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Antimicrobial Activities of Medicinal Plant *Rhamnus Virgata* (Roxb.) Batsch from Abbottabad, Nathia Gali, KPK, Pakistan

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ABSTRACT

Local people have used medicinal plants for generations in different areas. Unfortunately, many modern and even local communities don't have adequate awareness about the importance or value of medicinal plants. For that reason, this study was considered to survey and explore the uses of medicinal plants consumption based on their antimicrobial properties. For this purpose, *Rhamnusvirgata* is investigated based on scientific references. Antimicrobial activity was investigated in the bark of *Rhamnusvirgata.* The dried bark of *Rhamnusvirgata* was extracted using various solvents, including chloroform, Ethyl acetate and Deionized water. The whole work demonstrates that it serves as a potential antimicrobial crude drug and a source for natural compounds that act as new antibacterial and antifungal agents. It was effective against *Salmonella typhi, Shigella, Staphylococcus aureus, Pseudomonas aeruginosa,* and *E. coli.* Fungi showed little activity. The result indicates that *Rhamnusvirgata* is the best source of antimicrobial activity.

Keywords: *Rhamnusvirgata,* Antimicrobial activity, Chloroform, Ethyl acetate and Deionized water.

Introduction

Microbial resistance to antibiotics is rising due to excessive antibiotic use in human medicine. The problem of antibiotic resistance is not limited to the Pakistan subcontinent only but is a global problem. Antimicrobial resistance is a hidden pandemic that resides in between us. The discovery and development of penicillin in the 1900s gave medical science hope, but penicillin was quickly useless against the most susceptible bacteria. Bacterial antibiotic resistance is natural. Antibiotic resistance can be passed horizontally or vertically. Antibiotics may lose potency in 5 years due to rapid genetic changes in resistant bacteria (Bush, 2004). The ancient Ayurvedic texts Charaka Samhitha and Sushrat Samhitha (Chatterjee & Pakrashi, 1994) describe plant extracts as microbicides, insecticides, and various pharmacological medications. C. Asiatica has a high

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concentration of terpenoids, a chemical that aids in wound healing (Han, 1998), decreases diastolic blood pressure, and regulates blood sugar levels (Hawkins & Ehrlich, 2006). Plant medicines have historically been critical in the treatment of a wide variety of human ailments. According to the World Health Organization (WHO), traditional medicine is utilized to satisfy more than 80% of the world's population's basic health care needs (Zaika, 1988). Many of the plants used now were known to ancient cultures around the globe, and they were highly prized for their preservation and therapeutic properties. The antibacterial capabilities of plants and their components were studied in the late 19th century (Kitua & Malebo, 2004). Through testing and examination, man has been identifying noxious and therapeutic plants for centuries, and these plants are now being used in therapeutic procedures (Mitchell et al., 2001). Only a small proportion of the estimated 250'000-500,000 plant species have been investigated phytochemically, with the remainder screened biologically or pharmacologically. Numerous medicinal treatments have been developed from natural or synthetic compounds (Abbasi et al., 2019; Balunas & Kinghorn, 2005; Jacobs, Snoeijer, Hallard, & Verpoorte, 2004). The most familiar pathogenic bacterial strains are *Escherichia coli, Pseudomonas spp, Vibrio cholera, Shigella spp, Staphylococcus aureus* etc. Resistance to human pathogenic microorganisms has been widely reported on a global scale. Microbes have developed resistance to antibiotics as a result of their widespread usage. The adverse side effects of antibiotics on the host include allergy, aversion and, weakening of beneficial gut. Research on herbs and medicinal plants and their antimicrobial activity has been observed in several studies. Antibiotics are less effective as compared to antimicrobial substances in the healing of pathogenic infections. So, there is a need to isolate medicinal plants to use in herbal medicine because these medicinal plants have already been used as medicine in various diseases. These plant extracts may work as possible, reducing and stabilizing agents, obviating the need for several stages, lowering costs, and avoiding the use of harsh chemicals (Cerit, 2008).We examine medicinal plants in their many forms in this study, covering their history, current condition, and possible future usage as antimicrobial crude medications and as a source of natural chemicals for the development of novel anti-infection agents (Güven et al., 2020). According to World Health Organization (WHO) reports, 80 percent of the population living in developing countries is preferred traditional medicines with herbal origins for their basic health needs. Approximately 20 000 plant species are employed in the treatment of various ailments. According to industry estimates, plants currently provide at least 25 per cent of the active ingredients in pharmaceutically produced drugs (Ateyyat & Darwish, 2009). Thus, this study has led to examining the antimicrobial activity of the medicinal plant *Rhamnusvirgata*. The Rhamnaceae family consists of trees, shrubs, and vines in flowering plants, including Rhamnales (Sharma, 2010). The family has a worldwide distribution, mainly in the tropical, subtropical and temperate regions. Certain Rhamnus species exhibited insecticidal activity (Kosalec et al., 2013), anticancer activity (Ammar et al., 2009), antibacterial activity (Bhouri et al., 2011), antioxidant activity, and free radical scavenging ability (Finegold & Martin, 1982; Kubitzki, Rohwer, & Bittrich, 1990). The basic purpose of this study was to identify *Rhamnusvirgata*bark to use as medicinal plants. To isolate and characterize antibacterial and antifungal compounds from the bark of medicinal plant *Rhamnusvirgata.* The main purpose of this study is to explore *Rhamnusvirgata* with strong antibacterial and antifungal activity, which could act as a good candidate for the establishment of novel Phytomedicines.

