IN VITRO VALIDATION OF BIOLOGICAL AND CYTOTOXIC ACTIVITY OF METHANOL EXTRACT OF JORDANIAN ARTEMISIA HERBA-ALBA L.

Siwar Al-Qbilat, Omar Atrooz

Address(es):
1 College of Graduate Studies, Mutah University. Mutah-Jordan.
2 Department of Medical Laboratory Sciences, Faculty of Applied Medical Sciences, Al-Ahliyya Amman University, Amman 19328, Jordan.
3 Department of Biological Sciences, Faculty of Science, Mutah University. Mutah 61710, Jordan.

*Corresponding author: omihandd@gmail.com

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ABSTRACT

Artemisia herba-alba L. (A. herba-alba), growing in the Mediterranean regions, contains various phytochemical compounds with exceptional pharmacological and chemical properties. The present study aims to characterize chemical and biological activities of A. herba-alba’s methanic extract according to total protein, total phenols, antioxidant, anti-inflammatory, and anti-hemolytic activities. The study also evaluates the extract’s anti-proliferative activity of diverse cancer cell lines in vitro. Liquid chromatography-mass spectrometry (LC-MS) was used to conduct phytochemical analysis of A. herba-alba’s extract. The total phenolic amount, total proteins, anti-inflammatory, and anti-hemolytic activity was evaluated in vitro along side antioxidant activity by various spectrophotometric methods. LC-MS analysis of crude extract reveals that kaempferol, quercetin, geranyl acetate, dihydromyricetin, furanone, myricetin, quercetin, ellagic acid, limonene, eugenol, p-cymene, and naringin represent the major composition (81.5%). A. herba-alba’s extract showed 72.07% anti-oxidant activity through DPPH radical scavenging with IC50 value 0.67 µg/mL. Furthermore, the extract from A. herba-alba had a 68.71% anti-hemolytic impact and a 97.55% anti-inflammatory effect. These different bioactivities were linked and correlated to the total phenolic content in the extract (1.41 mg/ml). The correlation between phenolic contents and activity of DPPH scavenging, anti-inflammatory, and anti-hemolytic was expressed with Pearson correlation coefficient and found to be a strong correlation of the extract with R² close to 0.90. Furthermore, strong cytotoxic activity was shown by the extract against tested cancer cell lines. The IC50 values for lung cancer HCC95 was 7.0 µg/mL, breast cancer MDA-MB-231 was 6.9 µg/mL, prostate DU-145 was 6.8 µg/mL, and breast cancer 600MPE was 6.7 µg/mL. According to the research, the methanolic extract of A. herba-alba plant contains numerous phytochemicals, primarily phenolic compounds. The extract exhibits antioxidant, anti-inflammatory, and anti-hemolytic activities, as well as strong cytotoxic activity.

Keywords: Phenolic contents, Antioxidant, Anti-inflammatory, Anti-hemolytic, Anti-cancer

INTRODUCTION

Medicinal plants are considered significant in traditional medicine and are studied for their potential medicinal benefits. These plants have been utilized for their therapeutic properties and are a good source of biologically active compounds to treat various ailments (Van der Hoorn, 2008). When organisms consume plant-based diets, bioactive compounds can have an effect on specific biochemical and physiological processes within their bodies. These compounds have the potential to cause changes that can positively impact their health (Palma et al., 2002). Plants produce a wide range of secondary metabolites that have been discovered to regulate various organisms, viral proteases, and even humans. These compounds have been identified as having the ability to interact with and impact the biological processes of different organisms, including humans, and have been studied for their potential therapeutic uses (Blay and Pei, 2019). These secondary metabolites (particularly polyphenols) found in plants are considered to be essential components of human nutrition and are particularly interesting due to their unique biological properties (Cory et al., 2018). Few previous studies have been undertaken to examine the potential health benefits of polyphenols. Research has suggested that consuming moderate amounts of polyphenol-rich foods may help prevent the development of various health issues, such as colorectal cancer, diabetes, coronary heart disease, and liver problems. Studies have shown that these compounds can lower the risk of cancer progression, insulin resistance, neurodegenerative disorders, coronary heart disease, and osteoporosis. (Al-Khalid and El-Naas, 2012; Hussein and El-Ansary, 2019). The potential therapeutic properties of these chemical compounds found in plants are studied and these plants are used in various traditional medicines for centuries (Lin et al., 2016; Tanase et al., 2019).

