

Evaluation of the Anticoagulant Activities of *Cucumis melo* Rind Powder In Vitro: Preliminary Novel Findings

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Abstract

The first study of the anticoagulant effect of *Cucumis melon* rind powder extracts is herein reported. The anticoagulant potency of melon (*Cucumis melo*) rind powder (MRP) extracts has been explored using the cephalin kaolin time (TCK) test and the Quick time (TQ) test. The extraction was performed using three solvents (methanol, acetone, and n-butanol) at 80 %, in addition to distilled water. Results have shown that the aqueous extract had no anticoagulant capacity, while the methanolic and acetonetic extracts had heparin-like anticoagulant activity. The anticoagulant effect was observed on the endogenous, exogenous, and common pathways of coagulation with a highly marked effect on the exogenous pathway than on the endogenous pathway. Butanolic extract had a very significant ($p < 0.05$) anticoagulant activity which shows promise as an alternative anticoagulant therapy. This study, the first of its kind had evidenced the anticoagulant abilities of *Cucumis Melo* rinds. Therefore, this by-product could be deployed in medicine to manage and control bleeding and blood coagulation disorders.

Keywords: *Cucumis melo*, By-product, Rinds, Assessment, Anticoagulant activities

INTRODUCTION

Thrombosis and other blood clots with thrombus are the most feared complication of cardiovascular disease and among the major causes of death globally. Thrombosis is known to be related to large morbidity and mortality rate, likewise the 3rd most common cardiovascular disease after myocardial infarction and stroke, making it a major public health challenge. Thrombosis is caused by an imbalance between the body's coagulation and anticoagulant systems. Platelets and the coagulation cascade are the effective targets of antithrombotic methods, that harbor an inherent risk of bleeding. In addition, antithrombotic drugs cannot completely prevent thrombotic events, suggesting a third mechanism that has not yet been adequately addressed: the therapeutic gap due to inflammation [1-4]. Blood coagulation, also known as clotting, is the process of conversion of liquid blood into gel form. This process involves a series of complex chemical chain reactions characterized by the initiation, propagation, and termination phases of thrombin production. Anticoagulants, also known as blood thinners, can be used to prevent endovascular embolism or thrombosis, stroke, ischemic heart disease, deep vein thrombosis, pulmonary embolism, or other thrombotic diseases by affecting certain clotting factors during the clotting process by binding to those factors and preventing them from closing to phospholipid membranes tie [5]. Currently available antithrombotic agents are synthetic in origin and have potentially fatal side effects. To this end, efforts are focused on finding anticoagulants of natural origin

to cure thrombotic diseases. Several scientific reports have highlighted the existence of various anticoagulants derived from natural sources of plant/seed extracts that may be better therapeutic candidates to control and treat bleeding disorders [6-9]. In recent years, with the rapid increase in cardiovascular diseases, researchers have become increasingly interested in studying products of plant origin, known for their richness in bioactive molecules [10-14]. *Cucumis melo* and its by-products are a significant source of these molecules [15, 16]. Several biological activities have been attributed to melon (*Cucumis melo*) and its by-products, namely antioxidant [17], antiviral [18], antiradical, reducing hypertension, and nitric oxide (NO) induction activity [19]. According to our understanding, there are no scientific studies on the anticoagulant effects of melon by-products, the

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rinds. Therefore, this study aims to determine the anticoagulant potency of different powder extracts from the locally produced melon rind (Algeria).

MATERIALS AND METHODS

Plants Material

The canary-yellow melon fruits (*Cucumis melo* L.) were sold at a local market in the town of El-Kala, a small town in the District of El-Tarf, North-east of Algeria. The melons were washed and cleaned of adhering residues. After eating the pulp, the melon rinds were collected and dried on a clean line in the sun for a few days. The dried skins were ground in a household grinder (*Sonifer* brand) until a fine powder was obtained. The obtained MRP with a maximum particle size of 500 μm was stored in airtight bags at 4° C until analysis.

Preparation of Extracts

Several solvents were used for the extraction of phenolic compounds from MRP. These are methanol, acetone, n-butanol (all at 80%), and distilled water. The extraction was carried out by maceration of a quantity of 2 g of the MRP in 10 mL of each solvent based on the guidelines, slightly modified, laid by Derradji - Benmeziane *et al.* [20].

