Influence of solvent extraction on phenolic content, antioxidant and anti-inflammatory activities of aerial parts extract from Algerian Artemisia Herba-alba

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ABSTRACT

Artemisia Herba-alba is a commonly consumed medicinal and culinary herb in Algeria. This current study aimed to obtain the proper solvent for extracting bioactive compounds from Artemisa Herba-alba guided by phenolic content, flavonoid content, proanthocyanidins, antioxidant and anti-inflammatory activity. The results showed that ethyl acetate extract of exhibited the highest phytochemical composition (total phenolic content 92.29±3.25 mg GAE/g W, total flavonoids content 61.24±2.04 mg RT/g DW and proanthocyanidins 25.47±0.95 mg CAE/g DW), while butanol extract (BE), water extract (WE) and the minimum value in chloroform extract (CE). The best DPPH• scavenging capacity (IC₅₀ = 36.85±1.17 µm/mL), β-carotene (23.83± 0.53) and ferric reducing power (IC₅₀ = 14.71± 0.51 µm/mL) were observed in EAE followed by BE. Our study also revealed the high anti-inflammatory potential of EAE, BE, WE and CE. Ethyl acetate can be an efficient solvent for extracting antioxidants from Artemisa Herba-alba based on this study. All these biological activities are well correlated with the phenolic contents of these extracts. These findings demonstrate the remarkable potential of these plants as valuable source of antioxidants with exhibit original and interesting anti-inflammatory and anticancer capacities.

KEYWORDS: Artemisia Herba-alba, phytochemical, antioxidant, anti-inflammatory, Algeria

INTRODUCTION

Artemisia belongs to the Asteraceae family of plants, which consists of over 500 species distributed worldwide[1]. It is characterized by a wide range of morphological and phytochemical variability, which is associated with different geographical origins of the samples[2,3]. The genus displays a huge ecological plasticity, with species occurring from sea level to high mountains and from arid zones to wetlands, nown also as “desert wormwood” is a prominent plant of the Irano-Turanien steppes of Spain, North Africa and the Middle East[4]. It is one of the most widely used plants in the Algerian folk medicine; the aerial parts of Artemisia herba-alba are widely used in traditional medicine by many cultures since ancient times. Generally, essential oils of this plant exhibited wide range of bioactivity, due to the presence of secondary metabolites, acting through diverse modes of action. Is used for the treatment of diabetes, for its antihyperglycemic [5], hypoglycemic effect, antibacterial and antifungal activity[6], antiprotozoal effects[7], anti-inflammatory, anticancer activities[8], neuroprotective[9] and antiehelminthic[10]. Different compounds whose main components are essential oils and polyphenols manifest these diverse biological activities. Polyphenols play diverse roles such as functioning as antioxidant, antimicrobial, anti-allergic, anti-inflammatory and anticancer agents [11]. These compounds are the major low molecular weight bioactive components usually found in mushroom species, responsible for their antioxidant properties[12]. Phenolic compounds can play the role of antioxidants through different mechanisms, including terminating free radicals, reducing oxy-gen concentration, transforming primary products of oxidation into non-oxidant molecules, and acting as metal. Previous research has reported that phenolic composition is vastly influenced by biotic and abiotic factors[13], the potential of polyphenols to improve cardiovascular health through an array of actions including anti-inflammatory, antihypertensive and antiatherosclerotic activities. Bioactivities and bioavailability of plant polyphenols may be affected by interactions

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between these compounds and food matrix components\textsuperscript{[14]}. Oxidative stress, which is induced by free radical attack on cellular components by reactive oxygen species (ROS), has a major role in the development of many degenerative diseases. As a result, antioxidants protect against oxidative stress and, therefore, they are considered important in reducing the initiation and progression of these diseases. Endogenous antioxidant systems play a crucial function in combating oxidative stress, but dietary antioxidants are also important. Antioxidant compounds play an important role as health protective factors\textsuperscript{[15]}. They can delay or inhibit lipid oxidation by inhibiting the initiation or propagation of oxidizing chain reactions, and are involved in scavenging free radicals; many studies reported that the oxidation of lipids is substantially reduced by adding antioxidants to oils and fats. Propyl gallate (PG), butylatedhydroxyanisole (BHA), tertiary butylhydroquinone (TBHQ) and butylatedhydroxy-toluene (BHT) are some of the widely used synthetic antioxidants to retard oxidation and to prolong shelf life of foods. However, use of synthetic antioxidants has been limited in many countries because of their possible toxic properties for human health and the environment; adverse side effects of their use\textsuperscript{[16]}; use of such substances has been questioned due to their potential health risks and toxicity. Although synthetic antioxidants are used at low concentrations, there is a need for having antioxidants without side effects because the complications resulting from the long-term use of these compounds in man cannot be ignored. Extraction of phenolics from botanical sources is the first important step to exploit their industrial applications. Different solvent systems have been used for extraction of phenolics from plant materials. Water, ethyl acetate, ethanol, methanol, butanol and acetone are commonly used. Due to the chemical nature of these compounds in various plants, the extraction efficiency of phenolics mainly depends on the polarity of solvent as well as the species of plant materials used\textsuperscript{[17]}. To the best of our knowledge, information on the effect of solvents with various polarities on extracting phenolics from \textit{Artemisia Herba-alba} related to their antioxidant activities is still not available. In this study, efficiency of solvents including distilled water, ethyl acetate, chloroform and butanol in the extraction of antioxidant phenolics from aerial part of \textit{Artemisia Herba-alba} was investigated to obtain the best extraction solvent. The phytochemical composition, the antioxidant activity of the extracts was evaluated by in vitro methods (DPPH radical scavenging activity, \textit{β}-carotene radical scavenging activity and Hydroxyl radical scavenging and FRAP test) and the anti-inflammatory activity.

