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Assessment of Total Phenolic, Flavonoid, and Anthocyanin Contents of Amaranthus Retroflexus Extract and Its Inhibitory Effect on the Growth of Pseudomonas Aeruginosa

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Abstract

The use of medicinal plants in the treatment of bacterial infections has attracted increasing attention due to the growing resistance of bacteria to antibiotics and the adverse effects associated with their use. Therefore, this study aimed to determine the total Phenolic, Flavonoid, and Anthocyanin contents of Amaranthus Retroflexus extract and to evaluate its inhibitory effect on the growth of Pseudomonas aeruginosa. For this purpose, A. retroflexus plants were collected from an area near Tonekabon, Iran. The antioxidant compounds of the plant extract, including Phenolic, Flavonoid, and Anthocyanin compounds, were determined. In addition, the inhibitory effect of the extract on the growth of P. aeruginosa was evaluated at different concentrations. The results showed that the A. retroflexus extract was rich in antioxidant compounds, particularly Phenolic compounds, with a Total Phenolic Content (TPC) of 9.84±0.741 mg/g Dry Weight (DW). Furthermore, the Anthocyanin content of the extract was higher than its Flavonoid content. Regarding antibacterial activity, the extract showed minimal inhibition against P. aeruginosa at 800 and 1000 mg/mL. In contrast, the greatest inhibitory effects were observed at concentrations of 1400 and 1800 mg/mL. The antibacterial activity of A. retroflexus extract may be linked to its antioxidant compounds, suggesting potential for further investigation in pharmaceutical applications.

Keywords: Antioxidant, Amaranthus retroflexus, Pseudomonas aeruginosa, Antibacterial activity, Total phenolic content.

1 | Introduction

Antioxidants play an important role in protecting Biological systems and food products against the adverse effects of oxidative processes [1–3]. These compounds can neutralize free radicals, inhibit oxidative chain

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reactions, and consequently reduce oxidative damage. Oxidation control is particularly important in food, pharmaceutical, cosmetic, and healthcare products, where oxidative deterioration can adversely affect product quality, stability, safety, and shelf life. In Biological systems, excessive production of Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) can also disturb the balance between oxidants and endogenous antioxidant defenses, resulting in oxidative stress. Therefore, antioxidant compounds have received considerable attention because of their potential role in preventing or reducing oxidative damage and maintaining the stability of Biological and non-Biological systems [4–8]. Synthetic antioxidants, including Butylated Hydroxyanisole (BHA) and Butylated Hydroxytoluene (BHT), have traditionally been used to prevent oxidative deterioration in various products. However, concerns about the possible adverse effects of prolonged or excessive exposure to some synthetic antioxidants have increased interest in naturally derived alternatives.

Reported concerns regarding synthetic antioxidants include potential toxicological effects, such as hepatic enlargement and carcinogenic effects, which have encouraged researchers to investigate safer and more naturally occurring antioxidant compounds [5]. Consequently, there has been a growing interest in identifying plant-derived antioxidants that can provide effective antioxidant activity while potentially offering additional Biological properties. Plants are considered important natural sources of bioactive compounds, particularly secondary metabolites with antioxidant, antimicrobial, anti-inflammatory, and other Biological activities. Among these compounds, Phenolic compounds, Flavonoids, and Anthocyanins represent major groups of plant-derived phytochemicals that have attracted substantial scientific interest [9]. Their Biological activities are associated with their chemical structures and their ability to interact with free radicals, reactive species, cellular components, and other molecular targets. The presence and concentration of these compounds can vary considerably among plant species and may also be influenced by environmental conditions, geographical location, developmental stage, and extraction procedures. Therefore, characterization of the major antioxidant constituents of medicinal and potentially medicinal plants is an important step toward evaluating their possible applications [10].

