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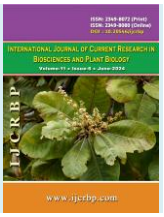

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Improvement of erythrocyte membrane stability by antioxidant active botanicals – A mechanistic approach

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Abstract

Erythrocytes, abundant in humans, are susceptible to oxidative stress, leading to membrane damage and hemolysis. This condition is prevalent in diseases like β thalasemmia, Glucose 6 dehydrogenase deficiency, and malaria. Six medicinal plants such as bark of *Albizia lebbeck*, endocarp of *Beta vulgaris*, Fruit skin of *Vitis vinifera*, Bark of *Thespesia populnea*, Fruit epicarp of *Punica granatum* and Leaves of *Euphorbia hirta* were investigated and evaluated for free radical scavenging activity and erythrocyte protective activity using their ethanolic extracts. Then, DPPH assay was carried out to all these six ethanolic extracts to study their antioxidant activity. The six ethanolic extracts were employed to prevent erythrocyte membrane oxidative damage caused by 2,2-azobis (2-amidinopropane) dihydrochloride (AAPH) in an ex vivo model. *Punica granatum* exhibited the highest scavenging activity (70.23±0.38%) compared to the other plants studied. Among the selected plants, *Albizia lebbeck* bark showed the highest levels of catalase (51.34±0.54%) and superoxide dismutase (SOD) (51.66±0.56%). The fruit epicarp of *Punica granatum* demonstrated the greatest inhibition of lipid peroxidation (72.66±0.82%). Additionally, it showed the highest percentage of free thiol levels (glutathione) in plasma (63.23±0.93%). All the selected plants displayed strong erythrocyte protective activity against hemolysis.

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Introduction

Erythrocytes, or red blood cells, are abundant in the human body and play a crucial role in oxygen transport. They contain haemoglobin, a metalloprotein with heme groups that bind oxygen (Chisté et al., 2014). However, due to their composition, including polyunsaturated fatty acids and high levels of oxygen and ferrous ions, erythrocytes are susceptible to redox activities that can generate toxic reactive oxygen species such as peroxyl radicals (ROO). These radicals can damage the erythrocyte membrane, leading to hemolysis and the loss of their oxygen transport function. While erythrocytes have an endogenous antioxidant system to counteract oxidative stress, it occurs when there is an imbalance between reactive oxygen species generation and the antioxidant defense system (Arbos et al., 2008).

Hemolysis is the premature destruction of erythrocytes in the bloodstream. Oxidative stress and the invasion of per oxidants or free radicals into the RBC membrane can cause this destructive process (Asgary et al., 2005; Chisté et al., 2014). Hemolysis is commonly observed in conditions such as sickle cell anemia, thalassemia, glucose6-phosphate dehydrogenase deficiency, hemolytic anemia and hemoglobinopathies (Sampath Kumar, 2011).

Medicinal plants are valuable sources of raw materials for medical preparations and can offer cost-effective treatment options for various clinical ailments. This study focuses on using extracts from *Albizia lebbeck, Beta vulgaris, Euphorbia hirta, Vitis vinifera, Punica granatum,* and *Thespesia populnea* to improve erythrocyte membrane stability. Fig. 1 depicts the work flow of this study.

