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Original Article

Antimicrobial Activity of *Senna didymobotrya* and *Thunbergia alata* Plant Extracts Against Selected Bacterial Clinical Isolates from Kericho Referral Hospital in Kenya

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Senna didymobotrya, Thunbergia alata, Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa, Bacterial infections, Minimum Inhibitory Concentrations (MICs), Antimicrobial activity.

ABSTRACT

Bacterial infections are distributed worldwide and cause deadly infectious bacterial diseases such as skin, soft tissue and respiratory tract infections, meningitis, and tuberculosis. Bacterial infections are very common and can be easily acquired since bacteria are ubiquitous. It has challenged modern healthcare providers; conventional drugs are costly and have side effects. Therefore, alternative remedies that are easily available, affordable, and effective are needed. This study was carried out to determine the antimicrobial activity of *Senna didymobotrya* and Thunbergia alata crude plant extracts against Staphylococcus aureus, *Streptococcus pyogenes* and *pseudomonas aeruginosa* common in Kericho County. Plant leaves of the two plants were sourced from two sites (Bomet and Kabianga), dried, milled into powder and solvent extracted using hexane, dichloromethane: methanol ratio (1:1) and methanol. Phytochemicals present in each plant extract were evaluated using standard laboratory procedures. Antimicrobial sensitivity testing, Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentration (MBC) were determined. Discs impregnated with standard antibacterial drugs were used as positive control. Leaves of *T. alata* and *Senna didymobotrya* collected from Bomet contained 7.41% and 10.4% while those from Kabianga contained 8.07% and 17.71% of extracts respectively, suggesting that site conditions do not influence the percentage of extracts. Leave extracts of *S. didymobotrya* and *T. alata* were found to be rich in alkaloids, flavonoids, terpenoids, glycosides and tannins irrespective of plant collection site, solvent of extraction. *S. didymobotrya* and *T. alata* plant extracts significantly inhibited growth of the exposed microbes in the following order: S. aureus, ≥*S. pyogenes* and≥ *P. aeruginosa* bacteria in comparison with commercial antibiotics (penicillin, chloromphenical, and erythromycin). The MIC values of the isolates ranged from 20×10-3 mg/ml to 4.8 ×10-3 mg/ml. However, bacterial inhibition by plant extracts showed re-growth of *S. pyogenes* after 36 hours, suggesting bacteriostatic nature. These results suggest that *S. didymobotrya* and *T. alata* leaves contain significant amounts of alkaloids, flavonoids, terpenoids, glycosides and tannins hence can be

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used as traditional medicine to manage *S. aureus, S. pyogenes* and *P. aeruginosa* bacteria found on human skin.

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INTRODUCTION

Humans and animals are susceptible to diseases associated with bacterial infections (Verbrugghe *et al.,* 2012). Bacterial infections are widely distributed worldwide and cause most of the deadly infectious bacterial diseases such as skin infection and soft tissue infections, respiratory tract infections, meningitis and tuberculosis (Russo *et al.,* 2016). Bacterial infections are very common and can be acquired easily since bacteria are ubiquitous. An infectious disease caused by bacteria exists in many forms and significantly affects human health. The sources of infectious diseases are vast, but in most cases arise from infectious microorganisms such as bacteria that can establish growth or replication in humans, harming specific systems of human body (Anderson & May, 1991). This has contributed to unsustainable socio-economic development following the emergence of antimicrobial resistant strains of pathogens to the available conventional (Kitonde *et al.,* 2014). It has become a challenge to modern healthcare providers to treat bacterial infections. This resistance is caused by exposure to microbes carrying resistant genes (Chalo, 2015). The problem of antibiotics resistance is not limited to Kericho County, it is a global issue. The indiscriminate and irrational use of antibiotics has created a unique challenge for human civilization due to microbe's development of antibacterial resistance to available drugs making it difficult to treat (Muteeb *et al*., 2023). To date there is no known method to reverse antibiotic resistance by bacteria, since it is a natural way in which bacteria adapt to antimicrobial agents. Antimicrobial agents are characterized according to their mechanism of action, that is, interference with cell wall synthesis, DNA and RNA synthesis, lysis of the bacterial membrane, inhibition of protein synthesis and inhibition of metabolic pathways (McManus, 1997). Bacteria may become resistant by antibiotic inactivation, target modification, efflux pump and plasmid efflux. The indiscriminate and irrational use of antibiotics nowadays has led to emergence of more lethal strains compared to the parent strain. The clinically available medicine is no longer effective against antibiotic resistant strains (Podolsky, 2015). Hence there is need to explore other remedies including use of medicinal plants which are within reach of many Kenyans. Many communities have been using medicinal plants to treat different ailments for many years, hence the need to investigate the efficacy of *Thunbergia alata* and *Senna didymobotrya* extracts for the

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potential treatment of bacteria that cause skin infections. Plant extracts have been known since ancient times to treat various ailments and are a better choice in the search of bioactive compounds.

