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UNRAVELING THE COMBINATIONAL APPROACH FOR THE ANTIBACTERIAL EFFICACY AGAINST INFECTIOUS PATHOGENS USING THE HERBAL EXTRACTS OF THE LEAVES OF *DODONAEA VISCOSA* AND FRUITS OF *RUBUS FRUTICOSUS*

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ABSTRACT

Dodonaea viscosa and *Rubus fruticosus* are two famous medicinal plants with a wide range of biological properties due to their unique phytochemicals. The main aim of the current study was to investigate the combined antibacterial potential of differential ratio of these two medicinal plants of *Dodonaea viscosa* and *Rubus fruticosus.* The leaves of *Dodonaea viscosa* alone and fruits of *Rubus fruticosus* were either used alone or in combinations were investigated for antimicrobial activities and these combinations included T1 (5mg/mL: 1.0mg/mL), T2 (4:1), T3 (3:1), T4 (2:1), T5 (leaves alone 1mg), T6 (1:5), T7 (1:4), T8 (1:3), T9 (1:2), T10 (fruits extract alone), T11 or control. The Agar well disc diffusion method was used for the analysis of antibacterial activities against human pathogens. The leaves of *D. viscosa* and fruits of *R. fruticosus* extract (5:1) displayed the maximum activities (29mm) against *K. pneumonia* and *S. aureus* (26mm) as compared to antibiotics control (28mm). Furthermore, 30.33mm of activity was observed against *P. aeruginosa* as compared to antibiotics (33.40mm). In the same way the highest activity of (33.67mm) was recorded against pathogenic strain of *S. typhi* in comparison with antibiotics (34mm). Their activity was also investigated by reversing their ratios or concentrations i.e., taking 1mg/mL of *D. viscosa* leaves extract and 5mg/mL of *R. fruticosus* fruit extract (1:5), and high activities were seen against *S. typhi* (26.67mm), *K. pneumoniae* (20.33mm), and *S. aureus* (15.33mm) respectively. It is concluded that that the combinational strategy of medicinal plants is an excellent source of alternative antimicrobials. The strategy needs further analysis and practices for the isolation of active compounds that are responsible for antimicrobial activity for better outcomes in future.

Keywords: Medicinal Herbs; *Dodonaea viscosa*; *Rubus fruticosus*; Antimicrobials; Pathogens

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1. INTRODUCTION

Microbial infections and antibiotic resistance are currently the major problems endangering society's health. In 2013, 9.2 million deaths from various infections were recorded, i.e., with 17% of all fatalities (WHO 2013). Natural, semi-synthetic, or synthetic substances that display antimicrobial action (kill or limit the development of bacteria) at quantities accessible in vivo are considered antimicrobial agents (Thapa and Chapagain 2020). It contains substances that are effective against viruses, fungi, bacteria, and protozoa (Tyagi et al. 2019; Giordani et al. 2020; Ti et al. 2021). Plants have capacity to synthesize aromatic chemicals; the vast majority of them are phenols or their oxygen-substituted variants (Bais et al. 2006). Plant-based substances can work independently or in tandem with antibiotics to increase their antibacterial activity against a variety of microorganisms (Cowanmm 1999). The vast majority of plant diseases, however, have an impact on plant development, reproduction and ultimately yield. The control of these illnesses in agriculture is quite challenging. A plant's innate immunity can halt the spread of diseases and its defensive responses at several levels. Some of these defenses are present before pathogen detection, whereas others are present following it (Jones and Dangl 2006). Knowing the processes of antibiotic resistance is therefore essential for coming up with remedies to stop the spread of antibiotic-resistant microorganisms (Walsh 2000).

Dodonaea viscosa Linn is one of the woody shrubs in the *Sapindaceae* family (AL-Oraimi and Hossain 2016). As it has been suggested that *Dodonaea viscosa* was initially discovered in Australia. It can be found in many temperate locations. comprising South America, in Africa, Mexico, Australia, New Zealand, India, the Virgin

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Islands, the Northern Marianas Islands, Florida, and Arizona. Akeake, Togovao, hop seed bush, and Jamaica switch sorrel are some of the common names of this species (Little, 1989). This plant has been identified in Salalah, Northern Oman, AL-Hamra, and AL-Jabal Al-Akhda (Hussain et al. 2013). Oman's people want to raise the plant. They have chosen commercially in a few years due to their potential antidote to many diseases. *Dodonaea viscosa* boasts a rich history of traditional medicinal uses, whispering promises of hidden therapeutic treasures, packs a powerful punch when it comes to biological activities (Malik et al. 2022; Urooj et al. 2023). *Dodonaea viscosa* scavenges harmful free radicals, protecting our cells from oxidative stress linked to chronic diseases (Mothana et al. 2010; Abbasi et al. 2011).

