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PHYTOCHEMICAL ANALYSIS OF *MORINGA OLEIFERA* LEAF EXTRACT AND ITS ANTIBACTERIAL ACTIVITY ON PUS FORMING BACTERIA *STAPHYLOCOCCUS AUREUS* FROM CLINICAL ISOLATES

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ABSTRACT:
The present study was aimed to investigate phytochemical constituents and antibacterial activity of aqueous extract of *Moringa oleifera* leaf belonging to the family *Moringaceae* on bacterial isolate *Staphylococcus aureus*. De-ionized distilled water was used to dissolve bioactive compounds of *Moringa oleifera* leaf for overnight to determined phytochemical constituents. The phytochemical analysis demonstrated the presence of tannins, glycosides, alkaloids, flavonoids and terpenoids are responsible for antibacterial activity. The antibiotic susceptibility test of *Staphylococcus aureus* was sensitive to Clindamycin and Erythromycin and was found resistance to Penicillin, Oxacillin, Cephalothin and Amoxyclav. The antibacterial activity of aqueous extract of *Moringa oleifera* against *Staphylococcus aureus* (gram positive bacteria) using agar well diffusion method in volumes of 300 and 500 µl/well. The results acquired from this study that aqueous extract of *Moringa oleifera* had antibacterial property against *Staphylococcus aureus* when obtained from a necessary volume.

Keywords:- Antibacterial activity, Phytochemical screening, Antibiotic susceptibility test, *Moringa oleifera*, *Staphylococcus aureus*.

INTRODUCTION :

Moringa (*Moringa oleifera Lam*) is a type of a local medicinal Indian herb belonging to the family of *Moringaceae,* also commonly known as "Drumstick-tree". *M. oleifera* is found in many tropical and sub-tropical regions. *Moringa* is also widely known and used for its health benefits (Satinder Pal Kaur Malhotra *et al.,* 2018). The leaves of *Moringa oleifera* are a good source of natural antioxidants due to the presence of different compounds such as ascorbic acid, flavonoids, phenolics and carotenoids (Shaymaa Rajab Farhan et al., 2021). *M. oleifera* constituents have been used for medicinal properties such as antioxidant activity, antimicrobial activity, blood pressure lowering effect, antitumor, anticancer, cholesterol lowering effect, antidiabetic, and antiobesity (Wulan Angestia *et al.,* 2020).

This plant has twelve other varieties of species and they are follows: *Moringa arborea, Moringa borziana, Moringa concanensis, Moringa drouhardi, Moringa pygmaea, Moringa rivae, Moringa ruspoliana and Moringa stenoprtala.* The classification of *Moringa oleifera* is started below (Ojeaga Imohiosen *et al.,* 2014).

Classification

Kingdom: *plantea*
Sub kingdom: *Tracheobionta*
Super division: *Spermatophyta*

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Division: *Magnoliophyta*
Subclass: *Magnoliopsida*
Order: *Capparales*
Family: *Moringaceae*
Genus: *Moringa*
Species: *Oleifera*

According to the World Health Organization 80% of the population in developing countries prefer to use herbal extract and their active components as traditional medicine therapy. The phytochemical analysis of *M. oleifera* showed its bioactive compounds with their pharmacological activity (Ehab Ali Found *et al.,* 2019). In traditional, it is important to bear in mind that the phytochemical contents present in leaves of *M. oleifera* depend on several factors such as geographical area where the plant is cultivated, type of soil, water and fertilizers, industrialization process and storage conditions (Unegbu, V. *et al.,* 2020). *Moringa* is wealthy in nutritional attributable to the presence of a spread of essential phytochemicals gift in its leaves, pods and seeds. In fact, Moringa provides 7times more vitamins C than oranges, 10 times more vitamin A than carrots, 17times more calcium than milk, 9times more protein than yoghurt, 15 more potassium than banana and 25 times more iron than spinach (Rockwood *et al.,* 2013). *Moringa* is rich in phytosterols like stigmasterol, sitosterol and estrogen production, which in turn malnutrition in children younger than 3years. About 6 spoonful of leaf powder can meet a woman's daily iron and calcium requirements, during pregnancy (Lakshimipriya Gopalkrishnan *et al.*, 2016).

