PHYTOCHEMICAL PROFILING OF *Artemisia herba-alba* Asso BY HPLC ANALYSIS AND INVESTIGATION OF THE ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES

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Abstract. The objectives of this study were to determine the extraction yield and phytochemical composition of *Artemisia herba-alba* (AHA) extract, as well as to investigate *in vitro* their antioxidant, and antibacterial activities. Antioxidant activity was carried out using DPPH radical scavenging test. The antibacterial potential of *A. herba-alba* against four bacterial strains was evaluated by agar diffusion and a modified broth macro-dilution method was used to measure the minimum inhibitory concentration (MIC). HPLC analysis identified six phenolic compounds in high concentration as folow; gallic acid 562.253 µg/g, chlorogenic acid 19159.806 µg/g, vanillic acid 776.255 µg/g, caffeic acid 512.314 µg/g, vanilin 4050.792 µg/g, coumaric acid 504.572 µg/g, rutin 6739.788 µg/g and quercetin 772.118 µg/g are all present. Additionally, the results demonstrated, the DPPH assay of free radical elimination yields a relatively poor antioxidant activity, with an average IC_{50} of 11.79 mg/mL. Additionally, employing the diffusion method on an agar medium, the antibacterial activity data demonstrate a considerable weakening in the species' inhibition. Regarding the minimum inhibitory concentration (MIC), we found that *Escherichia coli* exhibits the smallest zone of inhibition, which is equivalent to 10 mg/mL and *Staphylococcus aureus* exhibits the smallest zone of inhibition, which is equal to 25 mg/mL. On the other two species, *Klebsiella pneumoniae* and *Enterococcus faecalis*, the extract had the least effect. The correlation coefficients obtained with PCA (Principal Compound Analysis) are: *Staphylococcus aureus*: correlation coefficient = 0.935; *Escherichia coli*: correlation coefficient = 0.949; *Klebsiella pneumoniae*: correlation coefficient = 0.707; *Enterococcus faecalis*: absence of variability.

Key words: *Artemisia herba-alba*; phenolic compounds; HPLC analysis; antibacterial activity; antioxidant activity.

INTRODUCTION

A medicinal plant is a plant that has been utilized for treating illnesses from ancient times because of its curative qualities. Its components (leaf, stem, root etc.) can all be employed for healing, at least one of which. The foundation of both conventional and contemporary herbal therapy is these herbs. Their components, which are many and vary by species and have a wide variety of active substances, are what determines how effective they are [12, 15].

Twenty of the over 250 species of mugworts, a polymorphic genus, are found in the Maghreb and the Sahara (12 are in Morocco, 10 in Algeria, 6 in Tunisia, 5 in Libya, and 4 in Egypt) [23].

Triterpenes and sterols, two naturally occurring active components, are abundant in the composition of artemisia. These are typically compounds that act as antioxidants, like flavonoids and flavones. Thujones and other potent active compounds, like beta-caryophyllene, make up the essential oil itself. Along with tannins, resin, and inulin, artemisia also includes bitter compounds. Vitamins A, C, and several of the B groups are present in its leaves [32].

The plant is an antioxidant with beneficial anti-free radical capabilities (and their harmful effects on cells). Free radicals are necessary parts of our bodies. However, when they are present in excess, they hasten cellular aging and encourage the growth of cancer and cardiovascular disorders. As a result, antioxidant plants like mugwort aid in the body's removal of extra free radicals [1].

