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# Phenolic Profile, Antioxidant, and Antibacterial Activities of Extracts and Essential Oils of Algerian *Artemisia herba-alba* and *Thapsia garganica*

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ABSTRACT

*Artemisia herba-alba* and *Thapsia garganica* are two of the most famous and potent medicinal plants used in Algeria. This study aimed to investigate the phenolic profile, antioxidant, and antibacterial activities of extracts and essential oils of *Artemisia herba-alba* (AH) and *Thapsia garganica* (TG). The plant samples were extracted using four solvents with different polarities, while essential oils were obtained by the hydrodistillation method. Phytochemical screening was performed to identify various secondary metabolites present in the extracts. Total phenol, flavonoid, and tannin contents were determined following standard methods. Furthermore, the antioxidant activity was evaluated using FRAP and *β*-carotene bleaching assays. The antibacterial activity of the extracts and essential oils was evaluated by the agar well diffusion, and the disc diffusion assays, respectively. The results confirmed the richness of these plants in tannins (content ranging from 20.47 to 239.02 µg TAE/mg E) and flavonoids (1.47 to 83.89 µg QE/mg). The best ferric reducing antioxidant capacity was exhibited by the acetone extracts for both plants (A = 1.90 for AH extract and A = 0.83 for TG extract). The extracts prevented lipid peroxidation *in vitro*, with AH methanol extract exhibiting the highest activity with percentage inhibition of 90.01%. Furthermore, AH and TG extracts and essential oils exhibited good antibacterial activity against *E. coli*, *S. aureus*, and *P. aeruginosa* with inhibition zone diameters ranging from 9.5 to 20 mm. On the basis of these findings, AH aerial parts and TG roots appear to be good sources of bioactive compounds with antioxidant and antibacterial activities.

***Keywords:*** *Artemisia herba-alba, Thapsia garganica,* Antioxidant, Antibacterial, Secondary metabolites..

## Introduction

Oxidative stress results from an imbalance between the generation of free radicals such as reactive oxygen species (ROS) and the body's antioxidant defense systems. It is well known that oxidative stress is implicated in many diseases, including inflammatory disorders, cancer, neurodegenerative disorders, and atherosclerosis. Medicinal plants from various botanical families are an important source of antioxidants, and thus their addition to the diet can be effective in the prevention of illnesses related to oxidative stress.[1] Plant secondary metabolites such as tannins, terpenoids, alkaloids, and flavonoids have been reported to exhibit antibacterial properties.[2]

*Artemisia herba-alba* is a shrub from the Asteraceae family; it is common in Algeria, found in steppes and rocky pastures, and is locally called "Chih". The aerial parts of *Artemisia herba-alba* are traditionally used as anti-diarrheal, antidiabetic, and in the treatment of gastric disorders.[3] *Thapsia garganica* is a medicinal plant belonging to the *Apiaceae* family; it is widespread in Algeria, and locally called Derias or Bounafaa. This plant is traditionally used as an ointment to relieve rheumatism and as a diuretic.[4] Previous studies on these species focused mainly on the chemistry and biological activities of their essential oils, while there are limited studies on their phenolic extracts. The aim of this study was to investigate the antioxidant and antibacterial activities of essential oils and different solvents extracts of *A. herba-alba* and *T. garganica.* For this purpose, these extracts were characterized for their total content of polyphenols, flavonoids, tannins, and *β*-carotene/linoleic acid, as well as their antioxidant and antibacterial potentials in vitro.

## Materials and Methods

### *Chemicals*

The reagents used in this study were purchased from Sigma-Aldrich, Fluka and BioChem. Solvents were obtained from Sigma-Aldrich and AnalaR NORMAPUR.

### *Bacterial strains*

The bacteria strains used were *Escherichia coli* (ATCC22922), *Pseudomonas aeruginosa* (ATCC27853), and *Staphylococcus aureus* (ATCC22923). These bacteria were provided by Pasteur Institute in the province of M'sila, Algeria.

