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Extraction Yield, Phytochemicals Analysis, and Certain in Vitro Biological Activities of Artemisia Herba Alba Extracts

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ABSTRACT

The objectives of this study were to determine the extraction yield and phytochemical composition of different *Artemisia herba alba* (AHA) extracts, as well as to investigate *in vitro* their antioxidant, anti-inflammatory, anti-glycation, and antibacterial activities, also to study the effect of the solvent extraction on these characteristics. Four solvents with different polarities were used to prepare AHA extracts. Several *in vitro* tests were used in this study to evaluate the extract yield and phytochemical characteristics of the different AHA extracts, as well as to determine certain of their biological activities. Our findings showed that 80% aqueous ethanolic extract, significantly compared to other extracts, exhibited higher extraction yield (15.3%), higher phytochemical content (263.93 mg GAE/g E for total phenols), (40.94 mg QE /g E for total flavonoids), and (35.99 mg GAE/g E for total tannins), and higher Diphenyl-2-Picrylhydrazyl (DPPH) radical scavenging capacity (IC_{50}= 4.13 mg/mL) with no significant differences when compared to methanolic extract, as well as higher anti-glycation activity (IC_{50}= 3.96 mg/mL). However, the methanolic extract had a significant inhibitory effect against the majority of the tested bacteria compared to other extracts. While the anti-inflammatory potential of distilled water extract was significantly higher (IC_{50}= 2.96 mg/mL) compared to another extract. It is clear that the solvent type had a significant effect on the chemical and biological characteristics of AHA. The diversity and significance of *Artemisia herba alba*'s biological activities demonstrate its potential application in the pharmaceutical and food fields.

Keywords: Phytochemical composition, Antioxidant, Anti-inflammatory, Anti-glycation, Antibacterial.

INTRODUCTION

Since ancient times, medicinal plants have been widely recognized as an important source for the treatment of a range of diseases and could even be considered the origin of modern medicine (Parameswari *et al*., 2019; Bachir & Belhouala, 2021; Okpuzor *et al*., 2021). Even today, hundreds of plants are cultivated worldwide to obtain active substances for medicine,

pharmacy, cosmetics, and the food industry (Salmerón-Manzano *et al*., 2020). Nowadays, medicinal plants are very popular in many populations. Approximately 80% of the world's medicines (Parameswari *et al*., 2019) and most of the anticancer drugs are derived from plants or plant-based bioactive compounds (Calixto, 2019).

Phytochemicals such as phenolic acids, alkaloids, flavonoids, oligo-/polysaccharides, saponins, terpenoids, steroids, tannins, curcumin, xanthones, thiosugar derivatives, trigollin, chalcones, amino acids are naturally occurring in different parts of medicinal plants, like in leaves, fruits, roots, flowers, and seeds (Jamshidi-Kia *et al*., 2018; Parameswari *et al*., 2019). These bioactive compounds have emerged as innovative agents for the prevention of chronic diseases (Kang, 2021). Many plants have extractable bioactive compounds with a wide range of biological activity (Parameswari *et al*., 2019), including antioxidants, anti-inflammatory, antimicrobial, anticancer, antiviral, anti-allergic, immune-stimulatory, estrogenic (Đurović *et al.,* 2022), anti-hypertension, anti-glycation, and antidiabetic (Oraon *et al.,* 2017).

Artemisia herba alba, also known as "Shih" (Arabic name), is a greenish-silver perennial herb that belongs to the Asteraceae family (Al-Kharabsheh *et al*., 2017; Gacem *et al.,* 2020; Réggami *et al*., 2021). It grows in semi-arid and arid conditions and is widespread over the five continents, including South Europe, North Africa, North America, and the Middle East (Paolini *et al*., 2010; Asdadi *et al*., 2020; Ouguirti *et al*., 2021). This herb has a distinct odor as well as a bitter taste (Gacem *et al*., 2020). It is cheap and widely available (Asdadi *et al.,* 2020).

This plant is one of the most popular medicinal and aromatic plants extensively used in folk medicine by several cultures since ancient times for its medicinal properties (Younsi et al., 2016; Mohammed *et al.,* 2021; Réggami *et al.,* 2021), such as treating intestinal disturbances, stomach disorders, diarrhea (Seddik *et al*., 2010), colds, cough, bronchitis, neuralgias (Al-Kharabsheh *et al*., 2017), scorpion/snake bites, parasitic infections, hypertension (Younsi *et al*., 2016; Ouguirti *et al*., 2021), inflammation, diabetes mellitus (Rafiq *et al*., 2016; Asdadi *et al*., 2020; Réggami *et al*., 2021) and for the treatment of human and livestock wounds (Seddik *et al*., 2010; Qnais *et al.,* 2016). It has also been used in the cosmetic and food industries as a flavoring agent for tea and coffee (Rafiq *et al*., 2016; Ouguirti *et al.,* 2021).

This medicinal herb has a broad spectrum of phytochemicals or secondary metabolites (Nigam *et al*., 2019), like polyphenols, tannins, flavonoids, flavonols, terpenoids, coumarins, glycosides, sterols, polyacetylens (Choi *et al*., 2013), anthracenosids (Laouini *et al.,* 2018; Boukhennoufa *et al*., 2020) and alkaloids (Aljaiyash *et al*., 2018). These bioactive compounds have a variety of pharmacological and biological activities, including antidiabetic, antibacterial, antitumor, antimalarial, antioxidant, insecticidal, neurological (Younsi *et al*., 2016; Mohammed *et al.,* 2021), anti-allergic, anti-inflammatory (Laouini *et al*., 2018), leishmanicidal, spasmolytic properties (Paolini *et al*., 2010), anti-fungal, anti-mutagenic (Asdadi *et al*., 2020), antihepatoxic, choleretic, spasmolytic, antihelmintic, and antiphlogistic activities (Boukhennoufa *et al*., 2020).

