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RESEARCH ARTICLE
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ANTIBACTERIAL EFFICACY OF *MORINGA OLEIFERA* LATEX EXTRACT AGAINST PYOGENIC BACTERIA

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Abstract
Effective management of biofilm-related oral infectious diseases and increased resistance to antimicrobial agents and elevated virulence is now leading as a global challenge. *Streptococcus pyogenes* has been reported to be one of the most common and influential aerobic bacteria associated with leading cause of uncomplicated bacterial pharyngitis and tonsillitis. Due to prevailing use of antibiotics has been raising a real alarming issue, hence one alternative to overcome these problems is to use natural ingredients such as materials from plant extract such as *Moringa oleifera* latex. In the past, researchers paid only a little attention towards the plant extract which acts effectively against oral infection by latex.
This study is aimed to evaluate the antibacterial activity of the latex of *Moringa oleifera* against *Streptococcus pyogenes* from the provided pure cultured master plate. Latex (2gm latex – 2ml DMSO-Dimethyl sulfoxide) is given to dissolved with DMSO within 48 hrs of collection left undisturbed overnight. DMSO is previously tested for negative control.
Antibacterial Assay of Latex were calculated based on the inhibition zones using the Mueller–Hinton agar diffusion method (19.5–23.4 mm and 12–15 mm) , minimum inhibitory concentration (MIC) using Mueller–Hinton broth in a microdilution method (100% - 25%), and minimum bactericidal concentration (MBC) using nutrient broth and nutrient agar(100%and 50%) .
On witnessing the inhibitory results of M. oleifera latex can be considered one of the new infection-fighting strategies in controlling pyogenic bacteria responsible for oral infections, but further in vivo research and discovery of the mode of its action are immediately required to shed the light on the effects of latex against infection in buccal cavity instead of taking antibiotic pills, which might save the world from drug resistant bacterial pathogen

Keywords: *Moringa oleifera*, *Streptococcus pyogenes*, pharyngitis, tonsillitis.

Introduction:

A petrified documented serious problem against the harmful side effects and the drastically increasing of microorganisms getting resistance to synthetic antibiotics alarms the need of new strategies. In the past researchers paid only little attention to plant latex research. Despite the fact that novel classes and derivatives of antibiotics are steadily being synthesized by drug industries and handling to markets. The control of infectious diseases by microorganisms are severely threatened by the constant increase in the number of microorganisms developing resistance to antibacterial drugs[Cohen ,1992; Singer et al., 2003; Jazani et al .,2010] Such a terrifying fact is a cause of great concern, because new multi-resistant bacterial strains are transformed particularly in one with suppressed immunity. However, studying with one bacterial strain that is *Streptococcus pyogenes* is one of the most common aerobic bacteria associated with bacterial pharyngitis and tonsillitis. However, the prevailing use of

antibiotics has led to the development of antimicrobial resistance, which is a serious concern. This problem highlights the urgency of new strategies. Depending on the plant as the source of medicine is still prevalent in some rural underdeveloped regions of India, where traditional medical herbs play a major role in promoting primary healthcare. Natural plant extracts provide a constant inspiration of bioactive antimicrobial agents with low toxicity, a broad spectrum and good pharmacokinetics to be used clinically.

Since we are aware that India is rich in diversity. A number of plants are studied for their medical potential, which are used by traditional healers, herbal folklorists and Eastern Himalayas , Western Ghats and Andaman and Nicobar Islands. India is the greatest producer of medical herbs and is appropriately called the Botanical Garden of the World [Kattankulathur et al 2010] .,. Plants are the producers of various effective secondary metabolites which are the active ingredient of herbal medicines. There are too many herbs for which the medical value still remains to be investigated so that they can be replaced and used as an alternative for synthetic drugs. Since, some natural products are found to possess promising antimicrobial activity when given alone to Biofilms. [20]

Moringa oleifera , the so-called "Drumstick" or "Horseradish" is a plant with various medicinal properties that has been reported to act effectively against oral infections. It is distributed in Bundelkhand, and Uttar Pradesh, Tamil Nadu and Sub-Himalayan tracts. The plant is popularly known for abundance of latex in its branches and trunks, hence easily collected if wounded is made. Such a fact reinforcing the thought of latex is accumulated as their defence mechanism against insects and microorganisms.[Murugan . T . 2009]

