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Antimicrobial Compounds and Antimicrobial Activity of Extracts of *Thespesia garckeana* F. Hoffm. on *Candida albicans*, *Staphylococcus aureus* and *Escherichia coli*

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Abstract

Plant products as antimicrobials have received serious attention in the recent years. Herbal medicines form a significant role in African ethnomedicine. *Thespesia garckeana* F. Hoffm. is an important herbal medicinal plant used to treat or manage human diseases and ailments such as chest pains, cough, infertility and sexually transmitted infections. However, its antimicrobial compounds and activity analysis has not been evaluated. This study aimed at evaluating the phytochemical and antimicrobial activities of compounds in *Thespesia garckeana* leaves and bark extracts on growth of *C. albicans*, *S. aureus* and *E. coli*. The plant leaves and bark were collected from Mwingi, Kitui County of Kenya and transported to Maseno University department of Botany laboratory, where they were dried separately and crushed to obtain a fine powder. Crude plant extraction was done using soxhlet method with methanol and water solvents. Thin layer chromatography was used to purify the extracts and phytochemical screening was done using standard methods to determine the compounds present in the extracts. Thin layer chromatography was carried on bark and leaves extracts of *Thespesia garckeana*. Thin layer chromatography plate surface was coated with silica gel, and n-Hexane and Ethyl acetate used as solvent systems in the ratio 2:3 and 3:7 respectively. Antimicrobial activity was performed on test microorganisms using different concentrations of the plant extracts; 0 (distilled water), 90, 180, 270 and 360 mg/ml of the extract. Paper disc method was used for inoculation with three replications. Diameter of inhibition zones was measured using a transparent ruler. The data on growth inhibition was subjected to analysis of variance (ANOVA), and the treatments were separated and compared using LSD at $p<0.05$. The phytochemical compounds screening of leaves and stem bark extracts of *Thespesia garckean* revealed the presence of Tannins, Saponins, Cardiac glycosides, and Alkaloids. TLC using n-Hexane and Ethyl acetate ratio of 2:3 methanol leaves extracts produced 9 spots with Rf values of 0.94, 0.9, 0.81, 0.71, 0.35, 0.25, 0.2, 0.125 and 0.075 respectively. The stem methanol extracts produced 4 spots with Rf values of 0.99, 0.96, 0.9, and 0.06 respectively. Leaf methanol extracts using n-Hexane and Ethyl acetate in the ratio of 3:7 produced 8 spots with Rf values of 0.95, 0.89, 0.78, 0.46, 0.36, 0.3, 0.19, and 0.1 respectively. The stem bark methanol extracts produced 2 spots with Rf values of 0.97 and 0.94 respectively. Increase in extracts concentration of the leaves and stem bark of *Thespesia garckeana,* significantly inhibited the growth of *Candida albicans, Staphylococcus aureus* and *E. coli.* Methanol leaf and bark extracts had more inhibitory activity compared to the water extracts. The results from this study confirm the use of *Thespesia garckeana* in herbal medicine by traditional herbalists.

1. Introduction

Plants convert simple substances into complicated entities producing chemicals that are essential for human health **(Rahman *et al.,* 2018)**. The practice of traditional medicine is as old as the human race itself **(Kigen *et al.,* 2013; Mir *et al.,* 2013)**. Ethnobotanicals are potentially new sources of useful compounds for the development of chemotherapeutic agents **(Orakwelu, 2011)**. According to **Musyimi *et al.* (2008)** 70-80% of the people in the world depend on traditional herbal medicine for primary health care needs. The demand of herbal medicines is increasing. Plants are known to be the greatest sources of new antimicrobials (**Musyimi and Namnabah, 2021**). Medicinal plants are effective in treating diseases **(Wadankar *et al.,* 2011; Kiringe, 2006)**. Antimicrobial agents currently in use have many limitations **(Birru et al., 2017).** This has led to the use and search for drugs and dietary supplements derived from plants for prevention or treatment of diseases **(Orakwelu, 2011; Kaigongi, 2014)**. The medicinal values of plants lie on their phytochemical constituents **(Sujit and Amol, 2014)**. The most active of these bioactive constituents of plants are alkaloids, tannins, steroids, terpenoids, phenolics and other

