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Pomegranate – an Insight into its Antioxidative, Thrombolytic and Membrane Stabilizing Property

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Abstract
The present study is focused on exploring the antioxidative, thrombolytic and membrane stabilizing property of the peel and aril of pomegranate. Antioxidative property was deliberated based on their free radical scavenging ability in which the peel exerted higher radical scavenging potential. Thrombolytic potential of pomegranate was notably higher in aril (30.36%) than in peel (27.62%) while the standard streptokinase exhibited 58.38%. The membrane stabilizing activity was assessed under hypotonic and varying temperature conditions and compared with standard acetyl salicylic acid. The synergistic effect of the fruit could be further explored to be one of the best sources for atherothrombotic diseases.
Keywords: Pomegranate, Thrombolysis, Membrane stability, Free radical scavenging

1. Introduction:

Cerebral venous sinus thrombosis (CVST) is a common disorder that is often accompanied by significant morbidity and mortality (Chowdhury et al., 2011). Streptokinase, a standard in the study is a widely used fibrinolytic drug. All thrombolytic agents work by activating the enzyme plasminogen that clears the cross-linked fibrin mesh (Al-Mamun *et al.,* 2010). Selective thrombin inhibitors and antiplatelet agents are more potent, but their safety remains to be confirmed because they might cause serious bleeding complications along with reocclusion and reinfarction. Hence a plant based thrombolytic therapy, with its ability to produce rapid clot lysis, has long been considered as an attractive alternative (Baruah et al., 2006). Medicinal properties of plants have been investigated in the recent scientific developments throughout the world, due to their potent antioxidant activities, lesser or no side effects and economic viability (Kowti *et al*., 2010). Furthermore, the effect of any synthetic and herbal agents on the stabilization of erythrocyte membrane exposed to hypotonic solution has been studied extensively because the erythrocyte membrane resembles to lysosomal membrane and as such, the effect of drugs on the stabilization of erythrocyte could be extrapolated to the stabilization of lysosomal membrane (Omale and Okafor, 2008). Pomegranate, which is an important fruit of tropical and subtropical regions has been used for its medicinal purposes. It is also shown that pomegranate juice consumption reduces oxidative stress, atherogenic modification to LDL and platelet aggregation in humans and atherosclerotic apolipoprotein E–deficient mice. But the thrombolytic potential of the fruit is not fully exploited. Hence the present study focuses on antioxidative, membrane stability and thrombolytic property of the fruit.

2. Materials and Methods

2.1 Collection and preparation of fruit extract

The pomegranate fruit was procured from local market in Coimbatore and washed with water. The peel and pulp were homogenized separately with water and filtered. The extract was then used for further studies.

2.2 In vitro free radical scavenging activity

2.2.1 DPPH

Antioxidants react with DPPH and convert it to diphenyl-picryl hydrazine by donating its OH group. The degree of discoloration from purple to yellow colour was measured at 519nm as described by Mensor *et al*., 2001.

2.2.2 Nitric oxide

An aqueous solution of sodium nitroprusside spontaneously generates nitric oxide at physiological pH, which interacts with oxygen to produce nitrite ions that is measured at 546nm as explained by Green and Hill, 1984.

2.2.3 Superoxide

The extent of superoxide generation was studied on the basis of inhibition of the production of nitroblue tetrazolium formazon of the superoxide ion by the plant extracts and was measured spectrophotometrically at 560nm by the method of Winterbourn *et al*., 1975.

2.2.4 Hydrogen peroxide

H_2O_2 scavenging activity was measured in terms of a decrease in the absorbance at 230nm spectrophotometrically using the method of Ruch *et al.,* 1989.

2.2.5 Hydroxyl

Hydroxyl radicals are generated from a Fe^{2+}/ascorbate/EDTA/H_2O_2 system, which attacks deoxyribose and eventually produces thiobarbituric acid reactive substances (TBARS). The ability of the plant extracts to inhibit TBARS formation is measured spectrophotometrically at 532nm as described by Elizabeth and Rao (1990).

2.3 Determination of Clot Lysis

Experiment for clot lysis was carried out as reported earlier by Prasad et al., 2007. Streptokinase was used as a positive control and water as negative control.

*2.4 Membrane Stabilizing Activity***:**

The membrane stabilizing activity of the extractives was assessed by heat-induced and hypotonic solution-induced erythrocyte haemolysis as described by Shinde *et al*., 1999.

2.5 Statistical Analysis:

All the values are expressed as mean ± S.D. DMRT, t-test and one way analysis of variance were carried out.

