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RESEARCH ARTICLE

Antibacterial and antioxidant activities of *Alcea kurdica* flower, leaf and root aqueous and organic extracts

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Abstract

Global concerns are rising due to complications associated with the use of chemical agents and antibiotic resistance. Consequently, research focus has shifted towards the quest for effective agents of biological origin. The aim of the present study was to assess the antioxidant and antimicrobial potentials of aqueous and organic extracts derived from various parts of *Alcea kurdica*. Different parts of *A. kurdica* were obtained and prepared into leaf, flower and root powders. The powders were extracted with aqueous and organic solvents. The antimicrobial activity of these extracts was assessed against bacterial pathogens using the agar well-diffusion assay. Addi -tionally, the antioxidant effects of the extracts were evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) and resazurin dye scavenging assays. The results showed dose-dependent antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* for both the organic and aqueous leaf and floral extracts. Furthermore, an antioxidant effect (>80%) was also observed for the organic and water extracts of the flowers, leaves and roots of the plant at the highest concentration (500 µg/mL), as compared to ascorbic acid, which served as the positive control using both the DPPH and resazurin methods. The findings of this study highlighted that *A. kurdica* can be considered a rich source of potential antioxidant and antibacterial agents, warranting future investigation to identify its active ingredients.

Keywords

Alcea kurdica; DPPH; *Escherichia coli* ; resazurin; *Staphylococcus aureus*

Introduction

In recent years, there has been a growing emphasis on the search for effective natural drugs, driven by the widespread complications associated with the use of chemical drugs and their limited efficacy in treating certain diseases (1). This quest for natural remedies is a global phenomenon, with extensive research efforts underway worldwide.

In particular, third-world countries have a wealth of traditional medicinal knowledge that remains largely unexplored. The concern of losing these traditional knowledge systems is mounting, given the generation gap and the need for comprehensive research in this area (2). Additionally, the availability of herbs and their utilization have been affected by environmental degradation caused by human activities and the impact of global warming. This hypothetical situation is applicable to the Kurdistan region, which has witnessed tragic events over the past few decades, result-

ing in the destruction of nearly 4000 communities. In Kurdistan, ethnobotanical studies have been scarce and the society is undergoing rapid transformation, diminishing the importance of bio-cultural diversity and traditional herbal medicine (3). Understanding the use of medicinal plants and their role in local healthcare is essential for preserving traditional cultures and advancing modern drug development.

In fact, numerous studies have been conducted, leading to the development of novel drugs derived from medicinal plants. This ethnomedicinal practice helps elucidate the intricate relationship between nature and indigenous societies. Indigenous communities and their ethnomedical knowledge remain invaluable sources for reclaiming expertise in various fields, particularly medicine (4).

Alcea kurdica (Althaea) was initially discovered in Iraq in August 1841. The specimen was obtained from the Gara Mountain and has since been preserved in the herbarium of the Royal Botanic Gardens, Kew (5). Evidence suggests that *A. kurdica* was utilized for medicinal purposes by the extinct Neanderthal race, who are thought to have lived in Iraq some 60000 years ago (6). Studies have shown that the leaf and root of *A. kurdica* are useful in treating asthma, kidney pain and kidney stones (7). Furthermore, mucilage extracted from its floral parts has found extensive use as an anti-inflammatory for sore, oral and pharyngeal mucosa. Beyond its role as a sedative and diuretic, this plant has also been employed to address a wide array of ailments affecting the skin, respiratory, urinary and gastrointestinal systems (8). Due to the presence of flavonoids and other phenolic compounds, species of *Alcea* have also been shown to possess antibacterial, antioxidant and cytotoxic potentials (9). Based on these findings, researchers are currently interested in this plant to investigate its potential biological activities. Therefore, this study was aimed at investigating the antioxidant and antimicrobial potentials of the organic and water extracts from different parts of *A. kurdica*.