Moringa Oleifera is used in the medical field due to it's considerable medical potential components and pharmacological activities due to several studies of literature that have shown that it has a rich source of various phytochemical components, mostly glucosinolates and enhances microbial activities. It has many active components such as alkaloids, tannins, flavonoids, saponins and triterpenoids. The phytochemical analysis have proved many therapeutic activities including analgesic, anti-inflammatory, antidiabetic, cytotoxic, anti-cancer and hepatoprotective. However, little is known about the mechanism of antimicrobial activity of *Moringa oleifera* except for their activities against a range of microorganisms. Disk diffusion method was used for the evaluation of the antibacterial activity of *Moringa oleifera* latex extract and the significant distribution of inhibition zone appeared against *Streptococcus pyogenes* . Further on,

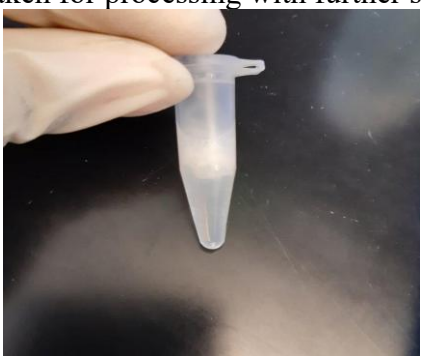
This investigation aimed to show the novel action of *Moringa oleifera* latex extract against oral pathogen *Streptococcus pyogenes* biofilm to prove better against antibiotics.

Materials and methods

Collection and preparation of the plant sample

The plant *Moringa oleifera* latex was collected by making wounds with a sharp sterilized knife. And waited for several hours until the latex started to come out of the wound. A spoon and box was sterilized prior to collection and the latex was picked up with the help of the spoon and kept in the box and covered with the lid. The sample was maintained in a tight container box for subsequent procedure.The sample used within 24 hours of collection. [Karim et al. 2011].

Preparation of the test extract:
At first the latex was cleaned with ethanol and distilled water for the removal of tree debris for several times. The sample latex was chopped off with sharp object into several small pieces and again it was washed off. The chopped pieces of latex was collected in a eppendorf and measured with several grams of 1000mg and 2000mg and diluted with DMSO and distilled water of 1ml or each concentration. The samples were vortex at least 2hr per day. After the samples gets dissolved into the DMSO and distilled water were taken for processing with further steps.



Materials: The materials used were Autoclave , Incubator, Biological safety Cabinet, Mueller Hinton Agar, Mueller Hinton Broth, Nutrient Agar plate, Agar agar, DMSO (Dimethyl sulfoxide), Iodonitrotetrazolium chloride (INT).

Bacterial preparation:
The tested microorganisms were subcultured for 24 hours at 37° C.

Antimicrobial activity assay :
1.Agar Well diffusion assay :
The antibacterial activities of latex were evaluated using agar well diffusion assay [7, 8]. In this method, 100 µL of each test organism, which was equivalent to a 0.5 McFarland standard, was inoculated on the MHA , it was spread on to the surface of the agar using a sterilized glass spreader. After 10 minutes of inoculation, the wells were prepared using a sterilized steel cork borer (1 cm in diameter). Wells were made on each plate, out of which three wells were loaded with each latex. Each test was done in triplicate. All the plates were then incubated aerobically at 35 ± 2°C for 16–20 h. (e antibacterial activities were evaluated by measuring the diameters of zones of inhibition (mm) against the test organism *Streptococcus pyogenes.*

2.Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)
2a) The previously prepared nephelometric flask used in the last experiment 100 µl of culture is added to a new nephelometric flask and OD is maintained at 0.08-0.1 Extract is prepared by dissolving 2 ml of Extract solvent (200 ul DMSO and 1800 ul sterile water), 10 test tubes are taken where 2 of them are marked positive and negative and the rest were marked according to dilution number In all the test tubes 2 ml of NB Broth is added. Then 100 ul of added to all of them. In the positive control 10 mg of antibiotic is dissolved in 2ml sterile water. Then 2 ml of extract is added to a test tube , mixed and from which 2 ml is transferred to the next one and serial dilution is performed for the next

7 test tubes this process was repeated. From the last test tube 2 ml is discarded and nothing is added in the negative control. Incubate the tubes at 37°C for 24 hours and growth is checked next day. Add 50 µl of 0.2 mg/ml INT (Iodonitrotetrazolium chloride) dye to each tube and incubate for an additional 30 minutes. Observe the color of the bacterial suspension in each tube; red indicates bacterial growth, while clear indicates bacterial inhibition. Determining the minimum concentration of the latex extract that inhibits bacterial growth, which is the MIC.

2 b) Take samples from the negative control, the tube with the MIC concentration, and the tube with the concentration one dilution higher than the MIC . Plate each sample onto a nutrient agar plate and incubate at 37°C for 24 hours. Count the number of bacterial colonies on each plate.

