Development and nutritional analysis of *taro* powder (*Colocasia esculenta* L. Schott) enriched with natural colorants

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ABSTRACT

Aims: To prepare an anthocyanin rich food product from Indigenous plant species.
Study design: Lab experiments based on CRD statistical analysis.
Place and Duration of Study: Department biochemistry and agricultural chemistry, Assam Agricultural university, Jorhat, Assam, India between June 2018 and January 2020.
Methodology: We collected anthocyanin rich plant samples *viz.* fruits of jamun (*S.cumini*L. and *S.fruticosum*Roxb. DC.), full-grown flowers of rose (*R.indica*L.) and studied the stability of total anthocyanin under pH, temperature and light. Taro powder was selected with low oxalic acid content to prepare an anthocyanin rich food product. The storage duration was also study biochemically for its commercial application.

Results:total anthocyanin content as 124.87±0.07 mg/100g (*S. cumini* L.) followed by 115.26±0.09 mg/100g (*R. indica* L.) and 91.41±0.09 mg/100 g (*S. fruticosum*Roxb. DC.). The anthocyanin pigments were found to be stable at an acidic pH (up to pH 5) and below 40º C temperature and activity was maintained under a light of 2500 lux for 6 hours. A novel food product was developed by immobilizing pigments extracted from *S. cumini* L., *S. fruticosumRoxb*. DC. and *R. indica* L. on *Colocasia esculenta* (L.) Schott (variety: Ahina) powder.
Conclusion: Anthcyanins act as potent antioxidant but its stability after extraction is a major concern. By applying different storage condition and suitable carrier material selction it can be stored for long.

Keywords: *Indigenous plants, Food colorants, Anthocyanin, Stability, Immobilization, Food product.*

1. INTRODUCTION

Food color industry is thought be increased up to 15 % in recent years. Natural colors like anthocyanin, betacyanin, carotenoids, betalins and chlorophyll etc. are now considered as substitutes for synthetic colorants. In terms of natural colorants, secondary metabolites mainly phenolic compounds are found to be rich in different colors due to its unique structural features. Structurally, anthocyanins are glycosides of polyhydroxy and polymethoxy derivatives of 2-phenylbenzopyrrile salts and are composed of an aglycone known as anthocyanidin. A carbohydrate residue (glucose, xylose, galactose, arabinose, rhamnose or rutinose) usuallyconnected to the anthocyanidin skeleton through the C3 hydroxyl organization in ring C. The functioning of anthocyanin is distinctlydepended on (i) Chemical structure, ring orientation and role of hydroxyl or methoxy groups (ii) Quantity of glycosyl, acyl groups, sugar acylation and the identification of acylating agent and (iii) Stability under varying pH, temperature, light and storage situations [17]. Anthocyanin as a compound is very unstable in natural condition but with proper selection of carrier material it can be stored for long duration. Taro powder do not have any anthocyanin compound if compared with high anthocyanin rich plant product like fruits and flowers of different indigenous. However high carbohydrate content of taro can be used to bind anthocyanin after proper extraction techniques and can stored long with proper study of storage condition.Provide a factual background, clearly defined problem, proposed solution, a brief literature survey and the scope and justification of the work done.

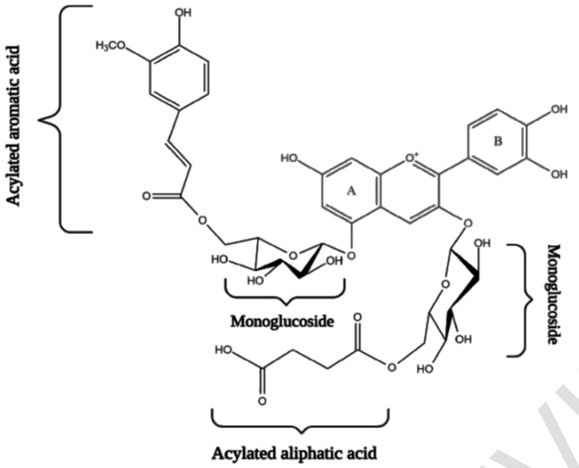

Fig 1: Basic structure anthocyanidin

2. MATERIAL AND METHODS

2.1 Chemical, reagentsand instruments

HPLC grade methanol, Milli-Q water (0.42 mm membrane filtered), ethanol and standards of anthocyanins as Delphinidin Chloride (CAS 28-53-0) and Pelagonidin chloride (CAS 134-04-3) were purchased from Sigma-Aldrich, India.Aluminium chloride, ascorbic acid, Bovine Serum Albumin (BSA), Folin Ciocalteu Reagent (FCR), hydrochloric acid (HCL), quercertin, nitric acid (HNO_3),sulphuric acid (H_2SO_4), anthrone and sodium carbonate (Na_2CO_3) were procured from Merck (India). All other chemicals used were of analytical grade. Instruments like Eutech pH meter (pH700), Waters 2487 HPLC (UV-Vis detector); Toledo ME204 weighing balance; Chemitomake:modelSpectrascan 2600 spectrophotometerand Hermle centrifuge 236s were majorly used.

2.2 Materials

Freshly ripped fruits of Jamun (*S.cumini*L. and *S.fruticosum*Roxb. DC.), Malabar spinach (*B.alba*L. and *B.rubra*L.) and full grown flowers of rose (*R.indica*L., *R.damascena*Mill. *andR.bracteata*J.C.Wendl.) were collected from different places of Jorhat, Assam (GPS: 26° 45' 0.00" -N and 94° 13' 12.00" -E). Samples were washed with distilled water, wrapped up in blottingpaper toremovewater and stored in refrigerated condition (4ºC). Matured tubers of *Colocasia esculenta* (L.) Schott (variety: Ahina)and fruits of Lemon cv. Assam lemon (*Citrus limon Burm*.) were collected from Horticultural orchard of Assam Agricultural University, Jorhat, Assam.

