Phytochemical properties and antioxidant activity of natural colourant extracted from Red tamarind *Tamarindus indica* var. rhodocarpa in Tamilnadu

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Abstract
Red tamarind unripen fruits, sources of excellent natural red colorant anthocyanins can be used in food, textile and cosmetics. Attempts have been made to extract the natural colourant from unripe fruits of red tamarind from 18 original sources at different districts of Tamilnadu using methanol and ethanol yielding maximum of 16.67 % and 17.5 % respectively. Anthocyanin content was estimated to 19.94 and 77.55mg/L in methanol extract and 10.023 and 34.268 mg/L in ethanol extract. The methanol and ethanol extracts of red tamarind were screened for chemical constituents and revealed the presence of secondary metabolites which included carbohydrates, phenols, tannins, flavonoids, alkaloids, terpenoids and anthocyanins and whereas saponins, steroids and glycosides were not detected.

The anthocyanin using UV spectral analysis was found to have 0.64 ppm. TLC analysis of the extracts revealed the presence of cyanidin-3-glycoside which was confirmed through HPLC analysis. Pelargonidin and delphinidin are the two more anthocyanins identified through HPLC analysis. Among the eighteen accession, Shivaraj Nagar (TVGSR 6) has maximum quantity of cyanidin-3-glycoside (1.187ppm), pelargonidin (0.1270ppm) and delphidin (0.1456ppm) compared to others. Antioxidant activity of the red tamarind extracts was found to have a range between 62% and 94%.The anthocyanins from red tamarind unripen fruits are rich in antioxidant properties with a wide scope for use in food and cosmetic applications.

Keywords: Red tamarind, TLC, HPLC, Phytochemical, Antioxidant activity.

Introduction

Colours have been always playing an important role in our day to day life as they give us the impression on fruits, foods, fabric and even medicines and strongly influence every moment of our lives.

Nowadays, interest on natural colourant is increasing on the global level due to their environment friendly nature. Plants are the reservoir of unique natural pigments that give them their colours. Natural colourant are extracted from renewable sources such as plant materials, insects, algae etc.

Though India is a seventh biggest country sharing two percent of land area on the earth, India is endowed with some 500 varieties of plants that can yield natural colours[1] but only a few sources were exploited commercially such as indigo, turmeric, annatto etc. The natural colourants have their own limitations like availability, colour yield, stability and toxicity. Hence, there is an emerging need to use anthocyanins because of desirable qualities in addition to its good stability at low pH and high temperature.

Anthocyanins are the most spectacular plant pigments which have raised a growing interest due to their extensive range of colours from orange to red, purple and blue in flowers, fruits and vegetables which have the potential to be incorporated as food colourant, innocuous and beneficial health[2-4]. In addition, industry is demanding natural pigments, especially natural red colors and anthocyanins are candidates for the introduction of these tone colorants. Hence exploration of anthocyanins from newer sources for use in food and cosmetics is warranted.

The red tamarind is a rare mutant which contains rosy red pigments which are responsible for its red colour and the unripen stage of the fruits provides anthocyanins[5,6]. The red pigment (anthocyanin) from the half-matured red variety tamarind could be used to impart a natural and attractive red colourant and hence there is ample scope for red tamarind to be used as a source of natural red food colourant in future[7].

The red fruited variety of tamarind taxonomically known as *Tamarindus indica* var. rhodocarpa (red tamarind) is one such species found in India. It is a rare variant with limited distribution in southern states of India. The unripen stage of the fruits provides anthocyanins. The anthocyanins from red tamarind unripen fruits are rich in antioxidant properties for use in food and cosmetic applications. The plethora of traditional uses has encouraged researchers to identify and isolate phytochemicals which are reported to have various desirable activities as natural colourants.

More than 100 phytochemical compounds namely phenols, flavonoids, alkaloids, terpenoids, tannins, saponins and steroids have been isolated from various parts of the plant. The great variety of underutilized fruits needs attention for scientific exploration. Hence, the present study aimed at profiling the phytochemical potential and antioxidant

activity of red tamarind ethanol and methanol unripen fruit extracts.

Material and Methods

Study material: The material selected for the study is the fruit of red tamarind, *Tamarindus indica* var. *rhodocarpa* in unripen stage. It has natural pigments in its unripen stage of fruits called anthocyanins which bear red colour depending on pH. Unripen fruits were collected from the 18red tamarind resources identified in different parts of Tamilnadu (Table 1). The collected fruits were labelled, transported to Chemistry and Bioprospecting Laboratory, Institute of Forest Genetics and Tree Breeding, Coimbatore for extraction of natural colourant.