The antimicrobial activities of *Moringa oleifera* leaves were investigated in vitro against bacteria, yeast and helminths pathogenic to man by disk-diffusion method. Antimicrobial is the act of killing or inhibiting or suppressing microorganism from their multiplication or growth (Ojega Imohiosen *et al.,* 2014). It is important to evaluate the antimicrobial properties *M. oleifera* leaves on some selected microorganisms and also to verify its phytochemical constituents (Abubakar Idris *et al.*, 2016). *Staphylococcus aureus* is one of the most opportunity pathogens associated with hospital and community acquired infections (Colombari V. *et al.,* 2006). *M. oleifera* inhibit Gram-positive and Gram-negative bacteria including *Staphylococcus aureus, Bacillus cereus, Escherichia coli, Salmonella enteronella enteritidis* and *Pseudomonas aeruginosa* (Fernanda Gomes *et al.,* 2018). *M. oleifera* has a broad safety margin for human and animal consumption (Sidney J Stohs *et al.,* 2015). Disk diffusion method was used for the evaluation of the antibacterial activity of *Moringa oleifera* extracts and the significant differences of inhibition zones appeared *P. aeruginosa, Enterococcus faecalis* and *Staphylococcus aureus* were used to assess the antibacterial effect of the extract (Makita C. *et al.*, 2015).

MATERIALS AND METHODS :

Materials used

Nutrient agar, Nutrient agar broth, Mannitol salt agar, MacConkey agar, Baird Parker agar and Muller Hinton agar, petri dishes, cotton swab, test tubes, microtips, antibiotic disk and distilled water was purchased from HiMedia Laboratories Pvt. Ltd., Mumbai.

Collection of plant

The plant material was collected from area of Nagpur and plant part (leaf) devoid of contaminant parts were carefully collected and kept in polythene bags which were then subsequently sealed. The stored specimens were thoroughly washed with tap water. They were shaded dried and ground with grinder to obtain course particle.

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Collection of bacterial isolates

Bacterial strains were obtained from Vishakha Clinical Microbiology Laboratory of Nagpur city. For the isolation, a loop full of above collected samples were streaked on the Nutrient agar and incubated at 37° C for 24 hrs. On next day, the individual colonies were detected based on the colony features suggested in the Nutrient agar brochure for discrimination and then sub-cultured to grow on selective and differential media i.e. Mannitol Salt agar, MacConkey agar and Baird Parker agar.

Preparation of M. oleifera leaf extracts

Aqueous Extract

Immerse 10g of *Moringa oleifera* leaf powder in 100 ml boiled de-ionized water for overnight. The aqueous fraction was separated using muslin cloth and filter through sterile whatman filter paper no. 01. The aqueous extract of *M. oleifera* leaves stored in refrigerator until used.

Phytochemical Screening of *Moringa oleifera* extract

The extract was subjected to phytochemical analysis to check the presence of bioactive compounds as per standard tests such as alkaloids, phenols, flavonoids, tannins, saponins, terpenoids, glycosides, reducing sugars, fats and oils, etc., present in the leaf extracts.

Saponins

Saponins were detected using the froth test. 1g of the sample was weighed into a conical flask in which 10ml of sterile distilled water was added and boiled for 5minutes. The mixture was filtered and 2.5 ml of the filtrate was added to 10 ml of sterile distilled water in a test tube. The test tube was stoppered and shaken vigorously for about 30 seconds. It was then allowed to stand to half an hour. Honeycomb froth indicated the presence of saponins.

Tannins

To a portion of the extract diluted with water, 3-4 drops of 10% ferric chloride solution is added. A blue colour is observed for gallic tannins and green colour indicates for catechol tannins.

Reducing sugars

To 0.5 ml of plant extracts, 1ml of water and 5-8 drops of Fehling's solution was added and heated over water bath. Brick red precipitate indicates the presence of reducing sugars.

