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Antimicrobial Activity of *Hibiscus sabdariffa* against Ocular Pathogens

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Abstract: The potential presence of naturally occurring antimicrobials in petals of flowers of *Hibiscus sabdariffa* L., (Malvaceae) was investigated against isolated eye pathogens. Owing to the usage of these flowers in common folklore medicine, the extracts of petals were screened for antibacterial activity against pathogenic microbes isolated from the eyes of eye infected persons. Bioactive compounds were extracted by cold extraction method, wherein Methanol, ethyl acetate and dichloromethane were used as solvents. The antibacterial activity of the extracts was assessed by agar well diffusion method. The study revealed that the extracts possessed antibacterial activity in a dose dependent manner. Among the tested flower extracts of Hibiscus *sabdariffa*, DCM extract showed better activity on most of the ocular pathogens tested. Hence the DCM extracts of petals of the flowers of *Hibiscus sabdariffa* can be used to discover antibacterial agent for developing new pharmaceuticals to treat eye infections.
Keywords: *Hibiscus sabdariffa* L., (Malvaceae), agar well diffusion method, Bioactive compounds, ocular pathogens.

INTRODUCTION

Plants are well known as a major source of modern medicines [1]. From ancient times, humans have utilized plants for the treatment or prevention of diseases, leading to the dawn of traditional medicine[2]. Extreme interest in plants with microbial activity has revived as result of current problems such as resistance associated with the use of antibiotics obtained from microorganisms [3].

Hibiscus sabdariffa L., (Malvaceae), commonly known as "roselle", is an important medicinal plant native to India and Malaysia, although it grows widely in the tropics and subtropics of both hemispheres and has become naturalized in many areas in Central America [4].

The plant is an annual, erect, herbaceous sub-shrub with a deep root system. The plant has fibrous stems, small branches, as well as bright red and acidic-tasting calyces. In folk medicine, an infusion from the calyces is used as a diuretic and to treat gastrointestinal disorders, liver diseases, fever, hypercholesterolemia, and hypertension [5]. Extracts from the calyces are reported to have a variety of therapeutic effects *in vivo* and *in vitro*, including anticancer and antioxidant properties [6-9]. Considering these facts, it is expected that the screening and scientific evaluation of the flowers of *H.sabadariffa* may provide novel antimicrobial compounds.

MATERIALS AND METHODS

Collection of Flowers

Fresh flowers of *Hibiscus sabdariffa* were collected from Turkapally village, R.R (Dt), Telangana, India, during the month of January and identified by Dr. S. Nirmala, head of the department of Pharmacognosy. Malla Reddy College of Pharmacy (Campus), Turkapally village, Telangana, India.

Extraction and fractionation [10]

Plant materials were successively extracted using solvents of increasing polarity (solvents such as EA, DCM + M was used for extraction was done) by cold extraction method. Stalk of flowers were removed to get petals alone. 2 kg of petals were soaked in 5 liter of each solvent viz., Methanol, DCM and Ethyl acetate in a separate air tight containing. These were allowed to stand at room temperature for 5 days, with occasional manual agitation of the container using a sterile glass rod at every few hours. The extracts were separately filtered using sterile Whatman No.1 filter paper. The resultant filtrates were then concentrated in a rotary evaporator (Laborator 4000- efficient, heidolph Germany) at 400 rpm/50^{0}C. Fifty ml of gummy extract

were obtained upon evaporation for each extract. The gummy extract was stored at 4^0C for further studies.

Fig-1: *H. sabadariffa* plant

Microorganisms

Five bacterial strains (viz., *Gardnerella vaginalis, Corynebacterium macbinleys, Staphylococcus agalactiae, Staphylococcus epidermidis and Bacillus cereus*) isolated from eye infected cases examined in Sarojini Devi Eye Hospital, Mehdipatnam, Hyderabad were used in the study. All the bacterial strains were from patients with eye diseases.

The bacteria were initially identified by streak plate method in blood agar medium and specifically identified at Royal Life sciences microbiological laboratory using enzyme assay method and maintained on nutrient agar slants at 4^0C.

Preparation of inoculums

Stock cultures were maintained at 4^oC 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 was incubated without agitation for 24 hrs at 37^oC. 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

Antibacterial activity of the methanol, DCM, Ethyl acetate extracts of petals of flowers *Hibiscus sabdariffa* were assayed using agar-well diffusion method of Kirby Bauer. The concentrated gummy petal extracts of 200 µg were dissolved in 1 ml of di methyl sulfoxide (DMSO) and dilutions of 25µl, 50µl and 75µl were prepared for each extract respectively, to assess the antibacterial activity. Approximately 10 ml of sterile Muller- Hinton Agar (MHA) was poured into sterile culture plates and allowed to set. About 1ml of 24 h old culture of five bacterial isolates were maintained in Muller- Hinton broth and stored in an incubator. Sterile cotton swabs were dipped in the bacterial suspension and evenly streaked over the entire surface of the agar plate to obtain uniform inoculums. The seed medium was then allowed to dry at room temperature for about 30 minutes. With the aid of a sterile well-cutter, wells of about 6 mm in diameter were punched on the plates. About 0.5 ml of each dilution of the extracts was dispensed into the wells and the plates were incubated at 37^0C for 24 h.

