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Physio-chemical and Phytochemical Characteristics of a Lesser-known Wild Edible Fruit of Mizoram, *Elaeagnus pyriformis* Hook. F. (Family Elaeagnaceae): Need for its Proper Domestication

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The lesser-known yet incredibly nourishing wild edible fruit *Elaeagnus pyriformis* Hook.f. is found in the north-eastern mountainous states of India. *Elaeagnus pyriformis* fruits were collected from three villages in Champhai District, Mizoram, with the goal of analysing their physio-chemical and phytochemical qualities in order to determine its potential for domestication. Measurements were made of the fruit's physical characteristics, including its length, diameter, weight, and moisture content. Juice was extracted, and total soluble solids (TSS), titrable acidity, ascorbic acid, reducing sugar, total sugar, non-reducing sugar, and sugar:acid ratio were among the chemical parameters that were examined afterward. The existence of several beneficial substances was determined by phytochemical screening. Physical and chemical differences between the samples from various villages were found to be significantly varied, according to the results. Ruantlang produced the highest titrable acidity, whereas Zotlang had the maximum fruit weight, length, TSS, reducing sugar, total sugar, non-reducing sugar, and flavonoid content. Fruit diameter, the ratio of sugar to acid, and the total phenolic content were all highest in Hmunhmeltha. The fruit's phytochemical examination identified flavonoids, terpenoids, alkaloids, tannins, and cardiac glycosides.

Keywords: Physio-chemical; phytochemical characteristics; edible fruit, phytochemical screening; fruits phytochemical; flavonoid.

1. INTRODUCTION

India has a varied agroclimatic zone that stretches from east to west and from north to south. Various plant species may be found in Mizoram's forests, and their fruits, seeds, tubers, shoots, and other parts are used extensively in the native diet. Various type of local wild fruits having abundance of nutritional and medicinal values are yet unexplored. Silverberry (*Elaeagnus pyriformis*) is one of the highly nutritious berry fruits found in the north eastern hilly states such as Mizoram, Meghalaya, Manipur, Sikkim, Nagaland and some parts of Arunachal Pradesh. *Elaeagnus pyriformis,* is locally called as Sarzûktê, Ramsarzûk, Zûktê in Mizoram. This fruit is really famous among locals during peak harvesting season (March- April) as it also provides a source of income due to its sweet and sour texture [1]. This fruit is consumed in the form of fresh fruit, processed in pickle, jam, jelly as well as leather [2]. Silverberry is rich source of vitamins, minerals, flavanoids and a very good source of essential fatty acids [3]. *Elaeagnus pyriformis* is a deciduous shrub often spiny which leaves are petiolate, flowers are hermaphrodite and are pollinated by bees which flowers during September-December and fruits are matured in March –April [4]. The mature fruits are pyriform in shape tapering towards the ends and fruits are pink coloured after ripening [1]. The study of these wild unexplored fruits is not only significant for identification of potential food sources but also to select promising types for domestication [5].

The current research deals with the analysis of fruits of Elaeagnus pyriformis Hook, f. collected from three different villages of Champai district of Mizoram for their physio-chemical properties.

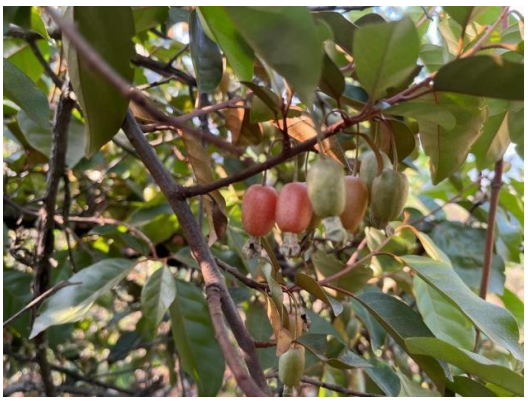

Fig. 1. Photo of the fruit, *Elaeagnus pyriformis*

2. MATERIALS AND METHODS

The sample of edible fruit, Sarzûk tê (Elaeagnus pyriformis) were collected in 2022-2023 from three villages namely, Zotlang, Ruantlang and Hmunhmeltha of Champhai District, Mizoram. The sample from Zotlang, Ruantlang and Hmunhmeltha. The specimens were bought to the department laboratory of the Horticultural College, Thenzawl, Mizoram for the analysis of Physiochemical properties.