The challenge of skin infections caused by bacteria has become a major health problem globally, regionally, and locally since such infections are nosocomial infections acquired in hospital facilities and have proved difficult to control. Recent reports indicate that *Staphylococcus aureus*, *Streptococcus pyogenes* and *pseudomonas* have become multi-drug-resistant pathogens and life-threatening.

2 MATERIALS AND METHODS

2.1 Collection of Plant Samples

Experimental design was used in this study. Mature *S. didymobotrya* and *T. alata* were selected in a simple random sampling manner from Bomet (0.31ºS, 35.2 º E 1981 m above sea level) and Kabianga regions (0.41ºS, 35.16ºE 1805 m above sea level) respectively.

Purposive sampling technique was used to pick the leaves of *S. didymobotrya* and *T. alata.* Fresh leaves of *S. didymobotrya* and *T. alata* were plucked separately from mature plants when both plants were at their flowering and fruiting stages. The collected leaves were placed in a sampling bag, labelled, and transported to the Botany laboratory at the University of Kabianga. Precautions were taken to ensure that the plants were not injured during the plucking of the leaves. In the laboratory, the leaves were air-dried in a shaded area for two weeks. A sample of the leaves was pressed using a plant press before taxonomic identification at the National Museum of Kenya.

2.2 Grinding of the Leaves

The dried leaves of both *S. didymobotrya* and *T. alata* were separately ground into a homogenized powder using a dry laboratory ball mill grinder (Lasany). The laboratory ball mill consists of a cylindrical shell rotating on a horizontal axis mounted on a sturdy mild frame. The ball mill is designed to withstand the rotation load of the mill, the charged medium and the material to be processed. The bulk dried leaves were loaded into the cylinder and fastened using the bolts. On turning the power on, the cylinder rotated, and the balls crushed the bulk dried leaves into fine powder. The powders were then separately extracted using hexane, dichloromethane: methanol (1:1), and methanol as follows:

2.3 Solvent Extraction of Phytochemical from *S. didymobotrya* and *T alata* leaves

The ground homogenized powder was separately treated with organic solvents of different polarities (starting from hexane, dichloromethane: methanol (1:1) and finally methanol) in a series method of solvent extraction as follows: 460g of the powder was put into a 2.5 litres reagent bottle, and then 1.5 litres of the solvent were added. The reagent bottle was tightly corked, swirled to ensure that the entire powder was submerged, and left to stand for 72 hours before filtration. The mixture was filtered using Whatman filter paper no. 1, and the filtrate collected into a conical flask. The filtrate was then evaporated under vacuum and dried to greenish-brown gummy semi-solid mass of constant weight (R1). The yield of the extract is evaluated as a percentage of the initial weight of the ground leaf powder.

The dry greenish-brown gummy semi-solid mass extract was put in a 10 ml beaker and covered with a parafilm, labelled for identification purposes and stored aseptically in a refrigerator (4 ºC) awaiting use. The residue was dried for subsequent extraction using solvents of a higher polarity.

2.4 Identification of Phytochemical in Plant Extracts

2.4.1 Test for Alkaloids

The extracts were tested for alkaloids by adding 5 ml of the extract in respective extracting solvent in a 10 ml test tube and 1 ml of Wagner's reagent was introduced, shaken for 1 min and allowed to stand. The appearance of reddish /brown precipitate signifies the presence of alkaloids.

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2.4.2 Test for Saponins

The extract was tested for saponins by mixing 2 ml of the extract with 6 ml of distilled water and shaken vigorously. Production of persistent foam for ten minutes indicates the presence of saponins.

2.4.3 Test for Flavonoids

The extracts were screened for flavonoids by mixing 2 ml of the extract with 2 ml of dilute sodium hydroxide (NaOH). An intense golden yellow precipitate indicated positive results for flavonoids.

