advantages including simple, popular equipment and rapid sample preparation.

4. Conclusion

In this study, we developed and validated a simple and reliable HPLC-DAD method for quantitative analysis of calycosin-*7-O-β*-D-glucoside and free astragaloside IV in the raw and honey-processed *Astragali Radix* samples. The developed HPLC-DAD method has met the criteria of the current ICH guidelines: specificity, linearity, accuracy, and recovery. Utilizing this HPLC method, we quantified the contents of calycosin-*7-O-β*-D-glucoside and free astragaloside IV in 10 samples of *Astragali Radix* and four samples of honey-processed *Astragali Radix*. The results showed that the calycosin-*7-O-β*-D-glucoside content varied from 0.016 - 0.032% in the *Astragali Radix* samples and 0.010 - 0.022% in the honey-processed *Astragali Radix* samples. The free astragaloside IV content varied from 0.016 - 0.066% in the *Astragali Radix* samples and 0.018 - 0.052% in the honey-processed *Astragali Radix* samples. However, the total content of astragaloside IV in *Astragali Radix* has not been determined by this method, so the analysis results of astragaloside IV in *Astragali Radix* samples in this study cannot be compared with regulations in Vietnamese Pharmacopoeia V ($\geq$ 0.04%). In the future, the developed HPLC-DAD method will be tested and applied to analyse the total content of astragaloside IV in *Astragali Radix* using an ammonia solution during sample preparation to apply in quality standardization of *Astragali Radix*.

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INVESTIGATION OF THE ANTITHROMBOTIC, ANTIOXIDANT ACTIVITY AND PHYTOCHEMICAL CONTENT OF THE AERIAL PART OF *OXALIS DEBILIS* KUNTH

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Summary

Investigation of the Antithrombotic, Antioxidant Activity and Phytochemical Content of the Aerial Part of *Oxalis debilis* Kunth

Oxalis debilis Kunth (OD) is traditionally used to treat various ailments, including blood stasis syndrome, despite the lack of scientific evidence. This study investigated the antiplatelet, anticoagulant, and antioxidant properties, as well as the phytochemical profile of OD aerial extracts. The aerial part of OD was extracted with methanol, and fractionated with *n*-hexane, ethyl acetate (EtOAc), and water. The antiplatelet and anticoagulant effects were tested with human blood samples. DPPH and ABTS assays were performed to examine the antioxidant capacity. The total flavonoid content (TFC) and total phenolic content (TPC) were quantified. The study indicated that the methanol extract and all fractions had potent antiplatelet effects. The water fraction had the highest antiaggregatory effect induced by adenosine diphosphate among fractions. In addition, the EtOAc and water fraction had anticoagulant effects. Free radical scavenging activities against both DPPH and ABTS were also observed for the MeOH extract, EtOAc, and water fraction. Moreover, the aerial OD was rich in TPC and TFC with the highest values observed in the methanol extract and the water fraction. These novel findings indicate the

promising value of aerial OD for discovering novel bioactive molecules and alternative treatment therapies for thrombotic disorders and oxidative stress-related diseases.

Keywords: *Antiplatelet, Anticoagulant, Antioxidant, Clotting time, Oxalis debilis.*

1. Introduction

The hemostasis plays an indispensable role in keeping the circulatory system functioning properly by preventing excessive blood loss following an injury. However, an imbalance in the hemostatic process might culminate in the growth of clots, obstructing blood vessels, resulting in a range of thrombotic conditions and the development of cardiovascular diseases (CVDs) [1]. Nowadays, a range of antithrombotic drugs is effective in reducing and preventing blood clots and platelet plug formation. However, they also pose challenges such as inflammation, comorbidities, and bleeding complications [2]. Due to the promising safety and healing properties, plant materials are a potential option to be considered for developing new antithrombotic drugs [3],[4].