The extraction efficiency is affected by the solvent extraction and its polarity, pH, temperature, time, and composition of the sample (Do *et al*., 2013). Because no single solvent can reliably extract all of the phytochemical and antioxidant compounds present in plant material, various phytochemicals are extracted in solvents of varying polarities. According to Nawaz *et al*. (2020), several studies report that the polarity of the solvent has a significant impact on the extract yield, the bioactive compound content, and the biological activities of plant material.

The characterization and biological activities of *Artemisia herba alba* have received less attention than those of other medicinal plants around the world. There are currently few studies examining the effects of various extraction solvents on extraction yield, phytochemical components, and biological activities of AHA extracts particularly *in vitro* anti-inflammatory and antimicrobial activity of AHA arterial parts. There are no published studies that investigate the *in vitro* antiglycation activity

of AHA extract as well. In Jordan, few reports investigated the characteristics and the biological activities of the arterial parts of AHA. Therefore, the objectives of this study were to determine the extraction yield and phytochemical composition of *Artemisia herba alba* extracts obtained by different solvents, as well as to investigate *in vitro* their antioxidant, anti-inflammatory, anti-glycation, and antibacterial activities, also to determine the effect of the solvent extraction on these characteristics.

Materials and Methods

Plant Material Preparation

The air-dried aerial part of *Artemisia herba alba* was purchased in May 2021 from a local herbal market in Amman, Jordan. It was ground using a mechanical blender (Moulinex Miller, France) and sieved until a fine powder was obtained. The sample was kept in glass jars, hermetically sealed, and stored away from light for later use.

Extraction Yield of *Artemisia herba alba*

Ten grams of sample powder were individually extracted with 100 mL of four organic solvents with different polarities, namely, 80% aqueous ethanol (A. ethanol), methanol, distilled water, and ethyl acetate in a rotary shaker (New Brunswick Scientific, USA) at 180 rpm for two hours and then in an ultrasonic bath (Bandelin Electronic-RK-103 H, Germany) at 37 °C for 15 minutes. The mixture was then filtered through Whatman No. 1 filter paper and the solvents were evaporated under vacuum using a rotary evaporator (Büchi, RE 121, Switzerland) at 38 °C and 120 rpm for 3 to 4 hours. The obtained extracts were stored in sterile dark vials in a refrigerator at 4 °C for later use (Hamadneh *et al*., 2018). The following equation was used to calculate the percentage of extract yield [EY]:

[EY] = (weight of dry extract/weight of dried plant sample) x100. (Equ 1)

Qualitative Detection of Phytochemical Constituents of *Artemisia herba alba* Extracts

The preliminary analysis of phytochemical constituents for the four AHA extracts was performed using the standard protocol described by (Akter, *et al*., 2018; Priyanka, *et al*., 2019; Chaikh & Patil, 2020) to identify the presence of phenolic compounds, flavonoids, alkaloids, saponins, tannins, carbohydrates, proteins, cardiac glycosides, and terpenoids**.** The presence of these bioactive compounds in the different AHA extracts in high quantity (+++), moderate quantity (++), or low quantity (+) was determined by the intensity of the color that appeared in the reaction mixture, and their absence (-) was determined by no change in the reaction mixture's color.

Quantitative Detection of Phytochemicals Content of *Artemisia herba alba* Extracts

Estimation of Total Phenols Content (TPC)

The total phenolic content present in the four AHA extracts was estimated using the Folin-Ciocalteu reagent according to the method described by Shirazi *et al*. (2014). Briefly, 100 µL of each extract was transferred into a test tube, followed by the addition of 500 µL of distilled water and 100 µL of Folin-Ciocalteu reagent, mixed well, and allowed to stand for 6 minutes. Then, 1 mL of 7% sodium carbonate (Na_2CO_3) and 400 µL of distilled water was added to the reaction mixture. The absorbance was measured using a UV/VIS spectrophotometer (SpectroScan 80D, China) at a wavelength of 760 nm after incubation for 90 minutes in darkness at room temperature. All the experiments were performed in triplicate. The phenolic content was calculated as mg of gallic acid equivalent (GAE) per g of extract; and the equation of the calibration curve was:

$Y = 0.0034x + 0.0373$, $R^2 = 0.9903$. (Equ 2)

Estimation of Total Flavonoids Content (TFC)

The total flavonoid content was determined using the aluminum chloride colorimetric method suggested by Kim *et al.* (2003). Precisely, 1 **mL** of each extract was transferred to a 10 **mL** volumetric flask containing 4 **mL**

of distilled water, followed by the addition of 300 µL of sodium nitrite ($NaNO_2$). After 5 minutes, 300 µL of 10% aluminum chloride ($AlCl_3$) was added. Then, after 6 minutes, 1 **mL** of 1 M sodium hydroxide (NaOH) was added, and the total volume was made up to 10 **mL** with distilled water. The reaction mixture was vortexed (ZX3 Vortex Mixer, Italy) and the absorbance was measured at 510 nm spectrophotometrically. All the experiments were performed in triplicate. The flavonoid content was expressed as mg of quercetin equivalent (QE) per gram of extract, and the calibration curve equation was:

$Y = 0.006x + 0.028$ $R^2 = 0.9996$ $(Equ\ 3)$

Estimation of Total Tannin Content (TTC)

