LEAF CONSTANT NUMBERS OF SELECTED *GARDENIA* SPECIES IN THAILAND

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ABSTRACT:

Background: *Gardenia* species is an important medicinal plant with significant ornamental and medicinal potential. Identification of medicinal plants is required to control the quality, efficacy as well as safety of individual plant materials. Leaf constant numbers are the important quantitative microscopic evaluations which can be used to identify and distinguish between some closely related species. Due to the lack of leaf constant data of *Gardenia* species in Thailand therefore, this study aimed to determine the leaf constant parameters in eleven *Gardenia* species in Thailand.

Methods: Fresh mature leaves of eleven *Gardenia* species in Thailand were evaluated for their leaf constants under microscope.

Results: To the best of our knowledge, this is the first report of leaf constant parameters in eleven *Gardenia* species in Thailand. The results of leaf constant parameters including stomatal number, stomatal index, palisade ratio, epidermal cell number, epidermal cell area and trichome number of eleven *Gardenia* species in this study reveal the individual characteristic and can be used to identification and distinguishing of these plant species.

Conclusions: Leaf constant numbers are suitable as a primary means for identification of medicinal plant and could provide useful supportive evidence for plant authentication.

Keywords: Gardenia; Leaf constant numbers; Stomatal index; Palisade ratio; Epidermal cell area; Trichome number

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INTRODUCTION

The genus *Gardenia* is a member of Rubiaceae family consisting of approximately 250 flowering plant species, native to the tropical and subtropical regions of Africa, Asia, Madagascar and Pacific islands [1, 2]. Twenty-two species of *Gardenia* have been found in Thailand, of these thirteen species are native including *Gardenia carinata* Wall. Ex Roxb., *Gardenia collinsiae* Craib, *Gardenia coronaria* Buch. Ham., *Gardenia elata* Ridl., *Gardenia griffithii* Hook. f., *Gardenia magnifica* Geddes, *Gardenia obtusifolia* Roxb. ex Hook. f., *Gardenia philastrei* Pierre ex Pit., *Gardenia saxatilis* Geddes, *Gardenia sootepensis* Hutch, *Gardenia thailandica* Triveng, *Gardenia truncata* Craib, *gardenia tubifera* wall. ex Roxb [3- 4].

The *Gardenia* species have high medicinal value and used in traditional system for the treatment of various ailments such as fever, hypertension, jaundice and ulcer of skin [5, 6]. Additionally, many species of *Gardenia* have been reported for a wide range of their pharmacological activities such as anti-inflammatory, anti-cancer, anti-HIV and anti-apoptotic [7-10]. Because of medicinal and scientific importance of *Gardenia* species, the identity, purity, safety and quality of these plant materials should be established for identification. One of the simplest and the economical methods is microscopic investigation [11]. Microscopic evaluation of leaf constants such as stomatal number, stomatal index and palisade

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ratio are frequency used for the evaluation of crude drugs as well as the medicinal plant samples [11]. However, no previous studies have been reported on the leaf constants of any *Gardenia* species in Thailand. Therefore, this present study aimed to determination of leaf constants including stomatal number, stomatal index, palisade ratio, epidermal cell number, epidermal cell area and trichome number of eleven *Gardenia* species in Thailand.

MATERIALS AND METHODS

Plant materials

Three individual of fresh mature leave of eleven *Gardenia* species namely *G. jasminoides*, *G. carinata*, *G. collinsiae*, *G. griffithii*, *G. lineata*, *G. obtusifolia*, *G. sootepensis*, *G. thailandica*, *G. taitensis*, *G. tubifera* and *G. vietnamensis* were collected from different locations throughout Thailand. Plant samples were identified by one of the authors (N.R.). The voucher specimens of the plant samples have been preserved at College of Public Health Sciences, Chulalongkorn University for future references.

Quantitative microscopy analysis of *Gardenia* species

Microscopic evaluation of leaf constants such as stomatal number, stomatal index, palisade ratio, epidermal cell number, epidermal cell area and trichome number were examined using the methods as described by Evans [12] with some modifications.

Leaves preparation

Fresh mature leave of plant samples were cleaned. The leaf portion between midrib and margin from the middle region was cut into small fragment (10 mm X 10 mm) and immersed in 3% sodium hypochlorite solution until chlorophyll was removed. The plant fragments were then cleared by gentry warming with choral hydrate solution (8 g/ml in distilled water) until the fragments were transparent. The cleared fragment sample was rinsed with distilled water and mounted in 50% glycerin on glass slide and observed under a light microscope (Zeiss Axio Imager A2) with an attached digital camera (Cannon Power shot A640) for the determination of leaf constants. Thirty fields per one individual plant sample were determined using AxioVision program.

