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REGULAR ARTICLE

In vitro assessment of the bioactive potential of *Artemisia campestris* L. fractions growing in Khenchela (Algeria)

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ABSTRACT

In order to valorize Algerian medicinal plants, this work aims to characterize quantitatively and qualitatively *Artemisia campestris* L. fractions, a medicinal plant from the Aures-Algeria area, and investigate its biological activities *in vitro*. During preliminary phytochemical screening, flavonoids, saponins, tannins, alkaloids, reducing compounds, and coumarins were discovered. Total polyphenols and flavonoids are greater in the n-butanolic fraction than in ethyl acetate and petroleum ether. The resulting antioxidant capability was assessed *in vitro* using three methods: DPPH free radical scavenging, hydrogen peroxide trapping, and iron reduction. The n-butanolic extract suppresses DPPH oxidation with an IC50 of 2.239±0.32mg/ml, which is comparable to that of standard (1.824±0.97mg/ml). Despite the fact that similar findings were seen in the neutralization of hydrogen peroxide and the iron chelating activity, The anti-inflammatory action was proven i*n vitro* by inhibiting protein denaturation and increasing HRBC membrane stability (Human Red Blood Cells). The n-butanolic fraction was more effective than diclofenac in preventing BSA degradation. It also inhibited membrane hemolysis in human erythrocytes by up to 83%. Activated partial thromboplastin and prothrombin times were used to analyze extrinsic and intrinsic coagulation pathways in *A. campestris* in order to determine its anticoagulant activity. The n-butanol fraction had the greatest impact on PT and aPTT lengthening, with 75.2s and 351s at 3 mg/ml concentration, respectively. The n-butanolic fraction of the *A. campestris* aerial part exhibited antioxidant, anti-inflammatory, and anticoagulant activities. As a result, it may be a viable natural resource for mitigating the impact of stress, which causes inflammatory and cardiovascular disorders.

1. Introduction

Artemisia L. is a genus of tiny shrubs and herbs native to northern temperate climates. It belongs to the large *Compositae* (*Asteraceae*) family, which has about 20,000 species and 1,000 genera. More than 500 species of *Artemisia* are part of the Anthemideae tribe, including *Artemisia campestris*

L (Bora and Sharma, 2011), known locally as "Tagouft," is a polymorphic plant with a large number of subspecies and variants used in North African recipes to treat various diseases. It has medicinal, pharmacological, and culinary qualities (Sijelmassi, 1993). Antivenom, antirheumatoid and antidiabetic characteristics are widely documented in traditional medicine for this species (Bnouham et al. 2002, Dib et al., 2016a), likely because of the presence and extravagance of bioactive metabolites such as flavonoids, chromones, acetophenones, coumarins, and essential oils (Vasconcelos et al. 1998, Pereira et al. 2018). Oxidative damage to bioactive systems is a common cause of many chronic illnesses, including cancer and heart disease. Hydrogen peroxide, superoxide anion, and hydroxyl radical are examples of reactive oxygen species (ROS). They have been associated with age-related health problems such as carcinogenesis and coronary heart disease (Marnett, 2000).

In living organisms, there are a lot of ways to keep the level of ROS and the problems it causes from getting too high. Antioxidants seem to be vital in the protection of a large variety of ailments and health problems since they defend against both direct ROS impacts and reactionary oxidative processes (Lekouaghet et al., 2020). Both reactions are inhibited by the synthetic antioxidants butylhydroxyanisole (BHA) and butylhydroxytoluene (BHT). Since their introduction to the food industry, questions have been raised about their safety and effectiveness because of their unstable and extremely volatile nature (Nabavi et al., 2008). Toxic chemicals and physical trauma may cause inflammation, which is a natural response to tissue damage. It is also associated with pain, as well as an increase in protein denaturation, an increase in vascular permeability, and membrane disruption. One of the most common treatments for inflammation is the non-steroidal anti-inflammatory drug (NSAIDs), but it has a number of side effects, the most serious of which is stomach irritation and the subsequent development of gastric ulcers (Ferrero-Millani et al., 2007). The biggest cause of death worldwide is cardiovascular disease. Coagulation problems may be caused by viral infections. Recently, a number of studies have indicated that patients with severe new coronavirus pneumonia exhibit coagulation malfunction (Hireche et al., 2021). Anticoagulants such as heparin and anti-vitamin K compounds have life-threatening adverse effects (Kishore, 2013). Thrombolytic therapy and mechanical treatments are typically used to treat thrombotic disorders. Slow and inadequate thrombolysis, such as the risk of hemorrhage, is a disadvantage of current thrombolytic therapy (Kumar et al., 2011 and Dib et al., 2017b). As a result, there is a growing need for novel remedies derived from plants, which constitute the major source of drugs for the treatment of many diseases. In this regard, this research has focused on the anti-oxidant, anti-inflammatory and, for the first time, anticoagulant properties of *Artemisia campestris* fractions.

