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Antimicrobial activity of *Abelmoschus esculentus* (flowers)

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Abstract

A large number of medicinal plants are claimed to be useful in treating skin diseases in all traditional system of medicine. The present study was carried out to investigate the antimicrobial effect of the compound isolated from the ethyl acetate fraction of flowers of *Abelmoschus esculentus*. This compound was shown to possess antimicrobial activity against bacteria and fungi. Four bacterial strains *Salmonella typhi*, *Escherichia coli*, *Enterococcus faecalis*, *Bacillus cereus* and two fungal strains *Curvularia lunata* and *Candida albicans* were tested by using disc diffusion method. The anti bacterial activity of the compound isolated from ethyl acetate fraction is almost comparable with standard *Chloramphenicol*. The antifungal activity is almost comparable with standard *Fluconazole*.

Keywords: *Abelmoschus esculentus*; antibacterial activity; antifungal activity; diffusion method; chloramphenicol; fluconazole

1. Introduction

Plants are a potential source of antimicrobial compounds and several researchers throughout the world are investigating the antimicrobial activity of medicinal plants, which are utilized in the traditional or alternative healthcare systems [1, 2]. Many of the plant materials used in traditional medicine are readily available in rural areas at relatively cheaper than modern medicine [3]. The antimicrobial activities of medicinal plants can be attributed to the secondary metabolites such as alkaloids, flavonoids, tannins, terpenoids that are present in these plants [4].

1.1 Plant description

Abelmoschus esculentus (Okra) is belonging to the family Malvaceae, it is an important vegetable crop grown mainly in the tropical or sub-tropical regions during summer and rainy season [5, 6]. It is widely grown in Africa, Asia, Southern Europe and America [7]. Okra is a rich source of many nutrients. A half cup of okra contains calories 25, dietary fiber 2g, protein 1.5g, carbohydrates 5.8g, vitamin A 460 IU, vitamin C 13mg, folic acid 36.5µg, calcium 50mg, iron 0.4mg, potassium 25mg, magnesium 46mg. Okra is subjected to attack by many insects and pathogens including fungi, viruses, mycoplasmas and nematodes [8-12].

2. Materials and methods

2.1 Collection of Flowers

Fresh flowers of *Abelmoschus esculentus* were collected from S. Pudur, Sivagangai (Dt), Tamil Nadu, India, during the month of February and identified by Dr. S. John Britto, Director, The rapinat Herbarium and Centre for Molecular Systematics (Authentication No. SS004 dated: 03/06/2016). St. Joseph's College (Campus), Triuchirappalli, Tamil Nadu, India.

2.2 Extraction and fractionation

Fresh flowers (3 kg) of *Abelmoschus esculentus* were extracted with 90% ethanol (5x500ml). The combined alcoholic extract was concentrated in vacuo and the aqueous extract was successively fractionated with petroleum ether (60-80 °C) (6x250 ml), Peroxide free diethyl ether (4x250 ml) and ethyl acetate (8x250 ml). Petroleum ether fraction and diethyl ether fraction did not yield any isolable material. Ethyl acetate fraction on concentration yielded a dry powder which was dissolved in DMSO to get various concentrations and were used for further study.

2.3 Antimicrobial procedure

2.3.1 Screening of antibacterial activity

2.3.1.1 Bacteria tested

Four bacterial strains were used throughout this investigation. All the bacterial cultures were

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obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India. The young bacterial broth cultures were prepared before the screening procedure.

2.3.1.2 Preparation of inoculums

Stock cultures were maintained at 4 °C on slopes of nutrient agar. Active cultures of experiment were prepared by transferring a loop full of cells from the stock cultures to test tube of Muller-Hinton Broth (MHB) that were incubated without agitation for 24 hrs at 37 °C. The cultures were diluted with fresh Muller-Hinton Broth to achieve optical densities corresponding to 2.0x10⁶ colony forming units (CFU/ml).

