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ORIGINAL ARTICLE

Antibacterial activity of *Citrus latipes* extracts using certain solvent

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ABSTRACT
The antibacterial activities of Citrus letipes extracts using certain solvents were examined in this study. The potential antibacterial characteristic of Citrus letipes is a lesser-known among other citrus species. The bioactive components from Citrus letipes were extracted using four distinct solvents: methanol ethanol, ethyl acetate and acetone. The antibacterial activity against bacterial strains was assessed using the agar well diffusion method. The outcomes demonstrated that all Citrus letipes extracts displayed antibacterial activity to various degrees. The strongest antibacterial effectiveness of citrus latipes with methanol extract was highest followed by ethanol, ethyl acetate and acetone extracts, which prevented the growth of tested bacterial strains at specific concentration. Methanolic extract exhibited a 20.1 mm maximal inhibition against Staphylococcus aureus and a 19 mm maximal inhibitory response to E coli at 100 µL concentration. The phytochemical analysis revealed the presence of a number of secondary metabolites, including as flavonoids, alkaloids, and phenolic compounds, which is effective as antibacterial properties.
Keywords: *Citrus letipes, Antibacterial, Solvent, Methanol Extract, Zone of inhibition.*

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INTRODUCTION

Over 80% of people in the globe consume herbal remedies to treat medical conditions, especially in infectious diseases and the medicinal plants have been used for therapeutic purposes for a long time. Traditional medicine can be used as a therapeutic natural agent due to the issue of bacteria resistance to the available existing antibiotics [1]. Oranges, mandarins, limes, lemons, grapefruits, and citrons are some of the varieties of citrus fruits, which are members of the genus Citrus and Rutaceae family. Color, sweetness, bitterness, and astringency are important organoleptic and commercial features of fruits. The majority of citrus species are consumed fresh, as juice ingredients, or as preserved segments [2]. Citrus fruit cultivated in tropical and subtropical regions of the world, *Citrus letipes* is the only source of citrus fruit used in trade. It is a species of the Rutaceae family and belongs to the orange subfamily Aurantioideae [3]. Citrus fruit has antibacterial, antifungal, antiviral, antidiabetic, and anticancer activity because it contains alkaloids [4]. *Citrus latipes* contains highest concentration of hespardin and narigingin which are major components of flavonoids [5]. Natural products are chosen in the modern world and have created new chemical variety as a result of their use as anti-microbial agents. There have been reports of using natural products for human health for a very long time [6]. *E.coli* is the most causative organism which is responsible for urinary tract infections (UTI). Compared to men, women are more likely to develop UTIs because of anatomical variations. It is believed that phytomedicines compounds are widely accepted by human body moreover citrus fruit are high in potassium content and vitamin c [7]. Objectives of this study to determine the antibacterial activity of *Citrus latipes* and their effectiveness against typically disease-causing bacteria.

MATERIAL AND METHODS

Collection of Material: Fruits are collected from local market of Shillong, Meghalaya and laboratory analysis has been done at microbiological laboratory of University of Science and Technology Meghalaya.

Peels preparation: Peels are removed from the fruit then cut into small pieces and air day in the sunlight for 5 continuous days.

Extract preparation: A 50g of powder were placed in the thimble, and 150 ml of ethanol were extracted from it over the course of 48 hours. Separate concentrations of the solvent extracts were performed under reduced pressure [8].

Phytochemical test: Test for carbohydrate (Molisch's test), Reducing sugars (Fehling's test), Tannins (Gelatin test), Flavonoid, Terpenes (Salkowski test) and Saponin were carried out using standard methods [9].

Test organisms: Test organism was isolated from urine sample and identify through series of biochemical test and culture on MaCconkey and Manitol salt agar.

Antibacterial activity

The antibacterial activity of the various plant extracts were evaluated using the agar-well diffusion method as per the standard procedure. Bacterial strains were put into Petri plates that contained 20 ml of Muller Hinton agar medium. The test organisms in the freshly seeded plate and using a well cutter, 10 mm-diameter wells were bored [10]. Plant extracts at quantities of 20, 50 and 100 µL were poured in this well. For positive and negative controls ciprofloxacin (20 µL) and distilled water (20 µL) were used respectively.

