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Antioxidant analysis of betacyanin extracted from Basella alba fruit

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Abstract: *Basella alba* fruit with dark blue skin and deep red violet flesh is a potential source of natural colorant. This study was aimed to evaluate the total betacyanin content, total phenol and to analyse the antioxidant activity against DPPH radical, superoxide anions, hydroxyl radical, metal chelating, hydrogen peroxide, FRAP, ABTS and deoxyribose degradation in a dose dependend manner. Betacyanin extracted from *Basella alba* fruit exhibited excellent antioxidant activity. It was therefore suggested that betacyanin could be beneficial in scavenging free radicals and our findings revealed that *Basella alba* have potential as good sources of natural antioxidant/nutraceutical compounds.
Key words: *Basella alba* fruit, betacyanin, total phenol, flavanoid content, antioxidants activity, free radicals scavenging effect.

Introduction

Natural pigments from plants have attracted great attention for their usefulness, not only in the food and cosmetic industries but also in nutraceutical and pharmaceutical developments[1-3]. The health-benefit properties of natural pigments have been focussed by many works, especially those of carotenoids and anthocyanins. Betalains, because of their relative scarceness in nature, have not been much explored as bioactive compounds, but some studies have indicated their potential as antioxidant pigments. These findings have helped to motivate utilisation of betalains as food colourants. Betalains are water-soluble nitrogen-containing pigments, which are synthesised from the amino acid tyrosine into two structural groups: the red-violet betacyanins and the yellow-orange betaxanthins. Betacyanins give red, blue or purple colors to certain flowers and fruits. They are commonly found in beets, amaranth, cacti and Swiss chard. Based on their chemical structures betacyanin can be classified into four kinds: betanin, amaranthin, gomphrenin and bouginvillein[3]. Betacyanins are water-soluble betalain pigments derived by glycosylation of betanidin, which can be considered as a condensation product of cyclodopa with betalamic acid[2]. It work as an antioxidant, scavenging up and neutrializing the damaging oxidative substances in our bodies. They have anti-inflammatory properties, and are also being studied for their protection against cancer. Since there is a rising demand for natural sources of food colorants with nutraceutical benefits, alternative sources of natural betacyanins are becoming increasingly important.

Oxidative damage in the human body plays an important causative role in disease initiation and progression. Antioxidants is a compound that can significantly delay or prevent the oxidation of substrate even if the compound is present in a significantly lower concentration than the oxidized substrate and can be recycled in the cells or irreversibly damaged[4]. The main characteristic of an antioxidant is its ability to trap free radicals. There

exist two types of antioxidants natural and synthetic. Natural antioxidants are classified according to their mechanism of action as chain-breaking antioxidants which scavenge free radicals or inhibit the initiation step or interrupt the propagation step of oxidation of lipid and as preventive antioxidants which slow the rate of oxidation by several actions[5-13]. Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) because of their possible activity as promoters of carcinogenesis[14], there is growing interest toward natural antioxidants from herbal sources[15-17]. Although almost all organism possess antioxidant defence and repair system to protect against oxidative damage, these system are insufficient to prevent the damage entirely[18]. Fruits and vegetables contain different antioxidant compounds like phenolic acids, ascorbic acid, polyphenols, carotenoids and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases.

Basella alba (family Basellaceae) known as Malabar spinach or cyclone spinach it is a fast growing vegetable, native to tropical Asia, probably originating from India or Indonesia and extremely heat tolerant. It is a climbing perennial plant that has short petioles, thick tender stems with circular to ovate leaves that are alternate and 5-12cms long. The leaves are thick, rugose, succulent and green to purple in color[19], its known for being rich in β-carotene and vitamin A[20] and used to treat catarrhal affections and to hasten suppuration. Decoction of the root relieves bilious vomiting. It is also a good source of vitamins and minerals[21]. The fruits of *Basella alba* is fleshy, stalkless, ovoid or spherical, 5-6 mm long, and purple when mature. The plant is reported to treat against laxative, inflammation, rubefacient, hemorrhages, skin diseases, sexual weakness, burns, ulcers, diarrhea and cancer. The present study was undertaken to evulate the betacyanin content from basella alba and to evaluate *in vitro* antioxidant property of extracts *Basella alba* using DPPH,hydroxyl and superoxide scavenging, reducing power, hydrogen peroxide scavenging, metal chelating, anti-ferric chloride hydrogen peroxide system, and deoxyribose degradations.

2. Materials and Methods

2.1 Sample collection

Basella alba fruit were collected from Coimbatore, Tamilnadu, India and stored in sealed polyethylene bags at -20°C until extraction.

2.2 Extraction

0.5 gm of *Basella alba* were treated with 10 ml acidified methanol.

And the mixture was centrifuged at 10,000 rpm for 10 min and supernatant was taken for analysis[22]

2.3 Analytical procedures

2.3.1 Flavanoids conformation test[23]

A. $Fecl_3$

1 ml of sample extraction was added with a small amount of $FeCl_3$**,** and results were observed.

B. $Alcl_3$

1 ml of sample extraction was added with 5% of $AlCl_3$ solution, and results were observed.

2.3.2 Conformation test for betacyanin[22]

A. 2M HCL

1 ml of sample extraction was added with 2ml of HCL for 5minutes at 100ºC, and the result were observed.

B. 2M NaoH

1 ml of sample extraction was added with 2ml of NaoH, and results were observed.

2.3.3 Total phenolic assay

Total phenolic compounds in betacyanin samples were quantified by using Foliciocalteu's method[24]. 50µl of Folin-ciocalteu's reagent (50% v/v) were added to 10µl of sample extract. It was incubated for 5 min. After incubation 50µl of 20 % (w/v) sodium carbonate and water was added to final volume of 400µl. Blank was prepared by replacing the reagent by water to correct for interfering compounds. After 30 min of incubation, the absorbance was measured using spectrophotometer at 760 nm.

