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Investigation of Phytochemical and Antioxidant Content of *Arctium lappa* Extract and its Antimicrobial Properties

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ABSTRACT

Objective: Plant secondary metabolites like phenols and flavonoids neutralize free radicals and are linked to antioxidant and cytotoxic activities. Due to rising drug resistance in pathogens such as *Salmonella paratyphi*, *Bacillus cereus*, *Escherichia coli*, and *Staphylococcus aureus*, medicinal herbs are increasingly explored for antimicrobial properties.

Method: This study evaluated the total phenolic content (TPC), total flavonoid content (TFC), antioxidant capacity, and antibacterial effects of *Arctium lappa*. TPC, TFC, and antioxidant levels were measured using Folin-Ciocalteu, Aluminum chloride, FRAP, and DPPH assays, respectively. Antibacterial activities were assessed by microdilution broth and disc diffusion methods to determine MIC50 and MBC values.

Results: Results showed that *A. lappa* ethanolic extract, prepared with diluted water and the boiling method, had the highest flavonoid content (7.9 ± 0.40 mg/g DW). The leaves and flowers extract, using methanol and the boiling method, had the highest phenolic content (62.13 ± 0.73 mg/g DW) and antioxidant capacity. The ethanolic extract of *A. lappa* leaves also demonstrated the strongest antibacterial activity, with an MBC of 0.140 mg/mL and an 8 ± 0.4 mm inhibition zone against all tested bacteria.

Conclusions: These findings suggest *A. lappa* bioactive compounds, particularly phenolics and flavonoids, offer promising potential for treating bacterial infections in pharmacognostic applications.

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Introduction

Using Herbal Medicines to treat diseases goes back hundreds of years in Iran, and there are Adequate scientific documents in this era. Medicinal plants have considerable Phytochemical, Antioxidant Content, and antimicrobial activities that can inhibit the growth of infectious microorganisms and degeneration and mutagenic factors. High interest in replacing chemical materials with natural ones has led to thousands of studies on natural resources. Experiments on different plant extracts resulted in the discovery of suitable natural substances for the treatment of various diseases (1,2).

The therapeutic properties of Herbal extracts and essential oils against microbial and non-microbial diseases have always been known. Many positive effects have been shown from different plant species against different microorganisms and Mutagenic factors. In recent studies, the antimicrobial properties of herbal products have attracted many researchers because of increased antibiotic resistance to microorganisms (2,3).
The choice of solvent is pivotal in the extraction of bioactive compounds from plants, directly impacting both the yield and quality of phytochemicals. Solvents with varying polarities selectively dissolve specific bioactive substances, influencing extraction efficiency and maximizing therapeutic potential (4). Polar solvents, such as methanol and ethanol, are highly effective in extracting hydrophilic compounds, including phenolic acids and flavonoids, which are well-known for their antioxidant properties (5). In contrast, non-polar solvents like isopropyl alcohol are better suited for isolating lipophilic compounds like triterpenoids (4). Increasing attention is also being given to natural deep eutectic solvents (NADES) due to their ability to enhance extraction efficiency in an environmentally friendly manner (6). Different solvents affect extraction efficiency and yield varying profiles of bioactive compounds from the same plant material, underscoring the need for precise solvent selection to achieve desired compound profiles. Advanced techniques like supercritical fluid and microwave-assisted extraction have been developed to further optimize extraction while minimizing solvent use, aligning with the growing demand for green and sustainable methodologies (6,7).
The species of the *Arctium* genus, also known as ‘burdock,’ comprise biennial herbs occurring in waste places, streams, and roadsides in temperate regions of Europe and Asia and in subtropical and tropical regions in North and South America; the genus is considered naturalized (8). The name of the genus comes from the Greek term ‘*arcteion*’, which means ‘bear,’ referring to the plant's natural characteristics characterized by hairiness. This genus includes eighteen recognized species, among which five are considered hybrid species due to the frequent outbreeding occurring between its allogamous representatives (9).
Arctium lappa has a long line of usage in traditional medicine due to the phytochemical, anti-inflammatory, bioactive, and Antioxidant properties of its primary and secondary metabolites (10). *A. lappa* has anti-bacterial, antifungal, diuretic, anti-oxidant, anxiolytic, anti-platelet aggregating, and HIV-inhibitory properties. Bacterial resistance to antibiotics is increasing and requires the introduction of groundbreaking and safe antibacterial substances (11).
The present investigation was undertaken to evaluate the phytochemical, antioxidant content, and antibacterial activity of Aquatic, Acetonic, and hydro-alcoholic extracts of *A. lappa* aerial parts on *Salmonella Paratyphi*, *Bacillus cereus*, *Escherichia coli*, and *Staphylococcus aureus* in laboratory conditions. Aquatic extract was prepared for its traditional and safe use in herbal medicine, acetonic extract for its efficiency in extracting less-polar phytochemicals, and hydro-

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alcoholic extract for its broad polarity that recovers both polar and moderately nonpolar bioactive compounds.

1. Materials and Methods

2.1. Chemicals and Reagents

Methanol, Ethanol, Folin-Ciocalteu reagent, Sodium carbonate, Gallic acid, Aluminum chloride, Potassium acetate, Quercetin, Mueller–Hinton broth, Mueller–Hinton agar, 2,4,6-tripyridyl-s-triazine (TPTZ), FeCl3, DPPH reagent, and other chemicals were purchased from Merck (12, 13).

2.2. Plant Material

Arctium lappa were provided from the Botanical Garden, Tehran, Iran, and were identified at Alzahra University herbarium. In this study, we used the aerial parts of *Arctium lappa.*

2.3. Sample Preparation for Total Phenolic and Flavonoid Assay

5 g of dried samples was added to each solvent, including 100 ml solvent such as 100 ml of 80% aqueous methanol, 100 mL of 80% aqueous ethanol, 100 mL of aceton, and 100 mL of distilled water. The maceration method was used for extraction. Samples were heated in a water bath for 60 min at a temperature of 70°C, then the extracts were centrifuged for 20 min at 2000 rpm (12, 13).

2.4. Determination of the Total Phenolic Compounds

Phenolic contents of samples were determined by the Folin–Ciocalteu method. 200 μL of the extract samples were added to 1 mL of 1:10 diluted Folin–Ciocalteu reagent. Then, after 4 minutes, 800 μL of saturated sodium carbonate (75g/L) was added. The absorbance at 765 nm was measured after 2 h of incubation at room temperature. The results were expressed as gallic acid equivalent (GAE) µg/g dry weight of crude extract (12, 13).