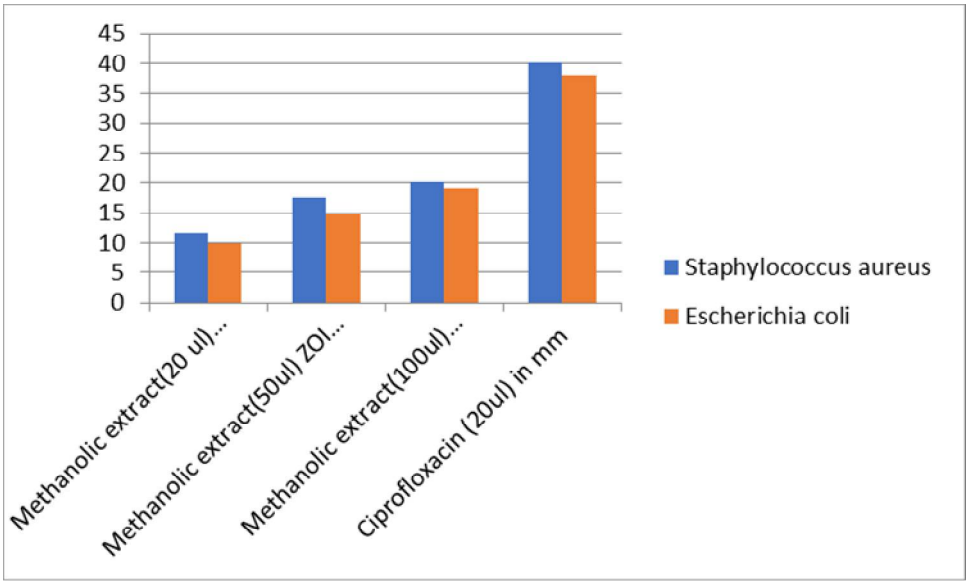
The plates were then incubated at 37 °C for 24 h. The test organisms in the freshly seeded plate and after 24 hours, the zone of inhibitions' diameter were measured in mm [9].

RESULT

Table No 1 Graph 1 show antibacterial activity of *Citrus letipes* peels using methanol extract effective against *Staphylococcus aureus and E coli.* Methanolic extract exhibited a 20.1 mm maximal inhibition against *Staphylococcus aureus* and a 19 mm maximal inhibitory response to *E coli* at 100 µL concentration.

Table No 1: Antibacterial activity using Methanol extract

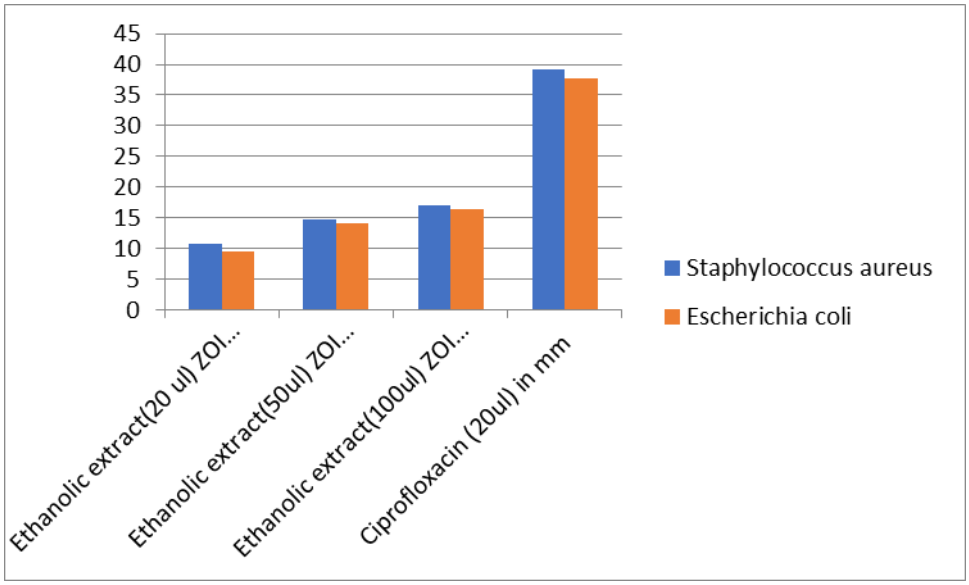
Pathogens	Methanolic extract (20 µL) ZOI in mm	Methanolic extract(50µL) ZOI in mm	Methanolic extract(100µL) ZOI in mm	Ciprofloxacin (20µL) in mm
Staphylococcus aureus	11.5	17.6	20.1	40
Escherichia coli	9.8	14.8	19	38



