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Anti-MRSA and cytotoxic activities of different solvent extracts from *Artemisia herba-alba* grown in Shubak, Jordan

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Abstract

Background: Globally, resistance to antimicrobial drugs is a major hazard to public health. Infections that were once easily treatable with antibiotics are becoming harder to control, leading to prolonged illnesses, increased mortality rates, and higher healthcare costs.

Aim: This study intended to assess the antimicrobial, specifically the anti-Methicillin resistant *Staphylococcus aureus* (MRSA), and anticancer properties of different extracts obtained from *A. herba-alba* (AHA).

Methods: The antibacterial tests of AHA were performed on two Gram-negative bacterial strains (*Escherichia coli* and *Klebsiella pneumonia*), two Gram-positive bacterial strains (Methicillin-resistant *Staphylococcus aureus* (MRSA), and Staphylococcus aureus). Initial screening for antibacterial activities was conducted using the well diffusion technique. Subsequently, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined through the broth-dilution assay. The anticancer test was carried out *in vitro* on a human colorectal carcinoma cell line (HCT-116) using MTT assay.

Results: Among all extracts, *n*-hexane extract of AHA was the most effective against *S. aureus* with the highest inhibition zone (24.67 mm ± 0.58) compared to standard antibiotic (erythromycin, 24.00 mm) followed by the methanolic extract against MRSA (24.00 mm ± 1.73). The methanol extract of AHA showed the highest antibacterial activity against MRSA. The results of MIC and MBC of the AHA methanol extract against MRSA were 1.17 ± 1.09 and 9.375 ± 0.0 mg/ml, respectively, demonstrating therapeutically significant antibacterial activity. Ethyl acetate extract has no antibacterial activity against *E. coli* and *K. pneumonia*. The findings indicated that the methanol extract of AHA exhibited the highest efficacy against the colorectal carcinoma cell line (HCT-116), with an IC_{50} value of 126.61 ± 13.35 µg/ml.

Conclusion: These findings suggest that the methanol extract of AHA could be considered as a potential agent to serve as a source of antibacterial and anticancer compounds.

Keywords: *A. herba-alba*, Antimicrobial activity, Cytotoxicity, Solvent extracts.

Introduction

Several critical illnesses are brought on by multidrug-resistant strains of pathogens such as *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii,* and *Pseudomonas aeruginosa* (Harding *et al.*, 2018; Hwang and Yoon, 2019; Lasko and Nicolau, 2020). Antibiotic-resistant bacteria (ARB) strains can cause various infections, such as gastritis, food poisoning, urinary tract infections, and encephalitis (Caneiras *et al.*, 2019; Huang *et al.*, 2022).

Due to the current constraints in treating these conditions with available medications, it is anticipated that they will surpass cancer as the primary cause of death by the year 2050. This is because conventional antibiotics may not effectively combat infections caused by ARB (Murray *et al.*, 2022).

Antimicrobial resistance (AMR) has been recognized as an important threat to public health systems worldwide, not just in developing countries. Drug-resistant microbial infections cause 23,000 deaths worldwide every year, including in developed countries like the United States. Similar facts exist in Europe, but they are much greater in developing countries of Asia, such as India, Latin America, and Africa (Davis *et al.*, 2017). Antibiotics can no longer be used to treat infectious infections, signaling an uncertain future for healthcare. AMR infection causes significant diseases, long-term

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hospital stays, greater healthcare expenses, higher second-line drug costs, and treatment failures (Thorpe *et al.*, 2018). For example, it has been estimated that antibiotic resistance costs more than nine billion euros annually only in Europe (Dixit *et al.*, 2019). In addition, the Centers for Disease Control and Prevention estimates that antibiotic resistance raises direct healthcare expenditures in the United States by an additional $20 billion annually, not including the estimated $35 billion in productivity losses (Dadgostar, 2019).

Due to a diminishing in antimicrobial discovery by the major pharmaceutical companies, the development of new antibiotics has been reducing quickly over the past few decades. Finding new alternatives, such as those derived from medicinal plants, is therefore interesting. Beneficial phytochemicals found in medicinal plants can be utilized in the prevention of disease and treatment of various ailments across the globe (Aanouz *et al.*, 2021). In addition, around three quarters (80%) of the Arab population uses herbal medicine for treatment and prevention (El-Dahiyat *et al.*, 2020).

Over the past few decades, medicinal plants have garnered significant attention in pharmacological research owing to their substantial content of polyphenols, quinines, flavonoids, and alkaloids (Adu-Amankwaah *et al.*, 2023). The bioactive compounds in these plants generally have anti-inflammatory (Živković *et al.*, 2020), cardioprotective, anti-carcinogenic (Mbuni *et al.*, 2020), antioxidant (Kaçar *et al.*, 2022), antiseptic, antibacterial, and antifungal properties (Elsonbaty *et al.*, 2020). Jordan's diverse geography and climate contribute to the presence of a wide variety of wild plants across the country (Al-Qura'n, 2009). There are approximately 868 genera and 142 families of plant species. There are 2,543 species of medicinal plants, and they are widely distributed over the country (Basheti *et al.*, 2017).

Artemisia herba-alba (AHA) ("desert wormwood" in English; "sheeh" in Arabic) is a medicinal and highly aromatic dwarf shrub that is typical of the steppes and deserts of North Africa (Tunisia, Algeria, and Morocco), Middle East (Egypt and Jordan), Southern Europe (Spain), and extends into the Northwestern Himalaya. AHA plant extract has long been utilized extensively in different countries in traditional medicine as soup or tea for the treatment of a variety of illnesses such as colds, coughing, intestinal disturbances, bronchitis, diarrhea, neuralgias arterial hypertension, reduces blood glucose levels (Wazaify *et al.*, 2011), and inflammations caused by fungal, bacterial, or viral infections (Jouad *et al.*, 2001; Laid *et al.*, 2008; Abu-Darwish *et al.*, 2015; Ouguirti *et al.*, 2021).

