Research Article
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In Vitro Antimicrobial Activity of Lemon Bark Extract Against *Salmonella*, *Shigella*, and *Escherichia coli*

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Abstract: The main objective of the study was the extraction and testing of the antimicrobial activity of the Lemon bark extract against the microorganisms involved in air and waterborne infections. The antibiotics are not very effective in the present day as the microorganisms are becoming resistant, the study concentrates on the antimicrobial activity and time ahead of the prophylactic potential of the lemon bark extract. Phytochemical analysis for the constituents of Lemon bark showed the presence of saponins, flavonoids and tannins in the aqueous form of solution. The potency test on microorganisms proved to be active against *Salmonella shigella* with the minimum inhibitory concentration of 100 mg/L was 16.27 mm, 150 mg/L was 16.43 mm and 250 mg/L was 16.73 mm. Meanwhile, for *Escherichia coli* for 100 mg/L was 13.20 mm, 100 mg/L was 13.10 mm and 150 mg/L was 13.13 mm.

Introduction

Lemon is a member of the Rutaceae family, a significant group of medicinal plants with promising potency for antibacterial and anticancer effects in crude extract (1). The most well-known member of the Rutaceae family is the citrus fruit known as Lemon, which is grown extensively and is a significant species (2). Although this plant has 160 citrus genera and is grown on every continent, Rutaceae is the most important one (3).

The lemon tree is a robust citrus tree with large, lanceolate, light green leaves and an articulated, short leaf stalk with a plain, non-winged edge. The first phases of development result in the budding leafy shoots having purple hues in a few days. The leaf blade that contains glands and flowers in clusters is where the essential oil is found. However, lemon trees are particularly well suited to the relatively dry subtropical climates of coastal regions, where variations in daily temperature show small differences but where the winter frost is very low because it is above the coastal foothills (4). The twigs, leaves, branches, and occasionally the trunks of lemon trees are affected by temperatures below -4 °C. Lemon trees have also been observed to suffer damage at temperatures between -1 and -2 °C when the tissues are very young and at -3 to -4 °C when the fruits and leaves are young adults (5). Lemon trees are primarily sensitive to cold.

These naturally occurring antimicrobials have been around for a long time and are gaining high significance, which is more critical in chemotherapy (6). They are biologically active substances that naturally occur in materials, and they indicate biological activity that has the potential to generate new drugs that are healthy for human beings.

The use of plant extract is due to the antimicrobial properties in which antimicrobial traits in plants enable for them to be used due to their secondary metabolism where the compounds are synthesised. An example is the phenolic compounds which are part of the phenolic compounds that are used to maintain human health (7).

Medicinal plants play a significant role in

maintaining the health of both individuals and the community. Their excellent medicinal value is attributed to the physiological actions of the human body, which are produced by chemical substances, and contain chemical compounds such as fats, oils, resins, oleoresins, and glycosides (8).

Lemon trees are described as being thorny and narrow in their botanical description. They can reach heights of 3.0 to 6.0 meters, with their dark green leaves set alternately along their stems. The Bush lemon, Variegated Pink, Lisbon, Eureka, Ponderosa, Yuzu, Vema, and Yen Ben are among the varieties of lemons that have smooth, porous skin. Some lemons have a pointy tip, while others have a circular shape at the base (9). Botanical terminology for plants: Order: Sapindales, Family: Rutaceae, Genus: Citrus, Species: *C. Limon*, Kingdom: Plantae, Angiosperms, Eudicots, Rosids (10).

Experimental Section

Materials and Methods

The phytochemical analysis was used to find the phytochemical constituent presence or absence of the lemon bark through different solvents, the phytochemical analysis of the Aqueous extract of the Lemon bark. To test the antimicrobial activity of the Lemon bark extract. The agar plates were set up in sterile glass Petri dishes, seeded with an inoculum of water and kept for 24 hours under incubation.

Collection of Plant Materials

The plant was identified at the Ministry of Green Economy and Environment, Forestry Department in central province Kabwe then collected the Lemon bark in the Zambia Compound of Mulungushi University area during December and January.

Preparation of Materials

The lemon bark was immediately cut into smaller pieces and dried. Then, masses representing 100 mg, 150 mg, and 250 mg which was soaked each in distilled water of volume 1.0 liter. The solutions of the extracts were allowed to stand for 24 hours away from the sun. After 24 hours, the solution was filtered on a Whitman number 1 filter paper in which the filtrate of extract obtained was then kept refrigerated in a sterile bottle for further study.

Phytochemical Analysis

Alkaloid Test

Dragendorff's test. An orange-red precipitate was formed by adding 1.0 mL of Dragendorff's reagent to 2.0 mL of extract, indicating the presence of alkaloids according to Chaudhary et al. 2010, (24).

Terpene Test

The Liebermann-Burchard was prepared according to Adu et al. 2019 but in brief 50 ml of acetic anhydride was pipetted into an amber glass vial and kept in an ice bath. After 30 minutes, 5 mL of concentrated sulphuric acid was pipetted and added carefully to the acetic anhydride in the vial. Then from the stock filtrate 1.0 mL was mixed and changes were observed (25).

