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Research Article

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Thrombolytic potential of *Punica granatum* and lipid profile: A correlation analysis

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ABSTRACT

Cardiovascular disease (CVD) remains the leading cause of death despite the identification of several CVD risk factors. A thrombus formed inside a blood vessel reduces the blood supply leading to a myocardial infarction. Hence the present study aimed at determining if a correlation exists between the thrombolytic potential and the lipid profile among normal, diabetic and hypertensive participants. The antioxidant potential of the fruit was gauged in terms of enzymic and non-enzymic antioxidants present in the fruit. It was found that there was no correlation between the thrombolytic potential of the fruit and the levels of cholesterol.

Keywords: *Punica granatum*, cholesterol, antioxidants, thrombolytic potential

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INTRODUCTION

CVD remains a leading cause of global morbidity and mortality despite identification of major cardiovascular risk factors and risk reduction via appropriate interventions [1]. Atherosclerosis, accumulation of lipids and macrophages/lymphocytes within the intima of large arteries, is the underlying reason for all cause of coronary heart disease, peripheral arterial disease and many cases of stroke [2]. Consistent evidences indicate that in India where healthcare infrastructure is limited in certain parts, there is a rise in saturated fat intake that results in an increase of serum cholesterol. This mount in serum cholesterol linearly increases the death rate due to cardiovascular diseases [3]. The thrombolytic potency of the aril and rind of the fruit had been recognised in our previous studies. So, the thrombolytic competence of *Punica granatum* in the blood with high cholesterol and other serum parameters is an indispensable task.

EXPERIMENTAL SECTION

i. Collection and preparation of extracts:

The fruit sample was collected from the Coimbatore city and certified by the Botanical Survey of India, Coimbatore. The voucher specimen was collected and maintained. Fresh fruits were collected, washed and homogenized using distilled water for the preparation of aqueous extract. It was then filtered using Whatmann No 1 filter paper and used for further study.

ii. Estimation of enzymic antioxidants:

The fruit samples were quantitatively analysed for the enzymic antioxidants, catalase, superoxide dismutase, peroxidase, polyphenol oxidase and glutathione peroxidase.

a) Catalase (CAT) - Catalase, is an enzyme that catalyses the decomposition of H_2O_2, water and oxygen. The activity of catalase was determined by Luck [4].

b)Peroxidase (POD) - Peroxidase activity of the samples was determined by the method proposed by Reddy *et al*. [5].
c) Polyphenol oxidase (PPO) - The method proposed by Esterbauer *et al*. [6] was used to assay polyphenol oxidase in the selected fruit sample.
d)Glutathione peroxidase (GPx) - Glutathione peroxidase activity was determined by the method of Rotruck *et al*. [7].
e) Superoxide dismutase (SOD) - The superoxide dismutase activity was estimated by the method of Misra and Fridovich [8].

iii. Determination of non-enzymic antioxidants:
Few of the non-enzymic antioxidants such as ascorbic acid, α-tocopherol, reduced glutathione, polyphenols and flavonoids were determined in the rind and aril of *P. granatum*.

a) **Ascorbic acid** is a water soluble antioxidant protects the biological system from oxidative stress. Ascorbic acid was estimated by the method of Roe and Kuether [9].
b) **Alpha-tocopherol** represents a spectrum of atleast eight independent lipophilic molecules (four tocopherols and four tocotrienols) with antioxidant activity. It was estimated by the method proposed by Rosenberg [10].
c) **Tannins** are water soluble polyphenols that are present in many plant foods. Determination of tannins was carried out by the method of Schanderl [11].
d) **Polyphenols** are commonly found in both edible and inedible plants, they have multiple applications in food, cosmetic and pharmaceutical industries. The content of polyphenols was determined by the method explained by Malick and Singh, [12].
e) **Flavonoids** are one of the most numerous and widespread group of phenolics in higher plants. The flavonoid content of the sample was determined by the method of Zhishen [13].

iv. Correlation between thrombolysis and HDL, serum cholesterol and protein in normal, hypertensive and diabetic participants:
The aril and rind of *P. granatum* were tested for their difference in thrombolytic efficacy among the normal, hypertensive and diabetic participants with respect to the serum cholesterol, HDL and protein level.

a) Estimation of Serum Cholesterol - Total cholesterol was estimated in serum obtained from the human blood samples used for thrombolytic activity following the kit method.
b) Estimation of Serum Protein - Serum protein was determined by the method of Lowry *et al.* [14].
c) Estimation of Serum High Density Lipoprotein (HDL) - HDL in serum was determined by kit method as proposed by **Burstein *et al.*** [15].

v. Determination of thrombolytic activity:
Thrombolytic activity of the plant extracts was determined using human blood samples by the method of Prasad *et al*. [16].

