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Antibacterial activity of *Moringa Oleifera* (drumstick) root bark

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ABSTRACT

The antibacterial activity of different extracts of M.oleifera root bark was investigated against Staphylococcus aureus, Escherichia coli, Salmonella gallinarum and Pseudomonas aeruginosa in vitro . Both the gram-positive and gram-negative organisms showed variable sensitivity to different extracts of M.oleifera root bark in organic solvents like methanol, acetone, ethyl acetate and chloroform and in inorganic solvent, water. In general, ethyl acetate and acetone extracts showed maximum antibacterial activity. The aqueous extract had minimum antibacterial activity against the test organisms.

Keywords: *Moringa oleifera*, antibacterial activity, inhibition zone.

INTRODUCTION

Bacteria are listed at first position among the microorganisms causing opportunistic diseases [1], innumerable antibacterial agents are currently employed in treating bacterial infections. However, the widespread and indiscriminate use of antibacterial agents resulted in development of drug resistance among many virulently pathogenic bacterial species [2]. Many of the currently used antibacterials are associated with adverse effects such as toxicity, hypersensitivity, immunosuppression, and tissue residues posing public health hazard. Further, the newer broad spectrum antibiotics are cost prohibitive and are not within the reach of poor Indian farmer. These disadvantages undermine the therapeutic utility of the currently

available antibacterials and thus necessitating the need for finding alternative remedies for treatment of bacterial diseases.

As the global scenario is now changing towards the use of non- toxic and eco-friendly products, development of modern drugs from traditional medicinal plants should be emphasized for the control of various human and animal diseases. *Moringa oleifera* (Drumstick) is one such plant which is reported to possess several medicinal properties. The different parts of this plant viz. leaves, stem bark, root bark, flowers, fruits and seeds are used in the indigenous systems of medicine for the treatment of variety of human ailments and some parts are also eaten as vegetable [3-4]. During recent years considerable work has been done to investigate the pharmacological actions of the leaves and seeds of *Moringa oleifera* on scientific lines but only limited work has been reported so far on antibacterial activity of *Moringa oleifera* root bark though it is reported to possess varied medicinal properties. Therefore, it was considered worthy to investigate the antibacterial activity of *Moringa oleifera* root bark.

EXPERIMENTAL SECTION

Plant Material:
Fresh root bark of *M. oleifera* (Drumstick) was procured in bulk in the month of March-April from near and around the Veterinary College campus, Anjora, Durg, India.

Preparation of extract:
Fresh root bark in bulk was locally obtained from *Moringa oleifera* plants. The barks were cleaned and shade-dried under a fan at room temperature. The dried root bark were ground into a fine powder with the help of an electrical grinder.. The powdered root bark *of M.oleifera* was processed to obtain cold extracts as described below:

Fifty gm of the powder was taken in each of the five 500 ml capacity conical flasks. Two hundred ml of water and organic solvents viz. methanol, acetone, ethyl acetate and chloroform were added separately to each flask and kept in a refrigerator for 72 hours for maceration. The contents of the flasks was shaken intermittently daily during the maceration period. After the end of 72 hr, the contents of the flasks were filtered through Whatman No.1 filter paper. The filtrates were taken in previously weighed evaporating petri dishes and kept under a fan. After complete evaporation of the solvents, the weight of the extracts was recorded. The extracts were transferred to screw cap vials to be preserved in a refrigerator for use whenever required.

Test Organisms
Pure cultures of pathogenic strains of *Escherichi coli*, *Staphylococcus aureus*, *Salmonella gallinarum* and *Pseudomonas aeruginosa* were obtained from the Post-Graduate Institute of Veterinary and Animal Sciences, Akola (MAFSU, Nagpur), where the bacterial cultures were originally obtained from Chandigarh (IMTCh). They were sub-cultured on nutrient agar and in nutrient broth and maintained in the Department of Veterinary Microbiology, College of Veterinary Science & AH, Anjora, Durg, India.

Test method
The different extracts obtained from the powder of *M. oleifera* were screened for antibacterial activity *in vitro* against the four bacteria mentioned above following disc diffusion method. [5-6].

Antibacterial assay

The blank discs of 6.25 mm diameter were punched from filter paper of uniform thickness and sterilized by heat.. The blank discs were separately impregnated with each of extract . The bacteria were grown in nutrient broth, incubated at 37^{O}C overnight. One ml of the broth culture of each bacterium was spread over the nutrient agar taken in glass Petri dishes aseptically. The extract impregnated discs and the reference antibiotic ciprofloxacin disc were placed on the inoculated nutrient agar in the Petri dishes and incubated at 37^{O}C. After 18 hr incubation the zones of inhibition of bacterial growth around the discs were measured.

Statistical method:

Fisher's t test [7] was used for statistical interpretation of relative zones of inhibition of bacteria by different extracts or ciprofloxacin.