The plant’s phytochemical composition plays a vital role in antioxidant, proteolytic, anti-inflammatory, and anti-hemolytic activities. The oxygen-centered free radicals produced by normal metabolic and physiological processes of human body may cause oxidative damage to biomolecules (lipids, proteins, etc.). This oxidative damage can lead to the development of chronic illnesses and has been linked to various health issues (Aiz et al., 2019; Moussa et al., 2019). Phyto-antioxidants are a specific type of phytounrient found in plants that are known for their ability to scavenge free radicals. This property makes them potentially valuable in preventing and treating diseases that are caused by the damage caused by free radicals (Spiegel, 2022). Inflammation is the body's natural response to harmful stimuli, such as tissue damage or allergies (Bzou et al., 2017). However, an uncontrolled and excessive inflammatory response can lead to a range of health issues. These can include allergies, cardiovascular dysfunction, metabolic syndrome, and many other illnesses. Therefore, it is important to manage and control inflammation to prevent disease progression (Saboob et al., 2019). Numerous plant species are known to manufacture specific chemical substances that possess the ability to either rupture or prevent the rupture of individual red blood cells (Kumar et al., 2011). The phytochemicals found in certain plants could potentially improve the condition of the red blood cell membrane, but it should be noted that some plants can cause significant adverse effects, such as hemolytic anemia (Zohra and Fawzia, 2014). Artemisia herba-alba L. (A. herba-alba) (Fig. 1) traditionally used in medicine to alleviate symptoms associated with different illnesses (Moufli and Eddoouk, 2012). During the analysis of this plant's phytochemistry, several compounds were identified including Acetate, cis-chrysanthenyl sesquiterpenes, monoterpenes, Herbaldin, flavonoids (cinealine and hispidulin). The plant’s aerial parts were found to have decreased toxicity (Ougderti et al., 2021). Commonly, A. herba-alba is used as a medicinal plant that is believed to possess healing properties for numerous health conditions such as respiratory infections, gastrointestinal disorders, diarrhea, neuralgias, diabetes, coughs, pneumonia, and hypertension. Research on the essential oils extracted from this plant has revealed that the diverse range of components present in the oils can lead to various biological actions, such as antioxidant, anithelmintic, anti-inflammatory, anti-bacterial, and antidiabetic effects (Dabe and Kefale, 2017).
This study aims to analyze the biological and chemical properties of Jordanian A. herba-alba's extract. Factors such as plant type, location, and environmental conditions may affect these properties. Specifically, the study focuses on evaluating the extract's effectiveness in terms of its total protein, total phenols, anti-inflammatory, antioxidants, and anti-hemolytic properties. Additionally, the study assesses the extract's ability to inhibit tumor cell growth in vitro.

MATERIAL AND METHODS

Study Material – Plant

A plant sample was collected in April 2022 from the Jordanian Madaba region (Elevation: 789.471, Latitude: 31° 43' 9.6267'' N, Longitude: 35° 47' 35.6748'' E). The plant was authenticated by the taxonomic Professor Saleh Al-Quran, Department of Biological Sciences, Mutah University, Jordan. The plant's aerial parts were then dried, crushed, and turned into a powder. Approximately 25 mg of the powdered plant material was soaked with 200 mL of 80% (v/v) methanol solution and stirred on a hotplate for 6 hours. Filter paper and gauze were used for filtering this prepared mixture. Once the solution was clarified via centrifugation at a rate of 1500 rpm for 20 minutes, a pure supernatant was obtained. The crude extract was obtained after evaporation of the solution. The yield of the dried plant extract was found to be 8.2 g/25 g of the powdered plant material used.

Determining the extraction yield

To determine the percentage yield (%) of the dried extract, the equation described by Dellavalle et al. (2011) was employed.

\[
\text{Yield (\%) = } \frac{\text{(DW} \times 100\text{)}}{\text{FW}}
\]

where DW is the dry weight of the plant powder, and FW is the weight of the plant powder.

