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Punica Granatum L (pomegranate) Leaves Extract Exhibits Antioxidant, Anticoagulant and Antiplatelet Properties

Research Article

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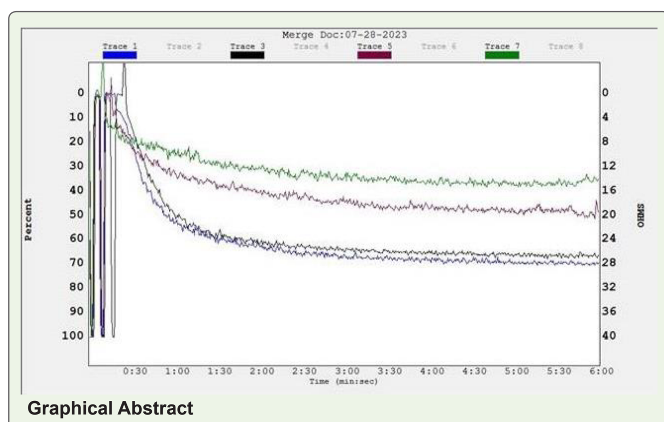
Abstract

Punica granatum L (pomegranate) belongs to *Lythraceae* family commonly known as pomegranate. The leaves of pomegranate are being extensively utilized in the folk medicine since long time. Various researchers reported the anti-inflammatory, anti-cholinesterase, anti-diabetic, anti-cancerous, cytotoxic, anti-bacterial and antibiotic properties. While, none of the studies claims the therapeutic utility of pomegranate in oxidative stress induced pathogenesis in specific thrombosis. Thus, the current study evaluates the antioxidant and antithrombotic properties *of Punica granatum* Leaves Aqueous Extract (PGLAE). Qualitative analysis of *PGLAE* exposed the presence of carbohydrates, tannins, alkaloids, flavonoids, steroids and polyphenols. *PGLAE* displayed about 95% of DPPH scavenging activity with an IC_{50} value of 4.5μg/ml. Interestingly *PGLAE* significantly (***p<0.0001) normalized the stress markers such as Lipid Peroxidation (LPO), Protein Carbonyl Content (PCC), Total Thiol (TT), endogenous antioxidant enzymes such as Superoxide dismutase (SOD) and catalase (CAT) in sodium nitrite induced oxidative stress in RBC. In addition, *PGLAE* caused strong anticoagulation in platelet rich plasma by extending the clotting time from control 181sec to 1120sec. The anticoagulant effect of *PGLAE* was also strengthened by APTT and PT tests. *PGLAE* was positive for only APTT test, revealed the identified anticoagulant effect of *PGLAE* could be due its interference in intrinsic pathway of blood coagulation cascade. *PGLAE* also exhibited antiplatelet activity by inhibiting ADP induced platelet aggregation with an inhibition percentage of 74%. *PGLAE* was nontoxic in nature as it was devoid of hemolysis of RBCs..

Keywords: Oxidative stress; Antioxidant; Anticoagulant; Antiplatelet properties



Graphical Abstract

Introduction

Pomegranate (*Punica granatum*), belongs to the family *Lythraceae* commonly known as pomegranate and it is native to Asia. Perhaps, it is broadly distributed throughout the India and major commercial crop of southern India. It is to note that fruits, fruit peel, flowers, seeds and leaves of pomegranate stores batteries of phytochemicals attributed to potential health benefits [1]. The fruit of pomegranate have been extensively consumed due to its immense therapeutic property. While, the pomegranate leaves have been used in the folk medicine since olden days to treat sore throat infections, thrush and urinary tract infections, fever, flu, and pneumonia [2,3]. Although, it is richest source of plethora of phytochemicals, researchers documented that pomegranate found contains high number of phenolic compounds

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responsible for antioxidant, antimicrobial, anti-inflammatory, anti-diabetic, and anticancer properties [4,5,6]. In addition, leaves also the key reservoir of phenolic compounds, fatty acids, and essential oils, responsible for radio-protective, peroxidation, inhibition of lipid oxidation and antioxidant property. Thus, leaves are also extensively used in the food industry for the production of preservatives [7,8]. Therefore, pomegranate leaves have been grasping the attention of scientists to validate their hidden therapeutic potential in managing various infectious and noninfectious diseases.