2.5. Determination of the Total Flavonoid Compounds

Extracts of samples (0.5 mL) were mixed with 0.1 mL of 10% aluminum chloride, 1.5 mL of 95% ethanol, 2.8 mL of distilled water, and 0.1 mL of 1 M potassium acetate. after incubation at room temperature for 30 min, the absorbance was measured at 415 nm with a spectrophotometer. The stock solution of quercetin was used to make a serial dilution of concentrations (10 – 100 μg/mL) in methanol to make the calibration curve (12, 13).

2.6 Antioxidant Activity Determined via the DPPH Assay

Assay employed for determination of the extract's ability to scavenge the DPPH radical was based on a protocol employed by Hatzidimitriou et al. (14). Briefly, 2.9 mL of a DPPH solution (0.1 mM in MeOH) was mixed with 0.1 mL of a methanolic extract (aliquot tested contained 15, 25, 35, or 50µg dry MOL decoction). Moreover, % DPPH inhibition was also estimated on the same dry extract weight basis (aliquot tested contained 35µg of dry extract) and on the same TP content basis (aliquot tested contained 5µg TP expressed as CAF) of herbal preparations. Standard solutions of CAF at concentrations of 20–160 mg/L were also tested. The absorption at 516 nm (A516) was recorded at the start of the reaction (t = 0) and after 30 min (t = 30). The results were expressed as % inhibition = [(A516 (t = 0) − A516 (t = 30)) × 100 / A516 (t = 0)], as well as CAF equivalents. All determinations were performed in triplicate at room

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temperature, and data are given as the mean ± standard deviation. IC_{50} value, the concentration able to scavenge 50% of the DPPH radical, was also determined (13, 14).

2.7 Antioxidant Activity Determined via the Ferric Reducing Ability (FRAP) Assay

The FRAP assay was carried out according to Benzie and Strain, with slight modifications, to determine the ferric reducing power capacity of the studied extracts. A mixture containing 3 mL of freshly prepared and prewarmed at 37 °C FRAP reagent and 50 µL of MOL extract (2 mg dry extract/mL MeOH) was incubated at 37 °C for 30 min, and the absorbance was then recorded at 593 nm. The same was applied for CAF solutions (125–1000 mg/L) to construct a standard curve. The ferric reducing ability of the examined extracts was expressed as CAF equivalents. Measurements were carried out in triplicate, and data are given as the mean ± standard deviation. The FRAP reagent contained 2.5 mL of a 10 mM TPTZ solution in 40 mM HCl and 2.5 mL of 20 mM FeCl3 × 6H2O and 25 mL of 0.3 M acetate buffer, pH 3.6 (13,14).

2.8 Determination of the Total Alkaloid Compounds

Mayer's reagent is used to analyze all types of alkaloids, prepared by dissolving 13.5 g of mercuric chloride and 5 g of KI in 1000 ml of distilled water. The HCL1% was added to the solution, and it was heated for 30 minutes. 2 mL of the final reagent was added to 5 mL of plant extract. Then, the absorbance at 470 nm was measured. The stock solution of Atropin, an alkaloid found in Aubergine, was used to make a serial dilution of concentrations (20 – 100 μg/mL) to make the calibration curve (15).

2.9 Determination of the Total Terpenoid Compounds

First 200 µl of herbal extracts were mixed with 2 ml of chloroform. The produced solvent was then vortexed, and then 100 µl of Sulfuric acid was added to the mixer. The final solution was incubated in the dark for 2 hours. Then, the absorbance at 538 nm was measured. The stock solution of Linalool, a terpenoid found in lemon, was used to make a serial dilution of concentrations (25 – 250 *m*g/mL) to make the calibration curve (16).

2.10. Antibacterial Activity

2.10.1. Sample Preparation for Antibacterial Assay

Samples (800 mg) from leaves and flowers were extracted with 80% ethanol (Sigma-Aldrich, USA) and diluted with 5 mL distilled water at 60°C for 3 h. The extracts were centrifuged at 3000 rpm for 15 min using a centrifuge (Eppendorf, Germany) and used to determine antibacterial properties (17).

2.10.2. Preparation of Microorganisms

Microorganism stock cultures were maintained at 4°C in nutrient agar (HiMedia, India). Active cultures were prepared by transferring a loopful of bacteria from the stock cultures to test tubes containing Mueller–Hinton broth (MHB, HiMedia, India) and incubating them at 37°C for 24 h in an incubator (Memmert, Germany). The cultures were diluted with fresh MHB to obtain densities corresponding to 2.0×10^6 colony-forming units (17).

2.10.3. Disc Diffusion Method

The disc diffusion method was used to investigate antimicrobial activity. Twenty milliliters of melted Mueller–Hinton agar (MHA, HiMedia, India) was poured into each sterile Petri dish to prepare the agar plates. After 10 minutes, the plates were solidified. Then, 0.1 mL of microorganism suspension was spread by the spread culture method and dried after 5 min. Forty microliters of extract were added to an 8 mm sterile disc. The disc was placed on the surface of the medium. After 10 min, the compounds were diffused, and the plates were

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incubated at 37°C for 24 h. Ciprofloxacin discs served as the positive control. Inhibition zones were observed at the end of incubation and measured with a ruler in millimeters (18).

2.10.4. Minimum Inhibitory Concentration (MIC_{50}) and Minimum Bactericidal Concentration (MBC)

1 mL of Tryptic Soy Broth was added to nine autoclaved tubes. One milliliter of the extract was added to the first and second tubes of the series; tube 2 was stirred, and 1 mL was transferred to tube 3. This serial dilution was repeated until tube 8. Then, 0.1 mL of the microorganism suspension was added to all tubes except tube 8. All tubes were incubated at 37°C for 24 h. Tubes 8 and 9 served as the positive and negative controls, respectively. The final extract concentrations were 160 mg/mL, 80 mg/mL, 40 mg/mL, 20 mg/mL, 10 mg/mL, 5 mg/mL, and 2.5 mg/mL. After 24 h of incubation, absorbance was measured at 600 nm using a spectrophotometer (Shimadzu, Japan) (17).

2.10.5. Scanning Electron Microscopy (SEM)

Pathogenic bacteria (S. aureus, E. coli) were treated for 24 h with each herbal extract at the respective MIC_{50} values. The bacterial samples were fixed with 2.5% glutaraldehyde in phosphate buffer (0.1 M, pH 7.4; Sigma-Aldrich, USA) for 1 h. Afterwards, each sample was washed 3–5 times at 5–10 min intervals with phosphate buffer, then dehydrated in ethanol (Sigma-Aldrich, USA) for 1 h. Samples were observed using a Scanning Electron Microscope (JEOL, Japan) to investigate morphological changes.

