World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

Check for updates

Morphological and anatomical characteristics of *Waltheria indica* (L.) growing on sandy soils of Binh Thuan province, Vietnam

Simphathai Mahaxay [1], Pham Van Ngot [1], Huynh Nguyen Van Anh [2] and Dang Thi Ngoc Thanh [2,*]

[1] *Faculty of Biology, HCMC University of Education, 280 An Duong Vuong Street, Ward 4, District 5, Ho Chi Minh City, Vietnam.*

[2] *Faculty of Natural Science Pedagogy, Sai Gon University, 273 An Duong Vuong Street, Ward 3, District 5, Ho Chi Minh City, Vietnam.*

World Journal of Advanced Research and Reviews, 2021, 09(01), 134–146

Publication history: Received on 02 January 2021; revised on 09 January 2021; accepted on 11 January 2021

Article DOI: https://doi.org/10.30574/wjarr.2021.9.1.0007

Abstract

Waltheria indica (L.) is a species in the list of medicinal plants in Vietnam. However, information about this species is still at the basic level. In order to provide more data on this medicinal plant, the present research has gone into further on the characteristics of the plant through morphological classification method, observing the thin slices of vegetative and reproductive organs and measuring with a reticule on the eyepiece and a stage micro-meter equipped for a light microscope. Combination of statistical analysis of botanical morpho-anatomical traits with characteristics of soil and climatic where the plants distribute was conducted to find ecological adaptation of the plants. The results showed that the plants growing in regions with different ecological conditions had significant differences in structural characteristics. This is the basis for collecting the medicinal plants *Waltheria indica* (L.) and continue to determine their pharmacological properties in the future.

Keywords: *Waltheria indica (L.)*; Binh Thuan province; Morpho-anatomical characteristics; Measurement with the light microscope

1. Introduction

Waltheria indica (L.) [synonymous nomenclature of *Waltheria americana* (L.)], is a medicinal plant of many African and South American countries used in treatment of pain, inflammation and some diseases such as dysentery, abscesses, epilepsy, anemia, asthma, erectile dysfunction [1]. *Waltheria indica* (L.) belongs to the family Malvaceae, and considered indigenous to tropical and subtropical America. Currently, this species has been found in nearly 100 other territories and countries, including Vietnam [2]. In 2013, Zongo et al. [1] made a comprehensive review of botanical characteristics, traditional medicinal uses, chemical composition, pharmacology and toxicity of *Waltheria indica*. Subsequent works (from 2016 - 2019) carried out research on this species including the aspects of antibacterial, anti-inflammatory, antioxidant, analgesic, toxicity to blood, liver, kidney, and male reproductive function (on rat) [3, 4, 5, 6]. In Vietnam, *Waltheria indica* was simply classified and described morphological in [7, 8]. The use of this plant in the traditional medicine mentioned in [7, 8] included cough, internal bleeding, oral thrush (caused by *Candida albicans*), and enhances fertility for women. There was also mentioned the ability to increase sperm production in remedies of South Africa [7]. Regarding the distribution area of *Waltheria indica* species in Vietnam, document [7] mentioned some kinds of abandoned, roadside, and plain soil. Some surveys showed that the species was distributed in North Central and Central Coast of Vietnam, including Phan Thiet, Binh Thuan [9, 10, 11]. The mentioned documents showed some information on toxicity, fertility enhancement, and treatment of some diseases such as syphilis and asthma that require more evidence. In Vietnam, scientific information on studies of adaptive morphological and anatomical characteristics as well

* Corresponding author: Dang Thi Ngoc Thanh
Faculty of Natural Science Pedagogy, Sai Gon University, 273 An Duong Vuong Street, Ward 3, District 5, Ho Chi Minh City, Vietnam.

as pharmacology of this species has not been published. Therefore, the present study will be the premise for the identification, collection and subsequent pharmacological studies of the plant *Waltheria indica*.

2. Material and methods

2.1. Samples collection and preparation

Binh Thuan belongs to the South Central Coast of Vietnam with geographical coordinates from 10°33'42" to 11°33'18" North latitude, from 107°23'41" to 108°52'18" East longitude. Figure 1 below illustrated the three sampling locations (star shapes) which were 3 communes bordering the East Sea: Binh Thanh commune in Tuy Phong district (1), Ham Tien commune (2) and Tien Thanh commune (3) in Phan Thiet City. The land here was mainly sandbanks and sand dunes that accounting for 18.22% of the province's natural area [12].

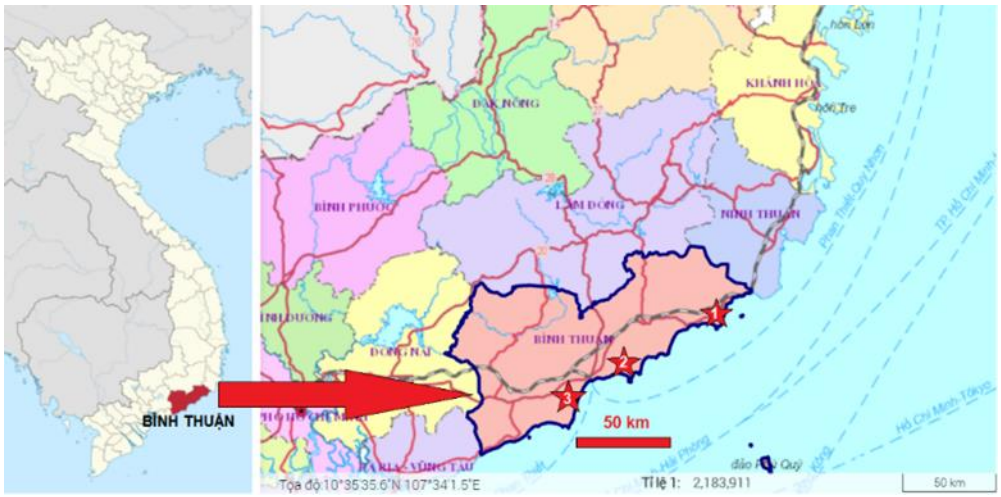

Figure 1 The map of Binh Thuan province [13] and the three sampling locations

Five points were randomly selected in each commune. Four plant samples and four soil samples were then collected at each site, making up 20 samples were taken per each commune. Soil was collected in 0 – 30 cm layer; each sample of the layer was taken at least 1 kg. Methods of collecting and handling samples were done according to Vietnamese National Standards TCVN 8551: 2010 and TCVN 7538-2: 2005TC [14]. Plant and soil samples were transferred into sterile plastic bags, sealed, labelled, refrigerated and transported immediately to the laboratory. Fresh plant samples were stored in a refrigerator at 5°C for use in morphology study, and then preserved by immersion in ethanol 70° solution for continued use in anatomy study.

