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Pharmacognostic Characterization and Standardization of the Aerial Parts of *Enhydra fluctuans* Lour. Collected in West Sumatra, Indonesia

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Abstract
The standardization of herbal raw materials is a critical prerequisite for ensuring the safety and reproducibility of traditional medicines. *Enhydra fluctuans* Lour. (Asteraceae), a semi-aquatic herb widely used in West Sumatra as a leafy vegetable and reported to possess antioxidant, thrombolytic, hepatoprotective, and antidiabetic activities. Despite these recognized bioactivities, comprehensive pharmacognostic data that would support the authentication, identification, and quality control of this species remain scarce. This study aimed to characterize the morphological, anatomical, and physicochemical properties of the aerial parts of *E. fluctuans*, and to establish standardization parameters following the guidelines of the Indonesian Herbal Pharmacopoeia. Morphological evaluation was conducted through macroscopic assessment of fresh plant material and simplicia, whereas anatomical structures were examined using transverse and longitudinal sections observed under a light microscope. Physicochemical analyses included determination of acid-insoluble ash and quantification of heavy metal contaminants (Cd and Pb) using gravimetric and spectroscopic methods. The leaves were found to exhibit both paracytic and actinocytic stomatal types, accompanied by glandular trichomes, while the stems showed bicollateral vascular bundles as a defining anatomical feature. The simplicia were characterized by their green coloration, distinctive odor, bitter taste, and the presence of dispersed prismatic calcium oxalate crystals. The non-specific parameters include total ash content, acid-insoluble ash, moisture content, and heavy metal contamination (Cd and Pb) met the acceptable limits defined by the Indonesian Herbal Pharmacopoeia. These findings strengthen its potential application as a safe and reliable herbal raw material for future pharmacological investigations and herbal product development.

INTRODUCTION

The use of medicinal plants has gained renewed global interest, particularly as sources of bioactive natural products for health and therapeutic applications. However, compared to synthetic pharmaceuticals, herbal medicines pose unique challenges due to inherent variability in chemical composition, which may be influenced by genetic, environmental, and processing factors (Kulkarni, 2025). Therefore, rigorous pharmacognostic evaluation and standardization are critical to guarantee the identity, purity, safety, and consistency of herbal raw materials before further pharmacological or clinical applications (Upadhyay et al., 2019).

Pharmacognostic study, involving morphological, anatomical (microscopic), and physicochemical analyses constitutes the foundational step for authenticating crude herbal drugs (Majid et al., 2021). Standardization ensures that herbal materials meet defined quality parameters (e.g., organoleptic, macroscopic/microscopic features, ash values, extractive values, absence of contaminants), thus minimizing batch-to-batch variation and preventing adulteration or misidentification (Chanda, 2014). Without such baseline data, the safety and efficacy claims for many botanical products remain questionabl, a concern increasingly highlighted in recent reviews calling for stricter quality control of medicinal plants (Kumar et al., 2023).

The semi-aquatic herb *Enhydra fluctuans* Lour. (Asteraceae) is commonly found in West Sumatra, Indonesia especially around springs, small rivers, and ponds and widely known locally as "Cikarau" (Delfita et al., 2022). It has a long history of use both as a leafy vegetable and in traditional medicine. Several ethnobotanical reports and pharmacological studies attribute to it various bioactivities including antioxidant, thrombolytic, hepatoprotective, and antidiabetic effects, as well as traditional uses to relieve inflammation (Delfita & Tjong, 2023; Delfita et al., 2021; Ali *et al*., 2013; Kuri *et al*., 2014). However, despite these pharmacological potentials, a comprehensive pharmacognostic and standardization study of *E. fluctuans*, especially for populations growing in Indonesia remains lacking.

Although some earlier reports from other regions (e.g. India) Deb *et al*., (2016) attempted to describe pharmacognostic aspects of *E. fluctuans*, the data heavy metal contamination was not reported. This heavy metal contamination must be present in the Indonesian herbal pharmacopoeia standards. Moreover, environmental and geographical variations may influence anatomical and chemical traits, making local validation essential. Given this gap, the present study aims to perform a detailed pharmacognostic evaluation (morphology, anatomy, microscopic features) and standardization (physicochemical parameters, purity, contaminants) of aerial parts of *E. fluctuans* collected from Jorong Tabek, Tanah Datar, West Sumatra. The resulting data are intended to serve as a reference for authentication and quality assurance, supporting safe and reproducible use of this herb as a medicinal raw material.

METHOD

Plant Material Collection and Authentication

Fresh aerial parts (young stems and leaves) of *Enhydra fluctuans* were collected from Tabek Village, Tanah Datar District West Sumatra, Indonesia. The plant material was taxonomically authenticated by a plant taxonomist at the Herbarium of the Department of Biology, Universitas Andalas, Padang. The collected aerial parts were washed, shade-dried at room temperature for 15 days, and subsequently cut into small pieces. The dried material was powdered using a mechanical grinder and sieved to obtain a uniform powder.