2. bation

2.11. Statistical Analysis

All data are the average of three times of analysis. ANOVA test by SPSS version 24 program was used to perform statistical analysis, and P-value < 0.05 and P-value < 0.01 were regarded as significant. Data were shown as means ± standard deviation (6, 7).

2. Results

3.1. Bioactive compounds and antioxidant activity in burdock fractions

In our study, the results for the Antioxidant and phytochemical composition of three burdock aerial parts extracts were summarized in the figures below. The highest Phenolic content using the Folin-Ciocalteu method was detected in the flower's acetic fraction, 31960 ± 160 µg/g DW ($p<0.05$), and the lowest content was detected in the stem's aqueous extract fraction, 5792 ± 60 µg/g DW ($p<0.05$) (Figure 1). However, the Leaf's ethanolic extract demonstrated the highest levels of total flavonoid content, 2034 ± 70 µg/g DW($p<0.05$), and the stem's aquatic extract showed the lowest flavonoid, 119.2 ± 10 µg/g DW ($p<0.05$) (Figure 2). The highest Antioxidant Activity using the FRAP method was detected in the flower's ethanolic fraction, 194.8 ±5 5 µM ($p<0.05$), and the lowest activity was detected in the stem's methanolic extract fraction, 87.9 ± 3.05 µM ($p<0.05$). As a polar solvent, it dissolves mainly lipophobic molecules (figure 3). The radical scavenging activity evaluated by the DPPH method showed the flower's methanolic extract had the highest RSA% with 95.4± 3.5% ($p<0.05$), and leaves aquatic extract with 58.8 ± 3.29 % ($p<0.05$) had the lowest RSA% (Figure 4). The highest Alkaloid content using the Mayer method was detected in the leaf's methanolic fraction, 1270 ± 44 µg/g DW ($p<0.05$), and the lowest content was detected in the stem's acetic extract fraction, with 112 ± 8 µg/g DW ($p<0.05$) (Figure 5). The highest Terpenoid content using the Salkowsky method was detected in the leaf's ethanolic fraction with 3026 ± 90 µg/g DW($p<0.05$), and the lowest

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content was detected in the stem's aqueous extract fraction, 366 ± 48 µg/g DW ($p<0.05$) (Figure 6).

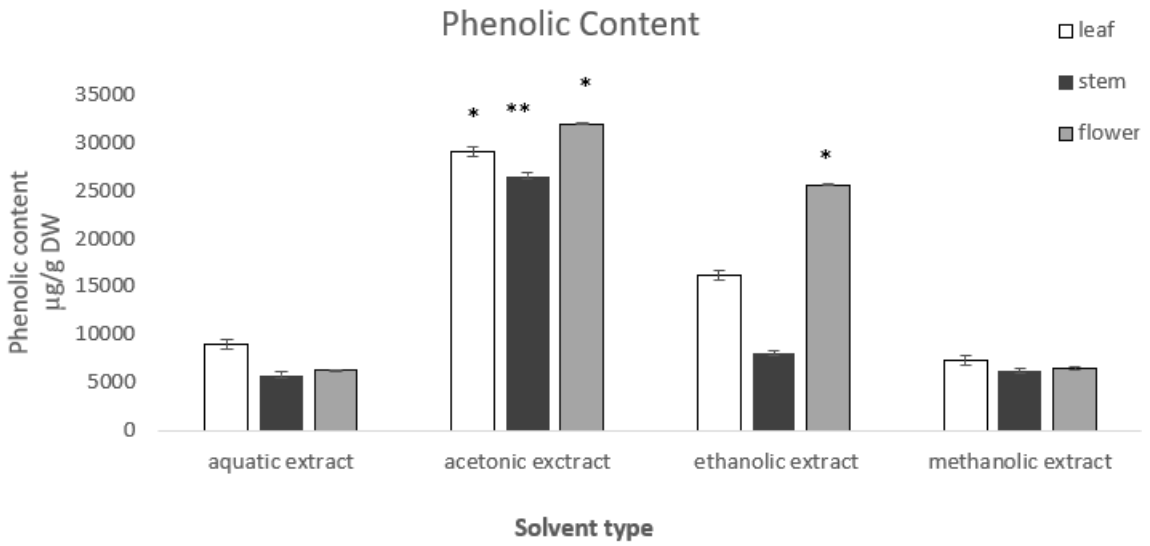

Figure 1: Investigation of phenolic content of aerial parts (leaf, stem, flower) of *A.lappa* aquatic, ethanolic, methanolic, and acetonic extracts using the Folin-Ciocalteu method (**, * means $p<0.05$,$p<0.01$).

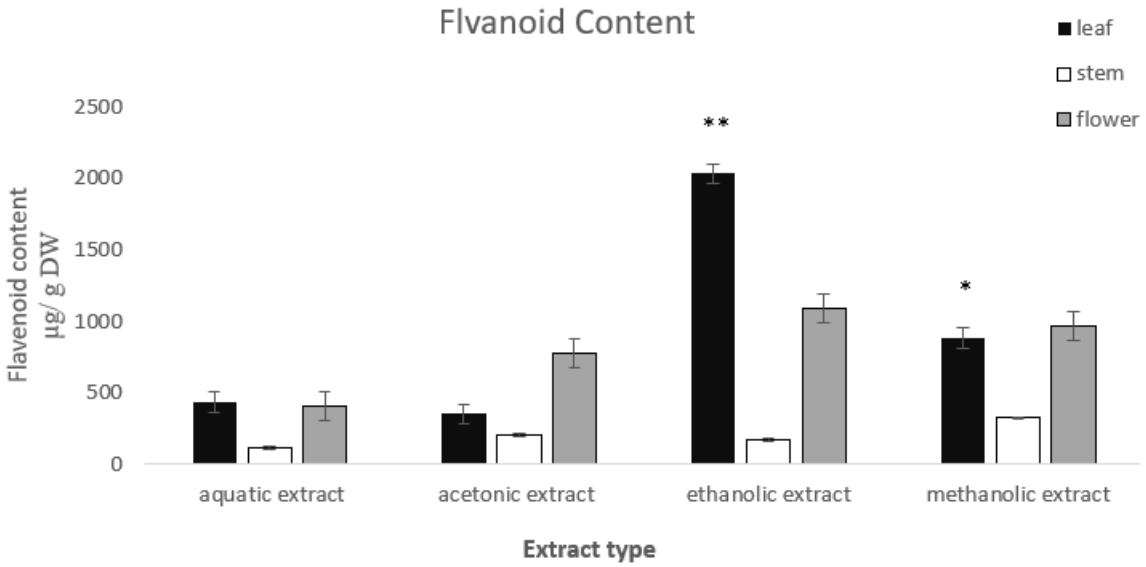

Figure 2: Investigation of flavonoid content of aerial parts (leaf, stem, flower) of *A.lappa* aquatic, ethanolic, methanolic, and acetonic extracts using the aluminum chloride method (** , * means $p<0.05$,$p<0.01$).

