Research Article

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Anti-inflammatory and antinociceptive features of *Bryonia alba* L.: As a possible alternative in treating rheumatism

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Abstract: *Bryonia* species have traditionally been used as a diuretic and laxative, to reduce edema and relieve joint pain. *B. alba* roots are used especially to treat rheumatic pain and applied to painful joints in Turkish folk medicine. *B. alba* roots were extracted with *n*-hexane, ethyl acetate (EtOAc), and methanol, successively. The carrageenan-induced hind paw edema model and the Whittle method were used to evaluate the anti-inflammatory activity of the extracts in mice. The *p*-benzoquinone-induced abdominal constriction test and tail flick test were used to evaluate the antinociceptive activity of the extracts in mice. DPPH-radical-scavenging activity, ABTS radical–scavenging activity, total antioxidant activity, and hydroxyl radical–scavenging activity were assessed. Furthermore, the total phenolic and total flavonoid contents were calculated for all the extracts. The EtOAc extract prepared from *B. alba* roots had the most promising anti-inflammatory, antinociceptive, and antioxidant activities. Moreover, it had high phenolic and flavonoid contents. Therefore, *B. alba* roots could be alternative treatment method for rheumatic disorders.

Keywords: Anti-inflammatory; antinociceptive; *Bryonia alba*; Cucurbitaceae.

1 Introduction

Rheumatoid arthritis is an inflammatory disease of unidentified etiology that has been recognized to occur since at least the 1800s [1]. It is the most severe of the inflammatory arthritis [2]. Recent evidence shows that rapid diagnosis and primary initiation of absolute disease-modifying treatment can delay or stop the development of the disease and permit patients to retain function that would otherwise be lost in this progressive and commonly disabling disease [3]. Even though several drugs are known to treat chronic inflammatory disorders, prolonged use of these agents should be avoided due to severe side effects. Therefore, discovery of safe and new anti-inflammatory agents with fewer side effects is needed [4]. Consequently, traditional medicines contribute to discover new drug leads.

*Bryonia* L. (Cucurbitaceae) genus comprises perennial herbs with annual climbing or trailing stems. *Bryonia* genus has four species, namely *B. multiflora*, *B. cretica*, *B. aspera*, and *B. alba*, in the flora of Turkey. In the present study, *B. alba* L. was selected as a material due to its folkloric usage. The stems of *B. alba* are usually glandular, sometimes sparsely spiculate or hairy, and rarely glabrous. The leaves of the plant are narrowly to broadly ovate–cordate. The petiole of *B. alba* is glabrous or sparsely spiculate [5]. *Bryonia* species traditionally have been used as diuretic and laxative, to reduce edema and relieve joint pain [6-8]. *B. alba* roots are used especially to treat rheumatic pain and applied to painful joints for their anti-inflammatory properties [7,9]. The roots also exert an anti-inflammatory effect in the inflammation of the serous tissues and are used for treating rheumatism, pneumonia, and cough in traditional medicine [10,11].

*Bryonia* species are also known to have biological activities such as analgesic, anti-inflammatory, antioxidant, antipyretic, antimicrobial, larvicidal and cytotoxic [18]. Their constituents exert cytotoxic and antitumor effects [18]. Previous studies reported
that \textit{B. alba} might display preventive atherogenic or antiatherosclerotic activities \cite{19}. Furthermore, there are some studies that prove the hepatoprotective activity of the root extract of \textit{B. alba} \cite{20} or even the protective activity for human cells against endogenous oxidative DNA damage.

Phytochemical studies have discovered that \textit{Bryonia} species contain a variety of phytochemicals including polyphenols, flavonoids, cucurbitane-type triterpene glycosides, sterols, saponins, alkaloids, and carbohydrates \cite{12,13}. Earlier phytochemical investigations of \textit{B. alba} revealed the presence of saponarin, isovitexin, vitexin, lutonarin, and highly oxygenated and unsaturated bitter compounds, for example, the tetracyclic triterpenoid named cucurbitacin, polyhydroxy unsaturated fatty acids, essential oil, wax, tannins, carbohydrates, and amino acids \cite{14-17}.