\textbf{MATERIALS AND METHODS}

\textbf{Plant material:} The aerial part of \textit{Artemisia Herba-alba} were collected from southeast of Algeria, state of El Oued on Mars 2015. The aerial part then separated from each other, washed and dried at room temperature. After, ground to a powder with a basic electric grinder and stored in the dark at room temperature before use. Then the powder was put in a hot air oven at 55 °C until complete drying. Depending on the physical characteristics of the samples, the time ranged from 18 at 24 h.

\textbf{Extraction of phenolic compounds:} The bioactive compounds were extracted according to the method described by Bebbar et al\textsuperscript{[18]}. 50 g of the aerial parts were extracted with 250 ml of water, ethyl acetate, butanol and chloroform for 5 h in Soxhlet. The extracts were filtered and evaporated under vacuum at 45°C before being dried and lyophilized for 10 h. the raw extract was stored at -40°C.

\textbf{Total phenolic content:} The total phenolic contents in all organs were determined by the folin-Ciocalteu method\textsuperscript{[19]}. Briefly, 100 µl of both the sample and the standard (gallic acid) of known concentrations were made up to 2.5 ml with water and mixed with 0.25 ml of 1N Folin-ciocalteu reagent. After 5 min, 2.5 ml of sodium carbonate aqueous solution (2%, w/v) was added to the mixture and was completed the reaction for 30 minutes in darkness at room temperature. The absorbance was read at 765 nm using a UV-visible spectrophotometer (Shimadzu UV-1800, Japan). For the blank the same protocol was used but the extract was replaced by solvent. The concentration of total polyphenols in the extracts was expressed as mg gallic acid equivalent (GAE) per g of dry weight using UV-Visible (Shimadzu UV-1800, Japan) and the equation of calibration curve: \(Y= 0.00778x, R^2 =0.991\), \(x\) was the absorbance and \(Y\) was the gallic acid equivalent. All
results presented are means (±SEM) and were analyzed in three replications.

**Total flavonoids**: Flavonoid content was determined by using a method described by Wen et al. [20]. Briefly, 0.25 mL of the extracts (supernatants after centrifuged mentioned above, 1 mL diluted to 2.5 mL) or rutin standard solution was mixed with 1 mL of distilled water in a test tube, followed by addition of 250 µL of a 5% (w/v) sodium nitrite aqueous solution. After 6 min, 250 µL of a 10% (w/v) aluminum nitrate aqueous solution was added and the resulting mixture was allowed to stand for a further 5 min before 0.5 mL of 1 mol/L NaOH in water was added. The mixture was made up to 2.5 mL by adding distilled water and mixed well. The absorbance at 510 nm was measured by using a spectrophotometer immediately. The results of triplicate analyses were expressed as mg of rutin equivalents of extractable compounds.

**Determination of proanthocyanidins**: Determination of proanthocyanidins content was determined using a spectrophotometric method [21]. A volume of 0.5 mL of ethanolic extract or standard (catechin) was added to the mixture of 3 mL of 4% vanillin- methanol (4%, v/v), 1.5 mL of hydrochloric acid and then vortexed. The resulting mixture was allowed to stand for 15 min at room temperature, the absorbance of each was measured at 500 nm using spectrophotometer (Shimadzu UV-1800, Japan). Total proanthocyanidin content was calculated as mg catechin equivalent (mg CTE/g) using the equation obtained from the calibration curve: \[ Y = 0.5617x, \quad R^2 = 0.9851 \] where \( x \) is the absorbance and \( Y \) is the catechin equivalent.