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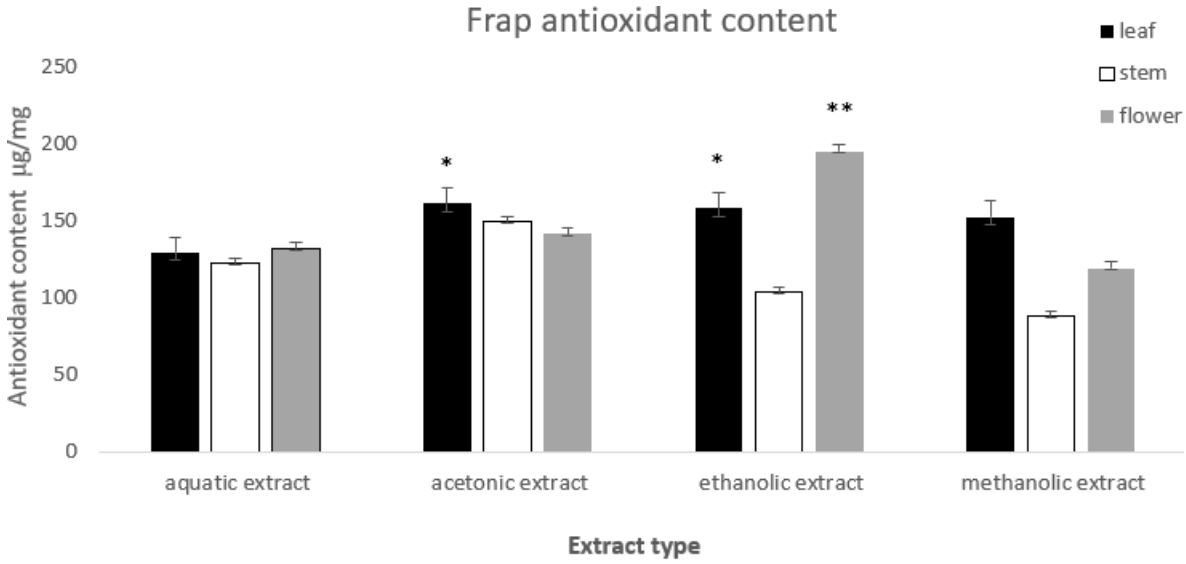

Figure 3: Investigation of Antioxidant content of aerial parts (leaf, stem, flower) of *A.lappa* aquatic, ethanolic, methanolic, and acetonic extracts using the FRAP method (**, * means $p<0.05$,$p<0.01$).

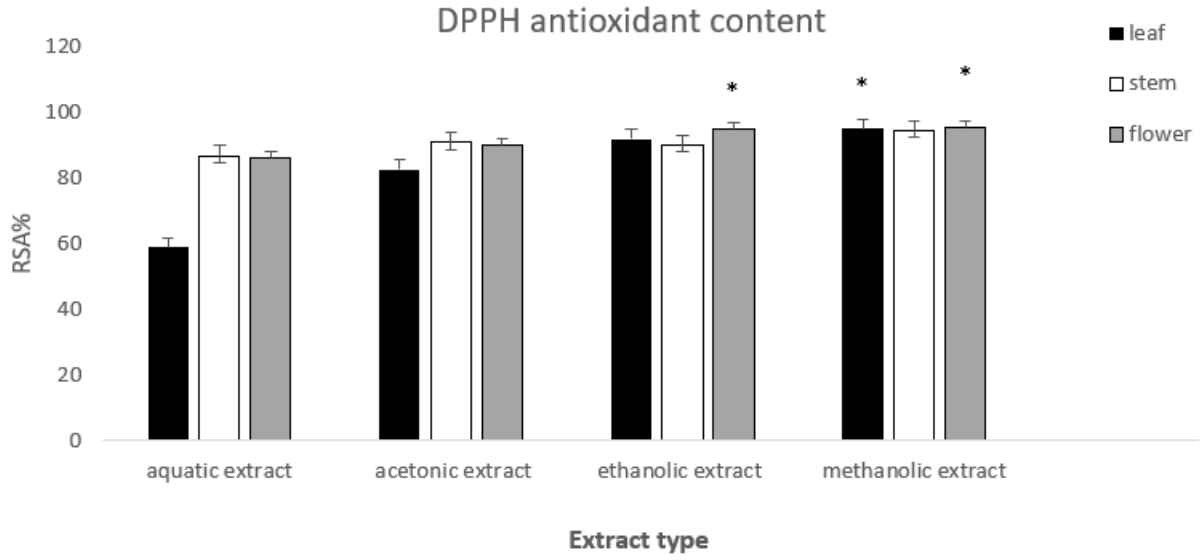

Figure 4: Investigation of Antioxidant content RSA% of arial parts (leaf, stem, flower) of *A.lappa* aquatic, ethanolic, methanolic, and acetonic extracts using DPPH method (**, * means $p<0.05$,$p<0.01$)

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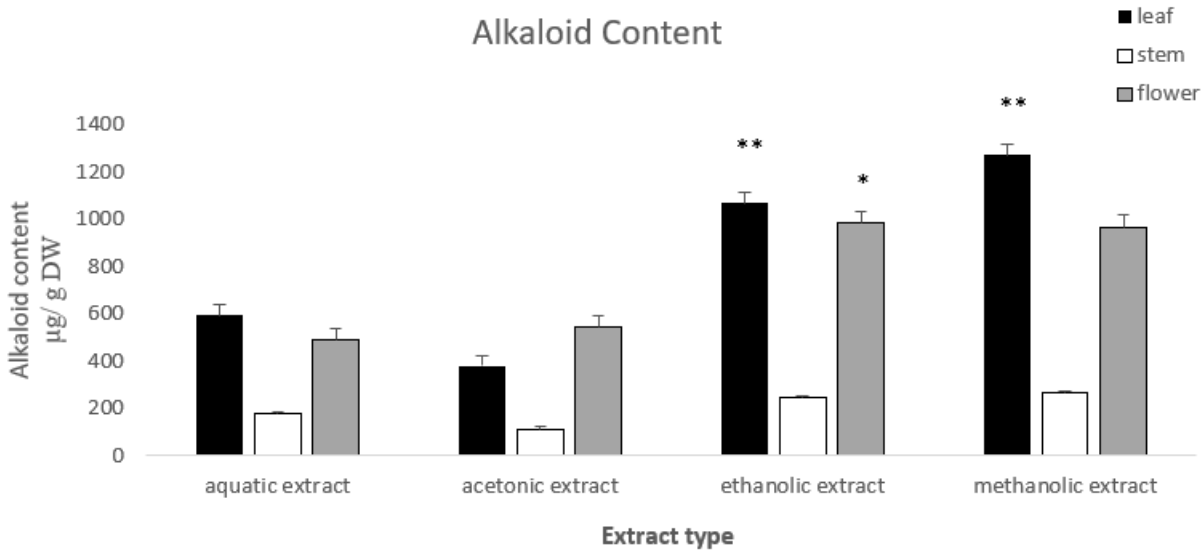