Melon Rind Powder Characterization

Anticoagulant Assay

The anticoagulant activity of the extracts and their main components was analyzed in vitro against both coagulation pathways (endogenous and exogenous) in a pool of normal platelet-free plasma using two global chronometric tests, the cephalin kaolin time (TCK) test and the Quick time (TQ) test expressed in seconds as applicated in the Biochemical laboratory of the Chadli Bendjedid hospital, EL-Tarf (Algeria).

Anticoagulant Activity Toward the Endogenous Pathway

The activity of the extracts was established on 100 μL of this plasma which is mixed with different volumes of the prepared extracts (10 μL , 20 μL , 30 μL). After incubating at 37° C for 15 minutes, add 100 μL cephalin kaolin to the mixture and incubate again at 37° C for exactly 3 minutes with stirring. The clotting time is then determined using a coagulometer by adding 100 μL of preheated calcium chloride (0.025M). In parallel, a control (without extract) is carried out under the same conditions. An elongation of TCK in the presence of polyphenols compared to the control indicates an anticoagulant effect.

Anticoagulant Activity against the Exogenous Pathway

The anticoagulant activity of the samples and their components against the exogenous pathway of coagulation was determined using a coagulation test called Quick Time

(TQ) (second) or prothrombin rate (%) which is the assay that explores factors II, V, VII, and X of the extrinsic pathway and the common coagulation pathway. Its principle is to measure the clotting time of platelet-poor plasma in the presence of calcium thromboplastin (a mixture of tissue factor and phospholipids). The factors of the exogenous pathway are therefore activated and the time that elapses until the formation of the clot is measured. A volume of 100 μL of platelet-poor plasma preheated for two minutes at 37° C is combined with different volumes (10 μL , 20 μL , 30 μL) of different extracts. Following the incubation of fifteen minutes at 37° C, 200 μL of calcium thromboplastin (preheated for a minimum of 15 minutes at 37° C.) is added to the mixture and then the coagulation time is recorded using a coagulometer. In parallel, a control is carried out under the same conditions without extract. A lengthening in clotting time with the availability of the extracts in comparison to the control reflects the activity of the anticoagulant.

Statistical Analysis

All measurements were carried out in duplicate, and data were expressed as mean \pm standard deviation and analyzed using the one-way analysis of variance (ANOVA) test. A value of P less than 0.05 ($p < 0.05$) was considered statistically significant.

RESULTS AND DISCUSSION

The anticoagulant activities of MRP are reported for the first time in this study. Therefore, the results of the current study are compared to the anticoagulant activity reported in other plant extracts.

Anticoagulant Activity Toward the Endogenous Pathway

Results for the anticoagulant capacity of different MRP extracts are presented in **Figure 1**. The data showed that both methanolic and acetonetic extracts exhibited anticoagulant activity, but only for 30 μL of extract, where it was observed that this volume exerted a significant anticoagulant activity ($p < 0.05$) with a respective TCK of 44 s and 43 s, i.e., a lengthening in clotting time of +6 s and +5 s for the methanolic and acetonetic extracts, respectively. The aqueous extract showed no anticoagulant potency as the respective recorded TCK values were 31.5 s, 34 s, and 38 s for 10 μL , 20 μL , and 30 μL , compared to the control which showed a TCK of 38 s. After a few minutes during the test, no blood clots formed in the presence of the butanolic extracts, confirming the potential anticoagulant effect of the molecules extracted with n-butanol towards the exogenous coagulation pathway.