Artemisia herba-alba, known as white mugwort or desert wormwood, is a plant with many traditional uses, particularly in North Africa, the Middle East and Asia. Used for centuries, it is renowned for its digestive properties, helping to relieve stomach upset, indigestion and bloating. Its antiparasitic properties make it a common remedy against intestinal infections [26]. Many reports ascribed various biological and/or pharmacological activities of the *A. herba-alba* in particular the antimicrobial activity [27, 37]. Therfore, its antimicrobial and antifungal properties make it useful for treating infections and disinfecting wounds. White mugwort is also used to soothe respiratory conditions such as coughs and bronchitis thanks to its expectorant effects [36]. In some cultures, it is used to reduce fever and treat malaria, and is also known for its anti-inflammatory and analgesic abilities, providing relief from joint and muscle pain [5]. It is sometimes used to manage and gynecological disorders, as well as as a general tonic to improve overall health [23]. Furher, it is also used in traditional medicine as an antispasmodic and in treatment of diabetes mellitus [2, 35, 38]. In Algeria, few reports investigated the characteristics and the biological activities of the arterial parts of *Artemisia herba-alba*. Therefore, the objectives of this study were to determine the extraction yield and phytochemical composition of *Artemisia herba-alba* extacts, as well as to investigate *in vitro* their antioxidant, and antibacterial activities.

MATERIALS AND METHODS

Chemicals

This investigation employed several analytical-grade compounds, including Folin-Ciocalteu reagent, gallic acid, ascorbic acid (Vitamin C), quercetin, tannic acid, sodium carbonate, aluminium chloride, 2,2-diphenyl-1-picrylhydrazyl (DPPH), potassium

dihydrogen phosphate (KH_2PO_4), dibasic potassium phosphate (K_2HPO_4), potassium ferricyanide ($C_6N_6FeK_3$), linoleic acid ($C_{18}H_{32}O_2$), β-carotene ($C_{40}H_{56}$), chloroform ($CHCl_3$), tween 40 ($C_{62}H_{122}O_{26}$), ferric chloride ($FeCl_3$), gallic acid ($C_7H_6O_5$), trichloroacetic acid (TCA), and dimethyl sulfoxide (DMSO). All chemicals and reagents were purchased from Sigma-Aldrich (USA) and were of the highest available purity.

Collection and identification of the plant

The Artemisia herba-alba plant material used in this study was harvested in March 2022 during the flowering period from the aerial portion of the plant in the town of Ain Maabed, located in the Wilaya of Djelfa, Algeria. This region is situated at latitude 34°48'17" North and longitude 3°7'46" East, covering an area of 328.02 km² with an elevation of 1038 meters. The climate in this area is classified as dry, chilly, and semi-arid. The plant material was authenticated by Pr. Chouikh Atef at the Department of Cellular and Molecular Biology, Faculty of Natural and Life Sciences, and a voucher specimen has been deposited in the herbarium under reference number DJF-2022-123, ensuring the authenticity of the plant material used in our research.

The aerial parts of Artemisia herba-alba were thoroughly cleaned and air-dried at room temperature in a well-ventilated, shaded area for two weeks to prevent the degradation of sensitive compounds. Once fully dried, the plant material was ground into a fine powder using a mechanical grinder. The powdered samples were then stored in airtight containers at room temperature, away from light and moisture, until further analysis.

Table 1. Bioclimatic Conditions of the Harvesting Zone for *Artemisia herba-alba* in Ain Maabed, Djelfa, Algeria

Parameter	Value
Location	Ain Maabed, Djelfa, Algeria
Latitude	34°48'17" N
Longitude	3°7'46" E
Elevation	1038 m
Area	328.02 km²
Climate type	Semi-arid
Temperature range	10°C - 30°C (average)
Annual precipitat	300 mm
Temperature range	10°C - 30°C (average)
Wind speed	10 km/h (average)
Humidity	45% (average)
Soil type	Sandy loam

Bacterial strains

The agar well-diffusion method was employed to assess the efficacy of the crude extract and the fractions isolated from *Artemisia herba- alba* against six pathogenic bacteria, including three Gram-positive bacteria (B.S: *Bacillus subtilis* ATCC 6633; S.A: *Staphylococcus aureus* ATCC 6538; L.I: *Listeria innocua* CLIP 74915) and three Gram-negative bacteria (E.C: *Escherichia coli* ATCC 8737; P.A: *Pseudomonas aeruginosa* ATCC 9027; S.T: *Salmonella typhimurium* ATTCC 14028). The Petri dishes were incubated for 24 hours at 37 °C. The inhibitory zone was characterized by a millimeter inhibitiondiameter. Three repitition were used for the antibacterial tests [4].

