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The effectiveness of essential oil extracted from alfalfa seeds for blood clotting

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RESEARCH ARTICLE

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ABSTRACT

The experiment was conducted on alfalfa seeds that were brought from Marjajah in the city of Touggourt. After the traditional extraction process, some of its physical properties were measured, including, refractive index, pH, with a yellowish green color, odor, prick, and transport value of 363 cm/S and on blood clotting. The results showed that the seed extract of alfalfa has an effect on blood clotting on the internal and external pathway by the prothrombin rate (TP) obtained that sample 1 has the largest clotting time of 22 seconds and by the time of cefalin kaolin (TCK), the highest coagulation time for sample 2 by 46 seconds. The prothrombin rate and the time of cefalin kaolin chronometer tests also show that alfalfa seed extract exercises an important anticoagulant activity compared to the two coagulation methods, because this activity is more pronounced towards the internal pathway that the external pathway passes, that is, the alfalfa seed extract is better than the normal witness and less than the positive witness heparin.

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1. Introduction

Health, disease, life, death, human food and medicine are all subjects that have captured human thought and experiences throughout history, but his battle with disease, that unknown enemy that raid him without permission, formed his eternal battle for survival perhaps the idea of discovering medicine from plants is one of the most distant ideas in order to confirm the relationship between integration between man and nature, whether in the early stages of human history, or in the advanced stages in which human medicine has been associated with scientists to the extent of their awareness of the philosophy of science on the one hand, and the extent to which their adventures lead in the direction to living creatures, from discoveries that help treat many diseases, by eating wild herbs and medicinal and aromatic plants, which are characterized by their pharmacological effectiveness and speed [1-7].

The vastness of the Algerian country, its geographical location, and its multiple climates (marine, continental and desert) have had a great impact not only on the intensity of plant diversity, but also on the composition of plants and giving them special characteristics, we chose alfalfa seeds, which are known as alfalfa, and which are cultivated abundantly in Algeria as the subject of our study, and this is the extent of their extensive uses, and the scarcity of practical studies of their properties that are used in the treatment, so we turn to the extraction of their essential oils chemical and biological.

2. Experimental

2.1. Studied plant material

The plant used in this study is the clover plant known scientifically as *Medicago sativa*, and the part used is seeds. Clover seeds are sown from the end of September 2018 to the beginning of October 2018, but the type varies according to the difference of seasons and the best ones are in October and the watering time is every three days to a week, and a month's duration is the maximum for the plant to appear and with the availability of water it can take only 15 days to appear. The quality of the seeds depends on the roots of the plant. If they are green and soft roots, this is an indication of the quality of the seeds. If the roots are yellow and dry, they are of lesser quality.

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2.2. Extracting essential oils

Essential oils are extracted by several different methods, the most important of which are distillation and organic solvent extraction [8]. In our study, we extracted essential oils from alfalfa seeds using the steam distillation method based on the principle of steam pressure to obtain the essential oil [8].

2.3. Oil yield

The yield of the essential oil extracted from the clover seed was calculated and the yield ratio of the extracted oil [6-9].

2.4. Physical properties

2.4.1. Refractive index

The index of refractive index is estimated at 20 °C in the case of oils and at 40 °C in the case of solid fats. The refractive index is measured by an Abbe-2WAJ Refractometer. The value of the index of refractive index calculated for the essential oils of alfalfa seeds is 1.3394, the value is between 1 and 2 and it is a value indicating the transparency of this oil and therefore the speed of light in this medium is fast and therefore it must be kept in vessels that do not allow the passage of light.

2.4.2. pH degree

pH is measured using a pH meter (TES-1380K) at 27 °C. The refractive index indicates the effect of these oils by light, and because essential oils are concentrated, so it is important to know the value of the pH of these oils, because the acid oils that cause sensitivity to light lead to risks including irritation of the skin, as well as the difficulty of conserving them. A score of 8.83 shows the quality of these oils.

2.4.3. Color

We notice that when the process of extracting the essential oils of alfalfa seeds is yellowish green. The color of the oil has traditionally been considered essential to assessing value because darker oils require additional costs to improve their color and because darker color is an indication of low oil quality [10].

2.4.4. Odor

We note the stench of prick, because the majority of volatile oils are characterized by its aromatic smell and its fresh flavor, due to the presence of some compounds with small partial weights and volatile rapidly at normal air temperatures.

2.4.5. Conductivity

The conductivity is measured using the Conductimetre (Mallette Volmatic EC) at a temperature of 25.5 °C. The value of conductivity is determined by 363 cm/µS. This value indicates the richness of these oils in mineral substances [10].