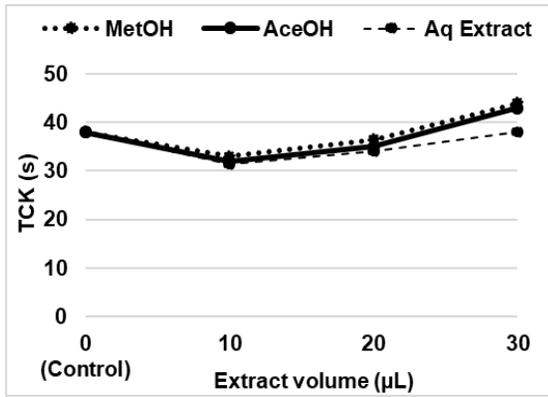


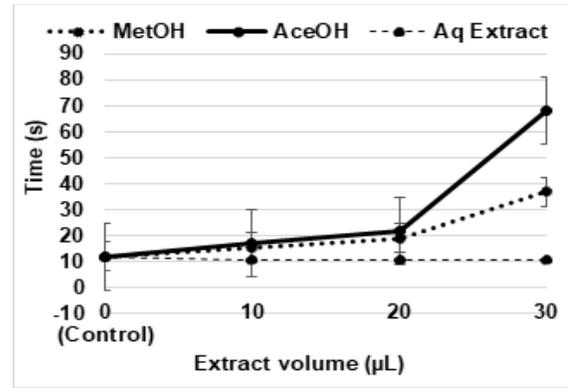
Figure 1. Anticoagulant activity towards the endogenous pathway of melon peel extracts

MetOH: methanolic extract; *AcOH*: acetonc extract; *Aq Extract*: aqueous extract.

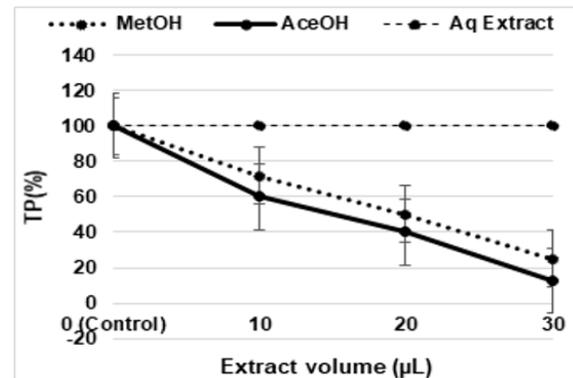
Contrary to what is reported in the current findings, Gholkar *et al.* [21] stated that the aqueous extracts from *Tulbaghia violaceae*, *Petroselinum crispum*, *Jatropha gossypifolia*, *Erigeron canadensis*, and *Bauhinia forficata* show a significant anticoagulant effect. The authors concluded that the aqueous extracts based on these plants can be used to treat cardiovascular diseases such as thrombosis. Similarly, Thoyajakshi and Poornima [3] noted that sweet seed radicle extract from *Lablab purpureus* (L.) possessed an anticoagulant activity by significantly prolonging plasma clotting time. The authors attributed this anticoagulant property to the protease (s) present in the aqueous extract of *Lablab purpureus* (L.) sweet seed radicle.

Anticoagulant Activity Toward the Endogenous Pathway

The results of the anticoagulant effect of various melon rind extracts are shown in **Figure 2**. It was observed that for the same concentration, the volumes tested were found to have a dose-effect relationship. In other words, the more the volume tested increases, the greater the anticoagulant activity. In fact, for 10 µL volume, a slight prolongation of the blood clot formation time (TQ) of +3.5 s and +5 s was observed with a respective prothrombin rate of 76 % and 60 % for the methanolic and acetonc extracts, respectively. By increasing the volume to 20 µL, the methanolic and acetonc extracts prolonged the TQs by +9 s and +10 s and reduced prothrombin levels to 45% and 40%, respectively. Surprisingly, the 30 µL volume had the most significant anticoagulant effect ($p < 0.05$) with a corresponding TQ prolongation of +25 s and +56 s and corresponding prothrombin levels of 25 % and 12.5 % for the methanolic and acetonc extracts, respectively.



a)



b)

Figure 2. Anticoagulant activity of melon peel extracts: top: coagulation time (TQ) (s), bottom: prothrombin rate (TP %)