2.3.4 Stability at variable pH

The betacyanin stability was tested by treating 1 ml of sample with 1 ml of pH 1.0 and 4.5 solutions. The color change was observed[25].

2.3.5 Determination of total betacyanin

The total amount of betacyanin content was determined by using pH differential method. A spectrophotometer was used for the spectral measurements at 210 nm and 750 nm[26]. The absorbance of the samples (A) was calculated as follows:

A= (Absorbance l vis-maxA 250-A750) pH 1.0-(Absorbance l vis-max A250-A750) pH4.5

Betacyanin pigment content (mg/liter) = (A X MW X DF X 1000)/ (e X 1).
Where,
Molecular weight of betacyanin (cyd-3-glu) = 449, Extraction coefficient (e) = 29,600, DF=Diluted factor[24]. 50 µl of Folin-ciocalteu's reagent (50% v/v) were added to 10µl of sample extract. It was incubated for 5 min. After incubation 50µl of 20 % (w/v) sodium carbonate and water was added to final volume of 400 µl. Blank was prepared by replacing the reagent by water to correct for interfering compounds. After 30 min of incubation, the absorbance was measured using spectrophotometer at 760 nm.

2.3.6 Total flavonoid content
The flavonoid content was determined according as the aluminum chloride colorimetric Method[27]. Briefly, aliquots of 0.1g of *Basella alba* sample was dissolved in 1 ml of deionized water. This solution (0.5 ml) was mixed with 1.5 ml of 95% alcohol, 0.1 ml of 10 % aluminium chloride hexahydrate ($AlCl_3$), 0.1 ml of 1 M potassium acetate (CH_3COOK), and 2.8 ml of deionized water. After incubation at room temperature for 40 min, the reaction mixture absorbance was measured at 415 nm against a deionized water blank on a spectrophotometer. Quercetin was used as a standard. Using a seven point standard curve (0-50mg/l), the levels of total flavonoid contents in *Basella alba* was determined in triplicate, respectively. The data was expressed as milligram quercetin equivalents (QE)/100 g fresh matter from fresh *Basella alba*.

2.4 Antioxidant assays
2.4.1 Scavenging activity of DPPH radical
Scavenging activity of betacyanin against DPPH radicals was assessed according to the method[28] with some modifications. Briefly, 0.1 mM DPPH-methanol solution was mixed with 1 ml of 0.1mM DPPH methanol solution. After the solution was incubated for 30 min at 25º C in dark, the decrease in the absorbance at 517nm was measured. Control contained methanol instead of antioxidant solution while blanks contained methanol instead of DPPH solution in the experiment. Ascorbic acid and BHT were used as positive controls. The inhibition of DPPH radicals by the samples was calculated according to the following equation:
DPPH-scavenging activity (%) = [1-(absorbance of the sample-absorbance of blank)/
absorbance of the control] ×100

2.4.2 Hydroxyl radical scavenging activity
The hydroxyl radical scavenging activity was determined according to the methods[29]. 0.1 ml of *Basella alba* betacyanin evaporated samples in the concentration of 1mg/ml, 10mg/ml, 50mg/ml, and 100mg/ml was taken in different test tubes. 1.0 ml of iron-EDTA solution (0.1% ferrous ammonium sulfate and 0.26% EDTA), 0.5 ml of DMSO (0.85% v/v in 0.1 M Phosphate buffer, pH 7.4) were added to these tubes, and the reaction was initiated by adding 0.5 ml 0f 0.22% ascorbic acid. Test tubes were capped tightly and heated on a water bath at 80-90 0c for 15 min. The reaction was terminated by the addition of 1 ml of ice cold TCA (17.5 %w/v).3 ml of Nash reagent (75g of ammonium acetate, 3 ml of glacial acetic acid, and 2 ml of acetyl acetone were mixed and raised to 1 L with distilled water) was added to all of the tubes and left at room temperature for 15 min for the color development. The intensity of the yellow color formed was measured spectrophotometrically at 412 nm against the reagent blank. The percentage of hydroxyl radical scavenging activity is calculated by using the formula:
% of hydroxyl radical scavenging activity=1-absorbance of sample/absorbance of blank×100

2.4.3 Determination of superoxide radical-scavenging activity
Superoxide radicals were generated by the method[30, 31], with some modifications all solutions were prepared in 0.05 M phosphate buffer (pH 7.8). The photo induced reactions were performed in aluminium foil-lined box with two 30W fluorescent lamps. The distance between the reaction solution and the lamp was adjusted until the intensity of illumination reached about 4000 lux. A 30µL aliquot of *Basella alba* betacyanin evaporated samples in the concentration of 1mg/ml, 10mg/ml, 50mg/ml, and 100mg/ml was mixed with 3ml of reaction buffer solution (1.3 mm riboflavin, 13 mM methionine, 63 µM nitro blue tetrazolium and 100µM EDTA, pH 7.8). The reaction solution was illuminated for 15 min at 25 º C. The reaction mixture, without sample, was used as a control. The scavenging activity was calculated as follows:
Scavenging activity (%) =
(1-absorbance of the sample/absorbance) ×100.

2.4.4 Metal chelating activity
The chelation of ferrous ions by the extract was estimated by the previous method[32] with slight modification and compared with that EDTA, BHT and that of ascorbic acid. The chelation test initially includes the addition of ferrous chloride. The

antioxidants present in the samples chelates the ferrous ions from the ferrous chloride. The remaining ferrous combine with ferrozine to form ferrous-ferrozine complex. The intensity of the ferrous-ferrozin complex formation depends on the chelating capacity of the sample and the colour formation was measured at 562 nm (Shimadzu UV-Vis 2450).