Fig. 1. Image of AHA plant from Shubak City (Jordan).

AHA belongs to the family Asteraceae, which comprises about 1,600 genera and more than 25,000 species (Nikolić and Stevović, 2015). The 20–50 cm tall, green perennial shrub AHA has tiny, hairy leaves. Although flowering occurs in September and lasts until December, the complete growth starts at the end of the summer with a fuzzy hair stem (Fig. 1) (Moufid and Eddouks, 2012).

Due to its diverse pharmacological and biological properties, AHA exhibits various beneficial effects, including its potential in antidiabetic (Bourebaba *et al.*, 2023), antimicrobial (Dif and Fatima Zohra, 2023), antitumor (Mohammed *et al.*, 2019), antimalarial, antioxidant (Mohammed *et al.*, 2021), insecticidal, and neurological activities (Boukraa *et al.*, 2022).

This study aims to examine the antibacterial properties of various extracts (including *n*-hexane, dichloromethane, ethyl acetate, and methanol) from AHA, focusing on their effectiveness against Methicillin-resistant *Staphylococcus aureus* (MRSA) as well as various types of both Gram-positive and Gram-negative bacteria. Furthermore, the study will assess their anticancer potential against colorectal carcinoma cell line (HCT-116).

Materials and Methods

Chemicals and reagents

All chemicals and reagents used were of analytical grade. Dichloromethane, ethyl acetate, methanol, and *n*-hexane, Mueller Hinton Broth (MHB), nutrient agar, dimethyl sulfoxide (DMSO), erythromycin, and cefotaxime were purchased from Merck (Mumbai, India) and Sigma-Aldrich (St. Louis, MO).

Collection and identification of plant material

Fresh plant AHA was harvested in the Shubak region of southern Jordan between March and April 2023. The herbarium specimen was verified by Professor Saleh Alquran (MU202309) from the Department of Biology, Faculty of Science, at Mutah University in Karak, Jordan.

Sample extraction

The procedure of extraction was carried out according to Ogbiko *et al.* (2018). The whole plant of AHA was rinsed with running water, dried under shade for 72 hours, and pulverized electric blender into powder form.

A Soxhlet extractor was used to extract the dried whole plant of AHA (50 g) using *n*-hexane, dichloromethane, ethyl acetate, and methanol as the solvents with growing polarity (each 500 ml). The obtained extracts were then dried using a rotary evaporator and kept for further analysis at 4°C in sealed glass containers. The extract was prepared at concentrations of 6% by dissolving in 10% DMSO (Stock solution = 60 mg extract/1,000 µl of 10% DMSO). For determination of plant extract yield (% Yield), the dried extracts' yield percentage (*w*/*w*) was estimated using the following formula:

Yield (%) = $A_1 \times 100/A_2$

where A_1 is the dry weight of the extract after solvent evaporation and A_2 is the weight of the dried whole plant powder.

Antibacterial activity of different solvents extracts of AHA

Bacterial isolates

In this study, the antibacterial of AHA extract was investigated against *Escherichia coli* (*E. coli*) American Type Culture Collection (ATCC 25922), *K. pneumonia* ATCC 200603, *S. aureus* ATCC 29213, and one clinical isolate: MRSA, OQ568766, was provided by the Jordan University Hospital, Amman, Jordan.

The strains included both Gram-negative as well as Gram-positive strains; for the antibacterial screening assay, all strains were initially sub-cultured in nutrient agar media and incubated at 37°C for 18 ± 2 hours.

Antibacterial screening

Agar well diffusion method

The antibacterial activity of methanol, ethyl acetate, dichloromethane, and *n*-hexane extracts was performed using the Agar well diffusion method according to the method of Bauer *et al*. (1966). Briefly, using a sterile swab, 100 µl of each test bacteria inoculated on Muller Hilton Agar was equivalent to a 0.5 McFarland standard. The wells were created using a sterilized cork borer (6 mm in diameter) into agar plates containing inoculums after 10 minutes of inoculation. After 24 hours of incubation at 37°C, the inhibitory zones (mm) were measured and the findings were compared to erythromycin (1.0 mg/disc). DMSO was employed as the negative control. The experiment was triplicate and the average values were calculated.

Determination of minimum inhibitory concentration

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of various extracts of AHA were assessed using the broth dilution method as outlined previously with certain modifications (Mushore and Matuvhunye, 2013). The assays were conducted on 96-well microtiter plates under aseptic conditions. For MIC evaluation in MHB, the extract working solution was serially diluted throughout the 96 wells to obtain final testing concentrations of 37.50, 18.75, 9.375, 4.69, 2.34, and 1.17 mg/ml with an optimum concentration of bacterial strains (10^8 CFU/ml) using 0.5 McFarland standard. In brief, all wells of the plate were filled with 90 µl of MHB, then the first column of the microtiter plate was filled with 100 µl of each solvent extract (100 mg/ml extract stock solution). A two-fold serial dilution was achieved by transferring 100 µl of diluents from the first column to the succeeding wells of each row. About 10 µl of bacterial suspension was added into each well, except for well 12. Each plate had a set of two controls: (a) positive control (11th wells) included test organisms only without test extract and (b) negative control (12 th wells) included only plant extract. The 96-well plates were incubated for 24 hours at 37°C in a temperature-controlled incubator. Values of MBC were determined by culturing wells' content with concentrations higher than or equal to MIC on agar plates. MBC is defined as the lowest concentration of extracts or antimicrobial agents that kills tested bacteria (no growth in the agar plate) after 24 hours at 37°C. The MBC values were determined by subculturing 15 µl of a microtitre plate on sterile nutrient agar plates. The growth of bacteria was measured after 24 hours of incubation at 37°C. The MBC was carried out in triplicates.