Oxalis debilis Kunth (OD) is a species from the genus *Oxalis,* in the family Oxalidaceae. Traditionally, the plant has been used to cure burns, ulcers, pharyngitis, emphysema, blood stasis, and skin boils. OD has been documented to have antidiabetic, antioxidant, hepatoprotective, and anti-inflammatory effects [5],[6]. This study assessed for the first time the antiaggregatory effect on platelet aggregation, anticoagulant, and antioxidant properties of the OD aerial part. Moreover, the total phenolic content (TPC) and total flavonoid content (TFC) were also quantified to evaluate its chemical profile.

2. Materials and methods

2.1. Materials

The OD plant was collected in Thanh Hoa, Vietnam in early February 2022 during the flowering period, and then identified and deposited at the Department of Life Sciences, University of Science and Technology (VAST).

Collagen and adenosine diphosphate (ADP) were bought from Chrono-Log Corp, United States. APTT (activated partial thromboplastin time) and PT (prothrombin time) were bought from Dade Behring Marburg GmbH, Marburg, Germany. Additionally, aspirin, ticagrelor, ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid), aluminum chloride ($AlCl_3$), ascorbic acid, DMSO (dimethyl sulfoxide), DPPH (2,2-diphenyl-1-pycrylhydrazyl), Folin and Ciocalteu's Phenol Reagent (FCR), gallic acid, and trolox were purchased from Sigma-Aldrich.

2.2. Preparation of aerial O. debilis extracts

The aerial part was thoroughly washed, dried, and then ground into a powder. Methanol (MeOH) 80% was used to extract 375 g of the powder to obtain 86.25 g of the total MeOH extract (OD.A.Me). Then, 52 g of the OD.A.Me extract was suspended in the water and successively fractionated with *n*-hexane and ethyl acetate (EtOAc). This process yielded three distinct fractions: 6.98 g of the ethyl acetate fraction (OD.A.E), 1.77 g of the *n*-hexane fraction (OD.A.H), and 43.26 g of the water fraction (OD.A.W).

2.3. The platelet aggregation test

The study obtained approval from the Ethics Committee, School of Medicine and Pharmacy, Vietnam National University, Hanoi, Vietnam (document number 02/2020/CN-HĐĐĐ). Blood was drawn from healthy individuals (18-35 years) who had not taken any medication in the previous 3 weeks, and had fasted for 8 hours. After being drawn into tubes that contained 3.2% sodium citrate (9:1, v/v), centrifugation at 170 g for 10 minutes or 1500 g for 10 minutes was done to acquire platelet-rich plasma (PRP) or platelet-poor plasma (PPP), respectively. The turbidimetric approach was employed to perform the platelet aggregation. Briefly, 450 µL PRP was incubated with 50 µL extracts at different concentrations at 37°C. After 3 minutes, an agonist (ADP 10 µM or collagen 2 µg/mL) was added to stimulate the platelet aggregation. The maximum amplitude was recorded. The aggregation inhibition percentage (%I) was determined by the following formula: $\%I = \frac{C-S}{C} \times 100\%$, where C and S are the maximum percentage of platelet aggregation of the negative control and tested samples, respectively. Ticagrelor (0.002 mg/mL) or aspirin (0.1 mg/mL) served as the positive control for ADP or collagen agonist, respectively. DMSO 0.1% served as the negative control. The antiplatelet assay was repeated three times [7],[8].

2.4. Anticoagulant assay

The coagulation tests, including PT and APTT, were evaluated following previously described methods [7],[8]. Briefly, 50 µL extracts

at different concentrations were added to 450 µL PPP, and then incubation of the mixtures at 37 °C was done for 3 min. The positive control was heparin at 2 IU/mL for PT, or heparin at 0.2 IU/mL for APTT. The negative control was DMSO 0.1%. Three duplicates of the experiment were carried out.

2.5. DPPH radical scavenging assay

First, 10 µL extracts were added into 190 µL DPPH 0.1 mM dissolved in methanol, and incubation of the mixtures at 37°C was performed for 20 min. Then, the measurement of absorbance at 517 nm was carried out. Ascorbic acid served as the positive control. The percentage inhibition (%I) of free radicals was determined using the below equation: $\%I = \frac{A - B}{A}$ × 100%, where A and B are the optical density of the negative control and the tested sample, respectively. Half - maximal inhibitory concentration (IC_{50}) denotes the concentration at which the sample's antioxidant activity is reduced by half [3],[4].

