Antibacterial screening of *Abelmoschus esculentus* (L.) Moench against the human pathogenic bacteria by using different solvents

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Abstract
Abelmoschus esculentus (l.) Moench which is commonly known as ladies finger belongs to the malvaceae family and it is widely cultivated throughout the world for its fibrous fruits which contain various nutrients. The antibacterial activity of *Abelmoschus esculentus* by using different solvents petroleum ether, ethanol, acetone and aqueous extract were examined against the human pathogenic bacteria *Staphylococcus aureus, Klebsiella pneumoniae, E.coli* and *Bacillus subtilis*. Antibacterial activity was investigated by Disc diffusion method. The *Abelmoschus esculentus* fruit extract showed effective zone of inhibition against the four bacterial pathogens. Therefore the *Abelmoschus esculentus* can be considered to be the promising source of antibacterial compounds.

Key words: *Abelmoschus esculentus,* Antibacterial activity, Different solvents, Disc diffusion method, Zone of inhibition

INTRODUCTION

Vegetables play a vital role in the improvement of the diet in mankind. Ladies finger (*Abelmoschus esculentus* L Moench) is one of the most important edible and nutritious vegetable crop. It belongs to the family Malvaceae, originating from tropical and subtropical Africa. The nutritional constituents of ladies finger include carbohydrate, protein, phosphorus, calcium, magnesium, iron, vitamin A and C with traces of vitamin B (Dilruba *et al*., 2009).

Ladies finger is a fruit vegetable, grown mainly for the pods. The tender fruits are used as vegetables either boiled or sliced and fried. Ladies finger pod contains 8.20 % carbohydrates, 2.10 % protein and a significant amount of riboflavin (Benchasri 2012). Ripe seeds contain edible oil that could be as high as poultry eggs and soybean (Akinfasoye and Nwanguma 2005).

MATERIALS AND METHODS

The *Abelmoschus esculentus* fruits were collected from the home garden. The fruits were dried under shade condition and cut into small pieces, pulverized in a grinder and store in sterile container for further use.

Test Organisms:

The test microorganisms used for antimicrobial analysis Gram positive *Staphylococcus aureus* (MTCC 6571), *Bacillus subtilis* (MTCC 1133) gram negative *E.coli* (MTCC 15223), *Klebsiella pneumoniae* (MTCC 33495) were collected from Microbial Type Culture Collection and Gene Bank (MTCC) Chandigarh. The bacterial strains were maintained on Nutrient Agar (NA).

Nutrient Broth Preparation:

Pure culture from the plates were inoculated into Nutrient Agar plate and sub cultured at 37ºC for 24 hours. Inoculum was prepared by aseptically adding the fresh culture into 2 ml of sterile 0.145 mol/L saline tube and the cell density was adjusted to 0.5 McFarland turbidity standard to yield a bacterial suspension of 1.5×108 cfu/ml. Standardized inoculum was used for Antimicrobial test.

Antibacterial Test:

Antibacterial activity was carried out by using disc diffusion method (Bauer *et al*., 1996). The medium was prepared by dissolving 38 g of Mueller-Hinton Agar Medium (Hi Media) in 1000 ml of distilled water. The dissolved medium was autoclaved at 15 Lbs pressure at 121 ^{0} C for 15 minutes (pH 7.3).

The autoclaved medium was cooled, mixed well and poured into Petri plates (25ml/plate). The plates were swabbed with pathogenic bacterial culture *Escherichia coli, Klebsiella pneumoniae, Bacillus subtilis* and *Staphylococcus aureus*. The Sample loaded disc was then placed on the surface of Mueller-Hinton Agar medium. The standard drug streptomycin 30 mcg concentration disc was used for positive control and empty sterile disc was used for negative control. The plates were

kept for incubation at 37°C for 24hours. At the end of incubation, inhibition zones were examined around the disc and measured with transparent ruler in millimetres. The size of the zone of inhibition (including disc) was measured in millimeters. The absence of zone inhibition was interpreted as the absence of activity (Kohner *et al.*, 1994; Mathabe *et al.*, 2006). The experiment was repeated triplicates.

RESULTS AND DISCUSSION

The antibacterial activity of *Abelmoschus esculentus* using Petroleum ether, Acetone, Ethanol, and Aqueous extract against bacterial pathogens gram positive *Staphylococcus aureus*, *Bacillus subtilis* and gram negative *E.coli* and *Klebsiella pneumoniae* were studied.

