Journal of Applied Horticulture, 24(1): 68-72, 2022

DOI: https://doi.org/10.37855/jah.2022.v24i01.13

Journal of Applied Horticulture
ISSN: 0972-1045

Physico-chemical variability in the fruits of Theichhungsen (*Haematocarpus validus* (Miers.) Bakh. F. ex. Forman)- A potential source of natural food colour

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Abstract

Haematocarpus validus is a promising fruit plant with an intense red fruit pulp that can be used as a natural food color source. The species grow in the wild in its natural state, and it has not yet been successfully domesticated on a large scale. Fruit samples of *H. validus* were collected from various locations for this study, and the morphology and biochemical properties of the fruit were investigated. Observations revealed that the morphological characteristics and biochemical content of fruits collected from different geographical locations differ substantially. Furthermore, the findings of this study will serve as a starting point for future scientific research into the fruit's potential, which includes serving as a natural source of food colour.

Key words: *Haematocarpus validus*, fruit size, red pulp, natural food colour

Introduction

Theichhungsen (*Haematocarpus validus* (Miers.) Bakh. F. ex. Forman) is an evergreen perennial woody climber, belonging to the family Menispermaceae. The plant grows wild in nature, climbing to other large trees or long support trees, and large-scale domestication and cultivation are not yet practiced. It is one of the promising and potential sources of nutrients, medicine, and also a potential source of natural colour owing to its vibrant blood red pulp.

H. validus, also known as blood fruit, is native to Southeast Asia and is mainly distributed in India, Bangladesh, Indonesia, Singapore, Thailand and Sri Lanka. The fruit grows wild in the Andaman and Nicobar Islands, Arunachal Pradesh, Mizoram, Tripura, Assam, and Meghalaya in India. The flowering time varies depending on the place, location, temperature and altitude. The species has been found to flower more than once a year under Andaman conditions, and the harvesting time is from April to August (Bohra *et al.*, 2016). In Bangladesh, vines produce flowers in mid- November to January, and the fruiting season is from May to August (Khatun *et al.*, 2014). The vines bloom in Meghalaya's hilly terrain from October to December, and the fruits are available in local markets from the last week of March to June (Sangma, 2016). The fruit is also known by different names to different people, such as Blood fruit (English), Khoonphal (Hindi), Roktogula/Lal-gula (Bengali), Thoyphal (Tripura), Tepattang (Garo), Sohsnam (Khasi) *etc.* In Mizoram, *H. validus* is commonly known as Theichhungsen, and also Theikhuangthlup, and is mainly distributed in Aizawl district, Lunglei district, Mamit district, Kolasib district and Champhai district, where they are found naturally occurring in the wild.

Even though *H. validus* is a less known fruit, it has lots of potential uses. Fruits are slightly acidic, sweet in taste when fully ripe and are eaten raw. Fruits and seeds are used for anemia and root mash is used to get relief from itching (Rahim *et al.*, 2015). Fruits are also used for preparing wines (Sangma, 2016). The fruits have a high concentration of anthocyanin, imparting a true blood red colour, which can be used as a natural colouring agent and a natural additive dye for food products (Singh *et al.*, 2014). The fruits are used as a dye in handicrafts and in the preparation of squash. Processed products like pickles and chutneys are also prepared from green fruits. Ripe fruits can also be dried and stored for future consumption (Bohra *et al.*, 2016).

Analysis and identification of phyto-chemicals is necessary for recognising the potential and chemical nature of indigenous wild edible fruits. Fruits of *H. validus* are found to be rich in iron, and seeds contain 0.11 mg 100 g^{-1} which is comparatively higher than most commercial fruits (Singh, 2013). Blood fruit was also found to contain nitrate, phytate, oxalate and saponin (Singh *et al.*, 2014). Khatun *et al.* (2014) reported total protein content as 0.6 gm 100 g^{-1}, carbohydrate 6.99 gm 100 g^{-1} and vitamin C 13.15 mg 100 g^{-1}. According to Bohra *et al.* (2019), the pulp of fully ripe fruits contained 8.76 mg/g of total anthocyanins, of which pelargonidin was the dominant anthocyanin, followed by cyanidin, peonidin and petunidin and hence could be a potential candidate for colouring the food and dye industries. The leaves also have a high content of choline that substantiates the ethnomedicinal use of its leaf and shoot extract as a hepatoprotective agent (Alex *et al.*, 2018a,b).

In Mizoram, *H. validus* has been found to grow in wild and semi-wild stages in forests, abandoned lands and home gardens.