2. Materials and Methods

2.1. Chemicals and Reagents

Several chemical reagents and solvents were used in our experiments, including methanol, n-butanol, petroleum ether, ethyl acetate, chloroform, toluene, acetone, acetic acid, glacial acetic acid, FeCl3, HCl, NaOH, NH4OH, I2, NaCl, AlCl3, Wagner's reagent, formic acid, dichloromethane, NaCl, dimethyl sulfoxide (DMSO), phosphate buffer saline (PBS), Tp (thromboplastin), TCK (cephalin Kaolin + $CaCl_2$), quercetin, gallic acid, and DPPH (2,2 diphenyl-1-picrylhydrazyl). The remainder of the chemicals and reagents were all of analytical quality.

2.2. Plant material and preparation of extracts

The aerial part (leaves and twigs) of the plant *Artemisia campestris L. was* collected in 2019 from M'toussa-Ain Touila (Khenchela region) (Lat: 35° 35'58 "S e Long. 7 ° 14'42" W) and identified by Dr. Zeraib Azzeddine, from the Faculty of Nature and Life Sciences of Khenchela University. The sample was dried, ground, and macerated (30g/100ml) in a hydromethanolic (30/70 methanol-water) solution for 24 hours at room temperature. With the regeneration of the solvent, this procedure was repeated three times. A rotary evaporator was used to concentrate the crude extract after that. Sequential extraction with increasing polarity solvents: petroleum ether (Petet), ethyl acetate (EtOAc), and n-butanol (BuOH) was used to partition the crude extract (Markham, 1982). Each fraction was weighed and represented as a percentage of the overall weight of air dried plant material. It was stored at 4°C until examined chemically and assessed for bioactivity.

2.3. Phytochemical Investigation

2.3.1. Phytochemical screening

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We used typical phytochemical tests to search for flavonoids, saponins, tannins, alkaloids, and reducing chemical compounds in the plant's crude extract (Edeoga, 2005).

2.3.2. Assessment of phenolic content

The phenolic content in extracts is determined utilizing Folin-Ciocalteau technique (Li et al. 2007). The liquids were combined for 4 minutes before incubation using 200 µl of each extract (dissolved in methanol) and 1 ml of Phenolphthalein (diluted at 10%). After the incubation period was completed, we added a solution of sodium carbonate (75 mg/ ml) to the mixture. The incubation period was 2 hours in the dark condition. A spectrophotometer calibrated to 765 nm was used to measure the absorbance. Gallic acid equivalent per mg of extract was used as the unit of measurement.

2.3.3. Assessment of flavonoids content

The aluminum trichloride method is used to measure the flavonoids in our extracts (Djeridane et al. 2007b). At the appropriate dilutions, 1 ml of each extract and the standard (dissolved in methanol) were added in equal amounts to a solution of $AlCl_3$ (2% in methanol). After 10 minutes of incubation, the absorbance of the samples was determined at 430 nm. Calculation of the flavonoid content was done using a calibration curve as the quercetin equivalent in µg per mg of extract.

2.4. In vitro evaluation of antioxidant activity

A single antioxidant test model should not be used to establish that a substance has antioxidant properties. FRAP (Ferric reducing antioxidant power), $DPPH^+$ (2,2-diphenyl-1-picrylhydrazyl) and other in vitro tests, depending on the substance used as a source of free radicals, are often used in practice to assess the antioxidant capacity of medicinal plants.

2.4.1. Radical-scavenging activity of DPPH

Stable 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) was used with minor modifications to measure the extract's free radical scavenging activity (Brand-Williams et al. 1995). The test sample (15 µl) was incubated for 30 minutes in the dark with 1.5 ml of DPPH reagent (100 µM) prepared in absolute methanol. A measurement of absorbance was taken at 517 nm. Ascorbic acid was the standard antioxidant. The percentage of free radical inhibition may be represented using the following formula:

I% = [(Abs Control Negative-Abs Sample) / Abs Control Negative] X 100

The IC50 value was established to be the sample concentration necessary to scavenge 50% of DPPH free radicals.

2.4.2. Hydrogen peroxide scavenging activity

The capacity to reduce hydrogen peroxide from the extract was tested using the Dehpour method (Dehpour et al., 2009). A 10mM hydrogen peroxide solution was prepared in phosphate buffer (pH 7.4). A 0.6ml hydrogen peroxide solution was treated with 1.4 ml of a diluted extract in distilled water. After 10 minutes, the absorbance of hydrogen peroxide at 230nm was measured in comparison to a buffer solution without hydrogen peroxide. The percentage of H_2O_2 trapped (I%) is calculated by the following formula:

$$I\% = [(A_o - A_1) / A_o] \times 100$$

The absorbance of the sample or standard solution was A1, whereas that of the control solution was A_o.

