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Antibacterial Properties of Ficus sycomorus Bark Extract Against Staphylococcus aureus and Escherichia coli

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Abstract

Objective*: To investigate the antibacterial properties of Ficus sycomorus bark extract against Staphyloccocus aureus and Escherichia coli.* ***Materials and methods****: This was a cross-sectional laboratory-based experimental study in which Staphylococcus aureus and Escherichia coli were cultured in the laboratory. Varying concentrations of F. sycomorus aqueous and ethanolic extracts were tested for antibacterial activity using the disc diffusion method. The sensitivity of the tested microorganisms to aqueous and ethanolic plant extracts was shown by zones of inhibition after incubation.*

Results*: Antibacterial activity was seen through zones of inhibition starting from 50 mg/ml upwards, with the zones of inhibition increasing as the doses increased. The highest observed zones of inhibition were seen with F. sycomorus 500 mg/ml for both aqueous and ethanolic extracts. A difference was however noted in that a larger zone of inhibition of 6.1mm was obtained with the 500 mg/ml ethanolic extract, as compared to the 5.0 mm observed with the aqueous extract of the same concentration, when tested against S. aureus. A 7.0 mm zone of inhibition was measured when 500 mg/ml F. sycomorus ethanolic extract was tested against E. coli whilst the 500 mg/ml aqueous extract resulted in a 6.8 mm zone of inhibition. Ciprofloxacin produced better antibacterial effects against S. aureus and E. coli with zones of inhibition of 7.5 mm and 10 mm compared to F. sycomorus extracts ($p<0.0001$).*

Conclusion*: The extracts of Ficus sycomorus displayed antibacterial activity against Staphylococcus aureus and Escherichia coli in a dose-dependent manner. The ethanolic extract produced better antibacterial properties against both S. aureus and E. coli than the aqueous extract.*

Keywords: Antibacterial properties; Escherichia coli Ficus sycomorus; Staphylococcus aureus

Introduction

Ficus sycomorus (*F. sycomorus*) is one of the plants that have been used in ancient history as well as modern times for various medicinal uses [1]. *F. sycomorus* has been said to possess antibacterial activities against *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) [2]. It is a genus of about 800 species in the family Moraceae [3]. The phytochemical constituents of *Ficus* for stem root and bark are saponins, tannins,

volatile oils, and Phenols [4]. Mixtures of such chemicals show a wide spectrum of biological effects and pharmacological properties more especially against *S. aureus* and *E. coli* [5].

S. aureus and *E. coli* are some of the most common bacteria and cause a number of diseases in the human body [6]. *Staphylococci* is a group of gram-positive bacteria that can cause a number of infectious diseases in various tissues of the body [7]. *Escherichia coli* is the type of species of the genus Escherichia that contains mostly motile gram-negative bacilli that fall within the family *Enterobacteriaceae*. It is the predominant non-pathogenic facultative flora of the human intestine [8].

Some studies have concluded that *Ficus* extracts exhibit antibacterial activity against selected microorganisms at different levels. These extracts exhibited the most significant antibacterial activity against *S. aureus*, *Proteus mirabilis* and *E. coli* [9]. *F. sycomorus* extracts showed antimicrobial activity against a wide range of bacteria including antibiotic-resistant species and fungal species [10].

E. coli is one of the most frequent causes of many common bacterial infections, including cholecystitis, bacteraemia, cholangitis, urinary tract infection (UTI), traveler's diarrhoea, and other clinical infections [11]. *S. aureus* can cause a range of illnesses, from minor skin infections to life-threatening diseases such as endocarditis, bacteraemia, and sepsis [12].

Diagnosis of *S. aureus* and *E. coli* infection is based on the collection of specimens from the infected site and stool sample respectively with microbiological testing of the sample in the laboratory [11].

Plant extracts have a variety of medicinal uses in humans and animals [13]. Discovery of lead compounds from plant extracts may help fight bacteria that have become resistant to antimicrobials [14]. Unfortunately, the discovery of antimicrobials derived from plants has declined in the last decade [14].

In Zambia, there are very few studies investigating the properties of plant extracts and to the best of our knowledge there is no published study investigating the antibacterial properties of *F. Sycomorus*. The purpose of this study was to investigate the antibacterial properties of *F. sycomorus* against *S. aureus* and *E. coli*. The study found that *F. sycomorus* has antibacterial properties against *S. aureus* and *E. coli* based on the zones of inhibition. Thus, this study contributed positively to the information on herbal plants of medicinal use in Zambia and the world at large.