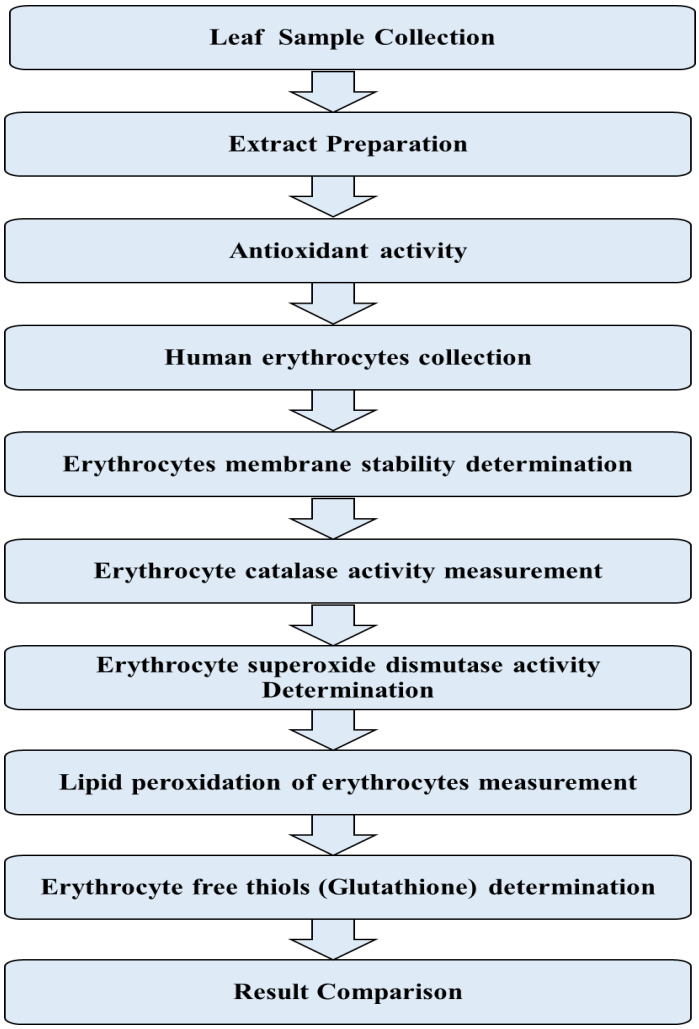

Fig. 1: Work flow.

Materials and methods

Collection of plant materials

Plant materials such as the leaves of *Euphorbia hirta*, bark of *Albizia lebback*, bark of *Thespesia populnea*, fruit endocarp of *Beta vulgaris*, fruit skin of *Vitis vinifera* and fruit epicarp of *Punica granatum* were collected from various parts of Kancheepuram district, Tamil Nadu, India. Fig. 2 shows the details of plant leaves collected.

Fig. 2: a. *Albizia lebbeck* (Bark), b. *Beta vulgaris* (Endocarp), c. *Euphorbia hirta* (Leaves), d. *Vitis vinifera* (Fruit skin), e. *Punica granatum* (fruit peel), f. *Thespesia populnea* (bark)

Preparation of plant extracts

The collected plant materials were washed and dried at room temperature. *Albizia lebbeck, Euphorbia hirta, Vitis vinifera, Punica granatum, and Thespesia populnea* were pulverized using a mechanical grinder. For extraction, 15g of the sample was macerated with 50 ml of ethanol for 24 hours under continuous agitation.

The extract of *Beta vulgaris* was prepared by adding chopped pieces to 70% ethanol (v/v) and subsequently filtered using Whatman No.1 filter paper and evaporated under vacuum at 40°C. The resulting dried extract was stored at room temperature (Fig. 3).

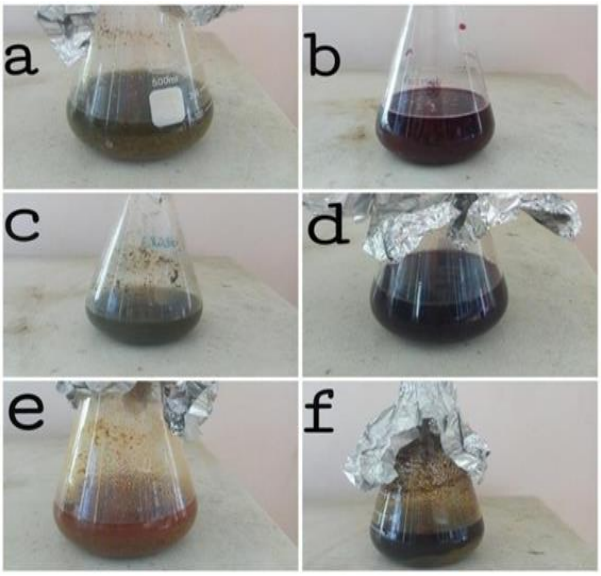

Fig. 3: a. *Albizia lebbeck* (Bark), b. *Beta vulgaris* (Endocarp), c. *Euphorbia hirta* (Leaves), d. *Vitis vinifera* (Fruit skin), e. *Punica granatum* (fruit peel), f. *Thespesia populnea* (bark)

Antioxidant activity of selected plant materials

DPPH radical scavenging activity was determined as previously described by (Kwak et al., 2015). The different amounts of each sample were combined with 190 l of 200 M DPPH in ethanol and incubated at 37 °C for 30 min. A spectrophotometer was used to measure the absorbance at 517 nm. The amount of DPPH radicals the sample is able to remove will be indicated by a decrease in absorbance. The positive control used was ascorbic acid. The DPPH radical scavenging activity can be calculated with equation 1.

$$DPPH\ radical\ scavenging\ activity(\%) = [((A\_Control - A\_Sample\ ))/A\_Control\ ] * 10 \quad (1)$$

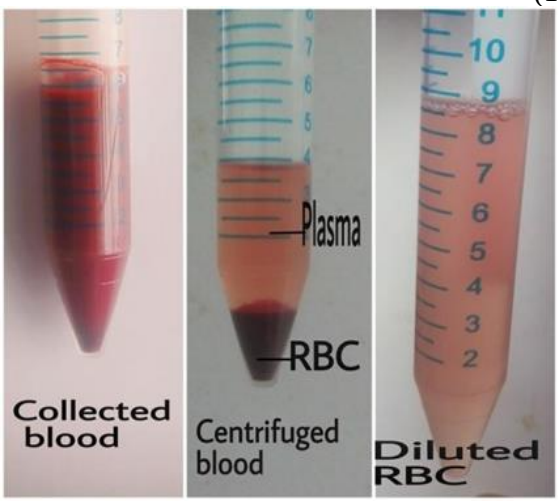

Fig. 4: Human peripheral blood in herpanized container.

