Chrysopogon zizanioides (L.) Roberty (Gramineae) Part I. Pharmacognostic Identification of Roots

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ABSTRACT

The purpose of this study is to identify the roots of yah faek (vetiver grass) or Chrysopogon zizanioides (L.) Roberty (syn. Vetiveria zizanioides (L.) Nash. ex Small) by taxonomic and pharmacognostic identification. Seven local cultivars of vetiver roots, viz., Srilangkha, Maehea, Indian, Yeepoon, Monto, Maeteay and Prarachataan were collected from similar habitats. Microscopic study revealed differences in the characteristics of parenchyma cells, collenchyma cells, cork cells, sclereid cells, vascular bundles and trichomes as well as starch grains, calcium oxalate crystals, oil granules, etc. This technique can be used to identify, characterize and distinguish the roots of yah faek.

Key words: Yah faek, Vetiver grass, *Chrysopogon zizanioides*, *Vetiveria zizanioides*, Pharmacognostic identification

INTRODUCTION

H.M. King Bhumibhol's visions on soil and water conservation strive enrich watersheds and prevent soil erosion. H.M. King Bhumibhol wants to use simple agricultural techniques and vetiver grass (*Chrysopogon spp.*) was brought in to conserve water and riverbank stability. Vetiver grass propagates easily, requires less aftercare, absorbs moisture in its roots, holds soil nitrogen and possesses ability to filter the toxins and chemicals which are otherwise discharged to rivers. Many countries in Asia use this grass to conserve bank soil erosion successfully. Though vetiver grass has many useful purposes, the negative effects of its distribution should be considered. The benefits of vetiver grass in other aspects, especially applied usages, should be studied to help balance nature.

The genus *Chrysopogon* (Gramineae) has a total of 26 species in the world, 2 of which are found in Thailand. The two vetiver grass in Thailand, "Yah faek don" - *Chrysopogon nemioralis* (Balan.) Holtt. Camus and "yah faek hawm" – *C. zizanioides* (L.) Roberty, are characterized by distinguished flower morphology and anatomy of their roots and leaves. The term *zizanioides* means riverside. *C. zizanioides* (L.) Roberty can grow in swamps and can endure 45 days in flood, but also grows on hills or mountains and resists drought for several months. Vetiver grass had rhizome buds which are used to propagate and easy to control. Germination by seeds is not common. This grass does not tend to become a noxious weed. Pruning techniques are applied to promote root and leaf growth and to retard and stunt the flowers that can cause outbreeding and mutations. Furthermore, this grass can grow vigorously on any kind of soil and local climate. Consequently, *C. zizanioides* (L.) Roberty is a species

which is very interesting to study. Thai villagers use vetiver grass as mulching material and to make mattresses, brooms, baskets, bags, fans, roof material, paper pulp and insect repellent in wardrobes. Yah faek hawm roots have volatile oil which is hot and spicy. Thai herbalists use vetiver grass to prepare medicine, viz., heart tonic, digestive tract cleaning, bloat relief, exhaustion relief and urine purity.

Some farmers have developed techniques to plant medicinal plants and aromatic trees in Malaysia and India by planting palm aroma (*Cymbopogon martini* Khus), *Chrysopogon zizanioides* (L.) Roberty, lemon grass *(Cymbopogon flexuosus* L.*)*, ocimum basil (*Ocimum sanctum* Linn.) and anise (*Pimpinella anisum* Linn.) in home gardens. These plants have high quality and are easy to extract their volatile oil compared to other regions in the world.

The identification of vetiver grass strains in Thailand is not yet clear. The names will follow the rules in the International Codes of Botanical Nomenclature: ICBN. The study of the taxonomic and pharmacognostic identification will help elucidate the vetiver strains systematically.

MATERIALS AND METHODS

Seven cultivars of vetiver grass - Srilaangkhaa, Maehae, Indian, Yeepun, Monto, Maeteay and Parachataan - were obtained from the Highland Development Office, Chiang Mai. The collection method and criteria are as follow :

- Collect plants that have leaves, flowers and fruits for identification
- Collect root and remove all dirt
- Collect the whole plant
- Choose about 5 blooming and just-budding flowers, with leaves and fruits if possible
- Choose about 5 young and ripen fruits with leaves
- If the specimen is not complete, collect more in other seasons with note attached
- Collect at least 5 duplicates, i.e.,
  - 2 duplicates for taxonomic identification
  - 3 duplicates for herbarium specimens
- Collect enough samples for studies in antimicrobial and anticancer activities

Preparation of vetiver powder

Vetiver powder is studied under a microscope. The powder is made from the root fibers from each cultivar. Vetiver roots were chopped into pieces, then dried in an oven at 40–60°C and ground to pass a no. 60 sieve. The microscopic characteristics of vetiver powder were studied, using a microscope.

Preparation of stain solutions

The stain solutions are solutions that are appropriate for each kind of cell to distinguish the tissue. The stain solutions for medicinal plant powder are specific to each constituent as described below :

1. Distilled water : tests parenchyma cells, starch, crystals and other basic cell components.
2. Picric acid in alcohol : dyes aleurone grains in yellow.

3. 2% Iodine solution : dyes starch grains in blue or violet, tragacanth in green and aleurone grains in yellow.

4. Sudan III in alcohol: dyes oil granules, olioresins, asafoetida and resins in orange.

5. 1-2% Phloroglucinol solution in alcohol + 20% hydrochloric acid: dyes lignin fibers and sclereids in pink or red, suberine and cutin in orange-red, e.g., collenchyma, epidermis. This solution may destroy the microscope lens.

6. Saturated aniline sulfate solution: dyes lignin fiber in pink. This solution does not present cells clearly, but saves the lens.

7. 75% Chloral hydrate solution: dyes cell structures (pollen grains, cell walls), by clearing the cell components, e.g., chloroplasts, starch grains, etc.

8. 10% Ferric chloride or ferric chloride T.S. : dyes tannin in green or blue-green, depending on types of tannin.

9. Ruthenium red solution: dyes mucilage or asafoetida in pink.

10. Tincture of alkana: dyes resins in red.

11. Diluted ammonia solution: dyes purgative herbs in gold and turns to red, under UV light, a glowing red-green.

12. 20% acid: reacts with chalk to produce CO_2

13. Lime water: dyes agar and tragacanth in yellow.

14. Alcohol: dyes *Acacia* in blue.

15. Some alkaloid reagents: react with some alkaloids to result in colors, crystals and precipitates, depending on the alkaloids and reagents used.

