Study of Phytochemical Composition on *Kaempferia parviflora* Wall. ex Baker*

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ABSTRACT

Kaempferia parviflora Wall. ex Baker belongs to the family Zingiberaceae. *Kaempferia parviflora* Wall. ex Baker is known as the nannwin net or sannwin net in Myanmar. *Kaempferia parviflora* Wall. ex Baker was collected from Kyaukse township, Mandalay Region. Morphological, histological and phytochemical studies of *Kaempferia parviflora* Wall. ex Baker were carried out, to get their correct identification. In morphological study, this plant was perennial rhizomatous herbs. Leaves were simple and alternate. The aerial pseudo-stem formed by leaf-sheaths. Inflorescences was tubular spike, axillary, with 1-2 flowers. In histological studies, stomata are present on both surfaces but numerous on lower surface than on the upper surface, tetracytic types. The vascular bundles of midrib and petiole are arranged in a crescent shape, collateral type**.** The vascular bundles of stem are arranged in a ring shape. In the phytochemical studies in the rhizome of *Kaempferia parviflora* Wall. ex Baker showed the presence of alkaloid, glycoside, flavonoid, phenol, polyphenol, lipophenol, saponin, tannin, terpene, steroid but reducing sugar is absent.

Keywords : *Kaempferia parviflora* Wall. ex Baker., morphological, histological, phytochemicals

1 INTRODUCTION

Zingiberacaeae family is the largest family of the order Zingiberales. It is widely distributed throughout the tropics particularly in Southeast Asia. In Southeast Asian region, several species of Zingiberaceae are used as spices, traditional medicines, flavoring agents and as source of certain dyes (Tewtrakul and Subhadhiasakul 2007).

Kaempferia parviflora Wall. ex Baker (Thai black ginger, Thai ginseng or Krachaidum) is a herbaceous plant in the family Zingiberaceae native to Thailand. In folk medicine of Suriname and Southeast Asia, rhizome is used for treatment of swelling, wounds and diarrhea, colic disorder, and as an aphrodisiac. Rhizomes of ginger root or *Kaempferia parviflora* Wall. ex Baker are used a food ingredients and made into wine (Nagahara *et al*. 2002).

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Zingiberaceae, *Kaempferia* is commonly grows in the tropical Asia. The genus *Kaempferia* L. (Zingiberaceae) is one of the most important medicinal plant. *Kaempferia* is a medium size genus with approximately 50 species mostly distributed from India, South China to Malaysia. *Kaempferia parviflora* Wall. ex Baker black ginger, Thai ginseng is an herbaceous plant in the family Zingiberaceae native Thailand. Rhizomes are employed against cough, stomachache, asthma and also as a vermifuge (Devi *et al*. 2016).

Scientifically, it is known as *Kaempferia parviflora* Wall. ex Baker in Japan it is commonly referred as "black turmeric" or "black ginger". In South East Asia region, especially in Thailand, people drink tea boiled from sliced black ginger as well as alcohol soaked with black ginger. Black ginger is commonly used a folk medicine for gastrointestinal complaints (Anonymous 2012).

Kaempferia parviflora Wall. ex Baker plant is a perennial herb with dark purple to black rhizome and these color lead to its rhizome. Its rhizome has been used as a folk medicine for the treatment of a wild variety of illness. There are many therapeutic function of *Kaempferia parviflora* Wall. ex Baker that have been reported in the antimicrobial, aphrodisiac effect, antigastric ulcer, anti-obesity effects and antioxidant effects (Wattanasri 2016).

Kaempferia parviflora Wall. ex Baker is one of the plants in the Zingiberaceae family originated from Thailand. In its origin, it is known as kra-chai-dam, Thailand ginseng, or black galingale. Recently, *Kaempferia parviflora* Wall. ex Baker has been reported to possess anti-mycobacterial, anti-plasmodial, anti-peptic ulcer and anti-viral protease effects as well as modulators of multi-drug resistance in cancer cells. Because of its pharmacological benefits and the increasing trend of herbal consumption in Indonesia, *Kaempferia parviflora* Wall. ex Baker is potentially developed in Indonesia (Rujjanawate *et al.* 2005).

The use of medicinal plants as herbal drugs is increasing rapidly. Ginger is the common name given to members of the Zingiberaceae family, a group of tropical, rhizomatous, herbaceous, perennial which have been gained much notoriety in the list of medicinal plants. The rhizome of species from this family are known to have many pharmacological values (Patanasethanont *et al.* 2007).