3. Results

3.1 in vitro Free radical Scavenging

3.1.1 DPPH (1,1-Diphenyl-2-picryl hydrazyl)

DPPH is a stable nitrogen centered free radical which can be effectively scavenged by antioxidants. The peel and aril of pomegranate were analysed for their DPPH scavenging ability and it was seen that peel of pomegranate exerted a greater inhibition (62.11%) when compared to the aril (44.84%). The results are shown in fig 1.

3.1.2 Superoxide

Inhibition of superoxide was done in the aril and peel extracts of pomegranate. The percent activity of superoxide scavenging of pomegranate peel was found to be 63.42 ± 3.48 which was higher than aril (40.89 ± 1.87) as shown in fig 1

3.1.3 Nitric oxide

Nitric oxide is an important bioregulatory molecule. If elevated it may bring about some undesired deleterious effects like renal dysfunction and tumor growth. So the nitric oxide scavenging capacity was determined. The fruit had remarkable nitric oxide scavenging ability. The peel exhibited 73.12 ± 3.89%, while the aril showed 53.76 ± 2.8% inhibition as represented in fig 1.

3.1.4 Hydrogen peroxide

Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. The aril and peel of the fruit was then analysed for their H_2O_2 scavenging ability and was found that peel showed higher inhibition of 79.76 ± 4.74 % while aril showed 39.87 ± 2.75 % inhibition only as shown in fig 1.

3.1.5 Hydroxyl

The hydroxyl radical scavenging showed a slight difference in their inhibition pattern. Inhibition by aril was more pronounced when compared to the peel. The results are shown in fig 1.

3.2 In vitro Clot Lysis

The aril and peel of pomegranate exhibited considerable thrombolytic activity of 30.36% and 27.62% respectively. The standard drug Streptokinase, a positive control exhibited the highest activity of 58.38% and water which was used as the negative control exhibited negligible clot lysis percent of about 4.49%. Statistical

representation of the effective clot lysis of the fruit extracts, Streptokinase and Water is represented in fig 2. The plant extracts showed lesser activity when compared to that of the standard streptokinase.

3.3 Membrane stability

The membrane stabilizing activity of aril and peel extracts of pomegranate was also determined. Significant protection against the lysis of human erythrocyte membrane induced by hypotonic solution and varying temperatures was exhibited by fruit extract when compared to the standard acetyl salicylic acid (Fig 3). In heat induced and hypotonic conditions, the aqueous extract of aril inhibited 41.13% and 21.72% respectively when compared to the standard which showed 53.27% and 67.9% inhibition. The peel extract also inhibited haemolysis with a percent of 49.8 and 30.06 respectively.

4. Discussion

The prevention of life style related atherothrombotic diseases such as myocardial infarction and stroke is an important and urgent social task in many developed countries. Epidemiological studies have provided irrefutable evidence for the causative role of inappropriate diet in the development and clinical outcome of thrombotic diseases (Yamamoto *et al*., 2007). Development of natural products from various plant and animal sources which have antiplatelet, anticoagulant, antithrombotic, and thrombolytic activity is being studied intensively (Prasad *et al*., 2007). Recently consumption of phytochemical rich plant foods, including whole grains, vegetables and fruits, was reported to reduce the risk for acute coronary disease incidence, which was closely associated with thrombosis (Ozben *et al* ., 2006).

Pomegranate which has been long considered as a folk medicine has a very impressive antioxidant capacity because of their tannins, polyphenols and anthocyanins. In addition to their antioxidative property, the fruit has also been explored for their enhanced role as diuretics, prokinetic agents, liver revivals, anti-parasitics and also in cosmetic industries (Ardekani *et al.,* 2011). So, the present study focused on evaluating the antioxidant, thrombolytic and membrane stability potential of the pomegranate.

The antioxidative property of the fruit was determined in terms of their ability to scavenge free radicals like DPPH, superoxide, nitric oxide, hydroxyl ion and hydrogen peroxide. It was found that the peel of the fruit exhibited higher scavenging ability when compared to the aril except in the case of hydroxyl ion scavenging. Free radicals are generated in several biochemical reactions in the body, which pose a threat in many diseases including cancer and cardiovascular diseases. The endogenous antioxidants are not sufficient in scavenging them and hence there is a need of dietary supplements (Li *et al., 2011*). The results of the study suggest that pomegranate could be used as an effective antioxidant in our daily diet. The thrombolytic activity of the aril was more pronounced when compared to the peel paving way for the use of pomegranate in herbal preparations for the treatment of many cardiovascular diseases. This property could be attributed to the antioxidant potential of the fruit (Sano *et al.*, 2005). The membrane stabilizing potential of the fruit evinced that they exhibit a reasonable stability as compared to the standard acetyl salicylic acid suggesting their use in herbal preparation.

In conclusion, the beneficiary effects of Pomegranate as available from the literature and our results indicate that the fruit can be effectively used in herbal formulations if further explored. Active principles responsible for thrombolysis are yet to be found out. Once found Pomegranate may be incorporated as a thrombolytic agent for the improvement of the patients suffering from Atherothrombotic diseases.