2.4.4 Test for Terpenoids

Terpenoids were tested by adding 1 ml of ethyl acetate to 5 ml of the extract followed by addition of 2 ml chloroform to the mixture and shaken vigorously. 3 ml of concentrated Sulphuric (H_2SO_4) acid was then carefully added. The reddish-brown coloration at the interface indicated the presence of terpenoids.

2.4.5 Test for Glycosides

Glycosides was tested as follows; 0.5 gm of the extract was dissolved in 2 ml glacial acetic acid containing two drops of 10% ferric chloride ($FeCl_3$) solution. 1 ml of concentrated Sulphuric acid was then added alongside under-layering the mixture. A brown ring at the interphase indicated the presence of glycosides.

2.4.6 Test for Tannins

Tannins were tested as follows; 0.5 gm of the extract was dissolved in 2 ml distilled water and four drops of ferric chloride reagent added. A blue-black precipitate indicates presence of tannins.

2.5 Media Preparation

The preparation of the media was done by following manufacturers direction for all media that was used in this study. The media was weighed using analytical balance and dispensed in 500 ml conical flask with the intended volume of distilled water. It was allowed to dissolve by swirling to mix while heating on a hotplate. The mixture was then sterilized by autoclaving at 121℃, for 15 minutes, then it was allowed to cool to 45℃. Then dispensed aseptically into the petri dishes and culture tubes under a biosafety cabinet. The media in the plates were allowed to solidify by closing the plates halfway.

2.6 Source of Test Bacterial Species

Bacteria species (*S. aureus, S. pyogenes* and *P. aeruginosa)* were obtained from Kericho Referral hospital as clinical isolates. The isolates were cultured in disposable plates on Eosin-Methylene Blue (EMB) agar and frozen in nutrient agar vials. The samples in the plates and vials were then transported to microbiology laboratory at the University of Kabianga. In the laboratory, the raw isolates were sub-cultured in nutrient agar plates for confirmatory tests.

2.6.1 Confirmatory test for S. aureus, S. pyogenes and P. aeruginosa

The clinical isolates were handled aseptically, and sub-cultured by inoculating onto the nutrient agar plates under a bio safety cabinet. The inoculated plates were then incubated at 37℃ for 24 hours after which growth of the micro-organisms was observed. Once the growth of microorganisms was observed, isolates were identified using the standard morphological and culture characteristic by performing Gram staining procedures followed by biochemical tests.

2.7 Antibacterial Sensitivity *of T. alata* and *S. didymobotrya* Leaf Extracts

The Kirby-Bauer technique was used to determine the antibacterial sensitivity of the plant extracts, against three bacterial strains (*S. aureus, S. pyogenes* and *P. aeruginosa*). The media used to evaluate antimicrobial sensitivity was Mueller Hinton Agar (MHA). Kirby-Bauer technique was performed by streaking bacterial inoculums to the surface of the plate (of 90 mm diameter) MHA. 50 mg of each plant extracts were weighed using the precision balance and dissolved in 10 ml of the extracting solvent. The paper discs were soaked in the mixture and allowed to stand for 20 minutes. The impregnated paper discs were aseptically placed on the surface of the inoculated plates. The plates were then incubated at 37° C for 24 hours.

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Paper discs (6 mm) of standard antibiotics (chloramphenicol 500 mg, penicillin 500 mg and erythromycin) were used as positive controls. The inhibition zones were measured and recorded in millimetres as the diameter of growth free zones around the discs using a clear ruler. Each extract and standard antibiotics were similarly tested independently and in triplicate.

3.0 RESULTS

The results obtained from the phytochemical screening of the leaf extracts, the antimicrobial sensitivity testing of the leaf extracts, commercial antibiotics and the determination of the minimum inhibitory and bactericidal concentration of *S. didymobotrya* and *T. alata* are presented in this section.

3.1 Extraction of phytochemicals from *T. alata* and *S. didymobotrya* leaves

The number of extracts from the leaves of *T. alata* from Bomet and Kabianga ecological sites using solvents of different polarities were as shown in *Table 1*.