Determination of minimal inhibitory concentration (MIC)

Using a modified broth macro-dilution approach, the MIC of *Artemisia herba-alba* crude extract and its fractions was assessed against the six previous bacteria. Each bacterial strain was divided into two sets of sterile test tubes (13 x 100 mm) with cotton plugs and 0.2 mL of bacterial inoculum. For 24 hours, each test tube was kept at 37 °C.

Calculation of yields in dry extracts

Methanol and water are combined in a 70/30 volume ratio for the maceration of *Artemisia herba-alba*. This maceration process is performed three times using fresh solvent for each extraction. Each maceration is agitated for 24 hours to ensure thorough extraction. After carefully decanting the three extracts onto Whatman filter paper, the resulting filtrates are combined. The combined filtrate is then evaporated using a rotary evaporator at a temperature range of 45 to 60 °C until all methanol is removed. Subsequently, the extract is dried in an oven at a temperature not exceeding 40 °C. The ratio between the mass of the dry extract obtained after evaporation and the original mass of the plant material employed is known as the yield of the *Artemisia herba-alba* extract. The following equation is used to compute this yield:

$$\text{Yield } \% = m_0 / m_1 \times 100$$

- m_0: the dry residue's mass, measured in grams.
- m_1: initial dry plant matter mass in grams.

Analysis of phenolic compounds by high-performance liquid chromatography (HPLC)

Using scanning equipment and high-performance liquid chromatography, the active components were discovered (HPLC). For the investigation of phenolic chemicals in crude extract, we utilized HPLC with UV-Vis type Shimadzu LC20 AL equipped with the universal injector (Hamilton 25l), an analytical column was a Shim-pack VP-ODSC18 (4,6 mm, 250 mm, 5 m), and UV-VIS detector SPD 20A type (Shimadzu). The reverse-phase chromatography studies were conducted using non-polar aliphatic residues, and the mobile phase comprised of gradient elution of a combination of acetonitrile and acetic acid (0.1%). The injection volume was 45 µL and the flow rate was 1 mL/min. The monitoring wavelength was 268 nm, and the sample and standard injection volume were 20 µL.

The retention duration and UV absorbance of various compounds were compared to those of the standards to identify them

Antioxidant activity

DPPH free-radical scavenging activity (DPPH)

The 1,1-diphenyl-2-picrylhydrazyl solution is made by dissolving 2.4 mg of DPPH in 100 mL of methanol. The same volume of each phenolic extract (or ascorbic acid as control) and of the prepared DPPH solution was mixed. The reaction mixture is stirred briefly, then allowed to cool to ambient temperature for 30 minutes

while being kept in the dark. At 517 nm, the absorbance of the reaction medium is measured and contrasted with that of the control (methanol-distilled water) [25, 48]:

Anti-radical activity % =

[(Abs control - Abs sample) / Abs control] x100

Antibacterial activity

As noted before, the agar diffusion method was used to assess the materials' antibacterial activity. Prior to usage, the bacterial strains were grown on nutrient agar for 24 hours at 37°C while in the stationary phase of growth. The suspension of bacterial cells is another factor (10^6 colony-forming units per mL). Were applied to Petri dishes containing Mueller Hinton agar (using sterile swabs). The discs (6 mm in diameter) were then immersed in 10 microliters of plant extract that were all dissolved in DMSO (5%, v/v) at different concentrations. Cephalexin (30 µg/mL) and (DMSO 5%, v/v) were used as a positive and negative control, respectively, and incubated for 24 hours at 37 °C [11, 36].

Determination of minimal inhibitory concentration (MIC)

MIC of extracts against the previous six bacteria was determined by an amended broth macro-dilution method. For MIC estimation, stock solutions of five different concentrations of each one of our samples. Additionally, were made in MHB broth that had been autoclaved. For each bacterial strain, two sets of sterile test tubes (13 x 100 mm) with cotton plugs and 0.2 mL of bacterial inoculum were set up. The ultimate volume of media in the flasks was 4 mL as a result of individually inoculating each test tube with 2 mL of each concentration of samples. As positive and negative controls, MHB with bacterial inoculum and MHB with all of the prior samples but without bacterial inoculum, respectively, were used. All test tubes were kept at 37°C for 24 hours [46].