Extraction of red tamarind unripe fruits: Unripen fruits of red tamarind collected from different red tamarind resources (Table 1) were processed by removing fruit shell, depulped and stored in containers at 4°C until used. 500 g of unripe fruit pulp of each resource were weighed and macerated in a motor and pestle and anthocyanin was sequentially extracted with methanol and ethanol over a period of 48 hours and then filtered. The filtrate was then subjected to rotary vacuum evaporator until solvents were completely removed and the residue form extract was weighed to quantify the yield. The extracts were stored in sterile amber bottles in a refrigerator at 4°C for further analysis.

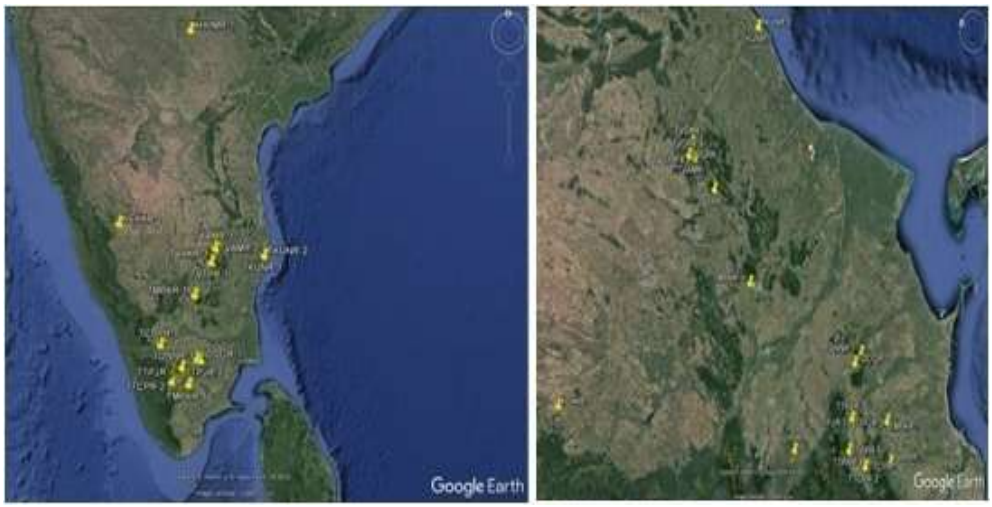

Figure 1: Red tamarind original genetic resources selected in Southern India

Table 1
Sources of red tamarind in different locations of Tamilnadu

S.N.	Original resources	Place of collection with tree number	Latitude	Longitude	Elevation
1.	TVGSR 1	Shivaraj Nagar Red - 1	N 12°56’02.70’’	E 78°42’12.80’’	380
2.	TVGSR 2	Shivaraj Nagar Red -2	N 12°56’02.60’’	E 78°42’12.30’’	379
3.	TVGSR 3	Shivaraj Nagar Red -3	N 12°56’02.70’’	E 78°42’14.00’’	379
4.	TVGSR 4	Shivaraj Nagar Red -4	N 12°56’03.00’’	E 78°42’14.80’’	377
5.	TVGSR 5	Shivaraj Nagar Red -5	N 12°56’03.00’’	E 78°42’16.30’’	378
6.	TVGSR 6	Shivaraj Nagar Red -6	N 12°56’03.00’’	E 78°42’17.40’’	379
7.	TVGSR 9	Shivaraj Nagar Red -9	N 12°56’02.90’’	E 78°42’17.50’’	380
8.	TVAKR-1	Kedampur Red - 1	N 12°50’24.80’’	E 78°42’54.80’’	413
9.	TVAKR-2	Kedampur Red - 2	N 12°50’15.10’’	E 78°42’35.10’’	336
10.	TVGMR-1	Mochampalli Red-1	N 12°56’86.50’’	E 78°48’76.20’’	305
11.	TVGPR 1	Peranampet Red - 1	N 12°56’04.50’’	E 78°42’20.80’’	357
12.	TTPJR-2	Jayamangalam Red - 2	N 10°06’16.40’’	E 77°36’18.10’’	272
13.	TTPJR-3	Jayamangalam Red - 3	N 10°06’17.60’’	E 77°36’17.60’’	272
14.	TTCPR-1	Rettapulipudhupatti Red-1	N 09°45’15.40’’	E 77°18’20.70’’	388
15.	TTCPR-2	Rettapulipudhupatti Red-2	N 09°46’05.80’’	E 77°18’40.8’’	353
16.	TCPPR 1	Pollachi Red - 1	N 10°39'12.00’’	E 77°02'03.60’’	333
17.	TTPPR 1	Bodi Red - 1	N 10°00'33.20’’	E 77°20'46.70’’	396
18.	TTPPR 2	Bodi Red - 2	N 10°00'30.80’’	E 77°20'42.10’’	373

Extraction of and quantification of anthocyanin: The extraction procedure of pigment estimation was followed as per the method described by Giusti and Wrolstad[8]. Frozen hand-peeled skin (in liquid nitrogen) from partially thawed fruit in liquid nitrogen was powdered using a stainless-steel warning blender and incubated overnight at 5˚C after blending with 25ml acetone. Repeat the process until a clear solution was obtained and filtered. To the filtrate, add 2 volumes of chloroform and store overnight at 5˚C. Collect the aqueous portion and dry in a vacuum rotary evaporator at 40˚C for 10 min, until all residual acetone evaporates.