### *Plant collection and identification*

Aerial parts of Artemisia herba-alba (AH) were collected in November, 2021, from Draa Elhadja region in M'sila-Algeria (PF6J+46C, M'Sila). The aerial parts were air-dried in the shade and pulverized. The roots of *Thapsia garganica* (TG) were collected in March, 2021 from Maadid region in M'sila-Algeria (RPHQ+C8J Maadid). The roots were separated, washed, peeled, dried, and pulverized.

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*Extraction of plant materials*

*Aqueous and organic solvent extracts preparation*

Aqueous extract of each plant was prepared as a decoction by heating a mixture of 10 g of plant material and 100 mL of distilled water at 70°C for 1 hour with constant agitation.[5] Organic solvent extracts were prepared following the method described by [6,] where 10 g of each plant material was defatted with n-hexane; the plant residue was then extracted with acetone, and after filtration, the material was further extracted with methanol. Plant materials were macerated for 48 hours in each of the solvent with constant agitation. The extracts were concentrated at reduced pressure, and the dried extracts were kept at +4°C until analysis.

*Essential oils extraction*

*A. herba alba* and *T. garganica* essential oils were obtained from 70 g of fresh plant material by hydrodistillation for 3 hours using Clevenger apparatus. The essential oils were preserved at 4°C until analysis.

*Phytochemical screening*

The phytochemical screening of the plants' extracts was carried out using standard methods as described by [7]. All extracts were prepared in DMSO at a concentration of 4 mg/mL and used to test for the presence or absence of saponins, tannins, flavonoids, quinones, and terpenoids.

*Determination of total phenol content*

Total phenol content in the extracts was determined using the Folin–Ciocalteu reagent according to the method described by [8] Briefly, 200 µL of each extract at a concentration of 2 mg/mL were mixed with 1 mL of diluted Folin–Ciocalteu reagent. (1:10). After the addition of 800 µL of sodium carbonate solution (75 g/L), the mixture was incubated at room temperature for 2 h in the dark. Absorbance was measured at 765 nm, and gallic acid (20 -100 µg/mL) was used as the standard for the calibration curve. Total polyphenols content was expressed as µg of gallic acid equivalents per mg of extract (µg GAE/mg E). All samples were performed in triplicate.

*Determination of total flavonoid content*

Total flavonoid content of the extracts was determined using the aluminum chloride colorimetric method as described by [9] One milliliter of 2% $AlCl_3$ solution was added to 1 mL of each extract (2 mg/mL). The reaction mixture was incubated at room temperature for 10 min in the dark, after which the absorbance was measured at 430 nm. Quercetin (10 - 50 µg/mL) was used as a standard, and total flavonoids content was expressed as µg of quercetin equivalents per mg of extract (µg QE/mg E). Each determination was done in triplicate.

*Determination of total tannin content*

The total tannin content of the extracts was determined using Folin–Ciocalteu reagent and tannic acid as standard, according to the method described by [10] Aliquots of 350 µL of each extract (2 mg/mL) were added to 1.5 mL of diluted Folin-Ciocalteu reagent (1:10). The mixture was agitated, followed by the addition of 1.5 mL of sodium carbonate solution. The reaction mixture was incubated at 45°C for 45 min in the dark. Absorbance was then measured at 765 nm, and a calibration curve was prepared using tannic acid (37.5 - 300 µg/mL). Total tannins content was expressed as µg of tannic acid equivalents per mg of extract (µg TAE/mg E). Each determination was carried out in triplicate.

*Determination of antioxidant activity*

*Ferric reducing antioxidant power (FRAP) assay*

The reducing power of the extracts was estimated using the method described by [11] Different concentrations of each extract (0.5 - 6 mg/mL) or ascorbic acid (10 - 80 µg/mL) were prepared in DMSO, and 500 µL of each solution was added to 1250 µL of phosphate buffer (0.2 M, pH 6.6) and 1250 µL of potassium ferricyanide (1%). After agitation and incubation at 50°C for 20 minutes, 1250 µL of trichloroacetic acid (10%) was added, and the mixture was centrifuged at 3000 rpm for 10 minutes. The supernatant (1250 µL) was mixed with 125 µL of distilled water and 250 µL of ferric chloride (0.1%). Absorbance was determined at 700 nm using a Shimadzu UV mini-1240 spectrophotometer (Japan), and each solution was repeated three times.