Seven cultivars of Vetiver root

Srilaangkhaa, Maehae, Indian, Yeepun, Monto, Maeteay, Parachataan

↓

Organoleptic identification

↓

7 cultivars

↙ ↘

Prepare herbarium specimens

Taxonomic identification and pharmarcognostic identification

↓

Herbarium specimens 7 of the cultivars

Process of microscopic characteristics of Vetiver root (see Appendix 1 in complete report)

Preparation of vetiver root powder

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Vetiver root samples

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Chop the roots into pieces and dry at 40-60°C

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Dried vetiver root samples

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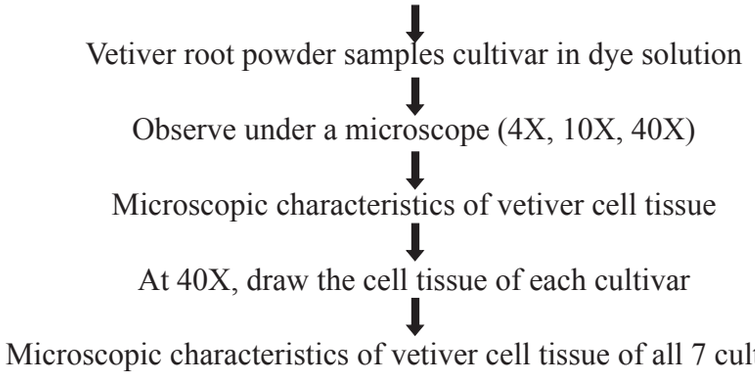
Grind and sieve through no. 60 mesh



RESULTS AND DISCUSSION

Taxonomic identification

Herbarium specimens of 7 cultivars of vetiver grass were deposited in the medicinal plant herbarium, Faculty of Pharmacy, Chiang Mai University, Chiang Mai and are coded as follow :

Srilaangkhaa root	Herbarium specimen No. 1: 009729
Maehae root	Herbarium specimen No. 1: 009730
Indian root	Herbarium specimen No. 1: 009731
Yeepun root	Herbarium specimen No. 1: 009732
Monto root	Herbarium specimen No. 1: 009733
Maeteay root	Herbarium specimen No. 1: 009734
Parachataan root	Herbarium specimen No. 1: 009735
Botanical name	*Chrysopogon zizanioides* (L.) Roberty
Synonym	*Vetiveria zizanioides* (L.) Nash. ex Small
Family	Gramineae
Thai common name	Faek hawm or Yah faek

Taxonomic identification of Vetiver grass

The external characteristics of 7 vetiver cultivars, Stem: perennial grass, thick and bushy, 1-2 m high, Basement spread out flat, Leaf sheath: compressed, tapering and acute, Blade: 45-100 cm long, 6-12 mm wide, erect, pale green, glabrous, dark green underneath, flower: yellow-grey or purple, stamen extruding, stigma lacking style, Seed: shriveled, root: no tap root, branched roots about 3 m deep.

Description of Chrysopogon zizanioides (L.) Roberty

A densely-tufted perennial grass. Rhizome branching with spongy aromatic roots. Culms stout, up to over 1.5 m, glabrous. Leaf sheath compressed, blades stiffish, narrowly- linear, acute, 30-90 cm long, 4-10 mm wide, erect, rigid, firm or somewhat spongy, usually glabrous, rarely more or less hairy downwards on the face, pale green, midrib slender, lateral nerves close, 6 or more on each side, margin spinously rough. Panicle oblong, up to over 30 cm long, very narrow; rachis stout, smooth; whorls 6-10 with up to 20 rays; branches oblique to suberect. Racemes up to 5 rarely 7.5 cm long, very slender; joints about as long as the sessile spikelets; pedicels similar, but shorter. Sessile spikelet, dorsally-compressed

awned; callus small, shortly-bearded. Glumes equal, thinly-chartaceous to membranous; lower 2-keeled, with narrow sharply-inflexed margins; upper boat-shaped, 3-nerved, acutely-keeled (lemma). Lower glume hyaline, upper a hyaline linear stipe. Palea 0 or very minute; lodicules 2, |minute, glabrous. Stamen 3. Stigma exserted laterally usually low down, longer than the styles. Pollen grain oblong, obtuse, dorsally slightly compressed. Use, the very aromatic roots are employed in perfumery and medicine.

Pharmacognostic identification of Vetiver grass

The internal characteristics of vetiver grass roots had both simple and compound starch grains in high quantities, also inside and outside of parenchyma cells. Oil granules and calcium oxalate crystals are also distributed inside and outside the cells. Large vessels, fiber cells, stone cells, sclereid cells and trichomes are slightly different among 7 cultivars. The common names of all cultivars derived from the places where they were first introduced in Thailand or the origin of the cultivar. All 7 cultivars under this study are from the Highland Development Office, Chiang Mai and all of the cultivars have characteristics of *Chrysopogon zizanioides* (L.) Roberty as shown in the following.

Figure 1. Morphological herbarium specimen of *Chrysopogon zizanioides* (L.) Roberty cv. Srilangkhaa.

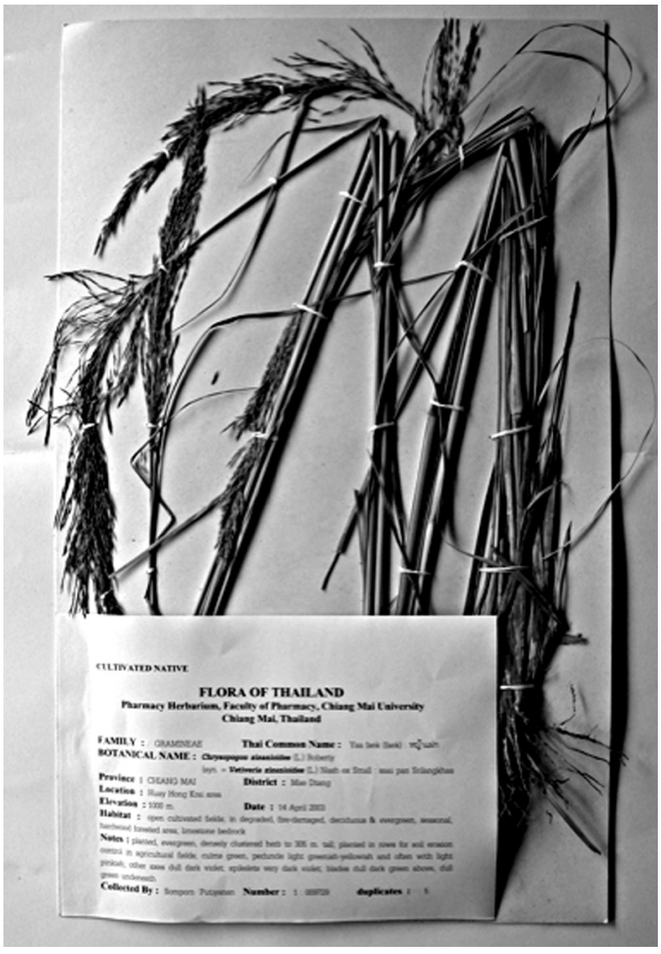
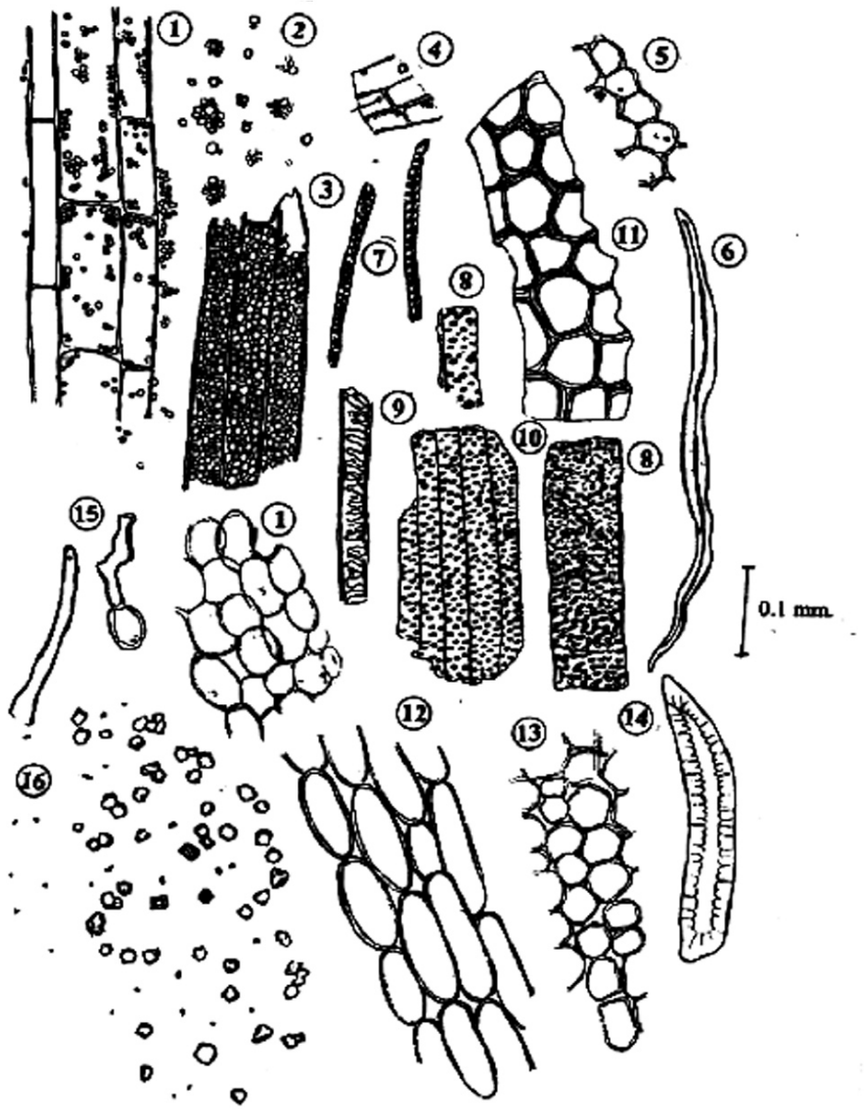