Numerous degenerative disorders that are chronic, like cancer, diabetes, and hypertension, have been linked with inflammation. All over the world, these health issues contribute to higher rates of illness and death. Aside from autoimmune diseases like Alzheimer's and arthritis, other inflammatory illnesses include viral, bacterial, and protozoal infections are also relevant (Borda and Koff 1992; Ahmad et al. 2011b). Many pharmacological studies have demonstrated the antibacterial, anti-inflammatory, analgesics antiviral, anti-ulcer, or anti-diarrheal qualities of this medicinal plant (Alagarsamy et al. 2007; Anilreddy 2009; Rajamanickam et al. 2010). Previous studies on phytochemistry revealed that *D. viscosa* contains secondary substances of the flavonoid and terpenoid types (Mata et al. 1991; Ortega et al. 2001; Sachdev and Kulshreshtha 1983; Fazal et al. 2012; Ahmad et al. 2012). The leaf decoction of *D. viscosa,* is now used as a mouthwash for toothaches (Qureshi et al. 2008), prevent HIV and the spread of *Candida albicans* (Patel and Coogan 2008), and leaf infusion treat malaria, (Ali et al. 2004; Fazal et al. 2012).

Blackberries (*Rubus fruticosus* L.) are a delicious and agood source of vitamins, minerals, and antioxidants (Marulandam et al. 2007). Blackberries are native to North America, South America, Asia, Europe, and Oceania, but they aregrown in many parts of the world (Hummer and Janick 2006). In northern parts of Pakistan's, it grows wild, including Chitral (Ahmad et al. 2006), Dir (Jan et al. 2008), Mansehra (Shah and Khan 2006), Malakand (Zabihullah et al. 2006), Kotli (Ajaib et al. 2010) where it is referred to by regional names Karwara (Ahmad et al. 2006; Zabihullah et al. 2006). *Rubus fruticosus* L. is a scrambling, perennial, deciduous, and perennial plant and can endure three or more seasons (Hummer and Janick 2006). Fruit is used to produce syrups, jams and other preserves (Chiej 1984). Additionally, the cooked root is utilized as food (Lust, 2014), and leaves are used to make tea (Phillips et al. 1987). Blackberries are agood source of vitamins C, K, and manganese, fiber and antioxidants. The antioxidants in blackberries have been shown to have a number of health benefits (Ahmad et al. 2014). Antioxidants are compounds that protect cells from damage caused by free radicals. Free radicals are molecules that can damage cells and contribute to the development of chronic diseases such as cancer, heart disease, and stroke (Ahmad et al. 2014; Fazal et al. 2023). Moreover, *R. fruticosus* is used to treat influenza (Shimizu et al. 2004) and its polyphenols inhibit influenza virus (Corao et al. 2002).

The type of flavonoids, phenolic acids, carotenoids, anthocyanins, vitamins and minerals, as well as the quantity and frequency of daily consumption, impact what makes fruits and vegetables immune-boosting and healthy and how long it takes the body to process and absorb these nutrients. Consequently, determining the presence of compounds such as antioxidants, the pigment known as vitamins and minerals is crucial to determining the nutritional value of a material (Vigliante et al. 2019). Reducing the risk of cancer, Protecting the heart from damage, Boosting the immune system, improving brain health, Reducing inflammation. Extensive evidence suggests that consuming more fruit reduces the chance of inflammation. Anthocyanins in fruit were found to be the trigger for the anti-inflammatory effects of the fruit in vivo in a mouse model (Dembinska-Kiec et al. 2008). Another study connectedg-type tannin to the inhibition of the hyaluronidase enzyme. Blackberry extract is part of an herbal formula for controlling cytokines in the management of inflammatory or immunological disorders (Randolph and Roh-Schmidt 2007). Immune, inflammatory, and metabolic problems can all be prevented and treated using it practically (Pergola et al. 2006). Therefore, the overall objective of the present study is to investigate the combined antimicrobial efficacy of *R. fruticosus* and *D. viscosa* both individually known for their potential. We aim to explore their synergistic symphony against microbes, moving beyond their individuals' effects and delving into their potential harmonious combinations. This investigation seeks to unlock a potent natural defense system, paving the way for sustainable solutions in the fight against microbial threats. Plant-based antimicrobial using dose-dependent effects to identify the optimal concentration for maximizing their natural and alternative anti-microbial influence.

2. MATERIALS AND METHODS

2.1. Ethical Approval

An ethical approval has been taken from the Ethical Approval Committee (EAC) of the Centre for Biotechnology and Microbiology (CBM) and Institution for conducting this experimentation.

2.2. Sampling

Dodonaea viscosa (Hop Bush) and *Rubus fruticosus* (blackberry) plants were collected from Swat following

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institutional guidelines. *Dodonaea viscosa* leaves were collected from plants grown in natural repositories of University of Swat. While, the fruits of *Rubus fruticosus* were collected from Kanju Township Swat. District Swat is not only famous for its beauty but also for the richness of medicinal herbs and plants utilized for decades in various ailments. The plants were brought to the main laboratory of the Centre for Biotechnology and Microbiology, University of Swat, Pakistan for further processing.

2.3. Sample Handling

The medicinal plants after collection were thoroughly washed and dried gently to separate the active parts that is traditionally used for the treatments of various infections. In *Dodonaea viscosa* the part of interest was the leaves that were separated in a sealed container for further investigation, while in the fruits of *Rubus fruticosus* were separated for antimicrobial activity and preserved in sealed plastic envelopes and placed in refrigerator.

2.4. Extraction of *Dodonaea viscosa*

The separated fresh leaves of *Dodonaea viscosa* were subjected to complete dryness initially and then grinded into fine powder. Soon after that, with the help of a digital balance the powder was weighed accurately and recorded. From the weighed plant powder exactly 1.0g was taken and was suspended in 15-20mL of ethanol for maximum and best solvent extraction.

