Pure Appl. Biol., 11(2):586-591, June, 2022
http://dx.doi.org/10.19045/bspab.2022.110059

Research Article

Antibacterial activity of *Chenopodium botrys* L. and *Chenopodium album* L. against growth promoting Rhizobacteria

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Citation

Hafsa Maria, Husna Begum, Hoor Shumail, Noreen Akhtar, Ayesha Pervaiz, Yousra Ayaz, Sara Khan, Madiha Khan, Shah Khalid and Syed Inzimam Ul Haq. Antibacterial activity of *Chenopodium botrys* L. and *Chenopodium album* L. against growth promoting Rhizobacteria. Pure and Applied Biology. Vol. 11, Issue 2, pp586-591.

http://dx.doi.org/10.19045/bspab.2022.110059

Received: 07/06/2021 Revised: 26/08/2021 Accepted: 04/09/2021 Online First: 10/09/2021

Abstract

Medicinal plants are considered as new resources for producing novel antibacterial agents that could act as alternatives to antibiotics. The purpose of present research was to evaluate the antibacterial profile of important medicinal plants i.e. *Chenopodium botrys* L. and *Chenopodium album* L. against two strains of rhizobacteria (*Pantoea agglomerans* and *Acinetobacter baumannii*). The antibacterial activity was evaluated by a modified agar well-diffusion method. Significant inhibitory effects were shown by crude extract and aqueous fraction on tested soil bacteria. The aqueous fraction revealed strongest antibacterial activity on *Pantoea agglomerans* followed by crude extract on *Pantoea agglomerans* on other hand n-hexane and chloroform exhibits mild to moderates activity on both strains. However, mild to moderate inhibitory effect was observed against *Acinetobacter baumannii* in response to all the extracts. Current findings revealed that *Chenopodium botrys* L. and *Chenopodium album* L. are rich source of natural antibacterial bioactive compounds, and the extracts/fractions of the plants possess potential as an antibacterial agent which could be useful in the search and development of new pharmaceutical agents.

Keywords: *Acinetobacter baumannii*; Antibacterial activity; Antibiotic resistant bacteria; *Chenopodium botrys* L; *Chenopodium album* L; *Pantoea agglomerans*

Introduction

The rhizosphere is the thin layer of soil that surrounds the root system **[1]**. Because of the presence of root exudates and rhizodeposits, the soil environment adjacent to the root system is a hotspot of microbial abundance

and activity [2-4]. The composition of root exudates is influenced by the physiological status and species of plants and microorganisms. Furthermore, rhizosphere microbial activity influences rooting patterns and the provision of essential nutrients to plants, improving the quality and amount of root exudates [5]. Plant growth-promoting rhizobacteria (PGPR) are a diverse group of bacteria found in the rhizosphere, on root surfaces, and in close vicinity to roots that can support plant development directly or indirectly [6]. PGPR enhance plant growth either directly by facilitating resource acquisition (nitrogen, phosphorus, and essential minerals), or indirectly by diminishing pathogen inhibitory effects on plant growth and development in the form of biocontrol agents, or indirectly by regulating plant hormone levels [7]. Plant growth-promoting rhizobacteria can promote plant growth, increase yield, reduce pathogen infection, and alleviate biotic and abiotic plant stress without conferring pathogenicity [8-10]. Rhizobacteria are the primary deriving forces in the recycling of soil nutrients, making them essential for soil fertility [7]. Chenopodium (Family Chenopodiaceae) is a weedy herb genus with over 200 species native to Pakistan, India, China, Europe, and North America [11]. The antibacterial potential of two significant weeds from the Chenopodiaceae family, *Chenopodium album* L. and *Chenopodium botrys* L., was investigated in this research. Fat-hen, bathua, vastukah, and chakvit are all names for *Chenopodium album* L. It is a polymorphous, common weed that grows in waste locations and in wheat, barley, mustard, and gram fields through the summer and winter, reducing yield [12, 13]. This plant is a neglected wild herb with antiviral, antifungal, anti-inflammatory, anti-allergic, antiseptic, and immunomodulating characteristics [14]. *Chenopodium album* L. is a rich source of minerals, antioxidants, and vital dietary ingredients, according to studies conducted around the world [15, 16]. *Chenopodium botrys* L. has been found in Pakistan's tropical and subtropical zones [17]. It is native to Europe and Asia, but it is also found in parts of North America. This herb is either an annual or a biennial [18]. *Chenopodium botrys* L. is a sticky, aromatic annual plant that smells like incense. *C. botrys* has been used for medicinal purposes in the past, however these therapeutic uses and health advantages are mostly based on folklore [19]. *Chenopodium botrys* has been used as an antispasmodic, anti-asthmatic, anthelmintic, and spice in traditional medicine. Antifungal and antibacterial properties of essential oil extracted from *C. botrys* have been reported [20].

