

Antimicrobial, Anticoagulant and Anticancer Effects of *Arum Palaestinum* Flowers Extracts

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Abstract

Background: Wild plants are amply utilized in traditional medicine and folkloric food worldwide. *Arum palaestinum* Boiss. (AP) is one of the wild Palestinian plants which leaves have a long history in the Middle Eastern countries as food and medicine. Herby, the current study aimed to evaluate the antimicrobial, coagulation cascade activities, and anticancer effects of (AP) flowers extract

Methods: The aqueous extract of (AP) flowers was screened on its antimicrobial activity using microdilution assay against eight pathogens. While, prothrombin time, activated partial thromboplastin time, and thrombin time tests were measured utilizing standard hematological methods. And Anti cancer effect was assessed by using Parameters of cell cycles and alph feta protein level that were investigated for (AP) flowers fractionated with aqueous, DMSO, and methanol

Results: The antimicrobial screening results revealed that the aqueous extract of (AP) has strong antibacterial effects against *P. vulgaris* and *E. faecium* compared with Ampicillin with MIC values of 6.25, 6.25 and 18 mg/ml, respectively. The aqueous extract of (AP) showed anticoagulant activity with significant prolonged results in aPTT and TT tests at high concentrations (50 mg/ml and 25 mg/ml) and slightly prolonged results in the PT test at a high concentration (50 mg/ml).

The anticancer results indicate a delay in cell cycle through decreased the cell proliferation rate following effects of the AP fractions. The delay in the S phase was in favor of the water fraction. Water and DMSO fractions while maintained the cells in the G2-M phase similar to the DOX, the flower extract in methanol accelerated the cells in the G2-M phase suggesting that (AF) flower extracts have anti-cancer properties. At the same time Aqueous extract decreased HCC aFP to 1.55-fold ($P=0.0008$). While DMSO and methanolic extract had no significant effects on HCC aFP levels, compared to control untreated cells of 2519.16 ± 198.1 ng/ml. This data show that (AF) aqueous solution is potent inhibitor of alpha-fetoprotein secretion (P -value <0.05), which indicates its anti-carcinogenic effects

Conclusion: These results showed that *the aqueous extract of (AP) plant* possesses bioactive components with antibacterial and anticoagulant properties, which may be exploited in the treatment of infectious diseases and blood coagulation disorders.

Introduction

Herbal medicine is one of the traditional therapeutic methods that many people have used in their communities to treat and prevent harmful diseases (1). This kind of treatment is based on the use of different plants parts such as leaves, flowers, seeds, bark, fruits, and roots in preparing of medicinal remedies to control or prophylaxis of disease and to find a cure for it, which later developed into synthetic or semisynthetic drugs, based on the assumption that they are safer, more potent, selective and come with few side effects (2). Herbal medicine has played the most important role in drug discovery, mostly because it is offering potential lead compounds, which result from several processes on plants including isolation, purification, and molecular characterization (3).

Thus, one of the lead sources is plant secondary metabolites that are derived biosynthetically from plant primary metabolites. These secondary metabolites are bioactive chemicals that include alkaloids, glycosides, phenolic compounds, tannins, and terpenoids which continue to be good model molecules in drug discovery due to their potential medicinal applications for the treatment of numerous conditions and diseases from migraine up to cancers (4). From the foregoing discussion, we see the importance of herbal medicine in helping humanity in discovering and developing medicines for many diseases, especially the intractable ones (5). The molecular process by which the body forms clots to avoid bleeding is the coagulation cascade. Platelets, endothelial cells, and leukocytes must be activated so that a suitable surface is provided for the adhesion of clotting proteins (6). Blood coagulation cascade reactions are propagated by complex enzymes containing a vitamin K-dependent serine protease and an accessory cofactor protein that are calcium-dependently assembled on the membrane surface (7). The traditional view of blood coagulation regulation consists of extrinsic and intrinsic pathways; extrinsic pathway depends on the initiation phase, include plasma factor VII/VIIa and transmembrane receptor tissue factor (TF), whereas intrinsic pathway requires amplification phase, that includes plasma FXI, FIX, and FVIII (8).

Antibiotics are usually used to treat various sorts of microbial infections and some microorganisms can develop more resistant strains by mutation or acquired resistance against some antibiotics. For example, most Gram (-ve) are inherently resistant to vancomycin. Bacteria are deemed resistant to an antibiotic if their development is not prevented by the maximum amount of the antibiotic that can be absorbed in the host dosage. However, microbial strains become resistant by the genetic alteration or/and the expression of proteins which usually occurred by the modification of target site, reduction of accumulation, or by the enzymatic inactivation (9).