Phytochemical analysis

Phytochemical Analysis was done by triple quadruple Liquid Chromatography Mass Spectrometer model LC-MS-S830, Shimadzu, Japan (BM-20A Control Bus Module, CTO-30A Column oven, LC-30AD Liquid Chromatography SIL-30 AC Auto Sampler, interface ESI, Column C-18 (4.6 x 25 mm, 5μm)). The solvents used in the eluent system for this experiment were composed of two components, namely 90% acetic acid-water (A) and 10% methanol (B). During the experiment, the elution gradient was maintained: an isocratic 10% B for 5 minutes, followed by a 10-100% B gradient over 20 minutes, 100% B for 6 minutes, and lastly, the column was re-equilibrated. A flow rate of 200 μL/min was utilized throughout the experiment. The scan Form was 50 to 1200 m/z and injection volume was 10 μL.

Determining total protein content

To measure the protein content, we used the Biuret method as recommended by Plummer (1988). To create the calibration standard curve, we prepared a series of concentrations (ranging from 0.1 to 1.2 mg/mL) of bovine serum albumin. The sample's absorbance was read at 540 nm using a Biotech Engineering Management Co. Ltd. spectrophotometer.

Determining total phenols content

For this mixture, a 0.1 mL of the extract's supernatant and 0.5 mL Folin Ciocalteu reagent was prepared and allowed to stay for 5 minutes at room temperature. Following this, 2.5 mL aqueous solution of sodium carbonate was mixed with the extracts. The samples were analyzed for their absorbance using a UV-visible spectrophotometer at 765 nm. The calibration curve was generated at varying concentrations of gallic acid and the results were presented in mg GAE/g (Gallic Acid Equivalent). The calibration curve of gallic acid was utilized to estimate the sample’s total phenolic content (Asfar et al., 2016).

DPPH radicals-scavenging assay

To perform the DPPH radicals-scavenging assay, 1.0 mL DPPH solution in methanol (60 μM) and 50 μl plant extracts were mixed and allowed to stay for 30 minutes at room temperature. The resulting mixture’s absorbance was measured against a blank sample of methanol solution at 517 nm. The experiment utilized gallic acid as positive control. The degree of inhibition was evaluated by formula given below (Bal et al., 2021).

\[
\text{Inhibition activity (\%) = } \frac{\text{(Ac-Ac)}}{\text{Ac}} \times 100\%
\]

Determination Anti-inflammatory activity

The method defined by Hemashree and Thangavelu (2018) was followed for evaluating anti-inflammatory activity. The test solution was prepared by combining 0.05 mL extract with 0.45 mL BSA. A control tube of the product was prepared using 0.05 mL sample and 0.45 mL distilled water, then adding 1.0mg/mL Diclofenac standard stock solution. The mixture was incubated at 37 °C for 20 minutes and then later at 70 °C for 10 minutes. After this incubation and adding 2.5 mL phosphate buffer, its absorbance was measured at 600 nm (Alamgeer et al., 2017).

Anti-Inflammatory activity (\%) = 100 - (AT - AP)/AC \times 100

where AT is the test solution; AP is the product control and AC is the control test. To determine the IC₅₀ value, the AAA Bioquest software was utilized. The IC₅₀ calculator was applied to obtain the half-maximal inhibitory concentration (IC₅₀) of the samples.

Determining Anti-hemolytic activity by H₂O₂ method

To conduct the experiment, different extract quantities (0.25 mL) were mixed with red blood cells (RBCs), 1.0 mL, and 1.25 mL of saline solution. After that, the mixture was maintained in an incubator at 37 °C for 10 minutes. Then, 0.25 mL of H₂O₂ was added to the solution, and it was incubated for another two hours at room temperature. Then, resulting mixture was centrifuged for 10 minutes at 3000 rpm, and the absorbance was measured at 540 nm. The formula for determining the anti-hemolytic activity was used in the experiment (Asfar et al., 2016).

Anti-hemolytic activity (\%) = (Ap - (As-Ac/Ap)) \times 100, where Ap is the control positive’s absorbance, Ac is the control negative’s absorbance, and As is the sample’s absorbance.