2.2. Field studies

The light intensity, temperature and humidity of the sampling site at the time of the survey were collected by using a light meter (Compact Light Meter LM-81LX) and an odometer Humidity and temperature (EXTECH RHT20). This data was recorded over 3 days of each sampling in Sep and Dec 2019, Jun and Sep 2020 combined with the hydro-meteorological forecast of the region at the website [15].

Density of plant (number of plants per 10 m^2) was recorded in the three sampling communes; 2 sites were selected randomly in each commune. At each site, 15 plants were randomly selected to determine the height of stems and the size of leaves. Growth cycle of the plant in the wild was also monitored for a year (10 plants per commune) to record the time points of germination, flowering and fruit setting.

2.3. Analyzing some physical and chemical criteria of soil

Soil texture particles and soil organic matter were analyzed according to the Vietnamese National Standards TCVN 8941-2011 and TCVN 8567-2010, respectively [14] by the Institute of Agricultural Science for Southern Vietnam, Ho Chi Minh City.

Soil salinity via electronic conductivity (EC) and soil acidity via pH_{H2O} and pH_{KCl} were determined according to the description of the Soil and Fertilizer Research Institute [16].

2.4. Analyzing morphological characteristics of plant

The shape and morphological features of the whole plant and the vegetative and reproductive organs corresponding to the different stages of development were described, recorded and photographed on site. Thereafter, the individual plant parts were further analyzed in the laboratory. Classification criteria and terminology were based on the literature [7, 8].

2.5. Analyzing anatomical characteristics of plant

The intermediate leaves (3rd and 4th leaves from the top of tree), primary and secondary stems and secondary roots were used to study anatomy. Thin slices of these parts were created using razorblade and were double stained with methylene blue and carmine alum. Determination of stomata type by imprinting with transparent nail polish. The quantity of stomata as well as the size of the composition of the plant organ was determined using a microscope equipped with eyepiece and stage micrometers (Olympus) [17, 18].

The stomata of the *Waltheria indica* leaves were difficult to study because of its dense trichomes. To perform the imprint, this hairy coat must first be removed. The leaves was dried at 60°C and fixed from the crumbling with transparent nail polish. When the polish dried, it made the hairs-attached film that was easily removed from the epidermis. Another nail polish was applied to this surface and allowed to dry to be peeled and examined under the microscope. This technique was currently not possible with the abaxial side of *Waltheria indica* because the trichomes coat was too adhesive to remove.

2.6. Processing statistics

All quantitative data were analyzed for One-way ANOVA (Analysis of Variance) and Least Significant Difference (LSD) with α=0.05.

3. Results and discussion

3.1. Field survey results

In 2019, the average annual temperature of Tuy Phong district and Phan Thiet city, Binh Thuan was 27.74°C and 27.75°C, respectively; the average annual rainfall was 734.6 and 866.8 mm, respectively; and the total annual sunshine hours was 3264.3 and 3114.8, respectively. Data in 2020 have not been summarized yet, but forecasted data in May 2020 showed that the temperature tends to increase by 0.5 – 1°C due to hot weather phenomenon. Rainfall was 10 – 25% lower in June and approximately the same period last year in September 2020 [15]. At the time of the survey, the average temperature was within the normal ranges. Average illuminance measured ranged from 52,000 to 100,000 lux; and the average humidity measured was 75 – 79%. The hot and windy climate of the central coast of Vietnam was characteristic of this place and was the condition for plants to exhibit adaptive properties (Figure 2).

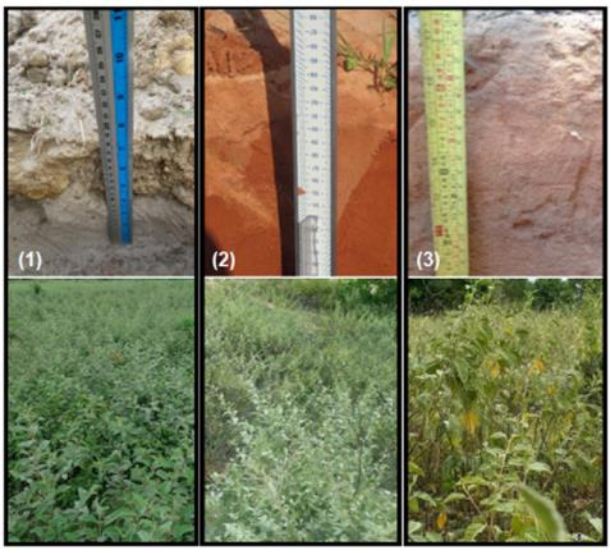

Figure 2 Soil profile (30 cm deep) and the landscape where *Waltheria indica* distributed
(1) Binh Thanh commune; (2) Ham Tien commune; (3) Tien Thanh commune

Regarding the characteristics of the soil where the samples were collected, the soil color of the three site was quite different (Figure 2) but the soil types were all sandy soils. Soil texture, salinity and acidity were shown in Table 1. It showed that the soil was nutrient poor, not salty, and was slightly acid to neutral. In particular, the soil in Ham Tien had very low organic matter content, below the detection threshold of the method used (*).

Table 1 Some physical and chemical criteria of soil samples

Criteria of soil		**Sampling location**		
		Binh Thanh	**Ham Tien**	**Tien Thanh**
Soil particles	Sand (%)	88	98	96
	Silt (%)	3	1	1
	Clay (%)	9	1	3
	Organic matter (%)	0.39	<0.3*	0.32
Soil acidity	pH_{KCl}	6.67	4.88	5.58
	pH_{H2O}	7.82	5.67	6.36
Soil salinity	EC (dS/m)	0.90	0.77	0.32

(*): LOQ (Limit of Quantitation) = 0.3

The indicators of density, height of stems and size of leaves of *Waltheria indica* plant in the 3 survey locations were shown in Table 2.

Table 2 Plant density, plant height and leaf size of *Waltheria indica* in the three survey locations.

Indicators		**Sampling location**		
		Binh Thanh	**Ham Tien**	**Tien Thanh**
Plant density (plants per 10 m2)		75.00 ± 11.31 ^{b}	29.00 ± 7.07 ^{a}	64.00 ± 15.56 ^{ab}
Plant height (cm)		79.10 ± 18.14 ^{b}	68.90 ± 10.61 ^{a}	86.37 ± 19.73 ^{b}
Leaf	Width of blade (cm)	4.26 ± 0,63 ^{c}	2.81 ± 0.43 ^{a}	3.49 ± 0.83 ^{b}
	Length of blade (cm)	6.25 ± 0.76 ^{b}	5.14 ± 0.75 ^{a}	6.08 ± 0.79 ^{b}
	Length of petiole (cm)	1.33 ± 0.30 ^{a}	1.31 ± 0.30 ^{a}	1.48 ± 0.53 ^{a}

Within the same row, different letters following the values were no significant difference at α = 0.05 in the LSD test (a <b).

