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Research Article

Stabilization of Food Colourant and Antimicrobial Activity in Fruit Extracts of *Basella rubra*. L

Pawar N [1], Shinde M [2], Junna L[1*]

[1]*Department of Biochemistry, Gulbarga University, Kalaburgi-585106, India*
[2]*Department Post Graduate studies and Research in Biochemistry, Tumkur University, Tumkur-572101, India*

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ABSTRACT
Betalains have potential as the natural colourant and functional food with pharmaceutical purposes in food applications. The total betalain content of *Basella rubra* L. grown at Kalaburagi region is evaluated for the first time. The present study is carried out to determine the pigment stability and antimicrobial activity of fruits of *Basella rubra*. Total betalain content of fruits was evaluated and it was found to contain a high amount of 48.15 mg betacyanins and sparing amount of 2.53 mg betaxanthins and total betalains account for 50.69mg/100g of fresh fruit pulp. The influence of different additives on the colour stability of pigment was evaluated. More than 80% pigment colour is stable in the presence of 5% sucrose at 4 °C under dark condition. The betalain extract of *Basella rubra* L fruits showed significant antimicrobial activity against *S.typhi, Aspergillus flavus, Microphomia fungi* and *Rhizopus stolonifer*. It is for the first time that the total betalain content and anti-microbial activity of *Basella rubra* cultivar grown in Hyderabad Karnataka region of Karnataka, India is reported.

Keywords *Basella rubra* L; Betalains; Pigment stability; Antimicrobial activity.

INTRODUCTION

The interest of the food industry in natural colourant replacing synthetic dyes has increased significantly over the decade, mainly due to safety issues[1]. 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[2]. Besides anthocyanins, carotenoids, and chlorophylls, betalains represent one of the four plant pigment classes commercially used for natural food colouring, allowing one to substitute synthetic colourants that are increasingly rejected by consumers. Betalain incidence is restricted to a few families of the plant order *Caryophyllales*, with the red beetroot (*Beta Vulgaris* L.) hitherto being the only betalainic plant exploited for commercial food colouring[3]. However, there is a demand for an alternative source other than the red beet because of the unfavourable earthy flavour caused by geosmin and pyrazine derivates, as well as high nitrate concentration associated with the formation of carcinogenic nitrosamine[4].

Basella fruit is a new source of red colour because it contains betalains which are the similar array of colour pigment found in beetroot. *Basella* spp. commonly known as Malabar spinach, spinach vine, or Ceylon spinach is a climbing perennial plant[5]. The fruits are purplish-red and fleshy. Their pigments are soluble in water and offer great tinctorial power, which makes them a potential source of natural dye[6]. Previous attempts have been made to the stability of betacyanins in the extract of *Basella rubra* L. fruits in relation to factors such as light, temperature and pH acting alone or in combination[7]. Hence, stabilizing the colour is of great industrial potential under various conditions in presence of different additives.

The development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from other sources including plants[8]. Screening of medicinal plants for antimicrobial activities and phytochemicals is important for finding potential new compounds for therapeutic use. Till date, there are no reports available concerning *B.rubra* fruits, which demonstrated antimicrobial activity.

The present study was undertaken with the dual purpose of ascertaining the content of the betalain pigment and pigment stability under various conditions in the presence of different additives and antimicrobial efficacy of fruit extract of *Basella rubra*. This article does not contain any studies with human or animal subjects.

MATERIALS AND METHODS

Collection of Fruits

The *Basella rubra* L plant was identified with the help of "*The Flora of Gulbarga district*"[9] and a voucher specimen is deposited in the Herbarium, Department of Botany, Gulbarga University, Kalaburgi (HGUG-136). The mature, healthy ripe fruits were collected during the month Oct-Nov 2014 from kitchen gardens of Kalaburagi, India. They were cleaned carefully and kept

**Author for Correspondence: jlshinde@rediffmail.com*

Table 1: Effect of temperature on colour stability of *B.rubra* betalains after 30 days of storage.

Temperature (ºC)	Pigment retained (%)
0	66.7
20	16.7
40	0
60	0
80	0

Table 2: Effect of pH on colour stability of *B.rubra* betalains after 30 days of storage at different conditions.

