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ANTIMICROBIAL ACTIVITY OF *MORINGA OLEIFERA* LAM., LEAF EXTRACT, AGAINST SELECTED BACTERIAL AND FUNGAL STRAINS

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ABSTRACT

Moringa oleifera Lam (Moringaceae) is a very useful tree in tropical countries. In folklore, and ayurvedic all parts of the tree used in different healing procedures for different diseases. This plant leaves are very good nutrient supplement for mall nutrition and also used as an antibiotic. Chloroform extract of plant leaves shows antibiotic property against wide range of pathogens like *Escherichia coli* (MTCC 443) (ZOI=08.8±1.0mm), *Pseudomonas aeruginosa* (MTCC 424) (ZOI=9.5±0.5mm), *Staphylococcus aureus* (MTCC3160) (ZOI=6.2±0.7mm) *Streptococcus* pyogenes (MTCC 442) (ZOI=7.0±0.5mm). *Aspergillus niger* (MTCC 1781) (ZOI=7.3±0.5mm), *Candida albicans* (MTCC 181) (ZOI=6.2±0.5mm) along with positive controls. So this plant extracts having good healing properties without side effects when compared with synthetic antibiotics.

KEY WORDS

Moringa oleifera, Chloroform extract of leaf, ZOI=Zone of inhibition, Antibacterial, Antifungal

INTRODUCTION

Antimicrobial resistance has become a global problem. Strategies to improve the current situation include research in finding new and innovative antimicrobials. Antibiotics and the chemotherapeutic agents have been of value in controlling many infections but they depend on judicious use to minimize the incidence of resistant forms [1]. Approximately 20% of the plants found in the world have been submitted to pharmacological or biological tests, and a substantial number of new antibiotics introduced on the market are obtained from natural or semi-synthetic resources [2]. In developing countries, due to the cost of efficient antimicrobials, a large proportion of the population utilizes medicinal plants for the treatment of infectious diseases. According to the World Health Organization's estimation, traditional healing provides the primary health care needs for a large majority (80%) of the population in Africa [3].

The "Moringa" tree is considered one of the world's most useful trees, as almost every part of the Moringa tree can be used for food or has some other beneficial properties. In the tropics, it is used as forage for livestock, and in many countries, it is used as a micronutrient powder to treat various ailments. The fruit of the tree is quite popular as a vegetable in Asia and African countries. In India and other parts of the country the fruit called as *drumstick.* The leaves contain more Vitamin A compared to carrots, more calcium than milk, more iron than spinach, more Vitamin C than oranges, and more potassium than bananas, and that the protein quality of Moringa leaves rivals that of milk and eggs [4]. The Moringa trees have been used to combat malnutrition, especially among infants and nursing mothers. The non-governmental organizations in particular-Trees for Life, Church World Service and Educational Concerns for Hunger Organization—have advocated Moringa as natural nutrition for the tropics [5]. Its leaves as food supplement, recommended for children with moderate malnutrition between the ages of 6 months to 5 years [6]. It has been claimed to have an unusually high content of calcium, iron, and vitamin A and high quality protein, and a low content of antinutrients (Natural or synthetic compounds that interfere with the absorption of nutrients) such as tannins and oxalates. The leaves have therefore been promoted as a potential low cost high quality food.

According to ethanobotanical studies its roots are bitter, acrid, thermogenic, digestive, carminative, anthelmintic, constipating, anti inflammatory, emmenagogue, diuretic, ophthalmic, expectorant and stimulant. They are useful in dyspepsia, anorexia, verminosis, diarrhea, colic, flatulence, paralysis, inflammations, amenorrhea, dysmenorrheal fever, strangury, vesicle and renal calculi. It is used in cough, asthma, bronchitis, pectoral diseases, splenomegaly, epilepsy and cardiopathy. Leaves are anti-inflammatory, anodyne, anthelmintic, ophthalmic and rich in Vitamin A and C. They are useful in scury, wounds, tumors, inflammation and helminthasis. Seeds are acrid, bitter, anodyne, anti-inflammatory, purgative, antipyretic and ophthalmic. They are useful in neuralgia, inflammations, intermittent fevers and opthalmopathy. Bark is regarded as an antiscorbic, and it exudes a reddish gum sometimes used for diarrhea [7].

