ISSN: 2986-9994

The Grant Medical Journals®

May, 2017; 02(04): 057-066 mds/16/1621/gmj

Medical Sciences
Original Research Paper

Comparative Study of Natural Phenolic Acids and Flavonols as Antiplatelet and Anti-Inflammatory Agents

Alexandra Moschona[1], Konstantinos D. Kyriakidis[2], Athanassios D. Kleontas[3], Maria Liakopoulou-Kyriakides[1]*

[1]Faculty of Chemical Engineering, Section of Chemistry, Aristotle University of Thessaloniki, Thessaloniki, 54124, Greece.
[2]Colchester Hospital, University Foundation Trust, Turner Road, Colchester, Essex, CO45JL, UK.
[3]Department of Cardiothoracic Surgery, Aristotle University School of Medicine, AHEPA University Hospital, Thessaloniki, Greece.

*****Corresponding author(s): Maria Liakopoulou-Kyriakides, Faculty of Chemical Engineering, Section of Chemistry, Aristotle University of Thessaloniki, Thessaloniki, 54124, Greece.
Email: markyr@eng.auth.gr

Accepted: 2017.03.13; Published: 2017.05.02;

ABSTRACT

The search for natural phenolic compounds from fruits, vegetables, as well as from their wastes, has recently gained importance, due to their beneficial effects on human health and their potential use as drugs. Here, three phenolic acids (gallic, ellagic, ferulic) and two flavonols (quercetin and kaempferol), isolated from wine and pomegranate wastes, and the standards were evaluated based on their radical scavenging activity, antiplatelet and anti-inflammatory activity, in vitro. Specifically, the phenolic compounds were tested for their antioxidant activity, using the DPPH method, for their effect on human platelet aggregation induced, by collagen, ADP and subsequently against cyclooxygenases 1 and 2 activities. It was found that all phenolic compounds presented high antiradical activity and inhibited both collagen and ADP-induced platelet aggregation, in a dose-dependent manner with IC50 values ranging from 60.0±0.5 to 112±1.1 μM for collagen and ADP. Ferulic acid, gallic acid and quercetin found to exert significant effect on COX-1 enzyme with IC50 values at 15.0±0.5, 9.0±0.1, 15.0±0.4 μM, respectively and kaempferol at a moderate level (IC50 58.0±0.9 μM). Ferulic acid, gallic acid, and kaempferol also inhibited COX-2 enzyme with IC50 values at 4.0±0.1, 14.0±0.3, 54.0±0.9 μM, respectively. Interesting, ellagic acid did not show any effect on both COX enzymes, whereas quercetin presented only anti-COX-1 activity at the concentrations tested. The study demonstrated the multifunctional role of the above natural compounds with all presenting high antiradical, and antiplatelet activity, while, differences and interesting data were obtained with respect to their anti-COX activity. The data showed the potential use of the most active compounds in dietary foods and pharmaceutical industries.

Keywords: phenolic compounds, antiplatelet activity, anti-inflammatory activity, collagen, ADP, COX enzymes

INTRODUCTION

The search for bioactive natural compounds, with multiple activities, such as antiplatelet and anti-inflammatory, and their isolation from plant sources has gained increasing importance nowadays, due to the growing worldwide concern about an alarming increase in the carcinogenicity of synthetic compounds. Natural products continue to provide greater structural diversity and offer great opportunities for finding novel bioactive compounds (Kandasamy et al., 2016).

Polyphenols are natural compounds found in most vegetables and fruits, being responsible for their taste and pigmentation. They represent a wide family of high-added value molecules, mainly known for their antioxidant properties, but also are reducing agents, and protect body tissues against oxidative stress and associated pathologies such as cancer, cardiovascular disease, allergies and inflammation (Mildner-Szkudlarz et al., 2010; Tapiero et al., 2002). Phenolic compounds are present in plant cell wall components, in fruits, such as pomegranate and several berries, tea, but also in agrochemical by-products, such as winery wastes and pomegranate peels. There have been several reports on the beneficial effects of these phenolic compounds against cardiovascular disease, cancer, antidiabetic and anti-carcinogenic effects (Favarin et al., 2013; Cerda et al., 2005; Berkban et al., 2015; Brenelli et al., 2013; Yilmaz and Usta, 2013). Some of the most known bioactive phenolic compounds with high antioxidant activity are ellagic acid, ferulic acid, quercetin, kaempferol, gallic acid, caffeic acid, etc. (Devi et al., 2015; Kumar and Pruthi, 2014; Singh et al., 2014; Gulcin 2006; Re et al., 1999).

Cardiovascular diseases, on the other hand, are the main cause of mortality and morbidity worldwide, accounting for nearly 30% of global deaths. Intravascular thrombosis, such as coronary, venous and arterial thrombosis, is cardiovascular diseases. The general pathogenesis of thrombosis includes platelet activation and so platelets play a very important role in cardiovascular diseases. Platelets are normally disc-shaped cells, which in the presence of a stimuli change into spiny spheres, bind to fibrinogen, aggregate and release their intracellular granules such as ADP (Jennings 2009). Platelet activation and aggregation are common factors in atherothrombotic events, and platelet aggregation might play a critical role in the atherothrombotic process (Chang et al., 2013; Al Awwadi et al., 2007). Patients with coronary heart disease tend to have increased platelet reactivity. Therefore, platelet aggregation inhibitors are still a promising approach for preventing thrombosis (Yu et al., 2016). It has been shown that platelet aggregation can be inhibited among others, by dietary fats and antioxidants, including phenolic compounds (Al Awwadi et al., 2007; Hubbard et al., 2003).

Despite the numerous reports on the beneficial effects to human health of natural phenolic compounds, a limited number of studies are dealing with antiplatelet or/and anti-inflammatory action of plant extracts and to a lesser extent, of specific phenolic compounds (Karlickova et al., 2016; Zhang et al., 2015; Mosawy et al., 2013; Chang et al., 2013; Lee and Kim, 2010; Navarro-Nunez at al., 2009).

