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ANTICOAGULANT ACTIVITY OF SOME *FICUS CARICA VARIETIES EXTRACTS GROWN IN ALGERIA*

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Abstract: *The hemostatic system is a complex balanced system that triggers clot formation to prevent blood loss after trauma. To block abnormal bleeding and maintain intravascular blood in a liquid state, in this study we investigated the possible anticoagulant effect of leaves and fruits extracts from some fig varieties grown in Algeria by determining the quick time (QT). The blood samples of the healthy individuals were used. For in vitro coagulation assays, the clotting times obtained in the presence of polyphenols in different extracts of F.carica samples indicate that they exert a high anticoagulant activity on the exogenous pathway of coagulation. Moreover, the polyphenolic extract of 'Roudane' variety presented a substantial increase in coagulation. Based on these preliminary results, it can be suggested that the fig polyphenolic extracts (FPE) of this varieties have anticoagulant activity that could be useful in preventing blood clots.*

Keywords: Anticoagulant, polyphenols, quick time, *Ficus carica* .L.

Introduction

Cardiovascular diseases are considered the leading causes of death today. More than 17.5 million people lost their lives victimized by causes directly or indirectly related to these disorders in 2012, representing 31% of all global deaths [1].

Hemostasis is a process of interaction between coagulation and anticoagulants that retain blood in the injured vascular system during the phases of the injury [2]. Interruption of the physiological process on hemostasis lead to blood flux regulation alteration and an intense activation of coagulant factors that result either in hemorrhage or thrombosis [3]. Without hemostasis, abnormal fibrin formation could result in the occlusion of the blood vessel causing disturbances mentioned above [4].

Anticoagulants are used for the prevention and treatment of severe thrombotic events. The most used are so far heparin and its derivatives and anti-vitamin K (AVK). Numerous clinical studies have demonstrated their action in the prevention and treatment of thromboembolic complications [5].

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The search for anticoagulant agents from natural herbal medicines is an area of considerable interest [6]. Many studies have been conducted to discover new natural products with coagulant or anti-coagulant/antiplatelet properties that could be more efficient and present a safer alternative than synthetic drugs [7, 8, 9].

The fig (At-Tin) has been of great importance as a source of human food since its earliest cultivation as a fruit tree. Seventy percent of the world's figs are produced in the Mediterranean countries where figs are an important part of the Mediterranean diet that is thought to be related to health and longevity [10]. It has been widely used to treat cardiovascular diseases in traditional medicine.

Despite its traditional usage, there is a lack of data to support the anticoagulant properties of *F.carica*. Therefore, it is time to explore alternative anticoagulants therapy. This study was conducted to evaluate the effects of leaves and fruits extracts of figs on blood coagulation by the determination of quick time (QT).

Material and Methods

Collection of Plant Materials

The leaves and fruits mature of ten varieties of *F.carica* : 'Bifer de tala amara'(V1), 'Avouacou'(V2), 'Alekak' (V3), 'Hamri'(V4), 'Karout'(V5), 'Roudane'(V6), 'Taranimt'(V7), 'Tameriout'(V8), 'Zreka'(V9), 'El fessi'(10), were collected from fifteen year old trees under natural agronomic treatment grown in ITAFV station (Technical Institute of Fruit Trees and Vine), Emdjez-Edchiche, Skikda- Algeria, between July and August of 2015, these varieties were identified by Belattar et al. [11]. The Plant material was collected early in the morning, without microbial contamination and without impact on the ecosystem. After harvest, the samples were cleaned, washed with running water, dried in a hot air oven at 40 ° C and reduced to a fine powder using an electric grinder (Moulinex). These powders were stored until the use for extract preparation.

Extraction of polyphenols

Dried powders of fig leaves and fruits (5g) were added to a mixture of ethanol and water (100 ml, 70:30 (v/ v)). The mixture was left in the dark for 5 days at room temperature. The suspension was threaded through a fine muslin cloth and then with filter paper of Whatman. The solvent was removed at temperature 60°C under reduced pressure in a rotary evaporator to dryness. They were conserved into sterile bottle and placed in a refrigerator until used for further analysis.

