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# EXTRACTS OF EDIBLE PODS OF *MORINGA OLEIFERA* LAM. (MORINGACEAE) AS NOVEL ANTIBACTERIAL AGENT AGAINST SOME PATHOGENIC BACTERIA

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## ABSTRACT

*Moringa oleifera* (Lam.) is well known for wide range of medicinal activities and contribution to dietary diversity. The present study focused on investigating the antibacterial potentiality of the crude extract and different solvent extracts of the pods of *M. oleifera* using agar well diffusion method against pathogenic gram negative bacterial strains namely *Pseudomonas fluorescens* and *Pseudomonas putida* and gram positive bacterial strains namely *Bacillus mycoides* and *Bacillus licheniformis*. The best result was showed by Petroleum ether extract against the said bacteria. M.I.C. of the aforementioned extract on gram negative bacteria *P. fluorescens* and *P. putida* were found to be 28.6 µg/ml and 32.0 µg/ml respectively while 39 µg/ml and 42 µg/ml values were obtained for gram positive bacteria *B. mycoides* and *B. licheniformis* respectively. The petroleum ether extract of *M. oleifera* pods may act as a novel and effective antibacterial agent.

**KEY WORDS:** ***Moringa oleifera*, Antibacterial activity, Minimum inhibitory concentration.**

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# INTRODUCTION

Bacteria are common inhabitants of both the surface and internal tissues of most organisms including plants, fishes and animals. The number of bacteria with reduced susceptibility to antibiotics along with various drug resistances is increasing day by day. The indiscriminate utilization of antibiotics, immunosuppressive drugs is worsening the situation. Moreover, in the developing countries, artificially produced chemical drugs are not only high- priced for the treatment of diseases but also often with enormous side effects. Besides, the bacterial diversity in any particular ecosystem is numerous[1]. Therefore, there is a need to search new eco- friendly strategies to manage microbial infections [2]. The demand on plant based therapeutics is rising in both budding and industrial countries due to the fact that those are natural products, non narcotic in nature, easily biodegradable with minimal ecological hazards, having no adverse side effects and easily available at reasonable prices. India is the largest producer of medicinal herbs and more or less 6,000 medicinal plants have been used in primary healthcare [3]. Nowadays, the researchers have discovered antibacterial properties from different parts of various botanicals against diverse pathogenic bacteria, [4,5,6,7]. Phytochemicals are also helpful to diminish vector borne diseases by reducing vector populations [8, 9, 10, 11]. However, only a few botanicals have enthused from the laboratory to field use, due to poor characterization and restriction of proper screenings. Therefore, to treat numerous diseases through herbal products, it is important to isolate the bioactive phytochemical compounds. *Bacillus licheniformis* is commonly allied with food spoilage and poisoning causing "ropy bread". Blemishing with this bacterium makes the bread sticky and stringy, developing a strong odour [12]. The issue of livestock abortions is the most serious environmental hazard identified in *B. licheniformis* [13]. *Bacillus mycoides* acts as a potent bacterial pathogen of channel catfish [14]. *Pseudomonas putida* was found to be associated with the ulceration on the dorsal surface of the fish causing 35% death of the rainbow trout[15]. *Pseudomonas fluorescens* affects the physiology of nerve cells and, in concurrence with recent clinical elucidation, suggests that *P. fluorescens* can behave as a pathogen[16]. It has also been reported to be an opportunistic pathogen in immune compromised fish like Koi[17]. The present study was carried out using an ethno pharmacologically important plant, *Moringa oleifera* (Lam) commonly known as Miracle tree or Horseradish tree or Ben oil tree [18]. It is a soft wooded, medium sized (10 m) widely grown, easily cultivable tree, found mainly in the tropical and subtropical regions [19]. All parts are used in for different nutritional and other purposes. The leaves possess highly nutritious value having a number of activities like anticonvulsant, antidepressant, antipyretic, anti-arthritic, anti-asthmatic, anti-inflammatory, analgesic, and neuro-protective in Alzheimer's disease [20, 21, 22]. Moreover the leaves of *M. oleifera* are used by the Indians in their herbal medicine as a hypocholesterolemic agent in obese patients [23]. The pods of *M. oleifera* can be cooked, or stored as a dried powder for many months reportedly without any major loss of its nutritional value [24]. The powdered form is utilized by the pregnant women and lactating mothers to improve their children's nourishment, especially in rural areas suffering from malnutrition [25]. *M. oleifera* seed has excellent coagulation properties for treating waste water [26]. *M. oleifera* pods were studied for free radicals scavenging abilities [27]. Over the past two-three decades, many reports have appeared in mainstream scientific journals recounting its nutritional and medicinal properties. Our present study concerns about the antibacterial potentiality of *M. oleifera* edible pods against few pathogenic bacteria viz. *B. licheniformis, B. mycoides, P. putida, and P. fluorescens* under laboratory condition. This is the first ever attempt to control the said bacteria with the petroleum ether extracts of *M. oleifera* edible pods as per our literature review is concerned.

