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ASSESSMENT OF PHYTOCHEMICAL AND ANTI-MICROBIAL ACTIVITY OF *MORINGA OLEIFERA LAM* AGAINST PATHOGENIC BACTERIA

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ABSTRACT

Antibacterial activities of aqueous, ethanol, petroleum ether and acetone extract of pod of *Moringa oleifera* (Shevga) were studied. The aqueous leaf extract of *Moringa oleifera* showed prominent antibacterial activities against *Psedudomonas aeruginosa, Salmonella typhimurium* and *E. coli.*, moderate activity against *Staphylococcus aureus*, *Salmonella typhi* and least activity against *Enterobacter aerogens* and *Proteus vulgaris*. The aqueous extract showed maximum the acetone and petroleum ether extract moderate and ethanol extract least antimicrobial activities. Phytochemical screening revealed the presence of glucosinolates, phenolic (flavonoids, anthocyanin, cinnamate, proanthocyanidin) leaves contain 4-(aL-rhamnopyranosyloxy) benzylglucosinolate, kaempfrol-3-glycoside sugar, D-Mannose and tannin. These antibacterial properties support its traditional use of leaves and pod in the treatment of enteric infection.

Key words: *Moringa oleifera, Psedudomonas aeruginosa, Salmonella typhimurium, E. coli, Staphylococcus aureus, Salmonella typhi, Enterobacter aerogens, Proteus vulgaris,* Infection.

INTRODUCTION

From time immemorial, men depend on plants as medicine. The plant kingdom represents a rich store house of organic compounds and many of which have been used for medicinal purposes and could serve as lead for the development of noval agent having good efficacy in various disorders.

In India, use of different parts of several vogue from ancient time and inherited traditionally. The root and bark of *Moringa oleifera* (Moringaceae) have been used in the

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indigenous system of medicine in India for the treatment of heart complaint, eye diseases[1]. The leave and seed extracts of *Moringa oleifera* show antimicrobial activity against *pseudomonas* and *Staphylococcus aureus*[2]. The presence of active principle flavonoids phenolic compound, glucosinotes, L-rhamnoside anisaldehyde were demonstarated in leaves[3]. The seed shows presence of phenylacetonitrite[4].

The enteric or diarrheal infection by water are major public health problems in developing countries and contribute to the death of 3 to 6.1 million children annually. Enteric bacteria comprised of *Salmonella spp., Shigella spp., Proteus spp., Klebsiella spp., E. coli, Pseudomonas spp., Vibrio cholerae* and *Staphylococcus aureus*, which are major etiologic agents of sporodic and epidemic diarrhea both in children and adult[5]. W.H.O 1993 reported that 85% population rely mainly on traditional therapies involving the use of plant extracts or their active constituents[6]. The use of medicinal plant in India contributes significantly in primary health and it is interesting to determine, whether actual pharmacological uses are merely based on folkore.

EXPERIMENTAL

Materials and method

The plants of *Moringa oleifera* were collected from Akola city and authenticated by Pharmacognosy Department, SGSPS, Institute of Pharmacy, Akola.

The fresh leaves/pod of *Moringa oleifera* were washed with water and the 0.5% $HgCl_2$, sterile water and grinded into mortar with pestle. The 50 g fresh leaves/pod were suspended in the 200 mL water and various popular organic solvents and extracted in Soxhlet apparatus and vacuum dried.

Amount of dry recovered powder extracts are recorded (50 g of original leaves and pod). This dry mass of various extract *Moringa oleifera* served for further experimentation.

Bacterial culture

The standard pathogenic bacterial cultures were procured from P.G. Department of Microbiology, Sant Gadge Baba Amravati University, Amravati,and used in the present study (Table 1). The bacteria rejuvenated in Muller-Hinton broth (Hi-media) at 37^{o}C for 18 hrs and then stocked at 40^{o}C in Muller-Hinton Agar subculture prepared from the stock for bioassay. A loopful culture was inoculated in 10 mL of sterile nutrient broth at 37^{o}C for 3-4

hos. Turbidity of culture was standardized to 10^5 CFU with the help of SPC and turbidometry.

Table 1: Bacterial culture used in study

Bacterial pathogen	MTC number
Escherichia coli (*E. coli*)	452
Staphylococus aureus (*S. aureus*)	87
Salmonella typhi (*S. typhi*)	733
Salmonella typhimurium (*S. typhimurium*)	98
Proteus vulgaris (*P. vulgaris*)	426
Pseudomonas aeruginosa (*P. aeruginosa*)	424
Enterobacter aerogens (*E. aerogens*)	111

Agar disc diffusion antibiotic activities

For antibacterial properties, 0.1 mi bacterial suspension of 10^5 CFU mL^{-1} was uniformly spread on Muller-Hinton plate to form lawn cultures. The aqueous, petroleum ether, acetone and ethanol extracts were prepared in their respective solvents in such a manner that ultimate amount (in dry form) in each disc came to 10 mg, 8 mg, 6 mg, 4 mg, and 2 mg. The blotting paper discs (8 mm diameter) were soaked in various diluted extracts, dried in oven at 50^0C to remove the excess of solvent and tested for their antibacterial activity against pathogenic bacteria by disc diffusion technique. After incubation of 24 hours at 37^0C, zone of inhibition of growth was measured in mm. Tetracycline 10 μg (Hi-Media disc) was used as positive control while disc soaked in various organic solvents and dried were placed on the lawns as negative control.

