

Research Article

Antibacterial activity of *Momordica charantia* L. and *Citrus limon* L. on gram positive and gram negative bacteria

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Abstract

The uses of herbal medicines are well recognized since the advent of mankind having very little side effects. Their use is a cheaper and quite affordable source of antimicrobial particularly in the rural areas. Some of the microbial strains are drug resistant and are always been a threat to human life. The herbal therapy is the best choice of remedy against the drug-resistant strains, which had opened the new roads for conventional use due to several antimicrobial phytochemicals and essential oils. *Momordica charantia* Linnaeus (L). and *Citrus limon* L. contain phytochemicals, which affect the bacteria, fungi, viruses, and parasites. The aim of this study is to examine the effects of *Momordica charantia* and *Citrus limon* peel extracts on gram-positive and gram-negative bacteria. Preparation of extracts in ethanol, methanol, and ether were prepared and activation of test cultures, antimicrobial assay by disc diffusion, well diffusion and spectrophotometry at 600 nm. The greater zones of inhibition were determined by ethanolic extract of *Momordica charantia* on the growth of *Bacillus cereus* and *Pseudomonas aeruginosa* whereas *Citrus limon* methanolic peel extract revealed the greater effects on *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Escherichia coli* at 15 and 20 µl concentrations respectively. The observations concluded that both the plant extracts have effects on bacteria that indicate that the preparations of these extracts up to pharmaceutical standard may be effectively used as antibacterial therapy against gram-positive and gram-negative bacteria.

Keywords: *Citrus limon* peel; G+ve and G-ve bacteria; *Momordica charantia*; Soxhlet extraction

Introduction

Antibiotics are being used for the remedial purpose of the infections in the prescribed doses. As long as the use of antimicrobial substances got the great significance, there were the issues of the emergence of antimicrobial resistance [1, 2]. Since a long time, before Christ, men depend upon the use of natural products, which are known the important source of medicines. The long use

of herbal extracts from medicinal plants and their products played a significant role to eliminate the infectious diseases and also possessing antioxidant, anti-inflammatory and anti-cancer potentials [3-6]. The use of allopathic and herbal medicines among the older populations was more important in chronic disease. It explored the great significance for clinical applications of the effectiveness and care of herbal medicines

alone and also with the combination of traditional drugs. World Health Organization (WHO) reported the use of medicinal plants for treatment in the form of synthetic and semisynthetic drugs. WHO also reported that 80 % of the global population uses this form of medicines [7, 8].

Momordica charantia, (Bitter gourd) a globally used vegetable is commonly called as Karela. It is significant tonic and laxative for remedy of the disorders of the gastrointestinal tract, disorders of skin, nose and bones and liver. Its effects are also known against blood cancer, malarial infection, dysmenorrhea, renal stone and anal hemorrhoids [9]. Bitter gourd as the food was used by people of tropical areas. Besides, the use as vegetable, the Karela leaves and fruits are being used in the form of tea, soups and also beer [10]. Citrus is a significant global fruit tree crop, belongs to the Rutaceae family possessing the phenolic compounds, vitamins, minerals and also carotenoids and have antioxidant activity [11-15]. Citrus fruits possess flavonoids that are considered as a protective agent against heart diseases and cancer and Ischemic stroke. *Citrus limon L.* is significantly used in small quantities, helps give taste to salads, various drinks and desserts. The health benefits of lemon include the maintenance of hypertension, iron absorption rising immunity, providing nutritional value including calories (17), 1.45 g of sugar, Iron (0.5g), folate (9µg), protein (0.64 g), fat (0.17g) potassium (116 mg), carbohydrate (5.41g), calcium (15 mg), zinc (0.05), magnesium (7 mg), phosphorus (13 mg) a rich source of vitamin C, which is required for the synthesis of collagen [16].

Materials and Methods

Preparation of crude extract in water

Crude extract of bitter gourd and lemon peels was prepared by mixing ten grams of powdered extract in 40 ml of the warm distilled water. Each flask was allowed for 20 minutes time at 80°C in water bath. After

mixing the test extracts were allowed to cool for 24 h. for percolation. Later on, the extracts were passed through double layered muslin cloth and the filtrate was allowed for centrifugation for thirty minutes at 5000 rpm. These extracts were again dissolved in 100 ml of distilled water mixed well and filtered by 0.2 mm size Millipore filter [17].