Materials and Methods

Collection of Plant Materials: *Alcea kurdica* was collected in May 2021 from natural fields in Duhok. The voucher specimen was deposited in the herbarium of the Department of Pharmacognosy, College of Pharmacy, Duhok University. The entire plant was cleaned, washed, cut and then shade-dried at room temperature in a well-ventilated room for 2 weeks. The dried leaves, flowers and roots were cut into distinct pieces and ground into powders using a mortar and pestle. These powders were then put into a tightly closed container in preparation for the extraction procedure.

Preparation of Extracts: The Soxhlet extraction method was used to extract the phytochemicals. Each of the dried plant parts (flowers, leaves and roots) was individually subjected to this process. Specifically, 50 g of dried plant powder was placed in a glass thimble and extracted using 500 mL of 70% ethanol over a 9h period. The resulting extract was collected, and its volume was reduced using a rotary evaporator. Subsequently, the extract was subjected to complete drying in an oven. The resulting dried extract was then dissolved in 250 mL. of ethyl acetate and mixed with an equal volume of water through 3 repetitions. The fractionated extracts were condensed, dried and appropriately labeled, including LEA (organic leaf fraction), LW (aqueous leaf fraction), FEA (organic flower fraction), FW (aqueous flower fraction), REA (organic root fraction) and RW (aqueous root fraction). Antibacterial Activity: The antibacterial activity of the test extracts (LEA, LW, FEA and FW) was assessed against 2 bacterial strains: *S. aureus* (Gram-positive bacteria) and *E. coli* (Gram-negative bacteria) using the agar well diffusion assay (10, 11). Briefly, plates of Muller–Hinton (MH) agar (Sigma) were aseptically prepared. Using sterile tips, 6 mm-diameter wells were punched on the agar plates after the organisms had been cultured. An aliquot of 200 µl of each test extract (LEA, LW, FEA or FW) at various concentrations was added to the wells and the culture was then incubated aerobically at 37 °C for 18h. Plates were cultured in triplicate. At the end of the incubation period, plates were checked for a lack of growth zone around each well and the diameter of the observed inhibition zone was measured (12-14).

DPPH Scavenging Assay: To assess the antioxidant activity, the scavenging capacity of the prepared extracts was assessed using a stable DPPH (2,2-diphenyl-1-picrylhydrazyl) technique (Sigma-Aldrich). When an electron is spared, DPPH has a dependable free radical associated with it. The test extract was combined with DPPH at a volume of 500 µl of each and the volume was completed to 2mL using absolute ethanol. The absorbance of each compound was measured at 517 nm (15, 16).

Resazurin Dye Scavenging Assay: Resazurin dye (Himedia, India) was used to test the extracts' ability to scavenge free radicals. Several concentrations of the extracts were prepared and combined with resazurin dye in the same volume. The absorbance at 600 nm of the mixtures was measured using distilled water as a blank solution (17).

Statistical Analysis: The data were statistically analyzed using the GraphPad Prism program (18). The results are presented as the mean±standard deviation of 3 experiments. A statistically significant difference was set at $p < 0.05$ (19, 20).

Results

Antibacterial activity

The antibacterial potential of the crude extracts from flowers and leaves (LW, LEA, FW and FEA) was assessed using the agar well diffusion method. The four test extracts exhibited a concentration-dependent ability to inhibit the growth of the reference strains of *S. aureus* and *E. coli*. However, there were no statistical differences in the zone of inhibition (measured in mm) among the different concentrations of the organic and water extracts (Fig. 1-4). At the highest test concentration (500 µg/mL), aqueous

leaf extract (LW) demonstrated a stronger ability to inhibit the growth of *E. coli* compared to *S. aureus* (Fig. 1). A similar trend was observed in the aqueous flower extract, as illustrated in Fig. 2. In contrast, at a concentration of 500 µg/mL, the floral organic extract showed the greatest effectiveness against *S. aureus* (Fig. 3). The maximum effect was observed for the organic leaf (LEA) extract against *E. coli* at 500 µg/mL (Fig. 4).