Pharmacognostic Evaluation

Macroscopic (Morphological) Examination

Macroscopic characters of fresh leaves and stems were examined following standard parameters (Evans, 2002) including size, shape, surface characteristics, leaf venation, presence of petiole, apex, margin, base, texture, color, aroma, and taste. Stem characteristics such as nodes, internodes, surface, branching pattern, and color were recorded.

Microscopic (Anatomical) Examination
For anatomical characterization, thin transverse sections of fresh leaves and stems were prepared by hand-sectioning, mounted in distilled water, and examined under a photo microscope (Olympus DP 22, Japan). Structural features recorded included upper and lower epidermis, stomatal type, parenchymatous tissues, and vascular elements.

Powder Microscopy of Simplicia
Microscopic evaluation of the powdered aerial parts involved observing diagnostic characters such as epidermal fragments, trichomes, vessel elements, parenchyma, and calcium oxalate crystals. All microscopic features were compared with established pharmacognostic references to confirm identity and diagnostic relevance.

Standardization Parameters
The standardization assessment included specific and non-specific parameters. Specific parameters consisted of identity confirmation, organoleptic characteristics, qualitative chemical profiling of the extract, and water- and ethanol-soluble extractive values. Non-specific parameters comprised total ash, acid-insoluble ash, moisture content, and heavy metal levels (Cd and Pb). All parameters were determined according to the procedures prescribed in the Indonesian Herbal Pharmacopoeia (Depkes, 2008).

Phytochemical Screening
Qualitative phytochemical screening of the extract and its fractions was performed following standard phytochemical investigation procedures. The metabolites evaluated included alkaloids, flavonoids, saponins, terpenoids, steroids, and coumarins, based on established protocols (Ghosal & Mandal, 2012).

Statistical Analysis
Pharmacognostic and standardization data of the aerial parts of *E. fluctuans* were analyzed descriptively and presented in tabular form.

RESULT AND DISCUSSION

1. RESULT

Pharmacognostic Characteristics
The general morphology of *Enhydra fluctuans* Lour. shows that the plant possesses slightly succulent stems, reaching approximately 30 cm in height, with branching occurring at the lower nodes, and the stems being slightly pubescent (Figure 1). The stems are green, cylindrical, hollow, and distinctly nodal. The surface is hairy, with denser trichomes around the nodes. The lower nodes bear adventitious roots. The leaves are green, odorless, simple, and sessile, with an opposite phyllotaxy. The leaves are linear in shape, measuring 3 to 7.5 cm in length. The leaf base is straight, the apex is acute, the margins are serrated, the surface is smooth, and the venation pattern is pinnate. The flowers are solitary and sessile, with a diameter of less than 1 cm. They are whitish-green in color and located in the axils of the leaves (Table 1).
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Figure 1. Aerial morphology of Cikarau (*E. fluctuans*)

Table 1. Macroscopic Characteristics of Cikarau (*E. fluctuans*)

No	**Paramter Assesed**	Literature
1.	General Morphology: The plant has slightly succulent stems, reaches approximately 30 cm in height, branches at the lower nodes, and has slightly pubescent stems.	The plant has slightly succulent stems, grows to about 30 cm in height, branches at the lower nodes, and is slightly pubescent with a faint aroma (Ali, Billah, et al., 2013).
2.	Stem: Green in color, cylindrical and hollow, possesses distinct nodes where leaves, branches, and flowers attach, lower nodes bear adventitious roots, stem surface is hairy, with denser trichomes around the nodes	The stem is slightly succulent, grows to 30 cm or more, branches at the lower nodes, is slightly pubescent, and emits a mild aroma (Ali, Billah, et al., 2013). The lower stem nodes produce adventitious roots. (Sarma, et al., 2014).
3.	Leaves Green in color, odorless, simple, sessile leaves with incomplete leaf structure, opposite leaf arrangement, linear leaves measuring 3 to 7.5 cm in length, leaf base straight, apex acute, margins serrated, surface smooth, venation pinnate.	Leaves are irregular, linear, measuring 3 to 5 cm in length; apex acute or obtuse; base straight; margins slightly serrated. (Ali, Billah, et al., 2013).
4.	Flowers Solitary, sessile flowers, less than 1 cm in diameter, whitish-green in color, flowers arise from the leaf axils.	Flowers are solitary and sessile, located in the leaf axils (Ali, Billah, et al., 2013. Flowers range from white to whitish-green (Sarma, et al., 2014)

Anatomical Characteristics

In the transverse section of the leaf taken through the midrib region, a compact rectangular-shaped epidermal layer is observed on both the upper and lower surfaces of the leaf. The upper epidermis is covered by a cuticle. The palisade parenchyma layer is located directly beneath the epidermis, consisting of elongated, densely packed cells rich in chloroplasts. The spongy parenchyma is distributed toward the inner region and is characterized by large intercellular spaces. The vascular tissues (xylem and phloem) contain their fundamental structural elements (Figure 2a).