The total tannin content was determined using the Folin-Ciocalteu assay suggested by Djemoui *et al*. (2019). Briefly, 100 µL of each extract was added to 750 µL of distilled water, 500 µL of Folin-Ciocateu reagent, and 1 **mL** of 35% sodium carbonate (Na_2CO_3). The total volume was made up to 10 **mL** with distilled water and then shaken vigorously. The reaction mixture was incubated for 30 minutes at room temperature, and the absorbance was measured at 725 nm. All the experiments were performed in triplicate. The tannin content was expressed as mg of gallic acid equivalent (GAE) per g of extract, and the equation of the calibration curve was:

$Y = 0.005x - 0.0343$ $R^2 = 0.9993$ $(Equ\ 4)$

Free Radical Scavenging Activity of *Artemisia herba alba* Extracts

The free radical scavenging activity in prepared AHA extracts was determined using the procedure described by Boukhennoufa *et al*. (2020). Overall, 50 µL of various concentrations (from 0.312 to 1 mg/ **mL**) of each extract in methanol was mixed with 1.95 **mL** of a 0.025 g/L methanolic solution of Diphenyl-2-Picrylhydrazyl (DPPH). The same procedure was applied to ascorbic acid **(**as a positive control). The negative control was prepared by adding 50 µL of methanol to 1.95 **mL** of the methanol solution of DPPH. All the mixtures were then incubated in a dark place at room temperature for 30 minutes. The absorbance was measured at 517 nm. The tests were carried out in triplicate. The percentage of inhibition [PI] of the free radical DPPH was calculated as follows:

[PI] = [(Abs sample – Abs control)/Abs control] x 100 $(Equ\ 5)$

In Vitro Anti-inflammatory Activity of *Artemisia herba alba* Extracts

The anti-inflammatory activity of the four AHA extracts was performed *in vitro* using the protein denaturation technique described by Kannadas *et al*. (2020). Briefly, 2 **mL** of different concentrations (from 0.375 to 12 mg/ **mL**) of each extract in distilled water were added to 2.8 **mL** of freshly prepared Phosphate Buffer Saline (PBS) (pH 6.4) and 0.2 **mL** of egg albumin (from the fresh egg). The same procedure was applied to diclofenac sodium (as a positive control). The negative control was prepared by replacing 2 **mL** of the sample with distilled water. The sample mixtures were then incubated (Gallenhamp incubator, China) at 37˚C for 15 minutes and then set in a water bath (GFL water bath, Germany) at 70 ˚C for 5 minutes. After the incubation time, the samples were allowed to cool down at room temperature, and the absorbance was measured at 660 nm. All the experiments were performed in duplicate. The percentage inhibition [PI] of protein denaturation was calculated using the following equation:

[PI] = [(Abs control – Abs sample) / Abs control] x 100. $(Equ\ 6)$

In Vitro Anti-glycation Activity of *Artemisia herba alba* Extracts

The anti-glycation activity of the four AHA extracts was performed *in vitro* following the serum bovine albumin-glucose model system suggested by Starowicz & Zielinski (2019) with some modifications. Serum Bovine Albumin (BSA) (10 mg/ **mL**), glucose (90 mg/ **mL**), and (from 0.375 to 1 mg/ **mL**) of each extract were dissolved individually in phosphate buffer (pH 7.4). Then, 1 **mL** of each prepared solution was mixed together in a 5 ml

polypropylene test tube. The positive control (aminoguanidine) (from 0.375 to 1 mg/ **mL**) was prepared by mixing 1 **mL** of each concentration with 1 **mL** of BSA and 1 **mL** of glucose, while the negative control was prepared by adding 1 **mL** of phosphate buffer to the mixture instead of the sample extract.

The tested solutions contain 0.01% sodium azide to prevent microbial development. The tubes were then closed and incubated for 72 hours at 37°C in darkness. After the incubation time, the fluorescence of advanced glycation end products (AGEs) was measured at excitation and emission wavelengths of 360 nm and 460 nm, respectively, using a Microplate Fluorensce Reader (BIO-TEK FLx800 Microplate Fluorensce Reader, USA). All the experiments were performed in duplicate. The percentage inhibition [PI] of AGEs formation by AHA extracts was determined using the following equation (FI: fluorescence intensity):

$$[PI] = \{1 - [(FI \text{ of extract}) / (FI \text{ of negative control})]\} \times 100 \quad (Equ\ 7)$$

Antibacterial Activity of *Artemisia herba alba* Extracts

The antibacterial activity of the four AHA extracts was determined according to the method employed by Akrout et al. (2009) and Younsi et al. (2016) by using an agar well diffusion test against various bacteria, including *Escherichia coli* (ATCC 25922), *Listeria monocytogenes* (ATCC 7644), *Salmonella Typhimurium* (ATCC 21292), *Salmonella Typhimurium* (ATCC 22876), *Staphylococcus aureus* (ATCC 25923), *Staphylococcus aureus* (ATCC 29213), *Enterobacter hormaechei* (ATCC 700323) and *Enterococcus casseliflavus* (ATCC 700327).

Briefly, 100 µL of bacterial suspensions (10^8 CFU/ **mL**) were spread on the Mueller Hinton agar (MHA) plates using a sterile cotton swab. Wells (6 mm in diameter) were made on each plate. Then, 100 µL of each freshly prepared AHA extract (100 mg/ **mL**) in 10% dimethyl sulfoxide (DMSO) and filter-sterilized using a micro-filter (0.2 µm) was dropped into each well. A standard disc containing gentamicin (10 µg /disc) was used as a positive control, while 100 µL of 10% DMSO was used as a negative control. These plates, after remaining at 4 °C for 2 **hours** were then incubated for another 24 **hours** at 37 °C. The diameter of the inhibition zone around discs or wells was finally measured. All tests were performed in triplicate.