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MATERIALS AND METHODS

Plant Material collection and powder preparation

Plant materials were obtained from Nathia Gali to GovernorHouse, region of Hazara division. Plant materials were shade dried and then mashed by using a grinder. The powder was then weighed on a balance, and it was 38g by weight and stored in an airtight container for further use.

Plant extraction

The fine particles or powder of the plant was processed for extraction by ethyl acetate, chloroform and deionized water.

Extraction from Chloroform

5g of shadow dried powder of *Rhamnusvirgata* was soaked in 100 ml of chloroform in a flask and covered with aluminium foil to avoid evaporation and prevent microorganisms' entry. After a week, the extract was filtered using a filter paper (Whatman filter) put the flask having filtrate on the hot plate to evaporate chloroform. After evaporation, add DMSO. Following plant extraction, the agar disc diffusion method was used to check the antibacterial activity of the extract against *Escherichia coli, Shigella and Salmonella typhi.*

Extraction from Ethyl acetate

For Ethyl acetate, a similar method was used for chloroform.5g of shadow dried powder of *Rhamnusvirgata* bark was soaked in 100 ml ethyl acetate in a flask and covered the flask with aluminium foil to prevent evaporation, and left it for a week. The extract was filtered using Whatman filter paper. The filtrate was refilter and put the flask on a hotplate (60C) to evaporate ethyl acetate. After evaporation, add DMSO. The dried extract was scratched and stored in a container. Following plant extraction, the antibacterial activity of the extract was determined using the agar disc diffusion technique against Escherichia coli, Shigella, and Salmonella typhi.

Extraction from Deionized water

5g of dried Powder of *Rhamnusvirgata* was soaked in 100ml of deionized water. After shaking well, the powder is mixed with water completely, and a solution is formed. Put a magnet into the solution after washing the magnet with deionized water. Put the flask on a hot plate at 100^0C for 5 minutes; after boiling, the temperature is decreased up to 80^0C for 30 minutes. Flask was then removed and allowed to cool. Whatman filter paper is used to filter the extracts. The filtrate is then centrifuged at 4000 rpm for 40 minutes. The supernatant was removed using a pipette and stored in a sterilized container at 40C. PBHM was transferred to Eppendorf and kept at 4C for antimicrobial activity. Then the filtered solution is stored in the serum cup.

Tested Microorganisms

Gram-positive bacteria (Staphylococcus aureus) and Gram-negative (*Escherichia coli, Shigella, SalmonellaTyphi and Pseudomonas aeruginosa)* bacteria and fungi (*Candida albicans*) were used for the study. Cultures were identified and maintained using classical diagnostic microbiology

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procedures (Boucher et al., 2009).

Positive and negative controls

Gentamycin, Cefoxitin, and Ciprofloxacin were used as positive controls for Gram-positive and Gram-negative bacteria, respectively. In contrast, Fluconazole served as a positive control for fungi, while solvent controls (deionized water and DMSO) were included in the experiment as negative controls.

Antimicrobial activity

The extracts' antimicrobial activities were studied using the agar well diffusion assay. The assay was performed based on the protocol suggested by the supervisor. The media used was Nutrient agar. When the agar was hardened, it was aseptically mixed with bacterial and fungal suspension and poured into sterile Petri dishes. Before streaking the plates with microorganisms (bacteria and fungus), wells in the media were made using a sterile borer. The plates are then sealed, labelled, and placed in a 37°C incubator. After 24 hours, each dish was examined for inhibitory zones. This was done using a ruler to measure the inhibitory zones. In this case, the clear growth inhibition zone was measured in mm.