Triplicates were maintained for each sample of the extracts respectively. For each bacterial assay control with standard antibiotic streptomycin was maintained. At the end of incubation period, inhibition zones formed on the medium were evaluated in mm.

RESULTS AND DISCUSSION

The data pertaining to the antibacterial potential of the petals of the selected flower extracts using methanol, DCM, Ethyl acetate extracts are presented in tables 1 and 2 and 3 respectively.

The extracts from *Hibiscus sabdariffa* petals showed inhibitory activity against all the five ocular bacterial isolates. The DCM extracts presented the highest activity and the extracts were able to inhibit all the five bacterial isolates viz., (*Gardnerella vaginalis, Corynebacterium macbinleys, Staphylococcus agalactiae, Staphylococcus epidermidis and Bacillus cereus).* The highest activity rate was recorded against *G. vaginalis.* On the other hand, the methanol and Ethyl acetate extracts exhibited their highest activity against *C. macbinleys*. Methanol extracts were also effective against all the bacterial isolates with highest against *Corynebacterium Macbinleys and lowest against Bacillus cereus* (Table 1).

Among the three solvents used, DCM is found to extract more potential compounds from the petals than ethyl acetate and methanol. The order of priority in solvents used ranges as

DCM > Ethyl acetate > Methanol

The extracts of the petals worked in a dose dependent manner, when the concentration of the extract was decreased the activity was also decreased. This was due to susceptibility of the species towards concentration of the extracts [11]. It has been reported that different solvents have different extraction capabilities and spectrum of solubility for the phyto-constituents [12, 13]. Our findings corroborate with the above reports that in the present study, DCM extract of Hibiscus sabdariffa possess more effective anti-bacterial activity than, ethyl acetate extracts and methanol extracts, thus signaling its broad spectrum of antibacterial activity. However, studies of Jeyaseelan *et al.* reported that flower extracts of *Allium Sativum* showed better inhibition on phytopathogens in the ethyl acetate, methanol and ethanol extracts than DCM and aqueous extracts [13].

CONCLUSION

The results of the present investigation clearly indicated that the antibacterial activity vary with the solvents used for the extraction of phytochemicals. The present study ascertains the value of plants used in ayurvedic medicine and it creates a considerable interest for the development of new drugs.

Further studies are being carried out to isolate and characterize the active compounds and to determine the toxicity and the optimum dose for treatment.

Table-I: Antibacterial activity of the methanol fraction of flowers of *Hibiscus sabdariffa*

S.No	OCULAR BACTERIAL ISOLATES	Zone of inhibition(mm)			
		25 µg/ml	50 µg/ml	75 µg/ml	Std drug Streptomycin 50mg
1	*Gardnerella vaginalis*	11	13	14	16
2	*Corynebacterium Macbinleys*	10	14	15	20
3	*Staphylococcus agalactiae*	10	10	11	18
4	*Staphylococcus Epidermidis*	10	11	14	17
5	*Bacillus cereus*	10	11	11	16

Table-2: Antibacterial activity of the ethyl acetate fraction of flowers of *Hibiscus sabdariffa*

S.No	OCULAR BACTERIAL ISOLATES	Zone of inhibition(mm)			
		25 µg/ml	50 µg/ml	75 µg/ml	Std drug Streptomycin 50mg
1	*Gardnerella vaginalis*	10	15	16	16
2	*Corynebacterium Macbinleys*	10	12	19	20
3	*Staphylococcus agalactiae*	10	11	12	16
4	*Staphylococcus Epidermidis*	10	11	11	17
5	*Bacillus cereus*	10	12	14	16

Table-3: Antibacterial activity of the dichloromethane fraction of flowers of *Hibiscus sabdariffa*

S.No	OCULAR BACTERIAL ISOLATES	Zone of inhibition(mm)			
		25 µg/ml	50 µg/ml	75 µg/ml	Std drug Streptomycin 50mg
1	*Gardnerella vaginalis*	12	14	18	16
2	*Corynebacterium Macbinleys*	11	12	16	20
3	*Staphylococcus agalactiae*	10	11	12	16
4	*Staphylococcus Epidermidis*	10	11	11	17
5	*Bacillus cereus*	10	10	11	16

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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