	Pigment colour retained (%)		
pH	Under Light at 25 ºC	Under Dark at 25 ºC	Under dark at 4 ºC
1	0	0	38
3	0	0	66
5	35	40	74
7	0	0	63
8	0	0	55

at -18 °C until further extraction.

Betalain Content

Extraction and Determination of Total Betalain Content

About 2 g of fruits were crushed _ with 100ml of ethanol (acidified to pH 2 with 1% citric acid) for 15 min at room temperature and left for 24 hours. The extract was filtered and quantified for betalains[10]. The total betalains in the fruit extract were carried out by Spectrophotometric method (UV-Vis spectrophotometer Labomade USA). The absorbance of fruit extract was measured at 477 nm and 535 nm. Measurements were made in triplicates. For quantitative analysis, the betalain content was calculated according to following equation:

BC (mg/g) = [A (DF) (MW)V/εLWd]

Where A is the absorption value at the absorption maxima of 535 nm and 477 nm for betacyanins and betaxanthins respectively, DF is the dilution factor, V is the solution volume (mL), Wd is the pulp weight (g), and L is the path length (1 cm) of the cuvette. The molecular weight (MW) and the molar extinction coefficient (ε) of betanin [MW=550 g/mol and 339 g/mol; ε= 60000 L/(mol cm) and 48000 L/(mol cm) in/H_2O] were applied in order to quantify the betacyanins and quantitative equivalents of the major betaxanthins respectively[11].

Large Scale Extraction of Betalains for Stabilization and Antimicrobial Activity

The colour stability and antimicrobial activities of fruit extract were investigated. The extraction was carried out as described by Ozela et al[6] with slight modification. 500 gms of mature fruits of *B.rubra* were soaked in 500 mL of acidic ethanol at pH 2 which was adjusted with hydrochloric acid. The soaked material was refrigerated for 48 hours. They were crushed gently and filtered through the muslin cloth; the filtrate was centrifuged at 5000 rpm for ten minutes. The supernatant was filtered through Whatman paper No.1. After filtration, chlorophyll was separated with 10 mL of a 1:1 mixture of ethyl ether and petroleum ether. The final extract of betalain obtained was concentrated to dryness. The dried extract was weighed and stored at -20°C in dark until used[6].

Betalains Stabilization

The effect of different physical factors like light, temperature, pH and different additives on the pigment stability of fruits of *B.rubra* was carried out as described previously by (Ozela et al[6], Reshmi et al[7], Hubbermann et al.[12]). In each solution, sufficient extract was used to obtain the absorbance reading between 0.9 and 1.5 at 535 nm. The study has been carried out for 30 days at three different conditions *viz* at room temperature (25 ± 1°C) in presence or absence of light and at cold-dark condition (4 °C).

Preparation of Pigment Solution

The solutions (0.5%) of acid, sugar, salt, hydrocolloid and amino acid were prepared. The solutions prepared and were stored in airtight, sterile transparent glass bottles for studying the light effect. Transparent glass bottles were placed between two fluorescent light tubes of 40 W, 2,500 Lux, corresponding to daylight, with a distance of 10 cm between the fluorescent light tubes and glass bottles, which were protected from any other kind of light source at a monitored temperature of 25 ± 1°C. The amber, airtight sterile glass bottles were used for storing the solution under dark at 25± 1°C and at cold- dark conditions at 4± 1°C. The colour intensity was recorded continuously every day up to seven days and then on the 15th day followed by 30th day spectrophotometrically at 535nm[6,9]. Colour retention was calculated according to the method of Tang et al[13] as follows:

[Chroma value at n is the days of storage / Chroma value at zero storage time] x 100

Antimicrobial Activity

Test Microorganism Seven bacterial strains namely *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Klebsiella pneumonia, Paenibacillus, Pseudomonas aeruginosa* and *Salmonella typhi* and three fungal strains namely *Aspergillus flavus, Microphomia fungi* and *Rhizopus stolonifer* from the microbial culture collection, Department of Biochemistry, Gulbarga University, Kalaburagi, India were used. The bacterial strains were maintained on the nutrient agar slants and the fungus strains were maintained on the potato dextrose agar slants at 4 °C until used.