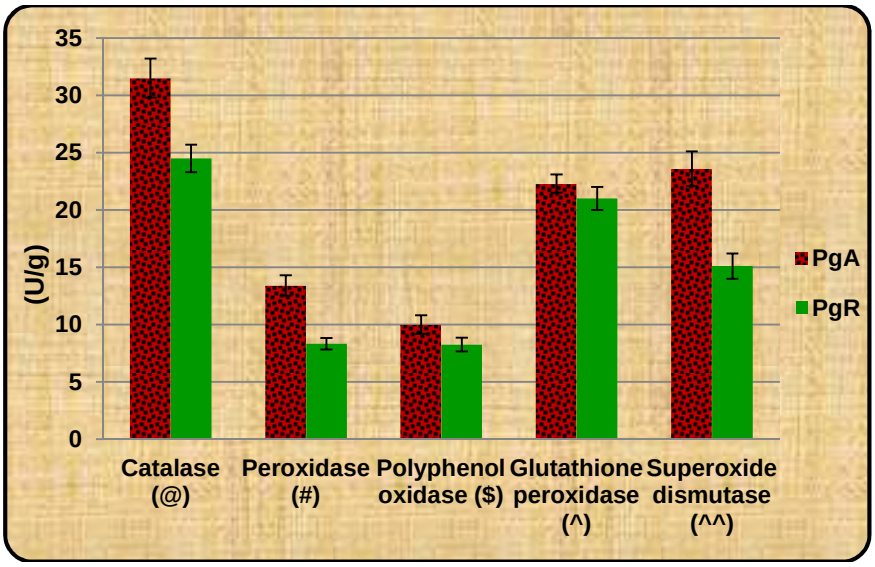
All the experimental procedures were initiated after reviewed by Institutional Human Ethic Committee (IHEC/14-15/BC/02).

RESULTS AND DISCUSSION

Natural antioxidant strengthens the endogenous antioxidant defenses from reactive oxygen species and restores an optimal balance by neutralizing the reactive species.

Enzymic antioxidants:
The aril and rind of *Punica granatum* were analysed for their enzymic antioxidants namely catalase, peroxidase, polyphenol oxidase, glutathione peroxidase and superoxide dismutase and the results are recorded in figure 1.

Figure 1: Levels of enzymic antioxidants of *Punica granatum*



PgA – *Punica granatum* Aril **PgR – *Punica granatum* Rind**

One Unit:

@ - Amount of enzyme required to decrease the optical density by 0.05 units

 # - Change in absorbance / min/ g of sample

$ - Amount of enzyme that catalyses the transformation of 1µ mole substrate / min

^ - nmoles of GSH consumed by the enzyme/min/g sample

^^ - The amount of enzyme that causes 50% reduction in the extent of NBT oxidation

The level of enzymic antioxidants was found to be higher in aril of pomegranate than the rind. Also, amount of catalase was higher than any other enzymic antioxidants that were studied. No significant difference was observed between glutathione peroxidase and superoxide dismutase content in aril extracts. Similarly, only a negligible difference was observed between the amount of peroxidase and polyphenol oxidase in rind extracts.

All the five enzymic antioxidants that were measured were seen elevated in the aril of the fruit than the rind. Among the different enzymic antioxidants that were analysed, catalase was found to be higher than the other antioxidants. *Curcuma caesia* and *Curcuma angustifolia* exhibited significantly higher catalase activity than superoxide dismutase [17]. This is in agreement with our findings where, the catalase activity was higher for both aril and rind of *Punica granatum*.

