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Study the effect of aqueous Extract of Moringa Oleifera plant leaves On Gram-positive bacteria

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Abstract
Background: *A growing therapeutic challenge is being presented by the significant rise in antibiotic resistance in recent years. Using plant-based antibiotic resistance inhibitors is one way to lessen antibiotic resistance. So this study aimed to evaluate the antibacterial properties of aqueous leaf extracts of Moringa Oleifera (M. Oleifera) plant against gram positive pathogenic bacteria staphylococcus aureus (S. aureus) isolated from wounds.* ***Materials and Methods:*** *Using the agar diffusion method, the antibacterial activity of Moringa oleifera leaf extracts was assessed in vitro. The disc diffusion method was then used to evaluate the antibacterial activity with sensitivity tests of certain antibiotic drugs. Using three concentrations (5,50, and 90%), the results demonstrated that all concentrations of Moringa Oleifera water extracts had different inhibitory effects on S. aureus bacteria.* ***Results****: The three concentration of aqueous leaf extracts of Moringa Oleifera plant had deferent inhibitory effects on staphylococcus strain. These results were compared with standard antibiotics which also showed variant sensitivity against S. aureus.* ***Conclusion:*** *These results provide valuable information that Moringa oleifera hold great promise as highly effective antibacterial agents.*
Key Word*:* ***Maringa Oleifera; leaves extract; S. aureus; diffusion method****.*

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I. Introduction

Since ancient times, medicinal plants—also known as medicinal herbs—have been found and utilized in conventional medical procedures. Or they are species of the plant kingdom, whose parts (flowers, leaves, roots, stems, fruits, or seeds) are directly used or used in some preparation as a medicine to treat a condition or disease. Plants synthesize hundreds of chemical compounds for various functions, including defense and protection against insects, fungi, diseases, against parasites[1] and herbivorous mammals[2].

The Sumerian civilization has the oldest known records of herbs, with clay tablets listing hundreds of medicinal plants, including opium. Dioscorides, a Greek physician who served in the Roman army, recorded more than 1000 medication recipes utilizing more than 600 medicinal herbs[3,4].

The Romans developed a rich herbal medicine legacy by building on and utilizing Greek expertise. Pedanius Dioscorides's "De Materia Medica" is a notable work that served as a thorough guide to medicinal herbs and had a lasting impact for generations[4,5]. Additionally, Pliny the Elder's "Naturalis Historia" contains valuable insights into Roman medical plant practices[6,7].

1.1 Phytochemical basis

Natural substances found in or derived from plants are known as phytochemicals[5,6]. While some phytochemicals are metabolites made to improve plant survival and reproduction, others are nutrients for the plant. Chemicals produced by primary or secondary metabolism in plants are known as phytochemicals[7]. The majority of phytochemicals, either alone or in diet, have uncertain or poorly characterized biological activity[8]. Essential nutrients are phytochemicals with known physiological functions[9].

1.2 Types of Phytochemical

- **Alkaloids**

Many medicinal plants include alkaloids, which are bitter-tasting substances that are abundant in nature and frequently harmful[6,7]. There are several classes with different modes of action as drugs, both recreational and pharmaceutical. Medicines of different classes include atropine, scopolamine, and hyoscyamine[10] (all from nightshade). See blow structure

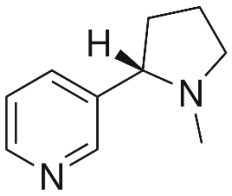

Figure. 1: The alkaloid nicotine from tobacco binds directly to the body's Nicotinic acetylcholine receptors, accounting for its pharmacological effects.

- **Glycosides**

Alexandrian senna, cascara, and rhubarb are among the medicinal plants that contain anthraquinone glycosides[11]. Plant-based laxatives made from such plants include senna, rhubarb and Aloe[12]. See figure 2 (Digoxin is used to treat infectious diseases, atrial fibrillation, atrial flutter and sometimes heart failure).

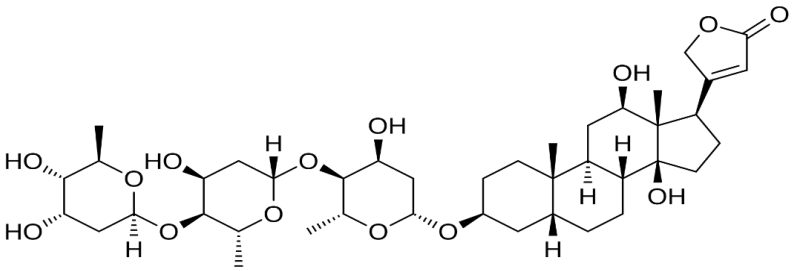

Figure 2: The glycosides from tobacco binds directly to the body's' Nicotinic acetylcholine receptors, accounting for its pharmacological effects.

