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Evaluation of Antimicrobial Properties of *Morus alba Grown* on Silkworm Litter

Manjula A C[*], Shivashankarappa L H[†], Asma Saqib[‡] , Vijay Deep [§] and Rohini Keshava [**]

Abstract:

Antibiotics provide the main basis for treating infectious diseases. Hence, there is an increase in the investigations on plants as a source of human disease management. *Morus alba* belonging to family Moraceae has been reported to possess several medicinal properties. In the present study the antimicrobial properties of mulberry plant grown in different conditions have been analysed. The main aim of the study was to compare the differences in the antimicrobial properties of mulberry grown on different organic manures such as farmyard manure and silkworm litter. The antimicrobial properties of aqueous, ethanolic and methanolic leaf extracts were tested against pathogenic bacteria such as *E.coli* and *Pseudomonas* and fungi such as *Aspergillus niger* and *Penicillium notatum*. Significant zone of inhibition were obtained for both bacterial pathogens when treated with extracts of mulberry grown on silkworm litter. Whereas with respect to fungi, FYM treated plant leaf extracts showed significant antifungal properties.

[*] Maharani Science College for Women (MSCW), Palace Road, Bengaluru, Karnataka, India; dracmanjulam3@gmail.com. (*Corresponding author)*
[†] (MSCW), Palace Road, Bengaluru, Karnataka, India
[‡] (MSCW), Palace Road, Bengaluru, Karnataka, India
[§] Maharani Lakshmi Ammanni College, Malleswaram, Bengaluru, Karnataka, India
[**] Department of Sericulture/Life Sciences, Bangalore University, Bengaluru, Karnataka, India

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Introduction

In developing countries like India, contagious diseases caused by microorganisms account for maximum health problems. The indiscriminate use of allopathic antimicrobial drugs has resulted in microbial resistance to these drugs. Hence researchers are keen on biologically active compounds isolated from plants for the elimination of pathogenic microorganisms because of the resistance that they have developed to antibiotics (Hunter and Reeves, 2002; Prashanth *et al.*,2003; Woldemichael *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).

The plants are being used from ancient times to cure diseases. Medicinal plants are immensely contributing to the primary health care of human beings from time immemorial. Medicinal plants are natural composite sources that act as new anti-infectious agents (Ushimaru *et al.*2007*)*. They are used against many ailments which includes infectious diseases caused by bacteria.

Morus alba has been reported to possess several medicinal properties. In traditional Chinese medicine, the fruit of mulberry is used to treat premature greying of hair, to "tonify" the blood, and to treat constipation and diabetes. The bark is used to treat cough, wheezing, edema, and to promote urination. It is also used to treat fever, headache, red dry and sore eyes. The root bark of mulberry has been used as a traditional medicine in Asian countries and exhibits antibacterial activity against food poisoning micro-organisms. Using activity against S. mutans in bioassay-guided fractionation of a methanol extract of dried root bark, and organic solvent fractions of this extract, the active antibacterial constituent was identified as kuwanon G. The compound displayed an MIC of 8 μg ml–1 against S. mutans, which was comparable to chlorhexidine and vancomycin (1 μg ml–1). Time-kill assays indicated that S. mutans was completely inactivated by 20 μg ml–1 kuwanon G within 1 min. while tested against other bacteria it was observed that the compound displayed preferential antimicrobial

activity against cariogenic (causing dental caries) bacteria. Electron microscopic examination of S. mutans cells treated with kuwanon G indicated that the mode of antibacterial action was inhibition or blocking of cell growth, as treated cells showed a disintegrated surface and an unclear cell margin (Kikuchi et.al. 2010). Hypolipidemic and antioxidant effects from freeze-dried powder of mulberry fruit have been reported (Zhang et.al. 2009). Neuroprotective effects of the mulberry fruit have been shown in *in vitro* and *in vivo* studies (Wang et.al. 2011). Albanol A, a compound isolated from the root bark extract of *M. alba*, is shown to be effective for treatment of leukemia (Kim et.al. 2010). The compounds Moracin M, steppogenin-4′-O-β-D-glucoside and mulberroside A isolated from the root bark of Morus alba L. have been reported to produced hypoglycemic effects (Nade et. al. 2009). Mulberroside A, a glycosylated stilbenoid, is reported to be useful in the treatment of hyperuricemia and gout (Naowaboot et.al. 2009a; Naowaboot et.al. 2009b). Methanol extracts of Morus alba roots have shown adaptogenic activity, indicating its possible clinical utility as an antistress agent (Nade et. al. 2009).

In the present study the antimicrobial properties of mulberry plant grown in different conditions have been analysed. The main aim of the study was to compare the differences in the antimicrobial properties of mulberry grown on different organic manures such as farmyard manure and silkworm litter.

Materials and methods:

Microorganisms: *Escherichia coli* and *Pseudomonas* procured from Victoria Hospital, Bangalore. The fungal species *Aspergillus niger* and *Pencillium notatum* were obtained from Department of Microbiology, Maharani's Science College for women, Bangalore. Mulberry leaves were obtained from the Mulberry garden at Maharani's Science College for women, Bangalore.

Preparation of Mulberry leaf Extracts:

Aqueous extracts: Young fresh leaves of the mulberry plants were collected from healthy plants. The leaves were properly washed under running tap water and rinsed with distilled water. The leaves were first cut into small pieces. 10 grams of the leaf material

was weighed and crushed with distilled water. It was then centrifuged at 10,000g for 10 min and the supernatant was collected. The pellets were washed twice with distilled water and pooled supernatants were used as source. The aqueous extracts of the plants were autoclaved at 121^{o}C for 15 min and cooled down to the room temperature before inoculating into the agar plate (Kunjal Bhatt et. al. 2003).