Statistical analysis

Excel 2010 was used to express the experimental results as a mean plus or minus the standard deviation for the assay and evaluation of biological activities. To evaluate the importance of these values, a one-way analysis of variance was added.

In order to determine the relationship between the concentration of the sample and the obtained zone of inhibition, a correlation test for antibacterial activity was conducted. Principal component analysis (PCA) is used to determine the correlation between the examined data (PCA). The STATISTICA program, version 6, was used to conduct these statistical analyzes.

RESULTS

Methanol extraction yield

The extracted substance has a yield of 16.62%, is light brown (yellow-brown), appears mushy, and dries up when exposed to air. But according to the research of [26], the extraction yield of *Artemisia herba-alba* with aqueous methanol (70/30) is only 13.3% for 10g of plant material, or 66.5% for 50 g of plant material.

Identification of phenolic compounds by high-performance liquid chromatography (HPLC)

The HPLC analysis of the aqueous crude extract of *Artemisia herba-alba* identified eight phenolic compounds out of nine reference standards from 65 peaks. The phenolic compound with the highest concentration was chlorogenic acid (19159.806 µg/g), followed by rutin (6739.788 µg/g) and vanillin (4050.792 µg/g). In contrast, lower concentrations of other phenolic compounds were detected, including vanillic acid (776.255 µg/g) and caffeic acid (512.314 µg/g). These results demonstrate a diverse phenolic profile in Artemisia herba-alba, with chlorogenic acid being the most abundant compound, highlighting its potential contribution to the plant's biological activity (Table 2).

Table 2. Characteristics of phenolic standards analyzed by HPLC

Compounds phenolic	Retention time (min)	Equation content	Concentration (µg/g extract)
Chlorogenic acid	13.392	y=21665x	19159.806
Caffiec acid	16.277	y=84066x	512.314
p-Coumaric acid	23.817	y=49495x	504.572
Gallic acid	5.29	y=54681x	562.253
Vanillic acid	15.53	y=65077x	776.255
Naringin	34.788	y=19379x	ND
Rutin	28.37	y=28144x	6739.788
Quercetin	45.047	y=45378x	772.118
Vanillin	21.46	y=58930x	4050.792

y: HPLC peak area, x: concentration (µg/mL), ND: not detected.

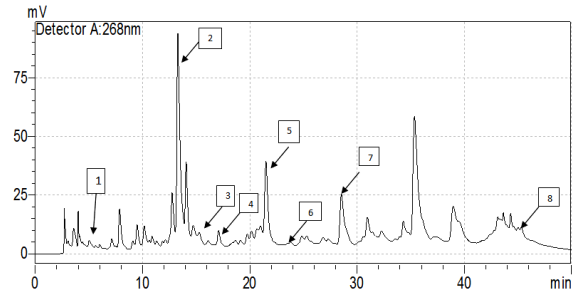

Figure 1. Chromatogram of an extract of *Artemisia herba-alba*, where 1: Gallic acid; 2: Chlorogenic acid; 3: Vanilic acid; 4: Caffiec acid; 5: Vanilin; 6: p-Coumaric acid; 7: Rutin; 8: Quercetin.

Evaluation of antioxidant activity

In this investigation, the scavenging abilities of our extract were determined using a technique based on the relatively stable radical DPPH (2,2-diphenyl-1-picryl hydrazyl). It has a 515 nm absorbance band. The antioxidant capacity of the extract of our investigated plant is calculated and compared using the activities of the compounds of the standards (table 3).

Table 3. Antioxidant capacity of *Artemisia herba-alba*

Extracts/standards	DPPH· (IC_{50}: µg/mL)
Artemisia herba-alba	11.79 ± 0.13
Ascorbic acid	0.0013±0.0019

Antibacterial activity

We investigated the antibacterial activity of our extracts using the agar diffusion method to assess their utility.