2.3 Preliminary analysis

2.3.1 Total moisture content

Moisture content was determined by of AOAC (1970) method. For this, approximately 10 g of sample was weighed in aluminium moisture boxes and dried in an oven at 54±2°C till constant weight. Total moisture content was expressed by following formula.

Moisture Content (g/100g sample) = $\frac{Initial\ weight\ (g) - Final\ weight\ (g)}{Weight\ of\ the\ sample\ \ (g)}$

2.3.2 Extractionofcolorant

Theextraction of colorantwas doneby grinding and maceration in 100 ml distilled water followed by filtration with muslin cloth [3]. Filtered samples werecentrifugedat10,000rpmfor15minutes.The supernatantwas again filteredwithWhatmanno.42filterpaper.The filtratewasstoredinrefrigeratedcondition (4ºC) forfurtheranalysis

2.3.3 Determination of total phenolic content

Qualitative analysis of phenolic content was done by the ferric chloride. The total phenolic content of extracts was determined using the Folin–Ciocalteu Reagent [23]. Gallic acid was used as standard and TPC was expressed as mg gallic acid equivalent weight per 100 g fresh mass.

2.3.4 Determination of total flavonoids

The total flavonoid content was determined by colorimetric method [6] and expressed in mg of Quecertin equivalent (QE)/100g of fresh weight.

2.4.1 Totalmonomericanthocyaninpigmentcontent

Total monomericanthocyaninpigmentcontent wasmeasuredby pHdifferentialmethod, which is basedonthestructuralchangeoftheanthocyaninchromophorebetweenpH1.0and4.5 [16]. Potassium chloride buffer (KCl, 0.025 M, pH 1.0) and sodium acetate buffer ($CH_3CO_2Na \cdot 3H_2O$ 0.4 M, pH 4.5) were prepared and diluted with extracted colorants (5:1). HCl (0.2N) was used to adjust the pH. After 15 mins of equilibration, the absorbance of two dilutions was read at 510 nm and 700 nm using a spectrophotometer. Results of total monomeric anthocyanin pigment content expressed as cyanidin-3-glucoside equivalents in mg/100g by the following formula:

Total monomeric anthocyanin pigment content = $\frac{(A \times MW \times DF \times 1000)}{\varepsilon}$

Where, A= $(A_{513}-A_{700})$ pH 1.0–$(A_{513}-A_{700})$ pH 4.5
MW= molecular weight of cyaniding-3-glucoside (449.2)
DF= Dilution factor
ε= Molar extinction coefficient (26,900 L mol^{-1} cm^{-1})

2.5.1 pH stability

The method by Selim *et al.* (2008) [22] with slight modifications was used to evaluate the stability of anthocyanin extract at varying pH. 2 ml extracted colorants mixed with buffers ranging from pH 1 to pH 10 were prepared and made up to 20 ml with the desired buffer. The test tubes were covered with aluminium foil and stored at room temperature for 2 hours.

2.5.2 Thermal stability

The thermal stability of extracted colorants was determined according to Amr and Al-Tamimi (2007) [24] with some modifications. 0.1 ml of extracted colorant was made up to 25 ml with 0.1 M (pH 3.5) citrate-phosphate buffer and kept in -4°C, 20°C, 40°C, 60°C, 80 °C and 100 °C for 6 hours. Absorbance was read at 520 nm and percentage retention of colorant was calculated using following equation:

Pigment retention = $\frac{Absorbance\ \ after\ \ heating}{Absorbance\ \ before\ \ heating} \times 100$

2.5.3 Light stability

Extracted colorants were prepared in equal proportion (1:1) in citrate- phosphate buffer (pH 3.5) in sealed test tubes [5]. To maintain average daylight condition (2500 lux), a square hardboard box (75 cm x 75cm x 75 cm) laden internally with aluminiumfoil.Two 40W bulbs were placed at 10 cm distance inside the box. Buffer mixed with colorants were incubated under artificial light at room temperature and readings were taken in-between 2h, 6h, 12h, 24 h, and 5days.

2.5 Development of an anthocyanin-rich solid food product

2.5.1 Preparation of *Colocasia esculenta* (L.) Schott (variety: Ahina) powder

Colocasia esculenta (L.) Schott (var. Ahina) powder was prepared by the method of Hazarika (2016)[12]. Pilled tubers were washed, cut into small pieces, air-dried at room temperature, ground to a fine powder and stored in a desiccator for further analysis. To immobilize colorant on edible carrier food material, the method given by Debnath, 2016 was followed. Powdered *Colocasia esculenta* (L.) Schott (variety: Ahina)was added to the concentrated dye in a ratio of 1:3 (w/v) to make it a viscous solution, then incubated at 4ºC overnight, further ground to powder and stored in a sealed clear glass bottle covered with aluminum foil in refrigerated condition (4ºC).

2.6 Nutritional analysis of carrier materials

Nutritional analysis of carrier material *viz*. *Colocasia esculenta* (L.) Schott (variety: Ahina)powder and Assam lemon beverage was done before and after immobilization of colorants extracted from selected plant samples.

2.6.1 Total phenol and total anthocyanin

Total phenolics and total anthocyanin content were determined by Folin Ciocalteau Reagent [23] and pHdifferentialmethod [16] respectively.

2.6.3 Total soluble protein

Total soluble protein was determined by Folin-Ciocalteu Reagent [7]. To 0.1 ml of the sample extract, 0.9 ml distilled water was added to make up the volume. 5 ml of alkaline copper solution was added, mixed well by vortexing and incubated at room temperature for 10 mins. After addition of 0.5 ml of Folin-Ciocalteu Reagent and readings were taken at 650 nm. The protein concentration was calculated out from the standard curve of BSA (Bovine Serum Albumin) and expressed in mg of protein content per 100 g on a fresh weight basis.