RESULTS

The whole work demonstrates the antimicrobial potential of *Rhamnusvirgata* bark extract against bacterial and fungal strains by using various solvents. Antifungal and antibacterial activities were examined by measurement of inhibitory zones. All extracts showed efficient antibacterial activity and very little antifungal activity. The zone of inhibition at 50µl concentration of chloroform and ethyl acetate was 2.0mm and 1.8mm, and 100µl concentration was 2.9mm and 2.4mm. Antifungal activity was examined using water extract, PBHM, chloroform and ethyl acetate extract at (50µl, 100µl). PBHM extract showed less antifungal activity, but significant activity was shown by ethyl acetate extract. The results suggested that Rhamnusvirgata extracts have antimicrobial activity against Gram-positive and Gram-negative bacteria due to the presence of active antibacterial components. The considerable suppression of bacteria implies that Rhamnusvirgata has antibacterial and antifungal chemicals capable of successfully inhibiting growth when extracted using ethyl acetate, chloroform, and deionized water as solvents. Table1: Antimicrobial activity of *Rhamnusvirgata* by Chloroform extracts.

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Table 1: Antimicrobial activity of *Rhamnusvirgata* by Chloroform extracts

CHLOROFORM EXTRACTS				
Tested Microorganism	**50µl CHLOROFORM EXTRACTS**	**100µl CHLOROFORM EXTRACTS**	**Positive control Antibiotics**	**Negative control DMSO**
E.coil	2.0mm	2.9mm	Ciprofloxacin=3.7mm	No
shigella	1.2mm	1.4mm	Cefoxitin=No	No
S.typhi	1.3mm	1.5mm	Cefoxitin=No	No
P.aeroginosa	1.8mm	2.1mm	Sulbactum=2.4mm	No
Candida albicans	1.7mm	2.0mm	Fluconazol=1.3mm	No

Figure 1: Zones of inhibition of *Rhamnusvirgata* by using Chloroform extract against *E. coli, S. Typhi, Aeruginosa, Shigella* and *Candida albicans.*

Table 2: Antimicrobial activity of *Rhamnusvirgata* by Ethyl acetate extracts.

ETHYAL ACETATE EXTRACTS				
Tested Microorganism	**50µl ETHYAL ACETATE EXTRACTS**	**100µl ETHYAL ACETATE EXTRACTS**	**Positive control Antibiotics**	**Negative control DMSO**
E.coil	1.7mm	2.4mm	Ciprofloxacin=3.5mm	No
Shigella	1.6mm	2.1mm	Cefoxitin=No	No
S.typhi	1.5mm	2.0mm	Cefoxitin=No	No
S.aureus	1.2mm	1.4mm	Gentmicin=1.8mm	No
P.aeroginosa	1.8mm	2.2mm	Sulbactum=2.4mm	No
Candida albicans	1.4mm	1.7mm	Fluconazol=1.7mm	No

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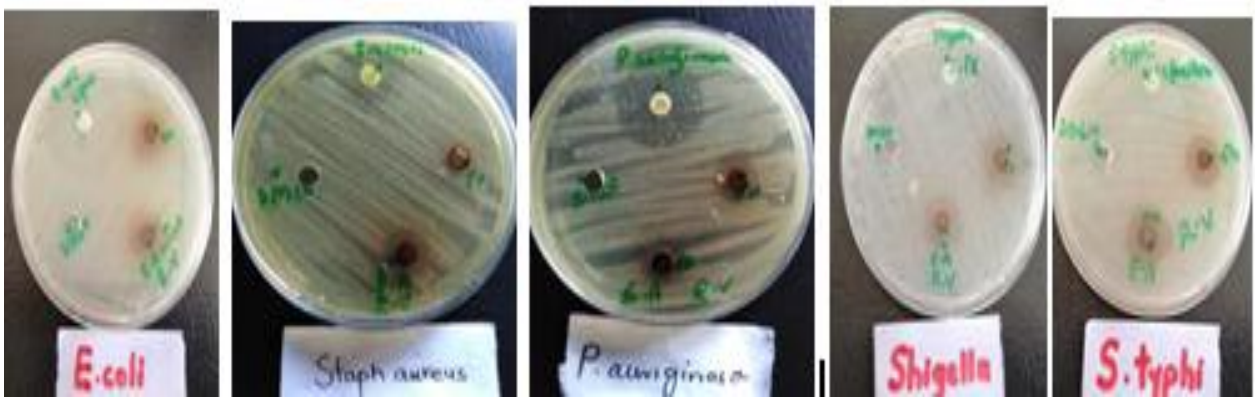