Results:

Agar well Diffusion Assay :

The antibacterial activity of Moringa species was examined in vitro by diffusion and dilution method against *Streptococcus pyogenes*. In this experiment DMSO extract showed the inhibitory property towards Bacterial isolates *S. pyogenes* and being most susceptible to DMSO extract at 1000 mg/ ml with zone of inhibition of 20 ± 0.5mm followed by 2000 mg/ml 23 ± 0.5 mm. The aqueous dissolved extract has a similar value of 19 mm and 22 ± 0.5 mm zone of inhibition at the concentration of 1000 mg/ml and 2000 mg/mp respectively.

The negative control was taken of DMSO and distilled water which did not inhibit the growth of *Streptococcus pyogenes*.

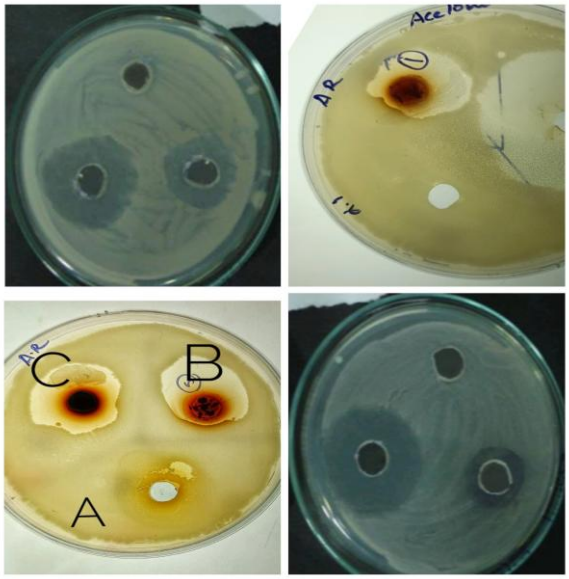


Fig : Above figures are clear zones due to proper filtration.
Latex was dissolved with DMSO and Sterilized water
A- DMSO + no extract 2000 ml concentration
B- DMSO + extract with 1000 ul /ml concentration
C -DMSO + extract with 2000 ul/ ml concentration

Minimum Inhibitory Concentration and Minimum Bactericidal concentration:

The activity of *Moringa oleifera* latex was assayed in vitro by the agar dilution method against *Streptococcus pyogenes*. The table demonstrates the highest and lowest value against *Streptococcus pyogenes.*

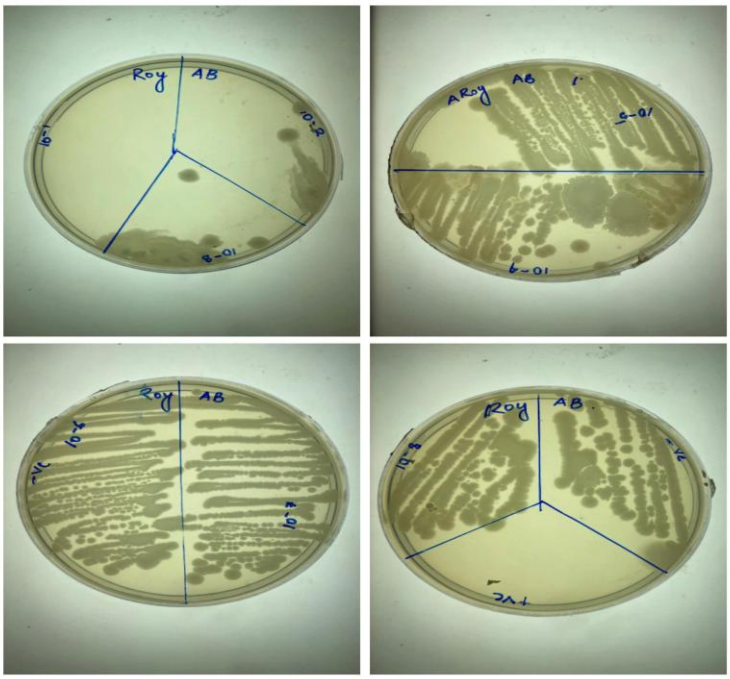


Fig: All the test tubes were plated on NA agar the dilution upto 3 power has shown no growth. Rest of the tubes has shown subsequent increase in growth.