Figure 2. Microscopic characteristic identification of powdered cv. Srilangkhaa root.



1) parenchyma cells	2) single and compound starch grains
3) parenchyma cells containing starch grains	4) oil globules
5) collenchyma cells	6) fiber cells
7) spiral vessels	8) bordered pored vessels
9) reticulate vessels	10) bordered pitted vessels
11) cork cells in surface view	12) cork cells in section view
13) parenchyma cell in section view	14) sclereids cells
15) trichomes	16) calcium oxalate crystals

Figure 3. Morphological herbarium specimen of *Chrysopogon zizanioides* (L.) Roberty cv. Maehae.

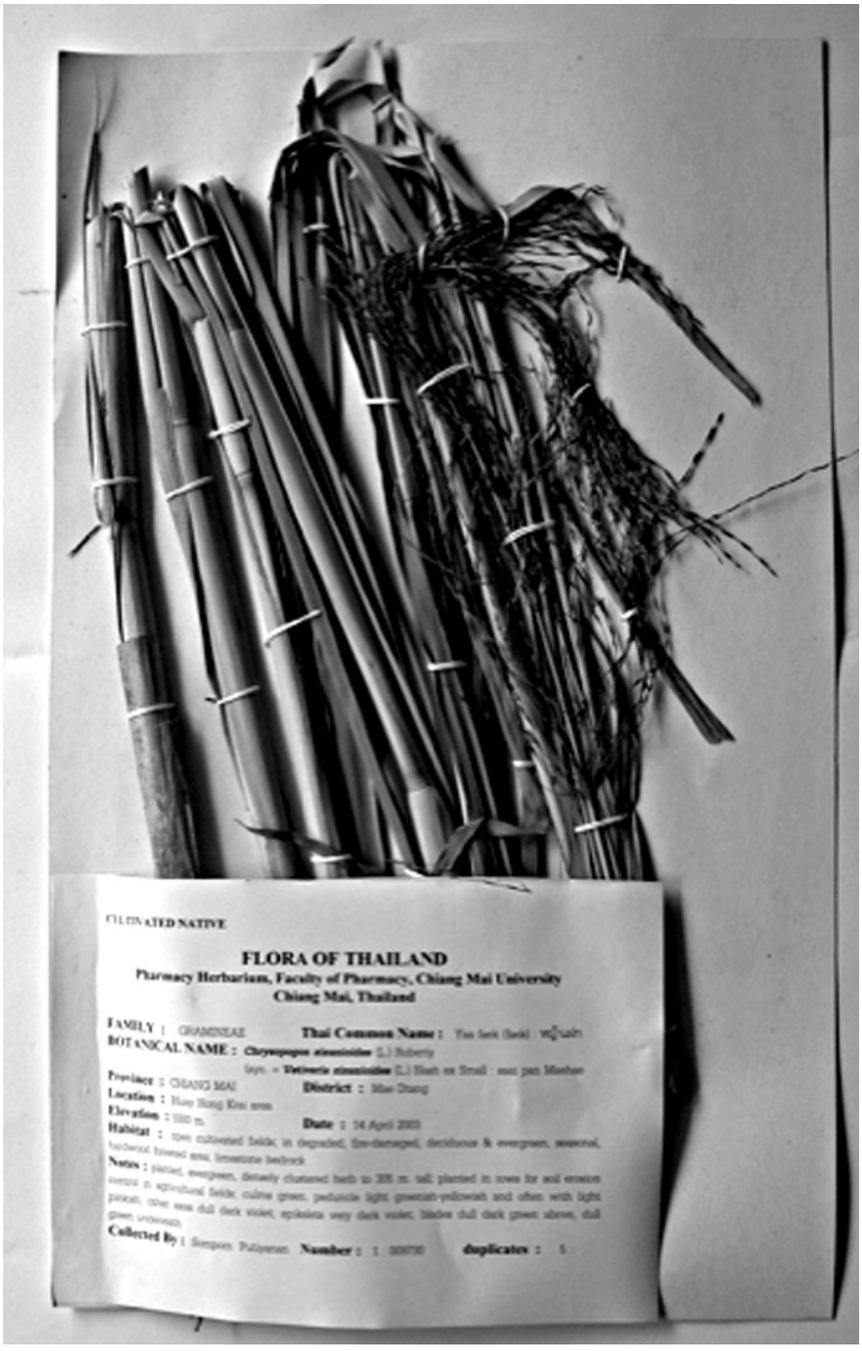
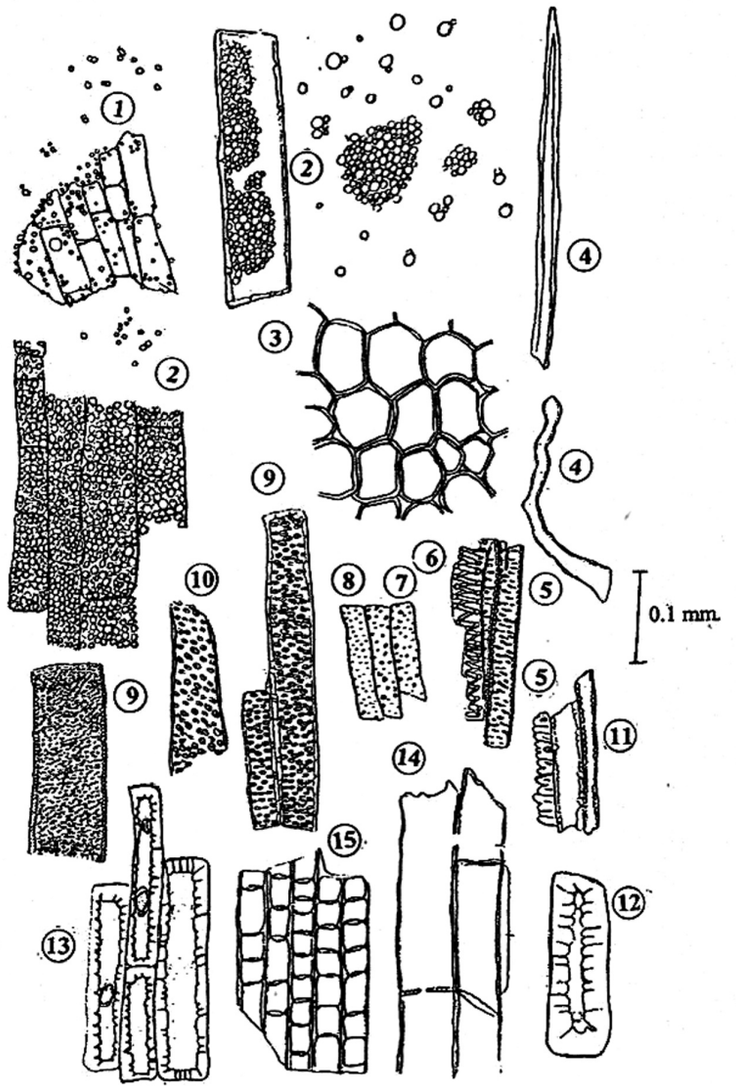