Human blood sample

Blood samples were drawn via vein puncture healthy volunteer donor (age 20-25 years old). The blood placed separately in containers sodium citrate or etilenediamine tetra-acetic acid (EDTA) as anticoagulants [12] to prevent the clotting process. To the 9 µl volume of blood, 1 µl volume of 3.2% sodium citrate solution (1/9 v) added to avoid natural coagulation process. Centrifugation (15 minutes at 2500 rpm) was carried out to separate the blood cells from plasma in order to obtain pure platelet plasma for quick time test.

Coagulantion assay

To determinate the influence of FPE on the coagulation activity, its extracts were evaluated *in vitro* by the exogenous coagulation pathway in normal plasma using the quick time (QT) test or prothrombin time (PT) test.100 µl of plasma, 50 µl of FPE were added together in a clean fusion tube and incubated for 180 seconds at 370C in water bath, followed by the addition of 200 µL of sodium chloride ($CaCl_2$) pre-warmed at 37°C for 10 min was added and the coagulation time was recorded with a digital coagulometer. Moreover, plasma alone was used as a control (no anticoagulant activity). The results were expressed in prolongation time compared to controls [13].

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Statistical analysis

All experimental values reflect an average of 3 experiments. Error bars indicate standard deviation (SD). Statistical analyses were performed using the SPSS 21 software. The parameters were compared using analysis of variance (ANOVA) between groups and values of $p \leq 0.05$ were considered statistically significant.

Results and Discussion

Recently it has been discovered that natural plant-derived chemicals are potential therapies for coagulation disorders and model molecules for the development of new drugs [14]. The antithrombolic plants could be an alternative for reducing the side effects from pharmaceuticals commonly used in thrembotic-related diseases [15].

On this basis the current investigation was conducted to evaluate the coagulation factors V, VII, and X in extrinsic coagulation pathway [16]. The effects of the FPE as an anticoagulant agent had been tested, using the principles of QT test in two healthy individuals. Normal QT ranged from 12 to 14 seconds, depending on the reagents used [17], and elongation from the negative control reflected the anticoagulant activity of the test material on the exogenous coagulation pathway.

The results of QT assays *in vitro* showed that FPE produced a significant increase in quick time as compared to control (Figure 1 and 2). The difference was highly significant between the extracts and the control ($p< 0.001$). For the plasma 1, the QT showed the highest clotting time for both extracts 'Alekake' and 'Roudane' leaves 53.33 ±1.52 s and 'Roudane' fruits 55.66 ± 1.52s, as compared to control (12.7 ±0.6 s), as showed Figure 1.

