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ANTIBACTERIAL ACTIVITY OF DYSPHAGIA AMBROCOIDES AND BERGINIA CILIATE AND THEIR SYNERGISTIC EFFECT AGAINST SELECTED BACTERIAL STRAINS

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ABSTRACT

Plants and plant-based substances are essential to microbial management programs, human health care, and animal health care. The development of innovative and potent antimicrobial drugs is currently crucial due to the increase in drug-resistant pathogens. Most people have thought about using medicinal herbs as a natural treatment for bacterial diseases. Medicinal plants can be used to make a variety of drugs. A substance with desired active characteristics that is extracted from plant tissue and usually treated with a solvent for a particular purpose is called a plant extract. In Khyber Pukhton Khuwa, there are numerous Dysphania ambrocoides (DA) and Berginia ciliate (BC) species. DA and BC are two traditional medicinal plants in Pakistan. This study aimed to ascertain the antibacterial activity of DA and BC extracts against four pathogenic bacteria: Salmonella typhi, Escherichia coli, Bacillus subtilus, and Staphylococcus aurous, using methanolic and distilled water extracts. The antibacterial activity of DA and BC was evaluated using the well diffusion and disc diffusion procedures. The synergistic effect of the combined extracts was evaluated using the same methods. Gram-negative bacteria were more effectively combated by them than gram-positive ones. The average zones of inhibition for Salmonella typhi 22, Escherichia coli 22, Staphylococcus aurous 16, and Bacillus subtilus 18.5 were calculated using the well diffusion method. In the disc diffusion method, the corresponding numbers for Salmonella typhi, Escherichia coli, Staphylococcus aurous, and Bacillus subtilis were 20–14, 19.3, and 17. Staphylococcus aurous, Salmonella typhi, Escherichia coli, and Bacillus subtilis were all effectively inhibited by the mixed extract. Extracts of Dysphania Ambrocoides leaves and Berginia ciliate roots were found to be potent antimicrobials. These findings suggest that DA and BC can be used as natural antimicrobials to treat bacterial infections either alone or in combination. Further research is needed to evaluate the extracts' mechanisms of action and safety and efficacy in vivo.

Keywords: *Antibacterial Activity, Drug-Resistant Pathogens, Synergistic Effects, Natural Antimicrobials*

INTRODUCTION

Antimicrobial agents play an important role in treating various infections. They are used in modern medicine and multiple industries. antimicrobial agents treat various infections such as pneumonia and wound infections. They are used in the cosmetic, pharmaceutical, and medical industries to prevent contamination. Anyhow, misuse of these antibiotics could cause bacteria resistance when released into the environment. These resistance bacterial genes could transfer into the bacterial population through plasmids. Plant-derived medicines have contributed largely to human health since antiquity; hence plants may be a source for the development of new novel antimicrobial compounds followed by subsequent clinical, pharmacological, and chemical studies [1]. For example, the most prominent anti-inflammatory agent acetylsalicyclic acid commercially known as the drug aspirin isolated from the bark of the willow tree Salix alba L.[2]. Quinine isolated from the bark of Cinchona pubescensVahl has been used traditionally to treat malaria, indigestion, fever, and mouth and throat disease for centuries [3].The roots of modern medicine lie in herbalism. It is estimated that 25 % of modern drugs are derived from herbal medicine [4]. Every ancient civilization has used plants for healing and in many cultures, the use of natural products has magical-religious significance with different points of view regarding the concepts of health and disease existing within each culture. According to the World Health Organization, today's herbal medicine is still the primary form of healing used by over 80 % of the world's population in developing countries [5]. In Bangladesh, approximately 75 % of the total population uses plant-originated medicine for fundamental health care [6] because they are more accessible to local people and comparatively safer and more affordable compared to orthodox or conventional medicine. Bangladesh is enriched with a vast plant diversity having about 450–500 plants with medicinal properties in traditional healthcare systems such as Ayurvedic, Unani, Hekimi, and other forms of folk treatments because of its favorable miscellaneous ecologic conditions such as temperate and humid weather and fertile alluvial soil. Moreover, Bangladesh has a national botanical garden, hilly tracks, and the world's largest mangrove forest [7] where thousands of medicinal plants flourish [8].

