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Botanical, Pharmacognosy, and Phytochemical Studies of Sintrong Leaves (*Crassocephalum crepidioides*) Growing in Tanjung Setia Village, West Coast, Lampung

Kajian Botani, Farmakognosi, dan Fitokimia Daun Sintrong (*Crassocephalum crepidioides*) yang Tumbuh di Desa Tanjung Setia Pesisir Barat Lampung

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Abstrak

Tanaman *Crassochepalum crepidioides* mempunyai khasiat antibakteri dan antiluka. Penelitian ini bertujuan untuk mengetahui profil botani, serbuk mikroskopis, skrining fitokimia, dan Kromatografi Lapis Tipis (KLT*)* daun *C. crepidioides* dari Desa Tanjung Setia Pesisir Barat Lampung, Indonesia. Metode meliputi pemeriksaan morfologi, serbuk mikroskopis, skrining fitokimia, dan Kromatografi Lapis Tipis (KLT). Hasil botani menunjukkan daun berbentuk lonjong, ujung meruncing, tulang daun menyirip, tepi bergerigi, permukaan berbulu halus, batang berkayu lunak, akar tunggang. Pengamatan mikroskopis ditemukan fragmen stomata. Skrining fitokimia positif terhadap alkaloid, flavonoid, saponin, tanin. uji KLT dengan lima titik (merah) dan nilai Rf rata-rata 0,21-0,97. Kesimpulan: uji kromatografi lapis tipis diduga menghasilkan bercak flavonoid pada nilai Rf 0,72-0,77.

Kata kunci: *Crassochepalum crepidioides, sintrong, antibakteri, Asteracea, Kromatografi Lapis Tipis (KLT)*

Abstract

Crassochepalum crepidioides plant has antibacterial and anti-wound properties. This study aims to determine the botanical profile, microscopic powder, phytochemical screening, and Thin Layer Chromatography (TLC). This study aims to determine the morphology of leaves, stems, roots, microscopic powder, phytochemical screening, and Thin Layer Chromatography (TLC) of *C. crepidioides* leaves from Tanjung Setia Village West Coast Lampung, Indonesia. Methods include morphological examination, microscopic powder, phytochemical screening, and TLC. The results of the botanical show oval-shaped leaves, pointed tips, pinnate veins, serrated edges, smooth leaf surface, soft wood stems, and tap roots. Microscopic observation found fragments of stomata. Positive phytochemical screening of alkaloids, flavonoids, saponins, and tannins. TLC test spot with five spots (red) and an average Rf value of 0.21-0.97. Concludes that the thin layer chromatography test is thought to produce flavonoid spots at an Rf value of 0.72-0.77.

Keywords: *Crassochepalum crepidioides, sintrong, antibakteri, Asteracea, Thin Layer Chromatography (TLC)*

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INTRODUCTION

Crassochepalum crepidioides belongs to the Asteraceae family (a subfamily of Asteroidae, with a shrubby habit. In Indonesia, this plant is known as sintrong and is often considered a weed, or some people use it as a vegetable. [1]. *C. crepidioides* is consumed as a salad in Australia because it contains protein, carbohydrates, fiber, and minerals. [2]. However, some studies report that *C. crepidioides* plants have the potential to be used as medicinal plants. The study showed that treatment with a potent leaf of ethanol extract from East Kalimantan Province with concentrations of 10%, 30%, 50%, 70%, 90%, and 100% could inhibit the growth of *Streptococcus mutans* with a diameter of 1.03 mm – 3.68 mm [3], while the concentration of ethanol extract of sintrong leaves of 30% from Sidoarjo, East Java, was able to inhibit *Salmonella typhii* by 9.2 mm and 10.82 mm [4]. This plant has also been formulated into liquid soap with concentrations of F1 2% and F2 3% and has been proven to inhibit *Staphylococcus aureus* by 14.2 mm—16.1 mm [5]. In addition, ethanol extract of young leaf tips of *C. crepidioides* (Benth) S. Moore) was shown to have activity with an IC50 value of 52.49 mg/ml (strong category) [6], with antioxidant and anti-inflammatory properties, *C. crepidioides,* at a dose of 50mg/kg/day, has been proven to heal wounds in mice within 3.5 days [7].

In African countries, *the C. crepidioides* plant is known as 'Ebolo,' it is used to treat indigestion and headaches. In the Democratic Republic of Congo, leaf sap is given to treat abdominal pain; in Uganda, it is used for stab wounds. The people of Tanzania use dried leaf powder to stop nosebleeds and suck it to treat sleeping sickness. Tannins found in the roots of plants are used to treat swollen lips [8].