But due to lack of awareness about the potential and knowledge of the health beneficial minerals and vitamins in this fruit, it is on the verge of extinction. Until now, proper exploration of the genetic variability and documentation of this fruit and its physico-chemical properties have not been made available.

Therefore, the present investigation has been carried out to analyse the physico chemical contents of the fruits collected from different parts of Mizoram, and to know the health compounds of *H. validus* and phytochemicals present in them.

Materials and methods

Collection of sample: The study was carried out during March to June, 2019. Fruits were collected from the wild in locations from different villages throughout Mizoram, *i.e.*, Buarpui, Tachhip, Seling, Rawpuichhip, Darlawn, Sesawng, Tlangnuam and Khuangleng (Table 1). The collected fruits were wrapped in paper in order to avoid loss of moisture during transportation to the laboratory. The freshly collected samples were then quickly blotted with blotting paper after washing with water. Fruit samples from each location were collected for further analysis and measurement of various parameters. The research was carried out at Mizoram University's Department of Horticulture, Aromatic and Medicinal Plants (HAMP) and Department of Biotechnology in Aizawl, Mizoram.

Morphology of fruit: Of the fruits collected from each location, 15 fruits were selected and the mean parameter value was used for the morphological analysis of the samples. The fruit and seed were measured for length and breadth, as well as the thickness of the peel. The weight of the fruit's skin, pulp, and seeds were measured using digital electronic balance.

Biochemical estimation: The total soluble solids (TSS) of the fruit was determined using hand refractometer calibrated in °Brix at room temperature. Total titratable acidity was determined by titrating the extracted juice against 0.1 NaOH (sodium hydroxide) solution using phenolphthalein as an indicator (A.O.A.C., 1995).

Reducing sugar and total sugar were determined by standard procedure of A.O.A.C (1995). Ascorbic acid content of fruit was estimated by 2,6 dichlorophenol indophenol dye titration methods (A.O.A.C., 1995; Ranganna, 1977) and expressed as ascorbic acid mg/100 g of fruit. The soluble protein was determined by the method of Lowry *et al.* (1951) using Bovine Serum Albumin (BSA) as standard protein. The total carbohydrate content was estimated by Anthrone reagent method (Yemm and Willis, 1954). Starch was estimated after perchloric acid digestion according to Hassid and Neufeld (1964). Anthocyanin content were determined by methanolic-HCI extraction and estimated as per Fuleki and Francis (1968). Total phenolic content was determined by Folin-Ciocalteu method (Singleton and Orthofer, 1999).

The data obtained were analysed following completely randomized design. The critical difference (CD) value was calculated at 5 % probability level for comparing different treatments (Panse and Sukhatme, 1985).

Results and discussion

The morphological data analysis (Table 2) and their variability (Table 4) shows that there were significant ($P<0.001$) differences in weight among the fruits collected from different locations in all fruit traits. The results showed that the mean fruit weight was 21.21 g ranging from 13.28 to 30.16 g with 24.09 % coefficient of variation; mean fruit length was 41.76 mm and fruit breadth was 31.55 mm; mean peel thickness is 3.71 mm and peel weight was 11.04 g, and mean weight of fruit pulp was 4.18 g (1.08 to 7.60 g). The fruits are single seeded, and average seed weight is 6.02 g (3.05 to 10.85 g); average seed length and diameter is 28.76 mm and 16.79 mm respectively.

Fruit samples collected from various villages also had significant difference morphologically. Fruits collected from Darlawn had the heaviest fruit weight (30.16 g) as well as pulp and peel weight, and also highest fruit and seed length and seed breadth. On the other hand, samples collected from Tachhip had the lightest pulp and seed weight, as well as the lowest fruit weight of 1.28 g. Buarpui fruits had the heaviest seed weight, but with lowest measure of fruit length and seed breadth, while the lowest measure of fruit breadth and seed length was found in Tachhip fruits.

Biochemical contents of the fruit as indicated in Table 3 and 4, showed that edible pulp of the fruit samples collected from different locations of Mizoram have an average TSS content of