2.6. ABTS radical scavenging assay

At room temperature, an equal volume of potassium peroxydisulfate (2.45 mM) and ABTS (7 mM) were mixed and incubated for 14 h in the dark. Ethanol was employed to dilute the obtained ABTS solution to reach an absorbance of 0.70 ± 0.05 at 734 nm. Then, 10 µL each sample at different concentrations and 190 µL diluted ABTS solution were mixed and incubated for 10 min. The absorbance was measured at 734 nm. Trolox and methanol served as the positive and negative controls, respectively. IC_{50} values were determined for tested samples [3],[4].

2.7. Determination of total phenolic content

The Folin-Ciocalteu assay was performed as previously described to quantify the TPC [9]. Briefly, 40 µL samples at different concentrations were added to 480 µL diluted Folin-Ciocalteu reagent. The mixture was then added with 480 µL Na_2CO_3 6%. The final solutions were incubated at 40°C for 20 min. After incubation, the absorbance at 765 nm was recorded. A standard curve was prepared using gallic acid at different concentrations. Finally, the TPC was computed using the formula below: $TPC = \frac{C_{GAE} \times 1000}{C_0}$, where TPC stands for milligrams of gallic acid equivalent per 1 gram of the sample (mg GAE/g), C_{GAE} represents the concentration of gallic acid equivalent (g GAE/mL) and C_0 represents the concentration of the sample (g/mL).

2.8. Determination of total flavonoid content

The TFC was quantified using the aluminum chloride colorimetric assay as described previously [9]. First, 240 µL extracts at different concentrations were mixed with 40 µL $NaNO_2$ 5% and 40 µL $AlCl_3$ 10%. After incubating for 6 min at 25°C, 400 µL NaOH 1 M and 280 µL ethanol 30% were added, and the mixtures were incubated for 15 min. The measurement of absorbance at 510 nm was carried out. A standard curve of quercetin at different concentrations was used. The TFC was determined using the following equation: $TFC = \frac{C_{QE} \times 1000}{C_0}$, where TFC stands for milligrams of quercetin equivalent per 1 gram of sample (mg QE/g), C_{QE} represents the concentration of quercetin equivalent (g QE/mL) and C_0 represents the concentration of the sample (g/mL).

2.9. Statistical analysis

Data were expressed as mean ± standard deviation (SD). An independent sample t-test was applied using Analysis Toolpak to determine the mean differences between groups. If the p-value was less than 0.05, it was judged as statistically significant.

3. Results and discussion

Antiplatelet aggregation effects of O. debilis extracts

All OD extracts suppressed ADP- and collagen-induced platelet aggregation and the antiaggregatory effect was concentration-dependent. The antiplatelet effect increased as the concentration increased from 0.5 to 2 mg/mL. At 2 mg/mL, the total MeOH extract and three fractions exhibited potent antiplatelet effects (%I ranged from 62.25% to 92.90% for ADP, and 96.95% to 99.4% for collagen). The water fraction OD.A.W at all tested doses showed high inhibitory effects, regardless of the activators used (%I ranged from 80.05% to 92.90% for ADP, and 93% to 99% for collagen). Furthermore, the MeOH extract OD.A.Me and the ethyl acetate fraction OD.A.E at 2 and 1 mg/mL also showed good inhibitory effects for both ADP and collagen agonists (%I ranged from 61.95% to 90.55% for ADP, and 97.15% to 99.4% for collagen). However, the *n*-hexane fraction OD.A.H had the lowest inhibitory impact

on platelet aggregation activated by ADP in comparison with other extracts ($p < 0.05$), while it strongly inhibited collagen-induced platelet aggregation at all concentrations (%I > 80%). Notably, in the case of ADP, OD.A.Me, and OD.A.W at 1 and 2 mg/mL had even higher inhibitory effects than ticagrelor ($p < 0.05$), while no significant difference was observed between OD.A.E at 2 mg/mL and the positive control ($p > 0.05$). In the case of collagen, there was no statistically significant difference in the antiplatelet effect between the positive control and OD.A.Me (at 1 and 2 mg/mL), OD.A.E (at 1 and 2 mg/mL), or OD.A.W (at 2 mg/mL) ($p > 0.05$) (Fig. 1).