Non-enzymic antioxidants:

Non-enzymic antioxidants namely vitamin C, vitamin E, tannins, polyphenols and flavonoids were quantified and the results are represented in table 1.

Table 1: Levels of non-enzymic antioxidants of *Punica granatum*

Antioxidants (mg/g)	PgA	PgR
Vitamin C	34.0 ± 1.47 *	28.5 ± 1.25
Vitamin E	6.5 ± 0.85 *	7.5 ± 0.97
Tannins	81.0 ± 2.80 *	60.0 ± 1.30
Polyphenols	140 ± 4.20 *	158 ± 5.20
Flavonoids	77.0 ± 2.10 *	70.5 ± 1.93

PgA – *Punica granatum* aril; PgR- *Punica granatum* rind

Among the non-enzymic antioxidants that were studied, vitamin C, flavonoids and tannins were found to be present in an excess amount in aril when compared with the rind. However, poylphenols and vitamin E were found to be increased in rind than aril. The high content of antioxidant activity in *Punica granatum* indicates that they may impart health benefits when consumed and should be regarded as a valuable source of antioxidants.

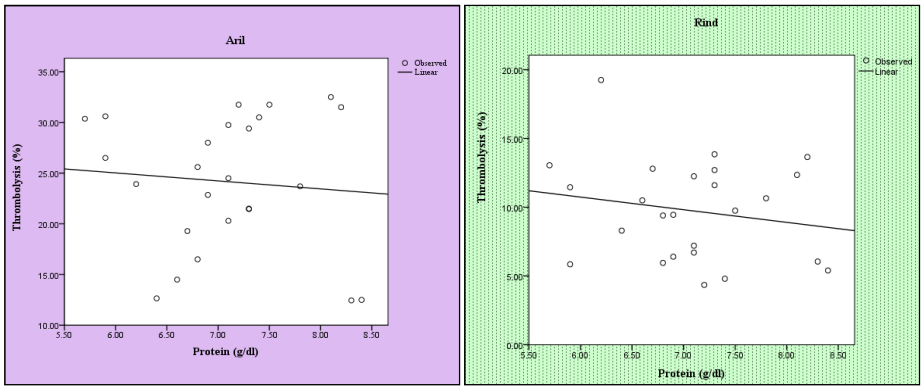
The high content of antioxidant activity in *Punica granatum* indicates that they may impart health benefits when consumed and should be regarded as a valuable source of antioxidants. Results from earlier studies have indicated that the fruit *Punica granatum* has potent thrombolytic activity. Hence, *Punica granatum* which exhibits good

antioxidant activity could serve as an excellent candidate for thrombolysis also, for the reason that they can quench the oxidative stress formed during ischemia and reperfusion.

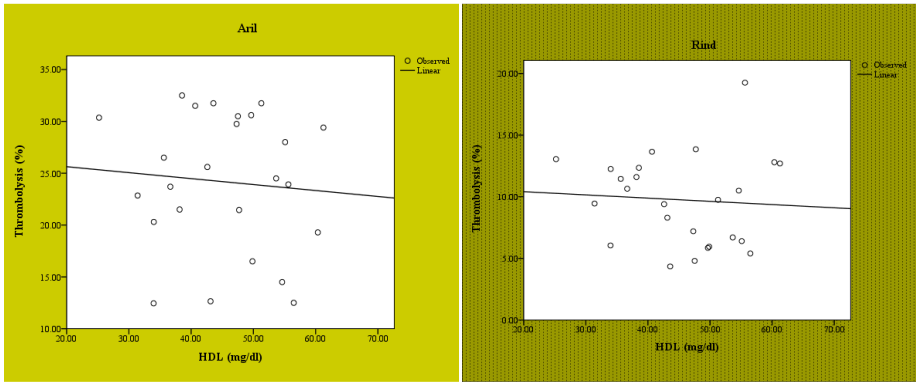
Comparison of lipid profile and thrombolytic activity of *Punica granatum*:

A correlation was made between the serum parameters and the thrombolytic efficiency of aril and rind of *Punica granatum*. The graphs are represented in figures 2, 3 and 4.