Here, phenolic fractions from red and white wine wastes and fresh pomegranate peels were analysed using HPLC and ESI-mass spectrometry and compared to their standards. The identified compounds were then obtained in large amounts by preparative HPLC for further use. More specifically, we evaluated three phenolic acids (ellagic acid, gallic acid and ferulic acid) and two flavonols (quercetin and kaempferol) (Scheme 1), for their antioxidant activity, their ability to inhibit human platelet aggregation induced by collagen and ADP, as well as their ability to inhibit the cyclooxygenases 1 and 2 (COX-1 and COX-2) enzymes.

MATERIALS AND METHODS

Materials

Wine wastes were kindly provided by “Ktima Gerovassiliou”, a winemaking factory in Epanomi (Thessaloniki, Greece) in the vintage 2015 and pomegranates was purchased from the local market of Thessaloniki. All samples were dried at 45 oC and extracted using the method described previously by Moschona et al. (2015).

A stock solution of phenolic compounds was prepared in ethanol at a concentration of 700 μM and stored in the refrigerator (-20 oC) for further analysis. Ellagic acid (2,3,7,8-tetrahydroxy-chromeno[5,4,3-cde]chromene-5,10-dione), ferulic acid (4-hydroxy-3-methoxycinnamic acid), gallic acid (3,4,5-trihydroxybenzoic acid) and quercetin (2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one) were also purchased from Sigma-Aldrich Cor. and kaempferol (3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one) was purchased from Fluka Biochimica. Liquid collagen type I extracted from rat tail tendons and solid adenosine diphosphate (ADP) were purchased from the EMD Millipore Corporation. All other reagents were purchased from Sigma-Aldrich Cor.

Identification by HPLC and ESI-MS

The phenolic mixtures obtained from wine and pomegranate wastes were analysed by HPLC and ESI-mass spectrometry. The analysis was carried out using a Thermo Finnigan Spectra HPLC system (San Jose, California) model UV 6000 LP, equipped with EZChromeElite software, Version 3.1.7., four Q-Grad pumps, a diode array detector (DAD).The wavelengths used were 280 and 365 nm. Separations were performed using a Grace Smart RP C-18 column (250x4.6 mm i.d.; 5 μm particle size). The volume injected was 20 μL. The mobile phase consisted of 2% (v/v) acetic acid in milli-Q water (eluent A) and 100% acetonitrile (eluent B) using a gradient program as follows: from 0 min, 100% A; 4 min, 85% A/15% B; 20 min, 60% A/40% B; 40 min, 45% A/55% B. Total run time was 40 min and the flow rate was 1 mL/min. Electrospray ionization mass spectrometry (ESI-MS) experiments were performed using a Thermo Fisher Scientific (Bremen, Germany) model LTQ Orbitrap Discovery MS. The experiments were run using a standard ESI source operating in a positive and negative ionization mode. The source voltage was 3.7 kV and the MS worked with a 300°C heated capillary.

DPPH radical scavenging assay

The free radical scavenging activity of phenolic compounds against DPPH (2,2-diphenyl-2-picrylhydrazyl hydrate) was determined spectrophotometrically (Blois 1958). The solution of DPPH was prepared, daily, by dissolving 0.1 g of solid

DPPH in 1 L ethanol and kept in the dark at 4 °C between measurements. The percent decrease in absorbance (equation 1) was recorded for each phenolic compound tested, at a concentration of 350 μM, and percent quenching of DPPH radical was calculated on the basis of the observed decrease in absorbance of the radical.

% Inhibition = [(ADPPH – AExtr)/ADPPH)] * 100 (1)

Procedure of the in vitro platelet aggregation experiments

Platelets were obtained from venous blood of 14 healthy donors (males and females). Volunteers, aged from 25-45 years old had not taken any medication within the last 15 days, other non-steroidal anti-inflammatory drugs (NSAIDs), such as aspirin, anti-depressants and contraceptive pills. The blood was immediately mixed with 3.8% sodium citrate solution and after that was centrifuged at 1000 rpm for 10 min to yield platelet rich plasma (PRP) and subsequently the remainder centrifuged at 4000 rpm for 10 min to obtain platelet poor plasma (PPP). Collagen at a concentration of 5 μg/ml, and ADP at 20 μM were used as aggregation agents. Platelet aggregation experiments were performed by a conventional photometric technique in a four channel aggregometer (Carat TX4 Platelet Aggregometer), at 37 °C, with continuous recording of light transmission, according to the method of Born (Born and Cross, 1963).

The aggregation was determined by recording the increase of light transmission and the calibration was performed using PPP (Sarigiannis et al., 2002). The results are expressed as antiplatelet activity % and calculated from equation (2) and the IC50 values (the concentration in μM that gave 50% inhibition) of each of the tested substances were determined.

Antiplatelet Activity % = (maximum aggregation of collagen - maximum aggregation of sample)/maximum aggregation of collagen*100% (2)

Anti-inflammatory activity

The anti-inflammatory activity of phenolic compounds was tested using COX (cyclooxygenases 1 and 2) activity assay kit (CAYMAN CHEMICAL, USA). The values were determined according to the manufacturer's instructions and the activity of samples on the enzymes, is expressed as percent inhibition (% inhibition). The assay measures COX activity (COX-1 and COX-2) utilizing the peroxidase component of cyclooxygenases. Briefly, heme and COX-1 (ovine) enzyme were added to test tubes containing COX reaction buffer. The mixture was vortex mixed and exposed to vehicle (CH3OH) or test substance in CH3OH for 10 min at 37 °C. This was followed by the addition of arachidonic acid with further incubation for 2 min. S stannous chloride solution was added to stop enzyme catalysis and the prostaglandins were quantified by enzyme immunoassay (EIA) (Nurtjahja-Tjendraputra et al., 2003).

DATA ANALYSIS

All experiments were run in triplicate and the results are expressed as mean ± standard deviation (SD) values (n=3). All data were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Identification by HPLC and ESI-MS

The HPLC profile of phenolic compounds extracted from wine and pomegranate wastes was recorded at 280 nm and 365 nm. Figure 1 gives the HPLC profile of red wine waste (a) and white wine waste (b) at 365 nm with identified compounds gallic acid (1), ellagic acid (2), ferulic acid (3), quercetin (4) and kaempferol (5). Identification was based on retention times, UV adsorption spectra of relative peaks and MS (data obtained, but not shown) compared with those of reference compounds, obtained as well (data not shown).