2.5. Extraction of *Rubus fruticosus*

The same procedure of extraction was also performed here but the fruits of the plants were separated to check its antimicrobial potential. They were gone through the same process of drying and grinding and then 1.0g of the fine powder was suspended in 15-20mL of ethanol to obtain the stock solution.

2.6. Media Preparation

To grow the necessary bacterium strains, a medium was prepared using a well-known concentration of the following components including nutrient broth (7.5g) + Agar (8.0g; solidification) and Distilled water (500mL). The steps involved in media preparation was given below

1. The manufacturer's dehydrated medium was dissolved in a suitable volume of distilled water, i.e., nutrient broth along with agar in 500mL of distilled water to make Nutrient agar.
2 The medium went through a sterilization process in an autoclave for 15 minutes at 121°C and 15 psi pressure.
3 The medium was later poured into petri plates under aseptic conditions in a laminar flow hood (LFH) to avoid contamination with partially open lids for the purpose of solidification.
4 Finally, the media was labelled properly with date, assigned batch numbers, and was placed in incubator for 24 h to check contamination if any.

2.7. Bacterial Strains Collection

Four of the common human pathogenic strains of bacteria were brought under observation against which the antibacterial activities of the desired plant extracts were investigated. These pathogenic strains were labelled as: B1: *Klebsiella pneumoniae*, B2: *Salmonella typhi*, B3: *Staphylococcus aureus*, and B4: *Pseudomonas aeruginosa*. These pathogenic strains were acquired from the Microbiology Lab of Pakistan Council for Scientific and Industrial Research (PCSIR), Peshawar, Pakistan.

2.8. The Restoration of Stock Culture

The stock cultures of pathogenic bacteria preserved at the main Laboratory of CBM were sub-cultured to cultivate fresh cultures for antimicrobial activities. Stock cultures were condensed; their revival was also necessary. To refresh the culture, a sterile cotton bud was dipped in the bacteria stock culture before being placed in a tube of sterile distilled water for an hour without being disturbed. Each bacterial strain was revived under sterile circumstances alone.

2.9. Preparation of Differential Concentrations of Medicinal Plant Parts

The leaves of *D. viscosa* and fruits of *R. fruticosus* were either used alone or in combination for the investigation of antimicrobial activities. Thèse combinations included were T1 (5mg/mL:1.0mg/mL), T2 (4:1), T3 (3:1), T4 (2:1), T5 (leaves alone 1mg), T6 (1:5), T7 (1:4), T8 (1:3), T9 (1:2), T10 (fruits extract alone), T11 or control according to the recent protocols (Ahmad et al. 2021; Ashraf et al. 2023).

2.10. Analysis of Antimicrobial Activity through Well Diffusion Method

Agar wells were produced in solidified agar that was commonly required for diffusion assays. A number of

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instruments, such as stainless steel (borer), were used to punch the Wells in the agar, however, for the current investigation, a cork borer was employed. It was held vertically to ensure uniform contact with the plate's base. Plant extracts were poured into the wells of each plate at varying concentrations or ratios to check the effect of the extract against each strain of the bacteria. Before their introduction into the plates a bacterial lawn was inoculated on the nutrient agar plate against whom the activity was going to be observed (Ahmad et al. 2021; Ashraf et al. 2023).

2.11. Statistical Analysis

Statistix 8.1 (USA) was used to determine the mean value and standard deviation. Plotting graphs were accomplished using the Origin Lab (8.5) program.

3. RESULTS

This study looked at the combined antimicrobial activities in fruits obtained from the *Rubus fruticosus* and leaves of *Dodonaea viscosa*. Antibacterial chemicals are those agents which inhibit the growth of certain bacteria. They are beneficial in preventing many diseases. Antimicrobial substances are frequently abundant in medicinal plants that have a wide range of biological effects. The antimicrobial potential of *Dodonaea viscosa* and *Rubus fruticosus* either alone or in combination were used in differential ratio against four types of pathogenic bacteria including *K. pneumoniae, S. aureus, P. aeruginosa* and *S. typhi*. *Dodonaea viscosa* and *Rubus fruticosus* either alone or in combination in differential ratio displayed variable zones of inhibition against these bacteria.