In vitro cytotoxicity evaluation

Cell culture and materials

The colorectal carcinoma cell line (HCT-116) purchased from the ATCC was used for the cytotoxic assay. These cells were cultured in minimum essential medium supplemented with 10% FBS, 100 units/ml of penicillin, 100 mg/ml of streptomycin, and 2 mM L-glutamine. The cultures were maintained in a controlled environment with a 5% CO_2 atmosphere at 37°C and subculture every 2 weeks.

(3- [4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) assay for cell viability

The cytotoxicity of the plant extract was established using (3- [4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) assay. The cells were exposed to varying concentrations of plant extracts (31.25, 62.5, 125, 250, 500, and 1,000 µg/ml) and allowed to incubate for 48 hours. After the incubation period, test samples were extracted from the wells and replaced with 200 µl of fresh medium. Subsequently, 20 µl of MTT solution (5 mg/ml in PBS) was added and incubated for 4 hours. At the end of the incubation period, the medium in each well was aspirated, and the formazan crystals that had formed were dissolved by adding 50 µl of DMSO to each well on the plates. The plates were gently agitated until the crystals were fully dissolved. The degree of MTT reduction was promptly determined by measuring the absorbance at 570 nm (Mosmann, 1983). The human umbilical vein endothelial cells (HUVECs) cell line was used as a normal cell line to assess the selectivity of extracts against these different cell lines.

The cell viability percentage was calculated using the following formula:

% Cell viability = (A – B)/(C – B) × 100%

where: A = absorbance of the test extract, B = blank absorbance, C = absorbance of the control.

The IC_{50} values, which signify the concentration at which 50% of the cells were eradicated, were derived through the examination of linear regression plots. These graphical representations illustrated the concentration of the test sample necessary to achieve a 50% reduction in absorbance in comparison to untreated cells.

Ethical approval

Not needed as this study does not involve experiments on animals or humans.

Results

Effects of various solvents on extraction yield

The impact of different polarity solvents including nonpolar (*n*-hexane), medium-polar (ethyl acetate and dichloromethane), and polar (methanol) on the AHA extraction rate was studied and the results are shown in Figure 2. About 50 g of AHA powder was extracted by a Soxhlet extractor and concentrated using a rotary evaporator. The final weights of the AHA extracts were 6.982, 3.945, 3.105, and 0.685 g for methanol, dichloromethane, ethyl acetate, and *n*-hexane extract, respectively. The results indicated that the methanol extract of AHA had the highest yield at 13.96%, followed by the dichloromethane extract at 7.87%, and the ethyl acetate extract at 6.21%. Conversely, the *n*-hexane extract had the lowest yield, which was 1.37%. Hence, the variation in extraction yields can be attributed to the differing polarities of the various compounds present in AHA.

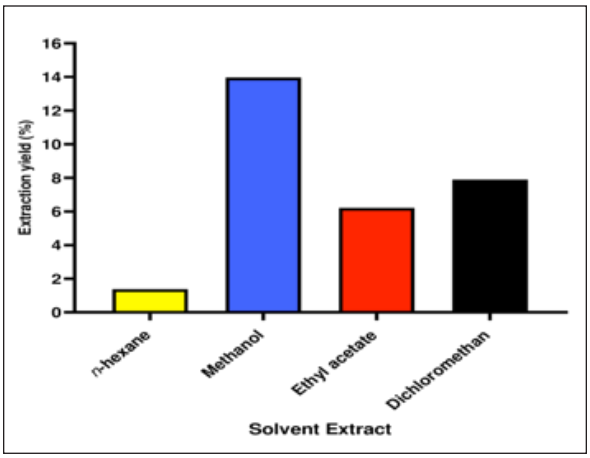

Fig. 2. Yield percentage of different solvents extract of AHA.

Antibacterial activity of different solvents extracts of AHA

Agar well diffusion assay

The antimicrobial efficacy of different extracts (*n*-hexane, methanol, dichloromethane, and ethyl acetate) derived from the whole parts of AHA was assessed based on the zone of inhibition against a range of pathogens. The results (zone of inhibition) were then compared to the activity of erythromycin (1.0 mg/disc). The detailed findings concerning antimicrobial activity are presented in Table 1.

The results of the present study clearly illustrate that erythromycin possesses a significant inhibitory effect against Gram-positive bacteria, including MRSA and *S. aureus*, with inhibition zones measuring 24 ± 0.00 and 34 ± 0.00 mm, respectively. These findings demonstrate that the crude *n*-hexane and dichloromethane extracts of AHA exhibit strong antimicrobial activity against all the examined microorganisms. In addition, all the extracts demonstrate potent antimicrobial activity against MRSA and *S. aureus*.

In this study, various extracts from AHA showed significant inhibitory effects against all bacterial isolates tested, except for *K. pneumonia* and *E. coli*. These two strains displayed resistance to the ethyl acetate extract and erythromycin, while *E. coli* also exhibited resistance to the methanol extract. The *n*-hexane and dichloromethane extracts of AHA demonstrated inhibitory effects against MRSA, *S. aureus*, *K. pneumonia*, and *E. coli*. In contrast, the methanol extract of AHA exhibited effectiveness against MRSA, *S. aureus*, and *K. pneumonia*, while the ethyl acetate extract of AHA was effective solely against Gram-positive strains, namely MRSA and *S. aureus*.

Table 1. Antimicrobial activity of AHA extracts against selected strains of Gram positive and Gram negative bacteria.

