International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 19-11-2024, Accepted: 20-12-2024, Published: 04-01-2025
Volume 10, Issue 1, 2025, Page No. 4-11

Antioxidant, anticoagulant, antiplatelet and anti-eryptotic (protection of RBCs) activities of *Acacia sinuata* leaves aqueous extract

Ramesha KL, Sujatha M, Shivakumar V, Devaraja S, Dr. Hemalatha MS*
Department of Food Science and Nutrition, Karnataka State Open University, Mysuru, Karnataka, India

Abstract
Acacia sinuata is the perennial, woody, large climbing shrub which grows on big trees belongs to the *Fabaceae*. It has been extensively used in the folk medicine since ancient time due to its arrays of medicinal qualities as it pacifies ulcer, heart burn, indigestion, constipation, skin disease, burning sensation, calculi, hemorrhoids, vitilligo, eczema and jaundice. While, none of the studies reported the protective role of *Acacia sinuata* extract on oxidative stress induced complications. Thus, present study proposes to scrutinize the protective role of *Acacia sinuata* Leaves Aqueous Extract (*ASLAE*) on oxidative stress induced RBCs damage and thrombosis. Preliminary qualitative phytochemical screening revealed the presence of carbohydrates, tannins, alkaloids, flavonoids, steroids and polyphenols. *ASLAE* showed 85% of DPPH scavenging activity with an IC_{50} value of 6.94 µg/mL. Interestingly ASLAE 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 RBCs. *ASLAE* displayed an anticoagulant effect by enhancing normal clotting time in platelet rich plasma from 120 sec to 800 sec. The anticoagulant effect of *ASLAE* was also strengthened by APTT and PT tests. *ASLAE* was positive for both APTT and PT test, revealed the identified anti-coagulant effect of *ASLAE* owing to intervention in intrinsic and extrinsic pathway of blood coagulation cascade. *ASLAE* also repressed both ADP and epinephrine induced platelet aggregation with an inhibition percentage of 70% & 65% respectively. *ASLAE* was non-toxic in nature as it was lacking hemolysis of RBCs and good in resolving oxidative stress induced pathogenesis and thrombotic disorders.

Keywords: Oxidative stress, antioxidant, anticoagulant and antiplatelet properties

Introduction
Cardio Vascular Diseases (CVD) such as coronary artery disease, stroke, hypertension, heart failure, rheumatic etiologies, congenital heart disease and peripheral vascular disease are considered as the multifactorial disorders represents 31% of deaths causing approximately 17.5 million deaths per year [1, 2]. The major basis of CVD is formation of thrombus (unwanted clot) in the arteries and vein due to over load of Reactive oxygen species (ROS) formed due to oxidative stress. At higher level ROS activates platelets, coagulation factors and induces atherosclerosis as well. In addition, ROS damage RBCs, WBC and platelets intern generates cellular ROS is the key cause for thrombosis responsible for heart attack and cerebral stroke [3]. RBCs transport gases (CO_2, O_2), metabolites, drugs, proteins and hormones. RBCs face lot of challenges by exposing to infectious agents, drugs and ROS [4]. The ROS induces eryptosis of (RBCs damage) results in membrane asymmetry and phosphatidyl serine externalization, eventually leads to prothrombotic condition [5]. Heme released from damaged RBCs seems to be cytotoxic that further elevates the release of hemoglobin that going to recruits nitric oxide which elicit further cellular damage, endothelial cell activation and platelets [6]. In addition, hemolysis encourages the expression of TNF- α that intern activates thrombomodulin a potent protein C activator [7]. However, RBCs protection play pivotal role in the supervision of life style diseases including oxidative stress and thrombosis. The anticoagulants and antiplatelet dugs play an effective role in the prevention and treatment of CVD. The anticoagulants, antiplatelets drugs, have been extensively used for treat thrombotic disorder unfortunately they cause lives threating side effects such as internal bleeding, birth defects and miscarriage [8, 9]. Hence, plant-based medicines which are an ancient method to treat many diseases can be explored.

Acacia sinuata (soap pod tree), belonging to the *Fabaceae* family, has been commonly known as one of the plants used in traditional herbal medicine for a long time [10]. Over the last decade, there has been a dramatic increase in interest in *Acacia sinuata* as a nutritional and medicinal product to treat a wide range of human disorders [11]. The plant leaves exhibit anti-inflammatory, anti-oxidant, anti-cholinesterase and cytotoxic properties, anti-bacterial and antibiotic properties [12]. Despite, the believed therapeutic potentials the defensive character of secondary Metabolites of *Acacia sinuata* Leaves Aqueous Extract (*ASLAE*) and their anti-oxidant and anti-thrombotic effect was least explored. Hence, the study was undertaken to study Antioxidant, anticoagulant, antiplatelet and anti-eryptotic effects.

Materials and methods
Reagents
1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,4-dinitrophenylhydrazine (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), Quercetin and 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 *Acacia sinuata* Leaves Aqueous Extract

Acacia sinuata leaves were collected from Tumkur local market. About 50 g of leaves were washed thoroughly, ground into powder with a grinder (SHARP, Japan), mixed with 500 mL of double distilled water. Centrifuged at 1500 g for 20 min at 15° C. Then the supernatant was collected. The obtained supernatant was concentrated using a vacuum evaporator (lyophilized). The dried *Acacia sinuata* Leaves Aqueous Extract (ASLAE) was stored at 4° C. Required amount of powder was weighed and dissolved in double distilled water and used for further studies.

Test for carbohydrates, Proteins, lipids

1 mL of ASLAE was treated with 2 drops of alcoholic α-naphthol solution in a test tube and development of purple/violet colored ring at the junction indicates the presence of carbohydrate. About 1mL of ASLAE was treated with 10 % NaOH solution followed by addition of 2 drops of copper sulphate solution. The formation of violet pink color indicates the presence of protein. 1 mL of ASLAE was treated with 0.5 N alcoholic potassium hydroxide (KOH) and added with 1 drop of phenolphthalein as indicator. The solution was heated in water bath for 1 h. the formation of white foam indicates the presence of lipids.

