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### ABSTRACT

Control and treatment of infection are one of the big challenges in medicine. The development of different strategies for the reduction and elimination of infection is an active field in various medicine and pharmacology. A unique formulation of "Disinfectant and Antiseptic Nano- compound using Ferula assa-foetida (NANO HING GUARD<sup>®</sup>)" attempts to increase the antiseptic power and reduce the adverse effects of chemical disinfectants and create stability using active ingredients including hydro-alcoholic extract of Ferula assa-foetida oleo-gum, zinc oxide nanoparticles which interact with each other, creating High-level antibacterial, antiviral, and anti-fungal properties.

The Ferula assa-foetida plant (Hing/Kuma), with its remarkable antimicrobial action against various bacteria, is a suitable agent for controlling bacterial, parasitic, fungal, and viral infections. Zinc oxide Nano-particles are non-toxic and biocompatible. Damage of DNA, cell membranes, and cellular proteins are considered various mechanisms for the antimicrobial activity of zinc oxide nanoparticles.

NANO HING GUARD<sup>®</sup> is used to disinfect and remove biofilm from all surfaces, halls, means of transportation, equipment in the food industry, livestock, poultry, aquaculture (disinfection of surfaces, tools, feeders, drinkers, and other equipment), slaughterhouses, agriculture, and related industries, healthcare centers such as hospitals, clinics, etc., public places, and households. This combination can be supplied as a solution, aerosol spray, or other standard methods. The results of Nano scale-analysis such as XRD, DLS, and TEM indicate the Nano- composition of the NANO HING GUARD<sup>®</sup>. The most important general advantages of this patent involve the use of native medicinal plants to replace disinfectant chemical compounds, the possibility of production on an industrial scale, non-resistant microorganisms, non-toxicity, environmental friendliness, strong effectiveness (Reduction 10<sup>7</sup> CFU of Microorganism), the non-destructive effect on buildings and equipment, its widespread use for disinfection in humans, livestock, poultry, and aquatic animals.

**KEYWORDS:** High- level Disinfectant, Antiseptic, Zinc Oxide nanoparticle, Ferula assa-foetida

### INTRODUCTION

Designing and formulation of a strong antiseptic and disinfectant compound based on plant materials and nanoparticles is essential due to the widespread prevalence of emerging or reemerging bacterial, viral, and endemic and pandemic diseases in society, increasing incidence of antibiotic resistance in bacteria, allergies, anaphylaxis shock in response to antibiotics, ineffectiveness, adverse environmental effects, and detrimental effects on tissues (such as skin and respiratory allergies, carcinogenesis, etc.) that occur following the use of commercial disinfectants based on chemical compounds. Researchers in various medicine, microbiology, and pharmacology fields are continually seeking new compounds to control, eliminate, and treat infections. Herbs have been used for centuries as a traditional and alternative medicine worldwide to treat infectious diseases. The World Health Organization has also recommended using natural substances to eliminate pathogens, which relieves concerns about the adverse effects of chemical compounds.

Hing/Kuma, scientifically named Ferula assa-foetida, is a medicinal plant of the genus Umbelliferae, whose primary origin is Iran and Afghanistan. Ferula assafoetida oleo-gum is obtained from incisions in the root or stem of Ferula assafoetida. The Ferula assa-foetida plant, with its remarkable antimicrobial action against various bacteria, is a suitable agent for controlling bacterial, parasitic, fungal, and viral infections. Sulfide nanomaterials and metal oxides have excellent antibacterial, antifungal, and antiviral properties. Hence, disinfectant compounds that contain these nanomaterials can express effective antibacterial, antifungal, and antiviral properties. Zinc oxide nanoparticles are non-toxic and biocompatible nanoparticles. Various mechanisms have been reported for zinc oxide's antimicrobial activity, including ROS release, which causes oxidative stress by damaging DNA, cell membranes, and cellular proteins, resulting in cell wall breakdown and, subsequently, leakage of cell contents, and eventually, cell death. Zn2+ release is another mechanism that can damage the cell membrane and penetrate intracellular contents. Abrasive ZnO also increases membrane permeability.

A unique formulation of "Disinfectant and antiseptic nanostructure compound using Ferula assa-foetida (NANO HING GUARD<sup>®</sup>)" attempts to increase the antiseptic power and reduce the adverse effects of chemical disinfectants and create stability using active ingredients including hydroalcoholic extract of Ferula assa-foetida oleo-gum, zinc oxide nanoparticles, saponin, ethylene diamine tetra acetic acid, hydrochloric acid, Acetic Acid which interact with each other, creating antibacterial, antiviral, and antifungal properties. The present product is completely recombinant and a High-Level Disinfectant. Reviewing the articles and published studies in reputable domestic and international journals, WIPO, Google Patent, FPO, LENS, and Iranian Patent Center, no similar study or patent was completed for the claimed compound making antiseptics and disinfectants, indicating an innovative effort in developing this product.

