



SCREENING OF ANTIBACTERIAL ACTIVITY OF TOTAL SOLUBLE PROTEIN OF MULBERRY VARIETIES

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ABSTRACT

Pathogenic bacteria are responsible for many human infections and if the growth of infectious bacteria is not checked, it may lead to serious health problems. Antibiotics are being used to check such bacterial infections. Plant extracts are also very important tool against such infections. Total Soluble Proteins of Mulberry plant belonging to the genera *Morus* of moraceae was used for the antibacterial assay. Heat Stable Proteins of three varieties of mulberry viz., M5, *Morus alba* and S13 were tested for the antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Bacillus subtilis* and compared with the antibiotic chloramphenicol. Area of zone of inhibition increased with the increase in the concentration of the HSP for all the microbes tested by all the mulberry varieties. Minimum inhibitory concentration against *Staphylococcus aureus* and *Pseudomonas aeruginosa* was recorded in S13 at 25 μ l. For *Escherichia coli* MIC was at 25 μ l by *Morus alba*. Whereas *Bacillus subtilis* was inhibited at MIC of 25 μ l of both S13 and M5 HSP. Maximum inhibition against *Staphylococcus aureus* was recorded at 100 μ l of S13, *Morus alba* was more effective against *E.coli* at 100 μ l whereas against *Bacillus subtilis* both S13 and M5 varieties were equally effective at 100 μ l.

Keywords: Pathogenic bacteria.

INTRODUCTION

The use of plant extracts with known antimicrobial properties can be of great significance in modern therapy. Mulberry belonging to genera *Morus* is used as sole food for silkworms but it also has several medicinal properties. Andallu et al., 2001 reported blood serum glucose reduction by mulberry which was used in the old Chinese herbal medicine. It also has the ability to reduce blood cholesterol and lipid levels, effective against arterial plaques, diuretic and expectorant. (Andallu and Varadacharyulu, 2003; Doi et al., 2000; Jang et al., 2002). Lokegaonkar and Nabar (2010) reported that leaf extract of *Morus alba* inhibited biofilm formation by of *streptococcus mutans* and *streptococcus sanguinis*. There is no report on the antimicrobial activity of heat stable proteins of M5, *Morus alba* and S13 varieties of mulberry. Considering the present day requirement for ecofriendly antibacterial drugs without any side effect, this study was carried out to evaluate the antibacterial potential of three varieties of mulberry viz., M5, *Morus alba* and S13 against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Bacillus subtilis*.

MATERIALS AND METHODS

The mulberry leaves of different varieties such as viz., M5, *Morus alba* and S13 were procured from the garden maintained in the Jnanabharathi campus, Bangalore University, Bangalore for the study. The healthy leaves were washed with tap water and then distilled water several times. Then the leaves were dried under shade. 10gms of leaves were grinded with prechilled acetone. The slurry obtained was filtered through whatmann filter paper. The extract was air dried and is stored at -4 $^{\circ}$ C until use.

Extraction of total soluble proteins (TSP)

1gm of sample was mixed well with extraction buffer containing Tris-EDTA and Thiol compounds and precipitated with 10% TCA. Then the slurry obtained was centrifuged at 15,000 rpm for 20min at 4 $^{\circ}$ C. From the supernatant TSP was extracted.

Extraction of heat stable proteins (HSP)

The TSP thus obtained was incubated at 70 $^{\circ}$ C for 10min and then centrifuged at 12,000 rpm for 20min at 4 $^{\circ}$ C to remove the precipitated heat liable protein.

Bacterial cultures

Bacterial cultures for the study, Viz., *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *staphylococcus aureus* (ATCC type)

were procured from Victoria hospital, Bangalore and maintained on nutrient agar medium.

Antibacterial activity assay

The bacteria under study were grown in Mueller Hinton agar media at 37 $^{\circ}$ C and maintained at 4 $^{\circ}$ C. Antibacterial assay was done by cup diffusion method (Perez et al., 1990). All the glasswares and media used for the assay was sterilized in autoclave at 121 $^{\circ}$ C and 15lb pressure for 15 minutes. The sterilized media was poured into sterilized petriplates and allowed to solidify at room temperature. Using sterile glass spreader 1000 μ l of bacterial suspension was spread on the solidified medium. Wells were bored in the medium using cork borer. Various concentrations of HSP viz., 25 μ l, 50 μ l, 75 μ l and 100 μ l was poured into the wells and incubated for 24hrs to 48hrs at 37 $^{\circ}$ C. Tris EDTA extraction buffer was used as control, and it was compared with standard chloramphenicol antibiotic.