MetOH: methanolic extract, *AcOH*: acetonc extract, *Aq Exact*: aqueous extract

At the level of this coagulation pathway, the acetonc extract was more potent than the methanolic extract. This could be attributed to the different nature and content of bioactive molecules extracted depending on the polarity and solubility of the phenolic compounds. Similar to the endogenous pathway, the aqueous extract showed no anticoagulant effect as the recorded time in the presence of the three volumes of 10 µL, 20 µL, and 30 µL was 11 s and the prothrombin level was 100 %, compared to the control which showed the same results. Our findings were not in agreement with those of Hmidani *et al.* [22] who found that concerning the anticoagulant effect, the aqueous extract of *Thymus* species (*Thymus atlanticus*, *Thymus zygis*, and *Thymus satureioides*) significantly ($p < 0.05$) increases the activated partial thromboplastin time, prothrombin time and thrombin time in a dose-dependent manner. In the presence of butanolic extract, no blood clots formed after a few minutes during the test. This demonstrated that butanolic extracts have a significant ($p < 0.05$) anticoagulant effect on the endogenous pathways. Hence, these effects could be attributed to the nature of the polyphenolic components present in this extract.

The clotting times obtained in normal plasma in the presence of methanolic and acetonic extracts showed that they have an anticoagulant effect on both coagulation pathways, with a larger influence on the exogenous compared to the endogenous pathway. This can be explained by the fact that the extracted metabolites from *Cucumis melo* rind powder using different solvents were able to inhibit exogenous pathway factors and/or common factors between the two coagulation pathways. Several previous studies have concluded that plant-based polyphenolic compounds can prevent platelet aggregation [1, 23, 24]. However, no studies had examined the anticoagulant potency of *Cucumis melo* rinds.

From these results, it is clear that the endogenous, common, and mainly the exogenous pathway of the coagulation cascade are significantly affected by MRP extracts (methanolic, acetonic, and butanoic). In this sense, previous studies have reported on the prolonging effect of the polyphenolic compound on the clotting time. The effect of the extraction methods on anticoagulant activities of the *Thymus atlanticus* aerial part was studied by Hmidani *et al.* [25]; They found that all *Thymus atlanticus* extracts tested had a significant inhibitory effect on both intrinsic and extrinsic coagulation pathways and decoction extraction method was the most appropriate than the Soxhlet and maceration methods. The authors added that the anticoagulant activity depends on the phytochemical composition of the plant such as flavonoids including rosmarinic acid and caffeic acid, the major polyphenols of *Thymus* aqueous extract in their study. Therefore, the difference in anticoagulant activity observed between the extraction methods studied could be attributed to their difference in phenols and flavonoid content. Our outcomes coincide with the findings of Alabdallat and Bin Dukhyil [26] where the authors stated that the methanolic extracts of *Artemisia herba-alba*, *Achillea fragrantissima*, and *Citrullus colocynthis* grown in Saudi Arabia were able to prolong the clotting time in the prothrombin time and partial thromboplastin time test. This prolongation of prothrombin time and partial thromboplastin time indicates the inhibition of the extrinsic and/or common pathway and the inhibition of the intrinsic and/or common pathway of coagulation demonstrating their anticoagulant activity. In one study, the corm, leaf, petal, and stigma ethanolic extracts from *Crocus sativus* were evaluated for blood clotting activity. It was demonstrated that the coagulation time of stigma extract (101.66 s) was almost equivalent to the standard drug, aspirin (101.66 s), suggesting a strong anticoagulant effect followed by petal extract (86.5 s). Leaf extract (66.83 s) represented a moderate inhibitory effect on coagulation activity, while corm extract (42.83 s) showed a neutral effect [27].

CONCLUSION

According to our understanding, this study is the first of its kind to report examining the anticoagulant activity of *Cucumis Melo* rinds. considering the aforementioned data analysis, it can be concluded that melon rind powder

exhibited an anticoagulant effect in vitro on the tow pathways of coagulation with a highly noticeable effect on the exogenous pathway than on the endogenous pathway. Therefore, it can be proposed that this potentially useful by-product matter could be valuable to explore as an anticoagulant drug and used as a drug candidate to treat coagulation-related diseases, therefore, replacing drugs like heparin, which is known to cause many unwanted complications like thrombocytopenia and risk of bleeding. However, to isolate the pharmacologically active components that contribute to the observed biological effects and to have a better knowledge of the safety of *Cucumis melo* rinds, further epidemiological investigations, laboratory investigations and clinical studies are required. It would also be desirable to study the mechanism of anticoagulant activity and study the effects of melon by-products on platelet activation and aggregation and ultimately develop medicinal formulations.

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