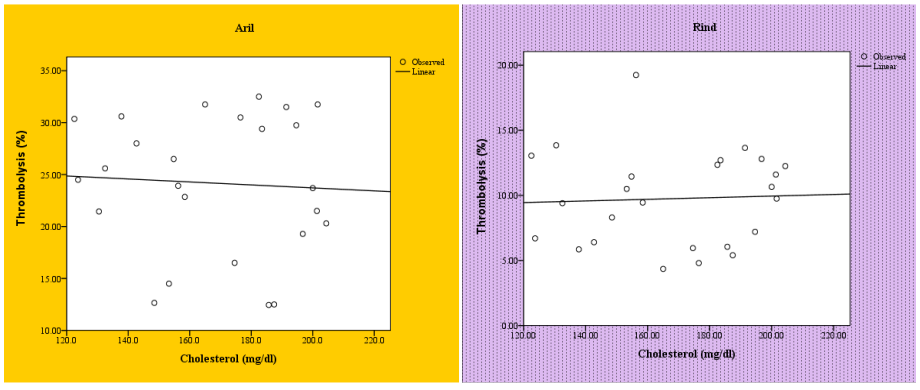
Figure 2: Correlation between lipid profile and thrombolytic activity of *Punica granatum* in healthy participants



r^2 – 0.008 p – 0.677 **r^2 – 0.035 p – 0.373**

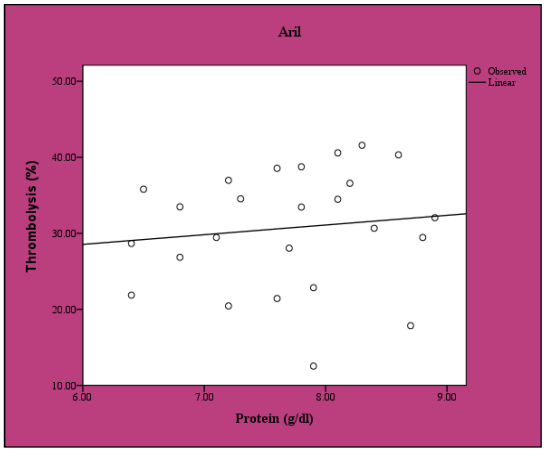


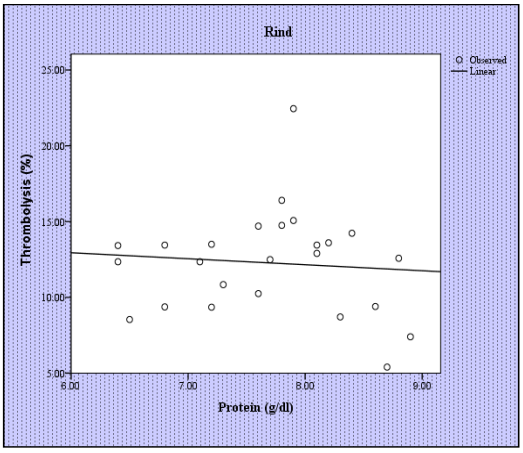
r^2 – 0.007 p – 0.692 **r^2 – 0.005 p – 0.743**



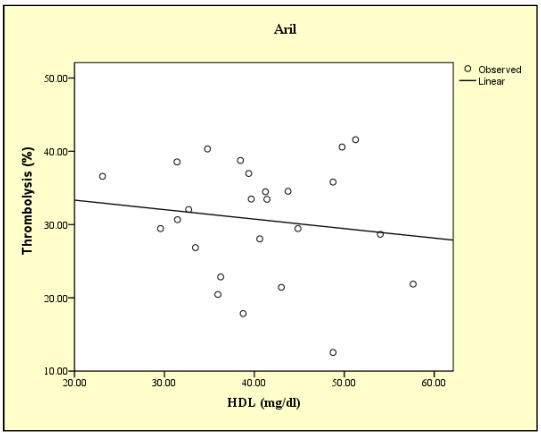
r^2 – 0.003 p – 0.783 **r^2 – 0.002 p – 0.828**