The equation between sample concentration and DPPH scavenging activity was used to obtain the IC_{50} value, or the sample concentration required to eliminate 50% of DPPH radicals.

Collection of human erythrocytes

Peripheral venous blood samples were collected from healthy volunteers using tubes containing EDTA-K3 as an anticoagulant (Fig.4 and Fig. 5). The collected blood samples (approximately 4ml) were then transferred to a sterile conic tube with PBS, centrifuged at 15000 rpm at 4℃ for 5 minutes. The pellet was centrifuged three more times under the same conditions after being rinsed with PBS and the supernatant was removed. The resulting erythrocytes were resuspended in the same buffer, and a 5% hematocrit suspension was prepared. Eosin dye was used to stain the erythrocytes and observed under compound microscope (45X). The suspension with the erythrocytes was kept on ice until further use (Chisté et al., 2014).

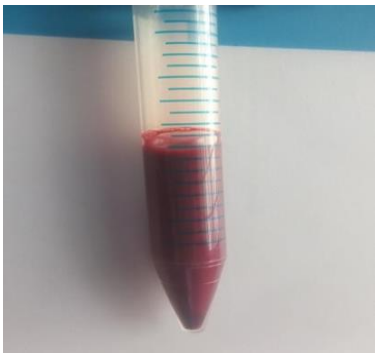

Fig: 5. Human erythrocyte collection a dilution.

Determination of erythrocytes membrane stability

Erythrocyte membrane stability was assessed by measuring the release of hemoglobin following membrane disruption caused by the hemolytic process. Different concentrations of the plant sample were added to erythrocytes in 48-well plates, followed by incubation at 37℃ for 30 minutes with gentle agitation. Subsequently,

APPH solution was added to all wells except the blank, and the mixture was incubated at 37℃ for varying time intervals. After incubation, the solution was transferred to 1.5 ml conic microtubes and centrifuged at 1500*g for 5 minutes at 4℃. The resulting supernatant was collected and placed in a 96-well plate, and the absorbance at 540nm was measured.

$$\% \ inhibition \ of \ hemolysis = \left\{\frac{[O.D_{Control} - O.D_{Sample}]}{[O.D_{Control}]}\right\} * 100 \quad (2)$$

O.D $_{sample}$ - is the absorbance value obtained from samples incubated with the tested crude ethanol extracts

O.D of blank- is the absorbance without AAPH and with crude ethanol extracts

O.D $_{control}$ – is the absorbance of wells with AAPH and in the absence of crude ethanol extracts (Chisté et al., 2014).

Measurement of erythrocyte catalase activity

Erythrocyte catalase activity was measured following the method outlined by (Gîlcă et al., 2009). Erythrocytes were diluted in 0.05 M potassium phosphate buffer (pH 7), and plant extracts of varying concentrations were added. The mixture was then incubated at 37℃ for 30 minutes. To initiate the reaction, 1 mM hydrogen peroxide was added and incubated for an additional 10 minutes. The samples were subsequently centrifuged at 5000 rpm for 2 minutes, and the absorbance of the supernatant was measured at 240 nm. The percentage of enzyme activity was determined by calculating the OD values using the blank (RBC and hydrogen peroxide). Ascorbic acid served as the standard.

$$\% \ of \ Catalase \ activity = \left\{\frac{[O.D_{Control} - O.D_{Sample}]}{[O.D_{Control}]}\right\} * 100 \quad (3)$$

Determination of erythrocyte superoxide dismutase activity

A 0.5 mL erythrocyte suspension was incubated with various plant extracts at different concentrations (10-50 µg) for 30 minutes at 30℃. 0.4 mL of 24 M NBT, 0.2 mL of 0.1 M EDTA, and 1 mL of 50 mL sodium carbonate were added. By adding 0.4 mL of 1 mM hydroxylamine hydrochloride, the reaction was started. Ascorbic acid was used as the reference standard for the plant extracts when measuring absorbance at 560 nm.