The aim of the present study was to evaluate the anti-inflammatory, antioxidant, and antinociceptive activities of \textit{B. alba} roots.

2 Experimental

2.1 Plant material

\textit{Bryonia alba} roots were collected from Cankırı, İlgaz in June 2016 and were identified by Prof. Dr. Hayri Duman from the Department of Botany, Faculty of Science, Gazi University. Voucher specimens are deposited in the Herbarium of Faculty of Pharmacy, Gazi University (GUEF3466).

2.2 Extraction procedure for the bioassays

Shade-dried roots (500 g) of \textit{B. alba} were successively extracted with 1500 mL of \textit{n}-hexane, ethyl acetate (EtOAc), and methanol (MeOH) at room temperature for 48 hours. The extracts were filtered and evaporated under reduced pressure at 40°C till dryness. The yields of each extract were 7.4% for \textit{n}-hexane, 13.2% for ethyl acetate, and 35.7% for methanol.

2.3 Determination of total phenolic content of the extracts

Total phenolic contents of the extracts were measured using reference methods including the Folin–Ciocalteu reagent and gallic acid as standard compounds \cite{23}. An aliquot of the extract solution (100 µL) containing 1 mg extract was taken into a volumetric flask. Distilled water and Folin–Ciocalteu reagent were added, and the flask was shaken thoroughly. Four milliliters Na$_2$CO$_3$ (75 g/L) was added, and the mixture was allowed to stand for 2 hours at room temperature with intermittent shaking. Then absorbance was measured at 765 nm. The same procedure was applied to standard gallic acid solutions of different concentrations (0.05, 0.1, 0.15, 0.25, and 0.5 mg/mL) to prepare the standard curve.

2.4 Determination of total flavonoid contents

The total flavonoid content of each plant extract was assessed using the method proposed by Zhishen et al. \cite{24}. In brief, each sample (1.0 mL) was mixed with 4 mL of distilled water and subsequently with 0.30 mL of a NaNO$_2$ solution (10%). After 5 minutes, 0.30 mL of an AlCl$_3$ solution (10%) was added followed by 2.0 mL of NaOH solution (1%). The absorbance was measured at 510 nm versus the blank after thorough mixing. The standard curve of rutin was prepared (10-100 mg/mL), and the results were expressed as rutin equivalents (mg rutin/g dried extract).

2.5 Animals

To assess the activities of the extracts, 120 mice were used for the anti-inflammatory and antinociceptive activities and each group included six rats. Swiss albino mice (20–25 g) were purchased from the animal breeding laboratories of Kobay A.Ş. (Ankara, Turkey). The animals left for 2 days for acclimatization to animal room conditions and were maintained on standard pellet diet and water ad libitum. Food was withdrawn on the day before the experiment, but free access to water was permitted. A minimum of six animals was used in each group, otherwise described in procedure. The present study was performed according to the international rules considering the animal experiments and biodiversity rights (Ethical Council Number: G.U.ET-05.004).

2.6 Preparation of test samples for bioassay

Test samples were given orally to test animals after suspending in a mixture of distilled H$_2$O and 0.5% sodium...
carboxymethyl cellulose (CMC). The control group animals received the same experimental handling as those of the test groups except that the drug treatment was replaced with appropriate volumes of the dosing vehicle. Indomethacin (10 mg/kg), morphine (10 mg/kg), and acetylsalicylic acid (ASA) (100 mg/kg) in 0.5% CMC was used as reference drug.

2.7 Anti-inflammatory activity

2.7.1 Carrageenan-induced hind paw edema model

The carrageenan-induced hind paw edema model was used for determining the anti-inflammatory activity of the extract. After 60 minutes, a test sample or dosing vehicle was orally administered. A freshly prepared suspension of carrageenan (0.5 mg/25 µL) in physiological saline (154 nM NaCl) was injected to the subplantar tissue of the right hind paw of each mouse. As the control, 25 µL of saline solution was injected into the left hind paw. Paw edema was then measured every 90 minutes for 6 hours after inducing inflammation. The difference in footpad thickness was measured using a gauge caliper (Ozaki Co., Tokyo, Japan). The mean values of treated groups were compared with those of a control group and analyzed using statistical methods. Indomethacin (10 mg/kg) was used as the reference drug [25].