2.1 Physical Parameters

The wild edible fruits were selected at random, and their average parameters were calculated in order to determine the physical characteristics of the fruits.

Fruit weight: Fruit weight was measured using an electronic weighing machine and expressed in gram (g)/milligram (mg).

Fruit length: Fruit length was measured by taking the longitudinal length between two poles of the fruits (30) with the help of Vernier Calipers and expressed in mm or cm.

Fruit diameter: The diameter of the fruit was taken at its broadest portion i.e., before cutting the fruits (30) horizontally and was measured with the help of Vernier Calipers and expressed in mm or cm.

Seed length: The length of the seed was taken at its broadest portion i.e., after cutting the fruit vertically and was measured with the help of Vernier calipers and expressed in mm or cm.

Seed diameter: The diameter of the seed was taken at its broadest portion i.e., after cutting the fruit horizontally and was measured with the help of Vernier Calipers and expressed in mm or cm.

Moisture Content: A microwave oven was used to measure the moisture content of the fruit. After the samples were weighed and heated to 35°C for a full day in a microwave oven, the dry mass was measured. The weighed samples can be dried until their ultimate mass, which is stated as a percentage (%), is consistent. The moisture content was calculated with the formula [6]:

$$\textbf{Moisture Content (\%)} = \frac{FW-ODW}{FW} x\ 100$$

Where, FW is Fresh Weight of Fruit

ODW is Oven Dried Weight of Fruit

2.2 Extraction of Juice

The selected fruit was crushed and grind with a grinding machine and soaked overnight in methanol for extraction of the fruit juice for further analysis. After soaking overnight, the juice was extracted by squeezing with muslin cloth and is filtered with filter paper as shown to clarify the juice and to remove the foreign materials. The filtered juices were subjected for rotary evaporator to remove the solvents. The dried extracts were then preserved in deep freezer at -20°C for further physio-chemical analysis. It was observed that 200g of the fruit extract gives 55ml of juice.

2.3 Chemical Parameters

Total Soluble Solids (TSS): Using pure fruit juice extract as the sample, the Zeiss Hand Brix refractometer was used to calculate the TSS of the fruit pulp. The results were given in percentage terms. Similar to this, the ASSOCIATION OF OFFICIAL ANALYTICAL COLLABORATION's standard procedures were used to estimate titrable acidity, reducing sugar, total sugar, and non-reducing sugar [6].

Titrable acidity: Ten grams of pulp were mashed with a pestle and mortar to estimate the titrable acidity. One hundred milliliters of distilled water was then added, and the mixture was filtered. Ten milliliters of the filtrate were titrated against 0.1% NaOH with an indicator of phenolphthalein. The following method was used to express titrable acidity as a percentage of anhydrous citric acid:

$$\text{Titrable acidity (\%)} = \frac{Titrate\ Value\ x\ Normality\ x\ Volume\ made\ up\ x\ Equ.wt\ of\ Citric\ acid\ x100}{Weight\ of\ sample\ x\ Aliquot\ \ x\ 100}$$

Ascorbic acid: The ascorbic acid concentration of the fruit pulp was estimated using the visual titration method [7], with the result represented in mg per 100 g. In order to attain this, 5 g of fruit pulp and 25 ml of 4% oxalic acid were mashed in a mortar. The resulting filtrate was then collected in a 50 ml volumetric flask and filtered through Whattman No. 1 filter paper. 4% oxalic acid was added to bring the volume up to 50 millilitres. Five millilitres of the extract were combined with five millilitres of oxalic acid, and the mixture was titrated against a standard 2, 6-dichlorophenol indophenol dye until the solution took on a pink hue that lasted for a minimum of fifteen seconds. The amount of ascorbic acid was calculated by using the dye factor as follows:

$$\text{Ascorbic acid (mg/100g)} = \frac{Titrate\ value\ x\ Dye\ factor\ x\ Volume\ made\ up}{Aliquot\ of\ extract\ taken\ x\ Volume\ of\ Sample\ taken}\ X\ 100$$

