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Research Article

Phenotypic Variation Profile of *Marsilea crenata* Presl. Cultivated in Water and in the Soil

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The aim of this research was to investigate possible phenotypic variation profile of *Marsilea crenata* Presl. cultivated in water and in the soil, to find alternative cultivation techniques to fulfill the increasing demand for pollutant-free plants. Phenotypic profile was investigated through macroscopic and microscopic examinations of all plant parts, and phytochemical screening on the ethanol extract of the leaves using thin layer chromatography technique was conducted to detect the terpenoid constituents. Results showed a variation of phenotypic profiles in the macroscopic examination caused by different cultivation methods. Phytochemical screening showed spots of terpenoid compounds with different color intensity. No differences were found in the microscopic examination of the leaves, petioles, and stems. Since major profile changes did not occur, *M. crenata* is recommended to be cultivated in water as its original habitat by providing a better caring and quality of water. Due to its phytoremediation property, it is necessary to grow *M. crenata* in pollutant-free water.

1. Introduction

Marsilea crenata Presl. is an aquatic plant and has been used through generations by people of Surabaya City, East Java Province of Indonesia, as vegetables. It is locally named semanggi, and people name it semanggi Surabaya, to correlate it with the name of the city.

Semanggi Surabaya itself is a popular dish from East Java Province and prepared from steamed semanggi leaves served with a special peanut sauce [1]. The leaves have been used empirically to prevent osteoporosis among postmenopausal women, but scientific research to investigate its health benefits is lacking. Study on the ethanol extract of the leaves using radioimmunoassay technique found its estradiol-like chemical contents. Research conducted in mice using ethanol extract of the leaves combined with axial weight-bearing exercise showed its antiosteoporotic activity. It showed its capacity in the prevention of imbalances of bone remodelling in human and mice [2].

Further investigation was carried out using a fraction of n-hexane leaf extract to study its antiosteoporotic activity in osteoporotic mice. Result showed improvement in trabecular femur and vertebrae bone masses of osteoporotic mice [3].

Further isolation and characterization of terpenoid compounds in n-hexane and petroleum ether leaf extracts were conducted [4]. Isolation of the active compound found some steroid compounds, and this opens the possibility of using *M. crenata* as a source of phytoestrogen [5].

In vitro study of n-hexane leaf extract using cell lines showed its antiosteoporotic activity [6].

The plant also contained various vitamins and minerals, such as calcium, iron, magnesium, Fe, and vitamins A and C [7]. The leaf and petiole were reported to contain protein, β-carotene, fat, and fiber [8].

A study was conducted on the plant grown in murky and contaminated waters in the rice field at Benowo District, East Java Province, and found high lead and mercury contents in the leaves. This was certainly caused by its phytoremediation property [1, 9]. The district itself was recognized as the source of the plants for commercial purpose. Therefore, research to study the growing method in the soil was conducted to find a better cultivation technique.

Result showed the ability of *M. crenata* to grow in the soil and was followed by standardization to find its specific and nonspecific parameters [10].

But, further study is still needed to find right cultivation techniques, in order to obtain pollutant-free plants.

The objective of the research was to study phenotypic variation profiles of *M. crenata* cultivated in water and in the soil. Phenotypic profiles were investigated through microscopic examinations of leaves, petioles, stems, and roots. Phytochemical screening was conducted to study the terpenoid constituents of the plant using thin layer chromatography technique [10].

2. Materials and Methods

2.1. Materials

2.1.1. Plant Materials. Plants were harvested from water and soil cultivations in April 2016. Plants harvested from the rice field at Benowo District, East Java Province, were being used as a control.

Determination was conducted at Purwodadi Botanical Garden, East Java, Indonesia.

2.1.2. Extract of Dried Powdered Leaves. Leaves were dried in an oven at 50°C and powdered and extracted with 96% ethanol in a sonic bath for 20 minutes.

2.2. Cultivation. Cultivation was conducted in South Surabaya City, East Java Province, Indonesia, in January 2016. Seeds were obtained from Benowo District, Surabaya.

(1) In water, cultivation was conducted in concrete pots size 58 × 49 × 16 cm filled with ready-made mixtures of soil and composts up to half height of the pots. Water was added to fill up the surface of the pots to provide muddy water as in their original habitat. It allowed settling for 24 hours before plant seedling was planted.