2.7.2 Acetic acid–induced increase in capillary permeability (Whittle method)

The effect of the test samples on the increased vascular permeability induced by acetic acid in mice was determined according to the Whittle method with some modifications. Each test sample was administered orally to mice in 0.2 mL per 20 g of body weight. Each mouse was injected with 0.1 mL of 4% Evans blue (Sigma) in saline solution (intravenously) at the tail 30 minutes after the administration. Then, 0.4 mL of 0.5% (vol/vol) acetic acid was injected intraperitoneally 10 minutes after the intravenous injection of the dye solution. The mice were killed by dislocation of the neck after 20 minutes, and the viscera were exposed and irrigated with distilled water, which was then poured into 10 mL volumetric flasks through glass wool. Each flask was made up to 10 mL with distilled water, 0.1 mL of 0.1 N NaOH solution was added to the flask, and the absorption of the final solution was measured at 590 nm. In control mice, a mixture of distilled water and 0.5% CMC was given orally in the same manner as described earlier. Indomethacin (10 mg/kg) was used as the reference drug [25].

2.8 Antinociceptive activity

2.8.1 p-Benzoquinone-induced abdominal constriction test

This method was performed on mice for determining antinociceptive activity. The mice were intraperitoneally injected with 0.1 mL per 10 g of body weight of 2.5% (wt/vol) p-benzoquinone solution in distilled water 60 minutes after the oral administration of a test sample. Control mice received an appropriate volume of dosing vehicle. The mice were then kept individually for the observation period, and the total number of the abdominal contractions (writhing movements) was determined for the following 15 minutes, starting 5 minutes after the p-benzoquinone injection. The data represented the average of the total number of writhes observed. The antinociceptive activity was then expressed as the percentage change from writhing controls. Acetylsalicylic acid at 100 mg/kg dose was used as the reference drug [25].

2.8.2 Tail flick test

The mice were detained in position with the tail extending out, and light heat was administered to the tail. Tail reaction times were then assessed 20, 40, and 60 minutes after vehicle or extract injection. A cut-off time of 10 seconds was used to avoid tissue damage [26]. Morphine at 10 mg/kg dose was used as the reference drug.

2.9 Statistical analysis

The results were expressed as mean ± standard error of the mean. The ANOVA test was performed to determine the significant differences among groups using GraphPad Prism 6.0.

2.10 Antioxidant activity

2.10.1 DPPH (2,2-Diphenyl-1-Picrylhydrazyl) radical–scavenging activity

The scavenging activity of B. alba extracts against DPPH radical was evaluated using the method proposed by Blois...
(1958) [27] with some modifications. In this method, 1 mL of B. alba extract (0.01 mg dw/mL) was mixed with 4 mL of 0.005 mg/mL DPPH methanol solution. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 minutes. The absorbance of the mixture was measured at 517 nm with ascorbic acid as reference. The ability to scavenge DPPH radicals was calculated using the following equation: DPPH radical–scavenging activity (%) = \[\frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100\], where \( \text{Abs}_{\text{control}} \) is the absorbance of DPPH radicals in methanol and \( \text{Abs}_{\text{sample}} \) is the absorbance of DPPH radical solution mixed with sample extract/standard. All determinations were performed in triplicate.

2.10.2 ABTS [2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate)] radical–scavenging assay

For the ABTS assay, the method proposed by Arnao et al. (2001) [28] was followed with some modifications. The stock solutions included 7mM ABTS solution and 2.4 mM potassium persulfate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 14 hours at room temperature in the dark. The solution was then diluted by mixing 1 mL of ABTS solution with 60 mL of methanol to obtain an absorbance of 0.706 ± 0.01 units at 734 nm using a spectrophotometer. Fresh ABTS solution was arranged for each analysis. Plant extracts (1 mL) were allowed to react with 1 mL of ABTS solution, and the absorbance was recorded at 734 nm after 7 minutes using a spectrophotometer. The ABTS-scavenging capacity of the extract was associated with that of ascorbic acid, and percentage inhibition was calculated as follows:

\[\text{Inhibition} \% = \left(1 - \frac{\text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}}\right) \times 100\]

where \( \text{Abs}_{\text{control}} \) is the absorbance of the negative control (without sample) at 532 nm, and \( \text{Abs}_{\text{sample}} \) is the absorbance of the reaction mixture containing the sample [30].