2.4.3. Ferric Reducing Antioxidant Power (FRAP)

Benzie's et al. (1996) approach was used to calculate the reducing power. For each extract concentration, 1 ml was mixed with 2.5 ml of buffer solution (pH 6.6; 0.2M) and 2.5ml of 1% potassium ferricyanide solution. The mixes were heated to 50° C for 30 minutes. The mixture was then treated with 2.5 ml of 10% trichloroacetic acid. For 10 minutes, the mixture was centrifuged at 3000 rpm. To 2.5 ml of distilled water and 2.5 ml of the supernatant from each concentration, we added 0.5 ml of FeCl3 (0.1%). The combination's absorbance was measured using a spectrophotometer at 700 nm. Vitamin C was used as a positive control, whereas extraction solvents were used as negative controls.

2.5. In vitro evaluation of anti-inflammatory activity

The anti-inflammatory effect of *A. campestris* extracts was demonstrated *in vitro* using the protein denaturation inhibition method and anti-hemolytic activity, which tests the membrane stability of HRBCs (Human Red Blood Cells).

2.5.1. Method of Bovine Serum Albumin (BSA) Denaturation

The *in vitro* anti-arthritic activity was studied using

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the albumin stability test (Rahman et al. 2012) at different concentrations. To make the standard solution, 0.45ml of BSA (0.5%) and 0.05ml of diclofenac sodium (0.05%) at varied concentrations are used.

Procedure

A 0.45 ml (0.5% w/v BSA) was combined with 0.05 ml of each extract and diclofenac sodium at various concentrations (50, 100, 250 µg/ml). After 20 minutes at 37°C, they were heated to 57°C for 3 min. When the aforementioned solutions have cooled, add 2.5 ml of phosphate buffer. A UV-Visible spectrophotometer was used to measure the absorbance at 660 nm. The control represents a 100% percentage of protein denaturation. The percentage inhibition of protein denaturation may be calculated as follows:

Inhibition percentage = 100-(Test solution ABS-Product control solution ABS)/Control test solution ABS

2.5.2. Anti-hemolytic activity (HRBC Membrane Stabilization Method)

Different extracts of *A. campestris* were tested for their anti-inflammatory effects on HRBC membranes *in vitro* using a spectrophotometric method (Yang, Z.G., *et al*. 2005). A healthy person's blood was drawn into EDTA vials and centrifuged at 1000 rpm for 5 minutes. Afterwards, the pellet was resuspended in saline solution after being rinsed three times in PBS (0.2 M; pH 7.4; 0.5%). Extracts (0.25–1 mg/ml in PBS) were added to erythrocyte solution and incubated for 20 minutes at room temperature. The resulting mixture should be supplemented with a 2% NaCl buffered saline solution. After 10 minutes of centrifugation at 1000rpm, the samples' absorbance at 540 nm was measured with a spectrophotometer. As a positive control, phosphate buffer saline was used. To figure out the percentage of hemolysis inhibition in each extract, we used this formula:

Inhibition percentage = (Ac-Aext/Ac) x100

A_{ext} was the absorbance of the extract or standard (Aspirin at 100µg), whereas A_c was the absorbance of the negative control.

2.6. In vitro evaluation of anti-coagulant activity

The extracts' anticoagulant activity was determined using the (Brummel-Ziedins et al. 2005) and (Pawlaczyk et al, 2009) techniques. External and internal clotting systems were tested using the activated partial thromboplastin time (aPPT) and prothrombin time (PT). The tests were performed on a fully automated coagulometer. It is made up of plasma that has been taken from healthy, untreated adults who have normal or similar TQ and TCK. Each volunteer's blood was injected into a solution of sodium citrate at a concentration of 3.2% and at a rate of one volume for every three volumes of blood that was drawn. After centrifuging the blood for 10 minutes at 3000 rpm, plasma devoid of platelets is obtained.

2.6.1. Assessment of the anticoagulant extrinsic pathway

The extrinsic pathway of coagulation's factors may be examined globally utilizing a fast coagulation test (TK) or a prothrombin time (PT). For this, 90 µl of standard plasma was mixed with 10 µl of each extract at various doses of 0.5–12 mg/ml produced in DMSO. Coagulation was started by adding 200 µl of thromboplastin that had been preincubated at 37°C for 15 minutes. The clotting time was tested once again using a coagulometer (Brummel-Ziedins et al. 2005).