To determine the IC₅₀ value, the AAA Bioquest software was utilized.

Cytotoxic activity

The cytotoxic activity was done in Smart Lab (Bn Khaldoon Street, Amman). Four types of human cancer cell lines were taken from the American Type Culture Collection (ATCC, Rockville, USA). The identification of cancer cell lines was done as breast cancer 600MPE (Invasive ductal carcinoma cancer cell line), breast cancer MDA-MB-145 (standard prostate cancer cell line), breast cancer MDA-MB-231 (Invasive ductal carcinoma cancer cell line), breast cancer HCC95 (Squamous carcinoma), and prostate DU-145 (standard prostate cancer cell line). The culture media for cancer cell lines were prepared using various reagents and materials obtained from Sigma Aldrich Chemie in Germany. The media were enriched with streptomycin and penicillin.

The plant’s extract was dissolved in a solvent mixture of RPMI 1640 and OptiMEM in a 1:1 ratio, following the protocol. The cells were cultivated in a humid environment at 37°C with 5% CO₂. The cytotoxic compound, cisplatin (in concentration ranging from 0.01 to 1000 μg/mL) was considered as a control for the anti-proliferative assay. The extract was weighed and dissolved in distilled water or ethanol to get a 100 mg/mL concentration and then diluted to obtain the desired concentrations between 1-10 μg/mL for injection.

Anti-proliferative assay (MTT assay)

Four kinds of cancer cell lines, namely breast cancer 600MPE, lung cancer HCC95, breast cancer MDA-MB-231, and prostate DU-145, were subjected to anti-proliferative tests. To conduct the tests, the cells were seeded at density of 1.0 x 10⁴/mL in 96-well plates. Different levels of A. herba alba's extract were introduced to the cultured cells. The MTT assay was employed for assessing the in-vitro cytotoxicity of the extract under examination following a period of incubation lasting between 72 and 96 hours. The cytotoxic activity was compared to the activity of cisplatin. The production of formazan was read at wavelength 540 nm. The IC₅₀ values were determined by analyzing the concentration–response curves (Atrooz et al., 2018).
Cytotoxic activity was measured using the formula given below:

\[
\text{Cell survival} (%) = \frac{\text{As}-\text{Ab}}{\text{Ac}-\text{Ab}} \times 100, \quad \text{where As is the sample’s absorbance, Ab is the blank’s absorbance, and Ac is the control’s absorbance.}
\]

Cell death (%) = 100 - Cell survival (%).

### Statistical Analysis

The experimental findings were acquired in three repetitions, and Microsoft Excel 2016 was utilized for representing data as the mean ± standard deviation (Mean ±SD). Linear correlation between TPC and A. herba-alba’s biological activities was calculated to estimate the relationship between them by using Microsoft Excel 2016 software.

### RESULTS

#### Extraction yield, and total content of proteins and phenols

The A. herba-alba’s methanolic extract was found to have an extraction yield of 32.8% (w/w) (Table 1). The Biuret method, a commonly used technique for protein quantification, was employed to determine the total protein content in A. herba-alba extracts. A. herba-alba extract was found to contain a protein concentration of 0.7132 µg/mL (Fig.4). The Folin-Ciocalteu method was used along with Gallic acid to calculate the total phenolic content (TPC) to be 176.25 ± 6.70 mg GAE/g.

### Phytochemical analysis

The phytochemical profile of methanol A. herba-alba’s extract was realized by LC-MS technique in Ibn Khaldoon Lab, Jordan. The compounds were identified according to the chromatographic and spectral data using retention times, molecular weight, and molecular formula in comparison with reference standards and literature data (Fig. 2). Different types of plant secondary metabolites are considered to be bioactive compounds with biological and pharmacological properties (Table 2). These compounds are accountable for various activities that include antioxidant, anti-inflammatory, anti-hemolytic, anticancer, and antimicrobial effects (Afasar et al., 2016).

### Biological activities

The use of DPPH radical scavenging technique revealed that the raw extract of A. herba-alba has an antioxidant activity of 72.07% ± 0.004 (Fig.3). IC\textsubscript{50} regression value was 0.6926 µg/mL (Fig.4).