Ethanolic and Methanolic extracts: Air dried plant materials were ground into a fine powder using a commercial blender. Powdered material was subjected to hot (Soxhlet) extraction using methanol and ethanol as solvents. For hot (Soxhlet) extraction, twenty five grams of dried powder of plant material was extracted with 250 ml of the solvent. The extracts were then distilled, evaporated and vacuum dried. The crude extracts thus obtained were used for assay of antibacterial activity (Kambli et.al. 2014).

Anti-microbial activity assay: Antimicrobial assay was carried out by well diffusion method (Perez *et al.,* 1990). The bacteria were grown in the Mueller Hinton agar media at 37^{0}C and maintained at -4^{0}C. The Sabouraud dextrose agar (SDA) was used for the fungi. The sterile media containing agar was poured into the sterilized Petri plates and allowed to solidify at room temperature. 100µl of microbial suspension was spread on the solidified medium. Wells were made in the medium using cork borer. Different concentrations of the extract were applied. The plates were then incubated for 24hrs at 37^{0}C. Inhibition zones formed on the medium were evaluated in mm. DMSO was used as the control. The standard antibiotic tetracycline was used as the positive control.

Results and discussion:

Table 1: Antibacterial activity of Mulberry leaf extracts.

	Escherichia coli				*Pseudomonas*			
Concentration of Extracts (µg/ml)	25	50	75	100	25	50	75	100
	Zone of Inhibition (mm)							
Sample 1 (Mulberry grown on silkworm litter)								
A (Ethanolic extract)	08	09	10	12	08	10	14	17
B (Methanolic extract)	00	10	12	14	15	12	15	13
C (Aqueous extract)	06	12	14	16	08	10	16	14
Sample 2 (Mulberry grown on FYM)								
A (Ethanolic extract)	00	06	09	12	08	06	10	12
B (Methanolic extract)	08	10	14	20	06	08	09	07
C (Aqueous extract)	12	14	19	17	03	05	13	15
Sample 3 (Untreated mulberry)								
A (Ethanolic extract)	10	15	17	19	08	10	12	15
B (Methanolic extract)	13	18	14	13	07	13	14	17
C (Aqueous extract)	12	11	10	10	08	12	16	14

Table 2: Antibacterial activity of standard antibiotic tetracycline.

Antibiotic	*Escherichia coli*	*Pseudomonas*
Tetracycline (10µg)	Zone of Inhibition (mm)	
	14	17

Table 3: Antifungal activity of Mulberry leaf extracts.

	Aspergillus niger				*Pencillium notatum*			
Concentration of Extracts (µg/ml)	25	50	75	100	25	50	75	100
	Zone of Inhibition (mm)							
Sample 1 (Mulberry grown on silkworm litter)								
A (Ethanolic extract)	08	09	10	12	00	15	17	10
B (Methanolic extract)	00	10	12	14	15	12	15	13

C (Aqueous extract)	06	12	14	16	08	06	12	10
Sample 2 (Mulberry grown on FYM)								
A (Ethanolic extract)	00	06	09	12	12	14	16	15
B (Methanolic extract)	08	10	14	20	08	10	14	19
C (Aqueous extract)	12	14	19	17	11	10	13	15
Sample 3 (Untreated mulberry)								
A (Ethanolic extract)	10	15	17	19	06	07	09	13
B (Methanolic extract)	13	18	14	13	10	13	14	15
C (Aqueous extract)	12	11	10	10	05	13	10	13

The leaf extracts of mulberry leaves was effective against all the bacteria studied with inhibition zone ranging from 8 mm to 20 mm with concentrations of 25, 50, 75 to 100 µg/ml of the leaf extracts. The highest zone of inhibition observed for *Escherichia coli* and *Pseudomonas* were 20 mm and 17 mm (Table 1). These were observed for the methanolic and ethanolic extracts of mulberry grown on FYM and mulberry grown on silkworm litter respectively. Untreated mulberry leaf extracts also recorded a zone of inhibition of 17 mm for *Pseudomonas*. Among the fungal species the highest zone of inhibition recorded were 20 mm and 19 mm for *Aspergillus niger* and *Pencillium notatum* respectively (Table 3). Both of these were recorded for the methanolic extracts of FYM treated mulberry. The highest zones of inhibition in all the cases were observed at 100 µg/ml concentration of the extracts. All the three extracts showed antimicrobial activities, however the highest zones were observed for the leaf extracts of mulberry treated with silkworm litter and FYM. Which suggests that mulberry treated with organic manures such as silkworm litter and FYM tend to have better antimicrobial properties. In addition, the zone of inhibitions for *Escherichia coli* and *Pseudomonas* on application of the standard antibiotic tetracycline were 14 mm and 17 mm respectively (Table 2). These results are comparable with those observed on the application of the leaf extracts on these organisms.

There is an ever increasing demand for mulberry as it is the sole food of silkworm. In addition, it has potent medicinal properties and can be effective source of natural therapeutics which help in overcoming the systemic side effects of synthetic drugs. Usage of

silkworm litter in the commercial cultivation of mulberry has several advantages as it enhances the quality and quantity of plants and reduces use of chemical fertilizers. Antimicrobial activity of protein extracts from different mulberry varieties has been reported (Manjula et.al. 2010). Increased growth rate, antioxidant activity and NPK content has been reported in medicinal plants grown on biofertilizers (Shuba et.al. 2013).

The overall results suggest mulberry to be a rich source of antimicrobial compounds and further phytochemical evaluation is essential to identify the specific compounds having the antimicrobial potential.

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