Kaempferia parviflora or Krachaidum (in Thai), also known as "Thai ginseng," is a medicinal plant in the family Zingiberaceae. It is found in tropical areas such as Malaysia, Sumatra, Borneo Island, and Thailand. Its rhizome has been long used as folk medicine for many centuries. A number of pharmacological studies of Krachaidum have shown the following properties: anti-inflammatory, antimutagenic, antidepressive, anticholinesterase, antimicrobial, anticancer, anti-peptic ulcer, cardioprotective, antiobesity activity, and aphrodisiac (Saokaew *et al*. 2016).

2 MATERIALS AND METHODS

2.1 Collection, Identification and Preparation of *Kaempferia parviflora* Wall. ex Baker

The specimens of *Kaempferia parviflora* Wall. ex Baker were collected from Moe Kaung village, Sintgaing Township, Kyaukse District, Mandalay Region. The collected plants were taxonomically identified with the help of references literature such as Hooker 1885 and Dassanayake 1987. The fresh specimens were pressed, dried and preserved for morphological studies.

IEEE-SEM, Volume 7, Issue 7, July-2019
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2.2 Histological studies of *Kaempferia parviflora* Wall. ex Baker

The fresh specimens were examined by preparing free hand sections and studied under microscope. The histological characters of fresh specimens of leaves and stems were prepared by the literature of Metcalfe and Chalk (1957), Esau (1965). The following reagents were used to examine the sections and powered samples.

1. Chloral hydrate solution for clearing agents.
2. Phloroglucinol and hydrochloric acid for testing lignin.
3. Concentrated sulphuric acid for testing calcium oxalate crystals.
4. Iodine solution for testing starch.

2.3 Preliminary phytochemical investigation of *Kaempferia parviflora* Wall. ex Baker

Preliminary phytochemical investigation was carried out at Department of Chemistry, Mandalay University, according to Harbone 1984. It was carried out for the rhizome of with a view to determine the constituents of alkaloids, glycosides, flavonoids, phenolic compounds, polyphenols, reducing sugars, saponins, steroids, tannin and terpene.

3 RESULTS

3.1 Morphological Studies of *Kaempferia parviflora* Wall. ex Baker

Scientific name - *Kaempferia parviflora* Wall. ex Baker
Myanmar name - Nannwin net, Sannwin net
Family - Zingiberaceae
Flowering period - September to December

Perennial rhizomatous herbs, 0.6-0.8 m high, rhizomes fleshy, branched, black. The aerial pseudo-stem formed by leaf-sheaths. Leaves simple, alternate, tuft base, 10.0-15.5 cm long, 5.0-12.5 cm wide; petioles long, 8-22 cm long, 0.3-1.0 cm wide, pale green, glabrous; leaf blade elliptic, acute at apex, green, glabrous on both surfaces; upper surface is green and lower surface is pale green. Inflorescence tubular spike, axillary, substanded by two bracts; lanceolate 3-4cm long. Flower bisexual, zygomorphic, epigynous, 4-6 cm long, white, center purple; pedicles 3.5-4.5 cm long. The most conspicuous part of the flower is two or three lobed lip labellum formed by the fusion of two staminode. Calyx tubular, glabrous or pubescent, apex acuminated split into equally lobes. Corolla tube thin and slender, white. Fertile stamen short; filament very short, flattened, anther dithecous, crested orbicular entire, about 1.5 cm long, yellow, basified connective prolonged, spur absent. Labellum emarginated, bright violet; Ovary inferior, pale yellow, pubescent, trilocular; style filiform, white. Fruit unknown.

Specimen examined : Kyaukse Township, Mandalay Region, Put Thaing Village

3.2 Histological studies of *Kaempferia parviflora* Wall. ex Baker

3.2.1 Lamina

In surface view of lamina, the upper epidermal cells and lower epidermal cells are covered with thin cuticle. The upper epidermal cells are parenchymatous, anticlinal thin-walled, straight, polygonal in shape, 30-54 µm long and 24-46 µm wide. The lower

IEEE-SEM, Volume 7, Issue 7, July-2019
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epidermal cells are parenchymatous, anticlinal thin-walled, straight, polygonal in shape, 35-68 µm long and 25-40 µm wide. Tetracytic type of stomata are present on both surfaces, numerous on lower surface than on the upper surface. The guard cells are reniform in shape.