## Materials and Methods

At first, for the production of NANO HING GUARD<sup>®</sup>, The hydroalcoholic extract of Ferula assa-foetida oleo-gum was prepared using the maceration method and hydroalcoholic solvent. Dissolving the zinc oxide nanoparticles (NAMAGO, Iran) in water is achieved by adding acids. After decreasing the zinc oxide solution's temperature, EDTA (Merck, Germany) is obtained according to the harvest formulation, and the solution is added while mixing. After proper mixing of the above compounds, the hydroalcoholic extract of Ferula assa-foetida is added. Finally, distilled water is added to finalize the solution volume. Characterization was done by dynamic light scattering (DLS), TEM, and XRD techniques. NANO HING GUARD<sup>®</sup> Antimicrobial activity was assayed against Gram-positive, Gram-negative bacteria, opportunistic fungi, Candida, and Spore bacteria using the well method and diffusion assay method. The minimum inhibitory concentration (MIC) was determined using the dilution method. The assayed compounds showed different degrees of antifungal and antibacterial activities, depending on the annealing temperature of preparation of these compounds. Antiviral activity Analyses of the claimed compound's effectiveness were was determined in terms of reduction in viral titers, measured by Haemagglutination (HA) by Using of Avian Influenza Virus (H9N2), Foot & Mouth Disease, Gumboro, Newcastle Disease (NVD). LD50/LC50 of above-mentioned mixture at dilution rate 1, 1/2, ..., 1/16 were injected in chorio-alantoic sac and egg Yolk.

# THE DISINFECTANT AND ANTISEPTIC NANO- COMPOUND USING THE FERULA ASSA-FOETIDA (NANO HING GUARD<sup>®</sup>)

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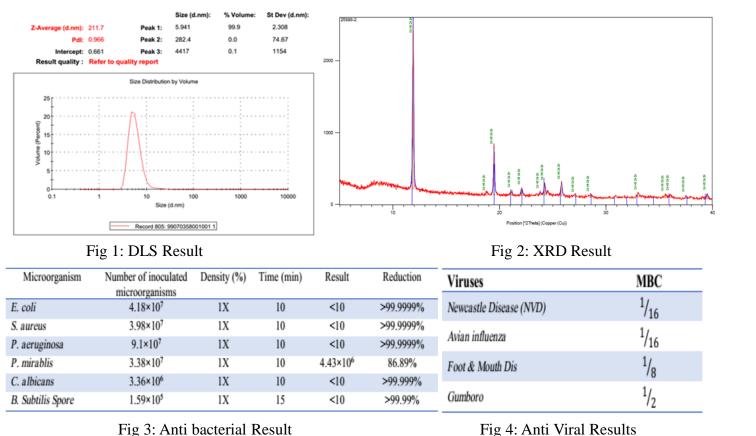
#### Results

Characterization data showed that accurate nano synthesis. Based on DLS data, the average size of synthesized nano-structures was 211.66 nm. The TEM imaging of the designed nanostructure was in accordance with the size obtained with DLS. The XRD analysis was shown in figure 2. According to this pattern, the characteristic peaks indicated its potent crystalline nature. These peaks are observable with high intensity in XRD pattern.

The test results of the effectiveness of the compound are presented using the well method. The effect of the claimed compound on Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa caused a 7-log reduction of strains. The effect of the claimed compound on the fungal strains of Candida albicans and Bacillus subtilis spore caused 6- and 5-log reduction, respectively. Therefore, according to the standard of disinfectant compounds, the claimed compound is categorized as a high-level disinfectant compound due to its ability to remove different bacteria, fungal, and bacterial spores, and viral categories.

Test results which were prepared by the Microbiology Laboratory of the Faculty of Veterinary Medicine, University of Tehran, after determining the effectiveness and complete removal of the affected strains with a 1X concentration of the claimed disinfectant compound, MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration) of this product were determined. MIC or MBC was measured by preparing a serial dilution from the original solution and evaluating its effect on the mentioned strains.

Test results for the effectiveness of the claimed compound on important viral categories in veterinary medicine (Newcastle virus, avian influenza, foot-and-mouth disease, and Gumboro virus). This report, which was prepared by the Microbiology Laboratory of the Faculty of Veterinary Medicine, the University of Tehran, MBC of this product was determined after determining the effectiveness and complete removal of the affected categories with a 1X concentration of the claimed disinfectant compound. MBC was determined by preparing serial dilution from the original solution and evaluating the effects on viral categories. Interestingly by all of the dilution making inactivate of Viruses but not effective on egg embryo.



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## CONCLUSIONS

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