2.6.4 Total starch content

Total starch content of *Colocasia esculenta* (L.) Schott (variety: Ahina) powder was determined by the anthrone method [21].0.2 g of sample was homogenized in 80% ethanol and centrifuged. To the extracted residue 5.0 ml of water and 6.5 ml of perchloric acid (52%) were added. After centrifugation, the supernatant was made up to 50 ml and 4 ml of anthrone reagent was added followed by heating in boiling water for 8 to 10 minutes. After cooling, the reading was taken at 630 nm.

2.6.7 Total Ash and minerals (Phosphorous and Iron)

The ash content of *Colocasia esculenta (var. Ahina)* powder and peel of Lemon cv. Assam lemon *(Citrus limon* Burm.) was determined by the method of AOAC (1970) [1]. Mineral solutions were prepared and analyzed for the determination of Phosphorous and Iron [2].

2.7 Evaluation after immobilization of colorants

After immobilization of colorants, *Colocasia esculenta* (L.) Schott (variety: Ahina)powder immobilized with colorants were evaluated for the change in total anthocyanin content (mg/100gm) and total starch content (%) with in storage period of one month (4ºC). Similarly, change in total anthocyanin content of Lemon cv. Assam lemon *(Citrus limon* Burm.*)* beverage mixed with colorants was also evaluated for same period of storage period (4ºC).

2.8 Statistical analysis

The data obtained from laboratory experiments were analyzed statistically. The experiment was laid out in Complete Randomized Design (CRD). All analysis was performed in triplicate and the average has been reported. The data were analyzed by one-way analysis of variance (ANOVA). The standard error of the mean difference (S. Ed.±) was calculated. The treatment means were compared among themselves by calculating critical difference (CD at P<0.05) as per the method of Panse and Sukhatrne (1978) [17].

The analysis of variance (ANOVA) was done with treatments and replications. The critical differences were calculated by the formula:

$$\text{CD} = t_{0.05,\ \text{error d.f.}} \times \text{S.Ed}$$

$$\text{Where, S. Ed} = \sqrt{\frac{2EMS}{r}}$$

3. RESULTS AND DISCUSSION

The design of experiment for present investigation is based on selection of experimental design by ISO 3534-3:1985 [13]. Accuracy (trueness and precision) of measurement methods and results were as per the guidelines of ISO 5725-1:1994 [14]. For all the recorded data we have taken three replications and standard deviation was in accordance with the replicated value.Out of seven selected plant samples flower of *Rosa indica* L. had highest total phenolic content of 1516.52±0.03 mg GAE/100g. In consideration of total flavonoid contentamong seven different plant samples, *Rosa damascena*Mill*. and fruits of S.cumini* L. had a higher amount of total flavonoid content of 262.87 ±0.07 mg QE/100g. (Table 1).Fruit extract of *S. cumini* L. (124.87±0.07 mg/100g) had the highest total anthocyanin concentration, followed by flower extract of *Rosa indica* L. (115.26± 0.09 mg/100g) and fruit extract of *S. fruticosum*Roxb. DC. (91.41± 0.09 mg/100g). The total anthocyanin content of Jamun fruit was found to be 195.58±6.15 mg/100g as reported by Ghosh *et al*. (2016). *Rosa indica* L. had total anthocyanin content of 115.26±0.091 mg/100g.Poonam (2016) [15] studied anthocyanin pigments in Indian rose types and discovered total anthocyanin concentration ranged from 2.14±0.12 mg/100g to 667.46±21.27 mg/100g.Characterization of anthocyanins in selected plant samples was done by the UHPLC method comparing with the retention time and absorbance peaks of two selected anthocyanin standards *viz* Delphinidin-3-glucoside (RT:10.713 minutes) and Pelargonidin -3-glucoside (RT:11.313 minutes). Pelargonidin-3-glucoside was present in a major amount followed by delphinidin-3-glucoside in extracted colorant of *S. cumini*L. and *S.fruticosum*Roxb. DC.fruits. On the other hand, delphinidin-3-glucoside was the major anthocyanin followed by pelargonidin-3-glucoside in the extracted colorant of *R. indica* L.

Species	Part used	Weight (g)	Length (cm)	Breadth (cm)	Moisture (%)	TPC (mg GAE/100 g)	TFC (mg QE/100g)	TAC (mg/100g)
1	Fruit	6.31±0.02	2.21±0.09	2.04±0.08	81.92±0.16	1149.28 ±0.07	262.87±0.07	124.87 ± 0.07
2	Fruit	2.56±0.06	0.64±0.03	0.69±0.06	78.48±0.23	741.25± 0.05	176.23 ±0.17	91.41 ± 0.09
3	Fruit	0.79±0.03	0.52±0.03	0.78±0.02	84.12±0.42	548.98± 0.42	184.16± 0.12	5.96 ± 0.06
4	Fruit	0.76±0.04	0.54±0.05	0.77±0.03	83.88±0.09	938.47± 0.83	292.52± 0.24	8.95 ± 0.11
5	Flower	4.03±0.04	3.83±0.09	3.30±0.06	81.37±0.16	1516.52± 0.03	172.91± 0.49	115.26 ± 0.09
6	Flower	4.73±0.02	4.07±0.08	3.47±0.02	76.65±0.17	1380.67 ±0.04	262.88± 0.05	2.36 ± 0.17
7	Flower	3.20±0.01	3.87±0.03	3.17±0.07	78.78±0.27	1347.23± 0.16	196.12± 0.89	0.66 ± 0.07

Table 1:Preliminary investigation of selected plant samples: *1) S. cumini* L., 2) *S. fruticosum*Roxb. DC., 3) *Basella alba* L., 4) *Basella rubra* L., 5) *R. indica* L., 6) *R. damascena* Mill. and 7) *R. bracteata* J.C. Wendl.