Phytochemical qualitative analysis

The obtained *Acacia sinuata* Leaves Aqueous Extract (*ASLAE*) was assessed for the existence of the preliminary phytochemicals. The analysis was done by following the standards methods of Harbone, *et al*. [13]

A few drops of saturated picric acid solution (Hager's reagent) were added to 1 mL of *ASLAE*. The formation of yellow precipitation indicates the presence of alkaloids. The test tube containing 1 % gelatin solution having sodium chloride was added with 1 mL of *ASLAE*. The formation of white precipitation indicates the presence of tannins. 1 mL of *ASLAE* was mixed with a few drops of acidic anhydride then boiled and cooled. The concentrated sulphuric acid was added by sides of the test tubes. The brown ring formation at the junction of the two layers indicates the presence of the steroids. 1 mL of *ASLAE* was treated with sulphuric acid and formation of orange color showed the presence of flavonoid in the mixture. About 1 mL of *ASLAE* was added with 5 % of ferric chloride solution and formation of deep blue or dark color indicates the presence of phenol. About 1 mL of *ASLAE* was hydrolyzed with concentrated sulphuric acid for 2 h on a water bath and filtered the solution. The filtrate was shaken with chloroform and added by 10 % ammonia. The formation of pink color indicates the presence of glycosides. 1mL of *ASLAE* was shaken with concentrated sulphuric acid and formation of yellow color at the lower layer indicates the presence of triterpenoids.

Determination of DPPH radical scavenging activity

The DPPH radical scavenging assay was carried out according to the method of Okoh *et al*. [14] The DPPH solution (0.04 mg/100 mL) was prepared by using 95 % of ethanol. The different concentration of ASLAE (5-30 µg) was preincubated with 150 µL of DPPH radical solution and the final volume was made up to 600 µL using 95 % ethanol and incubated for 30 min in dark at room temperature. The absorbance of the reaction mixture was measured spectrophotometrically at 517 nm. Ethanol was used to set the blank and ascorbic acid was taken as positive control. The scavenging activity was calculated using the formula given below.

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

The antioxidant activity of ASLAE was expressed as IC50. The IC50 value was defined as the concentration of the extract required for inhibiting the formation of DPPH radical by 50 percent.

Induction of oxidative stress in *RBCs* model by using $NaNO_2$.

The method of Luqman and Rizvi *et al*., [15] was used to induce oxidative stress in RBCs model using 10 mM sodium nitrite. Briefly, 1 mL of washed RBCs was pre-incubated with various concentrations of ASLAE (20-80 µg) for 30 min at room temperature in a clean Eppendorf tube. About 20 µL of 10 mmol/L NaNO2 was added to each Eppendorf tubes and further incubated for 90 min for room temperature. The reaction mixture without NaNO2 serves as positive control whereas NaNO2 treated reaction mixture without test sample serves as reference control. Finally, 2 mg/mL proteins from RBC lysate was taken from each tube and estimate the level of lipid peroxidation (LPO), protein carbonyl content (PCC), Total Thiol (TT) and antioxidant enzyme (superoxide dismutase and catalase) activity.

Evaluation of Lipid peroxidation (LPO)

The method of Ohkawa *et al*. [16] was followed to determine the LPO activity. Briefly, 100 µL stress induced RBCs lysate was mixed with 1.5 mL of acetic acids of pH 3.5 (20 %v/v), 0.2 mL of SDS (8% w/v) and 1.5 mL of thiobarbituric acid (0.8 % w/v). The reaction mixture was boiled at 60º C for 45 min. The reaction mixture was cooled and centrifuged at 2000 rpm for 10 min. The formed adducts were extracted by 3 mL 1-butanol. The formed TBA was measured spectrophotometrically (Thermo Scientific Biomate 6, USA) at 532 nm. Values were expressed in terms of malondialdehyde (MDA) equivalent to µmol MDA formed/mg of protein.

Determination of Protein Carbonyl Content (PCC)

The method of Levine *et al*. [17] was followed to quantify the protein carbonyl content. Briefly 100 µL NaNO2 treated RBCs and sample treated RBCs were taken in a clean test tube. Subsequently, an equal volume of 10 mM 2, 4-dinitrophenylhydrazine (prepared by using 2 mmol/L HCl) was added and incubated for 1 h at room temperature. Only 2 mmol/L HCl was used for blank. Using trichloroacetic acid (20 %, w/v) the reaction mixture was precipitated and centrifuged for 15 min at 1200 rpm. After repeated wash with acetone the final pellet was dissolved in 1 mL of tris buffer [20 mM pH 7.4 containing 0.14 M NaCl, 2 % SDS (w/v)]. The carbonyl content in the solution was quantified by monitoring using spectrophotometer at 360 nm and the results were expressed as µmol carbonyl groups/mg of protein.

Measurement of total thiols (TT)

The method of Zinellu *et al*. [18] was used to assess the total thiol content. Briefly, 100 µL of stress induced reaction mixture were taken in clean and dry test tubes. About 0.375 mL of Tris-HCl buffer (pH 8.2) was added and vortexed. Further 10 mM dithiol-bis-nitro benzoic acid (DTNB) and

1.975 mL of methanol were added and incubated for 30 min. the tubes were centrifuged for 10 min at 5000 rpm. The OD of supernatant was quantified by using spectrophotometer at 412 nm, and result was expressed as nmol of DTNB oxidized/mg protein.

