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In vitro study of: Human RBC membrane stabilization and antioxidant activity of *Thespesia populnea* L – Poovarasu

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Abstract
Thespesia populnea (L.) belongs to Malvaceae family, known as Poovarasu in Siddha literature, is a large avenue tree found in tropical and coastal region in Srilanka and India. The root bark, leaves, flower and fruits are useful in medical therapy such as scabies, psoriasis, eczema and ring worm infestation. The leaves are externally applied for the anti-inflammatory effect for condition like swollen joints. The purpose of the research is to find out the *in vitro* antioxidant potential and membrane stabilizing effect of *T. populnea* extract in human RBC model. Powdered samples (10 g) were extracted with 100 ml of solvents (hexane, ethyl acetate, methanol, ethanol and water) for 3 h at room temperature. The solvent was evaporated using a rotovapor and dry extract was obtained. The extract was then re-dissolved in water at 100 mg / ml ratio and used for the analysis of antioxidant activity through DPPH free radical scavenging assay. RBC membrane stabilization potential of plant extracts were investigated using human RBC model. Blood (2 ml) was drawn from volunteer in a heparinised tube and centrifuged at 2000 rpm for 10 min. The pellet (RBC cells) was washed twice with PBS (9 ml) and finally the pellet was re-suspended in 10 ml of PBS. The extract (500 µl) were added to 1 ml PBS, 1 ml of 3% H_2O_2 and incubated for 30 min. The contents were centrifuged and the supernatant was analyzed spectropmetrically. Based on the absorbance, the percentage of RBC membrane damage and inhibition of membrane damage were calculated. From the results, it is concluded that the aqueous extract of *T. populnea* bark showed appreciable antioxidant and ferric reducing capacities and effective in preventing RBC membrane damage.

Keywords: *Thespesia populnea*, extract, antioxidant, RBC, membrane

Introduction
Thespesia populnea (L) belongs to malvaceae family and known as Poovarasu according to Siddha literatures. It is a large avenue tree found in tropical and coastal region in Srilanka. The root bark, leaves, flower and fruits are useful in medical therapy such as scabies, psoriasis, eczema and ring worm infestation. The leaves are externally applied for the anti-inflammatory effect for condition like swollen joints. A poly herbal formula containing *T. populnea* is one of the ingredients for Alzheimer's disease therapy. *T. populnea* bark contains tannins, flavinoids, triterpenoids, Phytosterol, Glycosides protein, carbohydrate, lipids and fixed oil. In addition found to be presence Thespesone, Lupeol, Marsonone, Beta-sitosterol, Thespone, Kaempferol, Quinine, Quercetin, Gossypol and Rutin Inbaraj *et al*, 1999) [3]. The past research revealed that various phytoconstituents like butadion, escinol, esculetin, glyvenol, rutin flavinoids are responsible of stabilizing the erythrocyte membrane in the hypotonic haemolysis (Chaika and Khad Zhai, 1977) [21].
Erythrocyte have been used by a model system by a number of researchers for the study of interaction of drugs with membrane (Sessa and Weisman, 1968; Horie *et al*, 1979; Oyedapo and Famureva, 1995) [17],[19], [16]. RBC used as model cell by a several researchers to the study of interaction of drugs with membrane. It also has been reported that the production free radicals such as lipid peroxide and superoxide in various condition like heat induced stress haemolysis due to cell membrane de stabilization (Agarwal and Rangari, 2003). Flavinoids and triterpenoids and other phenolic compound are good scavangers for free radical due to their antioxidant properties (Smith *et al*, 1992; Repetto and Liesuy, 2002; Kumar *et al*., 2008) [24, 23, 22].

The potential health hazards and toxicity associated with synthetic antioxidant, turn our eye towards the search for antioxidants from natural sources for membrane stabilizing effect and antioxidant capacities in plant extract including DPPH, reducing power, superoxide radicals, hydrogen peroxide radicals, hydroxide radicals and lipid peroxidation assays. Therefore the aim and objective of the present study was to determine the phenolic content and to characterize the antioxidant and RBC membrane stabilization properties of different extracts of bark powder of *Thespesia populnea* L.