DPPH radical scavenging assay

In an attempt to rate these five phenolic compounds with respect to their antioxidant capacity the method of DPPH was used. Figure 2 gives the percentage of their antiradical activity, at a concentration of 350 μM. As it can be seen, their antiradical activity is with a decreasing order of gallic acid, ellagic acid, kaempferol, quercetin and ferulic acid. Among phenolic acids, gallic and ellagic acid showed potent DPPH radical scavenging activity. The results of antiradical activity of kaempferol and quercetin are in agreement with previous reports (Lee and Kim, 2010).

Platelet aggregation

Ellagic acid, gallic acid, ferulic acid, quercetin, and kaempferol, due to their high antiradical activity, were also tested for their effect on platelet aggregation, in vitro. Different inducers (collagen, ADP, arachidonic acid) activate different pathways and transduction signals and therefore each phenolic compound was tested using two aggregation agents, collagen, and ADP. Figure 3 shows that the effect of all phenolic compounds on human platelet aggregation in vitro, against both inducers, is dose dependent. At concentrations of 25-700 μM tested, all compounds prevented platelets from collagen-induced aggregation with IC50 values determined at 60.0±0.5, 112.0±1.1, 108.0±0.9 μM for the ellagic, ferulic and gallic acid and at 64.0±1.0 and 111.0±0.4 μM for kaempferol and quercetin, respectively.

Similar experiments were conducted using ADP as aggregation inducer. ADP contributes to platelet activation occurring both during protective haemostasis and during the formation of occlusive platelet-rich thrombi (Jennings 2009). As it can be seen from Figure 4, their inhibition, as in the case of collagen, is dose-dependent with IC50 values determined at 68.0±0.8, 78.0±0.7, 98.0±0.6 μM for the ellagic, ferulic and gallic acid and at 60.0±0.7, 74.0±0.9 μM for kaempferol and quercetin, respectively.

Table 1 gives the IC50 values of all phenolic compounds tested against collagen and ADP-induced platelet aggregation. As shown in Table 1, ellagic acid and kaempferol presented the best IC50 values against collagen (60.0±0.5 and 64.0±1.0 μM, respectively) and against ADP-induced platelet aggregation (IC50 values 68.0±0.8 and 60.0±0.7 μM, respectively).

The inhibitory effect of phenolic compounds using different platelet agonists has been also reported by Nardini et al. (2007), suggesting that phenolic compounds act at multiple sites and levels of the signalling cascade. As it can be seen, IC50 values obtained are slightly better in the case of ADP-induced.

Anti-inflammatory activity

Inflammation can cause local thrombosis, and thrombosis can amplify inflammation. Thus, we can regard anti-inflammatory therapies as potentially antithrombotic. In addition, antithrombotic treatment may suppress inflammation and help break the vicious cycle of the acute coronary syndromes by limiting the local and systemic amplification loops (Libby and Simon, 2001). Thus, the effect of the five phenolic compounds on both COX enzyme activities was also investigated. As it can be seen in Figure 5, ellagic acid had no effect against COX-1, while all the other compounds ferulic acid, gallic acid, quercetin and kaempferol were found dose-dependent against COX-1, at concentrations between 3-25 μM.

Additionally, kaempferol, by increasing its concentration, shows a linear behavior against the COX-1 enzyme, while the other three compounds (quercetin, gallic and ferulic acids), seem to reach saturation above the concentration of 25 μM. The IC50 value for the percentage inhibition of COX-1 was determined and found to be 9.0±0.1 μM for gallic acid, 15.0±0.5 μM for both ferulic acid and quercetin and 58.0±0.9 μM for kaempferol.

Figure 6 depicts the effect of different concentrations of phenolic compounds on inhibition of COX-2 activity. As it can be seen, ellagic acid and quercetin had no effect on COX-2 activity. Ferulic acid, gallic acid and to a lesser extend kaempferol were the only phenolic compounds that showed inhibition against COX-2 enzyme with IC50 values at 4.0±.01 μM for ferulic acid, 14.0±0.3 μM for gallic acid and 54.0±0.9 μM for kaempferol. Kaempferol, as in the case of a COX-1 enzyme, shows a linearly-dependent response, up to the concentration of 70 μM.

Table 2 gives the IC50 values of phenolic compounds against COX-1 and COX-2, as well as the ratio COX-2/COX-1. As Vane and Botting (1996) suggested, low COX-2/COX-1 ratios values indicate more potent COX-2 inhibitors.

Platelets prevent bleeding and mediate haemostasis at the site of a vascular injury. Platelet aggregation is one of the most important situations in the procedure of thrombus formation in response to rupture of an atherosclerotic plaque as well as they work as mediators of inflammation. Platelets are activated by collagen, adenosine diphosphate, serotonin, arachidonic acid etc. following different pathways. Collagen is a strong thrombogenic component of the subendothelium. In a vascular injury, collagen is exposed to circulating platelets, acts as a substrate for the adhesion of platelets and subsequently induces platelet activation (Roberts et al., 2004). During activation, collagen causes platelet shape change and promotes secretion by platelets of the contents of storage granules, which include ADP and ATP.

Furthermore, the ADP contributes to platelet activation by the release of thromboxane A2 from adherent platelets, enhances recruitment and aggregation to the primary plug, followed by platelet activation during protective haemostasis and pathologic thrombus formation (Jennings 2009). The ADP, acting via P2Y1 and P2Y12 receptors on the platelet surface amplifies the effects of the primary stimulus provided by the collagen (Wijeyeratne and Heptinstall, 2011).

It was found that all compounds, namely ellagic acid, ferulic acid, gallic acid, quercetin, and kaempferol inhibited platelet aggregation induced by both collagen and ADP, with ellagic acid being the dominant one (Table 1). Noticeable, the IC50 values obtained for the phenolic compounds presented were better than that of aspirin (IC50 150 μM, in our experiments). Ferulic acid gave almost the same IC50 value to that reported (66.3 μM) by Zhang et al. (2015) against ADP induction. It has been reported that quercetin inhibited ADP-induced rat platelet aggregation by 68.33±2.43%, at a concentration of 331 μM (Guo et al., 2014), while Bijak et al. (2014) reported a dose-depended decrease of the thrombin-induced platelet aggregation by quercetin. The slightly better results in terms of IC50 values, which were obtained using ADP as aggregation agent, than collagen, could be explained since ADP is considered a mild platelet agonist.