Preparation of aqueous extracts in solvents

The *Momordica charantia L.* and *Citrus limon L.* were purchased from local market of Jamshoro. Both vegetables were washed with tap water, surface was sterilized by 70% ethanol and rinsed with sterile double distilled water [18] and later on the vegetables were dried with Whatman filter paper and kept for one hour. The vegetables were peeled and allowed for 5-7 days in shadow to dry. The peeled skin of both vegetables were ground to powder and filtered to get fine powder and kept in freezer. The powdered samples (1 g) were added in 100 ml of ethanol, methanol and ether separately and extracted through the soxhlet apparatus for 24 h. at room temperature [19, 20]. Later on the extracts were allowed at 150 rpm agitation in rotary incubator for 24 h. for evaporation then filtered through Whatman No. 1 filter paper [20]. The concentrations were made by mixing respective solvents (100 ml) volume / volume in the filtered extracts.

Selection of bacterial isolates

Four different bacterial isolates; both Gram positive and Gram negative bacteria *Bacillus cereus*, *Staphylococcus aureus* (*S. aureus*), *Streptococcus pyogenes* (*Str. pyogenes*), *Bacillus cereus* (*B. cereus*), *Pseudomonas aeruginosa* (*Ps. aeruginosa*) and *Escherichia coli* (*E. coli*) were isolated and examined for the cultural characters, morphological characters and biochemical characters according to the methods of [18].

Antibacterial activity of test extracts

Disc diffusion method

The antibacterial activity was performed by the method of [21]. The broth culture of the test bacteria was maintained at 10^6 cells /ml using hemocytometer (Quijing-China) The bacterial cultures (100 μ L) were separately incubated at 37°C for 24 h to observe the colonies later on a small portion of colony was emulsified in nutrient broth and kept for 30 minutes to activate the test culture. Using sterile commercial swab the activated cultures were separately inoculated on Mueller Hinton agar plates (Oxoid). Filter paper discs of 6 mm size were prepared, sterilized and impregnated in crude extract and each aqueous extract for 5 minutes and later dried in air for 10 minutes and placed on the surface of the inoculated nutrient agar plates. Ampicillin disc of 10 μ g was used a positive control and 5% DMSO as negative control.

Agar well diffusion method

The broth culture of the test bacteria were inoculated by swab and three wells of 6 mm size were bores by sterile cork borer. A small volume of (1 μ l) of molten nutrient agar was poured in each bore of each agar plate. After solidification of nutrient agar in bored wells 0.3 ml of aqueous extract of ethanol, methanol and ether) of bitter gourd and lemon was poured allowed the plates for few minutes at room temperature and the plates were incubated for 24 h at 37°C. The diameters of antibacterial activities of water, ethanol, methanol and ether extracts were measured by zone of inhibition in mm [22].

Spectroscopic studies

Different volumes of aqueous extract of bitter gourd and lemon prepared in ethanol, methanol and ether were taken in 2, 10, 15, 20, 25 microliter (μ l) concentrations and mixed in 1 ml of the culture broth of each test culture in separate flasks. This suspension was mixed gently and absorption

was determined at 600 nm (A_{600}) by Hitachi U-1800 spectrophotometer.

Results

The effects of Ampicillin 10 μ g and Dimethylsulfoxide (DMSO) 5% were observed and positive and negative control respectively. The findings indicated varying zones of growth inhibition in positive control. Ampicillin showed greater effect on *Pseudomonas aeruginosa* strains (22 mm) followed by *Staphylococcus aureus*, *Bacillus cereus* whereas no zone observed by DMSO as negative control (Table 1). The test aqueous extract of *Momordica charantia* by disc diffusion method showed greater effect (3 mm) on *S. aureus* at 20 μ l, *B. cereus* 15 μ l and *Ps. aeruginosa* 5 μ l in water (Table 2), (16 mm) on *S. aureus* at 15 μ l, followed by (15.5 mm) *B. cereus* at 15 μ l and (14 mm) *Ps. aeruginosa* at 20 μ l in ethanol (Table 3) whereas (11 mm) on *B. cereus* at 20 μ l followed by (9 mm) *Str. pyogenes* at 20 μ l, (8 mm) *S. aureus*, *Ps. aeruginosa* and *E.coli* at 15, 20, 20 μ l in methanol respectively (Table 4) and (8 mm) *Ps. aeruginosa* at 25 μ l followed by (7 mm) *Str. pyogenes* at 20, *S. aureus* at 25 and (4 mm) *E. coli* at 20 μ l in ether respectively (Table 5).