An experiment was conducted for evaluating the extract’s anti-hemolytic activity to investigate whether they can counteract the oxidative damage caused to the erythrocyte membrane by H\textsubscript{2}O\textsubscript{2}, which targets red blood cells. The outcomes indicated that A. herba-alba exhibited significant and satisfactory maximum inhibition of hemolysis, with a percentage of 68.71±0.00071% (Fig.3). Furthermore, the result of IC\textsubscript{50} regression of A. herba-alba was 0.7132 µg/mL (Fig.4).

The crude extract of A. herba-alba demonstrated a high level of effectiveness in reducing inflammation, with a potency of 97.11% (Fig.3). While the results of IC\textsubscript{50} regression for extract of A. herba-alba revealed 1.4296 µg/mL (Fig.4).

### Table 1

<table>
<thead>
<tr>
<th>Extract</th>
<th>Yield (%)</th>
<th>Total protein (mg/mL)</th>
<th>Total Phenols (mg GAE/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. herba-alba</td>
<td>32.82±2.43</td>
<td>0.7118±0.035</td>
<td>176.25±6.70</td>
</tr>
</tbody>
</table>

Mean ±SD, n=3.

### Table 2

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Molecular formula</th>
<th>Molecular weight</th>
<th>Percentage(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Limonene</td>
<td>C\textsubscript{10}H\textsubscript{16}</td>
<td>164.2</td>
<td>5.5</td>
</tr>
<tr>
<td>p-Cymene</td>
<td>C\textsubscript{13}H\textsubscript{18}O</td>
<td>218.29</td>
<td>8.4</td>
</tr>
<tr>
<td>masticadavenic acid</td>
<td>C\textsubscript{30}H\textsubscript{36}O\textsubscript{2}</td>
<td>490.56</td>
<td>3.2</td>
</tr>
<tr>
<td>vetivencic acid</td>
<td>C\textsubscript{14}H\textsubscript{18}O</td>
<td>220.32</td>
<td>1.3</td>
</tr>
<tr>
<td>Furanone</td>
<td>C\textsubscript{4}H\textsubscript{6}O</td>
<td>72.07</td>
<td>2.1</td>
</tr>
<tr>
<td>artegenin</td>
<td>C\textsubscript{20}H\textsubscript{30}O</td>
<td>342.42</td>
<td>2.2</td>
</tr>
<tr>
<td>alpha curcumene</td>
<td>C\textsubscript{15}H\textsubscript{24}O</td>
<td>256.34</td>
<td>1.3</td>
</tr>
<tr>
<td>Cyclocadecane</td>
<td>C\textsubscript{20}H\textsubscript{40}O</td>
<td>320.72</td>
<td>1.3</td>
</tr>
<tr>
<td>Herbolide</td>
<td>C\textsubscript{40}H\textsubscript{60}O</td>
<td>560.83</td>
<td>3.2</td>
</tr>
<tr>
<td>Kaempferol</td>
<td>C\textsubscript{46}H\textsubscript{72}O</td>
<td>672.92</td>
<td>3.2</td>
</tr>
<tr>
<td>dihydroinolalo</td>
<td>C\textsubscript{26}H\textsubscript{40}O</td>
<td>342.42</td>
<td>1.3</td>
</tr>
<tr>
<td>Camphor</td>
<td>C\textsubscript{10}H\textsubscript{18}O</td>
<td>152.23</td>
<td>1.3</td>
</tr>
<tr>
<td>Viridene</td>
<td>C\textsubscript{16}H\textsubscript{22}O</td>
<td>256.34</td>
<td>1.3</td>
</tr>
<tr>
<td>Eugenol</td>
<td>C\textsubscript{10}H\textsubscript{18}O</td>
<td>152.23</td>
<td>1.3</td>
</tr>
<tr>
<td>Naringin</td>
<td>C\textsubscript{16}H\textsubscript{22}O</td>
<td>256.34</td>
<td>1.3</td>
</tr>
<tr>
<td>Quercetin</td>
<td>C\textsubscript{16}H\textsubscript{22}O</td>
<td>256.34</td>
<td>1.3</td>
</tr>
<tr>
<td>Geranyl acetate</td>
<td>C\textsubscript{40}H\textsubscript{60}O</td>
<td>560.83</td>
<td>3.2</td>
</tr>
<tr>
<td>Isocaryophyllene</td>
<td>C\textsubscript{14}H\textsubscript{20}O</td>
<td>224.32</td>
<td>1.3</td>
</tr>
<tr>
<td>Elemol</td>
<td>C\textsubscript{18}H\textsubscript{30}O</td>
<td>270.45</td>
<td>1.3</td>
</tr>
<tr>
<td>Butylated</td>
<td>C\textsubscript{18}H\textsubscript{30}O</td>
<td>270.45</td>
<td>1.3</td>
</tr>
<tr>
<td>Hydroxyloline</td>
<td>C\textsubscript{18}H\textsubscript{30}O</td>
<td>270.45</td>
<td>1.3</td>
</tr>
</tbody>
</table>

