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INHIBITION OF *STAPHYLOCOCCUS PASTEURI* USING *MORINGA OLIFERA* LEAVES EXTRACT

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Abstract:

In This study the antibacterial activities of aqueous ethanol, hexane and water extracts of leaf of *Moringa olifera* were examined. The solvent extracts were tested against some pathogenic bacteria isolated from different patients at Zagazig Hospital University (ZHU). The highest degree of antibacterial activity was shown by the aqueous ethanolic extract of leaf of *Moringa olifera* against pathogenic bacteria. 16srRNA used to identify the most sensitive bacteria. This bacterium which identified as *Staphylococcus pasteuri*MN368257.There is a great effect of *Moringa olifera* than different types of antibiotics against *Staphylococcus pasteuri* as the indicator organism. Transmission Electron Microscope (TEM) examination of the moringa leaves-treated bacteria showed the antibacterial action of moringa leaves against *Staphylococcus pasteuri* was manifested by signs of cellular deformation, partial and complete lysis of cell components.

Keywords: *Moringa olifera*, *Staphylococcus pasteuri*, ethanol extract, Antibacterial activity.

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Introduction:

Moringa oleifera is a perennial tree, still considered as among underutilized plant and falls under Moringaceae family. *Moringa oleifera* is grown for its nutritious pods, edible leaves and flowers and can be utilized as food, medicine, cosmetic oil or forage for livestock. Its height ranges from 5 to 10 m **(Padayachee and Baijnath, 2012).**The plant is also known as horse radish tree and drum stick tree. Other most important and valuable species of plant Moringa are *M.oleifera, M.arborea,*

M.drouhardii, M. ovalifolia, M. longituba, M. rivae, M. borziana, M. corcanensis, M. hildebrandtii, M. ruspoliana, M. stenopetala, M. peregrine, M .pygmaea. All plant parts are having remarkable range of some functional and nutraceutical properties **(Singh *et al*., 2012).**

Moringa oleifera is a small graceful tree with sparse foliage often planted in compounds or used in fencing in Nigeria. It resembles a leguminous species at a distance especially when flowering. Moringa is rich in nutrition owing to the presence of a variety of essential phytochemi-cals present in its leaves, pods and seeds.

Many pathogenic bacteria are known within Monera kingdom; due to cell wall structure and thickness, these pathogenic bacteria were divided into Gram negative and Gram positive. *Staphylococcus pasteuri* is a coagulase-negative, Gram positive organism which is emerging as an agent of nosocomial infections and a blood derivatives contaminant, though its role in causing human disease mostly remains controversial. Despite the paucity of isolates recovered, this bacterium has recently appeared to express resistance against several classes of antibiotic compounds, such as methicillin/oxacillin, macrolides, lincosamides, streptogramins, tetracyclines, chloramphenicol, streptomycin, fosfomycin, as well as quaternary ammonium compounds. **(Carretto, 2005)**.

Materials and Methods

1- Microorganisms and media used:

One hundred bacterial species belong to Gram- positive and Gram- negative bacteria were used in this study were procured from the Zagazig University Hospital (ZUH). from these species only one Gram- positive bacteria has inhibited by Moringa plant. This was as *Staphylococcus pasteuri* MN368257 was used for propagation of bacteria and in antibacterial bio assay experiments.

2- Identification of selected isolate:

Identification of selected isolate was confirmed by sequencing of partially amplified 16S rRNA gene. The DNA was extracted from bacteria following the protocol recommended by Sambrook & Russell (2001). 16S rRNA gene was sequenced using 5'-AGAGTTTGATCC TGGCTCAG-3' as forward primer and 5'-GGTTACCTTGTTACGACTT-3' as reverse primer. NCBI BLAST program (www.ncbi. nlm.gov/blast) and ClastalW2 program (https:// www.ebi.ac.uk/Tools/msa/clustalw2/) for sequence similarity and phylogenetic analyses was used to assess the similarities of the obtained 16S rRNA gene sequence in Genbank database.

3- Collection of Moringa plant:

Moringa oleifera leaves was collected from El-Shabanat village, Zagazig, Sharkia, Egypt, and identified by Botany Department, Faculty of Science, Zagazig University.