The extracted anthocyanin was purified by passing the aqueous extract through C-18 mini column previously activated with methanol followed by 0.01% aqueous HCl. Elute anthocyanins subsequently with methanol containing 0.01% HCl and concentrate the methanolic extract at 35˚C and dissolve the pigments in deionized water containing 0.01% HCl.

The purified anthocyanin was subjected to acid and alkaline hydrolysis. Saponify the purified pigments (2 ml) of red tamarind with 10% aqueous potassium hydroxide (10 ml) for 8 min at room temperature in the dark as per the method of Hong and Wrolstad[9].

Neutralize the solution with 2N HCl and purify the hydrolysate using a C-18 Sep-Pak cartridge. The monomeric anthocyanin content was quantified using the pH differential method. A Hitachi U-2000 UV spectrophotometer was used for spectral calibration at 510 and 700 nm respectively. Calculate the pigment content as cyanidin-3-glucoside (cyd-3-glc), using an extinction coefficient of 26,000 L cm^{-1} mg^{-1} and molecular weight of 448.8.

Qualitative phytochemical analysis: The methanol and ethanol extracts of the red tamarind unripe fruits were subjected to preliminary phytochemical screening to identify the presence of various secondary metabolites such as phenols, alkaloids, flavonoids, glycosides, carbohydrate, saponins, anthocyanin and tannins using standard methods[10].

Quantitative Phytochemical analysis: The methanol and ethanol extracts of the red tamarind unripe fruits were subjected to phytochemical quantification. The quantitative estimation of carbohydrates and total phenols was performed by the method described by Sadasivam and Manickam. Flavonoids were estimated by the method of Cameron et al.[11]

Chromatographic profiling of red tamarind unripe fruits:

Sample preparation: 10g of red tamarind unripe fruit pulp was soaked in 0.1% HCl in 100mL ethanol in a clean dry glass beaker for 24 hours. After 24 hours of incubation, the mixture was filtered through No.1 Whatmann filter paper. The filtrate was concentrated under reduced pressure on a rotary evaporator at 40°C until a syrup consistency was reached. The residue was redissolved in 10 mL ethanol and the extraction procedure was repeated twice. Purified anthocyanin was dissolved in ethanol for TLC analysis.

The purified sample in ethanol was separated by spotting on preparative silica gel-G plates coated with UV254 and UV356 binder (20 X 5cm, 250 µm with inorganic binder and UV 254) with 5 µl capillary pipettes in a TLC chamber using suitable mobile phase (n-Butanol: Acetic Acid: water (4:1:1)). The developed TLC plates were air dried and the chromatographic spots were visualized using ultraviolet lamps emitting at 254 and 365 nm. The separated individual anthocyanins fractions were eluted from TLC plates and purified in ethanol, filtered and stored at 4ºC until further HPLC analysis. Retardation factor (RF) value was calculated by using the formula:

Retardation factor (RF) =Distance travelled by solute (cm)/Distance travelled by solvent front (cm).

UV-Vis Spectrophotometer: Spectral measurements are important in the identification of many plant constituents, monitoring the chromatographic columns elutes during purification of plant products and for screening crude plant extracts for the presence of particular classes of compounds. The anthocyanin fragments separated in TLC were scanned between 700-200 nm and record the wavelength maxima and minima (λ max) and also record the OD of the same to confirm the group of compounds present in the eluted fragment.

HPLC analysis of red tamarind unripe fruit extract: High Pressure Liquid Chromatography (HPLC) is used to find the amount of a chemical compound within a mixture of other chemicals.

Sample preparation: 1.0 mg of red tamarind fruit extract was mixed with 1ml of mobile phase solution. The mixture was filtered through Varian Bond Elute C18 solid phase extraction to remove impurities. Then the filtrate was also degassed in ultra sonicator for 10 minutes and 20 µl of this sample was injected in HPLC system for separation and identification of anthocyanins present in the unripe fruit extract samples of red tamarind.

HPLC equipment with L-4000 UV detector, L-6200 Intelligent pump and RP-C18 column from Hitachi with Data Ace workstation was used in the present study. The solvent system containing phosphoric acid, acetic acid and acetonitrile in the ration 1.5:20:25 was used as the mobile phase and the flow rate was maintained at 1ml/min at a wavelength of 520 nm.

Antioxidant activity- DPPH radical scavenging ability: The DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical is widely used as the model system to investigate the scavenging activities of several natural compounds. To an aliquot of 2 ml of the extract of the red tamarind unripe fruits, add 1 mM of DPPH (prepared in ethanol) in a test tube.

