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Evaluation of the antibacterial potential of *Moringa oleifera* and *Azadirachta indica* against some pathogenic microbes: A comparative study

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Abstract

The efficacy of petroleum ether and chloroform leaf extracts of *Azadirachta indica* and *Moringa oleifera* were studied against *Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhimurium, Bacillus subtilis* and *Klebsiella pneumoniae* for varying concentration of extracts of 200mg/ml, 150mg/ml, 100mg/ml, 50mg/ml and 25mg/ml, using disc diffusion method. It was compared with gentamycin 150mg/ml as standard. The petroleum ether extract showed maximum and equal inhibition on *Pseudomonas aeruginosa* and *Bacillus subtilis,* followed by *Proteus vulgaris, Klebsiella pneumonia* and *Escherichia coli* in a descending order in both the extracts. *Salmonella typhimurium* was found to be resistant to petroleum ether extract of both plants. The chloroform extract showed maximum inhibition on *Pseudomonas aeruginosa, Proteus vulgaris* and equal zone of inhibition was shown by *Bacillus subtilis, Klebsiella pneumonia, Salmonella typhimurium* where as minimum zone of inhibition was recorded in *Escherichia coli.* Overall chloroform leaf extract exhibited better antimicrobial potential against pathogens. Results indicate the potential of these plants for further work on isolation and characterization of the active principle responsible for antibacterial activity and its exploitation as therapeutic agent.

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INTRODUCTION

Azadirachta indica (Neem) is a tree belonging to the mahogany family Moringaceae. It is a native to

Indian subcontinent, growing in tropical and semi-tropical regions. In India, Neem is known as "the village pharmacy" because of its healing versatility and it has been used in Ayurvedic medicine for more than 4,000 years due to its medicinal properties. The bark is well documented for antioxidant properties [1] and leaves are also found to possess antitumor potential [2], antidiabetic activity [3] and anti-inflammatory activity [4]. Its flowers are also known to contain antifertility properties [5, 6]. Neem extract has antibacterial as well as antiviral properties [7-9].

Moringa oleifera (family Moringaceae) is commonly known as Drumstick tree, indigenous to Northwest India. Most of the parts of the plant possess antimicrobial activity [10, 11]. They are well known for their pharmacological actions and are used for the traditional treatment of diabetes mellitus [12], hepatotoxicity [13], rheumatism and venomous bites and also for cardiac stimulation [14]. Leaves of *M. oleifera* have been used as antiulcer, diuretic, anti-inflammatory and for wound healing [15, 16]. They are also used to treat anxiety, diarrhea, inflammation of the colon, skin infections, scurvy, intestinal parasites and many other conditions [17]. The objective of this study was to evaluate the bactericidal effect of extracts of *M. oleifera* leaves and compared to the extracts of leaves of *A. indica* on some common pathogens.

MATERIALS AND METHODS

Selection of plant

The plant *M. oleifera* and *A. indica* were selected for the study. Its young leaves were collected from PG Department of Botany, TM Bhagalpur University, Bhagalpur, Bihar, India. The collected leaves were identified and authenticated by the Dr. Aloka Kumari (Women Scientist, DST, Govt. of India), Plants Systematics Research Centre, University Department of Botany, TM Bhagalpur University, Bhagalpur, Bihar, India.

Leaf extract

Petroleum ether extracts:

The completely shade dried plant leaves of *M. oleifera* and *A. indica* were ground in mortar and pestle and extracted in a percolator with 95% Petroleum ether separately and about 100 ml of petroleum ether per gram of plant leaves powder were used. The Petroleum ether extract was then dried under a reduced pressure at 40°C. The dried extract was stored in sterile bottles for further use.

Chloroform extracts:

The dried leaf powders of both the plants were ground in chloroform (1g/100ml) separately. The solvent was removed using a rotary vacuum evaporator at 40°C to give a concentrated extract which was then freeze-dried for further use.

Micro-organisms

The strains of *Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhimurium, Bacillus subtilis* and *Klebsiella pneumoniae* were used for the study.

Inoculum preparation:

For the antibacterial tests, micro-organisms were grown overnight in Luria Bertani Broth followed by incubation at 37˚C.

Antimicrobial screening

Agar disc diffusion assay

Following the Kirby-Bauer, the antibiotic sensitivity was tested against each strain [18]. The antibiotic (specific concentration) impregnated, disc absorbs moisture from the agar and antibiotic diffuses in to the agar medium. The rate of extraction of the antibiotic from the disc is greater than the rate of diffusion as the distance from the disc increases there is a logarithmic reduction in the antibiotic concentration. Zone of inhibition (ZI) of bacterial growth around each disc is measured and the susceptibility is determined [9].

Medium

3.8g of Muller Hinton Agar is added to 100 ml distilled water and autoclaved at 121°C for 15 minutes at 15 lbs and poured in sterile petri plates up to a

uniform thickness of approximately 4mm and the agar is allowed to set at ambient temperature and used [9].

Inoculums

The micro-organisms were inoculated in peptone medium and incubated at 37°C for 3-4 hours and used as inoculums.