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compounds **(Fasola and Iyamah, 2014)**. These have been found to produce a definite physiological action on human body **(Boadi *et al.,* 2015)**. The emergence of pathogenic micro-organisms that are resistant or multi-resistant to major classes of antibiotics has increased in recent years due to the indiscriminate use of synthetic antimicrobial drugs. Emerging and re-emerging infections and microbial drug-resistance pose a challenge to the global public health (**Musyimi and Namnabah, 2021**). The increasing failure of chemotherapies and antibiotic resistance exhibited by pathogenic microbial infections agents have led to the screening of several medicinal plants for their potential antimicrobial activity **(Alagesaboopathi, 2011)**. Most rural communities depend on traditional medicine for treatment of diseases **(Osazee *et al.,* 2013; Kigen *et al.,* 2013)**. Traditional medicine is widely used in Kenya and about 400 plant species have been recorded to be used in traditional remedies **(Kaigongi, 2014)**. In order to find novel antimicrobial agents with new modes of action, plants have been explored as sources for the identification of new and effective antimicrobials. Plant materials are important in the maintenance of human health as over 50% of all modern chemical drugs come from plant sources **(Osazee *et al.,* 2013; Kaigongi, 2014)**. Active components of the plants are now being investigated, extracted and developed into drugs with little or no negative effects or contra-indications **(Osazee *et al.,* 2013)**. Phytochemical compounds concentration in plants depend on the climate of the area and the ecological zone **(Musyimi *et al.,* 2008)**, very little information is available on the phytochemical compounds of *Thespesia garckeana* found in Kenya. Thin layer chromatography (TLC) is a quick, sensitive, and cheap technique used to determine the number of components in a mixture, verify the identity and purity of a compound, monitor the progress of the reaction, determine the solvent composition for preparative separations, and analyze the fractions obtained from column chromatography **(Cai., 2014)**. Thin layer chromatography (TLC) gives a quick review of the number of components in a mixture and gives the identity of compounds in a mixture when the Rf of the compounds are compared with the Rf of a known compounds (both run on the same TLC plate) **(Svendsen and Verpoorte, 1983)**.

Thespesia garckeana is a member of the *Malvaceae* family **(Maroy, 2017)**. According to **Ochokwu *et al.* (2015)** root decoction of *Thespesia garckeana* is drunk to relieve painful menstruation, as well as for coughs and chest pains. *Thespesia garckeana* is taken orally as remedy for Chest pains, Cough and Menstruation (**Mojeremane & Tshwenyane, 2004)**. Root and stem bark decoction of *Thespesia garckeana* is taken orally as remedy for sexually transmitted diseases such as gonorrhoea and syphilis; remedy for infertility and liver problems **(Kathembwa David personal communication, 2021)**. *Thespesia garckeana* has long been used by Mbeere community in Eastern Kenya region to treat chest pains and cough **(Keter and Mutiso, 2017)**. The plant is only regarded as a wild tree and its utilization in most parts of Kenya is poor hence the need for this study. Due to high cost of manufactured synthetic drugs in the market it's hard for local communities to access these drugs due to poverty. Besides, chemosynthetic drugs are associated with resistance, so more natural alternative drugs are required. There is need to investigate the potential antimicrobial activity and phytochemical constituents of plants. The essential values and uses of some medicinal plants has been worked out and published, but many of them remain unexplored in Kenya **(Sujit and Amol, 2014)**. Despite the penetration of conventional medicines, traditional medicine continues to be a feasible health care alternative for the majority of the Kenyan population **(Keter and Mutiso, 2017)**. Although *Thespesia garckeana* has been greatly tested on antimicrobial activity but very few studies have been conducted in Kenya especially on *Candida albicans*, *Staphyllococcus aureus* and *Escherichia coli*. There is scanty of information on the antimicrobial activity of *Thespesia garckeana* on *Candida albicans*, *Staphylococcus aureus* and *Escherichia coli* in Kenyan studies hence there is need to screen this plant against the three microbes. This study aimed at investigating the phytochemical and antimicrobial properties of leaf and bark extracts of *Thespesia garckeana* on *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*.

2. Material and methods

2.1 Plant collection, identification and preparation

Plant bark and leaves of *Thespesia garckeana* were collected from Mwingi, Kitui County-Kenya on July 2021 (plate 1). The leaves and stem bark of *Thespesia garckeana* were cleaned off the soil or dust by shaking, and were packaged inside carrier bags then transported to Maseno University, department of Botany for identification by the taxonomists. The leaves and stem bark were air-dried separately under shade for two weeks and pulverized using a wooden mortar and pestle to obtain a fine powdered-like texture (plates 2 and 3). According to WHO (1998), this is done to enhance the penetration of the extracting solvents into the plant cells, thus facilitating the release of the active principles. The pulverized plant samples were then stored in amber bottles and kept in a cool and dried environment under room temperature until it is was required for usage.

Plate 1 Showing *Thespesia garckeana F. Hoffm.* Plant growing wild in the dry areas of Mwingi, Kitui County in Kenya.

Plate 2 Powdered sample of stem bark.

Plate 3 Powdered sample of leaves.

