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Antibacterial Activity of Tamarindus indica Fruit Extracts against Staphylococcus aureus and Escherichia coli

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Abstract

Staphylococcus aureus and Escherichia coli cause a multitude of mild to serious infections in humans. Microorganisms such as Staphylococcus aureus and Escherichia coli have either developed resistance against the current treatments, or the current treatments cause unacceptable side effects. It is therefore imperative that research into new potential treatments is prioritized. Plants have shown to be a promising source of antibacterial agents. This study, therefore, aimed to determine the antibacterial activity of aqueous and ethanolic extracts of Tamarindus indica against Staphylococcus aureus and Escherichia coli. This was an in-vitro laboratory-based experimental study. Staphylococcus aureus and Escherichia coli were cultured in the laboratory. Different concentrations of aqueous and ethanolic extracts of Tamarindus indica 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. The aqueous and ethanolic extracts of Tamarindus indica exhibited activity against S. aureus and E. coli in a dose-dependent manner. The minimum inhibitory concentration was found to be 0.5 mg/ml against both micro-organisms. E. coli was more susceptible to both T. indica extracts with the 100 mg/ml dose giving a zone of inhibition of 14.8 mm ± 0.3 with the aqueous extract, and 13.5 mm ± 0.5 with the ethanolic extract. Testing of 100 mg/ml aqueous and ethanolic T. indica extracts against S. aureus resulted in maximum zones of inhibition of 8.5 mm ± 0.5. Tamarindus indica possesses dose-dependent antibacterial activity against Escherichia coli and Staphylococcus aureus.

Keywords: Antibacterial activity; Escherichia coli; Staphylococcus aureus; Tamarindus indica

Introduction

Staphylococcus aureus (*S. aureus*) is a gram positive pathogen that exists as normal flora on the skin, but has the ability to cause a range of conditions from minor skin and soft tissue infections to life threatening diseases such as infective endocarditis, toxic shock syndrome and osteomyelitis [1]. It is the major cause of hospitalizations at 18.8% with *Escherichia coli* (*E. coli*) following closely behind at 17.2% [2]. In the year 2017, over 119,000 *S. aureus* blood infections were reported in the United States with nearly 20,000 deaths occurring as a result [3]. In Zambia, a study carried out at the University Teaching Hospital, the country's largest referral centre reported that *S. aureus* was isolated in 30% of burns and 34% of bloodstream infections [4].

E. coli are gram negative pathogens which exist normally in the gut [5]. It is the leading cause of Urinary Tract Infections and has also been implicated as the one of the causative organisms of pelvic inflammatory disease, pneumonia, neonatal meningitis and diarrhoea [5]. *E. coli* made up 18% of the stool samples positive for bacterial pathogens belonging to children aged 0-59 months that were brought to the University Teaching Hospital in Zambia between December 2015 and April 2016 [6].

Several treatments are available for these two microorganisms; *S. aureus* infections can be treated by several classes of drugs that include, but are not limited to, beta lactams, sulphonamides, tetracyclines, glycopeptides [7] whilst *E. coli* treatment includes cephalosporins and fluoroquinolones [8]. Lately, however, there has been a rise in the resistance of *S. aureus* and *E. coli* to drugs [6,9]. In Zambia, particularly at the University Teaching Hospital, the prevalence of Methicillin Resistant *Staphylococcus aureus* among *S. aureus* isolates rose from 23% in 2003 to 47% in 2014 [9]. Resistance of diarrhoeagenic E coli to multiple drugs was also observed at the same hospital in a separate study [6]. In addition to antibiotic resistance being a global health problem, antibiotics also possess inherent side effects [10]. For instance, fluoroquinolones have a rare tendency to cause aortic aneurysm rupture and dissection, tendon rupture, tendonitis, amongst other events, as reported by the United States Food and Drug Administration [10].

Injudicious use of antibiotics has led to antimicrobial resistance [11], and penicillins, cephalosporins that are used to treat these bacteria have been reported to cause severe adverse reactions [12]. These two factors are just some of the reasons that have necessitated the discovery of new classes of drugs [13,14]. The newer drugs must be effective against these microorganisms and also exhibit fewer side effects. People in both developed and developing countries have turned to herbal medicines as they are more natural, effective, and possess fewer side effects, which has consequently increased their demand [15]. Herbal medicines have proven effective for several conditions ranging from non-communicable diseases such as diabetes [16] to infectious diseases such as those caused by *Staphylococcus aureus* [17].