**DPPH radical scavenging activity**: The radical scavenging activity using free-radical DPPH assay was carried out using the spectrophotometric method [22]. 1 ml aliquot of each extract was added to 0.5 mL of a DPPH ethanolic solution (7.8 mg DPPH in 100 mL ethanol 70 %). The mixture was vigorously shaken and left to stand in the dark for 30 min at room temperature. The antioxidant activity was then measured by using the calibration curve: \[ \text{DPPH Inhibition} \% = \frac{(A_t - A_o)}{A_o} \times 100 \] where \( A_t \) and \( A_o \) is the absorbance of control test after 30 min. \( A_o \) is the absorbance of the sample extract after 30 min. All results are means ±SD.

**β-Carotene linoleic acid bleaching assay**: The antioxidant capacity is determined by measuring the inhibition of the production of volatile organic compounds and the formation of conjugated diene hydroperoxides arising from linoleic acid oxidation [23]. Firstly, the β-Carotene was prepared by dissolving 2 mg of this reagent in 10 mL of chloroform. In the round-bottom flask are added 2 mL of β-Carotene solution, 40 mg of linoleic acid and 400 mg of Tween 80, after this preparation, the chloroform is removed at 40 °C using the rotary evaporator. The resulted mixture was added to 100 mL of distilled water (aerated) under vigorous shaking and protection from the light. A 4.8 mL of the last solution was transferred into different tube containing 0.2 mL of each extracts in ethyl acetate with different concentrations. A control sample was prepared of 0.2 mL ethyl acetate and 4.8 mL of β-Carotene reagent. The tubes were incubated at 50 °C for 2 h. the absorbance at 470 nm was measured, using UV-Visible spectrophotometer. The essay was carried out in triplicate and the results were provided as 50 % inhibition (EC\text{so} µg/ml). The antioxidant activity was calculated using the following equation:

\[ \% \text{ antioxidant activity} = \frac{(A_o - A_t)}{(A_o - A_{t0})} \times 100 \]

Where, \( A_o \) and \( A_t \) are respectively the absorbance calculated at zero time of incubation for simple extracts and control. \( A_o \) and \( A_{t0} \) are the absorbance measured after 2 h respectively for simple extract and control. All measurements were made in triplicate and averaged.

**Measurement of ferric reducing power (FRAP assay)**: The reducing power was determined by using FRAP assay [24]. Briefly, the FRAP reagent contained 2.5 mL of 10 mM tripyridyltriazine (TPTZ) in 40 mM HCl, 2.5 mL of 20 mM FeCl\text{3} and 25 mL of 0.3M acetate buffer (pH 3.6), was freshly prepared. A volume 0.2 mL, of extracts )various concentrations) or standard was mixed with 1.8 mL of freshly prepared FRAP reagent . The absorbance of each sample solution was subsequently measured at 595 nm. For the calibration curve, FeSO\text{4} \ was prepared in same solvent extraction in the range of 100–700 µM and Querecetin was used as positive controls . The results were expressed as mg/ml of Fe(II), using the equation obtained from the calibration curve of FeSO\text{4} \: Y = 6.908x, \ R^2 = 0.998.

Nitric oxide generation and determination by Griess reagent: Nitric oxide was produced from sodium nitroprusside. It interacts with oxygen to produce nitrite ion and determined by the use of Griess reagent [25]. A volume of 2 mL of sodium nitroprusside prepared in saline phosphate buffer (pH= 7.4) was added to 0.5 mL of different concentrations of plant extracts, BHT and querecetin. The mixture was set at 25 °C for 150 min. 0.5 mL of each sample from above solutions were added to 0.5 mL of Griess reagent (1% sulphanilamide, 2% H\text{3}PO\text{4} and 0.1% ACS reagent) and allowed to stand for 30 min. The absorbance of the chromophore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with naphthylethylene diamine was measured at 546 nm.
Statistical analyses: The data obtained in this study were expressed as the mean of three replicate determinations plus or minus the standard deviation (SD). Statistical comparisons were made with Student’s t test. P values <0.05 were considered to be significant.

