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Synergistic effect of *Euphorbia Milii* with Tannic Acid as a disinfectant against *Escherichia coli* and *Staphylococcus aureus*

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Abstract

Herbal disinfectant is the cheapest and most unique way to clean a surface. This study focused on the synergistic impact of *Euphorbia Milii* and Tannic acid as a disinfectant against microorganisms. The aqueous solvent extract of plant leaves was used mixed with tannic acid against *Staphylococcus aureus* (gram-positive) and *Escherichia coli* (gram-negative) bacteria tested by the disk diffusion method. Both bacterial species were isolated from the kitchen surface. Minimum Inhibitory Concentration (MIC) was recorded with an optical density at 600 nm using a UV-spectrophotometer, which showed inhibition of bacterial growth in a cultural broth mixed with extract of *Euphorbia Milii* and Tannic acid. According to the findings, the disinfectant showed a maximum zone of inhibition for *E. coli* (14 mm) and *S. aureus* (20 mm). The disinfectant activities of extract were tested and estimated using a time-kill analysis. Fourier transform infrared spectroscopy (FTIR) analysis was conducted to identify the chemical bond, giving information related to the active sites of chemical compounds present in disinfectants. Overall, this study reveals that *Euphorbia Milii* is an excellent candidate to formulate disinfection.

Keywords. *Disinfectant, Euphorbia, Minimum Inhibition Concentration, Spectrophotometer, Synergistic effect.*

INTRODUCTION

The bacterial spread of diseases occurs as a result of healthy infections fostered by the kitchen surface. It creates a crucial component during the disease's direct transmission. Disinfectants are chemicals or mixtures of substances that can be applied as antimicrobial agents on non-living things to eliminate germs in the environment (Padya and Doshi, 2017). There are two types of disinfectants on the market. chemically manufactured disinfectants and natural herbal disinfectants. Herbal disinfectants are now commercially accessible as a cost-effective and environmentally friendly option (Padya and Doshi, 2017 and Kaczmarek, 2010). Tannic acid is a biomaterial with biological and chemical characteristics, a natural tannin of phenolic acid molecules. Tannic acid has unique properties (Kaczmarek, 2010). *Euphorbia milii* is a flowering plant species in the Euphorbiaceae family. Researchers from all around the globe have studied the effects of different *Euphorbia* species on microbial infections (Saleem, 2019 and Singh, 2018 and Ismaila, 2017). Several types of *Euphorbia* have been used to treat infectious disorders, including warts, antimicrobials, and intestinal parasites. *Euphorbia milii latex* is known as the Crown of

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Thorns. This is the most common genus of the medicinal plant used globally. This plant is used for ornamental purpose and also contain excellent analgesic activity. This study will provide environmentally friendly and active disinfectants that kill these microbes and be used for domestic areas.

LITERATURE REVIEW

Basavegowda *et al*. 2020 investigated the antibacterial activity of several solvent extracts of E. *cotinifolia* leaves against certain human pathogenic microorganisms. They employed agar cup diffusion and minimum inhibitory concentrations with micro broth dilution (MIC). The inhibition zones measured against test microorganisms were 15.25-19.50 mm and 13.50-19.25 mm, respectively. Singh et al. (2018) investigated the antibacterial activity of Euphorbiaceae members against human infections using an ethanolic extract. They also assessed their phytochemical constituents. *Euphorbia milii, Euphorbia hirta, Euphorbia pulcherrima, Euphorbia tithymaloides, Euphorbia prostrata,* and *Emblica officinalis* are the species involved in their research. Narendra et al., 2015 investigated the antibacterial activities of hexane, ethyl acetate, acetone, and methanol extracts of *Euphorbia milii* (Euphorbia) flowers on *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, and *Proteus vulgaris* using the cup plate technique. Hexane, acetone, and methanol extracts reveal an open significant inhibitory zone for Staphylococcus aureus and *Bacillus subtilis* at 5g/ml concentrations.

RESEARCH METHODOLOGY

Preparation of disinfectant using Euphorbia milii extract and Tannic Acid
Euphorbia milii leaves were obtained from the USPCAS-W Garden of Mehran University, Jamshoro. The plants were harvested, cleaned with water, completely washed with distilled water, and dried. Dried leaves were pounded with a mortar and pestle and filtered through a muslin cloth. Tannic acid was measured and mixed with water.

Test organisms
As test organisms, two types of bacteria (*E. coli and S. aureus*) were used in this investigation. The bacterial strains were isolated from the USPCAS-W Kitchen and cultured on Muller-Hinton agar (MHA) for 24 hours at 37 °C incubation.

Antimicrobial properties of Euphorbia milii extract using the disk diffusion method
The disc diffusion technique was used to assess the antibacterial properties of *Euphorbia milii* leaves. Different concentrations of tannic acid mixed with plant leaves extract was administered to Whatman filter paper disks of 6 mm and dried. All tested disks were placed on MHA containing Petri plates having tested bacterial lawn. All Petri plates were incubated

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for 24 hours at 37°C. For each disk, the diameter of the inhibitory zone was measured and recorded.