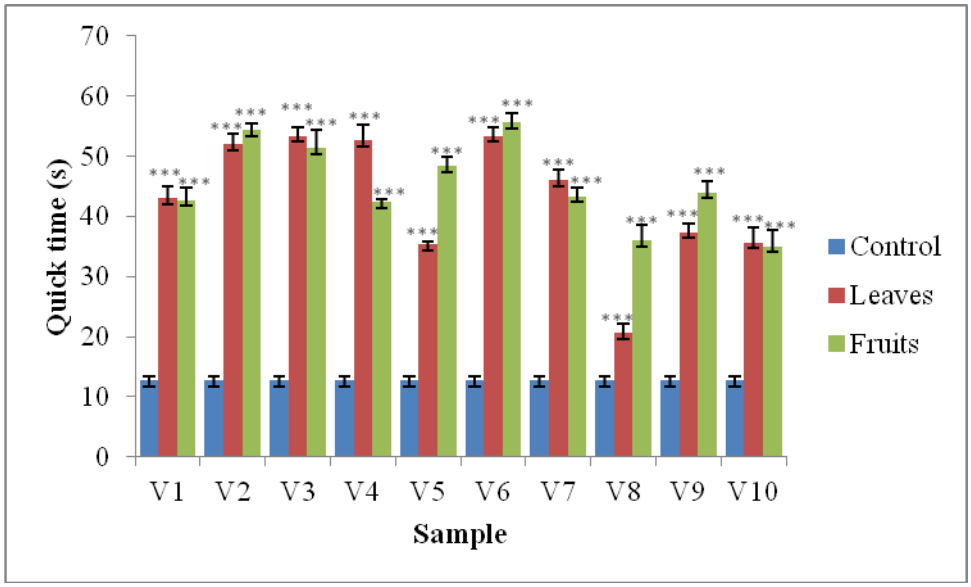

Fig.1: Comparison of effect of different FPE and the control on *in vitro* Anticoagulant activity (QT). V1, FPE of Bifer de tala amara; V2, FPE of Avouacou; V3, FPE of Alekak; V4, FPE of Hamri, V5; FPE of Karout; V6, FPE of Roudane; V7, FPE of Taranimt; V8, FPE of 'Tameriout; 'V9, FPE of Zreka; V10, FPE of El fessi. Values are expressed as mean ± SD of three independent experiments. Significant differences from the control are indicated as *** $P \leq 0.001$.

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Similarly, plasma 2 in the presence of FPE revealed anticoagulant activity, the maximum clotting time was observed with 'Roudane' leaves and fruits extracts for the 58.33±0.47s and 53.66±1.24 s respectively (Figure 1) and there were highly significant differences ($p < 0.001$) at volume comparing with the control (13.8±0.3 s).

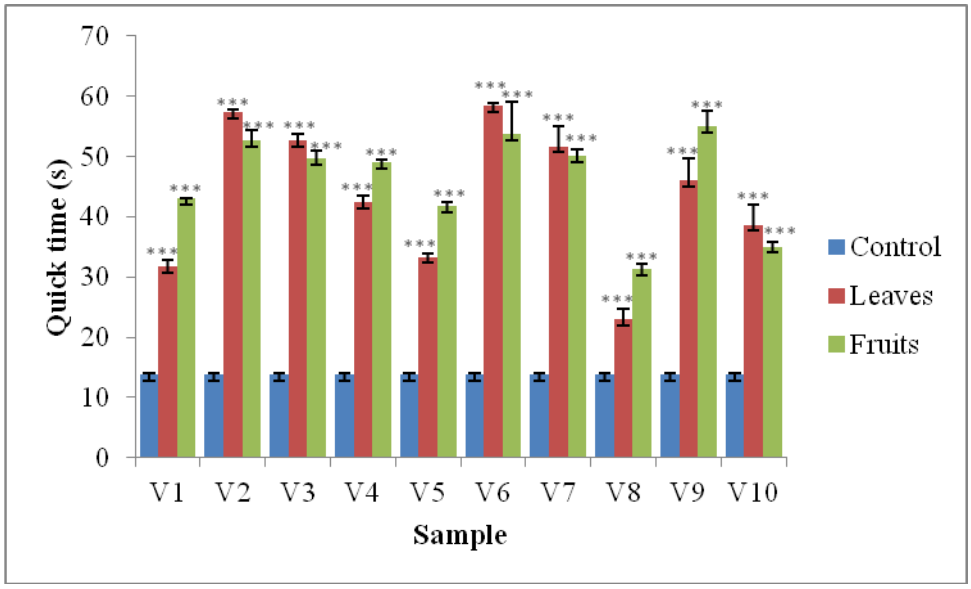

Fig.2: Comparison of effect of different FPE and the control on *in vitro* Anticoagulant activity (QT). V1, FPE of Bifer de tala amara; V2, FPE of Avouacou; V3, FPE of Alekak; V4, FPE of Hamri, V5; FPE of Karout; V6, FPE of Roudane; V7, FPE of Taranimt; V8, FPE of 'Tameriout; 'V9, FPE of Zreka; V10, FPE of El fessi. Values are expressed as mean ± SD of three independent experiments. Significant differences from the control are indicated as *** $P \leq 0.001$.

By comparing the coagulation times (TQ) in the two plasma under the influence of different varieties and plant organs (leaf and fruit), it appears that all the extracts are capable of exerting a significant anticoagulant activity ($p < 0.001$). The FPE of 'Roudane' exerts a great anticoagulant effect by comparing with all extracts.

In fact, polyphenolic compounds are an important group of organic secondary plant metabolites classified in several classes, including hydroxy benzoic acid, hydroxy cinnamic acid, coumarins, xanthones, stil-benes, lignans, anthraquinones and flavonoids [18]. It was found that the polysaccharide-phenol protein complex had anticoagulant and procoagulant effects in the blood coagulation system [19]. Further, Pawlaczyk et al. identified phenolic polysaccharides isolated from *Erigeron canadensis* as potentially useful anticoagulant and antiplatelet agents [19]. Accordingly, the anticoagulating activity of *F.carica*, measured in our study in terms of QT might be mainly due to its polyphenolic contents.