Materials and Methods

Samples collection

Chenopodium album L., and *Chenopodium botrys* L., were collected from Chakdara, Dir Lower, Khyber Pakhtunkhwa, Pakistan. The bacterial strains (*Pantoea agglomerans and Acinetobacter baumannii*) isolated from rhizosphere were obtained from the Department of Botany, Islamia College Peshawar, Pakistan.

Extract preparation

The aerial part of *Chenopodium album* L., and *Chenopodium botrys* L., were shade dried and powder with the help of an electric blender. Afterward, the plant material was soaked in 70% methanol and regularly shacked for maximum extraction for 7 days. The filtrated extract was evaporated under reduced pressure at 45°C using a vacuum pump and rotary evaporator after 7 days, yielding a semisolid mass of the extract, which was then dried on a water bath at 45°C. The thick pastes obtained are crude extracts of plant material. The crude methanolic extract was fractionated using a solvent-solvent extraction method. In a separating funnel, the crude methanolic extract was mixed with distilled water. Finally, 500 mL

of different solvents, such as n-hexane, chloroform, and aqueous, were partitioned one by one time, starting from a lower polar to a higher polar solvent. Each was repeated three times and different fractions were obtained. The dried fractions (crude extract, n-hexane, chloroform, and aqueous) were weighed and dissolved in sterile water, to prepare appropriate dilution to get the required concentration of about 100µg/ml, 300µg/ml, and 500µg/ml. They were stored in the refrigerator unless they were used for the experiment.

Antibacterial activity

The antibacterial activity was evaluated using the agar well diffusion protocol. For this purpose, bacterial inoculums were prepared through the colony suspension method. The prepared inoculums were swabbed three times on the nutrient agar plates at an angle of 60 degrees to ensure equal distribution of bacteria. After inoculation, 6mm wells were made in the plate with the help of a cork borer. The specific amount of each test sample was poured into a specific well and Ceftriaxone was used as control. The plates were incubated at 37° C for 18-24 hours. After incubation zone of inhibition were observed, measured, and compared.

Results

Different diameters of zone inhibition were seen against *Pantoea agglomerans* and *Acinetobacter baumannii* at concentrations of 100, 300, and 500g/ml of the fractions (crude extract, n-hexane, chloroform, and aqueous). Against both bacterial species, inconsistent results were obtained. In most cases, higher concentrations result in maximum inhibition. In this activity, a dose-dependent impact has been observed. The results are displayed in (Table 1).

Table 1. Antibacterial activity of the *Chenopodium album* L. and *Chenopodium botrys* L. extract against *Pantoea agglomerans* and *Acinetobacter baumannii*

Samples	Concentration µg/ml	*Chenopodium album* L.		*Chenopodium botrys* L.	
		Pantoea agglomerans	*Acinetobacter baumannii*	*Pantoea agglomerans*	*Acinetobacter baumannii*
Crude extract	100	2.5	-	1	0
	300	-	-	1.25	0
	500	2.06	2.05	1.5	2.05
n-hexane	100	2.1	-	1.766	1.433
	300	2.07	1.66	2.533	1.9
	500	2.33	2.33	3	1.733
Chloroform	100	-	1.45	1.6	0
	300	1.56	-	2	1.4
	500	1.53	1.63	2.3	3.733
Aqueous fraction	100	-	-	1.6	0
	300	-	1.8	1.9	0
	500	2.6	2.26	2.466	0
Ceftriaxone (Standard)	5.4	3	2.4	3	2.433