Determination of leaf constants

(i) Stomatal number and Stomatal index

The stomatal number is an average number of stomata per square millimeter of epidermis of the leaf. The stomatal index is a percentage of stomata as compared to all the epidermal cells in a same unit area of leaf. Stomatal index was calculated by using formula: Stomatal index = (S/S+E) x100, where, S= number of stomata per square millimeter in a given area of leaf, E= number of epidermal cells per square millimeter in the same area of leaf. The number of stomata and the number of epidermal cell on the lower surface of leaf in 1 square millimeter in each field were counted. The numbers of stomata were counted as stomatal number and the stomatal index using the above formula was calculated.

(ii) Palisade ratio

Group of four epidermal cells was traced under a microscope with a 40x objective lens magnification and 10x eyepiece lens. The palisade cells lying under the four epidermal cells were counted. Including the palisade cell which at least some of its area lies within the area of four epidermal cell was also count as 0.5 unit. The number of palisade cells obtained in each group divided by 4 gives the palisade ratio of that group.

(iii) Epidermal cell number and Epidermal cell area

Epidermal cell number is an average number of epidermal cell per square millimeter of leaf surface. The epidermal cell area is an average area of epidermal cell present in square millimeter. Epidermal cell area was calculated by using formula: Epidermal cell area = 1 mm^2/E, where, E= number of epidermal cells per square millimeter in a given area of leaf. The number of epidermal cell on upper surface of leaf in 1 square millimeter in each field were counted. The numbers of epidermal cells were counted as epidermal cell number and the epidermal cell area using the above formula was calculated.

(iv) Trichome number

Trichome number is an average number of trichome per square millimeter of epidermis of leaf surface. The number of trichome on lower surface of leaf in 1 square millimeter in each field were counted.

Data analysis

All leaf constant parameters were determined at least in thirty fields per one individual plant sample (ninety fields per each species) and the results expressed as mean ± standard deviation (SD).

RESULTS AND DISCUSSION

Plants are the importance sources of valuable phytochemical compounds. Approximately 25% of

