



## ANTIMICROBIAL POTENTIAL OF *BLEPHARIS PERSICA* (BURM. F.) O. KUNTZE (SEEDS)

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### ABSTRACT

Medicinal plants are the nature's gift to human being to make disease free healthy life. Herbal medicine is still the mainstay of about 75-80% of the whole population, mainly in developing countries, for primary health care because, better compatibility with the human body and fewer side effects. The use of phytochemicals with known antimicrobial properties can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted to prove their efficiency. In a search for phytochemical with antimicrobial activity the seed extract of *Blepharis persica* was evaluated against *Staphylococcus aureus* and *Escherichia coli* by agar disc-diffusion and micro dilution methods. The ethanolic (90%) extract showed significant effects with inhibition zones and minimum inhibitory concentration against *S.aureus* and *E. coli* in comparison of Tetracycline as standard drug for antimicrobial activity. The results demonstrate that the ethanolic (90%) extract of the seed of *B. persica* has significant antimicrobial activity and suggest that it may be useful in the treatment of infections.

**Key words:** Antimicrobial; Agar disc-diffusion method; Micro dilution method; Tetracycline.

### INTRODUCTION

Plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well being. There has been renewed interest in screening plants for novel biologically active compounds, particularly those that effectively intervenes the human ailments. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as communicable diseases (Saleh AI *et al.*, 2009). Nature has served as a rich source of medicinal plants for thousands of years and an impressive number of modern drugs has been isolated from natural antimicrobial agents with plant origin are not associated with side effects and have an enormous therapeutic potential to heal many infectious diseases (Odugbemi T, 2006).

*Blepharis persica* (Burm. f.) O. Kuntze.

(Acanthaceae) commonly known as Uttingana, Sunishannaka, Chaupatia and Borahu is a soft grey-pubescent perennial herb (Anonymous, 1996). It is indigenous to India (Punjab, Western Rajasthan, Malwa region of Madhya Pradesh), Pakistan, Iran, Africa (Thar) (Anonymous, 1986). It is used as purgative, tonic, aphrodisiac (Ayurvedic Pharmacopoeia, 2007), diuretic, expectorant and used in treatment of urinary discharges, leukoderma, ascites, disorders of liver and spleen. It contains saponin, tannins, flavonoids and glycoside (blepharin) (Pande M *et al.*, 2009; Gupta AK *et al.*, 2004). It has delayed seed dispersal and rapid germination property (Chatterjee A *et al.*, 1990). The traditional formulations are available in the name of Kumaryasava in Ayurvedic system and Majoon Bandkushad in Unani medicine system.

The present study is designed to investigate the antimicrobial activity of the seed extracts of *B. persica* against selected bacteria with the aim to establish the claimed biological activities of this plant.

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## MATERIAL AND METHODS

### Plant material

The seeds of the plant were collected in the month of April from Patiala, Punjab and authenticated by Dr. Sunita Garg, Chief Scientist, NISCAIR, New Delhi, India (Ref. No. - NISCAIR/RHMD/Consult/2013/2311/91 dated 13/09/2013). The seeds were shade dried, coarsely powdered and stored in an air tight container till use.

### Microorganisms used

The *Staphylococcus aureus* (NCTC 7447) as Gram +ve bacteria and *Escherichia coli* (NCTC 10418) as Gram -ve bacteria strains were employed for the present study. The microorganisms were maintained by sub-culturing and used at regular intervals in nutrient agar medium.

### Extraction

The plant material was extracted by cold maceration with ethanol (90%) and distilled water till exhausted completely. The extracts so obtained were freed off solvent under vacuum and used for further studies.

### Antimicrobial activity

The antimicrobial activity of *Blepharis persica* (seeds) was performed against *Staphylococcus aureus* (NCTC 7447) Gram +ve & *Escherichia coli* (NCTC 10418)-ve bacteria.

### Preparation of Standard Bacterial

The strains of *E. coli* and *S. aureus* were maintained on Muller Hinton agar (Beef, casein, acid hydrolysate, starch and agar) incubated at 37°C and stored in incubator till use.