3.1. The Combined Antibacterial Potential of *D. viscosa* and *R. fruticosus* against *K. pneumoniae*

Here the differential ratios of two medicinal plants (leaves and fruits alone or in combination) were investigated for antibacterial activities against *K. pneumoniae*. The T1 (*D. viscosa* in combination with *R.* fruticosus; 5:1) displayed 29mm zone of inhibition against *K. pneumoniae* as shown in (Fig. 1). T2 (*D. viscosa* in combination with *R. fruticosus* in the ratio of 4:1) exhibited 28.67mm zone of inhibition against pathogenic bacteria while, the T3 (*D. viscosa* in combination with *R. fruticosus* in the ratio of 3:1) has shown 22.67mm zone of inhibition against *K. pneumoniae* as shown in (Fig. 2). The T4 (*D. viscosa* in combination with *R. fruticosus* in the ratio of 3:1) displayed 17.67mm zone of inhibition against *K. pneumoniae* while, T5 (*D. viscosa* alone) exhibited 12.67mm zone of inhibition against pathogenic bacteria. The combination of *D. viscosa* with *R. fruticosus* in the ratio of 1:5 (T6) displayed 20.33mm zone of inhibition against the *K. pneumoniae.* The T7 (*D. viscosa* in combination with *R. fruticosus* in the ratio of 1:4) exhibited 15.33mm zone of inhibition against pathogenic bacteria while, T8 (*D. viscosa* in combination with *R. fruticosus* in the ratio of 1:3 displayed 13.33mm zone of inhibition against *K. pneumoniae*. T9 (*D. viscosa* in combination with *R. fruticosus* in the ratio of 1:2) displayed 12.67mm zone of inhibition against pathogenic bacteria while, T10 (*R. fruticosus* alone) exhibited 15mm zone of inhibition against *K. pneumoniae* (Figs. 1 and 2).

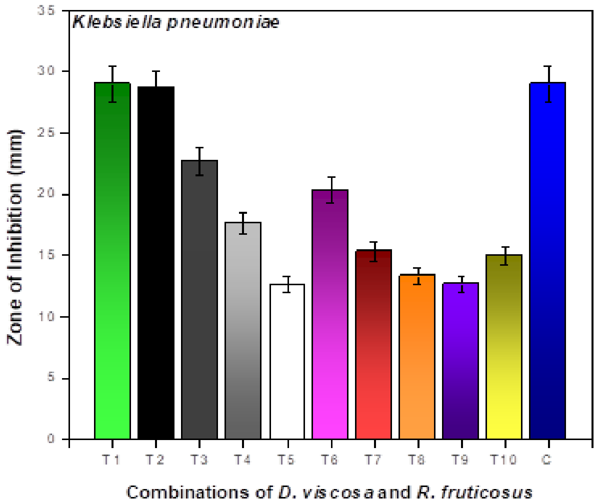

Fig. 1: Graphical representation of Combinational antibacterial potential of *D. viscosa* and *R. fruticosus* against *K. pneumoniae* and these combinations include T1 (5mg/mL: 1.0mg/mL), T2 (4:1), T3 (3:1), T4 (2:1), T5 (leaves alone 1mg), T6 (1:5), T7 (1:4), T8 (1:3), T9 (1 :2), T10 (fruits extract alone), T11 or control.

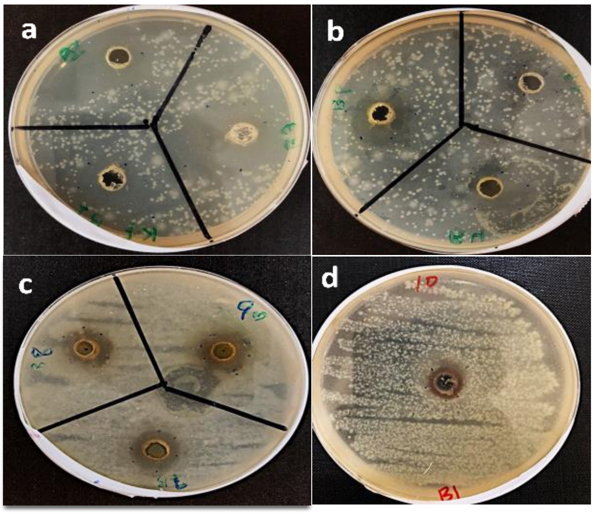

Fig. 2: Pictorial presentation of antimicrobial potential of the leaves of *D. viscosa* and fruits of *R. fruticosus* alone as (a) T1 (5mg/mL: 1.0mg/mL), T2 (4:1), T3 (3:1), (b) T4 (2:1), T5 (leaves alone 1mg), T6 (1:5), (c) T7 (1:4), T8 (1:3), T9 (1:2), and (d) T10 (fruits extract alone), T11 or control against *K. pneumoniae.*

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3.2. The Combined Antibacterial Potential of *D. viscosa* and *R. fruticosus* against *Staphylococcus aureus*

Here the differential ratios were tested for antibacterial activities against *Staphylococcus aureus.* The T1 (*D. viscosa* in combination with *R. fruticosus* in the ratio of 5:1) an optimum activity of 26.33mm zone of inhibition against *Staphylococcus aureus* as shown in (Fig. 3). The T2 (*D. viscosa* in combination with *R. fruticosus* in the ratio of 4:1) exhibited 20mm zone of inhibition against pathogenic bacteria while, the T3 (*D. viscosa* in combination with *R. fruticosus* in the ratio of 3:1) has shown 15mm zone of inhibition against *Staphylococcus aureus* as shown in (Fig. 4) comparatively lowered than T1 and T2. The T4 (*D. viscosa* in combination with *R. fruticosus* in the ratio of 3:1) displayed 13.67mm zone of inhibition against *Staphylococcus aureus* however, the T5 (*D. viscosa* alone) exhibited 11.67mm zone of inhibition against pathogenic bacteria. These results suggest that the leaves extract used alone presented lesser antibacterial potential than the combination of leaves and fruits extracts of medicinal herbs.