2.2 Phytochemical extraction

All work was done in accordance to the general guidelines and methodologies on research and evaluation of traditional medicine (WHO, 1998). The dried bark and leaves were extracted in Soxhelt apparatus by using 25ml of water and methanol for 48 hours and then were concentrated by evaporation. The extracts were used for phytochemicals analysis.

2.3 Preliminary phytochemical profiling

The bioactive constituents present in the methanol and aqueous extracts were screened for flavonoids, alkaloids, tannins, saponins, steroids, terpenoids and cardiac glycosides using standard procedures **(Harborne, 2002; Sofowora, 1993; Martinez *et al.,* 1998; Wall *et al.,* 1952)**. Phytochemical compounds were evaluated for the presence of various phytocompounds and quantity using a colour chart.

Test for Tannins
0.5g of dried powdered sample was boiled in 20ml of water in a test tube and filter. A drop of 0.1% ferric chloride was then added to the filtrate. An observation of brownish green of blue-black coloration would indicate a positive test.

Test for Saponins
2g of powdered sample was mixed in 20ml of distilled water in a boiling tube then placed in a hot water bath for 5 minutes and then filtered. 10ml of the filtrate was mixed with distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with three drops of olive oil and shaken vigorously and formation of an emulsion observed.

Test for flavonoids
About 5g of powdered plant sample was heated in a boiling tube with 10ml ethyl acetate over a steam bath for three minutes. They were then filtered and 4ml of the filtrate taken and shaken with 1ml of dilute ammonium solution. A yellow colouration observed would indicate a positive test for the flavonoids.

Test for Terpenoids
To 0.5g each of plant extract in a test tube, 2ml of chloroform was added followed by 3ml of 0.1M Sulphuric acid to form a layer. A reddish brown colouration of the interface formation would indicate a positive test for terpenoids.

Test for Steroids
2ml of acetic anhydride was added to 0.5g ethanoic extract of each sample with 2ml of 0.1M Sulphuric acid in a test tube. The colour change from violet to blue or green in the sample would indicate the presence of sterols.

Test for Cardiac glycosides
5ml of each extract in a test tube was exactly treated with 2ml glacial acetate acid containing one drop of 0.1% ferric chloride solution. To this, concentrated hydrochloric acid was added. A brown ring of the interface would indicate a deoxy-sugar characteristic of cardinolides thus determine a positive test. A violet ring would also appear below the rings while in the acetic acid layer or greenish ring may just gradually throughout the thin layer.

Test for alkaloids
A sample of 2g of dried plant extract was mixed with 40ml of 0.1M hydrochloric acid in a boiling tube and heated in a water bath for 10 minutes. The mixture was cooled and then filtered. To a portion of the filtrate, a few drops of Mayor's reagent were added. A slight turbidity of heavy precipitate was assumed to indicate the presence of alkaloids.

2.4 Thin layer chromatography (TLC)

Thin layer chromatography was carried on the two extracts (stem bark and leaves) of *Thespesia garckeana* according to **Musa *et al.* (2017)**. Using a glass TLC plate whose surface was coated with silica gel, the TLC plate was cut into a size of 5cm by 10cm; a mark was made in pencil about 1.5cm from the lower edge of the end of the plate which was the origin. The dissolved extracts were spotted on the line creating a distance of about 0.5 to 1cm between each spot, the distance prevent the spots from overlapping or mixing while separating. Solvent was prepared in the development chamber which makes the solvent system by mixing n-hexane and ethyl acetate in the ratio of 2:3 and 3:7 (v:v) respectively in which the spotted plate was placed in such that the liquid solvent does not touch the origin (line marked in pencil) and spotted with dissolved extract. The solvent which is the eluent moved through the plate and went up by capillary action of the plate carrying the compounds present in the extract which separates and appears as spots on the plate. The compounds that were closer to the origin showed less movement and are polar solvent was marked in pencil which marks the solvent front. The spots would be visible but if not, the spots were seen by use UV lamp to enhance visualization at different wavelengths of 365nm and 254nm.Then the retention factor was calculated using the formula proposed by **Musa *et al.* (2017).**

$$\mathbf{Rf} = \frac{\text{Distance travelled by the solute from the point of application to the center of spot}}{\text{Distance travelled by the solvent front}}$$

The retention factor **(Rf)** value essentially describes the distance travelled by the individual component.

2.5 Sources and maintenance of micro-organisms

Gram-positive organisms *Staphylococcus aureus,* Gram-negative organisms *Escherichia coli* and fungal yeast *Candida albicans* were obtained and confirmed from the department of Botany Maseno University. They were maintained on Mueller Hinton Agar (Oxford, UK) medium.