RESULTS AND DISCUSSION

In India, around 20,000 medicinal plant species have been recorded out of which more than 800 plants are traditionally used for treating human ailments (Kamboj, 2000). Bacterial infections are more prevalent in human population all across the globe. A large number of plants are reported to possess antimicrobial properties as seen in folk medicine and by medicinenem (Carvalho et al., 1999, Wiart et al., and Kaushik, 2003). As the bacterial strains are becoming resistant to the antibiotics already in use, there is a continuous need for newer antimicrobial agents preferably of plant origin to avoid side effects.

Commonly ethanolic or methanolic extracts are used as antibacterial agents and reports on the use of HSP of plants as antimicrobial agent is very few. Manjula and Shubha (2010) reported antimicrobial activity of heat stable protein of *Costus pictus* and Manjula et al., studied the antibacterial activity of HSP of DD, V1 and *M.indica* varieties of mulberry.

The study revealed (Table-1) the fact that S13 variety at 25 μ l recorded MIC against *P. aeruginosa* whereas *M.Alba* and M5 did not show inhibition at that concentration but at 100 μ l/l M5 was more effective than *M.alba* and S13. For *Staphylococcus aureus* also MIC was recorded in S13 HSP at 25 μ l/l and maximum inhibition was observed at 100 μ l/l. S13 was also effective against *Bacillus subtilis* at 25 μ l/l and it was slightly more effective than M5. MIC for *E.coli* was by *M. alba* at 25 μ l/l. There was a steady increase in the area of zone of inhibition with the increase in the concentration of HSP for all the three varieties tested for all the bacteria studied. The results proved that HSP of mulberry could be used as a potent antimicrobial agent against pathogenic bacteria studied.

Table 1: Varieties of Mulberry showing zone of inhibition in mm on pathogenic bacteria

Dilutions (extract µl/l)	Pseudomonas				Staphylococcus aureus				Escherichia coli				Bacillus			
	25	50	75	100	25	50	75	100	25	50	75	100	25	50	75	100
M5 (Zone of inhibition in mm)	0	8	12	19	0	9	11	12	0	10	11	14	6	9	12	16
S13 (Zone of inhibition in mm)	8	11	12	14	10	13	16	20	0	8	9	11	8	11	13	16
M.alba (Zone of inhibition in mm)	0	13	16	18	0	10	14	17	5	12	13	15	0	8	10	14
Cloramphenicol (+ve) (Zone of inhibition in mm)	20				15				15				10			

REFERENCES

- Andallu B., Suryakantham V., Lakshmi Srikanthi B., Reddy G.K. (2001) Effect of mulberry (*Morus Indica*) therapy on plasma erythrocyte membrane lipids in patients with type 2 diabetes. Clin. Chim. Acta. **314**, 47-53.
- Andallu B., Varadacharyulu N.C. (2003) Antioxidant role of mulberry (*Morus indica* L. cv. Anantha) leaves in streptozotocin-diabetic rats. Clin. Chim. Acta **338**, 3-10.
- Doi K., Kojima T., Fujimoto Y. (2000) Mulberry leaf extract inhibits the oxidative modification of rabbit and human low-density lipoprotein. Biol. Pharm. Bull. **23** (9), 1066-1071.
- Jang M.H., Kim H., Shin M.C., Lim B.V., Lee T.H., Jung S.B., Kim C.J., Kim E.H. (2002) Administration of folium Mori extract decreases nitric oxide synthase expression in the hypothalamus of rats. Jpn. J. Pharmacol. **90** (2), 189-192.
- Lokegaonkar, S. P and Nabar B.M (2010). Inhibition of Strptococcal biofilms using *Morus alba* leaf extract. J. of Microbial World. **12**(2): 161-167.
- Perez ,C., Paul,M and Bazerque, P. 1990.Antibiotics assay by agar-well diffusion method. Acta.Biol.Med.Exp.**15**:113-115
- Kamboj, V.P. 2000. Herbal medicine. Current Science **.78(1)**:35-39.
- Carvalho J.C.T., Vignoli, V.V., De Souza, G.H.B., Ujikawa, K.Neto,J.J and Acevedo, C. 1999.Antimicrobial activity of essential oils from plants used in Brazilian popular medicine. Acta Horticultrae.**501**: 77-81.
- Wiar, C., Mogana, S., Khalifah, S., Mahan, M., Ismail, S., Buckle, M., Narayana , A.K and Sulaiman, M. 2004. Antimicrobial screening of plants used for traditional medicine in the state of Peninsular Malaysia. Fitoterapia.**75(1)**: 68-73.
- Kaushik, P. 2003. Haridra(Turmeric): Antibacterial potential. Chowkhamba Sanskrit Series Office.1-124.
- Manjula, A.C and Shubha. 2010. Evaluation of heat stable proteins, total soluble proteins and antibacterial properties of *Costus pictus* D.Don. The Bioscan .**5(1)**:160-170.
- Manjula A.C., Shivashankarappa, L.H and Shubha 2011.Antibacterial activity of protein extracts of selected mulberry varieties. The Bioscan .In press.