Glycosides

25 ml of dilute sulphuric acid was added to 5 ml extract in a test tube and boiled for 15 minutes, cooled and neutralized with 10% NaOH, then 5 ml of Fehling's solution added. Glycosides are indicated by a brick red precipitate.

Alkaloids

2 ml of extract was measured in a test tube to which picric acid solution was added. An orange coloration indicated the presence of Alkaloids.

Flavonoids

4 ml of extract solution was treated with 1.5 ml of 50% methanol solution. The solution, 5-6 drops of concentrated hydrochloric acid was added and red colour was observed for flavonoids and orange colour for flavones.

Terpenoids

Four milligrams of extract were treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated solution of sulphuric acid was added slowly and red violet colour was observed for terpenoids.

Volatile oil

2 ml of extract was shaken with 0.1 ml dilute NaOH and a small quantity of dilute HCL. A white precipitate is formed if volatile oils are present.

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Phenols

To 2ml of extract, a few drops of ferric chloride solution was added. The appearance of a greenish yellow colour, confirms the presence of phenol.

Antibiotic susceptibility test

The bacterial strain is used to check the antibiotic susceptibility test on Muller Hinton agar using antibiotic disc. Streak on sterile Muller Hinton agar plate using cotton swab with the test organisms for lawn of growth and allowed it for 5 mins to dry then placed the antibiotic disc and incubated for 24 hours at 37°C. Antibiotic susceptibility test was evaluated by measuring the zones of inhibition against the test bacteria.

Agar well diffusion method

The antibacterial activity of aqueous leaf extract of *M. oleifera* against bacterial strain was studied by agar well diffusion method. The method was done on sterile solidified Muller Hinton agar plate, it was inoculated with 18 hours old broth culture of given organisms using sterile cotton swab horizontally and vertically in order to get a uniform microbial growth. Wells of 6 mm were punched in the agar and filled with plant extracts in volumes 300 and 500 µl/wells. The plates were incubated at 37° C for 24 hours. Antimicrobial activity was evaluated by measuring the zones of inhibition against the tested bacteria.

OBJECTIVE AND ANALYSIS :

The qualitative phytochemical analysis of *M. oleifera* leaf extracts were done to test for presence of phyto-constituents as per the methodology. The results of the phytochemical analysis of *M. oleifera* leaf extract using water are shown in Table 1.

The antibiotic susceptibility test of the bacterial isolates that is *Staphylococcus aureus* was subjected to their sensitivity and resistance pattern to commonly used antibiotic disc. As per the observation antibiotic sensitivity pattern of test bacterial isolate was sensitive to Clindamycin and Erythromycin and found resistance to Penicillin, Oxacillin, Cephalothin and Amoxyclav are shown in Figure 1 and Table 2.

The antibacterial activity of aqueous extract of *Moringa oleifera* leaf using agar well diffusion method, against gram positive bacteria *Staphylococcus aureus*. The zone of inhibition against *S. aureus* was 23mm and 25mm shown in Figure 2 and Table 3.

RESULTS :

The results of the phytochemical analysis of *Moringa oleifera* leaf extract using water are shown in Table 1. The phytochemical screening indicated the presence of tannins, flavonoids, glycosides, terpenoids and alkaloids in leaf extracts of *Moringa oleifera* that are responsible for antibacterial activity.

The result of antibiotic sensitivity pattern of test bacterial isolate was sensitive to Clindamycin with the zone of 28mm in diameter and Erythromycin with the zone of 25mm in diameter and was found resistance to Penicillin, Oxacillin, Cephalothin and Amoxyclav in Figure 1 and Table 2.

The result of antibacterial activity of *Moringa oleifera* against *Staphylococcus aureus* using agar well diffusion method was shown in Figure 2 and Table 3. The result shown that aqueous extract of *Moringa oleifera* had activity against bacterial isolate. The maximum zone of inhibition against *Staphylococcus aureus* was 25mm in diameter.