Reactive oxygen species (ROS) and Reactive nitrogen species (RNS) formed during oxidative stress are typically considered as harmful, toxic, mutagenic and carcinogenic [9]. Thus, oxidative stress is the key cause for life style diseases such as, diabetes mellitus, arthritis, cardiovascular complications (stroke, heart attack and thrombosis), cancer and thrombosis [10]. High frequency of deaths has been documented due to cardiovascular complications such as stroke and heart attack. Recent reports suggest that the thrombotic disorders are account for 1 in 3 deaths worldwide [11]. ROS and RNS at higher level not only alter the functions of platelets and coagulation factors but also damage the RBC and WBCs by generating RBC and WBC mediated ROS [12]. Generally, ROS induced eryptosis leads to membrane asymmetry, externalization of phosphatidyl serine and heme leakage turned out to into prothrombotic phase [13]. High rate of heme released from damaged red blood cells consider to be not only cytotoxic but also activates primary hemostasis (platelets) through endothelial damage by recruiting nitric oxide [14].

In a way, combitorial effect of ROS, RNS and cellular ROS are the major elicitors of thrombosis and other life style diseases [15]. Hence, it is an urgent need for the identification of new therapeutic agents for the treatment of oxidative stress and thrombosis. Perhaps, antithrombotic drugs that are currently available in the market tend to cause severe life-threatening side effects [16]. The plant-based medicines are also an ancient method to treat many diseases. Hence, current study aims to characterize key phytochemicals of *Punica granatum* Leaves Aqueous Extract and its antioxidant and antithrombotic properties.

Materials and methods

Reagents

1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,4-dinitrophenyl-hydrazine (DNPH), Trichloro acetic acid (TCA), 95% ethanol, Sodium nitrite (NaNO2), acetic acid, Thiobarbituric acid, Tetra Methyl Ethylene Diamine (TEMED), Ethylene Diamine Tetra Acetic Acid (EDTA), Hydrogen peroxide were purchased from Sigma Chemical Company (St. Louis, USA). Adenosine diphosphate (ADP) and Epinephrine were purchased from Sigma Chemical Company (St. Louis, USA). The human blood samples obtained from the healthy donors for the platelet-rich plasma (PRP).

Preparation of Punica Granatum Leaves Aqueous Extract (PGLAE)

Punica granatum leaves were collected from Komalapura village, Periyapatana taluk, Mysore district. About 50g of leaves were washed thoroughly, using grinder (SHARP, Japan), leaves were powdered. Then using 500ml of double distilled water the powder was mixed properly, centrifuged at 1500g for 20min at 15°C and collected the supernatant. Then the obtained supernatant was lyophilized. The dried *Punica granatum* Leaves Aqueous Extract (*PGLAE*) was kept at 4°C. Required amount of powder was weighed and dissolved in double distilled water for further studies.

Qualitative analysis

PGLAE was subjected for qualitative analysis using the method of Harbone et al., [17] as follows.

Test for carbohydrates, proteins, lipids: About 1ml of PGLAE was treated with few drops of alcoholic α-naphthol solution in a test tube. The appearance of violet ring at the intersection indicates the presence of carbohydrates. The PGLAE (1ml) was treated with 10% NaOH solution followed by the addition of few drops of copper sulfate ($CuSO_4$) solution. The development of violet pink color indicates presence of protein. About 1ml of PGLAE was treated with alcoholic KOH (0.5N) followed by the addition of 1 drop of phenolphthalein indicator. The solution was heated in water bath for 1h, the noticed white color foam indicates the presence of lipids.

Test for alkaloids, tannins, flavonoids and phenol: Briefly 1ml of PGLAE was added with a few drops of Hager's reagent (saturated solution of picric acid). The existence of alkaloids indicates the formation of yellow precipitation. About 1ml of PGLAE was received with 1% gelatin solution having sodium chloride. The development of white precipitate suggests the existence of tannins. About 1ml of *PGLAE* was mixed with a few drops of acidic anhydride thereafter boiled and cooled. A few drops of concentrated sulfuric acid were added by sides of the test tubes, the formation of brown ring at the intersection of the two layers represent steroids in the test tube mixture. Approximately, 1ml of *PGLAE* was added with sulfuric acid and formation of orange color indicates the presence of flavonoids. About 1ml of *PGLAE* was added with 5% ferric chloride solution and the development of deep blue or black color indicate the presence of phenol.

