REVIEW ARTICLE

Review Update on *Haematocarpus validus*

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Abstract

Haematocarpus validus, known as blood fruit due to its fruit's color, is a member of the Menispermaceae family and a critically endangered fruit crop that contains numerous nutraceuticals and traditional medicinal properties and is primarily found in South Asian countries. Various plant parts have been traditionally used to treat jaundice, anemia, itching, blood disorders, and body aches. Despite its many valuable properties, *H. validus* remains underutilized because its economic potential is still untapped. The goal of this review is to provide an overview of the plant's morphological characteristics, traditional usage, phytochemical profiles, and biological properties based on research data and analysis of the research findings. The morphological characteristics of the plant are thoroughly studied by individual observation by the authors, and they are validated by the Botanical Survey of India, Shillong. The current review provides exhaustive information on this wonderful plant so that the research breach can be taken for further rigorous investigation on its biological potential, genetic identity, sustainable propagation, and so on. Although the review provides comprehensive data on *H. validus* based on limited research findings, the lack of research on this plant is surprising and strongly recommends the need for extensive research on *H. validus* as a miraculous blood fruit woody climber.

Key words: Biological potentials, blood fruit, *Haematocarpus validus*, morphology, phenology, phytochemical compounds, woody climber

INTRODUCTION

Meghalaya is enriched with a unique diversity of indigenous fruit species of which most of them are less known and underutilized. *Haematocarpus validus* (Miers.) Bakh. f. ex Forman is one of them. *H. validus* belongs to the family Menispermaceae and is an endangered wild fruit crop with many nutraceuticals and traditional medicinal values. It is a perennial woody climber (liana) and usually grows in conditions such as dry environments and acidic soils.[1] At present, there are only two species in the genus: *Haematocarpus subpeltatus* Merr. and *H. validus* (Miers) Bakh. f. ex Forman.[2] *H. validus* is commonly called blood fruit, and locally, it is known by different names such as khoon phal, rakta phal, te'pattang, and roktogula.[3,4] After 100 years, *H. validus,* a highly endangered species, was rediscovered in India, adding a new place to the Indo-Myanmar biodiversity hotspot. *H. validus* is specifically found only in certain places in India (Meghalaya, Assam, Manipur, Tripura, Andaman, and Nicobar Island), Bangladesh, Sri Lanka, Indonesia, Thailand, Singapore, and Pakistan.[1,2,5,6] The fruit of *H. validus* is a good source of iron, and the indigenous people of the Garo Hills have been using it for treating anemic conditions and also for blood purification.[1,3,4] The fruit is also rich in antioxidants such as polyphenols, flavonoids, ascorbic acid, β-carotene, tannin, and anthocyanin. Due to its high anthocyanin content, the fruit is widely used as a natural colorant for food and beverages.[7] Other parts of this plant, such as the leaf, root, and shoots, are also used by the ethnic group of people for curing hepatic disorders and to relieve itching and pain.[8]

Despite the fact that the plant has promising therapeutic properties, little research has been conducted on it. This present review compiles all the fragmented data published on this plant in various journals since 2014. The current review describes morphological characteristics, geographical distribution, traditional uses, propagation, phytochemical profile, and pharmacological activities which will enlighten the significance of *H. validus* (Miers.) and will provide a new insight for researchers in the future.

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MORPHOLOGICAL CHARACTERISTICS

The flowers and leaves of *H. validus* were collected from Tura, West Garo Hills, district Meghalaya, on April 14, 2021, and authenticated by the Botanical Survey of India in Shillong, Meghalaya (BSI/ERC/Tech/2020-21/30). The voucher specimen of *H. validus* was deposited in the herbarium. *H. validus* is a perennial woody climber (liana) with a dark green glabrous branch. It spreads and grows up to 1000 m in height with the support of large trees like banyan or jackfruit trees in very dry and acidic soils. The morphological characteristics of the plant are given below:

Habit

Woody climber (liana) [Figure 1].

Bark

Rough, light grayish brown color, branches stout, wood consisting of consecutive layers of thin radiating plates.

Leaf

The leaves are 10–15 cm long, petiolate, light green (young leaves), dark green (mature leaves), elliptic, smooth and glossy, 3 veined, rigidly coriaceous, and pale glabrous beneath [Figure 2].

Stem

Glabrous, 3–6.6 cm in diameter, woody.

Inflorescence

Cauliflorous, axillary or supra-axillary, terminal panicle or raceme, staminate inflorescence, pistillate inflorescence not seen.

Flower

Tiny, odorous, greenish-white, unisex (dioecious), hanging [Figure 3].

