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The *in vitro* effect of different *Ammi visnaga* plant extract on human platelets from both vaccinated and unvaccinated individuals against COVID-19

Mounia Ammara[1], Loubna Khalki[2], Abdelhakim Bouyahya[3], Rania El Majnaoui[1], Naima Marzouki[4], Sanae Sayagh[4], Mounia Oudghiri[1], Younes Zaid[1,5*], Abdallah Naya[1]

[1]Department of Biology, Immunology and Biodiversity Laboratory, Faculty of Sciences Ain Chock, Hassan II University, Casablanca, Morocco

[2]Interdisciplinary Laboratory of Biotechnology and Health, Neurosciences and Cellular Physiology Team. Higher Institute of Biosciences and Biotechnology. Mohammed VI University of Sciences and Health, Casablanca, Morocco

[3]Laboratory of Human Pathologies Biology, Department of Biology, Faculty of Sciences, Mohammed V University, Rabat, Morocco

[4]Laboratory of Medical Biology, Hematology Department, Arrazi Hospital, CHU Mohammed VI, Marrakech, Morocco

[5]Materials, Nanotechnologies and Environment Laboratory, Department of Biology, Faculty of Sciences, Mohammed V University, Rabat, Morocco

**Corresponding author:* y.zaid@um5r.ac.ma

Abstract

The COVID-19 pandemic has sparked an increasing fascination in natural compounds that may regulate the process of platelet aggregation. Ammi Visnaga, a plant renowned for its use in traditional medicine, has been shown to have chemical components that may inhibit platelet aggregation. Additional study is required to ascertain the optimal dosage and any negative consequences of using Ammi Visnaga as a pharmaceutical agent to impede platelet aggregation. Platelets play a crucial role in the processes of hemostasis and blood coagulation. However, excessive activation of platelets may result in the development of blood clots, hence raising the risk of thrombosis. Viral vector vaccines designed for COVID-19 have shown significant efficacy in reducing the severity of the disease. However, there have been new reports linking these vaccines to sporadic cases of atypical thrombocytopenia syndrome (VITT). Scientists have shown considerable fascination in the medicinal possibilities of organic compounds, namely flavonoids derived from plants, owing to their abilities to reduce inflammation, combat oxidative stress, and protect the cardiovascular system. Previous research has shown the vasodilatory and anti-inflammatory effects of the flavonoids found in Ammi Visnaga. Nevertheless, the effect of these factors on platelet aggregation in persons who have been vaccinated against COVID-19 remains unknown and requires more exploration. The study hypothesized that the flavonoids found in Ammi Visnaga might regulate platelet aggregation by obstructing platelet activation pathways. This could possibly reduce the risk of thrombosis in those who have been vaccinated against COVID-19. The results might aid in the development of adjuvant drugs aimed at reducing the risks of vaccine-induced thrombosis, while using the health benefits provided by naturally occurring plant-derived compounds. The objective of the research was to evaluate the influence of Ammi Visnaga extracts on platelet activity in persons with both normal and hyper-reactive platelets, who had undergone COVID-19 immunization. Platelets underwent treatment with two distinct doses of water extract (WE 100mg/L and WE 300mg/L) as well as two distinct concentrations of flavonoids (100 mg/L and 300 mg/L). The findings indicated that the aqueous extract shown a more pronounced anti-aggregating effect in comparison to the flavonoid extract. This phenomenon may be linked to the possible synergistic interaction between the compounds included in the aqueous extract, as well as the wide range of active components, including quercetin.

Keywords: *Ammi Visnaga*, COVID-19, Platelets, Vaccines.

Full length article **Corresponding Author*, e-mail: younes_zaid@yahoo.ca

1. Introduction

The COVID-19 pandemic, resulting from the SARS-CoV-2 virus, has had a substantial influence on public health, compelling researchers and scientists to explore new therapeutic approaches aimed at mitigating the adverse consequences of this illness. Despite the significant contribution of vaccinations in combating the virus, there are ongoing worries around certain adverse effects, namely the potential occurrence of thrombosis and platelet [1].