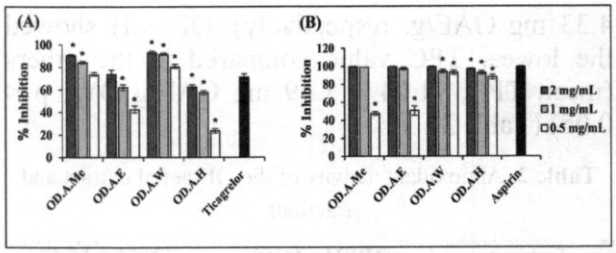

Fig.1. Percentage inhibition (%I) induced by (A) ADP, or (B) Collagen.
*: $p < 0.05$ when comparing extracts with ticagrelor 0.002 mg/mL (for ADP) or aspirin 0.1 mg/mL (for collagen), OD.A.Me: OD aerial methanol extract, OD.A.E: OD aerial ethyl acetate fraction, OD.A.W: OD aerial water fraction, OD.A.H: OD aerial *n*-hexane fraction

Anticoagulant activity of *O. debilis* extracts

The effects of OD extracts on PT and APTT are shown in Table 1.

Table 1. Anticoagulant effects of *O. debilis* extracts

Extracts	Concentration (mg/mL)	PT (s)	APTT (s)
OD.A.Me	4	12.00 ± 0.10 [a]	27.05 ± 0.45 [a]
	2	12.15 ± 0.35 [a]	26.75 ± 0.15 [a]
OD.A.E	**4**	12.05 ± 0.50 [a]	**31.00 ± 0.50** [a,b]
	2	12.30 ± 0.40 [a]	27.70 ± 0.80 [a]
OD.A.W	**4**	12.15 ± 0.75 [a]	**37.70 ± 1.20** [a,b]
	2	12.10 ± 0.60 [a]	27.40 ± 0.50 [a]
OD.A.H	4	12.20 ± 0.30 [a]	27.20 ± 0.40 [a]
	2	12.10 ± 1.00 [a]	27.20 ± 0.70 [a]
DMSO	0.1%	12.30 ± 0.40	27.90 ± 1.20
Heparin	2IU	28.45 ± 0.35	
Heparin	0.2IU		44.25 ± 1.35

[a] $p < 0.05$ when comparing between extracts and heparin, [b] $p < 0.05$ when comparing between extracts and DMSO, PT: prothrombin time, APTT: activated partial thromboplastin time, OD.A.Me: OD aerial methanol extract, OD.A.E: OD aerial ethyl acetate fraction, OD.A.W: OD aerial water fraction, OD.A.H: OD aerial *n*-hexane fraction

It was observed that both OD.A.E and OD.A.W at 4 mg/mL showed anticoagulant effects with significantly longer APTT compared to DMSO 0.1% (at 4 mg/mL, mean APTT for OD.A.E and OD.A.W, 31.00 ± 0.50 and 37.70 ± 1.20 vs. 27.90 ± 1.20 s, respectively, $p < 0.05$). However, they did not affect the PT parameter. Both OD.A.Me and OD.A.H did not have any significant difference in the clotting time compared with DMSO ($p > 0.05$). OD extracts exhibited lower APTT and PT than heparin at both tested doses ($p < 0.05$).