Extracts	Zone of inhibition (mm) (Mean ± SD)			
	Gram-positive bacteria		Gram-negative bacteria	
	MRSA	*Staphylococcus aureus*	*Klebsiella pneumonia*	*Escherichia coli*
n-hexane	14.67 ± 0.58	24.67 ± 0.58	9.33 ± 0.58	12.67 ± 0.58
Methanol	24.00 ± 1.73	22.67 ± 1.15	18.33 ± 0.58	NA
Ethyl acetate	21.00 ± 1.00	13.33 ± 2.89	NA	NA
Dichloromethane	22.67 ± 2.52	16.67 ± 0.58	8.67 ± 0.58	8.67 ± 0.58
Reference standard Erythromycin	24.00 ± 0	34.00 ± 0	NA	NA

(MRSA): Methicillin-Resistant Staphylococcus aureus; (NA): no activity.

In *n*-hexane extract, the maximum inhibition zone diameter was observed in *S. aureus* with a diameter of 24.67 ± 0.58 mm. Similarly, methanol extract showed a maximum inhibition zone with a diameter of 24.00 ± 1.73 mm in *MRSA* and 22.67.4 ± 1.15 mm in *S. aureus.* The rank of antibacterial activity of hexane extract against each bacterial strain is: *S. aureus* > MRSA > *E.coli* > *K. pneumonia*.

The dichloromethane extract of AHA showed considerable activity. Its activity against each strain is ranked according to MRSA > *S. aureus* > *E. coli* > *K. pneumonia.*

The antimicrobial activity assayed for AHA extracts showed an overall inhibitory effect against MRSA (24.00 ± 1.73 mm) with the methanol extract, 22.67 ± 2.52 mm with the dichloromethane extract, and 21.00 ± 1.00 mm with the ethyl acetate extract, comparing with erythromycin (24 ± 0.00 mm) as a positive control. High activity against *S. aureus* was found with *n*-hexane and methanol extracts (24.67 ± 0.58 and 22.67 ± 1.15 mm, respectively) (Table 1).

Determination of MIC

Table 2 demonstrates significant antibacterial activities of promising *n*-hexane, methanol, ethyl acetate, and dichloromethane crude extracts of AHA against test microorganisms reported as MIC. MIC is the term for the highest dilution or lowest concentration of extracts known to inhibit bacterial growth.

The MIC values of the *n*-hexane extract ranged from 2.34 mg/ml (*MRSA*) to 4.69 mg/ml (*S. aureus, K. pneumoniae*, and *E. coli*) and the methanol extract ranged from 1.17 mg/ml (*MRSA*) to 37.5 mg/ml *(S. aureus* and *K. pneumoniae*). Also, the MIC values of the ethyl acetate extract ranged from 2.34 (*MRSA*) to 9.375 mg/ml (*S. aureus* and *K. pneumoniae*), and for the dichloromethane ranged from 4.69 (*MRSA*) to 9.375 mg/ml (*S. aureus*, *K. pneumonia, and E. coli*) (Table 2). Methanol extract of AHA also demonstrated the highest antibacterial activity, and therefore highest antimicrobial activities, against clinical MRSA with MIC of 1.17 mg/ml, and a comparatively highest MIC value was observed for *S. aureus* and *K. pneumoniae* (MIC of 37.5 mg/ml).

Moreover, the ethyl acetate extract showed a MIC of 2.34 mg/ml against MRSA, and the dichloromethane extract showed a MIC of 9.375 mg/ml against all strains of microorganisms except MRSA (MIC of 4.69 mg/ml). These results demonstrate that the highest MIC (lowest antimicrobial activity) occurs when methanol is used as the solvent for extraction.

Based on the MIC values of the selected bacteria, the highest MIC (37.5 mg/ml) was found in the presence of methanol extract against *S. aureus* and *K. pneumonia*. On the contrary, the lowest MIC values were observed when methanol extract was tested with MRSA. On the other hand, all four solvent extracts (methanol, *n*-hexane, ethyl acetate, and dichloromethane) are potent antimicrobials against *MRSA* with the lowest MIC values, 1.17, 2.34, 2.34, and 4.69 mg/ml, respectively (Table 2).

Determination of MBC

The MBC is the lowest concentration of the several solvent extracts of AHA that kill 99.99% of bacteria. As shown in Table 2, the *n*-hexane extract showed antibacterial activity with MBC values ranging from 9.375 to 75 mg/ml. The MBC values for *n*-hexane extract were *S. aureus* = *E. coli* < MRSA < *K. pneumonia.* While the methanol extract showed antibacterial activity with MBC values ranging from 9.375 to 75 mg/ml. The MBC values for methanol extract were in MRSA < *E. coli* < *K. pneumonia* = *S. aureus.* Furthermore, ethyl acetate extract exhibited antibacterial activity with MBC values of 4.69–37.52 mg/ml. The MBC values of ethyl acetate extract were in MRSA ≤ *S. aureus* = *E. coli* < *K. pneumonia*. In contrast, the results revealed the antibacterial activity of dichloromethane extract

Table 2. MIC and MBC values of different solvent extracts of AHA against the tested microorganisms (mg/ml).