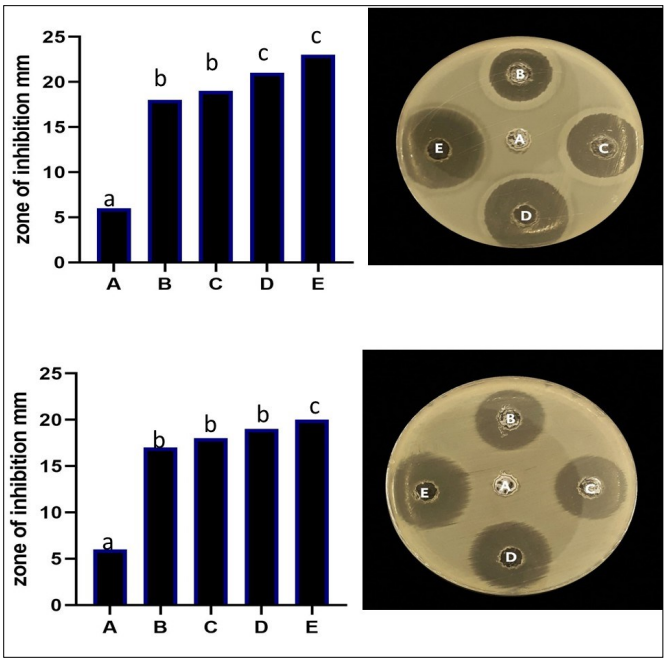
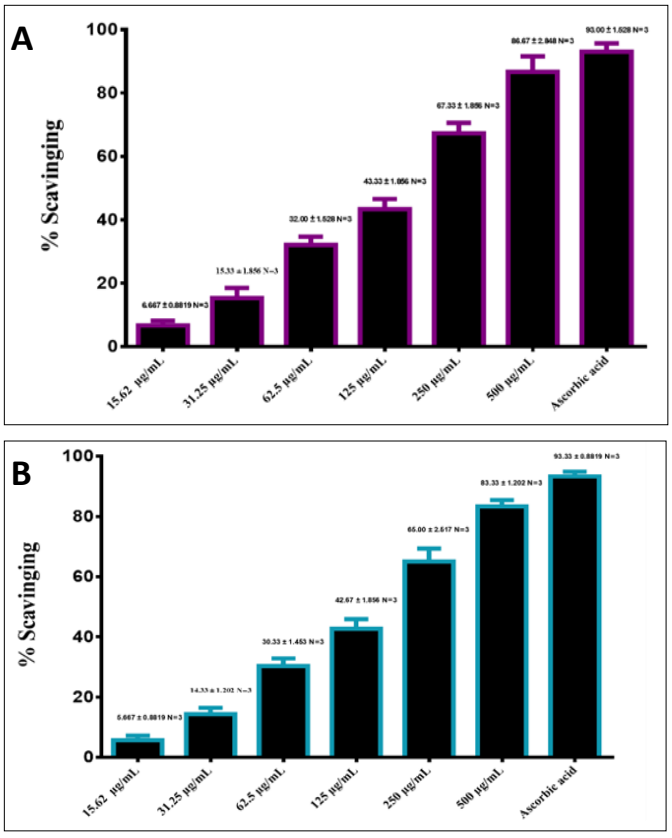


Fig. 1. Antibacterial activity of the aqueous leaf fraction (**LW)** against the test pathogens. **Right**: Representative figure of the zone of inhibition; **A**: Control; **B**: 62.5 µg/mL; **C**: 125 µg/mL; **D**: 250 µg/mL; **E**: 500 µg/mL; **Left**: Averaged zone of inhibition measured in mm; I: Activity against *E. coli*; II: Activity against *S. aureus*. Histogram bar with the small same letters indicate a non-significant (p<0.05) difference, while small different letters indicate a significant difference (p<0.05).