2.5. Anticoagulant activity of alfalfa seed oil

In vitro anticoagulant activity of alfalfa seed oil was evaluated using two coagulation pathways (internal and external pathway) global and chronometric tests (TCK and rapid time (TQ)). To assess the activity of anticoagulants, the following products were used: Thromboplastin (BIO-TP), thromboplastin buffer and cefalin choline rebuilding (BIO-CK).

2.5.1. Plasma preparation

Intravenously, two healthy, untreated adult volunteers, including normal and comparable TCK and TP were collected into the jackets tube (3.2% sodium citrate), and the blood was centrifuged for 3 minutes at 4000 rpm using FOTOFIX 32A (Hettich) ZentRifugen centrifuge to obtain poor plasma from platelets [11].

2.5.2. Evaluation of anticoagulant activity in relation to the external course

Rapid time test (TQ) allows exploration of the external course of thrombosis. TQ converted to prothrombin time is used to evaluate the activity of prothrombin complex factors with reference to 100% natural plasma [12-14]. This test consists of measuring the time it takes to form a fibrin clot at 37 °C when an excess of thromboplastin is added to the plasma [12-14]. Typically, the clot is formed from 11 to 12 seconds, which represents a fast time. TQ explores factors for external coagulation pathway: Factor VII, Factor X, Factor V, Factor II and fibrinogen [12-14]

2.5.3. Activating the coagulation factor

2.5.3.1. The method of work

Volume of 20 µL of alfalfa seed extract, in addition to 80 µL of standard plasma, which are incubated at 37 °C within 15 minutes. After incubation, add 200 µL of thromboplastin so that the coagulation time is measured using, and the results are expressed by the coagulation time in seconds (Figure 1) [15].

2.5.3.2. Evaluation of anticoagulant activity versus the internal course by a cefalin kaolin time test (TCK).

An evaluation of coagulation activity against the internal coagulation pathway was performed using TCK for partial coagulation, which allows exploration of plasma factor activity from the internal coagulation pathway. This test consists of measuring the coagulation time at 37 °C of depleted and extracted plasma in the presence of alternatives to phospholipid (cefalin) platelet factor 3 (F3P) from a stimulant to the communication system (Pricalicrine, molygalene, high molecular weight and factor XII) which is usually kaolin and calcium as a trigger, a clot is usually formed in 40 seconds [9].

A mixture of 80 µL of plasma and 20 µL of alfalfa seed extract are incubated at 37 °C for 15 minutes, then 100 µL of cephaline reagent are added, and the mixture is re-incorporated for 3 minutes in the incubator, followed by the addition of 100 µL of $CaCl_2$ to reclassify the plasma. Incubation time is measured using a stopwatch to form a clot of fibrin and the results are expressed by the clotting time (in seconds).

3. Results and discussion

The yield of the essential oil extracted from the clover seed was calculated and the yield ratio of the extracted oil was 4.61%. We note that the rate of oil yield for alfalfa seeds was considered and this is due to several factors, including heat, humidity, plant organics, plant harvest time, plant age, and growth phase [6-9].

Thrombosis occurs in three stages. The first stage is the stage of prothrombinase generation, in which the formation of the activated prothrombin complex is done in two ways, which are the external origin and the internal origin.

Table 1. The anticoagulant ability of alfalfa seed extract by the prothrombin rate.

Samples	Tromboplastin time (s)	Protombin rate (%)
Regular witness	11.5	100
Sample 1	22.0	37.1
Sample 2	15.0	64.4
Heparin (positive witness)	600<	Undefined

Table 2. The anticoagulant ability of alfalfa seed extract by cefalin kaolin time.

Samples	The time of cephaline kaolin (s)
Regular witness	28
Sample 1	48
Sample 2	46
Heparin (positive witness)	600<

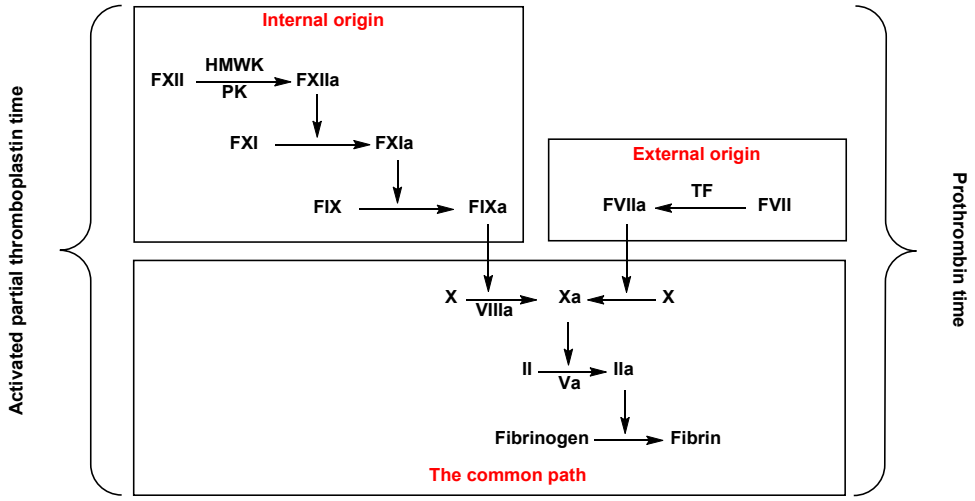

Figure 1. Activation of the clotting factor that ends in the generation of fibrin thrombus. HMWK: High molecular weight quinine generator; PK: Precursor of calcine; TF: Histological factor.