Figure 5: Investigation of Alkaloid content of arial parts (leaf, stem, flower) of *A.lappa* aquatic, ethanolic, methanolic and acetonic extracts using the Mayer method (** , * means $p<0.05$,$p<0.01$).

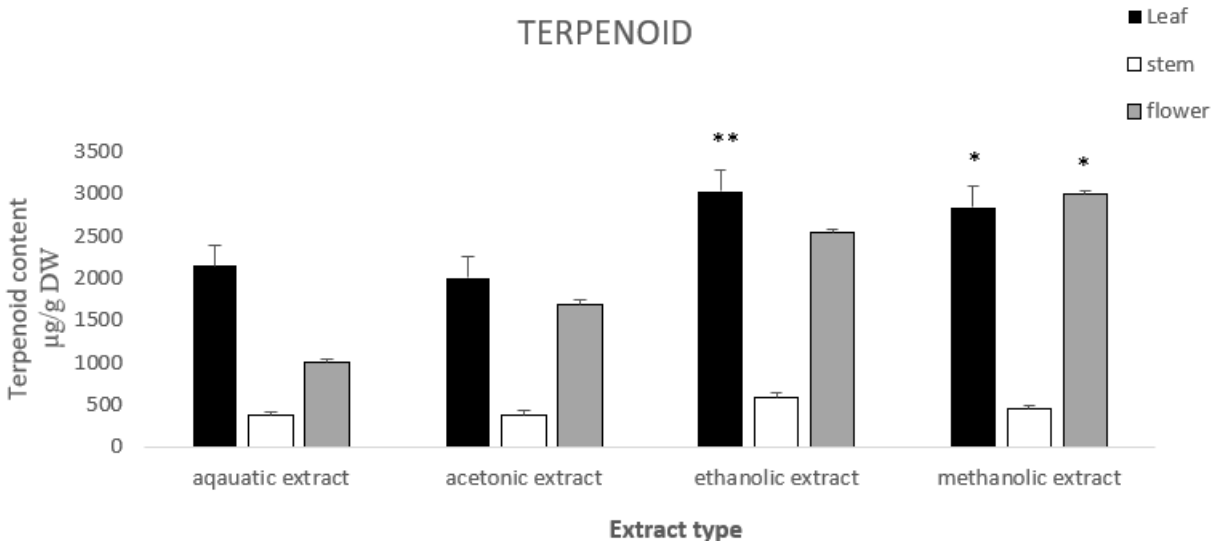

Figure 6: Investigation of Terpenoid content of aerial parts (leaf, stem, flower) of *A.lappa* aquatic, ethanolic, methanolic, and acetonic extracts using the Salkowsky method (**, * means $p<0.05$,$p<0.01$).

2.2. Antimicrobial activity

2.2.1 Antimicrobial Tests

Results showed that Ethanolic extract of *A. lappa* leaves had the highest inhibitory effect on *Staphylococcus aureus*, *Salmonella Paratyphi*, *Bacillus cereus*, and *Escherichia coli* (14 mm and 11.4mm, 12mm and 12mm, respectively) (Table 1). The MIC_{50} and MBC of *A. lappa* leaves extract against *Salmonella Paratyphi were 25000 and 40000 µg/ml*, respectively. Ciprofloxacin showed the highest inhibitory halo diameter of bacterial growth on *Staphylococcus aureus*, *Salmonella Paratyphi*, *Bacillus cereus*, and *Escherichia coli*. The MIC_{50} and MBC of *A. lappa* leaves extract against *Staphylococcus aureus* were 1250 and 20000 µg/ml, respectively. The MIC_{50} and MBC of *A. lappa* leaves extract against *Bacillus cereus* were 25000 and 40000 µg/ml, respectively. The MIC_{50} and MBC of *A.*

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lappa leaves extract against *Escherichia coli* were 25000 and 40000 µg/ml, respectively. (table 2, table 3). The inhibitory effect increased with higher concentrations.