Determination of Half Maximal Inhibitory Concentration (IC_{50})

The extract concentration providing 50% (IC_{50}) inhibition of the free radical DPPH, inhibition of the heat protein denaturation, and inhibition of the AGE formation was calculated from a plot of inhibition percentage versus extract concentration of the antioxidant, the anti-inflammatory, and the anti-glycation activity, respectively.

Statistical Analysis

All analytical determinations were performed at least in duplicates, and the results are expressed as mean ± standard deviation (SD). All statistical analyses were carried out using SPSS statistics software version 22 (IBM Corp., USA). One-way ANOVA and Duncan's multiple comparison tests were used to compare results with significant differences ($p \leq 0.05$). Values of $p \leq 0.05$ were considered significant.

Results and discussion

Extraction Yield of *Artemisia herba alba*

The extraction yield (%) of AHA extracts obtained by 80% A. ethanol, methanol, distilled water, and ethyl acetate is presented in Table 1.

Table 1: Extraction yield of AHA obtained with different solvents.

Artemisia herba-alba	**Extract yield (%)**			
	(80%) A. ethanol	Methanol	Distilled water	Ethyl acetate
	15.3	12.2	12.2	4.5

These results showed that 80% aqueous ethanolic extract had the highest extraction yield (15.3%), followed by methanolic extract (12.2%), distilled water extract

(12.2%), and ethyl acetate extract (4.5%), respectively. According to Amkiss *et al*. 2021, the extraction yield of AHA ethanolic extract is around 15.4% for 20 g of dry matter.

The extraction efficiency depends on a number of factors, such as the solvent used, the method of extraction used, the chemical nature and particle size of the phytochemical and the presence of interfering substances (Stalikas, 2007). The extract yield is affected by the solvent extraction and its polarity, pH, temperature, time, and composition of the sample. The combined use of water and organic solvent may enhance the extraction yield. Compounds such as proteins and carbohydrates other than phenolic compounds may be more solubilized in water/alcohol than in pure solvents (Do *et al*., 2013). This could explain why 80% aqueous ethanolic extract had high extraction yield than other pure organic solvents.

Qualitative Analysis of ***Artemisia herba alba*** **Extracts**

The presence or absence of the phytochemical compounds in the AHA extracts obtained by the four different solvents is shown in Table 2.

Table 2: Phytochemical analysis of AHA obtained by different solvents

Phytochemicals	(80%) A. ethanol	Methanol	Distilled water	Ethyl acetate
Phenols	+++[a]	+++	++[b]	+[c]
Flavonoids	+++	+++	++	+
Alkaloids	+++	+++	++	+
Tannins	+++	+++	++	+
Saponins	+++	+++	++	-[d]
Terpanoids	+++	+++	++	+
Carbohydrates	+++	+++	++	+
Proteins	+++	+++	++	+
Cardiac glycosides	+++	+++	++	-

[a]+++ present in high quantity, [b]++ present in moderate quantity, [c]+present in low quantity,[d]- absent.

The obtained data revealed a strong presence (+++) of phenolic compounds, flavonoids, alkaloids, saponins, tannins, carbohydrates, proteins, cardiac glycosides, and terpanoids in 80% aqueous ethanolic and methanolic extracts, whereas the distilled water extract showed a moderate presence (++) of these compounds. The ethyl acetate extract showed low presence to complete absence (+/-) of the phytochemicals. According to Benyahia *et al*. (2021), many studies have shown that the aerial section of the AHA plant is high in phytochemicals such as polyphenols, alkaloids, flavonoids, lactones, tannins, etc., whereas other studies have revealed that some of these bioactive compounds are absent in this plant. The presence or absence of phytochemical compounds in AHA extracts may be affected by the nature of the solvent used; thus, some organic solvents are more efficient than distilled water (Bencheqroun *et al*., 2012).

Quantitative Analysis of ***Artemisia herba alba*** **Extracts**

The total phenolic, flavonoid, and tannin contents (mean ± SD) in the four different AHA extracts are illustrated in Table 3, and their calibration curves are described in Appendix 1 (figures 1, 2, and 3).

Table 3: Total polyphenols, total flavonoids, and total tannins content in AHA extracts obtained by different solvents

AHA extracts	Total Polyphenols (mg GAE/g E)	Total Flavonoids (mg QE/g E)	Total Tannins (mg GAE/g E)
80% A. ethanol E	263.93±2.46[a]	40.94±1.45[a]	35.99±1.20[a]
Methanol E	207.85±1.55[b]	34.16±0.60[b]	30.86±0.34[b]
Distilled water E	131.48±0.61[c]	17.72±0.53[c]	22.79±0.41[c]
Ethyl acetate E	58.24±1.61[d]	7.83±1.20[d]	16.32±0.23[d]

[1] Values are the means of three independent replicate trails ± standard deviation.
[2] Treatment means within the same column without shared superscripts are significantly different ($p \leq 0.05$).
[3] E; Extract.

These findings indicate a significant ($p \leq 0.05$) difference in their contents. The total polyphenols (263.93 mg GAE/g E), total flavonoids (40.94 mg QE/g E), and total tannins (35.99 mg GAE/g E) were significantly ($p \leq 0.05$) higher in the 80% aqueous ethanolic extract than in the methanolic and distilled water extracts. While the lowest values of these bioactive compounds were found in the ethyl acetate extract.