$$\% \ of \ SOD \ activity = \left\{\frac{[O.D_{Control} - O.D_{Sample}]}{[O.D_{Control}]}\right\} * 100 \quad (4)$$

Measurement of lipid Peroxidation of erythrocytes

Plant extracts ranging from 10-50 µg (200 µL) were added to the erythrocytes. Fenton reagent was introduced to induce oxidation in the mixture of RBC and plant extract. Next, 1 mL of trichloroacetic acid was added to precipitate the RBC, followed by a 2-hour incubation at 50℃. After the incubation, the RBC was separated by centrifugation at 3000 rpm for 10 minutes at 5℃. The supernatant was collected and suspended in 1 mL of 0.67% thio barbituric acid (TBA). The absorbance value was measured at 532 nm.

$$\% \ inhibition \ of \ oxidation = \left\{\frac{[O.D_{Control} - O.D_{Sample}]}{[O.D_{Control}]}\right\} * 100 \quad (5)$$

Determination of erythrocyte free thiols (Glutathione)

Following protein precipitation, the levels of glutathione in erythrocyte lysate were measured using a solution comprising sodium chloride, metaphosphoric acid, and disodium ethylenediamine tetra acetic acid. The mixture was filtered after being left to stand for five minutes. The absorbance at 412 nm was measured following the addition of sodium phosphate solution and Ellman reagent.

$$\% \ Glutathione = \left\{\frac{[O.D_{Control} - O.D_{Sample}]}{[O.D_{Control}]}\right\} * 100 \quad (6)$$

Results and discussions

DPPH free radical scavenging activity

DPPH is a free radical that can be stabilized by accepting an electron or hydrogen radical, resulting in a diamagnetic molecule. The absorption of stable DPPH at 517 nm decreases in the presence of antioxidants. Fig. 6 demonstrates the dose response curve for the radical scavenging activity of ethanol extracts from various plants. The scavenging effect on DPPH increased with higher concentrations of the extracts. The antioxidant activity of the *Punica granatum* peel extract was found to be superior to other plant extracts. Results were compared to the standard ascorbic acid. The peel of *Punica granatum* extract showed the higher antioxidant activity than the other plant extract. The values of % scavenging of DPPH with respect to the different concentration of the plant extracts were tabulated in the Table 1 and Figs. 6 and 7.

Table 1. DPPH free radical scavenging activity of selected plant materials.

Concentration of the ascorbic acid used (µg).	% of Scavenging (Mean ± SEM)
10	63.56±0.72
20	71.39±0.19
30	72.27±0.34
40	75.21±0.75
50	82.40±0.17

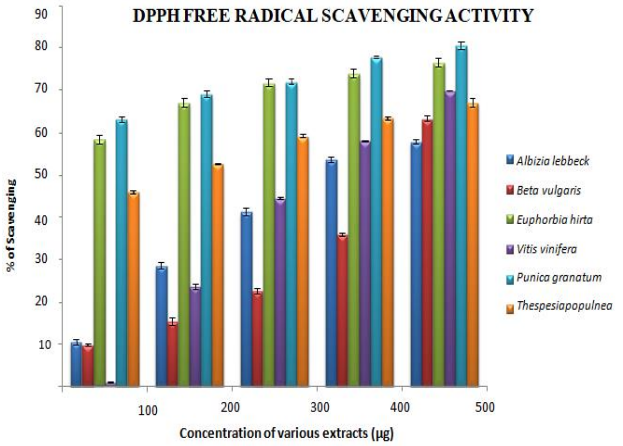

Fig. 6. DPPH free radical scavenging activity of selected plant extracts

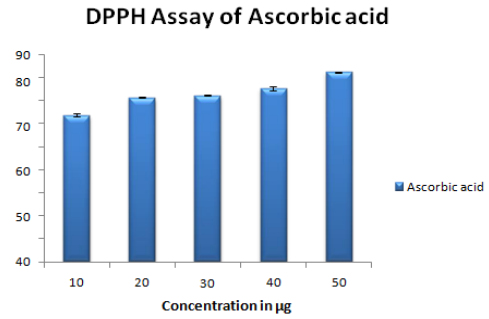

Fig. 7: DPPH free radical scavenging activity of Standard Ascorbic acid

Erythrocyte membrane stability assay

AAPH is a water-soluble azo compound commonly used to generate free radicals. The erythrocyte membrane contains vulnerable polyunsaturated fatty acids that are prone to peroxidation induced by free radicals. AAPH can be controlled to generate free radicals, making it useful for studying tissue damage and diseases like hemolysis (Ilavenil et al., 2011). The scavenging activities of the extracts were shown in the table, indicating that scavenging activity increased with higher extract concentrations. Ascorbic acid served as the standard, and the highest inhibition of hemolysis was observed with a concentration of 50 µg from the *Punica granatum* fruit peel (70 ± 0.38%). The microscopic images of normal erythrocytes and the lysed erythrocytes (APPH induced) were shown in Figs. 8 and 9. Fig. 9 represents the Erythrocyte Membrane Stability Assay of Ascorbic acid (Standard) and selected plant materials while table 3 depicts the Erythrocyte membrane stability assay of selected plant materials.