(2) In the soil, cultivation was conducted in clay pots size 63 × 26 × 18 filled with ready-made mixtures of soil and composts. Plants were watered twice a day (morning and afternoon).

All pots were exposed under full sunlight during their entire growth as in their original habitat in rice fields.

2.3. Phenotypic Profiles. Phenotypic profiles were studied through morphological and anatomical examinations. Morphological examination was conducted on leaf, petiole, stem, and root of the plant. Anatomical examination of the plant was conducted by observing the cross sections of leaf, petiole, and stem using light microscope.

2.4. Phytochemical Screening. Phytochemical screening was conducted by thin layer chromatography technique to study the terpenoid constituents of the leaves.

96% ethanol extract of powdered leaves from water and soil cultivations was used to detect terpenoid contents using n-hexane : ethyl acetate (4 : 6) as mobile phase. Anisaldehyde sulphuric acid was used as spray reagent.

Table 1: Morphology of leaves, petioles, stems, and roots of *M. crenata* cultivated in the soil and in water.

Leaf type	Compound, 4 leaflets on top of each petiole, cuneate, leaf tip truncate, leaf base acute/obtuse, margin integer
Petiole	Round, fine surface hair, light green
Stem	Round, fine surface hair, brown, grown above or just below the soil surface
Roots	Fibrous, appearing from each node, brown



Figure 1: Cultivation in water in concrete pots.



Figure 2: Cultivation in soil in clay pots.

3. Results and Discussions

3.1. Cultivation. Cultivation in concrete pots was shown in Figure 1. Figure 2 showed cultivation in clay pots. Figure 3 showed cultivation in the rice field.

3.2. Phenotypic Profiles

3.2.1. Morphological Examination. The plant has pinnately compound leaves with 4 cuneate leaflets, petioles rounded and finely hairy on the surface (Table 1). Morphological differences were observed through length and diameter of petioles, diameter of stems, length and density of roots, and distances between nodes of stems (Table 2). Figures 4, 5, and 6 presented different petiole's length of water and soil cultivated plants. Petiole was longer in *M. crenata* cultivated in soil compared to water cultivation (Figure 5). Figures 4 and 6 showed petioles of water cultivated plants in concrete pots that were relatively longer compared to water cultivation in the rice fields.

Figure 7 showed that differences of root profile grew from the stems, distances between nodes, diameter of stems, and length of petioles in water and soil cultivations. Fibrous roots appeared from each node of the stems, and each cultivation

Table 2: Morphological differences of leaves, petioles, and roots of *M. crenata* cultivated in the soil and water.

Plant part	Observation		
	Cultivation in water in concrete pots	Cultivation in the soil	Cultivation in water in rice field
Leaf			
Color	Light green	Light green	Light green
Diameter of compound leaves	3.5 × 3.5 cm	4.5–5 × 4.5–5 cm	3.0 × 3.0 cm
Size of leaf tip	1.7 cm	2.2 cm	1.7 cm
Size of leaf base	0.5 cm	0.7 cm	0.4 cm
Thickness	Thin	Thin	Thin
Petiole			
Length	12–15 cm	20–24 cm	11.5–15.5 cm
Diameter	Greater	Smaller	Greater
Roots	Long, vigorous, dense 8–17 cm	Short and sparse 2–5 cm	Short and sparse 3–8 cm
Distance between nodes of stems	1–3 cm	0.5–1 cm	5–5.5 cm



Figure 3: Cultivation and harvest in the rice field at Benowo District, East Java Province.

method showed different root appearances. Roots are longer and more vigorous in water cultivated *M. crenata* in concrete pots compared to water cultivation in the rice fields and in the soil. Figure 10 showed a different profile of roots in soil cultivation caused by a short node distance.

All cultivation methods yielded different distances between nodes of stems. Figure 9 showed that the longest distance occurred in water cultivation in the rice field, and the shortest was in soil cultivation (Figure 10). Nodes distances of water cultivation in concrete pots are shorter compared to the rice field cultivation (Figure 8).

Both cultivations in water showed same diameter of the petioles and stems (Figures 11 and 12), while smaller diameters were found in the soil cultivation (Figure 13).

Leaf size of both water cultivations was shown to be relatively smaller in diameter and length compared to soil cultivation (Figure 14).