Inflammation is a critical process for the protection against infection, but uncontrolled inflammation may contribute to tissue damage. As anti-inflammatory therapies limit thrombosis and reduce vascular inflammation, antithrombotic therapies may reduce vascular inflammation (Libby and Simon, 2001). As it is shown in Fig. 5 quercetin and ferulic acid had a highly potent effect against COX-1 enzyme (IC50 15.0±0.5 μM), whereas kaempferol a moderate one with IC50 at 58.0±0.9 μM. Interesting, ferulic acid showed also significant inhibition of the COX-2 enzyme (IC50 4.0±0.1 μM) and only kaempferol from flavonols possessed inhibitory activity against COX-2 (IC50 54.0±0.9 μM). Quercetin did not inhibit COX-2 enzyme at least at the concentrations tested, though in previous published results it is referred that most flavonoids, including quercetin, inhibit the COX-2 reaction, at higher concentrations (Lee and Kim, 2010).

Furthermore, ellagic acid did not show any inhibitory effect on both COX enzymes (Figs 5 and 6) while gallic acid, which is considered the monomer of ellagic acid, exerted a strong anti COX effect. Ellagic acid (Scheme 1) is a dilactone formed by the association of two molecules of gallic acid. Gallic acid had a significant effect against both COX-1 and COX-2 enzymes (Figs 5 and 6) with IC50 values at 9.0±0.1 and 14.0±0.3 μM, respectively. The lack of anti COX activity of ellagic acid may be attributed to its structure and specifically to the fact that its carboxyl groups are not free as in the case of gallic and ferulic acids. Furthermore, aspirin (Scheme 1), a well-known potent and representative COX inhibitor, has one aromatic ring and a free carboxyl group as gallic and ferulic acids, enhancing our observation, about the importance of the free carboxyl group in order for the phenolic acid to exert anti- inflammatory activity. Expansions of the study to more natural or synthetic phenolic compounds will clarify any structure-activity relationship.

As it has been mentioned, COX-1 and COX-2 represent key enzymes in the bioconversion of arachidonic acid to inflammatory prostaglandins. The difference between the two isoforms of COX enzyme is that COX-1 is expressed constitutively in almost all cell types, including platelets and those present in stomach, kidney, vascular endothelium, forebrain and uterine epithelium and is regulated as a housekeeping enzyme for various physiological functions, whereas COX-2 is inducible and expressed during tissue damage or inflammation in response to pro-inflammatory cytokines.

Thus, selective COX-2 inhibitors could reduce the undesired side effects arisen from inhibition of COX-1 activity (Blobaum and Marnett, 2007; Simmons et al., 2004; Brooks 2000; Williams et al., 1999). Based on the above, we attempted to rank the selectivity of our COX inhibitors by using the ratio COX-2/COX-1, as reported previously (Vane and Botting, 1996). Thus, ferulic acid followed by kaempferol and gallic acid, with ratio values at 0.27, 0.93 and 1.56 respectively, can be considered selective COX-2 inhibitors.

Moreover, anti-inflammatory therapies using NSAIDs have been associated with undesired cardiovascular side effects, such as heart attacks (Al-Saeed 2011). Thus, it is important for the compounds to possessing both anti-inflammatory and antiplatelet activity. Concluding, our results demonstrate that the specific phenolic compounds examined here, have a good inhibitory effect against two different platelet activation

inducers and three of them also showed strong anti-inflammatory activity.

CONCLUSION

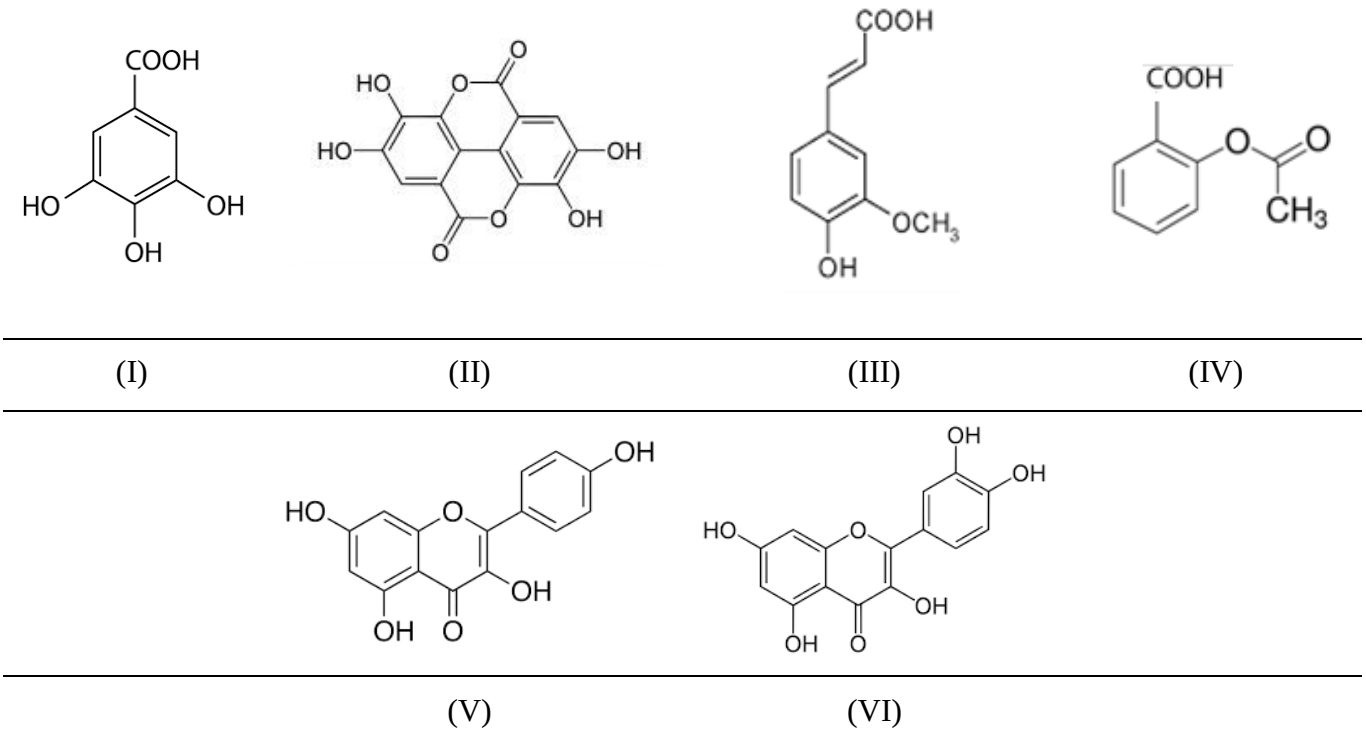
Our comparative study revealed interesting data regarding the source of the examined compounds, their potency, and functionality. All, compounds (phenolic acids and flavonols), presented both antioxidant and antiplatelet activity, with their potencies not interrelated. Differences were observed in the inhibition of COX enzymes, with ellagic acid lacking such activity and quercetin presenting only anti-COX-1 activity at the concentrations examined. The other three, ferulic acid, kaempferol and gallic acid inhibited both COX enzymes with selectivity towards COX-2. The data are very promising and the most active compounds may have used in dietary foods and in pharmaceutical industry. Though, in vivo experiments will enhance the above results.