In transverse section of lamina, mesophyll cells are differentiated into palisade parenchyma and spongy parenchyma. Palisade parenchyma consists of a two layer, oval shaped, thin walled, the cells 35-25 µm long and 15-20 µm wide. Spongy parenchyma consist three to five layers, irregular in shape, the cells 20-35 µm long and 15-28 µm wide. It contains calcium oxalate crystals.

Vascular bundles are embedded in the mesophyll cells, bundles collateral type. Xylem composed of spirally thickened vessel, tracheid, xylem fiber and xylem parenchyma cells. Phloem consists of sieve-tube, companion cell, phloem fiber and phloem parenchyma cell.

3.2.2 Midrib

In surface view of midrib, the epidermal cells are thin walled, parenchymatous, compactly arranged, polygonal in shaped, the cells 36-48 µm long and 28-48 µm wide. Tetracytic type of stomata are present.

In transverse section of midrib, epidermis is a single layer, the cells barrel in shape. Cortex consists of collenchyma and parenchyma. Collenchyma are two to three layers, the cells oval or rounded in shape, 24-36 µm long and 12-30 µm wide. Parenchyma are four to five layers, the cells oval or rounded in shape, 48-66 µm long and 30-40 µm wide.

Vascular bundles are arranged in a crescent shape, collateral type. Phloem are four to five layers, the cells 12-18 µm in vertical diameter and 18-26 µm in horizontal diameter, consists of sieve tube, companion cells, phloem fiber, phloem parenchyma. Xylem are arranged in radial row of three to four layers, it consists of vessels, tracheids, xylem fiber, xylem parenchyma.

3.2.3 Petiole

In surface view of petiole, the epidermal cells are thin walled, parenchymatous, polygonal in shape, the cells 30-42 µm long and 28- 34 µm wide cuticle. Stomata are present.

In transverse section of petiole, epidermis is a single layer, the cells barrel in shape. Cortex consists of collenchyma and parenchyma. Collenchyma are two to four layers, the cells oval or rounded in shape, 20-35 µm long, 12-36 µm wide. Parenchyma are three to five layers, the cells oval or rounded in shape,45-68 µm long, 32-48 µm wide. It contains calcium oxalate crystals.

Vascular bundles are arranged in a crescent shape, collateral type. Phloem are three to five layers, the cells 10-16 µm in vertical diameter and 15-25 µm in horizontal diameter, consists of sieve tube, companion cell, phloem fiber and phloem parenchyma. Xylem are arranged in radial rows of three to four layers, the cells 10-16 µm in vertical diameter and 15-25 µm in horizontal diameter. It contains of vessels, tracheids, xylem fiber and xylem parenchyma.

3.2.4 Stem

In surface view of stem, the epidermal cells are thin walled, parenchymatous, rectangular to polygonal in shaped, the cells 50-60 µm long and 25-40 µm wide with cuticle. It contains calcium oxalate crystals.

In transverse section of stem, epidermis is a single layer, the cells barrel in shape.

IEEE-SEM, Volume 7, Issue 7, July-2019
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Cortex consists of collenchyma and parenchyma. Collenchyma are two to four layers, the cells 32-48 µm long, 28-40 µm wide. Parenchyma are four to five layers, the cells oval or rounded in shape, 50-70 µm long, 30-45 µm wide. It contains few starch grains.

Vascular bundles are arranged in a crescent shape, collateral type. Phloem are three to five layers, the cells 8-14 µm in vertical diameter and 10-20 µm in horizontal diameter, consists of sieve tube, companion cell, phloem fiber and phloem parenchyma. Xylem are arranged in radial rows of two to three layers. It consists of vessels, tracheids, xylem fiber and xylem parenchyma.

3.2.5 Rhizomes

In transverse section of rhizome, epidermis is a single layer, the cells barrel in shape. Cortex consists of collenchyma and parenchyma. Collenchyma are three to four layers, the cells 30-45µm long, 25-35 µm wide. Parenchyma are four to five layers, the cells oval or rounded in shape, 60-82 µm long, 34-46 µm wide. It contains chloroplast and few starch grains.

Vascular bundles are arranged in a quadrangular in shape, collateral type. Phloem are three to six layers, the cells 7-13 µm in vertical diameter and 10-20um in horizontal diameter, consists of sieve tube, companion cell, phloem fiber and phloem parenchyma. Xylem are arranged in radial rows of two to three layers. It consists of vessls, tracheids, xylem fiber and xylem parenchyma.