2.6.2. Assessment of the anti-coagulant intrinsic pathway

The Kaolin Cephalin Time (TCK) is a test that allows the activity of endogenous plasma factors to be explored. It was evaluated according to the following steps: The platelet-poor plasma was incubated at 37°C for 15 minutes with 10µl of each extract at different doses of 0.5–12 mg/ml produced in DMSO. After 3 minutes at 37°C, the mixture was treated with a Kaolin cephalin solution. Then, 100 µl of an aqueous solution with 0.25 M $CaCl_2$ was added. The mixture was incubated for another 3 minutes before coagulation started. The time until the clot formed was then measured visually using the stopwatch (Pawlaczyk et al. 2009).

2.7. Statistical evaluation

All measurements were replicated three times, experimental results are expressed as the mean±SD. The IC50 values were determined through linear regression analysis.

3. Results

3.1. Phytochemical Analysis

The presence of major chemical groups such as

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flavonoids, saponins, tannins, alkaloids, reducing compounds, and coumarins was discovered in *A. campestris* L. species by approaches based on solubility tests, staining, and precipitation reactions. The presence of these active phytoconstituents may be responsible for biological activities and therefore be used as a source for future pharmacological research.

3.2. Extraction yield, total polyphenols and flavonoids contents

The percentage yields of dry plant material extraction, and even the total phenolic and flavonoid amounts, are described in Table 1. The gallic acid and quercetin calibration curves were used to figure out how many phenolic and flavonoid compound were in *Artemisia campestris* extracts.

	Petet	*EtocH*	*BuOH*
Extraction yield (w/w %of dry weight)	8.33± 0.22	3.33± 0.09	2± 0.12
Total phenolic content (µg EAG/mg)	47.25± 3.12	312.32± 10.22	468.74± 11.2
Flavonoid content (µg EQ/mg)	3.7± 0.8	11.3± 1.4	74.91± 5.1

Table 1: Yield of extraction and total polyphenols and flavonoids contents of extracts.

3.3. Antioxidant Activity

As indicated in figures 3, 4, 5, and 6, antioxidant activity of *A. campestris* extracts was demonstrated when tested for free DPPH, H_2O_2 scavenging activities, and the FRAP assay.

3.3.1. DPPH radical-scavenging activity

According to the data presented in figure 1, the percentage of reactive oxygen species inhibition increases with increasing concentration, either for ascorbic acid or for the various plant extracts. From the results presented in figure 2, the IC50 obtained for the n-butanolic fraction (9,1±0,09µg /ml) is much lower than those of ethyl acetate

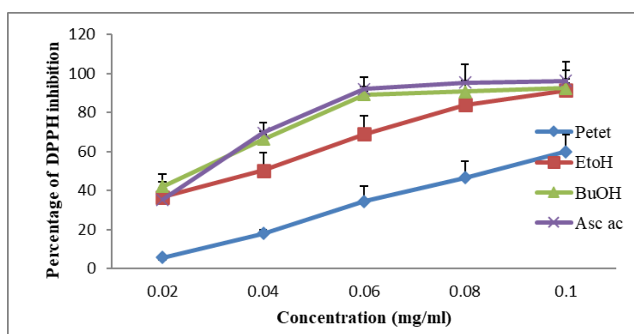

Figure 1: Percentage of DPPH radical inhibition as a function of extract concentration.

(18,7±0,13µg /ml) and petroleum ether extracts (42,5±0,21µg /ml) and even for ascorbic acid (11,2±0,23µg /ml) used as a reference molecule, and thus, a very high antioxidant activity.

3.3.2. Hydrogen peroxide scavenging

The IC50 values for H_2O_2-scavenging activity ranged from 43.9±0.16 to 47.4±0.3µg /ml in the extracts, while the value for ascorbic acid was 31.7±0.147µg /ml (figure 2).

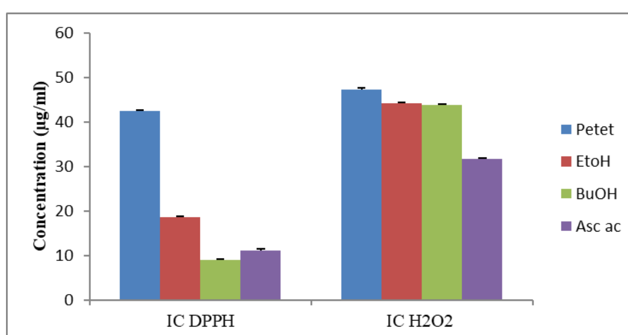

Figure 2: IC50 values of DPPH and H_2O_2 scavenging activity.

In a concentration-dependent way, all *Artemisia campestris* extracts scavenged hydrogen peroxide (figure 3).