# MATERIALS AND METHODS

### *Collection of Plant material*

The pods of *M. oleifera*, called "drumsticks", were properly collected from Burdwan district (23°16'N, 87°54'E), WB, India during spring (mid-March to mid- April 2013). They were taxonomically legitimated by Dr A. Mukherjee, Department of Botany, The University of Burdwan, Burdwan. The herbarium of the specimen has been kept in the Department of Zoology, The University of Burdwan, having the Voucher specimen no. GCZD- 09 Initially the pods were cleaned and rinsed with distilled water followed by drying on paper towel in the laboratory at 37 ± $^{o}$C for 24 h.

### *Test microorganisms*

Four bacterial strains namely, *P. fluorescens* (MTCC 103), *P. putida* (MTCC 1654), *B. mycoides* (MTCC 7343) and *B. licheniformis* (MTCC 530) were collected from Mosquito, Microbiology and Nanotechnology Research Units, Parasitology Laboratory, Burdwan. The bacteria were cultured in nutrient broth Hi-Media, M002 (Hi- Media Laboratories Limited Mumbai, India) at 37 °C and were maintained on nutrient agar slants at 4$^{0}$C with regular periods of subculture.

### *Antibiotics*

Antibiotic discs (Span Diagnostics Limited, Surat, India), of different concentrations were used during the present experiment. These were Amoxicillin (30µg), Kanamycin (30µg), Nalidixic acid (30µg) Chloramphenicol (30µg), Tetracycline (30µg), Norfloxacin (10µg), Gentamycin (10µg), Ampicillin (10µg), Penicillin G (10µg) and Ciprofloxacin (5µg).

### *Plant extracts preparation*

### *Crude extraction preparation*

Fresh pods of *M. oleifera* were rinsed in tap water followed by distilled water and soaked on a paper towel. Afterwards they were minced by mechanical grinder and the liquid was filtered by Whatman's no-1 filter paper. The filtrate was measured as stock solution (100% concentration) for the bioassay experiment. The concentrations (0.5%, 1.0%, 1.5%, 2.0% and 2.5%) were set up by adding up distilled water with the stock solution.

### *Differential solvent extraction*

For solvent extraction, fresh and clean pods of *M. oleifera* were air dried in shed for few days. 200 g dried pods were put into the column of the Soxhlet apparatus while 2 lit solvent was loaded into the solvent chamber in the ratio of 1:10. Three different solvents viz. petroleum ether, n-hexane and water were passed in a non-polar to polar fashion through the same column one after another respectively. The extraction period was 72 h for each solvent with maximum 8 h a day. Elutes were collected from chamber and made concentrated by evaporation in a rotary evaporator. The extractives were preserved at 4°C in a refrigerator for further bioassay.

### *Sensitivity Test*

Antibiogram was done by disc diffusion method [28] with commonly used antibiotics. The test microbes were removed from the slant aseptically with inoculating loops and transferred to 5.0 ml of sterile distilled water containing test tubes. Until the turbidity adjusted to 0.5 McFarland ($10^{8}$CFU/ml), sufficient inoculums were added. For each of the bacterium, one millilitre of the test tube suspension was added to the 15–20 ml of nutrient agar and transferred to the agar plate (9 cm in diameter). After cooling the inoculated agar plates at room temperature for 25 min, antibiotic sensitivity test discs were placed on the surface of solid agar. The plates were incubated for 24 h at 37° C. Clear zones of inhibition formed around the discs were measured and antibiotic sensitivity was assayed from the diameter of the inhibition zones (in mm). Zone diameters were interpreted as responsive, intermediate and resistant as per manufacturer's instructions.

### *Antibacterial Bioassay*

The antibacterial assay was conducted by agar well diffusion method [29]. The bacterial strains grown on nutrient agar at 37°C for 18 h were suspended in saline solution (0.85% NaCl) and adjusted to a turbidity of 0.5 Mac Farland standards ($10^{8}$ CFU/ml). The suspension was inoculated in 90 mm diameter Petri plates. Wells of 5 mm diameter were punched off after solidification

of the agar, and filled with 30 µl each of 2000 µg/ml extracts. The dissolution of the organic extracts was aided by 1% (v/v) dimethylsulphoxide (DMSO). DMSO was taken as control for solvent extracts and sterile distilled water was taken as control for crude extract. The plates were incubated for 24h at 37°C. Antibacterial activities were evaluated by measuring inhibition zone diameters. The experiments were repeated thrice.

