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Research Article

Comparative analysis of the possible radical scavenging, antibacterial and anti-inflammatory effects of several extracts in case of *Artemisia herba-alba* and *Juniperus communis* gathered from Algeria's mountainous regions

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Abstract

This research reports a comparative analysis of the possible radical scavenging, antibacterial and anti-inflammatory effects of several extracts in case of *Artemisia herba-alba* and *Juniperus communis* gathered from Algeria's mountainous regions. The plants extracts total phenolic acid content was ascertained using the Folin-Ciocalteu method, and their total flavonoid content was ascertained using the aluminum chloride colorimetric test. By estimating their ability to remove free radicals using the DPPH method and the ferric reducing antioxidant power (FRAP) assay, their antioxidant capacity was assessed. Disk diffusion was used to measure the antibacterial activity against five strains of bacteria, and a protein denaturation assay was performed to examine the anti-inflammatory activity. A high polyphenol content of 104.54 ±0.35 mg GAE/g was observed in the hydroethanolic extract of *Artemisia herba-alba*, while a high flavonoid content of 17.05 ±0.13 mg QE/g was found in the hydroethanolic extract of *Juniperus communis*. Both species extracts showed important antioxidant activity in two separate tests, where the hydroethanolic extracts demonstrated effective action. The crude extracts showed positive antibacterial activity, especially the hydroethanolic extracts against *Staphylococcus aureus* and *Klebsiella pneumoniae* with a super inhibition on concentration 20 mg/ml. In the anti-inflammatory assay, the hydroethanolic extract of *J. comminus* with IC_{50}: 23.58 ±0.02 µg/mL, has the most potent anti-denaturation effect on albumin. According to the study's findings, the hydroethanolic extracts in case of *Artemisia herba-alba* and *Juniperus communis* gathered from Algeria's mountainous regions are possible sources of phenolic compounds with important natural antioxidant, antimicrobial and anti-inflammatory characteristics that may be employed in pharmaceutical products.

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1. Introduction

Pharmaceutical industries, agri-food and cosmetics are all interested in aromatic plants (Daira et al., 2016). Because the various active compounds they contain —alkaloids, flavonoids, tannins, saponins, and essential oils —give them a variety of medical benefits in addition to their aromatic and culinary properties. They serve as an endless supply of the most potent folk cures and serve as the natural source of the majority of the pharmaceuticals that are currently prescribed (Larayetan et al., 2019).

Because Algeria is known for the diversity of its medicinal flora, which comprises hundreds of plant species, the use of herbal medicine is firmly ingrained in our culture (Dahmane et al., 2023). The so-called secondary metabolites that are accumulated by this plant material are a significant source of compounds like polyphenols and flavonoids, which are frequently utilised in medicine as antioxidants, anti-inflammatories, antibacterials, etc. (Roy et al., 2022).

Juniper falls under the Cupressaceae family, which has various species. The common Juniper (*Juniperus communis*) is a fragrant evergreen shrub with great therapeutic potential for treating both human and animal illnesses (Raina et al., 2019). The leaves of *J. communis* were found to be rich in a variety of chemical components, including Camphene, Verbenene, Myrcene, Limonene, Sabinene, α-Pinene, α-Thujene, and α-Terpinene, which were in charge of exhibiting varied pharmacological activity and treating a variety of ailments (Dhaka & Mittal, 2021). Numerous previous investigations support the biological effects of Juniperus *communis* (Šojić et al., 2017). The berries revealed a variety of pharmacological properties including an antioxidant (Höferl et al., 2014), antimicrobial (Peruč et al., 2018) and anti-inflammatory potential (Raina et al., 2019). In conventional medicine they are utilized as rheumatic, antiseptic, stomachic, diuretic, and cardiac medications (Stoilova et al., 2014).