Fruit

Fruits are drupes ovoid's ellipsoids, narrow and style scar near the base, grow in bunches, are stalked, and have smooth endocarps. The fruits are green before ripening and when it is fully ripened it appears dark red in color and is full of blood-red juice which are acidic and slightly sweet in taste. The average fruit is about 4–5 cm long and has about 18 g of peel weight, 6 g of flesh weight, and 2.01 mm of peel thickness [Figure 4].

Figure 1: Morphology (Habit) of *Haematocarpus validus*(Miers.) Bakh. f. ex Forman

Figure 2: Leaf characteristics of *Haematocarpus validus*(Miers.) Bakh. f. ex Forman

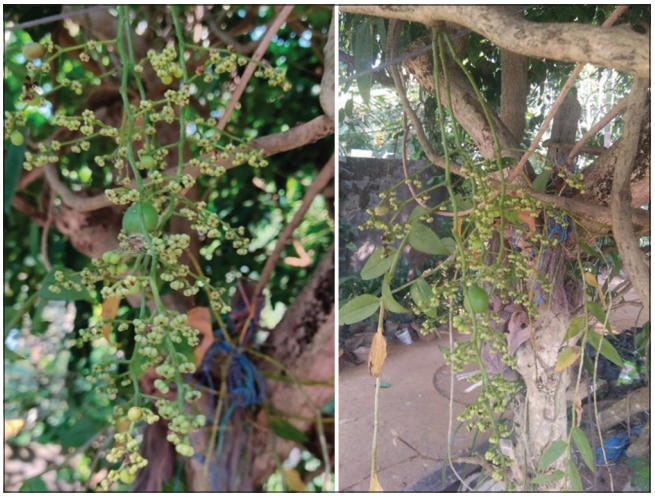

Figure 3: Flower characteristics of *Haematocarpus validus*(Miers.) Bakh. f. ex Forman

Seed

2–3 cm long, curved, non-endospermic, oblong, albumen absent, cotyledons fleshy, and thick. The seeds may be dispersed either by barochory (gravitational dispersal),

Figure 4: Fruit characteristics of *Haematocarpus validus* (Miers.) Bakh. f. ex Forman

zoochory (dispersal by birds or animals), or anthropochory (dispersal by humans).

PHENOLOGY PERIOD

The flowering time of *H. validus* varies in different places. The flowering period in Meghalaya (Garo Hills) and Bangladesh is from October to January, whereas in Andaman and Nicobar Island, the flowering occurs more than once a year. The fruits are available from March-June in Garo Hills and May-July in Bangladesh.[1,2,6,7]

VERNACULAR NAME

H. validus is known by various vernacular names in different geographical regions [Table 1].[6]

Table 1: Vernacular names of *Haematocarpus validus* (Miers.) Bakh. f. ex Forman

Vernacular name	Regions/language
Blood fruit	English
Te.pattang	Garo
Khoon phal	Hindi
Roktogula/Lalgula	Bengali
Thoyphal	Tirpura
Theichhung-sen	Mizoram
Ranguichi	Marma
Raktaphal	Tamil/Telugu/Malayalam
Sohsnam	Khasi & Pnar

GEOGRAPHICAL DISTRIBUTION

H. validus is usually found in Southeast Asian countries such as India, Bangladesh, Pakistan, Singapore, Indonesia, Laos, Thailand, and Sri Lanka. In India, it is mainly distributed in the states of Meghalaya, Assam, Andaman and Nicobar Islands, Mizoram, and Tripura.[1,2,5,6]

TRADITIONAL USES

H. validus has been used widely in traditional systems of medicine in various parts of India and in some other countries as well. The different parts of this plant have been used traditionally to cure ailments and diseases. The Chakma and Marma tribes of Chittagong Hill Tracts use the extracts of tender shoots and leaves as a curative measure of jaundice and root mesh for itching. They also use the leaf as an organic fertilizer.[1] *H. validus* fruits have a mildly acidic, sweet flavor and are eaten fresh. The fruit's high anthocyanin content gives it a true dark red color that can be used as a coloring agent and natural dye for foods such as jellies, yoghurts, and soft drinks. Since most synthetic-colored additives are carcinogenic, teratogenic, and may cause allergic reactions, using blood fruit's natural dye would be extremely beneficial in preventing health problems.[3] The residents of Mawlaiteng village (Meghalaya) believe that the blood fruit juice is their primary source of vitamin C during the winter season.[2] The Garo tribe of Meghalaya uses the iron-rich blood fruit for treating anemia and blood disorders. Ripe fruits are cut, soaked

overnight in a glass of water, and taken the next morning as medicine. The fruits are also used in the production of wine. In Tripura, it is used to make processed products like squash and also to dye local handicrafts.[6] Nicobarese tribes use a decoction of the leaves to relieve body aches, and the green fruits are used to make processed foods such as pickles and chutneys.[8,7]