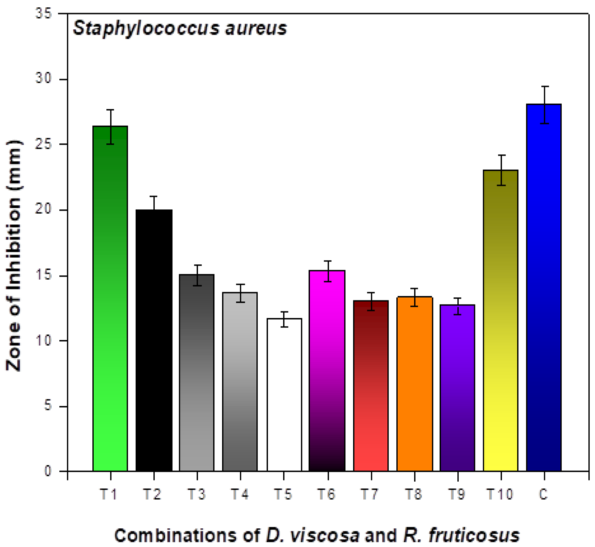

Fig. 3: Graphical representation of the combined antibacterial potential of *D. viscosa* and *R. fruticosus* against *Staphylococcus aureus* and these combinations include T1 (5mg/mL: 1.0mg/mL), T2 (4:1), T3 (3:1), T4 (2:1), T5 (leaves alone 1mg), T6 (1:5), T7 (1:4), T8 (1:3), T9 (1:2), T10 (fruits extract alone), T11 or control.

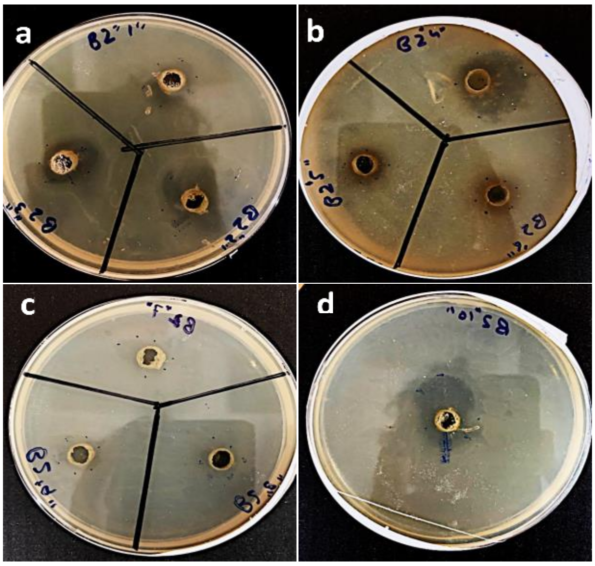

Fig. 4: Antimicrobial potential of the leaves of *D. viscosa* and fruits of *R. fruticosus* alone as (a)T1 (5mg/mL: 1.0mg/mL), T2 (4:1), T3 (3:1), (b) T4 (2:1), T5 (leaves alone 1mg), T6 (1:5), (c) T7 (1:4), T8 (1:3), T9 (1:2), and (d) T10 (fruits extract alone), T11 or control against *Staphylococcus aureus.*

Furthermore, the combination of *D. viscosa* with *R. fruticosus* in the ratio of 1:5 (T6) displayed 15.33mm zone of inhibition against the *Staphylococcus aureus.* The T7 (*D. viscosa* in combination with *R. fruticosus* in the ratio of 1:4) exhibited 13mm zone of inhibition against pathogenic bacteria while, T8 (*D. viscosa* in combination with *R. fruticosus* in the ratio of 1:3) displayed 13.33mm zone of inhibition against *Staphylococcus aureus*. Here the T9 (*D. viscosa* in combination with *R. fruticosus* in the ratio of 1:2) displayed 12.67mm zone of inhibition against pathogenic bacteria while, T10 (*R. fruticosus* alone) exhibited 23mm zone of inhibition against *Staphylococcus aureus* as shown in the Figs. 3 and 4. Here, the fruits extract alone has shown maximum antibacterial potential than combinations. In contrast, The T1, T2, T3, T4 also displayed excellent activities against the pathogenic strain that cause various infections in humans.

These results suggest that the combination of leaves and fruits were more effective than plants used alone. This combination displayed excellent activities against the pathogenic *Staphylococcus aureus* and can be used by various pharmaceutical industries to isolate the active ingredients from these plant parts for the formulation of active drugs against pathogenic microorganisms.

These results can also be exploited for the formulation of alternative drug against the highly resistant microbes that develop resistant to the commercially available antibiotics.

3.3. The Combined Antibacterial Potential of *D. viscosa* and *R. fruticosus* against *P. aeruginosa*

Here the differential ratios have shown excellent antibacterial activities against *P. aeruginosa*. The T1 (*D. viscosa* in combination with *R. fruticosus* in the ratio of 5:1) displayed 30.33mm zone of inhibition against *P. aeruginosa* as shown in (Fig. 5). The T2 (*D. viscosa* in combination with *R. fruticosus* in the ratio of 4:1) exhibited