Microorganisms	*n*-hexane	Methanol	Ethyl acetate	Dichloromethane	Cefotaxime
MIC					
MRSA	2.34 ± 1.25	1.17 ± 1.09	2.34 ± 1.36	4.69 ± 1.36	0.625
Staphylococcus aureus	4.69 ± 0	37.5 ± 0.0	9.375 ± 2.7	9.375 ± 2.71	<0.004
Klebsiella pneumonia	4.69 ± 1.36	37.5 ± 0.0	9.375 ± 2.7	9.375 ± 2.71	0.016
Escherichia coli	4.69 ± 0	4.69 ± 0.0	4.69 ± 0.0	9.375 ± 0.0	<0.004
MBC					
MRSA	18.75 ± 2.7	9.375 ± 0.0	4.69 ± 2.7	37.5 ± 0.0	0.625
Staphylococcus aureus	9.375 ± 0.0	75 ± 7.16	9.375 ± 2.7	18.75 ± 0.0	0.008
Klebsiella pneumonia	75 ± 0.0	75 ± 0.0	37.5 ± 0.0	37.5 ± 7.16	0.125
Escherichia coli	9.375 ± 1.36	18.75 ± 2.7	9.375 ± 0.0	18.75 ± 0.0	0.039

(MIC): minimum inhibitory concentration; (MBC): minimum bacterial concentration; (MRSA): Methicillin-Resistant Staphylococcus aureus.

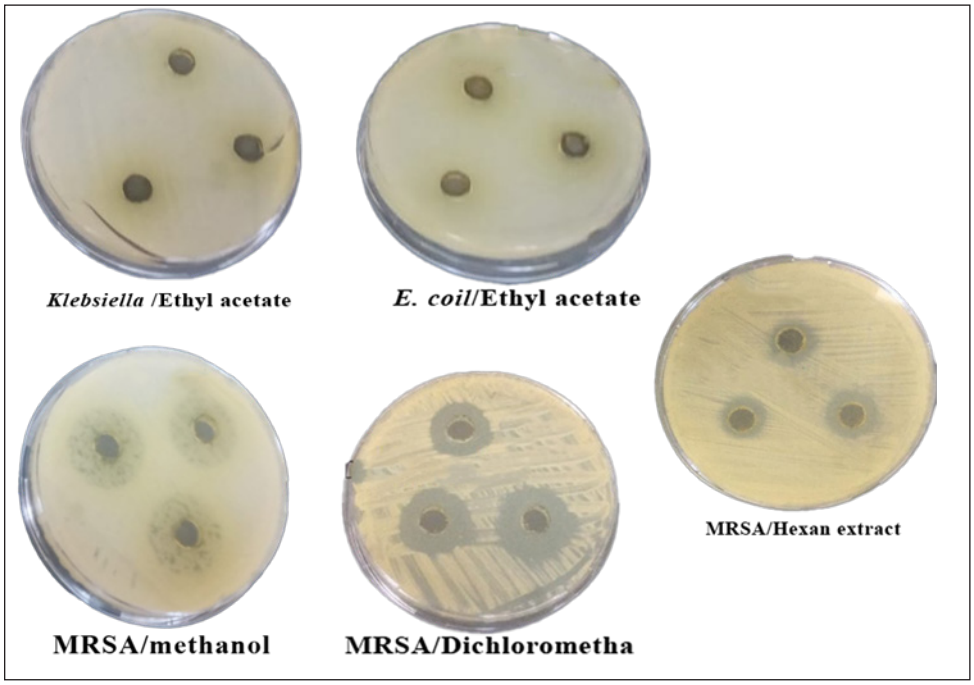

Fig. 3. Well diffusion of different extracts on different types of bacteria.

with MBC values ranging from 18.75 to 37.51 mg/ml. The MBC values of dichloromethane extract were in *S. aureus* = *E.coli* ≤ MRSA = *K. pneumonia*. Figures 3 and 4 provide visual representations of selected examples illustrating the MIC determined through the gel diffusion method and the MBC of various extracts tested against different bacterial strains.

Cytotoxicity of the plant extracts against HTC-116 and HUVEC cell lines

The MTT assay was used to assess the cytotoxic effects of different AHA extracts against a colorectal carcinoma cell line (HCT-116) in comparison to the HUVEC cell line. As depicted in Figure 5, it is evident that all extracts exhibit potential anticancer activity in a dose-dependent manner following a 48-hour treatment, the results are expressed as % inhibition. Table 3 illustrates the results of cytotoxic effects (IC50) of different extracts on HCT-116 and HUVEC cell lines. It displayed notable variations among the examined extracts, with the methanol fraction showing the highest effect (IC50 = 126.61 ± 13.35 µg/ml), followed by the ethyl acetate extract (IC50 = 259.83 ± 9.55 µg/ml), the dichloromethane extract (IC50 = 266.07 ± 1.61 µg/ml), and finally, the *n*-hexane extract (IC50 = 351.55 ± 8.39 µg/ml) compared with HUVEC cell line.

Discussion

The reports from the World Health Organization highlighting the growing prevalence of antibiotic-resistant bacteria have intensified the exploration of plant extracts with antibacterial properties for therapeutic purposes (Kebede *et al.*, 2021). As a member of the Asteraceae family, *Artemisia* is among several genera known for its richness in secondary metabolites and essential oils, which possess valuable therapeutic applications. In this aspect, AHA extracts have been shown a broad antibacterial action (Daoudi *et al.*, 2022; Ouchelli *et al.*, 2022).

The choice of an appropriate solvent is a critical step in the extraction of natural resources. Therefore, this study focused on evaluating both the extraction yield and the antibacterial efficacy of AHA using different organic solvents, including *n*-hexane, methanol, dichloromethane, and ethyl acetate.

The extraction yield differs depending on the choice of solvents. These variations in extraction yields can be attributed to the differing polarities of the solvents used. In the realm of phytochemical extraction techniques, methanol stands out as the most polar solvent commonly utilized. In the literature, it is often recommended to maximize the extraction of bioactive components from plants (Seo *et al.*, 2014).

The results showed that methanol, being the most polar solvent, yielded a higher extraction quantity compared to *n*-hexane, which is less polar in nature. On the other hand, the results suggest that AHA contains substances with higher polarity than the others, which aligns with the findings of Benmeziane *et al*. (2023), who noted that the extraction rate is influenced by the polarity of solvents.