Dysphania ambrosioides L. (Chenopodiaceae) is a Moroccan medicinal plant known locally as "M'Khinza." It is widely used in traditional medicine to treat numerous ailments, such as diabetes, digestive disorders, fever, fertility problems, immune disorders, hypertension, bronchitis, respiratory conditions, pharyngitis, cough, and flu [9]. The genus *D. ambrosioides* belonging to the family Amaranthaceae is an annual or short-lived perennial herb, covered with aromatic glandular hairs, and commonly known as Mexican tea. The plant height is 1.2 to 2.0 m, irregularly branched, and leaves oblong to lanceolate in shape up to 12 cm long.The greenish flower is produced in a branched panicle at the stem apex of the plant [10]. Remarkably, the species *D. ambrosioides* is widely distributed on the planet, and according to the WHO (World Health Organization), it is considered to be one of the most widely used medicinal plants in the world [11]. The species is native to South and Central America, with a wide distribution in Africa, Europe, Australia, and Asian countries. It is cultivated in the Philippines and Java for medicinal purposes [12].

While the unique antibacterial properties of Dysphania ambrosioides and Bergenia ciliata have been investigated, little is known about how well they cooperate to fight bacterial infections. Plant-based compounds can combine to increase effectiveness, decrease toxicity, and reduce resistance. This study aims to assess the antibacterial activity of Bergenia ciliata and Dysphania ambrosioides against certain bacterial strains, both separately and in combination. The results will aid in creating synergistic antibacterial drugs derived from plants.

2 Experimental section:

2.1 Chemicals:

The healthy, disease-free, root of Dysphania Ambrocoides (D. ambrocoides) and *bergenia ciliate* was collected from Torwal Tehsil Bahrain, District Swat KPK, and brought to the Department of Microbiology. Then washed with distilled water dried it (for 5 weeks) and ground it into fine powder.

2.2 Preparation for the plant Dysphania Ambrocoides (D. ambrocoides):

Methanol and distilled water were used to create extracts from 150 g of powdered leaves, using 25 g of powder for each extract. By Karthikon et al. (2009), 25 g of powdered leaves were soaked in 250 mL of

distilled water in a sterile 500 mL flask and allowed to sit at room temperature for 72 hours while being shaken at 150 rpm. Likewise, 25 g of powdered leaves were soaked in 250 mL of methanol under the same circumstances to create the methanolic extract. Following incubation, 125 mL of filtrate was obtained from the water extract after 28 hours of filtering through Whatman filter paper, and 200 mL from the methanolic extract after 2 hours. After centrifuging the filtrates for 7 minutes at 4400 rpm, the supernatants—which are regarded as 100% concentrated extracts—were gathered. Two grams of crude extract were obtained from the methanolic extract's open-air evaporation, whereas 2.5 grams were obtained from the water extract's evaporation in a hot air oven set at 60°C. Two grams of the methanolic extract and 2.5 grams of the water extract were dissolved in four milliliters of double-distilled water and 5 milliliters of double-distilled water, respectively, to create stock solutions (50 percent).

2.3 Preparation for the plant Berginia Ciliate (B. ciliate):

Methanol and distilled water were used to make extracts from 100 g of powdered Bergenia ciliata roots, with 25 g of powder for each extract. By Karthikon et al. (2009), 250 mL of distilled water or methanol was added to 25 g of powdered roots in sterile 500 mL flasks, and the mixture was shaken at 150 rpm for 72 hours at room temperature. Following incubation, 120 mL of the water extract and 200 mL of the methanolic extract were obtained after 30 and 4 hours of filtering through the Whatman filter paper, respectively. After centrifuging the filtrates for seven minutes at 4400 rpm, the supernatants—which are regarded as 100% concentrated extracts—were collected. While the methanolic extract was air-evaporated to produce 1.7 g of crude extract, the water extract was evaporated in a hot air oven set at 60°C to produce 1.5 g. 1.5 g of the water extract and 1.7 g of the methanolic extract were dissolved in 3 mL and 3.4 mL of double-distilled water, respectively, to create stock solutions (50%) in these volumes.