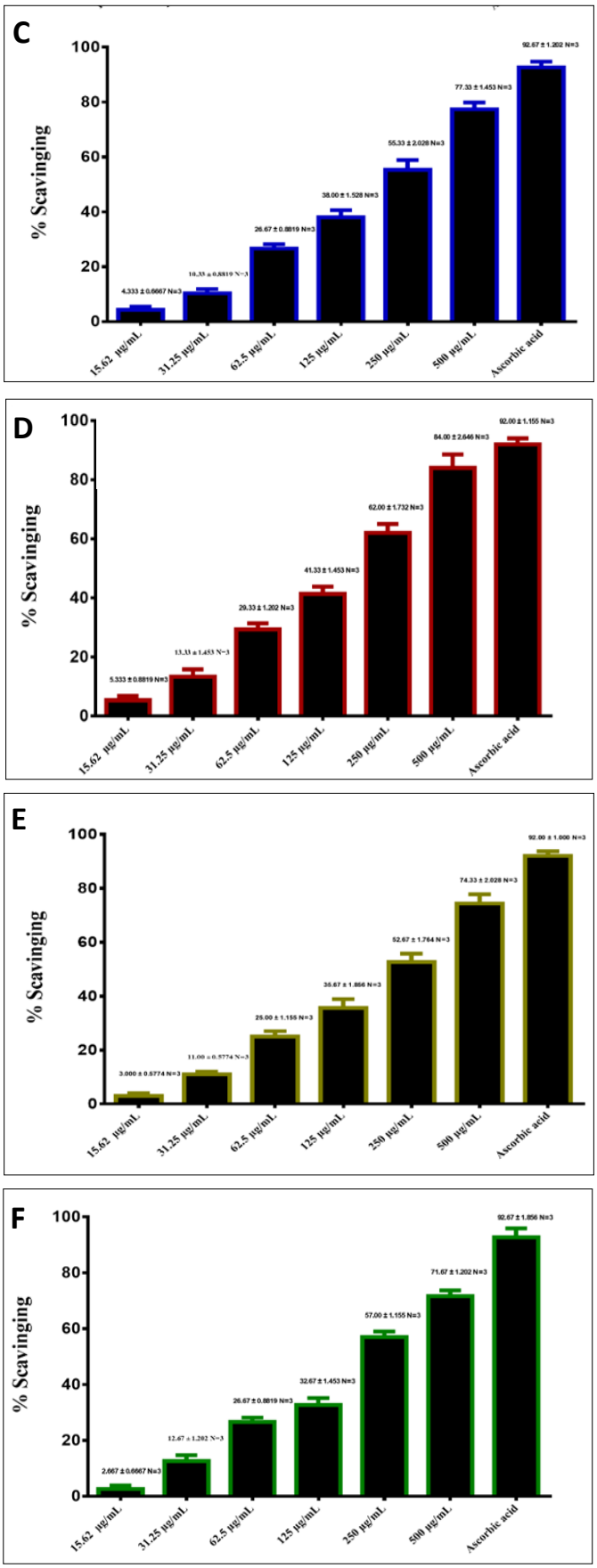


Fig. 2. Antibacterial activity of the aqueous flower fraction (**FW)** against the test pathogens. Right: Representative figure of the zone of inhibition; **A**: Control; **B**: 62.5 µg/mL; **C**: 125 µg/mL; **D**: 250 µg/mL; **E**: 500 µg/mL; **Left**: Averaged zone of inhibition measured in mm; **I**: Activity against *E. coli*; **II**: Activity against *S. aureus*. Histogram bar with the same letters indicate a non-significant (p<0.05) difference, while different letter indicate a significant difference (p<0.05).

