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

Siwar Al-Qbilat[1], Omar Atrooz[2,3*]

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

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* methanolic 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 alongside antioxidant activity by various spectrophotometric methods. LC-MS analysis of crude extract reveals that kaempferol, quercetin, geranyl acetate, dihydrolinalool, furanone, vetivenic acid, limonene, eugenol, p-cymene, and naringin represent the major composition (81.5%). *A. herba-alba's* extract showed 72.07 % antioxidant activity through DPPH radical scavenging with IC_{50} 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 R^2 close to 0.90. Furthermore, strong cytotoxic activity was shown by the extract against tested cancer cell lines. The IC_{50} 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-Anssary, 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 (**Aziz *et al*., 2019; Moussa *et al*., 2019**). Phyto-antioxidants are a specific type of phytonutrient 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 (**Bose *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 (**Saboon *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 (**Moufid and Eddouks, 2012**). During the analysis of this plant's phytochemistry, several compounds were identified including Acetate, cis-chryanthenyl sesquiterpenes, monoterpenes, Herbalbin, flavonoids (curvilineal and hispidulin). The plant's aerial parts were found to have decreased toxicity (**Ouguirti *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, anthelmintic, antispasmodic, antibacterial, and antidiabetic effects (**Dabe and Kefale, 2017**).

Figure 1 Photograph of *Artemisia herba-alba* L. from Madaba region in Jordan (Siwar Alqubelat, April 2022)

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' 9.6276" 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 an 80% (v/v) methanol solution and stirred on a hotplate for 6 hours, twice. 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/25g 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;
Yield (%)= (DW * 100)/ FW, where the FW is the weight of the plant powder, and the DW is dry weight of extract after evaporation.

Phytochemical analysis

Phytochemical Analysis was done by triple-quadrupole Liquid Chromatography Mass Spectrometer model LC-MS-8030, Shimadzu, Japan [BM-20A Control Bus Module, CTO-30A Column oven, LC-30AD Liquid ChromatographySIL-30 AC Auto Sampler, interface ESI, Column C-18 (4.6*25 cm, 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 was10 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 of 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 **(Afsar *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)**:
Inhibition activity (%) = (Ac-As) x 100/Ac, where Ac was the control's absorbance, and As was the sample's absorbance.
The half maximal inhibitory concentration values (IC_{50}), are defined as the concentration upon which DPPH radicals are scavenged at about 50%, and were calculated from concentration–response curves. The IC_{50} value is calculated using IC_{50} calculator by applying the AAA Bioquest software.

Determining 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 660 nm **(Alamgeer *et al*., 2017)**.
Anti-Inflammatory activity (%)= 100-(AT- (AP/AC)) x100,
where AT is the test solution; AP is the product control and AC is the test control. To determine the IC_{50} value, the AAA Bioquest software was utilized. The IC_{50} calculator was applied to obtain the half-maximal inhibitory concentration (IC_{50}) of the samples.

Determining Anti-hemolytic activity by H_2O_2 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. After that, 0.25 mL of H_2O_2 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 **(Afsar *et al*., 2016)**.
Anti-hemolytic activity (%) = (Ap – (As-Ac/Ap)) x 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 IC_{50} value, the AAA Bioquest software was utilized.

Cytotoxic activity

The cytotoxic activity was done in Smart Lab (Ibn 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-231 (Adenocarcinoma cell line), lung 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% CO_2. The cytotoxic compound, cisplatin (in concentration ranged 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 10^5/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 solution's absorbance was read at wavelength 540 nm. The IC_{50} values were determined by analyzing the concentration-response curves **(Atrooz *et al*. 2018)**.

Cytotoxic activity was measured using the formula given below:
Cell survival (%) = [As-Ab/Ac-Ab] x 100, 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 0.7118 ± 0.035 mg/mL (Table 1). 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.

Table 1 Approximate contents of extraction yield, total proteins, and total phenolic contents.

Extract	Yield (%)	Total protein(mg/mL)	Total Phenols(mg GAE/g)
A. herba-alba	32.82±2.43	0.7118±0.035	176.25±6.70

Mean ±SD, n=3.