Glycoside's test and Triterpenoid's test: The *PGLAE* (1ml) was hydrolyzed with concentrated H_2SO_4 for 2h in a water bath and filtered. The filtrate was shaken with chloroform followed by the addition of 10% ammonia. The formation of pink color indicates the amount of glycoside present in the sample. Briefly 1ml of *PGLAE* was added and shaken with concentrated sulfuric acid, the formation of color at the lower layer indicates the presence of triterpenoids.

Determination of the antioxidant activity by the DPPH assay

The method of Okoh et al., [18] was followed to carry out the DPPH radical scavenging assay. A DPPH radical solution was prepared by using 95% ethanol. The different concentration of *PGLAE* (5-30μg) was mixed with 150μL of DPPH radical solution and the final volume was made to 600μL by using 95% of ethanol and incubated for 30min at 37°C. The absorbance of the reaction mixture was measured by using spectrophotometer at 517nm. Ethanol was used as a blank and ascorbic acid was used as a positive control. The radical scavenging activity was calculated by using the given below formula.

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$$\text{DPPH scavenging activity (\%)} = \frac{(\text{Absorbance of control} - \text{Absorbance of the sample})}{(\text{Absorbance of control})} \times 100$$

Oxidative stress induction in HRBCs by using sodium nitrite ($NaNO_2$) inducer

The method of Luqman and Rizvi et al., [19] was followed to induce the oxidative stress in HRBCs model. In briefly, 1ml of washed RBC suspension was preincubated with different doses of *PGLAE* (20-80µg) for 30min in a clean test tube. Then 20µl of NaNO2 (10mmol/l) was added to the test tubes and further rested for 90min at 37°C. The NaNO2-untreated RBC was considered as positive control and NaNO2-treated RBC (absence of PGLAE sample) was taken as a reference control. Lastly, 2mg/ml of RBC lysate was taken from each tube and measured the level of lipid peroxidation (LPO), protein carbonyl content (PCC), Total Thiol (TT) and antioxidant enzyme (superoxide dismutase and catalase) activity.

Determination of Lipid peroxidation (LPO)

The method of Ohkawa et al., [20] was followed to estimate the lipid peroxidation. Briefly, 100 µL of (10mM) NaNO2 was mixed with RBCs incubated with *PGLAE* then about 1.5mL of acetic acid (pH 3.5, 20% v/v), SDS (8% w/v, 0.2mL) and 1.5mL thiobarbituric acid (0.8% w/v) added to the reaction mixture and were boiled at 45-60°C for 45min and centrifuged at 2000rpm for 10min. The formed adducts were extracted by using 3ml of 1-butanol. The TBRS (Thiobarbituric acid-reactive substance) in the supernatant was measured by spectrophotometrically (Thermo Scientific Biomate 6, USA) at 532nm. The values were stated in terms of malondialdehyde (MDA) corresponding to µmol MDA formed/mg of protein.

Determination of Protein Carbonyl Content (PCC)

The method of Levine et al., [21] was followed to assess the protein carbonyl content. Briefly 100µL of 10mM NaNO2 was treated with RBC preincubated with *PGLAE* in a clean test tube. Subsequently, an equal amount of 10mM 2,4-dinitrophenylhydrazine (DNPH) was added in a 2mmol/L HCl mixed well and left for 1h at room temperature. Only 2mmol/L HCl was used for blank. To obtain the precipitation of the reaction mixture, TCA (20%, w/v) was added and centrifuged at 1200rpm for 15min. the precipitate from the reaction mixture was cleaned using acetone and centrifuged for 15min at 2500rpm to get the final pellet. At the end, 20mM of 1ml Tris buffer [pH 7.4 having 0.14M NaCl, 2% SDS (w/v)] was used to melt the pellet and OD of the supernatant was estimated spectrophotometrically (Thermo Scientific Biomate 6 USA) at 360nm. The results were expressed as µmol carbonyl groups/mg of protein.

Measurement of total thiols (TT)

The method of Zinellu, et al., [22] was used to assess the total thiol content. In briefly, 100µl of 10mM NaNO2 was treated with RBCs which are preincubated with *PGLAE* in a clean test tube. About 0.375ml of 0.2M Tris-HCl buffer (pH 8.2) was added and vortexed. The reaction mixture was incubated for 30min by adding 10mM dithiol-bis-nitro benzoic acid (DTNB) and 1.975ml of methanol. The reaction mixture was centrifuged at 5000rpm for 10min. The samples' clear supernatants were collected and measured for photometric absorbance at 412nm, with the thiol content expressed as nmol of DTNB oxidized/mg protein.

