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The Disinfectant Properties of Suspensions of Herbal Extracts: An Antibacterial Study against *Escherichia Coli* and *Staphylococcus Aureus*

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ABSTRACT:

The herbal disinfection suspensions were tested on microorganisms isolated from the kitchen floor surface using the disc diffusion technique. Three plant extracts were prepared in an aqueous solvent, and their inhibitory zones were determined. Aak, Pomegranate, and dumb cane leaf extract mixture were used for making disinfectant. For *E. coli*, the plant combination disinfection 1, at a concentration of 1500 µl, revealed 15mm of zones. Maximum zones for disinfectant 2 were recorded at 750 µl, which was 12mm. At 3000 µl concentration, 16 mm zones were observed for disinfectant 3. At 1500 µl concentration, 15 mm of zones were seen for disinfectant 4. Zones of inhibition of *S. aureus* were reported to be 18 mm and 15 mm at 3000 µl for disinfectants 1 and 2. At 375 µl, 21 mm of zones were found for disinfectant 3. At 3000 µl concentration, 17 mm of zones were observed for disinfectant 4. Comparing E. coli and S. aureus may conclude that *S. aureus* has more potential inhibitory zones than *E. coli*. All of the disinfectants showed promising antibacterial activity against surface microorganisms.

Keywords: Zones of inhibition, Disinfectant, Disk diffusion method, Microbes, Concentration.

1 INTRODUCTION

Surface microbes transmission is more potent to the risk of contamination, non-living surface transmission to humans and the spread of pathogens. The surface plays an essential role in transmitting pathogens, and these transfers are made from person to person and object, leading to health problems. Disinfection is the best way to remove most or all of the harmful microorganisms from non-living things (Exner et al., 2004). Disinfection is a viable option for surface cleaning. Therefore, there are two types of disinfectants: chemical disinfectant (based on alcohol and has unfavourable effects on humans) and herbal disinfectant (made of plant material having no adverse effect on human health).

Aak plant, scientifically known as *Calotropis procera*, is a flowering plant from the family *Asclepiadaceae*. Aak plant is also called milkweed, is a perennial shrub or small perennial tree (Bilal et al., 2020). It is a subtropical evergreen widespread in Asia, Africa, and the Middle East. Milkweed is one of the most common species in Pakistan as it is surrounded by natural vegetation. *C. procera* has broad spectrum antibacterial effect as an antibacterial agent (Kumar *et al*., 2010; Ahmed *et al*. 2005). It produces a milky white latex with a variety of medicinal qualities (Saadabi et al., 2012). The blooms are tiny, and the gloves are hollow. A thick white sap pours from the leaves and stems when they are removed. The latex of milky weed is toxic. Aak plant has antibacterial, antifungal, and anti-inflammatory effects. Because it is easy to propagate and handle, this plant is commonly planted as an ornamental in arid or coastal regions. Because of its anesthetic effects, *C. procera* is particularly useful in medicine (Kawo *et al*., 2009), and in both traditional medical and veterinary drugs, treatments of this crude extracts of the plant are commonly utilized (Johnson

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et al., 2011; Kareen *et al*., 2008; Alencar *et al*., 2004; Dewan *et al*., 2000). *Aak* plant has also been utilized as an antimicrobial agent for all types of bacteria (Patil and Saini, 2012). Bilal et al., 2020 examined antibacterial activities of *C. procera* leaves with methanolic extract against *Escherichia coli, Pseudomonas aeruginosa, Enterococcus faecalis, Klebsiella pneumonia, Bacillus cereus, Proteus mirabilis,* and *Salmonella typhi* using the disc diffusion methods. The leaves extract was found to be effective in treating infections caused by *Proteus mirabilis, Pseudomonas aeruginosa,* and *Bacillus cereus.*

Pomegranate, scientifically often known as *Punica granatum,* is a member of the *Punicaceae* family and is extensively grown in subtropical and tropical environments. This is one of the oldest edible fruits in the world. It is a large, deciduous shrub found mainly in Asia. *P. granatum* extracts reduce periodontal disease by regulating dental vegetation (Ali *et al*., 2018). *P granatum* has antibacterial, antifungal, and antiviral properties. Alkaloids, glycosides, resins, essential oils, gums, and tannins are some of the unique compounds that give plants their therapeutic value (Banu *et al*., 2019). In the mediterranean environment, it is one of the most important horticultural fruits. The edible section of the fruit is high in saccharides, polyphenols, and vital minerals (Ozkan *et al*., 2003). *P granatum* is a herb, and the leaves are around 2 x 1 inch, elliptical, with white or red blooms, are often considered poisonous (double-flowered races), and have some vertical, frail branches. *P. granatum* has been used in traditional medicine for a variety of ailments. Many illnesses, high blood pressure, Hansen's disease, bronchiolitis, indigestion, and, notably, seem to respond well to different parts of this plant. Calcium oxalate, methypelletierine, tannic acid, Gallotannic acid, alkaloid, tannic acid, sugar, gallic acid, isopelletierine, psuedopelletierine, and pelletierine are all found in abundance in the plant (Ismail *et al*., 2012). Pomegranate is often used as a medication for anthelmintic to treat and prevent diseases such as diarrhoea, canker sores, giardiasis, acidity, abscess, bleeding, and respiratory ailments. It has also been used to treat fevers (Larrosa *et al*., 2010; Lee *et al*., 2012).