2.4 Preparation of mixed extract for the synergistic effect:

Using agar well diffusion and disc diffusion techniques, the antibacterial activity of extracts from Dysphania ambrosioides and Bergenia ciliata, both separately and together, was evaluated against four bacterial strains: Salmonella typhi, Escherichia coli, Staphylococcus aureus, and Bacillus subtilis. To investigate their synergistic effects, equal amounts of the methanolic and water extracts of both plants were made. The growth medium, nutrient agar, was made by dissolving 4.2 g of dried powder in 150 mL of distilled water, bringing the pH down to 7.4, and autoclaving it for 15 minutes at 121°C. Under a laminar flow hood, the medium was transferred into sterile Petri dishes and allowed to solidify. Following the bacterial culture's inoculation, 7 wells were formed on each plate for the agar well diffusion procedure. 20 µL of either single extracts, mixed extracts, or control solutions were added to each well. Methanolic and water extracts of D. ambrosioides were found in wells 1 and 2, B. ciliata methanolic and water extracts were found in wells 3 and 4, the combined extracts were found in wells 5 and 6, and double-distilled water was used as a negative control in well 7. Antibiotic discs containing ceftazidime, ciprofloxacin, or levofloxacin, depending on the bacterial strain, served as positive controls. A Digital Vernier Caliper was used to measure the zones of inhibition that resulted after incubating the plates for 24 hours at 37°C. Using the same procedure, the disc diffusion method substituted blank discs impregnated with the extracts for wells. To guarantee accuracy, every experiment was carried out three times, and the observed inhibitory zones were noted to assess the extracts' antibacterial and synergistic properties.

3 Results and discussion:

The antibacterial activity of extracts from Dysphania ambrocoides (DA) and Bergenia ciliate (BC), both separately and in combination, against four bacterial strains—Salmonella typhi, Escherichia coli, Staphylococcus aureus, and Bacillus subtilis—was assessed in this investigation. Methanolic and water extracts were used in the analysis, which was carried out using the well diffusion and disc diffusion techniques.

3.1 Antibacterial Activity Against Salmonella typhi

The highest zone of inhibition (28 mm) was seen in the synergistic methanolic extract (DABCME), which was followed by BCWE (27 mm). Comparatively less activity was displayed by individual water extracts (DAWE: 15 mm) and methanolic extracts (BCME: 25 mm; DAME: 14 mm). The inhibition zones for the control antibiotics cepro and levofloxacin were 19 mm and 15 mm, respectively.

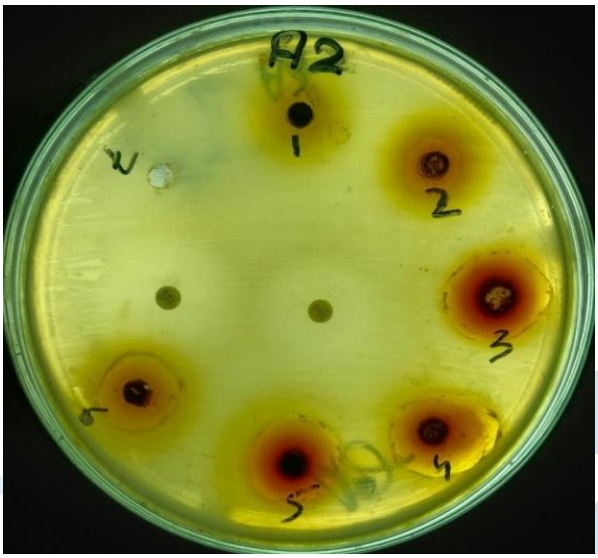

Figure 3.1: _Antibacterial activity of *Dysphania Ambrocoides* and *Bergenia Ciliate* against *Salmonella typhi*

Figure 3.1 represents the antibacterial activity of different extracts against Salmonella typhi is graphically represented by the "Zones of Inhibition of Dysphania Ambrocoides and Bergenia Ciliate against Salmonella typhi." It shows the distinct zones of inhibition that are seen around wells that contain various extracts on agar plates. The wells contain water and methanolic extracts of Bergenia ciliate (BCME and BCWE) and Dysphania ambrocoides (DAME and DAWE), as well as their synergistic mixtures (DABCME and DABCWE). There are also negative controls using double-distilled water (0 mm) and positive controls like Cepro (19 mm) and Levofloxacin (15 mm). The synergistic methanolic combination (DABCME) has the most antibacterial activity among the extracts, with the widest inhibition zone (28 mm). With a zone of 27 mm, the water extract of Bergenia ciliate (BCWE) comes in close second. Conversely, narrower zones are seen in individual extracts of Dysphania ambrocoides (DAME: 14 mm; DAWE: 15 mm), suggesting reduced activity. The antibacterial effects' specificity is confirmed by the negative control's lack of inhibition. The efficacy of the extracts, especially the synergistic combinations, in preventing Salmonella typhi growth is visually confirmed in this image.