Table 2 showed that the density and the plant height of *Waltheria indica* distributed in Ham Tien were the lowest, with 29 plants per 10 m^2, and 68.9 cm, respectively. The plant density in Tien Thanh and Binh Thanh was higher, at 64 and 75 plants per 10 m^2, respectively, with a statistically significant difference. Regarding leaf characteristics, the petiole length of plant samples collected at 3 locations did not differ. The width and length of leaf blades (lamina) of the plants collected in Ham Tien were the smallest, at 2.81 and 5.14 cm respectively, which were different statistically for the samples collected in Tien Thanh and Binh Thanh. Particularly, the plant height and lamina length of the plants growing at the 2 locations, Tien Thanh and Binh Thanh, had no statistically significant differences with α=0.05. The sparse density as well as the size of the plant parts were lowest of the *Waltheria indica* plants growing in Ham Tien could related to the soil characteristics of being poor in organic matter and slightly acidic rather than related to climatic conditions there.

For life cycle of *Waltheria indica*, field monitoring showed that during the dry season (from November to April next year), due to living in hot condition for many days, there was no rain, so most of plants died or lost all their leaves. When the rainy season came in May, some of the surviving plants sprouted. The few plants left over from last year proved that *Waltheria indica* was not an annual plant. The observation also showed most seedlings germinated from seeds to create a new generation of plants where their parents used to live. As described by [8], *Waltheria indica* plants bear fruits from November to June next year but the present study showed that since rain fell in May, a series of seedlings had sprouted

from the seed. This indicated the adaptation of the *Waltheria indica* plant's life cycle to the region's rainwater condition. How climatic conditions affect other plant characteristics was what would be analyzed next.

3.2. Morphological characteristics of the plant *Waltheria indica*

Classification position of *Waltheria indica* based on the document [7, 8] all showed that it belonged to the family Sterculiaceae. Compared to the general description [7, 8], the plants observed in the present study had several morphological adaptations. They were small shrubs, about 0.5 – 1 m high. When living for a long time and growing individually, the plants grew many branches spreading close to the ground due to the impact of strong winds (Figure 3). The root system was deep and widespread, adapting to arid, dehydrated soil conditions.

Figure 3 Morphology adapted to the living conditions of *Waltheria indica*

3.2.1. Morphological characteristics of vegetative organs

The plant *Waltheria indica* growing in the three locations had an average height of about 70 – 86 cm. All aerial parts of the plant covered with silky hairs (trichomes) (Figure 4 D). In some older plants, the stems, branches and petioles were slightly purple (Figure 4 E).

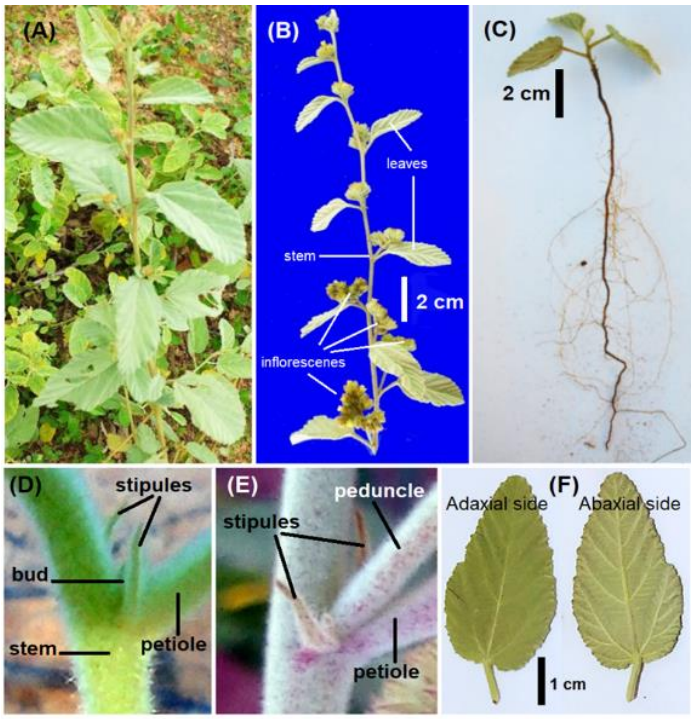

Figure 4 Morphology of *Waltheria indica* vegetative organs

Leaves of *Waltheria indica* were single, about 3 – 5 cm long and 2.5 – 3 cm wide, arranged in the alternate and spiral pattern (Figure 4 A, B, C). The leaves were green, covered with trichomes on both sides. The petiole was medium long

and had two attached stipules (Figure 4 D, E). The leaf blade (lamina) was elliptic or ovate-shaped with the margin was serrate and undulate. The venation was dichotomous and cross-venulate, slightly sunken on the adaxial but prominent on the abaxial of the lamina (Figure 4 F). In young plants that had just sprouted for a while, the roots had grown quite long relative to the stem's height (Figure 4 C). Trichomes mulch and root system growth were adaptive features of plant species distributed in the arid central region of Vietnam that have been reported [18, 19].

3.2.2. Morphological characteristics of reproductive organs

The flowers of *Waltheria indica* grew in dense clusters with very short pedicels making classification a bit difficult. There were documents that claimed type of *Waltheria indica*'s inflorescence was cyme or corymb [2]. However, based on its unusual inflorescence, the present study confirmed that was panicle pattern. Each inflorescence grew in the leaf axil, with a peduncle about 2 – 4 cm long, carrying many flowers. Flowers was small, hermaphrodite, pentamerous pattern, with or without bracts, with or without short pedicels. Calyx was green, hairy, fused at the base, and had 5 sepals about 3 – 4 mm long. It would coexist with the fruit to surround the fruit. The corolla was yellow, tabulate-shaped, about 4 – 5 mm long, the upper (free) parts of the 5 petals were twisted aestivation when not yet opened (Figure 5 A). The stamen set consisted of 5 filaments fusing to form a tube cover a pistil; and 5 antheres were opposite to the 5 petals. The ovary was superior, elongated with a hairy style that was about 3 mm long and terminated with a hairy stigma (Figure 5 B). The ovary had one chamber contained 1 – 2 ovules. Fruit was capsule, conical, about 2 – 3 mm long, containing 1 – 2 seeds. Seeds was ovoid-shaped, blackish brown, and 1.5 x 2 mm in size (Figure 5 E). For anomalous flowering, as noted above, the inflorescence had a panicle pattern (Figure 5 C). Flowers were larger with 5 large sepals, but not well developed petals. The fruits were also bigger than usual (Figure 5 D).