Tamarindus indica Linn (*T. indica* L.), of the Leguminosae family, is a medium sized tree located in Africa, India, the Caribbean and South America all the way up to Southern Florida [18]. It has many traditional uses in several countries and has been recorded by the British and American pharmacopoeias for its anti-pyretic, antiscorbutic, and laxative properties [18]. African slaves in Latin America used and influenced the use of *T. indica* in that region against infectious diseases most of which were intestinal conditions [19]. In Zambia, *T. indica* is used in conditions such as stomach disorders, constipation, intestinal worms, liver and gallbladder problems, dry eyes, colds and fever and even pregnancy-related nausea [20].

The aim of this research was therefore to validate the multiple reports of the use of *T. indica* for its antibacterial activity, particularly targeting *S. aureus* and *E. coli*.

Materials and Methods

Materials

Escherichia coli (ATC 25922), *Staphylococcus aureus* (ATC 25923), bacterial culture plates, swabs, cell lines, Cloxacillin, Levofloxacin, Dimethyl sulphoxide, thermometers, sterile saline, Seed pod of *Tamarindus indica* L.

Sample collection and plant identification

T. indica seed pods were collected from their natural habitat in the forest of Kazungula District of the Southern Province of Zambia in the month of March 2019. These pods were taken to the University of Zambia (UNZA), School of Agriculture in the Plant Science Department for botanical identification and authentication.

Laboratory equipment and personal protective equipment (PPE)

Culturing plates, test tubes, centrifugation machine, refrigerator, bacteria collecting swabs, universal transport media, gloves, laboratory coats and gowns, shoe covers, boots, respirators, face shields, safety glasses were the equipment used in this study.

Methods

Study type and design

The study was an in-vitro experimental laboratory-based study in which *T. indica* seed pods were tested for antibacterial activity against *E. coli* and *S. aureus*.

Study site and period

The study was carried out at the University Teaching Hospitals (UTH) in the Food and Drugs Control Laboratory, Lusaka, Zambia, from March 2019 to July 2019.

Preparation of *Tamarindus indica* fruit extract

Nwodo and colleagues' method was mimicked with slight variations: Fresh and ripe fruits of *T. indica* were cleaned by rinsing in distilled water to remove surface contaminants. Drying was done for 14 days after which they were ready for extraction using ethanol (96%) and distilled water. In this process, 50 g of fruit was weighed using analytical balance and placed into a beaker, and then 200 ml of ethanol (96%) was added. For the water extraction, 100 g of fruit was weighed using analytical balance and placed into a conical flask, and then 400ml of cold distilled water was added to mimic the traditional way of extraction. The samples were kept in a safe cupboard away from the light for a total of 72 hours while agitating every 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 evaporator at 37°C and packed into separate airtight containers at room temperature [18].

The percentage extractive yield of the aqueous and ethanolic extract was then calculated using the following formula:

%extraction yield = [weight of extract recovered / starting weight of the dry sample] x 100

Dilution of plant extracts

Each bioactive extract was made up to 100 mg/ml by dissolving 1 g of extract in 10ml of cold distilled water and was serially diluted to five concentrations (50, 10, 1.0, 0.5 and 0.25 mg/ml) using a weight: volume (w/v) ratio expressions.

Culturing, collection and preparation of the bacteria

Clinical strains of *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923) were obtained from the Microbiology at University Teaching Hospital, Lusaka, for the experiment.

E. coli and *S. aureus* were cultured and kept in the laboratory at conditions in a dark cupboard that allowed their growth 3 days. *E. coli* and *S. aureus* were provided the environment for their survival until the day the experiment was conducted.

Inoculum procedure

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 matched 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.

The Muller-Hinton agar plates were used and did not have excess moisture on the agar surface before inoculation and the plates were not excessively dry (wrinkled surface indicates excessive dryness). A sterile cotton swab was dipped into the bacteria suspension. Excess inoculum was removed by passing the swab inside the bacteria suspension tube. The media was then inoculated by swabbing 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 [21].

Disc diffusion method

Disc diffusion method was used for testing the antibacterial activities of the plant extracts. Levofloxacin 5 µg/ml and Cloxacillin 5 µg/ml were used as positive control (standard drug), while dimethyl sulphoxide (DMSO) was used as negative control to compare the results with that of experimental plant extracts. The agar plates were prepared as sterile glass petri dishes, seeded with innocula and kept for 24 hours under anaerobic conditions. Using a sterile cotton swab, the plate surface was covered in bacteria and the dried extract was then dissolved in 1 ml of DMSO using different masses of the plant extracts.