In addition to oxidative deterioration, microbial contamination represents another major challenge faced by the food, beverage, pharmaceutical, cosmetic, and healthcare industries. The presence and proliferation of pathogenic microorganisms can compromise product safety and quality and may result in significant public-health concerns. In the food industry, particular attention has been directed toward foodborne pathogens and their toxins because of their ability to cause gastrointestinal and systemic infections. Pathogenic microorganisms such as Escherichia coli, Staphylococcus aureus, Salmonella spp., and P. aeruginosa have been associated with a variety of infections and food-safety problems. Some of these microorganisms can cause gastrointestinal disturbances, including diarrhea and vomiting, whereas others may cause more severe infections, particularly in susceptible individuals [11]. The increasing resistance of bacterial pathogens to conventional antibiotics has further complicated the management of bacterial infections. Antibiotic-resistant microorganisms can result in increased treatment failure, prolonged illness, higher healthcare costs, and increased mortality compared with infections caused by susceptible microorganisms. Among clinically important antibiotic-resistant bacteria associated with healthcare-associated infections are several Gram-negative organisms, including members of the family Enterobacteriaceae and P. aeruginosa, as well as Gram-positive organisms such as Staphylococcus, Streptococcus, and Enterococcus species [6]. The emergence and dissemination of antimicrobial resistance have therefore become major challenges in infectious disease management and have stimulated extensive research aimed at identifying alternative antimicrobial agents [12].

Among the microorganisms of particular interest, P. aeruginosa is an important Gram-negative bacterium with considerable clinical relevance. Its ability to survive under diverse environmental conditions and its capacity to develop resistance to multiple antimicrobial agents make it an important target for investigations of alternative antibacterial compounds. The increasing prevalence of resistant bacterial strains has encouraged researchers to investigate natural products, particularly plant-derived compounds and extracts, as potential sources of new antimicrobial agents. Natural products may contain several bioactive constituents that act through different mechanisms and may therefore provide valuable candidates for further investigation [6].

In this context, medicinal and traditionally used plants have emerged as promising sources of naturally occurring antimicrobial compounds. The use of medicinal plants in infection management has received increasing attention because plants produce a wide range of secondary metabolites as part of their natural defense mechanisms. Essential oils, Phenolic compounds, Flavonoids, alkaloids, terpenoids, tannins, and other phytochemicals have been investigated for their potential antimicrobial properties. Consequently, considerable research has been conducted during the past decade to identify natural antimicrobial agents and to evaluate the Biological activities of plant extracts and essential oils [12]. In addition to their antimicrobial properties, many of these compounds exhibit antioxidant activity, suggesting that a single plant extract may possess multiple Biological functions.

Phenolic compounds constitute one of the most important groups of plant secondary metabolites and are widely distributed throughout the plant kingdom. Their antioxidant activity is largely associated with their ability to donate hydrogen atoms or electrons to reactive species and to stabilize free radicals through resonance mechanisms. Flavonoids, which constitute a major subgroup of Phenolic compounds, have also attracted considerable attention because of their diverse Biological activities. Depending on their chemical structure and degree of hydroxylation, Flavonoids can exhibit substantial radical-scavenging and metal-chelating properties. Anthocyanins, another important group of plant polyphenols, are responsible for many of the red, purple, and blue colors observed in plant tissues and have also been investigated for their antioxidant and other Biological activities.

The Biological activity of plant Phenolics and Flavonoids is not limited to their antioxidant effects. Numerous studies have demonstrated that Phenolic and Flavonoid compounds may also exhibit antimicrobial activity against a broad range of microorganisms [13]. Several mechanisms have been proposed to explain these compounds' antimicrobial effects. These mechanisms may include adsorption to and disruption of microbial cell membranes, modification of membrane permeability, interaction with cellular proteins and enzymes, interference with essential metabolic processes, and chelation or reduction of metal ions required for microbial growth. The combined action of different phytochemicals present in a plant extract may contribute to the overall antimicrobial activity of the extract. Therefore, the Biological effects of a crude plant extract cannot necessarily be attributed to a single compound and may result from interactions among several bioactive constituents.