Arum palaestinum Boiss. (AP) (Araceae) is a perennial herbaceous plant which native to the Levant and other countries located in the Mediterranean Basin. It grows up to 0.82 ft and blooms in the spring, between March and April months. The plant is well recognized by its dark purplish-black spadix enclosed by a reddish-brown spathe (10, 11). In traditional Palestinian medicine (AP) aqueous extract has been used for the treatment of Bacterial infection, cough, cancer, constipation, intestinal worms, skin infections, blood circulation disorders, and renal stones.

However, isoorientin, vitexin, luteolin, apigenin, quercetin-3-O-beta-glucoside, quercetin, esculin, ferulic and caffeic acids are the major recognized molecules in (AP) leaves (12). While phytol, phytol acetate, linolenic, linoleic, and hexadecanoic acids, were the major compounds in (AP) aerial parts (13, 14).

To the best of the author's knowledge, no previous studies were conducted to evaluate the antimicrobial and antiplatelet activities of (AP) flowers.

Material And Methods

Collection of the plant material

The flowers of (AP) plant were collected in April-May 2019 from the Tulkarem region in Palestine. The plant was characterized a pharmacognosist Dr. Nidal Jaradat in the herbal products laboratory at An-Najah National University. The plant was deposited in the Pharmacognosy Laboratory, Faculty of Medicine and Health Sciences at An-Najah National University (voucher specimen: Pharm-PCT-246). The study protocol complied with relevant international guidelines and legislation.

The collected flowers were washed with distilled water and later completely dried in shade at room temperature for 3 weeks. The dried parts were grounded into a fine powder using a mechanical blender and stored in tightly sealed special containers until use.

Preparation of (AP) sample

The dried (AP) flowers were chopped into small pieces and 100 g of (AP) flowers were boiled in 1 L of distilled water until the original volume is decreased to one fourth. After that, the decoction was filtered (Machrery-Nagel, MN 617 and Whatman no.1, USA) and the filtrate was placed into a freeze drier (Millrock Technology-BT85, China) apparatus until the aqueous extract turned into solid powder. The dried extract was then kept in a well-closed container for further use. In the next step, AP flower extracts were diluted with the water, DMSO and methanol solutions. To obtain the concentration of 0.1 mg/ml, 1 mg from each one of the preserved extracts was taken. Then it was diluted with 10 ml of the three solutions. Finally, 1 ml of the prepared dilution was obtained using a pipette. All preparations were packaged in suitable, locked containers and were sent to Hadassah Hospital Laboratories for further research.

Microbial isolates

The examined bacterial and fungal isolates were obtained from the American Type Culture Collection (ATCC). The selected species of microorganisms are frequently isolated in clinical settings in our region and some possess multidrug resistance. The isolates included three Gram-positive strains: *Staphylococcus aureus* (ATCC 25923), Methicillin Resistance *Staphylococcus aureus* (MRSA) a clinical strain, and *Enterococcus faecium* (ATCC 700221) and four Gram-negative strains: *Klebsiella pneumoniae* (ATCC 13883) *Proteus vulgaris* (ATCC 700221), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853). Meanwhile, the fungal isolates included *Candida albicans* (ATCC 90028).

Blood samples collection and preparation

Citrated blood samples were collected from five healthy volunteers. All participants were not under any medications especially anticoagulant, they were also non-smoker individuals. One part of sodium citrate was mixed with 9 parts blood to obtain a ratio equals 1:9. The citrated blood was centrifuged at 2500 rpm for 15 minutes to obtain citrated-PPP (platelet-poor plasma). Prothrombin time (PT), activated partial thromboplastin time (aPTT), and Thrombin time (TT) tests were conducted on plasma within 2 h of blood collection. All results were obtained by a digital coagulation analyzer (coagulation analyzer Coa-DATA 4004, Germany). All measurements were conducted in duplicate and 1% DMSO was used as a negative control (15).

Cell line

Hep 3B2.1-7 [Hep 3B, Hep-3B, Hep3B] was commercially purchased from ATCC (HB-8064). Cytotoxicity evaluation was carried out by using the HEP3B cell line, which has the same genotype, phenotype and features of hepatocellular carcinoma (HCC). These cells were taken from a Japanese person liver who had HCC and hepatitis B. HEP3B are characterized by the secretion of α -fetoprotein (α FP) that is considered a tumor marker. α FP were assessed by a commercially available ELISA kit from (R&D Systems, Inc., USA). HEP3B condition was accomplished by using RPMI-1640 medium enhanced with 1% penicillin, 1% streptomycin, 1% L-glutamine and 10% fetal bovine serum; it was adapted to pH 7.2 by Dulbecco's Phosphate Buffered Saline (DPBS). The growing process of the cells was performed at 37°C in ESCO cell-culture incubator which had a humidified atmosphere of 95% air and 5% CO₂ at 37° C.