Different concentrations of standard and (100-500 µg/ml) of *Basella alba* betacyanin samples in the concentration of 1mg/ml, 10mg/ml, 50mg/ml, and 100mg/ml were added to a solution of 100 µl $FeCl_2$ (1mM). The reaction was initiated by the addition of 250 µl ferrozine (1 mM). The mixture was finally quantified to 1.3 ml with methanol, shaken vigorously and left standing at room temperature for 10 min. after the mixture had reached equilibrium, the absorbance of the solution was measured spectrophotometrically. All the test and analysis were done in duplicate and average values were taken. The percentage inhibition of ferrous-ferrozine complex formation was calculated using the formula;
%= 1-As/Ac X 100. Where, '**Ac'** is the absorbance of the control, **'As'** is the absorbance of the sample.

2.4.5 Determination of reducing power
The reducing power was determined according to the method[33]. A 0.25ml aliquot of *Basella alba* betacyanin evaporated samples in the concentration of 1mg/ ml, 10mgml, 50mg/ml, and 100mg/ml was mixed with 2.5 ml of 200mM sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was then incubated at 50ºC for 20 min. After 2.5 ml of 10% trichloroacetic acid (w/v) were added, the mixture was centrifuged at 650g for 10 min. A 5ml aliquot of the upper layer was mixed with 5ml of distilled water and 1ml of 0.1% ferric chloride at 700nm was measured. A higher absorbance a higher reducing power.

2.4.6 Hydrogen Peroxide Scavenging Activity
A solution of hydrogen peroxide (40mM) was prepared in phosphate buffer (pH 7.4). 0.2 ml of various concentration of *Basella alba* anthocycanins (1, 10, 50, 100mg/ml) in 1.6ml phosphate buffer (pH 7.4) was added to 0.6ml of 40mM hydrogen peroxide solution. The absorbance value of the reaction mixture was recorded at 230 nm[34]. The percentage of hydrogen peroxide scavenging of *Basella alba* betacyanin was calculated using the following formula:
Hydrogen peroxide scavenging activity %
=[(absorbance of the sample- absorbance of the control)/ absorbance of the control]*100.

2.4.7 Estimation of anti-$FeCl_2$-H_2O_2 stimulated linoleic acid peroxidation
The effect of anti -$FeCl_2$-H_2O_2 stimulated linoleic acid peroxidation was determined by the method[35]. In brief, 0.2 ml of various concentration of *Basella alba* anthocycanins (1, 10, 50, 100mg/ml) were added to a solution of 0.1 M linoleic acid (0.2 ml), 2.0mM $FeCl_2$. H_2O (0.2ml) and 0.2M phosphate buffer (pH 7.4, 5 ml). The reaction mixture was incubated at 370 C for 24 h. After incubation, 0.2ml of BHA (20mg/ml), 1ml of thiobarbituric acid (TBA) (1%), and 1ml of trichloro acetic acid (TCA) (10%) were added to the mixture, which was heated for 30 min in a boiling water both. After cooling, 5ml of chloroform was added, and the mixture was centrifuged at 1000 x g to give a supernatant. Absorbance of the supernatant was measured using spectrophotometer at 532 nm.

2.4.8 Ferric reducing antioxidant power (FRAP) assay
FRAP assay was performed previous methods[36, 37] with some modifications. The FRAP assay reagent was prepared by adding 10 volume of 300mM acetate buffer, pH 3.6 (3.1g sodium acetate and 16ml glacial acetic acid), one volume of 10mM TPTZ prepared in 40mM HCL and one volume of 20 mM $FeCl_3$. The mixture was diluted to 1/3 with methanol and prewarmed at 37ºC. This reagent (3ml) was mixed with 0.1 ml diluted test samples similar to those used for ABTS assay. The mixture was shaken and incubated at 37ºC for 8 minutes and the absorbance was read at 593nm. A blank with only 0.1ml methanol was used or calibration. FRAP assay was expressed as inhibition percentage and was calculated using the formula**:**
FRAP assay % = 1-(test sample absorbance/blank sample absorbance) ×100.

2.4.9 ABTS free radical scavenging ability by the use of a stable ABTS radical cation
The free radical scavenging activity of betacyanin was determined by ABTS radical cation decolourisation assay[38]. ABTS is dissolved in water 10 7mM concentration($ABTS^{\cdot+}$) ABTS radical cation was produced by reacting ABTS stock solution with 2.45 mM potassium persulphate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12-16 hours dark before use. Because ABTS and potassium persulphate react stiochiometrically at a ratio of 1:0:5(mol/mol), this will result in incomplete oxidation of the oxidation ABTS. Oxidation of the

ABTS commenced immediately, but the absorbance was not maximal stable until more than 6 hours had elapsed. The radical was stable in this form for more than two days. Stock solutions of phenolics in ethanol, carotenoids in dichloromethane and plasma membrane antioxidants ion water were diluted such that 10µl when stored in dark at room temperature. Prior to use, the stock solution was diluted with 95% ethanol (1:89v/v) to an absorbance of 0.70(± 0.02) at 734 nm and equilibrated at 30°C exactly 6 minutes after initial mixing **(A_t).** One milliliter (1 ml) of diluted ABTS solution was mixed with 10 µl of extract of different strength (4.8µg/ml). The percentage decrease of absorbance at 734nm was calculated for each point and the antioxidant capacity of the rest compounds was expressed percent inhibition (% I) and Ic50 value was calculated from regression analysis. Quercetin (2-10mg/ml) was used as standard antioxidant. Percent inhibition of ABTS assay was calculated as:

ABTS assay % = (1-absobance of sample/ absorbance of control) ×100

3. Molecular antioxidant analysis

3.1 Determination of inhibitory effect on deoxyribose degradation

Inhibitory effect of the betacyanins on deoxyribose degradation was determined by measuring the reaction activity between either antioxidants or hydroxyl radicals (referred to as non-sitespecific scavenging assay) or antioxidants and iron ions (referred to as site-specific scavenging assay)[39]. For non-site-specific scavenging assay, a 0.1 ml aliquot of different concentration of betacyanin was mixed with 1ml of reaction buffer (100 µM $FeCl_3$, 104 µM EDTA, 1.5mM H_2O_2, 2.5mM deoxyribose, and 100µM L-Ascorbic acid, pH 7.4) and incubated for 1 h at 370 C. A 1ml aliquot of 0.5% 2-thiobarbituric acid in 0.025 M NaOH and 1 ml of 2.8% trichloroacetic acid were added to the mixture and it was heated for 30min at 800 C. The mixture was cooled on ice and the absorbance was measured at 532nm. Sitespecific scavenging activity, which represented the ability of betacyanins to chelate iron ions and interfere with hydroxyl radical generation, was measued using the same reaction buffer without EDTA. Percent inhibition of deoxyribose degradation was calculated as (1-absobance of sample/absorbance of control) ×100. Control-without sample.

Table 1. Phenolic composition, flavonoid and betacyanin content of *Basella alba* in acidified methanol solvent extraction

Basella alba solvent	**Total phenols (mg gallic acid Equ/g)**	**Total flavanoid (mg queracitin Equ/g)**	**Betacyanin (mg glucoside Equ/g)**
1.Acidified Methanol	750mg/g	350mg/g	15µg/g

Table 2. Antioxidant assay from *Basella alba* evaporated samples

Antioxidant assays	**Standards**		**Concentration(mg/ml)**				**IC^{50} Values**
	BHT	Ascorbic acid	**1mg**	**10mg**	**50mg**	**100mg**	
1.DPPH assay	95.8	96.6	19.1%	45%	54%	72%	60.82
2.Hydroxyl Scavenging activity	99.6	87.7	60.7%	76.1%	82.4%	82.6%	47.75
3.Superoxide radical activity	61	69.5	29.9%	31.7%	83.3%	83.5%	49.01
4.Reducing power	0.263	2.368	0.120	0.165	0.260	0.459	_
5.Deoxyribose degradation(site specific)	77.7	76.7	67.1%	74.1%	81.7%	85.7%	46.81
6.Deoxyribose degradation(non site specific)	79.1	75.5	10.3%	22.7%	74.3%	82.1%	51.81
7.Metal chelating	37.1	15.2	83.6%	79%	60%	50%	71.2
8.H_2O_2 scavenging	84	80	10%	44%	62.5%	92%	49.35
9.Anti-FeCl3	91	95	46%	66%	82%	87%	46.68
10. FRAP	-	-	25.3%	62.5%	65.5%	69.5%	57.93

4. Resuls and Discussion

4.1 Betacyanin Extraction

The total betacyanin was extracted by using acidified methanol as solvent system. Acidified methanol resulted significantly higher values of total betacyanin (Table 1). In previous studies they extracted betacyanin from *Basella rubra* by using 0.1% Hcl in ethanol as solvent[40].

4.2 Analytical Procedure

4.2.1. Flavanoid confirmation test

A. Fecl3

The presence of ferric chloride the acidified extract showed brown color which confirm the presence of flavanoids.

B. Alcl3

The presence of aluminium chloride the same extract showed dark color which confirm the presence of flavanoids.

4.2.2 Confirmation test for betacyanin

A. 2M Hcl

Basella alba betacyanin extract was unstable after adding 2M Hcl which confirm the presence of betacyanin

B. 2M NaoH

Basella alba betacyanin extract changes the color to yellow after adding 2M NaoH which confirm the presence of betacyanin.

4.3 Evaluation of total flavanoids

The total flavanoid content was evaluated to be 350mg/g (Table 1). The positive correlation was observed between total phenol and flavanoid suggest that total phenol are contributed mostly by flavanoids in fruits of *Basella alba*.

4.4 Determination of total phenolic content

The total phenolic content was observed to be 750mg/g in acidified methanol extract. Report has been provided regarding the total phenols and total flavanoids content in *Basella alba* leaf[41]. They also reported a high correlation between total phenol and total flavanoid content.

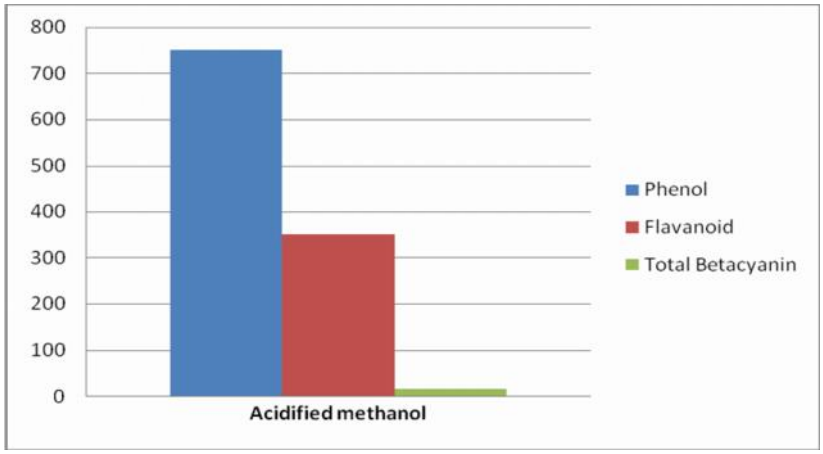
4.5 Stability at variable pH

The sample appear red color at pH 1 and color disappear at pH 4.5. They reported that the betacyanin are stable in low pH[42]. The results were found to be same in acidified methanol extract of *Basella alba* fruit.