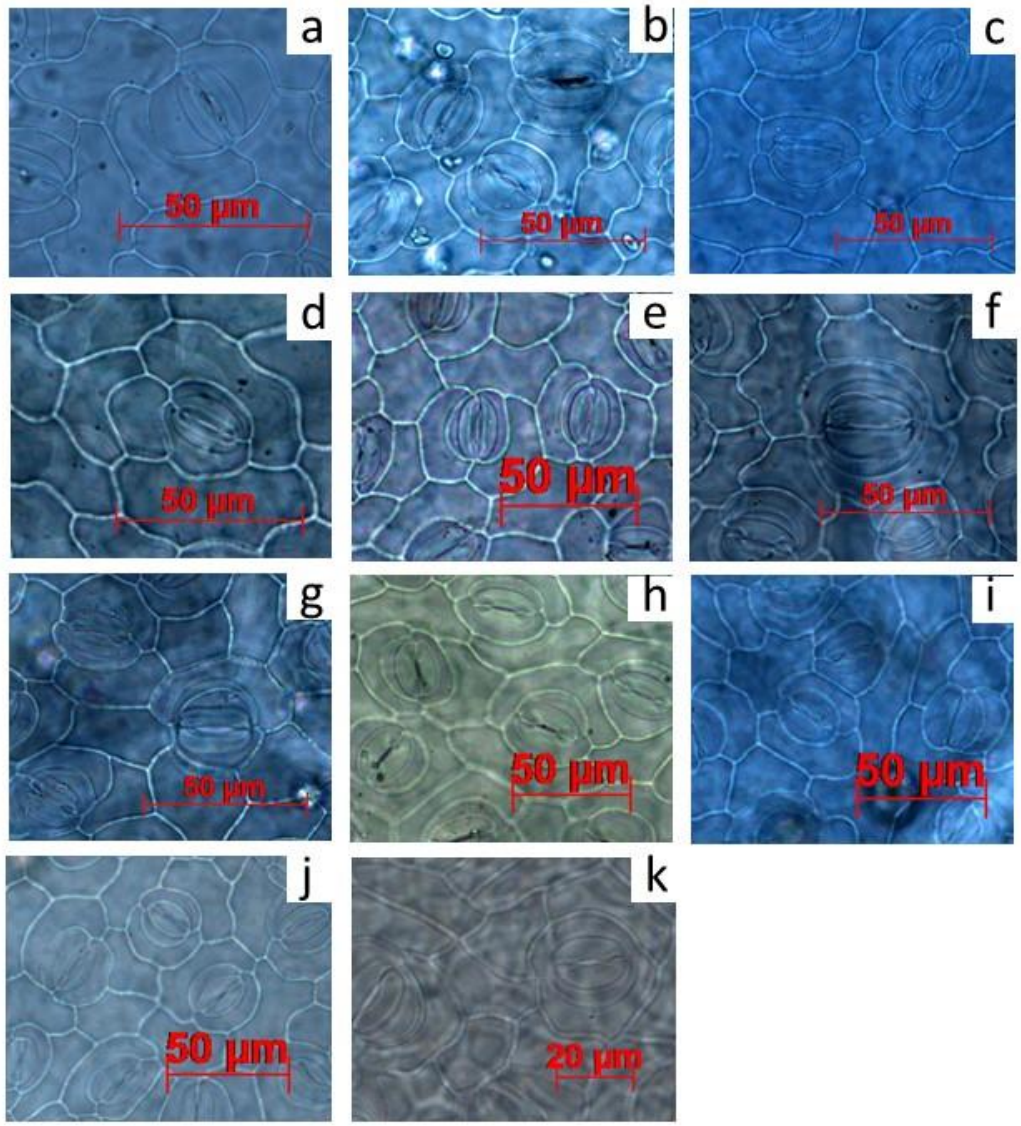

Figure 1 Photographs of paracytic type stomata of eleven *Gardenia* species: *G. carinata* (a); *G. collinsae* (b); *G. griffithii* (c); *G. jasminoides* (d); *G. lineata* (e); *G. obtusifolia* (f); *G. sootepensis* (g); *G. taitensis* (h); *G. thailandica* (i); *G. vietnamensis* (j); *G. tubifera* (k).

the modern medicines are derived from plants sources [13]. Therefore, medicinal plants identification and standardization are required to control the quality, efficacy as well as safety of individual plant materials.

Leaf constant is one of the important quantitative microscopic evaluations which can be used to identify and distinguish between some closely related species not easily characterized by qualitative microscopic evaluations [12]. Various parameters of leaf measurements are generally determined in plant materials including stomatal number, stomatal index, palisade ratio, vein-islet number, vein-termination number, epidermal cell area, epidermal cell number and trichome number. The mature fresh leaves of eleven *Gardenia* species were subjected to quantitative analysis for various leaf constants including type of stomata, stomatal number, stomatal index, palisade ratio, epidermal cell number, epidermal cell area and trichome number. The results were presented in Table 1-2 and Figure 1-4.

All of eleven *Gardenia* species showed the paracytic type of stomata as shown in Figure 1. The distribution of the stomata is hypostomatic in all eleven species (stomata occurring only on lower surface). In this study, the highest stomatal index (32.62±1.37) was found in *G. taitensis* and the lowest (17.24±0.67) in *G. tubifera* (Table 1). The

Table 1 Stomatal number, stomatal index and palisade ratio of eleven *Gardenia* species

Gardenia species	Stomatal number		Stomatal index		Palisade ratio	
	min-max	mean±SD	min-max	mean±SD	min-max	mean±SD
G. tubifera	400-575	466.39±33.34	15.69-19.17	17.24±0.67	3.25-4.75	4.12±0.34
G. carinata	312-484	380.49±30.05	15.56-24.41	19.58±1.48	3.62-5.75	4.60±0.52
G. vietnamensis	216-312	261.73±23.08	15.63-25.09	20.73±1.88	7.50-8.88	8.13±0.38
G. jasminoides	212-368	277.60±31.28	14.66-25.61	21.47±1.88	9.62-11.62	10.44±0.36
G. collinsae	376-444	405.73±11.38	21.93-25.06	23.59±0.50	9.75-11.50	10.49±0.42
G. griffithii	400-456	417.07±12.87	24.41-25.97	25.23±0.38	10.00-11.62	10.67±0.39
G. sootepensis	356-448	403.47±17.79	24.81-27.92	26.02±0.74	11.50-14.62	12.84±0.65
G. lineata	380-444	415.96±14.07	25.64-28.42	27.04±0.63	12.00-14.25	12.97±0.49
G. thailandica	508-588	537.91±18.85	27.79-29.30	28.40±0.33	12.12-14.62	13.30±0.46
G. obtusifolia	524-700	583.38±39.80	27.02-30.79	28.64±0.66	14.88-17.25	15.96±0.55
G. taitensis	292-344	319.29±12.69	29.80-35.56	32.62±1.37	16.62-21.00	18.80±0.88