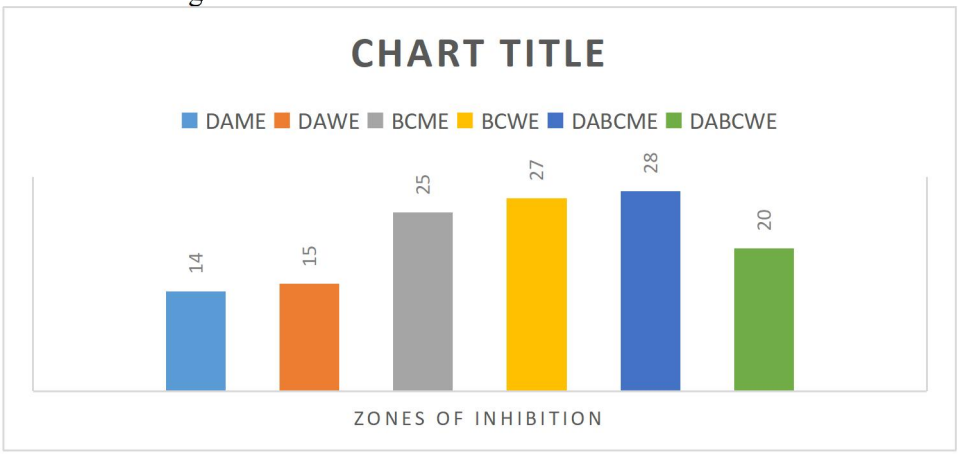

Figure 3.2: _Antibacterial activity of *Dysphania Ambrocoides* and *Bergenia Ciliate* against *Salmonella typhi*
In the form of a bar graph, represent the Antibacterial activity of Dysphania Ambrocoides and Bergenia Ciliate against Salmonella typhi" most likely depicts the antibacterial effectiveness of different extracts. It lists the zones of inhibition (in millimeters) against Salmonella typhi that are created by methanolic, water, and synergistic combinations of Dysphania ambrocoides (DA) and Bergenia ciliate (BC). The activity of individual extracts, synergistic combinations, and controls is graphically compared in the graph. With a zone of inhibition of 14 mm for the methanolic extract (DAME) and 15 mm for the water extract (DAWE), the separate extracts of Dysphania ambrocoides demonstrated moderate efficacy. On the other hand, the individual Bergenia ciliate extracts showed notable activity, with the water extract (BCWE) showing 27 mm and the methanolic extract (BCME) showing 25 mm. The water combination (DABCWE) demonstrated a moderate inhibition zone of 20 mm, whilst the methanolic combination (DABCME) demonstrated the largest inhibition zone of 28 mm, indicating robust antibacterial activity, among the synergistic combinations. Comparative controls are also included in the graph: the negative control, double-distilled water, exhibited no inhibition (0 mm), while the positive controls, Cepro (19 mm) and Levofloxacin (15 mm), demonstrated lesser activity than a number of plant extracts. When compared to individual Dysphania ambrocoides extracts and conventional antibiotics, the synergistic and Bergenia ciliate extracts' greater antibacterial activity is shown by the wider bars. The notion that Bergenia ciliate extracts and synergistic combinations are very effective against Salmonella typhi is supported by this visual representation.