Superoxide dismutase (SOD) activity

The method of Sundaram et al., [23] was followed to assess the SOD enzyme activity. In briefly, about 0.05mg protein from RBC lysate containing PGLAE (20-80µg/ml) with an agonist 10mM NaNO2 taken in a clean dry test tube. The reaction mixture was mixed with 1ml of 16mM phosphate buffer (pH 7.8) containing TEMED–EDTA (8mM/0.08mM) mixture. The decrease in absorbance was evaluated spectrophotometrically (Thermo Scientific Biomate 6 USA) at 406nm. The obtained result was expressed in U/mg of protein.

Catalase (CAT) activity

The procedure of Beers et al., [24] was followed asses the CAT enzyme activity. In briefly 0.05mg of protein lysate from RBC treated with PGLAE (20-80µg/ml) was taken in a clean, dry test tubes. The reaction mixture added with 1ml of 100mM phosphate buffer (pH 7.4) and 8.8mM H_2O_2. The optical density of the reaction mixture was measured spectrophotometrically at 240nm and the CAT activity was expressed as U/mg of protein.

Plasma re-calcification time

The assay was carried out according to the method of Quick et al., [25]. The crude *PGLAE* (2-12µg) was treated with PRP in 10mM 20µL tris HCl buffer of pH 7.4 for 1min at room temperature. The incubated mixture was treated with 20µL of 0.25M CaCl2 and the clotting time was recorded.

PT and APTT

As described by the method of Gangaraju S et al., [26] activated partial thromboplastin time and prothrombin time were carried out. The PGLAE (2-10µg/ml) was pre-incubated with 100µl of human citrated plasma for 1min. For APTT, 100µl of LIQUICELINE (Cephaloplastin derived from rabbit brain with phospholipids and ellagic acid preparation) was activated for 3min at 37°C. The clotting time was measured by adding 100µl of 0.02M CaCl2. For conduct Thromboplastin (PT), the clotting time was initiated by adding 200µl of PT reagent. The clot formation time was recorded in seconds.

Platelet aggregation

According to the method of Born [27], the turbidimetric approach was used inspect the impact of *PGLAE* on platelet function by using chronology dual channel whole blood optical lumi aggregation system. The varied concentration of *PGLAE* (20–60µg) and PRP (0.25ml) were preincubated and agonist was started by adding 10µM ADP and aggregation was monitored for 6min.

Direct hemolytic activity

Briefly, 1ml of erythrocyte and 9ml of PBS were mixed thoroughly. About 1ml of hematocrit suspension was incubated with PGLAE (50-200µg) at 37°C for 1h. The reaction mixture was added with 9ml of ice-cold PBS to terminate the reaction. The reaction mixture was centrifuged at 1500rpm for 10min. The release of hemoglobin in the supernatant was measured by spectrophotometer (Thermo Scientific Bio-mate 6, USA) at 540nm and percentage of hemolysis was calculated and plotted.

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Results

PGLAE exhibited antioxidant activity due to stored plethora of secondary metabolites

The initial screening of PGLAE was done by qualitative analysis of phytochemicals. The study revealed the presence of alkaloids, tannins, steroids, polyphenols, glycoside and triterpenoids (Table1). Thus, antioxidant activity of PGLAE was carried out, interestingly, PGLAE exhibited antioxidant activity by scavenging DPPH radical in a dose dependent manner. PGLAE showed about 70% radical with an IC_{50} value 4.94μg/ml (Figure 1).