In light of the aforementioned circumstances, there has been an increasing inclination towards the identification of natural substances capable of modulating the process of platelet aggregation. The plant Ammi Visnaga has a well-established history in traditional medicine. It has been shown that its chemical compounds, particularly khellin and visnagin, possess the ability to suppress platelet aggregation. However, further research is required to ascertain the most effective dose and any adverse effects associated with the use of Ammi Visnaga as a drug for inhibiting platelet aggregation [2]. Platelets are essential components in the processes of hemostasis and blood coagulation. However, an overstimulation of platelets might lead to the formation of blood clots, hence increasing the likelihood of thrombosis. Viral vector vaccines targeting COVID-19 have shown substantial effectiveness in mitigating the condition. However, there have been recent associations between these vaccinations and occasional occurrences of atypical thrombosis, referred to as vaccine-induced thrombocytopenia syndrome (VITT) [3]. Hence, it is crucial to analyze potential corrective strategies aimed at mitigating these adverse consequences. Researchers have shown significant interest in the therapeutic potential of natural chemicals, particularly flavonoids produced from plants, owing to their anti-inflammatory, antioxidant, and cardio-protective properties [4]. The vasodilatory and anti-inflammatory properties of the flavonoids included in Ammi Visnaga have been shown in previous studies [5]. However, their impact on platelet aggregation in individuals who have received the COVID-19 vaccine remains uncertain and requires more investigation. The objective of this research is to investigate the in vitro impacts of the aqueous extract and flavonoids obtained from Ammi Visnaga on platelet aggregation in both unvaccinated persons and those vaccinated with various COVID-19 vaccines. Gaining insight into the mechanisms by which these natural substances impact platelet aggregation might potentially facilitate the discovery of novel treatment approaches aimed at mitigating the potential dangers associated with vaccination-induced thrombosis. The hypothesis of this research was that the presence of flavonoids in Ammi Visnaga may have a regulatory effect on platelet aggregation by impeding platelet activation pathways, hence possibly mitigating the likelihood of thrombosis in persons who have received COVID-19 vaccination. It is plausible to consider that these chemicals may exhibit diverse platelet effects in individuals who have not had vaccinations and those who have, due to intricate connections between immunological and platelet systems. The present investigation aims to enhance comprehension of the possible impacts of Ammi Visnaga flavonoids and water extract on platelet aggregation, particularly within the framework of COVID-19 vaccinations. The findings have the potential to contribute to the progress of adjuvant medicines focused on mitigating the dangers of vaccine-associated thrombosis, while harnessing the health advantages offered by naturally occurring chemicals derived from plants.

2. Materials and methods

2.1. Collection of Blood

Blood samples were obtained from individuals who were in good health and did not consume any substances that might potentially impact the functioning or interfere with the activity of platelets. The blood samples were collected using blood collection tubes containing sodium citrate at a concentration of 2.2% with a volume ratio of 0.1. The process of generating platelet rich plasma (PRP) entails subjecting citrated blood to centrifugation at a velocity of 100g for a duration of 10 minutes. Platelet poor plasma (PPP) was produced by the process of centrifugation of platelet rich plasma (PRP) using 0.1 ul of PGE1 at a centrifugal force of 1500 g for a duration of 15 minutes [6]. The platelet pellet was washed using a modified version of Tyrode's buffer. Platelet counts were conducted under microscopic examination, and the concentrations of washed platelets were adjusted to $(250 \pm 25) \times 10^9$/L.

2.2. Extraction of Ammi visnaga samples

In this study, the Ammi Visnaga seeds were coarsely ground using an electric grinder. Subsequently, a quantity of 10g of the aforementioned sieved powder was immersed in 100 ml of distilled water. The solution was subjected to a temperature of 40°C in a water bath while being continuously stirred for a duration of 1 hour. Subsequently, the solution was let cool down thoroughly and subjected to several filtrations using Whatman paper. Subsequently, the solution was subjected to evaporation until complete desiccation under decreased pressure using a rotary evaporator operating at a temperature of 40°C. The specimen was retrieved by immersing it in distilled water, and afterwards, the concentrations were adjusted using distilled water as well. Flavonoids are a class of natural compounds found in many plant-based foods and beverages. They are known for their diverse range The method for obtaining the plant seed powder was identical to that used for the aqueous extract. After undergoing the process of sieving, a quantity of 10 grams of the powdered substance was afterwards introduced into a solution consisting of 300 milliliters of ethanol with a concentration of 70%. This mixture was left undisturbed for a period one full night. The crude extract underwent filtration followed by centrifugation at a force of 262 g for a duration of 30 minutes. The supernatants were subjected to concentration under decreased pressure at a temperature of 40°C in order to get the flavonoids. Subsequently, the flavonoid extract was retrieved using a 70% ethanol solution. The concentrations were subsequently adjusted to a solution consisting of 70% Ethanol.

