membrane and lipid bilayer embedded with proteins and porins (carrier proteins). These proteins allow passege of certain small molecules or ions either into or out of the cell periplasm. The active compounds may not be able to pass into the cells, making them inactive. The size of the porin channel particularly determines the size of the molecule that can pass through it and thus, the outer membrane serves as a barrier to the passage of many molecules and excludes many toxic compounds, and hence less sensitive to many extracts.However, the Gram-positive bacterium (*S. aureus*) has a relatively thick membrane consisting of layers of peptidoglycan, but regardless of its thickness, peptidoglycan is fully permeable to many substances including sugars, ions, and amino acids, and thus sensitive to most extracts (Nester *et al*., 2004).

All the *S. didymobotrya*extracts were inactive against *A. niger* (filamentous fungus) and *C. albicans* (yeast fungus). The inactive of the extracts against the two fungi, may be due to differences in cell wall composition. Based on Paiva, Gomes, Napoleao, Sa, Correia, and Coelho(2010) studies,yeast fungus cell wall contains polyssacharides and proteins, compared to chitin and glycan in the cell walls of filamentous fungi.The proteins expression of *C. albicans* function as a selective transport system to expell wastes and compounds that are deleterious to the cell. This functions as an efflux which is medically important in that, it allows micro-organisms to oust antimicrobial medications that are made to destroy them, and therefore render them inactive, according to Nester *et al.*, (2004). This could be one of the reasons why the plant extracts were ineffective against *C.albicans*. The

report of this study is in agreement with the findings of Masakazu, Firth, and Cannon (2010) who reported that expression of drug efflux pumps were responsible for the inactivity of drugs to *Candida* spp.

Minimum Inhibitory Concentration (MIC) is important to confirm resistance of micro-organisms to an antimicrobial agent and also monitor the activity of new antimicrobial agent, as reported by Das, Tiwari, and Shrivastava(2010). MIC of the plant extracts and standards ranged from 100mg/ 100µl to 0.02mg/100µl, with their inverse MIC of 0.01 mg/100 µl to 0.98 mg/ 100 µl. The organic extract of *S. didymobotrya* root and stem showed moderateactivity with inverse MIC of 0.16 each, as well as the standard antibiotic (streptomycin) against *S. aureus*. These extracts may be used for discovery of novel compounds at moderate concentrations with new mechanisms to combat bacterial strains unsensitive to drugs. This observation is collaborated by the studies of Mariita *et al*., (2010); Aiyegoro and Okoh (2009), who reported that bioactive extracts of medicinal plants at moderate concentrations are active against unsusceptible bacteria.

This study reports that flavonoids and terpenoids were highly present in all extracts that were screened for *S. didymobotrya* stem and root. Flavonoids have been associated with inhibition of cytoplasmic membrane functions as well as inhibition of of DNA gyrase enzyme and carrier protein activities, according to report by Paiva *et al*., (2010). This property of flavonoids may explain why the organic and aqueous crude extracts of root and stem were active against one of the four micro-organisms tested in this study.

Conclusion

The screening for antimicrobial activity of *Senna didymobotrya* extracts collected using available ethnomedical information, has verified that Kenyan medicinal plants have potential as new sources of antibacterial agents. Organic crude extracts of stem and root were active against one of the four microbial pathogens tested. This shows the potency of *S. didymobotrya* medicinal plant to treat bacterial diseases. Therefore, this study justifies the use of this plant by traditional healers/ or herbalists and thus may be the starting point of research as phytomedicine for the society's healthcare.

Findings of this study showed that flavonoids and terpenoids were present in great amounts in all the extracts screened, while sapogenins and quinones were present in sufficient amounts and were the four major classes of phytochemical compounds present in almost every extract screened. Alkaloids were moderately present in the stem extract. The presence of these classes of compounds in different crude extracts, suggests the richness of Kenyan medicinal plants with diverse phytochemicals. These chemicals can be used for research in development of new compound with antimicrobial activities for management of infectious diseases.

Recommendations

As only *in vitro* method was used in assessing the antimicrobial activity of the plant crude extracts, further investigations using bioassay guided fractionations are recommended to isolate and identify the pure compounds responsible for antibacterial activities of *S.* didymobotrya. Toxicology research

which is missing in this study is recommended for *S. didymobotrya*, in order to verify and document the safety of this medicinal plant to the society.