2.10.3 Total antioxidant activity (ferric-reducing antioxidant power, FRAP)

The FRAP assay was conducted according to the method proposed by Benzie and Strain (1996) [29] with some modifications. The stock solutions included 300mM acetate buffer (3.1 g C\(_6\)H\(_{12}\)NaO\(_6\) × 3H\(_2\)O and 16mL C\(_6\)H\(_{12}\)O\(_4\)), pH 3.6, 10mM 2,4,6-tripyridyl-s-triazine (TPTZ) solution in 40mM HCl, and 20mM FeCl\(_3\) × 6H\(_2\)O solution. The fresh working solution was made by mixing 25 mL of acetate buffer, 2.5 mL of TPTZ solution, and 2.5 mL of FeCl\(_3\) × 6H\(_2\)O solution and kept at 37°C before use. B. alba extracts (0.15 mL) were ready to react with 2.80 mL of the FRAP solution for 30 minutes in the dark. Readings of the colored product (ferrous tripyridyltriazine complex) were taken at 593 nm. The standard curve was linear for 0.015 and 0.15 mM Trolox. The results were expressed as mM TE/g dry mass. Additional dilution was required if the measured FRAP value was over the linear range of the standard curve. All determinations were made in triplicate.

2.10.4 Hydroxyl radical–scavenging assay

Hydroxyl radical–scavenging activity was measured as the ability of the extract to scavenge the hydroxyl radicals generated by the Fe\(^{3+}\)–ascorbate–EDTA–H\(_2\)O\(_2\) system. The reaction mixture in a final volume of 1.0 mL contained 100 µL of 2-deoxy-D-ribose (28mM in 20 mM KH\(_2\)PO\(_4\) buffer, pH 7.4), 500 µL of the extract at various concentrations (50–800 µg/mL) in buffer, 200 µL of (1.04mM EDTA and 200µM FeCl\(_3\)) (1:1, v/v), 100 µL of 1.0mM hydrogen peroxide (H\(_2\)O\(_2\)), and 100 µL of 1.0mM ascorbic acid. Test samples were kept at 37°C for 1 hour. The free radical damage forced on the substrate, deoxyribose, was measured using the thiobarbituric acid (TBA) test. Then, 1 mL of 1% TBA and 1.0 mL of 2.8% trichloroacetic acid were added to the test tubes and incubated at 100°C for 20 minutes. After cooling, the absorbance was measured at 532 nm against a blank containing deoxyribose and buffer. The scavenging activity on hydroxyl radicals was calculated as follows:

\[\text{Inhibition} \% = \left(1 - \frac{\text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}}\right) \times 100\]

where \( \text{Abs}_{\text{control}} \) is the absorbance at 532 nm of the reaction mixture containing the sample.

3 Results and Discussion

B. alba roots are used especially to treat rheumatic pain and applied to painful joints traditionally in Turkey [79]. Therefore, the present study was performed to evaluate the anti-inflammatory, antinociceptive, and antioxidant activities of \( n \)-hexane, ethyl acetate, and methanol extracts prepared from B. alba roots.

The paw edema induced by carrageenan has been used widely for assessing the anti-inflammatory action of steroidal and nonsteroidal drugs containing numerous chemical mediators, including serotonin, bradykinin,
Anti-inflammatory and antinociceptive features of *Bryonia alba* L...

Histamine, serotonin, and similar substances are released in the initial phase (90–180 minutes) of the inflammation. Prostaglandins, proteases, and lysosomes are activated in the later phase (270-360 minutes) [32]. The EtOAc extract of *B. alba* roots showed a statistically significant anti-inflammatory activity in carrageenan-induced hind paw edema model and acetic acid–induced increase in capillary permeability (Tables 1 and 2).