DISCUSSION :

The present study was carried out to determine antibacterial activity of *Moringa oleifera* leaf found in area of Nagpur city. This plant components and derivatives are known to be

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used to cure wounds, pain, ulcers, inflammation and liver diseases by traditionally. Microorganisms are important component of health-related illness or goodness as they affected the skin and many internal organs in humans and animals. Determination of antibacterial activity of *Moringa oleifera* plant, if any, becomes important in the light of influences on normal micro biota as well as inhibition of pathogenic organisms. For the determination of the antibacterial activity of *Moringa oleifera* aqueous extraction were attempted using established procedures. Test bacterial was isolated clinically from pus sample of human pathogen. The result was shown in Table 2 and Figure 1 was sensitive to Clindamycin and Erythromycin and was found resistance to Penicillin, Oxacillin, Cephalothin and Amoxyclav. Flavonoids and tannins have been reported to possess antibacterial activity, the antibacterial activity of flavonoids is due to their ability to complex with extracellular and soluble protein while tannins may be related to their ability to inactive amicrobial adhesions. Agar well diffusion was used to perform antibacterial activity of *Moringa oleifera* leaf against *Staphylococcus aureus* shown great antibacterial activity. The wells of agar were poured with the volumes 300 µl and 500µl which can be resulted into clear zone of inhibition with 25mm and 28mm in diameter. In present study, aqueous extract was found to possess antibacterial activity against *Staphylococcus aureus* in Table 3.

CONCLUSION :

Present study was carried out on sensorial properties, phytochemical, antimicrobial of *M. oleifera* leaves. Aqueous extract preparations of the plant tested against microorganisms showed higher antibacterial activity, this thus supports the fact that *M. oleifera* contain active phytochemicals and capable of inhibiting the growth of gram-positive bacteria. Flavonoids and tannins have been found to showed antibacterial activity because of their ability to complex with extracellular and soluble protein. The test organisms were analysed for their antibiotic sensitivity pattern. *S. aureus* was found sensitive to antibiotic Erythromycin and Clindamycin. It can be also concluded that the aqueous extract of *M. oleifera* leaf posses more antimicrobial activity by performing agar well diffusion method (23 and 25 mm) in a concentration dependent manner.

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Table 1: Phytochemical screening of *Moringa oleifera* leaf extract

Sr. No.	Tests	Aqueous extract
1	Saponins	Negative
2	Tannins	Positive
3	Reducing sugar	Negative
4	Glycosides	Positive
5	Alkaloids	Positive
6	Flavonoids	Positive
7	Terpenoids	Positive
8	Volatile oil	Negative
9	Phenol	Negative

Table 2: Antibiotic susceptibility test of *S. aureus*

Sr. No.	Antibiotic used	Diameter of zone	Interpretation
1	Penicillin (10unit)	No zone	Resistance
2	Oxacillin (1mcg)	No zone	Resistance
3	Cephalothin (30mcg)	No zone	Resistance
4	Clindamycin (2mcg)	28 mm	Sensitive
5	Erythromycin (15mcg)	25 mm	Sensitive
6	Amoxyclav (30mcg)	No zone	Resistance

Table 3: Antibacterial activity of *M. oleifera* leaf extract against *S. aureus* using agar well diffusion method

Sr. No.	Bacterial strain	Zone of inhibition (mm)	
		300 µl/well	500 µl/well
1	Bacterial isolate	23 mm	25mm

Fig 1: Antibiotic susceptibility test

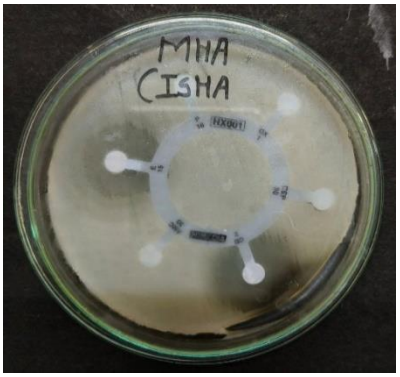

Fig 2: Agar well diffusion method

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