Antimicrobial test

The antimicrobial activity of (AP) flowers aqueous extract was assessed using the well diffusion method. The bacterial suspension was prepared by picking some colony of overnight agar culture of the test organism and adding it to a test tube containing 5 ml of nutrient broth, then the turbidity was compared with that of McFarland nephelometer tube No. 0.5 (1.5×10^8 cfu/ml); then it was diluted by taking 1000 μ l of suspension and it was added to 2 ml of nutrient broth (0.5×10^8 cfu/ml). The Minimum Inhibitory Concentration (MIC) is the lowest concentration of an antimicrobial that inhibits the growth of a microorganism after 18-24 hrs. The extract was subjected to serial broth dilution technique to determine their minimum inhibitory concentration for all tested microorganisms. The broth micro-dilution method was used to determine the antimicrobial activity of (AP). The plant extract was dissolved in 1 ml DMSO in a concentration of 100 % for aqueous extract. Then the final extract concentration was 100 μ g/ml. After that, each well was inoculated with microbial inoculums which were prepared in the same medium after dilution of standardized microbial suspension adjusted 0.5 McFarland scale. After well mixing, the 96-well micro-titration plates are incubated under 37°C for 24 h. For all bacteria we tested here, was conducted four controls including; 1- +ve control which contains media + bacteria; 2- -ve control which contains media only; 3- Extract control (extract + media): to be sure there is no contamination and turbidity and the changes are not occurred due to extract itself (so extracts were serially diluted in this control); 4- DMSO: no extract used. The established tests were conducted in triplicates (16).

Anticoagulant properties

Prothrombin Time (PT) test

The in-vitro method was used for the test. 100 μ l of pre-warmed plasma were incubated with 100 μ l plant extract for 5 minutes. All concentrations (50, 25, 5 and 1 mg/ml) from (AP) aqueous extract were tested. The clotting time was measured immediately after the addition of 200 μ l of pre-warmed thromboplastin reagent (Hemostat thromboplastin-SI. Human, Germany) (15).

Activated Partial Thromboplastin Time (aPTT) test

For this test, an in-vitro approach was used. For 5 minutes, 50 μ l of pre-warmed plasma were incubated with 50 μ l of plant extract. The concentrations of (AP) aqueous extract were evaluated at 50, 25, 5, and 1 mg/ml. The PTT reagent was then added and incubated for 3 minutes. The clotting time (aPTT, Human, Germany) was determined immediately after the addition of 50 μ l CaCl₂ reagent (15)

Thrombin Time (TT) test

The in-vitro technique was used for the test. 100µl of plant extract were incubated for 5 min with 100µl pre-warmed plasma. Different concentrations (50, 25, 5 and 1 mg/ml) from (AP) aqueous extract were tested. The clotting time was determined after 100 µl of pre-warmed thrombin reagent was added (Hemostat Thrombin Time. Human, Germany) (15).

Flow cytometry analysis

Following the culturing, the collected HEP3B cells were adjusted to 10^6 /ml in staining buffer (in saline containing 1% bovine albumin). The labeling of fragmented DNA with propidium-iodide (PI) and the staining of phosphatidylserine with annexin V-conjugated to Fluorescein isothiocyanate (FITC) were done according to the manufacturer's instructions for viability assessments and apoptosis. The apoptosis was then identified with annexin-V (+) but not propidium-iodide (-). Viable cells, on the other hand, were labeled with annexin-V (-) but propidium-iodide (-). In each experiment, unstained controls were used, such as IgG isotype controls and Fluorescence Minus One (FMO) controls. The use of propidium-iodide allowed for the investigation of the cell cycle by the quantification of DNA content. The HEP3B cells were fixed for at least 30 minutes in a cold 70% ethanol solution at 4°C. The cells were then rinsed twice in phosphate buffered saline (PBS). To dispose of the supernatant, it was calibrated to spin at 2000 rpm. The cells were treated with ribonuclease (50 I µl of 100 µg /ml RNase) to ensure that only DNA was stained. The cells were then stained with 5 µl of 50 µg Propidium iodide/100 ml and flow cytometry was used to evaluate them (Becton-Dickinson LSR II, Immuno-fluorometry systems, Mountain View, CA).(17)