*β-Carotene bleaching assay*

This assay was performed as described by [12] Briefly, 0.5 mg of *β*-carotene was dissolved in 1 mL of chloroform. One milliliter of the resulting solution was added to 25 µL of linoleic acid and 200 mg of Tween 40. The chloroform was then removed at reduced pressure, and 100 mL of distilled water saturated with oxygen were added with vigorous shaking. Aliquots of 2.5 mL of this mixture was added to 350 µL of each extract or the synthetic antioxidant BHT (positive control), both prepared at a concentration of 3 mg/mL. Absorbance at 490 nm was determined at time zero (t0) and then after 1 h, 2 h, 3 h, 4 h, 24 h, and 48 h of incubation at room temperature. DMSO, methanol, and distilled water were used as negative controls, and the percentage of inhibition was calculated using the following equation:

$$\% \text{ of inhibition} = (At/At0)\ X\ 100$$

Where; At is the absorbance of the test sample at time (t) and At0 is the absorbance of the test sample at a time t0.

*Determination of antibacterial activity*

Antibacterial activity of the extracts was evaluated using the agar well diffusion method. Briefly, 7 mm wells were formed aseptically in Mueller-Hinton agar, previously inoculated with a suspension of the test bacterial strains ($10^7$ germs/mL). A volume of 30 µL of each extract or DMSO (used to dissolve extracts) was then poured in the wells at different concentrations (100 and 200 mg/mL). After incubation at 37°C for 24 h, inhibition zones diameter around the wells was measured.[13]

*Statistical analysis*

Data were expressed as the mean ± SD of three independent experiments. All graphs were created using Microsoft Excel 2010 and phenols quantification was assessed by linear regression analysis.

## Results and Discussion

*Extraction yields and chemical composition*

The extraction yields for both plant extracts and essential oils are presented in Table 1. For the AH and TG extracts, yields were proportional to solvents' polarity, where the highest yield was obtained for the aqueous extract of TG. For the essential oils, yields were very minimal, with AH and TG yielding 1% and 0.4% essential oil, respectively. Essential oil from TG was characterized by a dark blue coloration.

**Table 1:** Yields of *Artemisia herba-alba* and *Thapsia garganica* extracts

| Extract | Yield (%) | |
|---|---|---|
| | *Artemisia herba-alba* | *Thapsia garganica* |
| Aqueous | 17.35 | 29.50 |
| Methanol | 09.56 | 03.60 |
| Acetone | 05.64 | 01.00 |
| Hexane | 02.12 | 00.80 |
| Essential oil | 01.00 | 00.40 |

The results of phytochemical screening are presented in Table 2. Flavonoids and quinones were detected in all the extracts. Tannins were detected in all extracts from AH and TG, except hexane extracts. Terpenoids were also present in the majority of the extracts, except the hexane and aqueous extracts of TG. Saponins were detected only in the aqueous extract of AH.

The total contents of phenols, tannins and flavonoids in AH and TG are presented in Table 3. Among the various extracts of AH, the aqueous extract had the highest content of total phenols (86.57 µg GAE/mg E). For this plant, the total phenol content was proportional to the polarity of the extracts. This observation aligns with that of [14] where the methanol extract of AH was found to contain high amount of total phenol with value of 43.61 µg GAE/mg E. For TG extracts, non-polar extracts were richer in phenols (acetone: 39.48 µg GAE/mg E, hexane: 25.37 µg GAE/mg E) compared to the polar extracts. According to [15] extracts prepared from the leaves, seeds, and roots of TG showed low

content of polyphenols, where the highest content was found in the seeds.