CONFLICT OF INTEREST

The authors confirm that there is no conflict of interest in this work.

ACKNOWLEDGEMENTS

The authors would like to thank the winery "Ktima Gerovassiliou" for providing wine waste samples of white and red vinification.
Part of this research work was supported by "11SYN_2_1992" action "COOPERATION 2011" of EYDE-ETAK funded by the Operational Program "Competitiveness and Entrepreneurship" (EPAN-II).



Scheme 1. Chemical structures of (I): gallic acid, (II): ellagic acid (III): ferulic acid, (IV): aspirin (acetylsalicylic acid), (V): kaempferol, and (VI): quercetin.

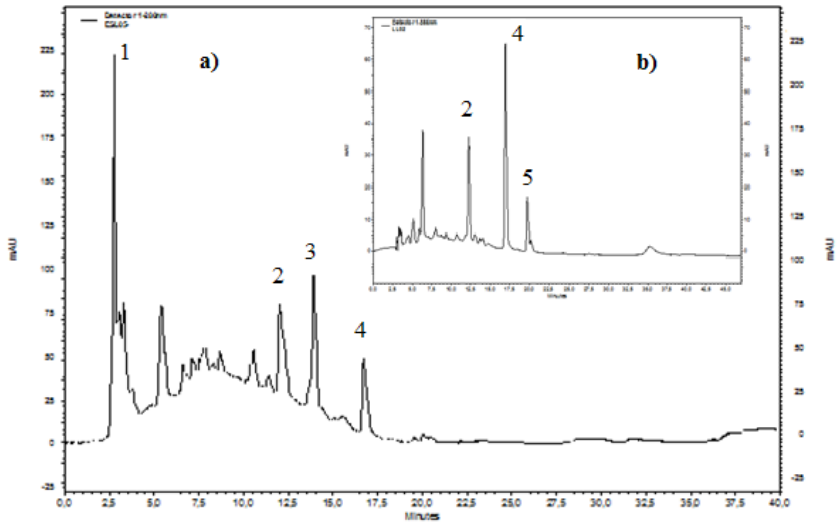


Fig. 1 HPLC analysis at 365nm of (a) red wine and (b) white wine wastes fractions: gallic acid (1) ellagic acid (2), ferulic acid (3), quercetin (4) and kaempferol (5).

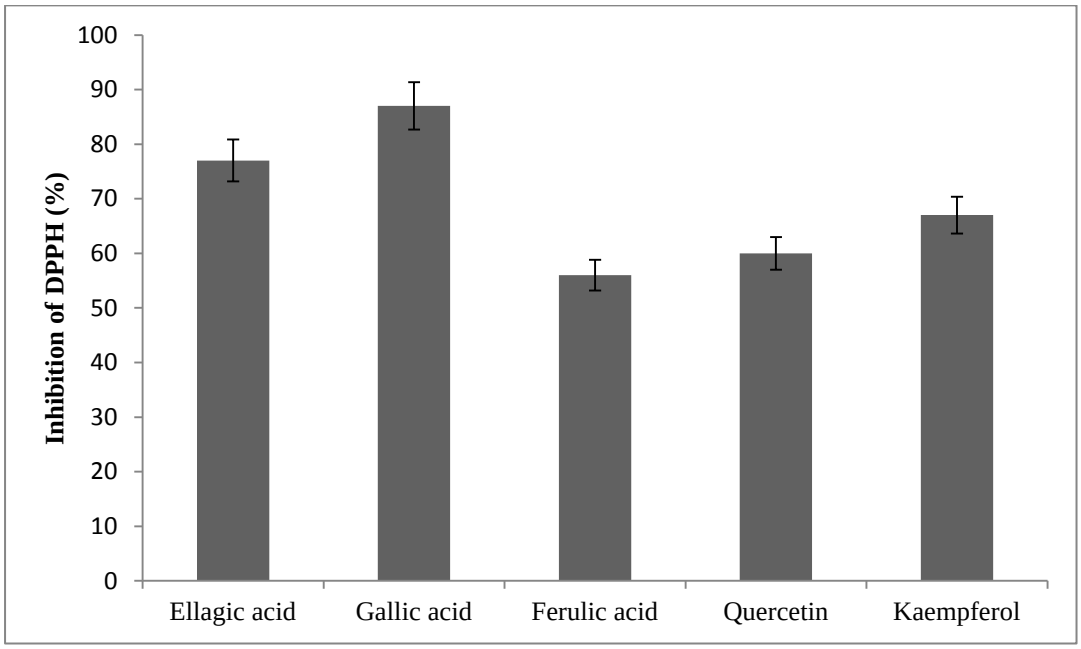


Fig. 2 DPPH radical scavenging activity of phenolic compounds.