Similarly, Dif *et al.* (2018) estimated the total polyphenols, flavonoids, and tannins in three AHA extracts in Algeria. They observed that the ethanolic extract was the most concentrated in the total phenolics and tannins (279.73 and 33.50 mg/mL respectively), whereas the methanolic extract had the highest amounts of total flavonoids (234.45 mg/mL). In contrast, Eddine *et al.* (2016) estimated the total polyphenols, flavonoids, tannins, and proanthocyanidins content in four AHA extracts. They revealed that ethyl acetate extract had the highest phenolics, flavonoids, tannins, and proanthocyanidins, followed by butanolic extract, water extract, and chloroform extract. The difference in results may be due to differences in the origin of the plant, type of solvent used, extraction method, extraction time, extraction temperature, and amount of plant used for extraction when compared to our results.

Many variables influence the phytochemical extraction, including extraction temperature, extraction time, plant origin and its particle size, the amount of the plant used for extraction (Chikezie *et al.,* 2015), and the extraction solvent and its polarity (Thouri *et al*., 2017). The polarities of polyphenols range from polar to non-polar, and the highest extraction of these bioactive compounds is generally obtained in polar solvents, which have a better efficiency of extraction as a result of stronger interactions (hydrogen bonds) between the polar sites of these compounds and the solvent (Liu *et al*., 2007; Thouri *et al*., 2017). This may explain why the 80% aqueous ethanolic extract had the highest amounts of these bioactive compounds compared with other organic solvent extracts.

DPPH Radical Scavenging Activity of *Artemisia herba alba* Extracts

The free radical inhibition percentages DPPH (mean ± SD) obtained by different AHA extracts at varying concentrations (from 0.03 to 1.00 mg/mL) and their IC_{50} values were reported in Table 4.

Table 4: The DPPH scavenging ability of AHA extracts obtained by different solvents at different concentrations and their IC_{50}

	Concentration (mg/mL)						IC_{50}
	0.03125	**0.0625**	**0.125**	**0.25**	**0.5**	**1**	
Ascorbic acid	24.67±0.63[a]	58.27±0.93[a]	86.49±0.42[a]	88.64±0.14[a]	89.53±0.08[a]	89.81±0.77[a]	01.74±0.01[d]
80% A. ethanol E	06.02±0.28[b]	12.83±0.45[b]	21.70±0.56[b]	40.84±0.16[b]	80.85±0.85[b]	86.36±0.21[b]	04.13±0.02[c]
Methanol E	05.48±0.21[b]	10.63±0.14[c]	21.76±0.96[b]	40.00±0.56[b]	77.00±1.09[c]	85.62±0.49[b]	04.31±0.04[c]
Distilled water E	01.22±0.32[c]	02.49±0.29[d]	05.50±0.21[c]	10.11±0.35[c]	19.57±0.66[d]	38.63±0.53[c]	11.58±0.13[b]
Ethyl acetate E	00.52±0.43[c]	01.37±0.49[e]	02.50±0.72[d]	05.67±0.86[d]	10.88±1.06[e]	20.25±1.47[d]	21.72±0.50[a]

[1]Values are the means of three independent replicate trails ± standard deviation.
[2]Treatment means within the same columns, without shared superscripts are significantly different ($p \leq 0.05$).
[3] E; Extract.

The results showed that the free scavenging capacity is proportional to the concentration of the AHA. Ascorbic acid (as a positive control) had significantly ($p \leq 0.05$) the highest scavenging activity, with an IC_{50} value of 1.74 mg/mL, followed by 80% aqueous ethanolic extract, with an IC_{50} value of 4.13 mg/mL. This concentration appeared insignificant ($p \leq 0.05$) when compared to the methanolic extract (IC_{50}= 4.31 mg/mL). Distilled water extract had an IC_{50}= 11.58 mg/mL, while ethyl acetate extract exhibited the lowest response (IC_{50}= 21.72 mg/mL). Jasim & El-Zayat (2019) reported that the methanolic extract of AHA had higher free radical scavenging activity (IC_{50} =0.06 mg/mL) than the aqueous extract (IC_{50}= 0.081 mg/mL), which differ from our results, and this difference could be attributed to the method used, extraction temperature, extraction time, plant origin and its particle size, or the amount of the plant used for the extraction.

In the current study, it was obvious that a significant correlation between phenolic content and free radical inhibition capacity particularly when the concentration of each extract increased. Polyphenols had the highest antioxidant proprieties (Tungmunnithum *et al*., 2018), and their total content was proportional to their antioxidant capacity (Do *et al.,* 2013). Several studies have found a positive correlation between total phenolic content and free-radical scavenging activity (Laouini *et al*., 2018).

***In -Vitro* Anti-inflammatory Activity of *Artemisia herba alba* Extracts**

The inhibition percentages (mean ± SD) of heat-induced denaturation of proteins obtained by four different AHA extracts at different concentrations (0.375 to 12 mg/mL) and their IC_{50} values were summarized in Table 5.

Table 5: *In-vitro* anti-inflammatory activity of AHA extracts obtained by different solvents at different concentrations and their IC_{50}

	Concentration mg/Ml						IC_{50}
	0.375	**0.75**	**1.5**	**3**	**6**	**12**	
Diclofinac sodium	21.51±0.34^{a}	31.65±0.85^{a}	42.75±0.38^{a}	67.30±0.15^{a}	90.03±1.07^{a}	90.03±1.07^{a}	2.94±0.02^{c}
80% A. ethanol	05.24±0.07^{c}	09.37±0.42^{c}	11.29±0.42^{c}	18.80±0.30^{c}	40.88±0.70^{c}	76.62±0.27^{c}	5.20±0.04^{b}
Methaolic E	02.26±1.27^{d}	05.16±0.11^{d}	10.55±0.30^{c}	19.19±0.46^{c}	38.43±0.23^{d}	65.66±0.07^{d}	5.80±0.03^{b}
Distilled water E	13.77±0.15^{b}	20.28±1.62^{b}	46.14±1.77^{b}	78.29±0.46^{b}	79.36±0.19^{b}	80.42±0.30^{b}	2.96±0.06^{c}
Ethyl acetate E	02.32±0.00^{d}	04.83±0.13^{d}	11.61±0.27^{c}	14.88±0.28^{d}	17.45±1.66^{e}	19.92±0.88^{e}	14.54±0.73^{a}

[1]Values are the means of three independent replicate trails ± standard deviation.
[2]Treatment means within the same column, without shared superscripts are significantly different ($p \leq 0.05$).
[3] E; Extract.