Reducing sugar

Fehling's copper reduction method was used to determine the reduction of sugar, and the results were expressed as a percentage. (Lane and Eynon, 1923) [8].

$$\text{Reducing Sugar (\%)} = \frac{mg\ of\ invert\ sugar\ (0.05)\ x\ Volume\ made\ up\ x\ 100}{Titre\ Value\ x\ Weight\ of\ sample}$$

Total sugar: A 25 ml portion of the sugar reduction solution was taken, and 2.5 ml of strong HCL was added. The mixture was then left overnight. Following 1% NaOH neutralization, the solution's volume was increased to 75 ml using distilled water, and it was then titrated against 10 ml of boiling Fehling's solution combination using methylene blue as an indicator.

From the value, percentage of total sugar was calculated as follows (Malakar et al., 2022):

Total Sugars = (Sucrose (%) + Reducing Sugar (%))

Sucrose (%) = (Total invert sugars (%) – Reducing sugar (%) X 0.95

Total Invert Sugar (%) =

$$\frac{mg\ of\ invert\ sugar\ x\ Volume\ made\ up\ x\ Volume\ of\ Stock}{Titre\ value\ x\ Weight\ of\ Sample\ x\ Aliquot\ taken}\ \text{x } 100$$

Non-reducing sugar: Non-reducing sugar was calculated out from the differences between total sugar and reducing sugar as (Malakar et al., 2022):

Non-reducing sugar = (Total sugar – reducing sugar) X 0.95

Sugar:Acid ratio: Sugar: acid ratio was calculated by dividing the total sugar with the titrable acidity in each treatment.

$$\text{Sugar: Acid ratio} = \frac{Total\ Sugar(\%)}{Titrable\ Acidity\ (\%)}$$

TSS: acid ratio: TSS: acid ratio was calculated by dividing the TSS with the titrable acidity in each treatment.

$$\text{TSS:Acid Ratio} = \frac{TSS}{Titrable\ Acidity\ (\%)}$$

All the above experiments were carried out 3 times and values are obtained by calculating the average of three experiments and data are presented as Mean±SEM.

2.4 Phytochemical Screening

The presence of various phytochemicals in E. pyriformis was detected using standard procedures described below:

Chemicals and reagents: Potassium iodide, bismuth nitrate, sulphuric acid, ferric chloride, hydrochloric acid, aluminium chloride, ammonium hydroxide, glacial acetic acid, chloroform, and olive oil.

Preparation of extracts: Five grams (5g) each of the plant powder was weighed and transferred into a beaker containing 200 ml of distilled. The mixture was heated on a hot plate with continuous stirring at 60°- 80°C for 30 minutes. The water extract was filtered through filter paper and the filtrate was used for the phytochemical analysis. The aqueous extracts were kept in refrigerator at 0°C until use.

Test for saponins: A vigorous shake was used to combine two milliliters of the extract with two milliliters of distilled water to determine whether saponins were present in the aqueous extract of various plants. The development of a steady, long-lasting foam suggested the presence of saponins. Three drops of olive oil were added to the foam, and it was violently shaken. The emulsion's creation verified that the extract contained saponins. (Banso and Adeyemo, 2006).

Test for tannins: Each aqueous extract (5 milliliters) was combined with a few drops of 0.1% ferric chloride to determine whether tannins were present in the various plant extracts. Tanning agents were detected by the development of a brownish green or blue-black tint. (Banso and Adeyemo, 2006).

Test for cardiac glycosides (Keller-Killani test): A solution of one drop of ferric chloride was added to two millilitres of glacial acetic acid, which was then applied to five millilitres of each extract. The mixture was then covered with one millilitre of concentrated sulfuric acid (H2SO4). A deoxy sugar, which is typical of cardiac glycosides, was suggested by the formation of a brown ring at the contact (Ayoola et al., 2008).