4.6 Determination of total betacyanin content

The total betacyanin content extracted by acidified methanol was found to be 15µg/g (Figure 1). Acidified methanol and acetone extraction resulted in significantly higher values for the total betacyanin content than the methanol and ethanol extract. Several authors reported aqueous acetone was better than various alcoholic solvents for fruit pro-cyanidin, betacyanin and other phenols[41]. They observed significant betacyanin interaction with aqueous acetone to form pyrano anthocyanidins which significantly lowered quantities of detectable betacyanins[44]. However, since acidified methanol preserves the extracted betacyanins in their original form better for longer duration[45]. It should be the solvent of choice for quantification and analysis of betacyanins.

Figure 1. Phenolic composition, flavonoid content and total betacyanin content of *Basella alba* in acidified methanol solvent extraction



5. Antioxidant Analysis

5.1 DPPH radical scavenging activity

Free radical scavenging is one of the known mechanism by which antioxidants inhibit lipid peroxidation[46, 47]. DPPH is a free radical compound and has been widely used to test the free radical scavenging ability of various samples. The ability of phenolic compound to quench reactive species by hydrogen donation was measured through the DPPH radical scavenging assay. DPPH radical scavenging activity of *Basella alba* fruit betacyanin, ascorbic acid and butylated hydroxytolulene (BHT) was shown in table 2. The betacyanin significantly inhibited the activity of DPPH radical n a dose depended manner. Antioxidant activity of sample extracted in acidified methanol is shown in figure 3. At 1mg/ml the scavenging effect where 19.1%, at 10mg/ml and 50mg/ml the scavenging effect where 45% and 54% respectively. While almost complete inhibition of DPPH radical activity was observed when anthoyanin was used at 100mg/ml. It shows that *Bsella alba* fruit betacyanin have strong hydrogen donating capacity and can be effectively scavenge DPPH radicals. These results indicate that radical scavenging capacity of betacyanin might be mostly related to there concentration of phenolic and hydroxyl group. The antiradical activity of phenolic compound depends on their molecular structure on the availability of phenolic hydrogen and on the possibility for stabilization of the resulting phenoxyl radicals formed by hydrogen donation[48, 49].

5.2 Hydroxyl radical scavenging

The hydroxyl radical is an extremely reactive free radical formed in biological systemand has been implicated as a highly damaging species in free radical pathology capable of damaging almost every molecule found in living cells. This species is consider as one of the quick initiator of lipid peroxidation process, attracting hydrogen atoms from unsaturated fatty acid[50]. Hydroxyl radial scavenging of betacyanin extract which increases with increasing concentration (figure 2). The extract exhibited the highest activity of 82.6% in 100mg/ml, 82.4% in 50mg/ml and 76.1% in 10mg/ml whereas 60.7% of inhibition noted as 1mg/ml respectively. This is similar to observations of several others who have reported as dose dependent activity in sesame coat, pomegranate peel and seeds and grape pomoce[27, 51, 29]

5.3 Superoxide anion scavenging acitivity

Superoxide anion radicals are produced by a number of cellular reactions, including various enzyme system such as lipoxygenases, peroxidase, NADPH oxidase and xanthine oxidase. Superoxide anion plays an important role in plant tissue and also involving in formations of other cell damaging free radicals[46]. In the present study superoxide radical was generated by illuminating a solution containing riboflavim. The relative scavenging effect of *Basella alba* betacyanin on superoxide radical are shown in table 2. The betacyanin extracts exhibided 83.5% scavenging acitivity at 100 mg per ml, 83.3% in 50mg/ml and 31.1% in 10mg/ml whereas 29.9% in 1mg/ml respectively. The result of superoxide radical anion scavenging acitivity of betacyanin extract, butylated hydroxyl toluene and ascorbic acid were shown in figure 3. The superoxide anion on radical scavenging acitivity might be due to the action of phenolic compounds. Also the flavanoid molecules with polyhydroxylated subsititution on the rings A and B and a free three hydroxyl substitution confired superoxide anion scavenging acitivity[31].

Figure 2. Antioxidant activity of *Basella alba*

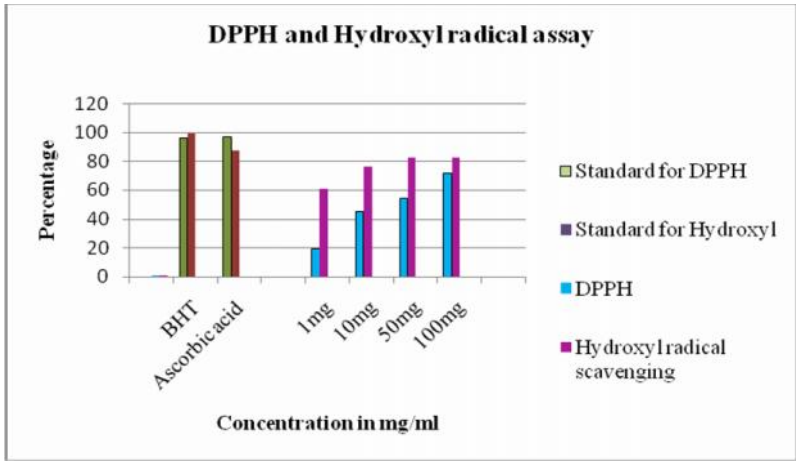
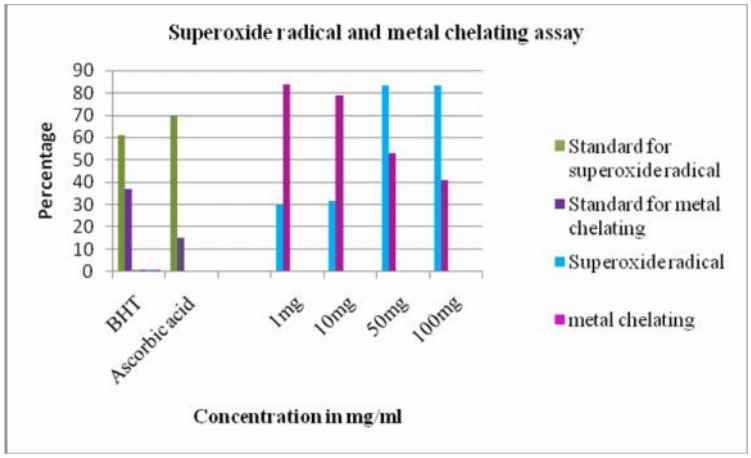