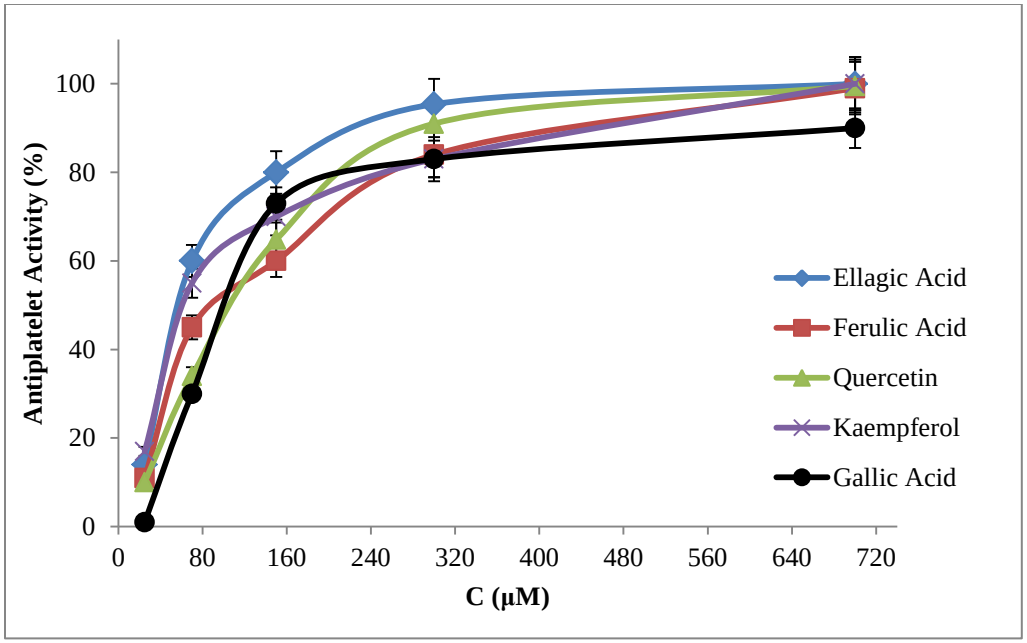


Fig. 3 Effect of concentration of phenolic compounds on human platelet aggregation *in vitro*, induced by collagen (5 µg/ml).

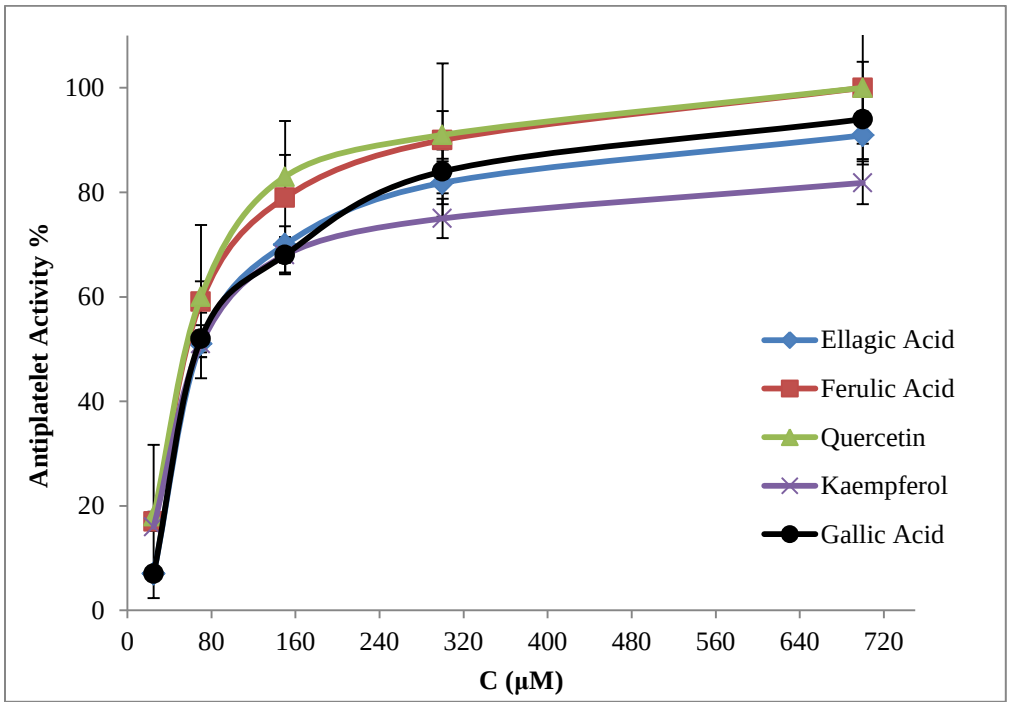


Fig. 4 Effect of concentration of phenolic compounds on human platelet aggregation *in vitro*, induced by ADP (20 µM).