Minimum Inhibitory Concentrations (MIC) of Euphorbia milii extract

The MIC of *Euphorbia milii* extract was also measured using a broth serial dilution technique, diluting in a series of test tubes containing Miller-Hinton broth and tested bacteria. The growth of bacteria was monitored using an optical density spectrophotometer set at 600 nm. MIC of *Euphorbia milii* was calculated in the presence and absence of Tannic acid was.

Time kill analysis

Time-killing analysis was carried out in broth culture medium using separate tubes containing 1 ml of the bacterial strain, 8 ml of Muller-Hinton broth, and 1 ml of *Euphorbia milii* extract mixed with tannic acid. The optical density was measured at various time intervals, particularly at 0, 15, 30, 45, 60, 75, and 90 minutes. Between optical density and time, a growth inhibition curve was plotted.

Fourier-transform infrared spectroscopy of Euphorbia milii extract

For the FTIR analysis, aqueous extracts of *Euphorbia milii* leaves were used. Ten mg of dried extract powder was encapsulated in a 100 mg KBr pellet to make clear sample tablets. The crushed plant sample was analyzed using an FTIR spectrometer (Shimadzu, IR Affinity 1, Japan) with a scanning range of 400 to 4000 cm^{-1} and a resolution of 4 cm^{-1}.

FINDINGS AND DISCUSSION

Antimicrobial effect of *Euphorbia milii* as a Disinfectant
The antibacterial activities of fresh *Euphorbia milii* leave extract were tested using the disc diffusion technique against *E. coli* and *S. aureus* bacteria at different concentrations. 6, 12.5, 25, 50, and 100 l. These microorganisms showed varying quantities of antibacterial capabilities for plant extract based on inhibitory zones. Figure 1 illustrates *E. coli* inhabiting zones of 10, 11, 11, 12, and 14 mm at doses of 6, 12.5, 25, 50, and 100 µl, respectively. *S. aureus* inhibited zones as wide as 16, 17, 17, 18, and 20 mm at a concentration of 6, 12.5, 25, 50, and 100 µl. As the concentration increased, the inhibition of microbes also increased.

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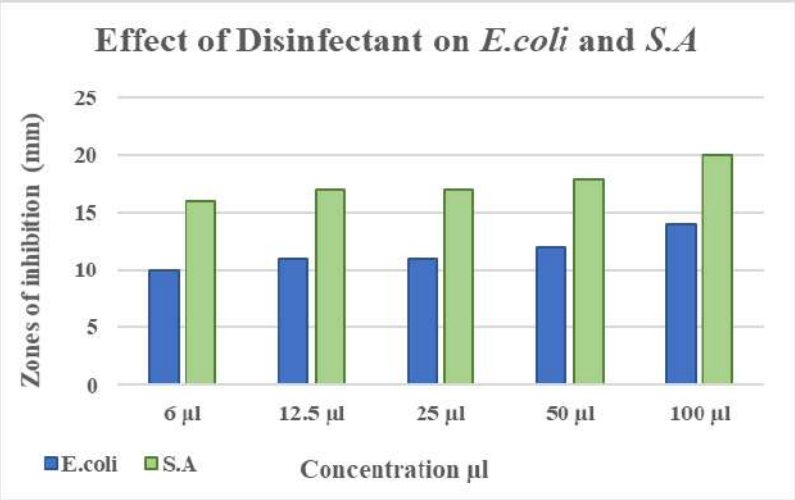

Figure 1. Antimicrobial effect of *Euphorbia milii disinfectant* on *E. coli* and *S. aureus*

Minimum Inhibitory Concentration of *Euphorbia milii* against Microbes

Figure 2 found that the concentration of *E. coli* at 125µl was increased while the growth, measured a 1.7OD, was decreased. The extract had a maximum growth on1.4 OD at 250 µl. It revealed a minimal reduction in growth at 0.2 and 0.1 OD at the concentration of 500 and 1000 µl, respectively.