Superoxide dismutase (SOD) activity

The method of Sundaram *et al*. [19] was followed to determine the SOD enzyme activity. About 0.05 mg of protein from the lysate (NaNO2-stress induced reaction mixture) was taken in a clean dry test tube and added with a 1 mL of reaction mixture consisting of phosphate buffer (16 mM, pH 7.8) and TEMED–EDTA (8 mM/0.08 mM). The decrease in the absorbance was monitored for 3 min in spectrophotometer. The results were expressed as U/mg of protein.

Catalase (CAT) activity

The catalase activity was assessed by the method of Beers *et al*. [20] Briefly, 0.05 mg of protein from the RBCs lysate was taken in clean and dry test tubes. About 1 mL of reaction mixture contains sodium phosphate buffer (100 mM, pH 7.4) and H2O2 (8.8 mM) was added to the RBCs lysate. The change in absorbance was monitored spectrophotometrically (Thermo Scientific Biomate 6 USA) at 240 nm for 3 min. The CAT activity was expressed as U/mg of protein.

Plasma re-calcification time

The method of Quick *et al*. [21] was used to conduct recalcification assay. Different concentration of ASLAE (20-120 µg) was incubated with 0.2 mL of 10 mM tris HCl (20 µL) buffer of pH 7.4 for 1 min at 37° C. The pre-incubated mixture was then treated with 20 mL of 0.25 M CaCl2 and the clotting time was recorded.

PT and APTT

Activated partial thromboplastin time and prothrombin time was done according to the method of Gangaraju S *et al*. [22] Different concentration of ASLAE (2-10 µg/mL) was pre-incubated with 100 µL each of normal citrated human plasma for 1 min. For APTT, 100 µL of LIQUICELINE (Cephaloplastin derived from rabbit brain with phospholipids and ellagic acid preparation) was activated for 3 min at 37° C. The clot was initiated by adding 100 µL of 0.02 M CaCl2 and the time taken for clotting was measured. For PT, the clotting was initiated by adding 200 µL of PT reagent (Thromboplastin). The time taken for the formation of the clot was recorded in seconds. The APTT ratio and the international normalized ratio for PT at each point was calculated from the values of control plasma incubated with the buffer for an identical period of time.

Platelet aggregation

The method of Born [23] was followed to conduct platelet aggregation method. Turbidimetric approach was used to examine the impact of ASLAE on platelet function using a chronology dual-channel whole blood/optical lumiaggregation system (Model 700, USA). ASLAE (10–30 µg) and PRP (0.25 mL) were pre-incubated and aggregation was started by adding agonists like ADP and epinephrine (10 µM) for 6 min, the aggregation was monitored.

Direct hemolytic activity

Packed human erythrocytes (1 mL) and PBS (9 mL) were thoroughly mixed. About 1 mL hematocrit suspension was incubated with various doses of ASLAE (50-200 µg) at 37^0 C for 1 h. The reaction was ended by adding 9 mL of ice-cold PBS and centrifuged for 10 min at 1000 rpm at 37^0 C. The amount of hemoglobin released in the supernatant was measured spectrophotometrically (Thermo Scientific Biomate 6, USA) at 540 nm and expressed as a percentage of hemolysis relative to 100 % lysis, with water serving as a positive control and PBS serving as a negative reference.

Results

Nutritional and Phytochemical qualitative analysis of *ASLAE*

The qualitative analysis of ASLAE was revealed the presence of key Nutritional and phytochemicals such as carbohydrates, proteins, alkaloids, tannins, steroids, flavonoids, phenols, glycosides and triterpenoids. The list of phytochemicals present in the ASLAE is tabulated in Table 1.

Table 1: Phytochemical analysis of *ASLAE*

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

DPPH radical scavenging activity of *ASLAE*

The anti-oxidant potential of *ASLAE* was analyzed by DPPH free radical scavenging method, ASLAE showed 85 % radical scavenging activity, with an average IC_{50} value of 6.94 µg/mL (Fig. 1).

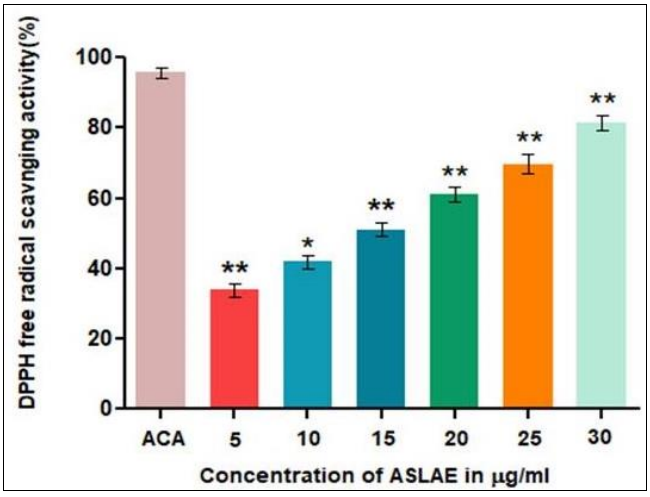

Fig 1: DPPH scavenging activity of ASLAE

Note: Anti-oxidant activity of ASLAE measured by DPPH method against the standard ascorbic acid.
**significant at $p < 0.001$ as compared with standard drug.

Oxidative stress in *RBCs* model by using $NaNO_2$ of *ASLAE*

As an indicator of lipid peroxidation, the level of malondialdehyde (MDA) was measured. In the case of $NaNO_2$-treated RBCs, there was an increased level of MDA which was observed in a significant manner (P< 0.001). Whereas, *ASLAE* treated RBCs the level of MDA was significantly (P<0.01) normalized as compared to the normal in a dose-dependent fashion (Fig. 2).