Table 1. Sources of *H. validus* fruits collected

Village	Latitude	Longitude	Rainfall (mm) monthly average (2019)	Temperature (°C) Mean annual maximum	Temperature (°C) Mean annual minimum	Soil Type
Buarpui	23°16'38.2"N	92°39'34.1"E	280.16	33.92	18.50	L.S. Humic Hapludults, F.L. Humic Hapludults, LS.Umbric Dystrochrepts,
Tachhip	23°34'22.1"N	92°42'35.4"E	445.52	29.65	14.67	F.L. Umbric Dystrochrepts, F.L. Humic Hapludults, L.S. Umbric Dystrochrepts
Seling	23°43'53.0"N	92°51'06.9"E	118.36	29.65	14.67	LS. Umbric Dystrochrepts, F.L. Humic Hapludults, L.S. Typic Hapludults
Rawpuichhip	23°47'19.7"N	92°33'55.7"E	258.32	29.34	15.66	LS.Typic Haplohumults, L.S. Typic Hapludults, F.L.Typic Dystrochrepts
Darlawn	24°01'14.2"N	92°55'22.6"E	158.75	29.65	14.67	F.L.Umbric Dystrochrepts, F.L. Humic Hapludults, L.S. Typic Hapludults
Sesawng	23°45'50.0"N	92°51'14.1"E	118.62	29.65	14.67	LS. Umbric Dystrochrepts, F.L. Humic Hapludults, L.S. Typic Hapludults
Tlangnuam	23°42'09.5"N	92°42'53.8"E	105.66	29.65	14.67	F.L. Umbric Dystrochrepts, F.L. Humic Hapludults, L.S. Typic Hapludults
Khuangleng	23°18'05.6"N	93°18'40.1"E	222.25	30.11	11.55	F.L. Typic Hapludults, F.L.Umbric Dystrochrepts, F.L. Humic Hapludults,

*Data collected from Department of Science & Technology, Govt. of Mizoram. L.S. = Loamy Skeletal, F.L.= Fine loamy

Table 2. Morphological parameters of *H. validus* fruits collected from different locations in Mizoram

Location	Peel weight (g)	Pulp weight (g)	Seed weight (g)	Fruit weight (g)	Fruit length (mm)	Fruit breadth (mm)	Seed length (mm)	Seed breadth (mm)	Peel thickness (mm)
Buarpui	10.85	2.63	10.85	23.64	32.46	31.58	26.7	15.11	4.38
Tachhip	9.15	1.08	3.05	13.28	46.30	30.79	22.51	15.84	4.56
Seling	13.79	4.83	4.56	23.46	41.08	31.59	26.66	17.22	4.82
Rawpuichhip	7.10	5.43	5.39	18.09	41.93	32.41	30.61	17.39	2.31
Darlawn	14.86	7.60	7.70	30.16	48.87	31.17	33.96	18.72	3.72
Sesawng	10.02	3.83	5.33	19.19	42.35	32.18	31.13	16.49	3.04
Tlangnuam	8.93	3.64	5.63	18.20	39.53	30.96	28.41	17.31	3.00
Khuangleng	13.65	4.37	5.62	23.64	41.56	31.68	30.10	16.22	3.82
Mean	11.04	4.18	6.02	21.21	41.76	31.55	28.76	16.79	3.71
$C.D_{0.05}$	1.11	0.64	0.61	1.74	1.82	1.56	1.02	0.44	0.37

Table 3. Biochemical constituents of *H. validus* fruits collected from different locations in Mizoram

Location	TSS (°brix)	Titratable Acidity (%)	TSS: Acid	Reducing sugar (%)	Total sugar (%)	Vitamin C (mg 100 g^{-1})	Protein (mg 100 g^{-1})	Carbohydrates (mg 100 g^{-1})	Starch (mg 100 g^{-1})	Anthocyanin (mg 100 g^{-1})	Phenols (%)
Buarpui	15.00	2.22	6.75	5.69	19.78	12.88	0.49	4.01	0.36	109.29	0.66
Tachhip	10.00	6.49	1.54	5.41	18.82	17.94	1.60	4.13	0.36	139.83	0.52
Seling	15.33	5.98	2.56	5.91	17.20	15.64	0.64	9.66	0.36	273.24	0.63
Rawpuichhip	14.33	5.29	2.71	8.34	21.29	12.88	1.24	8.74	0.36	177.52	0.65
Darlawn	15.66	2.22	7.05	9.29	20.56	9.66	0.84	6.29	0.36	198.57	0.69
Sesawng	15.66	6.15	2.55	6.69	21.77	7.82	0.64	4.90	0.35	250.50	0.64
Tlangnuam	13.00	2.90	4.48	8.22	22.59	10.58	0.63	4.01	0.35	167.81	0.65
Khuangleng	12.66	4.10	3.08	8.85	18.37	11.50	0.69	4.00	0.47	176.20	0.71
Mean	13.96	4.42	-	7.30	20.05	12.36	0.85	5.72	0.37	186.62	0.64
$C.D_{0.05}$	1.59	1.58	-	1.35	N/A	5.52	0.37	2.77	0.36	19.70	N/A

13.96 °Brix; total titratable acidity of 4.42 %, reducing sugar content of 7.30 % and total sugar content of 20.05 %. The fruit samples also contain 12.36 mg 100 g^{-1} of vitamin C; average protein content is 0.85 mg 100 g^{-1}, carbohydrate content of 5.72 mg 100 g^{-1}; starch content of 0.37 mg 100 g^{-1} and phenol content is 0.64 %. The fruit pulp contains high anthocyanin content *i.e.*, 186.62 mg 100 g^{-1} (109.29 to 273.24 mg 100 g^{-1}) from the collected samples and the coefficient of variation in anthocyanin content is 28.96 %.