PROPAGATION OF *H. VALIDUS*

Blood fruit grows well in dry environments and highly acidic soils, and its regeneration is mainly through seed germination. Fruits are harvested from the wild, but there is a lot of potential for encouraging their production in homestead gardens. A seed germination study was conducted as a first step toward small-scale domestication of this species.[7] The technique involves the extraction of seeds from fully ripened fruit and soaking the seeds in 0.1% thiourea or in 1000 mg/l of GA_3 solutions for 24 h which are then sown in coir pith as a substrate. The germinating seedlings are then raised in a polyhouse to protect them from the sun and rain. After 50 days of sowing, germination begins, and after 115 days, about 85% of the seeds germinate usually. Seedlings are then transferred to the polybags containing soil: FYM (1:1, v/v). Soaking seeds in thiourea (0.1%) or GA_3 (1000 mg/l) for germination and overall output may be recommended for obtaining healthy seedlings in large numbers.

Later, the effects of three different substrates (vermicompost, sand, and soil + vermicompost) on seed germination characteristics were studied.[9] According to the results, there were major variations between the treatments. Seed germination was substantially found to increase by 69% when vermicompost was used instead of the other substrates.

PHYSICAL CHARACTERISTICS

The physical parameters of the blood fruit have been investigated to find the most accurate models for predicting the mass of the blood fruit based on its physical characteristics.[10] According to their study, the various physical parameters of *H. validus* are mass (21.76 g), surface area (10.5 cm^2), firmness (14.77N), true density (1.010 g/cm^3), bulk density (0.980 g/cm^3), porosity (32.97%), length (3.563 cm), breadth (2.489 cm), thickness (2.51 cm), measured volume (9.324 cm^3), oblate spheroid volume (11.458 cm^3), ellipsoidal volume (11.597 cm^3), arithmetic mean diameter (2.854 cm), geometric mean diameter (2.813 cm), projected area perpendicular to length (6.930 cm^2), projected area perpendicular to width (4.828 cm^2), and projected area perpendicular to thickness (4.886 cm^2). Another study[11] investigated the effects of various treatment processes such as ultrasound, microwave, conventional thermal, and ultrasound-assisted microwave treatment on the sensory attributes (color, taste, flavor, and mouthfeel) of blood fruit juice samples using the fuzzy logic approach. The result obtained showed that the ultrasound-assisted microwave-treated juice was the most acceptable when compared to other treatments.

The qualitative properties of spray-dried blood fruit powder (BFP) and the determination of the sorption isotherm through model fitting were investigated.[12] Maximum yield was obtained with 30% maltodextrin (MD) at 150°C, with a 1.57% moisture content and water activity (*A*w) value of 0.3, respectively. Other physical properties of powder (BFP), such as hygroscopicity, bulk density, and solubility, were also determined in the same study. Morphological and mineral compositional studies were conducted using a field-emission scanning electron microscope. They also evaluated the differential scanning calorimetry, X-ray diffraction, and particle size distribution of BFP. It was observed that the moisture sorption isotherm study of BFP resulted in a good fit with the Peleg and GAB models.

PHYTOCHEMICAL PROFILE

There is very limited phytochemical information on *H. validus* available so far. *H. validus* contains a significant quantity of alkaloids, flavonoids, tannins, and anthocyanins that are essential for a variety of medicinal uses. The various secondary metabolites found in various parts of *H. validus* are described below:

Phytochemical profiling revealed that the three different parts of *H. validus* fruit, namely pulp, pericarp, and seed, are rich in antioxidants such as polyphenol, flavonoid, tannin, and anthocyanin.[3] The result showed that total polyphenols (Gallic Acid Equivalent, or GAE, 400 mg/100 g), flavonoids (Rutin Equivalent, or RE, 542 mg/100 g), tannins (Tannic Acid Equivalent, or TAE, 275.56 mg/100 g), and anthocyanins (Cynidine-3-Glucoside Equivalent, or C_3GE, 203.77 mg/100 g) were present in higher concentrations in pulp than the pericarp and seed. Maximum iron content (0.59 mg/100 g) is also found in the pulp, and beta-carotene content in the pulp is analyzed by reversed-phase high-performance liquid chromatography.