The simultaneous presence of antioxidant and antimicrobial compounds in plant extracts is particularly interesting from an applied perspective. In food systems, for example, natural antioxidant compounds can help limit oxidative deterioration and extend product shelf life, while antimicrobial constituents may contribute to controlling undesirable microorganisms. Accordingly, the incorporation or application of plant-derived bioactive compounds may provide an opportunity to address both oxidative and microbial challenges simultaneously [14]. Similar considerations have stimulated interest in the potential applications of plant extracts in pharmaceutical, cosmetic, nutraceutical, and food-related products.

A. retroflexus L., commonly known as redroot pigweed or wild amaranth, belongs to the family Amaranthaceae and is widely distributed in different regions. Although several Amaranthus species have traditionally been used as food or medicinal plants, the Biological properties of individual species can differ according to their phytochemical composition and environmental conditions. Plant species belonging to the genus Amaranthus have attracted scientific interest because they contain various biologically active constituents, including Phenolic compounds, Flavonoids, pigments, and other secondary metabolites. These characteristics make A. retroflexus a potentially valuable plant for investigating naturally occurring antioxidant and antimicrobial compounds [2].

The phytochemical composition of A. retroflexus may contribute to its Biological properties. In particular, Phenolic compounds, Flavonoids, and Anthocyanins may play important roles in its antioxidant potential. However, the concentration of these compounds can vary depending on several factors, including plant origin, environmental conditions, plant maturity, extraction method, and solvent characteristics. Therefore,

determining the total content of major antioxidant phytochemicals in an extract obtained from a specific geographical region can provide useful information for evaluating its Biological potential.

Furthermore, evaluating the antibacterial activity of A. retroflexus extract against clinically relevant bacteria such as P. aeruginosa can provide complementary information on the plant's potential Biological value. Establishing a relationship between the phytochemical composition of the extract and its antibacterial activity may also contribute to a better understanding of the possible role of plant-derived antioxidant compounds in microbial growth inhibition. Such investigations are particularly relevant in the context of increasing antimicrobial resistance and the continuing search for natural sources of Biologically active compounds [11].

Although plant-derived products cannot automatically be considered safe or therapeutically effective solely based on their natural origin, systematically evaluating their phytochemical composition and Biological activities is an important preliminary step toward assessing their potential applications. Determination of total Phenolic, Flavonoid, and Anthocyanin contents, together with experimental evaluation of antibacterial activity, can provide an initial profile of the Biological properties of a plant extract [15]. Further studies would subsequently be required to identify individual active compounds, elucidate their mechanisms of action, establish their safety profiles, and determine their practical efficacy in specific applications. The relationship between the antioxidant properties of plant-derived phytochemicals, their potential antibacterial mechanisms, and the rationale for investigating A. retroflexus is schematically illustrated in *Fig. 1*.

Therefore, the present study aimed to determine the total Phenolic, Flavonoid, and Anthocyanin contents of A. retroflexus extract collected from an area near Tonekabon, Iran, and to evaluate its inhibitory effect on the growth of P. aeruginosa. By examining both the major antioxidant phytochemical groups and the antibacterial activity of the extract, this study sought to provide an initial assessment of the Biological potential of A. retroflexus and its possible relevance as a natural source of bioactive compounds.