**Table 2:** Phytochemical constituents of different solvent extracts of *Artemisia herba-alba* and *Thapsia garganica*

| Phytochemical | *Artemisia-herba-alba* | | | | *Thapsia garganica* | | | |
|---|---|---|---|---|---|---|---|---|
| | Aq | MeOH | Ace | Hex | Aq | MeOH | Ace | Hex |
| Tannins | + | + | + | - | + | + | + | - |
| Saponins | + | - | - | - | - | - | - | - |
| Flavonoids | + | + | + | + | + | + | + | + |
| Terpenoids | + | + | + | - | - | + | + | - |
| Quinones | + | + | + | + | + | + | + | + |

(+): Presence, (-): Absence. Aq: Aqueous, MeOH: Methanol, Ace: Acetone, Hex: Hexane.

**Table 3:** Total polyphenol, flavonoid, and tannin contents of *Artemisia herba alba* and *Thapsia garganica* extracts

| Extract | | Total phenol content (µg GAE/mg E) | Total flavonoid content (µg QE/mgE) | Total tannin content (µg TAE/mg E) |
|---|---|---|---|---|
| *Artemisia herba-alba* | Aq | 86.57±0.09 | 11.10±0.06 | 112.05±0,63 |
| | MeOH | 35.31±2.14 | 32.21±0.46 | 239.02±4,35 |
| | Ace | 33.18±1.89 | 83.89±0.62 | 168.05±2,82 |
| | Hex | 10.29±1.13 | 16.23±0.99 | 39.38±0,37 |
| *Thapsia garganica* | Aq | 16.937±1.813 | 4.942±0.924 | 30.916±1.307 |
| | MeOH | 14.916±1.916 | 1.479±0.059 | 20.472±1.419 |
| | Ace | 39.489±0.044 | 17.27±0.419 | 83.888±2.911 |
| | Hex | 25.375±1.335 | 16.26±0.290 | 24.083±1.929 |

Values represent mean ± SD of triplicate determination. Aq: Aqueous, MeOH: Methanol, Ace: Acetone, Hex: Hexane, GAE: Gallic acid equivalents, QE: Quercetin equivalents, TAE: Tannic acid equivalents, E: extract.

With respect to the flavonoids content in AH extracts, the following order was observed: Acetone extract > Methanol extract > Hexane extract > Aqueous extract, where the acetone extract contained the highest content of total flavonoids with concentration of 83.89 µg QE/mgE. In the study of [16] methanol and aqueous extracts of AH were found to have flavonoid content of 47.97 and 31.86 µg QE/mg E, respectively. TG extracts contained lower flavonoids than AH extracts, where the highest amount was also found in the acetone extract with value of 17.27 µg QE/mg E. AH is characterized by high tannin content, which was confirmed in this study by the high amounts of this secondary metabolite in AH extracts, where methanol extract contained up to 239.02 µg TAE/mg E. However, tannins were less abundant in TG extracts, with the highest content of 83.88 µg TAE/mg E measured in the acetone extract.

*Antioxidant activity*

It is well known that the antioxidant activity of plant extracts cannot be established by performing only one type of antioxidant assay. The use of antioxidant tests based on different mechanisms, such as electron-transfer and proton-transfer is crucial. Therefore, in this study, ferric reducing capacity and lipid peroxidation prevention ability of AH and TG extracts were evaluated by means FRAP (electron-transfer based assay) and β-carotene bleaching assay (proton-transfer based assay).[17] the results obtained for both assays are presented in Figures 1 and 2.

