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Original Research Article

Antibacterial and Antioxidant Activities of Extracts from Selected Wild Plant Species Found in Jordan

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ABSTRACT

In the past decade, there has been a growing global interest in the antibacterial and antioxidant properties of plants, which are considered safe for use. This study aimed to evaluate the antibacterial and antioxidant activities, as well as the flavonoid and total phenolic contents, of three wild plant species found in Jordan: *Rubus canescens DC*, *Rumex vesicarus L*, and *Urtica pilulifera L*. The 70% methanol extracts of these plants were tested for their antioxidant activity using various assays, such as total phenols, free radical scavenging, and reducing power. Results showed that *Rubus canescens DC* extract had a higher percentage of total phenols and free radical scavenging activity, while *Rumex vesicarus* L extract had a higher percentage of reducing power. The extracts' antimicrobial activity was evaluated using a two-fold dilution method, including Minimum Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) activity. The extract of *Rubus canescens DC* showed higher antibacterial activity against the tested microorganisms, as measured by inhibition zones in plate-diffusion assays. These findings suggest that *Rubus canescens DC* could be a potential source of natural antioxidants and warrant further investigation of its therapeutic properties.

Keywords: Antibacterial, Antioxidants, Methanol extracts, *Rubus canescens* DC, *Rumex vesicarus* L and *Urtica pilulifera* L

Introduction

Plants are capable of synthesizing and storing a variety of primary and secondary metabolites, including diverse compounds with different structures and bioactivities .[1] Studies have demonstrated that plant-derived volatile aromatic substances, predominantly phenols, possess antimicrobial and antioxidant properties and have a role in the development of natural antimicrobial and antioxidant formulations.[2-4] Medicinal plants have been used since ancient times due to their antimicrobial, antioxidant, antiviral, anticarcinogenic, and antimutagenic properties.[5-13] Essential oils derived from plants have been used for centuries to prevent spoilage caused by microorganisms.[14] Many medicinal plants are rich sources of potentially bioactive compounds such as flavonoids, steroids, phenols, glycosides, alkaloids, tannins, and volatile oils, which can be found in various plant parts such as fruits, flowers, leaves, bark, seeds, and roots.[15] Because of their broad-spectrum antimicrobial properties, plant extracts can be utilized in various applications.[16] Additionally, plant parts such as fruits, leaves, stem, bark, or roots are considered to contain large amounts of the antioxidant compound.[17]
The antioxidant effect is primarily attributed to phenolic components such as phenolic acids and flavonoids.

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These compounds act as scavengers and interact with oxides and free radical (hydroperoxides) compounds, providing protection for vital components of living cells such as hormones, vitamins, and lipid membranes against oxidation damage.[18]
Jordan is home to approximately 2,543 plant species belonging to 142 families and 868 genera, with over 50% considered to be medicinal plants.[19] These plants are used both in the pharmaceutical industry and in traditional medicine.[20] The genus Rubus, which includes over 750 species, is known for its broad range of biological activities such as antimicrobial and antioxidant effects.[20-22] However, there is still a need for comprehensive research to further explore the potential medicinal applications of these plants. This study aimed to evaluate the antibacterial and antioxidant activity of *Rubus canescens DC* (known in Arabic as Ulaiq), *Rumex vesicarus* L. (known in Arabic as Hommaid, Homays, Hammad, Hanabay), and *Urtica pilulifera L*. (known in Arabic as Kurrays).

Materials and Methods

Collection of plants
During the months of March to May 2021, the leaves of *Rumex vesicarius*, *Rubus canescens DC*, and *Urtica pilulifera L*. were gathered from rural locations in Jordan figure 1, Voucher specimens 1, 3, 6, respectively. A botanist, Dr. Hamed Rabah Hamed Takruri, who is a Professor in Human Nutrition at the Department of Nutrition & Food Technology in the Faculty of Agriculture at the University of Jordan, identified the plant species in table 1.
The freshly collected plant leaves were rapidly enclosed in air-tight polyethylene (PE) bags to avoid any oxidation of their constituents and ensure their usability within 24 hours of collection