Table 1. Amount of extracts (%) from the leaves of T. alata and S. didymobotrya

Extracting solvents	Average (%) yield of *T. alata* extracts		Average (%) yield of *S. didymobotrya* extracts	
	Bomet	**Kabianga**	**Bomet**	**Kabianga**
Hexane	2.01	2.62	1.15	8.41
Dcm: Methanol (1:1)	3.19	4.33	8.22	7.92
Methanol	2.21	1.12	1.03	1.38
Total yield (%)	7.41	8.07	10.40	17.71

The yield of extracts from the Leaves of *T. alata* collected from Bomet ranged from 2.01% in hexane to 3.19% in DCM: Methanol giving a total yield of 7.41 % of extracts. Similarly, the yield of extracts from the leaves of *T. alata* collected from Kabianga ranged from 1.12% in methanol to 4.33% in DCM: Methanol giving a total yield of 8.07% of extracts.

The yield of extracts from the leaves of *S. didymobotrya* collected from Bomet ranged from 1.03-8.22% in methanol and DCM /methanol1:1 solvent respectively giving a total yield of 10.4% of extracts. Similarly, the yield of extracts from the leaves of *S. didymobotrya* collected from Kabianga ranged from 1.38-8.41% in methanol and hexane solvents respectively giving a total yield of 17.71% of extracts.

3.2 Profile of phytochemicals of *S. didymobotrya* and *T. alata* leaves

Table 2: Phytochemicals present in leaf extracts

Test reagent	Phytochemicals	Methanol				DCM: Methanol 1:1				Hexane			
		T. a		*S. d*		*T. a*		*S. d*		*T. a*		*S. d*	
		K	*B*	*K*	*B*	*K*	*B*	*K*	*B*	*K*	*B*	*K*	*B*
Wagner's reagent	Alkaloids	+	+	+	+	-	-	-	-	+	+	+	+
HCl + Mg + 10%H_2SO_4	Flavonoids	-	-	+	+	-	+	+	+	+	+	+	+
Distilled water	Saponins	+	+	+	+	+	+	+	+	+	+	+	+
Glacial acetic acid + $FeCl_3$ + conc H_2SO_4	Glycosides	-	-	-	-	-	-	-	-	+	+	+	+
$FeCl_3$	Tannins	+	+	+	+	+	+	+	+	+	+	+	+
Acetoaldehyde + conc. H_2SO_4	Terpenoids	+	+	+	+	+	+	+	+	+	+	+	+

Note: *T. a = Thunbergia alata*, *S. d = Senna didymobotrya*; K = Kabianga; B = Bomet; + = present; - = absent

The result of the phytochemical screening reveals that the extracts had Saponins, Tannins and Terpenoids irrespective of the site of collection and the solvent used. Hexane extracted most of the

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phytochemical in all the plants irrespective of site of collection, while Methanol extracted alkaloids, saponins, Tannins and terpenoids (*Table 2*).

3.4 Antimicrobial activity of the plant extracts

Table 3. Zones of bacterial inhibition (mm) against the plant extracts

Test Bacteria	Plant Extracts	Plant Collection Site			
		Kabianga		Bomet	
		Mean zone of inhibition in millimeters(mm)			
		S. didymobotrya	*T. alata*	*S. didymobotrya*	*T. alata*
S. pyogenes	Hexane	3.5	3.2	2.0	3.3
	DCM:Methanol(1:1)	3.2	3.0	2.8	3.0
	Methanol	4.8	3.8	4.5	3.3
P. aeruginosa	Hexane	3.1	2.5	1.3	2.8
	DCM:Methanol(1:1)	2.6	2.7	2.6	2.7
	Methanol	2.7	2.8	2.9	3.0
S. aureus	Hexane	3.0	2.8	1.6	2.9
	DCM:Methanol(1:1)	3.4	4.0	3.0	3.3
	Methanol	3.8	3.6	4.5	3.0

Table 4. Mean zones of bacterial inhibition (mm) against standard antibiotics

Antibiotic	Test bacteria		
	Mean zone of inhibition in millimetres (mm)		
	S. pyogenes	*P. aeruginosa*	*S. aureus*
Chloramphenicol	1.6	2.5	3.8
Penicillin	0.0	0.9	0.0
Erythromycin	2.1	2.1	3.5

From the antibacterial testing of the crude leaf extracts of *S. didymobotrya* and *T. alata* carried out on the selected bacterial pathogens (*S. pyogenes*, S. *aureus* and *P. aeruginosa*) the crude plant extracts was able to inhibit the bacteria pathogens on Mueller Hinton agar.