The test aqueous extract of *Momordica charantia* by agar well diffusion method revealed greater effect (3 mm) on *S. aureus* and *B. cereus* at 20 μ l and 25 μ l followed by (2.6 mm) *E. coli*, (1.8 mm) *Ps. aeruginosa* (1.7 mm) *Str. pyogenes* at 15, 20, 20 μ l in water respectively (Table 6), (16. mm) *S. aureus*, (16 mm) *B. cereus*, (13.5 mm) *Str. pyogenes*, (12.5 mm) *Ps. aeruginosa* and (10.4 mm) *E. coli* at 15, 15, 15, 20 and 25 μ l respectively in ethanol (Table 7) whereas (10, 8, 10, 8.2 9.2 mm) zone on *S. aureus*, *Str. pyogenes*, *B. cereus*, *Ps. aeruginosa* and *E.coli* at 20 μ l respectively in methanol (Table 8) and (9.4 mm) *E. coli*, (9 mm) *Ps. aeruginosa*, (8.2 mm) *Str. pyogenes*, (7 mm)

B. cereus and (6.2 mm) *S. aureus* at 25, 25, 20, 20 and 25 μ l respectively (Table 9).

On the other hand the aqueous extract of *Citrus limon* by disc diffusion method revealed (5, 5, 5 mm) *S. aureus*, *Str. pyogenes*, *Ps. aeruginosa* at 10, 20, 20 μ l and (4, 3 mm) zone on *B. cereus* and *E. coli* at 20, 20 μ l respectively in water (Table 10).

The greater zone on *Citrus limon* in ethanol extract was observed on *S. aureus*, *Str. pyogenes*, *Ps. aeruginosa*, *B. cereus* and *E. coli* (16, 16, 15.8, 15 and 11 mm) zone at 20, 25, 25, 25, 25 μ l respectively (Table 11) and in methanol the zone of growth inhibition of *Str. pyogenes*, *S. aureus*, *E. coli*, *B. cereus* and *Ps. aeruginosa* was observed 16, 15.5, 15, 14 and 12 mm at 20, 20, 20, 25 and 25 μ l respectively (Table 12) whereas test extract in ether showed 8.5, 7.6, 7.5, 7.5 and 7 mm zone of inhibition of *Ps. aeruginosa*, *Str. pyogenes*, *B. cereus*, *E. coli* and *S. aureus* at 25, 25, 20, 20 and 15 μ l concentration respectively (Table 13). The agar well diffusion method revealed greater

zone of inhibition of *Str. pyogenes*, *S. aureus*, *Ps. aeruginosa*, *E. coli* and *B. cereus* 6.2, 6, 5.7, 4.5 and 4 mm at 25, 20, 25, 20 and 15 μ l in water respectively (Table 14). The observation lemon extract in ethanol showed 14.2, 12.5, 15, 15 and 14.2 mm zones on the growth of *Ps. aeruginosa*, *E. coli*, *S. aureus*, *B. cereus* and *Str. pyogenes* at 20 μ l respectively (Table 15).

Citrus limon showed greater sensitivity zone 17.8, 17.5, 16.5, 11.4 and 10 mm zone on *S. aureus*, *E. coli*, *Str. pyogenes*, *Ps. aeruginosa* and *B. cereus* at 20, 25, 20, 20 and 20 μ l respectively (Table 16) whereas in ether 8, 8, 7.5, 7, and 5 mm zone on the growth of *B. cereus*, *E. coli*, *Ps. aeruginosa*, *Str. pyogenes* and *S. aureus* at 25 μ l concentration respectively (Table 17). Comparatively, the spectroscopic studies revealed the greater absorbance of *Momordica charantia* and *Citrus limon* at 15 and 20 μ l concentration respectively at A_{600} (Fig. 1 & 2) on the test bacteria.