Antimicrobial Screening The *in vitro* antibacterial activity of the betalain extract of *B. rubra* fruits was determined by agar disc diffusion method[14]. The test bacteria were cultured in nutrient broth at 37°C for 18 hours. Autoclaved Mueller Hinton agar (MHA) medium was cooled down to 40°C; 15mL of this medium was poured into a sterile Petri dish and allowed to set. 100µL inoculums suspension was swabbed uniformly and the inoculum was allowed to dry for 5 minutes. Varying concentrations of betalain extract (5mg/ml, 10mg/ml and 50mg/ml), positive control streptomycin (50 µg/100µL) and negative control ethanol were prepared. The extract and controls were loaded on 6mm sterile disc. The loaded disc was placed on the surface of the medium and the compound was allowed to diffuse for 5 minutes. Then the plates were kept for incubation at 37 °C for 24 hours. At

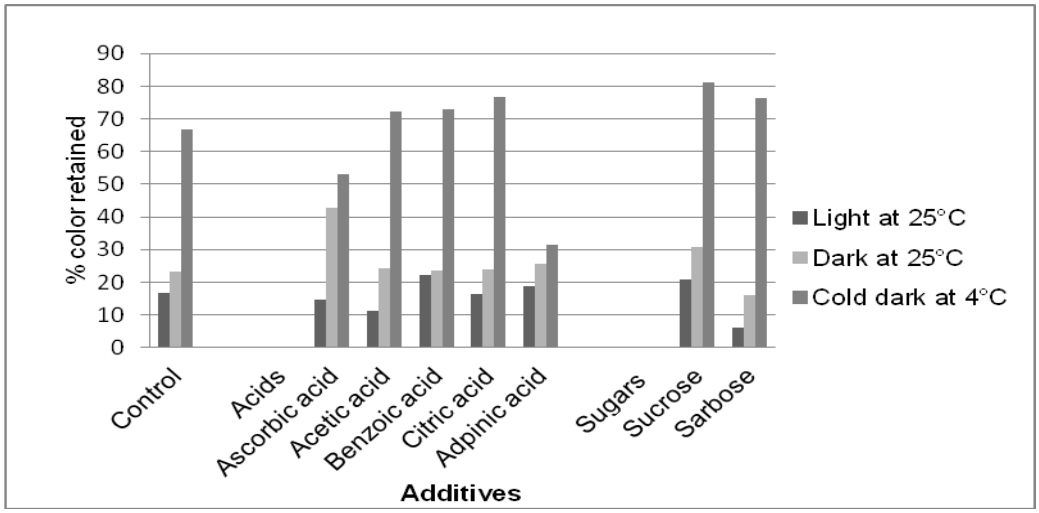

Figure 1: Effect of acids and sugars on colour stability of *B.rubra* betalains after 30 days of storage at different conditions.

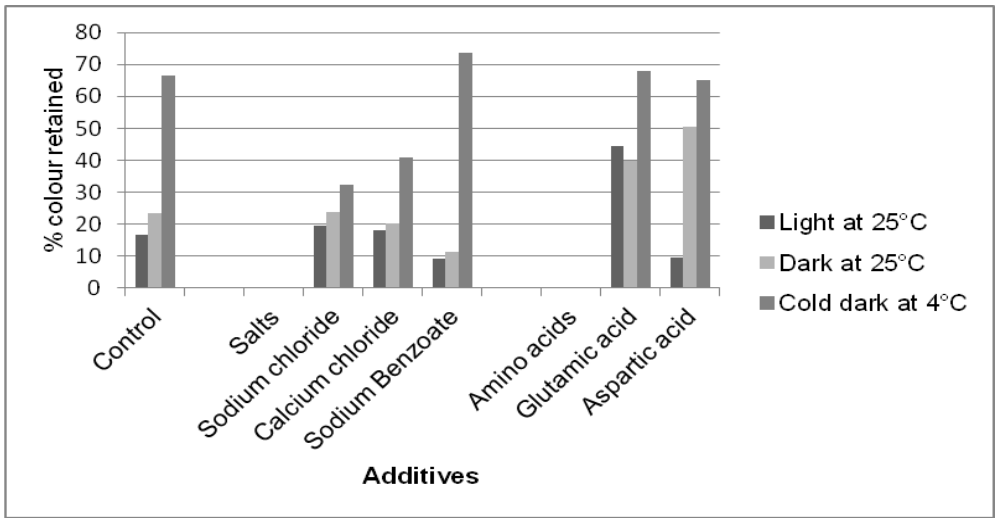

Figure 2: Effect of salts and amino acids on colour stability of *B.rubra* betalains after 30 days of storage at different conditions.