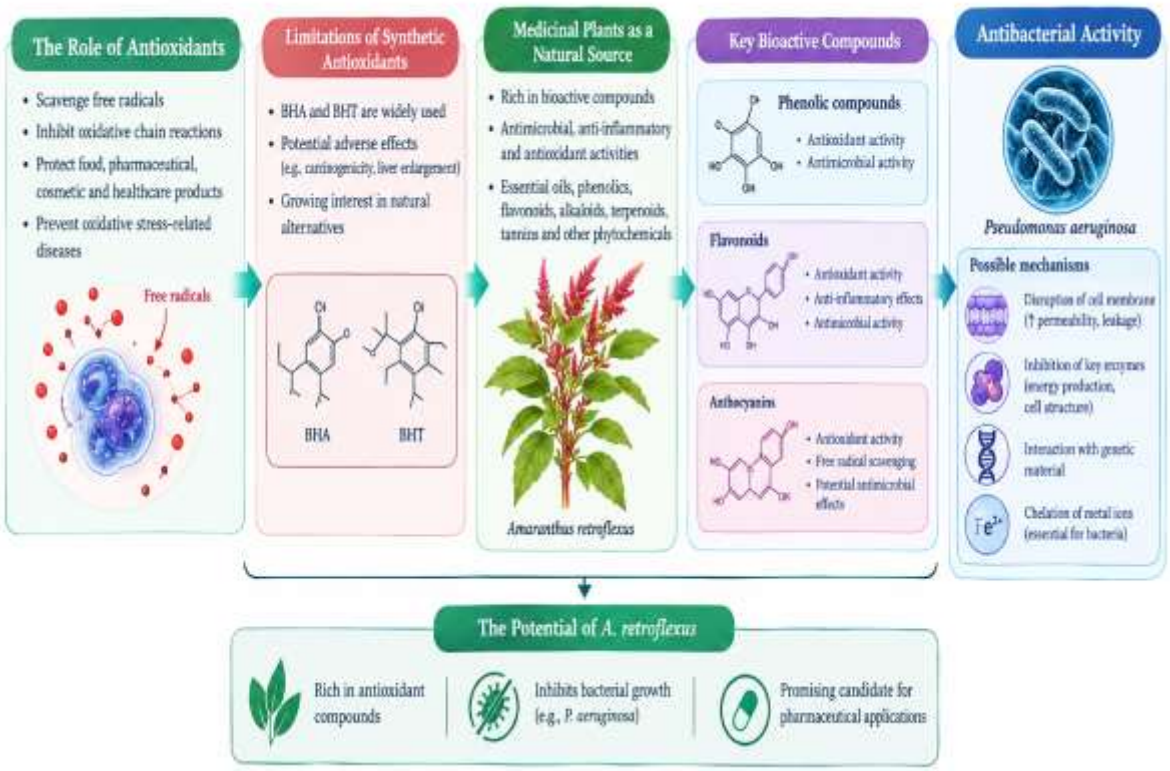


Fig. 1. Schematic overview of the rationale for investigating the antioxidant and antibacterial potential of A. retroflexus.

2 | Materials and Methods

2.1 | Plant Collection and Preparation of the Extract

The aerial parts of wild amaranth (A. retroflexus L.) were collected in spring 2026 from an area near Abbasabad, Iran. The collected plant materials were transferred to the laboratory and dried in the shade at ambient temperature with adequate air circulation to minimize direct exposure to sunlight and prevent potential degradation of light-sensitive phytochemicals. After drying, the plant material was ground into a fine, relatively homogeneous powder before extraction. For the preparation of the plant extract, 30 g of the dried powdered aerial parts were immersed in 200 mL of 80% methanol. The mixture was maintained at room temperature for 48 h to allow sufficient contact between the plant material and the extraction solvent. Following the extraction period, the mixtures were filtered to remove plant residues and obtain the methanolic extracts. The solvent was subsequently removed under reduced pressure using a rotary evaporator at a temperature below 40 °C. The relatively low evaporation temperature was intended to minimize thermal degradation of heat-sensitive bioactive compounds. The resulting crude extracts were collected and stored at 4 °C in a refrigerator until further analysis. The prepared extracts were then used to determine total Phenolic, Flavonoid, and Anthocyanin contents and to evaluate their antibacterial activity [12].

2.2 | Determination of Total Phenolic Content

The Total Phenolic Content (TPC) of the A. retroflexus extract was determined using the Folin–Ciocalteu colorimetric method. Briefly, mix 100 µL of the plant extract with 2 mL of 2% sodium carbonate solution, 2.8 mL of distilled water, and 100 µL of 50% Folin–Ciocalteu reagent. The reaction mixture was allowed to stand for 30 min to ensure adequate development of the colorimetric reaction. After incubation, the absorbance of the resulting solution was measured at 720 nm against an appropriate blank using a spectrophotometer. Gallic acid was used as the reference standard to prepare the calibration curve. The TPC of the extract was calculated based on the corresponding standard curve and expressed as milligrams of Gallic Acid Equivalents (GAE) per gram of dry plant material (mg GAE/g Dry Weight (DW)) [7].