It is clear that the inhibition percentage is proportional to the concentration of the AHA extracts. Diclofenac sodium (as a reference drug) and distilled water extract had the highest inhibition percentages with IC50= 2.94, and 2.96 mg/mL, respectively. The IC_{50} values for the 80% aqueous ethanolic and methanolic extracts were moderate (IC_{50}= 5.20 and 5.80 mg/mL, respectively), while the ethyl acetate extract had significant ($p \leq 0.05$) the lowest values (IC_{50} = 14.54 mg/mL).

Phytochemicals have recently been recognized as new natural compounds with potent anti-inflammatory properties. These bioactive substances, including phenolics, flavonoids, alkaloids, and terpenoids, may have an effective anti-inflammatory activity (Truong *et al*., 2021). Eddine *et al*. (2016) estimated in vitro the anti-inflammatory potential of aerial parts of Algerian AHA extracts obtained by ethyl acetate, butanol, water, and chloroform using a nitric oxide radical scavenging assay. They observed a positive correlation between anti-inflammatory activity and phenolic compounds. They revealed that the extract with the highest levels of phenolic content exhibited the highest *in vitro* anti-inflammatory activity by decreasing nitric oxide concentration in the reaction mixture. The strongest anti-inflammatory effect was observed for ethyl acetate (IC_{50} = 38.33 g/mL), which has the highest levels of phenolic content (92.29 mg GAE/g DW) compared to other extracts.

According to our findings, distilled water with a moderate phenols content had the highest percentage of inhibition of protein denaturation compared to other extracts. Various types of polyphenols may be degraded at different temperatures. However, it depends on the solvent type, pH, and treatment duration. At high temperatures, certain phenolic compounds may be destroyed (Antony & Farid, 2022). Various polyphenolic and non-polyphenolic components may contribute to the anti-inflammatory effect. This might explain why distilled water had the strongest anti-inflammatory potential.

In-Vitro Anti-glycation Activity of *Artemisia herba alba* Extracts

Glycation is a non-enzymatic reaction that occurs between reducing sugars and amino groups of proteins, producing advanced glycation end products (AGEs) (Kazeemet *et al.,* 2012), which cause lipid peroxidation, endothelial dysfunction, protein structural changes, and abnormal cellular activity (Intagliata *et al*., 2020). The inhibition percentages of AGEs (mean ± SD) by different *AHA* extracts at varying concentrations (0.0625 to 1 mg/mL) and their IC_{50} values were illustrated in Table 6.

Table 6: In vitro anti-glycation activity of AHA extracts obtained by different solvents at different concentrations and their IC_{50}

	Concentration mg/mL					IC_{50}
	0.0625	**0.125**	**0.25**	**0.5**	**1**	
Aminoguanidine	25.52±0.73[a]	35.93±0.73[a]	60.41±1.47[a]	93.75±1.47[a]	97.39±0.73[a]	02.29±0.04[e]
80% A. ethanol E	09.88±0.74[b]	20.31±0.73[b]	35.41±1.47[b]	53.62±0.70[b]	64.58±1.47[b]	03.96±0.09[d]
Methanol E	06.77±0.73[c]	14.58±1.47[c]	30.72±0.73[c]	50.52±0.73[c]	59.89±0.73[c]	04.86±0.8b[c]
Distilled water E	06.77±0.73[c]	15.10±0.73[c]	27.60±0.73[d]	34.89±0.73[d]	37.50±1.47[d]	05.78±0.16[b]
Ethyl acetate E	01.56±0.73[d]	05.72±0.73[d]	08.85±0.73[e]	16.66±1.47[e]	17.18±0.73[e]	14.18±1.37[a]

[1]Values are the means of three independent replicates trails±standard deviation.
[2]Treatment means within the same column, without shared superscripts, are significantly different ($p \leq 0.05$).
[3] E; Extract.

The anti-glycation activity is proportional to the concentration of the AHA extracts. Aminoguanidine (a reference drug) had significantly ($p \leq 0.05$) the highest inhibition percentage of AGEs (IC_{50}= 2.29 mg/mL), followed by 80% aqueous ethanolic extract with an IC_{50} value of 3.96 mg/mL then methanolic extract (IC_{50}= 4.86 mg/mL). The median values were found for distilled water extract (IC_{50}= 5.78 mg/mL), while ethyl acetate extract exhibited the lowest response (IC_{50}= 14.18 mg/mL).

Phytochemicals are the most researched class of compounds as candidates for anti-glycation (Odjakova *et al*., 2012). Several studies have revealed that plant extracts' anti-glycation capacity is significantly related to their phenolic content (Grzegorczyk-Karolak *et al*., 2016; Safari *et al*., 2018). There are no published studies dealing with the investigation of the antiglycation activity of AHA extract. Dearlove *et al*. (2008) revealed a high correlation between anti-glycation activity and the total phenolic amounts in twenty-four herbs and spices. On the other hand, Ramkissoon *et al.* (2012) reported that plant extracts' anti-glycation activities are not always contributed to their phenolic content or antioxidant properties.