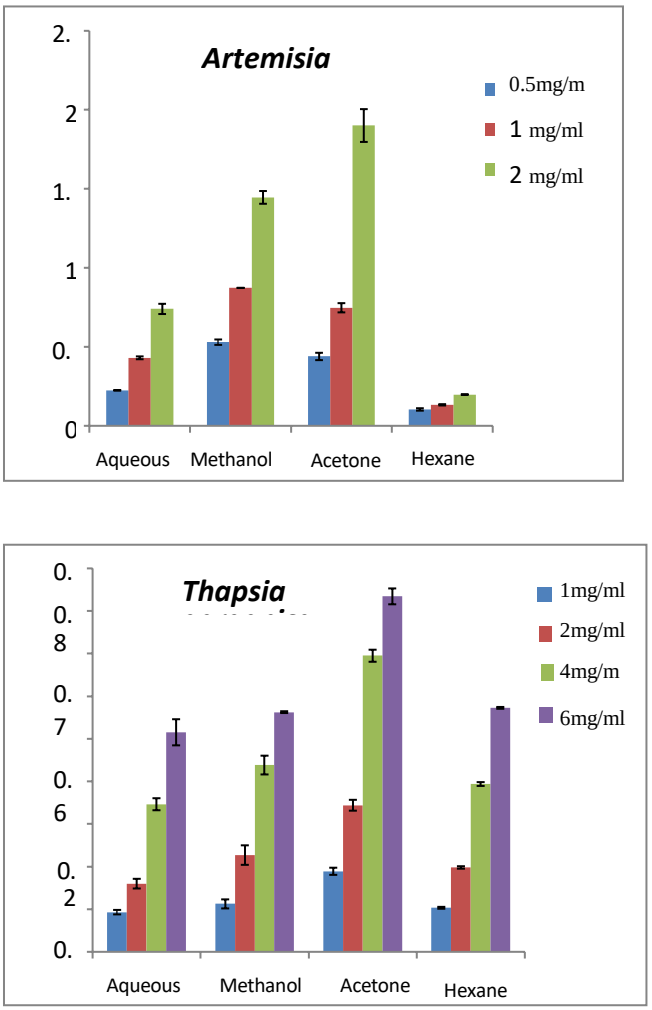


**Figure 1:** Reducing power of *Artemisia herba-alba* and *Thapsia garganica* extracts. Measured by FRAP assay. Data are expressed as mean ± SD of triplicate determination.

The FRAP assay is a simple test based on electron transfer, consisting of measuring the ability of substances to reduce potassium ferricyanide to the ferrocyanide form and thus causing colour change.[18]. According to the results obtained, AH and TG extracts, as well as the standard (ascorbic acid) exhibited a concentration-dependent iron-reducing power. The capacity of ascorbic acid to reduce iron was greater than that of the extracts, whose absorbance value was equal to 0.36 at concentration of 80 µg/mL. Among all AH extracts, the acetone extract showed the highest reducing capacity (A = 1.90) compared to the other extracts at the same concentration (2 mg/mL), this may be attributed to the flanoids content, which was also highest in the acetone extract of AH. In the study carried out by Ayad and colleagues,[16] aqueous and methanol extracts of AH exhibited $EC_{50}$ values of 532.36 and 249.88 µg/mL, respectively, in the FRAP assay.

Among TG extracts, the acetone extract, which had the highest phenolic content, showed the best reducing capacity (A = 0.83) compared to the

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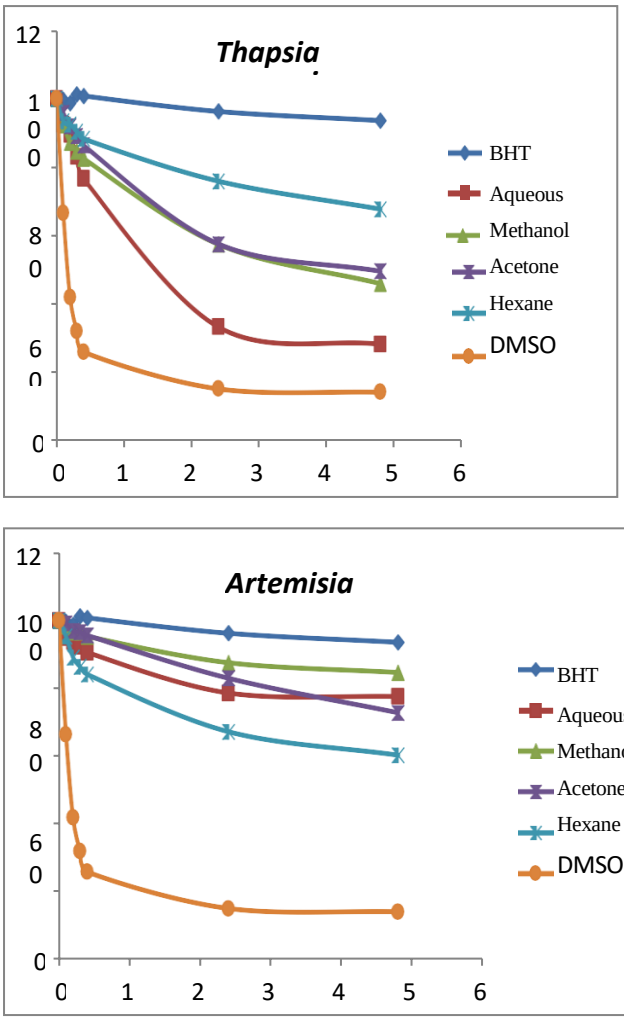