Radical scavenging activity of *O. debilis* extracts

In the DPPH test, OD.A.Me, OD.A.E, and OD.A.W showed radical scavenging activity (IC_{50}, 66.39 ± 1.46, 70.3 ± 0.83 and 77.28 ± 6.52 µg/mL, respectively). OD.A.H exhibited a modest antioxidant effect (mean IC_{50}, 192.79 ± 5.54 µg/mL, $p < 0.05$). All tested extracts had lower DPPH scavenging ability than ascorbic acid ($p < 0.05$) (Table 2).

In the ABTS assay, OD.A.Me showed the strongest radical scavenging activity (IC_{50}, 7.98 ± 0.79 µg/mL, $p < 0.05$). Both OD.A.E and OD.A.W also exhibited ABTS scavenging effects with IC_{50} of 12.94 ± 0.17 µg/mL and 16.78 ± 0.15, respectively. OD.A.H had the lowest antioxidant effect ($p < 0.05$). All extracts demonstrated the ability to scavenge free radicals, but their IC_{50} values were higher than that of trolox ($p < 0.05$) (Table 2).

Quantitative analysis of phenolic and flavonoid contents

The results showed that the TPC of aerial OD extracts ranged from 11.04 ± 1.59 to 55.19 ± 7.20 mg GAE/g. TPC was high in the total methanol extract (mean TPC, 49.69 ± 7.80 mg GAE/g). However, OD.A.E and OD.A.W exhibited the highest TPC (mean TPC, 55.19 ± 7.20, 51.00 ±

4.33 mg GAE/g, respectively). OD.A.H showed the lowest TPC value compared to the others (mean TPC, 11.04 ± 1.59 mg GAE/g DW, $p < 0.05$) (Table 3).

Table 2. Antioxidant activity of the OD aerial extract and fractions

Extracts	DPPH - IC50 (µg/mL)	ABTS - IC50 (µg/mL)
OD.A.Me	66.39 ± 1.46 [a]	7.98 ± 0.79 [b]
OD.A.E	70.30 ± 0.83 [a]	12.94 ± 0.17 [b]
OD.A.W	77.28 ± 6.52 [a]	16.78 ± 0.15 [b]
OD.A.H	192.79 ± 5.54 [a]	55.53 ± 0.14 [b]
Ascorbic acid	8.84 ± 1.30	
Trolox		4.71 ± 0.30

[a] $p < 0.05$ when compared to ascorbic acid, [b] $p < 0.05$ when compared to trolox, OD.A.Me: OD aerial methanol extract, OD.A.E: OD aerial ethyl acetate fraction, OD.A.W: OD aerial water fraction, OD.A.H: OD aerial *n*-hexane fraction

For the TFC assay, the highest TFC was observed in OD.A.E (mean TFC, 254.73 ± 9.36 mg QE/g DW, $p < 0.05$). In addition, OD.A.W showed a higher TFC value than OD.A.Me (mean TFC, 233.64 ± 9.07 vs. 147.64 ± 11.75 mg QE/g DW, $p < 0.05$). OD.A.H exhibited the lowest TFC value compared to the remaining extracts (mean TFC, 123.8 ± 5.02 mg QE/g DW, $p < 0.05$) (Table 3).

Table 3. Total phenolic and flavonoid content of the OD aerial extract and fractions

Extracts	TPC (mg GAE/g)	TFC (mg QE/g)
OD.A.Me	49.69 ± 7.80	147.64 ± 11.75 [b,c,d]
OD.A.E	55.19 ± 7.20	254.73 ± 9.36 [a,c,d]
OD.A.W	51.00 ± 4.33	233.64 ± 9.07 [a,b,d]
OD.A.H	11.04 ± 1.59 [a]	123.8 ± 5.02 [a,b,c]

[a] $p < 0.05$ when compared to OD.A.Me, [b] $p < 0.05$ when compared to OD.A.E, [c] $p < 0.05$ when compared to OD.A.W, [d] $p < 0.05$ when compared to OD.A.H, OD.A.Me: OD aerial methanol extract, OD.A.E: OD aerial ethyl acetate fraction, OD.A.W: OD aerial water fraction, OD.A.H: OD aerial *n*-hexane fraction.