2.6 Culture media

Mueller Hinton Agar (Oxford, UK) was prepared according to manufacturer's instructions, autoclaved and dispensed at 20ml per plate in 12 by 12cm petri dishes. Set plates were incubated overnight to ensure sterility before use.

2.7 Antimicrobial bioassays

Bioassays were prepared according to **Prescott *et al.* (1999)** and **Bonjar (2004)**. Plant extracts (stem bark and leaves) were diluted in distilled water and in methanol respectively to make a stock solution. Different concentrations of the plant extracts were prepared by diluting the stock extracts in water and methanol to make solutions of the following concentrations in triplicates: 0, 90, 180, 270 and 360 and a positive control of fluconazole on fungi and Amoxicillin on bacteria. Each labeled medium plate was uniformly inoculated with a test organism by using a sterile cotton swab rolled in the suspension to streak the plate surface in a form that lawn growth can be observed. Antimicrobial activity of the test agents was determined by measuring the diameter of the zone of inhibition. The experiment was arranged in a completely randomized design.

2.8 Disk method

Circular discs, 6mm diameter each was cut from a laboratory grade filter paper punch and each dipped in a known concentration of the plant extracts for about 2 minutes. According to **Musyimi *et al.* (2008)**. The diameter of inhibition zones was measured using a ruler and compared with the control paper disc.

2.9 Data Analysis

The data obtained from the study was subjected to analysis of variance (ANOVA) using SAS statistical package. Means were separated and compared at ($p<0.05$) for any significant difference between treatments and the micro-organisms.

3. Results

3.1 Phytochemical screening

Phytochemical screening of the leaves and stem bark extracts (table 1) showed that the leaves and stem bark extracts of *Thespesia garckeana* contained alkaloids, cardiac glycosides, saponins, flavonoids, and tannins. However, steroids and terpenoids were absent. It was evident that phytochemicals were more in the leaves than in the barks.

Table 1 Phytochemical screening of secondary metabolites present in leaves and bark extracts of *Thespesia garckeana.*

Test compound	Leaves	Stem bark
Tannins	+++	++
Saponins	+++	++
Flavanoids	++	+
Terpenoids	_	_
Cardiac Glycosides	++	+
Alkaloids	++	+
Steroids	_	_

Key: (+++) Highly present, (++) Moderately present, (+) Less present, (-) Not present. (Quantity of colouration was checked using a colour chart)

3.2 Thin Layer Chromatography (TLC)

Elution of the column with the n-hexane and ethyl acetate in 2:3 ratio led to isolation of nine fractions or spots of uncharacterized active compounds in leaves extracts (table 2). The leaf extracts recorded highest number of spots compared to bark extracts. The leaf extracts of ratio 3:7 recorded eight spots. The bark extracts of ratio 2:3 recorded four spots, and the bark extracts recorded least number of spots.

Table 2 The Rf values of active compounds isolated from the methanol plant extracts of *Thespesia garckeana* subjected to Thin Layer Chromatography (TLC) and ratio of n-hexane: ethyl acetate v/v as solvent systems.

No. of spots viewed at 365nm UV light	**Leaves** n-hexane and ethyl acetate ratio of **2:3 Rf values**	**Leaves** n-hexane and ethyl acetate ratio of **3:7 Rf values**	**bark** n-hexane and ethyl acetate ratio of **2:3 Rf values**	**bark** n-hexane and ethyl acetate ratio of **3:7 Rf values**
1	0.94	0.95	0.99	0.97
2	0.9	0.89	0.96	0.94
3	0.81	0.78	0.9	-
4	0.71	0.46	0.06	-
5	0.35	0.36	-	-
6	0.25	0.3	-	-
7	0.2	0.19	-	-
8	0.125	0.1	-	-
9	0.075	-	-	-

$$\textbf{Rf} = \frac{\text{Distance travelled by the solute from the point of application to the center of spot}}{\text{Distance travelled by the solvent front}}$$

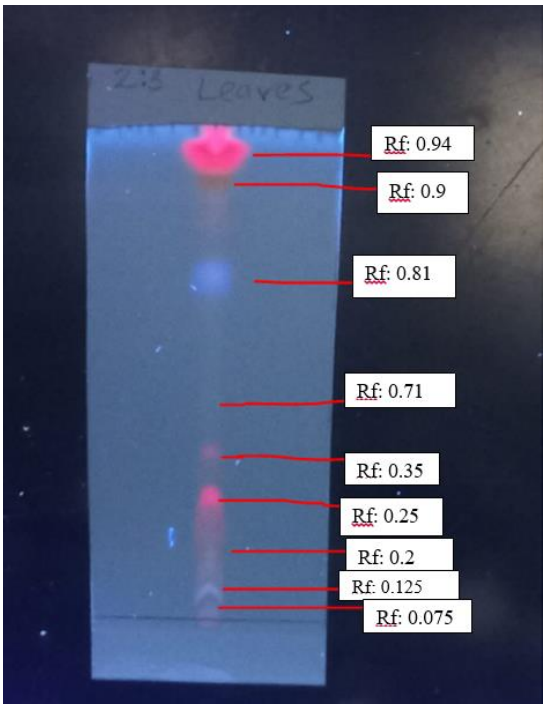

Plate 4 TLC plate spotted with leaves methanol extracts viewed at 365nm UV light at the ratio of 2:3 (n-hexane: ethyl acetate) respectively.