Test for flavonoids: Each plant extract's aqueous filtrate was combined with 5 ml of diluted ammonia solution, and concentrated H2SO4 was then added to identify the flavonoids. The appearance of a yellow tint suggested the existence of flavonoids. The yellow hue vanished as one stood. Each plant filtrate received a few drops of a 1% aluminum solution; the formation of a yellow tint showed the presence of flavonoids. (Dewanto et al.,2002)

Test for alkaloids: The presence of alkaloids in the aqueous extracts of the plants was determined by mixing 1 ml of each plant extract with Dragendorff's reagent. The formation of reddish-brown precipitate indicated the presence of alkaloids (Joshi et.al.,2013).

Test for steroids (Salkowski test): Each sample's 0.5 g ethanolic extract was first treated with 2 ml of acetic anhydride and then treated with 2 ml of sulfuric acid to detect the presence of steroids. Steroids were present in several samples because their hue changed from violet to blue or green. (Ayoola et al., 2008).

Test for terpenoids (Liebermann-Burchard test): Terpenoids in the aqueous extracts of different plants were identified by combining 5 ml of each extract with 2 ml of chloroform, followed by the gradual addition of 3 ml of concentrated sulfuric acid. This process led to the formation of a distinct layer. The appearance of a reddish-brown color at the interface between the layers indicated the presence of terpenoids. (Joshi et.al.,2013)

Total phenolic content: The total phenolic content of E. pyriformis extracts was determined using the Folin-Ciocalteau method (Chlopicka et al., 2012). Dilute extracts of both chloroform and ethanol (0.5 ml) at various concentrations were mixed with Folin-Ciocalteau reagent (5 ml, diluted 1:10 with distilled water) and aqueous Na2CO3 (4 ml, 1M). After allowing the mixture to stand for 5 minutes, the total phenols were measured at 754 nm using a double-beam UV/Vis spectrophotometer (Systronics model 2201). The total phenol content was expressed in terms of gallic acid equivalent (mg/g of dry mass), which serves as a common reference compound.

Total flavonoid content: The aluminum colorimetric method (Chang et al., 2002, Stankovic, 2011) was modified and used using quercetin as the standard to estimate the total flavonoid concentration. A quercetin calibration curve was created within the range of 0–200 µg/mL. In summary, 0.5 mL of extract and 0.5 mL of standard were put into separate test tubes, and then 10% aluminum chloride (0.1 mL), 1 M potassium acetate (0.1 mL), 80% methanol (1.5 mL), and distilled water (2.8 mL) were added to each and combined. The same procedure was followed to make a blank, except that 0.5 mL of distilled water was used in place of the sample or standard, and distilled water was also used in place of the same quantity of aluminum chloride. For thirty minutes, each tube was incubated at room temperature. The absorbance was measured at 510 nm with double beam UV/Vis spectrophotometer 2201 (Systronics). The flavonoid content was given in milligrams (mg) of quercetin equivalent (QE) per gram of extract.

2.5 Statistical Analysis

Fisher's method of analysis of variance (ANOVA) with completely randomized design was applied to the data collected from various observations during field experimentation and laboratory analysis. By computing the corresponding "F" value and comparing it with the relevant value of "F" at the five percent probability level, the significance and non-significance of the variance resulting from the various treatments were ascertained [9]. Through inter-treatment comparison, the crucial difference was computed at the 5% probability level. The formula below was used to determine the Standard Error Mean (S.E.M.). Treatments were classified as significant or non-significant at the 5 percent probability level based on the S.E.M. multiplied by the appropriate tabular values for Error Degrees of Freedom.