According to Nakagawa *et al.* (2002), green tea had a considerable anti-glycation effect and high antioxidant properties. However, Chen *et al.* (2011) suggest that *Astragalus membranaceus* extracts exhibit a substantial anti-glycation efficiency but with low antioxidant activity, while *Periploca sepium* showed a strong antioxidant activity but low anti-glycation capacity. In the current study, it was obvious that a significant correlation between phenolic content and anti-glycation capacity particularly when the concentration of each extract increased.

Antibacterial Activity of *Artemisia herba alba* Extracts

The antibacterial activity (mean value ± SD) of AHA extract obtained by different solvents against the selected bacterial strains using the agar well diffusion test is illustrated in Table 7. The results were expressed as the diameter of the inhibition zone (mm).

Table 7: Antibacterial activity of AHA extracted with various solvents.

	Zone of Inhibition (mm)							
	E. coli ATCC 25922	*L. monocytogenes* ATCC 7644	*S. Typhimurum* ATCC 21292	*S. Typhimurum* ATCC 22876	*S. aureus* ATCC 25923	*S. aureus* ATCC 29213	*E. hormaechei* ATCC 700323	*E. casseliflavus* ATCC 700327
Gentamicin	16.16±0.28[a]	15.50±0.28[a]	15.5±0.86[a]	08.50±0.28[a]	25.50±0.76[a]	13.00±0.76[a]	11.66±0.57[a]	09.33±.057[a]
80% A. ethanol E	01.49±0.573[c]	10.33±1.53[c]	11.00±1.73[b]	05.00±0.57[b]	08.00 ±1[c]	00.34±0.89[c]	08.16±0.28[c]	R
Methanol E	05. 00±1[b]	13 .00±1[b]	11.00 ±1[b]	01.33±0.57[d]	09 .00±1[bc]	08.50 ±0.5[b]	10.33±0.57[b]	R
Distilled water E	R	R	R	R	R	R	R	R
Ethyl acetate E	01.66±0.57[d]	09.33±0.57[c]	10.33±0.57[bA]	03.66±0.57[c]	09.33±0.57[b]	00.49±0.86[c]	10.00 ±1[b]	R

[1]Values are the means of three independent replicate trails ± standard deviation.
[2]Treatment means within the same column, without shared superscripts are significantly different ($p \leq 0.05$).
[3] E; Extract.

Our findings showed that gentamicin had a significant ($p \leq 0.05$) inhibition effect against all tested bacteria; strong activity was observed against *S. aureus* ATCC 25923 with an inhibition zone of 25.5 mm, whereas lower activity was seen against *S. Typhimurium* ATCC 22876 (08.5 mm). Methanolic, 80% aqueous ethanolic, and ethyl acetate extracts showed different inhibition levels against all tested bacteria except *E. casseliflavus* ATCC 700327. Distilled water did not show any effect against all selected bacteria.

The following results have been identified as not sensitive for diameters of ≤ 8mm, sensitive for diameters of 8–14mm, very sensitive for diameters of 14–20mm, and extremely sensitive for diameters of ≥20mm (Bellili *et al*., 2017). *L. monocytogenes* ATCC 7644 and *S. aureus* ATCC 29213 were both significantly ($p \leq 0.05$) sensitive to methanolic extract, with L. monocytogenes ATCC 7644 showing the greatest inhibition activity (13.00 mm). While *S. Typhimurum* ATCC 21292 was sensitive to methanolic, ethyl acetate, and 80% aqueous ethanolic extracts. E. *coli* ATCC 25922 and *S. Typhimurium* ATCC 22876, on the other hand, were not sensitive to all three extracts.

Gonelimali *et al*. (2018) reported that alcoholic extracts exhibit higher antimicrobial activity than aqueous extracts. Our results were consistent with those of Dahiya & Purkayastha (2012) who observed that among the 40 different extracts of eight plants, ethanolic and methanolic extracts were the most effective, with a significant inhibition effect against the majority of the bacteria strains tested. Water extracts, on the other hand, showed no inhibitory effect against any tested bacterial strain tested.

Similarly in the current study, distilled water extract did not show an inhibitory effect against the selected bacterial strains. This might be partially attributed either to the low concentration of antibacterial compounds or to the failure to extract effective antibacterial compounds. In addition, the discovered components (aromatic or saturated organic compounds) having a potential inhibitory effect on microorganisms were mostly obtained by ethanol or methanol extraction (Dahiya & Purkayastha, 2012). The combination of water and organic solvent could contribute to low antibacterial compound extraction in these extracts, resulting in a minimal inhibitory effect of 80% aqueous ethanolic extract.

Conclusion

Several *in vitro* tests were used in this study to evaluate the extraction yield and phytochemical characteristics of the different AHA extracts, as well as to determine some of their biological activities. Ours findings showed that the 80% aqueous ethanolic AHA extract significantly exhibited high extraction yield, high phytochemical composition, better DPPH scavenging capacity, and high anti-glycation activity compared to other extracts. While methanolic AHA extract had significantly higher antibacterial activity compared to other extracts. Whereas, distilled water extract exhibited higher anti-inflammatory activity, but failed to show any antimicrobial activity against all assayed bacteria compared to other extracts.The polarity of the solvent may significantly affect the extraction yield, the phytochemical content, and the biological activities of AHA extract. These results indicated that *Artemisia herba alba* has considerable antioxidant, anti-inflammatory, anti-glycation, and antibacterial activities. Further studies are needed to target the most important molecules responsible for these biological functions using advanced techniques, such as Fourier transform infrared spectroscopy, and nuclear magnetic resonance spectroscopy.