- **Polyphenols**

Polyphenols of several classes are widespread in plants, having diverse roles in defenses against plant diseases and predators[13]. They include hormone-mimicking phytoestrogens and astringent tannins[14]. Plants containing phytoestrogens have been administered for centuries for gynecological disorders, such as fertility, menstrual, and menopausal problems[15]. Among these plants are Pueraria mirifica[16], kudzu, angelica,fennel, and anise[17]. See figure 3.

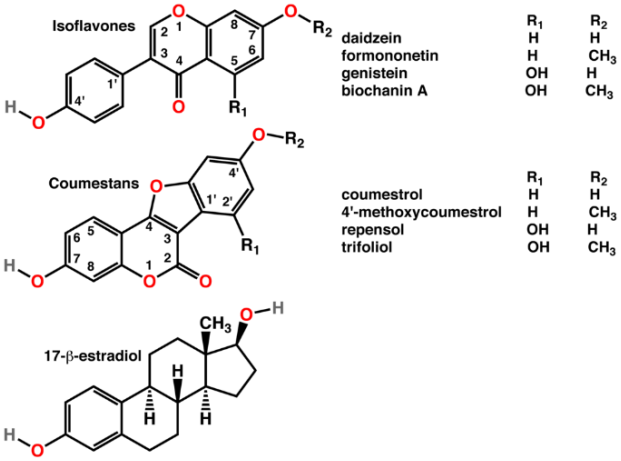

Figure 3: Polyphenols include phytoestrogens (top and middle), mimics of animal estrogen (bottom).

- **Terpenes**

Numerous medicinal plants[18] and resinous plants like conifers contain terpenes and terpenoids of various types. They deter herbivores and have a potent scent. They are useful in essential oils because of their scent, whether for aromatherapy or perfumes like rose and lavender[19,20,21], Some have therapeutic applications; for instance, thymol, an antibacterial, was formerly employed as a vermifuge[21] (anti-worm medication). See figure 4.

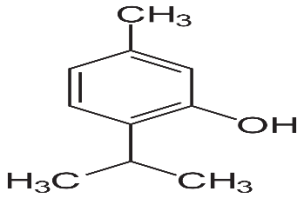

Figure 4: Terpenes compounds in ethanol and butanol extracts of seeds and leaves of *M. Oleifera*

1.3 Moringa Oleifera (M. Oleifera)

Plants could be used for multiple medicinal purposes. Moringa oleifera (MO) is considered the most famous plant used for this purpose Among these medicinal plants, the most effective is Moringa oleifera, a medicinal plant belonging to the Moringaceae family. The plant is known worldwide as the "miracle tree" due to its high nutritional value. Parts of the Moringa plant have had nutritional and medicinal importance since ancient times[22]. Moringa oleifera has been widely used and is distinguished by its unique composition of phytochemicals, such as phenolic acids, flavonoids, carotenoids, alkaloids, tannins, lectins, and terpenoids. These substances are characterized by their many properties, such as liver protection, antioxidants, and active antimicrobial properties. Researchers are targeting the development of drugs

II. Materials and Methods

2.1 Preparing leaf powder

1- Place the dry leaves in the grinder to make a very fine powder.
2- Transfer 25 g of powder to a beaker containing 100 ml of deionized water and by infusion overnight then it was filtered (through Whatman No.1 filter).
3- Filtrate the was pour it into dishes, and place it in the oven at 40°C for 24 hours.
4- After ensuring that it is completely dry, scrape off the dry layer of powder and collect it in special containers for later use.

2.2 Preparation of aqueous solution.

1- Different concentrations (5%, 50%, and 90%) of these aqueous solution were prepared and used in further processes.

2.3 Preparing of Culture media (Nutrient agar)

- **Prepare of Nutrient Agar?**

Suspend 28g of nutrient agar powder (CM0003B) in 1L of distilled water.
Mix and dissolve them completely. In our current experiment, 100 ml was used, so 2.8 g was dissolved instead of 28 g.
Sterilize by autoclaving at 121°C for 15 minutes.
Pour the liquid into the petri dish and wait for the medium to solidify. Be sure that you are preparing the agar in the clean environment to prevent any contamination**.**

2.4 Clinical Isolation

Bacterial isolates were collected from Alnukhba Lab/hilla city after being diagnosed with the Vitek device.