2.3.1.3 Antibacterial susceptibility test

The disc diffusion method (Bauer *et al.*, 1966) was used to screen the antibacterial activity. In-vitro antibacterial activity was screened by using Muller Hinton Agar (MHA) obtained from Himedia (Mumbai). The MHA plates were prepared by pouring 15 ml of molten media into sterile petriplates. The plates were allowed to solidify for 5 minutes and 0.1% inoculum suspension was swabbed uniformly and the inoculums were allowed to dry for 5 minutes. The compound of concentration 20mg/ml, 30mg/ml, 40mg/ml, 50mg/ml were loaded on 6 mm sterile disc. The loaded disc were placed on the surface of medium and the compound was allowed to diffuse for 5 minutes and the plates were kept for incubation at 37 °C for 24 hrs. At the end of incubation, inhibition zones formed around the disc were measured with a transparent ruler in millimeter. Standard antibiotic chloramphenicol of concentration 1mg/ml was used as positive control [13].

Table 1: Antibacterial activity of the compound isolated from the ethyl acetate fraction of flowers of *Abelmoschus esculentus*

S. No	Organisms	Zone of inhibition (mm)				
		Standard (Chloramphenicol)	Sample Concentration (mg/ml)			
			20	30	40	50
1	<i>Salmonella typhi</i>	17	00	00	18	24
2	<i>Escherichia coli</i>	17	00	07	18	25
3	<i>Enterococcus faecalis</i>	19	00	00	17	24
4	<i>Bacillus cereus</i>	18	00	09	17	26

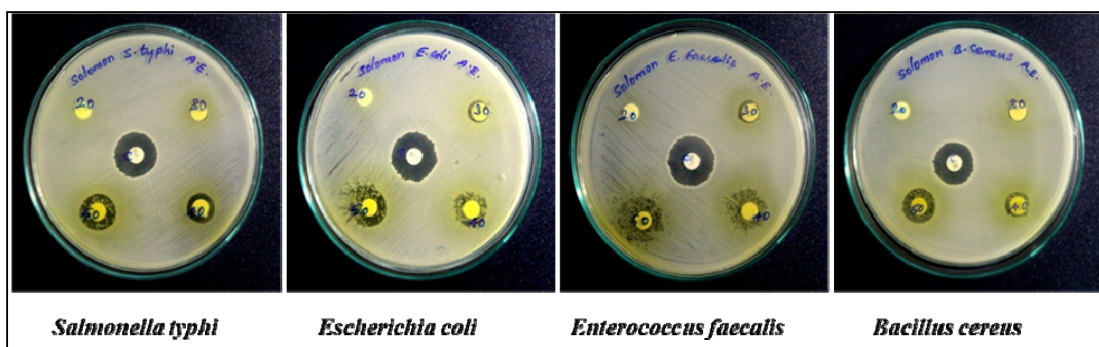
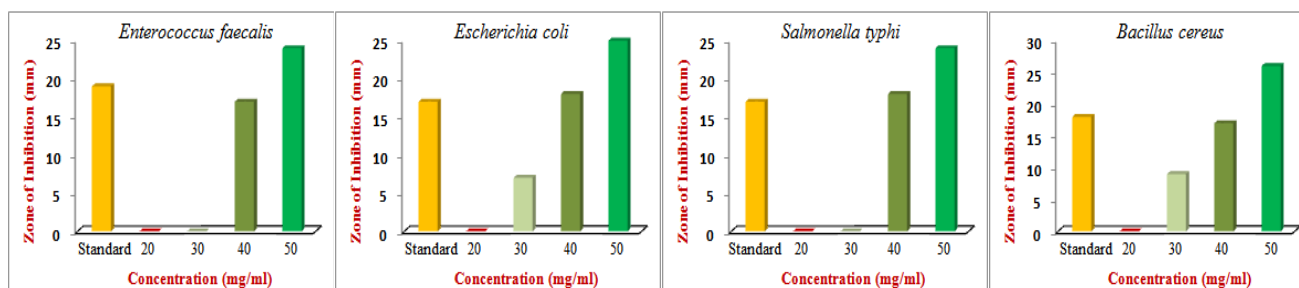


Fig 1: Antibacterial activity of the compound isolated from the ethyl acetate fraction of flowers of *Abelmoschus esculentus*



Graph No 1: Graphical representation of anti-bacterial activity of the compound isolated from the ethyl acetate fraction of flowers of *Abelmoschus esculentus* (Standard: Chloramphenicol, concentration 1 mg/ml)

2.3.2 Screening of antifungal activity

2.3.2.1 Culture Media

The media used for antifungal test was Sabouraud’s dextrose agar/broth of Hi media Pvt. Bombay, India.

2.3.2.2 Inoculum

The fungal strains were inoculated separately in Sabouraud’s dextrose broth for 6 hrs and the suspensions were checked to provide approximately 105 CFU/ml.