Materials and Methods

Materials

E. coli, *S. aureus* bacterial culture plates, swabs, ciprofloxacin disc, Dimethyl Sulphoxide (DMSO), thermometers, sterile saline. *F. sycomorus* bark part of the plant was collected from fresh live trees in April, 2019 from 10 miles in Chibombo district, Lusaka province of Zambia. Botanical identification and authentication of the plant were done at The University of Zambia (UNZA), School of Agricultural Sciences.

Preparation of the plant extracts

Fresh bark part of the plant was collected, washed using distilled water and cut into small pieces. It was further air-dried for 3 weeks away from direct sunlight and then ground into a powder using an MRE grinder. The sample was then subjected to maceration method.

Ethanol (96%) and distilled water were used as solvents for the extraction of crude extract. 100 g of bark powder was weighed using an analytical balance and placed into a round-bottomed flask, and then 750 ml of ethanol (96%) was added to the sample. 100 g bark powder was weighed using an analytical balance and placed into a round-bottomed flask, then 600 ml of cold distilled water was added. The sample was kept in a safe cupboard away from the light for a total of 72 hours while shaking it every-after 12 hours. The mixtures were then separately filtered using Buchner funnels and Whatman number one filter paper to obtain the filtrate. The filtrate was then reduced to a concentrated mass by drying using a temperature controlled rotary evaporator at 35°C and packed into separate airtight containers. The extracts were kept at a temperature of 4°C. The percentage yield was calculated as shown in Table 1. These extracts were then subjected to antibacterial screening against *E. coli* and *S. aureus*.

Culturing and collection of the bacteria

Clinical strains of *S. aureus* (ATCC 25923) and *E. coli* (ATCC 25922) were cultured in the Microbiology laboratory at the University Teaching Hospital (UTH) in Lusaka. The strains of bacteria were then collected and taken to the Food and Drugs Control Laboratory (FDCL) at UTH for further analysis and testing.

Preparation of bacteria Isolates

The bacteria strains were cultured and kept in the laboratory at conditions that allow their growth. The bacteria were kept in mosturised acidity environment at 37°C and with the right amount of food that support bacterial growth. *E. coli* and *S. aureus* were provided with the suitable environment for their survival and the susceptible cell line, until the day the experiment was conducted and the different amounts of plant extracts were used to obtain the results for the zone of inhibition of the bacteria.

Inoculum Preparation

Direct colony suspension was used. The bacteria inoculum suspension was prepared using sterile saline and turbidity compared with 0.5 McFarland turbidity standards. The turbidity was adjusted with saline until it matches that of 0.5 McFarland turbidity standards. This was done by holding the suspension and the 0.5 McFarland turbidity standards in front of a light source against a white background with contrasting black lines.

Inoculation

The Muller-Hinton agar plates were used for *E. coli* and the chocolate agar used for *S. aureus*. Precautions were taken ensuring the plates did not have excess moisture on the agar surface before inoculation and should not be excessively dry (wrinkled surface indicates excessive dryness). A wire loop was sterilised by heating using acetic flame until it was red hot and then cooled. The wire loop was dipped into the bacteria suspension until the loophole was full. The media was then inoculated by striking the agar surfaces in two directions at 90 degree angle to each surface and the third line at 45 degree angle and then it was allowed to stand for 20 minutes in order to facilitate absorption of excess inoculum before application of the test plant extracts.

Disc Diffusion Method

Disc diffusion method was done according Clinical and Laboratory Standards Institute (CLSI) guidelines of 2012 [16]. Disc diffusion method was used for testing the antibacterial activities of the two plant extracts. Ciprofloxacin 5 µg standard disc was used as a positive control, while DMSO was used as negative control to compare the results with that of experimental plant extracts.

Determination of the antibacterial activities

The antibacterial activities of positive control (Ciprofloxacin, 5 µg standard disc), negative control DMSO and different concentrations of two extracts (ethanol and aqueous) of *Ficus sycomorus* bark were investigated by using the disc diffusion method. Culture strains of *E. coli* and *S. aureus* were maintained on agar plates. The sensitivity of the tested pathogenic organisms to aqueous and ethanolic extracts were shown by zones of inhibition after incubation. The zones of inhibition were measured 12 times per isolate using a plastic ruler in mm. The concentration that gave the least zone of inhibition was recorded as the minimum inhibitory concentration (MIC) as given in Table 4.

