ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2019 Nov 30; 7(22):3790-3793.
https://doi.org/10.3889/oamjms.2019.505
eISSN: 1857-9655
***Herbal Medicine in Pharmaceutical and Clinical Sciences***

# Organic and Inorganic Analysis of *Rhaphidophora pinnata* (l.f.) Schott Water Extract

Masfria Masfria[1, 2*], Marianne Marianne[2, 3], Yade Metri Permata[1]

*[1]Pharmaceutical Chemistry Department, Faculty of Pharmacy, Universitas Sumatera Utara, Medan 20155, Indonesia; [2]Nanomedicine Centre of Inovation, Universitas Sumatera Utara, Medan 20155, Indonesia; [3]Pharmacology Department, Faculty of Pharmacy, Universitas Sumatera Utara, Medan 20155, Indonesia*

**Citation:** Masfria M, Marianne M, Permata YM. Organic and Inorganic Analysis of *Rhaphidophora pinnata* (l.f.) Schott Water Extract. Open Access Maced J Med Sci. 2019 Nov 30; 7(22):3790-3793. https://doi.org/10.3889/oamjms.2019.505

**Keywords:** Rhaphidophora pinnata; Water extract; Nano simplicia; Phytochemicals; Elements

***Correspondence:** Masfria Masfria. Pharmaceutical Chemistry Department, Faculty of Pharmacy, Universitas Sumatera Utara, Medan 20155, Indonesia; Nanomedicine Centre of Innovation, Universitas Sumatera Utara, Medan 20155, Indonesia. E-mail: masfria@usu.ac.id

**Received:** 25-Sep-2019; **Revised:** 17-Oct-2019; **Accepted:** 18-Oct-2019; **Online first:** 14-Nov-2019



**Funding:** This investigation was financially supported by the Ministry of Higher Education, Indonesia

**Competing Interests:** The authors have declared that no competing interests exist

**Abstract**

**BACKGROUND:** *Rhaphidophora* pinnata (L.f.) Schott is one of the Indonesian plants, has known as a medicinal plant. This plant is a vine; round stems have sticky roots and hanging roots. The leaves have been used as traditional anticancer in Singapore. Indonesian people have also used *R. pinnata* plants as a diuretic agent, anticancer and antibacterial. *R. pinnata* plants contain active substances from alkaloid compounds, flavonoids, saponins, tannins and triterpenoids/steroids.

**AIM:** The purpose of this study was to determine the organic and inorganic content of *Rhaphidophora pinnata* (L.f.) Schott water extract in the form of fresh leaves, micro simplicia and nano simplicia.

**METHOD:** The collected *R. pinnata* leaves are drained and grinded to make micro, and nano Simplicia powder of *R. pinnata* leaves. The size characterisation of *R. pinnata* leaves was analysing using Particles Size Analyzer. The water extract of *R. pinnata* leaves, micro simplicia, and nano simplicia *R. pinnata* leaves was made 10% (w / v) in water. Phytochemical screening of nano simplicia and nano simplicia water extract included an examination of alkaloid compounds, flavonoids, saponins, tannins, glycosides, and steroids/triterpenoids. Thin-layer chromatography analysis of water extract was analysed using TLC scanner. The element that contains in the water extract was analysed using atomic absorption spectrophotometry methods.

**RESULT:** The results of phytochemical screening of nano simplicia powder and nano simplicia water extract showed the presence of flavonoids, alkaloids, saponins, tannins, and glycosides. Eluent, which shows good elution is n-butanol: acetic acid: water (6: 2: 2). This eluent is used to elute polar and semipolar compounds and is very good for separating flavonoids. *R. pinnata* water extract contains the minerals potassium, sodium, magnesium and calcium.

**CONCLUSION:** *R. pinnata* water extract contains organic compounds in the form of flavonoids, alkaloids, saponins, tannins, and glycosides. The nano simplicia water extract showed more chemical content than other water extracts on the TLC plate by detection at a wavelength of 250 nm and 300 nm. The most element content in *R. pinnata* water extract is potassium, followed by magnesium, sodium, and calcium.

## Introduction

Based on hereditary experience, the use of herbal plants is considered effective enough to treat various kinds of diseases. *Rhaphidophora* pinnata (L.f.) Schott is one of the Indonesian plants, has known as a medicinal plant. This plant is a vine, round stems have sticky roots and hanging roots [1]. The leaves have been used as traditional anticancer in Singapore. Indonesian people have also used *R. pinnata* plants as diuretic agent [2], anticancer [3] and antibacterial [4]. *R. pinnata* plants contain active substances from alkaloid compounds, flavonoids, saponins, tannins and triterpenoids/steroids [5].