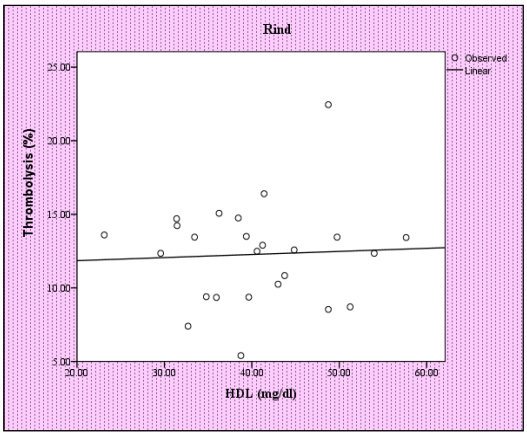
Figure 3: Correlation between lipid profile and thrombolytic activity of aril of *Punica granatum* in diabetic participants



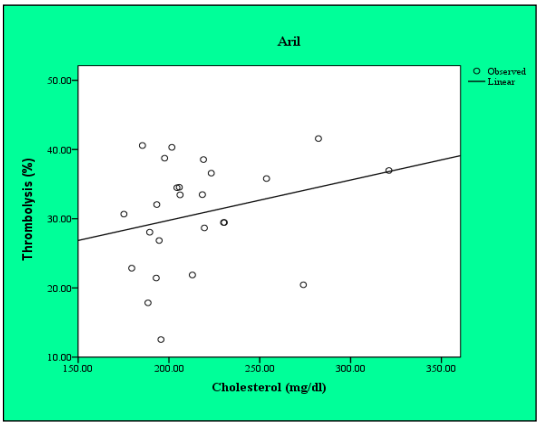


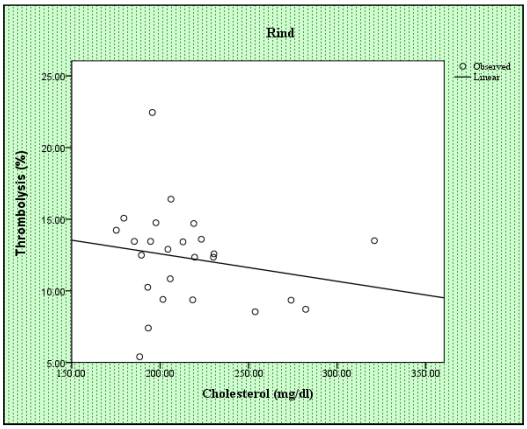
r^2 – 0.015 p – 0.557 **r^2 – 0.007 p – 0.681**





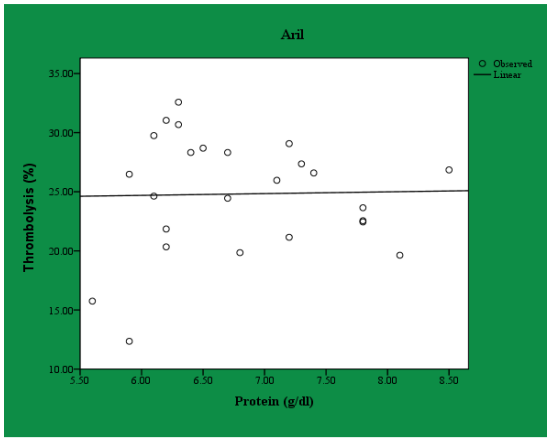
r^2 – 0.019 p – 0.511 **r^2 – 0.003 p – 0.811**