RESULTS AND DISCUSSION

During the past decades, traditional systems of medicine have become increasingly important in view of their safety. Several antibiotics are currently been used to treat a variety of infectious human diseases have limited antimicrobial spectrum and development of drug resistance in pathogen lead to serious ill effects. Plant extracts have broad spectrum antimicrobial activity, easily available, effective against human infection and also non-toxic to the human beings.

The present study was conducted to investigate antibacterial properties of pod of *Moringa oleifera.* Herbal medicine plays a fundamental role in tradition medicine in rural areas of India.

The amount of dry extracts recovered from various solvents are shown in Table 2. Antibacterial activities of aqueous, petroleum ether, acetone and ethanol extracts of leaves of *Moringa oleifera* were studied. The aqueous extract showed prominent antibacterial activities against *Pseudomonas aeruginosa, Salmonella typhimurium and Escherichia coli,* where as it was moderate against *Staphylococus aureus, Salmonella typhi* and least active against *Enterobacter aerogens and Proteus vulgaris.*

Table 2: Average recovery of dry extract from 50 g of Pod

Type of extract	Amount (g)
Aqueous	34.67
Petroleum ether	28.12
Acetone	29.64
Ethanol	30.16

The petroleum ether extract showed prominent antibacterial activities against *Pseudomonas aeruginosa, Salmonella typhimurium* and *Staphylococus aureus* where as it was moderate against *Salmonella typhi* and *Proteus vulgaris* and least against *Enterobacter aerogens, Pseudomonas aeruginosa* and *Escherichia coli*.

The acetone extract showed maximum activity against Escherichia *coli,* moderate against *Pseudomonas aeruginosa, Salmonella typhi* and *Proteus vulgaris* and least against *Salmonella typhimurium, Enterobacter aerogens* and *Staphylococus aureus.*

The ethanol extract showed maximum activity against *Salmonella typhimurium, Salmonella typhi* and *Pseudomonas aeruginosa,* moderate against *Escherichia coli* and *Proteus vulgaris* and least against *Enterobacter aerogens* and *Staphylococus aureus* (Table 3).

The aqueous extract showed maximum antibacterial activities against the tested bacterial pathogens due to higher solubility of antibacterial principle in aqueous as compared to organic solvents. Moreover, the pods are highly fibrous and it is difficult to ground it, and it may be difficult to extract the antibacterial principle by organic solvents.

Table 3: Antibacterial activity of *Moringa oleifera* Pod extracts against enteric pathogens (Zone of inhibition of growth in mm; average of 3 reading)

Bacterial pathogens	Aqueous extract			Petroleum ether extract			Acetone extract			Ethanol extract			Control		
	10 mg/disc	8 mg/disc	6 mg/disc	4 mg/disc	2 mg/disc	10 g/disc	8 mg/disc	6 mg/disc	4 mg/disc	2 mg/disc	10mg/disc	8 mg/disc	6 mg/disc	4 mg/disc	2 mg/disc	10 mmg/disc	8 mg/disc	6 mg/disc	4 mg/disc	2 mg/disc	Tetracycline	8 mg/disc	6 mg/disc	4 mg/disc	2 mg/disc
E. coli	24	21	20	19	17	20	18	16	15	14	30	27	24	20	17	24	23	18	14	10	16	-	-	-	10
S. aureus	21	19	17	16	14	25	21	19	17	16	21	19	17	15	14	22	20	17	15	14	17	-	10	-	11
S. typhi	21	17	16	14	12	22	20	17	16	15	26	21	18	17	15	26	25	20	16	10	18	-	10	11	10
S. typhimurium	25	22	21	18	17	27	25	23	20	17	21	18	15	18	12	26	23	20	18	11	15	-	11	12	10
P. vulgaris	20	19	17	15	14	22	19	16	14	12	26	21	19	17	15	23	20	17	14	12	16	-	-	10	-
P. aeruginosa	26	23	21	29	18	20	17	15	14	13	24	19	17	15	14	20	23	19	15	13	18	-	-	-	14
E. aerogens	20	18	15	14	13	20	17	15	14	13	16	17	15	14	13	22	20	16	14	10	15	-	-	-	10

The pods of *Moringa oleifera* contain various phytochemical components such as flavonoids, glycoside, phenolic compound, polysaccharide etc. (Table 4).

Table 4: Phytochemical analysis of *Moringa oleifera* pod

Phytochemical	Result
Alkaloid	Absent
Flavonoid	Present
Carbohydrate	Present
Glycoside	Present
Phenolic compound	Present
Nitriles isothiocynate	Present
Saponine	Absent
Volatile oil	Absent

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