Alpha Feto Protein (aFP) detection

aFP is produced whenever liver cells are regenerating. With chronic liver diseases, such as hepatitis and cirrhosis, aFP may be chronically elevated. Very high concentrations of aFP may be produced by certain tumors. This characteristic makes the aFP test useful as a tumor marker. Increased amounts of aFP are found in many people with the most common type of liver cancer called hepatocellular carcinoma and in a rare type of liver cancer that most commonly occurs in infants called hepatoblastoma. Secreted aFP concentrations in Hep3B cells culture medium were detected using Human alpha-Fetoprotein Quantikine ELISA Kit (R&D; DAFP00). Absorbance was measured at 450 nm using a Universal Microplate Reader

Statistical assessment

The obtained results of the studied (AP) aqueous extract were expressed as means \pm standard deviation (SD). Averaged data were compared using a *t*-test. The statistical significance was considered when the *p*-value was <0.05 .

Results And Discussion

Antimicrobial activity

The antimicrobial activity of (AP) crude aqueous extract was determined using micro-dilution assay against selected infectious pathogens belonging to the Gram-negative, Gram-positive and fungi strains.

Table 1
Antibacterial and antifungal MIC values of (AP) aqueous extract and positive controls (mg/ml)

	<i>S. aureus</i>	<i>E. faecium</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>K. pneumoniae</i>	<i>P. vulgaris</i>	MRSA	<i>E. faecium</i>	<i>C. albicans</i>
Source	ATCC 25923	ATCC 700221	ATCC 25922	ATCC 27853	ATCC 13883	ATCC 700221	Clinically diagnosed	ATCC-700221	ATCC-90028
Extract dissolved in D.W 100 mg/ml	3.125	6.25	6.25	3.125	6.25	6.25	6.25	6.25	R
Ampicillin	0.312	0.156	0.312	1.25	0.1	18	3.25	6.25	0
Ciprofloxacin	0.078	0.078	0.0156	0.0312	0.00125	0.15	12.5	14	0
Fluconazole	0	0	0	0	0	0	0	0	1.56

The results showed that the aqueous (AP) extract has antibacterial activity comparing with the positive antibacterial controls (Ampicillin and Ciprofloxacin) and has not antifungal activity compared with the positive antifungal drug Fluconazole as reported in Table 1.

In fact, the aqueous (AP) extract possessed an antibacterial effect against all the tested bacterial strains while this extract revealed a strong antibacterial effect against *P. vulgaris* compared with Ampicillin with MIC values of 6.25 and 18 mg/ml, respectively. Also has the same antibacterial activity against *E. faecium* with MIC values of 6.25 mg/ml. However, the antibacterial MIC values of (AP) were lower than Ciprofloxacin.

Anticoagulant properties

Table 2
Prothrombin Time (PT) results (in seconds) of (AP) aqueous extract

Sample	Participant 1 mean ±SD	Participant 2 mean ±SD	Participant 3 mean ±SD	Participant 4 mean ±SD	Participant 5 mean ±SD
Plasma only	13.25 ±0.05	13.1 ±0.1	14.55 ±0.05	12.25 ±0.05	12.2±0.1
Plasma + PBS	13.55 ±0.05	13.35 ±0.05	14.85 ±0.05	12.65 ±0.05	13.1±0.1
Plasma +1%DMSO	13.65 ±0.05	13.35 ±0.05	14.85 ±0.05	12.7 ±0.1	13.15±0.05
Water extract					
Plasma +50 mg/ml	19.5 ±0.1	18.85±0.05	18.95 ±0.05	17.0 ±0.1	18.35 ±0.05
Plasma + 25mg/ml	15.55 ±0.05	15.4 ±0.1	16.4 ±0.1	14.6 ±0.1	15.25 ±0.05
Plasma + 5 mg/ml	13.65 ±0.05	13.9 ±0.1	15.15 ±0.05	13.35 ±0.05	13.85 ±0.05
Plasma + 1 mg/ml	13.5 ±0.1	13.4 ±0.1	15.0 ±0.1	12.8 ±0.1	13.2 ±0.1

Table 3
Activated Partial Thromboplastin Time (aPTT) results (in seconds) of (AP) aqueous extract