The Asteraceae family includes the species *Artemisia herba-alba* (white mugwort), an aromatic and therapeutic plant (Nedjimi & Beladel, 2015). It was distinguished by a significant morphological diversity in respect to the regional ecological factors. *Artemisia herba-alba has* been shown to contain a number of secondary metabolites, the most significant of which are sesquiterpene lactones, including eudesmanolides and germacranolides. The flavonoids found in mugwort also exhibit a structural diversity, ranging from common flavonoids (flavonesglycosides and flavonols) (Ivănescu et al., 2021). In traditional medicine, Artemisia herba-alba is frequently used to treat stomach issues like diarrhea and abdominal pain. It is also used as a remedy for inflammation of the gastrointestinal tract (Abu-Darwish et a., 2015). Previous scientific studies have also proven the effectiveness of white mugwort as an antioxidant and antibacterial agent (Bordean et al., 2023).

In light of our interest in the therapeutic effects of plants. This study's objective was to examine the antioxidant, antibacterial, and anti-inflammatory properties in case of *Artemisia herba-alba and Juniperus communis* extracts, which were prepared using a variety of solvents.

2. Material and methods

2.1. Vegetal materials

Artemisia herba-alba was collected in septembre 2022 from the Kchida region (Batna, Algeria, 35°33´21" N 6° 10´ 26" E), and *Juniperus communis* in novembre 2022 from Wanza region (Tebessa, Algeria, 35° 57′ 00" N, 8° 08′ 00" E). The aerial parts are cleaned, dried, and ground. Then, it was stored at ambient temperature in a glass box until use. To obtain the extracts, the leaves (10 g) were macerated in 200 ml of distilled water and a hydroalcoholic solution of (ethanol : water= 80: 20) for 24 h at ambient temperature with fresh solvent every 24 hours, this procedure was carried out three times. The resultant mixture was then filtered, concentrated by evaporation under decreased pressure for at least 48 hours at a temperature below 40 °C, and kept until use.

2.2. Total phenolic contents (TPC)

1 ml of newly made Folin-Ciocalteu reagent (10 times diluted) and 8 ml of 7.5% sodium carbonate (Na_2CO_3) are combined with 200 µl of the extract. After 30 minutes in room temperature incubation, the entire mixture is measured at 765 nm using a spectrophotometer against a blank. Concentrations of polyphenols were inferred from the range of the linear calibration curve created using gallic acid (Jaradat et al., 2024).

2.3. Total flavonoid contents (TFC)

A 1 ml of the $AlCl_3$ solution (2% in methanol) was combined with 1 ml of each sample (made in methanol or distilled water). A spectrophotometer was used to measure the absorbance at 430 nm following ten minutes of incubation. With reference to the quercetin calibration curve, the findings are given in mg/g of dry plant matter equivalent of quercetin (Amira et al., 2022).

2.4. Radicals scavenging activity

2.4.1. Free radical cavenging

The DPPH technique was performed to conduct the antioxidant test. A 1 ml of various strengths of extract is mixed with 1 ml of DPPH methanolic solution (0.1×10^{-3} M). A negative control is simultaneously created by mixing 1 ml of methanol with a methanolic solution of DPPH. After 15 minutes of dark incubation at room temperature, the absorbance measurement is taken in comparison to a blank that has been created at 517 nm for every concentration. The test is done three times for each concentration. The positive control is a solution of ascorbic acid, whose absorbance was measured under the identical conditions as the samples. The findings were presented as an inhibition percentage (I%) (Țicolea et al., 2024).

$$I\% = [(\text{Abs controle} - \text{Abs test})/\text{Abs controle}] \times 100$$

Through linear regression, the IC_{50} values were visualized.