Vortex the mixture and incubate for 30 minutes in dark at room temperature with vigorous shaking. Ascorbic acid (vitamin C) at various concentrations (20 to 100mg/ml) was used as standard. Radical scavenging ability was calculated using the following formula. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity.

DPPH Scavenged (%) = [(A control– A test) / A control] × 100

where A control is the absorbance of the control reaction and A test is the absorbance in the presence of the sample of the extracts[12].

Results and Discussion

Extraction of natural pigment/anthocyanin: Nature has always dominated over synthetic colourants and gives more appealing look; as a result food poisoning was very common causing various health hazards. In the present study the natural colourant was extracted from unripe fruit of 18 red tamarind resources identified in different parts of Tamilnadu using methanol and ethanol yielding maximum of 16.67 % and 17.5 % respectively (Table 2). A dark red colour extract was obtained from the unripe fruit of red tamarind extracted with these solvents. Kaur et al[7] reported that the red pigment, anthocyanin was extracted from the half-matured red variety tamarind and used to impart a natural and attractive red colour and that there is ample scope for red tamarind to be used as a source of natural red food colourants in the near future.

Anthocyanins are the most spectacular plant pigments/ natural colourants which have raised a growing interest due to their extensive range of colours. During the past decade, interest in anthocyanins is getting augmented immensely because of its unique properties. The red tamarind is a rare variety which contains rosy red pigments which are responsible for its red colour[5,6]. The unripen stage of the fruits provides anthocyanins.

Mayavel et al[13] suggested that red tamarind is a good source of anthocyanins (Biocolourants) and has wide scope in food industry, pharmaceuticals, cosmetics and textile industries. Anthocyanin concentration was found to have a range between 240 and 245mg/lin pH differential method. However, the pH differential method is a simple laboratory method for determining the amount of anthocyanins followed by AOAC's strict validation process[14]. The plant grown region and its growth season have significant impact on the composition of anthocyanins both quantity and quality[15-16].

Nile et al[17] reported that 181.2 mg/ 100 g of total anthocyanin content was quantified in the grape variety Vidal Black and 716.4 mg/ 100 in Catawba. Forbes et al[18] reported that black raspberries own higher anthocyanin contents 400 mg/100 g of fruits than blackberries with 150 mg/100 g and the yellow raspberries provide 0–3.4 mg/100 g of anthocyanins.

The monomeric anthocyanin content ranged between 19.94 and 77.55mg/L in methanol extract and 10.023 and 34.268 mg/L in ethanol extract of the unripe fruits of red tamarind (Tale 3). The anthocyanin content of the unripen fruits of red tamarind is high (180 to 360 mg/g of unripe fruit) when compared to anthocyanin rich fruits like grapes (80-90 mg/g), cherry (70-75 mg/g) and jamun (120-130 mg/g)[18].

Table 2
Performance of organic solvents viz., ethanol and methanol for extraction of natural red colourant from red tamarind

S.N.	Tree Code	Place of collection	Extract yield (%)		% increase in yield in methanol extract
			Ethanol	Methanol	
1	TVGSR 1	Shivaraj Nagar Red - 1	2.87	3.89	35.54
2	TVGSR 2	Shivaraj Nagar Red -2	5.60	4.25	-
3	TVGSR 3	Shivaraj Nagar Red -3	8.03	8.35	4
4	TVGSR 4	Shivaraj Nagar Red -4	5.35	6.78	26.72
5	TVGSR 5	Shivaraj Nagar Red -5	8.08	7.05	-
6	TVGSR 6	Shivaraj Nagar Red -6	11.62	12.13	4.4
7	TVGSR 9	Shivaraj Nagar Red -9	9.40	11.98	27.44
8	TVAKR 1	Kedampur Red - 1	4.89	6.73	37.62
9	TVAKR 2	Kedampur Red - 2	3.53	4.25	20.39
10	TVGMR1	Mochampalli Red-1	5.54	7.68	38.62
11	TVGPR 1	Peranampet Red - 1	12.06	14.25	18.16
12	TTPJR 2	Jayamangalam Red - 2	13.78	16.78	21.77
13	TTPJR 3	Jayamangalam Red - 3	14.06	15.34	10
14	TTCPR 1	Rettapulipudhupatti Red-1	14.29	16.67	16.6
15	TTCPR 2	Rettapulipudhupatti Red-2	16.67	17.5	5
16	TCPPR 1	Pollachi Red - 1	12.89	14.18	10
17	TTPPR 1	Bodi Red - 1	9.32	11.02	18.2
18	TTPPR 2	Bodi Red - 2	10.43	11.45	9.8

Table 3
Total monomeric anthocyanin content in methanol and ethanol extracts of red tamarind unripe fruit