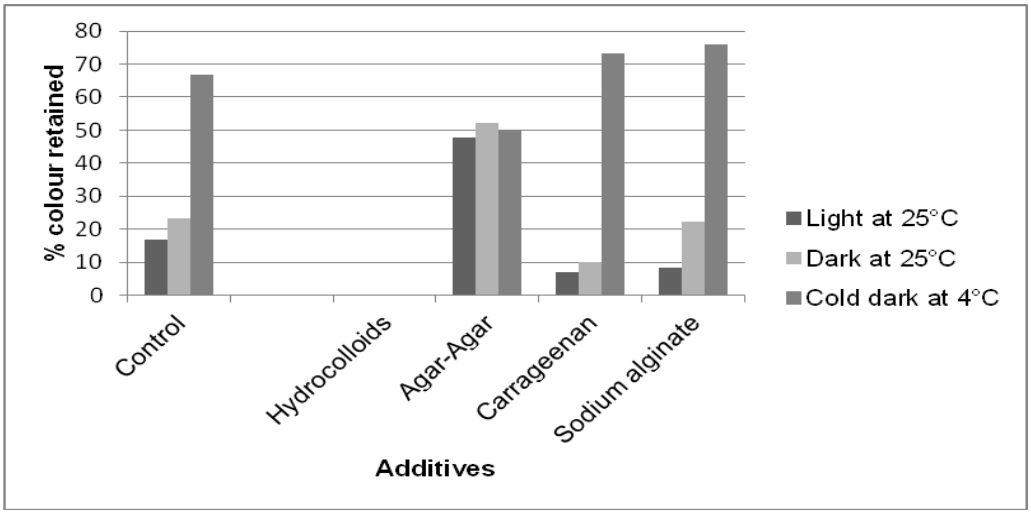

Figure 3: Effect of hydrocolloids on colour stability of *B.rubra* betalains after 30 days storage at different conditions.

the end of incubation, inhibition zone formed around the disc was measured with a transparent ruler in millimetre. The same procedure was followed for the fungal strains also.

Table 3: Antimicrobial activity of betacyanins extract against bacteria and fungi.

Microorganism	Zone of inhibition in mm
Bacterial strains	
Staphylococcus aureus	0
Klebsiella pneumonia	0
Salmonella.typhi	13
Escheria coli	0
Bacillus subtilis	0
Pseudomonas aeruginosa	0
Paenibacillus	8
Fungal strains	
Malassezia fungi	20
Aspergillus flavus	10
Rhizopus stolonifer	15

RESULTS AND DISCUSSION

The betacyanin and betaxanthine content are 48.15mg/100g and 2.53mg/100g respectively of fresh fruit pulp. Total betalains were 50.69 mg/100g fresh fruit pulp. The effect of different additives was analysed under three different storage conditions *viz.* under light and dark condition at room temperature and at the cold dark condition. The effects of light, temperature, and pH on the stability of betalains were also studied and it were found that the pigment colour of *B.rubra* fruits is stable at pH ≤5.0 in cold dark condition. Higher temperature and pH have shown colour destabilization effects. At 4 ºC the pigment was stable up to 66.70 % and as the temperature increased the pigment got decolourised (Table I). The effect of different pH range from 1 to 8 was studied and it was found that the pigment was decolourised in light and dark condition in all the pH tested except pH 5. Whereas, in cold dark condition the pigment was more stable and highest being at pH 5 (74%). The results are shown in Table II. Our results are in agreement with earlier findings which were reported by Ozela et al[6], and Kumar et al[15] for *B.rubra* betalains and Reshmi et al[7] for *Basella alba* and relationships between these effects and the decomposition of the pigments has always been observed, in the present study also.