Antioxidant activity

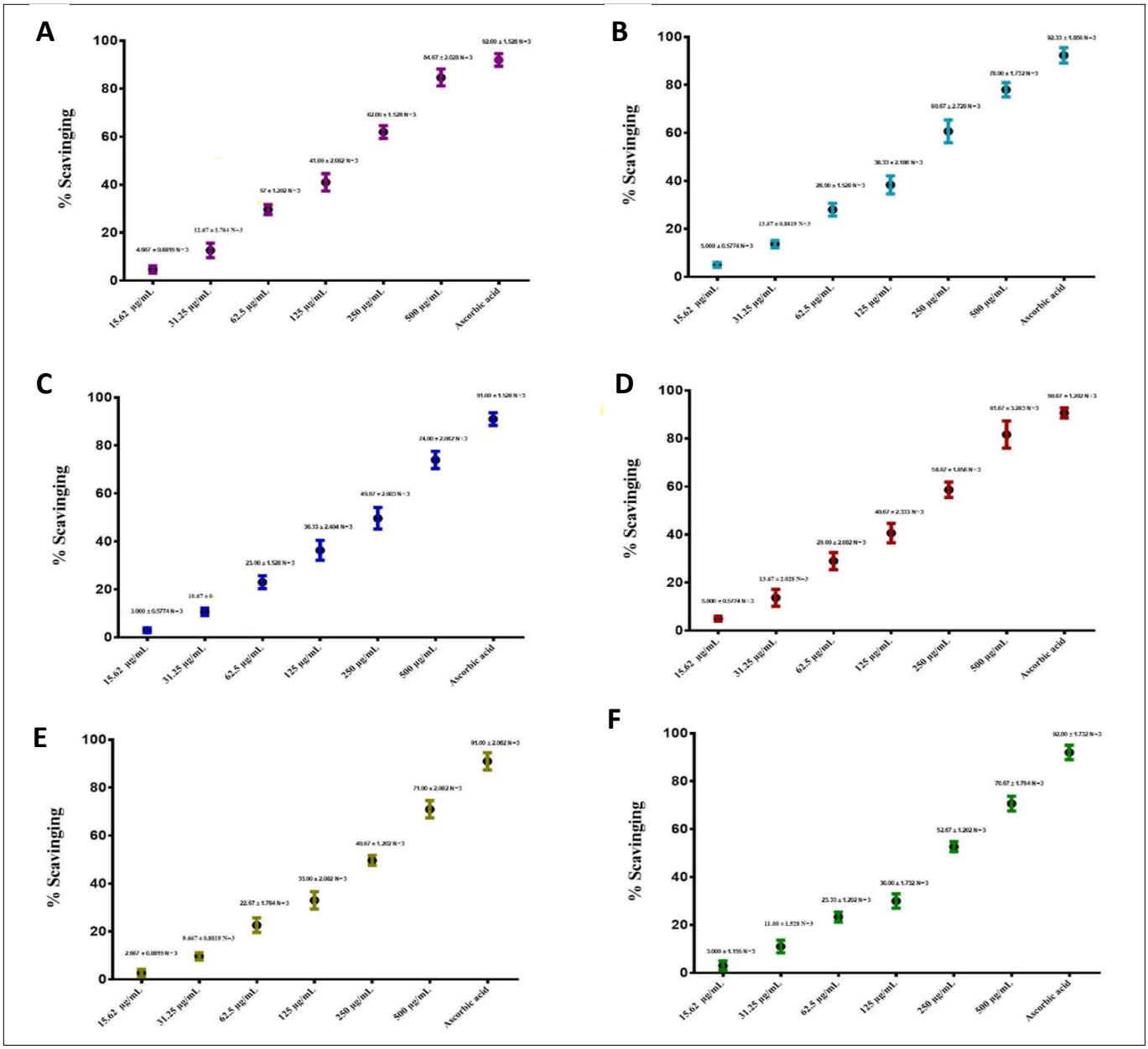


Fig. 3. Antibacterial activity of organic flower fraction (**FEA)** against the test pathogens. **Right**: Representative figure of the zone of inhibition; **A**: Control; **B**: 62.5 µg/mL; **C**: 125 µg/mL; **D**: 250 µg/mL; **E**: 500 µg/mL; **Left**: Averaged zone of inhibition measured in mm; **I**: Activity against *E. coli;* **II**: Activity against *S. aureus*. Histogram bar with the same letters indicate a non-significant ($p<0.05$) difference, while different letter indicate a significant difference ($p<0.05$).