3.2.2. Anatomical Examination. Anatomical examination through cross-section of the leaf, petiole, and stem was studied. Results showed that there were not any differences in anatomical profiles of *M. crenata* cultivated in water and soil. It showed that morphological differences did not cause



Figure 4: *M. crenata* cultivated in water.



Figure 5: *M. crenata* cultivated in the soil.



Figure 6: *M. crenata* cultivated in water in the rice field.



(a) (b) (c)

Figure 7: Petioles, stems, and roots of *M. crenata* cultivated in water in the pot (a), soil (b), and water in the rice field (c).



Figure 8: Roots of cultivated *M. crenata* in water in concrete pots.

any changes on the anatomical structures of leaves, petioles, and stems.

Leaf. Lower epidermis of the leaf showed a layer of epidermal cells with papillae (Figure 15).

Petiole. Petioles of *M. crenata* were composed of epidermis, aerenchyma, septa, inner cortex with parenchymatous cells, and vascular bundle (Figure 16).

Stem. Stems of *M. crenata* cultivated in the soil grew above or just below the soil surface, while those of water cultivation were emerged under the water. It composed of epidermis, aerenchyma, septa, inner cortex with sclerenchyma and parenchymatous cells, and vascular bundle (Figure 17). Aerenchyma was found in aquatic plants and provided aeration for the plan.

3.3. Phytochemical Screening. Phytochemical screening was conducted using thin layer chromatographic technique to study the terpenoid constituents of 96% ethanol extract of the leaves. Results showed that all ethanol extracts showed some violet spots of terpenoid compounds with the



Figure 9: Roots of cultivated *M. crenata* in water in the rice field.



Figure 10: Root of cultivated *M. crenata* in soil in a clay pot.

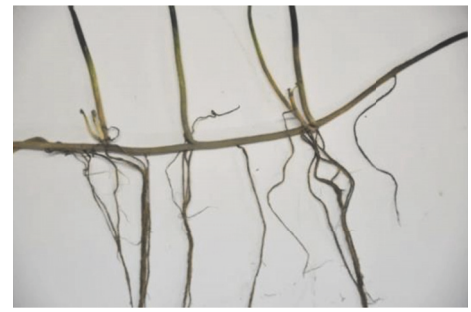


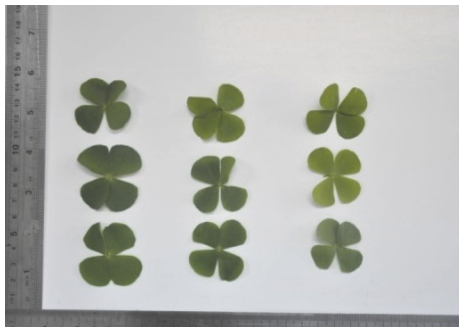
Figure 11: Stem, root, and petiole of *M. crenata* in water in concrete pots.



Figure 12: Stem, root, and petiole of cultivated *M. crenata* in water in the rice fields.



Figure 13: Stem, root, and petiole of cultivated *M. crenata* in the soil.



(a) (b) (c)

Figure 14: Leaves of *M. crenata* cultivated in the soil (a), in water in concrete pots (b), and in the rice field (c).

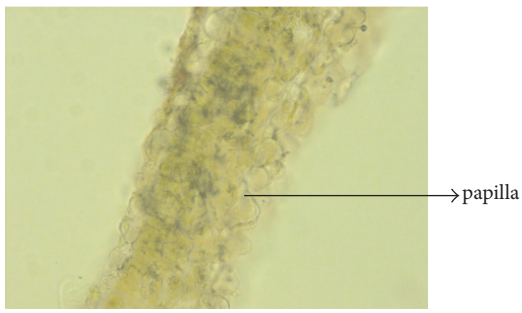


Figure 15: Cross section of *M. crenata* Presl. leaves showed papillae of lower epidermal cells using light microscope (400x).

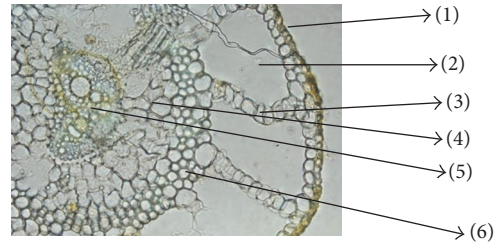


Figure 16: Cross section of the petiole of *M. crenata*. (1) = epidermis, (2) = aerenchyma, (3) = septum, (4) = parenchyma, (5) = vascular bundle, and (6) = inner cortex.