Figure 4. Microscopic characteristic identification of powdered cv. Maehae root.



1) parenchyma cells containing oil globules	2) parenchyma cells containing starch grains
3) parenchyma cells in section view	4) trichomes
5) reticulate vessels	6) spiral vessels
7) pitted tracheids	8) pitted vessels
9) borderd pitted vessels	10) bordered pored vessels
11) fiber cells	12) lignified fibro sclereids
13) sclereids	14) phloem
15) cork cell	

Figure 5. Morphological herbarium specimen of *Chrysopogon zizanioides* (L.) Roberty cv. Indian.

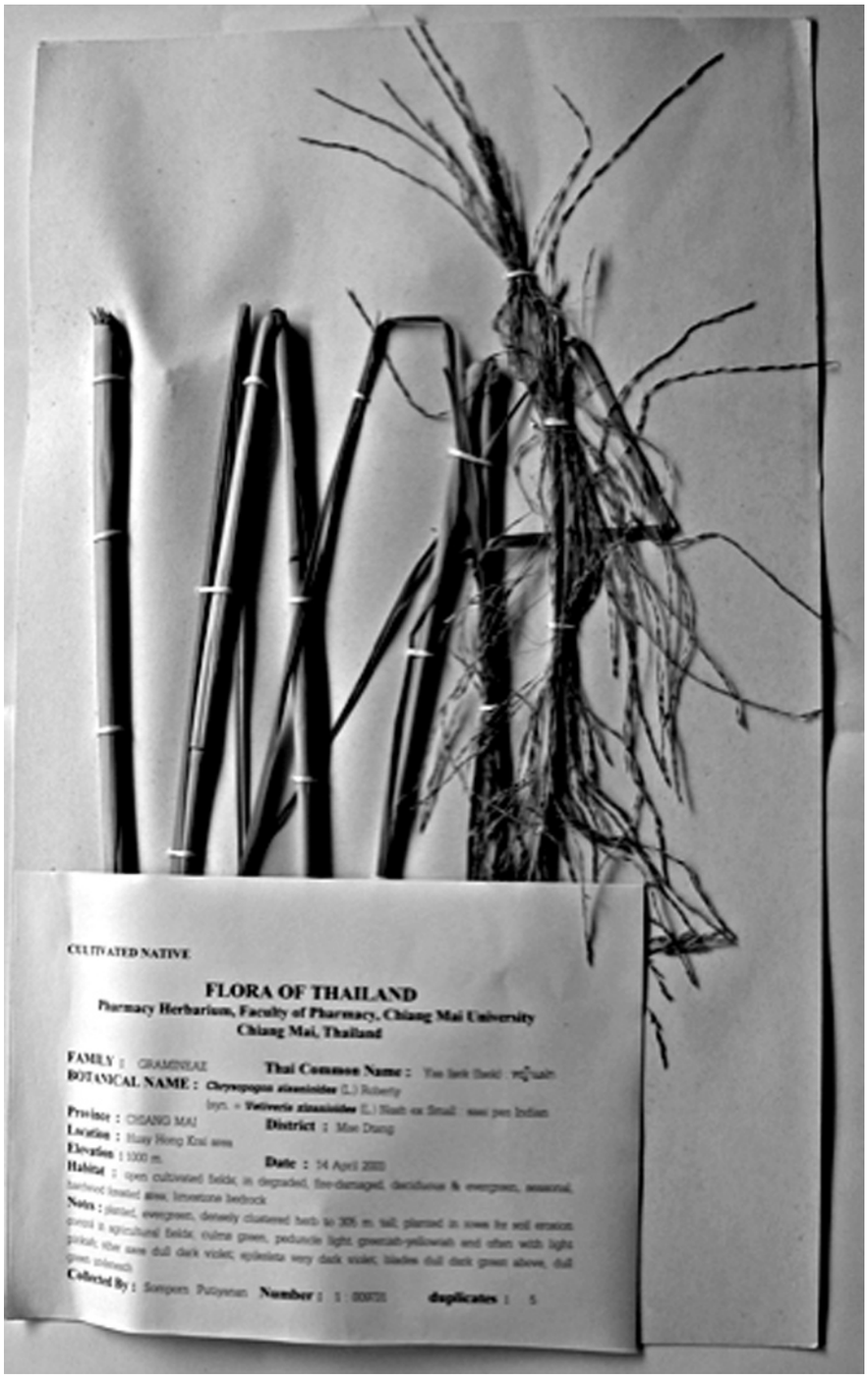
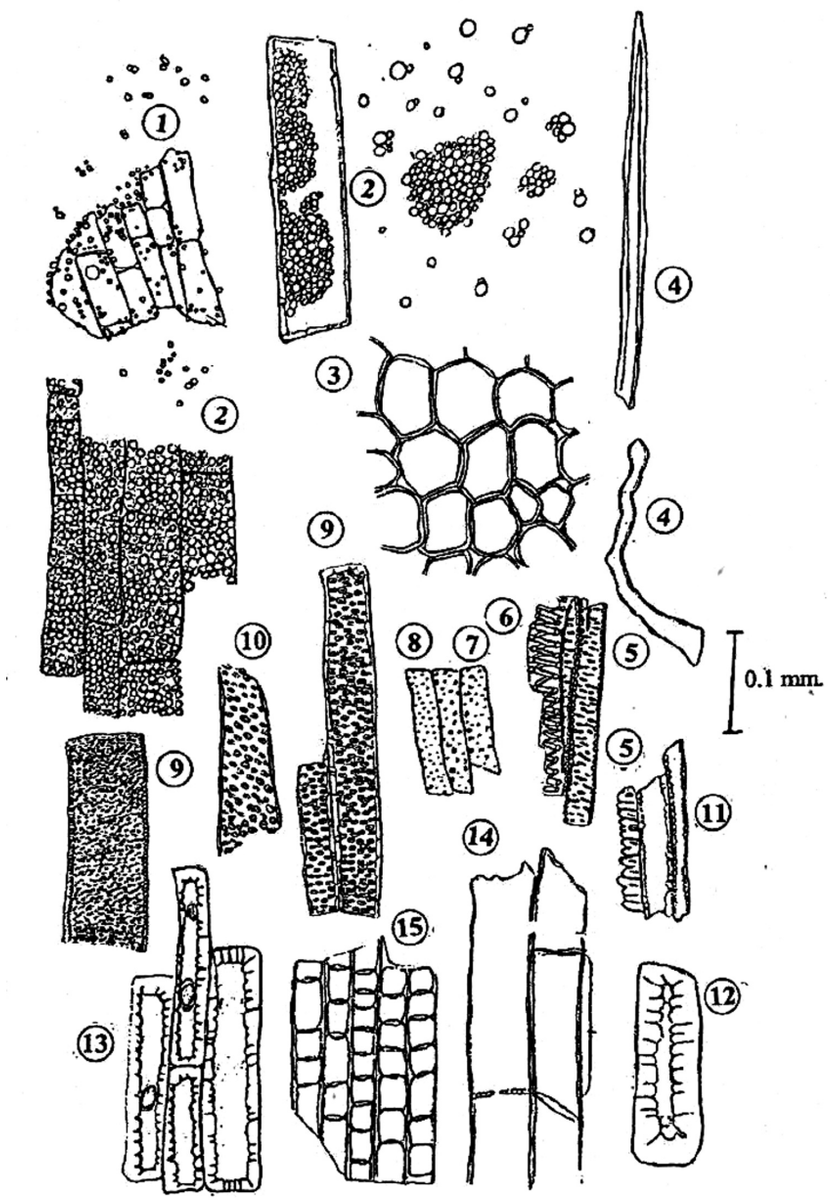