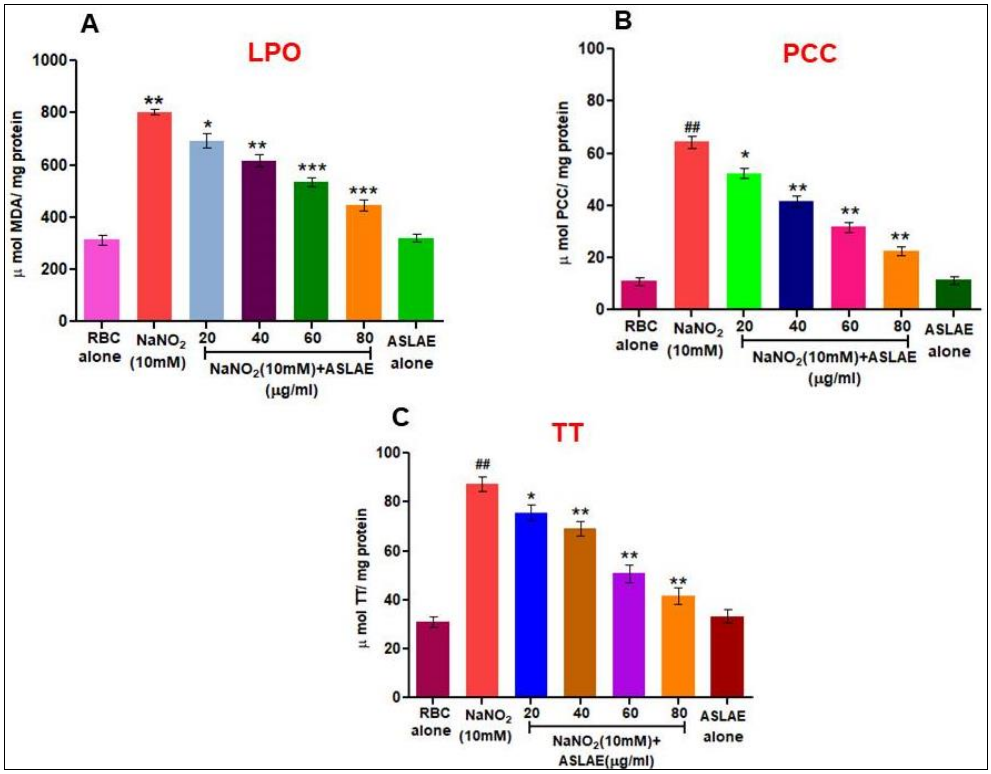

Fig 2: Effect of *ASLAE* on oxidative stress markers: (A) LPO (B) PCC (C) TT in RBCs

For determination of oxidative stress markers, NaNO2 (10 mmol/L) was used as an inducer. Data are presented as mean ± SD (n = 3). Significance was denoted by probability values where, p < 0.05 signifies *, p < 0.01 signifies **, p < 0.001 signifies ***/###; #: significant compared to NaNO2 (10 mM) treated RBCs.

Superoxide dismutase (SOD) and catalase (CAT) activity of *ASLAE*

ASLAE was found to regulate antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) activities on $NaNO_2$-treated RBCs. In the case of $NaNO_2$-treated RBCs, there was a significant (P < 0.01) decrease in the SOD activities (Fig. 3A). While *ASLAE* restore the SOD activity in RBCs in a dose-dependent manner with a statistically significant value at the concentration of 80 µg (P < 0.01). The *ASLAE* also restored the activity of CAT in a much better manner compared to SOD activity, as the statistically significant value was quite less than (P < 0.001) for RBCs at the concentration of 80 µg (Fig. 3B).

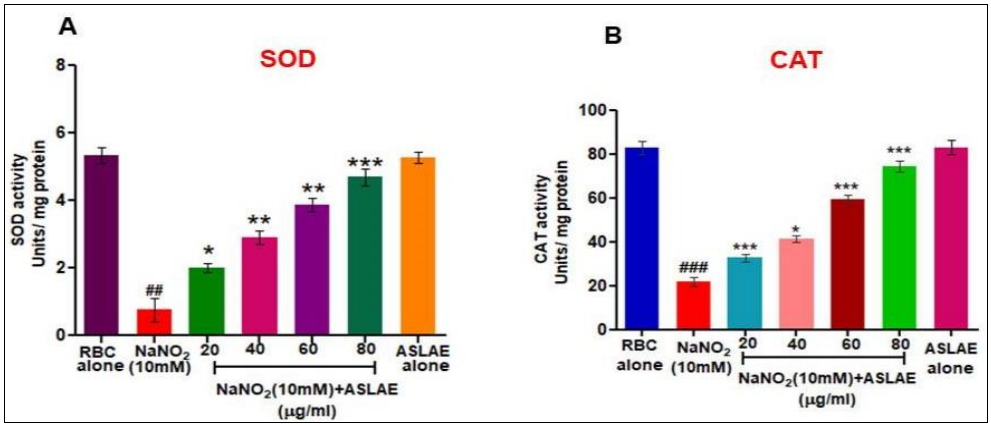

Fig 3: Effect of *ASLAE* on anti-oxidant enzymes: (A) SOD (B) CAT in RBCs

For determination of oxidative stress markers, NaNO2 (10 mmol/L) was used as an inducer. Data are presented as mean ± SD (n = 3). Significance was denoted by probability values where, p < 0.05 signifies *, p < 0.01 signifies **, p < 0.001 signifies ***/###; #: significant compared to NaNO2 (10mM) treated RBCs.

Plasma re-calcification time, PT and APTT of ASLAE

ASLAE caused anti-coagulation by enhancing the clotting time of PRP control 200 s to 800 s (Fig. 4A). *ASLAE* prolonged the clotting time of both APTT and PT which revealed its anti-coagulant effect was due to the interference in both intrinsic and extrinsic pathway of blood coagulation cascade (Fig. 4B).