Sample	Participant 1	Participant 2	Participant 3	Participant 4	Participant 5
	mean \pm SD				
Plasma only	25.3 \pm 0.1	26.2 \pm 0.1	30.45 \pm 0.05	27.95 \pm 0.05	29.15 \pm 0.05
Plasma + PBS	25.65 \pm 0.05	26.85 \pm 0.05	31.2 \pm 0.1	28.6 \pm 0.1	29.75 \pm 0.05
Plasma + 1%DMSO	25.6 \pm 0.1	26.9 \pm 0.1	31.35 \pm 0.05	28.7 \pm 0.1	29.8 \pm 0.1
Water extract					
Plasma +50mg/ml	>200	>200	>200	>200	>200
Plasma + 25mg/ml	185 \pm 4	166 \pm 3	179 \pm 5	147 \pm 6	163 \pm 4
Plasma + 5mg/ml	45.3 \pm .6	46.6 \pm 0.5	48.6 \pm 0.2	45.9 \pm 0.4	44.65 \pm 0.15
Plasma + 1mg/ml	31.6 \pm 0.2	33.5 \pm 0.3	37.7 \pm 0.2	34.85 \pm 0.25	32.1 \pm 0.1

Table 4
Thrombin Time (TT) results (in seconds) of (AP) aqueous extract

Sample	Participant 1	Participant 2	Participant 3	Participant 4	Participant 5
	mean \pm SD				
Plasma only	10.25 \pm 0.05	10.1 \pm 0.1	12.35 \pm 0.05	10.1 \pm 0.1	11.3 \pm 0.1
Plasma + PBS	10.45 \pm 0.05	10.55 \pm 0.05	12.6 \pm 0.1	10.35 \pm 0.05	11.65 \pm 0.05
Plasma + 1%DMSO	10.4 \pm 0.1	10.6 \pm 0.1	12.7 \pm 0.1	10.4 \pm 0.1	11.7 \pm 0.1
Water Extract					
Plasma +50mg/MI	38.9 \pm 0.2	41.7 \pm 0.1	39.7 \pm 0.2	35.1 \pm 0.2	36.7 \pm 0.1
Plasma + 25mg/MI	19.3 \pm 0.1	23.3 \pm 0.1	22.4 \pm 0.1	18.6 \pm 0.2	20.4 \pm 0.1
Plasma + 5mg/MI	10.85 \pm 0.05	11.25 \pm 0.05	12.85 \pm 0.05	10.4 \pm 0.1	12.15 \pm 0.05
Plasma + 1mg/MI	10.45 \pm 0.05	10.55 \pm 0.05	14.75 \pm 0.05	10.3 \pm 0.1	11.65 \pm 0.05

The *in-vitro* coagulation assays results showed that the aqueous extract of (AP) has prolonged aPTT and TT tests in a dose-dependent manner with the highest effect at 50 mg/ml. At the same time, a significant increase in PT test was observed only at a high concentration of plant extract (50 mg/ml) as observed in Tables 2-4. These tests are used to evaluate the coagulation cascade, PT is used for evaluation of the extrinsic and common pathways of the coagulation cascade. APTT for the intrinsic and common pathways, and TT for the conversion of fibrinogen to fibrin in the common pathway (17, 18). The results suggest that the inhibition of clotting factors in the intrinsic and common pathways because both aPTT and TT tests were prolonged significantly. The inhibition increased in these tests as the concentration of plant extract increased. PT was slightly prolonged, when a high concentration of plant extract was used, as a result of inhibition of the common pathway, since the main inhibition being in the intrinsic and common pathways.

These findings suggest either the presence of different active ingredients in this plant, at which the inhibitions of intrinsic and common pathways were observed or by acting on a common factor that inhibits both pathways. This factor may be through the protein C pathway, as this pathway is used to inhibit factor Va (in the common pathway) and factor VIIIa (in the intrinsic pathway). It is suggested that this plant may contain active ingredients that activate protein C to become the activated form (APC), then APC inhibits factors Va and VIIa, that's why prolonged results in the intrinsic and common pathways were seen (19). Also, it could be by the presence of an anti-thrombin substance (18).

Cytotoxicity

Arum palaestinum fractions inhibit DNA cell cycle of HEP3B cells

Parameters of cell cycles were investigated for (AP) flowers that were fractionated with aqueous, DMSO, and methanol. The incubation of these fractions of the (AP) flowers with HEP3B cells was achieved for 48 hours. Doxorubicin (DOX), is an anti-cancer drug known to inhibit cell proliferations and DNA arrest were used as control.