RESULTS AND DISCUSSION

Total phenolic, flavonoids and proanthocyanidins content:
The total phenolic content in four extracts ranged from 92.28 to 41.47 mg GAE/g DW, the EAE has the highest total phenolic content (92.29±3.25), and no significant difference was observed (p > 0.05), followed by butanolic extract (75.64±2.97), while, water extract (61.55±1.85) and CE had the lowest value at 52.94±2.04 mg GAE/g DW. The solvent efficiency on total flavonoid content, in ascending order, was CE < WE < BE < EAE. The highest total flavonoids was observed in EAE (61.24±2.04 mg RT/g DW) followed by BE (48.72±1.47 mg RT/g DW). Water extract and chloroform extract alone are relatively ineffective in extracting flavonoids with the contenting extracts ranged from 32.87±1.41 to 28.64±1.07 mg RT/g DW. As for proanthocyanidins, EAE exhibited the highest value at 25.47±0.95 mg CAE/g DW and the lower value in CE at 12.75±0.54 mg CAE/g DW.

β-Carotene: In β-Carotene linoleate model system free radical arises from oxidation of linoleic acid, attacked by the highly unsaturated β-Carotene molecules and causing decrease in absorbance at 470 nm. The presence of different antioxidants can hinder the extent of β-carotene bleaching by neutralization of the linoleate-free and other free radicals formed in the system [30]. The results of inhibition activity (50 %) of extracts and the positive control (BHT) were showed in Table 2, the high value obtained in EAE (IC50 = 23.83±0.53 µg/mL), WE (IC50 = 38.74±0.63 µg/mL), CE (IC50 = 47.24±0.71 µg/mL) and the lowest inhibition found in BE (IC50 = 51.28 ± 1.05 µg/mL). The interaction of a potential antioxidant with β-Carotene depends on organ extracts. The results indicated and supported that the presence of phenolic content with high concentration in the extracts of can moderately prevent the degradation of β-Carotene caused by radical reactions. Thus, consumption of such underutilization of the antioxidant can protect the oxidation and degradation of cellular macromolecules due to free-radical attacks [31].

Table 1: Total phenolic, flavonoid and Proanthocyanidins present in different solvent of aerial parts from Artemisia Herba-alba.

<table>
<thead>
<tr>
<th>Phytochemical</th>
<th>Solvents</th>
<th>Conc. (µg/ml)</th>
<th>Reaction time (min)</th>
<th>Antioxidant activity IC50 (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total phenolic content mg GAE/g DW</td>
<td>Ethyl acetate</td>
<td>92.29±3.25</td>
<td>5</td>
<td>14.56 ± 0.40</td>
</tr>
<tr>
<td>Total flavonoid content mg RT/g DW</td>
<td>Butanol</td>
<td>75.64±2.97</td>
<td>100</td>
<td>11.35 ± 0.21</td>
</tr>
<tr>
<td>Proanthocyanidins mg CAE/g DW</td>
<td>Water</td>
<td>61.55±1.85</td>
<td>5</td>
<td>12.44 ± 0.32</td>
</tr>
<tr>
<td>Total phenolic content mg GAE/g DW</td>
<td>Chloroform</td>
<td>52.94±2.04</td>
<td>100</td>
<td>23.83±0.53</td>
</tr>
<tr>
<td>Total flavonoid content mg RT/g DW</td>
<td></td>
<td>48.72±1.47</td>
<td>5</td>
<td>9.04 ± 0.49</td>
</tr>
<tr>
<td>Proanthocyanidins mg CAE/g DW</td>
<td></td>
<td>32.87±1.41</td>
<td>100</td>
<td>22.70 ± 1.12</td>
</tr>
<tr>
<td>Proanthocyanidins mg CAE/g DW</td>
<td></td>
<td>28.64±1.07</td>
<td>5</td>
<td>72.09 ± 1.78</td>
</tr>
<tr>
<td>Proanthocyanidins mg CAE/g DW</td>
<td></td>
<td>17.43±0.61</td>
<td>100</td>
<td>51.28 ± 1.05</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SD of triplicate measurements.

Scavenging activity of DPPH radical: The inhibitory concentrations of each extract and reference compound (BHT) required scavenging 50% of the DPPH radical, the IC50 values in antioxidant activity of three extracts were presented in figure 2. It shows that EAE, BE, WE, CE and BHT have important antioxidant potencies with IC50 values of 36.85±1.17, 44.08±1.94, 49.32±1.12, 57.07±1.96 and 31.61±0.87 µg/ml, respectively. Furthermore, EAE extracts showed to be the most potent free radical scavenger. The DPPH radical was widely used to evaluate the free-radical scavenging capacity of antioxidants [31]. The phenolic compounds are usually the major antioxidants in plant extracts [28]. Furthermore, a dietary intake of phenolics has been associated with reduced risk of different diseases, such as cancer, cardiovascular disease, diabetes, or atherosclerosis, probably due to their potent antioxidant properties [29].
The ferric reducing antioxidant power (FRAP) is often used to evaluate the ability of an antioxidant to donate an electron. The reducing power of EAE was the highest among the tested extracts, with an IC$_{50}$ = 14.71± 0.51 µg/mL, followed by BE, WE and CE (19.04± 0.45, 21.50± 0.74 and 23.75± 0.85 µg/ml) respectively (figure 3). The antioxidant activities of natural compounds are likely to exhibit mutual correlations with their reducing powers, potentially due to their hydrogen-donating ability. The presence of reducers converts the Fe$^{3+}$/ferricyanide complex to the ferrous form. Fe$^{3+}$ reduction is often used as an indicator of electron donating activity, which is a vital antioxidant mechanism of action of phenolic compounds. The presence of antioxidants in the sample would reduce the conversion of Fe$^{3+}$ to Fe$^{2+}$ by donating an electron, and Fe$^{2+}$ complex formation can be monitored by measuring the formation of Perl’s Prussian blue (Fe$_4$[Fe(CN)$_6$]$_3$) at 700 nm.