Coagulation is a process mainly due to the complex interaction of cellular and molecular components [20]. In this study, we presented *F.carica* as a potent anticoagulant by the prolongation of the clotting time in plasma-based QT assays. The QT is one of the most important tests to monitor coagulation and anticoagulant therapy and for the detection of blood-clotting disorders. A similar study was reported by Kale et al. in which the extract of *T. procumbens* ethanol leaf specifically reduced the clotting time

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[21]. Tarragon leaf extract showed inhibition of platelet aggregation, 60% adhesion to lamina coated plates, and decreased the 50% of protein secretion [22].Hydroalcoholic extract of four Medicinal plants *Newbouldia laevis* (leaves), *Annona Senegalensis* (leaves), *Cissampelos mucronata* (aerial part), *Cassytha filiformis* (aerial part) revealed the presence of coagulant properties [23]. Himour et al, also showed a reduced coagulation time of the methanolic extract of *Olea europeae* as a clotting time [24].

Conclusion

From the results found during evaluation of *in vitro* anticoagulant activity of different extracts of leaves and fruits of *F.carica*, evidently it is suggest that these fig varieties could be considered as a promising nutraceutical in the prevention of thrombotic disorders caused by various mechanisms thus supporting its traditional use.

Reference

[1]. WHO – World Health Organization, *Cardiovascular diseases,* **2015**. Available at: http://www.who.int/mediacentre/factsheets/fs317/

[2]. Sirridge, M.S.; Shannon, R., *Hematology Principles and Procedures*. 6Th ed. Philadelphia: Lea & Febiger. **1993**, *5*, 202-278.

[3]. Versteeg, H. H.; Heemskerk, J. W.; Levi, M.; Reitsma, P. H., New fundamentals in hemostasis. *Physiol. Rev.* 2013, 93, 327-358.

[4]. Ostrowski, A. P.; Valentini, S. A.; Pavanelli, M. F., Atividade anticoagulante do extrato aquoso, hidroetanólico e óleo essencial das folhas de *Tropaeolum majus*. *SaBios-Revista de Saúde e Biologia*. **2014**, *9*(2), 46-53.

[5]. Kortchinsky, T.; Vigue, B.; Samama, C. M., Antagonisation des héparines et des nouveaux anticoagulants. *Ann. Fr. Anesth. Reanim.*, **2013**, *32*, 37-49.

[6]. Li, Y.; Qian, Z. J.; Ryu, B.; Lee, S. H.; Kim, M. M.; Kim, S. K., Chemical components and its antioxidant properties in vitro: an edible marine brown alga, *Ecklonia cava*. *Bioorg. Med. Chem.*, **2009**, *17*, 1963-1973.

[7]. O'leary, K. A.; Pascual-Tereasa, S.; Needs, P. W.; Bao, Y. P.; O'brien, N. M.; Williamson, G., Effect of flavonoids and vitamin E on cyclooxygenase-2 (COX-2) transcription, *Mutat. Res. Fund. Mol. Mech. Mut.*, **2004**, *551*, 245-254

[8]. Bojić, M.; Debelijak, Ž.; Tomičić, M.; Medić-Šarić, M.; Tomić, S., Evaluation of antiaggregatory activity of flavonoid aglyconeseries, *Nutr. J*., **2011**, *10*, 73.

[9]. Mahajan, R.; More, D., Evaluation of anticoagulant activity aqueous and ethanolic extracts and their isolated phytochemicals of some medicinal plants, *Int. J. Pharm. Pharm. Sci*., **2012**, *4*, 498-500.

[10]. Trichopoulou, A.; Vasilopoulou, E.; Georga, K.; Soukara, S.; Dilis, V., Traditional foods: why and how to sustain them, *Trends Food Sci. Tech*., **2006**, *17*, 498-504.