Discussion

Platelet aggregation tests are crucial for evaluating bleeding disorders, thereby aiding in treatment determinations and mitigating complications associated with thrombosis. This is the first investigation of the antiplatelet aggregation activity of OD. A high value of percentage inhibition %I indicates a significant inhibitory impact on maximum platelet aggregation. Ticagrelor and aspirin are antiplatelet agents employed in the therapeutic management of cardiovascular patients. Ticagrelor works as a direct-acting $P2Y_{12}$ receptor antagonist, blocking ADP-induced signaling, while aspirin inhibits the COX-1 enzyme, thereby reducing thromboxane A2 production in response to collagen [10]. In the case of ADP agonist, both OD.A.Me and OD.A.W fractions at 2 mg/mL exhibit a more potent inhibitory impact on platelet aggregation than ticagrelor, while OD.A.E fraction at 2 mg/mL shows a strong antiplatelet property similar to ticagrelor. In the case of collagen agonists, OD.A.Me, OD.A.W, and OD.A.E at 2 mg/mL display comparable antiplatelet effects to aspirin. These results indicated that the methanolic extract and its fractions from aerial OD exhibited strong antiplatelet properties for both ADP and collagen agonists. This also suggests that bioactive compounds present in OD extracts might interfere with both ADP and collagen receptors and their antiplatelet effects in the extracts seem to be as strong as ticagrelor and aspirin. Notably, at all tested concentrations, all extracts had a higher %I in the case of collagen than in the case of ADP ($p < 0.05$). Inhibiting platelet aggregation induced by collagen directly prevents the first step of primary hemostasis, having a greater impact on overall platelet aggregation [11]. In addition, for the ADP agonist, the water fraction exhibited a stronger antiplatelet effect than other extracts, suggesting that water might be an effective solvent for extracting biologically active substances with antiplatelet activity from aerial OD. It was also possible that there were additive and synergistic interactions between bioactive substances in the water fraction that enhanced the antiplatelet activity of the fraction. Moreover, the water fraction, with high levels of phenolic compounds, may contain specific groups such as flavonoids, phenolic acids, and saponins, which have been determined to enhance antiplatelet activity [4]. Currently, the phytochemical constituents of OD have not been clearly identified. Further experiments are required to find bioactive molecules and clarify the mechanisms of antiplatelet action.

This study also reported novel findings on the anticoagulant effects of aerial OD extracts using

PT and APTT assays, which are routine tests used to assess coagulation disorders in clinical practice. These tests are also commonly applied to demonstrate the inhibitory impact of medicinal plants on the blood coagulation process [3],[4],[7],[8]. The PT test measures the effects of tested samples on the extrinsic coagulation factors, especially factors X, VII, and V, while the APTT test evaluates the influence of samples on the intrinsic coagulation pathway such as factors XII, XI, IX, and VIII [4]. The anticoagulant medication heparin primarily inhibits factors IIa and Xa, which impacts the extrinsic coagulation pathway. Additionally, heparin affects factors XIIa, XIa, IXa, and VIIIa, which are components of the intrinsic coagulation pathway [12]. Heparin also binds to antithrombin and inactivates thrombin. It reduces clot formation by interfering with several coagulation factors, leading to longer clotting time for PT and APTT tests [13]. Heparin exhibits the PT prolongation effect at higher concentrations than those causing APPT prolongation. Consequently, the concentration of heparin employed in the APTT and PT tests for this study is 0.2 and 2 IU/mL, respectively. Longer values than expected in these tests indicate the inhibitory effect on relevant coagulation factors. In this study, the ethyl acetate and water fraction significantly prolonged APTT. This suggests that bioactive components in these two fractions might affect factors XII, XI, IX, and VIII involved in the coagulation pathways. The total methanol extract and *n*-hexane fraction did not show any significant effect, possibly due to the low concentration of active components in these extracts or the interactions of active compounds with other substances present in these extracts that might affect the antiplatelet activity. Many previous studies reported the potential anticoagulant activity of medicinal plants [3],[4],[7],[8],[9],[14]. The possible effect on the intrinsic coagulation pathway of aqueous fractions derived from *Meriandra dianthera* leaves with significantly prolonged APTT was reported [14]. Some *Canna* species (*C. edulis*, *C. generalis and C. indica*) and their isolated compounds have been demonstrated for antithrombotic properties with high percentage inhibition of platelet aggregation and prolonged clotting times [3],[4],[7],[8],[9]. Screening plant extracts is known as a crucial initial step to guide further in-depth studies on the investigation of bioactive compounds and the mechanism of action. Furthermore, experiments should be implemented to evaluate the antithrombotic properties of plants belonging to the genus *Oxalis*.