2.4.2. FRAP assay

200 µl of each extract solution at various concentrations are combined with 2.5 ml of potassium ferricyanate solution ($K_3Fe(CN)_6$) produced in distilled water and 500 µl of phosphate buffer solution (0.2 M; pH = 6.6). After 20 minutes of incubation at 50°C, the mixture is taken out of the test tubes. After adding 2.5 ml of 10% trichloroacetic acid (TCA), centrifugation is carried out for 10 minutes at 650 rpm. Add 2.5 ml of distilled water and 0.5 ml of 0.1% FeCl3 to 500 µl of supernatant. The wavelength at which the absorbance is measured is 700 nm. The solvent is substituted for the extract to create a blank. Under the same circumstances, ascorbic acid is utilized as a positive control. The results were represented by the EC_{50} value (Asbabou et al., 2024).

2.5. Antibacterial activities

A 20 ml of Muller-Hinton agar is added to sterile Petri plates, and the dishes are incubated for 20 minutes. Following solidification, a 1 ml bacterial suspension containing 10^8 CFU/mL (Colony Forming Unit) was applied evenly across the surface of each culture medium (Shunying et al., 2005). Paper discs of 6 mm in diameter, sterile Whatman N°1, are impregnated with a volume of 5 µl of varying concentrations (2.5, 5, 10, and 20 mg/ml) and positioned on the solidified medium's surface. After that, the Petri plates are incubated for 24 to 48 hours at 37°C in an oven. *Escherichia* *coli* ATCC 25922, *Candida albicans* ATCC 14053, *Pseudomonas aeruginosa* ATCC 27853, *Klabsiella pneumoniae* ATCC 13883, and *Staphylococcus aureus* ATCC 25923 are the strains that were tested.

2.6. Anti-inflammatory activities (Albumin denaturation method)

For every combination, a reaction vessel was made up of 1 ml of the test extract, 1.4 ml of phosphate buffered saline solution, and 200 µl of egg albumin. As a negative control, distilled water was utilized rather than extract. After that, the mixtures were heated for five minutes at 70°C after being incubated for fifteen minutes at 37°C. The absorbance was measured at 660 nm following cooling (Osman et al., 2016)**.** Under the same operational circumstances, diclofenac sodium is utilized as a positive control. Three duplicates of the experiment were conducted. Following is the calculation of the percent inhibition of protein denaturation:

$$\text{Percentage protection against denaturation} = [(1 - \text{Abs Sample})/\text{Abs Control}] \times 100$$

2.7. Statistical analysis

Triplicate tests have been conducted on antioxidant capacity, antibacterial potential, and anti-inflammatory action. The findings are displayed as mean ± standard deviation (SD).

3. Results and discussion

3.1. Total phenolic and flavonoid contents

The *Artemisia herba-alba* TPC results showed that the hydro-ethanolic extract had a significant polyphenol concentration with 104.54 ±0.35 mg GAE/g compared to aqueous extract (58.89 ±0.22 mg GAE/g). For *Juniperus communis* the aqueous extract showed a greater value (103.80 ±0.30 mg GAE/g) than the hydroethanolic extract (78.11 ±0.27 mg GAE/g) (Table 1).

The overall flavonoid concentration was found to be lower in the *Artemisia herba-alba* aqueous and hydroethanolic extracts (4,88 ±0,075 and 2,06 ±0,03 mg QE/g, respectively) than the two *Juniperus communis* extracts (15.85 ±0.80 mg QE/g of aqueous extract and 17.05 ±0.13 mg QE/g of hydroethanolic extract) (Table 1).

The polarity of the extraction solvents utilized can be responsible for the modest differences in measured phenol and flavonoid levels that were noticed. Because phenols, flavonoids, and other secondary metabolites have varied degrees of polarity, different solvents extract diverse compounds from the plant matrix in various ways (Kaczorová et al., 2021). The drying and extraction conditions in terms of method, duration, temperature, particle size, solvent, number of extraction steps, expression of results, and geographic origin all contribute to the heterogeneity of the polyphenol content (Idoudi et al., 2023).