RT: Retention Time. %: percentage.

### Figure 2

LC-MS chromatogram (intensity mAU) of phytochemical profile of Artemisia herba-alba extract.

### Figure 3

The activity (%) of the antioxidant, anti-inflammatory and anti-hemolytic in the methanol crude extracts of A. herba-alba. Mean ±SD, n=3.
Anti-hemolytic activity of A. herba-alba extracts. Mean ±SD, n=3.

<table>
<thead>
<tr>
<th>Cancer cell line</th>
<th>IC₅₀ (µg/mL)</th>
<th>Anti-proliferative (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast cancer 600MPE</td>
<td>6.7±0.45</td>
<td>32± 1.70</td>
</tr>
<tr>
<td>Breast Cancer MDA-MB-231</td>
<td>6.9±0.64</td>
<td>23± 1.32</td>
</tr>
<tr>
<td>Lung cancer HCC95</td>
<td>7.0±0.76</td>
<td>30±2.03</td>
</tr>
<tr>
<td>Prostate DU-145</td>
<td>6.8±0.41</td>
<td>40±2.46</td>
</tr>
</tbody>
</table>

Mean: SD, n=3.

Correlation analysis

A linear correlation was established to demonstrate the association of the total phenolic content with DPPH scavenging, anti-inflammatory, and anti-hemolytic activity of A. herba-alba extracts. Good correlations have been reported between the various biological activities and the TPC. Fig. 5 explores the linear correlation between TPC and antioxidant, anti-hemolytic, anti-inflammatory, and anti-inflammatory activities. R² values were 0.9815, 0.8848, 0.9727, and 0.9279, respectively. Additionally, the linear correlation matrices between TPC and various biological assays were analyzed, revealing a strong and positive correlation between them (Table 4).

Table 4 Linear correlation between TPC and different biological activities assays by using Pearson correlation coefficient.

<table>
<thead>
<tr>
<th>TPC</th>
<th>DPPH</th>
<th>Anti-hemolytic</th>
<th>Anti-inflammatory</th>
<th>Cytotoxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPC</td>
<td>1.0</td>
<td>0.9815</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>DPPH</td>
<td>0.9815</td>
<td>1.0</td>
<td>0.9585</td>
<td>0.9116</td>
</tr>
<tr>
<td>Anti-hemolytic</td>
<td>0.8701</td>
<td>0.9585</td>
<td>1.0</td>
<td>0.7316</td>
</tr>
<tr>
<td>Anti-inflammatory</td>
<td>0.8848</td>
<td>0.8577</td>
<td>0.9116</td>
<td>0.7316</td>
</tr>
<tr>
<td>Cytotoxicity</td>
<td>0.9279</td>
<td>0.9551</td>
<td>0.8434</td>
<td>1.0</td>
</tr>
</tbody>
</table>

DISCUSSION

A. herba-alba has excellent pharmacological properties and has some biologically active molecules. In this study, the identification of the bioactive compounds in A. herba-alba’s extract was carried out by the LC-MS technique. It was found the major components (81.5%) include kaempferol, quercetin, geranyl acetate, dihydrodaurin, furanone, vetivinic acid, limonene, eugenol, p-cymene, and naringin (Table 2). Our findings were in good accordance qualitatively but differ quantitatively with previous studies, which indicate that the major constituents identified by different leaf extracts were monoterpenes, linalool, 2,4-dihydrolinalool, furanone, undecanal, a-pinene, tetradecanal, camphor, fenchol, and myrtenol (Tilaoui et al., 2011; Abdallah et al., 2015).