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 (**Afsar et al., 2016**).
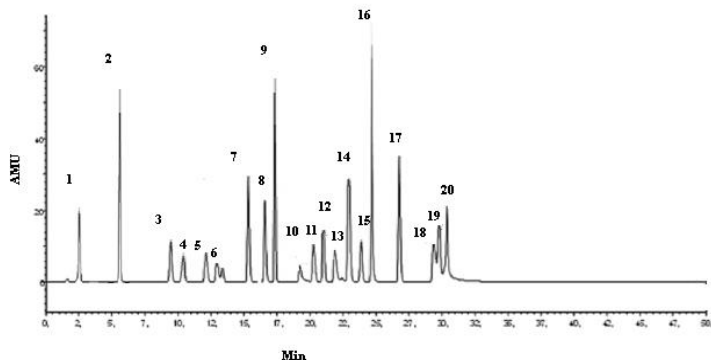

Figure 2 LC-MS chromatogram (intensity mAU) of phytochemical profile of *Artemisia herba-alba* extract.

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_{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_2O_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_{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_{50} regression for extract of *A. herba-alba* revealed 1.4296 µg/mL (Fig.4).

Table 2 Phytochemicals profiling of crude methanol extract of *A. herba-alba* using LC-MS technique.

Compounds	Molecular formula	Molecular weight	Percentage(%)
Limonene	$C_{10}H_{16}$	136.23	4.5
p-Cymene	$C_{10}H_{14}$	134.22	10.4
masticadienonic acid	$C_{30}H_{46}O_3$	454.7	2.3
vetivenic acid	$C_{15}H_{22}O_2$	234.33	5.4
Furanone	$C_4H_4O_2$	84.07	6.3
artemetin	$C_{20}H_{20}O_8$	388.4	0.7
alpha curcumene	$C_{15}H_{22}$	202.33	1.3
Cyclodecanone	$C_{10}H_{18}O$	154.25	0.2
herbolide	$C_{15}H_{22}O_4$	266.33	3.2
Kaempferol	$C_{15}H_{10}O_6$	286.24	17.2
dihydroLinalool	$C_{10}H_{20}O$	156.26	8.4
Camphor	$C_{10}H_{16}O$	152.23	1.3
Viridene	$C_{11}H_{18}$	150.26	2.1
Eugenol	$C_{10}H_{12}O_2$	164.2	5.5
Naringin	$C_{27}H_{32}O_{14}$	580.5	3.8
Quercetin	$C_{15}H_{10}O_7$	302.23	8.6
Geranyl acetate	$C_{12}H_{20}O_2$	196.29	11.4
Isocaryophyllene	$C_{15}H_{24}$	204.35	2.2
Elemol	$C_{15}H_{26}O$	222.37	1.8
Butylated Hydroxytoluene	$C_{15}H_{24}O$	220.35	3.3

RT: Retention Time. %: percentage.

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Evaluation of anti-proliferative potential by MTT assay

It was known that most anticancer drugs are cytotoxic to healthy cells. Based upon reports of the value of *A. herba-alba* in medical applications in Jordan, it was selected to be tested for cytotoxicity against different cancer cell lines. The cytotoxic results showed that *A. herba-alba's* methanolic extract has different inhibitory activity of cell proliferation (%) on breast cancer 600MPE, breast cancer MDA-MB-231, lung cancer HCC95, and prostate DU-145 (32,23,40, and 37%, respectively) (Table 4). The IC_{50} values of extract indicated the extract's effectiveness on the selected cancer cell lines at low concentrations (6.7, 6.9, 7.0, and 6.8 µg/mL) (Table 3).
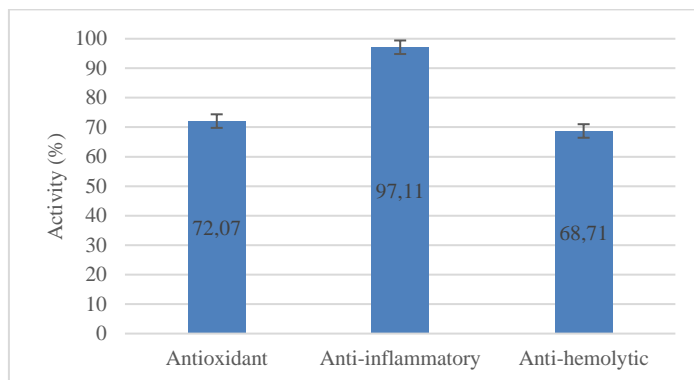