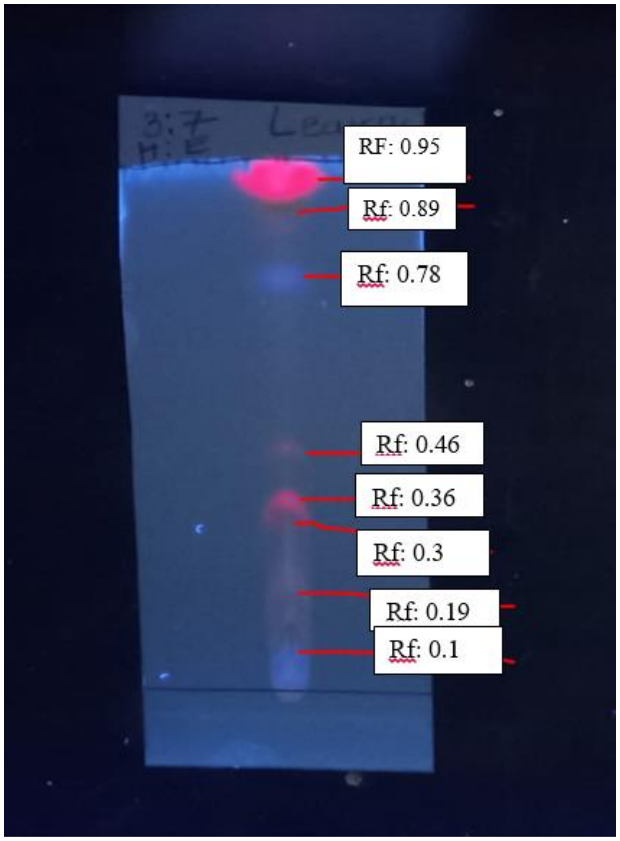

Plate 5 TLC plate spotted with leaves methanol extracts viewed at 365nm UV light at the ratio of 3:7 (n-hexane: ethyl acetate) respectively.

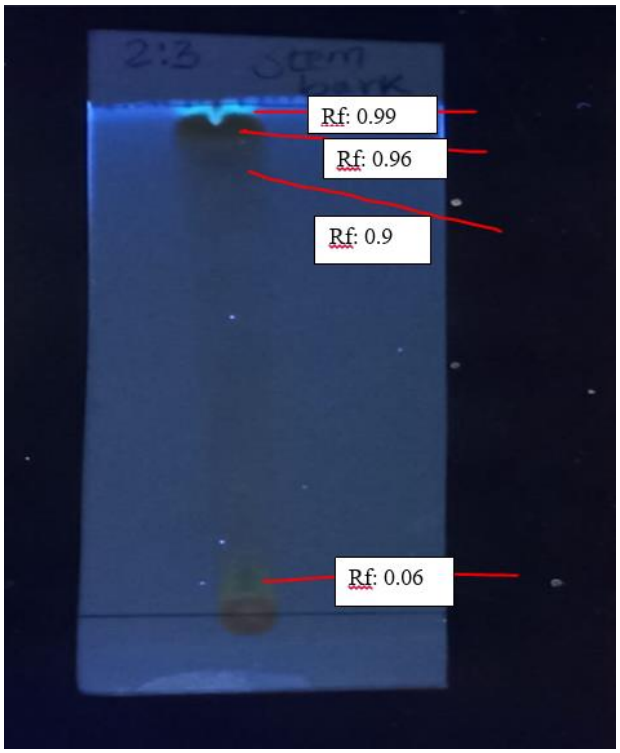

Plate 6 TLC plate spotted with stem bark methanol extracts viewed at 365nm UV light at the ratio of 2:3 (n-hexane: ethyl acetate) respectively.

