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Evaluation of Antimicrobial Activity of Citrus Leaves against Gram Positive and Gram Negative Bacterial Strains

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Abstract

In present study four bacterial strains, including *Escherichia coli* (Gram negative), *Pseudomonas aeruginosa* (Gram negative), *Staphylococcus aureus* (Gram positive), and *Bacillus subtilis,* were tested for antimicrobial activity of citrus leaf extracts in water, benzene, and methanol (Gram positive). According to the findings, *Staphylococcus aureus* (Gram positive) exhibited a 19 mm zone of inhibition in methanol extracts, followed by Bacillus subtilis (Gram positive) (16 mm). *Staphylococcus aureus* (Gram positive) showed zone of inhibition in benzene extracts was 17 mm, followed by *Bacillus subtilis* (Gram positive) (16 mm), and a similar pattern was seen in water extracts. Against *Pseudomonas aeruginosa* (a Gram negative bacteria), the zone of inhibition for gram negative bacteria was 12 mm in methanol extract, and a similar pattern was seen in benzene and water extracts.

Keywords: Antimicrobial activity, agar well diffusion assay, Citrus, concentration, phytochemicals, Staphylococcus aureus, methanol extract and minimum inhibitory concentration.

Introduction

In the flowering plant family Rutaceae, the Citrus is a species of small evergreen trees that is indigenous to Asia, particularly Northeast India (Assam), Northern Myanmar, or China. Lemons are a significant group of citrus. It is primarily renowned for its pulp and juice worldwide. Over the world, many citrus fruits are consumed as food or juice. Lemon can be utilised for a variety of health conditions, including those relating to the skin, weight reduction, digestion, constipation alleviation, eye care, scurvy, piles, peptic ulcer, respiratory disorders, gout, gums, and urinary disorders. It has anti-hyperglycemic action (Sen et al., 2011; Osfor et al., 2013; Oyedepot and Babarinde, 2013; Abdul et al., 2014; Kharjul et al., 2014), anticancer activity (Sen et al., 2012; Patil et al., 2009; Jomaa et al., 2012; Shanmugam et al., 2013), antiulcer activity (Rozza et al., 2011; Bhavitavya et al., 2012), anti-inflammatory and analgesic action (Sood et al., 2009; Negi and Anand, 2019) and antioxidant Activities (Chowdhury et al., 2009; Uddin et al., 2014). Lemon extract also has antimicrobial action (Pradeep et al., 2007; Dhiman et al., 2012; Guerra et al., 2013; Nada and Zainab, 2013; Shinka and Ndanusa, 2013; Madhuri et al., 2014; Yekeen et al., 2014; Akhtar et al., 2014; Mansour and Allem, 2016). The present investigation was performed to

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pp: 132 - 137

look the antimicrobial property of Citrus leaf extracts against two gram-negative bacteria, two gram-positive bacteria.

Materials and Methods

Plant Material: Leaves of Citrus plant were collected from local area and were identified by Head, Department of Botany, Government (P.G.) College, Bisalpur, Pilibhit.

Extraction: Using a Soxhlet apparatus, 10 g of finely ground, shade-dried Citrus leaves powder were extracted with 100 ml each of water, benzene, and methanol at a 1:10 ratio. The solvent extraction period was set at 4 hours daily for a total of 36 hours. The produced extract was taken out of the solvent chamber, and any extra solvent was rotary evaporated. The resultant residue was stored in a refrigerator for use in further studies.

Bacterial culture:

1. *Escherichia coli* (Gram negative),

2. *Pseudomonas aeruginosa* (Gram negative),

3. *Staphylococcus aureus* (Gram positive),

4. *Bacillus subtilis* (Gram positive)

Agar well diffusion method: To determine antibacterial activity, the Perez et al., (1990) published agar well diffusion assay was performed. 0.1 ml of diluted inoculums (2 x 108 CFU/ml) of the test organism were put to Muller-Hinton agar plates. Drilled wells in the agar with a 6 mm diameter were filled with 10 l of solvent blank (DMSO) and plant extract at a 10 mg/ml concentration. The plates were incubated at 37 °C for the entire night. The antibiotic amoxicillin was used as a positive control. The millimeter-sized zone of inhibition of test organism growth was determined for each well. Each test was carried out three times.

