e - ISSN - 2393 - 9672

European Journal of Environmental Ecology

Journal homepage: www.mcmed.us/journal/ejee

ESTIMATION OF PHYTOCHEMICALS AND DETERMINATION OF BETA CAROTENE IN *HAEMATOCARPUS VALIDUS* AN UNDERUTILIZED FRUIT OF ANDAMAN AND NICOBAR ISLANDS

D.R. Singh, Shrawan Singh and V. Shajeeda Banu

Division of Horticulture and Forestry, Central Agricultural Research InstitutePost Box No. 181, Garacharma (Post), Port Blair – 744 101, Andaman and Nicobar Islands.

Corresponding Author

D.R. Singh
Email :-drsinghhort66@gmail.com

Article Info

Received 24/05/2014; Revised 18/06/2014
Accepted 28/06/2014

ABSTRACT

Haematocarpus validus (Menispermaceae) has been reported herein for the first time from Andaman and Nicobar Islands. This iron-rich fruit is one among the lesser known and underutilized plant. Phytochemical profiling and estimation of anti-nutritional factors of three different fruit fractions *viz.*, pulp, pericarp and seed have been carried out and it was found that pulp invariably had the highest amounts of all the estimated phytochemicals; total polyphenol (400 GAE mg/100g), flavonoid (542 RE mg/100g), Tannin (275.56 TAE mg/100g) and anthocyanin (203.77 C_3GE mg/100g). Among the anti-nutritional factors, pulp contained lower amounts of nitrate, phytate, saponin and oxalate. The RP- HPLC chromatogram revealed its richness in beta carotenoid.

Keywords: *Haematocarpus validus*, Phytochemical profiling.

INTRODUCTION

Some of the bioactive compounds neutralize free radical species generated as a part of biochemical reactions in our body system [1]. Accessibility and availability of these bioactive compounds from dietary sources contribute significantly in traditional health system of tribal and rural population of developing world. Underutilized fruits and vegetables are commercially neglected but recognized as rich sources of nutrients and non-nutrient bioactive compounds [2,3]. These crops are very popular among local communities in many parts of the world and well accepted in their traditional diets and medicines. Nowadays, these are gaining significance in research and development programmes of many organizations. Andaman and Nicobar Islands are endowed with rich and unique diversity of indigenous fruit species [4] of which most of them are less known and under-utilized. Iron is one of the much talked dietary micronutrient for women and children health. It is being supplied to body through tablets, vegetables, fruits and other food items. In islands, one of the native and lesser-known fruit *Haematocarpus validus* which is commonly known as Khoon Phal/ Rakta Phal (Andaman grapes) by Bengali community of Andaman and Nicobar islands is very rich source of Iron. It was first time reported in Diglipur area of North Andaman. Menispermaceae which is known to contain plants rich in different alkaloids and are famous for their traditional medicinal usages. For many communities in developing countries, the major source of vitamin A in the diet is carotenoids especially beta carotene. Major advances have occurred in understanding the role and mechanisms of action of carotenoids. Carotenoids, with their highly reactive conjugated double bonds, act as free radical traps or antioxidants and may play an important role in quenching of toxic radicals. In view of the vital physiological functions of carotenoids, much attention has been focus on the determination of these pigments.

However, no scientific information is available for nutritional and phytochemical profiles of Raktaphal of Andaman Islands. Therefore, this study was conducted

with an objective to investigate the phytochemical profiles of Raktaphal. The present analysis highlights thedietary significance of Raktaphal and its commercial potential. The high anthocyanin content of the fruit gives the true dark red colour which can be used as coloring agent and as a natural dye for the food products. Typical applications include soft drinks, jellies and yoghurts. Since most synthetic coloured additives are carcinogenic, teratogenic and can cause allergic reactions, use of natural dye from khoonphal will be very much helpful in avoiding health risks in human. Several natural biocolorants, pigmentation including anthocyanin, additionally show antagonistic activity to certain bacteria, viruses and fungi thus protect food from microbial spoilage.

MATERIALS AND METHODS

Sample preparation

Analytical grade chemical reagents, 1,1-diphenyl 2 picrylhydrazyl (DPPH), Gallic acid, Anthrone reagent, Aluminum chloride and Formic acid were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Tannic acid, Ascorbic acid, conc. HCl, Sodium acetate buffer (pH= 1:4.5 ratio) and Sulphuric acid were purchased from Himedia (Himedia Lab. Pvt. Ltd., Mumbai, India). Methanol, Rutin, Folin-Ciocaulteu reagent, Potassium Chloride, Copper sulphate, Sodium hydroxide were purchased from Merck (Darmstadt, Germany) and Sodium acetate, Orthophosphoric acid, Chloroform, Petroleum ether, Acetone and Sodium carbonate solution were obtained from Rankem (RFCL Ltd., New Delhi). Methanol and Acetonitrile of HPLC grade purchased from Merck, β-carotene standard HPLC grade were purchased from Sigma-Aldrich.