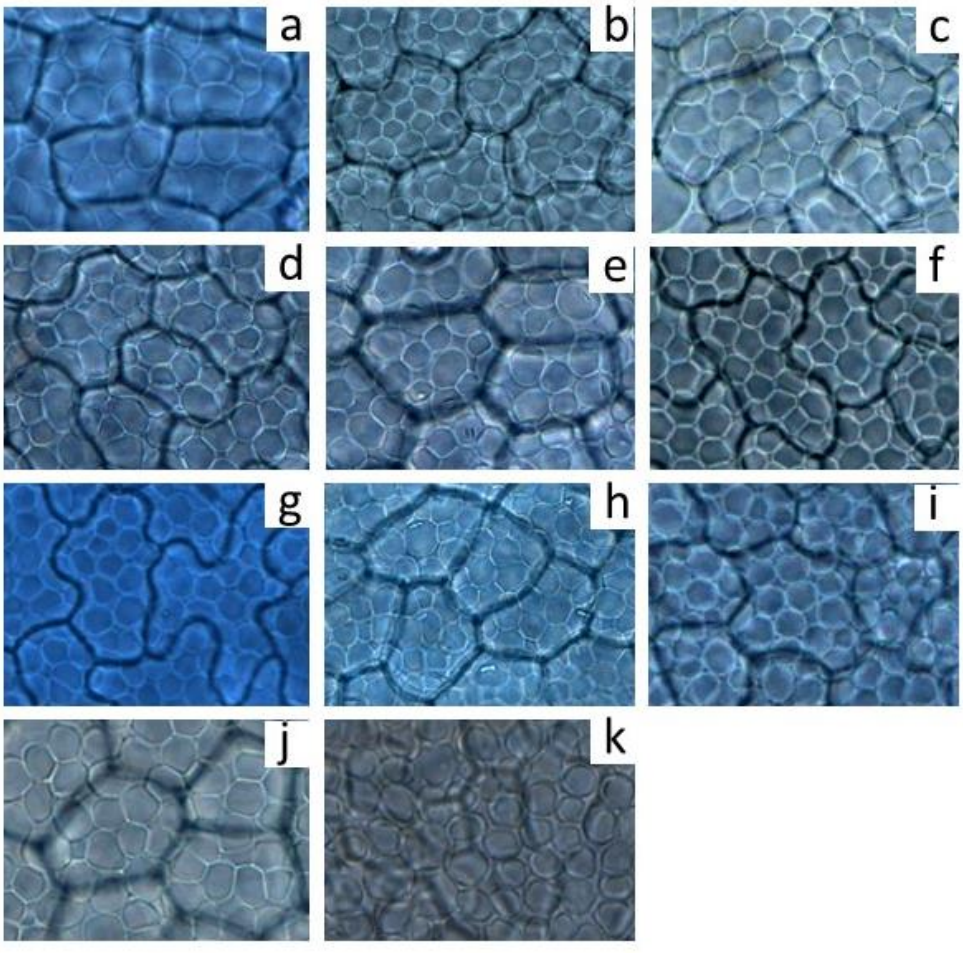

Figure 2 Photographs of palisade cells of eleven *Gardenia* species: *G. carinata* (a); *G. collinsae* (b); *G. griffithii* (c); *G. jasminoides* (d); *G. lineata* (e); *G. obtusifolia* (f); *G. sootepensis* (g); *G. taitensis* (h); *G. thailandica* (i); *G. vietnamensis* (j); *G. tubifera* (k).

stomatal number varies depend on species, leaf age, environmental condition and geographical sources where the plants were grown [14- 15]. The early study of stomatal number by Timmerman in 1927 showed that this value is usually useless for distinguishing between closely species because stomatal number varies considerably with the age of leaf and due to changes in climatic conditions [16].