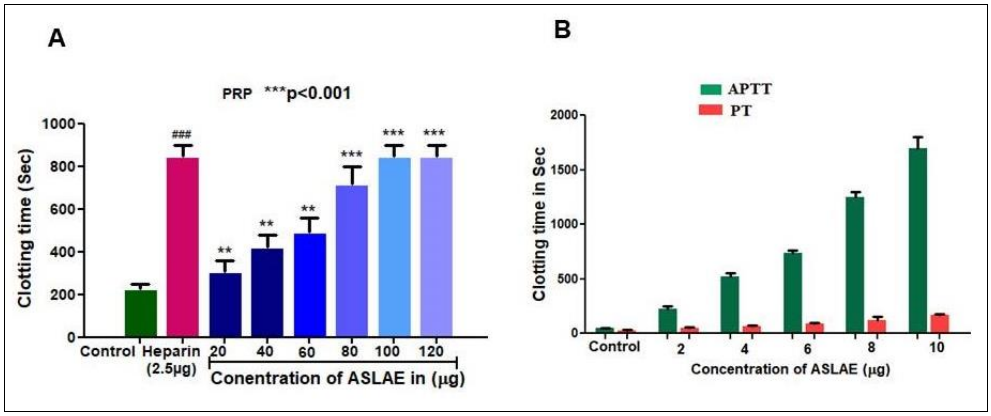

Fig 4: (A) Plasma re-calcification time of *ASLAE* (B) APTT and PT

ASLAE (2-12 µg) was pre-incubated with 0.2 ml of citrated human plasma PRP/PPP in the presence of 20 µL 10 mM Tris–HCl buffer (pH 7.4) for 1 min at 37 °C. 20 µL of 0.25 M $CaCl_2$ was added to the pre-incubated mixture and clotting time was recorded.

ASLAE (2-10 µg) was pre-incubated with 0.2 mL of normal citrated human plasma for 1 min at 37 °C. 100 µL of APTT reagent which was activated for 3 min at37 °C, was added. The clotting was initiated by adding 100 µL of 0.02 M $CaCl_2$ and the clotting time was measured. For PT: The clotting time was initiated by adding 200 µL of PT reagent to *ASLAE* (2-10 µg) the time taken for the visible clot was recorded in seconds.

Direct hemolytic activity of *ASLAE*

ASLAE inhibited ADP and epinephrine induced platelet aggregation of about 70 % and 65 % respectively at the concentration of 30 µg (Fig. 5A & 6A). The *ASLAE* did not harm the RBCs membrane. RBCs were damaged by positive control water but were protected by *ASLAE* (Figure.7)

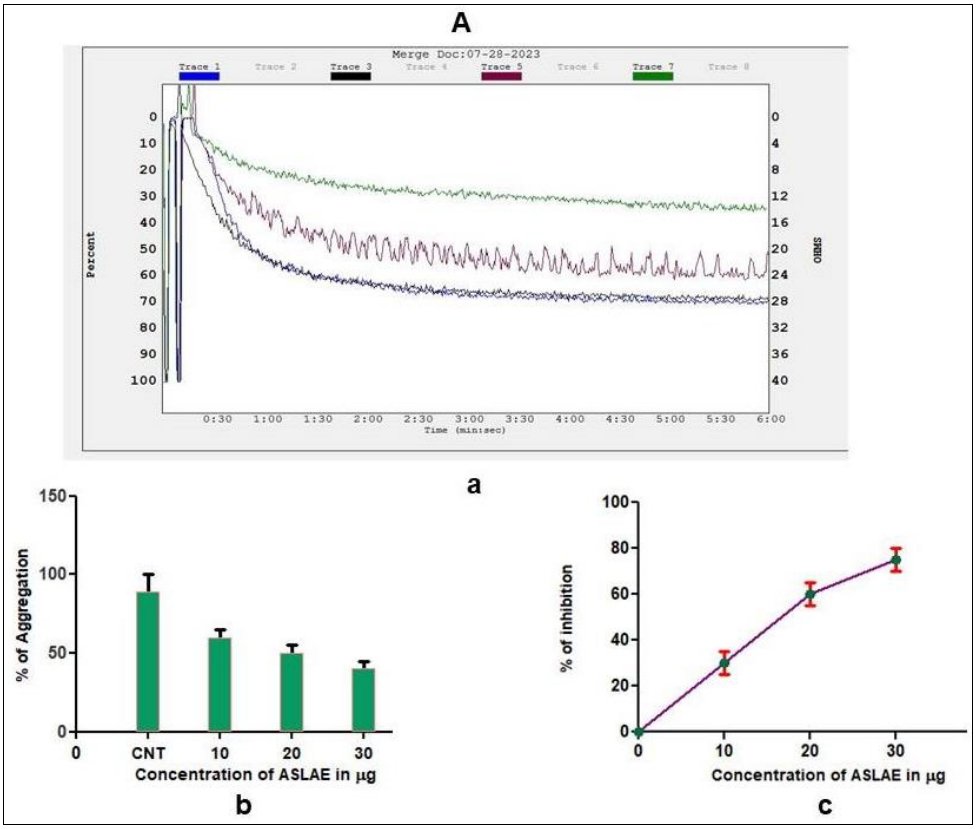

Fig 5: Inhibition of ADP induced platelet aggregation by *ASLAE*:

Aa: Traces of platelet aggregation Trace 1 (ADP 10 µM); Trace 2 (ADP 10 µM + 10 µg of ASLAE); Trace 3 (ADP 10 µM + 20 µg of ASLAE); Trace 4 (ADP 10 µM + 30 µg of ASLAE).

Ab: Dose dependent platelet aggregation %.

Ac: Dose dependent platelet aggregation inhibition %.