2.3. In Vitro Treatment Protocol

The platelets obtained from each patient were subjected to treatment with two different concentrations of WE (100 mg/L and 300 mg/L) and two different concentrations of flavonoids (100 mg/L and 300 mg/L). A volume of 50 microliters from each concentration was introduced into the treatment tube that contained the cleaned platelets. Prior to monitoring platelet aggregation, the incubation period lasted for a duration of 15 minutes at a temperature of 37°C.

2.4. Platelet Aggregation Measurement

Platelet aggregation was assessed using the SYSMEX CS-2500i optical aggregometer at the hematology laboratory located in the Mohamed VI University Hospital in Marrakech [7].

2.5. Statistical analysis

Statistical comparisons were conducted using a two-tailed Student's t-test with the assistance of GraphPad Prism for MacOs. Statistical significance was determined at a significance level of $P<0.05$.

3. Results

Figure 1 (a) provides evident indications of the anti-aggregating impact of three extracts (WE 100mg/L, WE 300mg/L, and F 100mg/L) on the platelets of an unvaccinated individual. Nevertheless, it is noteworthy that the flavonoid extract had the most pronounced impact, with an inhibition rate of roughly 60%. Figure 1 (b) demonstrates that the platelets of the anti-COVID19 vaccinated person exhibit an anti-aggregating effect when treated with three extracts (WE 100mg/L, WE 300mg/L, and F 300mg/L). In contrast, the extract containing flavonoids had the most notable impact, with an inhibition rate of around 60%. Figure 2 illustrate the percentage of platelet aggregation in two groups: V the vaccinated group V (n=9) and NV the unvaccinated group (n=9), both of which were not subjected to any kind of treatment. The process of aggregation was initiated using two agonists, namely ADP and collagen. Platelet aggregation produced by ADP and collagen was assessed in the untreated groups. Figure 2 (a) demonstrates that aggregation attains peak values of around 80% among the vaccinated cohort of patients, while the non-vaccinated group exhibits typical levels of aggregation. Figure 2 (b) Aggregation is a common occurrence across both cohorts of patients, those who have received vaccinations and those who have not. Figure 3 (a) The graph shows that the aqueous extract has a detectable anti-aggregating impact at a concentration of 300 mg/l for individuals who have not been vaccinated. On the other hand, it is impossible to determine if any anti-aggregating effects were caused by the four extracts that were employed in vaccinated people. Figure 3 (b) shows that the aqueous extract at a concentration of 300 mg/L and the flavonoid extract at a concentration of 100 mg/L both had a clear anti-aggregating impact for the individuals who were not vaccinated. However, none of the four extracts put through their paces in vaccinated volunteers showed any evidence of having a strong anti-aggregating impact.