Figure 2: Zones of inhibition of *Rhamnusvirgata* by using Ethyl acetate extract against *E. coli, S. Typhi, Aeruginosa, Shigella* and *Candida albicans.*

Table 3: Antimicrobial activity of *Rhamnusvirgata* by Water extracts

WATER EXTRACTS				
Tested Microorganism	**50µl**	**100µl**	**Positive control Antibiotics**	**Negative control DMSO**
E.coli	2.0mm	2.6mm	Ciprofloxacin=3.6mm	NO
S.typhi	1.6mm	2.1mm	Cefoxitin= NO	NO
S.aureus	1.1mm	1.2mm	Gentmicin=2.3mm	NO
Candida albicans	1.3mm	1.6mm	Fluconazol=1.3mm	NO

Figure 3: Zones of inhibition of *Rhamnusvirgata*by using water extracts against *S. Typhi, Pseudomonas aeruginosa, Shigella* and *E. coli.*

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Table 4: Antimicrobial activity of *Rhamnusvirgata* by Plant bio heavy materials (PBHM)

PBHM EXTRACTS				
Tested Microorganism	**50µl**	**100µl**	**Positive control Antibiotics**	**Negative controlD.H2O**
E.coli	2.0mm	3.6mm	Ciprofloxacin=3.9mm	NO
Shigella	1.6mm	1..7mm	Cefoxitin= NO	NO
S.typhi	1.7mm	1.8mm	Cefoxitin= NO	NO
S.aureus	1.4mm	1.6mm	Gentmicin=2.1mm	NO
P.aeroginosa	2.5mm	3.0mm	Sulbactum=3.6mm	NO
Candida albicans	1.6mm	1.9mm	Fluconazol=1.6mm	NO

Figure 4: Zones of inhibition of *Rhamnusvirgata* by using PBHM extract against *E.coli, S. Typhi, P.aeruginosa, Shigella* and *Candida albicans*