Figure 6. Microscopic characteristic identification of powdered cv. Indian root.



1) parenchyma cell containing single and compound starch grains	2) parenchyma cells containing oil globules
3) parenchyma cells in section veiw	4) lignified porous parenchyma cells
5) trichomes	6) fiber
7) bordered pored vessels	8) bordered pitted vessels and tracheids
9) reticulate vessels	10) cork cells in section veiw
11) lacunar collenchyma	12) calcium oxalate crystals

Figure 7. Morphological herbarium specimen of *Chrysopogon zizanioides* (L.) Roberty cv. Yee pun.

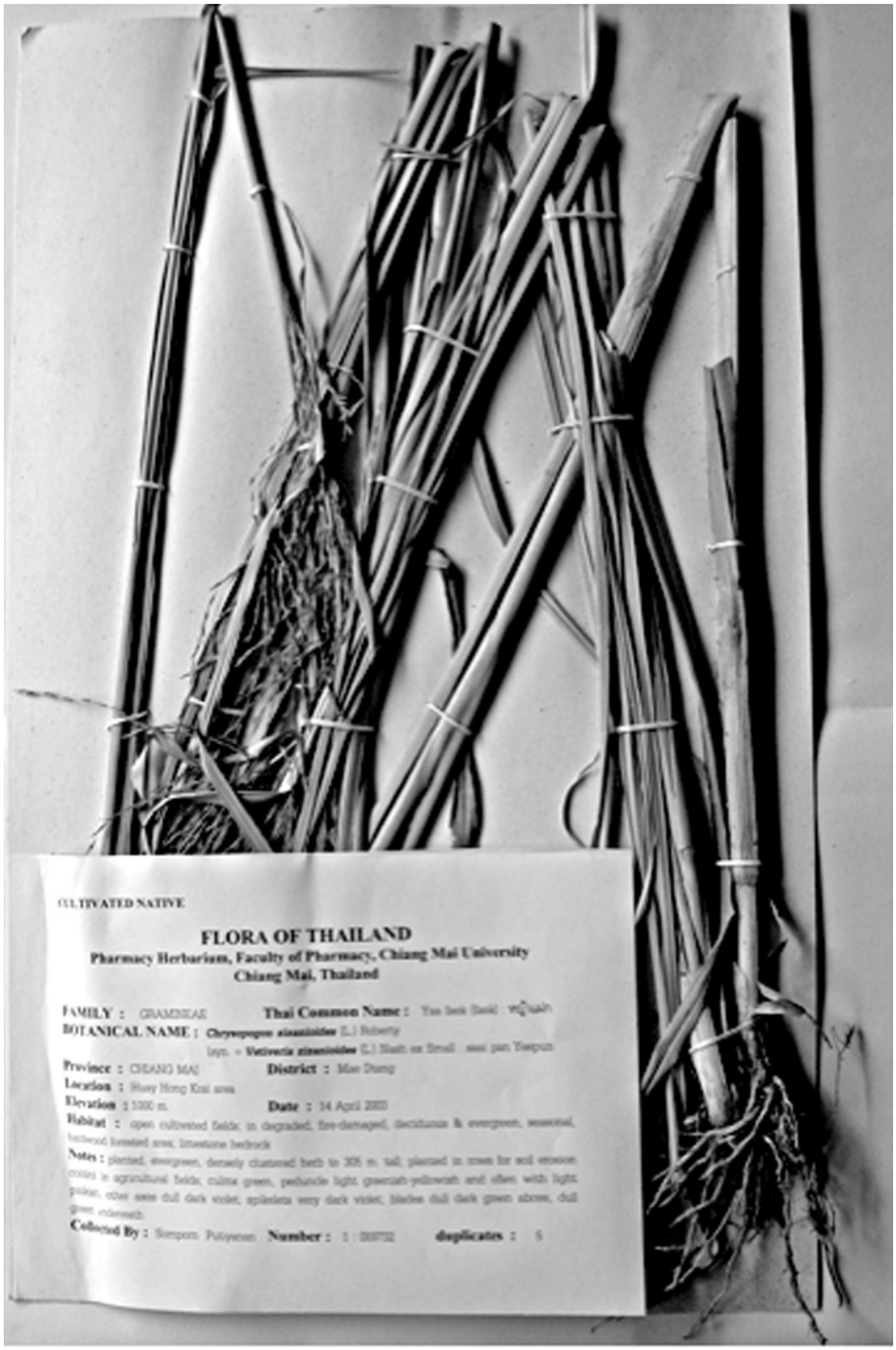
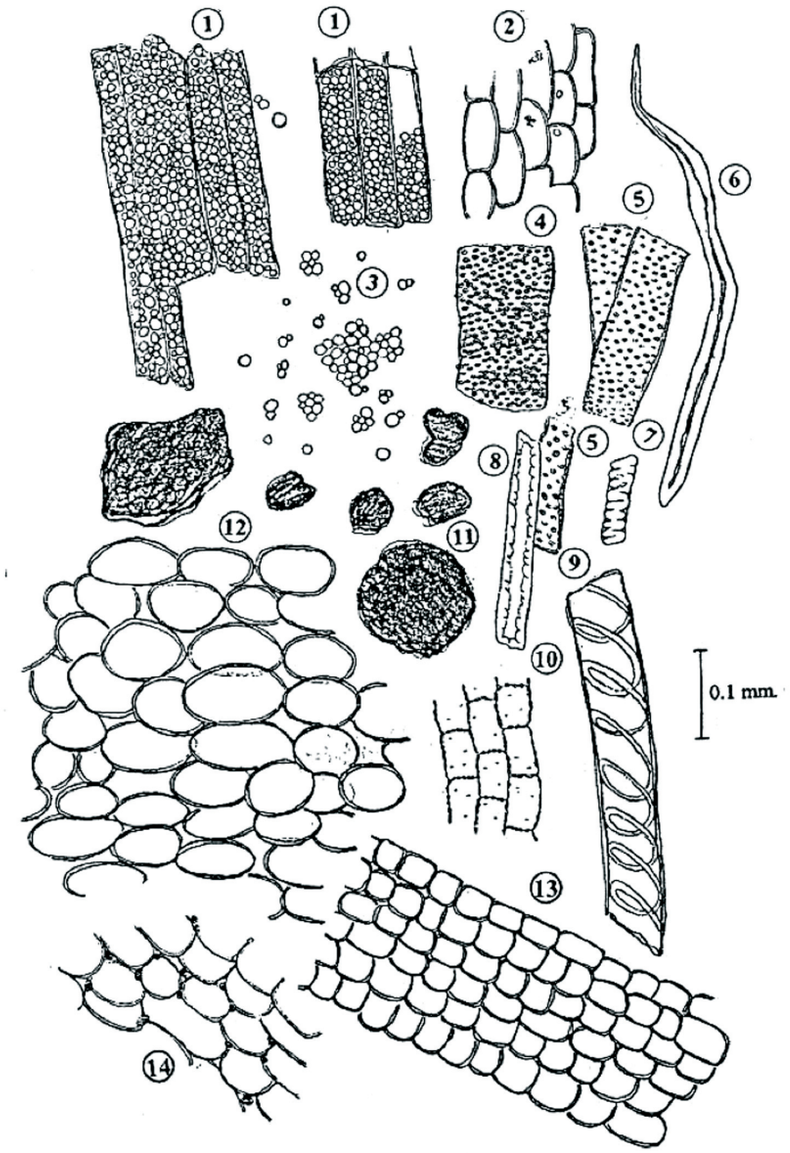