Tannins Test

The method for this was done according to Nigussie et al. 2021, the method in brief involved, 2.0 mL of filtrate stock solution of the lemon extract, a few drops of 10% Iron (III) chloride ($FeCl_3$) the pale yellow solution. The appearance of a blackish-blue colour could be the presence of Gallic tannins and a green-blackish could be the presence of catechol tannins which was the case (26).

Glycosides

The filtrate lemon extract stock solution 1.0 mL was pipetted and three drops of dilute sulphuric acid solution. Then 1.0 mL chloroform and 1.0 mL ether were added into the acidified lemon extract stock solution and shaken well. Then 3.0 ml ammonia was added and separated the organic layer. In the organic layer, the anticipated colour changes could have been pink, red or violet colour due to the presence of glycosides, however, it was just greenish blue (27).

Saponins

The solution was made by mixing 5.0 grams of the dried bark of lemon soaked in 5.0 mL of distilled water. Then warmed in the water bath, the formation of a persistent froth remained persistent even after adding 3 drops of olive oil, this was done according to the protocol of Tilaoui et al. 2021 (28).

Flavonoids

The lemon-dried crude 5.0 gram was measured and soaked 20.0 mL of 75% ethanol and soaked for 24 hours in a cool dry place. Then, after 24 hours it was filtered. Then 10.0 mL of the extract was used and 10 drops of hydrochloric acid (HCl) were added followed by 4 stripes of magnesium ribbons (4.0 mm). The colour of the changes was noted (28-29).

Antimicrobial Testing

To test the antimicrobial activity of the Lemon bark extract the agar plates were set up in sterile glass Petri dishes, seeded with inoculum of water and kept for 24 hours under incubation. Whiteman number 1 channel paper circles of 6 mm width were arranged and disinfected using an autoclave machine. The circles were impregnated with the various concentrates weakening focuses and were left for quite a while until the concentrates diffuse in them and dry. After drying, the concentrates were exclusively put onto the immunized Muller-Hinton agar, Xylose lysine deoxycholate agar and Eosin methylene blue agar medium with the assistance of sterile forceps

cautiously with sufficient dispersing between one another and were permitted to diffuse into the medium (11).

Materials for Antimicrobial Activity Test

Gram Stain Examination

The colony from the plate was picked by a flamed wire loop then the thick smear was made on a clean slide thereafter heat fixed the slide with a flame until the slide was dry (12). The slide was then added with crystal violet for 2 minutes, then rinsed with running water for 6 seconds, then flooded with Iodine solution for 2 minutes and rinsed for 6 seconds with running water. Lastly, the slide was flooded with decolourizer for 5 seconds then the smear was covered with safranin for 2 minutes and rinsed with running water for 6 seconds thereafter the slide was aired to dry before viewing under the microscope (13). The stained slide was viewed by the addition of immersion oil at the lens of X100 of the microscope to examine whether the bacterial organism was gram-positive or negative (14).

The antimicrobial activities of all microorganisms were tested for each type of culture media against the extract in which the bacteria for water borne were cultured, all bacteria were grown at 37 °C. The bacteria cultured were *S. shigella* on XLD and *E. Coli* which was cultured on EMB.

Result

The assay conducted in types of media, XLD Agar for *S. shigella* and EMB agar for *E. coli* bacterial strains using the aqueous extract for the minimum inhibitory concentrations are reported in **Table 1**. The results showed that the inhibition for *S. Shigella* depended on concentration of extract used. An extract with a concentration of 100 mg/L showed an inhibition zone of 16.27 mm average. Meanwhile, the inhibition for *E. Coli* showed an inhibition of 13.30 mm. The results suggest that the extract was more potent *S. shigella* than *E. coli* for same concentration. This is a trend also for the other concentrations.

Table 1. Zones of inhibition of lemon bark aqueous extract.

Bacterial strain	Concentration (mg/L)	Zone of inhibition (mm)
S. shigella	100	16.27
	150	16.43
	250	16.73
E. coli	100	13.20
	150	13.10
	250	13.13

The aqueous extract showing the zones of inhibition from each concentration of 100 mg/L, 150 mg/L and 250 mg/L on the medium of XLD for the organism *S. shigella* is given in **Figure 1**. The concentration of 100 mg/L had a zone of inhibition of 13.13 mm, 150 mg/L concentration had one inhibition of 13.10 mm and the concentration of 250 mg/L had one of inhibition 13.20 mm which showed a variation in the zone of inhibition as the concentration was increasing after 24 hours of incubation.

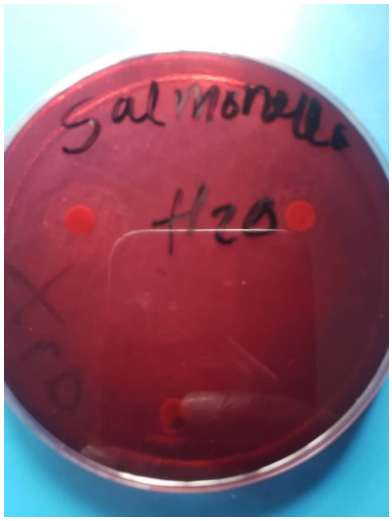

Figure 1. Zones of inhibition of lemon bark aqueous extract on *S. shigella*.

