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Standardization of anthocyanin extraction from Roselle (*Hibiscus sabdariffa* L.) calyces for edible colour

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Abstract
Extraction of anthocyanin from Roselle calyces for application in edible food colour was studied using different methods. The treatment ethanol acidified with 1.5 N HCl recorded highest anthocyanin content (1638.17 mg 100 ml^{-1}), TSS (28.40°B), total phenols (12.84 mg ml^{-1}), titratable acidity (10.83%) and total antioxidants (101.05 mg 100 ml^{-1}). The water activity was found to be highest (0.94) in the treatment of hot water extraction, while the pH was more (3.62) in treatment fermentation of calyces. The benefit cost ratio was found to be highest (24.88:1.0) in the treatment fermentation of calyces compare to all other treatments. The present investigations revealed that, the treatment of ethanol acidified with 1.5 N HCl was found to be the best with highest anthocyanin and total antioxidants with better benefit cost ratio among all other treatments which can be employed for large scale extraction of biocolour from Roselle calyces.

Keywords: Anthocyanin, total phenols, total antioxidants, cost: benefit ratio

Introduction
Colour is one of the most important quality attributes affecting the consumer's acceptance of food since it gives the first impression of food quality. The global demand for natural dyes is about 10,000 tonnes world over which is equivalent to one per cent of the world's synthetic dyes consumption and is expected to grow rapidly in near future. The recent ban on the use of azo dyes by European Union has also increased the scope for the use of natural dyes. Interest for bioactive compounds of a natural origin with a high antioxidant capacity has increased considerably in the past decades, mostly due to their potential in prevention of cancer, cardiovascular, chronic and neurodegenerative diseases.

Roselle (*Hibiscus sabdariffa* L.) a multi-use plant, belonging to the family *Malvaceae*, is widely distributed in tropical regions, especially in the Middle Eastern countries. It is generally considered as a medicinal plant. The calyces, also known as natal sorrel, (Anon., 1999; Mohamed *et al.* 2012) [4, 12] are potentially a good source of antioxidant agents such as anthocyanins and ascorbic acid. Roselle calyx is a rich source of dietary fiber, vitamins, minerals and bioactive compounds *viz.* organic acids, phytosterols and polyphenols. The phenol content in the plant consists mainly of anthocyanins like delphinidin-3-glucoside, sambubioside and cyanidin- 3- sambubioside contributing to their antioxidant properties. Its brilliant red colour and unique flavour makes it a valuable food product (Tsai and Ou, 1996; Mohamed *et al.* 2012) [21, 12].

Finding the most efficient extraction and separation method, as well as the full characterization of obtained bioactive compounds from natural matrices are a major challenge for researchers in the food, pharmaceutical, and cosmetic industry. The extraction efficiency of bioactive components from plant materials is affected by different factors, such as the extraction techniques, solvents, time, temperature, solvent-to-plant material ratio and many others. However, a suitable extracting method and solvent are crucial for ensuring an efficient extraction of the targeted nutraceuticals from plant material (Goli *et al.* 2005) [10]. Theoretically, the optimal extraction method should be simple, safe, reproducible, inexpensive and suitable for industrial application.

With this background the research was taken up to establish best extraction method of anthocyanin from Roselle calyces using different solvents, fermentation, enzymes and hot water extraction and analyzing the benefit cost ratio and correlation analysis of the different parameter influencing the anthocyanin content in the final extract.

Materials and Methods

The investigation was carried out at Department of Postharvest Technology, College of Horticulture, University of Horticultural Sciences,, GKVK Campus, Bengaluru, India. The raw material (calyces) required for the experiment was grown in the college experimental plot.

Extraction: Different solvents, enzymes, fermentation and hot water were used for extraction of anthocyanin from roselle calyces. Solvents like ethanol, hydrochloric acid, acetic acid and citric acid were used in the present study. Total 6 treatments were used to find out the suitable method for the optimum extraction of anthocyanin pigment from the roselle calyces. The experiment was conducted using Completely Randomized Statistical Design with 4 replications per treatment. The data were analysed statistically.