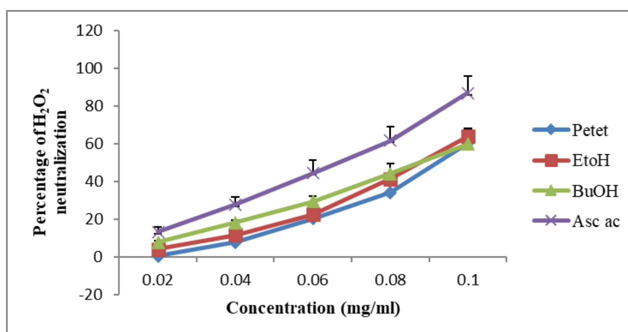

Figure 3: Percentage of H_2O_2 neutralization as a function of extract concentration.

3.3.3. Ferric Reducing Antioxidant Power (FRAP)

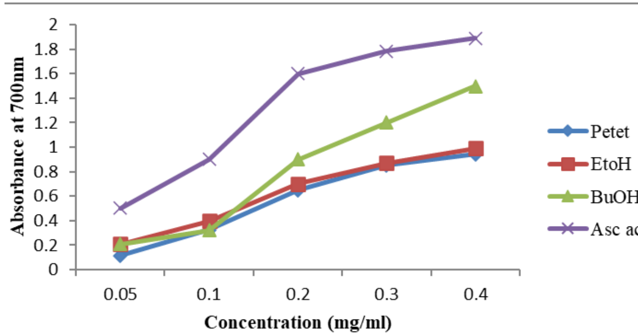

Figure 4: Extracts' reducing efficiency according to the FRAP test.

According to the results given in Figure 4 of the extracts' reduction potency, the n-Butanolic

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fraction has a moderate reducing power equivalent to that of ascorbic acid.

3.4. Anti-infammatory activity

3.4.1. Bovine Serum Denaturation Method

Figure 5 shows that the n-butaolic fraction of *A. campestris* at varied doses (50,100, and 250 µg/ml) gave excellent protection against protein denaturation, equivalent to diclofenac sodium at the same concentration.

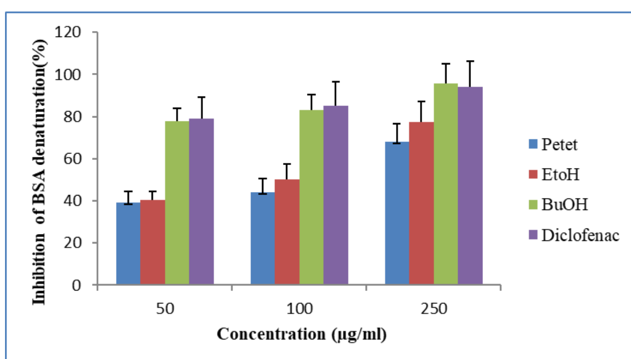

Figure 5: Effect of *A.campestris* extracts and diclofenac on BSA denaturation.

3.4.2. Anti-hemolytic activity

At doses ranging from 100µg/mL to 400µg/mL, all *A. campestris* extracts protect human erythrocyte membranes from hypotonic solution-induced lysis. At a concentration of 100 µg/ml, Petet extract had the lowest percentage inhibition of red blood cell hemolysis (8.07%), compared to 38.84% produced by aspirin at the same concentration (Figure 6). This is what they found: The BuOH fraction can stop the hemolysis of HRBCs in a dose-dependent way, with a very high significance of up to 83.97%.

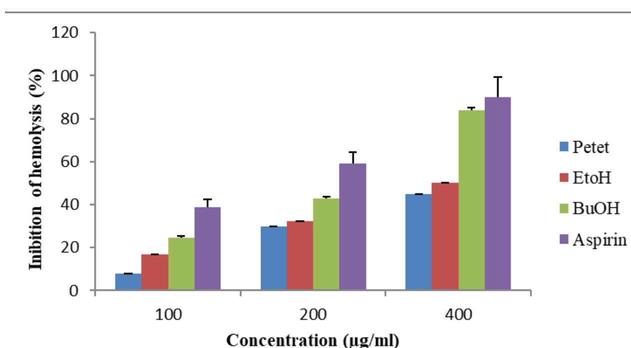

Figure 6: Antihemolytic effect of *A. campestris* fractions in comparison with a positive control (aspirin).

3.5. In vitro evaluation of anti-coagulant activity

PT and aPTT were used to assess the anticoagulant activity of *Artemisia campestris* L. extracts *in vitro* for both exogenous and endogenous pathways (Figure 7,8). The anticoagulant activity of the tested material is confirmed by a longer clotting time when compared to the control.

3.5.1. Evaluation of the anti-coagulant extrinsic pathway

The anticoagulant activity of the samples against exogenous coagulation was assessed using a coagulation assay called prothrombin level or prothrombin time (PT). Figure 7 shows the results, which reveal that PT was significantly prolonged at most of the concentrations tested (1.5, 3, 6, and 12 mg/ml) when compared to the control. If you take butanol at all concentrations, it has a much longer time for your blood to clot than the other extracts.

