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Screening for antimicrobial properties in crude extracts of *Amaranthus blitum* subsp. *oleraceus* (L.) Costea, a traditional medicinal plant

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Abstract: The increasing prevalence of antibiotic resistance and the emergence of novel pathogenic strains emphasize the need to investigate medicinal plants as accessible and cost-effective sources of potential antimicrobial agents. Species of *Amaranthus* have been used in traditional medicine for the treatment of various ailments. This study aimed to assess the *in vitro* antimicrobial effects of various extracts from the aerial and underground parts of *Amaranthus blitum* subsp. *oleraceus*. The extracts were tested for their effects on diverse microbial populations, including Gram-positive and Gram-negative bacteria, as well as *Candida albicans*, using the disk diffusion method. Crude ethanolic, acetonic, benzene and chloroform extracts were dissolved in 100% DMSO, while aqueous extracts were dissolved in distilled water to prepare stock solutions, which were adjusted to a concentration of 75 mg/mL for subsequent analysis. The results indicated that ethanol, acetone, and chloroform extracts from both aerial and underground parts exhibited antibacterial activity, particularly against *Bacillus anthracoides*, while ethanolic, acetonic, benzene, and chloroform extracts of both parts showed antifungal activity against *C. albicans*. All tested aqueous extracts exhibited very low antibacterial activity and did not show antifungal effect against *C. albicans*. *A. blitum* subsp. *oleraceus* has been cultivated as a vegetable crop for centuries in diverse regions worldwide. The observed antibacterial activity of its crude extracts indicates the presence of bioactive constituents, supporting the need for further phytochemical investigations and potential applications in both pharmacological and agricultural fields.

Keywords: *antibacterial activity, antifungal activity, disc-diffusion, inhibition zone, microorganisms, plant extracts*

INTRODUCTION

Since ancient times, humans have relied on nature to meet essential needs, including medicinal purposes. Plants have played a significant role in combating infectious diseases. In the modern era, the escalating problem of antibiotic resistance has renewed interest in plant-based therapeutics as potential alternatives or complements to conventional antibiotics. In 2017, the World Health Organization, in collaboration with researchers from the Division of Infectious Diseases at the University of Tübingen, Germany, applied a multicriteria decision analysis approach to publish a list of microorganisms with high levels of antibiotic resistance that need to be better managed [Talebi Bezmin Abadi et al., 2019]. The antimicrobial properties of the plants are attributed to their chemical constituents. Plants are a rich source of antimicrobials, with over 1340 species reported to possess antimicrobial activity and more than 30000 compounds isolated to date. Furthermore, it is estimated that 14–28% of higher plants are medicinal, and approximately three-quarters of bioactive plant-derived molecules were discovered through ethnomedicinal practices [Vaou et al., 2021]. Secondary metabolites, including major groups such as phenolics, terpenes, and nitrogen-containing compounds, enable plants to interact with the surrounding biotic and abiotic environment. At least five classes—glucosinolates, benzoxazinoids, terpenes, aromatics, and green-leaf volatiles—have been confirmed to function as key regulators in plant defense [Erb, Kliebenstein, 2020].

Amaranths are among the earliest cultivated vegetable crops, used as leafy vegetables, dye plants, ornamentals, and grains, and also found as weeds, in temperate, tropical and subtropical regions. The genus *Amaranthus* consists of approximately 74 annual species, exhibiting considerable morphological diversity and distinctly marked by monoecy and dioecy. Because they perform photosynthesis via the C4 pathway, amaranths easily adapt to challenging environmental conditions. Previous investigations have reported that amaranths have higher proximate nutrient content than commonly consumed edible crops, such as

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corn (*Zea mays* L.), buckwheat (*Fagopyrum esculentum* Moench), rye (*Secale cereale* L.), and rice (*Oryza sativa* L.), comparable nutritive characteristics to widely consumed vegetables, such as spinach (*Spinacia oleracea* L.), and nutrient content equivalent to some fodder crops, including maize, wheat (*Triticum* L.) and barley (*Hordeum vulgare* L.). Moreover, amaranths are rich in relatively rare amino acids, such as tryptophan and lysine, which could partially replace animal protein and enrich human diets with moderate-quality amino acids [Jimoh et al., 2022].