The details of the treatments are

T_1. 95% ethanol with 1.5 N HCl
T_2. 95% ethanol with 2% citric acid
T_3. 95% ethanol with 2% acetic acid
T_4. Distilled water with 0.2% pectinase
T_5. Fermentation of calyces
T_6. Hot water extraction

For the treatmentT_1 involving 95 per cent ethanol with 1.5 N HCl, the Due and Francis (1973) [8] method was adopted with little modifications. 50 g powdered roselle calyces was taken in Erlenmeyer conical flask to which 500 ml of solvent was added, the mixture was kept on hot water bath at 60°C for one hour, Later, the mixture was kept in refrigerator overnight. Next day the mixture was squeezed using muslin cloth to remove powder particles. Extracted liquid of 400 ml was concentrated using rotary evaporator (Make: HAHNSHIN Scientific Co., Model: HS-2005V) at 60°C, 40 rpm for 30minute until the final volume of 90 ml.The treatment T_2 involved mixing of 95 per cent ethanol with 2 per cent citric acid in the ratio of 85:15. The treatment T_3 was similar to T_2 except that citric acid was replaced by acetic acid. In the treatment T_4, 1g of pectinase was mixed in 500 ml of distilled water. For treatment T_5, the standard procedure of fermentation of calyces was adopted and for treatment T_6, dry powdered roselle calyces of 50 g was added to 500 ml of water and boiled at 100°C for 30 minute. Further, the extract was filtered through muslin cloth and was concentrated at 65°C, 40 rpm for 2 hours using rotary evaporator.

The anthocyanin extracted from each treatment was measured by reading optical density of the filtrate at 535nm using spectrophotometer (Make: SYSTRONICS, Model: UV/VIS Spectrophotometer 117).In addition, the physicochemical parameters like pH, Titratable acidity and water acitivity; functional properties like total phenols, total soluble solids and total antioxidants were determined.

Measurement of pH was done using pH meter (Make: Trans instruments Model: BO 3001) according to the method outlined in AOAC (2000) [5].The total titratable acidity of dried roselle calycesextract sample was determined by visual titration method (Ranganna, 1986) [16].The water activity of fresh calyces of was measured by Rotronichygrolab water activity analyzer (*aw*-HP23) according to method described by Manjula and Ramachandra, 2014. The total phenols were estimated through procedure given by Singleton and Rossi (1965) [20] and total soluble solids was determined using hand refractometer (Make: Erma Optical Works Ltd., Tokyo, Japan, 0-32°B range), and expressed as °Brix (Anon., 1984) [3], while the total antioxidants was estimated using FRAP method (Benzie and Strain 1996) [6].

Cost economics: Benefit-cost ratio of the extracted anthocyanin pigment was worked out for the best treatment, as the benefit cost ratio is an effective indicator of the commercial feasibility of the extraction of pigment,