Materials and methods

Sample collection

The *Thespesia populnea* bark sample was collected from Jaffna, Northern part of Srilanka and was authenticated by Dr. N. Ravichandran (Botanist), Research officer, Carism, Sastra University, Thanjavur, Tamilnadu, India.

Preparation of extract

Powdered samples (10 g) were extracted with 100 ml of solvents (hexane, ethyla acetate, methanol, ethanol and water) and kept for 3 h at room temperature. The extracts were then separated using Whatman No. 1 filter paper and used for further experiments. For antioxidant activity, the solvent was evaporated using a rotovapor (Make: Buchi, Model: R-300) and dry extract was obtained. The extract was then re-dissolved in water at 100 mg / mL ratio and used for the analysis.

Preliminary Phytochemical Screening

2.5gm of air dried powdered Thespesia poulnear digested in alchocol and allowed to stand for 24h.Then filter, filtrate was evaporated to dryness over a water bath. Collected residue screen qualitatively for the presence of following phytochemical constituents as per the standard protocol.

Total phenol content

The total phenolic content of extract was estimated according to the method of Suitably diluted sample (100 µl) was taken with 250 µl of Folin's-Ciocalteu reagent and 1000 µl of 5% of Na2CO3 was added and incubated for 30 min in dark. Then the absorbance was measured at 720 nm using Spectrophotometer. A calibration curve was prepared using standard gallic acid (16 – 100 mg/L; y = 0.0094x – 0.0585; R2 = 0.9939) and used to calculate the total phenolic content of the extract and the results were expressed as gallic acid equivalents (mg GAE / 100 g sample).

Antioxidant activity

The DPPH radical scavenging assay was used to analyze the antioxidant property of aqueous extract of the sample by following Sanchez-Moreno *et al*. (1998) [7] method. The extract (100 µl) was added to 0.9 ml of methanolic solution of DPPH (2.5 mg/100 ml) and the reactants were incubated at room temperature for 30 min in dark. Different concentrations of Butylated hydroxyanisol (BHA) were used as a standard and the solvent (distilled water) was used instead of extract in control. After 30 min, the absorbance was measured at 515 nm using a spectrophotometer and the radical scavenging activity of the extract was calculated and expressed on percentage basis.

Ferric reducing power

The reducing power of extract was determined according to the method of Oyaizu (1986) [8]. Samples (2.5 ml) in phosphate buffer (2.5 ml, 0.2 M, pH 6.6) were added to potassium ferricyanide (2.5 ml, 1.0%) and the mixture was incubated at 50 °C for 20 min. Trichloroacetic acid (2.5 ml, 10%) was added, and the mixture was centrifuged at 650 x g for 10 min. The supernatant (5.0 ml) was mixed with ferric chloride (5.0 ml, 0.1%), and then the absorbance was read spectrophotometrically at 700 nm. Based on the absorbency value, the ferric reducing power of extract was expressed.

Membrane stabilization potential

RBC membrane stabilization potential of plant extracts were investigated according to the methods proposed by Sakat *et al*. (2010). Human blood (2 ml) was drawn from volunteer in a heparinised tube and centrifuged at 2000 rpm for 10 min. The pellet (RBC cells) was washed twice with PBS (9 ml) and finally the pellet was re-suspended in 10 ml of PBS. The extract (500 µl) were added to 1 PBS, 1 ml of 3% H2O2 and incubated for 30 min. In normal control, 1 ml PBS was added instead of extract and in standard group, 1 ml of ascorbic acid was added instead of extract and in negative control only H_2O_2 was added. After incubation, the contents were centrifuged at 2000 rpm for 10 min and the supernatant was used to measure the absorbance at 520 nm. Based on the absorbance, the percentage of RBC membrane damage and inhibition of membrane damage were calculated.