Based on the results of empirical and scientific tests regarding the potential efficacy and antioxidant activity of *C. crepidioides*, it is necessary to introduce the plant profile of *C. crepidioides* academically. The most basic method of recognizing and identifying *C. crepidioides* can be done by studying botany, pharmacognosy, and phytochemistry. The botanical aspect is carried out by identifying the morphology of leaves, stems, and roots. The pharmacognosy aspect was carried out by microscopic observation of the identifying fragments, while the phytochemical aspect was carried out by phytochemical screening using color reagents and thin-layer chromatography (TLC). The novelty of this study lies in the sampling of *C. crepidioides* in Tanjung Setia Village, West Coast of Lampung, Indonesia. The results of this research are expected to provide the most basic information and reference on the learning of Botany.

METHODS

Sample preparation

The sample used in this study was Sintrong leaves (*Crassocephalum crepidioides*) from Tanjung Setia village, West Coast of Lampung. The sample was a fresh green leaf aged 3-4 months. Sample preparation includes determination, washing, wet sorting, drying, dry sorting, and making powder using a blender and then sieving.

Macroscopic and microscopic powder examination

Macroscopic examination of *C. crepidioides* leaf powder includes color, smell, and taste. Microscopic examination was performed by placing simplicial powder on an object glass, dripping with chloralhydrate, covering it with a cover glass, fixing it on a Bunsen flame, and then observing it with a 40x magnification microscope.

Extraction

Extraction was carried out for 3 days by placing 200 grams of simplicial powder (obtained from drying 3 kg of wet simplicial) in a container containing 1 L of 70% ethanol solvent (stirring every 1 day). Liquid extract is filtered. The liquid filtrate is evaporated with a rotary evaporator (temperature 50˚C, 60 rpm) to obtain a thick extract [29].

Screening phytochemistry

Phytochemical screening with color reagents includes alkaloids, flavonoids, saponins, and tannins. Alkaloid tests are performed with Meyer, dragendorf, and Wagner reagents. Flavonoid test with Mg and HCL reagents. Tannins with $FeCl_3$ reagents. Saponin with Aquades and HCl. Standard alkaloid solutions use caffeine; flavonoids use quercetin, and tannins use tea. The test sample solution and the comparison standard were prepared by dissolving 500 mg of the solution in 50 mL of 70% ethanol. Positive results of alkaloids with Meyer reagent if there is a white precipitate, dragendorf reagent orange precipitate, brown precipitate Wagner reagent, flavonoid positive if red/orange is formed, tannin positive if blackish-blue or blackish-green is formed, saponin positive if stable foam is formed for 5 minutes [28].

Thin-Layer Chromatography (TLC)

The KLT method is carried out by preparing a silica gel plate of 60 GF254, a capillary pipe, and a mobile phase (chloroform: dichloromethane: ethyl acetate (7:4:1). Before use, the plates are heated for 10 minutes at 100˚C, and elution of the eluents is carried out in the vessel. The test was carried out by extracting leaves on a plate, and then emulation was carried out using a chloroform solvent: dichloromethane: ethyl acetate (7:4:1). The illuminated plate was irradiated with UV 254 nm, UV 366 nm, and visible light [9].

RESULTS AND DISCUSSION

The plant determination test in this study was carried out at the National Innovation

Research Agency. Based on the results of the determination test Number: B-764/II.6.2./IR.01.02/3/2024, it shows that the sample used has the Latin name *Crassocephalum crepidioides* (Benth.) S. Moore with the family Asteraceae. The results of identifying the morphology of the leaves, flowers, stems, and roots of *Crassocephalum crepidioides* plants can be seen in Figure 1.

Figure 1. Morphology of *Crassocephalum crepidioides*

Figure 1 shows that *Crassocephalum crepidioides* has oval-shaped leaf morphology, tapered leaf tips, pinnacle leaf bones, serrated leaf edges, and a fluffy leaf surface. [10], The growth direction of perpendicular stems is soft and woody, with a green taproot [11]. The edges of the serrated leaves consist of the inner (sinus) and the outer parts (angulus) [12]. Fine hairs on the leaf's surface prevent excessive water loss [13]; *Crassocephalum crepidioides* has flowers with a tubular compound type, terminal florets, complete, and green-orange tips [14].