Table 4 exhibit the results of the antibiograms.

Table 4. Sensitivity (diameter of the inhibition zones in cm) of the tested bacterial strains in relation to the antibiotic

Bacterial strains	Zone of inhibition (average)
Staphylococcus aureus ATCC 25923	1.9± 0.7cm
Escherichia coli ATCC 25922	1.6± 0.5 cm
Klebsiella pneumoniae ATCC 13886	1.9± 0.6 cm
Enterococcus faecalis ATCC 51299	1.2± 0.4 cm
DMSO	Nill
Ciprpfloxicne	2.5± 0.7 cm

Figures 2, 3 and 4 provide an illustration of the evaluation of the antibacterial activity of the *Artemisia herba-alba* plant's extract. The agar diffusion method sensitivity test is extremely highly significant, with a P value of 0.001, according to the analysis of variance. The zones of inhibition presented by *Escherichia coli*, *Staphylococcus aureus, Klebsiella pneumoniae,* and *Enterococcus faecalis* against the antibiotic are, respectively, 1.6±0.5 cm cm, 1.9± 0.6 cm, 1.9± 0.7 cm, and 1.2±0.4 cm in diameter, showing a significant difference in the diameters of the zones of inhibition presented by the strains used against the antibiotic and the extract.

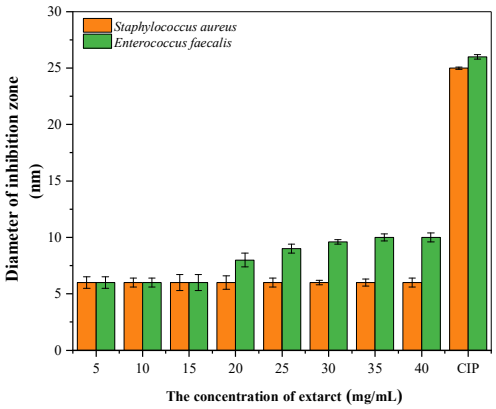

Figure 2. Gram-positive antibacterial activity of *Artemisia herba-alba.*

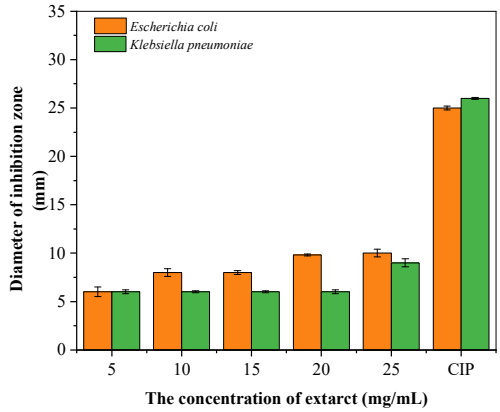

Figure 3. Gram-negative antibacterial activity of *Artemisia herba-alba*.