Graph 1: Antibiogram analysis of *Citrus letipes* using Methanol extract

Table No 2: Antibacterial activity using Ethanol extract

Pathogens	Ethanolic extract (20µL) ZOI in mm	Ethanolic extract (50µL) ZOI in mm	Ethanolic extract (100µL) ZOI in mm	Ciprofloxacin (20µL) in mm
Staphylococcus aureus	10.8	14.7	17	39.2
Escherichia coli	9.4	14.1	16.3	37.7



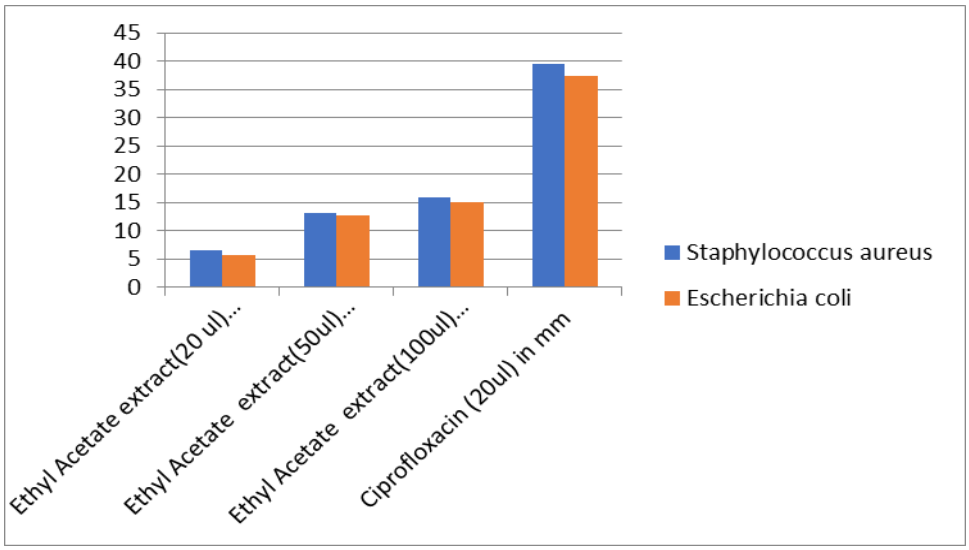
Graph 2: Antibiogram analysis of *Citrus letipes* using ethanol extract

Table No 2 Graph 2 show antibacterial activity of *Citrus letipes* peels using ethanol extract effective against *Staphylococcus aureus and E coli.*

Ethanolic extract exhibited a 17 mm maximal inhibition against *Staphylococcus aureus* and a 16.3 mm maximal inhibitory response to *E. coli* at 100 µL concentration.

Table No 3: Antibacterial activity using Ethyl acetate extract

Pathogens	Ethyl Acetate Extract (20µL) ZOI in mm	Ethyl Acetate Extract (50µL) ZOI in mm	Ethyl Acetate Extract (100µL) ZOI in mm	Ciprofloxacin (20µL) in mm
Staphylococcus aureus	6.4	13.1	15.9	39.6
Escherichia coli	5.7	12.6	15	37.3