RESULTS AND DISCUSSION

The result indicated that *E.coli* were sensitive to all the extracts studied. The organism was found to be equally sensitive to ethyl acetate, acetone and chloroform extracts and had the maximum antibacterial activity against *E.coli* among the extracts. The aqueous extract had minimum antibacterial activity against *E.coli*. The sensitivity of *E.coli* to methanol extracts was also similar to that of aqueous extract. *Staph. aureus* was sensitive to all the extracts studied. The ethyl acetate and acetone extracts had maximum antibacterial activity against *Staph. aureus* in comparison to the other extracts. *Staph. aureus* showed moderate and equal sensitivity to methanol and chloroform extracts in comparison to other extracts. The aqueous extract had minimum antibacterial activity against *Staph. aureus* in comparison to other extracts. The study revealed that *S. gallinarum* was also sensitive to all the extracts studied . The ethyl acetate and acetone extracts showed maximum antibacterial activity against *S. gallinarum*. The bacteria showed moderate and equal sensitivity to methanol and chloroform extracts. The aqueous extract had minimum antibacterial activity against the above bacteria. *P.aeruginosa* was also sensitive to all the extracts. The ethyl acetate, acetone and methanol extracts showed maximum and equal antibacterial activity against *P.aeruginosa*. The bacteria showed lower sensitivity to chloroform extract. The aqueous extract had minimum antibacterial activity against the above bacteria. However, ciprofloxacin which was considered as standard drug showed maximum activity against all the four test organisms.

The disc diffusion study revealed that both the Gram +ve and Gm –ve organisms showed variable sensitivity to different extracts of *M. oleifera*. In general, ethyl acetate and acetone extracts showed maximum antibacterial activity against *Escherichia coli, Staphylococcus aureus, Salmonella gallinarum* and *Pseudomonas aeruginosa*. The sensitivity of the above bacteria to methanol and chloroform extracts was in between the ethyl acetate/ acetone and aqueous extracts. The aqueous extract had minimum antibacterial activity against all the above bacteria among the extracts. However, ciprofloxacin showed highest antibacterial activity against all the test organisms.

The rough estimate of sensitivity pattern of the test bacteria against the extracts in comparison to ciprofloxacin is explained in Table 2. The test bacteria were categorized as very less sensitive (+), less sensitive (++), moderately sensitive (+++) and highly sensitive (++++) basing on the relative zones of inhibition. *Staph. aureus, S. gallinarum and P. aeruginosa* were moderately sensitive to all the extracts (except aqueous extract) and very less sensitive to aqueous extract whereas *E. coli* was less sensitive to ethyl acetate, acetone

and chloroform extracts and very less sensitive to methanol and aqueous extracts. All the bacteria were highly sensitive to ciprofloxacin.

The antibacterial potential of different extracts of *M. oleifera* specially of those of ethyl acetate and acetone extracts, demand further *in vitro* and *in vivo* studies to exploit their antibacterial action in the treatment of bacterial diseases of man and animals.

Table 1: Zones of inhibition of bacteria by extracts of M. oleifera root bark and ciprofloxacin

Extract	Zones of Inhibition (mm) ± SE*			
	E. coli	*Staph. Aureus*	*S. gallinarum*	*P. aeruginosa*
Methanol	09.66 ± 0.33[a]	15.66 ± 0.33	15.33 ± 0.66	17.00 ± 0.57
Acetone	13.33 ± 0.33	18.00 ± 0.57[a]	19.00 ± 0.57[a]	18.66 ± 0.33
Chloroform	13.00 ± 0.57	16.66 ± 0.33	16.00 ± 0.57	15.00± 0.57
Ethyl acetate	13.66 ± 0.33	19.66 ± 0.88[b]	20.00 ± 0.57[b]	19.00 ± 0.57[a]
Aqueous	08.33 ± 0.33[b]	08.33 ± 0.66[c]	07.66 ±0.33[c]	07.66 ± 0.33[c]
Ciprofloxacin	33.33 ±0.88[d]	30.66 ± 0.66[d]	29.33 ± 0.66[d]	29.00 ± 1.00[d]

** Mean of three observations*

b : Significantly higher than chloroform extract (P<0.05)

a : Significantly higher than chloroform extract (P<0.01)

c : Significantly lesser than methanol, acetone, chloroform and ethyl acetate extracts (P<0.001)

d : Significantly higher than all the extracts (P<0.001)

Table 2: Sensitivity pattern of bacteria to M. oleifera root bark extracts and ciprofloxacin

Extract Discs	Sensitivity Pattern			
	E. coli	*Staph. Aureus*	*S. gallinarum*	*P. aeruginosa*
Methanol	+	+++	+++	+++
Acetone	++	+++	+++	+++
Chloroform	++	+++	+++	++
Ethyl acetate	++	+++	+++	+++
Aqueous	+	+	+	+
Ciprofloxacin	++++	++++	++++	++++

+ : Very less sensitive (zone of inhibition between 5 and 10 mm)

++ : Less sensitive (zone of inhibition between 11 and 15 mm)

+++ : Moderately sensitive (zone of inhibition between 16 and 20 mm)

++++ : Highly sensitive (zone of inhibition more than 20 mm)

The present observation of antibacterial efficacy of *Moringa oleifera* root bark is also supported by the earlier observations of antibacterial activity of extracts of different parts *Moringa oleifera* [8-10].

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