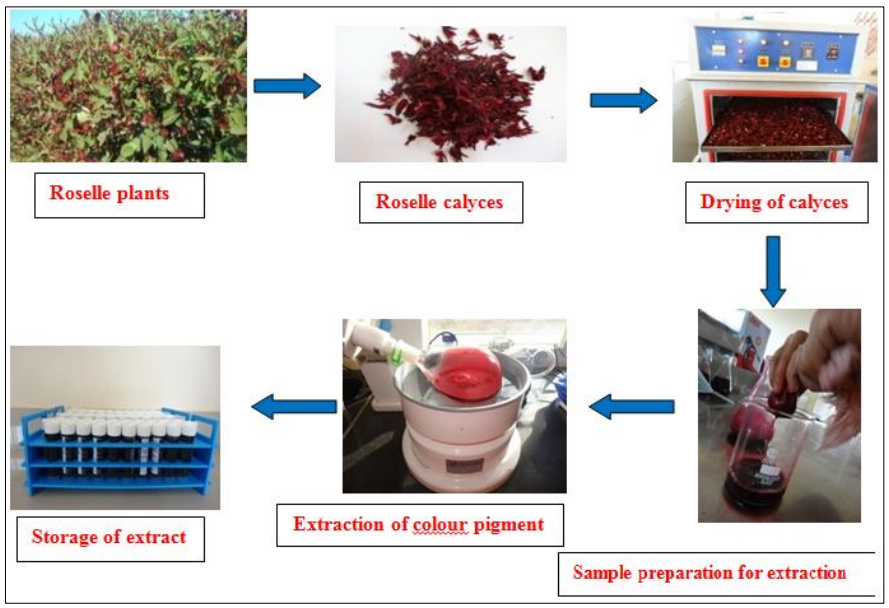


Fig 1: Flow chart of edible colour extraction from the Roselle calyces

Results and Discussion

Anthocyanin content

The extraction of anthocyanin from the roselle dried calyces by using different methods showed significant difference among the treatments. The highest anthocyanin content (1638.17 mg 100 ml^{-1}) was extracted from the treatment T_1(Ethanol with 1.5N HCl)as against 372.21 mg 100 ml^{-1} of anthocyanin from the treatment T_6 (hot water extraction) and

it was significantly superior over other treatments followed by treatments T_3(Ethanol with 2 per cent acetic acid) and T_2(Ethanol with 2 per cent citric acid) yielding 1311.24 mg 100 ml^{-1} 1241.27mg 100 $ml^{-1}$of anthocyanin respectively (Table 1 and Fig. 2). This may be because ethanol is a polar solvent which is effective in extraction of other polar compounds (Rivas-Gonzalo, 2003) [17].Addition of acids to water or ethanol increased the efficiency of anthocyanins extraction compared to distilled water alone. In general, HCl was more effective than citric acid and acetic acid because HCl serves to maintain a low pH, thereby providing a favourable medium for the formation of flavylium chloride salts from simple anthocyanins and improving the efficiency of anthocyanin extractions (Mattuk, 1998) [11]. Similar results were obtained by Abou-arab *et al.* (2011) [1] in Roselle and also by Oancea *et al.*(2012) [13] while working on extraction of anthocyanin from *Vaccinium corymbosum*. Extraction of pigments through fermentation proved to be much better than hot water extraction. The maintenance of low pH and addition of *Saccharomyces cervisiae* yeast during fermentation process facilitate higher extraction (Alobo and Offonry, 2009) [2].

pH and Titratable acidity

It is known science that, acidity and pH are inseparable. The pH is used as a scale to measure the acidity in the roselle extracts. An acid with pH value 1 is said to be very strong and as the pH value increases, acidity decreases. Ssignificant differences were observed among the treatments for the pH and titratable acidity. The pH of the extracted pigment was highest (3.62) in the treatment T_5 (fermentation of calyces) which was significantly different from other treatments (Table 1 and Fig. 2). It was followed by treatment T_4(Distilled water with 0.2 per cent pectinase)with pH of 2.34. The lowest (1.26) pH was found in the treatment T_1.The highest titratable acidity (10.83%) was observed in the treatment T_1which was statistically superior over all other treatments while the least titratable acidity (2.85%) was noticed in the treatment T_6. This may be due to use of 15 per cent 1.5 N HCl in the treatment leading to more acidic conditions that has facilitated better pigments extraction from Roselle calyces. The results are in comparison with findings of Abou-arab *et al.* (2011) [1] during analysis of Physico-chemical properties of natural pigments (anthocyanin) extracted from Roselle by using different solvents.