The plants that have been identified are then made of leaf simplicial powder. The results of the macroscopic powder examination of *Crassocephalum crepidioides* leaves can be seen in Figure 2.

Figure 2. Microscopic leaves powder of *Crassocephalum crepidioides*

Figure 2 shows that *Crassocephalum crepidioides* powder has a dark green color and a bitter taste. Each sample was then subjected to microscopic powder observation; the results can be seen in Figure 3.

Figure 3. Stomata of *Crassocephalum crepidioides*

Figure 3 shows the characteristics of the microscopic leaf powder, identifying fragments of *Crassocephalum crepidioides* leaves as stomata. However, the type of stomata cannot be determined. The leaves of *Crassocephalum crepidioides* have normocytic-type stomata with guard cells not surrounded by neighboring cells. [15]. Stomata are modifications of the leaf epidermis for the entry of carbon dioxide and the release of oxygen into the atmosphere, so the high density of stomata will increase the absorption of CO2 in the air [16]. Trichomes are another epidermal modification found on the leaves of *Crassocephalum crepidioides*, along with stomata. The morphology and distribution of trichomes can provide additional data to help identify medicinal plants appropriately [17]. The epidermal structure of the leaves of *Crassocephalum crepidioides* is uniseriate (consisting of a single layer) and rectangular-polygonal. The cuticle is not visible on the surface of the epidermis. It has mesophilic tissue in the form of an elongated cylindrical palisade and an irregularly spherical sponge [18]. Diagnostic features of leaf anatomy for standardizing raw materials for antipyretic drugs include the presence of anomocytic stomata, uniseriate multicellular trichomes, fibers with clear lumina, circular vessel type, single-layer palisade tissue, prism-shaped calcium oxalate crystals, and tannin-containing cells [19].

The powder is then extracted using a 70% ethanol solvent and evaporated to obtain a viscous extract. The yield of the condensed extract can be seen in Figure 4, while the percentage yield of the condensed extract can be seen in Table 1.

Figure 4. Viscous ethanol extract of *Crassocephalum crepidioides* leaves

Table 1. The percentage yield of the thick extract

Thick extract	Sample weight	Extract weight	yield (%)	Requirement (Farmakope Herbal Indonesia)
C. crepidioides	200 g	48.23 g	24.11 %	Not less than 8.7 %

Based on Figure 4, C. *crepidioides* thick extract has a blackish-brown color with a strong, distinctive odor. The percentage of condensed extract yield is 24.11%. The percentage of sample yield is based on the standard requirements of the herbal pharmacopeia, or not less than 8.7%. The results of the yield percentage in this study complement previous research on the ethanol fraction sample, with a weight of 800 grams, which produced a yield percentage of 109.70 grams (13.71%) [5].

The results of the thick extract were then qualitatively phytochemical screening using color reagents to test for the presence of alkaloids, flavonoids, tannins, and saponins. The phytochemical screening test in this study used a comparator (positive control) of caffeine as an alkaloid, quercetin as a flavonoid, and tea as a tannin. The results of the phytochemical screening test with color reagents can be visually seen in Figure 5, while the qualitative results can be seen in Table 2.