3.2. Antioxidant characteristics

The results are shown in Table 1 as the IC_{50} value for the Antioxidant characteristics of the investigated extracts. The findings demonstrated the capacity of several extracts to lessen DPPH radicals. Compared with the standard, the aqueous extracts of two studied plants showed weak antioxidant effects with IC_{50} 151.235 ±0.14 µg/mL for *A.herba-alba* and 101.06 ±0.19 µg/mL for *J.communis,* compared with hydroethanolic extraxts (*AhaA*: 69.44 ±0.17 µg/mL, Jc: 97.96 ±0.22 µg/mL).

FRAP assay was another antioxidant activity test used in this research. The results (Table1) showed that *J.communis* has the strongest ferric reducing antioxidant power, especially the hydroethanolic extract (49.49 ±0.14 µg/mL), while *A.herba-alba* exhibited a weak antioxidant effects where the hydroethanolic extract recorded the lowest EC_{50} value (72.46 ±0.09 µg/mL).

According to Khlifi et al. (2013) research**,** when comparing the antioxidant activity of extracts from *Ruta chalepensis L*. and *Peganum harmala L*, the extract from *A. herba-alba* showed the highest value (IC_{50}: 20.64 ±0.84 mg/L). *J. communis* plant has been widely employed in the food and beverage, pharmaceutical, and other industries because of its strong antioxidant action and polyphenol content (Tang et al., 2019). Owing to their repeatability and ease of operation. The antioxidant strength of pure molecules or unprocessed plant extracts is typically assessed using the DPPH and FRAP experiments. Other techniques are also employed, and they rely on the reactive oxygen species (ROS), which are oxidizing agents found in biological systems. It should be noted that antioxidants, whether they be natural or synthetic, can restrict the creation of reactive oxygen species, limit their ability to spread, or even destroy them, allowing for the prevention of diseases brought on by oxidative stress (Apak et al., 2007). The greatest antioxidant effects are often found in polyphenols with a high hydroxyl group count because these compounds can contribute more atoms to stabilize free radicals (Heim et al., 2002). Along with the previously mentioned factors, it should be noted that the antioxidant activity also depends on the concentration of the extracts, the components' structures, the antioxidants' nature, the evaluation method, how sensitive the antioxidants are to the test's temperature, and whether they are water- or fat-soluble (Falleh et al., 2008).

Table 1. Values of total phenolic, flavonoid contents, and in vitro antioxidant potential of different extracts of Artemisia herba-alba and Juniperus communis.

Plant extracts	***Total phenolic contents*** (mg GAE/g E)	***Total flavonoid contents*** (mg QE/g E)	***DPPH*** IC_{50} (µg/mL)	***FRAP*** $EC_{0,5}$(µg/mL)
Artemisia herba-alba				
Aqueous extract	58.89 ±0.22	4,88 ±0,075	151.23 ±0.14	60.24 ±0.12
Hydroethanolic extract	104.54 ± 0.35	2,06 ±0,03	69.44 ±0.17	72.46 ±0.09
Juniperus communis				
Aqueous extract	103.80 ±0.30	15.85 ±0.80	101.06 ±0.19	59.54 ±0.12
Hydroethanolic extract	78.11 ±0.27	17.05 ±0.13	97.96 ±0.22	49.49 ±0.14

3.3. Anti bacterial activity

It emerges from the results obtained (presented in Table 2) that all these extracts prove to be active against some microbial strains tested with different degrees, the increase in concentration of the extracts increases the diameter of the zone of inhibition. Significant antibacterial activity against *Klebsiella pneumoniae* and *Staphylococcus aureus* was demonstrated by the hydroethanolic extracts of the plants, as well as anti-candidiasis activity against *Candida albicans* with a super inhibition at concentrations of 20 mg/mL. *Pseudomonas aeruginosa* and *Staphylococcus aureus* were highly resistant to *A.herba-alb* aqueous extract, while *Escherichia coli* resisted the hydroethanolic extract of the same plant. *J. communus* aqueux does not possess antibacterial activity against *Pseudomonas aeruginosa* and *Candida albicans.* The results of a test using DMSO as a solvent (negative control) indicate that the solvent is appropriate and has no impact on the typical growth of any microbial strains.