Sample collection and preparation

Fresh and healthy fruits of Raktaphal were collected from Diglipur island of Andaman. The collected fruits washed with millipore water and small pieces were made on ice plate. For phytochemicals, the samples were prepared by repeated grinding of 2 g of respective samples with 10ml methanol till devoid of colour. Sample mixture was centrifuged at 5000 rpm for 10 min and filtered through Whatman No. 1 filter paper. The filtrate was concentrated by rotary evaporator and kept at -20^{o}C for further analysis.

Phytochemicals

The phytochemicals were estimated in fresh samples in three fruit fractions i.e, pulp, pericarp and seeds. Polyphenol was analysed by spectrophotometer at 700nm using Folin–Ciocalteau reagent (10%, v/v) with some modifications and expressed as gallic acid equivalent. Tannin content in samples was estimated by AOAC [5] and results were expressed as tannic acid equivalent. Flavonoids, were determined by standard process described by Sadasivam and Manikam [6]. Anthocyanin content was analysed using the pH differential method Flueki [7]. Results were expressed as mg of cynidine-3- glucoside equivalent. Anti-nutritional compounds are major concern for consumers and it was estimated in Raktaphal using standard titration methods described by Singh et al [8]andSaponin content was determined by AOAC [5].

HPLC analysis of beta carotenoid

The beta carotenoid was extracted from fruit pulp samples using the method of Olives Barba *et al* [9] with minor modifications. In brief, 2g sample was ground in 10ml of methanol and centrifuged at 5000 rpm for 20 min to separate the supernatant. The process was repeated till the sample was completely colourless. The extract was filtered through Whatman No. 1 filter paper and than through 0.45 µm membrane filter. 20µl sample was injected in RP-HPLC for analysis. The RP-HPLC (DIONEX, Ultimate 3000 series) conditions were C18 column and diode array detector (variable wavelength detectors VWD-3100 and VWD-3400). Mobile phase had methanol (solvent A) and acetonitrile (solvent B) in 90:10 ratio at a flow rate of 1.0 ml/min. The column temperature was 22°C and the absorbance was read at 450 nm.

RESULTS AND DISCUSSION

Phytochemicals

Phyto-chemical profiling in three different fruit fractions *viz.*, pulp, pericarp and seed revealed that pulp invariably had the highest amounts of all the estimated phytochemicals **(Fig.1).** Highest amount of total polyphenol (400 GAE mg/100g) was reported from pulp, followed by pericarp (341.04) and seed (249.55). The polyphenol content observed in investigated underutilized fruit species is in agreement with the findings of Ikram et al [10]. Total Flavonoids were found to be 542 RE mg/100g in pulp, whereas comparatively lower amounts found from pericarp and seed (290.45 & 285.66 respectively). Tannins are reported as strong free radical scavengers [11] and Raktaphal identified as potential source of tannins. Which was observed 275.56 TAE mg/100g in pulp followed by pericarp (239.51) and seed (181.85). Anthocyanins are water-soluble natural phenolics and possess colouring and therapeutic properties. In present study, the total concentration of anthocyanin in the investigated fruits found to be 203.77 C_3GE mg/100g in pulp followed by pericarp (164.41) and seed (102.17). The findings are similar to the observations made by Veberic et al [12]. Pulp is also found to be rich in iron contents (0.59 mg/100 g) and seeds contain 0.11 mg/100g which is comparatively higher than the fruit which are used in daily life *viz.*, mango (0.2mg/100g), apple (0.1mg/100g), guvava and cherries (0.3mg/100g) reported by Vinson [13].

Anti-Nutritional factors

Anti-nutrients are major limiting factors for utilization of underutilized fruits and vegetables. The *Raktaphal* was also found to contain nitrate, phytate, oxalate and saponin as shown in Table 1. The analysis of three different parts of *Raktaphal* showed the pulp which is edible potion had low nitrate and oxalate content. However, their quantities are in safe health limit for consumption. The seeds were comparatively had high amount of saponin which indicates its potential in industrial purpose.