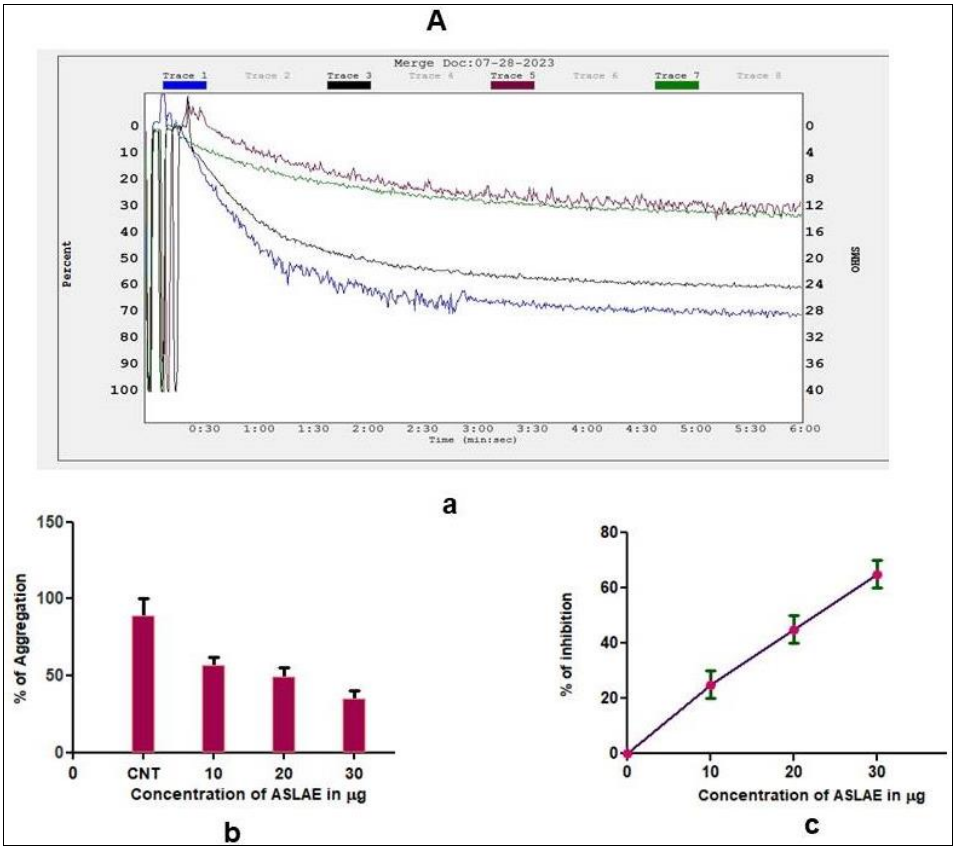

Fig 6: Inhibition of epinephrine induced platelet aggregation by *ASLAE*

Aa: Traces of platelet aggregation Trace 1 (epinephrine 10 µM); Trace 2 (epinephrine 10 µM + 10 µg of ASLAE); Trace 3 (epinephrine 10 µM + 20 µg of ASLAE); Trace 4 (epinephrine 10 µM + 30 µg of ASLAE).
Ab: Dose dependent platelet aggregation%.
Ac: Dose dependent platelet aggregation inhibition %.

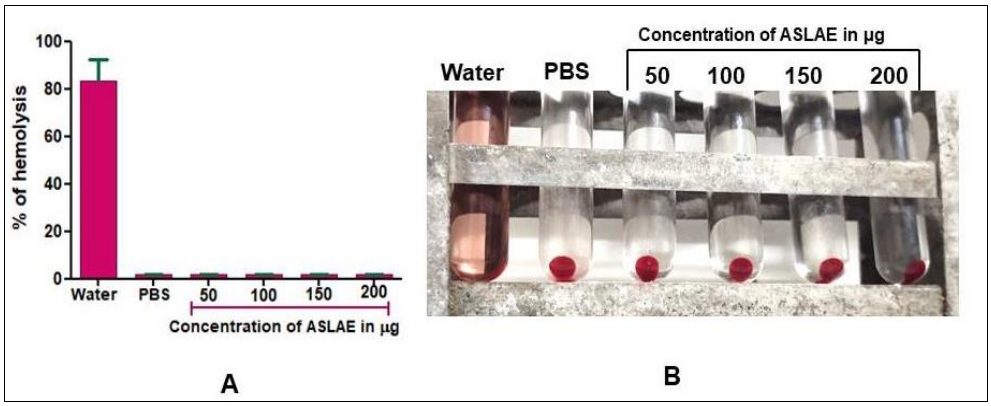

Fig 7: Direct Hemolytic assay of *ASLAE*

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

Discussion

Elevated level of free radicals (ROS) are the key exciters of all most all the life style disorders such as, atherosclerosis, diabetes, cancer and chronic inflammatory diseases, chronic obstructive pulmonary disease, neurodegenerative diseases, chronic kidney disease, rheumatoid arthritis, respiratory syndrome, hepatic diseases, lung diseases, cardio vascular complications and thrombosis [24]. In addition to that, ROS/RNS leads to colossal damage to RBCs and platelet function as a result they generate furthermore reactive oxygen species, they are not only causing thrombosis later on they cause colossal damage to vital organs and tissues that index high rate of mortality and morbidity. Practice of plant-oriented therapy is an ancient routine due to stored plethora of high quality of phytochemicals [25]. *Acacia sinuata* is a Mediterranean medicinal plant belongs to the family *Fabaceae*. It is a perennial, woody, large climbing shrub which grows on a big tree. Due to its stored array of medicinal qualities, it has been used from ancient time as a folk medicine and they are used to soothe vitiated pitta, skin disease, burning sensation, constipation, calculi, hemorrhoids, vitilligo and eczema [26]. In addition, it helps in