Data analysis

Repeated measures Analysis of Variance (ANOVA) was performed using Graphpad prism version 7.0 and determine and compare the antimicrobial activities using inhibition zones of the different concentrations of both the aqueous and ethanolic extracts of *F. sycomorus* to standard drug (ciprofloxacin 5 µg) against *S. aureus* and *E. coli*. Statistical significance was conducted at 95% confidence level ($p<0.0001$).

Ethical considerations

Ethical approval was done by the University of Zambia Health Sciences Research Ethics Committee (UNZAHSREC) on 6th May, 2019. The protocol ID was 20190217020. All procedures were done according to the stipulated laboratory guidelines.

Results

Table 1: Weight of the *Ficus sycomorus* extract, initial weight and calculated percentage yield of the plant.

***F. sycomorus* bark extract solvent used**	**Initial Weight of the plant in grams**	**Weight of the extract recovered in grams**	**Calculation of percentage yield**	**Percentage extraction yield**
Ethanol	100 g	14	14/100 × 100	14%
Water	100 g	18.3	18.3/100 × 100	18.3%

Table 2: Antibacterial properties of the ethanolic and aqueous extract of *F. sycomorus*.

Ficus sycomorus	**Concentration (mg/ml)**	**Zones of inhibition (mm)**	
		E. coli	***S. aureus***
Aqueous extract	20	0	0
	40	0	0
	50	3	2.2
	100	4.4	3.7
	200	5.2	4.7
	500	6.1	5
	Ciprofloxacin (5 µg)	7.5	5.8
Ethanol extract	20	0	0
	40	0	0
	50	3.1	4.5
	100	4.7	5.5
	200	5.4	5.7
	500	7	6.8
	Ciprofloxacin (5µg)	9.5	10

Note: The concentration, zones of inhibition of aqueous and ethanolic plant extract were recorded as shown in Table 2. Ciprofloxacin produced better antibacterial effects compared to the extracts ($p<0.0001$). The ethanolic extract produced better antibacterial activity compared to the aqueous extract

Table 3: Mean and standard deviations of the inhibition zones produced by different concentrations of both ethanolic and aqueous extracts of *Ficus sycomorus* against *S. aureus* and *E. coli*.

Bacteria	***Ficus sycomorus* bark extract**	**Mean ± SD of inhibition zones: Ethanol extract**	**Mean ± SD of inhibition zones: Aqueous extract**
Staphylococcus aureus	20 mg/ml	0	0
	40 mg/ml	0	0
	50 mg/ml	3.0 ± 0.1	2.2 ± 0.1
	100 mg/ml	4.4 ± 0.1	3.7 ± 0.3
	200 mg/ml	5.2 ± 0.2	4.7 ± 0.1
	500 mg/ml	6.1 ± 0.3	5.0 ± 0.2
	Ciprofloxacin (5µg)	7.5 ± 0.1	5.8 ± 0.1
Escherichia coli	20 mg/ml	0	0
	40 mg/ml	0	0
	50 mg/ml	3.1 ± 0.1	4.5 ± 0.1
	100 mg/ml	4.7 ± 0.2	5.5 ± 0.1
	200 mg/ml	5.4 ± 0.1	5.7 ± 0.2
	500 mg/ml	7.0 ± 0.1	6.8 ± 0.1
	Ciprofloxacin (5µg)	9.5 ± 0.2	10.0 ± 0.1

Table 4: Minimum inhibitory concentrations of both aqueous and ethanolic extracts against *S. aureus* and *E. coli*. MIC was 50 mg/ml for both extracts.

Bacteria	Extract type	MIC
S. aureus	Ethanolic	50 mg/ml
	Aqueous	50 mg/ml
E. coli	Ethanolic	50 mg/ml
	Aqueous	50 mg/ml

Discussion

In our study, it was discovered that the *F. sycomorus* extract had antibacterial properties with different activities on the different bacteria. Ciprofloxacin 5 µg produced zones of inhibition of 10 mm for *S. aureus* and 9.5 mm for *E. coli*. These were all higher than that of the *Ficus* extract. This is because the ciprofloxacin was in its pure antibacterial form with 100% active ingredient.