Figure 1 shows zones of inhibition of each plant extract on the test microorganisms.

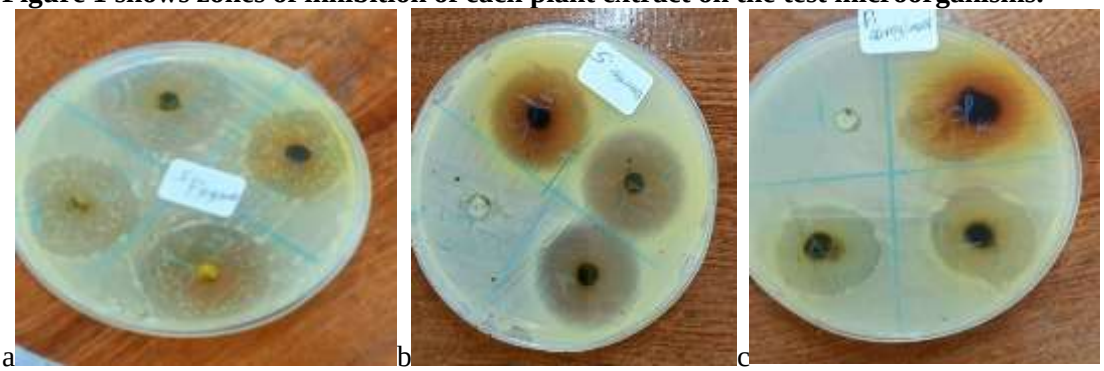

Figure 1: Zones of inhibition of a) *S. pyogenes* b) *S. aureus* c) *P. aeruginosa* in all plant extracts.

The commercial antibiotics were tested against each micro-organism and the results showed that penicillin is less active on all the three micro-organisms since the zone inhibited ranged from 00 mm to 0.9 mm. The gram-positive *S. aureus* and gram-negative *P. aeruginosa* showed a higher zone of inhibition on the plate with

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chloramphenicol as compared to those with erythromycin and penicillin (*Figure 2*).

Figure 2: Zones inhibited by the commercial antibiotics

The zones of inhibition exhibited by the commercial antibiotics are smaller compared to those inhibited by the plant extracts.

3.5 Minimum Inhibitory Concentration

The tubes were observed for the presence of turbidity; the turbidity of the tubes indicates the amount of microbial growth with the least turbid tubes correlating with the absence of microbes. The tube with no plant extract was opaque and turbid because the microbes were able to flourish. The lowest concentrations where no turbidity was observed were determined and noted as the minimum inhibitory concentration (Parvekar *et al.*, 2020). The table below shows the values in millilitres of the minimum inhibitory concentration.

Table 5. MIC Values (mL) of Plant Extract against Test Organisms

Test bacteria	Plant extracts	Plant collection site			
		Kabianga		Bomet	
		MIC values in millilitres (mg/ml) $\times 10^{-3}$			
		S. didymobotrya	*T. alata*	*S. didymobotrya*	*T. alata*
S. pyogenes	Hexane	4.8	4.8	4.8	8.0
	DCM:Methanol (1:1)	8.0	4.8	4.8	8.0
	Methanol	8.0	8.0	8.0	8.0
P. aeruginosa	Hexane	8.0	8.0	8.0	8.0
	DCM:Methanol (1:1)	20	20	20	20
	Methanol	20	20	20	20
S. aureus	Hexane	20	20	8.0	8.0
	DCM:Methanol (1:1)	20	8.0	8.0	8.0
	Methanol	20	20	20	20

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Figure 3. MIC of T. alata and S. didymobotrya plant extracts against S. pyogenes