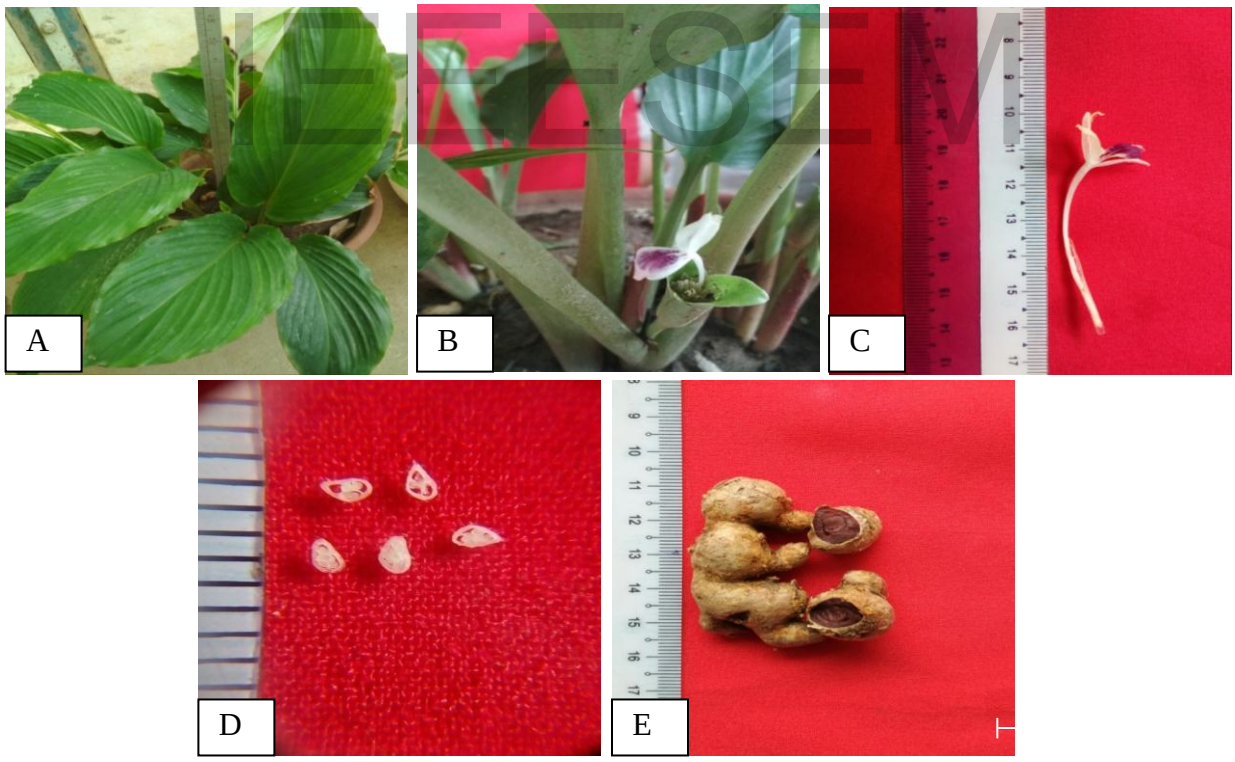

Figure 1. Morphological Studies of *Kaempferia parviflora* Wall. ex Baker
A. Habit B. Inflorescence C. Flower
D. C S Ovary E. Rhizome