21mm zone of inhibition against pathogenic bacteria while, the T3 (*D. viscosa* in combination with *R. fruticosus* in the ratio of 3:1) has shown 21mm zone of inhibition against *P. aeruginosa* as shown in Fig. 6. The T4 (*D. viscosa* in combination with *R. fruticosus* in the ratio of 3:1) displayed 13mm zone of inhibition against *P. aeruginosa* while, T5 (*D. viscosa* alone) exhibited 13.67mm zone of inhibition against pathogenic bacteria. These results suggest that the combination of leaves and fruits were more effective than plants used alone. This combination displayed excellent activities against the pathogenic *Staphylococcus aureus.* The combination of *D. viscosa* with *R. fruticosus* in the ratio of 1:5 (T6) displayed 12.67mm zone of inhibition against the *P. aeruginosa*. Furthermore, the T7 (*D. viscosa* in combination with *R. fruticosus* in the ratio of 1:4) exhibited 23.33mm zone of inhibition against pathogenic bacteria while, T8 (*D. viscosa* in combination with *R. fruticosus* in the ratio of 1:3) displayed 15.33mm zone of inhibition against *P. aeruginosa*. Herein, the T9 (*D. viscosa* in combination with *R. fruticosus* in the ratio of 1:2) displayed 14.33mm zone of inhibition against pathogenic bacteria while, T10 (*R. fruticosus* alone) exhibited 21.67mm zone of inhibition against *P. aeruginosa* as shown in the Figs. 5 and 6.

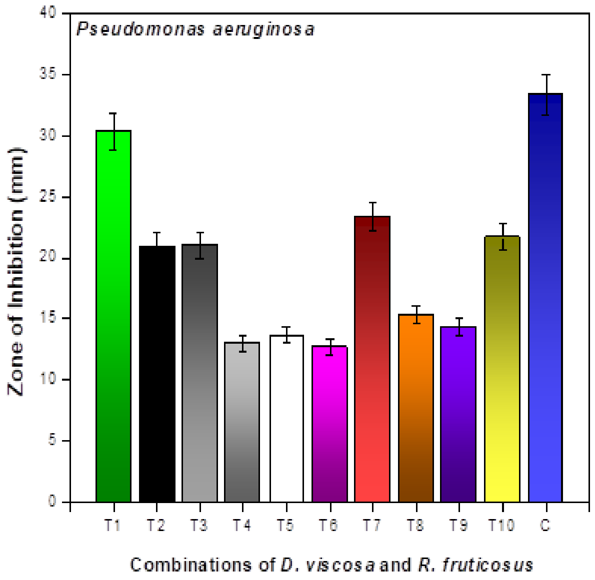

Fig. 5: Graphical representation of Combinational antibacterial potential of *D. viscosa* and *R. fruticosus* against *P. aeruginosa* and these combinations include T1 (5mg/mL: 1.0mg/mL), T2 (4:1), T3 (3:1), T4 (2:1), T5 (leaves alone 1mg), T6 (1:5), T7 (1:4), T8 (1:3), T9 (1:2), T10 (fruits extract alone), T11 or control.

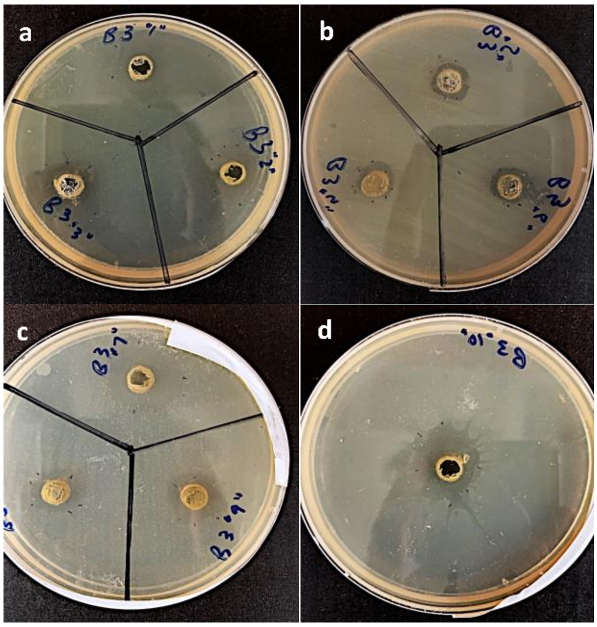

Fig. 6: Pictorial presentation of antimicrobial potential of the leaves of *D. viscosa* and fruits of *R. fruticosus* alone as (a)T1 (5mg/mL: 1.0mg/mL), T2 (4:1), T3 (3:1), (b) T4 (2:1), T5 (leaves alone 1mg), T6 (1:5), (c) T7 (1:4), T8 (1:3), T9 (1:2), and (d) T10 (fruits extract alone), T11 or control against *P. aeruginosa*

3.4. The Combined Antibacterial Potential of *D. viscosa* and *R. fruticosus* against *S. typhi*

Here the differential ratios of two medicinal plants and the plants leaves and fruits alone were investigated for antibacterial activities against *S. typhi.* The T1 (*D. viscosa* in combination with *R. fruticosus* in the ratio of 5:1) displayed 36.67mm zone of inhibition against *S. typhi* as shown in (Fig. 7). Moreover, the T2 (*D. viscosa* in combination with *R. fruticosus* in the ratio of 4:1) exhibited 21mm zone of inhibition against pathogenic bacteria while, the T3 (*D. viscosa* in combination with *R. fruticosus* in the ratio of 3:1) has shown 17mm zone of inhibition against *S. typhi* as shown in Fig. 8. The T4 (*D. viscosa* in combination with *R. fruticosus* in the ratio of 3:1) displayed 13mm zone of inhibition against *S. typhi* while, the T5 (*D. viscosa* alone) exhibited 26mm zone of inhibition against pathogenic bacteria (Ashraf et al. 2023).