The use of gallic acid as the calibration standard allows the Phenolic content of the extract to be expressed in a standardized form and facilitates comparison of the results with those obtained in other studies employing the Folin–Ciocalteu assay.

2.3 | Determination of Total Flavonoid Content

The Total Flavonoid Content (TFC) of the plant extract was determined using an aluminum chloride-based colorimetric assay. For this purpose, 500 µL of each extract was mixed with 1.5 mL of 80% methanol, 100 µL of 10% aluminum chloride solution, 100 µL of 1 M potassium acetate solution, and 2.8 mL of distilled water.

The resulting mixtures were thoroughly mixed and allowed to stand for 40 min to allow the formation of the Flavonoid–aluminum complex. Subsequently, the absorbance was measured at 415 nm against the corresponding blank. Quercetin was used as the standard compound to prepare the calibration curve. The TFC was calculated using the standard curve and expressed as milligrams of Quercetin Equivalents (QE) per gram of dry plant material (mg QE/g DW) [4]. This procedure provides an estimation of the overall Flavonoid content of the extract based on its reaction with aluminum chloride and enables the results to be expressed relative to a known Flavonoid standard.

2.4 | Determination of Total Anthocyanin Content

The total Anthocyanin content of the plant material was determined using an acidified methanol extraction procedure. Briefly, 0.02 g of the dried plant tissue was thoroughly ground in a porcelain mortar with 4 mL of

1% hydrochloric acid in methanol. The resulting mixture was kept at 4°C for 24 h to facilitate the extraction of Anthocyanin pigments into the acidified methanolic solvent.

After extraction, the samples were centrifuged at 13,000 rpm for 10 min. The resulting supernatant was carefully separated from the precipitated plant material. The absorbance of the supernatant was subsequently measured at 530 and 657 nm against a blank containing 1% hydrochloric acid in methanol. The absorbance values obtained at these wavelengths were used to calculate the Anthocyanin content according to the following *Eq. (1)* [9]:

$$A=A_{530}-(0.25\times A_{657}), \quad (1)$$

where A represents the corrected absorbance of the extract, and the subscripts indicate the wavelengths (nm) at which the absorbance measurements were performed. The measurement at 657 nm was used to correct for background interference, particularly that associated with non-Anthocyanin pigments, while the absorbance at 530 nm primarily reflects the contribution of Anthocyanin pigments. The corrected absorbance was subsequently used for the determination of Anthocyanin content according to the method described in the cited reference [9].

2.5 | Evaluation of Antibacterial Activity by the Disk Diffusion Method

The antibacterial activity of the aerial-part extract of A. retroflexus was evaluated using the disk diffusion method. Initially, the test microorganism, P. aeruginosa, was activated in nutrient broth under appropriate culture conditions. Following activation, a bacterial suspension corresponding to 0.5 McFarland turbidity was prepared. According to the experimental protocol, a 0.5 McFarland suspension corresponds to approximately 1.5×10^8 bacterial cells/mL. For the antibacterial assay, 0.1 mL of the prepared bacterial suspension was uniformly spread over Mueller–Hinton agar plates to obtain an even bacterial lawn. Sterile paper disks served as carriers for the plant extract. Different concentrations of the A. retroflexus extract, including 400, 600, 800, and 1000 mg/mL, were prepared for evaluation. A volume of 30 µL of each extract concentration was applied to the corresponding sterile paper disks. The disks were subsequently placed carefully on the surface of the inoculated Mueller–Hinton agar plates. Following placement of the disks, the plates were incubated under suitable conditions for the test microorganism for 24 h. After incubation, the antibacterial activity of the extract was evaluated by measuring the diameter of the inhibition zones surrounding the disks. The diameter of each inhibition zone was measured in millimeters (mm). Larger inhibition zones indicated greater inhibition of bacterial growth under the experimental conditions [13]. The disk diffusion assay provided an initial assessment of the A. retroflexus extract's ability to inhibit P. aeruginosa growth. The antibacterial activity observed at different extract concentrations was subsequently considered in relation to the phytochemical composition of the plant extract, particularly its Phenolic, Flavonoid, and Anthocyanin contents.