PGLAE regulates NaNO2-induced oxidative stress marker *in-vitro*

The level of lipid peroxidation was measured as liberation of malondialdehyde (MDA) content. In case of $NaNO_2$ treated RBCs the level of MDA was significantly (P< 0.001) increased. Whereas in PGLAE treated RBCs, the level of MDA was significantly (P< 0.001) normalized as compared to normal RBCs alone in a dose dependent manner (Figure 2A). Similarly, the protein carbonyl content in $NaNO_2$ treated RBCs was elevated compare to the normal. But in case of PGLAE treated RBCs there was a significant (P<0.001) decrease in the level of carbonyl content was noticed (Figure 2B). Similarly, the total thiol content was also increased in $NaNO_2$ treated RBCs, however, in PGLAE treated RBCs there was a significant decrease in the level of thiol content was identified (Figure 2C). Furthermore, PGLAE was also normalized the anti-oxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) activities in NaNO2-treated RBCs. In the case of NaNO2-treated RBCs, there was a significant (P< 0.01) decrease in the SOD and CAT activities (Figure 3A) (Figure 3B). Perhaps, PGLAE restored the SOD and CAT activity in a dose-dependent manner with a statistically significant value at the concentration of 80μg (P< 0.01& P< 0.001) respectively.

PGLAE exhibited anticoagulant property by interfering with intrinsic and extrinsic pathway of blood coagulation

PGLAE exhibited anti-coagulation by increasing the clotting time of PRP control 200s to 1010s (Figure 4A). *PGLAE* delayed the clotting time of both APTT and PT suggesting its anti-coagulant effect was due to the interference in both intrinsic and extrinsic pathway of blood coagulation cascade (Figure 4B).

Table 1: Phytochemical analysis of PGLAE

Preliminary test	Results
Carbohydrates	+
Proteins	+
Lipids	–
Alkaloids	+
Tannins	+
Steroids	+
Flavonoids	+
Phenols	+
Glycosides	+
Triterpenoids	+

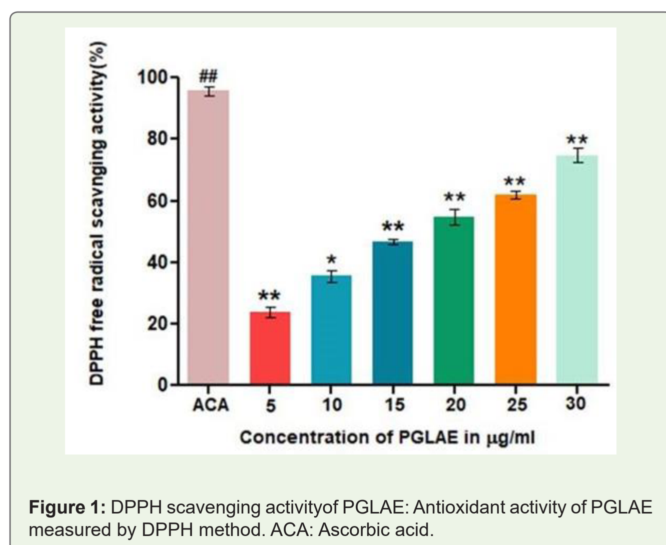

Figure 1: DPPH scavenging activityof PGLAE: Antioxidant activity of PGLAE measured by DPPH method. ACA: Ascorbic acid.

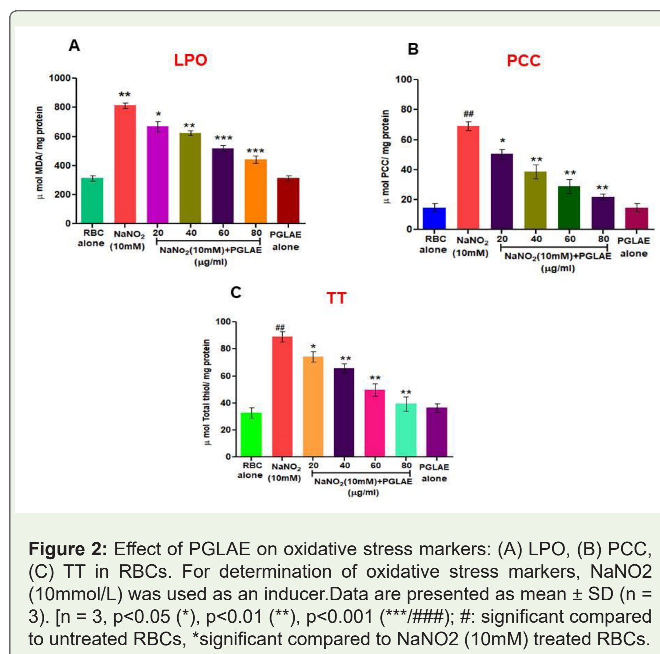

Figure 2: Effect of PGLAE on oxidative stress markers: (A) LPO, (B) PCC, (C) TT in RBCs. For determination of oxidative stress markers, NaNO2 (10mmol/L) was used as an inducer.Data are presented as mean ± SD (n = 3). [n = 3, p<0.05 (*), p<0.01 (**), p<0.001 (***/###); #: significant compared to untreated RBCs, *significant compared to NaNO2 (10mM) treated RBCs.