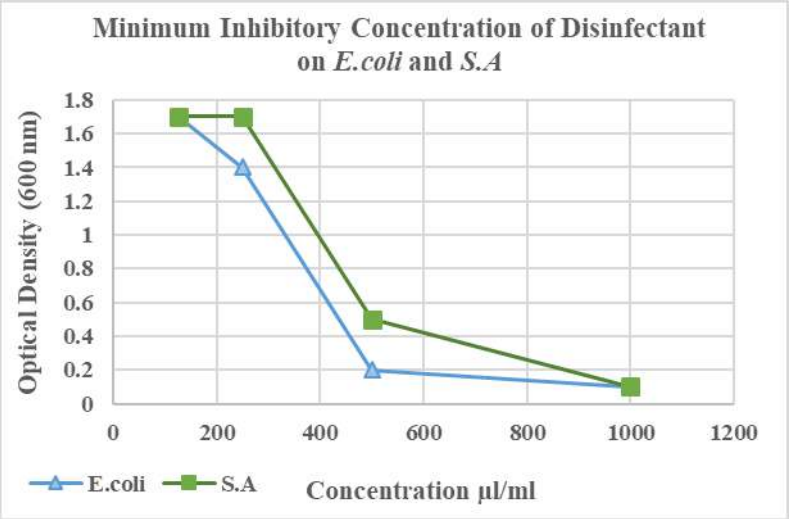

Figure. 2 MIC of *Euphorbia milii* against on *E. coli* and *S. aureus*

At 125 µl, the density of *S. aureus* began to rise, and the growth was observed slowing at 1.7 OD. A maximum increase in growth was measured at the concentration of 250 µl at 1.7 OD. In comparison, a minimum decrease in the growth of S. aureus was observed at the concentration of

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500 and 1000 µl of the extract with OD of 0.5 and 0.1, respectively. The growth rate of both of the microbes began to reduce as the concentration raised.

Time kill analysis

The growth of *E. coli* and S. aureus was significantly decreased from 0 to 15 minutes and stayed steady till 90 minutes, as observed in a time-kill study, shown in Figure 3. The result revealed that the mixture had good activity as a disinfectant.

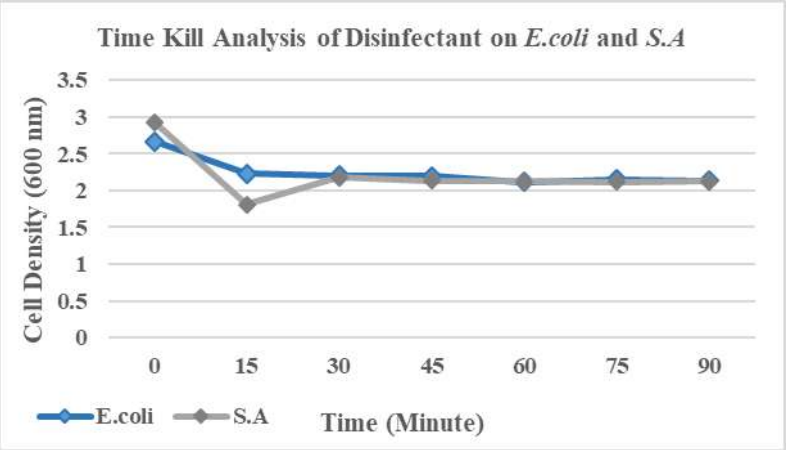

Figure. 3 Time kill analysis on *E. coli* and *S. aureus*

Fourier-transform infrared spectroscopy of *Euphorbia Milii* leaves extracts

The Fourier Transform Infrared Spectrophotometer (FTIR) was used to identify chemical bonds (functional groups) in compounds presented in Euphorbia milii extracts that displayed different characteristic peak values.

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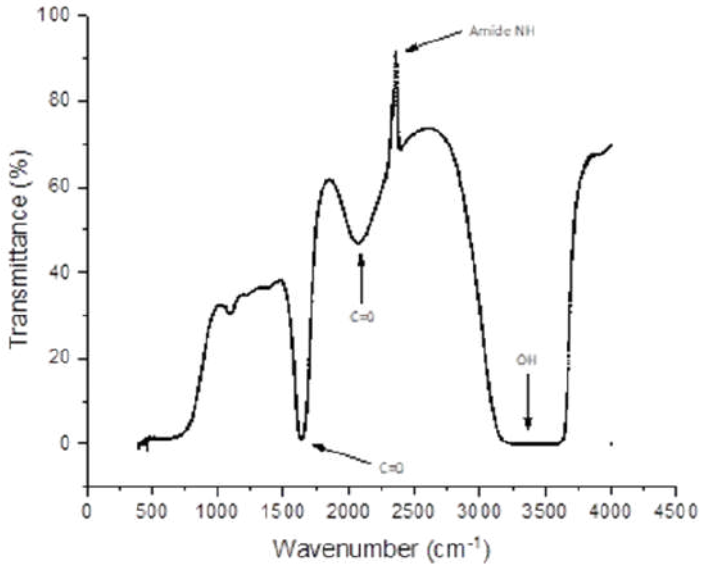

Figure. 4 FTIR of *Euphorbia Milii* leaves extracts

The FTIR spectrum of *Euphorbia Milii* leaves extracts showed the broadband at 3287 $cm^{-1,}$ identified as the OH group. The brand at peak 1223 cm^{-1} showed the presence of –C-O- bond.

CONCLUSION

The capacity of disinfectants to boost antibacterial characteristics is critical. In this study, we discovered that *Euphorbia Milii* leaves were effective against surface microorganisms at various doses in combination with tannic acid. The most potent inhibitory zones were determined, with varied doses suggesting the plant's most possible active against microbes. The lowest inhibitory concentration was also determined, indicating that various disinfectant concentrations had distinct effects on particular bacteria. To produce natural remedies for various general concerns and conquer many public issues, research on the phytochemicals found in these plants must be done.

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