2.6 | Statistical Analysis

All experimental data were obtained from three replicates and are presented as mean ± Standard Error (SE). Data calculations and table preparation were performed using Microsoft Excel 2010. The phytochemical analyses and antibacterial assays were used to characterize the antioxidant and antibacterial properties of the A. retroflexus extract.

The experimental results were subsequently compared descriptively across the evaluated extract concentrations to assess changes in antibacterial activity. The results are presented in the corresponding tables and figures of the study.

3|Results and Discussion

3.1|Total Phenolic, Flavonoid, and Anthocyanin Contents of the Aerial-Part Extract of Amaranthus Retroflexus

The phytochemical analysis showed that the aerial-part extract of wild amaranth (A. retroflexus L.) contained considerable amounts of antioxidant-related phytochemicals, particularly Phenolic compounds. Among the three phytochemical groups evaluated in this study, Phenolic compounds represented the predominant group detected in the extract. The TPC of the A. retroflexus extract was determined to be 9.84±0.741 mg GAE per g DW. In comparison, the total Flavonoid and Anthocyanin contents were 0.426±0.001 mg QE per g DW and 0.619±0.012 mg/g DW, respectively (*Table 1*). These findings indicate that the aerial parts of A. retroflexus contain a measurable number of Phenolic compounds together with Flavonoids and Anthocyanins. The relatively higher TPC compared with the measured Flavonoid and Anthocyanin contents suggests that Phenolic compounds may constitute an important component of the phytochemical profile of the investigated extract. It should be noted that the TPC determined by the Folin–Ciocalteu method represents the overall reducing capacity attributed to Phenolic constituents under the assay conditions and should not necessarily be interpreted as the concentration of individual Phenolic compounds. The presence of Flavonoids and Anthocyanins in the extract further demonstrates the phytochemical diversity of the aerial parts of A. retroflexus. These secondary metabolite groups have been widely investigated because of their potential antioxidant and Biological activities. Therefore, the simultaneous detection of Phenolic compounds, Flavonoids, and Anthocyanins provides a preliminary indication of the potential Biological value of the plant extract and provides a basis for further evaluation of its antibacterial properties.

Table 1. Total Phenolic, Flavonoid, and Anthocyanin contents of the aerial-part extract of A. retroflexus. Data are presented as mean ± SE.

Plant Material	TPC (mg GAE/g DW)	TFC (mg QE/g DW)	Total Anthocyanin Content (mg/g DW)
Aerial parts	9.84±0.741	0.426±0.001	0.619±0.012

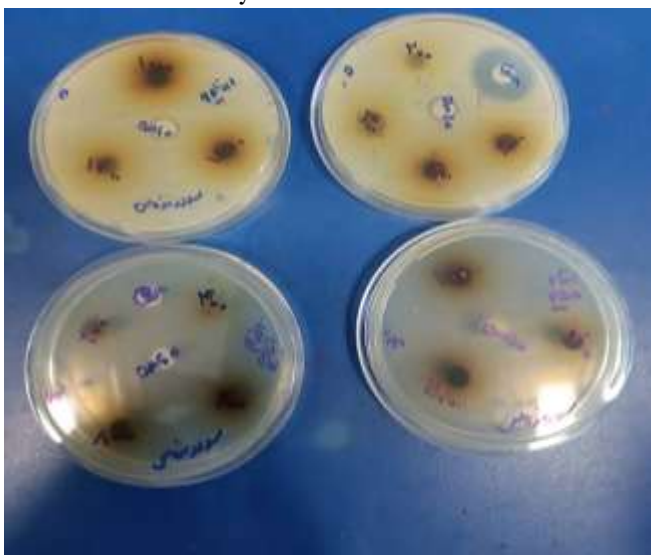
3.2|Antibacterial Activity of Amaranthus Retroflexus Extract Against Pseudomonas Aeruginosa