Sample preparation
The leaves of the chosen plants were dried by placing them in a clean, dark place at room temperature 25ºC. After drying, the plants were ground into a powder in a blender and sieved to ensure uniform particle size. The plant powder, 10 mg of each was soaked in 70% methanol at 25ºC for a week, and then placed on a shaker (GFL mbH 3005, Germany) at 150 rpm for 24 hours. The mixture was filtered through filter paper and then passed through a 0.45um filter (IKA RV10B, Germany) using a vacuum pump (Vacuubrand ME 4NT, Germany) at 40°C, and 130 rpm to completely remove the solvents. The resulting crude extracts were stored in dark glass bottles with a cap in a refrigerator at 8°C until used in the assay.[23]

Total phenols determination
To determine the total phenolic content, the crude extract (0.5 mL) was mixed with Na2CO3 (2 mL) and Folin-Ciocalteu reagent. A standard compound, Gallic acid, was used for comparison. After incubating the mixture at room temperature for 30 minutes, the absorbance was measured at 765 nm.[24]

Free radical scavenging activity
The DPPH reagent was mixed with different concentrations (200, 100, 50, 25, 12, and 6 µg/mL) of each plant crude extract (CE), as well as with positive control, Butylated hydroxytoluene (BHT) at a concentration of 60 mg/mL in methanol. After a 30-minute incubation period at room temperature, the absorbance was measured at 517 nm using a Biotek spectrophotometer.[25,18]

Screening of reducing power of Crude extracts
Before treatment with trichloroacetic acid, the crude extract was combined with phosphate buffer and potassium ferricyanide and incubated for 30 minutes at 50°C. As a reference, ascorbic acid was used. The absorbance of the samples, standard, and negative control was measured at 700 nm.

Antibacterial activity
Bacterial strains and culture conditions
Strains of *Listeria monocytogenes*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* were acquired from the University of Jordan Hospital Laboratory and the central laboratory of the Jordanian Ministry of Health located in Amman. The strains underwent oxidase and catalase tests to confirm their identity, as shown in Table 2. Before the experiment, the strains were cultured and then transferred to broth media individually.[26]

Screening of Antibacterial activity
The bacterial cultures were allowed to grow for 18 hours and then diluted to achieve an inoculum of 10^5 colony forming units per milliliter (CFU/mL). A volume of 100µL of the inoculum was spread onto pre-dried Tryptic Soy Agar plates. Agar well diffusion assay (AWDA) was employed to evaluate the antimicrobial activity of the plant extracts. For comparison purposes, antibiotic discs were used as positive controls.[27, 28]

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)
The MIC values of the crude extracts were determined using the 96-well micro-dilution method. A 100µL of each bacterium was added, and a serial dilution of each crude extract was prepared. After incubating for 24 hours, the absorbance was measured at 600 nm. The MBC was determined using the pour plate technique, where 20µL aliquots from clear wells were plated. The lowest concentration *of Rubus canescens DC* (CE) that showed no visible growth on the agar plates was considered the MBC.

Statistical analysis
The GraphPad Prism ANOVA was utilized to determine any statistical variations between the control group and the different treatment groups. Subsequently, the Dunnett's post hoc test was applied. A p-value less than 0.05 was the accepted threshold for statistical significance in all analyses.

Results and Discussion

The data revealed that *Rubus canescens DC* contained higher amounts of phenolic compounds than *Rumex vesicarus L* and *Urtica pilulifera L*, as shown in Figure 2. In terms of scavenging activity, *Rubus canescens DC* (CE) showed a stronger effect compared to the positive control BHT, while the crude extracts of *Rumex vesicarus L*. and *Urtica pilulifera L*. showed a relatively similar effect, as illustrated in Figure 3. The results indicated that the crude extract of *Rubus canescens DC* had a higher reducing power compared to the crude extracts of *Rumex vesicarus L* and *Urtica pilulifera L*, as demonstrated in Figure 4 when compared to ascorbic acid.
Aromatic plants, including *R. canescens DC*, have been found to possess diverse biological activities such as antimicrobial and antioxidant properties.[29] The study by Assafiri et al. also revealed that *R. canescens DC* contains high phenolic content and that phenolic compounds play a major role in the antioxidant capacity of these plants.[29,30] In addition, our results showed that *R. canescens DC* (CE) had stronger scavenging activity than BHT, while the crude extracts of *Rumex vesicarus L* and *Urtica pilulifera L* exhibited similar scavenging activity. The reducing power results were compared to ascorbic acid, and it was found that *R. canescens DC* (CE) had higher reducing power than *Rumex vesicarus L* (CE).