A. herba-alba’s extract has potential for antioxidant activity and has a high percentage of DPPH radical scavenging 72.07%, and the IC₅₀ of A. herba-alba extract (0.693 µg/mL) was less as compared to other plant extracts, which means that potency of this plant is higher at lower concentrations, while its toxicity is relatively lower. These findings are in line with the research conducted by Amkiss et al. (2022). The extract was found to contain a high concentration of phenolic compounds (248.56 ± 11.05 µg GAE mg⁻¹ extract) and exhibited significant DPPH-radical-scavenging activity, as reported by Liu et al. (2018).

The crude extract of A. herba-alba was found to have a significant quantity of phytochemical compounds. The total phenolic content was determined to be 176.25±6.70 mg g⁻¹ extract, using gallic acid as a reference. The findings of the current study align with those of Ouguirti et al. (2020), which indicated that A. herba-alba leaf extract contains a significant amount of phenols, flavonoids, and polyphenolic compounds. Analysis of the linear correlation between TPC and DPPH scavenging, anti-inflammatory, and anti-hemolytic activity of A. herba-alba extracts was expressed and found to have a strong correlation. The results of this study indicate a robust correlation between TPC and the biological activity of A. herba-alba methanol extracts. These significant relationships highlight the vital role of phenolic components in the biological properties exhibited by the plant. Different cultivars may contain different types and quantities of phenolic chemicals in their extracts, which can result in different biological effects. It was not possible to find any previous research that explored the connection between the total phenolic content (TPC) of A. herba-alba extracts and their anti-inflammatory, anti-hemolytic, and cytotoxicity properties. However, there has been research that found a correlation between TPC and DPPH scavenging. Results of current study are consistent with few of the previous studies conducted by Indradi et al. (2017), and Amkiss et al. (2022). These studies stated that the total phenolic content of Alba species was shown to have a significant relationship with antioxidant activity (%) and a negative association with their IC₅₀ of DPPH scavenging abilities (Tilaoui et al., 2011; Amkiss et al., 2022).

There are several secondary metabolites in plants with a variety of biological functions (Kumar et al., 2017). Such activities include antioxidant, anti-inflammatory, anti-hemolytic, proteolytic, anti-proliferative, and antimicrobial (Leong and Shui, 2002; Nagai et al., 2003). Phytochemical analysis of plant extracts is commercially important in the pharmaceutical and food industries (Radha and Laxmi Priya, 2015). The analysis of the methanol extract from A. herba-alba demonstrated existence of a considerable quantity of 20 phytoconstituents. The extract has high phenolic content and showed good varied biological activities.

Our study emphasizes the biological potential of A. herba-alba extract. The substantial amounts of phytoconstituents including kaempferol, quercetin, geranyl acetate, dihydrodaurin, furanone, vetivinic acid, limonene, eugenol, p-cymene, and naringin may be accountable for these antioxidant, anti-hemolytic, cytotoxic, and anti-inflammatory effects (Haritha et al., 2014; Bourguet et al., 2016; Ouguirti et al., 2021). Furthermore, the observed biological effects may stem from the combined and cooperative actions of the biomolecules involved, instead of from any individual and clearly defined components (Dagne et al., 2000).
CONCLUSION

The study concluded that A. herba-alba’s methanolic extract has a promising source of phytochemical compounds (particularly, bioactive phenols). Furthermore, high activities of anti-oxidant, anti-hemolytic, anti-inflammatory and cytotoxic activities were demonstrated in A. herba-alba extracts. This study is the first one to establish the linear correlation between TPC and above biological activities. According to the findings, the methanol extract of A. herba-alba is a source of a variety of phytochemicals that enhance its pharmaceutical and marketing values.

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