There are more than 20 elements needed by the body, and one of the biggest sources is from plants. Many of them are metals: such as sodium, potassium, calcium and magnesium, present in considerable concentrations, and are known as 'bulk elements'. Indeed, these four cations constitute nearly 99% of the metal ion content of the human body. Others, such as cobalt, copper, iron, manganese, molybdenum, and zinc, are known as 'trace elements', with dietary requirements that are much lower than bulk elements; however, they are no less inseparable for human life [6].

In recent times, the boundary between inorganic and organic has become clearer because many inorganic compounds contain organic ligands, and many organic compounds contain metal. This is the first study to analyse the organic and inorganic content in R. pinnata leaves water extract. The organic content was analysed by phytochemical screening and thin-layer chromatography technique. As well as the inorganic content was the finite macro minerals analysis using atomic absorption spectrophotometry methods.

## Material and Methods

The materials used in this research were *R. pinnata* leaves, calcium, potassium, sodium and magnesium standard solution and another reagent. Those chemicals and solvents used were analytical grade and are commercially available from Merck. *R. pinnata* leaves were cleaned and dried, then crushed to obtain micro simplicia and nano simplisia powder. The sample identification was carried out by the Bogorinese Herbarium Division of the Biology Research Center-LIPI Bogor. The nano simplicia powder was analysed by LIPI (Lembaga Ilmu Pengetahuan Indonesia) using particle size analyser.

The tools used in this study were glassware, hot plate (Fisons), desiccator, pH paper, Whatman filter paper no.42, light microscope (Boeco), analytical balance (Boeco), oven, infusion pan, a set of Atomic Absorption Spectrophotometers (Hitachi Z 2000) with air-acetylene flame complete with calcium cathode lamps, a set of Thin Layer Chromatography (Densitometer), TLC Silica Gel 60 F 254 aluminium sheets 20 x 20 cm (Merck) and furnaces (Stuart).

The collected *R. pinnata* leaves are washed continuously by water from the impurities and drained at a temperature of 40-60°C to dry. Then the simplicia of *R. pinnata* leaves were grinded using a blender then stored in a tightly closed container, protected from heat and light. The simplicia powder of *R. pinnata* leaves then smoothed using nanotechnology with a High Energy Ballmill. Nano-simplicia *R. pinnata* leaves were prepared at PUSPITEK Gd. 410 Serpong-South Tangerang Technology Incubator Hall. The characterisation of *R. pinnata* leaves nano-simplicia using Particles Size Analyzer (PSA) was carried out at PUSPITEK Gd. 410 Serpong-South Tangerang Technology Incubator Hall.

The water extract of *R. pinnata* leaves, micro-simplicia *R. pinnata* leaves, and nano-simplicia *R. pinnata* leaves were made in three different concentration 10% in water, using infused technique according to Indonesian Pharmacopeia 4th Edition [7]. Phytochemical screening of nano-simplicia and nano-simplicia water extract *R. pinnata* leaves included examination of alkaloid compounds, flavonoids, saponins, tannins, glycosides, and steroids / triterpenoids.

Thin-layer chromatography analysis of fresh leaf water extracts, micro and nano simplicia from R. pinnata leaves were carried out using 3 types of eluent mixtures namely n-butanol: acetic acid: water (6: 2: 2) [8]; n-hexane: ethyl acetate (6: 4) [9] to detect flavonoids, and petroleum ether: ethyl acetate: acetic acid (6: 3: 1) to detect phenol derivatives [10].

Testing of mineral content in water extracts is carried out by Atomic absorption spectro-photometric methods, with wet destruction for sample preparation. Testing of mineral content begins with the creation of a series of concentrations for the four minerals measured, and then the regression was calculated and then used to calculate the mineral content in the sample solution with different dilutions for each mineral and repetition 6 times. Analysis of potassium, calcium, magnesium and sodium were quantitatively measured using atomic absorption spectrophotometry at wavelengths of 766.5 nm; 422.7 nm; 285.2 nm; and 589.0 nm using an air-acetylene flame. The data obtained were tested statistically using Student T, with $t < t$ table, at 99% confidence intervals with a value of $\alpha = 0.01$.

## Results

Results of phytochemical screening of nano-simplicia powder and nano-simplicia water extract presented in Table 1.

