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Antibacterial activity of Indian medicinal plant- *Moringa oleifera* against MRSA and *Klebsiella* Spp. (ESBL) which are commonly isolated bacteria in hospital environments

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Abstract
Multi drug resistant bacteria have been increasing in the world in a more than expected pace. Medical fraternity and researchers are trying their best to find newer antibiotics to fight against such superbugs but not finding newer ones or the process is tedious. Nature has provided many a medicinal plants which may be used as a supplement along with moderately sensitive antibiotics which can eradicate multidrug resistant bacteria. *Moringa oleifera* leaves and bark extracts have been tested against multi drug resistant bacteria. There is a lacunae here to make use of these plant products and find newer molecules to treat multidrug resistant bacterial infections as the results are encouraging. *Moringa oleifera* ethanol extracts have shown good zone of inhibition with MRSA (25mm) and *Klebsiella* Spp. (21mm).

Keywords: ESBL: extended spectrum beta lactamase, MRSA: methicillin resistant staphylococcus aureus, spp: species. CLIA: clinical laboratory institute, MIC: minimum inhibitory concentration, MBC: minimum bactericidal concentration

Introduction
Plant materials remain an important resource to combat serious diseases in the world. Due to presence of most important bioactive constituents which are alkaloids, tannin, flavonoids and phenolic compounds [1]. Advances in identifying new sources of natural products with antimicrobial activities and expanding antibiotic chemical diversity are providing chemical leads for new drugs [2]. Nature has been a source of medicinal agents for thousands of years and about 80% of the world's population rely on traditional medicines for their primary health care [5].

Multi-drug resistant strains of MRSA and *Klebsiella* Spp. *(ESBLs)* are widely distributed in hospitals and are increasingly being isolated from community acquired infections [4] all this has resulted in severe consequences including increased cost of medicines and mortality of patients.

Antibiotic resistance is the ability of bacteria or other microbes to resist the effects of an antibiotic. Antibiotic resistance occurs when bacteria change in some way that reduces or eliminates the effectiveness of drugs, chemicals or other agents designed to cure or prevent infections. The bacteria survive and continue to multiply causing more harm. There are numerous plants and natural products which have antifungal, antibacterial and antiprotozoal activities that could be used either systemically or locally [3]. Neem leaves extract/paste is one of the commonly used antiviral agents used in chicken pox.

Indiscriminate use of antibiotics promotes development of antibiotic-resistant bacteria by mutation along with other mechanisms. Every time a person takes an antibiotic, sensitive bacteria are killed, but resistant germs may be left to grow and multiply.

Materials and Methods
MRSA was isolated from wound swab of an inpatient from Male Medicine ward and *Klebsiella* Spp. was isolated from sputum sample of a patient suffering from upper respiratory tract infection admitted in D.Y. Patil Hospital, Nerul. Navi Mumbai. Samples were collected and transported to Microbiology Laboratory by taking aseptic precautions and

processed for culture on Blood agar, chocolate and McConkeys agar. Biochemical tests were performed on the isolates for identification and confirmation. Antibiotic sensitivity by Kirby-Bauer disc diffusion test was performed to look for susceptibility pattern of each isolate. Plants/parts were collected, authenticated by experts, washed, dried and powdered. These products were subjected to Soxhlelet extraction. Once extracted theses are stored at 4-8 ^{0}C in leak proof containers for further use. Final concentration of the extracts adjusted to 1g/ml.

Pic 1: *Moringa oleifera* (Leaves)

Pic 2: *Moringa oleifera* (Bark)

Disk diffusion test: In this method the standardized bacterial isolate (0.5N McFarland standard) was spread on an Mueller-Hinton agar plate by lawn culture and then paper discs containing specific concentration of antibiotics are placed and incubated at 37 ºC overnight. If the isolate is susceptible to the antibiotic, it does not grow around the disk thus forming a zone of inhibition. Strains resistant to an antibiotic grow up to the margin of the disk. The diameter of zone of inhibition was measured from one end to another end via the disc with the help of a scale. Zone of inhibition is measured in millimetres (mm). In this study along with sensitive and resistant antibiotic discs, sterile filter paper discs containing 50μLs of plant extract was used and tests were performed according to CLSI guidelines. For MRSA Vancomycin disc and Oxacillin as sensitive and resistant antibiotics respectively.

Quantitative Method

Micro broth dilution tests: A polystyrene tray containing 96 wells was filled with small volumes of serial two-fold dilutions of different antibiotics. The inoculum suspension and standardization is done according to 0.5 McFarland standard. The bacteria was inoculated into the wells and incubated at 37 ºC overnight. MIC and MBC is determined in microbroth dilution test.

Methicillin-resistant Staphylococcus aureus (MRSA) [13] is a bacterium that causes infections in different parts of the body. One of the most common bacteria isolated from Hospital acquired infections. It is tougher to treat than most strains of Staphylococcus aureus because it's resistant to some commonly used antibiotics. Until now Vancomycin is the drug of choice but there is increase in borderline resistance to Vancomycin too.