Anatomically, the leaves of E. fluctuans possess stomata with subsidiary cells arranged parallel to the long axis of the guard cells, forming four or more subsidiary cells arranged radially around the stomatal pore. In other words, the stomata exhibit both paracytic and actinocytic types (Figure 2b). Stomatal density is higher on the upper epidermis than on the lower epidermis. Glandular trichomes are present on both the upper and lower epidermal surfaces (Figure 2c).

The anatomical structure of the stem of E. fluctuans consists of a thin epidermal layer, with the outer wall covered by a cuticle. Beneath the epidermis lies the ground tissue (cortex), composed of parenchyma and collenchyma cells. Numerous young parenchyma cells rich in chloroplasts are present, along with large air spaces within the cortex. Transverse sections show that the arrangement of the vascular tissues is bicollateral, in which the xylem is positioned between the outer and inner phloem (Figure 3).

Macroscopic Characteristics of the Simplicia

Based on the macroscopic examination, the simplicia is in the form of powder, green in color, with a characteristic odor and a bitter taste (Table 3).

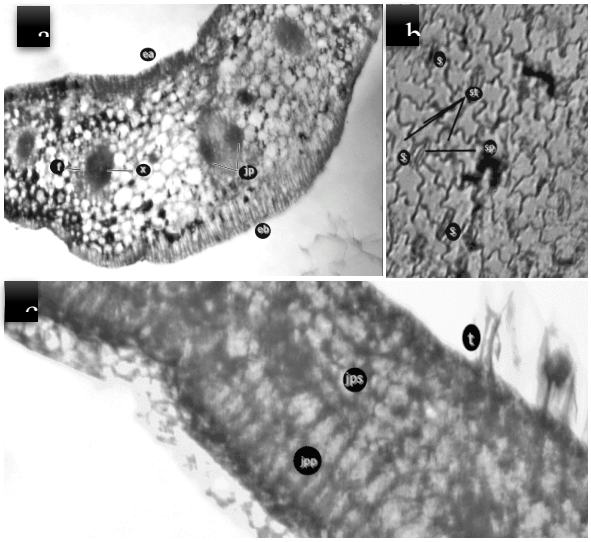

Figure 2. Anatomical characteristics of Cikarau leaves (*E, fluctuans*) a, c = transverse sections of the leaf of E. fluctuans; b = leaf surface section of E. fluctuans. ea = upper epidermis; f = phloem; x = xylem; jp = vascular bundle; eb = lower epidermis; S = stomata; st = subsidiary cell; sp = guard cell; jpp = palisade parenchyma; jps = spongy parenchyma; t = trichomes. Magnification 400×.

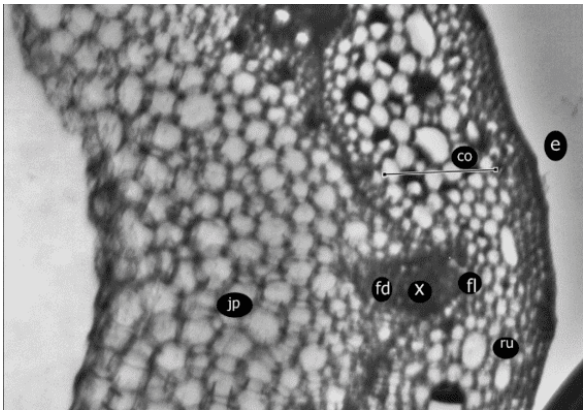

Figure 3. Transverse section of the stem of E. fluctuans. e = epidermis; co = cortex; fl = outer phloem; fd = inner phloem; x = xylem; jp = parenchyma tissue; ru = air spaces. Magnification 400×.

Microscopic Characteristics of the Simplicia

Microscopic examination of the powdered simplicia revealed the presence of various diagnostic fragments, including parenchyma cells, oil cells, trichomes, vascular bundle sheaths, palisade parenchyma, phloem fibers, sieve cells, laticiferous cells, epidermal cells, oil globules, and calcium oxalate crystals (Figure 4).

