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Antimicrobial Activity of Hibiscus Vitifolius (Flowers)

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ABSTRACT

This study was performed to evaluate the antimicrobial activity of *Hibiscus vitifolius*. The ethyl acetate fraction of *Hibiscus vitifolius* were shown to possess an antimicrobial activity against bacteria and fungi, viz. Six bacterial strains were *S. typhi, E. coli, E. faecalis, B. cereus, B.Substilis, Lacto bacillus* and two fungal strains *C.lunata, C.albicans* by using disc diffusion method. The anti bacterial activity of ethyl acetate fraction is almost comparable with standard solvent control *Chloramphenicol*. The anti fungal activity of ethyl acetate fraction extract is almost comparable with standard solvent control *Fluconazole.* Further studies are highly needed for future drug development.

Keywords: *Hibiscus vitifolius*, antimicrobial activity

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INTRODUCTION

Plants have been used for the treatment of diseases all over the world before the advent of modern clinical drugs. Natural phytochemicals are known to contain substances that can be used for therapeutic purposes or as precursor for the synthesis of novel drugs. Nearly 50% modern drugs are of natural products origin and as such these natural products play an important role in drug development in pharmaceutical industry. Plants remain the most common source of antimicrobial agents [1, 2]. Many aromatic plants have been used traditionally in folk medicine as well as to extend the shelf life of foods, showing inhibition against bacteria, fungi and yeast [3].Medicinal plants are renewable sources therefore farmers get encouraged to include them in traditional agriculture [4].

The genus Hibiscus (Malvaceae) consists of about 200 species, mainly distributed in tropical and subtropical region of the world. There are about 40 species grown in India and many are valued as ornamental plants and cultivated in gardens. Some species are used for medicinal properties[5,6]. Hibiscus vitifolius Linn., (syn. Fioria vitifolia (L.) Mattei), which is a perennial shrub widely distributed in India. It is used in the treatment of contraceptive, pulsating anterior fontanelle in babies, kidney problems [7,8,9,10]. The present study was undertaken to evaluate the antibacterial potentials and phytochemical analysis of H.vitifolies flowers extract against some selected bacterial species with the possible use as a genuine antimicrobial agent in pharmacological industries.

MATERIALS AND METHOD

Collection of Flowers

Fresh flowers of Hibiscus vitifolius were collected from Z.Suthamalli, Ariyalur(Dt), Tamil Nadu, India, during the month of August and identified by Dr.S.John Britto, Director, The rapinat Herbarium and Centre for Molecular Systematics (Authentication No. DP001 dated: 06/11/2015). St.Joseph's College(Campus),Trichy. Tamil Nadu, India.

Extraction and fractionation

Fresh flowers (2 kg) of Hibiscus vitifolius 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^{0}C) (6x250ml), Peroxide free diethyl ether (4x250ml) and ethyl acetate (8x250ml). 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.

Antimicrobial procedure

Screening of antibacterial activity

Bacteria tested:

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

Preparation of inoculums:

Stock cultures were maintained at 4^{o}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^{o}C. The cultures were diluted with fresh Muller-Hinton broth to achieve optical densities corresponding to $2.0x10^{6}$ colony forming units (CFU/ml).

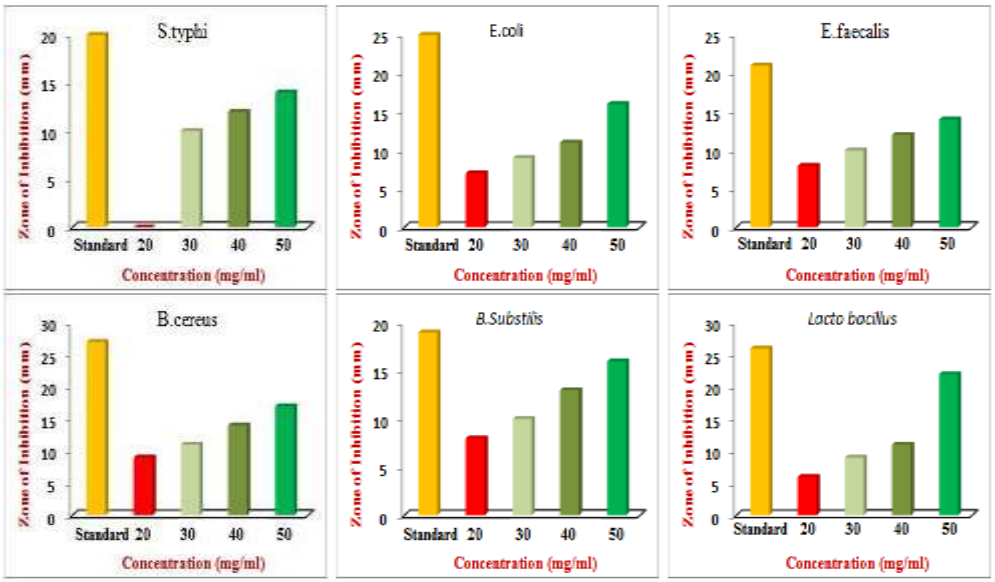
Antibacterial susceptibility test:

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 10mg/ml, 20mg/ml, 30mg/ml, 40mg/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^{o}C for 24 hrs. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter. Standard antibiotic chloromphenicol of concentration 1mg/ml was used as positive control[11].