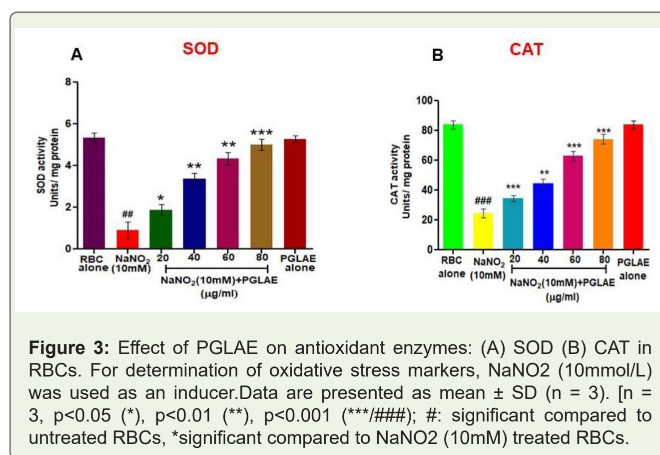

Figure 3: Effect of PGLAE on antioxidant enzymes: (A) SOD (B) CAT in RBCs. For determination of oxidative stress markers, NaNO2 (10mmol/L) was used as an inducer.Data are presented as mean ± SD (n = 3). [n = 3, p<0.05 (*), p<0.01 (**), p<0.001 (***/###); #: significant compared to untreated RBCs, *significant compared to NaNO2 (10mM) treated RBCs.

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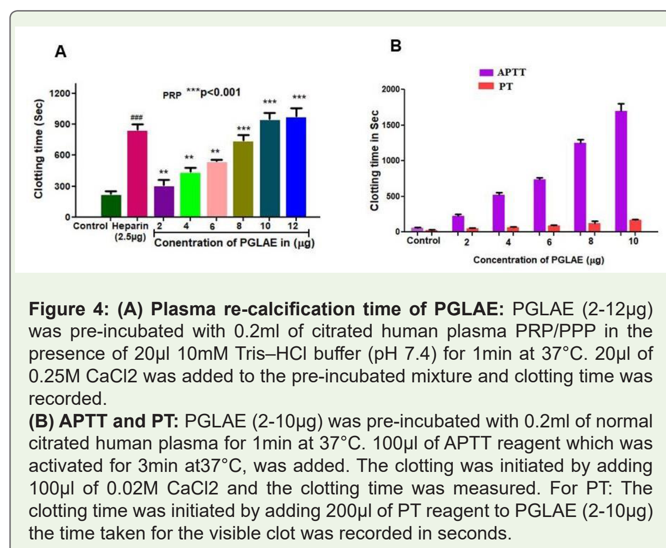

Figure 4: (A) Plasma re-calcification time of PGLAE: PGLAE (2-12µg) was pre-incubated with 0.2ml of citrated human plasma PRP/PPP in the presence of 20µl 10mM Tris–HCl buffer (pH 7.4) for 1min at 37°C. 20µl of 0.25M CaCl2 was added to the pre-incubated mixture and clotting time was recorded.
(B) APTT and PT: PGLAE (2-10µg) was pre-incubated with 0.2ml of normal citrated human plasma for 1min at 37°C. 100µl of APTT reagent which was activated for 3min at37°C, was added. The clotting was initiated by adding 100µl of 0.02M CaCl2 and the clotting time was measured. For PT: The clotting time was initiated by adding 200µl of PT reagent to PGLAE (2-10µg) the time taken for the visible clot was recorded in seconds.

PGLAE exhibited anti-platelet activities

PGLAE was scrutinized for platelet aggregation properties by using ADP as an agonist. *PGLAE* inhibited ADP induced platelet aggregation of platelet rich plasma in a concentration dependent manner with the aggregation inhibition of 70% at the concentration of 60µg (Figure 5).