Results and Discussion

Table 1: Preliminary phytochemical screening of *Thespesia populnea* bark Symbol (+) indicates presence and (–) indicates absence of compounds

S. No.	Phytochemicals	Results
1	Alkaloids	-
2	Flavonoids	-
3	Phenols	+
4	Proteins	+
5	Sterols	+
6	Carbohydrates	+
7	Glycosides	-
8	Terpenoids	+
9	Starch	-

The phytochemical screening of the *T. populnea* extract revealed the presence of phytochemicals such as phenols, proteins, sterols, carbohydrates and terpenoids.

Total phenolic content

Among three different solvent extracts of *T. populnea*, methanol extract was found to exhibit higher level of total phenolic compounds (1518 mg GAE / 100 g), which is followed by ethanol (1347 mg GAE / 100 g) and water extract (1145 mg GAE / 100 g) (Figure 1).

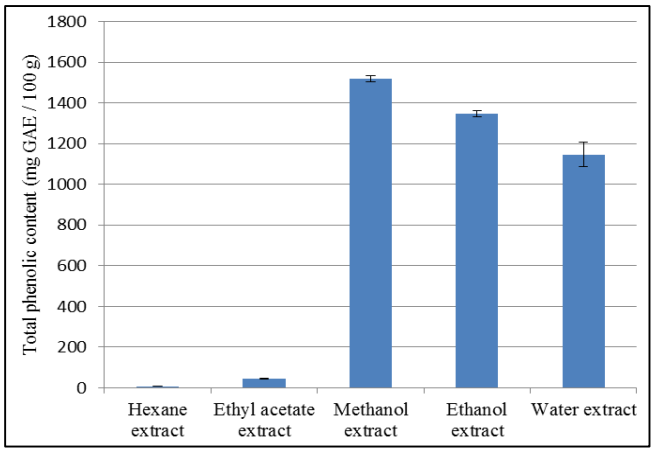

Fig 1: Total phenolic content of different solvent extracts of *Thespesia populnea* bark

Antioxidant activity

Antioxidant activity of solvent extracts of *T. populnea* was analyzed using DPPH radical scavenging assay and the results revealed that the aqueous extract has higher level of antioxidant power (IC-50 = 9.37 mg/ml) when compared to ethanol (IC-50 = 75 mg/ml) and methanol extracts (IC-50 = 354 mg/ml) (Figure 2). The antioxidant power of aqueous extract of *T. populnea* was appears to be comparable to that of standard gallic acid (IC-50 = 2.34 mg/ml). The aqueous extract actually contained lower level of total phenols and methanol extract showed higher phenolic content, but the antioxidant activity of these extracts were in contrast to their phenolic content. Hence, there is no relationship between the phenolic content and antioxidant activity of *T. populnea* bark extracts. Use of aqueous extract in traditional system of medicine was confirmed by its higher antioxidant power.

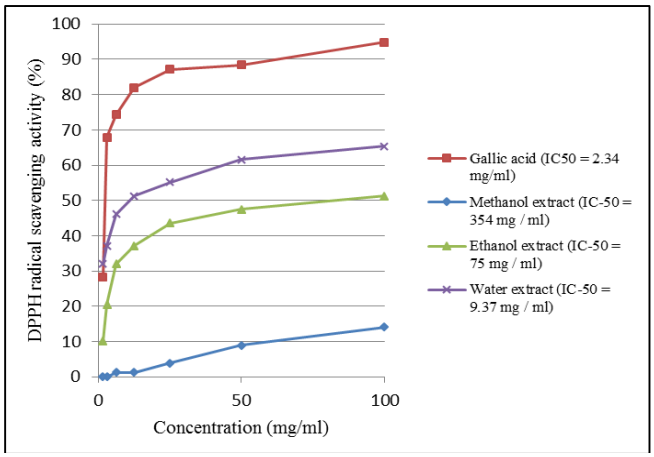

Fig 2: Antioxidant activity of different solvent extracts of *Thespesia populnea* bark

