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ANTIBACTERIAL ACTIVITY OF PROTEIN EXTRACTS OF SELECTED MULBERRY VARIETIES

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ABSTRACT
Mulberry is a member of the family Moraceae belongs to the genus *Morus*. The leaves are used to feed silkworms. It is a rich source of proteins. The plant has also been found to possess therapeutic value for many diseases. Protein extracts of selected mulberry varieties viz., *Morus indica,* V1, and DD was examined against different pathogenic bacteria using cup diffusion method. The heat stable protein extracts of these varieties exhibited varying degrees of inhibitory activity against different bacteria viz., *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis.* Among the 3 varieties tested V1 variety showed more significant antibacterial activity against *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Escherichia coli* whereas DD was more effective against *Staphylococcus aureus*.

INTRODUCTION

India has a wealth of medicinal Plants which has been used by our ancestors against various ailments from time immemorial. It is a big repository of medicinal plants that are being utilised in traditional medicine (Chopra *et al.*, 1956). Medicinal plants generally produce many secondary metabolites which constitute an important source of microbicides, pesticides and many pharmaceutical drugs. The effects of plant extracts on bacteria have been studied by various researchers in different parts of the world (Reddy *et al.,* 2001; Gallo *et al*., 2006, Karthikumar *et al.,* 2007). Mulberry reduces blood serum glucose which was used in the old Chinese herbal medicine (Andallu *et al.,* 2001). It has the ability to reduce blood cholesterol and lipid levels, fight against arterial plaques, diuretic and expectorant. (Andallu and Varadacharyulu, 2003; Doi *et al.,* 2000; Jang *et al.,* 2002). Increasing interest in the health benefits of various plant extracts has led to the investigation of mulberry protein extracts for their antibacterial activity. In the present study protein extract of 3 mulberry varieties viz., *Morus indica,* V1 and DD were evaluated for antibacterial activity.

MATERIALS AND METHODS

Collection of samples

The mulberry leaves of different varieties such as V1, DD, *Morus indica* were procured from the garden maintained in the Jnanabharathi campus, Bangalore University, Bangalore for the study.

The healthy leaves were washed with distilled water several times, shade dried. About 10g of leaves were blended with prechilled acetone. The slurry obtained was then filtered through whatmann filter paper by adding chilled acetone over the funnel. The extract was air dried and is stored in sealed condition at -40ºC until use.

Extraction of total soluble proteins (TSP)

1g of sample was stirred with extraction buffer containing Tris-EDTA and Thiol compounds and precipitated with 10% TCA. The slurry was centrifuged at 15,000 rpm for 20min at 4ºC. The supernatant was taken and the volume was measured. The TSP was quantified at 280nm and aliquot was kept in the refrigerator.

Extraction of heat stable proteins (HSP)

The TSP was incubated at 70ºC for 10min and then centrifuged at 12,000 rpm for 20min at 4ºC to remove the precipitated heat liable protein. The protein content was determined by Lowry's method (Lowry *et al*., 1951).

Bacterial cultures

E.coli, Pseudomonas aeruginosa, Bacillus subtilis and *staphylococcus aureus* (ATCC type) were procured form Victoria hospital, Bangalore and maintained on nutrient agar medium.

Antibacterial activity assay

The bacteria were grown in Mueller Hinton agar media at 37ºC and maintained at 4ºC. Study of Antibacterial activity was done by cup diffusion method (Perez *et al.,* 1990). The media was sterilised and poured into the sterilized Petri plates. It was allowed to solidify at room temperature. 1000 μL of bacterial suspension was spread on the solidified medium using sterile glass spreader. Wells were bored in the medium

Table 1: Different varieties of Mulberry showing zone of inhibition in mm

	Pseudomonas				*Staphylococcus aureus*				*Escherichia coli*				*Bacillus*			
Dilutions(extract µL/L)	25	50	75	100	25	50	75	100	25	50	75	100	25	50	75	100
DD(Zone of inhibition in mm)	0	10	12	16	0	9	13	15	0	8	10	12	0	6	8	11
V1(Zone of inhibition in mm)	11	15	18	21	7	9	9	12	0	11	16	19	9	10	13	15
M.indica (Zone of inhibition in mm)	0	0	7	10	7	9	11	13	0	7	9	10	0	7	8	11
Cloramphenicol (+ve) (Zone of inhibition in mm)	20				15				15				10			

using cork borer. The HSP is used for study. Different concentration viz., 25 µL, 50 µL, 75 µL and 100 µL of extracts was poured into the wells and incubated for 24hr to 48hr at 37ºC. Tris EDTA extraction buffer was used as control and it was compared with standard chloramphenicol antibiotic.

RESULTS AND DISCUSSION

Antibiotics provide the main basis for treating infectious diseases. Hence there is an increased need in the investigation of plant as a source of human disease management (Prashanth *et al.,* 2001*;* Woldmichael *et al.,* 2003*).* The continuous evolution of bacterial resistance to antibiotics has necessitated the search for novel and effective antimicrobial compounds (Fagbemi *et al.,* 2009).

According to the table 1 V1 variety at 25 µl/L showed inhibition to *P. aeruginosa* and as the concentration increased area of zone of inhibition was also increased. Whereas DD and *M .indica* did not have any inhibition at that concentration. Area of zone of inhibition proportionately increased with increase in concentration. *Morus indica* recorded MIC at 75 µl/L and no inhibition was observed at 25 µl/L and 50 µl/L. Both V1 and *Morus indica* recorded MIC at 25 µl/L for *Staphylococcus aureus* and area of zone of inhibition increased with increase in concentration. Whereas DD at 100 µl/L recorded zone of inhibition of 15mm which is similar to the standard antibiotic and greater than V1 and *Morus indica*.

For *E.coli* protein extract of all the three varieties at 25 µl/L could not inhibit the growth but at 50 µl/L there was zone of inhibition which increased with increase in concentration. V1 recorded Minimum inhibitory concentration at 25 µl/L for *Bacillus subtilis* whereas DD and *Morus indica* did not show any inhibitory effect at that concentration. At 50 µl/L DD and *Morus indica* showed inhibition.

For *Pseudomonas aeruginosa, Bacillus subtilis* and *Escherichia coli* V1 recorded maximum area of zone of inhibition at 100 µl/L which was significantly higher than other varieties and chloremphenicol antibiotic. All the above results show V1 has maximum inhibitory activity against *Pseuomonas aeruginosa, Bacillus subtilis* and *Escherichia coli* compared to other varieties. DD at higher concentration of 100 µl/L showed inhibitory activity against *Staphylococcus*. At 100 µl/L of leaf protein extract zone of inhibition was maximum for all the bacteria tested. This is in accordance with the report of Manjula and Shubha (2010) on *Costus pictus*.

Since the above results indicate that the protein extract of the three varieties of mulberry have a broad spectrum antibacterial activity against bacterial species tested, it can be explored as a potential natural antibacterial source in pharmaceuticals.

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