Conflict of interest

The authors declare that there are no conflicts of interest.

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Appendix

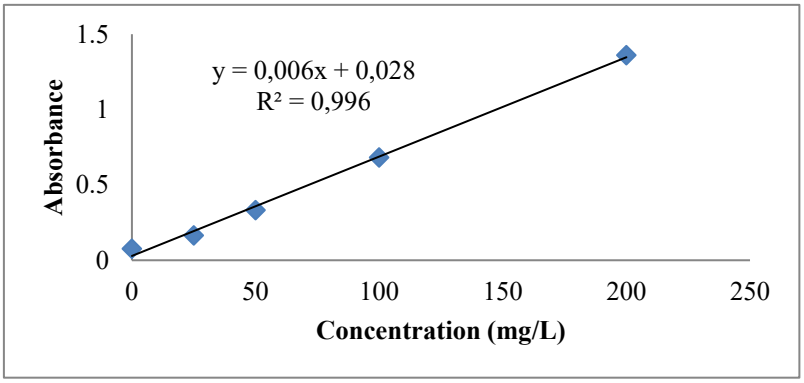

Figure 1: Standard curve of quercetin (TFC).

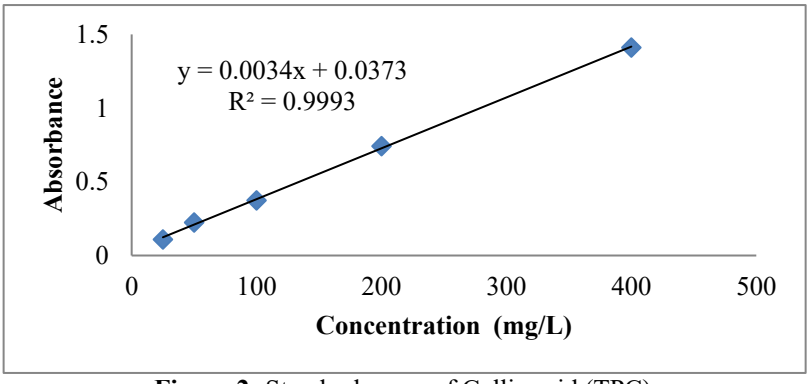

Figure 2: Standard curve of Gallic acid (TPC).

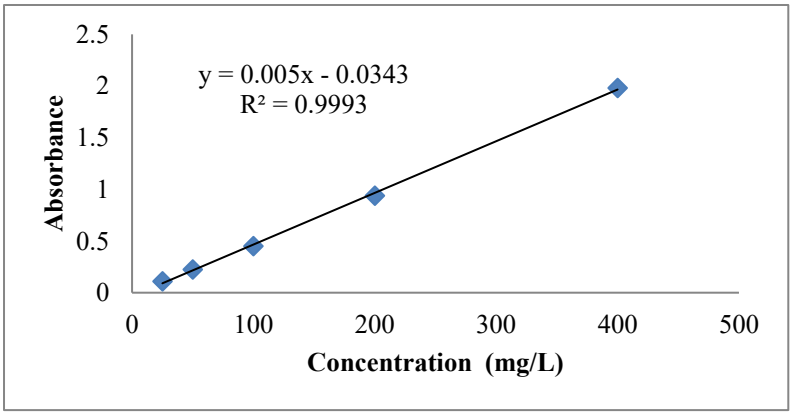

Figure 3: Standard curve of Gallic acid (TTC).

إنتاجية الاستخلاص وتحليل المواد الكيميائية النباتية وبعض الأنشطة البيولوجية المخبرية لمستخلصات نبات الشيح ألبا

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ملخص

تهدف هذه الدراسة الى تحديد إنتاجية الاستخلاص والتركيب الكيميائي النباتي لمستخلصات الشيح(AHA) المختلفة، وكذلك التحقق من أنشطتها المضادة للأكسدة والمضادة للالتهابات والمضادة للجلايكيشن والمضادة البكتيريا في المختبر، أيضًا لدراسة تأثير الاستخلاص بالمذيب على هذه الخصائص. تم استخدام أربع مذيبات مختلفة الأقطاب لتحضير مستخلصات الشيح. تم استخدام العديد من الاختبارات في المختبر في هذه الدراسة لتقييم محصول المستخلص والخصائص الكيميائية النباتية لمستخلصات AHA المختلفة، وكذلك لتحديد بعض أنشطتها البيولوجية. أظهرت النتائج التي توصلنا إليها أن مستخلص الإيثانول المائي (80 %) أظهر بشكل كبير مقارنة بالمستخلصات الأخرى، محصول عالي من الاستخلاص (15.3 %)، ومحتوى كيميائي نباتي مرتفع) 263.93 ملجم/GAE جم E لإجمالي الفينول) (40.94 ملجم /QEجم E لإجمالي الفلافونويد)، و (35.99ملجم/GAE جم E لإجمالي التانين)، أفضل قدرة على كسح الجذر ثنائي فينيل-2-بيكريل هيدرازيل (DPPH) مع عدم وجود فروق ذات دلالة إحصائية عند مقارنتها بمستخلص الميثانول، بالإضافة إلى نشاط مرتفع مضاد للجلايكيشن (3,96 ملجم/مل. (IC50 =من الواضح أن نوع المذيب كان له تأثير كبير على الخصائص الكيميائية والبيولوجية للشيح. يوضح تنوع وأهمية الأنشطة البيولوجية للشيح إمكانية تطبيقها في المجالات الصيدلانية والغذائية.

الكلمات الدالة: التركيب الكيميائي النباتي، مضاد الأكسدة، مضاد الإلتهاب، مضاد للجلايكيشن، مضاد البكتيريا