Table I: Antibacterial activity of the compound isolated from the ethyl acetate fraction of flowers of Hibiscus vitifolius

S.No	Organisms	Zone of inhibition(mm)				
		Standard	Sample Concentration (mg/ml)			
		(Chloramphenicol)	20	30	40	50
1	*S.typhi*	20	0	10	12	14
2	*E.coli*	25	7	9	11	16
3	*E.faecalis*	21	8	10	12	14
4	*B.cereus*	27	9	11	14	17
5	*B.substilis*	19	8	10	13	16
6	*Lacto bacillus*	26	6	9	11	22

Figure 1: Antibacterial activity of the compound isolated from the ethyl acetate fraction of flowers o*f Hibiscus vitifolius*



Graph 1: Graphical representation of anti bacterial activity of the compound isolated from the ethyl acetate fraction of flowers of *Hibiscus vitifolius*. (Standard: Chloramphenicol, concentration 1 mg/ml)

Screening of antifungal activity

Culture Media

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

Inoculum

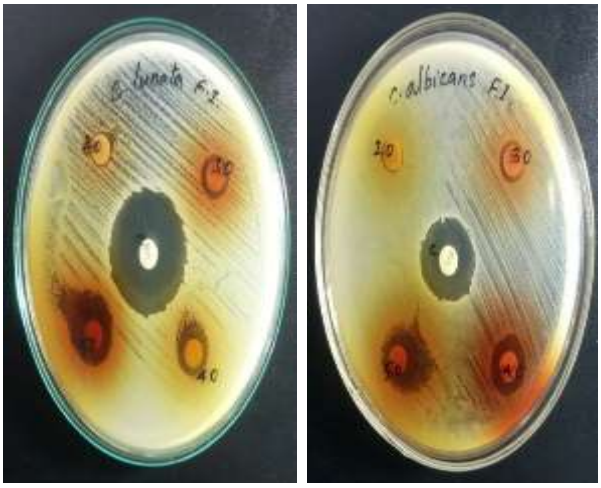
The fungal strains were inoculated separately in Sabouraud's dextrose broth for 6 h and the suspensions were checked to provide approximately 105 CFU/ml.

Determination of antifungal activity

The agar well diffusion method (Perez, 1993) 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 (Fucanazole, concentration 1 mg/ml) was used as positive control and fungal plates were incubated at 37^{o}C for 72 hrs. The diameters of zone of inhibition observed were measured.

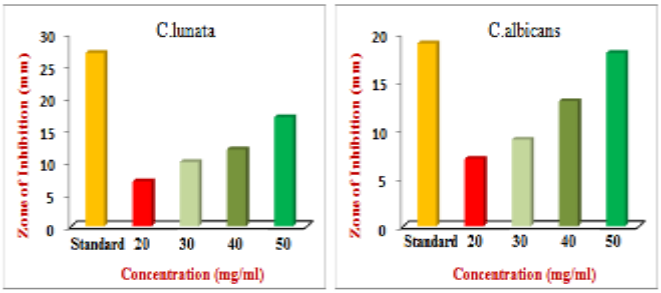
Table II: Antifungal activity of the compound isolated from the ethyl acetate fraction of flowers of *Hibiscus vitifolius*

S.No	Organisms	Zone of inhibition(mm)				
		Standard	Sample Concentration (mg/ml)			
		(Fluconazole)	**20**	**30**	**40**	**50**
1	*C.lunata*	27	7	10	12	17
2	*C.albicans*	19	7	9	13	18



C.lunata **C.albicans**

Figure II: Antifungal activity of the compound isolated from the ethyl acetate fraction of flower of *Hibiscus vitifolius*



Graph .2: Graphical representation of antifungal activity of the compound isolated from the ethyl acetate fraction of flowers of *Hibiscus vitifolius.* (Standard: Fluconazole, concentration 1 mg/ml

RESULTS AND DISCUSSION

Hibiscus vitifolius flowers exhibited significant antimicrobial activity when compared with standard drug. It is evident from the data presented in Table I that the compound isolated from the ethyl acetate fraction of possesses antibacterial activity. The disc diffusion method result showed the zone of inhibition for 20 mg/ml as 0 mm, 7 mm, 8 mm, 9 mm, 8 mm and 6 mm, for 30 mg/ml as 10 mm, 9 mm, 10 mm, 11 mm, 10 mm and 9 mm, for 40 mg/ml showing 12 mm, 11 mm, 12 mm, 14 mm, 13 mm, and 11 mm, for 50 mg/ml as 14 mm, 16 mm, 14 mm, 17 mm, 16 mm and 22 mm, for the test sample against S. typhi, E.coli, E.faecalis, B.cereus, *B.substilis and Lacto bacillus* respectively when compared with standard drug *Chloramphenicol* showing 20 mm, 25 mm, 21 mm, 27 mm, 19 mm and 26 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 7 mm, 7 mm, for 30 mg/ml as 10 mm, 9 mm, for 40 mg/ml as 12 mm, 13 mm, for 50 mg/ml as 17 mm, for the test solution against C.lunata, C.albicans respectively when compared with standard drug *Fluconazole* showing 27 mm, 19 mm of inhibition respectively.

CONCLUSION

The antimicrobial studies support its traditional uses and may prove to be useful for clinical evaluation and development of commercial drugs. *Hibiscus vitifolius* biological activity will definitely give fruitful results. So it can be recommended as a plant of phyto pharmaceutical importance. These *H.vitifolies* plant extract can be used to formulate the new antibacterial drugs against the diseases.

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