Water activity (a_w)

Water activity is the measure of effective concentration of water in a substance. It is the ratio of vapour pressure of water in the extract to the vapour pressure of pure water at same temperature. Water activity is based on a scale of 0 to 1.0 with pure water having a water activity of 1.00. Usually products that contain low per cent of moisture and high extractants will have less water activities. The water activity differed significantly among the treatments. The least water activity (0.80) was observed in the treatment T_1(Ethanol with 1.5N HCl).However, the calyces treated with ethanol with 2 per cent citric acid had water activity of 0.81$a_w$which was on par with the treatment T_1. The highest water activity of 0.94a_w was recorded in treatment T_6) followed by 0.92$a_w$in the treatment T_5which was statistically on par with treatment T_4 (Distilled water with 0.2% pectinase) with water activity of 0.92a_w. This is due to use of water for extraction led to more moisture in extractants compared to ethanol solvents.

Total phenols

The total phenolic content significantly differed among the treatments. It was highest (12.84 mg GAE ml^{-1}) in the treatment T_1(Ethanol with 1.5N HCl), which was significantly superior over all other treatments while the treatment T_5(Fermentation of calyces) yielded 9.88mg GAE $ml^{-1}$of total phenolic content (Table 1 and Fig.2). The lowest total phenols (2.29 mg GAE ml^{-1}) were in the treatment T_6 (Hot water extraction).The difference is probably due to the characteristics of the solvent used in the extraction. As the polarity of the solvent increases, higher extraction yields of total extractable phenolic compounds were obtained (Esa *et al.*2010) [9]. The results are in agreement with those reported (Shil *et al.* 2005) [19] that the ethanol is good solvent for polyphenol extraction and is safe for human consumption and by Abou-arab*et al.*(2011) [2] in roselle by using different solvents.

Total soluble solids

The data revealed that the TSS varied significantly among the treatments (Table 1 and Fig.2). The highest TSS (28.4 °B) was observed in the treatment T_1 (Ethanol with 1.5N HCl) which was statistically superior over all other treatments, followed by treatments T_2(Ethanol with 2 per cent citric acid) and T_3 (Ethanol with 2 per cent acetic acid) with 26.75 °B and 25.83 °B respectively. The lowest (5.65 °B) TSS was observed in the treatment T_6 (Hot water extraction). These findings were in accordance with Abou-arab *et al.*(2011) [1] who works on analysis of physico-chemical properties of natural pigments (anthocyanin) extracted from roselle by using different solvents. High solids is due to acidified ethanol not only extracts the anthocyanin pigments, along with it also helps in extraction of higher total sugars, organic acids, proteins, phenols which give stability to extractants.

Total antioxidants

The total antioxidantactivaty was highest(101.05 mg GAE 100 ml^{-1}) in the treatment T_1(Ethanol with 1.5 N HCl) and least(23.72 mg GAE 100 ml^{-1})was observed in the treatment T_6- hot water extraction (Table 1 and Fig.2). This vast difference is probably due to the characteristics of the solvent used. This phenomenon can be explained by a change in polarity of the antioxidant compounds due to particular solvent used for extraction. As the polarity of the solvent increased, higher extraction of total soluble solids was noticed (Table 1) which comprising of ascorbic acid, flavonoids, α-tocopherol, and other phenolic compounds, carotenoids, amino acids, peptides, proteins, might also play a significant role in antioxidants activities (Shahidi, 2000; Esa *et al.* 2010) [18, 9]. Christian and Jackson (2009) [7] also reported that the high antioxidant activity observed in the roselle could be due to the high ascorbic acid content of roselle. Similar results were obtained by Abou-arab *et al.* (2011) [1].