Benmeziane *et al.* (2023) reported that the AHA methanol extract yields approximately 12.2%, while the ethyl acetate extract yields around 4.5%. In contrast,

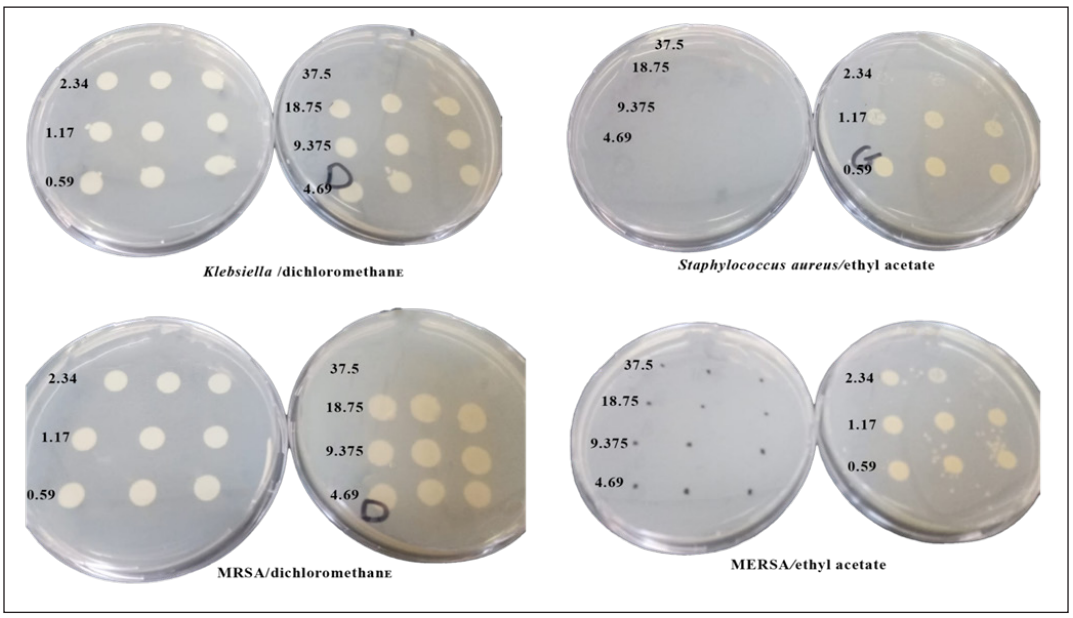

Fig. 4. MBC of different extracts on different types of bacteria.

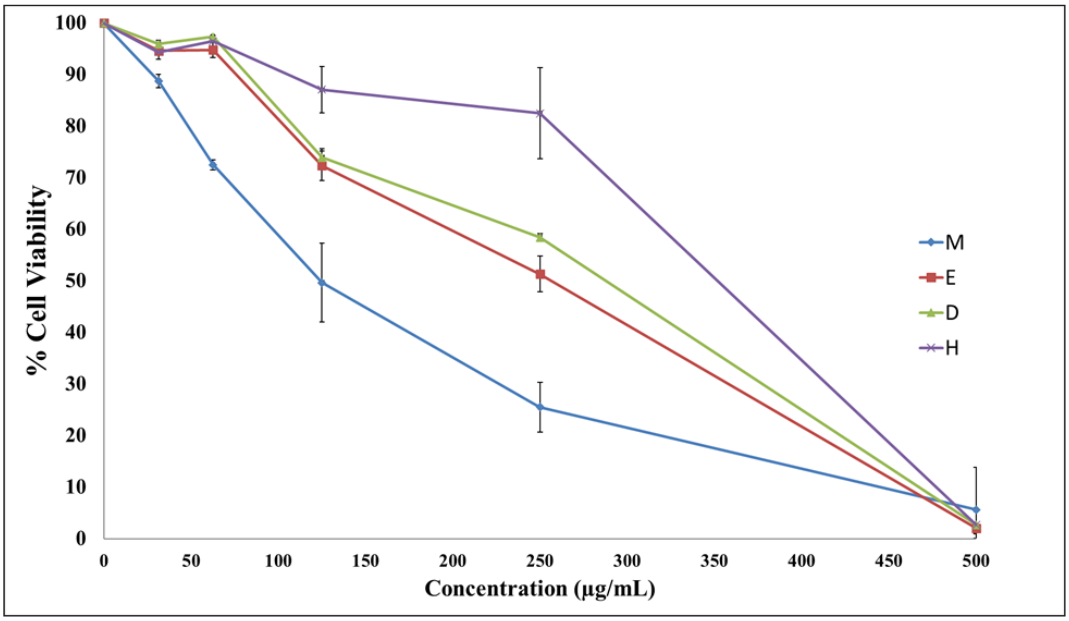

Fig. 5. Cytotoxic effect of different AHA extracts against HCT-116 cell line. M = methanol extract, E = ethyl acetate extract, D = dichloromethane, H = *n*-hexane extract. All values are expressed as mean ± SD, *n* = 3.

Table 3. Cytotoxic effects of different extracts on HCT-116 and normal cell line (HUVEC).

Cell name	Methanol	Ethylacetate	Dichloromethane	*n*-hexane	5 –fluorouracil (positive standard)
HTC-116	126.61 ± 13.35	259.83 ± 9.55	266.07 ± 1.61	351.55 ± 8.39	4.61 ± 0.17
HUVEC	>450	>490	>400	>413	–

(HUVEC): Human umbilical vein endothelial cells.

the present study demonstrated relatively higher yields, with methanol extract at 13.96% and ethyl acetate extract at 6.21%.