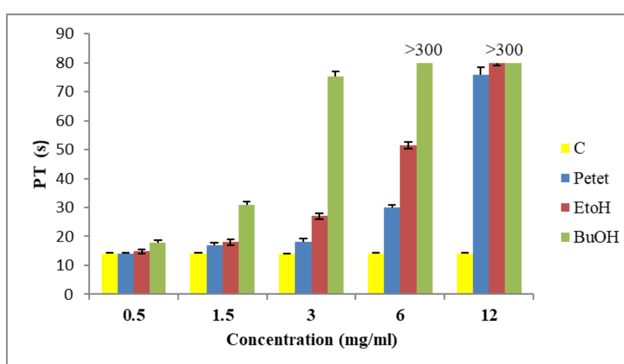

Figure 7: Prothrombin time of normal human plasma treated with *A.campestris* fractions at different concentration.

3.5.2. Evaluation of the anti-coagulant intrinsic pathway

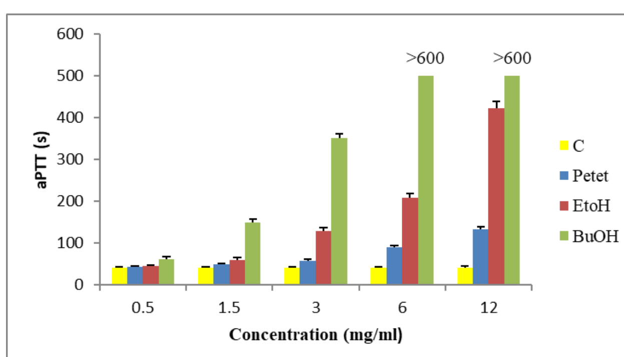

Figure 8: Activated partial thromboplastin time of normal human plasma treated with *A. campestris* fractions at different concentration.

4. Discussion

The Algerian steppe is depicted as a huge regional strip ranging from the Tunisian border to the Moroccan border, stretching over 1000 km long and 300 km wide and covering a total area of 20 million hectares. The aromatic plants of the family *Asteraceae* (*Compositae*) constitute by far the most

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important the most important family in our territory (Djellouli, 1990). The present study focuses on valuing *Artemisia campestris*, a species of this native family. The phytochemical investigation revealed the existence of therapeutically effective phytoconstituents such as flavonoids, saponins, tannins, alkaloids, reducing compounds and coumarins, However, saponins and coumarins were absent in the identical plant gathered from the Djelfa region (Saihi, 2011). The method of extraction, the nature of the solvent, the particle diameter of the sample, the storage time and circumstances, the existence of storage conditions, the presence of interfering compounds and/or a delipidation phase, all have an impact on the extraction of polyphenols from plants (Chaudhary et al., 2015). The use of water followed by fractionation against polar solvents may favor the extraction of polar chemicals such as polyphenols and, therefore, affect the biological activity of the sample. More polar chemicals were obtained using aqueous extractions under reflux followed by liquid-liquid fractionation. Extraction yields ranged from 2 to 8.33% on average. Ethyl acetate is used to get mono O-glycosides and some di O-glycosides, but n-butanol is used to get the most polar flavonoids (di-o-glycosides, triglycosides, and tetra-glycosides). The colorimetric Folin-Ciocalteu technique, one of the ancient methods for determining the amount of phenolic compounds in medicinal herbs and foods (Blasa et al. 2007). In the Folin-Ciocalteu technique, gallic acid is the most frequently used reference (Maisuthisakul et al. 2008). However, the n-butanolic fraction presents the highest content of 468.737±83.049 µg EAG /mg compared to the other fractions, ethyl acetate and petroleum ether, with 319±50.119 and 47.25±3.12 µg EAG /mg respectively. In a study on eleven medicinal herbs, including *Artemisia campestris*, Djeridane et al. (2006a) calculated flavonoids concentrations in 70% and 80% (v/v) ethanolic extracts to be 7.46 and 5 mg ER/g, respectively. When the extraction is performed with a 50% alcoholic solution, this concentration can exceed 450 mg GAE/g of extract (Akrout et al., 2011). This difference in content can be explained by the geographical location of the plant species, its maturity, genetics, climate, harvesting period, drying conditions, metabolite content of each species (metabolism), and the nature and polarity of the solvent used for extraction or fractionation (Iloki-Assanga et al., 2015). Bennour et al.(2020) found that evaporating the solvent in an oven at 40 °C was the optimal evaporation method for extracting phytochemicals from *M. oleifera* leaves, utilizing a cold maceration method with 70% aqueous methanol as the solvent. The phenolic compound group is one of the most extensively dispersed and ubiquitous groups in plants, flavonoids, tannins, and phenolic acids are the most common phenolic elements found in plants as antioxidants (Pobłocka-Olech et al., 2016). The aluminum trichloride method is used to quantify flavonoids, with quercetin serving as the standard. Flavonoids are the most important polyphenolic class, with over 5000 chemicals already reported (Gomez-Caravaca et al., 2006). The n-butanolic extract has the highest flavonoid content (74.91±5.1µg EQ/mg of extract), followed by the ethyl acetate extract (11.3±1.4µg EQ/mg), and finally the petroleum ether extract (3.7±0.8µg EQ/mg). Djeridane et al. (2006a, 2007b) determined the concentration of flavonoids in ethanolic extracts at 70% and 80% (v/v), estimated at 7.46 and 5 mg ER/g respectively. However, Akrout et al. (2011) estimated this value to be 56.31 mg ER/g extract when extraction is performed with a 50% alcoholic solution. While Saoudi et al. (2010) found a content of 131.89 mg RE/g aqueous extract of the leaves, Djeridane et al. (2006a) discovered that flavonoids account for 98 percent of total polyphenols in the aerial part of plants, which is not surprising given that the aerial part is important for solar radiation protection (Ryan et al., 2002) and that this group of phenolic compounds is involved in the coloration of leaves and flower petals (Havsteen, 2002 and Gervaise, 2004). Today, there is a great deal of interest in examining the antioxidant activity of plant extracts or foods to discover whether they have therapeutic characteristics, which is one of the most important criteria for drug development (Ghani et al., 2019). By reducing excess free radicals, antioxidants are needed as an additional defense mechanism for our bodies to heal conditions such as aging, cancer, inflammation, cardiovascular and neurological diseases (Moskovitz et al., 2002). Antioxidant testing may be divided into methods based on electron transfer processes and those based on hydrogen atom transfer reactions due to the diversity of antioxidants and their reactivity The scavenging ability of physiologically relevant oxidants has been tested in several ways (Huang et al., 2005 and Hayouni et al., 2007). DPPH (2,2-diphenyl-1-picrylhydraza) radical scavenging is one of the in vitro techniques used, and it is effective,