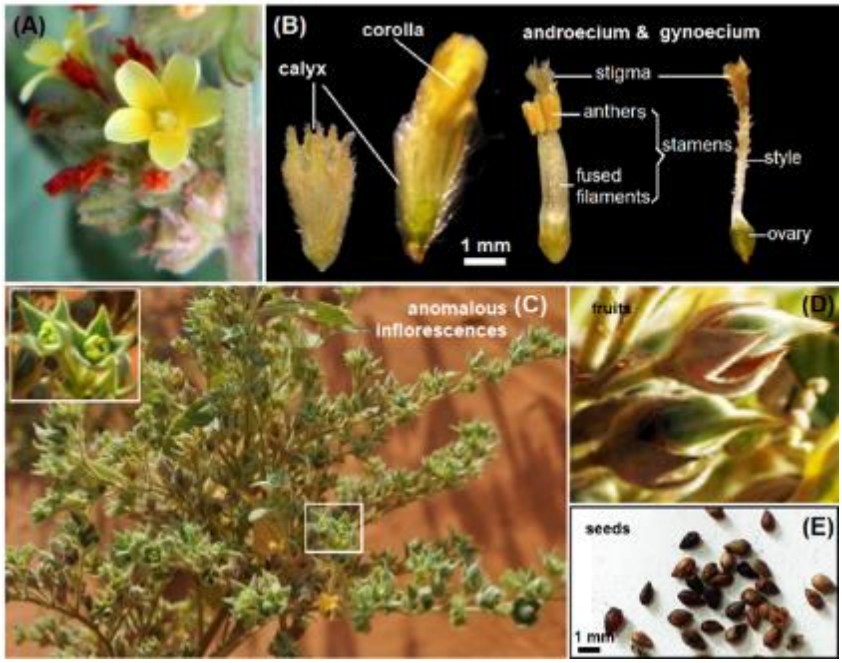

Figure 5 Morphology of *Waltheria indica* reproductive organs

The plant *Waltheria indica* was known by many different Vietnamese names such as "ké rừng", "hoàng tiền", "xà ba". Although the synonym *Waltheria americana* has been recognized, the name of the family which it belongs to has not been agreed. Documents in Vietnam and some other countries' documents still classified *Waltheria indica* in family Sterculiaceae [7, 8, 9, 10, 11] instead of family Malvaceae as the publication of [20]. These indicated the need to re-describe some of the other morphological and biological features of this plant as the present study done to assist in more accurate identification and collection within the country. In addition, the detection of unusual flower clusters helped to determine the type of inflorescence of *Waltheria indica*. However, the cause of this unusual flowering was not mentioned in the existing literature.

3.3. Anatomical characteristics of the plant *Waltheria indica*

3.3.1. Anatomical characteristics of stems

The cross-section of primary stems were oval to nearly round with wavy edge. The structure and dimensions of the parts that made up the stems were shown in Figure 6 and Table 3 below.

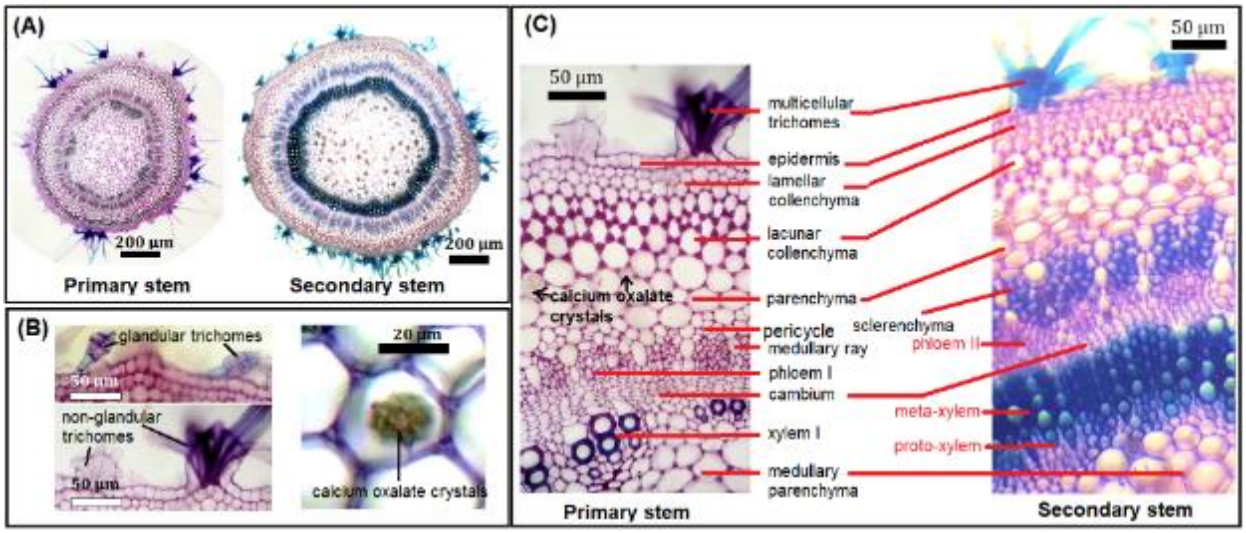

Figure 6 Anatomy of *Waltheria indica* stems
(A) Cross-section; (B) Some of specific structure; (C) Detailed structure

Table 3 Dimensions of components of the primary stems (n = 20)

Components	Sampling location		
	Binh Thanh	**Ham Tien**	**Tien Thanh**
Cuticle	3.66 ± 0.26 [a]	4.64 ± 0.73 [b]	3.31 ± 0.42 [a]
Epidermis	13.95 ± 2.57 [a]	12.17 ± 1.83 [a]	14.94 ± 9.50 [a]
Cortex collenchyma	48.59 ± 14.48 [a]	70.29 ± 25.28 [b]	55.31 ± 11.93 [a]
Cortex parenchyma	49.22 ± 11.43 [a]	54.30 ± 8.04 [b]	55.22 ± 14.79 [b]
Pericycle	20.49 ± 4.51[b]	27.00 ± 5.39 [c]	14.33 ± 8.58 [a]
Phloem	38.85 ± 11.60 [a]	42.83 ± 6.40 [a]	37.64 ± 5.89 [a]
Cambium	20.79 ± 7.36 [a]	34.72 ± 6.84 [b]	32.66 ± 7.87 [b]
Xylem	67.05 ± 13.71 [b]	56.80 ± 7.98 [b]	47.52 ± 11.13 [a]
Medullary parenchyma	234.01 ± 42.9 [a]	234.73 ± 20.00 [a]	243.46 ± 31.60 [a]
Total	469.59 ± 74.42 [a]	525.37 ± 26.67 [a]	503.66 ± 48.92 [a]

Thickness calculated in radius of cross-section (µm). Number of samples: n = 20.
Within the same row, different letters following the values were no significant difference at α = 0.05 in the LSD test (a <b<c).