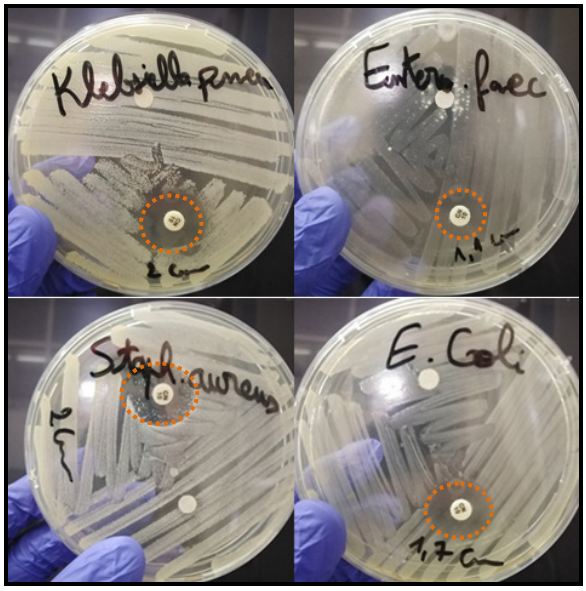

Figure 4. Results of the bacterial strain susceptibility test

Determination of MIC by dilution method on agar medium

On the most active extracts, a MIC concentration margin was also determined using the dilution procedure on an agar medium. *Escherichia coli* and *Staphylococcus aureus* species required minimum inhibitory concentrations in our extract of 10 mg/mL and 25 mg/mL, respectively. According to Marrif *et al.* (1995) [34], the MIC value for *Escherichia coli* was 2.125 mg/mL and 1.062 mg/mL for *Staphylococcus aureus*. In contrast to what our data indicated, the results revealed a significant antibacterial activity. *Escherichia coli* and *Staphylococcus aureus* readings from Marrif *et al.* (1995) [34] were much lower than our values for both species, which were restricted to the ranges of 0.038 mg/L to 0.311 mg/L and from 0.155 mg/L to 0.624 mg/L, respectively.

Correlation test of antibacterial activity

We note a positive correlation between the concentration of the extract and the diameters of the zones of inhibition obtained through the results of the correlation test applied to the extract samples. This is true for all the studied species except for *Enteroccocus faecalis*, which exhibited no activity. *Staphylococcus aureus:* correlation coefficient = 0.935; *Escherichia coli:* correlation coefficient = 0.949; *Klebsiella pneumoniae:* correlation coefficient = 0.707; *Enterococcus faecalis*: absence of variability.

DISCUSSION

The great range of pharmacological activities of medicinal plants are mostly attributed to their phytochemical components [8]. Biologically active substances are recognized to be chemical components in plants or crude extracts. Secondary metabolites components include some chemical compounds. They are directly responsible for antioxidant, antibacterial, antifungal and anticancer activities [17]. The yield of chemical groups that are more frequently employed in

medicine was calculated in this study using specific extractions of *Artemisia herba-alba* aerial sections. This study found that utilizing a different technique to extract the plant's leftovers with 100% methanol yielded a result of 19%, which is very identical to our results. Gill Burt (2010) [24] found that when other *Artemisia vulgaris* species of the Asteraceae family were studied, the yield of extracting the aerial parts with an aqueous methanol solution at 70% for 10 days while sometimes agitating the mixture was only 5.5%. In comparison to our maceration result, this value is substantially lower.

As a result, the yield rate varies depending on the extraction method and the plant portion. The composition and polarity of the solvents used for the extraction can explain this discrepancy [8, 42]. Additionally, it is affected by a number of variables, including pH, temperature, extraction duration, and sample makeup [16]. This explains the poor yield of our maceration process, which takes the longest time compared to other extraction methods, and may be caused by the degradation of some natural chemicals like polyphenols. The longer the extraction period, the lower the yield [18, 47].

Flavonoids are also thought to be anti-inflammatory, anti-tumor, anti-carcinogenic, hypo-tensive, diuretic, and antioxidant. We first differentiate the derivatives of benzoic acid, made up of a skeleton of seven carbon atoms, such as vanillic acid and gallic acid, among the compounds discovered by HPLC in the extracts of plants dried according to different modes. In parallel, phenol acids produced from C3-C6 cinnamic acid include caffeic acid and chlorogenic acid. Antioxidant metabolites include vanillic acid, gallic acid, and caffeine acid [14, 22].

Ascorbic acid, gallic acid, and chlorogenic acid levels in dried fruits are surprisingly high after drying, according to Pannaratn et *al*. (2010) [44]. The presence of phenolic acids has a substantially greater influence on the antioxidant potential of *Haloxylon scoparium* and *Aristida pungens* extracts. Gallic acid and caffeic acid are these two taxa's primary phenolic components. Rutin, also known as quercetol 3-O-rutinoside, is a diglycoside of quercetin, a flavonol-like flavonoid that is reasonably prevalent in nature and well-known for its exceptional antibacterial properties. The chemical composition of phenolic compounds in plants, which ranges from simple to highly polymerized molecules, determines their solubility. Phenolic acids, phenylpropanoids, anthocyanins, and tannins can all be found in various concentrations in plant materials [45]. The wide range of physicochemical parameters affecting the extraction of polyphenols is caused by this structural diversity. The polarity of the solvent used also has an impact on the solubility of phenolic compounds. As a result, it is highly challenging to establish a procedure that could effectively extract all of the plant's phenolic components [33].