Figure 3 The activity (%) of the antioxidant, anti-inflammatory and anti-hemolytic in the methanol crude extracts of *A. herba-alba*. Mean ±SD, n=3.
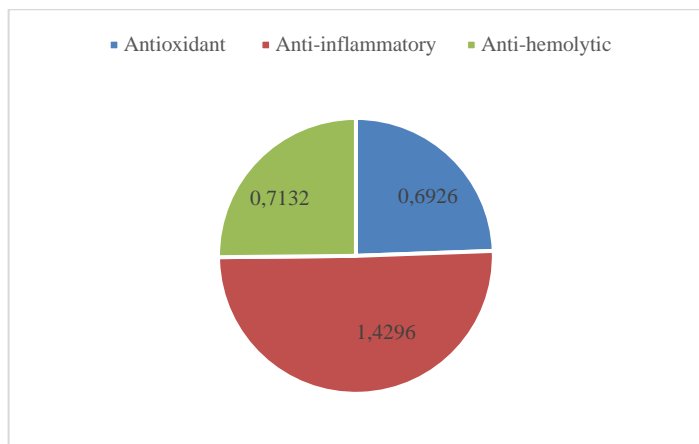
Figure 4 IC_{50} values (µg/mL) of DPPH scavenging, anti-inflammatory, and anti-hemolytic activity of *A. herba-alba* extracts. Mean ±SD, n=3.

Table 3 Anti-proliferative activity (%) and IC_{50} (µg/mL) of *A. herba-alba* extract on different cancer cell line. Results are compared to the control Cisplatin.

Cancer cell line	IC_{50} (µg/mL)	Anti-proliferative (%)
Breast cancer 600MPE	6.7±0.45	32± 1.70
Breast Cancer MDA-MB-231	6.9±0.64	23±1.32
Lung cancer HCC95	7.0±0.76	30±2.03
Prostate DU-145	6.8±0.41	40±2.46

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, antiproliferative, and anti-inflammatory activities. R^2 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).
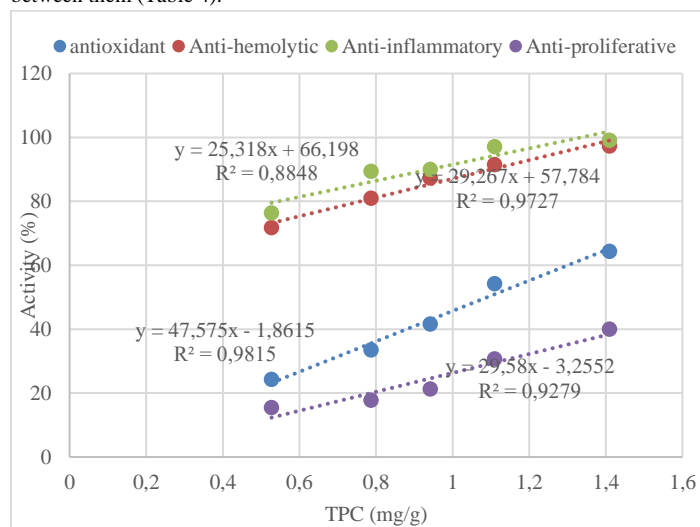

Figure 5 Correlation between the amount of TPC and antioxidant, anti-inflammatory, anti-hemolytic and anti-proliferative activity of the methanol crude extract of *A. herba-alba*.

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

	TPC	DPPH	Anti-hemolytic	Anti-inflammatory	Cytotoxicity
TPC	1				
DPPH	0.9815	1			
Anti-hemolytic	0.8701	0.9585	1		
Anti-inflammatory	0.8848	0.8577	0.9116	1	
Cytotoxicity	0.9279	0.9551	0.8434	0.7316	1

DISCUSION

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, dihydrolinalool, furanone, vetivenic 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 leave extracts were monoterpenes, linalool, 2-tridecanol, 2-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 IC_{50} 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 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 IC_{50} 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 Laxmipriya, 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, dihydrolinalool, furanone, vetivenic acid, limonene, eugenol, p-cymene, and naringin may be accountable for these antioxidant, anti-hemolytic, cytotoxic, and anti-inflammatory effects (**Haritha et al., 2014; Bourgou 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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