The combination of *D. viscosa* with *R. fruticosus* in the ratio of 1:5 (T6) displayed 26.67mm zone of inhibition against the *S .typhi,* however, the T7 (*D. viscosa* in combination with *R. fruticosus* in the ratio of 1:4) exhibited 22.67mm zone of inhibition against pathogenic bacteria, furthermore, the T8 (*D. viscosa* in combination with *R. fruticosus* in the ratio of 1:3) displayed 21.33mm zone of inhibition against *S. typhi* and the T9 (*D. viscosa* in combination with *R. fruticosus* in the ratio of 1:2) displayed 18.67mm zone of inhibition against pathogenic bacteria. While the T10 (*R. fruticosus* alone) exhibited 20mm zone of inhibition against *S. typhi* as shown in Figs. 7 and 8.

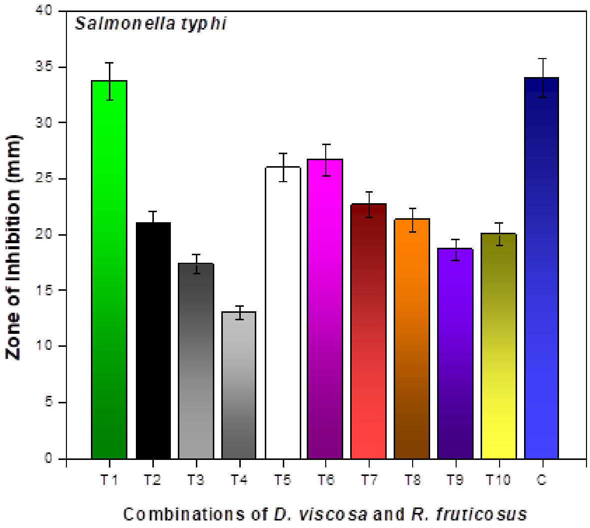

Fig. 7: Graphical representation of Combinational antibacterial potential of *D. viscosa* and *R. fruticosus* against *S. typhi* and these combinations include T1 (5mg/mL: 1.0mg/mL), T2 (4:1), T3 (3:1), T4 (2:1), T5 (leaves alone 1mg), T6 (1:5), T7 (1:4), T8 (1:3), T9 (1:2), T10 (fruits extract alone), T11 or control.

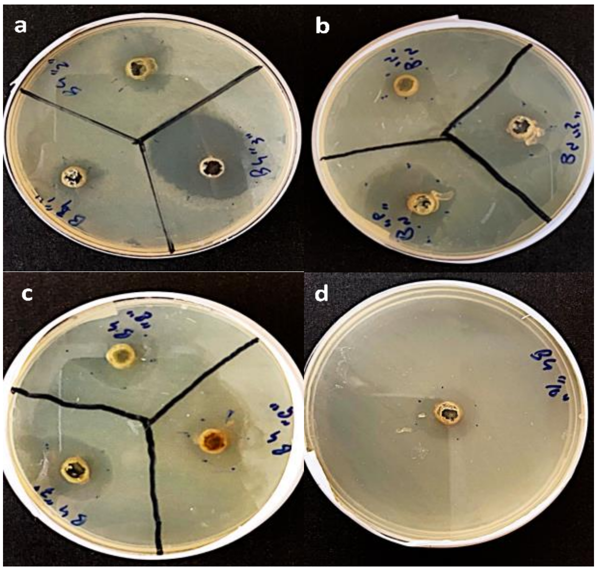

Fig. 8: Pictorial presentation of antimicrobial potential of the leaves of *D. viscosa* and fruits of *R. fruticosus* alone as (a)T1 (5mg/mL: 1.0mg/mL), T2 (4:1), T3 (3:1), (b) T4 (2:1), T5 (leaves alone 1mg), T6 (1:5), (c) T7 (1:4), T8 (1:3), T9 (1:2), and (d) T10 (fruits extract alone), T11 or control against *S. typhi.*

These results suggest that the combination of leaves and fruits were more effective than plants used alone. This combination displayed excellent activities against the pathogenic *Staphylococcus aureus* and can be used by various pharmaceutical industries to isolate the active ingredients from these plant parts for the formulation of active drugs against pathogenic microorganisms.