The antibacterial activity of the aerial-part extract of A. retroflexus was evaluated against the Gram-negative bacterium Pseudomonas aeruginosa using the disk diffusion method. Gentamicin was used as the positive control, whereas Dimethyl Sulfoxide (DMSO) was used as the negative control. The use of these controls allowed the antibacterial response associated with the plant extract to be distinguished from the activity of a reference antibiotic and from any possible effect of the solvent, respectively.

The results showed that the extract did not produce a detectable inhibition zone against P. aeruginosa at concentrations ranging from 200 to 1000 mg/mL under the experimental conditions. In contrast, increasing the extract concentration to 1400 and 1800 mg/mL resulted in detectable inhibition of bacterial growth. The inhibition-zone diameters were 2.00±0.001 mm and 4.00±0.091 mm at 1400 and 1800 mg/mL, respectively (*Fig. 1*). The increase in inhibition-zone diameter with increasing extract concentration indicates a concentration-dependent antibacterial response within the tested range. The absence of a detectable inhibition zone at lower concentrations suggests that the concentration of bioactive constituents reaching the bacterial cells may have been insufficient to produce measurable growth inhibition under the disk diffusion assay conditions. At higher concentrations, however, the extract exhibited a measurable inhibitory effect against P. aeruginosa. The positive control, gentamicin, produced a substantially larger inhibition zone, with a diameter of 30 mm (*Fig. 1*). However, the A. retroflexus extract demonstrated detectable antibacterial activity at the two highest concentrations evaluated; its inhibitory effect under the present experimental conditions was considerably lower than that observed for the reference antibiotic. This comparison indicates that the extract

should be considered a potential source of antibacterial compounds rather than an equivalent substitute for conventional antibiotics based solely on the present assay. The antibacterial activity observed at higher extract concentrations may be associated, at least in part, with phenolic compounds, Flavonoids, Anthocyanins, or other phytochemicals present in the aerial parts of A. retroflexus. Plant Phenolics and Flavonoids have been reported to interact with bacterial cellular structures and may interfere with membrane integrity, enzyme activity, and other essential cellular processes. However, the present results do not establish a direct causal relationship between any individual phytochemical group and the observed antibacterial effect. Identification of the specific compounds responsible for the antibacterial activity would require further phytochemical characterization and mechanistic studies. The results demonstrate that the aerial-part extract of A. retroflexus possesses measurable antibacterial activity against P. aeruginosa, particularly at the higher concentrations tested. The combination of a relatively high TPC and detectable antibacterial activity suggests that this plant may represent a potential source of bioactive phytochemicals worthy of further investigation.

Fig. 2. Antibacterial activity of A. retroflexus extract against P. aeruginosa determined by the disk diffusion method.



4 | Conclusion

The increasing prevalence of bacterial resistance to conventional antibiotics has intensified the search for new antimicrobial compounds and alternative therapeutic resources. In this context, medicinal plants represent an important and promising source of bioactive compounds that may provide candidates for the development of novel antimicrobial and pharmacological agents. The present study therefore investigated the aerial parts of wild amaranth (A. retroflexus) collected from its natural habitat, with particular emphasis on its antioxidant constituents and antibacterial activity against Pseudomonas aeruginosa [16–18].

The results demonstrated that the methanolic extract of the aerial parts of A. retroflexus contained considerable amounts of antioxidant-related phytochemicals, particularly Phenolic compounds. The TPC was 9.84±0.741 mg GAE/g DW, whereas the total Flavonoid and Anthocyanin contents were 0.426±0.001 and 0.619±0.012 mg/g DW, respectively. The relatively higher Phenolic content compared with the other measured phytochemical groups suggests that Phenolic constituents may represent an important component of the chemical profile of the investigated extract. However, these colorimetric measurements estimate total compound classes and do not identify individual Phenolic, Flavonoid, or Anthocyanin molecules. Therefore, further chromatographic and spectrometric analyses would be required to determine the specific compounds responsible for the observed Biological properties.