### *Determination of Minimum Inhibitory Concentration (MIC)*

MIC was determined by dilution method as described by the National Committee for Clinical Laboratory Standards 1993 [27]. The cultures were diluted in Müeller-Hinton broth at a density adjusted to turbidity of 0.5 MacFarland standards. Equal volume of 0.5 ml of each extract (by serial dilutions from the suspension of n-hexane and petroleum ether plant extract stock solution) and nutrient broth were mixed in test tubes. Specifically 0.1 ml of standardized inoculums ($5 \times 10^5$ CFU/ml) was added to each tube. The tubes were incubated at 37°C for 24 h. Two control tubes were maintained for each test batch. These included antibiotic control (tube containing extract and the growth medium without inoculums) and bacterium control (the tube containing growth medium, physiological saline and the inoculums). The lowest concentration (highest dilution) of the extract that produced no visible bacterial growth (no turbidity) when compared with the control tube was regarded as MIC.

### *Statistical analysis*

The results are presented as mean ±SD. The data were analysed by using Excel and Easy plot software.

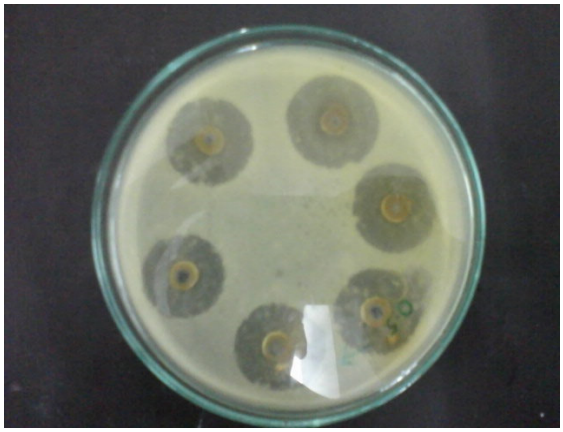
## RESULTS

Antibiogram assay of the test Gram positive and Gram negative bacterial strains were depicted in Table1 against some broadly used antibiotics. All the given data are calculated by taking the mean value of three sets of observations and rounded off for the sake of convenience. *P. fluorescens*, *P. putida, B. licheniformis* and *B. mycoides* strains were found to be resistant to several antibiotics like Ampicillin (10 µg), Penicillin-G (10 µg) etc. Susceptibility of all bacteria to Chloramphenicol (30 µg) was highest among all the antibiotics tested excluding *B. licheniformis*, which exhibited maximum sensitivity against tetracycline (30 µg). The petroleum ether extracts exhibited maximum antibacterial activity against *P. fluorescence,* followed by *P. putida, B. licheniformis* and *B. mycoides* (Table II).

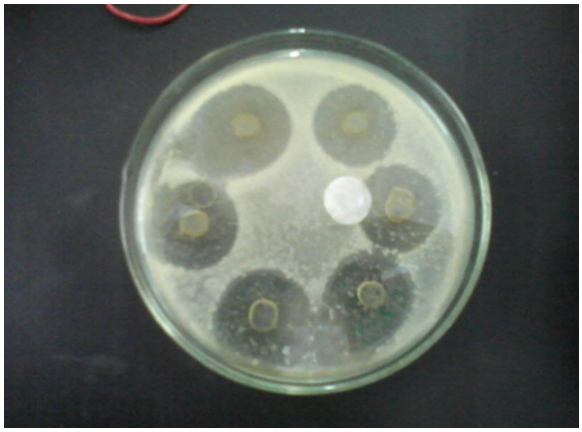
## DISCUSSION

The greatest antimicrobial activity was shown by petroleum ether extract in comparison to other solvent extracts. This verdict of result is fascinating, due to the fact that for the treatment of a bacterial infection, decoction of the plant parts is employed in the traditional method whereas, as per present study, preparing an extract with an organic solvent has revealed to provide a better antibacterial bustle. These explanation may be accepted for two reasons, firstly, presence of petroleum ether improves the nature of biological active components and its action; secondly, the stronger extraction capacity of PET could have produced a huge number of active constituents accountable for antibacterial activity. The prospective for developing antimicrobials from botanicals appears rewarding, as it will show the way for the development of a phytomedicine to act against microbes. Hence the pods of *M. oleifera* may be used to evaluate the bioactive natural products that will escort to the advancement of new pharmaceuticals. Thus, such a screening of various natural products and recognition of its active fractions must be well thought as a fruitful approach. Previously, Bhattacharya *et al.* [30] elucidated an array of 35 different bio-active compounds from the ethanol fraction of *M. oleifera* leaves. The extracts of pods were very much effective in concern to antibacterial potentiality; however, in vivo study on this medicinal plant is necessary to conclude several factors like toxicity of the active constituents, their side effects and diffusion in different body parts. The antimicrobial actions can be made superior if the active components are purified and sufficient dosage resoluted for proper administration.