Method

The bacterial suspension was inserted with a sterile cotton swab. It was then rotated and compressed against the wall of the test tube so as to express the excess fluid. The surface of Muller Hinton Agar plate was inoculated with the swab. The swab was passed three times over the entire surface to ensure that the growth was uniform and confluent (or semi confluent). Standard disc of Gentamycin 150 mg/ml, 6 mm in diameter were used as positive control and the solvent used for preparing extract was used as negative control. The plates were incubated overnight at 37°C for 18-24 hours. Antimicrobial activity was evaluated by measuring zone of inhibition by using Hi Media zone scale.

DETERMINATION OF MEDIUM INHIBITORY CONCENTRATION

Micro dilution assay

The minimum inhibitory concentration was defined as the lowest concentration of the compound to inhibit the growth of micro-organisms [19]. Varying concentrations of the extracts (200mg/ml, 150mg/ml, 100mg/ml, 50mg/ml and 25mg/ml) were prepared. Standardized test organism of controls (0.1ml) was equally set up by using solvents and test organisms without extract. The minimum inhibitory concentration was recorded by the tube with least concentration of extract without growth after incubation.

RESULT

The findings of this study show maximum inhibition in *P. aeruginosa, P. vulgaris and B. subtilis* followed by *K. pneumoniae and E. coli* in the descending order when tested against 150mg/ml Gentamycin. The maximum zone of inhibition was produced by Petroleum Ether extract (PET) of *A. indica* against *P. aeruginosa* and equal in *P. vulgaris*, *B.* subtilis. It was found minimum inhibition in *E. coli.* Petroleum Ether leaf extract of *Moringa oleifera* show maximum inhibition in *P. aeruginosa* followed by *P. vulgaris and B. subtilis* whereas maximum inhibition was observed in *E. coli* (Table:1) Chloroform leaves Extract of *A. indica and M. oleifera* were compared with 150 mg/ml Gentamycin as standard. Chloroform extract of *A. indica* and *M. oleifera* shows maximum inhibition on *P. aeruginosa* followed *P. vulgaris* and an equal inhibition on *K. pneumonia, B. subtilis, S. typhimurium* and minimum inhibition on *E. coli* in descending order and in agreement with similar trend (Table:2).

DISCUSSION

Synthetic drugs used as antimicrobial agents have various side effects. Hence, herbal products can be used as an alternative to such synthetic drugs to minimize side effects. Azadirachta indica leaves possessed good antimicrobial activity. The extracts of Neem are well documented for medicinal purposes, could be useful for the growth inhibition of the carcinogenic bacterium, S. sobrinus [20]. Moringa oleifera leaves are also found to be a parallel alternative to A. indica. These antimicrobial principles are actually the defensive mechanism of the plants against different pathogens. It is speculated that the antimicrobial activities of bioactive compounds depend on interactions between their lipid components with the net surface charge of microbial membranes. Furthermore, the drugs might cross the cell membranes, penetrating into the interior of the cell and interacting with intracellular sites critical for antibacterial activity [21, 22]. Petroleum ether extract as well as chloroform extract of both leaves showed antimicrobial activity which certainly indicates that these extracts contain higher concentration of active antimicrobial agents

(Table:1,2). These may include alkaloids, glycosides, volatile oils or tannins [23-25]. The present study established M. oleifera leaves as one of the strong alternative as antimicrobial agent. The chloroform extracts of Moringa oleifera leaves were found to act as a better antimicrobial agent than petroleum ether extract. Further research work is suggested to identify and isolate the bioactive principle present in leaves responsible for antimicrobial properties of M. oleifera.

Table 1: *In vitro* activity of leaves of *Moringa oleifera* and *A. indica* in Petroleum Ether (PET) extract against some common pathogens

S. N.	Name of Pathogens	Gentamycin 150mg/ml (Std.)	PET Extract (*A. indica*)	PET Extract (*M. oleifera*)
1	*Escherichia coli*	11mm	8mm	5mm
2	*Pseudomonas aeruginosa*	16mm	12mm	9mm
3	*Salmonella typhimurium*	13mm	-	-
4	*Proteus vulgaris*	16mm	11mm	8mm
5	*Klebsiella pneumoniae*	14mm	10mm	7mm
6	*Bacillus subtilis*	15mm	12mm	8mm

Table 2. *In vitro* activity of leaves of *Moringa oleifera* and *Azadirachta indica* in Chloroform extract against some common pathogens

S. N.	Name of Pathogens	Gentamycin 150mg/ml (Std.)	Chloroform Extract (*A. indica*)	Chloroform Extract (*M. oleifera*)
1	*Escherichia coli*	11mm	7mm	6 mm
2	*Pseudomonas aeruginosa*	16mm	14mm	11mm
3	*Salmonella typhimurium*	13mm	9mm	8mm
4	*Proteus vulgaris*	16mm	11mm	10mm
5	*Klebsiella pneumoniae*	14mm	9mm	8mm
6	*Bacillus cereus*	15mm	9mm	8mm

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