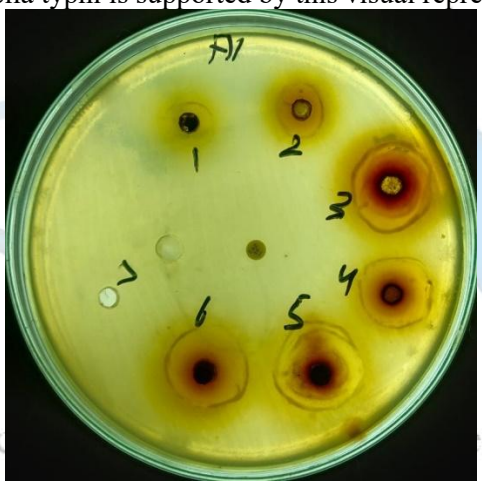

Figure3.3: Antibacterial activity of *Dysphania Ambrocoides* and *Bergenia Ciliate* against *Escherichia coli*
In order to illustrate the antibacterial effectiveness of different extracts, "Figure 3.3 Antibacterial activity of Dysphania Ambrocoides and Bergenia Ciliate against Escherichia coli" graphically represents the zones of inhibition on agar plates. Superior antibacterial activity was demonstrated by the biggest zones of inhibition (27 mm) displayed by the synergistic methanolic extract (DABCME) and the methanolic extract of Bergenia ciliate (BCME). While the methanolic and water extracts of Dysphania ambsrocoides had smaller zones (14 mm each), the water extract of Bergenia ciliate (BCWE) also showed substantial activity (23 mm). While the positive control (Ceptaxime) displayed a zone of 19 mm, which was smaller than that of the synergistic and Bergenia ciliate extracts, the negative control (double-distilled water) displayed no inhibition, indicating the specificity of the antibacterial actions. The potential of Bergenia ciliate extracts and synergistic combinations as powerful antibacterial agents against Escherichia coli is highlighted in this figure.

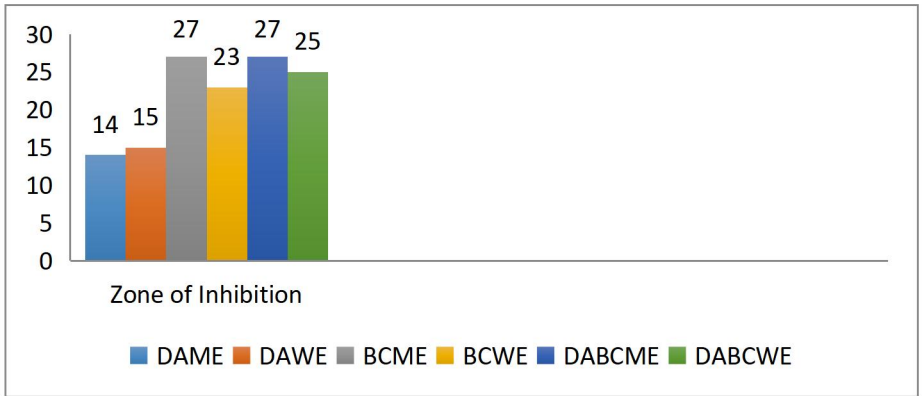

Figure 3.4: Antibacterial activity of *Dysphania Ambrocoides* and *Bergenia Ciliate* against *Escherichia coli*

The antibacterial activity of different extracts against Escherichia coli is shown in terms of inhibition zones (in millimeters) in Figure 3.4 "Antibacterial activity of Dysphania Ambrocoides and Bergenia Ciliate against Escherichia coli." The maximum inhibition zones (27 mm) were shown by the synergistic methanolic extract (DABCME) and the methanolic extract of Bergenia ciliate (BCME), suggesting potent antibacterial activity. While the extracts of Dysphania ambrocoides showed reduced activity, with both methanolic (DAME) and water extracts (DAWE) creating zones of 14 mm, the water extract of Bergenia ciliate (BCWE) also displayed substantial activity (23 mm). In comparison to individual extracts, the synergistic water extract (DABCWE) demonstrated a zone of 25 mm, indicating its increased potency. While the negative control (double-distilled water) exhibited no action, the plant extracts' activity—especially that of the synergistic and Bergenia ciliate extracts—surpassed that of the positive control (Ceptaxime, 19 mm). The higher antibacterial ability of Bergenia ciliate extracts and synergistic combinations against E. coli is demonstrated in this figure.

	DAME	DAWE	BCME	BCWE	DABCME	DABCWE
Zones of inh	22	18	19	17	24	20
	2.5	4.4	2			
	3.5	1.8	3			
	4.5	2.8	5			