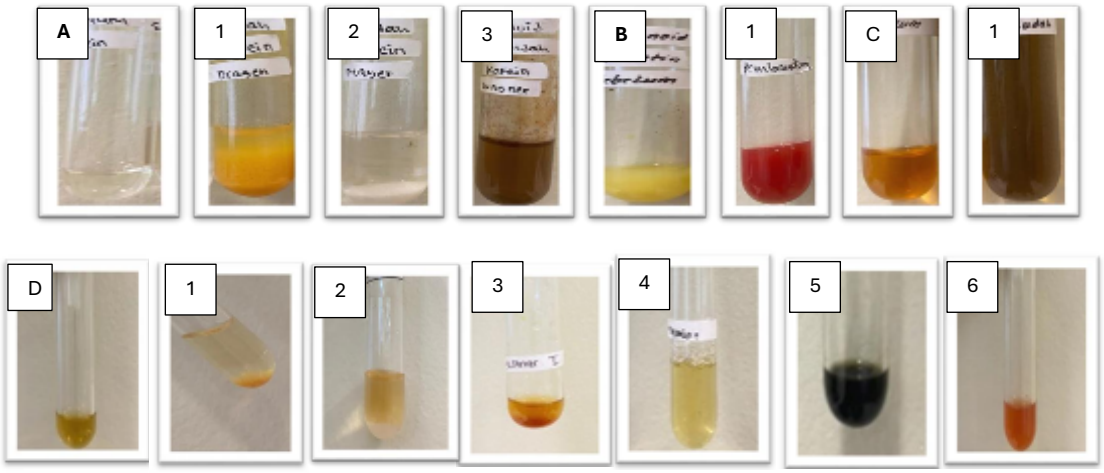

Figure 5. Comparator of phytochemical screening tests. A. caffeine (alkaloid). 1 Dragendorf test (orange precipitate). 2. Meyer test (White precipitate), 3. Wagner (brown precipitate). B. Quesertin (flavonoid). 1. Flavonoid (orange) test. C. Tea (tannin). 1 Tannin test (Blackish green). D. extract C. *crepidioides leaves* before being dipped in a color reagent. 1. Dragendorf test. 2. Meyer test. 3. Wagner test. 4. Saponin test. 5. Tannin test. 6. Flavonoid test.

Table 2. Phytochemical screening test

Senyawa	*C. crepidioides*	Reagen	Indicator
Alkaloid	+	Mayer	White precipitate
	+	Dragendorf	Orange precipitate
	+	Wagner	brown precipitate
Flavonoid	+	HCL +Mg	Orange
Saponin	+	Aquadest	Stable foam 1-2 minutes
Tannin	+	$FeCl_3$ 1%	Blackish green

(+) : (-): none

Based on Figure 5. The phytochemical screening test for alkaloids is carried out with a caffeine comparator (control positive); after the Dragendorf test is positive and produces orange precipitation, the Meyer test is positive and produces white precipitation, the Wagner test produces brown precipitation, for the flavonoid test is carried out using comparator and positively produces an orange color. In contrast, the tannin test uses a tea comparator, with positive results for an orange color. Blackish green, then, concerning the control results, phytochemical screening tests were carried out for *C. crepidioides* ethanol extract. As per Table 2, both positive test samples contain alkaloids, flavonoids, tannins, saponins, and phenols. This study's results differ from previous studies, which showed that the ethanol extract of *C. crepidioides* leaves from India was positive for alkaloids and flavonoids. Still, negative tannins, saponins, and phenols were proven to inhibit *S. aureus* by 10.83 mm and MRSA by 11.84 mm, while hot water extract produced antioxidant activity between 26% and 78% [20].

The results of other quantitative analysis on ethanol extract of *C. crepidioides* leaves from Wawonggole Village, Konawe Regency, Southeast Sulawesi Province, showed flavonoid and tannin levels of 6.751% and 0.43%, resulting in an inhibition zone in *E.coli* of 12.2 mm (strong), 12.67 mm (strong), 19.15 mm (very strong), and 20.85 (very strong) at concentrations of 5%, 10%, 20%, 30% [18]. A review of *C. crepidioides* contains Pyrrolizidine alkaloids (PAs) (a group of natural alkaloids) under the name jacobine as protection from herbivores. [2]. Research on the water fraction of *Crassocephalum crepidioides* in Nigeria yielded a total phenolic of 15.34 mgGAE/g, flavonoids of 2.29 mg, tannins of 2.39 mg, and DPPH activity of 16.42% [21]. Some secondary metabolite compounds that have antioxidant, antibacterial, anti-inflammatory, and antidiabetic activity include Sinapic acid, 3-Feruloylquinic acid, Dihydroquercetin, Malic acid, Hexose-hexose-Nacetyl, gallic acid, which is evidenced by the inhibiting activity of α-amylase at a dose of 20 µg/ml of 45.81, 50 µg/ml of 56.47, 100 µg/ml of 67.28, 250 µg/ml of 77.94, 500 µg/ml of 81.69. This activity is better than acarbose (a drug that lowers blood glucose levels in people with type 2 diabetes) [22].

This study also conducted a thin-layer chromatography test using a comparator as a control for flavonoid compounds. The results of the thin-layer chromatography test observed in this study were the band distance and Rf value using UV 254 nm and UV 366 nm. The mobile phase is chloroform dichloromethane ethyl acetate (7:4:1) with 3x replication testing. The results of the thin-layer chromatography test can be seen in Tables 3 and 4.