In another study,[5] six alkaloids were isolated from the leaf and fruit extracts of *H. validus* using a high-resolution liquid chromatograph mass spectrometer. These compounds are sinomenine, (S)-reticuline, ambelline, metanephrine, ecgonine, and choline. The choline content of the leaf and fruit extracts of *H. validus* was estimated using high-performance thin-layer chromatography.[13] They have reported that the leaf extract had a very high choline content (>500 mg/100 g dry weight) than the fruit extract (197.81 mg/100 g dry weight). Following, a study was conducted to determine the functional group region and fingerprint region of *H. validus* leaf and fruit crude extracts using Fourier-transform infrared spectroscopy.[14] Based on their findings, it was deduced that the chemical structure of the compounds in leaf and

fruit crude extracts had functional group and fingerprint spectra that were more or less identical. The functional groups region of the spectra contained alcohols and phenols (OH-stretch), alkanes (aliphatic C-H stretching band), alkenes (-C=C- stretch), and 1^0 amines (N-H bend), while the fingerprint regions represented aromatics (C-C stretch-in ring, C-H loop), aromatic amines (C-N stretch), alcohols, carboxylic acids, esters, ethers (C-O stretch), and alkyl halides (C-Cl stretch, C-Br stretch). Again, the total phenol, flavonoid, and alkaloid content of the leaf and fruit extracts of *H. validus* was reported.[15] In their report, it was found that the leaf extract had a higher total phenol and total flavonoid content (113.33 4.41 mg GAE/g and 67.93 2.01 mg Quercetin Equivalent or QE/g) than the fruit extract (86.67 2.88 mg GAE/g and 59.63 1.49 mg QE/g), and the total alkaloid content of both leaf and fruit extracts was about 2.72 ± 0.08% and 1.93 ± 0.06%, respectively.

The photosynthetic pigments (chlorophylls and total carotenoids) were isolated from the leaf extracts of blood fruit using two different solvent systems (acetone and dimethylsulfoxide).[7] Their result showed that the dimethylsulfoxide solvents isolated higher contents of chlorophylla (0.405 mg/g), chlorophyllb (0.132 mg/g), total chlorophylls (0.537 mg/g), chlorophylla: chlorophyllb (3.059 mg/g), and total carotenoids (0.071 mg/g) than the isolation used by the acetone solvent. Subsequently, anthocyanins such as pelargonidin, cyanidin, peonidin, petunidin, malvidin, and delphinidin were isolated from the pulp of the blood fruit.[16] The most abundant anthocyanin was found to be pelargonidin, which was followed by cyanidin, peonidin, petunidin, malvidin, and delphinidin. They also studied the physiochemical parameters of the blood fruit pulp, which include the total soluble solid (17.0 B), moisture (90.12%), pH (3.01 acidic), and total phenolic content (13.44 mg GAE/100 g). The phenolic compounds from the blood fruit juice powder using high-performance liquid chromatography were studied[17], and the isolated compounds were resveratrol and a group of phenolic acids (gallic acid, protocatechuic acid, chlorogenic acid, ferulic acid, vanillic acid, o-coumaric acid, p-hydroxy benzoic acid, rutin, daidzein, epigallocatechin, catechin, gallocatechin gallate, apgenin 7-O neohesperidoside, and quercetin). Bioactive compounds such as 2-bromotetradecane (0.18%), tetracosane (1.64%), myristic acid (19.91%), palmitic acid (12.39%), eicosane (9.13%), hexadecanoic acid, 4-[(trimethylsilyl)oxy] butyl ester (7.45%), dodecane 4, 6-dimethyl (6.34%), decane 1, 9-bis[(trimethylsilyl)oxy] (5.17%), octadecane (4.38%), heptadecane (4.35%), trimethylsilyl ester (3.06%), stearic acid (2.47%), and bis (2-ethylhexyl) phthalate (1.72%) were isolated from the ethyl acetate extracts of *H. validus*.[18]

NUTRITIONAL COMPOSITION

Blood fruit is rich in micro- and macronutrients such as proteins, carbohydrates, fats, ash, crude fiber, vitamin C, carotenoids, β-carotene, iron, copper, zinc, manganese, calcium, magnesium, carbon, potassium, phosphorous, and sulfur. Blood fruit is well known for its richness in iron, as it consists of about 0.59 mg/100 g of iron, which is slightly higher than the iron content of commonly consumed fruits such as apples (0.1 mg/100 g), mangoes (0.2 mg/100 g), and cherries (0.3 mg/100 g). Therefore, consumption of blood fruit can aid in the treatment of diseases caused by iron deficiency.[1,6] The blood fruit also contains anti-nutritional factors such as nitrate, phytate, oxalate, and saponin.[3]

BIOLOGICAL POTENTIAL OF *H. VALIDUS*

Antimicrobial Activity

The thermosonicated extracts of blood fruit in water, methanol, ethyl acetate, and acetone demonstrated significant antimicrobial activity against four strains of food-poisoning bacteria, such as the Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus cereus*) and Gram-negative bacteria (*Escherichia coli* and *Salmonella typhi*).[18] They also performed the minimum bactericidal concentration (MBC) test to determine the antimicrobial activity against all four strains of bacteria (*S. aureus*, *B. cereus*, *E. coli,* and *S. typhi*). The result obtained showed that the thermosonicated ethyl acetate extract was very effective against the pathogenic microorganisms, with an MBC value of 1.5 mg/mL.