Table 3 above showed that the primary stem of *Waltheria indica* plant obtained at Ham Tien had the highest diameter, although the difference (in radius) was not statistically significant. However, it could be seen that the structures related to solidity and protection both increase in size and the difference was statistically significant; in which the size of the cuticle, cortex collenchyma, and pericycle were 4.64, 70.29, and 27.00 µm, respectively. Difference between epidermis, phloem and medullary parenchyma of *Waltheria indica* at 3 sampling locations was not statistically significant. The shorter and stiffer stems of *Waltheria indica* plants growing in Ham Tien were probably the feature adapted to the more sparsely densities of them here in order to increase their resistance to coastal winds.

Going from the outside, the epidermis consisted of a layer of small, irregular cells. The outside of the epidermis had a thin cuticle and many stellate protect multicellular trichomes and many mace-shaped glandular multicellular trichomes (Figure 6 B). Inside the epidermis, there were 6 – 8 layers of polygonal cells of cortex collenchyma with the size of cells growing from the outside to the inside that incuded 2 – 3 layers of lamellar collenchyma with a little chloroplasts outside and 4 – 5 layers of lacunar collenchyma inside (Figure 6 C). The next layer was parenchyma with large spherical large cells that sometimes contained spiked ball-shaped calcium oxalate crystals. (Figure 6 B). In the primary stem of *Waltheria indica* plant, the endodermis was not clearly distinguished.

The stele occupied most of the cross section, including the sclerenchyma, the medullary rays, the primary vascular bundles and the medulla (pith). The pericycle consisted of 3 – 4 layers of irregular polyhedron cells that separated by medullary rays (Figure 6 C). The primary medullary ray consisted of rows of parenchyma cells arranged in a radial direction, larger than the sclerenchyma and phloem I cells. The primary stem of *Waltheria indica* had many vascular bundles arranged into a nearly continuous, including the primary phloem (phloem I) on the outside and the primary xylem (xylem I) on the inside. Between the xylem and phloem was the cambium (proto-cambium) consisting of many undifferentiated small cuboid cells arranged close together. The medulla located in the centre of the stem, was composed of large, oval or spherical parenchyma cells with small intercellular spaces. There were reserve starch granules and calcium oxalate crystals in some of these cells.

The primary structure of *Waltheria indica* stem only existed for a short time, then it was changed to the secondary structure due to activity of cork cambium and vascular cambium. The action of cork cambium formed the tough outermost layer of the stem including cork cells (bark) in outside, and phelloderm inside. The bark, cork cambium, and phelloderm formed the structure called periderm that substituted for the epidermis of young plants. The bark consisted of 5 – 7 layers of suberized cells arranged close together. The cork cambium consisted of a layer of cuboid cells fit close together. The phelloderm consisted of 10 – 12 layers of parenchyma cells that contained some chloroplasts. Inward from the periderm, the pericycle in the primary structure turned into the sclerenchyma to increase the rigidity of the stem. The vascular cambium to create secondary vascular bundles including the secondary phloem to the inside and secondary phloem to the outside. The secondary phloem was stratified. This has not been previously reported [19] but this type of phloem was a common feature found in plants of the family Malvaceace [21, 22]. The secondary xylem consisted of wooded cells arranged in a continuous ring. Primary xylem was pushed inward, alternating with the pith. The medullary parenchyma also contained some starch granules and calcium oxalate crystals like in the cortex parenchyma (Figure 7).

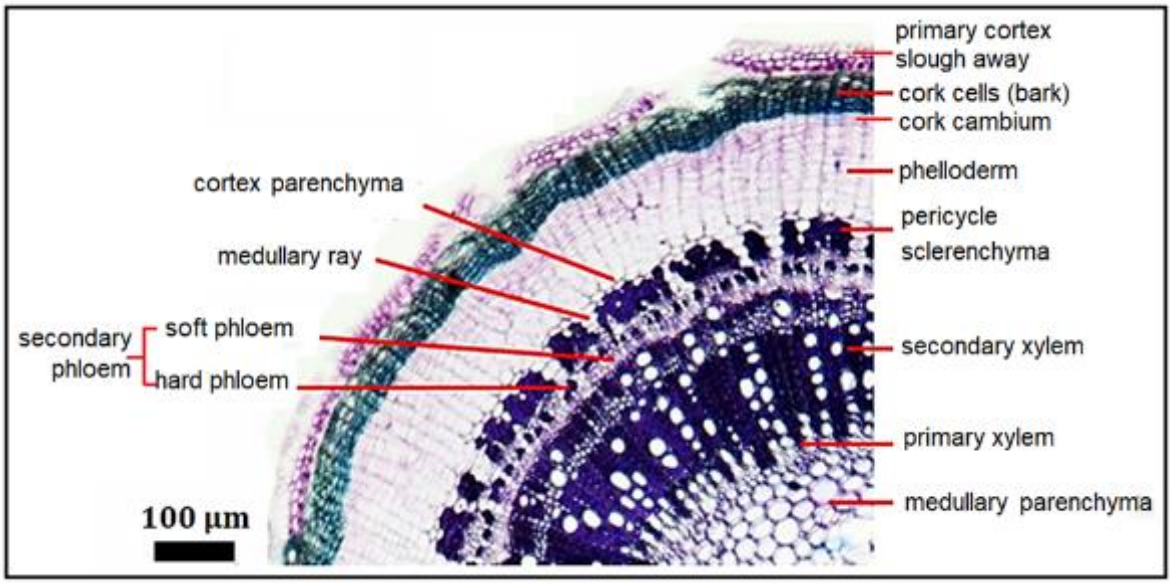

Figure 7 Structure of the secondary stem of *Waltheria indica* in the cork-forming stage

3.3.2. Anatomical characteristics of leaves

The cross section of the leaf blade showed the egg-shaped midrib (mid vein) clearly on the abaxial side (Figure 8 A). This figure also showed the dense stellate trichomes that made the imprinting technique difficult to perform. The abaxial of the midrib (from lower to upper) had a structure consisting of layers similar to the primary structure of the stem. However, on the adaxial side, going from the outside to the inside included the epidermis, collenchyma and parenchyma

in contact with the xylem of the vascular bundle (Figure 8 B). For the leaf blade (lamina), the adaxial epidermis consisted of irregular cells that was larger than the abaxial epidermis. Similarly, the cuticle on the adaxial was thicker than the abaxial. Stomata distributed on both sides. The protect trichomes had more quantity than the gladulary ones. These structures helped to protect the leaves from evapotranspiration in dry habitats. Especially, the stellate trichomes helped create a micro-atmosphere around the leaf surface by trapping water vapor released from the leaf.