The aqueous extract showing the zones of inhibition from each concentration of 100 mg/L, 150 mg/L and 250 mg/L on the medium of EMB for the microorganism *E. coli* is given in **Figure 2**. The concentration of 100 mg/L had a zone of inhibition of 16.27 mm, 150 mg/L concentration had one of inhibition 16.43 mm and the concentration of 250 mg/L had one of inhibition 16.73 mm which showed an increase in zone of inhibition of as the concentration was increasing after 24 hours of incubation.

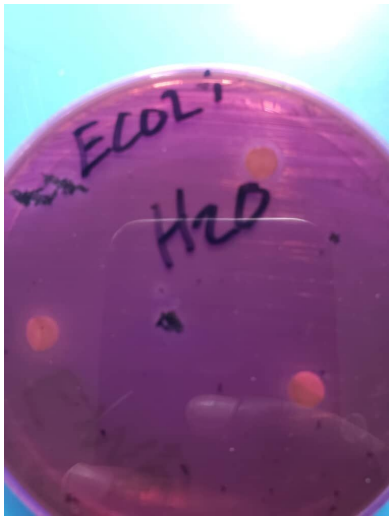

Figure 2. Shows the zones of inhibition of E. Coli.

Table 2. Phytochemical analysis of lemon bark aqueous extracts.

No	Phytochemical constituent	Reagent/Method	Colour change	Conclusion
1	Alkaloids	Dragendorff reagent	Buff- colour	-
2	Terpenes	Liebermann- Burchard	Clear	-
3	Tannins	10% FeCl3	Green	+
4	Glycosides	Bontrager Test	Bluish-green	-
5	Saponins	Foam forming test	Frothing	+++
6	Flavonoids	70% HCl-(Shinoda test)	Pinkish-brown	++

Note: (+) means the level of occurance.

The results of the phytochemical analysis of the aqueous extract is given in **Table 2**. The different phytochemical constitutes showed that the extra had tannins, saponins and flavonoids. The intensity of the extracts showed that there were relatively more flavonoids and saponins. Otherwise, tests for alkaloids, terpenes and glycosides did not seem to be present in the extract.

The above table shows the phytochemicals through different solvents showing presence in excess or less or absent. In terms of intensity of presence it was found that the presence of various phytochemical constituents were as in **Table 2**.

The analysis of the gram stain results is given in **Table 3**. The gram-negative strains are rod-shaped with the colour pink. All stains of the isolates showed the presence of gram-negative bacteria.

Table 3. Gram stain of isolated microorganisms.

Gram stain	Gram-negative
Shape	Rods
Colour	Pink
Number of stains	2

Discussion

The study was necessary to know the minimum inhibition concentration (MIC) of the Lemon bark extract against the isolates of the waterborne bacteria to establish whether antimicrobial activity occurs. The MIC of three concentrations of the extract was used and, in the experiment, it was found that the MICs did inhibit the bacterial strains as expected (15).

The lemon bark extract was extracted by obtaining the bark of lemon cut in pieces then soaking it in distilled water for 24 hours then filtering using the filter paper and measuring the concentration of 100 mg/L, 150 mg/L and 250 mg/L that was put on paper disc then dried them (16-19).

The Lemon bark extract showed antimicrobial activity against the gram-negative bacteria that's S. shigella and E. coli that were the isolated organisms, this is an important finding as these organisms cause a spectrum of diseases in humans such as Typhoid fever and Diarrhoea that can be bloody or prolonged (20). The simple use of Lemon bark extract can prevent such types of infections and help in the keeping of good health (21). It is important to point out that exercise, good diet and good personal hygiene are also needed (22).

The phytochemical constituents of the bark of the lemon which were the glycosides, saponins, flavonoids, and tannins are metabolites that can be used towards achieving a defence mechanism against many microorganisms (23).

Conclusion

The Lemon bark extract showed the following phytochemical tests positive: tannins, saponins, and flavonoids. When investigated, for antimicrobial activity, by way of zones of inhibition for *S. shigella* and *E. coli*. The Lemon bark showed antimicrobial activities against *S. shigella* and *E. Coli* with the minimum inhibitory concentration of 100 mg/L, showing an inhibition zone of 16.27 mm, 150 mg/L with an inhibition zone of 16.43 mm, and 250 mg/L with an inhibition zone of 16.73 mm.

Against *E. coli* for 100 mg/L with an inhibition zone of 13.20 mm, 10 mg/L with an inhibition zone of 13.10 mm, and 150 mg/L with an inhibition zone of 13.13 mm. It was generally observed that the lemon bark showed a relatively higher inhibition zone against *S. shigella* than *E. Coli* for the same concentration of lemon bark concentrations.

Declarations

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Conflict of Interest

The authors declare no conflicting interest.

Data Availability

The unpublished data is available upon request to the corresponding author.

Ethics Statement

Not applicable.

Funding Information

Not applicable.

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