Clearly, the *n*-hexane extract displayed notable efficacy against all the tested microorganisms, particularly showing strong inhibitory effects against the *S. aureus* strain, with an impressive inhibition zone diameter of 24.67 mm. Moreover, the findings revealed that all extracts exhibited antimicrobial activity against the MRSA strains. Among these extracts, the methanol extract demonstrated the most significant inhibition zones, measuring 24 mm, a result comparable to the effectiveness of erythromycin. Furthermore, the results revealed that all extracts exhibited activity against the MRSA strains, with the highest level of activity observed in the methanol extracts. This particular extract demonstrated the most substantial inhibition zones, measuring 24 mm, a level of effectiveness comparable to that of erythromycin.

These findings are consistent with the outcomes of previous studies conducted by Khan *et al.* (2022), who also reported that *n*-hexane and methanol solvent extracts exhibited the most potent antimicrobial activities.

Dilabazian and Na'was (2019) conducted a study on the antibacterial properties of *Artemisia verlotiorum* methanol extract against 5 MRSA strains and 13 methicillin-sensitive *S. aureus* strains (MSSA) using the well agar diffusion assay. They observed that the extract effectively inhibited both groups of *S. aureus* (MRSA and MSSA). This outcome is likely attributed to methanol's ability to dissolve a higher quantity of secondary metabolites, which could enhance its antimicrobial potential.

In general, Gram-positive bacterial strains were inhibited by all extracts while Gram-negative bacteria were inhibited only by *n*-hexane and dichloromethane extracts. This result was consistent with other studies, which showed that *n*-hexane-based plant extracts had higher antibacterial activity compared to the other solvents extract (Hussain *et al.*, 2022). Also, this result is consistent with those of the previous investigations of Mohammed *et al.* (2021). Ahameethunisa and Hopper (2012) studied the antimicrobial activity of *n*-hexane extracts of *Artemisia parviflora* which showed maximum activity against *S. aureus*, *E. coli,* and *K. pneumoniae*.

This study demonstrated that *K. pneumoniae* and *E. coli* were resistant to AHA ethyl acetate extract. In a previous study, six organic *Artemisia nilagirica* solvents were tested against 15 phytopathogens and clinically significant standard reference bacterial strains. The results revealed that all the extracts had inhibitory activity against Gram-positive and Gram-negative bacteria, with the exceptions of *K. pneumoniae*, *Enterococcus faecalis*, and *S. aureus*, which showed these bacteria, were resistant to *A. nilagirica* extracts.

In research by Hussain *et al.* (2022), it was shown that *A. rutifolia* extracts in methanol, ethyl acetate, and *n*-hexane are rich in flavonoids and phenols, and that all of the studied extracts effectively inhibited the development of both Gram-positive and Gram-negative bacteria across a broad spectrum.

More accurate data on antibacterial activity were obtained by determining the MIC and MBC values in Table 2. Except for the ethyl acetate extract of AHA against *S. aureus* (9.375 mg/ml), all plant extracts in the current investigation exhibited MBC values about two times more than their equivalent MICs. This suggests that the extract had both bacteriostatic and bactericidal effects at the same dose.

According to MIC and MBC values (a very low MIC exhibits strong antibacterial effects), when compared to cefotaxime, methanol, ethylacetate, and *n*-hexane extracts exhibited a higher antibacterial effect against MRSA (MIC = 1.17 ± 1.09 mg/ml, MBC = 9.375 ± 0.0 mg/ml), (MIC = 2.34 ± 1.36 mg/ml, MBC = 4.69 ± 2.7 mg/ml), and (MIC = 2.34 ± 1.25 mg/ml, MBC = 18.75 ± 2.7 mg/ml), respectively, whereas *S. aureus* and *K. pneumonia* were the most resistant with a high MIC and MBC values of AHA by utilizing methanol extract (MIC = 37.5 ± 0.0 mg/ml, MBC = 75 ± 7.16 mg/ml), (MIC = 37.5 ± 0.0 mg/ml, MBC = 75 ± 0.0 mg/ml), respectively. These values are quite comparable to those reported by Bertella *et al.* (2018), using an additional chemotype of AHA essential oils against *S. aureus* strains (MRSAA1, MRSAB1) with MIC and MBC of 5 and 10 mg/ml, respectively.

In our study, the highest antibacterial activity (MIC = 1.17 ± 1.09 mg/ml and MBC = 9.375 ± 0.0 mg/ml) against MRSA was recorded for methanol extract. The antibacterial activity against MRSA methanol extract, Echeverría *et al.* (2017), confirmed the substantial antimicrobial activity exhibited by the methanolic extract of *Artemisia absinthium*, suggesting that the bioactivity of flavones and flavonoids are potential antibacterial agents is responsible for antibacterial activity, also, the same author reported the spectrum of activity of flavones was more against Gram-positive bacteria because of the wide range of lipophilicity and cell lysis.

In addition, other investigations of *Artemisia* species' *in vitro* antimicrobial activity have shown findings that are comparable to those of the current study. The methanolic extract of *Artemisia campestris* and AHA grown in Southern Algeria had a negative inhibitory effect on *E. coli and K. pneumonia* (Bakchiche *et al.*, 2022). In addition, Mashraqi *et al.* (2023) reported on negative inhibitory effect on *S. aureus* by using methanol extract of AHA and *A. absinthium*.

Consequently, *n*-hexane was more effective against all of the tested bacteria; this may be because the active chemicals are more soluble in hexane, this was also noticed by Abu-Darwish *et al.* (2015). There have been

several researches on the antibacterial and antioxidant properties of Artemisia species across the world and it is thought that these plants produce important secondary metabolites that have therapeutic benefits against illness (Erel *et al.*, 2012; Javid *et al.*, 2015). Hexane extract is a very effective inhibitor for clinical pathogenic bacteria, as shown by the results of the zone of inhibition and MIC investigations (Ahameethunisa and Hopper, 2012).