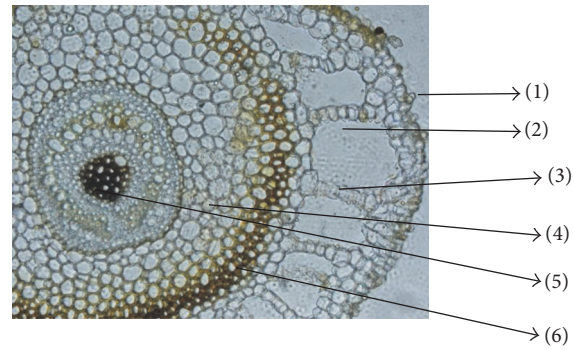


Figure 17: Cross section of the stem of *M. crenata*. (1) = epidermis, (2) = aerenchyma, (3) = septum, (4) = parenchyma, (5) = vascular bundle, and (6) = sclerenchyma.

same Rf values, where soil cultivated leaves showed more intensive colors of spots with Rf values of 0.98, 0.93, and 0.43 (Figure 18).

Research found a phenotypic variation profile caused by different cultivation methods of *M. crenata*. Such variation showed plant's response to changes through environmentally induced shifts of its phenotype [11, 12]. It could be seen from the sizes of petioles, stems, roots, and leaves. Water cultivation in concrete pots showed longer and more vigorous roots compared to water cultivation in the rice field and soil. This was caused by better caring that give better growing environment compared to cultivation in the rice field in murky and contaminated waters at Benowo District, where farmers used pesticides [13]. Longer, denser, and more vigorous roots proved the plant's ability to enhance nutrient uptake [14]. Soil cultivation showed longer petioles, shortest distance between nodes, and more intensive spot colors of terpenoid compounds in the ethanol extract of the leaves. Such accumulation of metabolites was possibly caused by stresses occurring during the changing of cultivation method. This indicated the secondary metabolite's role in the adaptation to the changing of environment and overcoming stress conditions [13].

Since major profile change did not occur, it is indicated that *M. crenata* was best cultivated in water as its original habitat as an aquatic plant.

4. Conclusion and Recommendation

It is concluded that *M. crenata* was best cultivated in water as its original habitat as an aquatic plant. Cultivation should be conducted by giving a special attention to better caring using pollutant-free water. For safety reasons, it is not recommended to use the plant's phytoremediation property for water or soil cleaning purposes [15, 16]. This was based on the fact that people consumed the leaves as vegetable, and vegetable rich in heavy metal could cause serious health problems in humans [17, 18]. Further research is still needed to find a better cultivation method for obtaining pollutant-free plants.

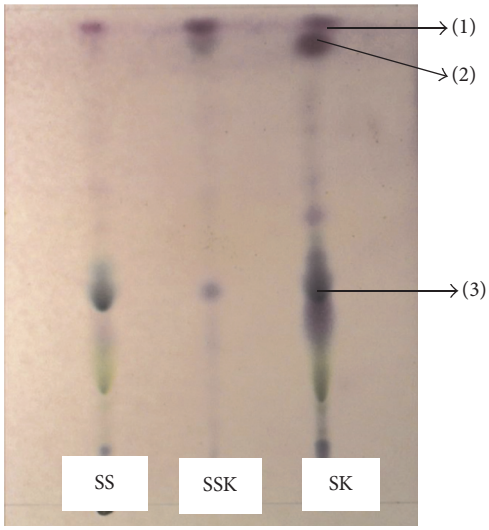


Figure 18: Thin layer chromatogram of 96% ethanol extracts of powdered leaves of *M. crenata* from water cultivation in the rice field (SS), water cultivation in concrete pots (SSK), and soil cultivation (SK). Rf 1 = 0.98, Rf 2 = 0.93, and Rf 3 = 0.43. Stationery phase: TLC silica gel 60 GF 254. Mobile phase: n-hexane : ethyl acetate = 4 : 1. Spray reagent: anisaldehyde sulphuric acid.

Conflicts of Interest

The authors stated that there are no conflicts of interest regarding the publication of this paper.

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