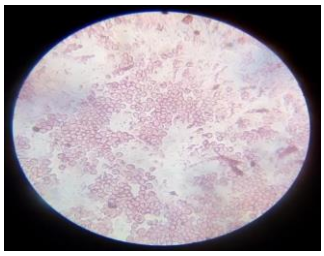

Fig. 7: Microscopic view of normal erythrocytes

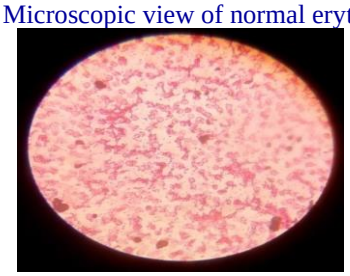

Fig. 8: AAPH induced lysed erythrocytes

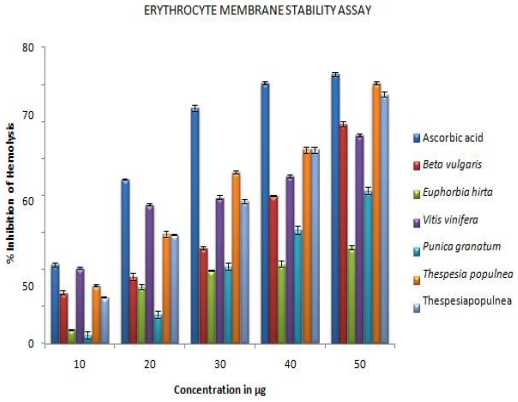

Fig. 9: Erythrocyte Membrane Stability Assay of Ascorbic acid (Standard) and selected plant materials.

Measurement of catalase activity of erythrocytes

Hydrogen peroxide, a by-product of biochemical reactions, acts as a weak oxidizing agent and can directly inactivate enzymes by oxidizing thiol groups. It can easily penetrate cell membranes and react with Fe^{2+} and Cu^{2+} ions, generating hydroxyl radicals that can cause toxic effects. Consequently, the catalase enzyme plays a vital role in regulating the accumulation of hydrogen peroxide within cells[10]. Catalase activity of selected plant materials and standard ascorbic acid was

depicted in table 4. Catalase activity on erythrocytes of standard Ascorbic acid and various selected plant extracts was given in Fig. 10.

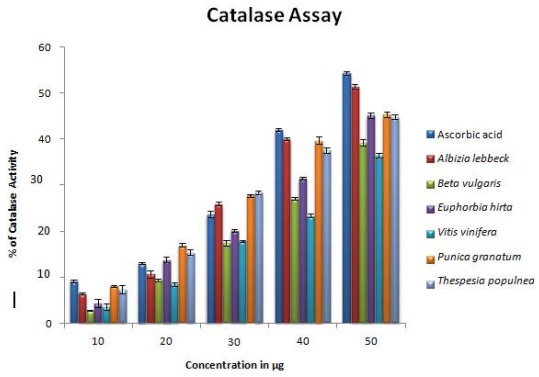

Fig. 10: Catalase activity on erythrocytes of standard Ascorbic acid and various selected plant extracts

Measurement of SOD activity on erythrocyte

Superoxide dismutase (SOD) is an antioxidant enzyme abundantly present in erythrocytes, serving as a crucial defense against oxygen toxicity. Plant extracts were found to enhance SOD activity in human erythrocytes. Among the extracts tested, the bark of *Albizia lebbeck* exhibited the highest SOD activity at a concentration of 50 µg, followed by the bark of *Thespesia populnea*, fruit epicarp of *Punica granatum*, leaves of *Euphorbia hirta*, and endocarp of *Beta vulgaris* (Hseu et al., 2008).

Table 4 depicts the SOD activity of selected plant materials and standard ascorbic acid while Fig. 11 gives the SOD Activity on erythrocytes of on erythrocytes of Ascorbic acid (Standard) and selected plant extracts.

Table 2. DPPH free radical scavenging activity of selected plant materials.