Estimation of minimum inhibitory concentration: A stock solution of extract (20 mg/ml) was made by dissolving 100 mg of each dry extract in 5 ml of extracts. From this stock, 2-fold serial dilutions of 10, 5, 2.5, 1.25, and 0.625 mg/ml were made. These concentrations were used to determine the stocks and the minimal inhibitory concentration. The broth dilution method was employed to determine the Minimal Inhibitory Concentration (MIC). Briefly, 6 test tubes were filled with 2 ml of nutritional broth and 0. 1 ml of the prepared concentration of each extract. The test tube containing the extract and the suspension of nutritional broth was then given 0.1 ml of standard inoculum. Following a secure corking, each test tube was incubated for 24 hours at 37 °C. After that, they were examined to determine whether any growth was apparent or not. The lowest concentration at which there was no appreciable microbiological growth was called the MIC (Esimone et al., 2012).

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pp: 132 - 137

Results and Discussion

Antimicrobial activity of Citrus leaf extracts in water, benzene and methanol was evaluated against four microorganisms such as *Escherichia coli* (Gram negative), *Pseudomonas aeruginosa* (Gram negative), *Staphylococcus aureus* (Gram positive), *Bacillus subtilis* (Gram positive). The results shown in Table-1 indicates that zone of inhibition were larger against gram positive bacterial strains than gram negative bacterial strains. *Staphylococcus aureus* (Gram positive) showed 19 mm zone of inhibition followed by *Bacillus subtilis* (Gram positive)(16 mm) in methanol extracts. In benzene extracts zone of inhibition were of 17 mm against *Staphylococcus aureus* (Gram positive) followed by *Bacillus subtilis* (Gram positive) (16 mm) and similar pattern was in water extracts. In case of gram negative bacteria zone of inhibition was 12 mm against *Pseudomonas aeruginosa* (Gram negative) in methanol extract and similar pattern was observed in benzene and water extracts. The Minimum Inhibitory Concentration (MIC) value against *Escherichia coli* (Gram negative), *Pseudomonas aeruginosa* (Gram negative), *Staphylococcus aureus* (Gram positive), *Bacillus subtilis* (Gram positive), was also determined using methanolic extracts of all the samples. The test was conducted in accordance with the prescribed protocol, and the outcomes are displayed in Table 2. The findings showed that S. aureus had the lowest and most significant MIC value (1.02±0.03), whereas amoxicillin had a MIC of 0.23±0.03mg/ml. Citrus leaves are rich in phytochemicals which provide it antimicrobial and other medicinal properties. Citrus lemon leaves oil was very strong essential oil as an anti-bacterial activity against Gram-negative (*E. coli and P. aeruginosa*) and Gram- positive (*B. cereus and S. aureus*). *C. sinensis* essential oil inhibited the *Aspergillus niger* growth; in addition, the lemon essential oil antibacterial activity was reported against various bacteria Gram-negative and Gram-positive (Baratta et al. 1998).

Conclusion: The oil extracted from *Citrus* leaves appears to be a good source of antibacterial agent. The oil from *Citrus* leaves has the potential to be used as a natural food preservative that can prevent the growth of many bacterial strains.

Table 1: Antibacterial activity of different leaf extracts of *Citrus*

		Concentration of Plant Extracts										
		Water			**Benzene**			**Methanol**			**DMSO**	**ANTIBIOTIC (Amoxicillin)**
		Zone of Inhibition (in mm)										
S. No.	**Bacteria**	**25**	**50**	**100**	**25**	**50**	**100**	**25**	**50**	**100**		
T1	*Escherichia coli* (Gram negative) .	-	6	09	-	8	11	-	8	10	0	18

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pp: 132 - 137

T2	*Pseudomonas aeruginosa* (Gram negative)	-	8	10	-	8	11	-	09	12	0	18
T3	*Staphylococcus aureus* (Gram positive)	-	10	12	7	11	17	10	15	19	0	24
T4	*Bacillus subtilis* (Gram positive)	-	9	14	7	10	16	9	14	16	0	24

Table 2: Determination of MIC value of different leaf extracts of *Citrus*

S. No.	Bacterial Strains	MIC value (mg/ml) of Methanol Extracts	Antibiotic (Amoxicillin)
T1	*Escherichia coli*	2.3±0.05	1.43±0.04
T2	*Pseudomonas aeruginosa*	3.06±0.1	0.22±0.02
T3	*Staphylococcus aureus*	1.02±0.03	0.23±0.03
T4	*Bacillus subtilis*	1.10±0.03	1.02±0.02

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