Nitric oxide is an abundant reactive species that acts as an important biological signaling molecule in a large variety of diverse physiological processes, including neurotransmission, blood pressure regulation, defense mechanisms, smooth muscle relaxation and immune regulation. Although many substances may participate in the anti-inflammatory activity, phenolic compounds have been largely recognized as natural molecules with anti-inflammatory effects. Positive correlations have been found between phenolic compounds and anti-inflammatory effects. In the present study, it was also observed that the extract with the highest anti-inflammatory activity showed the highest levels of phenolic content. NO generated by endothelial cells, macrophages and neurons is involved in the regulation of various physiological processes. Oxygen reacts with the excess NO to generate nitrite and peroxy nitrite anions, which act as free radicals. The decrease in the concentration of the NO radical was due to the antioxidant activity of the phenolic compounds. NO is a potent pleiotropic mediator of various physiological processes such as smooth muscle relaxing, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is a diffusible free radical which plays many roles as an effector molecule in diverse biological systems, including neuronal messenger, in vasodilatation and antimicrobial and antitumor activities.

The scavenging activity of the extracts against nitric oxide was calculated. All extracts down-regulated NO production with IC$_{50}$ < 100 µg/mL. The strongest effect was observed for the EAE with an IC$_{50}$ = 38.33 ± 0.72 µg/mL. Regarding the other extracts, BE (IC$_{50}$ = 43.53 ± 0.95 µg/mL), WE (IC$_{50}$ = 46.55 ± 0.85 µg/mL) and CE (IC$_{50}$ = 51.84 ± 0.97 µg/mL, the results were shown in figure 4. Nitric oxide is an abundant reactive species that acts as an important biological signaling molecule in a large variety of diverse physiological processes, including neurotransmission, blood pressure regulation, defense mechanisms, smooth muscle relaxation and immune regulation. Although many substances may participate in the anti-inflammatory activity, phenolic compounds have been largely recognized as natural molecules with anti-inflammatory effects. Positive correlations have been found between phenolic compounds and anti-inflammatory effects. In the present study, it was also observed that the extract with the highest anti-inflammatory activity showed the highest levels of phenolic content. NO generated by endothelial cells, macrophages and neurons is involved in the regulation of various physiological processes. Oxygen reacts with the excess NO to generate nitrite and peroxy nitrite anions, which act as free radicals. The decrease in the concentration of the NO radical was due to the antioxidant activity of the phenolic compounds. NO is a potent pleiotropic mediator of various physiological processes such as smooth muscle relaxing, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is a diffusible free radical which plays many roles as an effector molecule in diverse biological systems, including neuronal messenger, in vasodilatation and antimicrobial and antitumor activities.
CONCLUSION

In this study, several in vitro assays were applied to evaluate the phenolic content, flavonoid content, proanthocyanidins, antioxidant an anti-inflammatory activities of aerial part extract from Algerian *Artemisia Harba-alba* obtained by ethyl acetate, butanol, water and chloroform. Results indicate that the four extract exhibit different antioxidant activities in different test systems. Moreover, the contents of phenolic content, flavonoids and proanthocyanidins as well as antioxidant activity varied according to the solvent utilization. All the extracts were proven to be notable antioxidants DPPH, β-carotene and strong reducing power. A correlation between the quantity of phenolics and antioxidant activity indicates that phenolics significantly contributed to the antioxidant capacity of four extracts. However, other antioxidant substances may also play an important role in the antioxidant capacity of plants. The data reported in this work confirmed the traditional application of *Artemisia Harba-alba* in the treatment of inflammatory disorders. Chemical composition should be further studied by using more advanced techniques, such as LC-MS, multiple mass spectrometry and nuclear magnetic resonance spectroscopy.

Conflict of Interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

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