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Figures and tables

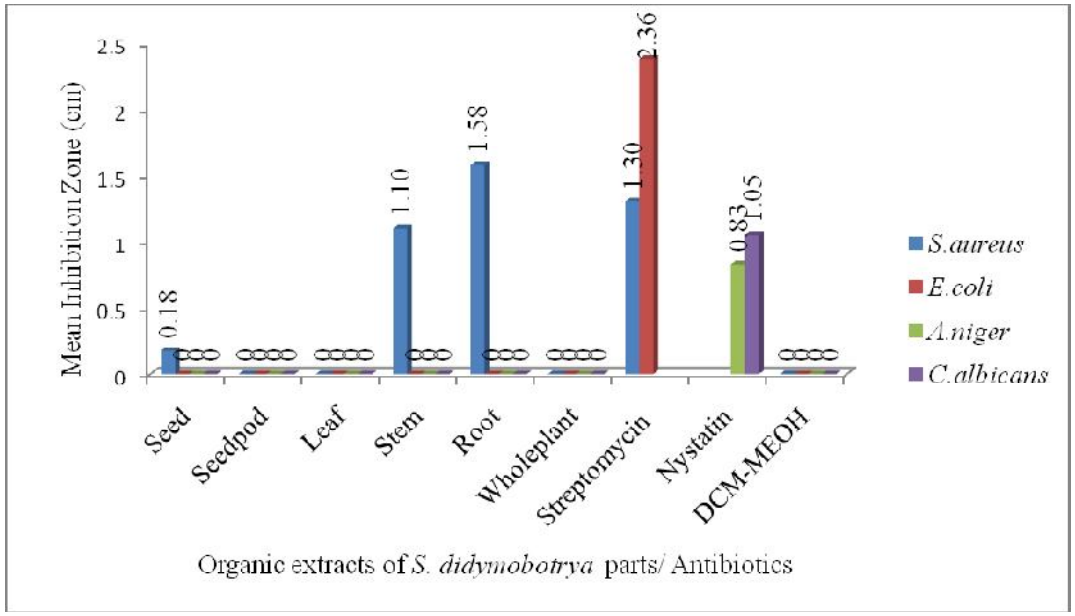

Figure 1. Mean inhibition zones of organic extracts of *S. didymobotrya* parts compared with Streptomycin and Nystatin at 100mg/ 100µl

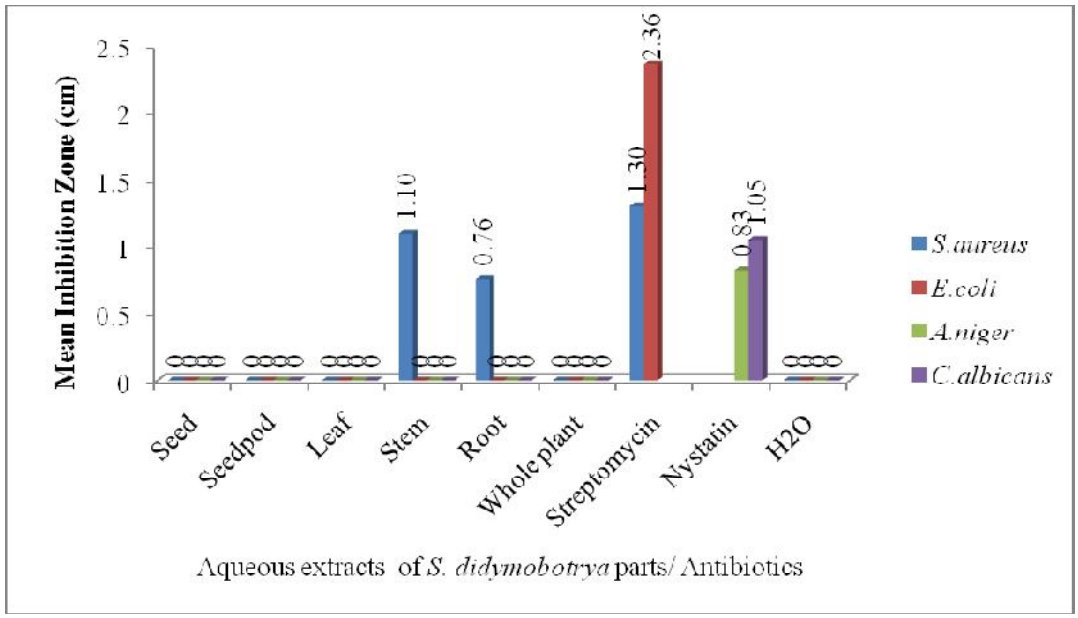

Figure 2. Antimicrobial activity of aqueous extracts of *S. didymobotrya* parts compared to Streptomycin and Nystatin at 100mg/ 100µl