Antioxidant Activity

The scavenging activity of the leaf and fruit extracts of *H. validus* was studied using the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay.[15] The result obtained showed that the scavenging activity was highest in the leaf extract (77.88 ± 1.29%) than the fruit extract (72.21 ± 0.86%) at a concentration of 200 µg/mL, which was comparable with ascorbic acid (10 mg/mL methanol) as a standard. The antioxidant activity of various solvent-assisted blood fruit extracts was studied by DPPH, oxygen radical absorbance capacity, 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid), and ferric antioxidant power assay.[18] Blood fruit extracts were made using a variety of solvents, including water, ethyl acetate, methanol, and acetone. These extracts were divided into two groups: Those that were thermosonicated (treated) and those that were not (untreated). In their results, the treated ethyl acetate extract showed the strongest antioxidant activity in all four assays.

DNA Protection Ability

The ability of leaf and fruit extracts of *H. validus* to protect pUC18 plasmid DNA from the harmful effects of hydroxyl radicals produced by Fenton's reagent was tested by DNA nicking assay. The result obtained showed remarkable protection against DNA damage caused by hydroxyl radicals

produced during Fenton's reaction, as there was no nicking of the supercoiled form of the pUC18 plasmid.[15]

Hepatoprotective Activity

H. validus also possesses hepatoprotective activity due to the presence of a high content of choline in the leaf.[13] The methanolic extract of *H. validus* leaf and fruit is used for quantifying the choline content by using high-performance thin-layer chromatography spectro-densitometric analysis. The leaf extract contained a very high choline concentration, confirming the ethnomedicinal use of the blood fruit's leaves as a hepatoprotective agent. Further studies are required *in vivo* or *in vitro* to confirm this finding.

DISCUSSION AND CONCLUSION

The current review provides a brief overview of the morphology, traditional use, phytochemical profile, and chemical constituents of *H. validus*. According to the web-based data, research reports, and personal observation, it was discovered that the plant is regarded as a critically endangered fruit crop and has its origins in traditional medical systems. The fruits of *H. validus* are very nutritious and are consumed raw by the people of the Northeast, specifically the people of Garo Hills, Meghalaya. Phytoconstituents such as polyphenol, flavonoid, ascorbic acid, β-carotene, tannin, and anthocyanin have been reported by various researchers[3,15,17] in different extracts of *H. validus*. Polyphenols are rich in the leaves and pulp of the fruit, which might be responsible for their good pharmacological activity. Blood fruit (*H. validus*) has important bioactive compounds, antioxidant properties, and some essential minerals, which play a significant role in human nutrition and traditional medicine for treating arthritis, jaundice, hypertension, cancer, etc.[12]

H. validus is mostly found in forest areas and is rarely cultivated, but it has been found to have good therapeutic potential and is used as a remedy for various ailments. Hence, more research on this plant is the need of the hour to uncover the hidden areas and their practical pharmacological and clinical applications, which can be used for the benefit of mankind. Different parts of *H. validus* exhibit curative effects for jaundice, body aches, itching, anemia, blood disorders, and other ailments, although scientific support for these claims has yet to be generated. Information and studies on sustainable methods of propagation, agricultural techniques, crop improvement, utilization, conservation, and the nutritional and therapeutic potential of this particular fruit species are quite limited and need further investigation. It is also critical to increase the acceptance and marketability of this fruit by raising awareness of its nutritional benefits. The plant can also be subjected to bioprospecting, which might aid in the development of new therapeutics and the preservation of traditional medical systems, as well as the preservation of biological and cultural diversity by demonstrating their medical, economic, and social values.

The current review provides exhaustive information on this wonderful plant so that the research breach can be taken for further rigorous investigation on its biological potential, genetic identity, sustainable propagation, and so on. Although the review provides comprehensive data on *H. validus* based on limited research findings, the lack of research on this plant is shocking and strongly recommends the need for extensive research on *H. validus* as a miraculous blood fruit woody climber.

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