4. Discussion

In a previous study, they were able to notice a change in the hematological platelet parameters in rats following 28 days of oral gavage with the aqueous extract of the Ammi Visnaga plant at three distinct doses: 300, 600, and 1000 mg/kg (Table 1) [8]. Ammi visnaga is classified under the botanical family Apiaceae and is recognized as a herbaceous plant with therapeutic properties. A. visnaga is often regarded as a weed and is used in several countries for its medicinal properties in traditional herbal medicine for a range of uses. The examination of historical documents has shown a multitude of therapeutic attributes associated with A. visnaga, establishing it as a widely used remedy for a diverse range of afflictions. The plant is used both in its natural form as a herb and as an ingredient in the manufacturing of several herbal medicines. These medications are employed in the treatment of renal colic, ureteric stones, angina pectoris, coronary arteries, cardiovascular problems, and asthma. Moreover, it is used as a traditional remedy for the treatment of vitiligo and psoriasis [9]. It is also known as Khella, and has been found to have effects on platelet activation. It has been reported that visnagin, a compound extracted from the plant, stimulates platelet activation, aggregation, and proliferation [10]. Additionally, the ether and ethanolic extracts of Ammi visnaga have shown inhibitory effects on platelet-activating factor-induced asthmatic reactions [11]. The primary objective of this research was to assess the impact of A. Visnaga extracts on platelet function in individuals with both normal and hyper-reactive platelets from volunteers who had received COVID-19 vaccination. Based on the findings of our study, it can be inferred that the aqueous extract exhibited a greater anti-aggregating impact compared to the flavonoid extract (Figure 3). This observation may be attributed to many possibilities, including the potential synergistic interaction among the molecules present in the aqueous extract, in contrast to the isolated flavonoid extract. The potential mechanism behind the antiplatelet aggregation action of the aqueous extract of A. Visnaga might be attributed to its diverse array of active ingredients, such as quercetin. Quercetin, being the most prevalent flavonoid in the plant, exhibits scavenging properties against both reactive oxygen species (ROS) and reactive nitrogen species (RNS) [12]. According to Navarro-Nuñez et al., there is evidence suggesting that quercetin is associated with the suppression of platelet aggregation mediated by TxA2 and a decrease in kinase activity [13]. For instance, the inhibitory effects of quercetin on the many signaling pathways enhanced by the collagen receptor GPVI have been reported by Hubbard et al. (2003). According to the same study, it was shown that quercetin had inhibitory effects on collagen-induced platelet aggregation through suppressing the process of Syk tyrosine phosphorylation [14]. In their study, Oh et al., (2012) found that quercetin demonstrated anti-platelet effects when exposed to collagen, ADP, or thrombin. This was achieved through various mechanisms, including the inhibition of collagen-induced platelet secretion, suppression of intracellular calcium mobilization, inhibition of GPIIb/IIIa activation, elevation of cAMP levels, and enhancement of vasodilator-stimulated phosphoprotein (VASP) activation. Ultimately, these actions resulted in the suppression of mitogen-activated protein kinase (MAPK) phosphorylation [15]. Nevertheless, it appears that the anti-platelet effects of plants rich in flavonoids can be attributed to their ability to interfere with various processes at different sites and levels. These include inhibiting the production of reactive oxygen species (ROS), reducing oxidative stress, preventing the reduction of nitric oxide (NO), modulating the arachidonic acid (AA)-thromboxane pathway, as well as affecting receptors such as ADP, thrombin, collagen, and aIIbb3 integrin [16]. However, further research is needed to fully understand the mechanisms and potential therapeutic applications of Ammi visnaga in platelet activity.