Ficus sycomorus aqueous extracts of 500 mg/ml resulted in the largest zones of inhibition of 6.1 mm in ethanol and 5.0 mm in aqueous for *S. aureus*. This showed that ethanol was a better extractor of the phytochemicals needed for inhibiting *S. aureus* as compared to water. These findings could also be due to the fact that ethanol is more polar and hence yielded polar constituents than water. This explains the higher zones of inhibition in ethanol than water. In a study done in Damascus, Syria, it was found that the zones of Inhibition by methanol extract of *F. Sycomorus* L. ranged between 11.5 and 21.5 mm [17]. This investigation showed that the leaf extract of acetone and methanol possesses a higher antibacterial activity than that of the present study of ethanol plant extract [17]. This may be as a result of differences in phytochemical compounds being extracted by methanol and acetone as compared to the ethanol used in this study. The part of the plant that was used was the leaf, which could have different phytochemical constituents from the bark of the plant and these have more antibacterial activities. Geographical setting could be another reason for the difference in the activity as there is a climate difference between Zambia and Syria. Differences in environmental or climate factors that subject the plant to having different concentrations of phytochemicals that are responsible for the antibacterial activity of *F. sycomorus*. Another study in Nigeria showed that antibacterial activity of *F. sycomorus* stem bark extracts (250 mg/ml) against test organism *S. aureus*. The zone of inhibition was 16.5 mm using agar diffusion method18. This was way more than what was found in our study and this could have been due to the different methods used. Our study utilised the disc diffusion method while Adeshina et al. 2009 used the agar diffusion method [18].

The antibacterial properties of *F. sycomorus* bark extract against *E. coli* was determined using the disc diffusion method and the results show larger zones of inhibition in aqueous extract than in ethanol extract. This could indicate that water extracted the phytochemicals needed to inhibit *E. coli* more than ethanol though at concentration 500 mg/ml, ethanol had a higher zone of inhibition (7.0 mm) than aqueous (6.8 mm). In a similar setting of a study done in India, the methanolic stem extract showed maximum antibacterial activity against *E. coli* of 7 mm zone of inhibition at concentration of 500 mg/ml, using disc diffusion method [19]. These results are very similar to the ethanol extract done in our study of 7.1 mm zone of inhibition. This could be because of the similar method and concentration used, on a similar part of the plant. On the contrary, a study done in India reviewed that methanol extract of *F. sycomorus* (MIC, 0.156 to 5 mg/ml; minimum inhibitory concentrations, 0.313 to 5 mg/ml) showed strong antibacterial activity against bacteria, including *E. coli* [20]. It is proved that *F. sycomorus* plants could act as a natural antibacterial agent [20]. The antimicrobial activity in our study were less than that a similar study done by Joseph and Justin Raj [20]. The differences in the findings could be attributed to differences in the geographical locations. The differences in climate between Zambia and India could have affected the phytochemical constituients and hence variations in the antibacterial properties. Another reason for the differences could have been in the difference in extraction media method employed. The results of these two studies could also show that

methanol could be a better extractor than ethanol and water.

The zones of inhibition were measured, and it was found that the MIC was 50 mg/ml in ethanol and water for both *S. aureus* and *E. coli*. On the contrary, Odunbaku and colleagues found that the ethanol leaf extract of *Ficus* had a MIC of 300mg/ml against *E. coli* and 700 mg/ml against *S. aureus* [21]. The MIC found by Odunbaku and colleagues was too high compared to our study, the high MIC could be attributed to the *Ficus* species having lesser concentration of the phytochemicals. The methanol extract from wood of *Ficus* aerial roots inhibited the growth of tested microorganisms, with MIC value of 39.1 mg/ml for *E. coli* and 78 mg/ml for *S. aureus* [22]. The MIC for *E. coli* was low probably because the wood of the roots have more phytochemicals responsible for antibacterial activity compared to the bark of the stem. In the literature, various criteria are applied to determine the susceptibility of extracts and isolated compounds as microbial inhibitors. According to the South African journal of Botany, Teinkela and colleagues stated that antimicrobial activity of a crude plant extract has been defined as significant with MIC below 100 mg/mL, moderate with MIC between 100 mg/mL and 625 mg/mL, and low with MIC values more than 625 mg/mL [22].

The minimal antibacterial activity obtained in this study can further be attributed to deviations in geographical locations, rain pattern and season of harvesting the plants. Geographical location has been reported to influence the chemical constituents of plant extracts of the same genus found in different environment as suggested by Olusesan and colleagues [23]. The same plant grown under different conditions can give rise to total different phytochemical constituents [24, 25].

Conclusion

In conclusion, the plant extract of *Ficus sycomorus* displayed antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* in both Ethanolic and aqueous extract. The ethanolic extract showed a better antibacterial effect than the aqueous extract. The zone of inhibition varied suggesting the varied degree of efficacy in different concentrations, showing that the antibacterial activity was concentration dependent.

Conflicts of Interest

None declared.

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