clotting of blood, liver disorder especially very effective for jaundice. The qualitative analysis revealed the presence of secondary metabolites such as alkaloids, tannins, steroids, polyphenols, glycosides and triterpenoids [27]. ASLAE exhibited potent anti-oxidant property by scavenging DPPH radicals. At room temperature, DPPH accept electrons and convert it into stable diamagnetic molecules. The reducing ability of DPPH free radicle is determined by decrease in the optical density and slight diminish of blue/purple color to pale yellow/colorless solution. The antioxidant molecules quench the free radicals by donating hydrogen/electron [28]. The result suggesting that ASLAE reduce the ROS compare to resultant hydrazine by responding with hydrogen donor in the antioxidant principle. ROS can damage RBCs and hence linked to thalassemia, sickle cell anemia, diabetes, sepsis, hepatic and renal insufficiency, Wilson's disease, hemolytic uremic syndrome, hypophosphatemia, G6PD-deficiency and chronic kidney disease. High level of MDA and protein carbonyl content may decrease the antioxidant enzymes which is the key indication of oxidative stress. The ASLAE significantly reduce the level of sodium nitrite induced oxidative stress such as LPO, PCC, TT and re-establish the antioxidant enzymes activity such as SOD and catalase in RBCs. Thus, *ASLAE* could offer better management of oxidative stress induced thrombosis [29].

The circulation of elevated ROS in blood may cause abnormality in RBCs that alter the structure of both RBCs and platelet membranes results in the formation RBCs and platelet derived ROS. Interestingly, ASLAE displayed anti-coagulant and anti-platelet activity, supports its multitude therapeutic potential on stress induced pathophysiologies. Several phytochemicals impart to contribute anti-coagulant and anti-platelet properties [30]. Most importantly, ASLAE enhanced the clot generation progression of APTT and PT, suggests its anti-coagulant activity could be due to its action on both intrinsic and extrinsic pathway of blood coagulation. ASLAE exhibited non-toxic property as it did not rupture the red blood cells. The present study validates the *Acacia sinuata* Leaves Aqueous Extract (ASLAE) defensive effect on RBCs, against oxidative stress along with beneficial role on thrombosis.

Conclusion

In the present study report the preliminary examination of phytochemicals present in the *Acacia sinuata* Leaves aqueous Extract (ASLAE) and examine the biological effect of ASLAE. The ASLAE showed anti-oxidant activity by inhibiting DPPH radicals and anticoagulant activity by delaying the clotting time in PRP of human blood plasma. Moreover, ASLAE exhibited antiplatelet activities by inhibiting ADP and epinephrine induced platelet aggregation. Interestingly, ASLAE non-toxic to RBCs since it does not cause the RBCs lysis. So, the ASLAE has good in resolving oxidative stress indued pathogenesis and thrombotic disorders. The observed properties might be owing to the occurrence of active phytochemicals. Therefore, purification and characterization of phytochemicals is of future research interest.

References

1. Liu L, Gao Q, Zhang Z, Zhang X. Elsholtzia Rugulosa: Phytochemical Profile and Antioxidant, Anti-Alzheimer's Disease, Antidiabetic, Antibacterial, Cytotoxic and Hepatoprotective Activities. Plant Foods Hum Nutr,2022:77(1):62-67. doi:10.1007/s11130-021-00941-4.
2. Bergendi L, Beneš L, Ďuračková Z, Ferenčik M. Chemistry, Physiology and Pathology of Free Radicals. Life Sci,1999:65(18–19):1865-1874. doi:10.1016/S0024-3205(99)00439-7.
3. Singh R, Devi S, Gollen R. Role of Free Radical in Atherosclerosis, Diabetes and Dyslipidaemia: Larger-than-life. Diabetes Metab Res,2015:31(2):113-126. doi:10.1002/dmrr.2558.
4. Carvalho C, Moreira PI. Oxidative Stress: A Major Player in Cerebrovascular Alterations Associated to Neurodegenerative Events. Front Physiol,2018:9:806. doi:10.3389/fphys.2018.00806.
5. Cooper LT, Baughman KL, Feldman AM, Frustaci A, Jessup M, Kuhl U, *et al*. The Role of Endomyocardial Biopsy in the Management of Cardiovascular Disease: A Scientific Statement from the American Heart Association, the American College of Cardiology, and the European Society of Cardiology. Circulation,2007:116(19):2216-2233. doi:10.1161/CIRCULATIONAHA.107.186093.
6. Vilesgonzalez J. Atherothrombosis: A Widespread Disease with Unpredictable and Life-Threatening Consequences. Eur Heart J,2004:25(14):1197-207. doi: 10.1016/j.ehj.2004.03.011.
7. Leopoldino AAO, Bertolini FM, Mesquita JVD, Cousin E, Passos VMDA. Increasing Burden of Osteoarthritis in Brazil from 2000 to 2017-- Results from the Global Burden of Disease Study (GBD), 2017. Acta Fisiátr,2020:27(2):76-81. doi:10.11606/issn.2317-0190.v27i2a169409.
8. Prandoni P, Milan M, Vedovetto V, Sarolo L, Adamo A, Bilora F. Department of Cardiothoracic and Vascular Sciences, Vascular Medicine Unit, University Hospital of Padua, Italy. Venous and Arterial Thrombosis: Is There a Link?. Int J Hematol Res,2015:1(2):29-34. doi: 10.17554/j.issn.2409-3548.2015.01.4.
9. Evans NS, Ratchford EV. Vascular Disease Patient Information Page: Venous Thromboembolism (Deep Vein Thrombosis and Pulmonary Embolism). Vasc Med,2014:19(2):148-150. doi: 10.1177/1358863X14529007.
10. Madamanchi NR, Hakim ZS, Runge MS. Oxidative Stress in Atherogenesis and Arterial Thrombosis: The Disconnect between Cellular Studies and Clinical Outcomes. J Thromb Haemost,2005:3(2):254-267. doi:10.1111/j.1538-7836.2004.01085.x.
11. Kiefmann R, Rifkind JM, Nagababu E, Bhattacharya J. Red Blood Cells Induce Hypoxic Lung Inflammation. Blood,2008:111(10):5205-5214. doi: 10.1182/blood-2007-09-113902.
12. Mohamed A, Bhardwaj H, Hamama A, Webber C. Chemical Composition of Kenaf (*Hibiscus Cannabinus* L.) Seed Oil. Ind Crops Prod,1995:4(3):157-165. doi:10.1016/0926-6690(95)00027-A.
13. Seca AML, Silva AMS, Silvestre AJD, Cavaleiro JAS, Domingues FMJ, Neto CP. Chemical Composition of the Light Petroleum Extract of Hibiscus Cannabinus Bark and Core. Phytochem Anal,2000:11(6):345-150. doi:10.1002/1099-1565(200011/12)11:6<345: AID-PCA540>3.0.CO:2-T.