**Table 1: Results of phytochemical screening of nano-simplicia powder and nano-simplicia water extract**

| No. | Screening | Results | |
|---|---|---|---|
| | | Simplicia | Extract |
| 1. | Flavonoid | + | + |
| 2. | Alkaloid | + | + |
| 3. | Saponin | + | + |
| 4. | Tannin | + | + |
| 5. | Glycoside | + | + |
| 6. | Steroid/Triterpenoid | + | – |

(+) positive: Contains a class of secondary metabolites; (–) negative: Does not contain a class of secondary metabolites.

Analysis of the content of organic compounds in water extracts was carried out using a thin layer chromatography method, and the resulting plate was scanned using a densitometer or TLC scanner. The elution results from the three water extracts with different eluents can be seen in Figure 1.

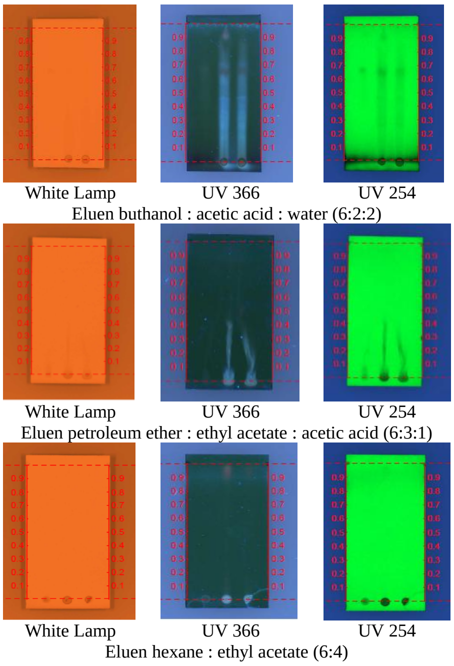



*Figure 1: Elusion of R. pinnata water extract*

The detection result of TLC of water extract (Eluent: butanol: acetic acid: water (3: 1: 1)) using densitometer can be seen in Figure 2 and Table 2-3.

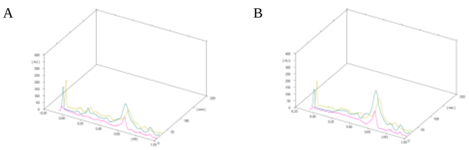
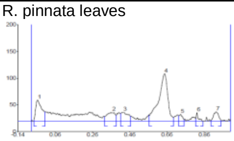
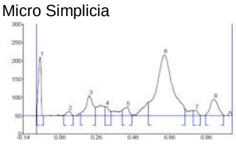
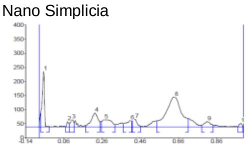



*Figure 2: The detection result of the TLC at wavelength 250 nm (A) dan 300 nm (B)*

The detection result of TLC of water extract (Eluent: butanol: acetic acid: water (3: 1: 1)) using extract using a densitometer at a wavelength of 250 nm can be seen in Table 2.

**Table 2: The detection results of TLC water extract using a densitometer at a wavelength of 250 nm**

| Waters extracts | | RF | Area | % Area |
|---|---|---|---|---|
| R. pinnata leaves  | 1 | 0.06 | 1039.7 | 16.59 |
| | 2 | 0.32 | 585.1 | 9.22 |
| | 3 | 0.41 | 530.9 | 8.37 |
| | 4 | 0.56 | 3504.2 | 55.23 |
| | 5 | 0.72 | 177.8 | 2.80 |
| | 6 | 0.81 | 133.8 | 2.11 |
| | 7 | 0.90 | 373.6 | 5.89 |
| Micro Simplicia  | 1 | 0.07 | 1989.0 | 10.69 |
| | 2 | 0.08 | 274.1 | 1.47 |
| | 3 | 0.17 | 1733.8 | 9.32 |
| | 4 | 0.30 | 492.8 | 2.65 |
| | 5 | 0.41 | 641.1 | 3.45 |
| | 6 | 0.54 | 11534.9 | 62.02 |
| | 7 | 0.79 | 296.8 | 1.60 |
| | 8 | 0.86 | 1637.2 | 8.80 |
| Nano Simplicia  | 1 | 0.06 | 1908.3 | 14.74 |
| | 2 | 0.07 | 152.8 | 1.18 |
| | 3 | 0.09 | 318.6 | 2.46 |
| | 4 | 0.17 | 1355.4 | 10.47 |
| | 5 | 0.26 | 1001.8 | 7.74 |
| | 6 | 0.37 | 529.9 | 4.09 |
| | 7 | 0.42 | 503.6 | 3.89 |
| | 8 | 0.55 | 6562.1 | 50.68 |
| | 9 | 0.78 | 486.4 | 3.76 |
| | 10 | 0,97 | 129,2 | 1,00 |

The research obtained the inorganic content of R. pinnata leaves, micro and nano simplicia water extract for potassium, calcium, sodium and magnesium minerals can be seen in Table 4.