### Disc diffusion assay

A modified disc-diffusion method was adapted used for antimicrobial susceptibility testing. The dried plant extracts were dissolved in sterile water, to reach a final concentration of 20 mg/ml and sterilized by filtration by 0.22 µm Millipore filters. The media used were Muller Hinton agar for the bacteria. The discs (6 mm in diameter) were impregnated with 10 µl of the extracts (200µg/disc) at a concentration of 20 mg/ml and placed on the inoculated agar. The antimicrobial (µg/disc) from tetracycline (30 UI/disc) was used as positive reference standard to determine the sensitivity of the tested microbial strains. The inoculated plates were incubated at 37°C for 24 h for bacterial strains. Antimicrobial activity was evaluated by measuring the zone of inhibition against the tested organisms. All inhibition assays and controls were made in triplicate (Mohaddese M et al., 2013).

### Micro dilution method

The 96-well plates were prepared by dispensing 50 µl of Mueller–Hinton broth for bacteria, into each well. A 50 µl from the stock solution of tested extracts (concentration of 200 mg/ml) was added into the first row of the plate. Then, twofold, serial dilutions were performed by using a micropipette. The obtained concentration range was from 100 to 0.1953 mg/ml, and then added 10 µl of inocula to each well except a positive control. Plant extract with media was used as a positive control and inoculum with media was used as a negative control. The test plates were incubated at 37°C for 18 h. After 18 h 50 µl of a 0.01% solution of 2, 3, 5- triphenyl tetrazolium chloride (TTC) was added to the wells and the plate was incubated for another hour. Since the colourless tetrazolium salt is reduced to red colored product by biological active bacteria, the inhibition of growth can be detected when the solution in the well remains clear after incubation with TTC. MIC was defined as the lowest sample concentration showing no color change (clear) and exhibited complete the inhibition of growth (Gutterman Y, 1972).

## RESULTS & DISCUSSION

The antimicrobial activity of plant material ethanolic (90%) and Aqueous extracts were determined against two microorganisms i.e. *Staphylococcus aureus* as Gram +ve and *Escherichia coli* as Gram -ve determined by agar diffusion and micro dilution methods.

### Disc Diffusion Method

The antimicrobial effect of the extracts was observed against the tested microorganisms by agar diffusion method was reported in Table 1 and graphical representation in Fig. 1.

In this method, the bacterial inoculum is adjusted to certain concentration, inoculated onto the entire surface of a Mueller-Hinton agar (MHA) plate with a sterile cotton-tipped swab to form an even lawn. The paper disks impregnated with antibiotic solution was placed on the surface of each MHA plate using a sterile pair of forceps. Then the plates were incubated aerobically and the diameter of zone inhibition was measured by a ruler or caliper. Based on the diameter of the inhibition zone and the results are then assigned to three categories, susceptible, intermediate, or resistant. The bigger the diameter of the inhibition zone, the more susceptible is the micro-organism to the antimicrobial (Murray PR, 1995). In agar diffusion method, the minimum acceptable zone of inhibition was 7mm. The ethanolic (90%) extract of *B. persica* showed zone of inhibition against *Staphylococcus aureus* - 9.4±0.5mm & *Escherichia coli* - 8.3±0.5mm. The aqueous extract had showed mild antimicrobial activity as compared to the ethanolic (90%) extracts with respect to zone of inhibition of tetracycline

as standard against *Staphylococcus aureus* -  $24.2 \pm 0.5$  mm & *Escherichia coli* -  $26.3 \pm 0.5$  mm.

### Micro Dilution Method

The antimicrobial effect of the ethanolic (90%) and aqueous extracts was observed against the tested microorganisms by Micro dilution method was reported in Table 2 and graphical representation in Fig. 2.

In micro dilution method, two-fold serial dilutions of an antibiotic made in Mueller-Hinton agar (MHA) medium and then bacterial suspensions were inoculated on the MHA using a Cathra replicator with 1 mm pins. The advantages of agar dilution include the ability to simultaneously test the susceptibility of a number of bacteria in one plate and the ability to test susceptibility of fastidious organisms since the agar with supplements is able to adequately support the bacteria

growth. Moreover, as mentioned above, the test results yield MIC values for testing bacteria ((Murray PR, 1995). The antimicrobial agents are expressed its potency as minimum inhibitory concentration (MIC) in this method. The method was carried out in a broth dilution test, in which a specific amount of bacteria was added to the serial dilution of antimicrobial agents in broth wells. After incubation, bacterial growth was indicated The ethanolic (90%) extract of *B. persica* was able to produce minimum inhibitory concentration against *Staphylococcus aureus* - 0.575mg/ml & *Escherichia coli* - 0.725mg/ml. Tetracycline as standard had minimum inhibitory concentration against *Staphylococcus aureus* - 0.025mg/ml & *Escherichia coli* - 0.01mg/ml (Zgoda JR and Porter JR, 2001; Dickert H et al., 1981; Clinical and Laboratory Standards Institute, 2009).