IEEE-SEM, Volume 7, Issue 7, July-2019
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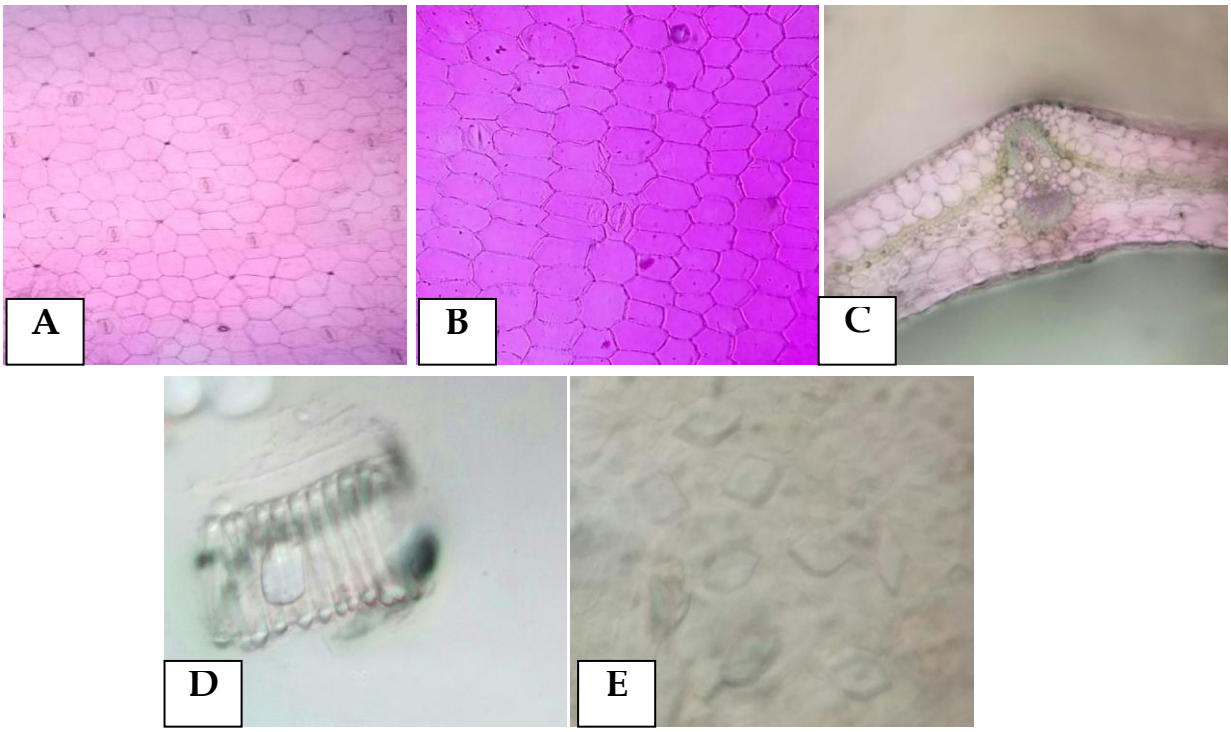

Figure 2. Histological studies of lamina and powder
A. Lower surface of lamina B. Upper surface of lamina
C. Transverse section of lamina D. Vessel
E. Crystal