**Table 4: Mineral levels of potassium, calcium, sodium and magnesium in a 10% concentration of water extract**

| Minerals | Mineral levels of water extract (µg/ml) | | |
|---|---|---|---|
| | R. pinnata leaves | Micro-simplicia | Nano-simplicia |
| Potassium | 973.07 ± 21.505 | 2888.00 ± 2.6502 | 4373.00 ± 17.416 |
| Magnesium | 37.87 ± 0.7638 | 253.27 ± 0.5132 | 346.57 ± 1.3317 |
| Sodium | 16.89 ± 0.3257 | 30.90 ± 0.1241 | 36.28 ± 0.2125 |
| Calcium | < LD | 74.92 ± 1.2331 | 83.49 ± 0.4239 |

LD (Limit detection = 0.01 µg/ml).

## Discussion

The purpose of phytochemical screening is to find out the secondary metabolites found in simplicia and extract. The results of the phytochemical screening of nanoparticle powder showed the presence of flavonoids, alkaloids, saponins, tannins, glycosides, and steroids/triterpenoids. The results of the phytochemical screening of *R. pinnata* water extract showed the presence of flavonoids, alkaloids, saponins, tannins, and glycosides.

Elution is carried out using several types of eluents namely, n-butanol: acetic acid: water (6: 2: 2); petroleum ether: ethyl acetate: acetic acid (6: 3: 1); and n-hexane: ethyl acetate (6: 4). Eluent, which shows good elution is n-butanol: acetic acid: water (6: 2: 2). This eluent is used to elute polar and semi-polar compounds and is very good for separating flavonoid derivatives. Eluent of h-hexane: ethyl acetate (6: 4) is the mobile phase used to separate semi-polar and non-polar compounds. Using this eluent can be seen in Figure 2, the results of the TLC visually only show 1 stain with Rf approaching 1.

From the results of TLC detection using a densitometer, each water extract showed absorption at several tested wavelengths, the absorption of which has the most optimal results at length 250 and 300 nm. The number of compounds successfully separated by the mobile phase of n-butanol: acetic acid: water (6: 2: 2) for each extract varies in number and percentage. In general, it can be seen that an increase in the number of strains detected from fresh leaf water extracts, macro simplicia and nano simplicia. This is presumed because the particle size affects the amount of substance being absorbed during the extraction process. The smaller the particle size, the contact surface area between the material and the solvent will be greater so that with the same time and concentration different amounts of compounds are obtained.

To determine or analyse the mineral content of a material or sample, it must be degenerated / digest first. The usual method is dry ashing or wet digestion. In this study, the sample was prepared using wet digestion because the sample examined is a liquid material that is water extract. This method is considered to be more practical, the tools needed are simple, and the testing time is relatively shorter compared to dry ashing methods [11]. After the destruction is complete, the sample is diluted and analysed using atomic absorption spectrophotometer.

Calcium has antagonistic properties against potassium and magnesium. Increasing the concentration of calcium in the soil will reduce the concentration of potassium and magnesium [12]. In the study of potassium levels in extracts of water is very much greater than calcium and sodium so that it is hoped that this plant can give a dieresis effect and help the dissolution process of calcium stones found in the kidneys.

Potassium can be a diuretic, so sodium excretions from body fluids increase. This mineral can also change the activity of renin-angiotensin by reducing renin secretion, which causes a decrease in angiotensin II, which plays a role in vasoconstriction. The work of Na-K pumps that function to pump cell fluids and transfer potassium and sodium ions in and out of cells is also influenced by the high amount of potassium in extracellular fluid, this causes the greater secretion of sodium ions and can reduce blood pressure [13].

Potassium intake in a person can affect blood pressure. Low intake of potassium will lead to an increase in blood pressure, whereas a high intake of potassium will cause a decrease in blood pressure. Increased potassium intake can reduce systolic and diastolic blood pressure due to a decrease in vascular resistance. Vascular resistance is caused by dilation of blood vessels and an increase in water and sodium loss from the body, the results of sodium and potassium pump activity. Ideal potassium intake is 4.7 g/day and can be obtained from fruits and vegetables that contain high potassium [13].