Fruits from different parts of Mizoram also showed significant difference in biochemical contents. Darlawn fruits had the highest TSS:acid ratio and also highest reducing sugar content. indicating that these fruits are sweet with low acidity. Fruits from Buarpui had the lowest protein and anthocyanin content, Sesawng fruits recorded the lowest vitamin C and starch concentrations. Tachhip fruit samples have the lowest TSS: acid ratio and also phenols, but contains highest protein and vitamin C (17.94 mg 100 g-1). Highest anthocyanin content (273.24 mg 100 g-1) is obtained from Seling fruits, that could make it the best lot for colour extraction.

With literature survey, we noted differences in fruit size when compared to previous findings. Although observations show that the fruits have an average

Table 4. Variability in physico-chemical parameters

Parameter	Mean	Minimum	Maximum	Range	Coef.Var.
Peel weight (g)	11.04	7.10	14.86	7.76	25.05
Pulp weight (g)	4.18	1.08	7.60	6.52	46.28
Seed weight (g)	6.02	3.05	10.85	7.80	38.87
Fruit weight (g)	21.21	13.28	30.16	16.88	24.09
Fruit length (mm)	41.76	32.46	48.87	16.41	11.57
Fruit breadth (mm)	31.55	30.79	32.41	1.62	1.79
Seed length (mm)	28.76	22.51	33.96	11.45	12.17
Seed breadth (mm)	16.79	15.11	18.72	3.61	6.63
Peel thickness (mm)	3.71	2.31	4.82	2.51	23.54
TSS (°Brix)	13.96	10.00	15.66	5.66	14.12
Titratable Acidity (%)	4.42	2.22	6.49	4.27	40.63
TSS:Acid	3.84	1.54	7.05	5.51	53.58
Reducing sugar (%)	7.30	5.41	9.29	3.88	21.20
Total sugar (%)	20.05	17.20	22.59	5.39	9.19
Vitamin C (mg 100 g^{-1})	12.36	7.82	17.94	10.12	26.34
Protein (mg 100 g^{-1})	0.85	0.49	1.60	1.11	44.81
Carbohydrates (mg 100 g^{-1})	5.72	4.00	9.66	5.66	40.20
Starch (mg 100 g^{-1})	0.37	0.35	0.47	0.12	10.82
Anthocyanin (mg 100 g^{-1})	186.62	109.29	273.24	163.95	28.96
Phenols (%)	0.64	0.52	0.71	0.19	8.78