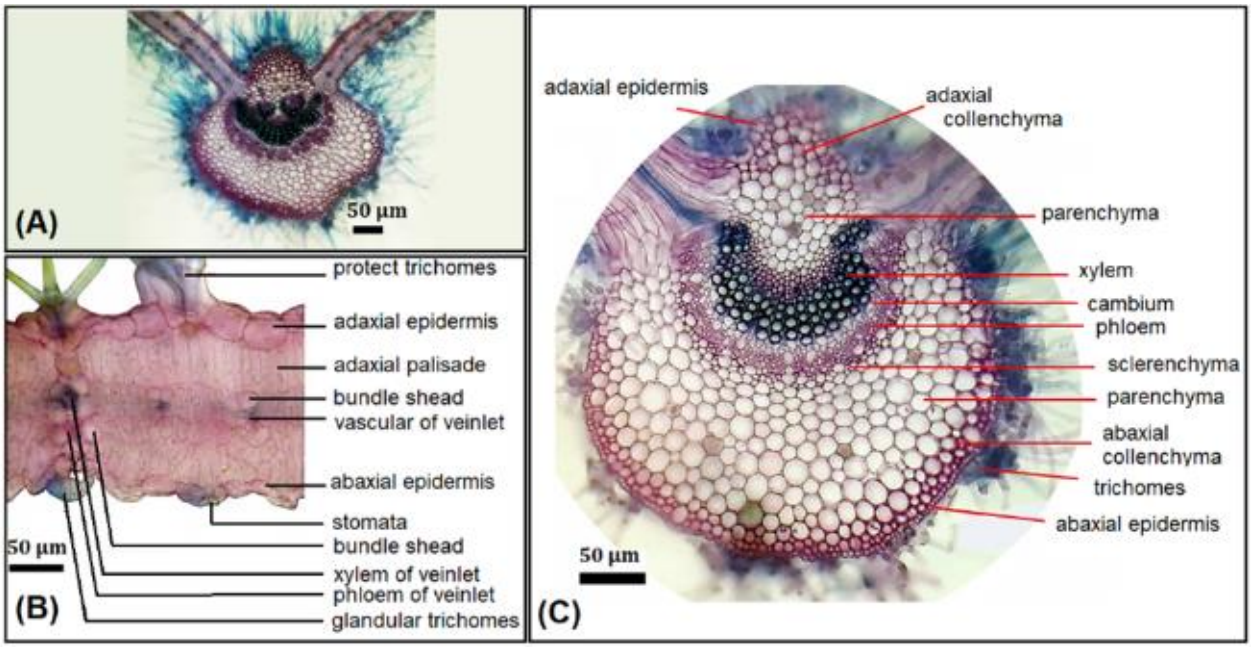

Figure 8 Anatomy of *Waltheria indica* leaf

The homogeneous leaf flesh consisted of the whole palisade mesophyll with 4 layers of cuboid tightly arranged cells, perpendicular to the epidermis. The first layer adjacent to the adaxial epidermis was longer than the other 3 layers, differentiating clearly. Scattered in the tissue were spike ball-shaped crystals of calcium oxalate. The veinlet consisted of a layer of pericycle covering the vascular bundle with the xylem at the adaxial and the phloem at the abxial (Figure 8 C).

For the stomata on the abaxial side, Figure 9 A showed that it was anisocytic type with guard cells between two larger subsidiary cells and one distinctly smaller one. This was as described by [23]. However, the density of stomata was quite large, 402.61 ± 27.03 per mm^2. The stomata arranged around the base of stellate trichomes (red dots) forming a rather special pattern (Figure 9 B). As noted above, the pattern of the stomata on the adaxial surface was not observed.

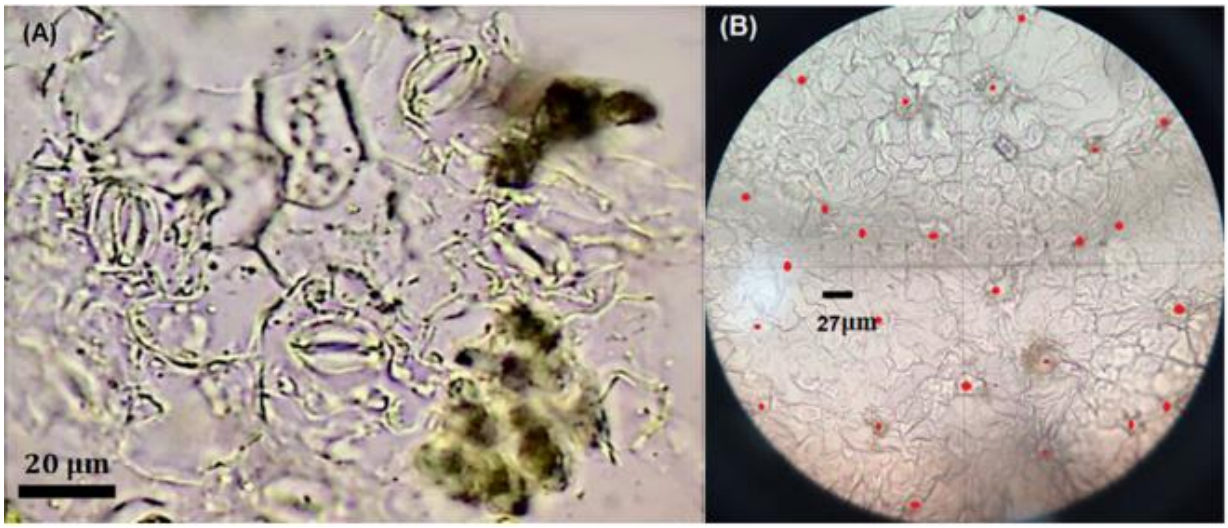

Figure 9 Stomata pattern on the abaxial surface of *Waltheria indica* leaf

In terms of the size of the parts, Tables 4 and 5 below would present the results of statistical analysis of *Waltheria indica* leaves at the 3 sampling locations.