The antibacterial assay showed that the extract did not produce a detectable inhibitory zone against P. aeruginosa at concentrations up to 1000 mg/mL. In contrast, measurable inhibition was observed at 1400 and 1800 mg/mL, producing inhibition zones of 2.00±0.001 and 4.00±0.091 mm, respectively. Although this

activity was considerably weaker than that of the positive control gentamicin, which produced a 30-mm inhibition zone, the results indicate that the extract possesses a measurable antibacterial effect at relatively high concentrations. The concentration-dependent increase in the inhibition zone also suggests that the amount of extract applied may influence its antibacterial activity.

The relatively limited susceptibility of P. aeruginosa to the plant extract may be associated, at least in part, with the structural and physiological characteristics of this Gram-negative bacterium. Unlike Gram-positive bacteria, Gram-negative bacteria possess an additional outer membrane surrounding the peptidoglycan layer. This outer membrane, which contains lipopolysaccharides and has a relatively hydrophilic surface, can restrict the penetration of various antimicrobial compounds. In addition, enzymes and other resistance mechanisms located in the periplasmic space and cell envelope can contribute to the degradation, modification, or exclusion of potentially harmful compounds. These characteristics may reduce the susceptibility of P. aeruginosa to plant-derived antimicrobial constituents and may partly explain why relatively high extract concentrations were required to produce measurable growth inhibition.

Plant extracts and essential oils can exert antibacterial effects through several mechanisms. One important mechanism involves interaction with the phospholipid bilayer of the bacterial cell membrane, altering membrane permeability and causing leakage of intracellular components. Plant-derived compounds may also interfere with enzymes involved in cellular energy production and the synthesis of essential structural components. In addition, some phytochemicals can interact with cellular proteins and nucleic acids, thereby disrupting essential cellular processes. The antibacterial activity of Phenolic compounds is particularly influenced by the number and position of hydroxyl groups, which can affect their interaction with bacterial cellular components. Phenolic compounds may interact with the bacterial cell envelope and form complexes with cellular proteins, potentially altering membrane integrity and inhibiting microbial growth. They may also interact with sulfhydryl groups or proteins and consequently interfere with the activity of essential enzymes.

Considering the relatively high TPC detected in the A. retroflexus extract, these compounds may contribute to the antibacterial activity observed in the present study. Flavonoids and Anthocyanins may also contribute to the extract's overall Biological activity through complementary mechanisms. Nevertheless, the present results do not establish a direct causal relationship between a specific phytochemical group and antibacterial activity because the extract was evaluated as a complex mixture. Identifying individual bioactive constituents and evaluating their antibacterial effects would therefore be necessary to clarify the molecular basis of the observed activity [19–21].

The findings indicate that A. retroflexus can serve as an accessible plant source of antioxidant-related phytochemicals, particularly Phenolic compounds. The methanolic extract also exhibited inhibitory activity against P. aeruginosa at high concentrations, although its antibacterial effect was substantially lower than that of gentamicin. These findings suggest that A. retroflexus may be a worthwhile candidate for further pharmacological and phytochemical investigation. Future studies should focus on the isolation and identification of its major bioactive compounds, determination of their individual and synergistic antimicrobial effects, evaluation against a broader range of pathogenic microorganisms, and assessment of their mechanisms of action and safety. Such investigations could provide a more comprehensive understanding of the plant's therapeutic potential and determine whether its bioactive constituents have practical applications as natural antimicrobial or antioxidant agents.

Authors' Contributions

All research activities and manuscript development were conducted by the author, who also approved the final manuscript.

Data Availability

All data supporting the reported findings in this research paper are provided within the manuscript.

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Conflict of Interest

The author declares that in this paper, there is no conflict of interest in this paper.

Consent for Publication

The author has provided their consent for the publication of this manuscript.

Ethics Approval and Consent to Participate

This research did not involve human participants or animals.

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