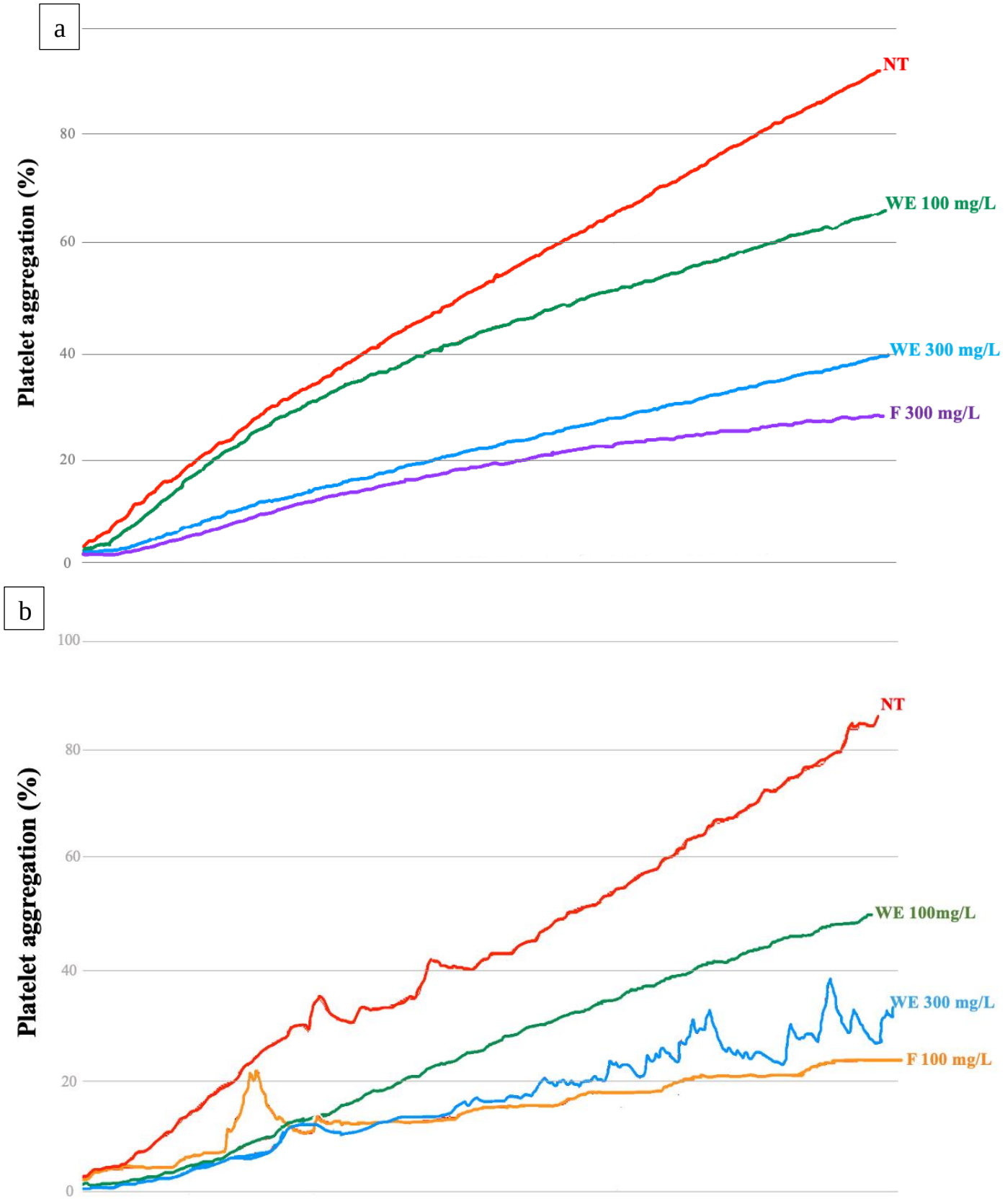

Figure 1: Original tracings of two participants (a) Percentage of collagen induced platelet aggregation within an unvaccinated volunteer, (b) Percentage of collagen induced platelet aggregation within a vaccinated volunteer.