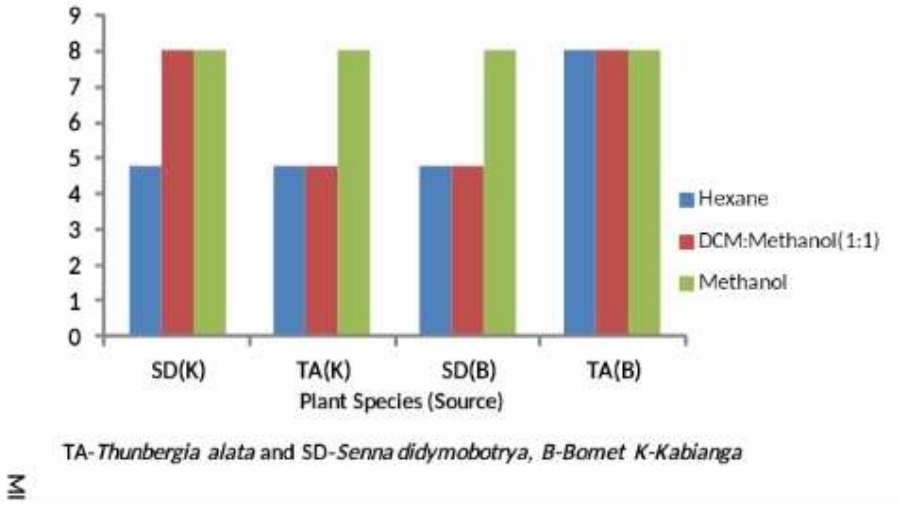

Figure 4. MIC of T. alata and S. didymobotrya plant extracts against P. aeruginosa