Conc. of the extract used(µg)	% of Scavenging (Mean ± SEM)					
	Albizia lebbeck (Bark)	*Beta vulgaris* (Endocarp)	*Euphorbia hirta* (Leaves)	*Vitis vinifera* (Skin)	*Punica granatum* (Epicarp)	*Thespesia populnea* (Bark)
100	10.51±0.60	9.87±0.29	58.26±0.19	0.92±0.19	63.11±0.71	45.90±0.36
200	28.57±0.94	15.35±0.84	67.10±0.79	23.63±0.62	69.14±0.82	52.60±0.19
300	41.26±0.88	22.51±0.65	71.76±0.21	44.42±0.30	71.97±0.60	59.16±0.41
400	53.61±0.64	35.96±0.36	73.93±0.41	58.00±0.11	77.82±0.31	63.37±0.28
500	57.85±0.47	63.26±0.64	76.52±0.30	69.87±0.09	80.58±0.84	67.02±0.92

Table 3. Erythrocyte membrane stability assay of selected plant materials.

Conc. of the extract used (µg)	% Inhibition of Hemolysis (Mean ± SEM)						
	Ascorbic acid (Standard)	*Albizia lebbeck* (Bark)	*Beta vulgaris* (Fruit Endocarp)	*Euphorbia hirta* (Leaves)	*Vitis vinifera* (Fruit Skin)	*Punica granatum* (Fruit Epicarp)	*Thespesia populnea* (Bark)
10	21.21±0.65	13.67±0.51	3.72±0.19	20.33±0.4	2.30±0.11	15.66±0.18	12.53±0.15
20	44.32±0.23	17.96±0.9	15.23±0.63	37.48±0.34	7.76±0.39	29.63±0.79	29.41±0.17
30	63.56±0.86	25.78±0.47	19.67±0.27	39.41±0.58	20.81±0.42	46.12±0.49	38.36±0.47
40	70.23±0.35	39.84±0.13	21.36±0.78	45.16±0.41	30.61±0.51	52.16±0.86	52.25±0.92
50	72.71±0.55	59.18±0.71	25.81±0.56	56.22±0.39	41.22±0.61	70.23±0.38	67.24±0.56

Table 4. SOD activity of selected plant materials and standard ascorbic acid.

Conc. of the extract used (µg)	% of SOD Activity (Mean ± SEM)						
	Ascorbic acid (Standard)	*Albizia lebbeck* (Bark)	*Beta vulgaris* (Endocarp)	*Euphorbia hirta* (Leaves)	*Vitis vinifera* (Fruit Skin)	*Punica granatum* (Fruit Epicarp)	*Thespesia populnea* (Bark)
10	18.55±0.56	12.45±0.67	8.98±0.56	11.77±0.70	7.61±0.99	7.77±0.12	13.45±0.31
20	26.80±0.44	19.99±0.51	17.51±0.19	17.67±0.44	19.32±0.59	22.38±0.31	24.90±0.39
30	33.15±0.62	28.56±0.34	27.88±0.22	22.38±0.16	21.55±0.33	30.45±0.42	36.19±0.46
40	47.87±0.71	39.67±0.78	38.40±0.60	35.08±0.73	31.87±0.21	43.99±0.93	45.7±0.44
50	56.44±0.83	51.66±0.56	48.12±0.56	48.48±0.55	41.43±0.45	49.56±0.44	50.5±0.61

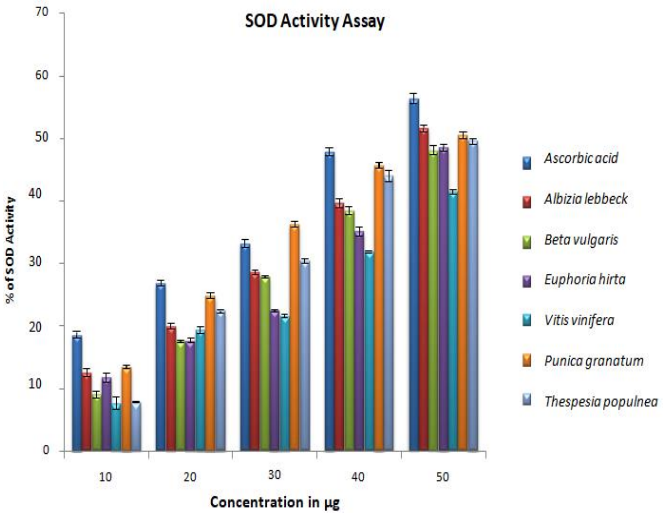

Fig. 11: SOD Activity on erythrocytes of on erythrocytes of Ascorbic acid (Standard) and selected plant extracts

Measurement of lipid peroxidation in erythrocytes

Lipid peroxidation is a proposed mechanism for cell injury and death caused by oxidative damage (Hseu et al., 2008). In this study, plant extracts and ascorbic acid were evaluated for their activity as inhibitors of lipid peroxidation and hemolysis. The extract from Albizia bark demonstrated the highest activity in preventing lipid Peroxidation compared to other plant extracts. Table 5 gives the overview of lipid peroxidation of erythrocytes and standard ascorbic acid while Fig. 12 represents the Lipid Peroxidation assay on erythrocytes of Ascorbic acid (standard) and selected plant extracts.