Dieffenbachia, whose common name is Dumb-cane, is a long, green, perennial herb growing at 1 to 1.5 meters. It belongs to the *Araceae* family, which is the typical household plant. It is the oldest monocotyledon family, with over 105 genera and 3000 species of herbaceous monocotyledons. They primarily grow inside; however, they grow outside in some regions. Ganiyat *et al.* (2011) studied the dieffenbachia leaves and stemmed using essential oil as a solvent. Dumb cane shows a positive response towards *E. coli* and *staphylococcus aureus.* These mostly grow indoors, with occasional exceptions. The leaves of these species are 20 to 40 centimetres long, broad, and sharply lanced on both sides and are dark to glossy green on both sides. The tips are thin, and the base is circular. There is an inflorescence structure on this plant. It includes deadly alkaloids and toxic sap toxin glycosides. The toxic effects have been linked to calcium oxalate crystals and free nitrogen. Dieffenbachia species are ubiquitous planters seen in the outdoors of homes, offices, and supermarkets across the world. Tissue culture is currently used to disperse several Dieffenbachia species, making it easier to absorb species (Elsheikh *et al*., 2013). Indoor plants, frequently regarded as decorative plants, were discussed in detail by Jayaprada Rao C *et al*., 2015. These plants contain antibacterial qualities that allow them to survive harmful bacteria. These plants are adept at controlling humidity in enclosed spaces, preventing microbial proliferation and survival.

Methanolic extracts of Dieffenbachia spps and Cordyline spps have been discovered to monitor various microbial

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species. They inhibit the growth of *Saccharomyces cerevisiae ,S. aureus, and Corynebacterium diphtheria*.

This study aims to identify natural plants with antibacterial activity against bacteria isolated from a contaminated surface and develop various combinations of disinfectants with the antibacterial properties of selected plants discussed above. The different mixtures of the plants as disinfectants might be used for domestic and commercial floor cleaners.

2 METHODOLOGY

2.1 Preparation of disinfectants using plant extract

Aak, Pomegranate), and leaves of Dumb cane were collected from the Garden of USPCAS-W, Mehran University, Jamshoro. The plants were picked, then cleaned with water to remove dirt, then completely washed with distilled water, and finally dried. The dried leaves were then crushed with a pestle and mortar and pestle and filtered through a muslin cloth.

2.2 Test organisms

This investigation used two types of bacteria as test organisms, namely *E. coli and S. aureus*. The bacterial strains were isolated from kitchen surface samples of the USPCAS-W and cultured at 37 °C incubation for 24 hours on the Muller-Hinton agar (MHA).

2.3 Antimicrobial properties of disinfectant extract through the disk diffusion technique

The antibacterial properties were evaluated using the disc diffusion method of Aak, Pomegranate and dumb cane leaves. Different concentrations of plant leaf extract were mixed and administered on 6 mm Whatman filter paper disks and kept under a UV lamp to dry. All tested disks were put on MHA with Petri plates having tested bacterial media. All these plates were incubated for 24 hours at 37°C. The diameter of the inhibitory zone for each disk was determined and recorded in millimetres (mm).

2.4 Mixture disinfectants of selected plant extracts

To prepare the different disinfection ratios, each disinfection was mixed in the quantity of 1 ml. To prepare the

disinfection 1, Aak (1ml) and Pomegranate (1ml) were mixed, while for disinfection 2, Aak (1 ml) and dumb cane (1ml) were mixed in 1:1. For disinfection 3, Pomegranate (1ml) was mixed with dumb cane (1ml), and for disinfection 4 Aak (1ml), Pomegranate (1 ml), and dumb cane (1ml) were mixed toghater to prepare the disinfections. All of the disinfection were diluted in 2 fold concentrations. The extract suspensions were loaded on disk and tested via antimicrobial susseptibity test.

3. RESULTS AND DISCUSSION

The disc diffusion method was utilized to evaluate the antibacterial activity of 4 different type of disinfections containing Aak, Pomegranate, and dumb cane active against *E. coli* and *S. aureus*. The antibacterial activities of these disinfections were examined by the disk diffusion method against *E. coli* bacteria at varying concentrations: 375, 750, 1500, and 3000 µl.