Figure 3. Antioxidant activity of *Basella alba*



5.4 Metal chelating activity

Ferrozine can form complexes with Fe^{2+} but in the presence of chelating agent, the complex formation is disrupted with the result that the red color at the complex is decreased. Measurement of color reduction therefore allows estimation of chelating activity of the coexisting chelator[52]. In this assay the betacyanins extracts interfere with the formation of ferrous and ferrozine complex, suggesting they have chelating activity and can capture ferrous ion before ferrozine. Betacyanins extracts registered the highest metal chelating activity at 100 mg/ml which are comparably higher to the positive standards ascorbic acid and butylated hydroxyl toluene respectively (Table 2). It was previously reported that metal chelating activity in some medicinal plants for evaluating their antioxidant activity[53].

5.5 Reducing power

It has been reported that reducing power is associated with antioxidant activity and may serve as significant reflection on the antioxidant activity[27, 54]. Betacyanins from *Basella alba* fruit exhibited a higher reducing power than butylated hydroxyl toluene and ascorbic acid, suggesting that strong electron-donating capacity (table 2). The reducing power of *Basella alba* fruit betacyanin at 1 mg/ml, 10 mg/ml, 50 mg/ml, and 100 mg/ml were 0.120, 0.165, 0.260, 0.459 respectively. The reducing properties are generally associated with the presence of reductones[55], which have been shown to exert antioxidant action by breaking the free radical chain by denoting a hydrogen atom[56]. The reducing power of *Basella alba* fruit is probably due to the presence of phenolic compounds which might act as electron-donor.

Figure 4. Antioxidant activity of *Basella alba*

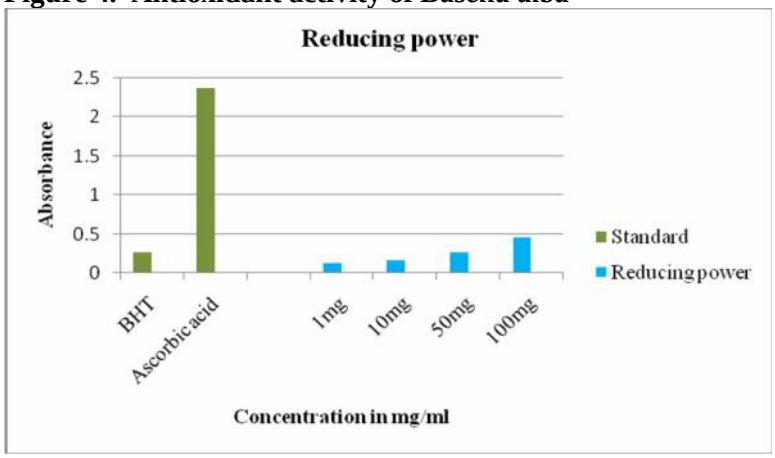
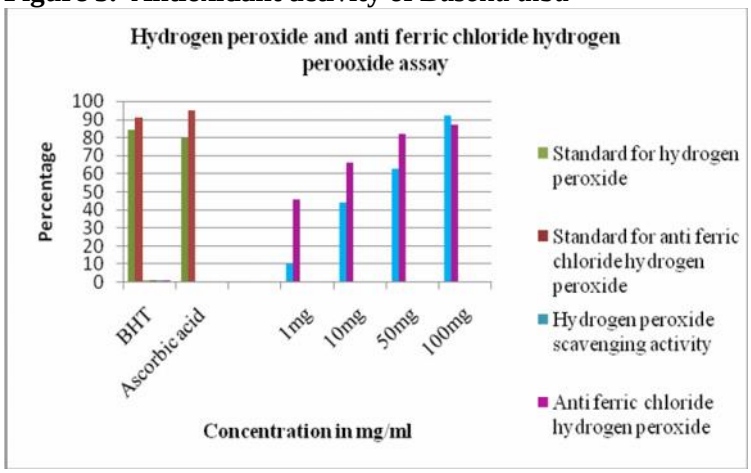


Figure 5. Antioxidant activity of *Basella alba*



5.6 Hydrogen peroxide scavenging activity

Hydrogen peroxide scavenging activities of the betacyanin extracts, BHT and ascorbic acid was measured at 230nm. Hydrogen peroxide scavenging activities of the betacyanin extracted by using acidified methanol were 10% at 1 mg/ml, 44% at 10 mg/ml, 62.5% at 50 mg/ ml and 92% at 100 mg/ml respectively. BHT (84%) and ascorbic acid (80%) had higher hydrogen peroxide scavenging activity than betacyanin extracts (table 2). It was reported earlier that the extract were capable of scavenging hydrogen peroxide in concentration depend manner. Although hydrogen peroxide itself is not reactive, it can sometimes cause cytotoxicity by giving rise to hydroxyl radicals in the cell[57]. Thus removing H_2O_2 is very important throught food system[58].