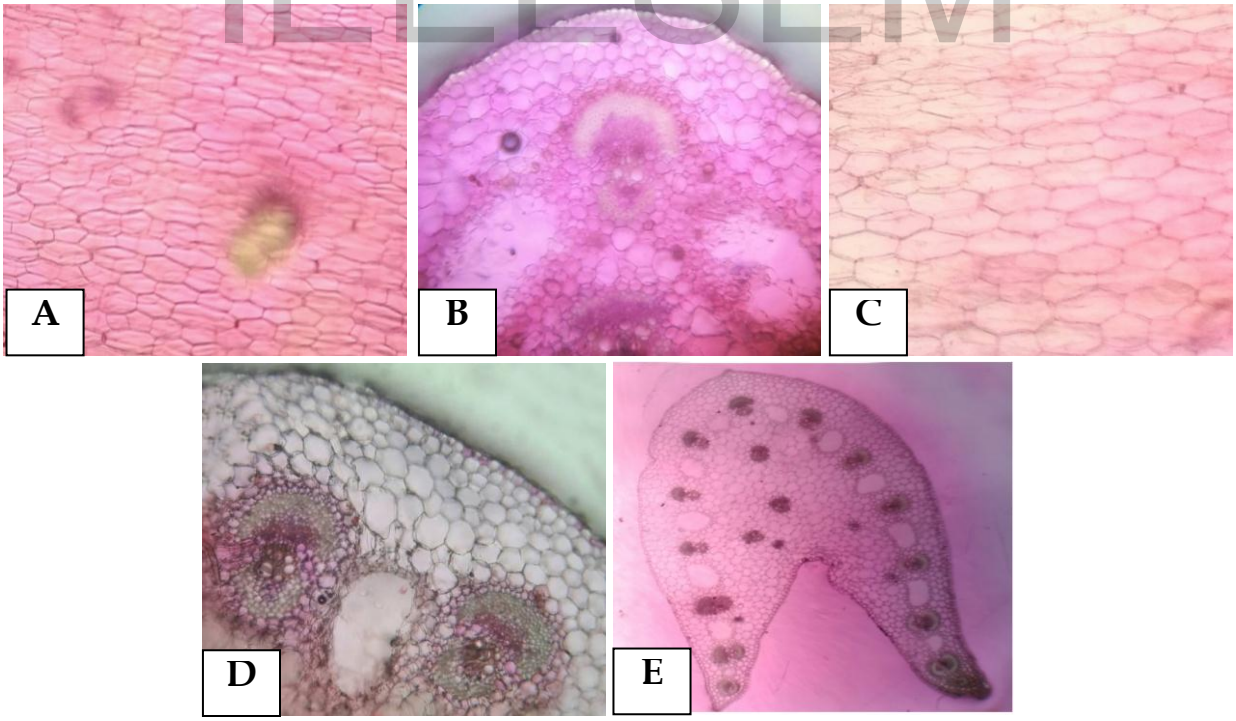

Figure 3. Histological studies of midrib and petiole
A. Surface view of midrib B. Transverse section of midrib
C. Surface view of petiole D. Transverse section of petiole
E. Outline of petiole

IEEE-SEM, Volume 7, Issue 7, July-2019
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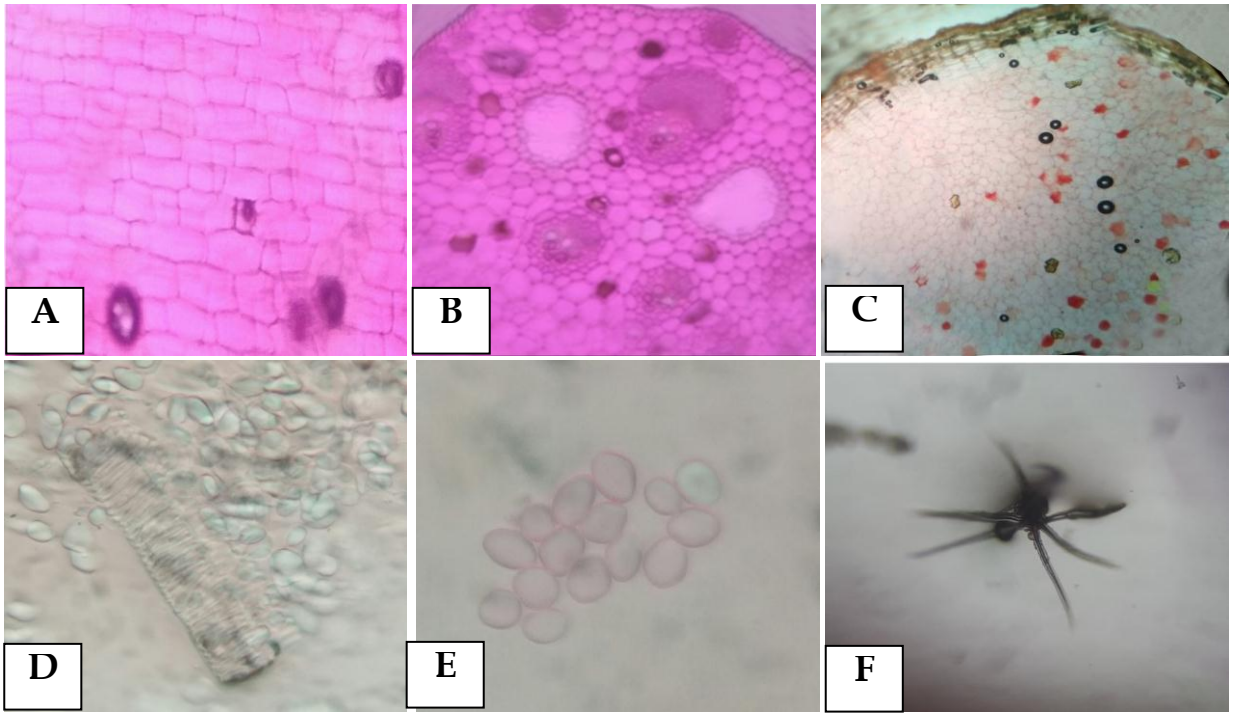

Figure 4. Histological studies of stem and rhizome powder
A. Surface view of stem B. Transverse section of stem
C. Transverse section of rhizome D. Vessel
E. Starch F. Stellate hair

3.3 Preliminary Phytochemical Properties of *Kaempferia parviflora* Wall. ex Baker

Preliminiary phytochemical properties were carried out for the *Kaempferia parviflora* Wall. ex Baker with a view to determine the presence of the constituent of alkaloid, glycoside, flavonoid, phenol, polyphenol, lipophenol, saponin, tannin, terpene and steroid. However, reducing sugar is absent. The results were present in Table 1.