4- Solvent extracts preparation:

Different solvents (200 mL) hexane, ethanol 70% and distilled water using magnetic stirrer at room temperature were used for extracted 20g of each sample individually and followed by filtration through What man no.1 filter paper. The residues were re-extracted under the same conditions, and then hexane combined

filtrates were evaporated in a rotary evaporator (BüCHI-water bath-B-480) below 40°C. Ethanol 70% and distilled water extracts were freeze- dried (Thermo- electron Corporation – Heto power dry LL 300 Freeze dryer). To determine the yield, the dried extracts after evaporation of solvents were weighed and stored at -20°C until analysis carried out**.**

5- Screening moringa plant extracts for their antibacterial activities:

Disc diffusion method: Moringa leaves hexane; water and ethanolic extract were tested against pathogenic bacteria by the Kirby-Bauer disk-diffusion method. The indicator bacteria were swabbed on the surface of agar plates. Then, filter paper discs were soaked in leaf extracts for 15 min and placed onto the agar plates previously seeded with the indicator bacteria. After incubation for 24 h, inhibition zones diameter (IZD) were measured by mm ruler after subtracting the diameter of the filter paper disc **(Bauer *et al*., 1966).**

6- Transmission Electron Microscopy (TEM) analysis:

Staphylococcus pasteuri MN368257, was selected for TEM examination. This bacteria was grown in nutrient broth incubated at 37 ˚C to reach about 10^6 CFU mL -1 . The values of about 50 µg/mL of ethanolic leaf extract was added to *Staphylococcus pasteuri* cell suspensions respectively except controls and incubated at 37 ˚C for 4 h.. Ultrathin sections were prepared for investigation by TEM.

Perfusion or immersion fixation of the tissue occured using a modified procedure **(Karnovsky, 1965).** The cells were left overnight at 4° C, then washed 3 x for 15 min in 0.1 M sodium phosphate buffer + 0.1 M sucrose and postfixed 90 min. in 2 % sodium phosphate buffered osmium tetroxide pH 7.4. Then washed 3 x for 15 min in 0.1 M sodium phosphate buffer pH 7.4 and dehydrate 2 x 15 min: 50 % ethanol (in distilled water). Then contrasted overnight using 70 % acetone + 0.5 % uranyla cetate + 1 % phosphotungstic acid at 4° C, 2 x for 15 min. 80 % ethanol, 2 x 15 min. 90 % ethanol, 2 x for 15 min. 96 % ethanol, 3 x 20 min. 100 % ethanol and 2 x 15 min. acetone. Then 30 min. 2 : 1 acetone : Epon mixture, 30 min. 1 : 1 acetone : Epon mixture ,30 min. 1 : 2 acetone : Epon mixture, Epon pure solution overnight at 4° C and finally new fresh Epon solution. After that they were put in incubator for 48 h. at 65° C for polymerization and cut with an ultra microtome set to 50 - 100 nm section thickness.Then rinse sections to grids or gelatine-covered one-whole grids made of cooper or nickel. Post contrasting of sections were carried out as reported previously: 10 min. 8 % uranyl acetate and 5 min 0.7 % leadcitrate + 0.9 % sodium citrate after drying for 15 min sections may be investigated in a transmission electron microscope **(Reynolds, 1963).** Ultrathin sections were observed at 80 kV using a JEOL 2100 TEM at 80 KV at EM Unit, Mansoura University, Egypt.

Results and Discussion:

The present study was conducted to obtain preliminary information on the antibacterial activity of hexane, water and ethanol extracts of *Moringa oleifera* Lam. leaves in Zagazig, Egypt against some pathogenic bacteria, only one pathogenic bacteria is sensitive and identified by 16srRNA **(Figure 1).** The disc diffusion method was applied to be used in this study. The ethanolic extract has greater antibacterial activity than hexane and water extracts **(Figure 2)** and **(Table 1)**.This result is interesting because in the traditional method of treating a bacterial infection, decoction of the plant parts or boiling the plant in water is employed whereas,

according to present study, preparing an extract with an organic solvent was shown to provide a better antibacterial activity, Ethanol extract of fresh leaves showed the antibacterial effect against the tested Gram-positive bacteria (*Staphylococcus pasteuri*) and their respective diameter zones of inhibition were 63, for leaves. But no inhibitory effects of hexane and water extracts of leaves were noticed. These results disagree with the results that obtained by **(Mashiar *et al.*, 2009)** were reported that ethanol extract of *Moringa olifera* leaf has no inhibitory effect on genus Staphylococcus.