Table 1. Determination of negative (5% DMSO) and positive control (Ampicillin 10 μ g) on test bacteria

Bacteria	Positive control	Negative control
	Ampicillin (10 μ l) zone size (mm)	DMSO (5%)
<i>Staphylococcus aureus</i>	20	00
<i>Streptococcus pyogenes</i>	18	00
<i>Bacillus cereus</i>	20	00
<i>Pseudomonas aeruginosa</i>	22	00
<i>Escherichia coli</i>	16	00

Table 2. Determination of antibacterial activity of *Momordica charantia* in water by disc diffusion method

Test bacteria	Zone size (mm)				
	Concentration of extracts (μ l)				
	5	10	15	20	25
<i>Staphylococcus aureus</i>	00	00	00	03	03 ^{±1}
<i>Streptococcus pyogenes</i>	00	00	02	02	02
<i>Bacillus cereus</i>	02	02	03	03	03
<i>Pseudomonas aeruginosa</i>	03	02 ^{±1}	03	03	03
<i>Escherichia coli</i>	00	00	00	00	02 ^{±1}

Table 3. Determination of antibacterial activity of *Momordica charantia* in ethanol by disc diffusion method

Test bacteria	Zone size (mm)				
	Concentration of extracts (µl)				
	5	10	15	20	25
<i>Staphylococcus aureus</i>	06	14	16	16 ^{±1}	16
<i>Streptococcus pyogenes</i>	05	08	13	14	14.5
<i>Bacillus cereus</i>	08	12	15.5	15.5	15.5
<i>Pseudomonas aeruginosa</i>	06	08	12	14	14
<i>Escherichia coli</i>	04	04 ^{±1}	07	09	09

Table 4. Determination of antibacterial activity of *Momordica charantia* in methanol by disc diffusion method

Test bacteria	Zone size (mm)				
	Concentration of extracts (µl)				
	5	10	15	20	25
<i>Staphylococcus aureus</i>	03	05	08	08	08
<i>Streptococcus pyogenes</i>	02	04	07	09	09
<i>Bacillus cereus</i>	04	05	09	11	11
<i>Pseudomonas aeruginosa</i>	02	04	07	08	08 ^{±1}
<i>Escherichia coli</i>	04	06	07	08	09

Table 5. Determination of antibacterial activity of *Momordica charantia* in ether by disc diffusion method

Test bacteria	Zone size (mm)				
	Concentration of extracts (µl)				
	5	10	15	20	25
<i>Staphylococcus aureus</i>	00	03	04	05	07
<i>Streptococcus pyogenes</i>	03	04	05	07	07
<i>Bacillus cereus</i>	02	04	04	05	06
<i>Pseudomonas aeruginosa</i>	04 ^{±1}	05	06	07	08
<i>Escherichia coli</i>	02	02	3.5	04	04

Table 6. Determination of antibacterial activity of *Momordica charantia* in water by agar well diffusion method

Test bacteria	Zone size (mm)				
	Concentration of extracts (µl)				
	5	10	15	20	25
<i>Staphylococcus aureus</i>	00	00	02	02	03 ^{±1}
<i>Streptococcus pyogenes</i>	00	00	00	1.7	1.6 ^{±1}
<i>Bacillus cereus</i>	00	00	02	03	03
<i>Pseudomonas aeruginosa</i>	00	01	01	1.8	02
<i>Escherichia coli</i>	00	02	2.6	2.6	2.5 ^{±1}

Table 7. Determination of antibacterial activity of *Momordica charantia* in ethanol by agar well diffusion method

Test bacteria	Zone size (mm)				
	Concentration of extracts (µl)				
	5	10	15	20	25
<i>Staphylococcus aureus</i>	08	11	16.5	16.5	16.3
<i>Streptococcus pyogenes</i>	07	10	13.5	13.2	13.2
<i>Bacillus cereus</i>	09	11	16	16	16
<i>Pseudomonas aeruginosa</i>	05	07	09	12.5	12.5
<i>Escherichia coli</i>	04	04 ^{±1}	07	10	10.4

Table 8. Determination of antibacterial activity of *Momordica charantia* in methanol by agar well diffusion method

Test bacteria	Zone size (mm)				
	Concentration of extracts (µl)				
	5	10	15	20	25
<i>Staphylococcus aureus</i>	07	8.2	09	10	10
<i>Streptococcus pyogenes</i>	04	5.5	07	08	08
<i>Bacillus cereus</i>	6.5	7.2	09	10	10
<i>Pseudomonas aeruginosa</i>	05	5.8	07	8.2	8.2
<i>Escherichia coli</i>	05	6.4	7.3	9.2 ^{±1}	9.2