Ahameethunisa and Hopper (2010, 2012) studied the antibacterial activity of different solvent extracts of *Artemisia parviflora* grown in India (e.g., methanol, ethanol, chloroform, *n*-hexane, petroleum ether, ethyl acetate, and acetone) showed inhibitory effect against Gram-positive and Gram-negative bacteria. The results revealed that the *n*-hexane extract of *A. parviflora* showed a high level of antimicrobial activity for all the microbes tested with the MIC value of 32–64 mg/ml. Methanol and ethyl acetate extracts of *A. parviflora* showed moderate antimicrobial activity, due to the presence of higher concentrations of active antimicrobial agents such as terpenoids, phenolics, and volatile oils, in *Artemisia* species (Hussain *et al.*, 2022).

It is well-established that the efficacy of plants is linked to their composition of bioactive compounds. Consequently, numerous studies have been conducted on the whole plant of AHA, employing various solvents and essential oils, to explore the presence of these bioactive compounds. One study conducted by Hudaib and Aburjai (2006) reported that the main components of the essential oil from the aerial parts of AHA from Jordan were *trans*-sabinyl acetate (5.4%), germacrene D (4.6%), α-eudesmol (4.2%), and caryophyllene acetate (5.7%) (Hudaib and Aburjai, 2006). Also, Amkiss *et al.* (2021) showed that the major phytocomponents identified in AHA ethanolic extract using GC–MS were Anobin (37.30), a- Santonin (36.53), and Alkhanin (36.02). Moreover, another study done by Tilaoui *et al.* (2011) showed that the major chemical composition of the essential oil of aerial parts of AHA was Bisabolone oxidea (17.55) and Farnesene epoxide, Ea (17.08). They also reported that the major compounds identified in this plant were β-Thujone (25.1), α-Thujone (22.9), and 1,8-Cineole (20.1).

In addition, terpenoids are among the most commonly encountered active antimicrobial compounds discovered in plants. Research has demonstrated that terpenoids possess antimicrobial properties against bacteria, including strains that are both susceptible to and resistant to antibiotics. This is mostly due to their capacity to encourage cell rupture and to hinder the production of protein and DNA (Álvarez-Martínez *et al.*, 2021).

Continuously searching for new natural anticancer compounds within medicine plants and traditional foods is a promising strategy for discovering a new anticancer agent that is safe and able to overcome the multidrug resistance developed against conventional chemotherapy. Accordingly, the present research assessed the cytotoxic effects of AHA extracts against colorectal carcinoma cell lines (HCT-116). Our results indicate that the different solvent fractions displayed significant inhibitory effects on the growth of HCT-116 cells in a dose-dependent manner. Among the tested extracts, the methanol fraction exhibited the highest cytotoxic effects (IC_{50} = 126.61 ± 13.35 µg/ml), followed by ethyl acetate (IC_{50} = 259.83 ± 9.55 µg/ml), dichloromethane (IC_{50} = 266.07 ± 1.61 µg/ml), and *n*-hexane extracts (IC_{50} = 351.55 ± 8.39 µg/ml). 5-Fluorouracil, an anticancer drug, was utilized as a positive control to evaluate and compare the anticancer efficacy of various extracts. Furthermore, the HUVEC normal cell line affirmed the selectivity of various extracts, as all of them demonstrated no activity against HUVEC at concentrations exceeding 400 µg/ml.

Our findings align with those of Khlifi *et al.* (2013), who reported that methanol extract from AHA caused a reduction in cell viability of various human cancer cell lines, including human bladder carcinoma RT112, human laryngeal carcinoma Hep2, and human myelogenous leukemia K-562. In addition, Mohammed *et al.* (2019) demonstrated the significant cytotoxic and anti-proliferative effects of AHA methanolic extract on the e human hepatocellular carcinoma (HepG2) cell line. To the best of our knowledge, there is currently no existing study that has conducted a comparative analysis of the anticancer potential of AHA extracts obtained using solvents of varying polarities. The current study findings have revealed noteworthy disparities in the cytotoxic effects of the examined fractions. These differences can be ascribed to the unique bioactive compounds present in each extract, a characteristic that is predominantly influenced by the selection of the extraction solvent (Purnamasari *et al.*, 2019). It is noteworthy that the cytotoxic impact of AHA was enhanced by increasing solvent polarity, suggesting that its active anticancer constituents are polar compounds. In prior studies, an evaluation of the chemical composition of the methanolic extract from AHA revealed that the polar sesquiterpenes, artemisinin, is among its primary constituents (Maggar, 2012). It was recently found that artemisinin has anticancer effects against primary colon cancer (SW480) and lymph node metastatic (SW620) (Otto-Ślusarczyk *et al.*, 2021). Furthermore, the study of (Jakovljević *et al.*, 2020) showed that the chlorogenic acid and quercetin-3-O-glucopyranoside of AHA methanolic extract was the most abundant polyphenolic compounds which are characterized for their anticancer properties (Maiyo *et al.*, 2016).

Conclusion

The methanol extract exhibited the most potent effects against MRSA and HCT-116, while the AHA hexane

extract demonstrated the most effective antibacterial action against *S. aureus*. Future research efforts should emphasize on isolating particular chemical constituents from AHA and give priority to assessing their cytotoxic effects on mammalian cells. If certain elements prove to be safe, it would be intriguing to explore their potential therapeutic roles in addressing specific diseases, potentially combining traditional remedies with pharmaceutical treatments.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Author contributions

RA, SD, MA, FN, and SS concept and design the proposal. SA, SA, MA, FN, and OA collect the required data. RA, SD, SS, OA, SA, MA, and FN analyzed the collected data and wrote the manuscript. All authors revised and approved the final manuscript.

Data availability

All data generated and analyzed are included in this research article.

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