Mohammed et al. (2021) found that the fractions of extracts of *A. herba-alba* showed varying degrees of antibacterial activity against the bacteria (*Staphylococcus aureus, Escherichia coli*). By using the disc diffusion method, crude leaf organic extracts of the Himalayan plant J. communis in the solvents chloroform, methanol, hexane, and ethanol, as well as aqueous extracts, revealed antibacterial potential against a variety of drug-resistant bacteria species, including *Agrobacterium tumefaciens, Escherichia coli* (Sati & Joshi, 2010). According to Shamsudin et al. (2022), phenolic chemicals, flavonoids, and steroids have the ability to prevent the growth of microbes at specific concentrations. It has been demonstrated that lipophilic flavonoids break down microbial membranes by making membrane lipids more fluid. Microorganism toxicity can be caused by the quantity and location of hydroxyl groups on the aromatic ring of phenolic substances (Cowan, 1999). Crude extracts can be employed in food systems to stop the growth of foodborne bacteria, extending the shelf life of processed foods. These extracts have broad-spectrum antibacterial activity against positive and negative gram bacteria.

Table 2 . Zone inhibition diameters of Artemisia herba-alba and Juniperus communis.

Plant extract	Concentrations (mg/mL)	Inhibition zone (mm)				
		E.coli	***P. aeruginosa***	**K. pneumoniae**	***S. aureus***	***C.albicans***
Aq (*Aha*A)	20	10 ±1.04	NA	12 ±1.25	NA	12 ±0.76
	10	8 ±0.28	NA	18 ±1.5	NA	11 ±1.32
	5	NA	NA	8 ±0.76	NA	NA
	2.5	NA	NA	NA	NA	NA
Hydroeth (*Aha*A)	20	NA	11 ±2.02	9 ±1.15	13 ±1.5	13 ±2.84
	10	NA	7 ±0.28	9 ±0.5	12 ±2.51	12 ±2.36
	5	NA	NA	8 ±0.5	9 ±1.15	12 ±1.32
	2.5	NA	NA	NA	9 ±0.86	10 ±1.04
Aq (Jc)	20	11 ±2.08	NA	10 ±1.52	10 ±1.32	NA
	10	10 ±1.73	NA	10 ±0.57	NA	NA
	5	NA	NA	NA	NA	NA
	2.5	NA	NA	NA	NA	NA
Hydroeth (Jc)	20	7 ±0.28	15 ±1.60	11 ±1.73	21 ±1.15	13 ±2.02
	10	NA	10 ±1.04	8 ±0.5	15 ±1.52	12 ±0.76
	5	NA	NA	7 ±0.28	12 ±2.08	10 ±1.73
	2.5	NA	NA	NA	10 ±1.32	8 ±0.57
DMSO		0	0	0	0	0

NA = no activity

3.4. Anti-inflammatory activity

Proteins may get denatured when they are subjected to heat or chemicals. As a result, their physiological qualities are lost and their molecular structure is altered. Inflammatory disorders are known to be associated with tissue protein denaturation (Ruiz-Ruiz et al., 2017). In the current study, *A. herba alba and J. communis* extracts' in vitro anti-inflammatory efficacy was estimated by measuring the denaturation of egg albumin. According to Table 3, for both the aqueous and hydroethanolic extracts, the percentage inhibition of egg albumin denaturation increased in a concentration-dependent manner. Maximum inhibition provided by diclofenac is greater than 97.5%. The hydroethanolic extract of *J. comminus*, with IC_{50}: 23.58 ±0.02 µg/mL, might have the strongest ability to prevent albumin from becoming denaturated, followed by the aqueous extract of *A.herba alba* (IC_{50}: 32.24 ±0.02 µg/mL). While the hydroethanolic extract of the same plant (IC_{50}: 58.22 ±0.02 µg/mL) showed the lowest inhibitory activity. In comparison with diclofenac (IC_{50}: 21.7 µg/mL), All of the extracts exhibited a lesser ability of preventing albumin denaturation (Figure 1).