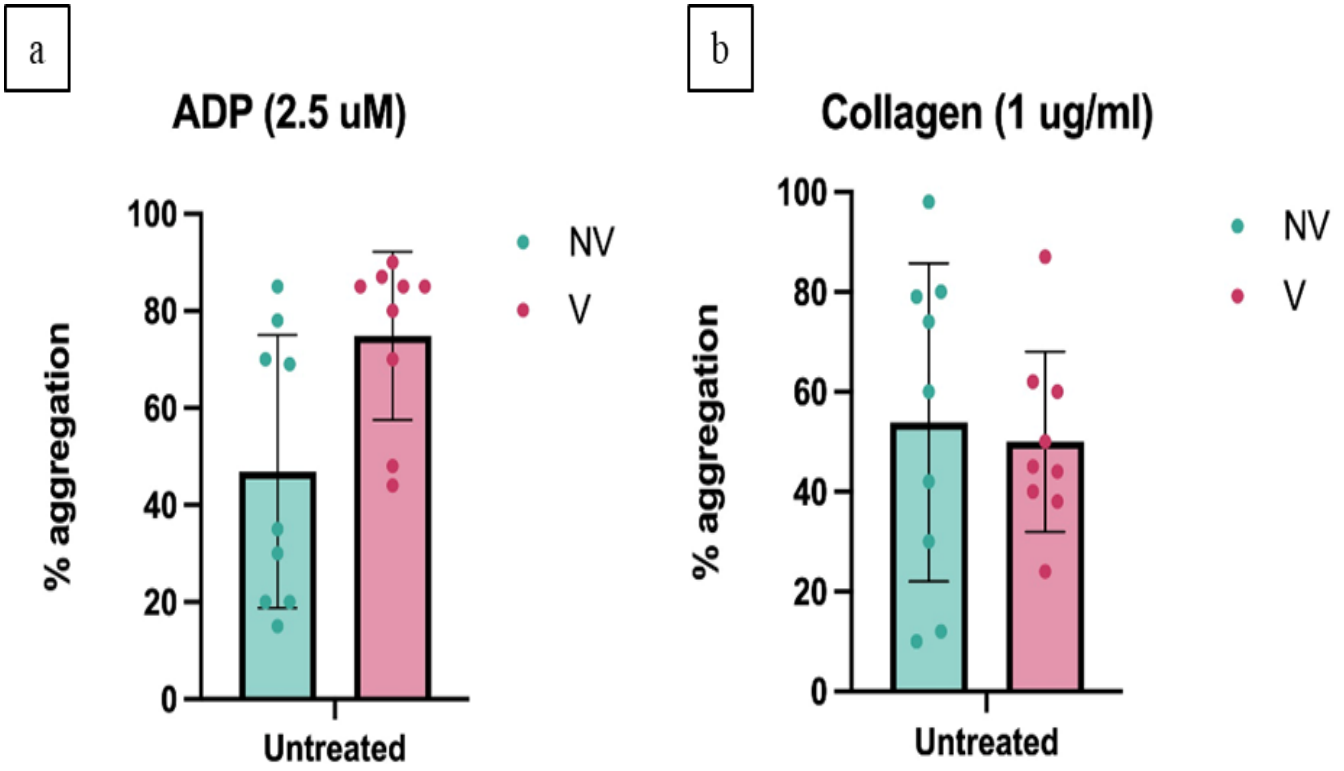

Figure 2: Non treated platelets' percentage aggregation comparing non-vaccinated and vaccinated patients induced by different agonists ADP (a) and Collagen (b).