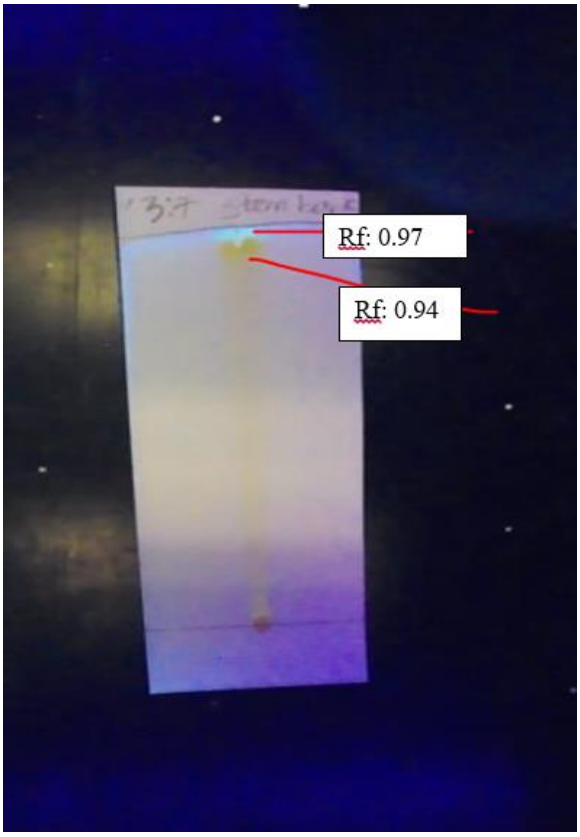

Plate 7 TLC plate spotted with stem bark methanol extracts viewed at 365nm UV light at the ratio of 3:7 (n-hexane: ethyl acetate) respectively.

3.3 Antimicrobial Assays

Water and methanol leaf and bark extracts of *Thespesia garckeana* plants represented good activity against the test microbes (table 3). There was no significant differences ($p>0.05$) between the water extracts and methanol extracts (table 4). There was no significant difference ($p>0.05$) between the plant part used either the leaves or the barks. There was a significant difference ($p<0.05$) between the extract concentration whereby the more concentrated extracts showed a greater diameter of growth inhibition. The antibiotics (positive control) exhibited a greater zone of diameter inhibition. There was a significant difference ($p<0.05$) between the two microorganisms, *Candida albicans* and *E. coli and Staphylococcus aureus.* Whereby diameter growth of *Staphylococcus aureus* was more inhibited compared to *Candida albicans* and *E. coli.*

Table 3 Mean growth inhibition zones of the microbes using methanol and water extracts *Thespesia garckeana* leaves and stem bark extracts.

Test microbe	***Leaf Extract***	***Concentration***	***Diameter of growth inhibition (mm + s.e)***
S. aureus	Methanol extract	0mg/ml	6.00 ± 0.00
		90 mg/ml	8.00± 0.33
		180 mg/ml	10.33± 0.33
		270 mg/ml	11.00± 0.58
		360 mg/ml	15.00± 0.58
		Positive control (Amoxicillin)	32.00± 0.58
	Water extract	0mg/ml	0.00
		90 mg/ml	8.33± 0.33
		180 mg/ml	11.00± 0.58
		270 mg/ml	14.33± 0.88
		360 mg/ml	16.00± 0.58
		Positive control (Amoxicillin)	32.00± 0.58
E. coli	Methanol extract	0mg/ml	6.00 ± 0.00
		90 mg/ml	7.67± 0.33
		180 mg/ml	10.00± 0.00
		270 mg/ml	11.00± 0.58
		360 mg/ml	16.00± 0.58
		Positive control (Amoxicillin)	32.33± 1.45
	Water extract	0mg/ml	6.00 ± 0.00
		90 mg/ml	7.33± 0.33
		180 mg/ml	8.00± 0.58
		270 mg/ml	12.00± 0.58
		360 mg/ml	15.00± 0.58
		Positive control (Amoxicillin)	32.33± 1.45
C. albicans	Methanol extract	0mg/ml	6.00 ± 0.00
		90 mg/ml	7.33± 0.33
		180 mg/ml	9.00± 0.00
		270 mg/ml	10.00± 0.58
		360 mg/ml	14.00± 0.58
		Positive control (fluconazole)	33.67± 1.85
	Water extract	0mg/ml	6.00 ± 0.00
		90 mg/ml	7.00± 0.00
		180 mg/ml	8.00± 0.58
		270 mg/ml	12.00± 0.58
		360 mg/ml	15.00± 0.58
		Positive control (fluconazole)	33.67± 1.85
Test microbe	***Bark Extract***	***Concentration***	***Diameter of growth inhibition (mm + s.e)***
S. aureus	Methanol extract	0mg/ml	6.00 ± 0.00
		90 mg/ml	10.67 ± 0.88
		180 mg/ml	10.33 ± 0.33
		270 mg/ml	12.00 ± 0.58
		360 mg/ml	13.66 ± 0.88
		Positive control (Amoxicillin)	32.00 ± 0.58
	Water extract	0mg/ml	6.00 ± 0.00
		90 mg/ml	8.00 ± 0.58
		180 mg/ml	13.33 ± 0.88
		270 mg/ml	13.00 ± 1.15
		360 mg/ml	15.33 ± 0.33
		Positive control (Amoxicillin)	32.00 ± 0.58