S.N.	Tree code	Place of collection	Anthocyanin content mg/L	
			Methanol extract	Ethanol extract
1	TVGSR 1	Shivaraj Nagar Red – 1	34.648	23.183
2	TVGSR 2	Shivaraj Nagar Red -2	35.896	18.116
3	TVGSR 3	Shivaraj Nagar Red -3	37.199	20.077
4	TVGSR 4	Shivaraj Nagar Red -4	32.702	17.935
5	TVGSR 5	Shivaraj Nagar Red -5	30.871	25.788
6	TVGSR 6	Shivaraj Nagar Red -6	49.064	22.725
7	TVGSR 9	Shivaraj Nagar Red -9	33.002	20.998
8	TVAKR 1	Kedampur Red - 1	19.938	10.023
9	TVAKR 2	Kedampur Red - 2	33.093	14.008
10	TVGMR 1	Mochampalli Red-1	37.391	12.568
11	TVGPR 1	Peranampet Red - 1	36.189	26.952
12	TTPJR 2	Jayamangalam Red – 2	77.553	26.598
13	TTPJR 3	Jayamangalam Red - 3	73.041	27.265
14	TTCPR 1	Rettapulipudhupatti Red-1	65.808	34.268
15	TTCPR 2	Rettapulipudhupatti Red-2	68.332	23.044
16	TCPPR 1	Pollachi Red - 1	64.617	26.632
17	TTPPR 1	Bodi Red - 1	21.403	14.478
18	TTPPR 2	Bodi Red – 2	27.815	13.172

The red tamarind accessions of Pollachi, Jayamangalam, Shivaraj Nagar and Rettapulipudhupatti were found to have high anthocyanin content than others.

Phytochemical constituents of unripe fruit extract of red tamarind: To classify the types of organic constituents present in the unripe fruit of red tamarind, preliminary phytochemical screening tests were carried out. The methanol and ethanol extracts of red tamarind screened phytochemically for their chemical constituents revealed the presence of secondary metabolites which includes carbohydrates, glycosides, phenols, tannins, saponins, flavonoids, alkaloids, terpenoids, steroids and anthocyanins. Phenols were observed to be more in methanol extract; flavonoids in ethanol extract; tannins and anthocyanins were recorded more in both ethanol and methanol extracts compared to other metabolites. Phytochemical screening of the *Curcuma longa*, natural dye confirmed the presence of saponins, tannins, flavonoids and alkaloids[20].

Among the phytochemicals screened, phenols, flavonoids, tannins and anthocyanins were in large quantity in red tamarind unripe fruit extract than the others. Kaur et al[7] reported that the extract of pomegranate is rich in phytochemical compounds. Pomegranates contain high levels of flavonoids and polyphenols[21]. The phytochemical screening of *Spathode acampanulata* the dye yielding flower revealed the presence of tannins, cardiolides and terpenoids in both ethanol and NaOH extract and saponins, tannins, cardiac glycosides, cardiolides and terpenoids only in ethanol extract.

Primary and Secondary metabolites of red tamarind unripe fruits: The plethora of traditional uses has encouraged researchers to identify and isolate phytochemicals which are reported to have various desirable activities as natural colourants. There is tremendous scientific interest on exploration of natural colourant which exhibits high biodegradability, low toxicity and compatibility with the environment. The phytochemicals present in the unripe fruits of red tamarind were quantified and presented in table 4.

The carbohydrate quantified for the unripe fruit methanol and ethanol extracts of 18 accessions of red tamarind ranged from 8.397 to 25.622 mg/g in ethanol extracts and 7.009 to 23.038 mg/g in methanol extracts. Ethanol extract of Shivaraj Nagar Red-9 (23.827 mg/g), Peranampet Red-1 (24.33 mg/g) and Rettapulipudhupatti Red-2 (25.622 mg/g) and methanol extracts of Rettapulipudhupatti Red-2 (21.746mg/g), Shivaraj Nagar Red-9 (22.081mg/g) and Jayamangalam Red – 2 (23.038 mg/g) showed high carbohydrate content. The carbohydrate and protein contents were high in red tamarind with maximum range of 8.6g-12.61g.

Rowayshed et al[22] reported that the fruit extracts of grapes and strawberry have carbohydrate content range between 8.81mg/ml and 75mg/ml. The carbohydrate present in the natural colourant decreased the water activity around the anthocyanin and thus protected it from changes in structure and thereby decreased the change in colour[23]. In the present study the phenol content ranged from 0.27 to 0.92 mg/g in ethanol extracts and from 0.54 to 0.93 mg/s in methanol extracts.

Overall in many accessions, phenol content was comparatively high in methanol extracts which proved that

this solvent extracts more of the phenols than ethanol. Avocado seed can be used as a potential source of colorant due to 77% phenolic content (mg/g Gallic Acid Equivalents) in the colored extract and the utilization of phenolics for formation of coloured pigments[24]. *Thymus moroderi* was used as a food colorant because it came with potential polyphenols content[25].

Polyphenols, carotenoids and chlorophylls are natural pigments that are derived from raw materials. Flavonoids are the most important largest group of plant pigments ranging from pale yellow (isoflavones), deep yellow (chalcones, flavones, flavonols, aurones), orange (aurones) to reds and blues (anthocyanins)[26]. Total flavonoids of unripe fruit pulp extracts of red tamarind were estimated.