Table 1: Bacterial strains used in this study.

No.	Name of microorganism	Origin	Properties	Catalase test	Oxidase test
1	*Pseudomonas aeruginosa*	ATCC 27853	G-ve, rod-shaped, no arrangement	G+ve	G+ve
2	*Escherichia coli*	ATCC 25922	G-ve, rod-shaped, no arrangement	G+ve	G+ve
3	*Staphylococcus aureus*	ATCC 29213	G+ve, cocci, cluster	G+ve	G+ve
4	*Listeria monocytogenes*	ATCC 7644	G+ve, rod-shaped,	G+ve	G+ve
5	*Salmonella typhimurium*	ATCC 21292	G-ve, motile by peritrichous flagella.	G+ve	G+ve

Table 2: Inhibitory zone in Millimeter (Mm) measured by caliper caused by tested extracts against different microbial strains.

Name of microorganism	*Rumex vesicarus* L.	*Rubus canescens* DC	*Urtica pilulifera L.*	Antimicrobial agent (control)
Pseudomonas aeruginosa	0 ± 0.0	4 ± 1.2	1 ± 0.3	23 ± 0.4 (Amikacin)
Escherichia coli	0 ± 0.0	7.25 ± 1.34	3.75 ± 0.5	19 ± 0.32 (Ampicillin)
Staphylococcus aureus	4.25 ± 1.8	14.5 ± 2.34	1.25 ± 0.4	15 ± 0.24 (Penicillin)
Listeria monocytogenes	0 ± 0.0	17 ± 3.5	8.5 ± 3.4	17 ± 0.6 (Streptomycin)
Salmonella typhimurium	1 ± 0.5	15 ± 2.5	0 ± 0.0	18 ± 0.5 (Ampicillin)

Results are mean ± SD (n = 3-4 independent replicates)

The reducing power value is an important indicator of antioxidant activity and indicates the ability of compounds to act as electron donors.[31] Therefore, our findings suggest that the high concentration of phenolic compounds in *R. canescens DC* contributes to its strong radical scavenging activity and reducing power.

The results of the study showed that *R. canescens DC* (CE) had significant antimicrobial activity against various microorganisms, including *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Listeria monocytogenes* (ATCC 7644), and *Salmonella typhimurium* (ATCC 21292) as compared to *Rumex vesicarus L.* and *Urtica pilulifera L.* (CEs) Table 3. The inhibition zones of the tested strains increased with increasing antimicrobial activity of *R. canescens DC* (CE). The screened (CEs) showed relatively lower antimicrobial activity compared to positive controls, except for *R. canescens DC* against *Staphylococcus aureus* and *Listeria monocytogenes*, which had higher antimicrobial activity than the control antibiotic. Negative controls had no significant effect on microbial strains, except for *S. aureus* and *L. monocytogenes. R. canescens* DC (CE) had a minimum inhibitory concentration (MIC) value of 2.5 µg/ml against *S. aureus*, 1.25 µg/ml against *S. aureus and L. monocytogenes*, and 0.15625 µg/ml against *P. aeruginosa, E. coli* and *S. typhimurium*, indicating its strong inhibitory effect against these microorganisms. These findings demonstrate the promising antimicrobial potential *of R. canescens DC* (CE) against various pathogenic microorganisms.

The efficacy of various compounds in killing bacterial strains was measured using the minimal bactericidal concentration (MBC) method. In this study, the bactericidal effect of *R. canescens DC* extract against selected bacterial strains was tested, and the lowest concentration that was lethal to the bacterium was recorded as the MBC. The MBC values for *R. canescens DC* extract were determined, and it showed the most promising killing effect of 0.625 µg/mL against S. aureus and L. monocytogenes, 0.3125 µg/mL against L. monocytogenes alone, and 0.15625 µg/mL against *P. aeruginosa, E. coli*, and *S. typhimurium*, as presented in Table 3.