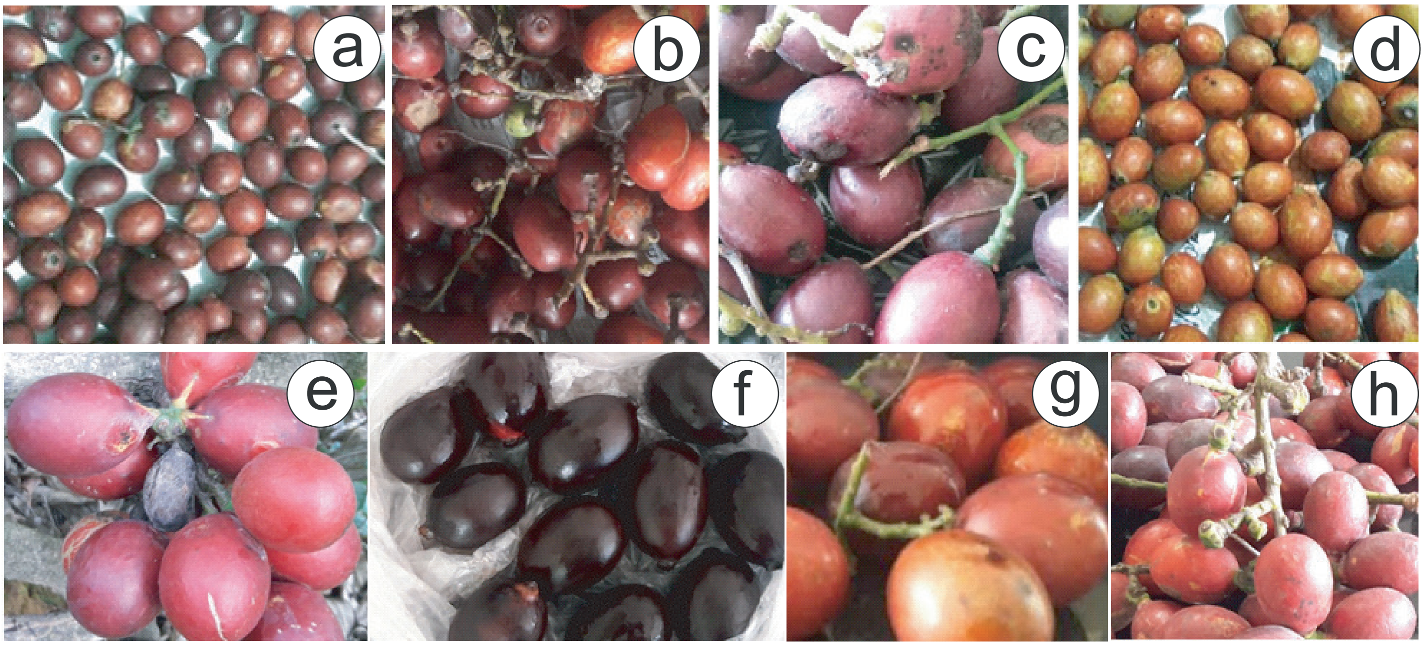

Fig. 1. Variability of *Haematocarpus validus* germplasms collected from different villages *i.e.*, (a) Rawpuichhip, (b) Tachhip, (c) Khuangleng, (d) Buarpui, (e) Sesawng, (f) Darlawn, (g) Tlangnuam and (h) Seling

individual fruit weight of 21.21 g (13.28 to 30.16 g), reports from Bangladesh, Andaman Islands, and Garo Hills have fruits with individual fruit weights ranging from 12.7 to 30.85 g. Also, the edible pulp weight differs, ranging from 1.08 to 7.60 g (Mizoram), 6 g (Bangladesh), 2.9 g ± 0.2 (Andaman Islands), and 3.21-8.79 g (Garo hills of Meghalaya) (Rahim *et al.*, 2015; Bohra *et al.*, 2019; Sangma, 2016). Differences in morphological characters are due to differing environmental conditions prevailing from where fruits are gathered. Physical characteristics were found to vary widely in the jamun, hatkora and mango (Hazarika *et al.*, 2013; Prakash *et al.*, 2010; Singh *et al.*, 2010).

Previously reported biochemical constituents of the fruit showed slight variations. To our current findings, our research shows that edible pulp has total soluble solids (TSS) of around 13.96 ^{o}Brix (10.00 to 15.66 ^{o}Brix) and a titratable acidity of 4.42 %, reports from Andaman Islands have TSS of 17 °Brix and acidic pH of 3.01 (Bohra *et al.*, 2019). Such variations have been observed in "hatkora" (Hazarika *et al.*, 2017), citrus accessions (Singh and Singh, 2003) and in sweet cherry (Shrivastav *et al*., 2014). Individual genotypes' differing genetic makeup could cause fruit TSS content to vary (Hazarika *et al.*, 2017). In this study, the total phenolic content of *H. validus* fruits was 0.64 %. The phenolic compounds are known to have antioxidant properties, and so blood fruit may have use in the food processing industries (Bohra *et al.*, 2019). In this study, the anthocyanin content was found to be 186.62 mg 100 g-1 (109.29 to 273.24 mg 100 g^{-1}). Earlier reports also showed total anthocyanin content of 8.76 mg/g and 203.77 C3GE mg/ 100 g in the pulp (Bohra *et al.*, 2019; Singh *et al.*, 2014). Anthocyanin provides the true blood-red colour, making it an excellent natural colouring agent and additive dye for food products. The dominant anthocyanin in *H. validus* fruit pulp, Pelargonidin, has medicinal potential for further exploration (Bohra *et al.*, 2019).

We can thus conclude that there is significant difference in morphological characteristics and biochemical contents of *H. validus* fruits collected from different locations, and there are variations in the physico chemical parameters of *H. validus* fruits reported from Mizoram with that of other places. However, regardless of locations, the fruit is found to be rich in anthocyanin content which makes it a potential source of natural colour for use in the food industries.

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Received: August, 2021; Revised: October, 2021; Accepted: October, 2021