Reducing power

Ferric reducing power of *Thespesia populnea* bark extracts was analyzed and the results are shown in Figure 3. In this assay, Fe (III) is reduced to Fe (II) by the antioxidant compound through electron transfer. The reduced Fe (II) forms the Pearl's blue complex, which can be measured at 700 nm. Among the samples, aqueous extract of *T. populnea* exhibited higher ferric reducing power (74.45%) when compared to ethanolic extract (62.37%) and methanolic extract (55.83%). Even though the methanol extract has more amount of polyphenols, highest level of ferric reducing power was noted in ethanolic extract, which indicates the type of phenolic compounds extracted with methanol and water were different and aqueous extract is more suitable to enhance the iron reducing power of *T. populnea*.

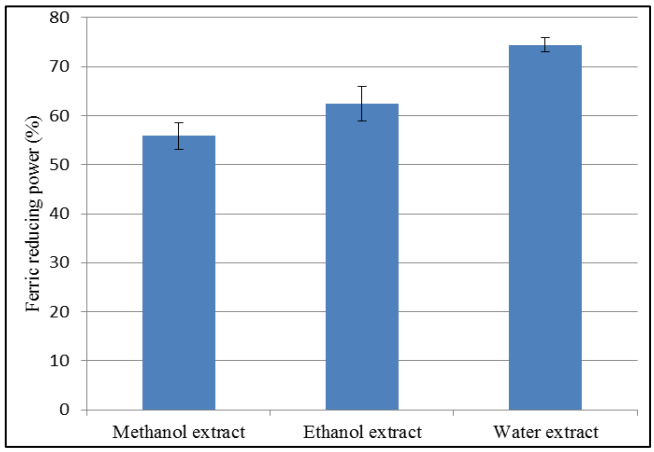

Fig 3: Ferric reducing power of *Thespesia populnea* bark

Membrane stabilization potential

RBC membrane stabilization power of extracts of *T. populnea* was given in the Figure 4. In this assay, oxidative stress was induced in RBC cells using H2O2 through the production of hydroxyl radicals and the membrane damage was measured colorimetrically and the inhibition capacity of the extracts was determined. Among different solvent extracts of *T. populnea*, ethanol extract exhibited higher level of membrane stabilization effect (103.43%), which is followed by aqueous extract (82.84%) and aqueous extract (51.96%) and the synthetic standard ascorbic acid (41.67%) (Figure 4).

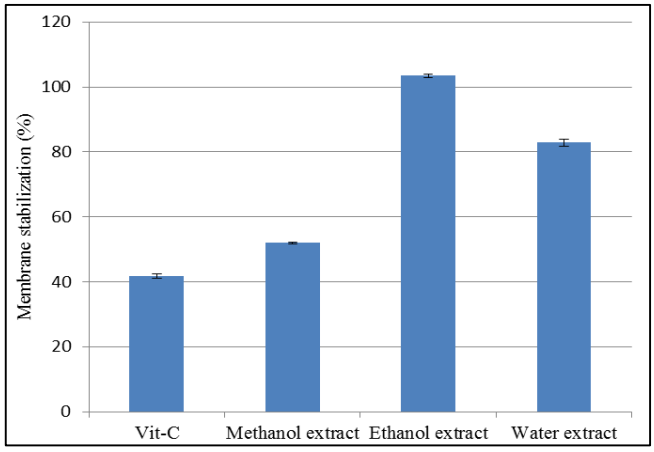

Fig 4: RBC membrane stabilization potential of solvent extracts of *Thespesia populnea* bark

Conclusion

Among the different solvents of *Thespesia populnea* bark, methanol extract was found to contain higher amounts of total phenolic compounds, but it exhibited poor antioxidant power. At the same time, the aqueous extract of *T. populnea* bark showed appreciable antioxidant power, even though it has lower phenolic content when compared to other extracts. Ethanol extract of *T. populnea* was noted to be effective in preventing RBC membrane damage, which was caused by oxidative stress. So, the ethanolic and aqueous extracts of bark of *T. populnea* warrants further *in vivo* studies to prove their antioxidant power.

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