In Figure 1, DOX treated cells decreased G1 phases from 65.3 ± 7.8 in untreated cells to $54\pm 5.1\%$ ($p=0.01$). (AP) fractions also shifted the G1 populations to $51.3\pm 3.1\%$, $52\pm 2.2\%$ and $50\pm 2.4\%$ in the aqueous, DMSO and Methanol fractions, respectively ($p<0.05$ in all groups). Moreover, (AP) fractions phase shifted S phase population which is in charge of DNA replication from $17.7\pm$ in both HEP3B and DOX treated cells to $9.5\pm 2.6\%$, $12.1\pm 1.9\%$ and $12.6\pm 1.5\%$ in the aqueous, DMSO and Methanol fractions, respectively ($p<0.05$ in all groups). Same pattern of results were obtained in the G2-M phase populations. (AP) fractions phase inhibited G2-M population; in which Mitosis commonly takes effect, from 23.7 ± 1.5 in the untreated cells to $6.35\pm 1.9\%$, $8.1\pm 3.1\%$ and $19.7\pm 5\%$ in the aqueous, DMSO and Methanol fractions, respectively ($p<0.05$ in all groups). DOX-treated cells had $7.4\pm 1.8\%$ of population in the G2-M phase. Overall data indicate a delay in cell cycle through decreased the cell proliferation rate following effects of the AP fractions. The delay in the S phase was in favor of the water fraction. Water and DMSO fractions while maintained the cells in the G2-M phase similar to the DOX, the flower extract in methanol accelerated the cells in the G2-M phase. In conclusion, these data might imply that (AF) extracts have anti-cancer properties.

(AF) extracts promote cell death by apoptosis

The next step was to see if (AF) flower extracts could disrupt DNA content and, as a result, cause programmed cell death (apoptosis). To study this, it was discovered that cells undergoing apoptosis had their phospholipid phosphatidylserine (PS) transferred from the inner face of the plasma membrane to the cell surface; thus, the presence of PS on the cell surface can be used to identify apoptotic cells. PS was stained using a fluorescent compound of annexin-V, a protein known to have a high affinity for PS, as described in the materials and techniques section. After then, flow cytometry analysis was performed in order to detect the PS. Cells were also stained with propidium iodide (PI), a dye that can only enter the cell when the plasma membrane is damaged. Positive for PS but negative for PI, early apoptosis was assessed. Nonetheless, it was observed that cells in the late apoptotic and necrotic stages were positive for both PS and PI.

Figure 2 demonstrates that the untreated cells (HEP3B cells alone), which are considered the control cells, maintain a baseline apoptotic cell population of $39\pm 5.3\%$. It also represents that cells receiving treatment with aqueous, DMSO and methanol fractions of the (AF) flower had a baseline apoptotic cell population of $57.3\pm 2.5\%$, $20.6\pm 3.9\%$ and $18.3\pm 1.5\%$, respectively. As can be observed, DMSO as well as the methanol fraction had diminished apoptosis compared to that of the untreated cells. While the population of the late apoptotic/necrotic cells was recognized to increase in all the different fractions of the (AF) flower to $54.3\pm 4.7\%$, $76.3\pm 4.5\%$, $79.7\pm 3.05\%$, for the aqueous, DMSO and methanol fractions respectively, compared to $41.7\pm 1.5\%$ in the untreated cells. All results showed statistically significant between all tested groups. Generally, the given data propose that the (AF) flowers shift the cells to necrosis by prolongating the G2-M phase of the HEP3B cell cycle and confirm anticancer potentials of the (AF) fractions.

(AF) flower extracts decrease alpha-fetoprotein (αFP) secretion from HEP3B cells

αFP a tumor marker and a major protein that is synthesized in the fetal liver has been speculated to be the fetal analog of serum albumin. Usually, it has very low levels in healthy adults, however high levels of it might hint different types of cancers such as liver cancer. Figure 3 shows the averages of declined to αFP to 1625.8 ± 64.8 ng/ml, 2340 ± 65.6 ng/ml and 22493.3 ± 88.4 ng/ml after processing aqueous, DMSO and methanol treatments, respectively, compared to the average of the untreated cells of 2519.2 ± 198.1 ng/ml. *Arum palaestinum* flower extract made in water decreased HCC αFP to 1.55-fold ($P=0.0008$). While flower extracts made in DMSO and methanol had no significant effects on HCC αFP levels, compared to the average of the untreated cells of 2519.16 ± 198.1 ng/ml. This data show that *Arum palaestinum* aqueous solution is potent inhibitor of alpha-fetoprotein secretion (P -value <0.05), which indicates its anti-carcinogenic effects

Conclusion

The current work is regarded as a preliminary examination for the future creation of naturally occurring antiplatelet and antibacterial medicines, despite the fact that certain synergistic or antagonistic effects remain unknown. The anticancer mechanism of (AP) aqueous extract and isolated compounds, as well as a large-scale manufacture, must all be further researched.

List Of Abbreviations

AP *Arum palaestinum* Boiss.