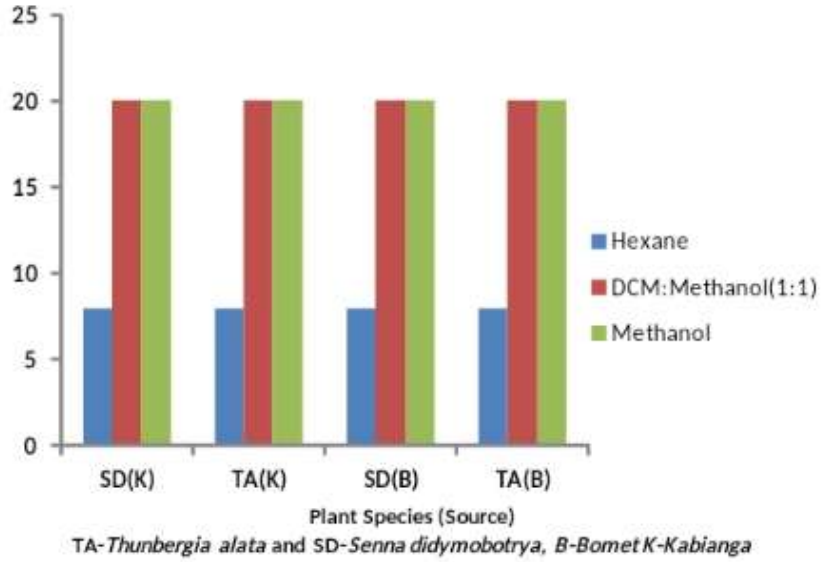

Figure 5. MIC of T. alata and S. didymobotrya plant extracts against S. aureus

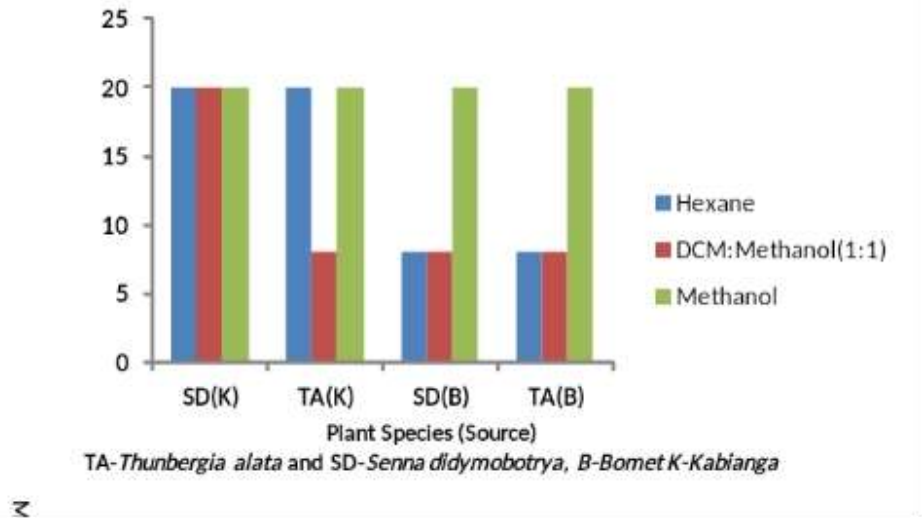

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Table 5 and Figures 3,4 and 5 show the MIC values (mg/ml) ×10-3 of *S. didymobotrya* and *T. alata* plant extracts against *S. pyogenes, S. aureus* and *P. aeruginosa.* Irrespective of plant species, methanolic extracts inhibited *S. aureus* and *P. aeruginosa* at 20 ×10-3 (mg/ml), however lower MIC values ranging from 4.8- 8.0 ×10-3 (mg/ml) were observed in *S. pyogenes*. Such results indicate that these plant extracts could be bacteria static necessitating the evaluation of MBC. The most interesting activity was obtained from *S. didymobotrya* from the two regions.

3.6 Minimum Bactericidal Concentration (MBC)

The Minimum bactericidal was determined from the broth dilution test resulting from the MIC tubes by subculturing a loopful of the bacterial suspension from the MIC tubes on Nutrient agar. The lowest concentration of the extract at which no growth was observed was recorded as the MBC. But after 48 hours there was growth on the plates which were seeded with *S. pyogenes.* This meant that the plant extracts were bacteriostatic at some point not allowing it to grow but the bacteria were kept at their stationary phase When cultured on the plates with nutrients, the bacteria were able to form colonies.

4 DISCUSSIONS OF RESULTS

Antimicrobial resistance is a problem that continues to challenge the healthcare sector in many parts of the world both in developed and developing countries. The emergence and spread of multidrug-resistant pathogens have threatened the current antibiotic therapy. This has demanded for the search of a new source of antimicrobial activity of different medicinal plant extracts against human pathogens that cause skin infections. *S. didymobotrya* and *T. alata* contain various phytochemicals from different classes which have antimicrobial activity (Ndegwa *et al*., 2022). Hence the present study was carried out to evaluate the antimicrobial sensitivity testing of the two plant extracts as antimicrobial agents against *S. aureus, S. pyogenes* and *P. aeruginosa* isolates. The plant extracts exhibited good antimicrobial activity towards the tested bacterial isolates. *S. pyogenes* was only suppressed and not completely eradicated by the plant extracts as was exhibited by the antimicrobial sensitivity tests after 36 hours.

Literature studies show that *T. alata* chloroform stem extract has some antibacterial activity against *Pseudomonas aeruginosa* while a significant antibacterial activity of higher concentration of ethanolic leaf extract has been observed against *Salmonella typhi.* Methanolic crude extract of *Thunbergia grandiflora* leaves have significant microbial activity against some Gram-positive and Gram-negative bacteria (Mbachu & Moronkola, n.d.). Methanolic extract of the flower of *Thunbergia grandiflora* showed antibacterial activity against *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Bacillus cereus, Proteus mirabilis* and *Streptococcus pyogenes* due to the presence of phenols, alkaloids and flavonoids (Sultana *et al*., 2015). *S. didymobotrya* leaves and pods have been evaluated and found to contain a number of anthraquinone derivatives e.g., emodin, chrysophanol, physcione and knipholone. Other compounds isolated from leaves are aloe-emodin, rhin and small quantities of dianthrone, emodin, catechinic, tannis, flavonoids and aloe-emodin B-glucoside (Nzuki, 2016).

Anthraquinones exhibit other biological effects including diuresis, vasorelaxation and induction of muscular contractions, antioxidant properties as well as antibacterial and antifungal activities. Emodin is known to be a feeding deterrent against a wide range of organisms (Izhaki, 2002). The roots of *S. didymobotrya* has been used in the treatment of venereal diseases, jaundice, fever, and backache as reported by (Nyamwamu et al., 2015), while the leaf infusion/decoction has been used in the treatment of bacterial diseases, fibroids, and diarrhoea (Singh & Singh, 2010). The stem has also been evaluated for its biological use and found to have preservative effect on milk (Kawanga, 2017). The current study shows some significant variations in the phytochemical's contents, like Alkaloids, flavonoids, Saponins,

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Terpenoids and glycosides. The variation is due to several environmental factors such as climate and altitude as mentioned by (Pant et al., 2021).

The phytochemical screening of crude plant extracts of *S. didymobotrya* and *T. alata* revealed the presence of alkaloids, flavonoids, saponin, tannin and terpenoids. These classes of phytochemicals are known to show curative activity against several pathogenic micro-organisms and this could explain the reason why it has been used majorly by traditional herbalists to treat a wide range of ailments (Saxena *et al*., 2013).