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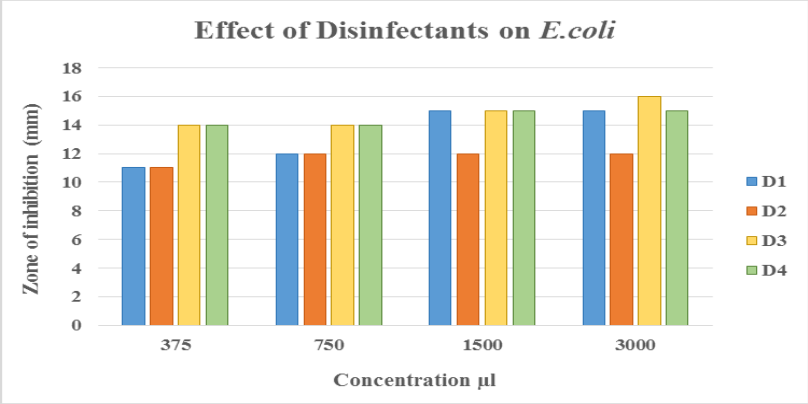

Figure. 1: Effect of herbal disinfectants on *E.coli*

3.1 Antibacterial activity of different disinfections on *E. coli*

As shown in figure 1, *E. coli* displayed different quantities of antibacterial capabilities for plant extract established on inhibitory zones. Figure 1 demonstrates zone of inhabition for *E.coli* at minimum concentration was 11mm, whereas maximum zone of inhibition was 15 mm for Disinfectant 1 made up of Aak and pomegranate suspension. For disinfectant 2 that was included Aak and dumb cane suspension, was observed with minimum zones of inhibiton 11mm and maximum zone of inhibiton was 12 mm. For disinfectants 3, which was made from Pomegranate and dumb cane suspension, the antibacterial activity was observed at 14mm at minimum level where as 16 mm at maximum level. The disinfectant 4 was made up of Aak, Pomegranate, and dumb cane suspension; and showed minimum activity at 14mm and maximum activyt at 15 mm.

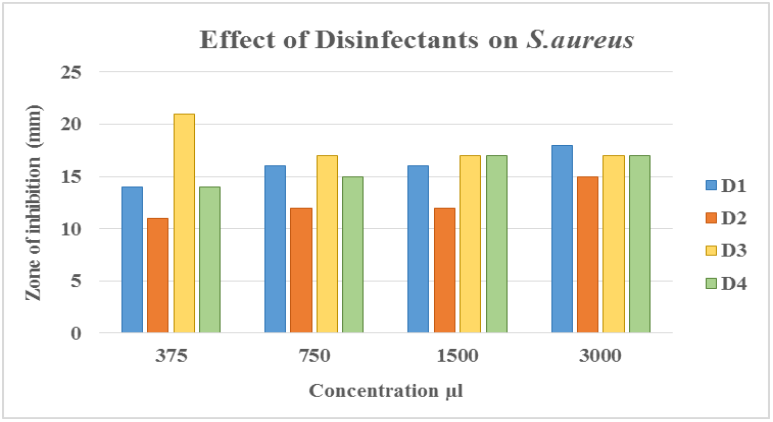

Figure. 2: Effect of herbal disinfectants on *S.aureus*

3.2 Antibacterial activity of different disinfections on *S. aureus:*

To test the antibacterial activity of different suspensions of disinfections sam e combination were used and tested via disk diffusion technique at various concentraions such as 375, 750, 1500, and 3000 µl. The ativity for disinfection 1 against *S. aureus* was minimum at the 14 mm and maximum at 18 mmas shown in figure 2. Whareas, disinfectant 2 compositions showed maximum zone of inhibiton at 11mm while , 15 mm was recorded as maximum. For disinfectant 3, the antibacterial activity was obsed against *S. aureus* at 21mm, whereas the maximum zone of

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inhibiton was recored at 17 mm. For disinfectant 4, the minimum zones was at 14mm whereas the maximum was at 17 mm.

We observed that all of the disinfectants gave potential activity against both of the selected microbes. that indicates that these disinfectants are major candidate to formulation of herble disinfectios against surface microbes. Comparing the bacteria, *S.aureus* showed better performance than *E.coli*. According to the microbial analysis disinfectant 4 had higher inhibitory action than the other. Aak, Pomegranate, and dumb cane had stronger antibacterial action against microbes.

4. CONCLUSION

The ability of disinfectants to enhance antibacterial attributes is critical. In the current study, we found that all disinfectant formulas were useful against surface microorganisms at different doses. The most potent inhibitory zones were observed, with different doses indicating that these herbal disinfectants are most active against microbes. In a comparison of both microbes, *S.aureus* yields better outcomes than *E.coli*. All of the disinfections used in this study showed excellent antibacterial activity against surface microorganisms. To generate natural remedies for different general concerns and conquer many public issues, research on the phytochemicals observed in these plants' disinfectants must be done. The disinfectant was very effective and increased the antibacterial activity against microorganisms.

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