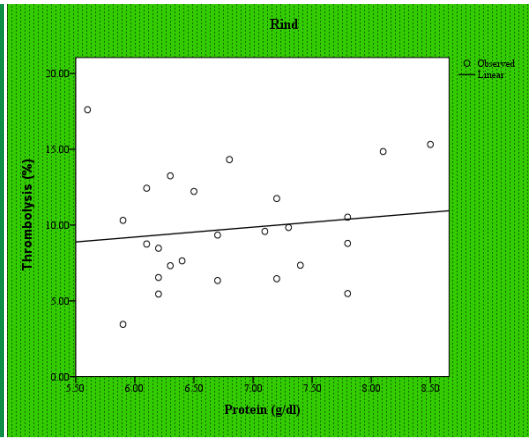




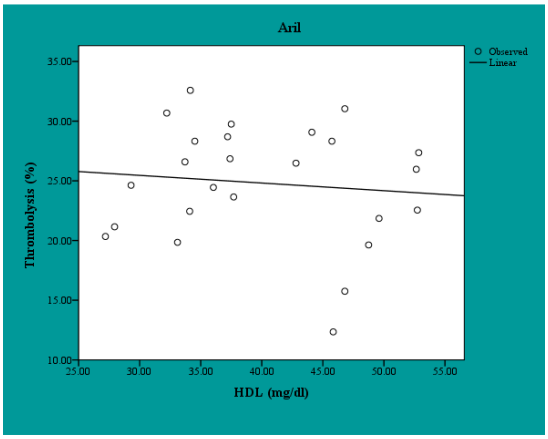
r^2 – 0.068 p – 0.207 **r^2 – 0.038 p – 0.353**

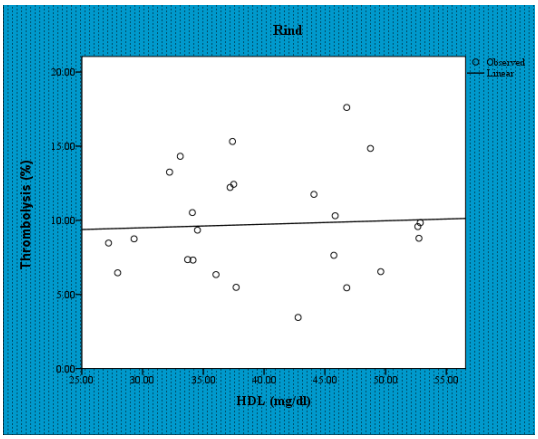
Figure 4: Correlation between lipid profile and thrombolytic activity of aril of *Punica granatum* in hypertensive participants



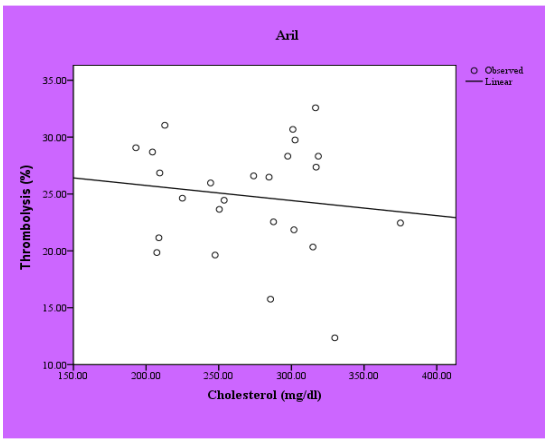


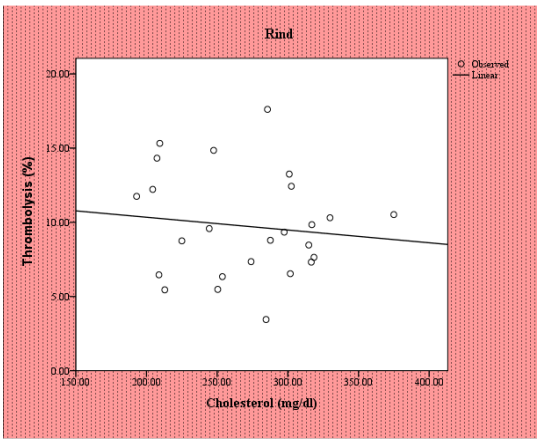
r^2 – 0.001 p – 0.911 **r^2 – 0.021 p – 0.493**





r^2 – 0.011 p – 0.617 **r^2 – 0.003 p – 0.798**





r^2 – 0.017 p – 0.531 **r^2 – 0.014 p – 0.573**

From the results it is understood that the clot lysing ability of both the aril and rind of *Punica granatum* is unrelated to the level of cholesterol in patients and the cause of thrombus formation also.

CONCLUSION

Lifestyle diseases like diabetes, cardiovascular diseases, hypertension etc is very widespread in present day's world. The above results and with the analysis of the available literatures, the fruit *Punica granatum* gains significance as a potent antioxidant and a thrombolytic agent. The results also further strengthen the ability of the fruit to lyse the clot irrespective of the cause of clot formation and the cholesterol levels. At the other end of the spectrum, it is essential to identify the compound/compounds present in *Punica granatum* responsible for thrombolysis.

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