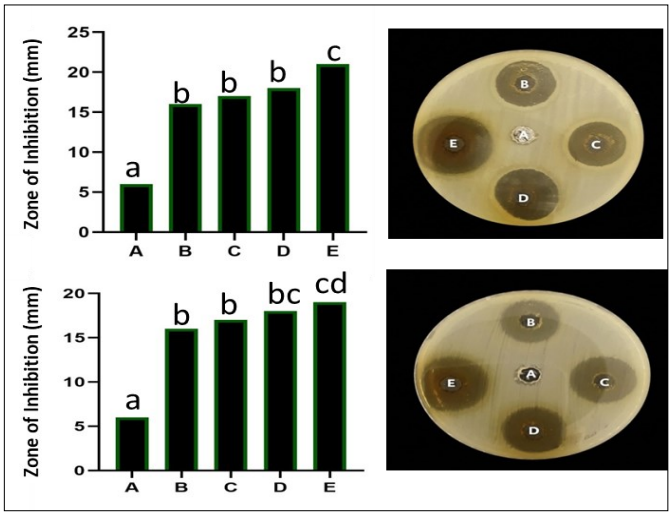


Fig. 4. Antibacterial activity of organic leaf fraction (**LEA)** against the test pathogens. **Right**: Representative figure of the zone of inhibition; **A**; Control; **B**: 62.5 µg/mL; **C**: 125 µg/mL; **D**: 250 µg/mL; **E**: 500 µg/mL; **Left**: Averaged zone of inhibition measured in mm; **I**: Activity against *E. coli*; **II**: Activity against *S. aureus*. Histogram bar with the small same letters indicate a non-significant ($p<0.05$) difference, while small different letters indicate a significant difference ($p<0.05$).

DPPH Scavenging Assay: The DPPH assay was used to evaluate the antioxidant capacity of each extract and the results are expressed as a % of inhibition compared to ascorbic acid, used as the positive control. The overall trend was an increase in the % of antioxidant activity with rising concentrations of the extracts, as presented in Fig. 5A-F. As shown in Fig. 5A, the antioxidant activity of the organic flower extract (FEA) is highest (> 80%) at the maximum test concentration (500 µg/mL). Compared to other extracts, floral aqueous extract (FW) and leaves organic extract (LEA) showed a higher proportion of antioxidant activity (>80%) (Fig. 5B and D). A similar observation of a concentration-dependent increase in antioxidant activity was made when antioxidant activity was also determined with the resazurin assay (Fig. 6A-F).

Discussion

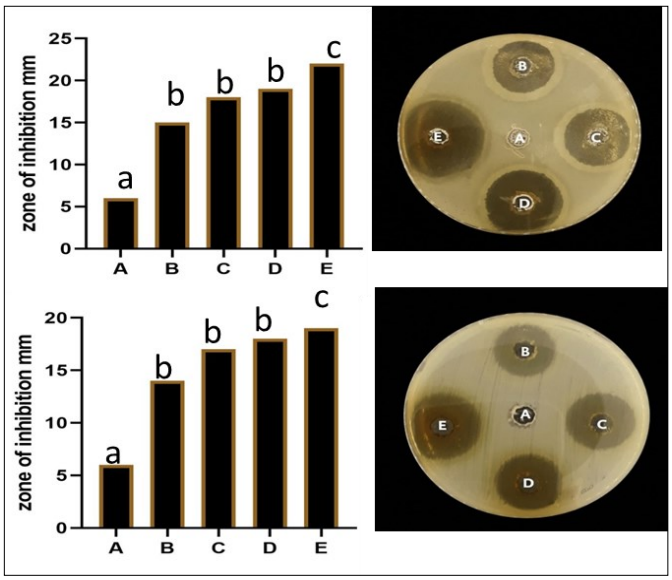


Fig. 5. Antioxidant activity of the extracts using DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. The results are represented as the mean±SD of three independent replicates. **A**: FEA; **B**: FW; **C**: LW; **D**: LEA; **E**: REA; **F**: RW. Histogram bar with the small same letters indicate a non-significant ($p<0.05$) difference, while small different letters indicate a significant difference ($p<0.05$).