5.7 Estimation of anti-$Fecl_2$-H_2O_2 stimulated linoleic acid peroxidation

Iron salts are thought to react with H_2O_2 called the Fenton reaction, to make hydrogen radicals which bring about peroxide reaction of lipids[35]. The effect of betacyanin extracts of *Basella alba* on the formation of malonaldehyde (MDA) from linoleic acid is show in table 2. As the concentration of the antioxidant extracts increased the formation of MDA decreased. A dose dependent MDA inhibition in linoleic acid oxidation was evident. Acidified methanol extraction showed a higher inhibition of MDA ranging from 46% to 87% at 1 mg/ml to 100 mg/ml than BHT and ascorbic acid (figure 5). FTC method was at primary stage of linoleic acid peroxidation. The peroxide reacts with ferrous chloride to form a ferric chloride pigment. In this method the concentration of peroxide decrease as the antioxidant activity increases. The percentage inhibition was based on the absorbance in FTC method (table 2). It showed total peroxide value produced by oxidation of linoleic acid. The higher absorbance value indicate the lower level of antioxidant[59].

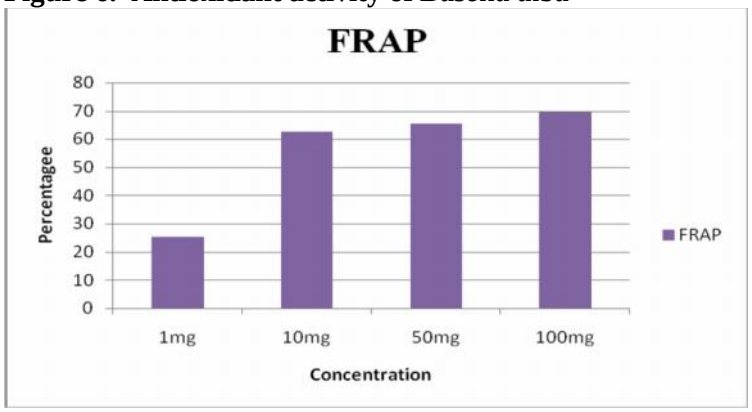
5.8 Ferric reducing antioxidant power (FRAP) assay

The FRAP assay measures the ability of antioxidant to reduce the ferric 2, 4, 6-tripyridyl-S-triazine complex [Fe (III)-$T_PT_Z^2$]$^{2+}$ to intensely blue colored ferrous complex [Fe (II-$(T_PT_Z)^2$]$^{2+}$ in acidic medium. FRAP values are calculated by measuring the absorbance increase of 593nm and relating it to a ferrous ion standard. The reduction capacity of a plant extract may serve as a significant indicator of it potential antioxidant activity[60]. The betacyanin extract exhibited higher antioxidant capacity and the percentage inhibition was shown in table 2. Figure 6 shows the reducing power of the Quercetin and Trolox. The IC^{50} value was found to be 57.93.

5.9 ABTS assay

ABTS is a method based on reduction of 2,2´-azinobis (3-ethylbenzothiozoline sulphonate) radical. Although $ABTS^{\cdot+}$ have been widely used to measure the antioxidant capacity of natural extract based on their ebility to reduce radical cation, the reaction of $ABTS^{\cdot+}$ with free radical scavenges present in the test sample occur rapidly and can be accessed by following the decrease in the sample absorbance at 734nm (figure 7). The highly significant correlation were observed between the total antioxidant capacity and phenolics content[61]. The major type and represtative component of phenolic compound idientified in the present study mainly included polyphenolic compounds example flavanoids.

Figure 6. Antioxidant activity of *Basella alba*



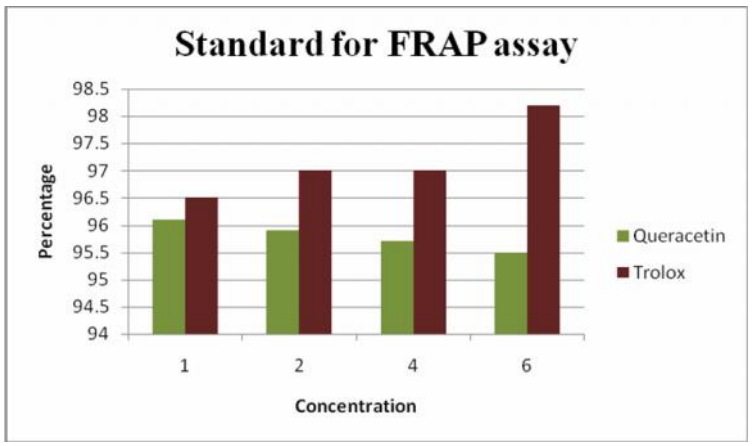
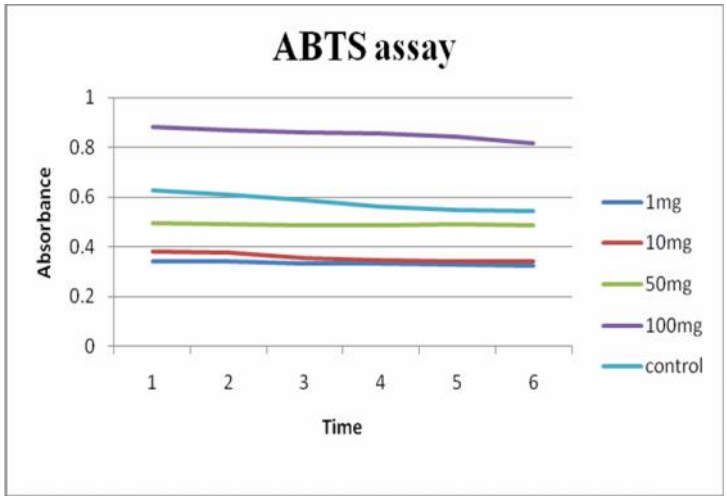
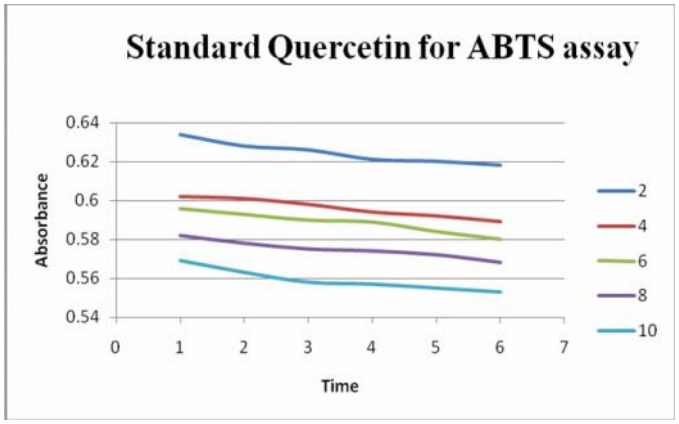
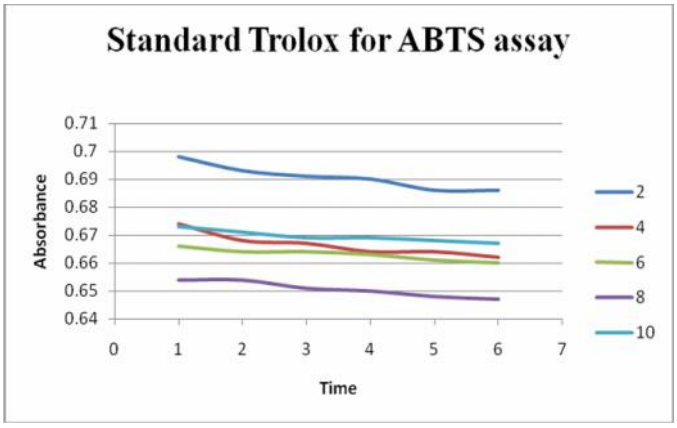