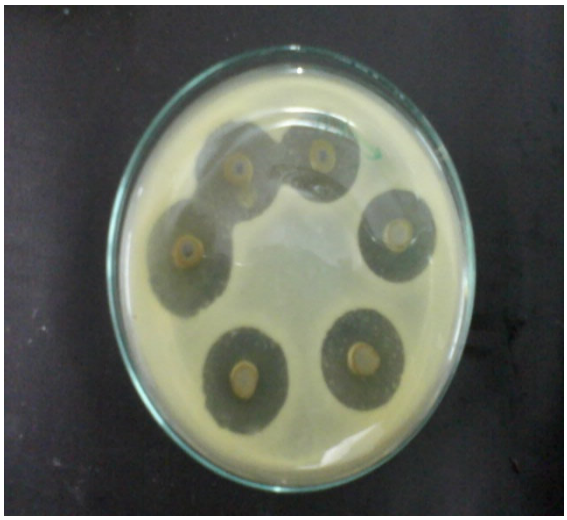
**Figure 1**
***Susceptibility of four reference bacterial strains to different concentrations of pods of Moringa oleifera in nutrient agar***



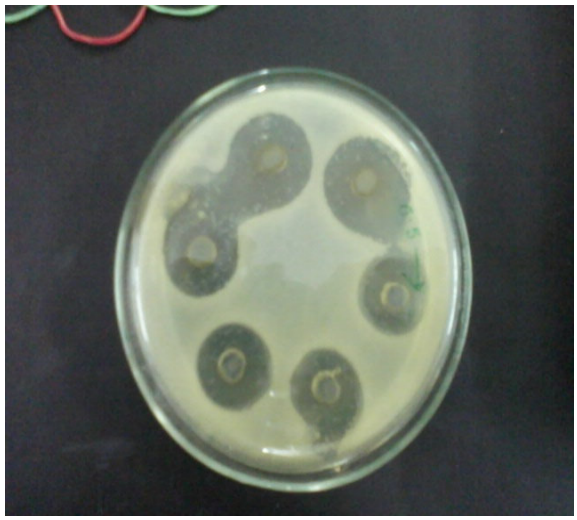
***Bacillus licheniformis***



***Bacillus mycoides***



***Pseudomonas fluorescens***



***Pseudomonas putida***

**Table 1**
***Susceptibility of four reference bacterial strains to some antibiotics in nutrient agar***

| Antibiotics (µg/ml) | Diameter of the inhibitory zones (mm) | | | |
|---|---|---|---|---|
| | *P. fluorescence* | *P. putida* | *B. mycoides* | *B. licheniformis* |
| Amoxycillin (30) | 15 | 0 | 0 | 9 |
| Kanamycin (30) | 27 | 13 | 11 | 22 |
| Chloramphenicol (30) | 30 | 25 | 20 | 22 |
| Nalidixic acid (30) | 0 | 11 | 0 | 20 |
| Tetracyclin (30) | 12 | 0 | 0 | 25 |
| Gentamycin (10) | 19 | 16 | 17 | 20 |
| Norfloxacin (10) | 24 | 16 | 8 | 0 |
| Ampicillin (10) | 0 | 7 | 0 | 0 |
| PenicillinG (10) | 0 | 0 | 0 | 0 |
| Ciprofloxacin (5) | 19 | 22 | 17 | 11 |

**Table 2**
***Antimicrobial sensitivity assay of crude and petroleum ether extracts of pods of Moringa oleifera***

| Pod extracts | Diameter of the inhibitory zones (mm) | | | |
|---|---|---|---|---|
| | *P. fluorescence* | *P. putida* | *B. mycoides* | *B. licheniformis* |
| Crude extract of pods | 17 | 9 | 11 | 14 |
| Solvent extract of pods | 29 | 28 | 21 | 23 |
| Sterile distilled water | 0 | 0 | 0 | 0 |
| Dimethylsulphoxide | 0 | 0 | 0 | 0 |

**Table 3**
***Minimum inhibitory concentration of bioactive fraction of Petroleum ether from pods of Moringa Oleifera***

| Bacterium | M.I.C. Value (µg/ml) |
|---|---|
| *Pseudomonas fluorescens* | 28.6 |
| *Pseudomonas putida* | 32 |
| *Bacillus mycoides* | 39.0 |
| *Bacillus licheniformis* | 42.0 |

## CONCLUSION

The pod extract of *M. oleifera* were proved to be a bioactive antibacterial agent which could be used for treatment of various diseases in future. On the other hand, isolation of individual compounds and their biological activities require desires to be uncovered further to enhance its pharmacological importance and open new opportunity in the field of research.

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