Assafiri *et al*. demonstrated that extracts from *R. canescens DC* have potent bactericidal properties against both Gram-positive and Gram-negative bacteria.[29] Phytochemical screening of the extracts revealed the presence of various compounds including flavonoids, anthraquinones, glycosides, steroids, triterpenoids, tannins, terpenoids, alkaloids, and total phenol. These compounds are known to possess antibacterial activity. Terpenes, in particular, have been identified as being responsible for the strong antibacterial activity against both Gram-positive and Gram-negative bacteria.[32] In this study, *R. canescens DC* extract was found to be effective against all tested bacterial strains, namely *S. aureus, L. monocytogenes*, and *S. typhimurium*, except for *Pseudomonas aeruginosa* and *E. coli*.

The findings of this study are consistent with those of Panizzi *et al*., who observed inhibition zones in response to a crude methanolic extract of *Rubus ulmifolius*.[33] The antibacterial properties of flavonoids were found to be more effective against Gram-negative bacteria than Gram-positive bacteria, as reported by Gyawali and Ibrahim in 2014.[34] There is a functional relationship between the composition of plant extracts and their antibacterial properties, as demonstrated by these authors.[35] Plant phenolic compounds, such as the presence of the–OH group and allylic side chain, are important contributors to the antimicrobial activity of plant extracts against microorganisms.[36] The mechanism of action of plant extracts against bacteria varies depending on the plant, the portion of the plant used, and the type and concentration of compounds present in the extract. A number of potential mechanisms of action have been proposed, including the inhibition of specific biochemical pathways, enzyme inactivation, increased membrane permeability, and the presence of lipophilic compounds like terpenoids that cause membrane disruption.[37] The minimal bactericidal concentration (MBC) is a measure of an extract's ability to kill bacteria. The bactericidal activity of *R. canescens DC* extracts was tested against selected bacteria strains, and the MBC was defined as the lowest concentration that was lethal to the bacterium

R. canescens DC Plant (Assafiri *et al*., 2020).

Urtica pilulifera L (Jaradat *et al*., 2015).

Rumex vesicarius L (Rechinger, 1954)

Figure 1: The plant species (*Rumex vesicarius, Rubus canescens* DC and *Urtica pilulifera* L)

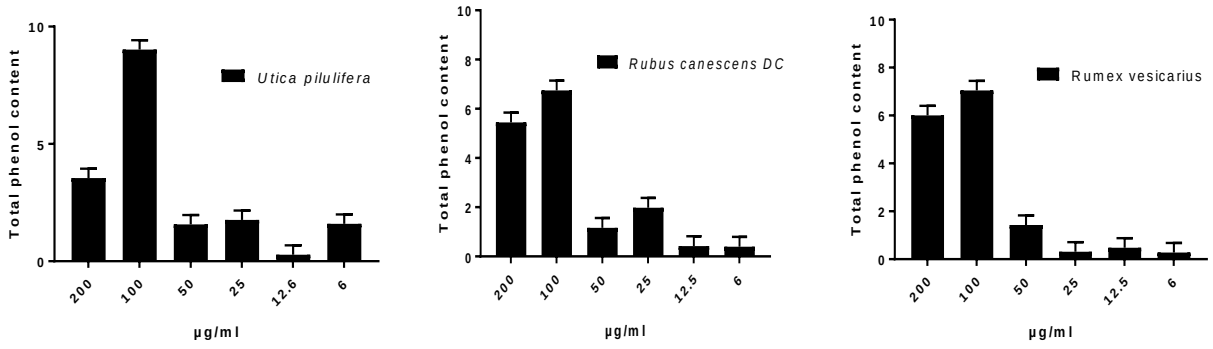

Figure 2: The phenolic contents of Crude extracts of plants. The results were expressed as Gallic acid equivalent and expressed as means ± SD (n = 3-4 independent replicates).