Cost economics

Effect of different extraction methods on cost benefit ratio of extracted anthocyanin pigment were analyzed (Table 2). The net returns of Rs.23,641 was obtained from the treatment fermentation of calyces which recorded highest benefit cost ratio (B:C) of 24.88:. However, the lowest B:C ratio of 3.94:1 was obtained from the distilled water with 0.2 per cent pectinase treatment with net returns of Rs.17,571. This variation in the benefit cost ratio is mainly because of the low

capital requirement during extraction of anthocyanin in the fermentation of calyces compare to all other treatments and higher pigments recovery among the treatments. However, calyces treated with ethanol acidified with 1.5 N HCl (T_1) recorded the maximum total returns (Rs.36,855) compared to all other treatments which is due to the solvent used in the treatment facilitates in maximum recovery of pigments from roselle calyces. These results are in accordance with the results of Rajeswari *et al.*(2014) [15],wherein they obtained *Monascus* pigment from fermentation.

Correlation studies

Correlation studies on effect of extraction method on different parameter in relation to anthocyanin in roselle extract are presented in the Table 3. Correlation is a measure of association between more than one character and it operates the relationship between dependent and independent characters.

In the present study,the dependent variable was anthocyanin and it was related to many different independent parameters.It was more of positive relation than negative correlation,. Anthocyanin exhibited positive and significant association with total antioxidants (0.81), total phenols (0.77), titratable acidity (0.73), total soluble solids (0.66), while, anthocyanin showed negative and significant correlation with water activity (-0.51) and pH (-0.50). The results are in confirmation with the findings of Olaya *et al.* (2009) [14].

Table 1: Effect of extraction methods on different parameters of extractant at T_0

Treatment	Anthocyanin(mg 100 ml^{-1})	pH	Titratable Acidity(%)	TotalPhenols (mg GAEml^{-1})	TSS (°B)	Total Antioxidants (mg GAE 100 ml^{-1})	Water activity (a_w)
Ethanol acidified with 1.5N HCl (85:15)	1638.17^{a}	1.26^{d}	10.83^{a}	12.84^{a}	28.40^{a}	101.05^{a}	0.80^{d}
Ethanol with 2% citric acid	1241.27^{c}	2.31^{bc}	8.33^{c}	6.99^{c}	26.75^{ab}	84.71^{c}	0.81^{c}
Ethanol with 2% acetic acid	1311.24^{b}	2.22^{c}	9.73^{b}	8.11b^{c}	25.83^{b}	89.41^{b}	0.82^{c}
Distilled water with 0.2% pectinase	956.09^{e}	2.34^{bc}	6.53^{d}	6.04^{c}	18.80^{c}	82.55^{d}	0.92^{b}
Fermentation of calyces	1093.68^{d}	3.62^{a}	3.52^{e}	9.88^{b}	24.98^{b}	57.11^{e}	0.92^{b}
Hot water extraction	372.21^{f}	2.39^{b}	2.85^{f}	2.29^{d}	5.65^{d}	23.72^{f}	0.94^{a}
S.Em±	2.15	0.03	0.15	0.34	0.71	0.50	0.00
CD at 5%	6.40	0.12	0.49	1.05	2.14	1.51	0.01

T_0 = Time zero

Table 2: Effect of different extraction methods on benefit cost ratio of anthocyanin extract

Treatments	Dried Roselle (kg)	Roselle cost (Rs)	Chemicals cost (Rs)	Miscellaneous cost (Rs)	Total cost (Rs)	Anthocyanin content (mg 100 ml^{-1})	Total returns l^{-1}(Rs)	Total returns	Net returns (Rs)	B:C ratio
T_1	1	350	3306	100	3756	1638.17	20475	36855	33099	8.81:1
T_2	1	350	3290	100	3740	1241.27	15512	27921	24181	6.46:1
T_3	1	350	3270	100	3720	1311.24	16387	29496	25776	6.92:1
T_4	1	350	4005	100	4455	979.60	12237	22026	17571	3.94:1
T_5	1	350	500	100	950	1093.68	13662	24591	23641	24.88:1
T_6	1	350	100	100	550	372.21	4650	8370	7820	14.21:1

Note: Based on the concentration of anthocyanin in the extract, the cost ranges from Rs. 5000-25000 l^{-1}. The cost of extract fixed at Rs.5000 having anthocyanin concentration of 400 mg 100 ml^{-1}.