[11]. Belattar, H.; Yahia, A.; Nemli, S.; Ates, D.; Erdogmus, S.; Ertan, B.; Himour, S.; Hepaksoy, S.; Tanyolac, M.,B., Determination of the Population Structure of Fig Genotypes from Algeria and Turkey Using Inter Primer Binding Site-Retrotransposon and Simple Sequence Repeat Markers. *Agricultural Sciences (AS)*, **2007**, *8*, 1337-1357.

Corresponding author: h.belattar@centre-univ-mila.dz

DOI: 10.2478/asn-2019-0010

[12]. Gonzalez-Covarrubias, V.; Dane, A.; Hankemeier, T.; Vreeken, R. J., The influence of citrate, EDTA, and heparin anticoagulants to human plasma LC–MS lipidomic profiling, *Metabolomics*, **2013**, *9*, 337-348.

[13]. Kokate, C. K., *A text book of Practical pharmacognosy* .5th Edition,Vallabh Prakashan New Delhi: **2005**, 107-111.

[14]. Lee, W.; Yang, E. J.; Ku, S. K.; Song, K. S.; Bae, J. S., Anticoagulant activities of oleanolic acid via inhibition of tissue factor expressions, *The Korean Society for Biochemistry and Molecular Biology*, **2012** *18*, 390-395.

[15]. Chaves, D. S. A.; Costa, S. S.; Almeida, A. P.; Frattani, F.; Assafim, M.; Andzingali, R. B., Metabólitos secundários de origem vegetal: uma fonte potencial de fármacos antitrombóticos, *Quim. Nova*., **2010**, *33*, 172-180.

[16]. Sivrikaya, A.; Baran, H.; Abusoglu, S.; Ozturk, B.; Vatansev, H.; Ali, U., Effect of Gender and Age on the Prothrombin Time (PT), Activated Partial Thromboplastin Time (aPTT) Levels and International Normalized Ratio (INR), *Int. J. Mevlana Med. Sci*., **2013**, *1*, 27-30.

[17]. Caquet, R., Antioxidant properties of spices, herbs and other sources. New York. Springer science, Business Media: tailored for a challenging environment, *Microbiology and molecular biology reviews*, **2004**, *70* (2), 344.

[18]. Manach, C.; Scalbert, A.; Morand, C.; Rémésy, C.; Jimenez, L., Poly-phenols: Food sources and bioavailability, *Am. J. Clin. Nutr*., **2004**, *79*, 727–47.

[19]. Pawlaczyk, I.; Czerchawski, L.; Kuliczkowski, W.; Karolko, B.; Pilecki, W.; Witkiewicz, W.; Gancarz, R., Anticoagulant and anti-platelet activity of polyphenolic-polysaccharide preparation isolated from the medicinal plant Erigeron *canadensis* L., *Thromb. Res*., **2011**, *127*, 328–40.

[20]. Hoffman, M.; Monroe, D. M., Coagulation 2006: A modern view of hemostasis, *Hematol. Oncol. Clin. North Am*., **2007**, *21*, 1-11.

[21]. Kale, M. A.; Shahl, S. R.; Somani, V. G.; Shamkuwar, P. B.; Dhake, A. S., Hemostatic activity of the leaves of *Tridax procumbens* Linn., *Int. J. Green Pharm*., **2008**, *2*, 54-5.

[22].Yazdanparast, R.; Shahriyary, L., Comparative effects of *Artemisia dracunculus*, *Satureja hortensis* and *Origanum majorana* on inhibition of blood platelet adhesion, aggregation and secretion, *Vasc. Pharmacol*., **2008**, *48*(1), 32–7.

[23]. Dandjesso, C.; Klotoé, J. R.; Dougnon, T.V.; Sègbo, J.; Atègbo, J. M.; Gbaguidi, F., Fah, L; Fanou, B ;Loko, F; Dramane, K., Phytochemistry and hemostatic properties of some medicinal plants sold as anti haemorrhagic in cotonou markets (Benin) , *Indian Journal of Science and Technology*, **2012**, *5*(8), 3105-3109.

[24]. Himour, S.; Belhain, H.; Bouketta, M., Anticoagulant activities of *Olea europaea* leaves and fruit extract, *European Scientific Journal*, **2017**, *13*(30), 90-95.

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Corresponding author: h.belattar@centre-univ-mila.dz

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