Table 9. Determination of antibacterial activity of *Momordica charantia* in ether by agar well diffusion method

Test bacteria	Zone size (mm)				
	Concentration of extracts (µl)				
	5	10	15	20	25
<i>Staphylococcus aureus</i>	00	02	05	5.8	6.2
<i>Streptococcus pyogenes</i>	04	05	07	8.2	8.2
<i>Bacillus cereus</i>	3.7	4.5	06	07	07
<i>Pseudomonas aeruginosa</i>	04	4.8	06	07	09
<i>Escherichia coli</i>	05	6.8	7.5	8.5	9.4

Table 10. Determination of antibacterial activity of *Citrus limon* in water by disc diffusion method

Test bacteria	Zone size (mm)				
	Concentration of extracts (µl)				
	5	10	15	20	25
<i>Staphylococcus aureus</i>	03	05	05	05	05
<i>Streptococcus pyogenes</i>	02	3.5	04	05	04 ^{±1}
<i>Bacillus cereus</i>	03	3.5	3.5	04	04
<i>Pseudomonas aeruginosa</i>	01	02	03	05	05
<i>Escherichia coli</i>	02	1.5	02	03	03

Table 11. Determination of antibacterial activity of *Citrus limon* in ethanol by disc diffusion method

Test bacteria	Zone size (mm)				
	Concentration of extracts (µl)				
	5	10	15	20	25
<i>Staphylococcus aureus</i>	06	11	12	16	16
<i>Streptococcus pyogenes</i>	07	10	12	14	16
<i>Bacillus cereus</i>	06	11	12	13.5	15
<i>Pseudomonas aeruginosa</i>	04	06	13.5	15	15.8
<i>Escherichia coli</i>	05	07	08	9.5	11.2

Table 12. Determination of antibacterial activity of *Citrus limon* in methanol by disc diffusion method

Test bacteria	Zone size (mm)				
	Concentration of extracts (µl)				
	5	10	15	20	25
<i>Staphylococcus aureus</i>	09	11.4	13.5	15.5	15.5
<i>Streptococcus pyogenes</i>	08	12.5	14.3	16	16
<i>Bacillus cereus</i>	5.5	07	8.6	11	14
<i>Pseudomonas aeruginosa</i>	05	06	08	9.5	12
<i>Escherichia coli</i>	08	10	12	15	15

Table 13. Determination of antibacterial activity of *Citrus limon* in ether by disc diffusion method

Test bacteria	Zone size (mm)				
	Concentration of extracts (µl)				
	5	10	15	20	25
<i>Staphylococcus aureus</i>	05	05	07	07	07
<i>Streptococcus pyogenes</i>	5.8	6.5	07	6.7	7.6
<i>Bacillus cereus</i>	04	06	6.8	7.5	7.5
<i>Pseudomonas aeruginosa</i>	05	5.8	07	08	8.5
<i>Escherichia coli</i>	03	05	6.8	7.5	7.5

Table 14. Determination of antibacterial activity of *Citrus limon* in water by agar well diffusion method

Test bacteria	Zone size (mm)				
	Concentration of extracts (µl)				
	5	10	15	20	25
<i>Staphylococcus aureus</i>	2.5	4.8	5.2	06	06
<i>Streptococcus pyogenes</i>	03	3.5	4.3	5.5	6.2
<i>Bacillus cereus</i>	03	3.5	04	04	04
<i>Pseudomonas aeruginosa</i>	02	03	3.8	05	5.7
<i>Escherichia coli</i>	02	02	3.4	4.5	4.5

Table 15. Determination of antibacterial activity of *Citrus limon* in ethanol by agar well diffusion method

Test bacteria	Zone size (mm)				
	Concentration of extracts (μ l)				
	5	10	15	20	25
<i>Staphylococcus aureus</i>	06	10.8	12	14.2	14.2
<i>Streptococcus pyogenes</i>	05	7.5	11	12.5	12.5
<i>Bacillus cereus</i>	5.4	08	12.5	15	15
<i>Pseudomonas aeruginosa</i>	6.5	08	13.7	15	15
<i>Escherichia coli</i>	06	09	13	14.2	14.3 ^{±1}

Table 16. Determination of antibacterial activity of *Citrus limon* in methanol by agar well diffusion method