Amaranthus blitum subsp. *oleraceus* (L.) Costea (syn. *A. lividus* L.) occurs in diverse regions worldwide and is commonly known as wild amaranth, pigweed, purple amaranth or livid amaranth. It is used as a traditional medicine against lung disorders in Nigeria. The plant's fluid extract is applied internally as an astringent for the treatment of ulcerated mouth and throat. It also has applications in Ayurveda (known as Vaspaka, Marisa, etc., in Sanskrit) where is utilized to relieve vata, cold and cough, and to control bile secretion [Nehal et al., 2016]. *A. blitum* subsp. *oleraceus* is an annual, glabrous plant, 17-40 cm high, with dark-green leaves. Fruits are ellipsoid, indehiscent, and seeds measure 1-1.2 mm in diameter. The species is mainly distributed in Samur-Davachi, Kur-Araz, and Lankaran lowlands of Azerbaijan, often found along riversides and roadsides in weedy areas [Flora of Azerbaijan, 1952]. In Azerbaijan, 11 species are found two of which are cultivated [Asgarov, A., 2016]. The aim of this study was to assess the antimicrobial effect of different extracts obtained from the roots and aerial parts of *A. blitum* subsp. *oleraceus* on selected microorganisms.

MATERIALS AND METHODS

Plant material. The aerial and root parts of *A. blitum* subsp. *oleraceus* were collected during the summer of 2024 from Javad village, Sabirabad district, Azerbaijan (40°01'27.28″ N, 48°26'47.16″ E; 11 m below sea level). A voucher specimen was deposited in the Herbarium of the Institute of Botany (BAK29763), Baku, Azerbaijan after identification according to Flora of Azerbaijan [1961]. All collected materials were stored at room temperature, protected from direct contact with sunlight until further use.

Preparation of extracts. All the collected parts were chopped into small pieces. 9.4 g of finely chopped roots were extracted separately in a flask with different solvents using the cold maceration method [Adunola et al., 2015]. For this purpose, 80 mL of 96% ethanol, 100 mL of acetone, 205 mL of distilled water, 80 mL of chloroform, and 70 mL of benzene were used. The homogenate was filtered through filter paper and concentrated by solvent evaporation using a rotary evaporator. This process yielded: 30 mg of dry extract with acetone (0.32%), 1.6 g with distilled water (17.02%), 60 mg with chloroform (0.64%), 30 mg with benzene (0.32%), and 370 mg with ethanol (3.94%). For the extraction of the aerial parts, 24 g of raw material was treated with 505 mL of 96% ethanol, 12 g with 240 mL of acetone, 18 g with 420 mL of distilled water, 220 mL of chloroform, and 200 mL of benzene. This process yielded: 30 mg of dry extract with acetone (0.25%), 4.01 g with distilled water (22.28%), 170 mg with chloroform (0.94%), 170 mg with benzene (0.94%), and 0.54 g with ethanol (2.25%). The highest extract yield was obtained with distilled water, followed by ethanol, indicating a greater solubility of the extractable components in polar solvents. Stock solutions were prepared by dissolving the dry extracts at 75 mg/mL. For those obtained with distilled water from both roots and aerial parts, 30 mg were dissolved in 400 µL distilled water; for the extracts obtained with other solvents, 30 mg were dissolved in 400 µL DMSO.

Antimicrobial activity. Microorganisms and growth conditions. The following microorganisms were chosen to assess antimicrobial activity: *Staphylococcus aureus* Rosenbach, MRSA (Methicillin-resistant *Staphylococcus aureus*), *Escherichia coli* Migula, *Klebsiella pneumoniae* (Schroeter) Trevisan, *Pseudomonas aeruginosa* (Schröter) Migula, *Bacillus anthracoides* Cohn and *Candida albicans* (C.-P. Robin) Berkhout. All microbial strains were supplied by the Department of Medicinal Microbiology and Immunology at Azerbaijan Medical University. Bacterial strains were cultured on Mueller Hinton agar, while *C. albicans* was cultivated on Sabouraud dextrose agar. Cultures were incubated at 37°C. For inoculum preparation, a suspension of each test strain was adjusted to match the visual turbidity of a 0.5 McFarland standard. The microbial stock suspensions were concentrated and incubated for 24 h prior to the experiment.