Potassium and calcium are minerals that are needed by our body. Calcium is useful in the formation and maintenance of bones and teeth. But if the level of calcium is excessive in the blood, then when it is excreted, calcium tends to bind to oxalate ions and uric acid to become a mass in the kidney glamorous. Potassium in the human body is important in delivering nerve impulses and releasing energy from protein, fat, and carbohydrates during metabolism. Potassium enters the body from the intestinal tract by diffusion through the capillary wall and active absorption [14]. Then potassium enters the cells also by diffusion and requires active metabolic processes. Potassium is removed through urine by secretion and filtering. Potassium also plays an important role in the delivery of nerve impulse to muscle fibres and also in the ability of the muscles to contract [11].

In conclusion, R. Pinnata water extract contains organic compounds in the form of flavonoids, alkaloids, saponins, tannins, and glycosides. The nano simplicia R. pinnata water extract showed more chemical content than other water extracts on the TLC plate with the developer n-butanol: acetic acid: water (6: 2: 2) to detect flavonoids followed by detection at a wavelength of 250 nm and 300 nm. The most element content in R. pinnata water extract is potassium, followed by magnesium, sodium, and calcium. Further research is needed to see the diuresis and anti nephrolithiasis effect from R. pinnata so that its utilisation in the treatment of kidney stones is scientifically proven.

## References


1. Heyne K. Tumbuhan Berguna Indonesia. I. Badan Litbang Kehutanan, editor. Jakarta: Yayasan Sarana Wanajaya, 1987.

2. Masfria, Dalimunthe CA, Syafridah. Pemeriksaan kandungan mineral pada daun ekor naga (Rhaphidophora pinnata (l.f.) schott) secara spektrofotometri serapan atom. 2012; 11(2).

3. Masfria, Hafni A. Cytotoxicity of "Ekor naga" leaf (Rhaphidophora pinnata (Lf) Schott) chloroform extract against T47D cancer cells. Int J PharmTech Res. 2014; 6(4):1327-33.

4. Masfria. Antibacterial activity of ethyl acetate and ethanol extract of Rhaphidophora pinnata (L.f) schott leaf against four types of bacteria. Int J ChemTech Res. 2015; 8(6):905-14.

5. Fernandez MAM, Wiratmini NI, Ermayanti NGA. The Effect of Rhaphidophora pinnata, Schott Leaf Extract on The Uterus Development of Ovariectomized Mice (Mus musculus). Biologi. 2015; 19(2):74-80.

6. Crichton RR. Biological Inorganic Chemistry. 2nd ed. Amsterdam: Elsevier, 2012.

7. Ditjen POM RI. Farmakope Indonesia [Internet]. 4th ed. Departemen Kesehatan Republik Indonesia. Jakarta: Departemen Kesehatan RI, 1995. Available from: https://doi.org/10.6066/jtip.2013.24.2.121. https://doi.org/10.6066/jtip.2013.24.2.121

8. Marliana SD, Suryanti V. Skrining fitokimia dan analisis kromatografi lapis tipis komponen kimia buah labu siam (Sechium edule Jacq. Swartz.) dalam ekstrak etanol. Biofarmasi. 2005; 3(1):26-31. https://doi.org/10.13057/biofar/f030106

9. Putra RT, Lukmayani Y, Kodir RA. Isolasi Dan Identifikasi Senyawa Flavonoid Dalam Tumbuhan Lamun Cymodocea Rotundata. Pros Penelit Spes Unisba. 2015; 444-50.

10. Medić-Šarić M, Jasprica I, Smolčić-Bubalo A, Mornar A. Optimization of chromatographic conditions in thin layer chromatography of flavonoids and phenolic acids. Croatica Chemica Acta. 2004; 77(1-2):361-6.

11. Fitriani NLC, Walanda DK, Rahman N. Determination of Potassium (K) and Calcium (Ca) Content in Chayote (Sechium Edule) and The Effects With its Growth Soil Ni. J Akad Kim. 2012; 1(November):174-80.

12. Shear CB, Crane HL, Myers AT. Nutrient Element Balance: Response of Tung Trees Grown in Sand Culture to Potassium, Magnesium, Calcium, and Their Interactions. Technical Bulletin. Washington, 1953:1085.

13. Tulungnen RS, Sapulete IM, Pangemanan DHC. Hungungan Kadar Kalium dengan Tekanan Darah Pada Remaja di Kecamata Bolangitang Barat Kabupaten Bolaang Mongondow Utara. J Kedokt Klin. 2016; 1(2):37-45.