Whatman number 1 filter paper discs of 6mm in diameter were prepared and sterilized using autoclave machine. The discs were impregnated with the different dilutions of concentrations extracts and left for some time until the extracts diffused in them and dried. Negative control was also prepared as 1.0 ml of DMSO to plain disc and was left to dry. After drying, the extracts, DMSO and the levofloxacin discs were individually placed onto the inoculated Muller-Hinton agar medium with the help of sterile forceps carefully with adequate spacing between each other and were allowed to diffuse into the media. The plates were incubated at 37˚C for 24 hours [22].

Determination of the antibacterial activities

The different concentrations of the aqueous and ethanolic extracts of *T. indica*, culture strains of *Escherichia coli*, *Staphylococcus aureus* was maintained on agar plates. The sensitivity of the tested pathogenic organisms to aqueous and ethanolic extracts was shown by zones of inhibition after incubation. The zones of inhibition were measured using a plastic ruler in mm. For each concentration of the extract, the zone

of inhibition was measured three times to minimize the error and the mean was recorded. The statistical analysis was then performed.

Data analysis

Analysis of data was done using Statistical Packaging for Social Sciences (SPSS) version 21. The activity of *T. indica* against the test microorganisms was measured in millimetres. Statistical significance was conducted at 95% confidence levels with a $p < 0.05$.

Ethical considerations

The study was undertaken after due approval of the study protocol by the University of Zambia Health Sciences Research Ethics Committee. The Protocol ID was 20190217135 and IORG of 0009227.

Table 1: Percentage yields of the Ethanolic and Aqueous extract of the Seed Pod of *T. indica.*

Plant name and solvent	Initial weight of the extract in grams	Weight of the extract recovered in grams(g)	Calculation of percentage yield	Percentage of extraction yield
Tamarindus indica Linn, (Ethanol)	50.16 g	25.79 g	25.79 g/50.16 g x 100	51.4%
*Tamarindus indica Linn (*Aqueous)	50.97 g	23.65 g	23.65 g/50.16 g x 100	46.4%

Table 2: Mean zones of inhibition of levofloxacin and negative controls in millimetres (mm).

Positive Control (Levofloxacin Discs)	Positive Control (Cloxacillin Discs)	Negative Control (Dimethyl Sulphoxide)	
Escherichia coli	*Staphylococcus aureus*	*Escherichia coli*	*Staphylococcus aureus*
29.0 ± 0.50	30.0 ± 0.50	0.0	0.0

Table 3: Mean zones of inhibition of bacterial growth by *T. indica L.* extracts in millimetres (mm).

Extract Concentration (mg/ml)	Ethanol Extract (mm)		Aqueous Extract (mm)	
	Escherichia coli	***Staphylococcus aureus***	***Escherichia coli***	***Staphylococcus aureus***
0.25	0	0	0	0
0.5	1.2 ± 0.1	0.50 ± 0.1	1.9 ± 0.6	0.6 ± 0.1
1	4.4 ± 0.1	1.6 ± 0.1	5.0 ± 0.1	1.6 ± 0.1
5	6.0 ± 0.1	2.2 ± 0.1	6.6 ± 0.1	2.3 ± 0.1
10	6.5 ± 0.1	3.7 ± 0.1	7.3 ± 0.2	3.7 ± 0.1
50	10.1 ± 0.1	4.1 ± 0.1	12.3 ± 0.3	4.4 ± 0.2
100	13.5 ± 0.5	8.5 ± 0.5	14.8 ± 0.3	8.5 ± 0.5

Results

Percentage yields of the extract

Table 1 shows different samples of the seed pod of *T. indica* were weighed using an electronic top pan balance for ethanolic and aqueous extracts. The ethanolic extract had a higher percentage yield of 51.4% compared to 46.4% of the aqueous extract.