Yield: 1800 ml of roselle extract kg^{-1} of dried calyces

T_1: Ethanol acidified with 1.5N HCl (85:15), T_2: Ethanol with 2% citric acid, T_3: Ethanol with 2% acetic acid, T_4: Distilled water with 0.2% pectinase, T_5: Fermentation of calyces and T_6: Hot water extraction

Table 3: Correlation studies on effect of extraction methods on different parameters in relation to anthocyanin content in Roselle extract

Parameters		1	2	3	4	5	6	7
1	Water activity	1						
2	Total soluble solids	-0.74*	1					
3	Acidity	-0.90*	0.70*	1				
4	pH	0.61*	-0.13	-0.74*	1			
5	Total antioxidants	-0.79*	0.83*	0.92*	-0.58*	1		
6	Total phenols	-0.58*	0.64*	0.62*	-0.49*	0.71*	1	
7	Anthocyanin	-0.82*	0.94*	0.85*	-0.41*	0.93*	0.81*	1

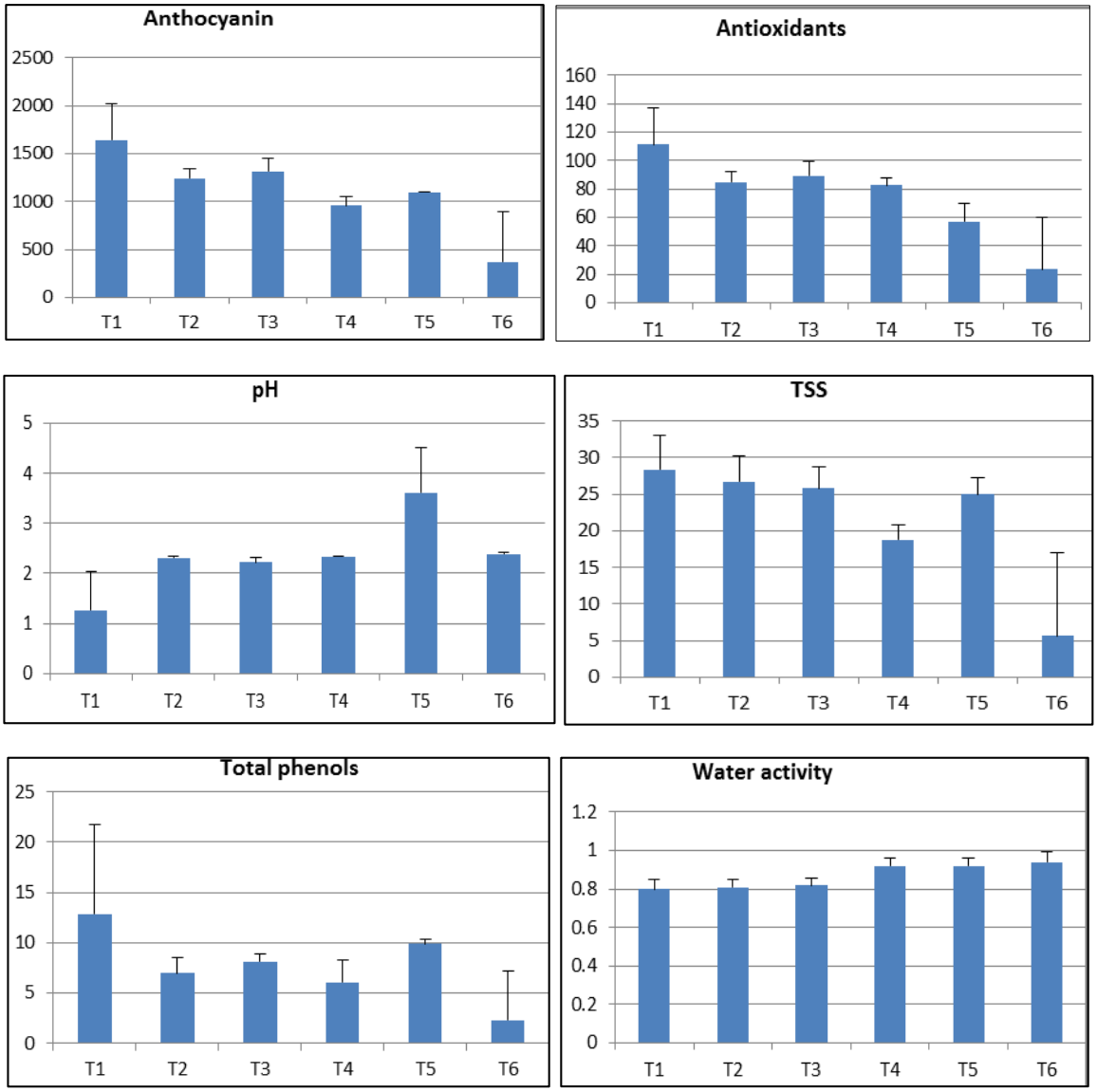
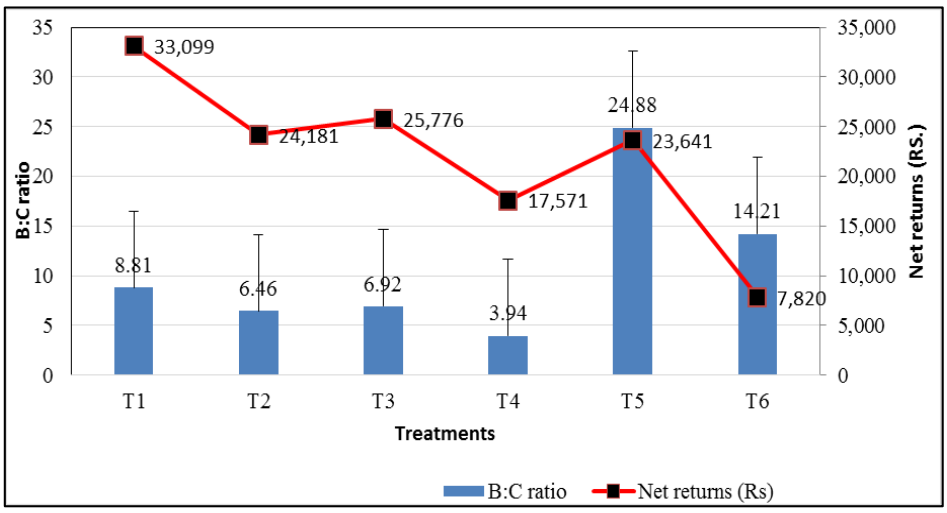