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simple, and predictable, and it may sequester free radicals (Fabri et al., 2011). A lower IC50 value indicates that an antioxidant's ability to scavenge free radicals is more active (Chen et al., 2013). As a control, ascorbic acid was utilized, the n-butanolic fraction of *A. campestris* showed the greatest free radical scavenging activity in the present study. Numerous investigations have revealed that *A. campestris* essential oils have good antioxidant activity by scavenging DPPH radicals (Gherib, 2009 ; Saihi, 2011 ; Dib et al., 2017b). According to Boudjouref (2011), the ethanolic extract of *Artemisia campestris* has the most active antiradical activity of the three. The search for new anticancer agents by the SRB method revealed the activity of methanolic extract of *A. campestris* stems against some lineages responsible for epithelial ovarian cancer (EOC), which are (OVCAR-4, FOUV-1, and COV-362), where normal epithelial cells (HOE) and carboplatin (CBPT) were used as negative and positive controls, respectively (Maafia, 2019). According to our findings, the antiradical activity of the extracts is depending on their total polyphenol and flavonoid concentration. Indeed, phenolic elements, particularly flavonoids, are known as potential antioxidants that may accumulate radical oxygen atoms and reactive oxygen forms (Javanovic et al., 1994). Flavonoids have a low redox potential, which allows them to be thermodynamically able to reduce free radicals by transferring hydrogen atoms from hydroxyl groups to free radicals, this makes them scavengers (Douaouya and Bouzerna, 2016).

In the body, hydrogen peroxide may be transformed into oxygen and water and reactive species (-OH) can be produced that may lead to ADN damage even if hydrogen peroxide itself is not a highly reactive compound. The elimination of H_2O_2 from food systems is thus of paramount importance (Rj et al., 1989). As a result, plant extracts' ability to scavenge hydrogen peroxide was investigated to see if they showed the same pattern of activity as the ability to reduce OH radicals. In a concentration-dependent way, *Artemisia campestris* fractions scavenged hydrogen peroxide. This H_2O_2 piégeage can be attributed to their ability to give H_2O_2 electrons and therefore neutralize it in water (Ebrahimzadeh et al., 2009). Furthermore, another technique for testing antioxidant activity was the Ferric Reducing Antioxidant Power (FRAP) method, which is based on the presence of reductant compounds in the extracts and causes the complex Fe3+/ ferricyanure to be reduced to ferrous form, allowing the amount of polyphénols involved in the redox reaction to be determined (Amarowicz et al., 2004, Bougandoura and Bendimerad, 2012). In fact, n-butanolic extract outperformed the other extracts. This might be explained by its high content of phenols and flavonoides, which play a key role in the Fenton reaction's chélation of transition metals (Valko et al., 2007).