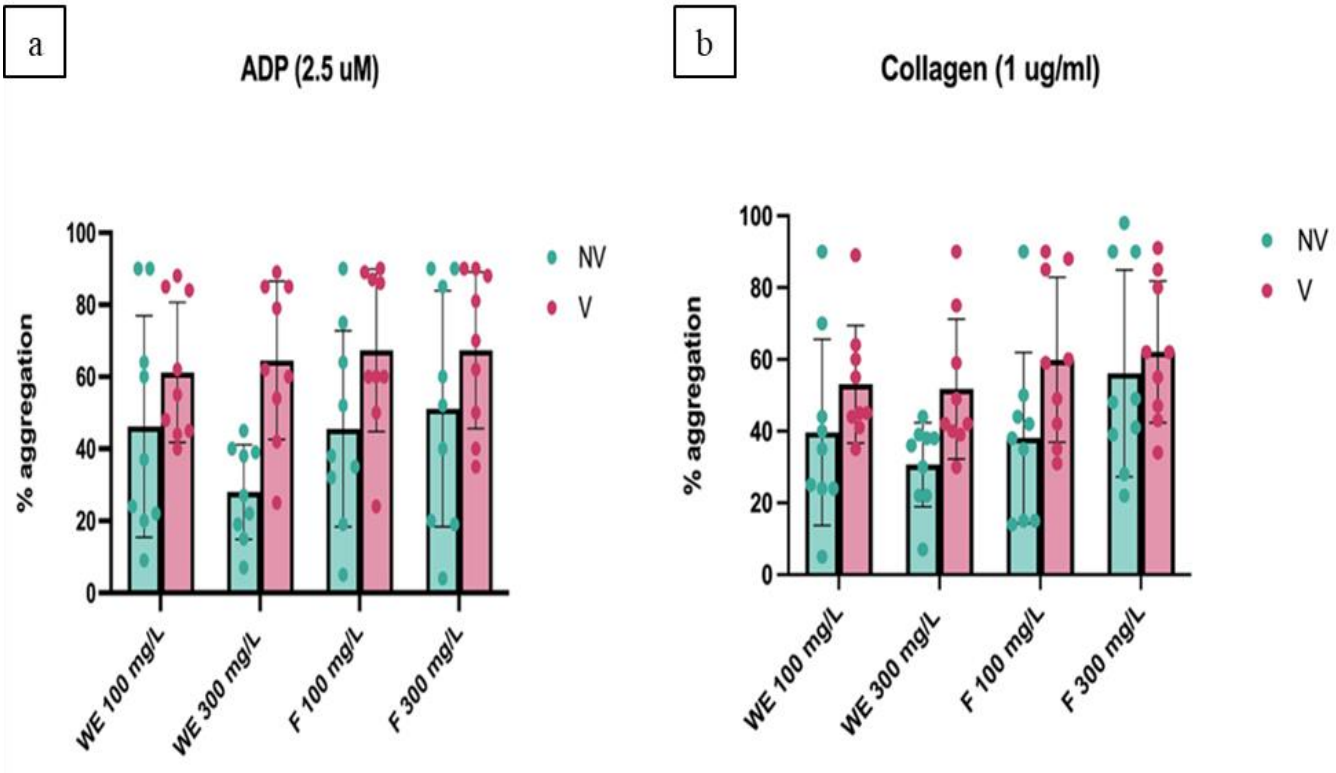

Figure 3: Effects of different extracts including water extract and flavonoids on platelet aggregation of non-vaccinated and vaccinated patients induced by different agonists ADP (a) and Collagen (b).

Table 1: Hematological platelet parameters.

Parameters	Doses (mg/kg)		
	300	**600**	**1000**
PLT (10^3/ul)	1020,25 ± 64,11	985,50 ± 167,95	1067,75 ± 240,00
PDW (fl)	10,55 ± 1,95	8,77 ± 0,33	9,65 ± 0,80
MPV (fl)	7,95 ± 0,55	7,50 ± 0,27	7,77 ± 0,33
PCT %	0,83 ± 0,10	0,74 ± 0,14	0,82 ± 0,16

5. Conclusions

Our study revealed a notable impact of the aqueous extract on the platelet aggregation of individuals who received the anti-COVID19 vaccine, as shown by their platelets' sensitivity in comparison to those who did not get the vaccine. The aqueous extract exhibited a more pronounced anti-aggregating activity compared to the flavonoids, perhaps attributed to the synergistic interactions between the separated components.

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Conflicts of Interest
The authors declare no conflict of interest.

References

[1] A. Greinacher, T. Thiele, T. E. Warkentin, K. Weisser. (2021). A Prothrombotic Thrombocytopenic Disorder Resembling Heparin-Induced Thrombocytopenia Following Coronavirus-19 Vaccination. Research Square preprint. 1–8.

[2] N. Khalil, M. Bishr, S. Desouky, O. Salama. (2020). Ammi Visnaga L., a potential medicinal plant: A review. Molecules. 25 (2): 1–18. https://doi.org/10.3390/molecules25020301

[3] M. Franchini, G. M. Liumbruno, M. Pezzo. (2021). COVID-19 vaccine-associated immune thrombosis and thrombocytopenia (VITT): Diagnostic and therapeutic recommendations for a new syndrome. European Journal of Haematology. 107 (2): 173–180. https://doi.org/10.1111/ejh.13665

[4] M. W. Arafah, B. Almutairi, M. Al-Zharani, A. A. Alkahtane, F. O. Al-Otibi, D. Ali, W. M. Alghamdi, I. S. Alanazi, N. H. Aljarba, N. M. Alhoshani, N. S. AL-Johani, N. Alkeraishan, A. Alhenaky, S. Alarifi, S. Alkahtani. (2021). The protective effect of Ammi visnaga extract against human hepatic cancer. Journal of King Saud University - Science. 33 (6): 101540. https://doi.org/10.1016/j.jksus.2021.101540