The specific components of Moringa preparations that have been reported to have hypo-tensive, anticancer, and antibacterial activity include 4-(4'-*O*-acetyl-α-L-rhamnopyranosyloxy)benzyl isothiocy-anate, 4-(α-L-rhamnopyranosyloxy)benzyl isothiocy-anate, niazimicin, pterygospermin, benzyl isothiocyanate, and 4-(α-L-

rhamnopyranosyloxy) benzyl glucosinolate. It is also rich in a number of vitamins and minerals as well as other more commonly recognized phytochemicals such as the carotenoids (including β-carotene or pro-vitamin A)[8].

MATERIALS AND METHODS

(a) *Plant collection*:

Plants were collected between the month of June and July 2010 in the Sagar nagar area Visakhapatnam, A.P, India. Plant leaves were initially dried in an air-conditioned, dehumidified room, then further dried in an oven at ca. 40ºC for a total of seven days, and then finally ground to a fine powder.

(b) *Preparation of Extracts*:

Petroleum ether extracts:

The dried plant leaves of moringa was ground and extracted in a percolator with 95% Petroleum ether. About 100 ml of Petroleum ether per gram of plant eaves powders was used. The Petroleum ether extract was dried under a reduced pressure at 40°C. The dried extract was stored in sterile bottles until further use. Before testing, 10 mg of dried extract was dissolved in 1 ml of Petroleum ether.

Chloroform extracts:

In chloroform (1g/100ml) the dried leaf material was ground. The solvent was removed using a rotary vacuum evaporator at 40 °C to give a concentrated extract, which was then frozen and freeze-dried until use. Before testing, 10 mg of dried extract was dissolved in 1 ml of chloroform.

Micro organisms used:

Standardized strains from the (MTCC) Microbial Type Culture Collection and Gene Bank Institute of Microbial Technology Sector 39-A, Chandigarh – 160036, INDIA were used in bioassays. The Gram-positive bacteria were *Staphylococcus aureus* (MTCC 3160) *Streptococcus* pyogenes (MTCC 442). The Gram-negative bacterium was *Escherichia coli* (MTCC 443), *Pseudomonas aeruginosa* (MTCC 424). And fungal strains were *Aspergillus niger* (MTCC 1781), *Candida albicans* (MTCC 181), *Candida tropicalis* (MTCC 1406). The bacterial strains were maintained on L.B agar (Luria and barteni agar) medium and the fungal strains were maintained on SDA (Sobouraud's dextrose agar) at 4ºC.

Inoculum preparation:

For the antibacterial tests, organisms were grown overnight in Luria Bertani Broth followed by incubation at 37ºC. For antifungal tests, organisms were aseptically inoculated on Petri dishes containing autoclaved, cooled and settled SDA medium. The Petri dishes were incubated at 31 °C for 48 h grown 48 hrs on Sabourauds Dextrose Agar (SDA) [9].

Antimicrobial Susceptibility Testing:

The antimicrobial activities were found by using a modified agar well diffusion method [10].

Agar Diffusion Assay:

0.2 ml of each of the seeded broth containing 10^7 test organisms was inoculated on the plates of solidified agar and spreaded uniformly [11]. Wells of approximately 4mm in diameter and 2.5mm deep were made on the surface of the solid medium using a sterile borer [12] and filled with 20 µL of the plants extracts and Petroleum ether and chloroform as a blank. The concentration of the extracts employed was 20 µg/ml. Simultaneously Ampicillins was used as a positive control for bacterial strains *S. aureus, P. aeruginosa, E. coli* and *S.* pyogenes Nystatin was used as a positive control for fungal strains (*A.niger, C. albicans*) at a concentration of 1 µg/ml respectively [13]. The dilution medium for the positive controls was sterile distilled water. The test was carried out by triplicates. The bacterial plates were incubated at 37°C for 24 hrs, and the fungal plates were incubated at 31°Cs for 48 hrs [9].

Minimal inhibitory concentration (MIC) evaluation:

The MIC was evaluated on plant extracts that showed antimicrobial activity. This test was performed at four concentrations of each extract (6.3, 12.5, 25, 50 µg/ml) by the same modified agar well diffusion method[10]. All the experiments were conducted with a minimum of 3 replicates per strain. The experiments were repeated thrice. All values are expressed as means ± standard deviation.