PGLAE displayed non toxicity to RBCs

The *PGLAE* did not rupture the RBCs cell membrane, whereas, water a positive control ruptured the RBCs and generated the ghost red blood cells revealed its nontoxic nature (Figure.6).

Discussion

Higher level of free radicals generated during biological oxidation found to elicit cytotoxicity, as they damage proteins, lipids and DNA [28]. Thus, oxidative stress is the key cause for the pathogenesis of all types of life style diseases such as, diabetes, thrombosis, cancer, and autoimmune diseases and neurological syndromes. The life style diseases have been managed by non-steroidal, anti-inflammatory and steroidal drugs. It is to note that the said drugs trigger severe side effects such as headache, painful periods, sprains and strains, colds, flu and arthritis with long term pain [29]. Thus, researchers thriving to explore an alternative therapy. Perhaps, drugs discovered from medicinal plants appear to be promising as they are devoid of cellular toxicity but having high range of therapeutic index. Although, medicinal plants have been exploited since ancient time to treat several ailments, several such medicinal plants have not been validated scientifically. Thus, in the current study antioxidant and antiplatelet activity of Pomegranate (*Punica granatum)* leaves was undertaken. Pomegranate (*Punica granatum*) belongs to the *Lythraceae* family and it has been extensively used traditional herbal medicine [30].

The various parts of pomegranate found to exhibit anti-inflammatory, anti-oxidant, anti-cholinesterase, and cytotoxic properties, anti-bacterial and antibiotic properties were reported

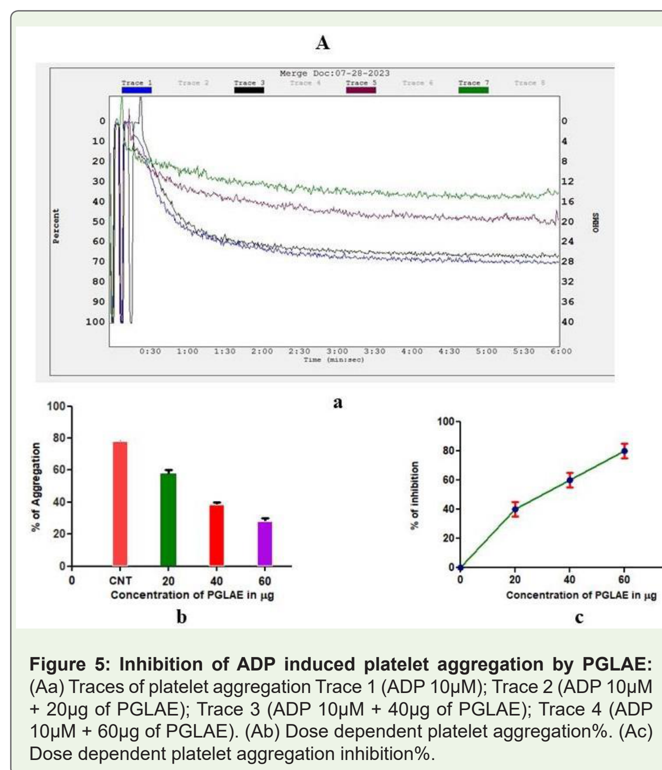

Figure 5: Inhibition of ADP induced platelet aggregation by PGLAE: (Aa) Traces of platelet aggregation Trace 1 (ADP 10µM); Trace 2 (ADP 10µM + 20µg of PGLAE); Trace 3 (ADP 10µM + 40µg of PGLAE); Trace 4 (ADP 10µM + 60µg of PGLAE). (Ab) Dose dependent platelet aggregation%. (Ac) Dose dependent platelet aggregation inhibition%.