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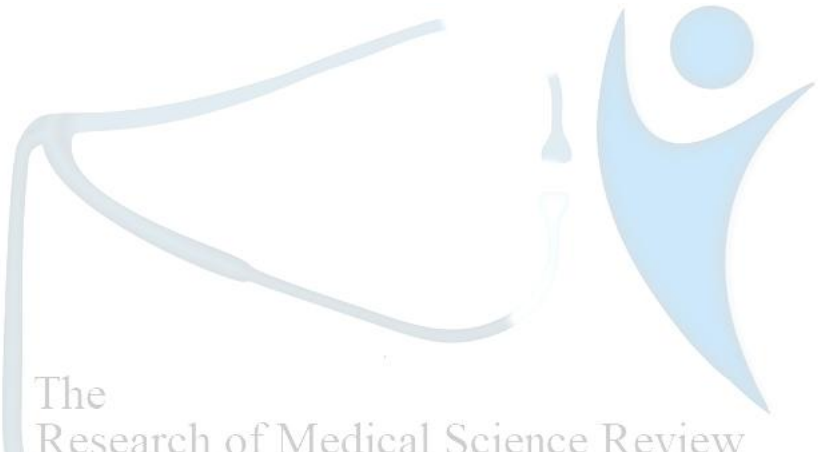

Figure 3.5: Antibacterial activity of *Dysphania Ambrocoides* and *Bergenia Ciliate* against E. coli

The antibacterial activity of Bergenia ciliate and Dysphania ambrocoides against E. coli is shown in Figure 3.5. Both plants' methanolic and water extracts displayed different inhibitory zones, with the methanolic extracts demonstrating greater efficacy. When compared to individual extracts, synergistic combinations (DABCME and DABCWE) demonstrated increased activity, suggesting a potent synergistic impact. Comparable to the positive control, the methanolic combination (DABCME) had the greatest inhibition (27 mm).

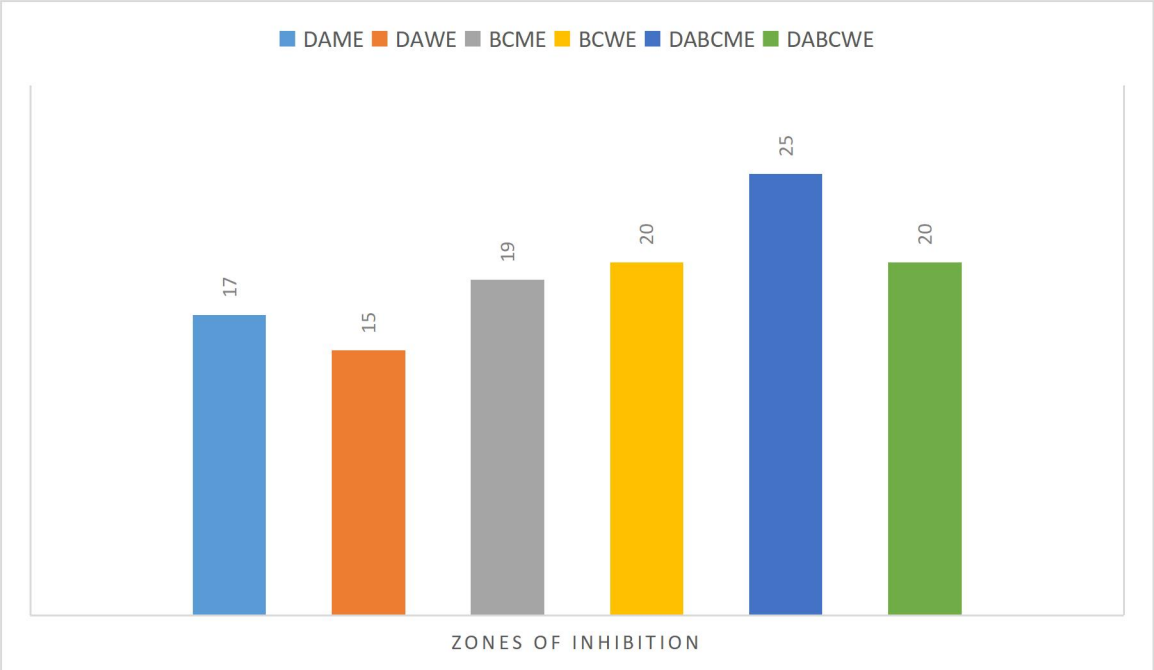

Figure 3.6: Antibacterial activity of *Dysphania Ambrocoides* and *Bergenia Ciliate* against *Staphylococcus aurous*

The antibacterial activity of Bergenia ciliate and Dysphania ambrocoides against Staphylococcus aureus is depicted in Figure 3.6. Both D. ambrocoides extracts and the synergistic combinations (DABCME: 18 mm, DABCWE: 14 mm) were outperformed by the Bergenia ciliate extracts (BCME and BCWE), which showed the greatest inhibition (22 mm and 18 mm, respectively). Every extract outperformed the 8 mm positive control.