E. coli	Methanol extract	0mg/ml 90 mg/ml 180 mg/ml 270 mg/ml 360 mg/ml Positive control (Amoxicillin)	6.00 ± 0.00 8.00 ±0.58 8.67 ±0.33 11.00 ±0.58 15.67± 0.88 32.33± 1.45
	Water extract	0mg/ml 90 mg/ml 180 mg/ml 270 mg/ml 360 mg/ml Positive control (Amoxicillin)	6.00 ± 0.00 7.33 ±0.33 8.67 ±0.33 10.33 ±0.33 15.00± 0.58 32.33± 1.45
C. albicans	Methanol extract	0mg/ml 90 mg/ml 180 mg/ml 270 mg/ml 360 mg/ml Positive control (fluconazole)	6.00 ± 0.00 7.33 ±0.33 8.33 ±0.33 9.33 ±0.33 15.00± 0.58 33.67± 1.86
	Water extract	0mg/ml 90 mg/ml 180 mg/ml 270 mg/ml 360 mg/ml Positive control (fluconazole)	6.00 ± 0.00 7.33 ±0.33 8.33 ±0.33 9.33 ±0.33 15.00± 0.58 33.67± 1.86

Table 4 Antimicrobial activity of *Thespesia garckeana* leaves and stem bark water and leaf extracts, comparison of methanol and water extracts on *Staphylococcus aureus, E. coli* and *Candida albicans*

		Growth inhibition diameter (mm)
Microbes	*Staphylococcus aureus*	14.26a
	E. coli	13.54b
	Candida albicans	13.38b
	LSD	***0.41***
Solvents	Methanol	13.65a
	Water	13.81a
	LSD	*0.33*
Plant parts extracts	Stem bark	13.76a
	Leaves	13.69a
	LSD	***0.33***
Extract concentration	0mg/ml	6.00f
	90mg/ml	7.86e
	180 mg/ml	9.50d
	270 mg/ml	11.28c
	360 mg/ml	15.06b
	Positive control	32.67a
	LSD	***0.57***

Means with the same letter are not significantly different. Data presented are means of three replicates.

4. Discussion

Searching for antibacterial and antifungal activity of the secondary metabolites from medicinal plants is a spotlight to date **(Zuo *et al.,* 2008)**. In Kenya, traditional medicines play a major role in primary healthcare and upkeep of rural communities (**Kokwaro, 2009; Kisangau and Kokwaro, 2004**). Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids, phenolic compounds, and flavonoids, which have antimicrobial properties **(Duraipandiyan *et al.,* 2006)**. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on human body **(Musyimi *et al.,* 2008)**. The most important of these bioactive compounds are alkaloids, flavanoids, and tannins **(Edeoga, 2005)**. Data from preliminary phyotochemical screening confirm the presence of alkaloids, flavonoids, and anthraquinones and absence of steroids and terpenoids. These secondary metabolites are well recognised for their antioxidant, antifungal and antibacterial effects in line with previous findings from other studies **(Tan *et al.,* 2008; Bianco *et al.,* 2015; Kouadri, 2018)**. Several spectroscopic and chromatographic analytical methods have been developed for standardization of products from medicinal plants, which include thin layer chromatography, to guarantee their quality, efficacy and safety **(Rajani *et al.,* 2016)**. The leaf extracts recorded highest number of spots compared to bark extracts. Different Rf values indicate of the polarity of the compounds which helps in selecting a particular solvent system **(Biradar and Rachetti, 2013)**, for separating pure compounds present in different fractions. In this present study thin layer chromatography confirmed the presence of potentially active metabolites. Compounds with high Rf values in less polar solvent systems have low polarity while those with low Rf values have high polarities. Thin layer chromatography gives semi quantitative information about the main active constituents present in a plant **(Banu and Nagarajan, 2014)**. The bioassay test should be sensitive, reliable and prompt **(Marston and Hostettmann, 2015)**. The type of extraction method, duration of extraction, temperature and polarity of the solvent used influence the quality and concentration of bioactive components isolated from the raw materials **(Nantitanon *et al.,* 2010)**. Both