Shivaraj Nagar resources (2, 4 and 9) showed high flavonoid content than other resources and the methanol extract of TVGSR 4 was found to have high quantity of flavonoids 9.25 mg/g and TVGSR 6 was found to be low of 1.24mg/g and ethanol extracts of TVGSR 9 were found to have high quantity of flavonoids (8.26mg/g) and minimum of 0.44mg/g in TTPPR 2. Flavonoids are the major colouring component of flowering plants and being phytochemicals, humans and animals cannot synthesize flavonoids[27]. The plant-rich flavonoids are responsible for food colour, taste, prevention of fat oxidation and protection of vitamins and enzymes[28]. Flavonoids are garnering interest in the food, beverage and supplement industries because of their natural red-purple colorant properties. *Pterocarpus santalinus* (Red Sandalwood) is also a plant source of flavonoid dye[29]. Tannins are the most vital ingredients necessary for dyeing and improve the affinity of fibres towards different dyes.

It was quantified in unripe fruits pulp extracts of red tamarind and it was found that methanol and ethanol extract of TTCPR-1, Rettapulipudhupatti were found to have high quantity of tannin for about 15.81mg/g and 15.62 mg/g. Tannins play very important role in dyeing with natural dyes. By mixing with different natural dyes it gives different shades like yellow, brown, grey and black. *Acacia catechu*[30], *Terminalia chebula*[31], *Punica granatum*[32] and *Quercus infectoria*[33] are plant sources for tannins.

Characterization of anthocyanins in the unripe fruit extracts of red tamarind: Anthocyanins present in various parts of plants may be characterized using various chromatographic and spectral analysis. In the present work the unripe fruit extract of red tamarind was subjected to TLC separation, the separated and purified fractions were subjected to UV scan and the phytoconstituents present were quantified in HPLC.

Table 4
Estimation of metabolites in ethanol and methanol extracts of *T. indica* var. rhodocarpa

S. N.	Tree code	Place of collection	Carbohydrate content (mg/g)		Phenol content (mg/g)		Flavonoid content (mg/g)		Tannin content (mg/g)	
			Ethanol extract	Ethanol extract	Ethanol extract	Ethanol extract	Methanol extract	Methanol extract	Methanol extract	Methanol extract
1.	TVGSR 1	Shivaraj Nagar Red - 1	14.641	0.76	1.74	8.08	7.86	1.54	0.78	16.77
2.	TVGSR 2	Shivaraj Nagar Red -2	9.21	0.90	7.01	15.77	15.27	7.41	0.93	7.153
3.	TVGSR 3	Shivaraj Nagar Red -3	8.397	0.72	3.63	14.18	13.89	2.34	0.80	11.148
4.	TVGSR 4	Shivaraj Nagar Red -4	12.105	0.81	7.79	14.74	14.55	9.25	0.84	16.22
5.	TVGSR 5	Shivaraj Nagar Red -5	12.846	0.60	2.34	2.06	1.91	1.39	0.65	18.277
6.	TVGSR 6	Shivaraj Nagar Red -6	19.688	0.59	4.68	11.22	10.84	1.24	0.65	17.272
7.	TVGSR 9	Shivaraj Nagar Red -9	23.827	0.89	8.26	14.64	14.18	8.95	0.80	22.081
8.	TVAKR 1	Kedampur Red - 1	14.255	0.32	1.07	8.20	10.40	2.38	0.54	13.995
9.	TVAKR 2	Kedampur Red - 2	11.411	0.75	4.02	10.45	10.55	4.50	0.73	7.009
10.	TVGMR 1	Mochampalli Red-1	16.253	0.27	1.15	8.25	11.22	3.98	0.55	11.291
11.	TVGPR 1	Peranampet Red - 1	24.33	0.73	4.43	13.32	13.61	3.52	0.64	15.622
12.	TTPJR 2	Jayamangalam Red - 2	19.736	0.69	3.93	8.64	8.0	4.69	0.66	23.038
13.	TTPJR 3	Jayamangalam Red - 3	13.301	0.69	4.33	7.96	8.18	4.94	0.70	18.11
14.	TTCPR 1	Rettapulipudhupatti Red-1	15.622	0.92	4.21	15.81	15.61	4.24	0.91	8.54
15.	TTCPR 2	Rettapulipudhupatti Red-2	25.622	0.92	3.11	15.59	15.30	4.46	0.91	21.746
16.	TCPPR 1	Pollachi Red - 1	12.703	0.75	3.67	15.25	15.42	2.01	0.71	10.574
17.	TTPPR 1	Bodi Red - 1	14.234	0.72	4.54	9.18	9.23	3.31	0.76	11.674
18.	TTPPR 2	Bodi Red – 2	17.942	0.55	0.44	9.32	9.49	4.72	0.71	7.559