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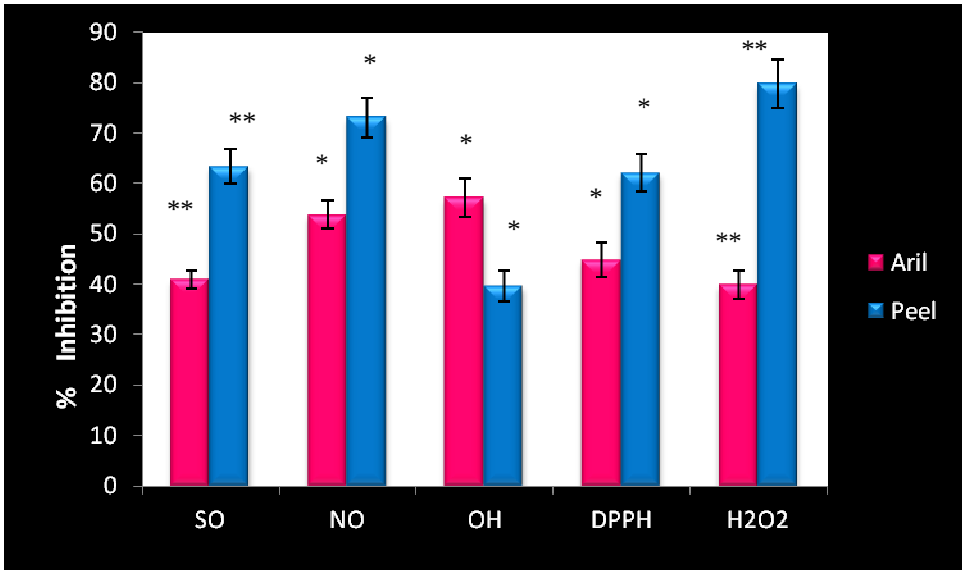
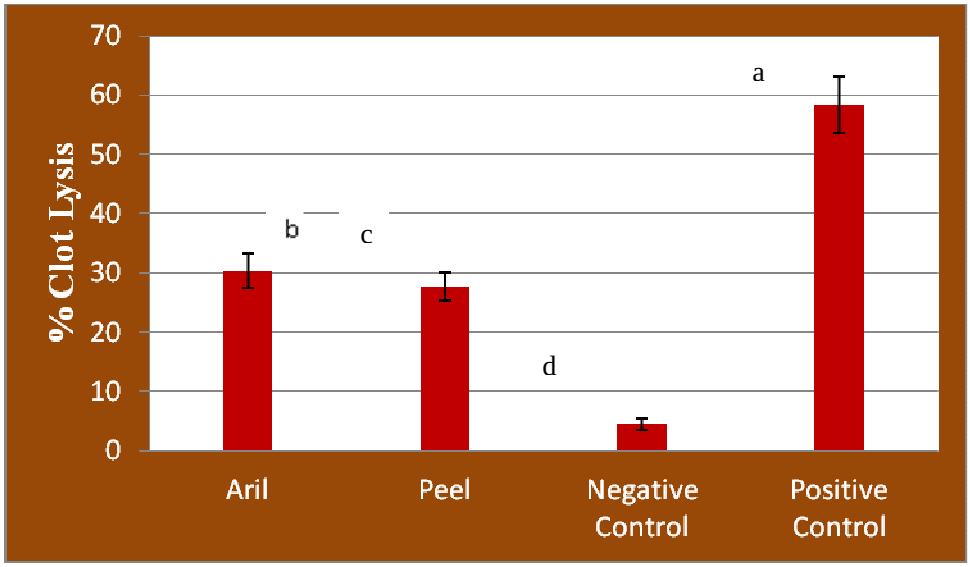
Fig 1 Free radical Scavenging of Pomegranate aril and peel



Values are mean ± SD of six samples in each group. Significant at *- t<0.05 ; ** - t<0.01

Fig 2: Percent Clot Lysis by Pomegranate



Values are mean ± SD of six samples in each group. Different superscripts are significant at 5% (p<0.05) by DMRT

Fig 3 Membrane stabilizing activity of aril and peel of Pomegranate

Sample	Concentration (mg/mL)	% Inhibition	
		Heat Induced	Hypotonic solution induced
Aril	1.0	41.13 ± 1.62 a*	21.72 ± 1.31 a*
Peel	1.0	49.8 + 1.59 b*	30.06 ± 0.97 b*
Acetyl salicylic acid	0.1	53.27 ±1.87 c*	67.9 ± 1.93 c*

Values are mean ± SD of six samples in each group. * - significant at p<0.05