3. RESULT AND DISCUSSION

The data of the physical characterstics of the fruit in three villages viz Zotlang, Ruantlang and Hmunhmeltha is shown in **Table 1**. The maximum fruit weight and length was observed in Zotlang 1.79 mg and 17.28 mm followed by 1.35 mg and 14.39 mm in Ruantlang respectively. While the lowest fruit weight (1.26 mg) and fruit length (11.68 mm) was observed in Hmunhmeltha. However, Hmunhmeltha recorded fruits with maximum diameter (13.62mm) followed by Ruantlang(12.75 mm)_ and Zotlang(11.53 mm). Moisture content of fruit collected from Zotlang(66.24%) was maximum while fruits of Hmunhmeltha(59.87%) recorded the lowest moisture content. The longest seed length was observed in Zotlang(15.64 mm) and lowest was recorded in Ruantlang(23.98mm).While, seed diameter was maximum in Hmunhmeltha(9.33 mm) followed by Ruantlang (8.85 mm) and Zotlang(6.21 mm) as shown in **Table 1**.

The results obtained from physio-chemical analysis of the fruit sample from three villages is depicted I **Table 2.** The chemical characteristics analysis of ***Elaeagnus pyriformis*** shows maximum TSS in Zotlang(15.86 Brix) and minimum I Hmunhmeltha(13.65 Brix). The maximum Reducing sugar (17.075%), Total sugar(15.98%), Non-reducing sugar (4.89%), and TSS:acid ratio (13.73%) was detected in Zootlang. The lowest Reducing sugar (14.05%), Total sugar (11.2%), Sugar-Acid ratio(8.27%) was observed in Ruantlang. Fruit collected from Hmunhmeltha recorded the maximum Sugar:Acid ratio (15.26%). The maximum Titrable acidity (1.381%) was found in the fruits collected from Ruantlang as show I **Table 2.**

The phyto-chemical analysis of the fruits shows maximum Phenol content **in** Hmunhmeltha (1.80 mg/g of extract) followed by Zotlang(1.14 mg/g of extract) and lowest phenol content was found in Ruatlang(0.77 mg/g of extract). While, the maximum content of Flavonoid was found in Zotlang (0.42 mg/g of extract) followed by Hmunhmeltha (0.08 mg/g of extract) with the lowest flavonoid content was found in Ruatlang (0.07 mg/g of extract) as depicted in **Table 2.**

The phytochemical analysis of the wild edible fruit *Elaeagnus pyriformis* shown in Table 2 indicates that the extracts of the fruit screened showed the presence of terpenoids, flavonoids and tannins, whereas saponin and steroids were not detected in the fruit. Cardiac glycosides and alkaloids were also detected on the fruit.

The parameters shows Zotlang village with the highest in fruit length, weight, moisture content, TSS, reducing sugar, total sugar content, TSS: Acid ratio and flavonoid. Titrable Acidity and Ascorbic Acidity were highest from the sample from Ruantlang village. The most of fruit diameter, Sugar: Acid ratio, total phenolic content were observed from the third village, Hmunhmeltha of Champhai District. Phytochemical analysis of the fruit showed the presence of a number of medicinal active secondary metabolites. After testing of its significance, the physical and biochemical parameters are all found to be significant.

The physical parameters viz., the fruit weight, fruit length, fruit diameter, seed length, seed diameter and moisture content of the fruit was found to be highly significant. The biochemical parameters viz., Titratable acidity, Ascorbic acidity, TSS, Non-Reducing sugar and phytochemical parameter of flavonoid content shows that sample from Zotlang village is at par with the sample from Zotlang villageand Hmunhmeltha village. Whereas, the biochemical parameters viz., Reducing Sugar and Sugar: Acid ratio shows that the samples from Zotlang village and Ruantlang village are at par with

Table 1. Physical characteristics of *Elaeagnus pyriformis*

Villages Vi	Fruit weight (in mg)	Fruit length (in mm)	Fruit diameter (in mm)	Seed length (in mm)	Seed diameter (in mm)	Moisture content (in %)
Zotlang	1.79	17.28	11.53	15.64	6.21	66.24
Ruantlang	1.26	11.68	12.75	13.98	8.85	63.68
Hmunhmeltha	1.35	14.39	13.62	14.53	9.33	59.87
MEAN	**1.47**	**14.46**	**12.64**	**14.72**	**8.13**	**63.26**
SEd(±)	**0.03**	**0.81**	**0.11**	**0.06**	**0.08**	**0.19**
C.D(0.05)	**0.08**	**2.24**	**0.31**	**0.17**	**0.22**	**0.53**