TF Transmembrane receptor tissue factor

ATCC American Type Culture Collection

Citrated-PPP Citrated -platelet-poor plasma

PT Prothrombin Time

aPTT Activated Partial Thromboplastin Time

TT Thrombin Time

HCC Hepatocellular Carcinoma

αFP α-fetoprotein

DPBS Dulbecco's Phosphate Buffered Saline

MIC Minimum Inhibitory Concentration

FITC Fluorescein isothiocyanate

PI Propidium-Iodide

FMO Fluorescence Minus One

PBS phosphate buffered saline

APC Activated Protein C

DOX Doxorubicin

PS Phatidylserine

MRSA Methicillin Resistance *Staphylococcus aureus*

Declarations

Ethics approval and consent to participate

Ethical approval for the current study was obtained from the *Institutional Review Board (IRB) of An-Najah National University* (approval number: Med.Dec 2021/8). The collection of the plant material complied with the WHO Guidelines for the Assessment of Herbal Medicines and Legislation. Written informed consent was obtained from all participants. The consent form which contains details about the research and contacts of investigators. Volunteers' willingness to participate in the investigation was documented by the signing of the written informed consent. We confirm that all methods were carried out in accordance with relevant guidelines and regulations. Furthermore, we confirm that all experimental protocols were approved by the IRB of *An-Najah National University*.

Consent to publish

Not applicable

Availability of data and materials

The data used to support the findings of this study are included in the article.

Competing interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Authors' Contributions

All research is done by the authors.

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Figures

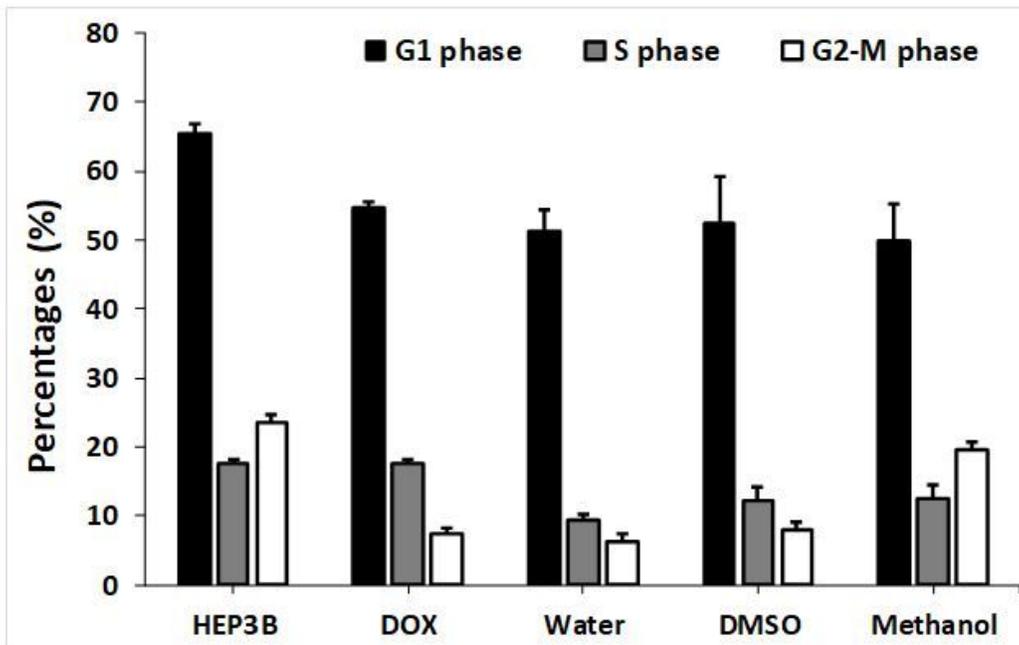


Figure 1

The proportion of cells in the cell cycle phases after the processing of the different fractions of the (AP) flower extracts.