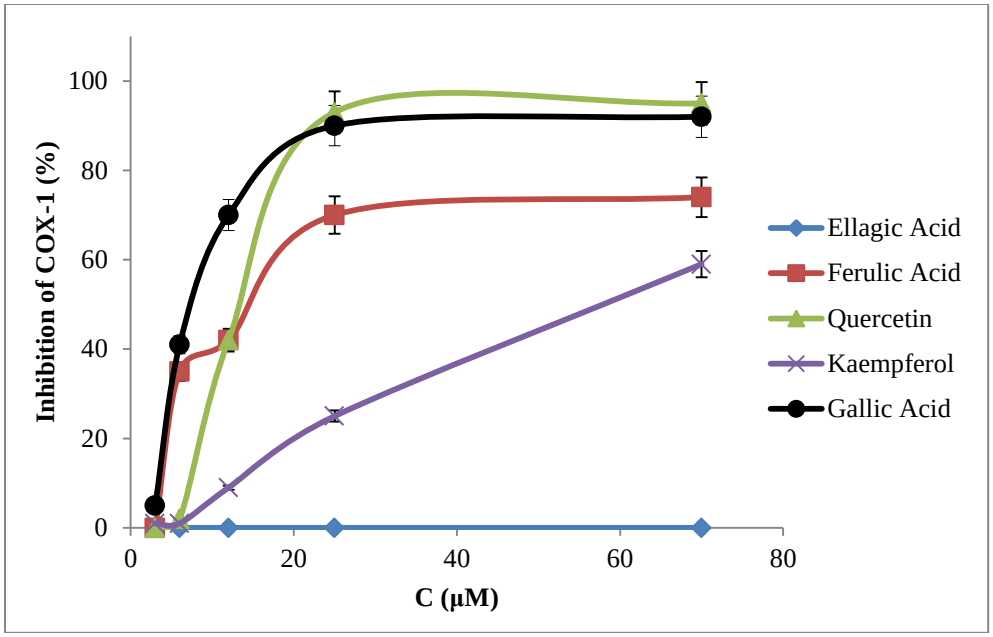


Fig. 5 Anti COX-1 effect of ellagic acid, ferulic acid, gallic acid, quercetin and kaempferol at different concentrations.

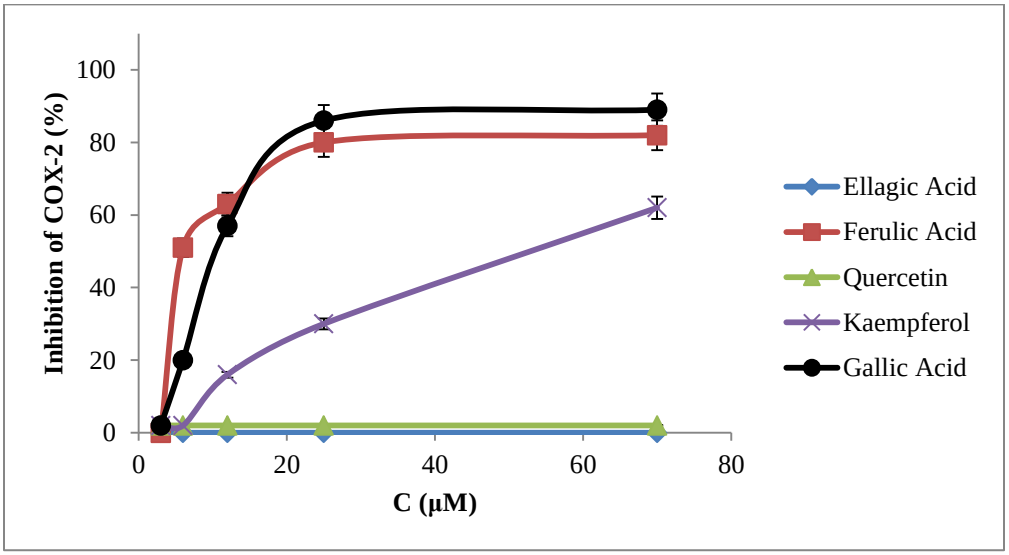


Fig. 6 Anti COX-2 effect of ellagic acid, ferulic acid, gallic acid, quercetin and kaempferol at different concentrations.

Table 1. IC_{50} values of antiplatelet activity of phenolic compounds against collagen and ADP.

	IC_{50} value (µM)	
Phenolic compounds	**Collagen**	**ADP**
Ellagic acid	60.0±0.5	68.0±0.8
Gallic acid	108.0±0.9	98.0±0.6
Ferulic acid	112.0±1.1	78.0±0.7
Quercetin	111.0±0.4	74.0±0.9
Kaempferol	64.0±1.0	60.0±0.7

Table 2. IC_{50} values of anti-inflammatory activity of phenolic compounds against COX-1 and COX-2.

	IC_{50} value (µM)		
Phenolic compounds	**COX-1**	**COX-2**	**COX-2/COX-1**
Ellagic acid	0.0±0.0	0.0±0.0	0.00
Gallic acid	9.0±0.1	14.0±0.3	1.56
Ferulic acid	15.0±0.5	4.0±0.1	0.27
Quercetin	15.0±0.4	0.0±0.0	0.00
Kaempferol	58.0±0.9	54.0±0.9	0.93

REFERENCES

1. Al Awwadi N A, Borrot-Bouttefroy A, Umar A, Saucier C, Segu MC, Garreau C, Canal M, Glories Y, Moore N (2007) Effect of Armagnac fractions on human platelet aggregation in vitro and on rat arteriovenous shunt thrombosis in vivo probably not related only to polyphenols. Thromb Res 119: 407-413
2. Al-Saeed A (2011) Gastrointestinal and cardiovascular risk of nonsteroidal anti-inflammatory drugs. Oman med j 26:385-391
3. Berkban T, Boonprom P, Bunbupha S, Welbat JU, Kukongviriyapan U, Kukongviriyapan V, Pakdeechote P, Prachaney P (2015) Ellagic acid prevents L-NAME-Induced hypertension via restoration of eNOS and p47phox expression in rats. Nutrients 7:5265-5280
4. Bijak M, Ziewiecki R, Saluk J, Ponczek M, Pawlaczyk I, Krotkiewski H, Wachowicz P, Nowak P (2014) Thrombin inhibitory activity of some polyphenolic compounds. Med Chem Res 23: 2324-2337
5. Blobaum AL, Marnett LJ (2007) Structural and functional basis of cycloxygenase inhibition. J Med Chem 50(7):1425-1441
6. Blois MS (1958) Antioxidant determinations by the use of a stable free radical. Nature 181:1199-1200
7. Born GVR, Cross MJ (1963) The aggregation of blood platelets. J Phys 168:178-195
8. Brenelli de Paiva L, Goldbeck R, Dantas dos Santos W, Marcio Squina F (2013) Ferulic acid and derivatives: molecules with potential application in the pharmaceutical field. Brazilian J Pharm Sci 49(3):395-411
9. Brooks PM (2000) Experimental and clinical pharmacology COX-2 inhibitors. A Pres 23(2):30-32
10. Cerda B, Tomas-Barberan FA, Espin JC (2005) Metabolism of antioxidant and chemopreventive ellagitannins from strawberries, raspberries, walnuts, and oak-aged wine in humans: identification of biomarkers and individual variability. J Agric Food Chem 53:227-235
11. Chang Y, Chen WF, Lin KH, Hsieh C-Y, Chou DS (2013) Novel bioactivity of ellagic acid in inhibiting human platelet activation. Evid-Based Compl Alt 2013:1-9
12. Devi KP, Malar DS, Nabavi SF, Sureda A, Xiao J, Nabavi SM, Daglia M (2015) Kaempferol and inflammation: from chemistry to medicine. Pharmacol Res 99:1-10
13. Favarin D C, Teixeira M M, Lemos de Andrade E, Freitas Alves C, Chica J EL, Sorgi CA, Faccioli LH, Rogerio AP (2013) Anti-Inflammatory effects of ellagic acid on acute lung injury induced by acid in mice. Mediators Inflamm Hindawi Pub Corp 2013:1-13
14. Gulcin I (2006) Antioxidant activity of caffeic acid (3,4-dihydroxycinnamic acid). Toxicology 217:213-220
15. Guo C, Liu S, Guo Y, Yin Y, Lin J, Chen X, Sun MZ (2014) Comparative function-structural analysis of antiplatelet and