Test bacteria	Zone size (mm)				
	Concentration of extracts (μ l)				
	5	10	15	20	25
<i>Staphylococcus aureus</i>	05	8.7	13	17.8	17.8
<i>Streptococcus pyogenes</i>	7.5	12	14	16.5	16.5
<i>Bacillus cereus</i>	5.5	07	08	10	10
<i>Pseudomonas aeruginosa</i>	06	7.5	8.5	11.4	11.4
<i>Escherichia coli</i>	08	10	13	17.4 ^{±1}	17.5

Table 17. Determination of antibacterial activity of *Citrus limon* in ether by agar well diffusion method

Test bacteria	Zone size (mm)				
	Concentration of extracts (μ l)				
	5	10	15	20	25
<i>Staphylococcus aureus</i>	02	03	03	3.5	05
<i>Streptococcus pyogenes</i>	04	05	5.5	07	07
<i>Bacillus cereus</i>	4.5	05	6.4	7.5	08
<i>Pseudomonas aeruginosa</i>	03	3.7	05	7.5	7.5
<i>Escherichia coli</i>	04	05	6.5	7.2	08

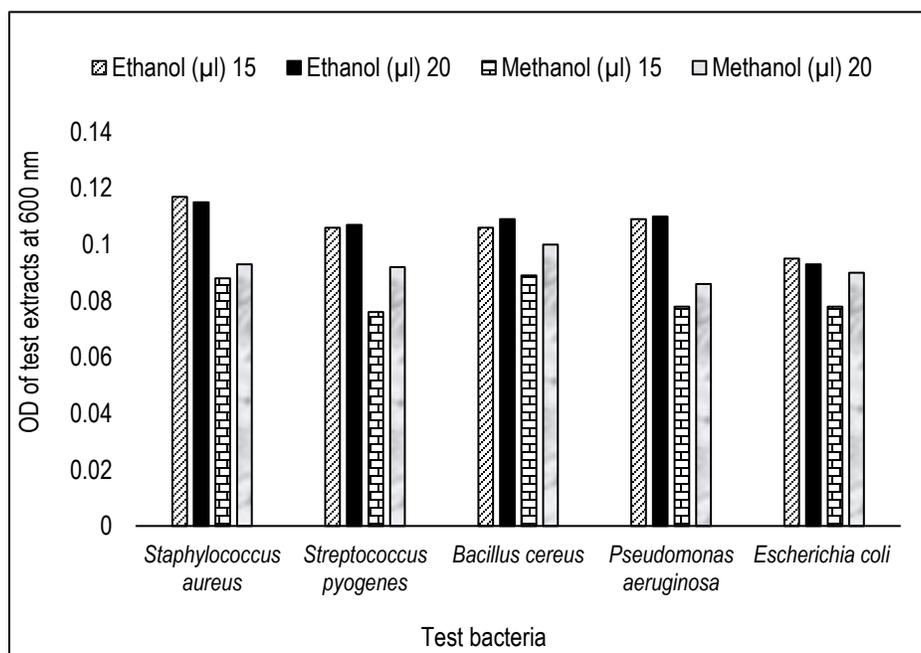


Figure 1. Spectroscopic studies of *Momordica charantia* extract in ethanol and methanol at 15 and 20 µl concentration at 600 nm