Figure 7. Antioxidant activity of *Basella alba*





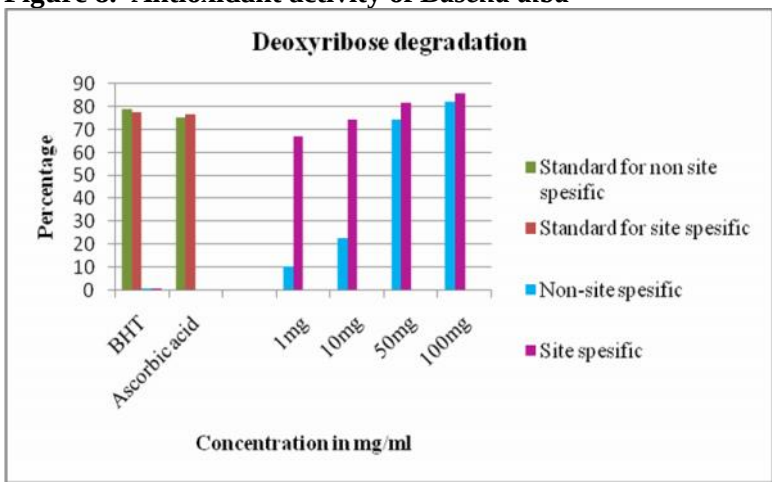


6. Moleculars assays

6.1 Inhibitory effects of deoxyribose degradation

Hydroxyl radical can be formed by the Fenton reaction in the presence of reduced transition metals such as Fe^{2}, and H_2O_2, which is known to be the most reactive of all the reduced forms of dioxygen and is thought to initiate cell damage *In vivo*[62]. To determine whether litchi betacyanins reduce hydroxyl radical generation by chelating metal ions or by directly scavenging hydroxyl radicals, the effects of the betacyanins or hydroxyl radical generated by Fe^{3+} ions were analyzed by determining the degree of deoxyribose degradation. The concentration-dependent inhibition of hydroxyl radical induced deoxyribose degradation by betacyanins in both the site-specific and non-site specific assays using the same concentration, relatively greater antioxidant activity was observed in the site specific assay than in the non-site specific assay (figure 8), implying that the betacyanins inhibited deoxyribose degradation mainly by chelating metal ions rather than by scavenging hydroxyl radical directly. In this system, betacyanin extracts exhibited a stronger concentration dependent inhibition of deoxyribose oxidation. It was earlier reported that molecules that can inhibit deoxyribose degradation are those that can chelate iron ions and render them in active or poorly active in a Fenton reaction[63]. In the present study, in another system, we demonstrated the iron chelating ability of the betacyanin extracts. It is likely that the chelating effect of *Basella alba* betacyanins on metal ions may be responsible for the inhibition of deoxyribose oxidation. Iron, a transition metal is capable of generating free radicals from peroxides by the Fenton reaction and is implicated in many diseases[64]. Fe^{2+} has also been shown to produce oxyradicals and lipid peroxidation and reduction of Fe^{2+} concentration in the Fenton reaction would protect against oxidative damage. Similar results were reported for extracts of opuntia ficus-indica var. Saboten[39] and *Hypericum perforatum* L.[65]

Figure 8. Antioxidant activity of *Basella alba*



7. Conclusion

From the results it can be concluded that *Basella alba* has high amount of betacyanins content on its fruit. Reactive oxygen species plays a crucial role in a wide range of common diseases and age related degenerative conditions including cardiovascular diseases, inflammatory conditions and neuro degenerative diseases such as Alzheimer's disease, mutations and cancer. So antioxidant capacity is widely used as a parameter to characterize food or medicine plant and their bio-active components. In this study, the antioxidant activity of the betacyanins extracted from *Basella alba* fruit was evaluated and it showed very strong antioxidant activity.

Thus these results suggest that betacyanin extract from *Basella alba* fruit can be used as antioxidant material, food addictives.

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