14. Okoh SO, Asekun OT, Familoni OB, Afolayan AJ. Composition and Antioxidant Activities of Leaf and Root Volatile Oils of *Morinda Lucida*. Nat Prod Commun,2011:6(10):1934578X1100601. doi:10.1177/1934578X1100601032.
15. Luqman S, Rizvi SI. Protection of Lipid Peroxidation and Carbonyl Formation in Proteins by Capsaicin in Human Erythrocytes Subjected to Oxidative Stress. Phytother Res,2006:20(4):303-306. doi:10.1002/ptr.1861.
16. Ohkawa H, Ohishi N, Yagi K. Assay for Lipid Peroxides in Animal Tissues by Thiobarbituric Acid Reaction. Anal Biochem,1979:95(2):351-358. doi:10.1016/0003-2697(79)90738-3.
17. Levine RL, Williams JA, Stadtman EP, Shacter E. [37] Carbonyl Assays for Determination of Oxidatively Modified Proteins. In: Methods in Enzymology. Elsevier, 1994, 346-357. doi:10.1016/S0076-6879(94)33040-9.
18. Kare SK, Vinay V, Maresz K, Prisk V, Vik H. Tamarindus Indica Seed Extract-Based Botanical Compositions Alleviate Knee Pain and Improve Joint Function in Mild-to-Moderate Osteoarthritis: A Randomized, Double-Blind, Placebo-Controlled Clinical Study. Evid Based Complement Alternat Med,2022:2022:1-14. doi:10.1155/2022/2226139.
19. Beers RF, Sizer IW. A Spectrophotometric Method for Measuring the Breakdown of Hydrogen Peroxide by Catalase. J Biol Chem,1952:195(1):133-40. doi:10.1016/S0021-9258(19)50881-X.
20. Quick AJ, Stanley-Brown M, Bancroft FW. A Study of the Coagulation Defect in Hemophilia and in Jaundice. Thromb Haemost,1980:44(01):2-5. doi:10.1055/s-0038-1650068.
21. Born GVR. Aggregation of Blood Platelets by Adenosine Diphosphate and Its Reversal. Nature,1962:194(4832):927-929. doi: 10.1038/194927b0.
22. Sies H. Oxidative Stress: Oxidants and Antioxidants. Exp Physiol,1997:82(2):291-295. doi:10.1113/expphysiol.1997.sp004024.
23. Hybertson BM, Gao B, Bose SK, McCord JM. Oxidative Stress in Health and Disease: The Therapeutic Potential of Nrf2 Activation. Mol Aspects Med,2011:32(4-6):234-246. doi: 10.1016/j.mam.2011.10.006.
24. Chalo DM, Lukhoba C, Fidahussein DS, Nguta JM. Antimicrobial Activity, Toxicity and Phytochemical Screening of Selected Medicinal Plants of Losho, Narok County, Kenya. J Nat Prod Biochem,2017:15(1):29-43. doi:10.13057/biofar/f150106.
25. Srinivasa C, Kengaiah J, Nandish SKM, Ramachandraiah C, Hanumegowda SM, Shivaiah A, *et al*. Caesalpinia Crista Coat Extract Protects Red Blood Cell from Sodium Nitrite-Induced Oxidative Stress and Exhibits Antiplatelet Activity. Blood Coagul Fibrinolysis,2020:31(5):293-302. doi:10.1097/MBC.0000000000000913.
26. Kengaiah J, Nandish SKM, Ramachandraiah C, Srinivasa C, Shivaiah A, Sannaningaiah D. *In-Vitro* and *in-Vivo* Studies of Tamarind Seed Edible Extract Reveals Anti-Oxidative, Anticoagulant, Antiplatelet Events. Asian J Pharm Pharmacol,2019:5(6):1104-1116. doi:10.31024/ajpp.2019.5.6.5.
27. Hanumegowda SM, Srinivasa C, Shivaiah A, Venkatappa M, Santhosh S, R R, *et al*, Sannaningaiah D. Antioxidant and Antithrombotic Activities of Kenaf Seed (*Hibiscus Cannabinus*) Coat Ethanol Extract in Sprague Dawley Rats. Appl Biochem Biotechnol,2023:195(2):772-800. doi:10.1007/s12010-022-04144-8.
28. Girish TK, Vasudevaraju P, Prasada Rao UJS. Protection of DNA and Erythrocytes from Free Radical Induced Oxidative Damage by Black Gram (*Vigna Mungo* L.) Husk Extract. Food Chem Toxicol,2012:50(5):1690-1696. doi: 10.1016/j.fct.2012.01.043.
29. Davidović-Plavšić B, Kukavica B, Škondrić S, Jimenez-Gallardo C, Žabić M. Wild Garlic Extract Reduces Lipid Peroxidation in Terbuthylazine-Treated Human Erythrocytes. Biomarkers,2021:26(7):617-624. doi:10.1080/1354750X.2021.1953598.
30. Thushara RM, Hemshekhar M, Paul M, Shanmuga Sundaram M, Shankar RL, Kemparaju K, *et al*. Crocin Prevents Sesamol-Induced Oxidative Stress and Apoptosis in Human Platelets. J Thromb Thrombolysis,2014:38(3):321-330. doi:10.1007/s11239-014-1056-7.