antiradical activities of flavonoid phytochemicals. J Anim Plant Sci 24:926-935
16. Hubbard GP, Wolffram S, Lovegrove JA, Gibbins JM (2003) The role of polyphenolic compounds in the diet as inhibitors of platelet function. Proc Nutr Soc 62(2):469-78
17. Jennings LK (2009) Mechanisms of platelet activation: need for new strategies to protect against platelet-mediated atherothrombosis. Thromb Haemostasis 102(2):248-257
18. Kandasamy S, Ramu S, Aradhya SM (2016) In vitro functional properties of crude extracts and isolated compounds from banana pseudostem and rhizome. J Sci Food Agric 96:1347-1355
19. Karlickova J, Riha M, Filipsky T, Macakova K, Hrdina R, Mladenka P (2016) Antiplatelet effects of flavonoids mediated by inhibition of arachidonic acid based pathway. Planta Med 82:76-83
20. Kumar N, Pruthi V (2014) Potential applications of ferulic acid from natural sources. Biotech Rep4:86-93
21. Lee JH, Kim GH (2010) Evaluation of antioxidant and inhibitory activities for different subclasses flavonoids on enzymes for rheumatoid arthritis. J Food Sci 75(7):212-217
22. Libby P, Simon DI (2001) Inflammation and thrombosis: The clot thickens. Circulation 103:1718-1720
23. Mildner-Szkudlarz S, Zawirska-Wojtasiak R, Gośliński M (2010) Phenolic compounds from winemaking waste and its antioxidant activity towards oxidation of rapeseed oil. Int J of Food Sci Tech 45:2272-2280
24. Mosawy S, Jackson DE, Woodman OL, Linden MD (2013) Treatment with quercetin and 30,40-dihydroxyflavonol inhibits platelet function and reduces thrombus formation in vivo. J Thromb Thrombolys 36:50-57
25. Moschona A, Ziagova MG, Iliadou A, Liakopoulou-Kyriakides M. (2016) Optimization studies for selective recovery of phenolics from wine wastes. Agric Sci 7:187-195
26. Nardini M, Natella F, Scaccini C (2007) Role of dietary polyphenols in platelet aggregation. A review of the supplementation studies. Platelets 18(3):224-243
27. Navarro-Nunez L, Rivera J, Guerrero JA, Martínez C, Vicente V, Lozano ML (2009) Differential effects of quercetin, apigenin and genistein on signalling pathways of protease-activated receptors PAR1 and PAR4 in platelets. Brit J Pharmacol 158:1548-1556
28. Nurtjahja-Tjendraputra E, Ammit AJ, Roufogalis BD, Tran Van H, Duke C C (2003) Effective anti-platelet and COX-1 enzyme inhibitors from pungent constituents of ginger. Thromb Res 111:259-265
29. Roberts D, McNicol A, Bose R (2004) Mechanism of collagen activation in human platelets. J Biol Chem 279:19421-19430
30. Sarigiannis YM, Stavropoulos GP, Liakopoulou-Kyriakides MT, Makris PE (2002) Novel synthetic RGD analogs incorporating salicylic acid derivatives show antiplatelet activity in vitro. Lett Pept Sc 9:101-109
31. Simmons DL, Botting RM, Hla T (2004) Cyclooxygenase isozymes: The biology of prostaglandin synthesis and inhibition. Pharmacol Rev 56(3):387-437
32. Singh M, Kaur M, Silakar O (2014) Flavones: An important scaffold for medicinal chemistry. Eur J Med Chem 84:206-239
33. Tapiero H, Tew K D, Nguyen Ba G, Mathé G (2002) Polyphenols: do they play a role in the prevention of human pathologies? Biomed Pharmacother 56:200-207
34. Vane JR, Botting RM (1996) Mechanism of action of anti-inflammatory drugs. Scand J Rheumatol, 25(suppl 102): 9-21
35. Wijeyeratne YD, Heptinstall S (2011) Anti-platelet therapy: ADP receptor antagonists. Br J Clin Pharmacol 72(4):647-657
36. Williams CS, Mann M, DuBois RN (1999) The role of cyclooxygenases in inflammation cancer, and development. Oncogene 55:7908-7916
37. Yu G, Wang F, Zhang B, Fan J (2016) In vitro inhibition of platelet aggregation by peptides derived from oat (Avena sativa L.), highland barley (Hordeum vulgare Linn. var. Nudum hook F.), and buckwheat (Fagopyrum esculentum Moench) proteins. Food Chem 194:577-586
38. Yılmaz B, Usta C (2013) Ellagic acid-induced endothelium-dependent and endothelium-independent vasorelaxation in rat thoracic aortic rings and the underlying mechanism. Phytother Res 27:285-289
39. Zhang PX, Lin H, Qu C, Tang YP, Li NG, Kai J, Shang G, Li B, Zhang L, Yan H, Liu P, Duan JA (2015) Design, Synthesis, and In Vitro Antiplatelet aggregation activities of ferulic acid derivatives. J Chem Hindawi Pub Corp 2015:1-7