Table 1. Preliminary phytochemical tests for rhizome of *Kaempferia parviflora* Wall. ex Baker

No	Constituents	Extract	Test Reagent	Observation	Result
1	Alkaloid	Water	Dragendroff's reagent Wagnar reagent	Greenish Greenish	+
2	Glycoside	Water	10%$(CH_3CO)_2$ Pb	Brick red ppt.	+
3	Flavonoid	Ethanol	Conc. HCl, Mg	Reddish brown	+
4	Phenol	Water	10%$FeCl_3$	Reddish brown	+
5	Polyphenol	Ethanol	1%$FeCl_3$,$K_3Fe(CN)_6$	Greenish brown	+
6	Lipophenol	Water	0.5N KOH	Red solution	+
7	Reducing Sugar	Water	Benedict's solution	Green yellow	_
8	Saponin	Water	Shake	Froth	+
9	Tannin	Water	10%$FeCl_3$	Reddish brown	+
10	Terpene	Ethanol	$(CH_3CO)_2O$,$CHCl_3$, conc H_2SO_4	Reddish	+
11	Steroid	Ethanol	$CHCl_3$,Conc.H_2SO_4	Two layer red ppt.	+

(+) = present (-) = absent

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4 DISCUSSION AND CONCLUSION

Kaemperia parviflora Wall. ex Baker is widely cultivated throughout the tropical region of Myanmar. It is one of the species in Zingiberaceae family. In the present work, the morphological and histological characters of *Kaemperia parviflora* Wall. ex Baker.

In morphological studies, *Kaemperia parviflora* Wall. ex Baker is perennial rhizomatous herbs. The stems are aerial pseudo-stem formed by leaf-sheaths. The leaves are alternate, simple, leaf blade, elliptic, acute at apex, green glabrous on both surface. These characters are similar to those given by Lawrence 1965.

For *Kaemperia parviflora* Wall. ex Baker Inflorescences are tubular spike axillary. Flowers are white, center purple, bisexual, zygomorphic. These characters are similar to those given by Dassanayake 1987.

For *Kaemperia parviflora* Wall. ex Baker, ovary is inferior, pubescent, trilocular, axile placentation. Style is filiform, white. Which are in agreement with those given by Dassanayake 1987.

In the histological studies, *Kaemperia parviflora* Wall. ex Baker the leaves are dorsiventrally, tetracytic type of stomata are present on both surfaces of the lamina but usually more numerous on the lower surface than on the upper surface. The epidermal cells are parenchymatous, anticlinal thin walled, straight, polygonal in shape. These characters are agreed with those given by Metcalfe and Chalk 1957.

In the midrib of *Kaempferia parviflora* Wall. ex Baker, the epidermal cells are thin walled, parenchymatous, compactly arranged, polygonal in shaped. Cortex consists of collencyma and parenchyma. The vascular bundles are arranged in a crescent shaped, collateral type. These characters are agreed with those given by Esau 1965.

In the petiole, *Kaempferia parviflora* Wall. ex Baker, the epidermal cells are thin walled, parenchymatous, compactly arranged, polygonal in shaped. Cortex consists of collencyma and parenchyma. The vascular bundles are arranged in a crescent shaped, collateral type. These characters are agreed with those given by Metcalfe and Chalk 1975.

In the stem, *Kaempferia parviflora* Wall. ex Baker, the epidermal cells are thin walled, parenchymatous, compactly arranged, polygonal in shaped. Cortex consists of collencyma and parenchyma. The vascular bundles are arranged in a crescent shaped, collateral type. These characters are similar to those given by Esau 1965.

According to the results, phytochemical studies on the rhizome of *Kaemperia parviflora* Wall. ex Baker showed the presence of a wide of secondary metabolites such as alkaloid, glycoside, flavonoid, phenol, polyphenol, lipophenol, saponin, tannin, terpene and steroid. Reducing sugar are absent.

In Myanmar, a large number of medicinal plants are found as natural resources. Local people are identifying plants based mostly on morphological characters, but they cannot be able to identify the dry parts of the medicinal plants.

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