The findings of the DPPH test are shown in Table 2, given as the concentration that inhibits 50% of the radicals (IC_{50}).

When compared to ascorbic acid, our plant extract *Artemisia herba-alba* had very little antioxidant activity. This could be because methanol, which makes up around 75% of the solution, causes phenolic components to precipitate out [47]. We point out that whereas Khennouf et *al*. (2010) [29] reported an IC_{50} value of 0.0329 mg/mL by researching the antioxidant impact of *Artemisia herba-alba*, which is significantly lower than the value we discovered, Bouchara et *al*. (2021) [10] recorded an IC_{50} equal to 140.09 mg/mL. The absence of phenolic components in the extract, particularly flavonoids, which are known to be potential antioxidants with the capacity to scavenge free radical species and reactive oxygen species, may be the cause [7]. Due to their low redox potential, flavonoids have been shown to have a scavenger effect by thermodynamically lowering free radicals (R•) by transferring hydrogen atoms from hydroxyl groups [12, 13]. The presence of polyphenols, flavonoids, and tannins were analyzed using main components analysis, which revealed a substantial positive correlation > 0.9, or close to 1, between the three parameters. Our extract has a very poor antioxidant activity, it might be said.

We can qualitatively estimate the antibacterial efficacy of *Artemisia herba-alba* extract against four bacterial strains using the agar diffusion method, including *Escherichia coli, Klebsiella pneumoniae*, Gram-positive *Enterococcus faecalis*, and *Staphylococcus aureus*. A volume of plant extract at a certain concentration on discs, and measuring the diameters of the zone of inhibition, in millimeters (mm), of the discs. This approach relies on the ability of the active ingredients of the extracts to migrate within a Petri dish filled with a stable nutritional solution (Mueller Hinton) [28, 40].

The antibacterial inhibition power is reflected in these sizes. Regarding DMSO, it is found that this substance has no impact on the examined bacterial strains. Any inhibitory impact that is shown is therefore a result of the extracts' active ingredients. The dimensions of the tablets for all species correspond to the diameters of the zones of inhibition in the extract. The results for the extract were equal to 8mm for *Escherichia coli* at 10 mg/mL (the dosage of the antibiotic Gentamicin employed) (half of the antibiotic inhibition zone). However, at this dose (10 mg/mL), the extract exhibited no activity against the three species *Staphylococcus aureus, Klebsiella pneumoniae* and *Enterococcus faecalis*. In comparison to the antibiotic, the extract appeared to have variable degrees of antibacterial activity against the microorganisms tested at the other concentrations, according to the data. With the exception of *the Enterococcus faecalis* strain, which is resistant to the plant's studied bactericidal and bacteriostatic effects? According to the research of Mighr et *al*. (2010) [35] and Mohamed et *al*. (2010)

[36], the two pathogenic strains of bacteria (*Staphylococcus aureus* and *Escherichia coli*) showed the strongest antibacterial activity, while the bacterium *Klebsiella pneumoniae* showed weak activity in the presence of a single concentration of 25 mg/mL. The study by Bonnaillie et *al*. (2012) [9] and Khennouf et *al*. (2010) [29] supported these findings, indicating similar antibacterial effects against these strains. Additionally, the extract had no effect on the bacterium *Enterococcus faecalis*.

Our findings concur with those of Achat (2013) [2], however they are inconsistent with those of Hameed et *al*. (2014) [25] and Mighri et *al*. (2010) [35]. These findings might indicate that *Klebsiella pneumoniae* and *Enterococcus faecalis* were the two strains most resistant to the extract. The bacteria that responded to the extract with the greatest sensitivity were *Staphylococcus aureus* and *Escherichia coli*, respectively. Therefore, it can be said that the extract exhibited antibacterial activity: weak to nonexistent against *Klebsiella pneumoniae* and *Enterococcus faecalis*, respectively, and weak to nonexistent against *Staphylococcus aureus* and moderate to strong against *Escherichia coli*.