HPLC analysis of beta carotenoid

Fruits were analyzed for their beta carotenoid content because it is the precursor of vitamin A and eaten in both raw and cooked form by humans in daily life [14]. Raktaphal found to be very rich in beta carotene. The standard chromatogram of beta carotene is presented in fig 2 (a) and RP-HPLC chromatogram for beta carotene content in Raktaphal is presented in fig 2 (b).

Table 1. Anti-Nutritional factors (mg/100g) in *Haematocarpus validus*

Fruit Fraction	Anti-Nutritional Factors (mg/100g)			
	Nitrate	Phytate	Oxalate	Saponin
Pulp	16.25	422.68	34.95	85.56
Pericarp	25.00	506.83	39.82	85.28
Seed	19.58	415.83	33.82	100.06

Fig.1. Phytochemicals (mg/100g) in *Haematocarpus validus*

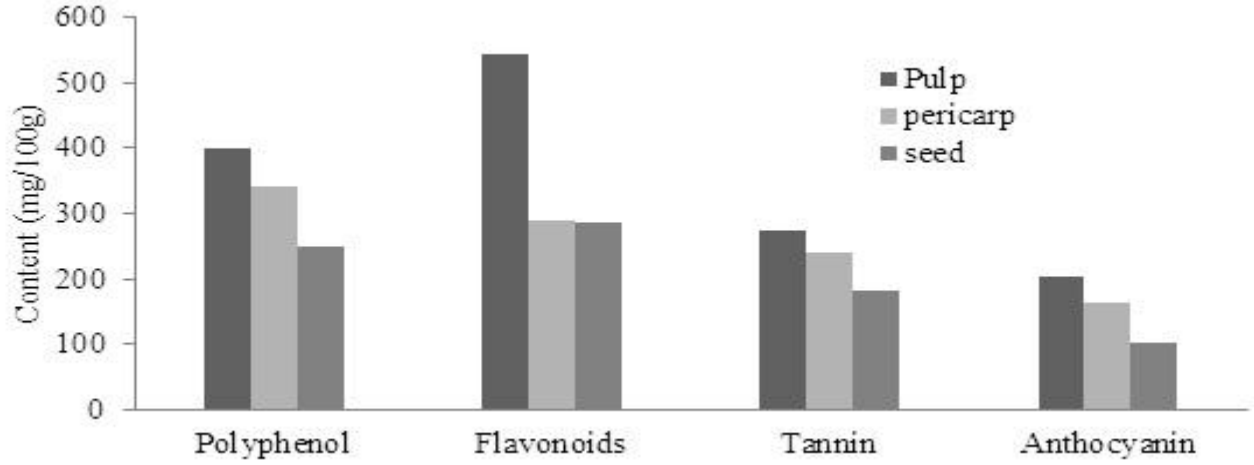


Fig. 2. HPLC chromatogram of beta carotene and content in *Haematocarpus validus*

Fig 2(a)- standard beta carotenoid

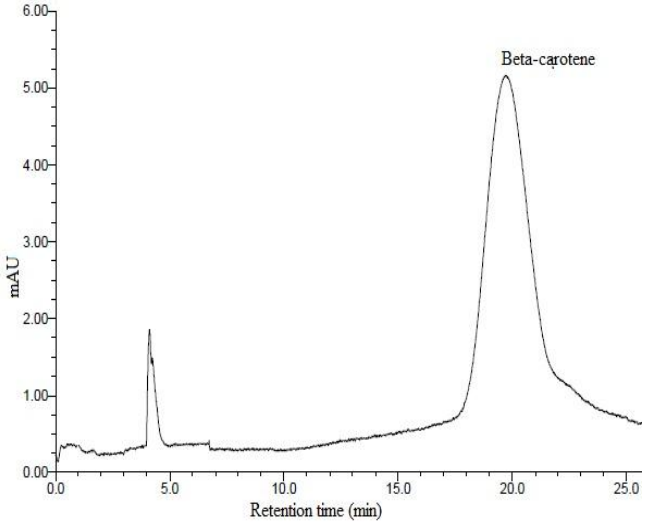
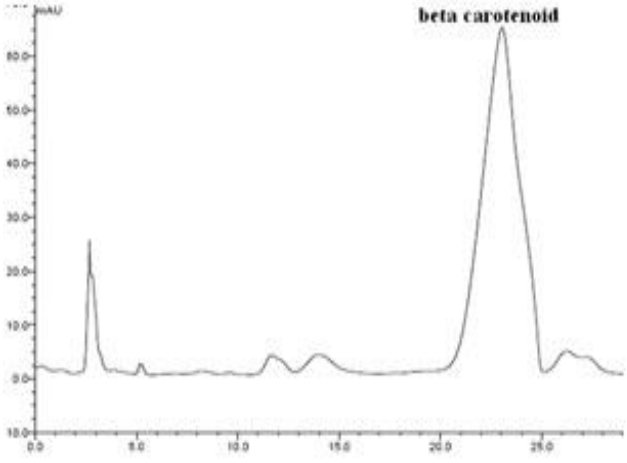