Mean zones of inhibition of the standard drugs

Cloxacillin and Levofloxacin were used as positive controls and were tested against *S. aureus* and *E. coli* respetively with Cloxacillins having a zone of inhibition at 30 mm and Levofloxacin at 29 mm (**Table 2**). DMSO was used as a negative control and no antibacterial activity was observed against both *S. aureus* and *E. coli.*

Mean zones of inhibition of *Tamarindus indica Linn.* extracts in millimetres (mm)

Table 3 shows that three repeat measurements were done for each concentration of ethanolic and aqueous extracts of *T. indica Linn.* against *E. coli*, and *S. aureus*. The Mean Zones of Inhibition were recorded as shown in the table below. All the values were expressed as ± Standard Error Means (SEM). The highest zone of inhibition was demonstrated with the highest concentration of the ethanolic extract against *E. coli* measuring 13.5 mm (SD=0.5). This was followed by the aqueous extract with a the maximum of 14.3 mm and both the aqueous and ethanolic extracts inhibiting *S. aureus* with the maximum zone of inhibition for both being 8.5 mm.

Discussion

The objective of the study was to determine the activity of *Tamarindus indica* against *Staphylococcus aereus* and *Escherichia coli*. As can be observed from the results displayed in **Table 3**, *T.indica* aqueous and ethanolic extracts had activity against these microorganisms, which appeared to be dose-dependent. Another result of notice is that *E.coli* was more susceptible to *T.indica* than *S. aureus* was, for both the aqueous and ethanolic extracts. The highest susceptibility was observed with *E.coli* to 100mg/ml, the highest tested dose of aqueous extract of *T.indica*, giving a zone of inhibition of 14.8 mm. The minimum inhibitory concentration of both the aqueous and ethanolic extracts of *T.indica* against *E. coli* and *S. aureus* was found to be 0.5 mg/ml.

Similar studies have found that *T. indica* fruit extracts possess activity against *E. coli* [23,24]. Interestingly, other studies found *T.indica* fruit had activity against *E.coli* but none against *S. aureus* [25,26]. It is therefore fair to say that studies thus far have revealed differences in antimicrobial activity of *T. indica*. An explanation for the differences in the antibacterial activities between studies performed in the past as well as the current study could be the difference in phytochemicals and/or their concentrations. Previous studies have afterall demonstrated that plants grown in different environments may have varying chemical constituents with different concentrations [27,28].

There are also differences between the current study and those previously published in the Minimum Inhibitory Concentration (MIC). The present study found that the MIC for both the aqueous and ethanolic extracts of *T. indica* against both *E.coli* and *S. aureus* was 0.5 mg/ml, another study that determined that antibacterial activity found that the MIC against the same strain of the bacterial organisms was 75 mg/ml [19]. This huge difference could be explained by the different phytochemicals and/or their concentrations in the plant parts used. Whereas the current study used fruit to determine antibacterial activity, the comparator study used dried leaves to give MIC of 75 mg/ml. In fact, the comparator study also determined activity against *S. aureus* and *E.coli* using fresh leaves and found that there was no activity against *E.coli* and the MIC was over 150 mg/ml for *S.aureus*.

A study conducted in Nigeria performed phytochemical analysis on the fruit pulp and found that it possessed saponins, tannins, alkaloids and attributed antibacterial activity to the presence of these phytochemicals [24]. Another study revealed the presence of flavonoids, saponins, alkaloids and tannins [26]. It is possible that some or all of these phytochemicals could have caused antibacterial activity against the tested organisms, with the possibility of working in a synergistic manner.

T. indica has been found to be active against other microorganisms including *Bacillus subtilis, Enterococcus faecalis, Pseudomonas aeruginosa* [19], and *Burkholderia pseudomallei* [29]. Clearly *T. indica* has high medicinal value and has the potential to yield valuable and efficacious antibiotics in this era of antibiotic resistance. It is therefore of paramount importance that testing be taken to the next level.

Conclusion

The current study has revealed that *T. indica* aqueous and ethanolic extract inhibit *S. aureus* and *E. coli* in a dose-dependent manner. The MIC in this study was found to be 0.5 mg/ml for both the ethanol and aqueous extract against both microorganisms. Therefore, this study supports the use of *T. indica* as an antibacterial plant. Thus, a lot of research must be conducted to help discover drugs from plant sources.

Ethical approval

Ethical approval was granted by the University of Zambia Health Sciences Research Ethics Committee (Protocol ID: 20190217135, IORG no: 0009227, IRB no: 00011000).

Conflict of interest

The authors declare no conflict of interest

Author contribution

All authors contributed to proposal development, write up, data collection, data analysis, manuscript writing, editing and approved the final version of the manuscript.

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