The extract contains bioactive chemicals, which are responsible for its antibacterial activities. These consist of alkaloids, saponins, flavonoids, and tannins [17]. As a result, the difference in antibacterial activity between extracts from the same plant and those from other plants is caused by the latter's variable chemical composition [19]. For *Staphylococcus aureus*, the inhibitory zones had widths ranging from 6 to 10 mm when the extract concentration is 25 to 40 mg/mL. Where as for *Escherichia coli*, these zones range between 6 and 10 in the presence of 5 to 25 mg/mL. As a result, the extract's activity changes proportionally with concentration.

However, the diameters of the zones of inhibition depend on a variety of variables, chiefly: the ability of substances (present in the extracts) to diffuse in the agar medium; the antimicrobial strength of the diffused substances; the growth and metabolic activity of the microorganisms in the medium [6, 32]; and the date of harvest, which can cause very significant variations in chemical composition and activity [3]. The investigation of plant extracts' antibacterial activity should be guided by a set of broad factors. The defining of common parameters, such as plant material, methods employed, growth medium, and microorganisms examined, is of the utmost importance [47]. Some researchers have hypothesized that the antimicrobial components of plant extracts (terpenoids, alkaloids, and phenolic compounds) interact with the enzymes and proteins of the microbial cell membrane, disrupting it to allow a flow of protons to the outside of the cell and causing cell death, or may inhibit enzymes required for amino acid biosynthesis [24, 26].

Other studies have explained that these plant extracts' inhibitory effects result from their hydrophobic properties, which enable them to interact with the proteins and mitochondria of microbial cell membranes and disrupt their structures and alter their permeability [20, 48].

The findings demonstrate that our extract has both Gram-negative and Gram-positive activity, which has been supported by a number of studies including those by Mohammed et *al*. (2021) [38]. However, the formation of the zone of inhibition for the Gram- strain of the bacterium *Escherichia coli* began at a concentration of 5 mg/mL of the extract, whereas the Gram+ strain of the bacterium *Staphylococcus aureus* began to emerge at a concentration of 25 mg/mL.
In line with the findings of Amor et *al*. (2019) [2] and Mwambete et *al*. (2009) [41] Gram-negative bacteria are therefore more resistant to our extract than Gram-positive bacteria. This might be because Gram-negative bacteria have an outside membrane rich in polysaccharides that prevents the cell wall from being permeable to lipophilic substances, while Gram-positive bacteria only have an exterior peptidoglycan layer that is not a reliable permeability barrier [21, 43].

The phenolic chemicals included in the extract, among other things, may contribute to these variations. As we already indicated, our extract only contained small levels of these chemicals. Flavonoids offer a wide range of antibacterial properties. In fact, depending on the type of bacteria, they fight a wide variety of them with varying vigor. Flavonoids have the ability to stop the growth of a variety of bacteria, including *Staphylococcus aureus, Escherichia coli, Enterococcus faecalis, Enterobacter cloaceae, Heliotropium sinuatum, Proteus mirabilis* and others [4, 37, 49]. The extensive therapeutic benefits of tannins, similar to those of other types of polyphenols, including their anti-infective properties, make them quite popular [30, 31]. A significant variance in the extraction method, components, and bacterial strains utilized in various investigations may be the cause of the substantial range in the MIC of *Artemisia herba-alba* extract. Additionally, the MIC of various plant extracts may vary due to the chemical composition of those extracts and the volatile nature of those elements [12, 39, 41, 48].

Our study showed that *Artemisia herba-alba* extract exhibits significant antibacterial activity against various bacterial strains, indicating its potential as a source of bioactive compounds. Rutin, vanillin, and chlorogenic acid were identified as key contributors to this activity. However, contaminants in the crude extract may have reduced its effectiveness. Further research is essential to evaluate in vivo efficacy, understand bacterial resistance mechanisms, and assess the extract's potential toxicity and synergistic effects with conventional antibiotics.

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Conflict of interest. There is no actual or potential conflict of interest in relation to this article.

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