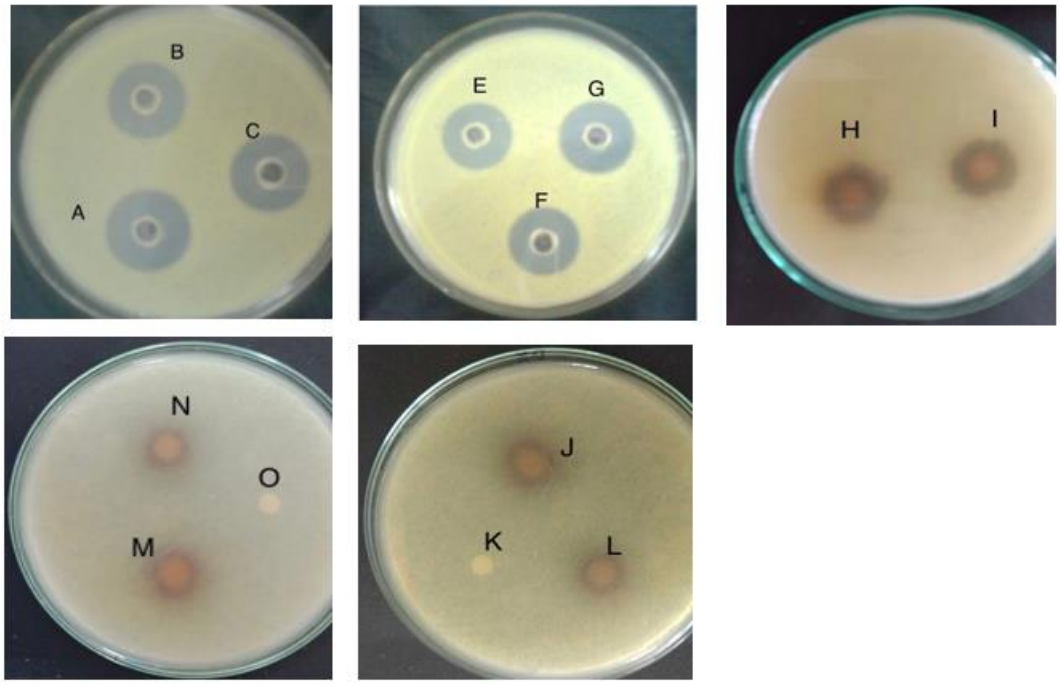

Figure 7: Antimicrobial activity of *A. lappa* aerial parts extracts using the disc diffusion method. (A) Leaves ethanolic extract against *Staphylococcus aureus,* (B) leaves aquatic extract against *Staphylococcus aureus, (C)* flower ethanolic extract against *Staphylococcus aureus,* (E) Leaves ethanolic extract against *Bacillus cereus,* (F) leaves aquatic extract against *Bacillus cereus,* (G) flower ethanolic extract against *Bacillus cereus,* (H) Leaves ethanolic extract against *Salmonella Paratyphi,* (I) leaves aquatic extract against *Salmonella Paratyphi,* (J) Leaves ethanolic extract against *Escherichia coli,* (K) ethanol negative control against *Escherichia coli,* (L) flower ethanolic extract against *Escherichia coli,* (M) Leaves Aquatic extract against *Escherichia coli,* (N) leaves aquatic extract against *Escherichia coli,* (O) aquatic negative control against *Escherichia coli.*

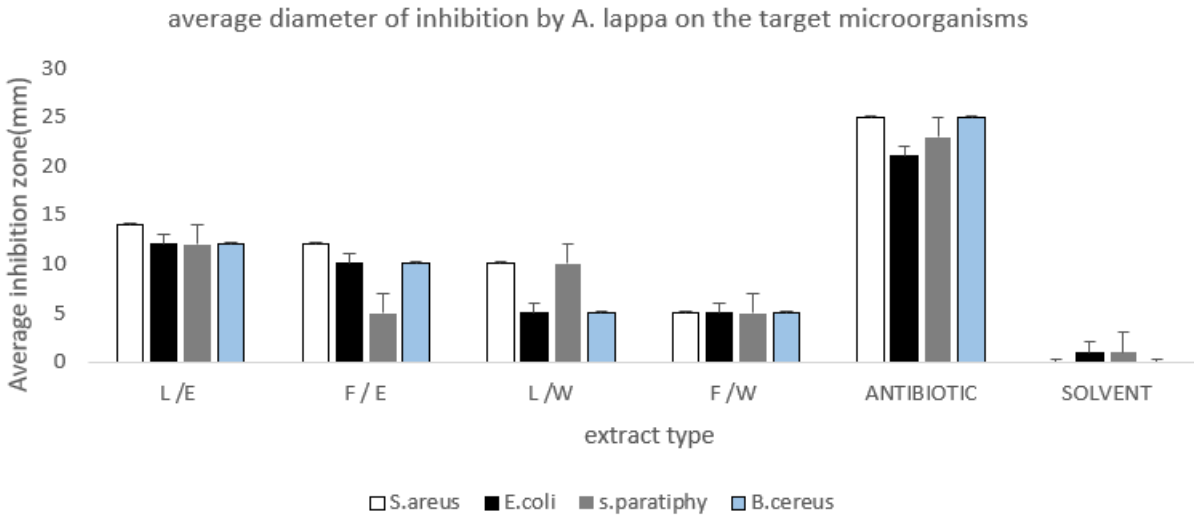

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Figure 8: Investigation of average diameter of inhibition by *A. lappa*'s flower and leaf ethanolic and aqueous extract against *Bacillus cereus*, *Escherichia coli*, *Salmonella paratyphi*, and *Staphylococcus aureus* using the disc diffusion test on Muller-Hinton agar. (L: Leaf, F: Flower, E: ethanol, W: Water, Solvent (negative control): Ethanol and water, Antibiotic (positive control): Ciprofloxacin

Table 1: Average diameter (mm) of inhibition halos produced by *A.lappa* phases on the target microorganism in the Agar-diffusion test.

Plant extract	***S. aureus***	***E. coli***	***S. paratyphi***	***B. cereus***
***A. lappa* (leaves/ ethanol extract)**	14	12	12	12
***A. lappa* (flower/ ethanol extract)**	12	10	5	10
***A. lappa* (leaves/ water extract)**	10	5	10	5
***A. lappa* (flower/water extract)**	5	5	5	5
Positive control (Ciprofloxacin)	25	21	22	25
Negative control (Ethanol/Water)	0	1	2	0

Table 2: The MIC_{50} content of *A.lappa* phases on the target microorganism in the Agar-diffusion test. (Unit: µg/ml)

Plant extract	***S. aureus***	***E. coli***	***S. paratyphi***	***B. cereus***
***A. lappa* (leaves/ ethanol extract)**	1250	2500	2500	2500
***A. lappa* (flower/ ethanol extract)**	2500	5000	10000	5000
***A. lappa* (leaves/ water extract)**	5000	10000	5000	10000
***A. lappa* (flower/ water extract)**	10000	10000	10000	20000

Table 3: The MBC content of *A.lappa* phases on the target microorganism in the Agar-diffusion test. (Unit: µg/ml)

Plant extract	***S. aureus***	***E. coli***	***S. paratyphi***	***B. cereus***
***A. lappa* (leaves/ ethanol extract)**	20000	40000	40000	40000
***A. lappa* (flower/ ethanol extract)**	40000	80000	160000	80000
***A. lappa* (leaves/ water extract)**	80000	160000	80000	160000
***A. lappa* (flower/ water extract)**	160000	160000	160000	160000

3.2.2. SEM **(Scanning Electron Microscopy)**

SEM images showed differences in cell structures between Extract-treated bacteria and the non-treated control bacteria. Non-treated cells were intact and showed a smooth surface, as can be seen in figure 9 A and figure 10 A, while bacterial cells treated with the herbal extract underwent considerable structural changes, as can be obviously discriminated in figure 9 –A' and figure 10 –A'. SEM observations confirmed the damage to the structural integrity of the cells and considerable morphological alteration to all tested Gram-positive and Gram-negative bacteria.