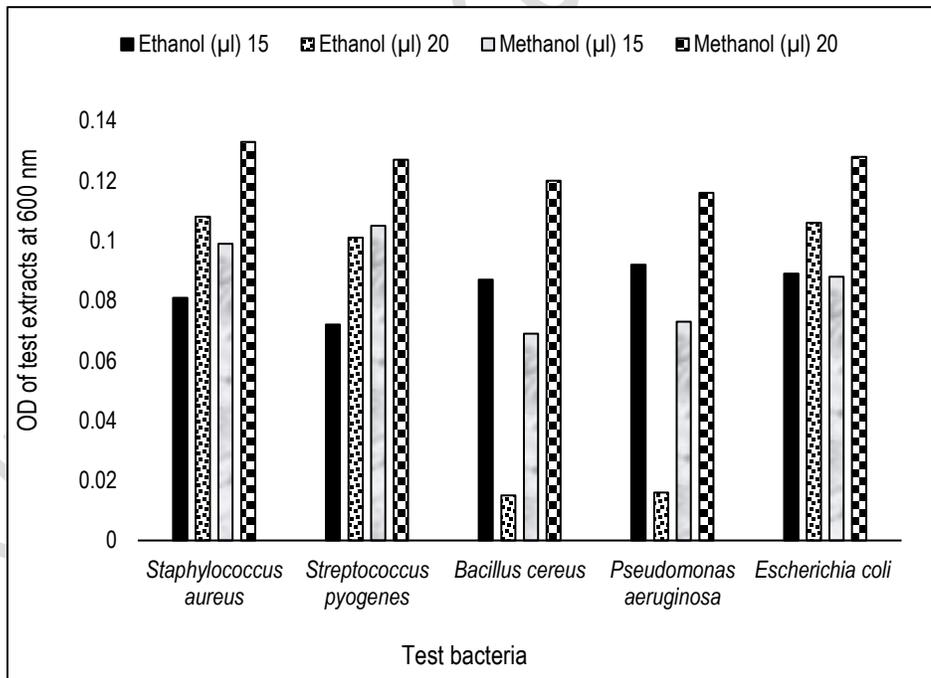


Figure 2. Spectroscopic studies of *Citrus limon* extract in ethanol and methanol at 15 and 20 µl concentration at 600 nm.

Discussion

All test strains were examined for their sensitivity against the positive control (Ampicillin) and negative control (5% DMSO) that revealed sensitivity of ally test strains to ampicillin antibiotic with varying zone sizes whereas DMSO showed no effect on all test strains (Table 1). Bitter gourd peels when dissolved in water revealed insignificant effect on test strains not more than 4-5 mm zone of inhibition by disc diffusion method and agar well diffusion methods (Table 2, 6) whereas in extract in ethanol showed antibacterial effects on test bacteria at varying concentrations. The greater effects were observed on *S. aureus* and *B. cereus* at 15µl concentration by disc diffusion and agar well diffusion methods (Table 3, 7).

The lemon peel extract in methanol showed the significant effects on a test bacteria. The greater effect of lemon extract in methanol was observed on *Staphylococcus aureus*, *E.coli*, *Streptococcus pneumoniae* and *Pseudomonas aeruginosa* respectively by agar well diffusion method. The agar well diffusion method showed the greater zones as compared to the disc diffusion method due to the percolation of the aqueous extracts more rapidly in the agar medium. The spectroscopy of two concentration (15, 20 µl) of extracts in ethanol and methanol of *Momordica charantia* and *Citrus limon* was done to compare the greater optical density of test bacteria at A_{600} .

The aqueous extracts of bitter gourd in water, methanolic and ether revealed the lesser effects on the test bacteria compared to the ethanolic extracts, which has considerable bacteriostatic or bactericidal may be due to higher concentration of dissolved antimicrobial compounds and their polarity including the momordin, alpha- and beta-momorcharin, cucurbitac in B1 and oleanolic acid. On the other side, the limonene as major constituent in lemon also

inhibit bacterial growth. The reason of variable antibacterial effects of ethanolic and methanolic extracts on bacteria are due to their cell wall composition including the peptidoglycan and phospholipid layer in Gram positive and Gram negative bacteria respectively. The antibacterial effect may be due to lipid bilayer, where the extract penetrates and disintegrates the membranous structure of bacteria [23-27]. Our findings at A_{600} essentially confers the effects of *Momordica charantia* and *Citrus limon* at 15, 20 µl concentrations on test bacteria.

Conclusion

It is concluded that the test extracts prepared in ethanol and methanol are more effective than the extracts in water and ether on the test bacteria. Bitter gourd extract showed higher antibacterial activity against Gram negative bacteria *Staphylococcus aureus* and *Bacillus cereus* at 15µl concentration. The lemon peel extract in methanol showed the greater effects on *Staphylococcus aureus*, *E.coli*, *Streptococcus pneumoniae* and *Pseudomonas aeruginosa* respectively 20 µl concentration. It is also concluded that the agar well diffusion method is more reliable method than the disc diffusion method. The spectroscopic studies confers the effects of *Momordica charantia* and *Citrus limon* at 15, 20 µl respectively.

Authors' contributions

Conceived and designed the experiments: AA Noor & Z Ahmed, Performed the experiments: Z Ahmed, Analyzed the data: AA Noor, Contributed materials/ analysis/ tools: Z Ahmed & AA Noor, Microbiology Research Laboratory, Institute of Microbiology, Wrote the paper: Z Ahmed.

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