Both in presence or absence of light, the temperature had a significant impact on the stability of anthocyanin pigment. The anthocyanin concentration was tested for 6 hours at 20°C, 40°C, 60°C, 80°C and 100°C, and it was observed that anthocyanin degradation was less at lower temperatures (below 40°C). More than 50% pigment retention up was found for *R. indica* L. (111.65 mg/100g) at 40°C. However, at 60°C, 50% pigment retention was observed for *S. cumini* L. (68.72 mg/100g) and *S. fruticosum*Roxb. DC. (75.34 mg/100g) (Fig. 3). In contrast, fast degradation rate was found above 60°C for 6 hours. The rapid decomposition of anthocyanin at higher temperatures could be attributed to the hydrolysis of the 3-Glycoside structure, which protects unstable anthocyanin. Anthocyanin acylation contributes to their stability as temperatures rise [9]. The per cent pigment retention of extracted colorants showed similar results. Per cent pigment retention of extracted colorants was found to be greater than 50% at 60°C temperature for both the fruits of jamun (55 and 82.41 %). Whereas, flower extract of *R. indica* L. have the percentage pigment retention reduced as the temperature increased. (Table 2. a).

After one hour of incubation at each pH (1-10), the total anthocyanin content was measured. At pH 2 the maximum total anthocyanin content for extracted colorants from plant samples was observed as 122.14 (*S. cumini* L.), 90.57 (*S. fruticosum*Roxb. DC.) and 99.38 (*R. indica* L.) expressed in mg/100g. With rising pH the total anthocyanin content steadily dropped, however more than 50% of total anthocyanin was retained up to pH 4. As the pH was raised higher, the overall anthocyanin content declined up to 2.92, 1.36 and 1.93 mg/100g (Table 2.a). In acid solutions, some anthocyanins were red, violet or purple in neutral solutions and blue in alkaline pH solutions. Anthocyanin molecules in solution were found to be in a state of equilibrium between the colored cationic form and the colorless pseudo base. It became protonated and formed a positive ion or cation at low pH, deprotonated as pH increased, and formed a negative ion or anion at high pH (Roobha*et al.*,2011).

The stability of extracted colorants under light was investigated by incubating sample extracts for 2 hours, 6 hours, 12 hours, 24 hours, and 5 days under 2,500 lux light intensity (Table 2 c.). Light exposure for 2 hours (2500 lux) resulted in pigment retention of 92.61 % (115.61 mg/100g), 90.12% (82.38 mg/100g) and 87.12 % (101.21 mg/100g) in extracted colorants from *S. cumini* L., *S. fruticosum*Roxb. DC. and *R. indica* L. However, with increasing light exposure duration, TAC fell drastically. 24 hours of light exposure results in less than 10 % of pigment retention i.e., 9.87% (12.33mg/100g), 4.51 % (4.13 mg/100g) and 8.09 % (9.33 mg/100g). To retain the TAC, extracted colorants must be mixed with some carrier material that increase its self-life. Stability of anthocyanin under light rely upon the presence of oxygen, interactions with other components (sugars and ascorbic acid), hydrolyzation at glycoside linkages to produce chalcone, alpha diketones and velocity of free sugar formation [18][17].*S.cumini*L.(124.87±0.07mg/100g),*S. fruticosum*Roxb. DC. (115.26±0.09mg/100g)and*Rosaindica*L. (91.41±0.09mg/100g) were found with higher total anthocyanin content (Table 1) fromthespectroscopicdeterminationandUHPLCcharacterizationof anthocyanins. Extracted colorantsfromthese three sampleswere subjectedforimmobilizationoninertcarriermaterialto developasolidanthocyaninrichfoodproduct.(Fig. 2).

a.Sample	**4ºC**	**20ºC**	**40 ºC**	**60 ºC**	**80 ºC**	**100 ºC**
***S. cumini* L.**	124.87 (100)	119.68 (95.84)	77.29 (61.89)	68.72 (55)	35.65 (36.16)	19.02 (15.23)
***S. fruticosum*Roxb. DC.**	91.41 (100)	89.24 (97.58)	82.85 (90.63)	75.34 (82.41)	45.38 (49.64)	11.26 (12.31)
***R. indica* L.**	115.26 (100)	113.58 (98.54)	111.65 (96.86)	8.23 (7.1)	7.63 (6.61)	3.68 (3.19)
CD$_{(0.05)}$	0.073 (0.08)	0.0124 (0.35)	0.073 (0.35)	0.06 (0.06)	0.08 (1.06)	0.074 (0.06)
SE(d)	0.034 (0.03)	0.006 (0.16)	0.033 (0.16)	0.025 (0.02)	0.037 (0.49)	0.035 (0.02)
SE(m)	0.024 (0.02)	0.004 (0.11)	0.023 (0.11)	0.018 (0.01)	0.265 (0.35)	0.024 (0.01)

b.Sample	**pH 1**	**pH 2**	**pH 3**	**pH 4**	**pH 5**	**pH 6**	**pH 7**	**pH 8**	**pH 9**	**pH 10**
***S. cumini* L.**	102.36 (81.97)	122.14 (97.81)	118.96 (95.26)	110.93 (88.83)	84.23 (67.45)	55.24 (44.23)	26.44 (21.17)	14.67 (11.74)	4.54 (3.63)	3.65 (2.92)
***S. fruticosum* Roxb. DC.**	69.35 (75.86)	90.57 (99.08)	88.44 (96.75)	82.51 (90.26)	42.62 (37.16)	37.16 (40.65)	19.21 (21.01)	12.04 (13.17)	3.05 (3.33)	1.25 (1.36)
***R. indica* L.**	91.47 (79.35)	99.38 (86.22)	112.45 (97.56)	106.86 (93.71)	85.98 (74.59)	74.88 (64.96)	55.02 (47.73)	18.86 (16.36)	7.44 (6.45)	1.93 (1.67)
CD$_{(0.05)}$	0.072 (0.051)	0.056 (0.045)	0.06 (0.067)	0.063 (0.12)	0.067 (0.78)	0.066 (0.098)	0.07 (0.098)	0.061 (0.53)	0.062 (0.064)	0.069 (0.028)