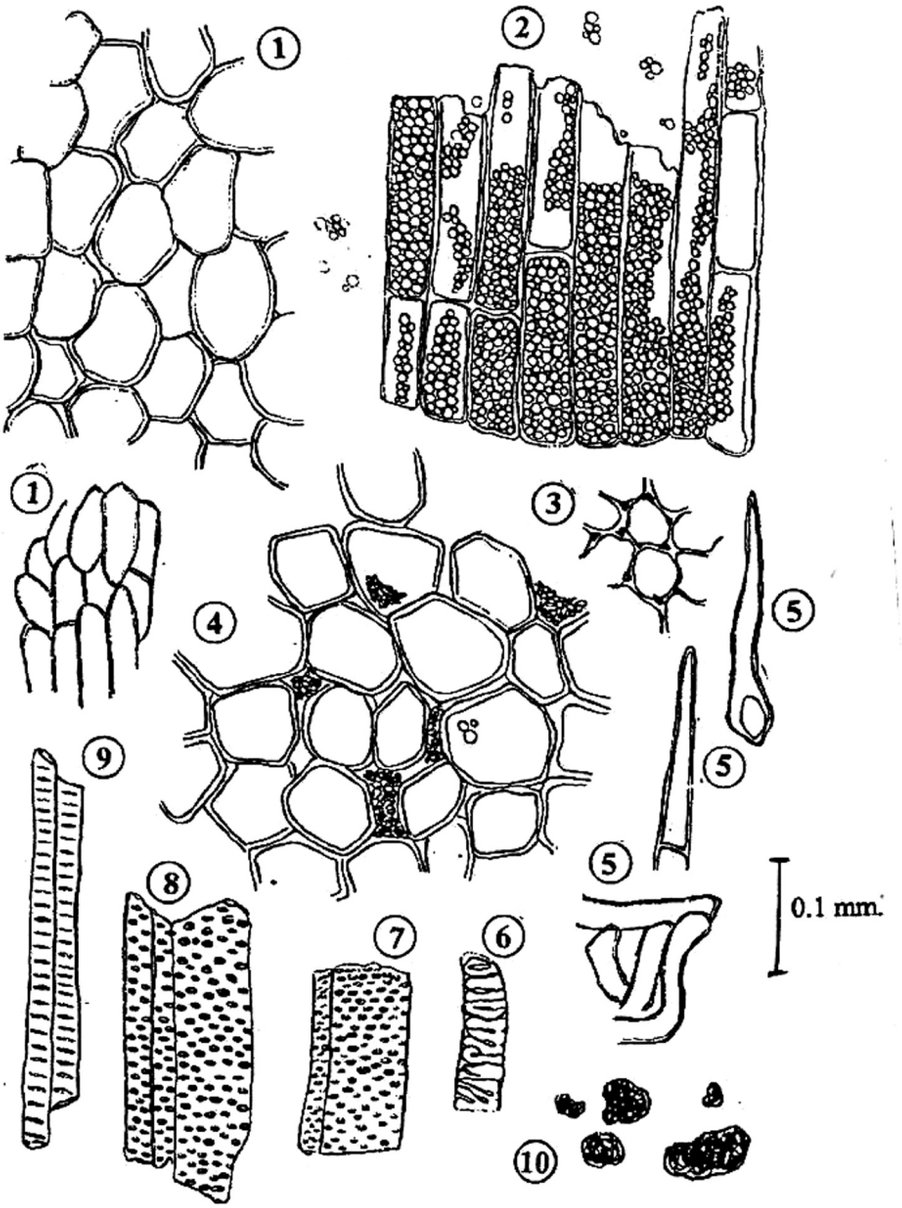
Figure 8. Microscopic characteristic identification of powdered cv. Yeepun root.



1) parenchyma containing starch grains	2) parenchyma cells
3) simple and compound starch grains	4) bordered pitted vessels
5) bordered pored vessels	6) fiber
7) reticulate vessels	8) sclereids
9) spiral vessels	10) lignified xylem parenchyma
11) brown pigments	12) cork cell in section view
13) cork cell	14) angular collenchyma cells

Figure 9. Morphological herbarium specimen of *Chrysopogon zizanioides* (L.) Roberty cv. Monto.

Figure 10. Microscopic characteristic identification of powdered cv. Monto root.



1) parenchyma cells	2) parenchyma containing starch grains
3) angular collenchyma cells	4) cork cell in surface view
5) trichomes	6) reticulate vessels
7) bordered pitted vessels	8) bordered pored vessels
9) scalariform vessels	10) brown pigments masses

Figure 11. Morphological Herbarium Specimen of *Chrysopogon zizanioides* (L.) Roberty cv. Maeteay.