4. DISCUSSION

The use of plants in traditional medicine has increased interest in ethno-plant concentrates all over the world in recent years (Chassagne and Quave 2021; Julsrigival et al. 2021; Radovanović et al. 2023). Indeed, the World Health Organization (WHO) estimates that 70% of people in various countries use traditional medicine to treat various ailments (Li et al. 2020). Active phytochemicals found in medicinal plants have a variety of biological functions that can benefit human health through the pharmaceutical and alimentary sectors, but they also have significant value in the cosmetic, perfume, and agrochemical industries. Phenylpropanoids, terpenoids, and the most notable alkaloids are just a few of the secondary metabolites found in these plants that are being studied for therapeutic development (Geissman 1963; Ahmad et al. 2011a; Hussain et al. 2015; Majeed et al. 2021; Ashraf et al. 2023). In the current study the antimicrobial potential of *Dodonaea viscosa* and *Rubus fruticosus* either alone or in combination was used in different ratio against four types of pathogenic bacteria including *K. pneumoniae, S. aureus, P. aeruginosa* and *S. typhi.*

The initial treatment a combination of *D. viscosa* and *R. fruticosus* was taken in (5:1) against all of the four strains in which the largest zone of inhibition was observed against *S. typhi* (33.67mm), followed by *P. aeruginosa* (30.33mm), *S. aureus (*26.33mm), and *K. pneumoniae* (29.00mm). Activity of *D. viscosa* was also examined separately against the mentioned strains of bacterial pathogens in which high activity was observed against *S. typhi* (26.00mm) followed by *P. aeruginosa* (13.67mm), and *K. pneumoniae* (12.67mm). Their activity was also investigated by reversing their ratios or concentration i.e., taking 1mg/mL of *D. viscosa* and 5mg/mL of *R. fruticosus* (1:5), a high activity of inhibition or zone of inhibition was seen against *S. typhi* (26.67mm) followed by *K. pneumoniae* (20.33mm), *S. aureus* (15.33mm), and *P. aeruginosa* (12.67mm).

Antibacterial activity of *R. fruticosus* was also examined separately in the current study to identify its effectiveness, the investigation revealed a high activity of the plant extract against *S. aureus* (23.00mm) followed by *P. aeruginosa* (21.67mm), *S. typhi* (20.00mm), and *K. pneumoniae* (15.00mm). Similar study was performed by Riaz et al. (2011) to investigate the antibacterial properties of an indigenous medicinal herb Rubus fruticosus fruit, leaves, root, and stem. The Kirby-Bauer technique was used to screen crude methanolic extracts against eight bacterial species such as *E. coli, Salmonella typhi, Streptococcus aureus, Proteus mirabilis, Micrococcus luteus, Citrobacter*, *Bacillus subtilis,* and *Pseudomonas aeruginosa.* The leaves showed a zone of inhibition at the lowest

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dose level, while the root extract produced results comparable to the standard Ampicillin used. Minimum inhibitory concentration (MIC) values against bacteria were determined; the stem extract has 20g against all strains tested, while the rest has different MIC values against different strains. On a MIC scale, the order of potency is stem>root>leaves>fruit. The purpose of the study of Orpin et al. (2018) was to examine the antibacterial effects of *Dodonaea viscosa* (*D. viscosa*) leaves extracts. Some Enterobacteriaceae were studied at Federal University Dutsinma utilizing various solvents such as ethanol, petroleum ether, and distilled water. The agar well diffusion technique was employed to test the inhibitory activities of these extracts at four different concentrations: 1000, 2000, 3000, and 4000g/mL.

The study discovered that aqueous extracts of *D. viscosa* have a greater percentage yield of 20.46% when compared to ethanolic and petroleum ether extracts. It also demonstrates that saponins, tannins, and phenols were found in all three extracts, whereas flavonoids were detected in both the aqueous and ethanolic extracts and alkaloids were present exclusively in the ethanolic extract. The results also reveal that the *D. viscosa* leaf extract has some antibacterial activity against several of the test microorganisms. *D. viscosa* leaves have a high concentration of alkaloids, glycosides, and saponins, said by (Giri 2001). The antibacterial activity results demonstrate that the methanolic extract of *D. viscosa* leaves has promising action against both bacterial strains tested at higher doses. I.e. 1000g/mL of methanolic extract of *D. viscosa* was taken for analysis against *E. coli* and *S. aureus* in which efficient activity was observed against both of the strains through their zone of inhibition 14mm and 15mm respectively.

A dose of 500mg/mL of methanolic extract of *D. viscosa* was also examined in which 13mm zone of inhibition was observed against *S. aureus and* 12mm was observed against *E. coli.* Hence, all these investigations and approaches illustrates that whatever the advancement and technologies the world is going to experience the fact is that medicinal plants would always be a good priority and a drug of choice to avoid the risk of resistance and to ensure the safety of everyone's life around the world. Therefore, a proper survey, analysis over the utilization of medicinal plants is the need of the day that must be practiced.

5. CONCLUSION

It is conducted that for the current results that the combinational strategy is excellent source of natural antimicrobials as alternative to antibiotics. Most of the combinations displayed ideal activities against the selected pathogenic microorganisms as compared to control. It is recommended that these plants may need further investigation for the isolation of active compound that is responsible for antimicrobial activity. The fruits of *Rubus fruticosus* are the rich source of phytochemicals and antioxidants. However, the active component which plays a key role in inhibiting the growth of microbes needs to be explored in future studies. Similarly, the active phytochemical in the leaves of *D. viscosa* may be isolated for various drugs formulation against the pathogenic bacteria.

Author's Contribution

Muhammad Haroon Ur Rashid, Mehwish, and Hafsa Wahab performed the experimentation, Sajjad Ahmad, and Liaqat Ali analyzed the data through statistical software's, Nisar Ahmad design the experiment, supervision and finalized the manuscript for submission, Hina Fazal provided the bacterial cultures, and Murad Ali supervised the work, and reviewed the manuscript. All authors have seen the final version of the manuscript and approved it.

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