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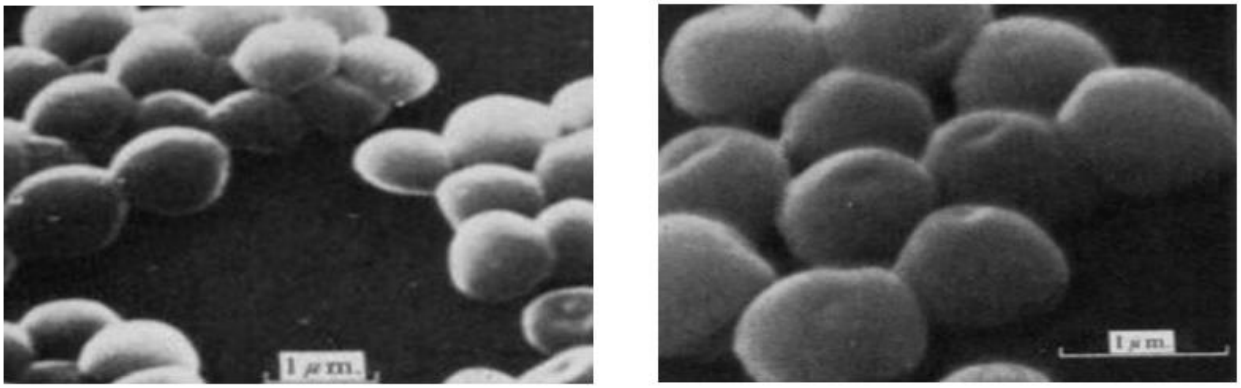
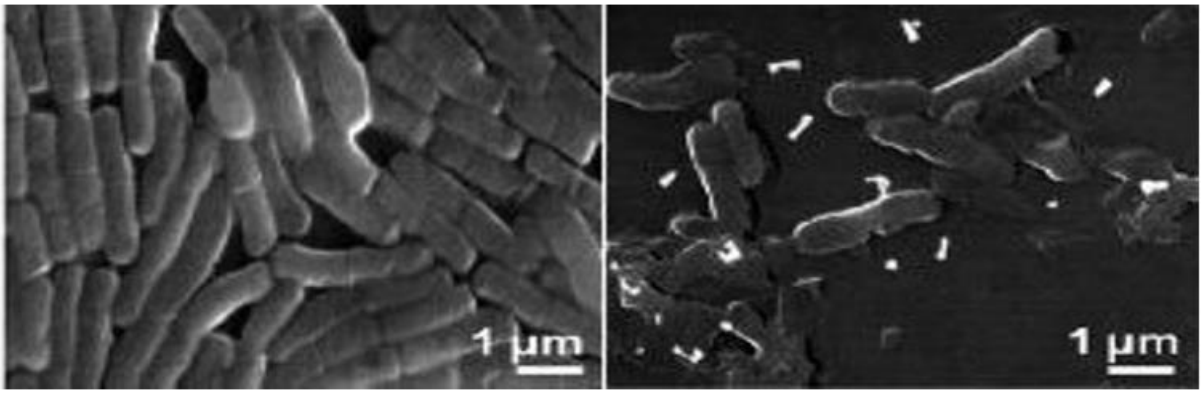


(A) (A')

Figure 9: SEM micrographs of (A) intact untreated bacterial cells of *Staphylococcus aureus*, (A') damaged bacterial cells of *Staphylococcus aureus* treated with *A. lappa* leaves ethanolic extract.



(A) (A')

Figure 10: SEM micrographs of (A) intact untreated bacterial cells of *Escherichia coli*, (A') damaged bacterial cells of *Escherichia coli* treated with *A. lappa* leaves ethanolic extract.

2.3. Discussion

Arctium lappa, more commonly recognized as burdock or bardana, is a highly significant medicinal plant with a long history of use, deeply rooted in ancient healing traditions across diverse cultures and societies (8). The leaves of this plant contain a wide variety of bioactive compounds, including phenolic, flavonoid, alkaloid, and terpenoid substances. However, while the roots and seeds of *A. lappa* have been the primary focus of previous research, there remains a relatively limited number of studies addressing the therapeutic potential of its leaves. This gap in knowledge motivated our decision to specifically investigate the aerial parts of *A. lappa* in the present study (20,21).

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This study highlights the phytochemical richness and biological activities of *Arctium lappa* aerial parts, revealing how solvent type and plant organ significantly influence both the concentration of bioactive compounds and their functional effects. By systematically evaluating phenolics, flavonoids, alkaloids, terpenoids, antioxidant potential, and antimicrobial activities, our results provide a detailed picture of the therapeutic value of this traditional medicinal plant.

The analysis of phenolic compounds (Figure 1) showed that the flower's acetonic extract contained the highest levels, while the stem's aqueous extract had the lowest. These results reinforce the importance of solvent polarity in determining extraction efficiency. Previous studies have similarly reported that organic solvents such as acetone and ethanol are far superior to water in releasing phenolic constituents (5,22). Phenolics are widely recognized for their strong antioxidant and antimicrobial properties (10,11), and their abundance in flower acetonic extracts helps explain the high antioxidant capacity observed in subsequent assays. Comparable work by Nascimento et al. (2018) also found that phenolic-rich extracts of *A. lappa* roots had potent antioxidant activity, further supporting our observations.

The measurement of flavonoids (Figure 2) revealed that ethanolic leaf extracts contained the highest concentrations. Flavonoids are among the most bioactive plant secondary metabolites, contributing not only to free radical scavenging but also to antibacterial and anti-inflammatory actions. The strong presence of these compounds in leaf ethanolic extracts is consistent with the findings of Jahanban-Esfahlan et al. (2018), who demonstrated that ethanol is particularly effective in recovering flavonoids from *A. lappa* leaves (22). A similar pattern was observed in other medicinal plants, where ethanol consistently outperformed aqueous solvents in extracting flavonoids (Do et al., 2014). This suggests that ethanol may be the solvent of choice when the therapeutic goal is to maximize flavonoid recovery.

The antioxidant potential of the extracts, assessed by both FRAP and DPPH methods, confirmed these phytochemical trends. Figure 3 shows that the flower's ethanolic fraction exhibited the highest ferric reducing antioxidant power, while Figure 4 demonstrates that the flower's methanolic fraction displayed the strongest radical scavenging activity. These results establish a direct link between polyphenolic content and antioxidant performance, a relationship that has been consistently described in the literature (10,11,23). For example, Aroca et al. (2020) reported that *A. lappa* extracts rich in phenolic acids had superior antioxidant effects, and Petkova et al. (2022) noted similar outcomes in root extracts, which showed that sequential extraction of *A. lappa* roots has also demonstrated the presence of distinct phytochemical fractions, with non-polar triterpenes in hexane and phenolic acids in ethyl acetate. Notably, the ethyl acetate fraction exhibited the strongest antioxidant activity, while all fractions displayed antimicrobial effects against pathogens such as *Salmonella*, *E. coli*, *S. aureus*, and *B. cereus*. These findings highlight the potential of burdock root extracts as sources of bioactive compounds for antimicrobial applications (21,24). By comparing our results with these findings, it becomes clear that both plant organ and solvent type influence the yield of antioxidant metabolites. Interestingly, although the flower's acetonic extract contained the highest total phenolics, the ethanolic and methanolic extracts were superior in antioxidant assays, suggesting that qualitative differences in phenolic composition, rather than absolute quantity, may play a key role.