Furthermore, the EtOAc extract displayed antinociceptive activity in the *p*-benzoquinone-induced writhings in mice model (Table 3). Acetic acid and *p*-benzoquinone mouse models are simple and fast for the screening of antinociceptive activity [33]. Furthermore, they involve complex mechanisms, including the production of pro-inflammatory cytokines and opening of ion channels [34,35]. Acetic acid–induced writhing mechanism depends on the activation of peritoneal macrophages and mast cells, which leads to the release of cytokines, such as tumor necrosis factor alpha and interleukin (IL)-1β, as well as sympathomimetic amines and eicosanoids [36]. However, the *p*-benzoquinone-induced writhing model depends on the cytokines IL-18, interferon gamma, and endothelin-1 [35]. Nevertheless, the acetic acid and *p*-benzoquinone models share nociceptive mechanisms such as prostanoids, other cytokines such as IL-33 [37], susceptibility to opioid treatment [35,38], and mitogen-activated phosphatidylinositol 3-kinase and protein kinase [39]. In addition, none of the extracts obtained from *B. alba* roots showed any activity in the tail flick test in the present study (Table 4). The tail flick test usually is conducted to appraise the analgesic activities of opioids [40]. In our study, since any extract obtained from the roots of *B. alba* did not show any activity in the tail flick test, it was concluded that *B. alba* roots did not show their analgesic activities through the opioid receptors.

The EtOAc extract showed strong DPPH and ABTS radical–scavenging activity when the antioxidant parameters of the extracts were evaluated (Table 5). Furthermore, when compared to the extracts according to phytochemical contents, the EtOAc extract was found

### Table 1: Effects of test materials on carrageenan-induced hind paw edema in mice.

<table>
<thead>
<tr>
<th>Material</th>
<th>Dose (mg/kg)</th>
<th>90 min (×10⁻² mm) ± S.E.M.</th>
<th>180 min (×10⁻² mm) ± S.E.M.</th>
<th>270 min (×10⁻² mm) ± S.E.M.</th>
<th>360 min (×10⁻² mm) ± S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>48.6 ± 5.8</td>
<td>52.6 ± 7.3</td>
<td>58.4 ± 6.2</td>
<td>67.9 ± 5.1</td>
</tr>
<tr>
<td><em>n</em>-Hexane</td>
<td>0.1</td>
<td>45.3 ± 4.7</td>
<td>46.9 ± 8.2</td>
<td>55.3 ± 7.5</td>
<td>58.4 ± 6.9</td>
</tr>
<tr>
<td>EtOAc</td>
<td>0.1</td>
<td>37.5 ± 4.2</td>
<td>39.4 ± 5.4</td>
<td>40.6 ± 4.8*</td>
<td>42.3 ± 4.2**</td>
</tr>
<tr>
<td>MeOH</td>
<td>0.1</td>
<td>42.1 ± 5.6</td>
<td>44.5 ± 6.1</td>
<td>52.7 ± 5.9</td>
<td>55.6 ± 7.3</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>10</td>
<td>35.4 ± 3.5</td>
<td>38.4 ± 2.8*</td>
<td>37.7 ± 3.1**</td>
<td>34.2 ± 2.4**</td>
</tr>
</tbody>
</table>

*p < 0.05; **p < 0.01; ***p < 0.001 significant from control.

### Table 2: Effects of test materials on increased vascular permeability induced by acetic acid in mice.

<table>
<thead>
<tr>
<th>Material</th>
<th>Dose (mg/kg)</th>
<th>Evans blue concentration (mean ± S.E.M.) (µg/mL)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>10.6 ± 1.3</td>
<td></td>
</tr>
<tr>
<td><em>n</em>-Hexane</td>
<td>0.1</td>
<td>9.7 ± 2.1</td>
<td>8.5</td>
</tr>
<tr>
<td>EtOAc</td>
<td>0.1</td>
<td>6.7 ± 0.9</td>
<td>36.8*</td>
</tr>
<tr>
<td>MeOH</td>
<td>0.1</td>
<td>9.1 ± 1.6</td>
<td>14.2</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>10</td>
<td>5.5 ± 0.4</td>
<td>48.1***</td>
</tr>
</tbody>
</table>

*p < 0.05; **p < 0.01; ***p < 0.001 significant from control.