TEM Image Analysis

TEM images of moringa leaves-treated bacteria given in **(Figure 3)** show various signs of cellular deformation, indicating on direct disruptive action of moringa leaves on the cell wall and cell membrane. *Staphylococcus pasteuri* intact cells treated with 25 µg /mL of moringa leaves showed evidently reduced relative contents after 4 h of incubation at 37°C and high mortality rates. The bacteria escaping the death were characterized by different manifestations of deformation, such as cell shrinkage, cell membrane wrinkles and pore formation as well as some emptiness of cellular live materials. The analysis of TEM images indicated that moringa leaves caused total degeneration of cell membranes, cell swelling, and vacuole formation and finally completes lysis of cell components.

A mixture of aqueous ethanolic moringa leaves extract with Vancomycin (30 mcg), Tetracycline (30 mcg), Amoxicillin (25 mcg) has less effect on *Staphylococcus pasteuri* than leaves without antibiotics **(Table 2 and Figure 4).**

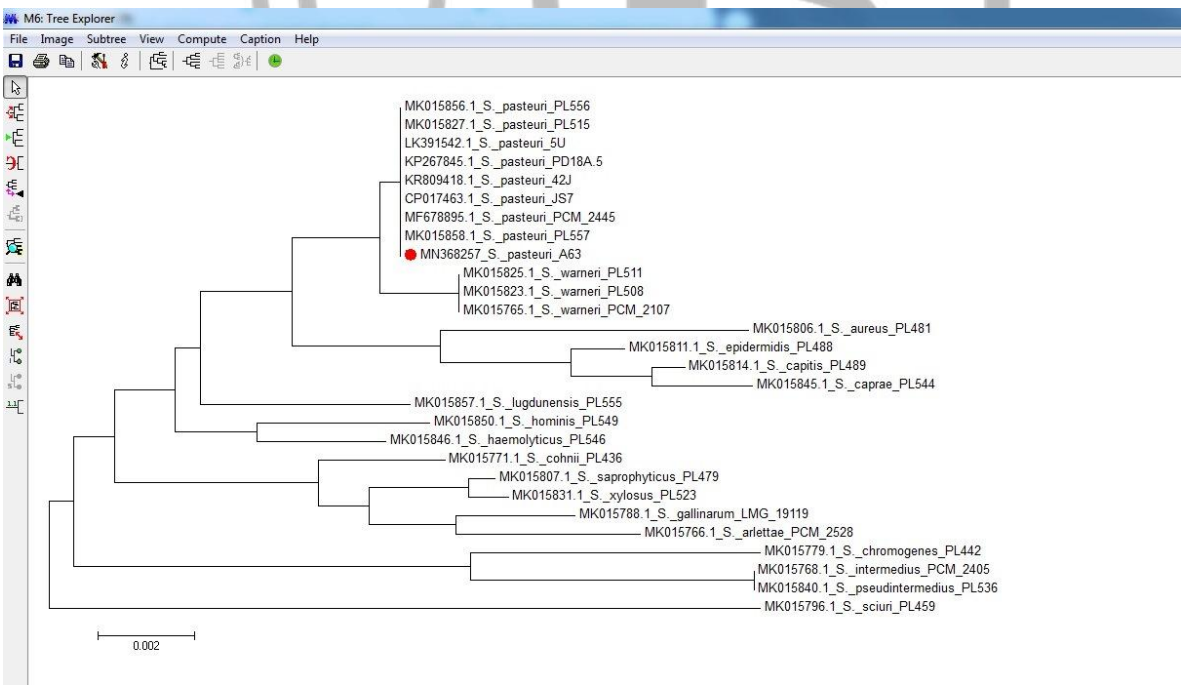

Figure 1: Phylogenetic tree of *Staphylococcus pasteuri* MN368257

Table (1): Antibacterial activity of leaves of *Moringa olifera* extract against *Staphylococcus pasteuri* by disc diffusion method.

Values of inhibition zones are means of three replicates. (-): No inhibition zone, extracts of **H**: Hexane; **EA**: ethyl alcohol; **W**: Water.

Figure 2: Antibacterial activity of aqueous ethanolic extract of moringa leaves.