**The physical characterstics of Elaeagnus pyriformis was found to be significant at 0.05*

Table 2. Chemical Characteristics of *Elaeagnus pyriformis*

Villages	Physio-Chemical Properties								Phyto-Chemical Properties	
	TSS (ºBrix)	Titrable acidity (%)	Ascorbic acid (mg/100g)	Reducing sugar (%)	Total sugar (%)	Non-reducing sugar (%)	Sugar: Acid ratio (%)	TSS: acid ratio (%)	Phenol (mg/g/extract)	Flavooid(mg /g/extract)
Zotlang	15.86	1.107	15.86	17.07	15.98	4.89	14.07	13.73	1.14	0.42
Ruantlang	14.01	1.381	22.04	14.05	11.2	1.74	8.27	11.82	0.77	0.07
Hmunhmeltha	13.65	1.165	15.86	16.41	12.68	1.65	15.26	9.376	1.80	0.08
Mean	14.51	1.22	17.92	10.56	13.29	2.76	12.54	11.65	1.24	0.19
SEd(±)	0.60	0.07	1.66	0.38	0.26	0.43	0.64	0.57	0.06	0.01
C.D(0.05)	1.67	0.20	4.61	1.05	0.71	1.19	1.78	1.57	0.16	0.04

**The chemical characteristics of Elaeagnus pyriformis was found to be significant at 0.05%*

Table 3. Preliminary phytochemical analysis of wild edible fruit of Mizoram

Tests	*Elaeagnus pyriformis*		
	Zotlang	Ruantlang	Hmunhmeltha
Saponin	-	-	-
Tannin	+	+	+
Cardiac glycosides	+	+	+
Flavonoids	+	+	+
Alkaloids	+	+	+
Steroids	-	-	-
Terpenoids	+	+	+

**+ indicates Present; - indicates Absent*

the samples from Hmunhmeltha. The biochemical and the phytochemical parameters are also found to be very significant. Result shows variation among genotypes which was in accordance with the finding of [1]. The fruit have various antioxidant properties but has lesser contents as compared to that of *Elaeagnus latifolia*, which is an early variety and are slightly bigger in size. The total phenolic content and total flavonoid test are said to be at par. Flavonoids are present in all vascular plants and about ten classes of them are recognized [10] which comprises of more than 4000 polyphenolic compounds [11]. They play several important roles in the life of plant life like stimulation, protection, flavouring, pigmentation and in plant-micro-organism communication [12]. (Kumar *et al,* 2013) reported flavonoids to have different biological activites like antioxidant, hepatoprotective, antibacterial, anti-inflammatory, anticancer, antiviral in their review. The study showed the content flavonoid which could be responsible for their various uses. The phytochemical screening of *E.pyriformis* has alkaloids, tannins, saponins, steroids, flavonoid contents and have been reported to elicit physiological and biochemical response in the human body which was also reported by [13]

4. CONCLUSION

We can conclude that the fruit, *Elaeagnus pyriformis* Hook.f. (Sarzûktê) collected from three different locations of Champhai possess a slight difference in their physical and chemical properties. This study has made contribution in revealing the goodness and natural nutrient contents of the fruit. The study shows the presence of tannins, cardiac glycosides, flavonoids, alkaloids and terpenoids which have been found to possess numerous medicinal properties.

The utilization of medicinal plants proves beneficial not only for indigenous communities but also extends to processing and exportation. There is a growing demand for medicinal plants, emphasizing the importance of identifying species with both medicinal and nutritional value. Preserving these plants is crucial to prevent their extinction. The North-East region of India boasts diverse flora and fauna, thriving in favourable climate conditions suitable for cultivating various fruit crops. The development of improved plant varieties has contributed to success stories, enhancing the country's economic status. Wild edible fruits from this region not only offer delightful Flavors but also exhibit potential health benefits. It is imperative to analyse their nutritional content thoroughly, shedding light on their diverse properties. This understanding encourages the recognition and conservation of these natural resources, instilling a sense of value and importance among people.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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