Determination of free thiol group (Glutathione) in plasma

The main antioxidant defense in stored erythrocytes is GSH. Nevertheless, it may also be involved in the oxidative alteration of membrane proteins and lipids, which may weaken the membrane skeleton and jeopardize the survival of erythrocytes. Intracellular oxidant scavenging within the cytosol relies on GSH and glutathione peroxidase to eliminate low micromolar levels of hydrogen peroxide and lipid hydroperoxides.

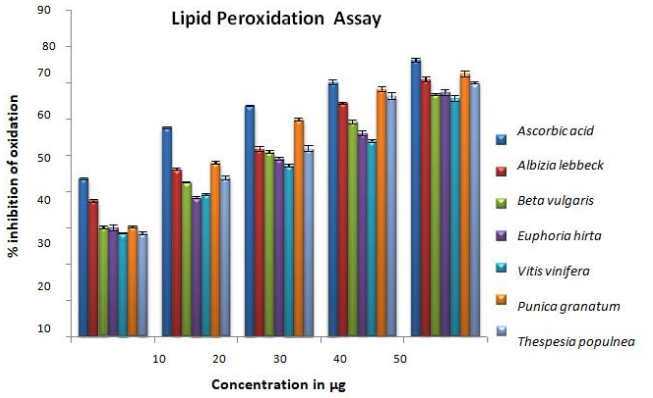

Fig. 12: Lipid Peroxidation assay on erythrocytes of Ascorbic acid (standard) and selected plant extracts.

GSH oxidation can occur directly through radical attack or indirectly through repair processes involving the reduction of oxidized membrane protein thiol groups[12]. The selected plant extracts have shown efficacy in replenishing the depleted endogenous antioxidant GSH in erythrocytes caused by AAPH treatment (Mikstacka et al., 2010).

Incubation with these plant extracts resulted in a concentration-dependent increase in GSH levels. Among the selected plant materials, the maximum free thiol level was observed in the bark of *Albizia lebbeck* at a concentration of 50 µg (63.11±0.28), surpassing Vitis vinifera with approximately 51±0.47 at 50 µg. Table 6 represents the Free thiol level in erythrocytes while Fig. 13 gives the results of the erythrocytes free thiol level assay.

Table 5. Lipid peroxidation of erythrocytes and standard ascorbic acid

Conc.of the extract used (µg)	% Inhibition of oxidation (Mean ± SEM)						
	Ascorbic acid (Standard)	*Albizia lebbeck* (Bark)	*Beta vulgaris* (Endocarp)	*Euphorbia hirta* (Leaves)	*Vitis vinifera* (Fruit Skin)	*Punica granatum* (Fruit Epicarp)	*Thespesia populnea* (Bark)
10	43.56±0.34	37.56±0.45	30.12±0.29	29.99±0.88	28.55±0.19	30.34±0.28	28.51±0.44
20	57.67±0.37	46.12±0.22	42.55±0.18	38.33±0.27	39.19±0.34	48.07±0.41	43.88±0.56
30	63.79±0.13	51.79±0.71	51.01±0.46	49.01±0.41	47.22±0.49	60.01±0.32	51.90±0.79
40	70.22±0.76	64.55±0.29	59.34±0.55	56.12±0.55	53.91±0.33	68.31±0.69	66.35±0.90
50	76.34±0.55	70.99±0.66	66.89±0.31	67.45±0.71	65.76±0.78	72.66±0.82	70.11±0.21

Table 6. Free thiol level in erythrocytes.

Conc. of the extract used(µg)	% free thiol level (Glutathione) (Mean ± SEM)						
	Ascorbic acid (Standard)	*Albizia lebbeck* (Bark)	*Beta vulgaris* (Endocarp)	*Euphorbia hirta* (Leaves)	*Vitis vinifera* (Fruit Skin)	*Punica granatum* (Fruit Epicarp)	*Thespesia populnea* (Bark)
10	29.34±0.45	17.65±0.53	9.69±0.82	11.25±0.44	7.33±0.38	20.23±0.58	18.45±0.61
20	37.71±0.89	33.45±0.72	22.59±0.59	19.33±0.92	20.18±0.57	39.31±0.26	27.81±0.45
30	43.55±0.99	40.90±0.39	31.45±0.37	29.88±0.36	31.23±0.26	48.91±0.88	39.38±0.71
40	56.34±0.67	59.10±0.81	48.19±0.83	47.29±0.94	42.94±0.96	52.45±0.25	43.99±0.82
50	68.45±0.63	61.11±0.28	54.83±0.93	59.41±0.89	51.38±0.47	63.23±0.93	58.22±0.81