Bacteria	Inhibition zone diameter (mm)	
Staphylococcus pasteuri	Solvent extracts	Leaves
	EA	63
	H	—
	W	—

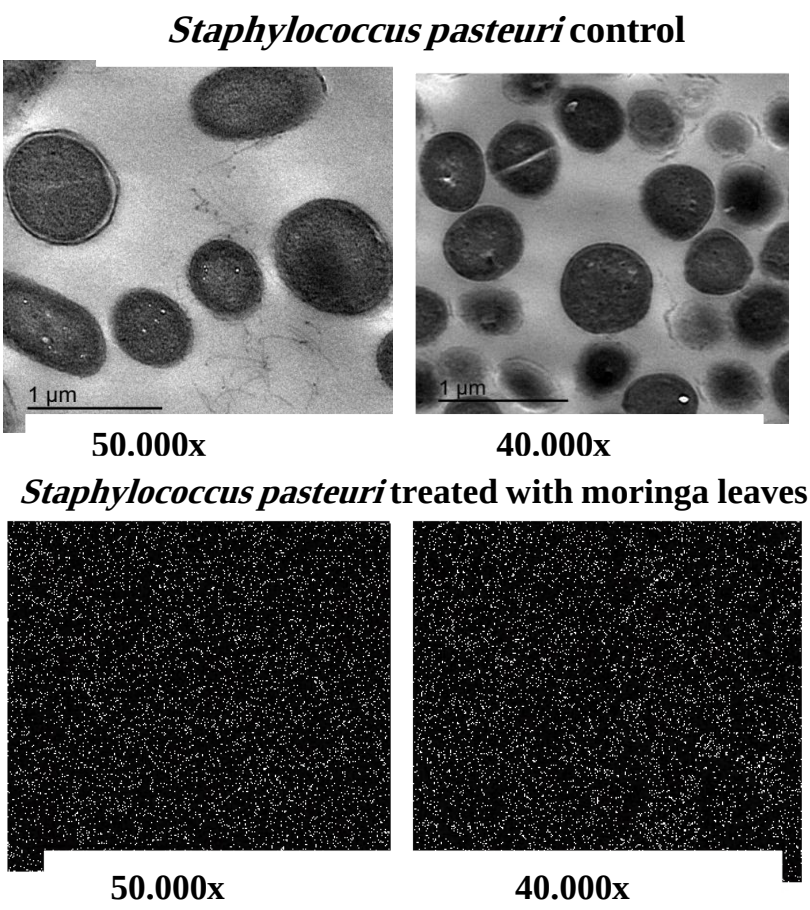

Figure 3: TEM of *Staphylococcus pasteuri* treated with moringa leaves compared to untreated control bacteria.

Table (3): Antibacterial activity of *Moringa olifera* leaves extract against *Staphylococcus pasteuri* compared to different types of antibiotic by disc diffusion method.

Concentration (µg ml^{-1})	Inhibition zone diameter (mm)
A=Vancomycin (30 mcg)	25
B=Tetracycline (30 mcg)	30
C=Amoxicillin (25 mcg)	-ve
D=Vancomycin (30 mcg) + moringa leaf extract	35
E=Tetracycline (30 mcg) + moringa leaf extract	30
F=Amoxicillin (25 mcg) + moringa leaf extract	60
G=Moringa leaf extract	63

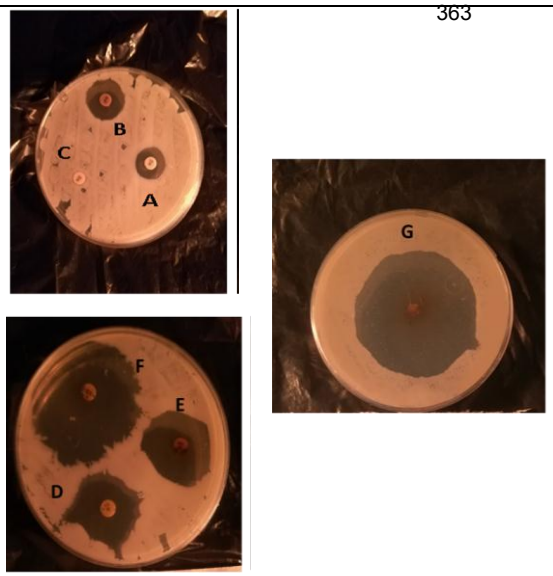

Figure 4: Antibacterial activity of mixing of moringa leaf extract and different types of antibiotics against *Staphylococcus pasteuri*

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