In vitro antimicrobial activity of extracts. The disk diffusion assay was used to assess the antimicrobial activity of the different *A. blitum* subsp. *oleraceus* extracts [Bauer et al., 1966]. A cotton swab was employed to uniformly distribute the bacterial and fungal inocula over the surface of the respective nutrient media in 90-mm Petri dishes. Sterile paper disks, 6-mm in diameter, were loaded with 7 µL of the prepared stock

solutions and subsequently placed onto the inoculated agar surfaces. The Petri dishes were then incubated at 37°C for 18–24 hours. The antimicrobial activity was determined by measuring the diameters of the inhibition zones (in mm) formed around the disks after incubation. Negative control disks containing 7 µL of distilled water and 100% DMSO were included to verify that the solvents did not exert any antimicrobial effects.

RESULTS AND DISCUSSION

The present research assessed the antimicrobial potential of *A. blitum* subsp. *oleraceus* against five bacterial strains and one fungal strain. Table 1 illustrates the effects of both root and aboveground part extracts prepared using multiple solvents together with the inhibition zone diameter caused by negative controls. The crude ethanolic root extract of *A. blitum* subsp. *oleraceus* exhibited inhibitory activity against all tested microorganisms except MRSA, for which DMSO produced a 7 mm inhibition zone. Although the crude acetone extract of roots showed 10 mm inhibition zone against MRSA, 11 mm inhibition zone against *S. aureus*, 8 mm inhibition zone against *E. coli* which 7 mm of these effects were attributed to the DMSO, suggesting that the extracts' intrinsic antibacterial activity was relatively weak. All extracts showed antifungal activity against *C. albicans*, except the crude aqueous root extract. With regard to the aboveground parts, the crude acetone extract showed slightly higher activity against *K. pneumoniae* with an inhibition zone of 9 mm, compared to 7 mm for the aqueous extract, both surpassing the negative control of 6 mm. The strongest activity among all strains was observed against *B. anthracoides*, with the crude chloroform extract inhibiting growth by 13 mm and the ethanolic extract by 11 mm. Benzene was used as a solvent to extract non-polar compounds from the plant material. The crude benzene extract of aerial parts exhibited antimicrobial activity against *B. anthracoides* and *C. albicans*. Benzene extracts of *Combretum malabaricum* (Bedd.) Sujana, Ratheesh & Anil Kumar (syn. *Quisqualis indica* Blanco) have also been reported to show antimicrobial activity against *S. aureus*, *P. aeruginosa*, *K. pneumoniae*, *Bacillus subtilis*, *B. cereus* [Bhangale, Qureshi, 2020]. All extracts, except the crude chloroform extract, showed only minimal activity (7 mm) against *P. aeruginosa*, indicating a weak inhibitory effect. Extracts from aboveground parts, except the aqueous extract, demonstrated antifungal activity against *C. albicans*, with inhibition zones ranging from 8 to 9 mm; the chloroform extract was the most effective at 9 mm. While as a negative control, DMSO is included, further dilution could reduce potential solvent-related effects. Overall, the crude ethanolic root extract exhibited a broader spectrum of antimicrobial activity, affecting a greater range of microorganisms. A similar pattern was observed in our previous research on various *Lactuca* L. species (*L. serriola* L., *L. tatarica* (L.) C.A. Mey, *L. saligna* L.) [Shukurlu et al., 2022; Shukurlu, Muradova, 2022; Shukurlu et al., 2024].

A concentration of 525 µg/disc was used in this study to assess antimicrobial activity. Although this concentration is relatively high, it falls within the range of concentrations (500-2000 µg/disc) previously tested for solvent extracts (i.e. methanol, n-hexane, ethyl acetate, butanol, water) in disc diffusion assays [Khan et al., 2017; Ali et al., 2017].