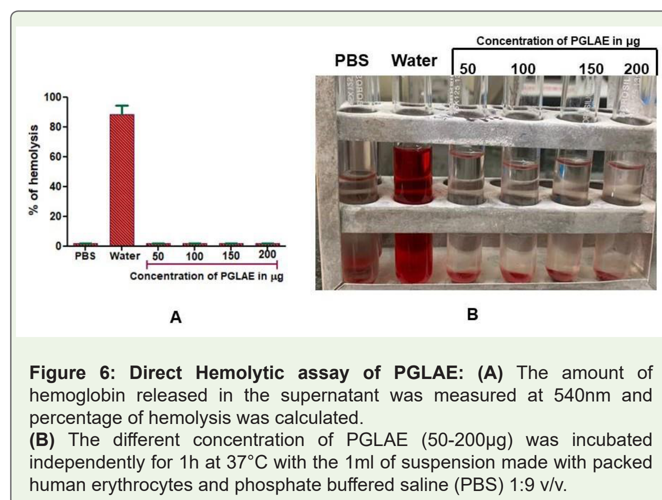

Figure 6: Direct Hemolytic assay of PGLAE: (A) The amount of hemoglobin released in the supernatant was measured at 540nm and percentage of hemolysis was calculated.
(B) The different concentration of PGLAE (50-200µg) was incubated independently for 1h at 37°C with the 1ml of suspension made with packed human erythrocytes and phosphate buffered saline (PBS) 1:9 v/v.

[31]. According to our qualitative tests pomegranate leaves gave positive results for the secondary metabolites such as alkaloids, tannins, steroids, polyphenols, glycosides, and triterpenoids. During oxidative stress there could be a massive decrease in the production of endogenous antioxidant machineries, which has to be managed by providing antioxidant load externally.

Interestingly *PGLAE* exhibited potential antioxidant property by quenching DPPH free radical. Plant extract such as, *Hyptis fasciculata, Orbignya speciosa* [30], *Paeonia suffruticosa, Cinnamomun cassia* [31] found to exhibit antioxidant activity. Free radicals stored in the body affect all the cells, however, red blood cells are more sensitive and often

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undergo eryptosis by free radicals, generates RBC mediated reactive oxygen species which are not only damage WBC and platelets but also linked to thalassemia, sickle cell anemia, diabetes, sepsis, hepatic and renal insufficiency, Wilson's disease, hemolytic uremic syndrome, hypophosphatemia, G6PD-deficiency, chronic kidney disease and heart attack [32]. Our findings revealed that PGLAE normalized the sodium nitrite-induced stress markers such as LPO, PCC, TT, SOD, and CAT in RBCs. Therefore, PGLAE may be a better candidate in the management of oxidative stress induced pathogenesis. Plants extract such as, sorghum extract protects RBC from sodium nitrite-induced oxidative stress [33].

ROS and ROS mediated cellular ROS alters hemostasis (platelets, coagulation cascade and fibrinolysis) is main cause for thrombosis what is known as formation of unusual clot in the arteries and veins [34]. Antithrombotic drugs (anti-coagulant, antiplatelet and fibrinolytic) have been currently using in the management of thrombotic drugs. The recent reports suggests that the said antithrombotic drugs triggers side effects such as passing blood urine, passing blood in the poo, bruising, prolonged nosebleeds, vomiting blood and severe backpain [35]. Hence their utility is also considered to be the bottleneck. Importantly, PGLAE showed anticoagulant property by extending the clotting time of platelet rich plasma (PRP).

In addition, PGLAE also extended the clotting time of both APTT & PT clearly indicates that the examined anticoagulant potential of PGLAE is due to the interference both intrinsic and extrinsic pathway of blood coagulation cascade non-specifically. The plant extract such as *Angelica shikokiana* [36], *Cyclopia subternata* [37], *Thymus atlanticus* [38] exhibited anticoagulant activities. Platelets are the major components of connective tissue play an important role in maintaining fluidity of the blood. Often their damage by ROS, bacteria, toxins and drugs activates them to initiate a clot [39]. Thus, platelet activation contributes immensely in progression of thrombotic disorders (heart attack and stroke) [40]. Antiplatelet interventions ought to be the treatment option for the prevention of thrombotic disorder [41]. Surprisingly, *PGLAE* also inhibited agonist ADP triggered platelet aggregation of human platelet rich plasma confirms its antiplatelet activity. Plant extract such as, *Selaginella bryopteris* [42] *Lindera obtusiloba* [43] and *Leuzea carthamoides* [44] exhibited antiplatelet activities. Moreover, *PGLAE* was unable to damage RBC compare to positive control water revealed its non-toxic nature.

Conclusion

In conclusion, we for the first time explored the potential therapeutic role PGLAE. PGLAE through its antioxidant potential ameliorates sodium nitrite oxidative induced RBC damage. In addition, *PGLAE* showed anticoagulant and antiplatelet properties. Hence, purification and characterization of the actives principles is of great future interest.

Conflict of interest

The authors declare that there are no conflicts of interest

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