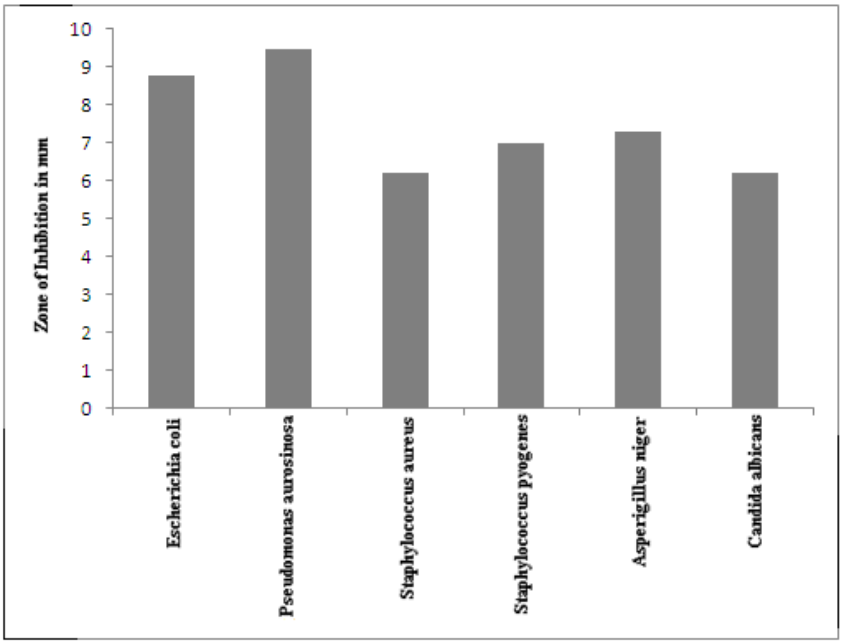
RESULTS AND DISCUSSION

Chloroform extract zone of inhibition against *E.coli* was 8.8±1.0 mm, and positive control (Ampicillin) zone of inhibition was 6.2±0.5 mm. Chloroform extract zone of inhibition against *Pseudomonas aeruginosa* was 9.5±0.5 mm, and positive control was 5.5±0.5 mm. The chloroform inhibition zone against *Staphylococcus aureus* was 6.2±0.7 mm, and positive control was 5.5±0.5 mm, and the zone of inhibition against *Streptococcus* pyogenes was 7.0±0.5 and positive control zone of inhibition was 6.0±0.5.

The chloroform extract zone of inhibition against *Aspergillus niger* was 7.3±0.5 mm, and positive control (Nysatisn) was 5.2±0.8 mm. In the case of *Candida albicans* chloroform extract the zone of inhibition was 6.2±0.5 mm, and the positive control was 5.0±0.6 mm.

Graph 1

Antimicrobial Susceptibility testing against different microorganisms



The Ampicillin was taken as a positive control for the Gram negative and Gram positive bacteria because it's broad-spectrum antibiotic activity. Compare to this, our plant extract also shows broad-spectrum antibiotic activity against Gram positive and Gram negative bacteria.

Nystatin is a polyene antibiotic (polyene antibiotics are a class of antimicrobial polyene compounds that target fungi) used for the treatment of Candida infections was taken as a positive control against the three fungal strains. The

Chloroform extract of leaves showed good activity against *A.niger* and *C.albicans*.

Petroleum ether extract doesn't show any zone of inhibition against all tested bacterial and fungal strains. There was no zone of inhibition around the blank wells in all plates (filled with chloroform and petroleum ether). According to our results chloroform extract of leaves shows good inhibition against gram positive, gram negative bacteria and fungi along with positive controls.

CONCLUSION

The leaf extracts of *Moringa oleifera* showed varying antimicrobial activity on wide range of microorganisms. The extract was more effective than traditional antibiotics to combat the pathogenic microorganisms studied. The chance to find antimicrobial activity was more apparent in chloroform than petroleum ether extracts of the same plants. The plant could be a source of new antibiotic compounds. Further work is needed to isolate the secondary metabolites and study of metabolic interchanges in bacterial metabolic pathways when applying this extract

This *in vitro* study demonstrated that folk medicine can be as effective as modern medicine to combat pathogenic microorganisms. The use of these plants in folk medicine suggests that they represent an economic and safe alternative to treat infectious diseases.

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