Klebsiella Spp. is a type of Gram-negative bacteria that can cause different types of healthcare-associated infections, including pneumonia, bloodstream infections, urinary tract infections, wound or surgical site infections, and meningitis. One of the threats is the development of *Klebsiella pneumoniae* carbapenemases producing strain, where it is resistant to broad-spectrum antibiotics belonging to carbapenems family. *Klebsiella* Spp. also is one of the commonly isolated bacteria in Hospital acquired infections [14]. For *Klebsiella* Spp., Imipenem and Ampicillin is used as the sensitive and resistant antibiotic control respectively.

Table 1: showing traditional uses of the plant/parts.

Sl. No	Name of the Plant/Part	Traditional Uses
1	*Moringa oleifera* (Leaves)	Antioxidant, Antidiabetic, Hepatoprotective, Antibacterial
2	*Moringa oleifera* (Bark)	Antioxidant, Antidiabetic, Hepatoprotective, Antibacterial

Results and Discussion

Table 2

Plant/plant parts	Name of bacterial Strain with Extraction solvent	MIC	MBC	DDT in mm
MRSA				
Moringa oleifera(Leaves)	Ethanol	187.5	375.5	25 ± 0.5
	Distilled water	93.8	187.5	11± 0.5
Moringa oleifera (Bark)	Ethanol	93.8	187.5	18± 0.5
	Distilled water	46.9	93.8	12± 0.5
Klebsiella Spp.				
Moringa oleifera(Leaves)	Ethanol	31.25	62.5	24.6± 0.5
	Distilled water	15.62	31.25	10 ± 0.5
Moringa oleifera (Bark)	Ethanol	187.5	375.0	21± 0.5
	Distilled water	46.9	93.8	8± 0.5

Note: Antibiotic disc used as a sensitive control is Imipenem which is 17mm for *Klebsiella* Spp.

The results show that ethanol extracts of *Moringa oleifera* leaves as well as bark, have shown zone of inhibition bigger than the control antibiotic (Imipenem-17mm). With these results there is a scope for us where we can combine traditional therapy with antibiotic treatment which shall give a better results.

Whole of the world is looking forward to tackle the issue of multidrug resistance by inventing newer antibiotic molecule. In the Southeast Asia traditional medicines are used in abundance along with Allopathic antibiotics. As bacteria are acquiring resistance for these synthetic molecules, it is the need of the hour is to find newer molecules or alternate solutions. The disc diffusion techniques, MIC of above used medicinal plants prove encouraging and promising results. In an era where new molecules are not produced due to increased antibiotic resistance.

Moringa oleifera is a good source of various phytochemicals like alkaloids, flavonoids, carbohydrates, glycosides, saponins, tannins, Terpenoids and has exhibited a good antifungal activity [8].

Moringa oleifera has highest antibacterial activity and aqueous extract of both the plants has lowest activity. Molecular level studies to find the antibacterial agent/molecule which when subjected to animal experiments, Bioavailability/bio equivalence studies and clinical research will be able to bring about the natural antibiotics in these antibiotics.

The antimicrobial activity of the extracts tested, which reveal bioactivity on organisms such as *E. coli, S. aureus, P. aeruginosa, S. typhi, S.typhimurium* and *E. aerogenes* is encouraging as these organisms range from pathogenic and toxigenic organisms liable to cause food – borne illnesses to spoilage-causing organisms liable to spoil food products. The control of these organisms by the extracts in foods would reveal the potentials of these extracts as preservatives [9].

As human beings we need to understand and believe in new integrated research which comprises of allopathic medicine (clinical and diagnostic microbiology, pharmacology), ayurveda and biomedical research can bring about a change to fight against multidrug resistance.

Ethanol was the best solvent comparing with water and methanol for Moringa leaves as its extract showed the best antimicrobial activity [10].

Both the leaf and bark extracts of Moringa collected from the Saudi Arabian region possess anti-cancer activity that can be used to develop new drugs for treatment of breast and colorectal cancers [11]. The human and animal as well as in vitro studies described in the preceding text indicate that various preparations of *M. oleifera* leaves and other plant parts possess a wide range of physiological and pharmacological activities [12].

Time and again we should grow and test more plant extracts with all possible drug resistant bacteria which can show us a better understanding, why we have to go and choose medicinal plants.

Conclusion

In an era of emergence of multidrug resistant bacteria where newer antibiotics have become scarce, naturally available medicinal plants can be the answer along with moderately sensitive antibiotics. Basic research targeted to detect antibacterial activity clearly shows the positive results and will be useful if more research results in newer antibiotic molecule. *Moringa oleifera* which is easily cultivated, can be used as a vegetable and which is medicinal plant with multiple properties. It is proved to be a good antibacterial agent against multi drug resistant bacteria like *MRSA* and *Klebsiella Spp.*

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