TLC separation of the unripen fruits extracts of red tamarind revealed two different spots by comparing with their corresponding standards bands in the sample column showing that first one is Cyanidin-3-glucoside and second one is Pelargonidin. Separation and identification of anthocyanins extracted from the Mulberry fruit by TLC method were also reported[34]. The purified anthocyanins eluted through TLC when subjected to wave length scanning in UV visible spectrophotometer between 200 and 800 nm showed λ_{max} in the range of 220-240 and 200-220 respectively which confirmed the presence of anthocyanins in red tamarind. The anthocyanin pigment from *Ficus Padana* Burm.f has two absorption peaks at 278 nm in UV range and another is λmax = 526 nm in visible range which showed the presence of anthocyanin compounds.[35]

HPLC is a tool for identifying and quantifying individual anthocyanin in a sample. Anthocyanins were identified by their respective retention times and characteristic chromatogram. 12 anthocyanins by HPLC analysis of the extracts from the Ayamurasaki cultivar of sweet potato and twenty six anthocyanin pigments were identified in purple sweet potato cell cultures. Cyanidin, pelargonidin and delphinidin are the major anthocyanins identified in red tamarind through chromatographic analysis. Cyanidin 3-glucoside was also reported as the major anthocyanin of Jabuticaba *(Myrciaria cauliflora*), a tropical fruit[37].

In Bilberry, five major anthocyanidin a glycones: delphinidin, cyanidin, petunidin, peonidin and malvidin were reported through HPLC analysis. Anthocyanins are polyphenols that exist in plants as red pigments. Sigurdson et al[35,36] also reported that Phycocyanin from the blue algae and Spirulina (*Arthrospira platensis*) are the only source of blue colour and so far, no anthocyanin-based blue colourant is commercially available.

In unripe fruit extract of red tamarind, the anthocyanins, Cyanidin 3-glucoside, pelargonidin and delphinidin were quantified high in Rettapulipudhupatti Red-1 (TTCPR-I) and Pollachi-1 (TCPPR-I). Cyanidin 3-glucoside was quantified high in Rettapulipudhupatti Red-2 (TTCPR-2), pelargonidin and delphinidin in Pollachi 1 (TCPPR-I). Similarly the anthocyanins identified in grapes were malvidin-3-glucoside, delphinidin-3-glucoside, petunidin-3-glucoside, cyaniding-3 glucoside and peonidin-3-glucoside on the basis of their retention times [17]. Anthocyanins are the group of flavonoid natural colorants that give a variety of shades from orange-red (pelargonidin) in reddish to blue-violet (delphinidin) colour of grapes and blueberries[41].

Delphinidin-3-rutinoside is the major component reported in the reddish color berries and cyanidin-3-rutinoside in the black ones[42]. Pelargonidin appears as red-coloured pigment in nature and it differs from most of the anthocyanidins [43]. Pelargonidin gives red hue to few fruits and berries[44]. The authentic anthocyanins reported in red tamarind account for its red natural colourant.

Antioxidant activity: Antioxidant activity of red tamarind extracts (methanol and ethanol) was made for eighteen accessions and it was found that methanol extract of TVGSR (Shivaraj Nagar) accession has 62% antioxidant activity than ethanol extract of TVGSR. In Pudhupatti, accessions such as TTPJR 2 and TTPJR 3 have antioxidant activity of 92% than the ethanol extract having 80% activity. TTCPR 1 and TTCPR 2 of Rettapulipudhupatti exhibited 94% and 82% antioxidant activity of methanol and ethanol extract; methanol extract of Rettapulipudhupatti has high antioxidant activity. Pollachi accession TCPPR 1 showed highest antioxidant activity comparatively than the other accessions of ethanol extract of about 84% but methanol extract of Pollachi has high antioxidant activity than ethanol extract of 92% respectively (Fig. 2).