Table 3. Thin-layer chromatography test of ethanol extract *of Crassocephalum crepidioides* leaves

Spots	Rf	Visible light	254 nm	366 nm
1	0.21	Green	dark	Red
2	0.27	Green	dark	Red
3	0.48	Green	dark	Blue
4	0.61	Green	dark	Red
5	0.72	Green	dark	Blue
6	0.77	Green	dark	Red
7	0.97	Green	dark	Red

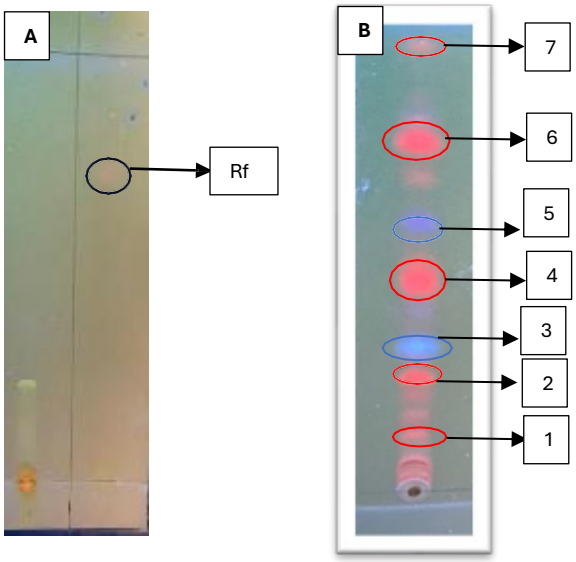

Figure 6. A. control positive (quercetin). B. Ethanol extract of *Crassocephalum crepidioides* leaves (366 nm)

Based on table 3 and figure 6. The results of the KLT test of the ethanol extract of *Crassocephalum crepidioides* leaves obtained seven spots with red and blue colors. The average Rf value of the ethanol extract of *Crassocephalum crepidioides* leaves was 0.21-0.97. As for the nine spots from the two test samples, spots 5 and 6 produced an average Rf value of 0.72 and 0.77. The average Rf value is close to the quarantine Rf value of 0.71 at 366 nm UV. Quercetin is a flavonoid compound that can form color complexes. The flavonoid spots produced on the KLT plates are blue and pink with an average Rf value of 0.72-0.77, so it is suspected that spots 5 and 6 are flavonoid compounds. This is to the results of the KLT test of methanol extract of *Crassocephalum crepidioides* leaves using the mobile phase of acetonitrile, ethyl acetic acid, and ethanol (35:5:15) which produces flavonoid and phenol patches of red color at a wavelength of 366 nm, after derivatization by spraying of flavonoid $AlCl_3$ reagents produces a yellow color, while spraying with NP reagents produces phenols with a brown color, in addition to the total result of flavonoids 65.21 mg and phenol 26, 78 mg can effectively inhibit nitric oxide production [23].

The presence of flavonoids in *C. crepidioides* leaf extract was proven to function as an antioxidant by lowering malondialdehyde (MDA) at 37.5 mg/Kg/BB in rats. [24]. In addition, the content of flavonoids and phenols makes *C. crepidioides* leaves a source of phytochemicals for preventing diabetes mellitus [24]. This is proven in a study of giving *C. crepidioides* leaf extract doses of 150 mg and 300 mg to produce antidiabetic effects by lowering blood glucose levels and β-pancreatic cell protection for 14 days in Wistar rats [25]. Some flavonoid actions as antidiabetics include suppressing glycogen phosphorylase enzymes, alpha-glucosidase, and alpha-amylase. All these enzymes function to break down complex carbohydrates into simple carbohydrates (monosaccharides), which cause increased blood glucose levels. Inhibition of these enzymes by flavonoids can help reduce blood glucose levels and strategies in managing diabetes therapy [26]. The principle of flavonoids as antioxidants can inhibit free radical oxidation reactions. Flavonoids will donate hydrogen ions to neutralize free radicals. The antioxidant strength of flavonoids depends on the number of hydroxyl groups (-OH). The greater the number of hydroxyl groups, the higher the antioxidant activity, so it prevents lipid peroxidation and reduces malondialdehyde (MDA) levels [24]. In addition, flavonoids can inhibit bacterial growth by disrupting the integrity of membranes and cell walls, preventing protein and nucleic acid synthesis, and blocking the formation of virulence factors and bacterial biofilms [27].

CONCLUSION

The morphological characteristics of the *C. crepidioides* plant are in the leaves and flowers, with positive phytochemical screening containing alkaloids, flavonoids, tannins, saponins, and phenols. Confirmation of the thin-layer chromatography test is suspected to produce flavonoid patches at an Rf value of 0.72-0.77.

CONFLICT OF INTEREST

I hereby declare that there is no conflict of interest in writing this scientific work.

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