[5] G. V. Anrep, M. R. Kenawy, G. S. Barsoum. (1949). The coronary vasodilator action of khellin. American Heart Journal. 37 (4): 531–542. https://doi.org/10.1016/0002-8703(49)91131-X

[6] Y. Zaid, F. Puhm, I. Allaeys, A. Naya, M. Oudghiri, L. Khalki, Y. Limami, N. Zaid, K. Sadki, R. B. El Haj, W. Mahir, L. Belayachi, B. Belefquih, A. Benouda, A. Cheikh, M. A. Langlois, Y. Cherrah, L. Flamand, F. Guessous, E. Boilard. (2020). Platelets Can Associate with SARS-Cov-2 RNA and Are Hyperactivated in COVID-19. Circulation Research. 127 (11): 1404-18.

[7] Y. Zaid, F. Guessous, F. Puhm W. Elhamdani, L. Chentoufi, A. C. Morris, A. Cheikh, F. Jalali, E. Boilard, L. Flamand. (2021). Platelet reactivity to thrombin differs between patients with COVID-19 and those with ARDS unrelated to COVID-19. Blood advances. 2021. 5 (3): 635-639. doi: 10.1182/bloodadvances.2020003513.

[8] A. Ez-zahir, N. Abdallah, S. Nadia, M. Farida, B. Khalid, O. Mounia. (2020). In vivo anti-psoriasis, anti-inflammatory activities and oral toxicity studies of aqueous extract from seeds of Ammi visnaga L. International Journal of Research in Pharmaceutical Sciences. 11 (SPL4): 1025–1037. https://doi.org/10.26452/ijrps.v11ispl4.4239

[9] J. Malik, R. Gandhi, P. Vishwavidyalaya. (2020). Recent updates on Phytoceuticals used in Pancreatitis. April.

[10] A. Bartáková, M. Nováková. (2021). Secondary metabolites of plants as modulators of endothelium functions. International Journal of Molecular Sciences. 22 (5): 1–27. https://doi.org/10.3390/ijms22052533

[11] A. E. Al-Snafi. (2016). A Review of Medicinal Plants with Broncho-Dilatory Effect-Part 1. Scholars Academic Journal of Pharmacy. 5 (7): 297–304. https://doi.org/10.21276/sajp.2016.5.7.6

[12] H. S. Mutlag. (2017). Dose Dependent Anti-inflammatory Effect of Ammi majus Alcoholic Extract in Rat: Chronic Study. Iraqi Journal of Pharmaceutical Sciences. 21 (1): 82–86. https://doi.org/10.31351/vol21iss1pp82-86

[13] L. Navarro-Núñez, J. Rivera, J. A. Guerrero, C. Martínez, V. Vicente, M. L. Lozano. (2009). Differential effects of quercetin, apigenin and genistein on signalling pathways of protease-activated receptors PAR 1 and PAR 4 in platelets. British Journal of Pharmacology. 158 (6): 1548–1556. https://doi.org/10.1111/j.1476-5381.2009.00440.x

[14] G. P. Hubbard, S. Wolffram, J. A. Lovegrove, J. M. Gibbins. (2004). Ingestion of quercetin inhibits platelet aggregation and essential components of the collagen-stimulated platelet activation pathway in humans. Journal of Thrombosis and Haemostasis. 2 (12): 2138–2145. https://doi.org/10.1111/j.1538-7836.2004.01067.x

[15] W. J. Oh, M. Endale, S. C. Park, J. Y. Cho, M. H. Rhee. (2012). Dual roles of quercetin in platelets: Phosphoinositide-3-kinase and MAP kinases inhibition, and cAMP-dependent vasodilator-stimulated phosphoprotein stimulation. Evidence-Based Complementary and Alternative Medicine. https://doi.org/10.1155/2012/485262

[16] C. Faggio, A. Sureda, S. Morabito, A. Sanches-Silva, A. Mocan, S. F. Nabavi, S. M. Nabavi. (2017). Flavonoids and platelet aggregation: A brief review. European Journal of Pharmacology. 807: 91–101. https://doi.org/10.1016/j.ejphar.2017.04.009