Fig 2: Effect of extraction methods on anthocyanin, total antioxidants, pH, TSS, total phenols and water activity of extractant at T_0



Note: Figures in the parenthesis indicate net returns in rupees

T_1: Ethanol acidified with 1.5N HCl (85:15), T_2: Ethanol with 2% citric acid, T_3: Ethanol with 2% acetic acid, T_4: Distilled water with 0.2% pectinase, T_5: Fermentation of calyces and T_6: Hot water extraction

Fig 3: Effect of different extraction methods on benefit cost ratio of anthocyanin extract

Conclusion

From the present investigations, the treatment of ethanol acidified with 1.5 N HCl was found to be the best as it yielded highest anthocyanin and total antioxidants compare to all other treatments. The procedure with slight modification over the earlier method described by Due and Francis (1973) [8] can be used for large scale extraction of biocolour from roselle calyces. Though the cost economics was found to be highest in treatment involving fermentation of calyces, the retention was poor after storage. Thus the ethanol acidified with 1.5 N HCl found to be best with respect to extraction and cost benefit ratio.

References

1. Abou-arab AA, Abu-salem FM, Abou-arab EA, *et al*. Physico-chemical properties of natural pigments (anthocyanin) extracted from roselle calyces (*Hibiscus subdariffa*). J Am. Sci. 2011;7:445-456.
2. Alobo AP, Offonry SU. Characteristics of coloured wine produced from roselle (*Hibiscus sabdariffa*) calyx extract. J Inst. Brew. 2009;115:91-94.
3. Anonymous. Official Method of Analysis. Ed. Sioney Williams Association official Analytic Inc. Virginia 14th Ed, 1984, 423-462.
4. Anonymous. The trusted herbal anti-aging. Herbal remedies using roselle (*Hibiscus sabdariffa*). 1999. http://www.ageless.co.za/rosella.htm.
5. AOAC. Official methods of analysis of the association of official analytical chemists 17th Ed. Association of Official Analytical Chemists, USA. 2000.
6. Benzie IFF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay. Anal. Chem. 1996;239:70-76.
7. Christian KR, Jackson JC. Changes in total phenolic and monomeric anthocyanin composition and antioxidant activity of three varieties of sorrel (*Hibiscus sabdariffa*) during maturity. J. Food Comp. Anal. 2009;22:663-667.
8. Due CT, Francis FJ. Anthocyanins of roselle (*Hibiscus sabdariffa* L.). J Food Sci.1973;38:810-812.
9. Esa NM, Fong SH, Amin I, Chew LY *et al*. Antioxidant activity in different parts of Roselle (*Hibiscus sabdariffa* L.) extracts and potential exploitation of the seeds. Food Chem. 2010;122:1055-1060.
10. Goli AH, Barzegar MS, Mohammad A, *et al*. Antioxidant activity and total phenolic compounds of pistachio (*Pistachio vera*) hull extracts. Food Chem. 2005;92:521-525.
11. Mattuk HI. Studies on the utilization of natural colorants extracted from some plant sources. Egypt J Appl. Sci. 1998;13:286-303.
12. Mohamed BB, Sulaiman AA, Dahab AA, *et al*. Roselle (*Hibiscus sabdariffa* L.) in Sudan, Cultivation and their uses. Bull. Environ. Pharmacol. Life Sci. 2012;1:48-54.
13. Oancea S, Stoia M, Coman D. Effects of extraction conditions on bioactive anthocyanin content of *Vaccinium corymbosum* in the perspective of food applications. Procedia Eng. 2012;42:489-495.
14. Olaya CM, Castano MP, Garzon GA, *et al*. Stability of anthocyanins from *Rubus glaucus Benth* and *Solanum betaceum* cv. Dark-red strain as affected by temperature, storage time and water activity. Acta Biol. Colomb. 2009;14:143-156.
15. Rajeswari TR, Ponnusami V, Sugumaran KR, *et al*. Production of *Monascus* pigment in low cost fermentation. Int. J Chem. Tech. Res. 2014;6:2929-2932.
16. Ranganna S. Handbook of analysis and quality control for fruit and vegetable products. Second edn. Tata McGraw-Hill Pub Co New Delhi, India,1986, 1138.
17. Rivas-Gonzalo J. Analysis of polyphenols. In Methods in Polyphenols Analysis. Royal Society of Chemistry, Athenaeum Press, Ltd., Cambridge, U.K. 2003, 95-98.
18. Shahidi F. Antioxidants in food and food antioxidants. Nahrung. 2000;44:158-163.
19. Shil J, Nawaz H, Pohorly J, Mittal G, Kakuda Y, Jiang Y, *et al*. Extraction of polyphenolics from plant material for functional foods-engineering and technology. Food Rev. Int. 2005;21:139-166.
20. Singleton VL, Rossi JA. Acolorimetry of total phenolics with Phosphomolybdic-phosphotungstic acid reagents. American J Enol. Viticult. 1965;16:144-158.
21. Tsai J, Ou M. Colour degradation of dried roselle during storage. Food Sci. 1996;23:629-640.