Discussion

Medicinal plants are an important field of traditional medicine in many parts of the world. Over the last two decades, there has been a rise in research on natural materials as potential sources of novel antibacterial medications. To discover the source of the therapeutic benefits, many extracts from traditional medicinal herbs have been investigated [21]. Some medicinal plants

used in traditional medicine are useful in treating bacterial and oxidative stress-related diseases [22, 23]. For new and re-emerging infectious diseases, there is an ongoing and urgent need to identify new antimicrobial agents with diverse chemical structures and unique mechanisms of action. In this regard, the antibacterial activity of the medicinal plants *Chenopodium botrys* and *Chenopodium album* L. was tested using three fractions (n-hexane, chloroform, and aqueous) and crude extract. According to the results of the investigational study, *C. botrys* contains practically all the key categories of bioactive chemicals, as evidenced by phytochemical screening. As a result, the existence of these secondary metabolites may be responsible for the plant's pharmacological potential. Foroughi [24] investigated the chemical composition of *Chenopodium botrys* L. essential oil as well as its antibacterial activities. Their findings show that at a concentration of 0.007 g/ml, *C. botrys* essential oil inhibits the growth of *Escherichia coli* and *Staphylococcus aureus*. Ullah [23] investigated the antibacterial and phytochemical properties of crude methanolic extract and successive fractions of *C. botrys* aerial parts using DPPH, ABTS free radical scavenging, and antibacterial and phytochemical tests. They showed that the *n*-hexane, chloroform, ethyl acetate, and aqueous fractions of *C. botrys* showed antibacterial activity towards *S. aureus, K. pneumoniae*, and *P. aeruginosa*. Among all fractions, the ethyl acetate and chloroform fraction were most effective against *K. pneumoniae* and *P. aeruginosa* in comparison to the standard antibiotic used. Similarly, chloroform fraction was most active against *S. aureus* followed by ethyl acetate and *n*-hexane. These studies support our results that the plant is used in traditional medicine and suggest that *C. botrys* possess compounds with good antibacterial properties. Kaur [25] investigated the antibacterial activity of *Chenopodium album* L. leaves extract using various solvent extracts of *Chenopodium album* L. leaves, including methanol, acetone, and chloroform extracts. The effects of the various extracts on the test organisms *Lactobacillus*, *Bacillus subtilis*, and *Escherichia coli* were studied. According to these findings, alkaloids and flavonoids are the chemicals responsible for antibacterial activity in higher plants. Furthermore, secondary metabolites including tannins and other phenolic compounds are classified as active antimicrobial agents.

Conclusion

It is concluded from this study that the fractions (n-hexane, chloroform, and aqueous) and the crude extract of *C. botrys* L. and *C. album* L. possess remarkable antibacterial activity against rhizobacterial strains i.e., *Pantoea agglomerans* and *Acinetobacter baumannii* comparable to that of the selected reference antibiotic (Ceftriaxone). As a result, it's believed that these plants could be a natural antibacterial agent due to their high concentration of bioactive chemicals. These plants could be studied further to isolate and characterize novel, cost-effective antibacterial chemicals. The bacterial inhibition of these plants was mainly due to their chemical components, therefore further studies should be carried out to reveal other significant compounds to control the rising issue of antibiotic resistance by the development of novel medicines or in combination with folk medicines to increase their effectiveness.

Authors' contributions

Conceived and designed the experiments: S Khalid & H Shumail, Performed the experiments: H Maria, H Begum, N Akhtar, A Pervaiz & Y Ayaz, Analyzed the data: H Maria & H Begum, Contributed materials/ analysis/ tools: S Khan & M Khan, Wrote the paper: S Khalid & SIU Haq.

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