SE(d)	0.033 (0.017)	0.025 (0.089)	0.026 (0.032)	0.031 (0.051)	0.033 (0.049)	0.032 (0.073)	0.031 (0.049)	0.028 (0.018)	0.029 (0.064)	0.032 (0.048)
SE(m)	0.023 (0.011)	0.018 (0.13)	0.018 (0.011)	0.021 (0.017)	0.022 (0.007)	0.022 (0.014)	0.022 (0.014)	0.019 (0.008)	0.02 (0.01)	0.023 (0.014)

c. Sample	**2500lux(2h)**	**2500lux(6h)**	**2500 lux (12h)**	**2500lux(24h)**	**2500lux(5days)**
***S. cumini* L.**	115.65 (92.61)	86.55 (69.31)	32.21 (25.79)	12.33 (9.87)	0.94 (0.75)
***S. fruticosum*Roxb. DC.**	82.38 (90.12)	67.31 (73.63)	26.93 (29.46)	4.13 (4.51)	0.62 (0.67)
***R. indica* L.**	101.21 (87.12)	81.07 (70.33)	53.08 (46.05)	9.33 (8.09)	0.07 (0.60)
CD$_{(0.05)}$	0.07 (0.09)	0.186 (0.067)	0.06 (0.071)	0.06 (0.019)	0.063 (0.011)
SE(d)	0.032 (0.101)	0.087 (0.043)	0.027 (0.121)	0.028 (0.021)	0.029 (0.07)
SE(m)	0.022 (0.013)	0.061 (0.02)	0.019 (0.013)	0.019 (0.015)	0.02 (0.01)

*Values were expressed in average of three replications

Table 2: Effect of temperature (a), pH (b) and light exposure duration (2500 lux) (c) on TAC of extracted colorants from fruits of *S. Cumini* L, *S. fruticosum*Roxb. DC. and flowers of *Rosa indica* L. Within optimum condition i.e., 40 ºC (temperature), pH 4 and exposure of light (2500 lux) up to six hours resulted in more than 50 % pigment retention

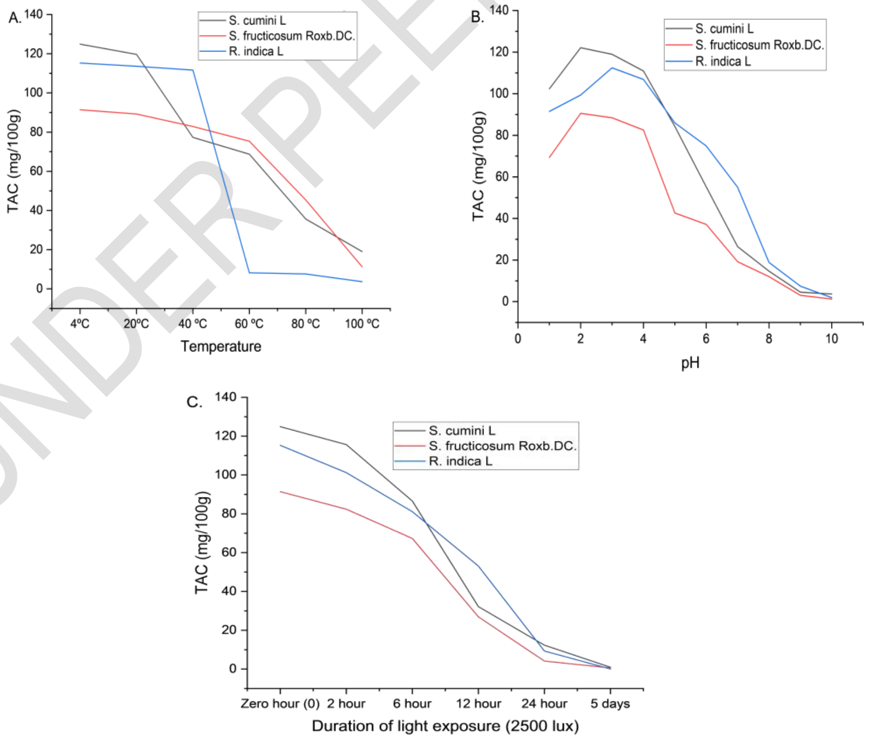

Figure 2: Effect of A. temperature B. pH and C. duration of light exposure (2500 lux) on extracted colorants obtained from fruits of *S. cumini* L., *S. fructicosum*Roxb.DC. and flowers of *R. indica* L.

Total phenol, total anthocyanin, total soluble protein, total starch, total ash, phosphorus and iron content in raw *Colocasia esculenta* (L.) Schott (variety: Ahina) were found to be 46.78±0.19 mg GAE /100g, 0.84±0.32 mg/100g, 0.86±0.16%, 92.63±0.64%, 1.83±0.09%, 28.79±0.13 mg/100g and 7.63±0.38 mg/100g, respectively (Table 3). Total soluble protein, total starch content, total ash content as well as minerals *viz*. Phosphorus and iron were found to be in similar range in both raw and colorant mixed final product of *Colocasia esculenta* (L.) Schott (variety: Ahina) (Table 3). The total phenol content and total anthocyanin content of the final food product was found to be higher than raw *Colocasia esculenta* (L.) Schott (variety: Ahina). The final product isolated from *S. Cumini* L., *S. fruticosum*Roxb. DC., and *Rosa indica* L. found with increased total phenol content of 71.83±0.08 mg GAE/100g, 49.42±0.14 mg GAE/100g, and 69.54±0.35 mg GAE/100g. In comparison to 0.84 mg/100g in raw *Colocasia esculenta* (L.) Schott (variety: Ahina) powder, total anthocyanin concentration in final product blended with colorants was found to be 44.76±0.70 mg/100g (*S. Cumini* L.), 35.66±0.67 mg/100g (*S. fruticosum*Roxb. DC.) and 48.71±0.39 mg/100g (*Rosa indica* L.) (Table 4.)