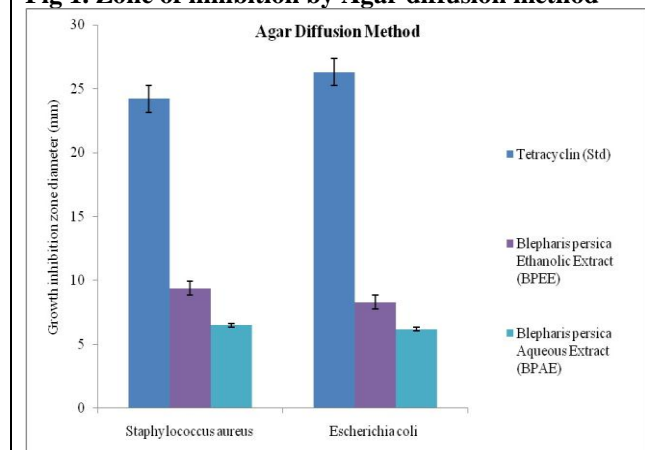
**Table 1. Inhibitory effect of samples in Agar diffusion method**

Test Sample	Extracts	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>
		Zone of Inhibition	
<i>B. persica</i> (Seed Extract)	Ethanolic Extract (BPEE)	$9.4 \pm 0.5$ mm	$8.3 \pm 0.5$ mm
	Aqueous Extract (BPAE)	$6.5 \pm 0.5$ mm	$6.2 \pm 0.5$ mm
Tetracycline	Reference Standard	$24.2 \pm 0.5$ mm	$26.3 \pm 0.5$ mm

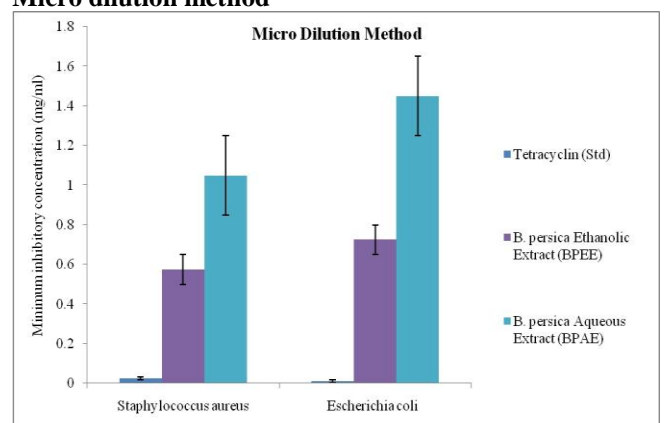
**Table 2. Inhibitory effect of Samples in Microdilution Method**

Test Sample	Extracts	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>
		Minimum inhibitory concentration	
<i>B. persica</i> (Seed Extract)	Ethanolic Extract (BPEE)	0.575 mg/ml	0.725 mg/ml
	Aqueous Extract (BPAE)	1.05 mg/ml	1.45 mg/ml
Tetracycline	Reference Standard	0.025 mg/ml	0.01 mg/ml

**Fig 1. Zone of inhibition by Agar diffusion method**



**Fig 2. Minimum inhibitory concentration (mg/ml) by Micro dilution method**



### CONCLUSION

With increasing microbial resistance to antibiotics as well as with growing interest in eco-living lifestyles, increased attention is being paid to the natural antimicrobial compounds. Antimicrobial substances are abundant in the nature but have encountered problems

because of the diversity of criteria and techniques employed for evaluation. Nature has served as a rich source of medicinal plants for thousands of years and an impressive number of modern drugs has been isolated from natural antimicrobial agents with plant origin are not associated with side effects and have an enormous

therapeutic potential to heal many infectious diseases. The research on plants as natural antimicrobial has been carried out by *in-vitro* method. The ethanolic (90%) and aqueous extracts of the seed of *B. persica* showed the significant antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli* in agar diffusion and micro dilution methods. This present study indicated that *B. persica* might be applicable in natural medicine and food as a source of antibacterial products. Further studies aimed at the isolation and identification of active substances from the extract which exhibited antimicrobial

activity need to be undertaken to confirm the findings.

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#### CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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