The result obtained from antibacterial activity of *Abelmoschus esculentus* by using petroleum ether, acetone, ethanol, and aqueous extract were presented in Table-I, Plate-I and Figure-I.

Table - I. Antibacterial activity of *Abelmoschus esculentus* against bacterial pathogens using different solvent extracts.

No	Bacterial Pathogens	Zone Of Inhibition (mm)				
		Streptomycin	Petroleum ether	Acetone	Ethanol	Aqueous
1	*Staphylococcus aureus*	27.66±0.47	16±0.66	19±0.66	18.66±0.66	17.66±0.22
2	*Bacillus subtilis*	21±0.66	11.66±0.22	14.66±0.88	14.33±0.22	13.33±0.88
3	*Klebsiella pneumoniae*	22.33±0.22	7.66±0.47	16.66±0.22	15.66±0.22	15.33±0.22
4	*E.coli*	25.33±0.22	21.33±0.81	18.66±0.22	18.33±0.81	NZ

*** Each value is a mean of three data after subtracting the standard disc value 5mm *NZ- No zone, *mm- Millimetre**

Figure - I. Antibacterial activity of *Abelmoschus esculentus* against bacterial pathogens using different solvent extracts.

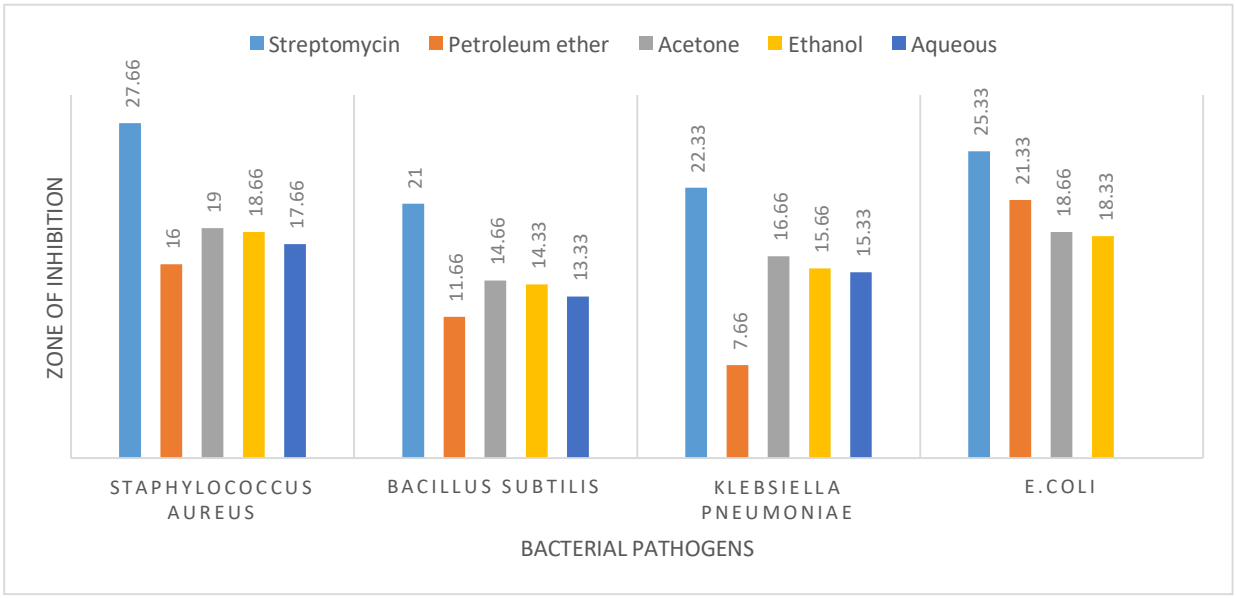
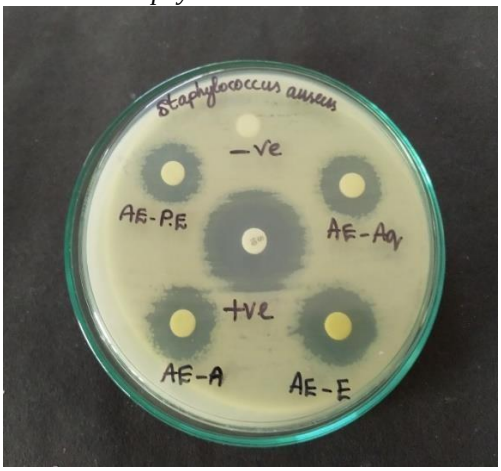