Parameters	**Nutritional analysis of raw Taro powder**	**Nutritional analysis of final product mixed with colorants**		
		***S. Cumini* L**	***S. fruticosum*Roxb. DC.**	***Rosa indica* L.**
Total phenol content (mg GAE /100g)	46.78±0.19	71.83±0.08	49.42±0.14	69.54±0.35
Total anthocyanin content (mg/100g)	0.84±0.32	44.76±0.70	35.66±0.67	48.71±0.39
Total soluble protein (%)	0.86±0.16	0.84±0.28	0.82±0.14	0.84±0.52
Total starch content (%)	92.63±0.64	91.98±0.42	92.14±0.06	91.86±0.09
Total ash content (%)	1.83±0.09	1.79±0.14	1.80±0.45	1.80±0.24
Phosphorus (mg/100g)	28.79±0.13	28.77±0.05	27.61±0.22	26.84±0.17
Iron (mg/100g)	7.63±0.38	7.42±0.12	6.99±0.08	7.09±0.32

*Values were expressed in average of three replications

Table 3: Nutritional comparison of Final Food Product from *Colocasia esculenta* (L.) Schott (variety: Ahina) with raw powder.

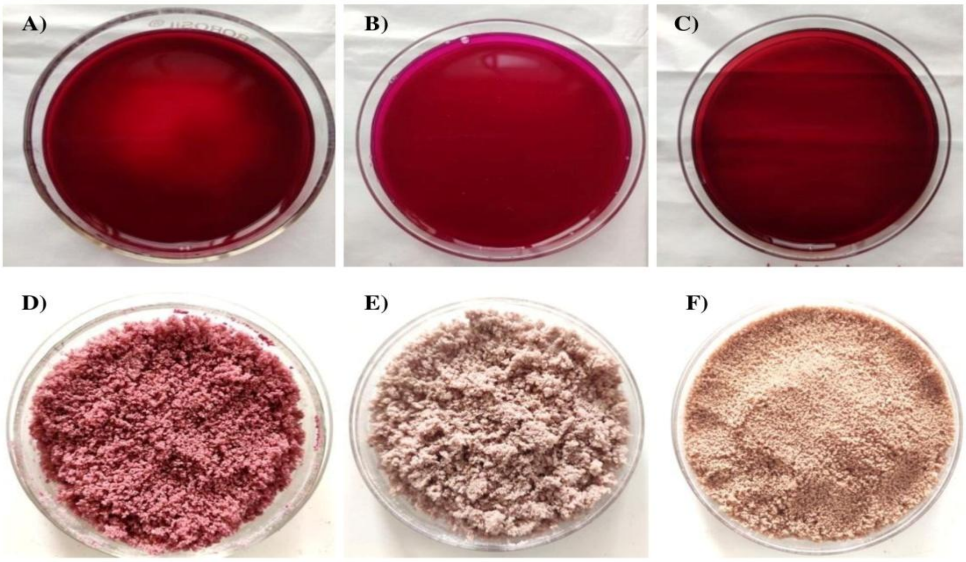

Figure 3: Concentrated colorants extracted from A.*S. cumini*l. B. *S. fruticosum*roxb. DC. C. *R. indica* L. which were later mixed and immobilized on taro powder. Figure D, E and F represents the taro powder with concentrated colorants from *S. cumini*L., *S. fruticosum*roxb. DC. and *R indica* L.

4. CONCLUSION

Bioavailability and nutritional diversity in colorants of different indigenous and wild plant sections have been indicated in previous publications. On the basis of chemical attributions, the source of colorants varies from plant to plant. In terms of food coloring, anthocyanin extraction and stability are key concerns. Although the majority of the colorants are phenolic compounds, extraction in an aqueous medium yields the highest extraction percentage for food usage. The total anthocyanin concentration of the fruit extracts of *S. cumini* L. was found to be the highest among seven plant samples, with a value of 124.87±0.078 mg/100g, followed by *R. indica* L. (115.26±0.091 mg/100g) and *S. fruticosum*Roxb. DC. (91.41±0.097 mg/100g). High total anthocyanin concentration suggested a greater stability of total anthocyanin extracted from plant samples in the acidic range up to pH 4. Best stability was obtained with temperature treatment of 40ºC for 6 hours. 2,500 lux light intensity applied up 2 hours of incubation resulted in lowest degradation of anthocyanin content. The starch content of *Colocasia esculenta* (L.) Schott (variety: Ahina) in the final food product was unaffected by the addition of colorants. The total anthocyanin content of final products was determined to be 39.42 mg/100g (*S. cumini* L.), 31.33 mg/100g (*S. fruticosum*Roxb. DC.) and 44.51 mg/100g (*Rosa indica* L.) after one month of refrigerated storage. Similarly, enriched Assam lemon beverage with extracted colorants results in better stability and stable pH for color optimization.