Other classes of bioactive compounds were also noteworthy. Figure 5 revealed that methanolic leaf extracts contained the highest alkaloid levels, while Figure 6 showed that ethanolic leaf extracts were particularly rich in terpenoids. Both classes of compounds have been linked to antimicrobial, cytotoxic, and even anticancer effects (17,18). Alkaloids can disrupt protein synthesis and enzyme function, while terpenoids are known to interfere with bacterial membrane integrity. These findings suggest that the strong antibacterial activity of ethanolic

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leaf extracts is likely due to a synergistic effect of multiple phytochemical groups, rather than phenolics and flavonoids alone. Comparable results were reported by Lou et al. (2016), who showed that terpenoid-rich fractions of *A. lappa* had remarkable antibacterial effects in food preservation systems (25).

The antimicrobial assays provided direct confirmation of these biochemical observations. As shown in Figures 7 and 8, ethanolic leaf extracts consistently produced the largest inhibition zones against all four pathogens tested, while aqueous extracts were significantly less effective. This solvent-dependent difference has been widely reported in antimicrobial studies of *A. lappa* and other herbs (18,22,26). Gram-positive bacteria, including *Staphylococcus aureus* and *Bacillus cereus*, were clearly more sensitive than Gram-negative strains such as *E. coli* and *Salmonella Paratyphi*. The reduced susceptibility of Gram-negative bacteria is well understood: their outer membrane provides an additional permeability barrier that limits the diffusion of lipophilic phytochemicals (19,27,28). In contrast, the absence of this barrier in Gram-positive bacteria makes them more vulnerable to plant-derived antimicrobials. These structural differences explain why, despite similar concentrations of bioactive compounds, Gram-negative bacteria often require higher doses for growth inhibition.

The MIC and MBC results (Tables 2 and 3) supported these trends by showing that ethanolic extracts inhibited bacterial growth at much lower concentrations than aqueous extracts. For example, ethanolic extracts of leaves exhibited MIC values as low as 1250 µg/mL against *S. aureus*, whereas aqueous extracts required much higher doses. These findings are consistent with those of Pirvu et al. (2017), who demonstrated that *A. lappa* leaf extracts increased the efficacy of common antibiotics against both Gram-positive and Gram-negative bacteria (18). Similarly, Sultana et al. (2018) reported that ethanolic extracts of *A. lappa* leaves exhibited strong antibacterial activity, particularly against *S. aureus* (26).

The SEM analysis provided valuable mechanistic evidence to complement the antimicrobial assays. Figures 9 and 10 show that untreated bacterial cells retained a smooth and intact morphology, whereas cells treated with ethanolic extracts displayed disrupted membranes and irregular surfaces. These structural damages confirm that phytochemicals in *A. lappa* act directly on bacterial cell walls and membranes, compromising their integrity and ultimately leading to cell death. Such observations are in agreement with previous studies, including those of Bouhaddou et al. (2017) and Lou et al. (2016), who described similar morphological alterations in bacteria treated with plant-derived antimicrobials (25,29). The visualization of these effects underscores the therapeutic promise of *A. lappa* extracts, as they appear to operate through a physical mechanism that is less likely to be overcome by conventional resistance pathways.

Taken together, these results demonstrate that ethanolic extracts of *A. lappa* leaves, which are especially rich in phenolics, flavonoids, alkaloids, and terpenoids, possess the strongest antioxidant and antimicrobial activities among the tested extracts. This outcome is in agreement with Jahanban-Esfahlan et al. (2018), who highlighted the crucial influence of extraction methods on the biological activity of *A. lappa* (22). Although the activity of these extracts did not reach the level of Ciprofloxacin, their broad-spectrum effects remain significant, particularly given the urgent need for alternative strategies to address antibiotic resistance. Moreover, the combination of different classes of bioactive compounds suggests potential for synergistic actions, which could be further explored in future studies.

Overall, the findings of this study strengthen the case for considering ethanolic extracts of *A. lappa* leaves as promising candidates for natural therapeutic development. By demonstrating a clear relationship between phytochemical composition, antioxidant activity, and antibacterial effects, our results not only validate the traditional use of *A. lappa* but also provide a scientific

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foundation for its application in modern pharmacology. Future research should focus on isolating and characterizing the specific compounds responsible for these effects, as well as evaluating their safety and efficacy in clinical settings. The therapeutic promise of medicinal plants has been further validated by other scientific studies, which similarly report that natural plant products inhibit the growth of *S. Paratyphi*, *B. cereus*, *E. coli*, and *S. aureus* (11,23,24,30). When comparing the effects of different medicinal plants, our results clearly indicate that ethanolic extracts prepared from *A. lappa* leaves showed strong antimicrobial activity against a broad range of microorganisms. This outcome suggests that ethanolic extracts of *A. lappa* leaves may serve as an effective natural alternative to conventional synthetic medications currently in use.

3. Conclusions

Arctium lappa, commonly known as burdock, has exceptionally high concentrations of a wide range of different bioactive substances, such as, but not limited to, a large number of phenolic compounds, a variety of alkaloids, a large number of terpenoids, and a wide range of flavonoids, which together offer a very promising and productive path for further research and practical application in the area of pharmacological studies that are specifically focused on treating bacterial infections and, at the same time, reducing the potentially harmful effects that are linked to mutagenic factors that can pose serious and substantial health risks to people. However, further research is still needed to fully determine the safe and efficient use of these plant extracts in therapeutic settings so that their full potential can be realized. Furthermore, investigating the modes of action and possible synergistic effects with other substances may improve our comprehension and utilization of these natural therapies.

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Authors' contributions
All Authors contributed to the conception of the work, conducting the study, revising the draft, approving the final version of the manuscript, and agreed on some aspects of the work. All Authors contributed to the conception of the work, approval of the final version of the manuscript, and agreed on some aspects of the work.

Conflict of interest
Authors declare no conflict of interest. Ethical considerations
Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the authors. This work has been performed in culture medium, so ethical committee approval was not needed.

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