DAME	DAWE	BCME	BCWE	DABCME	DABCWE
17	15	19	20	25	20
2.5	4.4	2			
3.5	1.8	3			
4.5	2.8	5			

To resize chart data range, drag lower right corner of range.

Figure 3.7: Antibacterial activity of *Dysphania Ambrocoides* and *Bergenia Ciliate* against *Bacillus subtilius*
The antibacterial activity of Bergenia ciliate and Dysphania ambrocoides against Bacillus subtilis is seen in Figure 3.7. The agar well diffusion method was used to quantify the activity, and the outcomes were expressed as zones of inhibition. Bergenia ciliate's methanolic extract (BCME) showed the largest inhibitory zone among the various extracts, followed by its water extract (BCWE), suggesting strong antibacterial activity. Both the methanolic (DAME) and water-based (DAWE) extracts of Dysphania ambrocoides exhibited modest zones of inhibition. Although they were less successful than the individual extracts of Bergenia ciliate, the synergistic combinations of the extracts (DABCME and DABCWE) showed increased antibacterial activity when compared to the extracts of Dysphania ambrocoides. While the negative control (distilled water) had no activity, the positive control (a common antibiotic) displayed a smaller inhibitory zone than the majority of plant extracts. This implies that Bergenia ciliate has a more potent antibacterial impact on Bacillus subtilis and that extracts work better together, though not as well as they do alone.

Table 1: Antibacterial activity of *Dysphania Ambrocoides* and *Bergenia Ciliate* against *Bacillus subtilius*

S NO	Extracts(separately)	Zones of inhibition in mm	Extracts(synergested)	Zones of inhibition in mm	Averages
01	DAME	16	DABCME	21	17.3
02	DAWE	13	DABCWE	20	17
03	BCME	15			
04	BCWE	17			
Controls Standard antibiotic (+) Tetracyclin:8mm Double distilled water(-): oo					

The antibacterial activity of Bergenia ciliate and Dysphania ambrocoides against Bacillus subtilis is compiled in Table 1, which also compares the zones of inhibition for the extracts used alone and in combination. The methanolic extract of Bergenia ciliate (BCME) exhibited the largest inhibitory zone among the different extracts, followed by its water extract (BCWE), indicating strong antibacterial activity. With smaller zones of inhibition, the methanolic (DAME) and water (DAWE) extracts of Dysphania ambrocoides demonstrated modest antibacterial activity. When the extracts were mixed, the synergistic methanolic extract (DABCME) and water extract (DABCWE) showed more inhibition than the separate extracts of Dysphania ambrocoides, but they were not as effective as the separate extracts of Bergenia ciliate. While the negative control (distilled water) exhibited no inhibition, the positive control (a common antibiotic) showed less action than the plant extracts. According to this research, Bergenia ciliate is more efficient than Dysphania ambrocoides against Bacillus subtilis. Additionally, while synergistic combinations can enhance antibacterial activity, they might not be able to surpass the most effective individual extract.

Conclusion:

According to the study, Dysphania ambrocoides and Bergenia ciliate have strong antibacterial properties against a range of bacterial pathogens, such as Salmonella typhi, Bacillus subtilis, Staphylococcus aureus, and Escherichia coli. Bergenia ciliate had the most antibacterial activity among the studied extracts, especially its methanolic extract, which continuously displayed the largest zones of inhibition. When compared to separate extracts of Dysphania ambrocoides, the synergistic mixtures of extracts from both plants had stronger antibacterial activities, suggesting possible synergistic interactions. According to these results, Bergenia ciliate and Dysphania ambrocoides may be useful natural sources of antibacterial agents that can be used to fight off infections that are resistant to drugs. The study also emphasizes the importance of investigating plant-based antibacterial tactics as sustainable and environmentally acceptable substitutes for synthetic antibiotics. More research is advised to understand the precise mechanisms of action of these plant extracts, improve extraction methods, and assess their safety and effectiveness in vivoore research is advised. Furthermore, expanding the manufacturing of these bioactive substances and examining their potential for synergy with currently available antibiotics could pave the way for novel therapeutic strategies in the treatment of microbial infections.

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