Colorants extracted from	**1 day**		**2 days**		**4 days**		**6 days**		**12 days**		**30days**	
	TAC (mg/100g)	TSC (%)	TAC (mg/100g)	TSC (%)	TAC (mg/100g)	TSC (%)	TAC (mg/100g)	TSC (%)	TAC (mg/100g)	TSC (%)	TAC (mg/100g)	TSC (%)
***S. Cumini* L**	44.60 (99.64)	91.97 (99.98)	44.57 (99.57)	91.93 (99.94)	44.43 (99.26)	91.84 (99.84)	43.98 (98.25)	91.76 (99.76)	42.72 (95.44)	90.68 (98.58)	39.42 (88.06)	90.07 (97.92)
***S. fruticosum*Roxb. DC.**	35.63 (99.91)	92.12 (99.97)	35.58 (99.77)	92.08 (99.93)	35.47 (99.46)	92.01 (99.85)	35.12 (98.48)	91.98 (99.82)	34.75 (97.44)	91.52 (99.32)	31.33 (87.86)	89.96 (97.63)
***Rosa indica* L.**	48.70 (99.97)	91.86 (100)	48.67 (99.91)	91.85 (99.98)	48.59 (99.75)	91.83 (99.96)	48.56 (99.69)	91.76 (99.89)	46.23 (94.91)	91.25 (99.35)	44.51 (91.38)	90.84 (98.88)
CD$_{(0.05)}$	0.066 (NS)	NS (NS)	0.103 (NS)	NS (NS)	NS (0.110)	0.053 (0.064)	NS (0.032)	NS (NS)	NS (0.12)	0.061 (NS)	0.082 (NS)	0.065 (0.012)
SE(d)	0.027 (0.012)	0.018 (NS)	0.029 (0.015)	0.014 (0.051)	0.017 (0.032)	0.021 (0.03)	0.016 (0.013)	0.019 (0.12)	0.017 (0.021)	0.021 (NS)	0.033 (0.078)	0.026 (0.08)
SE(m)	0.019 (0.007)	0.012 (0.078)	0.021 (0.012)	0.010 (0.032)	0.012 (0.017)	0.015 (0.102)	0.011 (0.01)	0.013 (0.041)	0.012 (0.014)	0.015 (NS)	0.023 (0.021)	0.018 (NS)

*Values were expressed in average of three replications

Table4:Change in TAC (mg/100g), Total starch content (%) and per cent degradation of taro powder mixed with extracted concentrated colorants from *S. Cumini* L, *S. fruticosum*Roxb. DC. and *Rosa indica* L. during storage up to 30 days at 4° C.

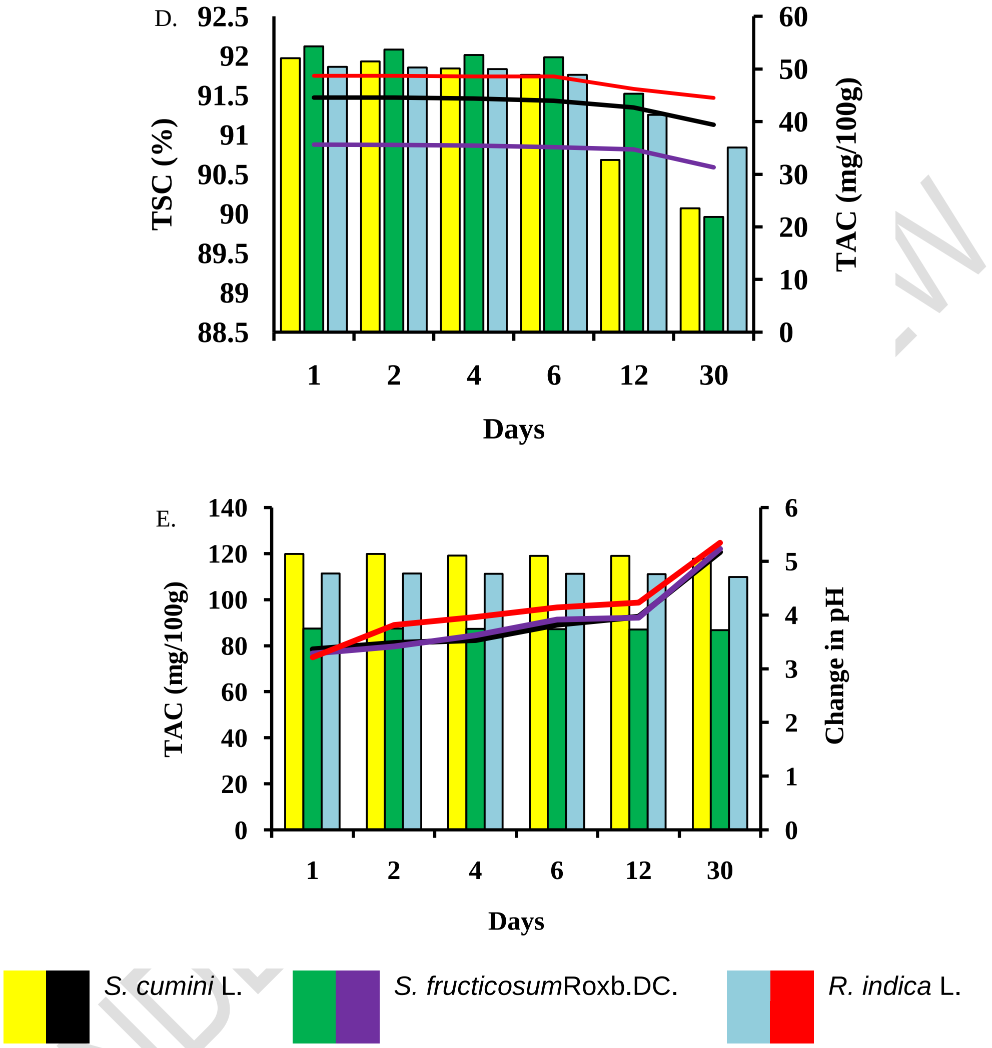

Figure 4. : Graphical representation (D) of change in TSC (%) and TAC (mg/100g) in final food products of taro powder mixed with concentrated colorants extracted from fruits of *S. cumini* L., *S. fructicosum*Roxb.DC. and flowers of *R. indica* L. during one month of storage period shows decreasing pattern of TSC and TAC with increasing time. Figure 4. represents the change in TAC (mg/100g) and pH of prepared beverage enriched with extracted colorants from selected plant samples. pH was found to be increased with decreasing TAC in prepared beverage during one month of storage period.