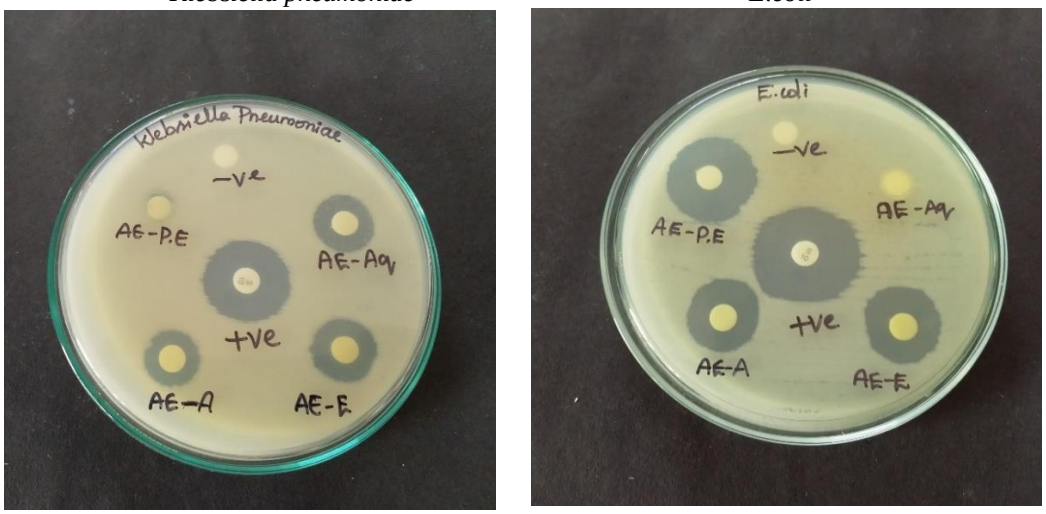
Plate - I. Antibacterial activity of *Abelmoschus esculentus* against bacterial pathogens using different solvent extracts.

Staphylococcus aureus



Bacillus subtilis

Klebsiella pneumoniae *E.coli*



*Ae - *Abelmoschus esculentus*, P.E - Petroleum ether, E- Ethanol, A- Acetone, Aq-Aqueous

The petroleum ether extract of *Abelmoschus esculentus* showed maximum activity against the pathogen *E.coli* (21.33±0.81)mm followed by *Staphylococcus aureus* (16±0.66)mm, *Bacillus subtilis* (11.66±0.22)mm and lowest zone of inhibition against the pathogen *Klebsiella pneumoniae* (7.66±0.47)mm. In acetone extract of *Abelmoschus esculentus* showed maximum against the pathogen *Staphylococcus aureus* (19±0.66)mm followed by *E.coli* (18.66±0.22)mm *Klebsiella pneumoniae* (16.66±0.22)mm and lowest zone of inhibition against the pathogen *Bacillus subtilis* (14.66±0.88)mm. In ethanol extract *Abelmoschus esculentus* showed highest zone of inhibition against the pathogen *Staphylococcus aureus* (18.66±0.66)mm followed by *E.coli* (18.33±0.81)mm, *Klebsiella pneumoniae* (15.66±0.22)mm and lowest zone of inhibition against the pathogen *Bacillus subtilis* (14.33±0.22)mm In aqueous extract *Abelmoschus esculentus* showed highest zone of inhibition in *Staphylococcus aureus* (17.66±0.22)mm followed by *Klebsiella pneumoniae* (15.33±0.22)mm and lowest zone of inhibition against the pathogen *Bacillus subtilis* (13.33±0.88)mm. There is no zone of inhibition in *E.coli*.

The *Abelmoschus esculentus* fruit extract had antibacterial activity against the gram positive bacterial pathogen *Staphylococcus aureus* (Sri lestari *et al.*, 2023). Pereira JA *et al.*, 2007 discussed that the ethanol extract of *Abelmoschus esculentus* against *E.coli* and *Klebsiella pneumonia*e was examined by disc diffusion method based on the presence and absence of zone of inhibition. The ethanol extract of *Abelmoschus esculentus* showed inhibitory properties against the bacterial pathogens *E. coli and Klebsiella pneumoniae.* Results of research have revealed that the *Abelmoschus esculentus* extract can inhibit the growth of the two microorganisms. The present study was correlated with the above findings.

CONCLUSION

The results obtained from this study considering the bacterial activity using assay of different solvent extracts from the vegetable *Abelmoschus esculentus*. It clearly revealed significant antibacterial activity against all the tested human pathogenic bacteria and it should be thoroughly being investigated for natural antibiotic properties.

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