Previously, it was documented that methanolic extracts of the aerial parts of *A. blitum* subsp. *oleraceus* showed inhibitory effects against *E. coli* and *Salmonella enterica* serovar typhi (*S. typhi* Eberth) [Nehal et al., 2016]. The methanolic extract of the aerial parts of *Amaranthus tricolor* L. (red amaranth) exhibited modest antibacterial activity against *S. typhi* and *Listeria monocytogenes* Murray [Jahan et al., 2022]. As a polar solvent, ethanol can efficiently extract a wide range of both polar and nonpolar bioactive constituents such as lipids, fatty acids, phenolic compounds, and terpenoids, while offering the additional advantage of being safe for pharmaceutical uses [Lee et al., 2024]. Crude extracts of *Amaranthus* plants contain bioactive compounds such as alkaloids (betacyanins and betaxanthins), polyphenols (flavonoids, steroids, catechuic acid, and tannins), terpenoids (cerasinone and norecasantalic acid), and saponins [Hassan et al., 2024]. The methanolic extract of *A. tricolor* L. leaves showed antibacterial activity against *E. coli*, *S. aureus* and *P. aeruginosa*. The ethyl acetate extract demonstrated antibacterial effect against *E. coli*, *S. aureus*, *P. aeruginosa* and *K. pneumoniae*. The chloroform extract exhibited notable activity against *E. coli*, *S. aureus*, *K. pneumoniae*, and inhibited *S. mutans*. The petroleum ether extract showed activity against *E. coli*, *S. aureus*, *K. pneumoniae* and *E. faecalis* [Pulipati et al., 2014]. Phenolic compounds identified in *A. blitum* subsp. *oleraceus* include quercetin, isoquercetin, hyperoside, rutin, kaempferol, myricetin, apigenin, catechin, and naringenin [Sarker et al., 2024]. According to another study, *A. blitum* subsp. *oleraceus* grown in Turkey contained naturally occurring antioxidant compounds. It is one of the most popular leafy vegetables consumed in west Black Sea region

Table 1. The antimicrobial potential of various solvent-derived extracts from the roots / aboveground parts of *A. blitum* subsp. *oleraceus.*

Test microorganisms	Inhibition zone diameters (mm) of plant extracts at 525 µg/disc concentration					Negative controls	
	Crude aqueous extract	Crude ethanolic extract	Crude acetone extract	Crude benzene extract	Crude chloroform extract	Distilled water	DMSO
Klebsiella pneumoniae	8 / 7	7 / –	– / 9	6 / –	– / –	6	6
Bacillus anthracoides	– / –	8 / 11	6 / 10	– / 10	10 / 13	–	–
Staphylococcus aureus	7 / 7	8 / 9	11 / 9	8 / 7	7 / 8	7	7
Escherichia coli	7 / 7	9 / 8	8 / 7	8 / 7	9 / 9	–	7
Pseudomonas aeruginosa	– / 7	7 / 7	– / 7	7 / 7	7 / –	–	–
Candida albicans	– / –	9 / 8	11 / 8	9 / 8	10 / 9	–	–
MRSA	– / –	7 / 8	10 / 7	8 / 8	– / 8	6	7

Note: Values represent the diameter of inhibition zones (mm). Overall, one fungus and six bacterial strains have been tested.

of Turkey, locally known as "dari mancari" [Ozsoy et al., 2009]. The dichloromethane and methanol extracts of *A. blitum* subsp. *oleraceus* demonstrated radical-scavenging activity [Amornrit, Santiyanont, 2016]. The water extract showed promising α-glucosidase and α-amylase inhibitory activity [Hasbal Çelikok et al., 2022]. Phytochemical analysis revealed the presence of phytol and β-sitosterol, gallic acid, caffeic acid, quercetin, vanillin, and kaempferol in the leaf extract. The extract was also found to reduce the number of dividing cells and exhibited an anti-proliferative effect [Durhan et al., 2022].

Limitations. The present study provides preliminary screening data using the disk diffusion method, which has limited sensitivity for crude plant extracts, particularly against yeast such as *Candida albicans*. The relatively high extract concentration tested (525 µg/disc) and the weak inhibition zones close to the disk diameter should be interpreted with caution. Additionally, the use of 100% DMSO as a solvent resulted in slight antimicrobial activity, which may have partially influenced the results for some microorganisms. Positive controls and quantitative assays such as minimum inhibitory concentration (MIC) determination was not included and should be considered in future studies to better assess antimicrobial potency and active compound profiles. Moreover, as replicates and statistical analyses were not included, the inhibition zone data are presented as a qualitative result.

CONCLUSION

The findings suggest that *A. blitum* subsp. *oleraceus* may contain bioactive compounds with potential therapeutic value, particularly against antibiotic-resistant microorganisms. Further studies are necessary to isolate these compounds and optimize extraction methods to maximize their efficacy.