Acids Different acids were selected to determine their influence on the colour stability under three different storage conditions. Several acids; acetic acid, citric acid, ascorbic acid, benzoic acid or adipinic acid were tested. During storage, the initial pink colour of the samples in the presence of different acids was markedly reduced. However, after 30 days of storage in citric acid under dark at cold stabilized 76.8% of the colour, which is higher as compared with other acids. The pigment degradation was rapid at room temperature in the presence of light and also under a dark condition as compared to cold (4 ºC) under dark condition (Figure I).

Sugars The influence of sucrose and sorbose were investigated in the presence and absence of light and in cold-dark condition. It was found that 5% sucrose had high influence in stabilizing the pigment under cold-dark condition. 81% of colour is retained after 30 days of incubation (Figure I). 21% more pigment was retained as compared with the control (66.7%). Similar findings were reported for anthocyanins from black currant and elderberry concentrates[12], blueberry[16] and frozen strawberries[17]. Stabilization of pigment colour due to sugar addition may be caused by lowering the water activity since water activity was reported to influence anthocyanin pigment stability[12] or it may due to the inhibition of enzymatic reactions or the hindering of different condensation reactions by sucrose at cold condition[17]. The increase in colour values upon addition of sugar into *B.rubra* fruit samples has been reported earlier[15].

Salts The colour stability was determined in the presence of different salts. Sodium chloride, calcium chloride and sodium benzoate were tested for stability. It was found that sodium chloride and calcium chloride has no stabilizing effect on the pigment in the presence and absence of light (Figure II). Similar findings were reported for black currant and elderberry anthocyanins[12]. But, in presence of sodium benzoate 73% colour was retained in cold under dark condition. Similar findings were reported by Kumar et al[15] for aqueous extract of *Basella rubra* fruit in salt sodium chloride.

Amino acids Two amino acids glutamic acid (monosodium glutamate) and aspartic acid were tested for their influence on betalain colour (Figure II). A marked effect was found for glutamic acid, 44% colour is retained in light condition. Aspartic acid showed 50% stabilizing effect under dark condition. Glutamic acid is used as the flavour enhancer and food additive. Both the tested amino acids exhibited a marked co pigmenting effect for colour stability.

Hydrocolloids The influence of three hydrocolloids (1% w/v) sodium alginate, agar-agar, and carrageenan on the colour stability was studied (Figure III). It was observed that agar-agar stabilizes the colour of pigment up to 50 % under all the three storage conditoins up to 30 days. Whereas, in the presence of sodium alginate and carrageenan there is a decrease in the pigment colour at room temperature in the presence and absence of light. However, under cold at dark condition both the hydrocolloids helps to retain the colour. 75% and 73% of the colour was retained in presence of sodium alginate and carrageenan respectively.

Antimicrobial Activity The antimicrobial activity of the betalain extract of *B.rubra* was evaluated against bacterial and fungal strains for the first time. The results indicated that the betalain extract showed antimicrobial activities against gram-negative bacteria *Salmonella typhi* with a zone of inhibition 13mm and all three tested fungi strains viz., *Microphomia fungi*, *Rhizopus stolonifer*, *Aspergillus flavus* (Table III). The extract showed the highest activity against *Microphomia fungi* and MIC value of 50 mg/ml. However, no activity was showed against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Klebsiella pneumonia, Paenibacillus* and *Pseudomonas aeruginosa*.

The *in vitro* antioxidative activity of betalains has been considered to be responsible for the protection against chronic diseases *in vivo*. Therefore, it is desirable to stabilize betalains in food systems and to enrich foods with these natural pigments. The results of our study

clearly indicate that betalain content and colour strength was highest in *B.rubra* fruits. The *B.rubra* betacyanins were most stable at low pH (≤5.0) in the presence of additives such as sucrose, and hydrocolloids under cold dark condition. Hence, the pigment from *B.rubra* fruits can be used in cold food products like ice-creams, jelly, desserts and cold beverages. The betalains of *B.rubra* fruits also showed antibacterial and antifungal activity.

Further, it can be concluded that the betalains from the *B.rubra* fruits can be efficiently used as a natural colourant in selected foods especially those require cold storage and as a natural antibiotic against few fungal and bacterial strain as it is a cheap and easily cultivable plant.

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