2.3.2.3 Determination of antifungal activity

The agar well diffusion method (Perez) was modified. Sabouraud’s dextrose agar (SDA) was used for fungal cultures. The culture medium was inoculated with the fungal strains separately suspended in Sabourauds dextrose broth. A total of 8 mm diameter wells were punched into the agar and filled with sample solution and solvent blanks (hydro alcohol and hexane). Standard antibiotic (Fluconazole, concentration 1 mg/ml) was used as positive control and fungal plates were incubated at 37 °C for 72 hrs. The diameters of zone of

inhibition observed were measured.

Table 2: Antifungal activity of the compound isolated from the ethyl acetate fraction of flowers of *Abelmoschus esculentus*

S. No	Organisms	Zone of inhibition(mm)				
		Standard (Fluconazole)	Sample Concentration (mg/ml)			
			20	30	40	50
1	<i>Curvularia lunata</i>	17	00	00	16	22
2	<i>Candida albicans</i>	18	00	00	16	22

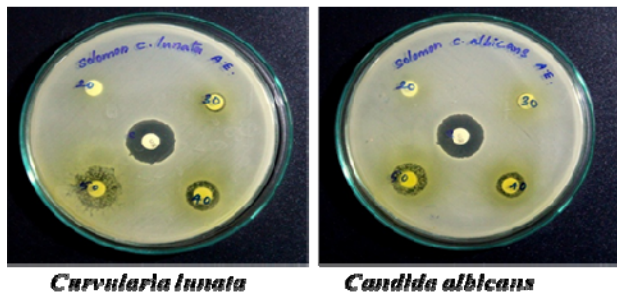
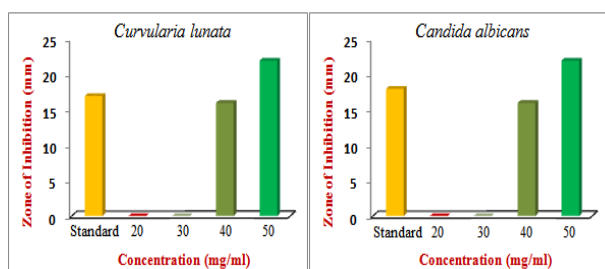


Fig 2: Antifungal activity of the compound isolated from the ethyl acetate fraction of flowers of *Abelmoschus esculentus*



Graph 2: Graphical representation of anti-fungal activity of the compound isolated from the ethyl acetate fraction of flowers of *Abelmoschus esculentus* (Standard: Fluconazole, concentration 1 mg/ml)

3. Results and discussion

In the present study, *Abelmoschus esculentus* flowers were screened for antimicrobial activity and compared with standard drug. It is evident from the data presented in Table I that the compound isolated from the ethyl acetate fraction of *Abelmoschus esculentus* flowers possesses antibacterial activity. The disc diffusion method result showed the zone of inhibition for 20 mg/ml as 0 mm, 0 mm, 0 mm and 0 mm, for 30 mg/ml as 0 mm, 7 mm, 0 mm and 9 mm, for 40 mg/ml showing 18 mm, 18 mm, 17 mm and 17 mm, for 50 mg/ml as 24 mm, 25 mm, 24 mm and 26 mm, for the test sample against *Salmonella typhi*, *Escherichia coli*, *Enterococcus faecalis* and *Bacillus cereus* respectively when compared with standard drug chloramphenicol showing 17 mm, 17 mm, 19 mm and 18 mm zone of inhibition respectively.

Then it is evident from the data presented in Table II that the test sample possesses antifungal activity. The disc diffusion method result showed the zone of inhibition for 20 mg/ml as 11 mm and 12 mm, for 30 mg/ml as 16 mm and 18 mm, for 40 mg/ml as 21 mm and 21 mm, for 50 mg/ml as 29 mm and 28 mm for the test solution against *Curvularia lunata*, and *Candida albicans* respectively when compared with standard drug Fluconazole showing 20 mm and 18 mm of inhibition respectively.

4. Conclusion

The antimicrobial and antifungal activity of the compound isolated from the ethyl acetate fraction of flowers of

Abelmoschus esculentus may be due to the presence of flavonoids. Flavonoids are known to exhibit antimicrobial activity through formation of a complex with the bacterial cell wall. From the results, it is evident that the compound with higher concentration could be used as an alternative for antibiotics.

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