Inflammation is caused by the denaturation of proteins, which leads to their loss of function (Nargund et al., 1993). As a result, Alzheimer's disease as well as rheumatoid arthritis may be treated with medications that limit denatured protein aggregation production and protein condensation (Saso et al., 2001). The capacity of *A. campestris* fractions to suppress protein denaturation has been examined in this context. In comparison to the other fractions, the n-butanolic fraction was shown to be the most efficient, and the inhibitory effect of BSA on thermal denaturation was dose-dependent. The outcomes of this extract are comparable to those of sodium diclofenac, a popular anti-inflammatory prescription. Inflammatory disorders may be caused by denaturation of proteins *in vivo*, which might lead to the production of autoantigens. A possible process of denaturation is the modification of electrostatic, hydrogen, hydrophobic, and disulfide linkages, which retain the three-dimensional structure of proteins (Chatterjee et al., 2012). Polyphenols in *Artemisia campestris*, like flavonoids and tannins, help make it an anti-inflammatory food (Sangeetha, 2011). The most abundant cells in the human body are erythrocytes, which possess several biological and physical properties and thus have been frequently used for drug delivery (Kumar, 2011). Toxic substances such as hypotonic liquid may injure red blood cells (RBCs), heat, methyl salicylate, or phenylhydrazine, the membranes lyse, causing hemolysis and hemoglobin oxidation (Feirrali et al., 1992). In the HRBCs membrane stabilization experiment, the anti -inflammatory effect was dose related. The BuOH fraction elicited a stronger response, with a percentage reduction in red cell hemolysis equivalent to that of aspirin. The erythrocyte membrane was stabilized by all *A. campestris* extracts examined, particularly n-butanolic, which inhibited hypotonia-induced lysis. To minimize the inflammatory response, stabilizing the lysosomal membrane, which is comparable to the erythrocyte membrane, is essential. This is because it stops

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activated neutrophils from releasing lysosomal components such as lytic enzymes and active mediators of inflammation, which cause tissue damage upon their extracellular release (Yurugasan et al., 1981; Vadivu and Lakshmi, 2008). On the other hand, hypotonicity-induced hemolysis may result from cellular shrinkage caused by osmotic loss of electrolytes and intracellular fluid components. The extract has the ability to inhibit or accelerate the flow of these intracellular components (Yang et al., 2010). Flavonoids and tannins, alone or in combination, may be responsible for the impact of the anti-inflammatory extract/fraction (Sudharshan et al., 2010). Coagulation was proven to be a crucial lethal component throughout the COVID-19 pandemic's progression (Ackermann et al., 2020 and Schulman, 2020). Determining the active ingredient in plants might also help researchers find safer alternatives to anticoagulants. In this context, utilizing prothrombin and activated partial thromboplastin times, the effects of *A. campestris* fractions on human blood coagulation have been examined *in vitro*. In this study, a prolonged aPTT was seen at all concentrations of extracts. This means that these extracts may have an inhibitory or deficient effect on intrinsic pathway coagulation factors (Hireche et al., 2021). The n-butanolic fraction was shown to be the most potent extract. It is possible, however, that this extract might have an effect on factors Xa and Va, or on the interaction between thrombin and the fibrinogen substrate, which would prevent the formation of thrombosis. An inhibitor of proteases has been found in *A. campestris*, limiting the production of active factors such as Xa, Va, and andthrombin from zymogens in the organism (Félix -Silva et al., 2014). The anticoagulant pathway may be stimulated by the binding of the active agent to antithrombin III, producing a conformational shift in protein C (PC), resulting in the activation of protein C (APC), which inhibits factors Va and VIIIa (factor Va and VIIIa cofactors) with its cofactor (protein S). Anticoagulants may be found in secondary metabolites generated by plants (Luo et al., 2017).

5. Conclusion

To conclude, the antioxidant, anti-inflammatory, and anticoagulant properties of the n-butanolic fraction of the *A. campestris* aerial part were found. As a result, it might be a valuable natural resource for lowering the effects of oxidative stress, which can lead to inflammatory and cardiovascular disorders. Furthermore, the results of this study are preliminary, and they are just the beginning of the search for physiologically active natural compound. As a consequence, this research generates a wide range of experimental perspectives. In reality, more research is needed in a number of areas, including more efficient separation techniques (HPLC, LC/MS, GC/MS...) for qualitative characterization of flavonoids and evaluating and testing these active compounds *in vivo* for additional biological features in the context of pharmacological and industrial applications.

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