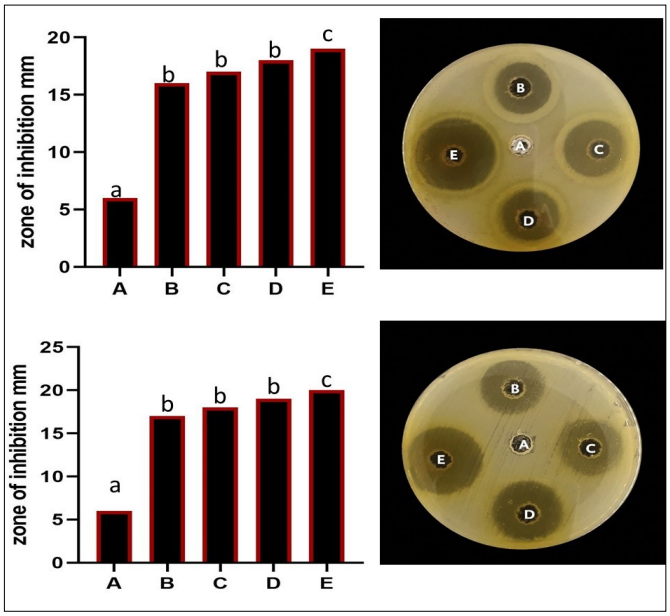


Fig. 6. Antioxidant activity of the extracts using resazurin assay. The results are represented as the mean±SD of three independent replicates. **A**: FEA; **B**: FW; **C**: LW; **D**: LEA; **E**: REA; **F**: RW.Histogram bar with the small same letters indicate a non-significant ($p<0.05$) difference, while small different letters indicate a significant difference ($p<0.05$).

In the present study, *A. kurdica* effectively inhibited the growth of reference strains, with *S. aureus* representing Gram-positive bacteria and *E. coli* representing Gram-negative bacteria. These findings suggest that *A. kurdica* can be employed as a potential source of effe-ctive antimicrobial agents. These findings are consistent with several prior studies (21-23). For instance, hydro-alcoholic extracts of *A. rosea* demonstrated antimicrobial activity against test-resistant strains of *Klebsiella pneumoniae* and *Streptococcus pneumoniae* using the disk diffusion and broth microdilution methods (21). In another study, ethyl acetate extract of *A. rosea* (100 mg/mL) was shown to inhibit *E. coli* (zone of inhibition = 28±1.56 mm) and *S. aureus* (25±01.58 mm), followed by *K. pneumoniae* and *Proteus vulgaris* with inhibition zones of 18±0.74 mm and 13±0.12 mm respectively (22). Similarly, antibacterial activity was reported for flower and leaf aqueous extracts of *A. arebelensis* against resistant *S. aureus* isolates (23). *Alcea digitata* also studied and showed antimicrobial activity against strains of Gram-positive and Gram-negative bacterial isolates (24).

Phenolic content has been shown to mediate antimicrobial activity in addition to the protective antioxidant effect. Contrary to the findings in this study, *Acer heldreichii* and *A. apterocarpa* did not exhibit any inhibi-tory effects on the growth of test strains (25, 26). The alcoholic leaf extract of *A. arebelensis* did not show an antimicrobial effect, while its aqueous extract was able to inhibit the growth of resistant *S. aureus* isolates (27). Furthermore, the antimicrobial activity of aqueous leaf extracts from *A. kurdica* was found to be modest against various microbial isolates such as *E. coli*, *Pseudomonas aeruginosa*, *Candida albicans*, *Cladosporium macrocarpum* and *Fusarium oxysporium*. However, no antimicrobial effect was reported against *S. aureus*, *Bacillus subtilis*, *Enterobacter aerogenes*, *Salmonella enterica* or *Fusarium solani* (28).