Table 4 Dimensions of components of the midrib (n = 20)

Components	Sampling location		
	Binh Thanh	**Ham Tien**	**Tien Thanh**
Adaxial cuticle	3.77 ± 0.51 [a]	4.63 ± 0.66 [b]	4.20 ± 0.87 [a]
Adaxial epidermis	15.35 ± 3.04 [b]	13.92 ± 3.02 [a]	11.51 ± 2.75 [a]
Adaxial collenchyma	86.90 ± 21.00 [a]	124.91 ± 13.85 [c]	101.95 ± 22.28 [b]
Adaxila parenchyma	80.19 ± 36.72 [a]	126.89 ± 34.01[c]	105.20 ± 31.79 [b]
Xylem	112.20 ± 12.29 [b]	98.56 ± 18.72 [a]	101.35 ± 12.90 [a]
Cambium	17.70 ± 3.49 [a]	28.02 ± 5.75 [b]	25.71 ± 7.95 [b]
Phloem	31.42 ± 9.91 [a]	49.84 ± 10.03 [b]	51.65 ± 14.76 [b]
Sclerenchyma	4.51 ± 0.15 [a]	4.60 ± 0.66 [a]	4.70 ± 0.78 [a]
Abaxila parenchyma	119.48 ± 24.46 [a]	191.15 ± 28.00 [b]	174.12 ± 35.55 [b]
Abaxial collenchyma	86.70 ± 29.96 [a]	73.05 ± 17.32 [a]	80.20 ± 33.37 [a]
Abaxial epidermis	14.46 ± 3.17 [a]	12.39 ± 2.17 [a]	19.54 ± 31.07 [a]
Abaxial cuticle	3.55 ± 0.15 [a]	4.75 ± 0.87 [b]	3.71 ± 0.78 [a]
Total	585.37 ± 94.75 [a]	741.81 ± 62.43 [a]	694.35 ± 72.52 [a]

Thickness calculated in whole cross-section (μm). Number of samples: n = 20. Within the same row, different letters following the values were no significant difference at α = 0.05 in the LSD test (a <b<c).

Table 5 Dimensions of components of the lamina (n = 20)

Components	Sampling location		
	Binh Thanh	**Ham Tien**	**Tien Thanh**
Adaxial cuticle	4.85 ± 1.30 [a]	4.22 ± 1.15 [a]	4.97 ± 1.71 [a]
Adaxial epidermis	15.82 ± 3.32 [a]	14.63 ± 4.01 [a]	19.51 ± 5.04 [a]
Palisade mesophyll	119.04 ± 23.00 [b]	96.89 ± 18.62 [a]	114.36 ± 10.87 [b]
Abaxial epidermis	10.36 ± 1.94 [a]	9.79 ± 1.57 [a]	10.81 ± 2.29 [a]
Abaxial cuticle	4.23 ± 1.20 [a]	3.95 ± 1.08 [a]	3.48 ± 1.69 [a]
Total	154.30 ± 25.27 [b]	129.49 ± 19.63 [a]	153.14 ± 11.82 [b]

Thickness calculated in whole cross-section (μm). Number of samples: n = 20.
Within the same row, different letters following the values were no significant difference at α = 0.05 in the LSD test (a <b<c).

For the midrib, similar to that of the primary stem, the structures related to the firmness of *Waltheria indica* leaves collected in Ham Tien had the greatest thickness and the differences were statistically significant. In which, the sizes of cuticle and collenchyma were 4.36 and 124.91, respectively. Although there was a non-statistically significant difference in the size of the entire midrib of the samples collected at the three locations, the increase in size difference of the adaxial structures such as cuticle, collenchyma and parenchyma showed their role of creating lift of the leaves. The parenchyma growth also enhanced the leaf's ability to store water, which was an adaptive feature of drought tolerant plants. Meanwhile, the difference in the size of the abaxial parts was not statistically significant. In addition, the smallest size of the palisade mesophyll in the samples collected in Ham Tien (96.89 ± 18.62 [a]) resulted in a decrease in leaf blade thickness while the other components were unchanged. Thin and small leaves were features associated with the nutrient deficiency condition of the soil compared to the other 2 locations, and this difference was statistically significant.

3.3.3. Anatomical characteristics of roots

The secondary root micro-anatomy of *Waltheria indica* had a nearly circular shape, including the secondary cortex, the vascular cambium and the stele. The secondary cortex included periderm and several layers of soft and hard phloem that was stratified and interrupted by medullary rays (Figure 10).

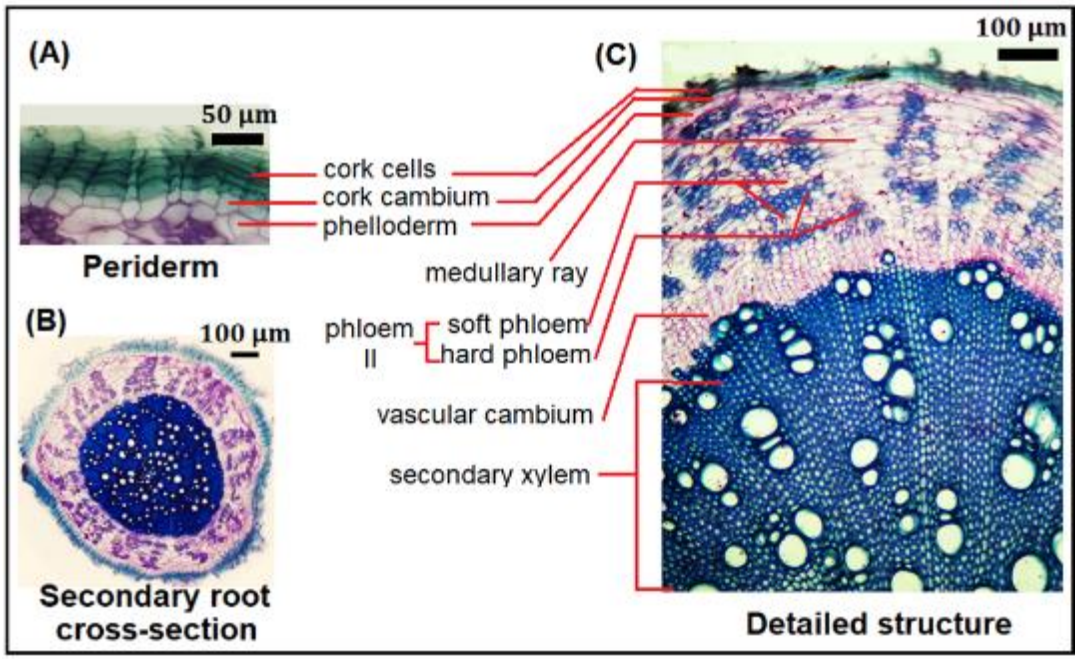

Figure 10 Anatomy of *Waltheria indica* secondary root

This secondary structure of the root was quite similar to that of the stem except some features such as the thin phelloderm consisted of only 1 – 2 cell layers, the stratification of the secondary phloem was clear and the secondary xylem occupied all the medullar. The dimensions of components of the root shown in Table 6.

Table 6 Dimensions of components of the secondary root (n = 20)

Components	Sampling location		
	Binh Thanh	**Ham Tien**	**Tien Thanh**
Periderm	65.24 ± 13.58 ^{b}	63.23 ± 11.83 ^{b}	52.50 ± 9.65 ^{a}
Secondary phloem	109.40 ± 3.18 ^{a}	163.45 ± 3.84 ^{c}	129.85 ±10.41 ^{b}
Vascular cambium	11.87 ± 2.58 ^{a}	13.96 ± 3.29 ^{a}	18.65 ± 4.73 ^{a}
Secondary xylem	163.86 ± 23.36 ^{a}	312.65 ± 78.92 ^{c}	276.94 ± 32.36 ^{b}
Total	360.87 ± 42.97 ^{a}	563.79 ± 94.48 ^{c}	488.43 ± 49.61 ^{b}

Thickness calculated in radius of cross-section (µm). Number of samples: n = 20.
Within the same row, different letters following the values were no significant difference at α = 0.05 in the LSD test (a <b<c).