Antioxidant experiments are popular and attractive due to their contributions to the treatment of linked diseases such as CVDs, cancer, and aging [15],[16],[17]. Notably, antioxidants may indirectly contribute to the prevention of thrombotic events by mitigating oxidative stress, as they influence platelet activation, coagulation, and fibrinolysis, interfering with normal hemostatic function [4]. The results in the present study showed that the aerial extracts of OD collected in Vietnam possess not only potential antithrombotic properties but also the ability to scavenge free radicals and protect against oxidation. In comparison to the DPPH assay, the IC_{50} values for the ABTS assay were lower for all extracts and fractions, which might be explained by the higher sensitivity of the ABTS in comparison with the DPPH [18]. A previous study on OD and *O. corniculata* in India revealed that OD had a higher antioxidant ability than *O. corniculata* (mean IC_{50} for DPPH assay, 25.82 ± 0.68 vs. 73.67 ± 0.91 μg/mL) [19]. It was also found that OD leaf extracts in *in vivo* rat models decreased oxidative damage and inhibited lipid peroxidation in the diabetic liver via increasing enzymatic antioxidant activity [5]. With its potential antioxidant property, OD is a promising candidate for further research and drug development for oxidative stress-related disorders.

The biological effects of polyphenols and flavonoids, such as their anticancer, neuroprotective, antiviral, and antioxidant activities are receiving attention [5],[20],[21]. Furthermore, polyphenol flavonoids may help prevent atherosclerosis, platelet adhesion, and aggregation. For example, luteolin, quercetin, and kaempferol, which are classified as flavonoids, may potentially influence the functionality of factors VIII, IX, XI, and XII within the intrinsic coagulation pathway [22]. This study indicated

that phenolics and flavonoids were found in all extracts, especially in the ethyl acetate and water fraction and they might contribute to the observed antiplatelet and anticoagulant effects of aerial OD. Polyphenols and flavonoids were also reported to be present in the hydro-alcoholic extract of OD leaf collected in India with TPC and TFC of 56.34 ± 2.09 mg GAE/g and 45.22 ± 2.31 mg QE/g, respectively [5]. Additionally, the TPC of the whole plant of OD was found to be significantly greater than that of *O. corniculata*, another plant in the same genus (52.15 ± 1.54 vs. 32.34 ± 1.36 µg GAE/mg) [19]. The presence of high TPC and TFC in OD aerial extracts might contribute significantly to its biological activities. The aerial part of OD could be a promissory natural resource to explore bioactive flavonoids and phenolic compounds. Further experimental investigations are also necessary to elucidate the role of polyphenols and flavonoids present in OD and their antithrombotic potential.

4. Conclusion

This study demonstrated novel findings on antiaggregatory and anticoagulant effects of the aerial part of OD, while also highlighting its antioxidant potential. The aerial OD extracts showed potent antiplatelet effects induced by both ADP and collagen. Moreover, the water and ethyl acetate fractions showed anticoagulant activity. The aerial OD extracts were shown to have high amounts of phenolics and flavonoids. Therefore, OD is a potential candidate for searching for natural bioactive ingredients and developing alternative strategies to prevent and treat thrombotic disorders and oxidative stress-related diseases. Future studies are required to investigate biologically active compounds and clarify their mechanisms of action.

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