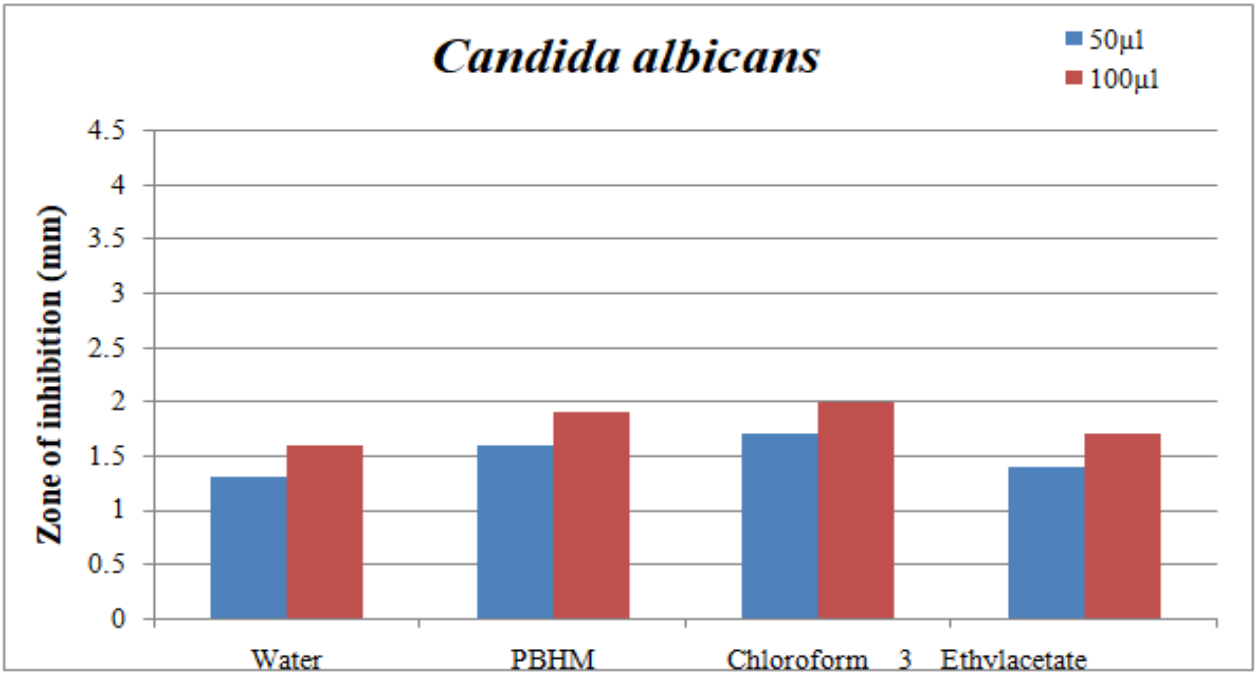

Figure 5: Water, PBHM, Chloroform, Ethyl acetate extracts of *Rhamnusvirgata* bark activity against *Candida albicans.*

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DISCUSSION

Antimicrobial resistance is a major concern for the healthcare industry in many nations, both developed and developing. The emergence and spread of multidrug-resistant diseases are a significant threat to the effectiveness of current antibacterial treatment. As a result, researchers have turned to plants, which produce various bioactive chemicals with recognized medicinal capabilities (Hassawi & Kharma, 2006; Romero et al., 2005; Talbot et al., 2006). This investigation has been carried out to determine the antibacterial activity. of *Rhamnus virgata* bark against *E.coli, Staphylococcus aureus, Shigella, Pseudomonas aeruginosa, Salmonella typhi and Candida albicans using* chloroform, ethyl acetate and deionized water extracts collected from Nathia Gali on the way to Governor House, region of Hazara division for this study. Even though certain extracts showed significant antibacterial activity against the various tested bacterial isolates, numerous extracts demonstrated marginal antibacterial activity against the test bacterium. Best results are observed by chloroform and PBHM fractions in the case of *E. coli*. The zone of inhibition was 2.0mm and 2.9mm, while the zone against antibiotic ciprofloxacin, which was used as a positive control, was 3.7mm. Ethyl acetate fractions showed the highest activity of 1.7mm and 2.4mm. Still, in the case of *Rhamnus virgata,* ethyl acetate fraction showed significant inhibition against E. coli compared to other bacterial strains. In contrast to a study conducted in Jordan, which discovered that the plant P. lanceolata was ineffective against Candida albicans, this study found that Differential results due to differences in environmental conditions and genetic variation among plant species could be caused by variations in quality or composition of the same plant species, which could be caused by variations in a plant's quality or content (Getie et al., 2003). When we compared it to previously published data from Ethiopia, D. viscose was shown to have antibacterial action against S. aureus p. erogenous and Candida albicans (Al-Ghamdi, 2007). Other studies did not show effective antibacterial effects against S. aureus. The antimicrobial activity of R. nervous against bacteria of Gram-positive and Gram-negative of S. aureus, P. aeruginosa, and P. mirabilis was found to be inhibited at the concentration (60 mg/ml), which is consistent with previous findings from Ethiopia and Saudi Arabia against S. aureus, S. pyogenes, and P. aeruginosa, respectively, and disagreed with previous findings against C. Albicans (Ali, Jülich, Kusnick, & Lindequist, 2001). Among the bacteria tested, S. aureus, P. aeruginosa, P. mirabilis, and Candida albicans were the most susceptible to W. somnifera's plant extract. This agrees with a prior report from Yemen on the subject (Ali et al., 2001). Whereas the extract of P. crisp was the least antibacterial active, it was only active against S. aureus and P. aeruginosa. Itdid not affect P. amirabilis and Candida albicans. This finding was in contrast to that reported from Somalia, which found that the extract of P. crispa was the least antibacterial active (Foudah, Alam, Soliman, Salkini, & Yusufoglu, 2016).

CONCLUSION

The present study is all about medicinal plant use, and the main focus was on the therapeutic importance of *Rhamnusvirgata*. For this purpose. *Rhamnusvirgata* bark was tested for antibacterial and antifungal properties. The bark of *Rhamnusvirgata* was used after extraction by various solvents (Chloroform, Ethyl acetate and Deionized water). All the extracts were checked for antimicrobial activity against different bacteria and fungi. All the extracts showed good results.

The study indicates that *Rhamnusvirgata* is a medicinal plant used for therapeutic purposes.

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