The statistical results showed that the development of secondary cortex of *Waltheria indica* roots collected in Ham Tien. The development of components such as periderm, phloem and xylem caused the roots diameter to increase (563.79 ± 94.48 ^{c}). Meanwhile, the differences of vascular cambium sizes were not statistically significant. The enhanced root development helped the plant adapt to the drought and nutrient deficient conditions there.

4. Conclusion

This study had shown the need for unification for identification and taxonomy (up to the family level) of *Waltheria indica* in Vietnam. Morphological and anatomical analysis showed that the plant had features that were adapted to the dry,

sunny and windy conditions of Binh Thuan province. In which, the plants growing in Ham Tien had many different anatomical details compared to the plants growing in the other 2 locations, probably due to the poor soil nutrient condition of this place.

Compliance with ethical standards

Acknowledgments

The authors would like to thank Nguyen Quoc Vu and Nguyen Thai Minh Chau for their assistance in the study.

Disclosure of conflict of interest

There is no conflict of interest.

References

[1] Zongo F, Ribuot C, Boumendjel A, Guissou I. Botany, traditional uses, phytochemistry and pharmacology of *Waltheria indica* L. (syn. *Waltheria americana*): a review. J Ethnopharmacol. 2013 Jun 21; 148(1): 14-26.

[2] Plants of the World Online [Internet]. London: Royal Botanic Gardens Kew; © 2017 [cited 2020 Oct 26]. Available from http://plantsoftheworldonline.org/taxon/.

[3] Yougbare-Ziebrou MN, Lompo M, Ouedraogo N, Yaro B, Guissoun IP. Antioxidant, analgesic and anti-inflammatory activities of the leafy stems of *Waltheria indica* L. (Sterculiaceae). Journal of Applied Pharmaceutical Science. 2016 Feb; 6(02): 124-129.

[4] Koma OS, Fatokun OA, Theophilus OA. Phytochemical Screening and in Vitro Antimicrobial Activity of Waltheria Indica Linn Leaf Extracts. Biomedical Sciences. 2017 Oct 6; 3(5): 86-93.

[5] Hamidu JL, Ayo JO, Adelaiye AB, Abubakar MS. Effects of ethanolic extract of *Waltheria indica* aerial parts on some liver and kidney function indices in albino rats. African Journal of Biotechnology. 2018 May 9; 17(19): 626-632.

[6] Basiru A, Akorede GJ, Soetan K, Olayemi FO. Adverse reproductive effects of ethanolic root extract of *Waltheria indica* in male Wistar rats. J Complement Integr Med. 2019 Aug 21; 6(4).

[7] Pham Hoang Ho. Plants used as medicinal herbs in Vietnam. HCMC: Youth Publishing House; 2006. (Language: Vietnamese)

[8] Vo Van Chi. Vietnamese Medicinal Plants Dictionary, Volume 1. HCMC: Medical Publishing House; 2018. (Language: Vietnamese)

[9] Hoang Xuan Thao, Le Tuan Anh, Ngo Thi Diem My. Floral diversity of the sand dune areas of Trieu Phong and Hai Lang district, Quang Tri province. In: Editorial board of the Proceeding of the 7th National Scientific Conference on Technology and Biological Resources (on 2017 Oct 20) eds. Scientific report on ecology and biological resources. Ha Noi: Natural and Applied Sciences Publishing House. 2017; 936-946.

[10] Hoang Xuan Thao, Nguyen Thi Kim Ngan. Floral diversity of the sand dune areas of Hai Thien commune, Hai Lang district, Quang Tri province. Hue University Journal of Science: Earth Science and Environment. 2017 Apr; 126(1A): 67-79.

[11] Ho Dac Thai Hoang, Le Thai Hung, Truong Thi Hieu Thao, Tran Khuong Duy, Le Thai Thuy Nhi. Diversity of plant species in Doi Hong, Phan Thiet city, Binh Thuan province. Hue University Journal of Science: Earth Science and Environment. 2018 Dec; 127(4A): 73-86.

[12] Binh Thuan Portal [Internet]. Binh Thuan: Binh Thuan Provincial People's Committee; © 2018 [cited 2020 Oct 26].

[13] Administrative map system [Internet]. Hanoi: Government Electronic Port of Vietnam. © 2016.

[14] Vietnamese National Standards - Technical regulations [Internet]. Hanoi: Portal electronic of the Ministry of Agriculture and Rural Development; © 2005 [cited 2020 Oct 26].

[15] Hydro-meteorological news [Internet]. Hanoi: Vietnam Meteorological Hydrological Administration. © 2015 [cited 2020 Oct 26].

[16] Soils and Fertilizers Research Institute. Handbook of soil, water, fertilizer, crop analysis. Hanoi: Agriculture Publishing House. 1998.

[17] Tran Cong Khanh. Practice of plant morphology and anatomy. Hanoi: University and Professional High School Publishing House. 1981.

[18] Pham Van Ngot, Nguyen Thi Thu Ngan, Quach Van Toan Em. Study on adaptive characteristics of some plants in the sandy soil areas of Phan Thiet city, Binh Thuan province [Final report of scientific projects facility]. HCMC: Ho Chi Minh City University of Education. 2017.

[19] Duong Tien Thach, Phan Thi Dieu. Morphological and anatomical features related to adaptibility of some coastal rock plants in Nhon Ly commune, Quy Nhon city, Binh Dinh province. Journal of Science, Can Tho University. 2018 Aug; 54(6A):20-28.

[20] The Angiosperm Phylogeny Group, Chase MW, Christenhusz MJM, Fay MF, Byng JW, Judd WS, Soltis DE, Mabberley DJ, Sennikov AN, Soltis PS, et al. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. Botanical Journal of the Linnean Society. 2016 May. 181(1): 1-20.

[21] Vo Van Chi. The Dictionary of Common Plants. Hanoi: Science and Technology Publishing House. 2003.

[22] Medicinal Plants - Malvaceace [Internet]. Ho Chi Minh City: Faculty of Pharmacy - University of Medicine and Pharmacy, HCMC; [cited 2020 Oct 26].

[23] Aworinde DO, Ogundairo BO, Osuntoyinbo KF, Olanloye OA. Foliar epidermal characters of some sterculiaceae species in Nigeria Bayero Journal of Pure and Applied Sciences. 2012 June; 5(1): 48-56.