Fig 2(b)- beta carotenoid content in *Haematocarpus validus*



CONCLUSION

Consumption of this iron-rich fruit will help to overcome diseases which are caused due to low hemoglobin such as anemia and also will be effective in enhancing the immune system of the body. Structuredinterviews with coastal and tribal populace are imperative for understanding the traditional medicinal uses of underutilized fruits would serve in formulation of nutrition-rich feed additives. The study highlighted the potential of Raktaphal as natural antioxidants like polyphenols, tannin, flavonoids and anthocyanins. The RP- HPLC identification of beta carotene in Raktaphal supports to be a source of iron. Further, fatty acid profiling of the seeds and photochemical analysis in different parts and stages shall increase its economic value which would be beneficial for the island's community.

DECLARATION

The authors declare that they have no conflict of interest.

ACKNOWLEDGEMENT

The authors Thank Shri Manindramistry, custodian farmer, Diglipur, North Andaman for sparing the fruits for analysis. The authors also acknowledge the Director, Central Island Agricultural Research Institute, Port Blair, for his kind support for financial and laboratory facilities.

REFERENCES

1. Halliwell B. (1994). Free radicals, antioxidants, and human disease, curiosity, cause or consequence? *Lancet*, 344, 721–724.
2. Larson RA. (1988). The antioxidants of higher plants. *Phytochemistry*, 27, 969–978.
3. Singh S, Singh DR, Salim KM, Srivastava A, Singh LB, Srivastava RC. (2011). Estimation of proximate composition, micronutrients and phytochemical compounds in traditional vegetables from Andaman & Nicobar Islands. *Int J Food SciNutr*, 62(7), 765–773.
4. Abraham Z, Senthilkumar R, Joseph John K, Sharma TVRS, Nair NV, (2008). Unnikrishnan M. Collection of plant genetic resources from Andaman and Nicobar Islands. *Genet Resour Crop Evol*, 55, 1279–1289.
5. AOAC. (1995). Official methods of analysis. Association of official analytical chemists, 16th ed. American Public Health Association, Washington.
6. Sadasivam, S Manikam A. (1996). Biochemical methods. Wiley Eastern Ltd, Madras, India.
7. Fuleki T, Francis FJ. (1968). Quantitative methods for anthocyanins, Extraction and determination of total anthocyanin in cranberries. *J Food Sci*, 33, 72–77.
8. Singh S, Singh DR, Shajeeda-Banu V, Salim KM. (2012). Determination of Bioactives and Antioxidant Activity in *Eryngiumfoetidum*L, A Traditional Culinary and Medicinal Herb. ProcNatlAcadSci India, *Sect B BiolSci*, 83(3), 453–460.
9. Olives-Barba AL, Huratado CM, Mata SMC, Ruiz FV, Lopez STM. (2006). Application of a UV Vis detection-HPLC method for a rapid determination of lycopene and beta carotene in vegetables. *Food Chem*, 95, 328–336.
10. Ikram EHK, Eng KH, Jalil AMM, Ismail A, Idris S, Azlan A, et al. (2009). Antioxidant capacity and total phenolic content of Malaysian underutilized fruits. *J Food Comp Anal*, 22, 388–393.
11. Mishra J, Singh RD, Jadon VK, Gausain MP, Aradhana. (2010). Assessment of phenolic components and antioxidative activities of Phaseolus vulgaris L. *Int J Integ Biol*, 9, 26–30.
12. Veberic R, Colaric M, Stampar F. (2008). Phenolic acids and flavonoids of fig fruit (*Ficus carica* L.) in the northern Mediterranean region. *Food Chem*, 106, 153–157.
13. Vinson JA, Xuehui S, Zubik L & Bose P. (2001). Phenols antioxidant quantity and quality in food, fruits, *Journal of Agricultural Food Chemistry*, 49(11), 5315-5321.
14. Benedich A and GS Higdon. (2004). Biological actions of Beta carotene. *Am J Nat*, 32(2), 225-230.