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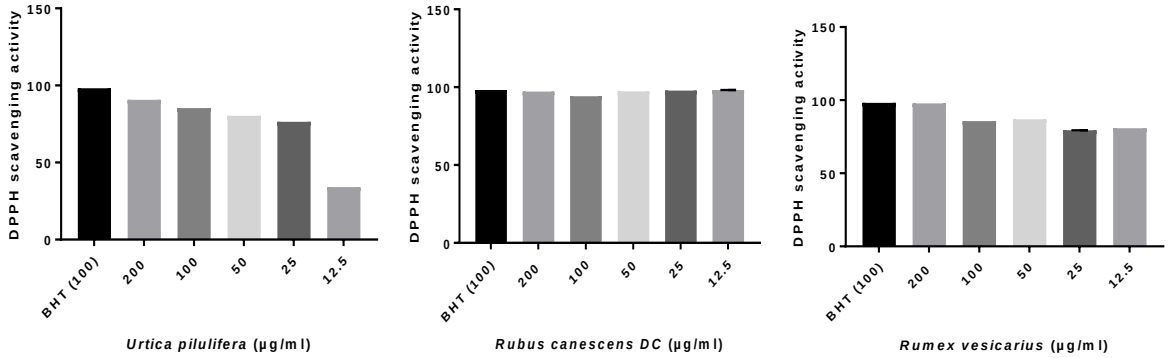

Figure 3: The antioxidant activities of *Urtica pilulifera* L, *Rubus canescens* DC and *Rumex vesicarus* L. and. The results represent percentage of DPPH scavenging activity. BHT was used as positive control at 100 µg/ml. Results are expressed as means ± SD (n = 3-4 independent replicates).

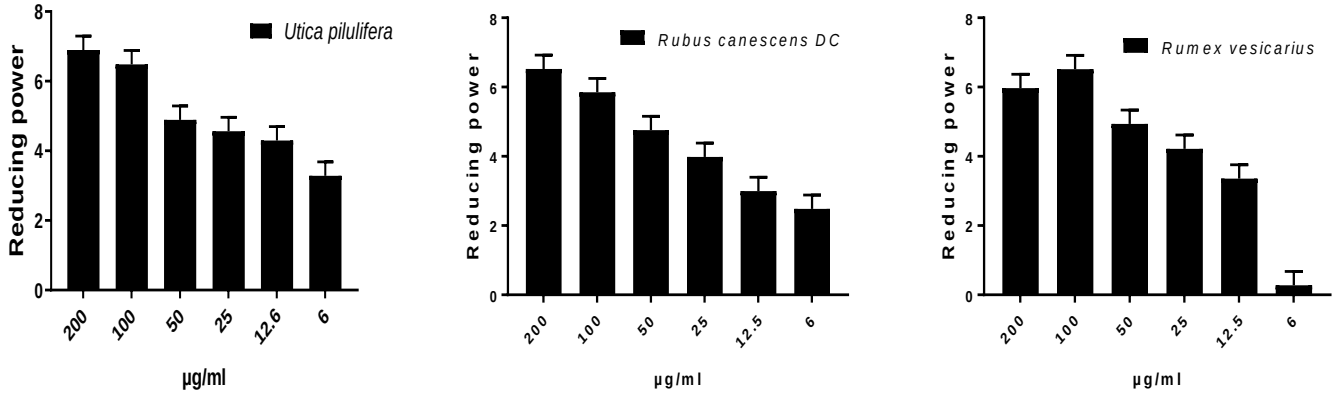

Figure 4: Reducing power of Crude extracts of plants. The results were expressed as ascorbic acid equivalent and expressed as means ± SD (n = 3-4 independent replicates).

Table 3: MIC and MBC values (µg/ml) of *Rubus canescens* DC extract against different bacterial and strains.

Bacterial Strains	MIC (µg/mL)	MBC (µg/mL)
Pseudomonas aeruginosa	0.3125	0.625
Escherichia coli	0.3125	0.625
Staphylococcus aureus	0.625	1.25
Listeria monocytogenes	0.625	1.25
Salmonella typhimurium	0.08	0.15625

Conclusion

This study investigated the antimicrobial and antioxidant activities of extractions from *Rubus canescens DC, Rumex vesicarius L*, and *Urtica pilulifera L*. The results showed that *R. canescens DC* extraction had significantly higher levels of phenolic compounds and demonstrated greater antioxidant and antimicrobial activity compared to the other plant extracts tested

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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