Consuming fruits and vegetables with high antioxidant capacity has been linked to a number of health benefits, including a lower chance of developing chronic diseases. This association has been reported in the literature consistently. To accomplish this, a study was performed to understand the efficacy of selected plant material extracts such as Leaves of *Euphorbia hirta*, Bark of *Albizia lebback*, Bark of *Thespesia populnea*, Fruit endocarp of *Beta vulgaris*, Fruit skin of *Vitis vinifera* and Fruit epicarp of *Punica granatum* against oxidative stress of erythrocytes. Oxidative stress occurs due to the release of free radicals and it can be prevented by antioxidant system of the body. Even the erythrocytes have its own robust systems to protect itself against oxidative image and hemolysis including CAT, SOD, glutathione, etc. DPPH free radical scavenging activity was done primarily to study the antioxidant activity of the selected plant extracts and the maximum antioxidant activity was noted in the peel of *Punica granatum* of about 80.58±0.84 % of free radical scavenging against ascorbic acid as a positive control. The other plant extracts also showed the considerable scavenging activity.

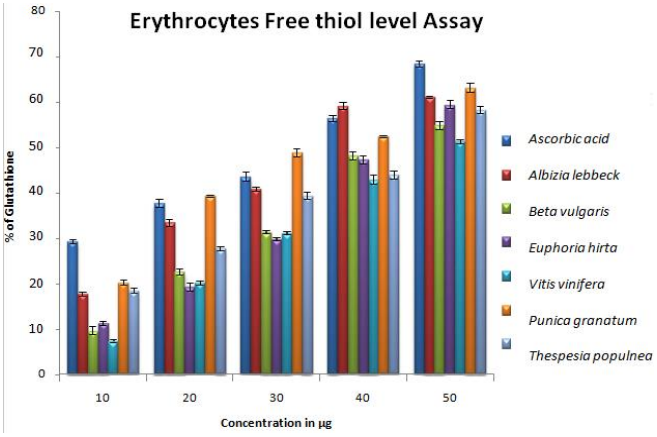

Fig. 13: Erythrocytes free thiol level assay.

Then the erythrocyte membrane stability assay was performed to study the protective role of selected plant materials against AAPH induced hemolysis. The highest percentage inhibition of hemolysis was showed by the *Punica granatum*'s fruit peel at concentration of 50µg (70.23±0.38) among the other selected plant extracts. The increase of CAT activity or SOD activity can explain the selected plant extract protection against AAPH induced hemolysis. The maximum activity was observed in *Albizia lebbeck* (Bark) for both the enzyme activity such as 51.34±0.54% of CAT activity and 51.66±0.56 % of SOD activity among the other selected plant extracts. Apart from the ascorbic acid, the plant extract showed its efficiency against inhibition of lipid peroxidation *Albizia lebbeck* bark extract showed maximum activity in preventing the lipid peroxidation. In addition, free thiol level of erythrocyte was calculated. GSH is the major non-enzymatic antioxidant, regulator of intracellular redox homeostasis and is ubiquitously present in all cell types. *Punica granatum*'s peel showed the highest level of free thiol level (63.23±0.93). In conclusion, the selected plant materials efficiently protected the human erythrocytes against oxidative injury to prevent the hemolysis which is the major condition in many diseases.

Conclusions

This study aimed to evaluate the antioxidant potential of selected plant materials (*Albizia lebbeck, Beta vulgaris, Euphorbia hirta, Vitis vinifera, Punica granatum, Thespesia populnea*) and their protective effects against oxidative stress on erythrocytes. DPPH free radical scavenging activity revealed that *Punica granatum* peel exhibited the highest antioxidant activity (80.58±0.84%) compared to ascorbic acid. The erythrocyte membrane stability assay demonstrated the protective role of the selected plant materials against APPH-induced hemolysis, with *Punica granatum* showing the highest inhibition of hemolysis at a concentration of 50 µg

(70.23±0.38%). Increased CAT and SOD activity indicated the efficacy of the selected plant materials against AAPH-induced hemolysis, with *Albizia lebbeck* displaying the highest activity (51.34±0.54% and 51.66±0.56%). *Albizia lebbeck* also exhibited maximum activity in preventing lipid peroxidation, while *Punica granatum* peel showed the highest level of free thiol content (62.23±0.93%). Overall, the results suggest that the selected plant materials effectively protect erythrocytes from oxidative damage and prevent hemolysis, which is significant in various diseases.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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