extracts had broad spectrum activity in that they inhibited growth of *S. aureus, C. albicans* and *E. coli.* This could be due to the presence of active components. The results clearly showed that tested organisms were susceptible to the bark and leaf extracts of *Thespesia garckeana*. Moreover, some plants may exhibit broad-spectrum of antimicrobial effects, which possibly control the impairments associated with multidrug-resistant microbes **(Kenneth-Obosi, 2017)**. Antimicrobial activity due to saponins have been demonstrated to inhibit bacterial colonization, lower surface tension of extracellular medium and lyses of bacterial membranes **(Al-Bayati and Al-Mola, 2008)**. Saponins, tannins steroids and terpenoids have antibacterial and antifungal activity **(Emitaro *et al.,* 2020)**. Gram negative bacteria are less susceptible to antimicrobial agents than Gram positives, as a result of the phospholipidic composition of their bacterial membrane. Tannins act by complexing bacterial proteins, interfering with protein adhesion, inactivating enzymes and disrupting bacterial membrane **(Siebert and van Wyk, 2015)**. Glycosides are responsible for antimicrobial activity. Results on the antimicrobial activity of the leaves and stem bark extracts of *Thespesia garckeana* against the test microorganisms at different concentrations revealed that inhibition zone diameters increased with increase in extract concentration. This implies that the more the concentration of the extract, the higher the biological activity against the test organism. Flavonoids have been used as scavengers of superoxide anions, anti - inflammatory and antimicrobial agent (**Nirosha *et al.,* 2019; Hossain *et al.,* 2013**). Plants with tannins are known to have anti- inflammatory effects on the mouth and also treat diarrhea (**Musyimi and Namnabah, 2021**). The biological functions of alkaloids, saponins and their derivatives are very important (**Stary, 1998**).

The variation in the concentration of the phytochemicals in the leaves and stem bark extracts could be attributed to the type of solvent used and the response of each individual part to biotic and abiotic factors. Polar solvents are better extractors of phytochemicals than non polar solvents. The concentration of bioactive compounds in each plant part depends on the environmental conditions, age of the plant and solvents used in extraction **(Borges *et al.,* 2018; Musyimi *et al.,* 2008)**. Differences in phytocompounds of plants could be associated with the geographical and environmental factors of the region whre the plants are grown. The phytochemical screening results agree with those of **Ikewuchi and Ngozi (2009)**. Leaves and bark extracts had the same activity. This could be due to the occurrence of the same active compounds. The study on antimicrobial activity was in agreement with **Birru *et al. (*2017)** who used barks of *Thespesia garckeana* to test for antimicrobial activity. The curative properties of medicinal plants have been attributed to the presence of alkaloids, flavonoids, phenols, saponins, steroids in plants (**Britto and Sebastian, 2011**).

The antimicrobial activity of the stem bark and leaves extracts have a wide range of activities because different concentration of the extract inhibited the growth of *Candida albicans, Staphylococcus aureus* and *E. coli*, this could probably be due to the active ingredients in the extracts which interfered with pathogen cell functioning hence arresting the growth **(Emitaro *et al.,* 2020**). **Breijyeh *et al.* (2020)** indicate that gram-positive bacteria are usually more susceptible to antimicrobial agents than gram negative bacteria. phytochemicals flavonoids, saponins, tannins and alkaloids are active against Gram positive and Gram negative bacteria. Inhibitory activity of secondary metabolites emanates from the sequential inhibition of the biochemical pathway, inhibition of protein synthesis, and disintegration of the outer membrane and altering the reproductive system of the pathogen **(Ramirez-Gomez *et al.,* 2019)**. Phenolic compounds act as antimicrobial agents. Since methanol and water extracts had the same activity on the test microbes, the two solvents can be used for extraction of the phytochemicals from the leaves and the stem barks of this plant. Secondary metabolites have the potential as bactericidal, bacteriostatic, or fungicidal effect against human pathogens **(Dholaria and Desai, 2018; Motaleb, 2011)**. Data from our results reveal the great potential of *Thespesia garckeana* plants for therapeutic treatment, in spite of the fact that they have not been completely investigated. Further studies should be undertaken to unveil the exact mechanism of action and reveal the drug targets of the microorganisms tested.

4. Conclusion

From this study the following it can be concluded that the leaves and bark of *Thespesia garckeana* are rich in phytochemicals especially the tannins, saponins, flavonoids, cardiac glycosides and alkaloids, thus are of medicinal value. TLC revealed that leaves methanol extracts had more spots compared to bark methanol extracts. Antimicrobial testing showed that both methanol and water extract of leaves and bark of *Thespesia garckeana* had a broad spectrum activity against *E. coli, S. aureus and C. albicans.* This study has confirmed the antimicrobial potency of the plant extracts, and explains why the plant is used by the traditional healers and the possibility to use the plant as a suitable alternative to synthetic antimicrobial agents.

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Conflict of Interest

The authors report no conflicts of interest. The authors are responsible for the content of the paper.

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