The in-vitro antimicrobial susceptibility testing presented in *Table 3* showed the zones inhibited by the plant extract in mm against *S. pyogenes, S. aureus* and *P. aeruginosa*. The plant extracts exhibited considerable zones of inhibition against the test micro-organisms, the highest being *S. aureus* 4.8 mm in methanolic plant extract of *S. didymobotrya* from Kabianga region. Its level surpassed the degree of commercial antibiotics. The lowest was 1.3 mm on *P. aeruginosa* hexane extract from *S. didymobotrya* from Bomet region. Although these plant extracts were sensitive to *S. pyogenes* with inhibited zone of 4.00 mm, the plant extract seemed to be bacteriostatic and not bactericidal. This is because after 36 hours there was growth of the micro-organism on the MHA plate seeded with *S. pyogenes* and with all the plant extract in test. *S. pyogenes* displayed some level of resistance.

However, in comparison with the reference antibiotics especially Chloramphenicol, *S. didymobotrya* plant extract exhibited a much higher antimicrobial activity against *S. aureus.* The zones of inhibition inhibited by most antibiotics against some of the reference bacteria were found to be equal or close to those of plant extract. Although in a study carried out by (Musau & Wanjiru, 2020) their methanolic crude extract showed a big zone of inhibition on both *S. aureus* and *P. aeruginosa* at 24.0 mm and 25.5 mm respectively, contrary to what was found in this study. This variation in zones of inhibition could be attributed to the fact that the plant extract from the two regions could have had different concentrations of the phytochemicals. Due to different ecological zones and different environmental factors. This was categorically stated by (Pant *et al.,* 2021) in his literature that plants of the same species grown in different environment have different concentration of a particular secondary metabolite. This research agrees with his findings because the plants from the Bomet region showed a large layer of foam when tested for Saponins compared to those from the Kabianga region. This is because plants must produce a specified amount of phytochemicals to overcome environmental stress.

From the results of MIC presented in *Table 5,* it was observed that the greatest activity of extracts against *S. aureus* and *P. aeruginosa* was 4 ml as its MIC while MBC of 6 ml was noted similarly. The results on inhibition of bacterial growth have shown that the extracts are active at high concentrations and inactive at low concentrations. Thus, the study findings suggest that the inhibition of bacterial growth activity is dependent on the concentration. The activity of the extracts against the test micro-organisms may indicate a greater zone of inhibition at a higher concentration. This is an important observation to be considered when formulating a therapeutic substance that will be active against multidrug-resistant organisms.

The victory of traditional medicine may be attributed to the administration of large doses of high concentration over a long period of time. This study agrees with the study done by *(Ojo et al.*, 2022) on the concentration of the drug administered to the Swiss albino rat infected with *P. aeruginosa* and *S. aureus* over a period of 10 days.

5 CONCLUSIONS

In this study, the evaluation of the antimicrobial activity of the crude extracts of the two plants on *S. aureus, S. pyogenes* and *P. aeruginosa* revealed great information on the medicinal importance of the plant extracts over infections caused by the test organisms under this study at low concentrations. This can therefore be concluded

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that the Leaf extracts of *S. didymobotrya* and *T. alata* possess antibacterial activities which can inhibit the growth of some bacterial isolates. Thus, at the end of this study, it was found that the leaf extracts of the two plants was able to inhibit the growth of *S. aureus*, *S. pyogenes* and *P. aeruginosa* at diameters ranging from 1.3 to 4.8 mm which was the best result for this study. The traditional use of the two plants was confirmed as a potential therapeutic agent against commonly acquired skin infections and other ailments as has been used before worldwide. The plants being bactericidal against *S. aureus* and *P. aeruginosa* are of great value. Based on this study, it is therefore recommended that further study can be carried out on the leaves of the two plants especially the phytochemical constituents so that the main active compounds that inhibited growth of the bacterial isolates can be extracted, purified, and used in pharmaceutical industries. The two plants have the potential to be used in the development of new phytopharmaceuticals. Finally, since the study conducted was based on crude extracts, further studies can be conducted in this direction based on specific phytochemicals. Also, other solvents can be used including aqua in the extraction of the two plants to determine their effectiveness levels.

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