### Table 3: Effects of the extracts against *p*-benzoquinone-induced writhings in mice.

<table>
<thead>
<tr>
<th>Material</th>
<th>Dose (mg/kg)</th>
<th>Number of writhings (mean ± S.E.M.)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>39.5 ± 7.4</td>
<td></td>
</tr>
<tr>
<td><em>n</em>-Hexane</td>
<td>0.1</td>
<td>35.8 ± 6.8</td>
<td>9.4</td>
</tr>
<tr>
<td>EtOAc</td>
<td>0.1</td>
<td>26.0 ± 4.3</td>
<td>34.2**</td>
</tr>
<tr>
<td>MeOH</td>
<td>0.1</td>
<td>34.4 ± 6.1</td>
<td>12.9</td>
</tr>
<tr>
<td>Aspirin</td>
<td>100</td>
<td>21.5 ± 2.2</td>
<td>45.6***</td>
</tr>
</tbody>
</table>

*p < 0.05; **p < 0.01; ***p < 0.001 significant from control.
to have the highest total phenolic and flavonoid contents (Table 6).

*B. alba* has demonstrated its significance in folk medicine mainly as purgative and mostly due to cucurbitacin content [41-43]. Besides cucurbitacins, which are the most significant compounds presented in *B. alba* extract, other compounds in the extract, such as lectins, flavonoids, and lipids, also act synergistically to increase the biological activity [44]. Previous studies showed the significance of cucurbitacins isolated from *B. alba*, particularly cucurbitacins B, D, E, I, and their derivatives, owing to their therapeutic effect or adjuvant management [45-47]. Cucurbitacins J, K, and L are found in significant amounts in the roots [41,48] and exert cytotoxic effects [41]. Other studies demonstrated the antiatherosclerotic and atherogenic activities of trihydroxyoctadecadienoic acids in *B. alba* [49]. Meanwhile, a few studies demonstrated the hepatoprotective activity of the root extract [10] on human cells contrary to endogenous oxidative DNA damage, although its capability to protect against oxidative DNA damage could not be verified [50]. *B. alba* roots are also known for their adaptogenic activity, which is related to their cucurbitacin content and which improves the sensitivity to stress due to the effects of eicosanoids and corticosteroids. Gupta et al. [51] reported that the chloroform extract of *B. laciniosa* leaves had anti-inflammatory activity and inhibited peritoneal leukocyte migration in mice. Konopa et al. [48] isolated cucurbitacins from *B. alba* having cytotoxic and antitumor effects. Ukiya et al. [52] isolated cucurbitane-type triterpenoids from *B. dioica* roots. All isolated compounds were tested for their anti-inflammatory activity. The tested compounds displayed marked anti-inflammatory effects, with 50% inhibitory doses (ID$_{50}$) of 0.2–0.6 mg. In fact, *B. alba* roots also increased nitric oxide (NO) concentration in humans, which might be responsible for the adaptation

**Table 4:** Effects of the extracts on tail flick test.

<table>
<thead>
<tr>
<th>Material</th>
<th>Dose (mg/kg)</th>
<th>Time after injection (mg/kg)</th>
<th>0 min</th>
<th>20 min</th>
<th>40 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td></td>
<td>3.4 ± 1.1</td>
<td>3.3 ± 0.9</td>
<td>3.5 ± 1.3</td>
<td>3.2 ± 0.8</td>
</tr>
<tr>
<td>n-Hexane</td>
<td>0.1</td>
<td></td>
<td>3.6 ± 0.9</td>
<td>3.8 ± 0.3</td>
<td>3.6 ± 0.7</td>
<td>3.5 ± 0.9</td>
</tr>
<tr>
<td>EtOAc</td>
<td>0.1</td>
<td></td>
<td>3.1 ± 1.2</td>
<td>3.1 ± 0.6</td>
<td>3.3 ± 0.2</td>
<td>3.2 ± 0.3</td>
</tr>
<tr>
<td>MeOH</td>
<td>0.1</td>
<td></td>
<td>3.3 ± 1.8</td>
<td>3.7 ± 0.8</td>
<td>3.4 ± 0.5</td>
<td>3.1 ± 0.6</td>
</tr>
<tr>
<td>Morphine</td>
<td>10</td>
<td></td>
<td>3.4 ± 1.2</td>
<td><strong>6.2 ± 0.2</strong></td>
<td><strong>5.5 ± 0.7</strong></td>
<td><strong>5.3 ± 0.1</strong></td>
</tr>
</tbody>
</table>