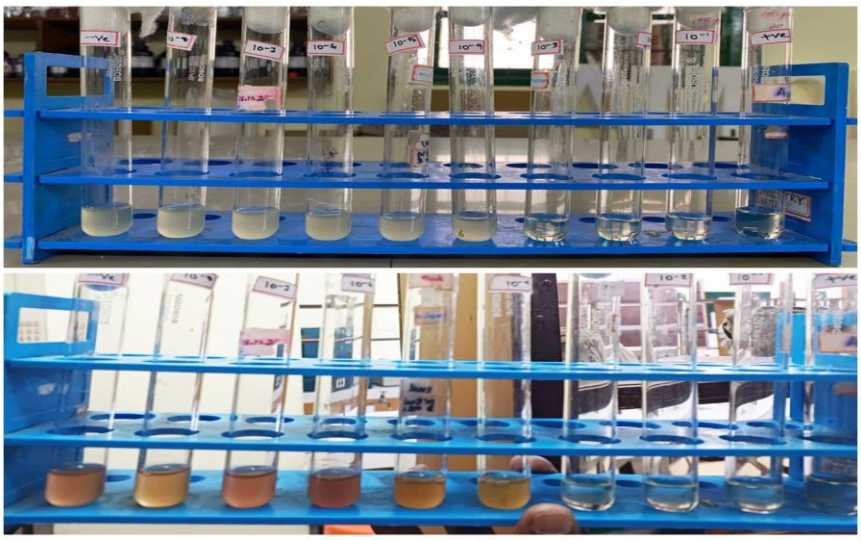


Fig: The test tubes were turbid until 10–³ clear (no turbidity due to bacterial growth) but in addition to INT dye bacterial growth was observed in test tubes clearly and with negative control.

Discussion

The research for new antibacterial agents from a natural ororganic source has become a very crucial endeavor, considering the escalating the levels of antibiotic resistance. One of the efforts in this research is focused on the use of Moringa latex, which is widely available in India just to exclude the antibiotics for oral pathogen *Streptococcus pyogenes*. Traditional medicine has been practiced worldwide, including in Indonesia, and other countries for centuries. The agar well diffusion methods were used in this study due to their routine use as a quantitative method in clinical laboratories.

In the agar well diffusion and broth dilution method, 100 µL of organism was inoculated on the MHA. The wells were prepared using a sterilized teel cork borer (1 cm diameter). Wells were made on each plate, out of which three wells were loaded with each of latex. All the plates were then incubated at 35 ± 2°C for 16–20 h.Antibacterial activities were evaluated by measuring the diameters of zones of inhibition (mm) against the organism *Streptococcus pyogenes*.

The results obtained revealed that DMSO extract was the best extracting solvent for a fraction with antibacterial activity. Generally, the aqueous extracts showed similar activity on the isolate. The minimal inhibitory concentration (MIC) for the latex extract was 1000mg/ml to 2000mg/ml , while in the case of Minimum Bactericidal Concentration (MBC) of the latex DMSO was 2000 mg/ml.

The zone of inhibition were obtained as DMSO extract has 23± 0.5mm (2000 mg/ml); 20± 0.5 mm(1000 mg/ml) in case of sterilized water and latex (1000 mg/ml) 19±0.9mm and 22 ± 0.5 mm (2000 mg/ml).Chemically the latex of *Moringa oleifera* is composed of several phytochemical components which were exclusively proved in various studies includes proteolytic enzymes and cardenolides , alkaloids, glucosinolates, flavonoids, saponins, triterpenoids and tannins with potent anthelmintic properties and bactericidal activity [Deepak 1995, Debey and Jagannatha,2003] . The phytochemical analysis of *M. oleifera* showed its bioactive components , with their pharmacological activities . *Moringa oleifera* has a broad safety margin for human and animal consumption [Muhammad Evy prastiyanto et al,2020].To the best of our knowledge, this is the first report dealing with latex and oral pathogens. Synergy research in Phytomedical has established itself as a new key activity in recent years. It is the one main aim of this experiment to find a scientific rationale for the therapeutic superiority of herbal drugs derived from traditional medicine. [Williamson 2001 ; Rosato et al ., 2007 ; Wagner and Ulrich - Merzenich , 2009]

Conclusion:

Increase in resistance to commercially available antibiotics projects a major dilemma in the treatment of bacterial infection throughout the world. Based on the above investigation it can be concluded that latex of *Moringa oleifera* can be a potential source for herbal drug preparations against pathogenic bacteria. But advanced in vivo research and discovery investigating the mode of its action are necessary to throw light upon the effects. Needless to say , latex are less potent anti- infectives than conventional antibiotics. Future optimization of these products through structural alteration might allow the development of potent pharmaceutical drugs with active agents. Since it was seen that the crude extract manage to kill upto certain measure of *Streptococcus pyogenes* . We can conclude that advanced technologies should be adapted to get more information about natural resources. Or else the day is too far where no antibiotics will be left to kill any microbe.

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