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Göy pəncərin (*Amaranthus blitum* subsp. *oleraceus* L. Costea) müxtəlif hissələrindən əldə edilmiş xam ekstraktların antimikrob fəallığının dəyərləndirilməsi

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Antibiotiklərə qarşı rezistentliyin artması və yeni patogen ştammların meydana çıxması, potensial mikrobəleyhinə vasitələrin əlçatan və iqtisadi cəhətdən səmərəli mənbəyi kimi dərman bitkilərinin tədqiq edilməsinin vacibliyini vurğulayır. *Amaranthus* növləri ənənəvi təbabətdə müxtəlif xəstəliklərin müalicəsində istifadə olunmuşdur. Bu tədqiqatın məqsədi *Amaranthus blitum* subsp. *oleraceus* növünün yerüstü hissələrinin və köklərinin müxtəlif ekstraktlarının *in vitro* mikrobəleyhinə təsirini qiymətləndirməkdir. Ekstraktların Qram-müsbət və Qram-mənfi bakteriyalara, həmçinin *Candida albicans* da daxil olmaqla müxtəlif mikroorqanizmlərə təsiri disk diffuziya metodu ilə qiymətləndirilmişdir. Xam etanol, aseton, benzol və xloroform ekstraktları 100% DMSO-da, sulu ekstraktlar isə distillə olunmuş suda həll edilərək işçi məhlulları hazırlanaraq sonrakı analizlər üçün 75 mq/ml konsentrasiyaya çatdırılmışdır. Nəticələrə əsasən, yerüstü hissələrdən və köklərdən etanol, aseton və xlorofromla əldə edilən ekstraktlar, xüsusilə *Bacillus anthracoides* əleyhinə antibakterial fəallıq sərgiləmişdir, həmçinin hər iki hissələrdən əldə edilən etanol, aseton, benzol və xloroform ekstraktları *Candida albicans* əleyhinə antifunqal təsir nümayiş etdirmişdir. Sınaqdan keçirilən bütün sulu ekstraktlar çox zəif antibakterial fəallıq göstərmişdir və *Candida albicans* əleyhinə heç bir antifunqal təsir müşahidə edilməmişdir. *Amaranthus blitum* subsp. *oleraceus* əsrlər boyu dünyanın müxtəlif regionlarında tərəvəz bitkisi kimi becərilmişdir. Xam ekstraktlarda müşahidə olunan antibakterial fəallıq *Amaranthus blitum* subsp. *oleraceus* bitkisində bioloji fəal komponentlərin mövcudluğunu göstərir və əlavə fitokimyəvi tədqiqatların aparılaraq, həm farmakoloji, həm də aqrar sahələrdə tətbiqlərin həyata keçirilməsini məqsədəuyğun edir.

Açar sözlər: *antibakterial fəallıq, antifunqal fəallıq, disk-diffuziya, inhibisiya zonası, mikroorqanizmlər, bitki ekstraktları*

Оценка антимикробной активности сырых экстрактов различных частей щирица синеватая (*Amaranthus blitum* subsp. *oleraceus* L. Costea)

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Рост распространенности устойчивости к антибиотикам и появление новых патогенных штаммов подчеркивают необходимость изучения лекарственных растений как доступных и экономически эффективных источников потенциальных антимикробных веществ. Виды рода *Amaranthus* использовались в традиционной медицине для лечения различных заболеваний. Целью данного исследования была оценка *in vitro* антимикробных действий различных экстрактов надземной и подземной части *Amaranthus blitum* subsp. *oleraceus*. Экстрак-

ты анализированы на разнообразные микробные популяции, включая грамположительные и грамотрицательные бактерии, а также *Candida albicans*, с использованием метода диско-диффузии. Сырые этанольные, ацетоновые, бензольные и хлороформные экстракты растворялись в 100% DMSO, а водные экстракты – в дистиллированной воде для приготовления рабочих растворов с концентрацией 75 мг/мл для последующего анализа. Результаты показали, что этанольные, ацетоновые и хлороформные экстракты как надземной, так и подземной частей проявляли антибактериальную активность, особенно против *Bacillus anthracoides*, тогда как этанольные, ацетоновые, бензольные и хлороформные экстракты обеих частей показали противогрибковую активность против *Candida albicans*. Все исследованные водные экстракты проявили очень слабую антибактериальную активность и не оказывали противогрибкового эффекта против *Candida albicans*. *Amaranthus blitum* subsp. *oleraceus* которые культивируются как овощное растение на протяжении веков в различных регионах мира. Наблюдаемая антибактериальная активность сырых экстрактов свидетельствует о наличии биологически активных компонентов в *Amaranthus blitum* subsp. *oleraceus*, что обосновывает дальнейшие фитохимические исследования и применение этих экстрактов как в фармакологии, так и в сельском хозяйстве.

Ключевые слова: *антибактериальная активность, противогрибковая активность, диско-диффузионный метод, зона ингибирования, микроорганизмы, растительные экстракты*