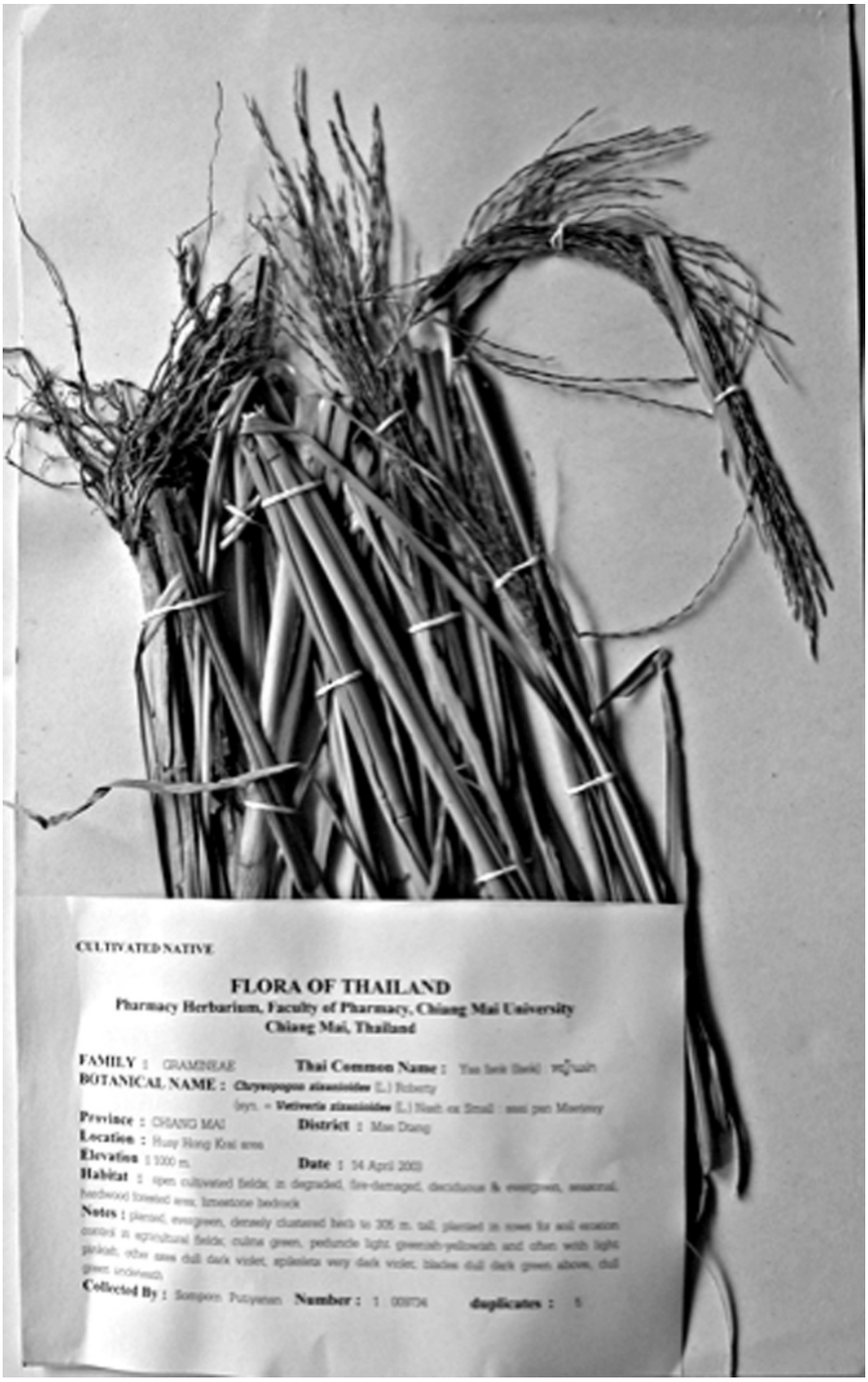
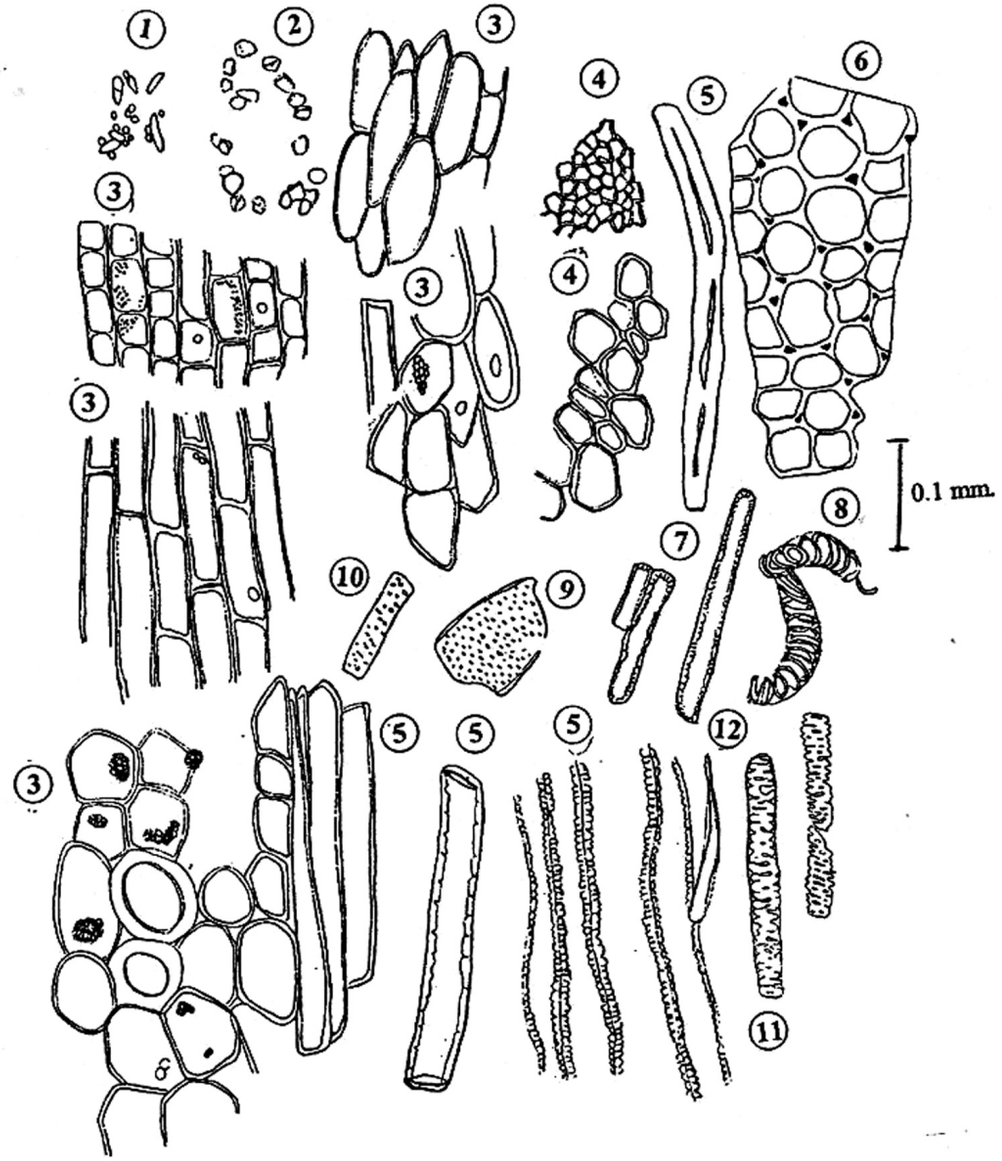


Figure 12. Microscopic characteristic identification of powdered cv. Maeteay root.



1) starch grains	2) calcium oxalate crystals
3) parenchyma cells	4) cork cells
5) fiber cell	6) angular collenchyma cells
7) sclereid cells	8) reticulate vessels
9) bordered pitted vessels	10) bordered vessels
11) reticulate vessels	12) trichomes

Figure 13. Morphological herbarium specimen of *Chrysopogon zizanioides* (L.) Roberty cv. Prarachataan.