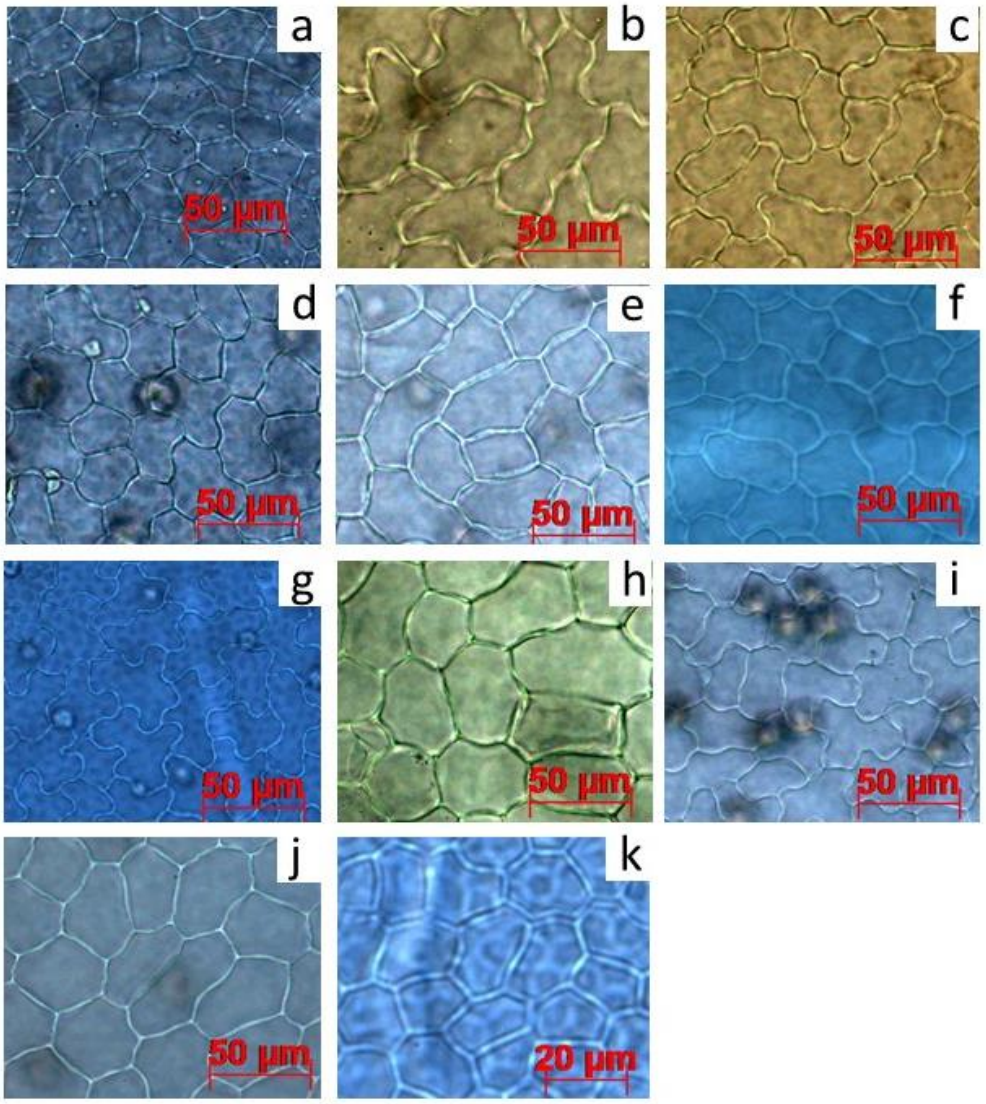

Figure 3 Photographs of epidermal cells of eleven *Gardenia* species: *G. carinata* (a); *G. collinsae* (b); *G. griffithii* (c); *G. jasminoides* (d); *G. lineata* (e); *G. obtusifolia* (f); *G. sootepensis* (g); *G. taitensis* (h); *G. thailandica* (i); *G. vietnamensis* (j); *G. tubifera* (k).

However, stomatal index is highly constant for a given species which is not affected by age of plant, size of leaf and environmental conditions [12, 17]. Stomatal index is useful in differentiation of closely related species and also for detection of adulterants.

The other importance leaf constants parameters is palisade ratio, it can be determined on fine powders but cannot applicable to determine on monocot leaves, as the differentiation in mesophyll cell is not possible in monocot plants. This value does not alter based on geographical variation and differs from species to species. Therefore, this value remains constant for a given plant species and is also useful diagnostic feature for characterization and identification of different plant species [15, 17]. In this study, the largest palisade ratio was found in *G. sootepensis* (18.80±0.88) and the lowest was found in *G. tubifera* (4.12±0.34) (Table 1). The palisade cell of eleven *Gardenia* species was showed in Figure 2.