**Figure 2:** *β*-carotene bleaching kinetic in the presence of *Artemisia herba-alba* and *Thapsia garganica* extracts and controls. Each value represents the mean of triplicate determination

other extracts at a concentration of 6 mg/mL, followed by the hexane and methanol extracts with very close values (0.59 and 0.56, respectively) and finally the aqueous extract (A = 0.51), which showed the lowest reduction capacity. According to the study of [19] on extracts of TG leaves, the best reducing activity obtained was A = 0.78 at a concentration of 1 mg/mL.

The reducing potential of the extracts could be linked to the presence of molecules capable of donating electrons. The interaction of polyphenols with transition metals plays an important role in the antioxidant properties of plant, especially for flavonoids, which have, from a structural point of view, several potential sites.[20]

The β-carotene bleaching assay is widely used to evaluate the antioxidant activity of bioactive compounds because β-carotene is extremely susceptible to free radicals and hydroperoxide derivatives resulting from the oxidation of linoleic acid.[21] These radicals attack the chromophore of β-carotene, resulting in a discoloration of the reaction mixture, which results in a decrease in absorbance at 490 nm. The presence of antioxidants could prevent oxidation and thus discoloration of this molecule by neutralizing these radicals.

The kinetics of β-carotene bleaching was carried out in the presence of AH and TG extracts and an antioxidant butylated hydroxy toluene (BHT) which was used as the positive control. In general, the anti-lipid peroxidation activity of AH extracts was higher than that of TG extracts. After 24 h of incubation, the highest antioxidant activity (inhibition %) was exhibited by AH methanol extract (90.01%), which was the closest to BHT (96.23%). AH acetone and aqueous extracts gave inhibition percentages of 82.96% and 78.20%, respectively, while the hexane extract showed the lowest activity among the AH extracts with percentage inhibition of 67.17%. Previous study has reported the antioxidant activity of AH essential oil by the β-carotene bleaching assay, where the $RC_{50}$ value was equal to 0.87 mg/mL.[22] The antioxidant activity of AH extracts in this test is proportional to their tannins content. This group of secondary metabolites is known for its lipid peroxidation inhibitory activity.[23] For TG extracts, the best antioxidant activity was exhibited by the hexane extract with an inhibition percentage of 75.82%, followed by the acetone and methanol extracts with very close values (57.43 and 57.30%, respectively), while the aqueous extract gave the lowest oxidation inhibition (33.11%). According to a study carried out by [24] the antioxidant activity of methanol extract of *T. garganica* leaves was evaluated by the β-carotene bleaching test, where an $IC_{50}$ of 123.55 µg/mL was recorded. In the present study, the most active extract was the non-polar hexane extract, which may likely be due to the higher ability of the hexane extract to penetrate the lipophilic phase used in this assay, in comparison to the other extracts.[18]

*Antibacterial activity of AH and TG essential oils and extracts*

The antibacterial activity of the essential oils (EO) and extracts from both plants was evaluated by the disc diffusion method for essential oils and the agar well diffusion method for the extracts. Antibacterial potential was evaluated by measuring the diameters of the inhibition zones, and the results obtained are shown in Table 4.