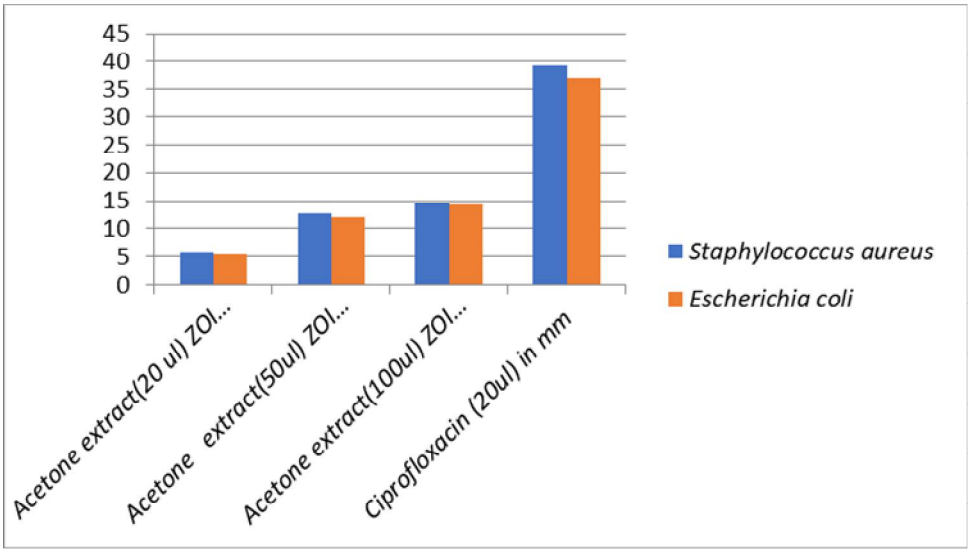
Graph 3: Antibiogram analysis of *Citrus letipes* using ethyl acetate extract

Table No 3 Graph 3 show antibacterial activity of *Citrus letipes* peels using ethyl acetate extract effective against *Staphylococcus aureus and E coli.*

Ethyl acetate extract exhibited a 15.9 mm maximal inhibition against *Staphylococcus aureus* and a 15 mm maximal inhibitory response to *E coli* at 100 µL concentration.

Table No 4: Antibacterial activity using acetone extract

Pathogens	Acetone extract (20µL) ZOI in mm	Acetone extract (50µL) ZOI in mm	Acetone extract (100µL) ZOI in mm	Ciprofloxacin (20µL) in mm
Staphylococcus aureus	5.8	12.7	14.5	39.3
Escherichia coli	5.3	12.1	14.3	37.1



Graph 4: Antibiogram analysis of *Citrus letipes* using acetone extract

Table No 4 Graph 4 show antibacterial activity of *Citrus letipes* peels using acetone extract effective against *Staphylococcus aureus and E coli.*

Acetone extract exhibited a 14.5 mm maximal inhibition against *Staphylococcus aureus* and a 14.3 mm maximal inhibitory response to *E coli* at 100 µL concentration.

Antibacterial activity of *Citrus letipes* using different solvent Methanol extract show highest Zone of inhibition followed by Ethanol extract, Ethyl acetate and acetone compare to the traditional antibiotic Ciprofloxacin.

DISCUSSION

The plant kingdom continues to be a useful and accessible resource for primary healthcare and alternative healthcare systems. Plant species contains various compounds which have high medical value as well as these plants may be a useful as an antibacterial agent because of their potential antibacterial chemicals.

In this study, plant extract was prepared from dried materials and agar well diffusion method was employed to measure the antibacterial activity of plant extracts against the certain bacteria. The minimum inhibitory concentration (MIC) of the extract was also determined using the broth dilution method, which had previously been tested in earlier study [9]. Methanolic, Ethanolic, ethyl acetate and acetone extract exhibit 20.1, 17, 15.9, 14.5 mm zone of inhibition against *Staphylococcus aureus* whereas 19, 16.3, 15, 14.3 zone of inhibition seen against *E coli* at 100 µL concentrations. This has been close resemblance to the different in findings may be due to presence of flavonoids, alkaloids and other phytochemical different composition. *Citrus letipes* and its methanolic extract were found to have the strongest antibacterial effects against *Staphylococcus aureus* and *E coli*, followed by Ethanol, ethyl acetate and acetone extract.

CONCLUSION

The antibacterial susceptibility assay results provided encouraging proof of the Citrus fruit's ability to fight against microorganism. Thus, MICs assays can confirm that the chemical has antibacterial properties and provides dependable indicator of the medication concentration needed to restrict the development of

microorganisms. These traditional species can be exploited as a possible source of medications against many diseases since phytochemical analysis identifies the components that are responsible for a plant's antibacterial activity. These findings may useful for research and the creation of innovative antimicrobial therapies by highlighting the medicinal potential of *Citrus letipes* as a natural source of antibacterial compounds.

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