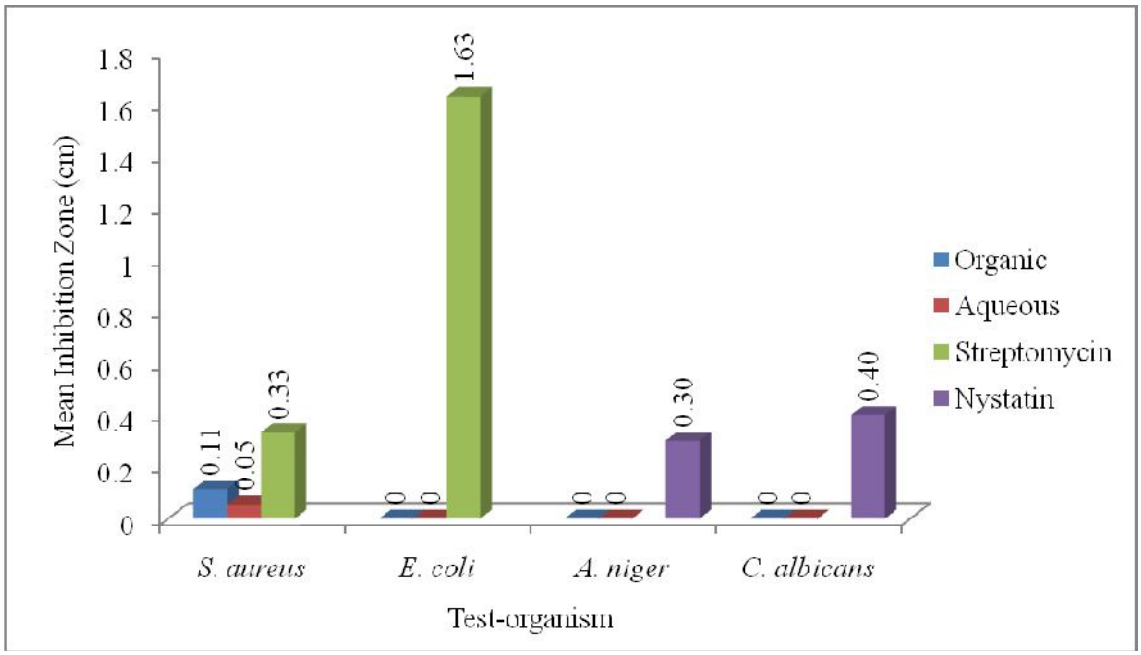

Figure 3. Susceptibility of test-organisms to organic and aqueous extracts of *S. didymobotrya* and antibiotics

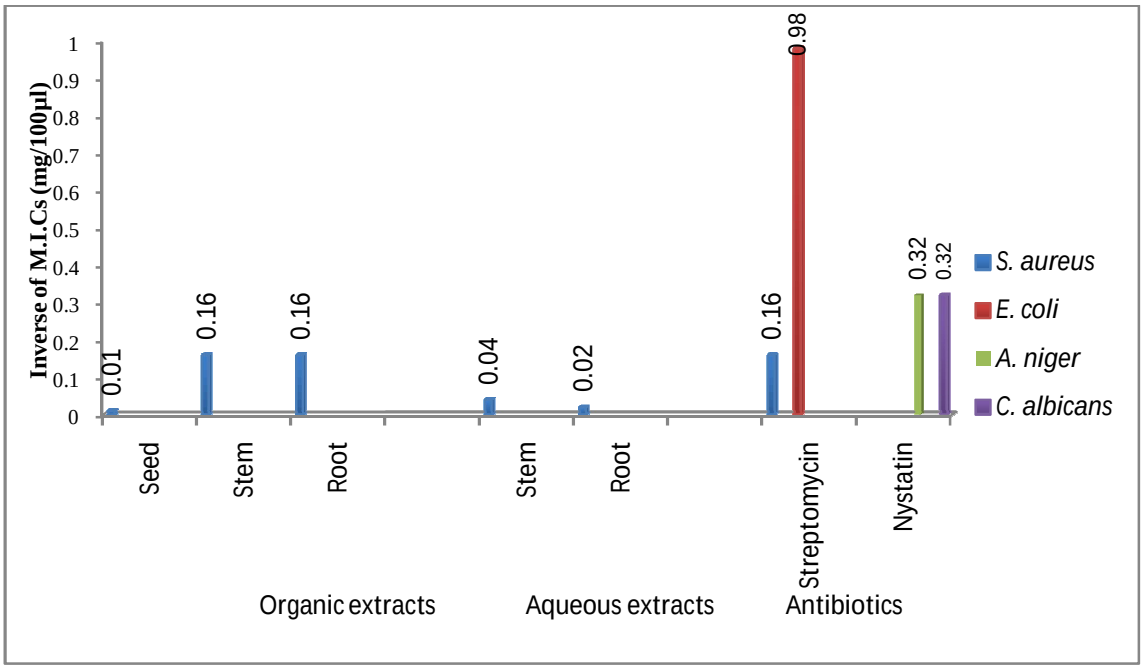

Figure 4.MICs for active organic and aqueous extracts of *S. didymobotrya*, compared to streptomycin and nystatin

Note: Lower inverse of MICs indicate low activity while high inverse of MICs represent high activity.

Table 1. Five classes of compounds screened presentin*S. didymobotrya* extracts (stem androot)

	Five classes of compounds screened present				
Extracts	**Alkaloids**	**Sapogenins**	**Terpenoids**	**Quinones**	**Flavonoids**
Stem	+	++	+++	+++	+++
Root	_	++	+++	+	+++

Key: +++= highly or greatly present; ++ = moderately or fairly present; + = less present (trace amounts); - = Not present

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