*p < 0.05; **p < 0.01; ***p < 0.001 significant from control.

**Table 5:** Antioxidant activity of the extracts obtained from *B. alba* roots.

<table>
<thead>
<tr>
<th>Extracts</th>
<th>FRAP (µM TE/g dw)</th>
<th>DPPH %</th>
<th>ABTS %</th>
<th>OH %</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-Hexane</td>
<td>9.1 ± 0.4a</td>
<td>28.9 ± 0.2a</td>
<td>35.4 ± 0.3a</td>
<td>11.3 ± 0.8a</td>
</tr>
<tr>
<td>EtOAc</td>
<td>66.3 ± 0.8c</td>
<td>82.6 ± 0.4c</td>
<td>87.1 ± 0.2c</td>
<td>26.5 ± 0.5c</td>
</tr>
<tr>
<td>MeOH</td>
<td>38.7 ± 0.6b</td>
<td>53.2 ± 0.5b</td>
<td>62.8 ± 0.3b</td>
<td>21.9 ± 0.4b</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>81.2 ± 0.2c</td>
<td>92.1 ± 0.7c</td>
<td>96.8 ± 0.6c</td>
<td>56.8 ± 0.3c</td>
</tr>
</tbody>
</table>

Values with different letters are significantly different at p < 0.05; All determinations were performed in triplicate (mean ± S.E.M.)

**Table 6:** Total phenolic, flavonoid, and tannin contents of the extracts.

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Total Phenol Content (mg of GA/g of extract)</th>
<th>Total Flavonoid Content (mg of RU/g of extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-Hexane</td>
<td>13.91 ± 0.37a</td>
<td>18.67 ± 0.30a</td>
</tr>
<tr>
<td>EtOAc</td>
<td>44.35 ± 0.54b</td>
<td>54.71 ± 0.47b</td>
</tr>
<tr>
<td>MeOH</td>
<td>31.54 ± 0.86a</td>
<td>26.49 ± 0.38a</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM of three replicates
of humans to heavy physical workout [42]. In contrast, the production of the same NO molecule, which is responsible for many inflammatory processes, is inhibited by \textit{B. alba} roots by inhibiting the activity of inducible NO synthase, which might be the mechanism underlying the anti-inflammatory effect [53,54].

It has been reported that the EtOAc extract has high phenolic content in the present study. A previous study conducted by Ambriz-Pérez et al. [55] exhibited that phenolic compounds work like nonsteroidal anti-inflammatory drugs (NSAIDs), furthermore some NSAIDs inhibit pro-inflammatory mediators besides cyclooxygenase by inhibiting their activity or gene expression. Besides, some phenolic compounds can up/down regulate transcriptional factors, like nuclear factor-kB, in inflammatory and antioxidant pathways [55].

The anti-inflammatory, antinociceptive, and antioxidant activities of \textit{B. alba} roots could be attributed to its phenolic compounds and cucurbitane-type triterpenoids.

### 4 Conclusions

In the present study, EtOAc extract prepared from \textit{B. alba} roots showed statistically significant anti-inflammatory, antinociceptive, and antioxidant activities. Therefore, \textit{B. alba} roots might serve as an alternative treatment for rheumatic disorders. In further analysis, bioassay guided fractionation and isolation studies should be conducted for discovering potential new anti-inflammatory agents.

**Conflict of interest:** Authors declare no conflict of interest.

### References


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