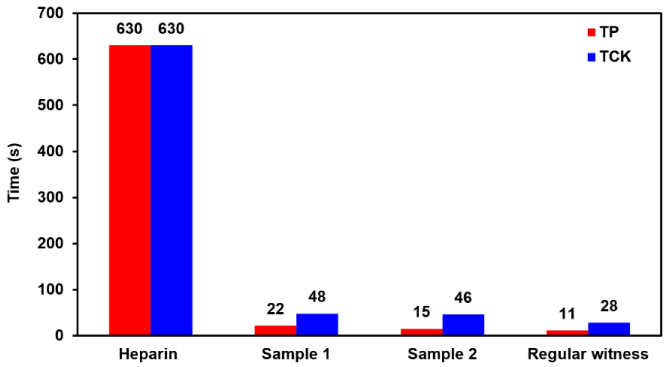

Figure 2. TP and TCK values for the studied alfalfa seed extract.

The second stage is the throbbing step, in which the formation of thrombin from prothrombin is guaranteed during the stimulation activity of prothrombinase. Either the third stage is fibroma so that it leads to the formation of insoluble fibrin from fibrinogen.

The efficacy of anticoagulants of alfalfa seed extract in the laboratory against the internal and external coagulation pathways was evaluated using two TCK and TQ chronometric tests, respectively (Table 1). The results obtained in the table show that sample 1 has the largest clotting time with 22 seconds, followed by sample 2 which lasted 15 seconds. The normal witness gives a rapid time of 11.5 seconds and is low compared to alfalfa seed extract while heparin (positive control) gives a clotting time greater than 600 seconds. It is noticeable that the level of prothombin with natural control is 100%, but levels of prothombin caused by clover seed extract are low, as it was 37.01 and 65.04%.

The results show that the highest clotting time for sample 1 is 48 seconds, followed by sample 2 by 46 seconds. Since alfalfa seed extract is high compared to the regular witness (28 seconds), while heparin, which is a positive control, gives clotting time greater than 600 seconds (Table 2).

The chronometer test (TP Series 1) and TCK (Series 2) also show that alfalfa seed extract has an important anticoagulant activity compared to the two coagulation methods, because this activity is more pronounced towards the internal pathway that the external pathway passes, that is, alfalfa seed extract is better than the control. The normal and lower than positive mark are heparin (Figure 2).

These results coincide with what Dr. Enas Malkawi (2019) indicated that alfalfa contains vitamin 'K' that contributes to blood clotting when wounds are injured, and it is worth noting that the daily requirement of this vitamin is 129 micrograms for

men, 90 micrograms for women, and meets one cup of alfalfa, 13% of the recommended daily amount of vitamin 'K' [10-15].

4. Conclusion

This work aims to study alfalfa seed extract by studying some chemical and physical properties and assessing biological activity (blood clotting). We extracted essential oils from alfalfa seeds in the traditional way, and obtained a yellowish-green oil, aroma, and yield. The yield was at a reasonable rate of 4.61% and measured some of its physical properties represented by the refractive index value of 1.3349. This is evidence of oil transparency, and in terms of measuring the degree of pH, its value is estimated at 8.83, and it is an alkaline value that, if analyzed with the value of the refractive index, indicates the quality of this and the oil. With regard to the chemical property represented in the conductivity, it was estimated at a value of 363 cm/µS. This is evidence of the richness of oils in mineral substances. Procedure and track external by the prothrombin rate obtained that sample 1 has the largest clotting time with 22 seconds, and by the time of cefalin kaolin that the highest clotting time for sample 2 is 46 seconds, that is, to perform an important activity compared in two coagulation methods, alfalfa seed extract is better than normal control and less than positive control heparin.

Based on the results obtained, alfalfa seeds can be considered as a source about me with compounds of biological importance, because the activity of its essential oils in its ability to coagulate blood in addition to its enrichment with mineral compounds, which was indicated by the value of the index of refraction.

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Disclosure statement

Conflict of interests: The authors declare that they have no conflict of interest.
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Sample availability: Samples of the compounds are available from the author.

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