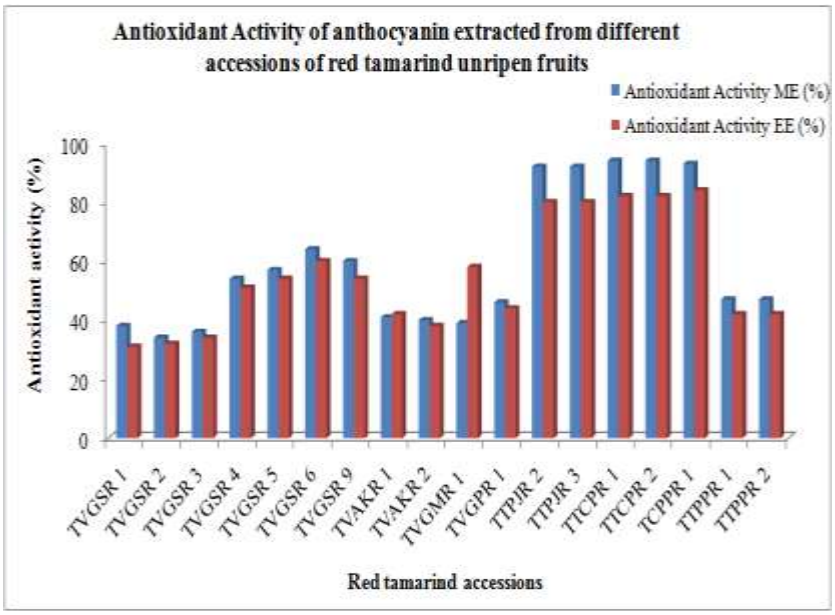

Figure 2: Antioxidant activity of anthocyanin of red tamarind unripen fruits

Table 5
Quantification of anthocyanin in different accessions of red tamarind unripen fruits

S.N.	Accession Nos.	Name of the Compound	Retention time (Mins)	Quantity of compound (ppm)
1.	TVGSR 1	Cyanidin	1.280	0.4020
		Pelargonidin	1.192	0.265
		Delphidin	3.066	0.0893
2.	TVGSR 2	Cyanidin	1.280	0.1041
		Pelargonidin	1.192	0.0399
		Delphidin	3.066	0.0833
3.	TVGSR 3	Cyanidin	1.280	0.1106
		Pelargonidin	1.192	0.0432
		Delphidin	3.066	0.0703
4	TVGSR 4	Cyanidin	1.280	0.1151
		Pelargonidin	1.192	0.0464
		Delphidin	3.066	0.0700
5.	TVGSR 5	Cyanidin	1.280	0.1404
		Pelargonidin	1.192	0.0522
		Delphidin	3.066	0.0664
6.	TVGSR 6	Cyanidin	1.280	1.1876
		Pelargonidin	1.192	0.1270
		Delphidin	3.066	0.1456
7.	TVGSR 9	Cyanidin	1.280	0.4203
		Pelargonidin	1.192	0.0880
		Delphidin	3.066	0.0894
8.	TVAKR 1	Cyanidin	1.280	0.1506
		Pelargonidin	1.192	0.0589
		Delphidin	3.066	0.0758
9.	TVAKR 2	Cyanidin	1.280	0.2158
		Pelargonidin	1.192	0.0568
		Delphidin	3.066	0.0818
10.	TVGMR 1	Cyanidin	1.280	0.2035
		Pelargonidin	1.192	0.0740
		Delphidin	3.066	0.0569
11.	TVGPR 1	Cyanidin	1.280	0.2806
		Pelargonidin	1.192	0.0642
		Delphidin	3.066	0.0657
12.	TTPJR 2	Cyanidin	1.280	1.934
		Pelargonidin	1.192	0.5879
		Delphidin	3.066	1.1355
13.	TTPJR 3	Cyanidin	1.280	2.7697
		Pelargonidin	1.192	1.0967
		Delphidin	3.066	0.7406
14.	TTCPR 1	Cyanidin	1.280	2.2983
		Pelargonidin	1.192	1.3028
		Delphidin	3.066	1.1426
15.	TTCPR 2	Cyanidin	1.280	3.5828
		Pelargonidin	1.192	1.0709
		Delphidin	3.066	0.8238
16.	TCPPR 1	Cyanidin	1.280	2.5061
		Pelargonidin	1.192	1.4075
		Delphidin	3.066	1.5596

17.	TTPPR 1	Cyanidin	1.280	0.3302
		Pelargonidin	1.192	0.1408
		Delphidin	3.066	0.4353
18.	TTPPR 2	Cyanidin	1.280	0.3878
		Pelargonidin	1.192	0.1870
		Delphidin	3.066	0.2240

It was found that berries are rich in phenolic compounds and thus the radical scavenging activity can be attributed to the combined effects of anthocyanins and phenolic compounds[45]. Antioxidant activity of red tamarind extracts was found to have range between 62% and 94%. The anthocyanins from red tamarind unripen fruits are rich in antioxidant properties for use in food and cosmetic applications.

Conclusion

Anthocyanins are the important polyphenolic compounds present in Red Tamarind. Accessions of Pollachi, Peranampet, Jayamangalam and Rettapulipudhupatti were found promising and showed high extract yield and anthocyanin content as well. Phytochemical screening showed the presence of phytochemical in the unripe fruit extract of red tamarind. Characterization of anthocyanins using TLC and HPLC analysis revealed the presence of three major anthocyanins pigments viz. cyanidin-3-glycoside, pelargonidin, delphinidin.

The red colour of the unripen fruit extracts of red tamarind is due to presence of cyaniding-3-glycoside with higher quantity of 94.21%. From the result, it is understood that the Shivaraj Nagar, Jayamangalam, Rettapulipudhupatti and Pollachi accessions have more antioxidant activity and hence the extract of red tamarind may be considered as a potential pigment for use in food and cosmetic application.

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