According to the results, all extracts of TG did not show any activity against the bacterial strains used in the study. However, AH extracts showed a good activity against *E. coli* and *S. aureus*, while *P. aeruginosa* was resistant to these extracts.

**Table 4:** Antibacterial activity (Inhibition zone diameter in mm) of extracts and essential oils of *A. herba-alba* and *T. garganica*.

| Bacterial strain | Extracts concentrations (mg/mL) | | | | | | Essential oil | | Gentamicin 10 µg/disc |
|---|---|---|---|---|---|---|---|---|---|
| | AH methanol | | AH acetone | | AH hexane | | AH | TG | |
| | 100 | 200 | 100 | 200 | 100 | 200 | | | |
| *E. coli* | NA | NA | 09.5 ± 0.70 | 13.5 ± 0.70 | NA | NA | 16.0 ± 2.00 | 15.0 ± 0.00 | 25.0 ± 1.00 |
| *S. aureus* | 10.5 □0.70 | 15.5 ± 0.70 | 18.0 ± 2.82 | 18.5 ± 0.70 | NT | 15.0 ± 0.00 | 15.66 ± 2.08 | 20.0 ± 0.00 | 35.33 ± 0.57 |
| *P. aeruginosa* | NA | NA | NA | NA | NA | NA | 16.33 ± 4.50 | NA | 20.5 ± 4.94 |

Values represent mean ± SD of triplicate determination. AH: *Artemisia herba alba*, TG: *Thapsia garganica*, NA: No activity, NT: Not tested.

For the *E. coli* strain, AH acetone extract at concentrations of 200 and 100 mg/mL was less effective than gentamicin (25 mm); the inhibition zone diameters shown by AH extracts for this strain varied from 9.5 to 13.5 mm. Furthermore, higher inhibition zones ranging from 10.5 to

18.5 mm were exhibited by AH extracts against *S. aureus*, where the acetone extract was the most active, while gentamicin gave an inhibition zone diameter of 35.33 mm against this strain.

On the other hand, EOs from AH and TG were very active against all bacterial strains tested. Both EOs gave a very close inhibition zones against *E. coli* (16 mm for AH and 15 mm for TG). *S. aureus* was more sensitive to TG essential oil with an inhibition zone diameter of 20 mm, while AH essential oil gave an inhibition zone diameter of 15.66 mm against this strain. Only EO from AH showed an activity against *P. aeruginosa* with an inhibition zone diameter of 16.33 mm.

It is well known that gram positive bacteria are more sensitive to bioactive molecules from plants, which is due to the absence of the outer membrane.[25] Several authors have explained the antibacterial activity of polyphenols and terpenes by the modification of cell membranes permeability. Flavonoids can also act by inhibiting both energy metabolism and DNA synthesis, thus affecting protein and RNA synthesis. In the case of Gram-positive bacteria, a modification of intracellular pH as well as interference with the energy-generating system (ATP) has been reported.[26]

## Conclusion

The present study was focused on the phenolic profile, antioxidant, and antibacterial properties of *A. herba-alba* and *T. garganica* from Algeria. Phytochemical profiling confirmed the richness of these species in flavonoids, tannins, terpenoids, and quinones. Quantifications of phenols showed that both plants are very rich in tannins and flavonoids, especially the AH extracts. Furthermore, a good ferric reducing potential was exhibited by the extracts, especially acetone extracts. A very important anti-lipid peroxidation capacity was also exhibited by the extracts, where the methanol extract of AH displayed a very close activity to BHT. Both extracts and essential oils from AH and TG exhibited good antibacterial activity against *E. coli*, *S. aureus*, and *P. aeruginosa*. Therefore, AH and TG extracts can be considered as promising sources of natural antioxidant and antimicrobial compounds for biological and pharmaceutical applications.

## Conflict of Interest

The authors declare no conflict of interest.

## Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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