The ability for some extract constituents, such as flavonoids, phenolic acids, and tannins, to interact with the aliphatic areas around the lysine residues on albumin molecules and prevent it from denaturing owing to heat has been demonstrated by research (Williams et al., 2008). More investigation is necessary, nevertheless, to completely comprehend the precise mechanisms by which these chemicals are able to inhibit denaturation. Dhaka and Mittal (2021) and Khlifi et al. (2013) confirmed that *A.herba-alba* and *J. communis* extracts possess anti-inflammatory activities.

Table 3. The percentages of protein denaturation inhibition by Artemisia herba-alba and Juniperus communis

Concentration (µg/mL)	Inhibition of protein denaturation (%)				
	Aq(*AhaA*)	**Hydroeth(*AhaA)***	**Aq(JC)**	**Hydroeth(Jc)**	**Diclofenac sodium**
10	40.83	26.66	34.16	44.16	-
50	55	55	59.16	56.66	54.16
100	73.33	67.5	74.16	73.33	76.66
150	78.33	79.16	82.5	82.5	90.83
200	89.16	85.83	92.5	91.66	97.5

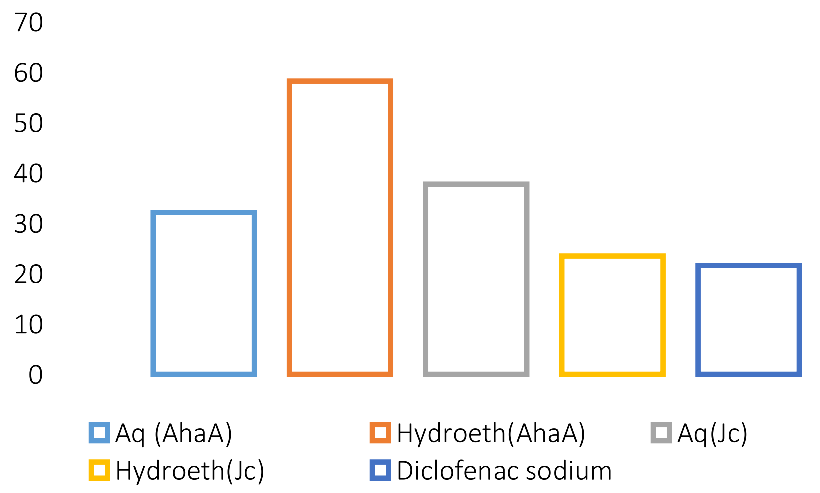

Figure 1. IC$_{50}$ values of anti-inflammatory activity of Artemisia herba-alba and Juniperus communis extracts

4. Conclusion

The findings of this study prove that hydroethanolic extracts in case of *Artemisia herba-alba* and *Juniperus communis* gathered from Algeria's mountainous regions have significant in vitro antioxidant, antibacterial, anti-inflammatory effects. The results discovered the presence of total phenolics and flavonoids, which enhance its various biological activities. These conclusions support the traditional use of mountainous *Artemisia herba-alba* and *Juniperus communis* in the treatment of inflammatory diseases and bacterial infections. They may allow the development of pharmaceutical drugs to treat all disorders associated with the studied activities, such as inflammatory gastrointestinal diseases. However, supported in vivo research is required to understand the exact mechanisms of inhibiting free radicals, antibacterial and anti-inflammatory activities.

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Author's declaration and contribution

There is no actual or potential conflict of interest in relation to this article. Each author contributed to the study's concept and design. The authors read the final draft of the work, provided feedback on earlier iterations, and gave their approval.

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