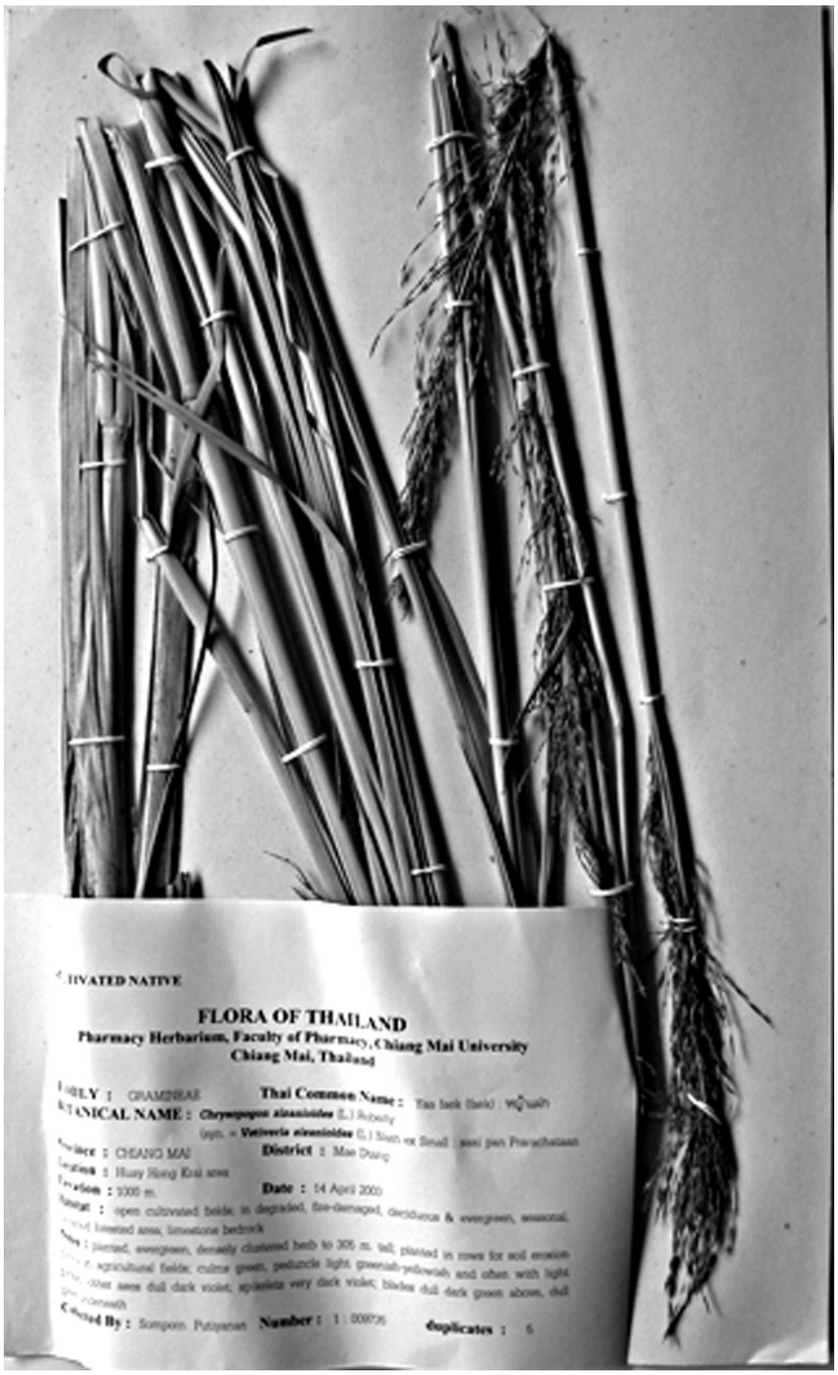
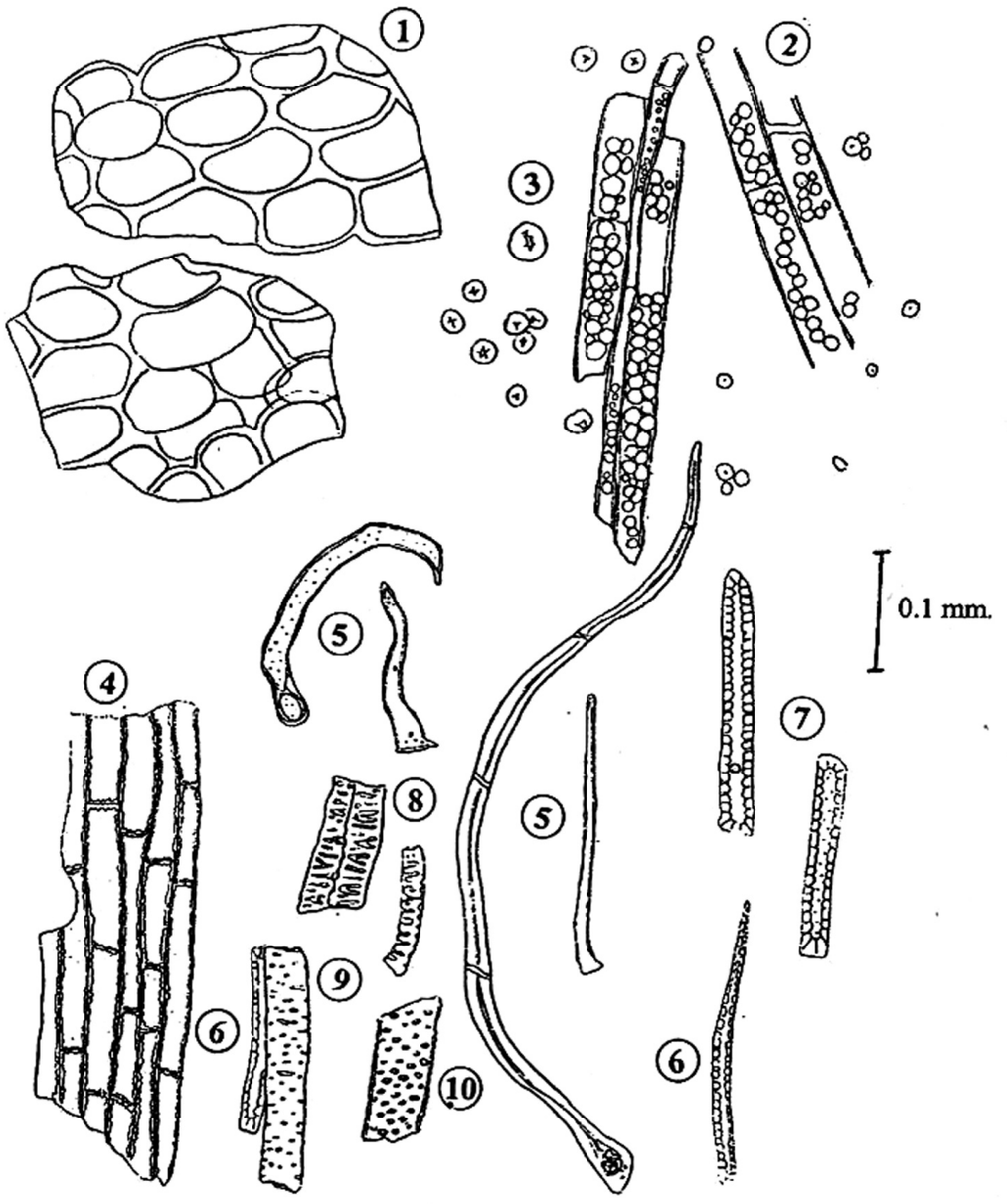


Figure 14. Microscopic characteristic identification of powdered cv. Prarachataan root.



1) parenchyma cells in section veiw	2) parenchyma cell containing single and compound starch grains
3) simple and compound starch grains	4) lignified porous parenchyma cells
5) trichomes	6) fiber cells
7) sclereids cells	8) reticulate vessels
9) bordered vessels	10) bordered pored vessels

CONCLUSION

Pharmacognostic study, using macroscopic and microscopic characterization of *Chrysopogon zizanioides* (L.) Roberty demonstrated the differences in the cell shape, detail and size of each tested strain after detecting by microscope. This technique could be applied in the systematic identification of the roots of different strains. Subsequent study on antimicrobial activity and cytotoxicity of the root extracts will be reported in the upcoming part.

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