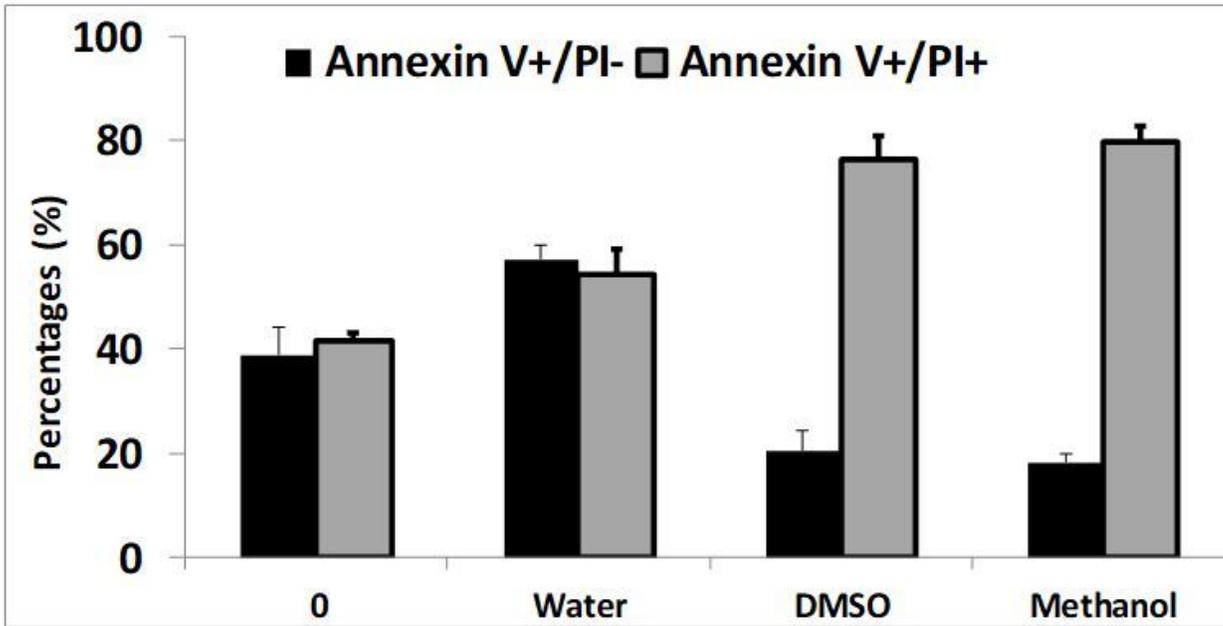


Figure 2

The apoptotic cell population and the population of the late apoptotic/necrotic cells following treatment with (AF) flower extracts

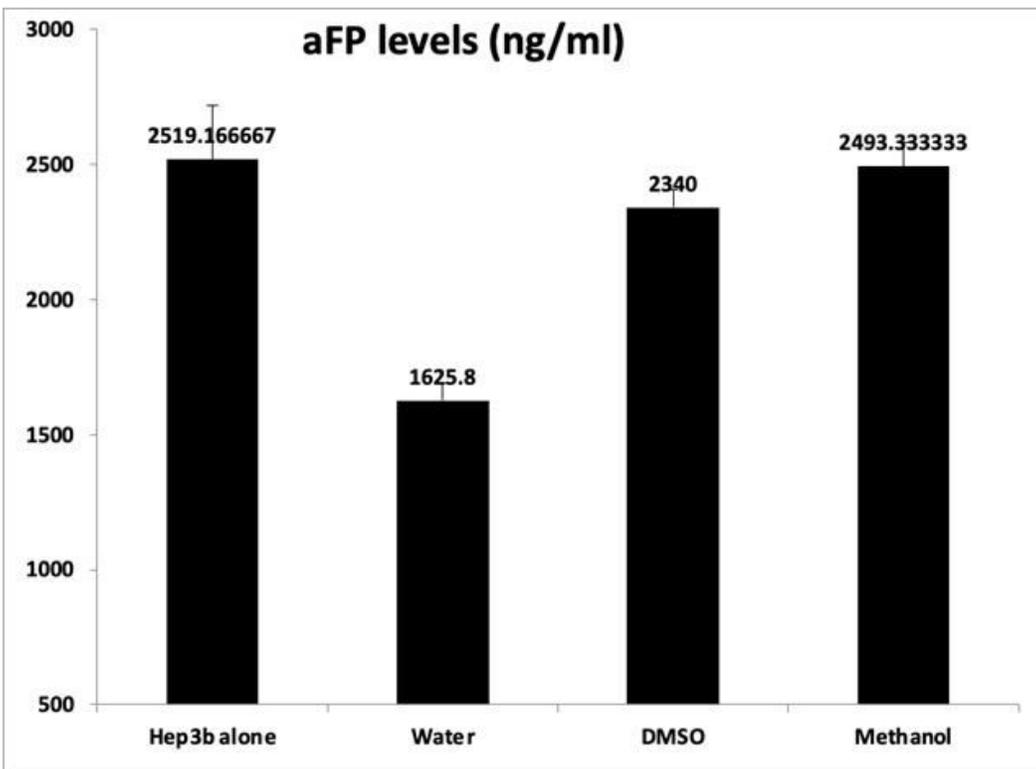


Figure 3

Averages of the secretion of the aFP from the HEP3B cells following treatment from the different fractions of the (AF) flower.