The radical scavenging potential of the 6 test extracts in the current study might be attributed to their phenolic contents. This assumption has been drawn from earlier research by Gorinstein and coworkers (29, 30). Moreover, the total phenolic contents of *Alcea* species have been linked to their antioxidant activity (31, 32). For instance, flower, leaf and seed extracts of *A. hyrcana*, *A. fasciculiflora* and *A. rosea* have demonstrated high antioxidant activity (30, 33, 34). It's noteworthy that dihydrokaempferol and kaempferol-3-O-(6″-(E-coumaroyl))-β-d glucopyranoside isolated from *A. rosea* exhibit significant antioxidant activity (35). In addition, extracts from *A. setosa* revealed moderate antioxidant activity with a DPPH scavenging assay (36). The flower extract of *A. pallida* also demonstrated antioxidant activity that was correlated with its phenolic content (37). However, there can be variations in antioxidant activity attributed to different factors. For instance, when compared to 95 other plant extracts, the aqueous extract of *A. acaulis* and *A. apterocarpa* exhibited relatively lower antioxidant activity, likely due to their lower phenolic content (38). Furthermore, it's worth noting that the choice of solvent can influence antioxidant activity, with chloroform and ethanol-water (1:1 v/v ratio) extracts often exhibiting stronger DPPH radical scavenging capacities compared to other extracts of the same plant component (39).

The information regarding the compounds found in *Alcea kurdica* is limited and only a few attempts have been made to identify its ingredients. In a study conducted in 2022, various compounds were detected in the root extract, including gallic acid, syringic acid, chlorogenic acid, quercetin, 1-undecyne, 3-cyclohepten-1-one, 2,3-dihydro-3,5-dihydroxy-6-methyl 4H-pyran-4-one, methyl nonanoate, nonanoic acid, pyrogallol, 2-vinyl-9-[beta-D-ribofuranosyl] hypoxanthine, hexadecanoic acid, 1-methyl -2-methylenecyclohexane, 9,12-Octadecadienoic acid, cis-bicyclo[3.3.0]oct-2-ene, methyl ester 1,3,5,2,4-trithia (3-Siv) diazepine-6-carboxylic acid (40), sterols including

Beta-Sitosterol, Campesterol and Stigmasterol (41). In contrast, leaf extracts contained compounds like gallic, chlorogenic and caffeic acids (42).

Flavonoids including apigenin, resveratrol, rutin, silybin and sterols including β-sitosterol, stigmasterol were identified in both flowers and leaves (43). Antioxidants activity had been reported in different *Alcea* species such as *A. hyrcana* (33), *A. rosea* (22), *A. setosa* (34).*A. fasciculiflora* (38), *A. dissecta* (37), *A. pallida* (44).

On the other hand, antibacterial activity has been reported in different *Alcea* species, such as *A. rosea* (35), *A. arebelensis* (23) and *A. digitata* (24).

Conclusion

Plants have been and will continue to be regarded as valuable sources of natural compounds with beneficial effects on the human body. The findings of this study reveal that 6 extracts from the various parts of *A. kurdica* exhibit both antioxidant and antibacterial activities. Therefore, it is strongly recommended that further research, especially involving animal studies, be conducted to investigate the specific compounds responsible for these observed effects as well as explore other potential health benefits that may be derived from this plant.

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Authors' contributions

DHA, SRA: study conception and design; DHA, SRA: data collection: SRA, EJK: analysis and interpretation of results; DHA, EJK: draft manuscript preparation, All authors reviewed the results and approved the final version of the manuscript.

Compliance with ethical standards

Conflict of interest: **:** Authors do not have any conflict of interests to declare.

Ethical issues: None

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