The epidermal cell of eleven *Gardenia* species were showed in Figure 3 and the epidermal cell number, epidermal cell area and trichome number were showed in Table 2. *Gardenia tubifera* exhibited the highest epidermal cell number (4261.0±80.04) and the lowest epidermal cell area (234.7±4.39 µm^2) whereas *G. collinsae* exhibited the lowest epidermal cell number (439.6±17.19) and the highest epidermal cell area (2278.5±89.48 µm^2). Epidermal cell areas are relatively constant within a

Table 2 Epidermal cell number, epidermal cell area and trichome number of eleven *Gardenia* species

Gardenia	**Epidermal cell number**		**Epidermal cell area (μm^2)**		**Trichome number**	
species	min-max	mean±SD	min-max	mean±SD	min-max	mean±SD
G. tubifera	4100.0-4400.0	4261.0±80.04	227.27-243.90	234.7±4.39	8-16	11.11±2.92
G. carinata	1940.0-2316.0	2119.3±70.09	431.78-515.46	472.4±15.36	-	-
G. griffithii	1184.0-1652.0	1420.3±112.61	605.33-844.59	708.4±54.88	-	-
G. thailandica	1264.0-1396.0	1331.8±34.67	716.33-791.14	751.4±19.59	-	-
G. obtusifolia	1084.0-1404.0	1262.7±61.57	712.25-922.51	793.8±39.07	-	-
G. jasminoides	884.0-1116.0	1028.2±53.63	896.06-1131.22	975.2±52.87	-	-
G. lineata	940.0-1124.0	1015.3±36.81	889.68-1063.83	986.1±34.97	-	-
G. sootepensis	804.0-936.0	887.1±27.06	902.53-1243.78	1076.1±82.61	-	-
G. taitensis	748.0-912.0	826.5±46.65	1096.49-1336.90	1213.8±67.98	-	-
G. vietnamensis	652.0-772.0	714.0±28.54	1295.34-1533.74	1402.7±56.17	-	-
G. collinsae	400.0-476.0	439.6±17.19	2100.84-2500.00	2278.5±89.48	12-28	17.95±3.71

Figure 4 Scanning electron microscope of trichome of *Gardenia sootepensis*: non glandular, unicellular trichome occurred on lower surface

narrow range for each species that allows a correct identification even though some degree of overlapping with closely related species. These values were used as a taxonomic tool for the identification of plant materials such as *Stanhopea* species (Orchidaceae) [18].

Trichomes have been defined as epidermal protuberances that founded on the leaves, stems, flowers, fruits, seeds, petals, stalks and peduncles of plants [12, 19]. The morphological characters of trichomes and their density are taxonomic important for identification of plant samples [20-21]. Trichomes number was induced variation by seasonal and environmental factors [22]. Distribution of non-glandular trichomes, unicellular and simple unbranched on over the lower surface of leaf were observed only in *G. sootepensis* and *G. tubifera* (Figure 4 and Table 2). In this study, trichome number can be used to separated eleven *Gardenia* species into 2 groups, group 1 consists of two species (*G. sootepensis* and *G. tubifera*) which has densely of trichome distribution on lower surface of leaf whereas group 2 has no trichome distribution on the lower surface of leaf consisting of nine species of *Gardenia* (*G. jasminoides*, *G. carinata*, *G. collinsiae*, *G. griffithii*, *G. lineata*, *G. obtusifolia*, *G. thailandica*, *G. taitensis*, and *G. vietnamensis*). The high density of trichomes on the lower surface of *G. sootepensis* and *G. tubifera* is of taxonomic interest and could be used to delimit the taxon from the other species. Kemka, C.I. used trichome density to classified eight *Crassocophalum* species [23].

In recent study, this is the first report of leaf constant parameters of the stomatal number, stomatal index, palisade ratio, epidermal cell number, epidermal cell area and trichome number of eleven *Gardenia* species in Thailand. These values could be used for identification and separation of *Gardenia* species from each other.

CONCLUSION

Leaf constant parameters are suitable as a primary means of identification of a sample and can

provide very useful supportive evidence. The established on leaf constant like stomatal number, stomatal index, palisade ratio, epidermal cell number, epidermal cell area and trichome number of eleven *Gardenia* species under examined can serve as an important tool for the identification parameters of this plant. These parameters can make a positive evaluation and identification when taken together with other factors. Additionally, these parameters may be useful as quality control standards.

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