2.5 Antibiotic discs

A group of antibiotic discs was used for comparison. See below list
1- Netilmicin (NET) 30 mcg, Levofloxacin (LE), Penicillin G (P), Amphotericin B (AMB), Tobramycin (TOB) and Optochin (OP).

- **2.6 Evaluation of antibacterial activity of aqueous solution of Moringa Oleifera on positive bacteria by using of Disk diffusion method.**

1- Bring dishes prepared in the manner mentioned above.
2- Make small holes or wells in the planting media, with a radius of about 5 mm.
3- Inoculation of culture media with the bacteria targeted in this current study by using of Zone inhibition.
4- Place the antibiotic discs and then place 75 microliters of the required concentration in the central well.

III. Results

1. Study of antimicrobial activity of aqueous extract (5% and 50%) of M. Oleifera plant leaves by using of Zone inhibition compared to standard antibiotic disc.

The current study showed that there is a weak and limited effect of the aqueous extract with 5% concentration on the other hand there was significantly affecting with 50 % of Moringa plant leaves extract on gram positive bacteria (S. aureus). As well as the results also showed that the effect of the 5% concentration of the extract was much less than the effect of the two (Le and NET) antibiotics, while the effect of the 50% concentration was similar to them. See below figure 5.

A: 5 % **B: 50 %**

Figure 5: Show the effect of concentrations A: 5% and B: 50% of aqueous extract of Moringa plant leaves on gram positive bacteria (Staphylococcus aureus).

2. Study of antimicrobial activity of aqueous extract (5% and 90) of Moringa Oleifera by using of Zone inhibition % compared to standard antibiotic disc.

The effect of aqueous solution for the Moringa Oleifera plant leaves demonstrated in figure 6. When using a higher concentration (90%) of Moringa leaves extract, the inhibition was much greater compared to the effect of the 5% and 50% concentrations. On the other hand, the effect of the 90% concentration of the Moringa extract was much greater compared to the effect of the two (Le, TOB and NET) antibiotics. Also It was found the is no effect of remaining antibiotics (P, OP, and AMB) on S. aureus bacteria.

A: 50 % **B:** 90 %

Figure 6: Show the effect of concentrations A: 50% and B: 90% of aqueous extract of Moringa plant leaves on gram positive bacteria (Staphylococcus aureus).

IV. Discussion

The increasing frequency of pathogenic germs that are resistant to the more recent or contemporary antibiotics developed during the past three decades has raised concerns, as shown by Valarmathy et al., 2020. Because they can accomplish the same goal with fewer adverse effects than synthetic antimicrobials, plant-based antimicrobials have immense therapeutic potential. M. oleifera is a highly prized plant with a remarkable array of therapeutic applications and a high nutritional content[24]. Antibacterial Activities of Moringa Oleifera has been shown in different studies. Using the Disc agar diffusion technique, Bukar et al 2010 evaluated the antibacterial activity of Moringa oleifera leaf One Gram-positive bacterium (Staphylococcus aureus). The result of M. Oleifera leaf extracts show that it had activity against four gram positive bacterial isolates.

In this study, we evaluated the antimicrobial activity of Moringa Oleifera leaf extracts against pathogenic bacteria included or represented in *Staphylococcus aureus* isolated from chronic wounds infections. The results obtained showed that all concentrations of methanolic extracts of Moringa oleifera leaves extract had high inhibitory effects on S. aureus. These results are consistent with the results of Talreja, et.al in (2010). Also in this study it was found that the effect activity is directly proportional to the concentration. Other researchers have observed that Moringa oleifera leaf extracts had varying antibacterial efficacy against various pathogens. Chloroform extract of Moringa oleifera Lam. plant leaves had antibiotic properties against a variety of pathogens, including Escherichia coli (MTCC 443), Pseudomonas aeruginosa (MTCC 424), and Staphylococcus aureus (MTCC 3160), according to Devendra et al. (2011) and *Streptococcus pyogenes* (MTCC442).

These effects of Moringa Oleifera may result from the components found in its leaves such as protein, lipids, minerals, vitamins, tannin, favonoids, saponins, phenolic acids, isothiocyanate, and others are active components isolated from Moringa Oleifera. The pharmacological and medicinal aspects of these compounds. The cultivation processes and storage of plant parts have a great influence on the contents of these bioactive compounds which may afectits application and use[28].

V. Conclusion

These results provide valuable information that Moringa oleifera hold great promise as highly effective antibacterial agents.

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