REFERENCES

1. AOAC official methods of analysis. 10th Ed. Association of Analytical Chemists, Washington D.C. .1970.

2. AOAC official methods of analysis, 13th Ed. Association of Official Analytical Chemists, Washington, D.C.1980.

3. Abdulrahman FI, Inyang I, Abbah, J, John AL, Amos S. &Gamaniel K. Effect of aqueous leaf extract of Irvingiagabonensis on gastrointestinal tract in rodents.Indian J. Exp. Biol.2004; 42:787-91.

4. Ameya G, Tsalla T &Getu F. Antimicrobial susceptibility pattern, and associated factors of Salmonella and Shigella infections among under five children in Arba Minch, South Ethiopia. Ann. Clin. Microbiol. Antimicrob. 2018;17: 1. https://doi.org/10.1186/s12941-018-0253-1.

5. Amr AK & Al-TamimiEK.Stability of the crude extracts of Ranunculus asiaticusanthocyanins and their use as food colorants. Int. J Food Sci. Tech.2007; 42(8):985 – 991.

6. Chia-Chi Chang, Ming-Hua, Y., Hwei-Mei, W. &Jiing-Chuan, C. Estimation of Total Flavonoid Content in Propolis by Two Complementary Colorimetric Methods.J. Food Drug Anal.2002;10(3):178-182.

7. Chitnis N, Pemberton P &Yukich J. Theory of reactive interventions in the elimination and control of malaria. Malar J. 2019; 18:266-269.

8. Debnath, I.Phytochemical analysis and antimicrobial activity of Aparajita (Clitoriaternatea L.) against rice pathogens. M.Sc. (Biochemistrty and Agricultural Chemistry) thesis, Assam Agricultural University, Jorhat, Assam.2016.

9. Devi P, Saravanakumar M & Mohandas S. African the effects of temperature and pH on stability of anthocyanins from rd sorghum (Sorghum bicolor) bran.2012; J. of Food Sci. 6(24): 567-573.

10. El-GharablyAMA.Characterization of anthocyanin pigments extracted from grape skins and its potential uses as antioxidant and natural food colorants. J. Home Econ.2005; 15: 51–70

11. Ghosh P, Pradhan RC, Mishra S, Patel AS & Kar A.Physicochemical and nutritional characterization of Jamun (*SyzygiumCuminii*). Curr. Res. Nutr. Food Sci.2017; 5 (1):25-35.

12. Hazarika I. Biochemical characterization of some Colocasia (*Colocasia esculenta* L.) germplasm. M.Sc. (Biochemistrty and Agricultural Chemistry) thesis, Assam Agricultural University, Jorhat, Assam.2016.

13. ISO 3534-3. Statistics — Vocabulary and symbols — Part 3: Design of experiments,1985.

14. ISO 5725-6. Accuracy (trueness and precision) of measurement methods and results — Part 6: Use in practice of accuracy values.1994

15. Kumari P. Isolation and characterization of anthocyanin pigments from Indian Rose varieties as a potential source of nutraceuticals. division of floriculture and landscaping. ICAR - Indian Agricultural Research Institute New Delhi 110012. retrived from http://krishikosh.egranth.ac.in/handle/1/5810069960.2016.2016.

16. Lee J, Durst R &Wrolstad R. Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: Collaborative Study. J. of AOAC Int. 2005; 88:1269-1278.

17. Miguel MG and Albano SM. Biological activities of extracts of plants grown in Portugal. Industrial Crops and Products.2011; 33:338-343.

18. Panse VG and Sukhatme PV. Statistical Methods for Agricultural Workers. Indian Council of Agricultural Research Publication.1985; 87-89.

19. Ramalingam R, Palmurugan S &Shanmugapriya D. Effect of temperature, light, pH on the stability of anthocyanin pigments in *Cocculus hirsutus* fruits. Int. J. Multidiscip. Res. Mod. Educ.2016; 2(2):91-96.

20. Roobha J. Marappan S., Aravindhan KM & Devi PS. The effect of light, temperature, pH on stability of anthocyanin pigments in Musa acuminata bract. Res. Plant Biol.2011; 1: 5-12.

21. Sadasivam S.& Manickam A. Biochemical Methods for Agricultural Sciences. Wiley Eastern Ltd., New Delhi.1992.

22. Selim KA, Khalil KE, Abdel-Bary MS & Abdel-Azeim N.A. Extraction, encapsulation and utilization of red pigments from Roselle (Hibiscus sabdariffa L.) as natural food colorants. In: 5th Alexandria Conference of Food and Dairy Science and Technology, Alexandria, Egypt. 2018; 7-20.

23. Singleton VL & Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J. Enol. Vitic.1965;16:144-158.

24. AmrAK and Al-Tamimi EK (2007). Stability of the crude extracts of Ranunculus asiaticus anthocyanins and their use as food colourants. Int. J Food Sci. Tech.2007; 42(8):985 – 991.

ABBREVIATIONS

TAC: Total Anthocyanin Content**,** TPC: Total Phenolic Content**.** TFC: Total Flavonoid Content**,** TSS: Total Soluble Sugar**,** TSP: Total Soluble Protein**,** EMS: Error Mean Square**,** GAE: Gallic Acid Equivalent**,** QE: Quecertin Equivalent