Table 2. Macroscopic characteristics of simplicia

No	**Parameter Assesed**	Result
1.	Appearance	Powder
2.	Color	Green
3.	Odor	Distinctiv
4.	Taste	Bitter

Microscopic Characteristics of the Simplicia

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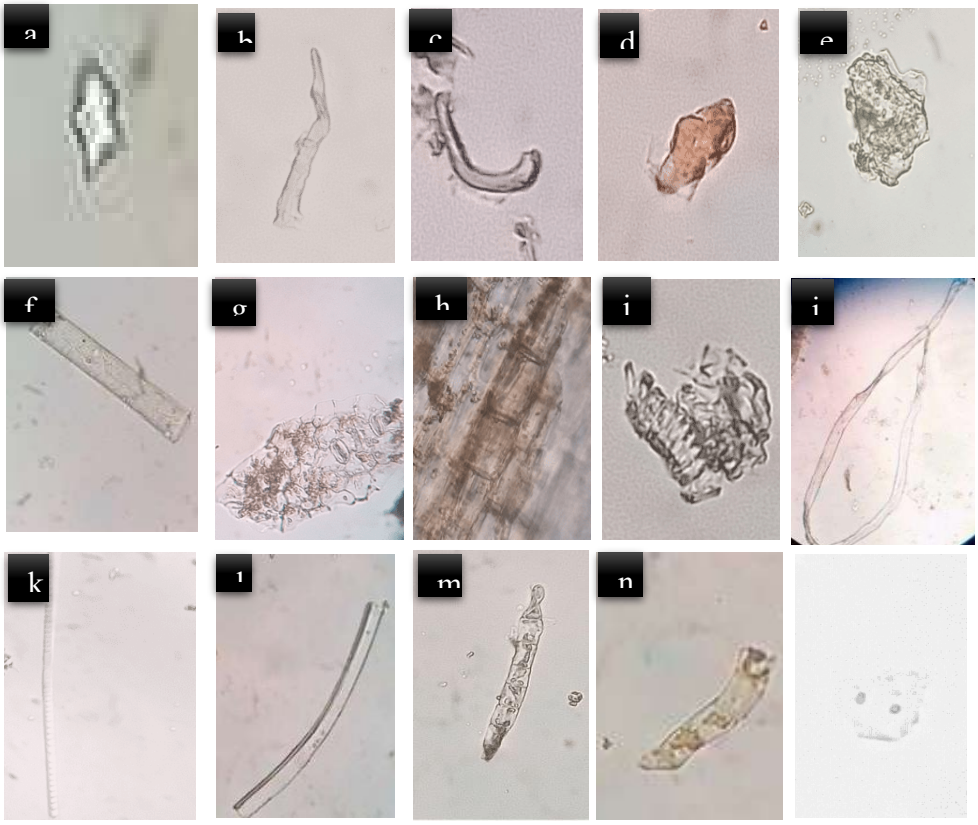

Figure 5. Microscopic characteristics of Cikarau (*E. fluctuans*) simplicia. a = calcium oxalate crystals; b, c = trichomes; d = oil cells; e = laticifer cells; f = mature sieve cells; g = epidermal cells; h = parenchyma cells; i = palisade parenchyma; j = phloem fibers; k = sieve cells; l = collenchyma; m, n = sieve vessels; o = oil globules. Magnification 400×.

Standardization of the Aerial Parts of Cikarau (*E. fluctuans*)

The standardization of the aerial parts of Cikarau (*E. fluctuans*) was carried out by evaluating its specific parameters, which included extract identity, organoleptic characteristics, solvent-soluble constituents, and the preliminary phytochemical profile of the extract and its fractions. The extract used in this study was a concentrated preparation obtained from the aerial parts (herba) of *E. fluctuans*, known locally as Cikarau in Minangkabau (West Sumatra) and Godobos in Javanese regions. The botanical identity was confirmed as *E. fluctuans* Lour., and the plant material consisted exclusively of aerial parts.

The organoleptic evaluation comprised the assessment of color, consistency, odor, and taste. These characteristics were documented for both the ethanol extract and its respective fractions, and the results are summarized in Table 3. Additionally, the extract and fractions were analyzed for their solvent-soluble constituents to support the establishment of quality parameters in accordance with pharmacognostic and pharmacological standards.

Table 3. Organoleptic properties of ethanol extract and E. Fluctuans fraction and soluble compound content.

No	Paramter Assesed	Result
1.	Color	
	Ethanolic extract	Dark deep green
	n-Hexane Fraction	Dark green and glossy
	Ethyl acetate Fraction	Brown
	n-Butanol Fraction	Brown
2.	Odour	
	Ethanolic extract	distinctive odor
	n-Hexane Fraction	distinctive odor
	Ethyl acetate Fraction	distinctive odor
	n-Butanol Fraction	distinctive odor
3.	Appearance	
	Ethanolic extract	Paste
	n-Hexane Fraction	Paste
	Ethyl acetate Fraction n-Butanol Fraction	Paste with the presence of a thin brown-colored fluid, a concentrated solution with crystal-like deposits visible at the bottom of the container (when undisturbed).
4.	Taste	
	Ethanolic extract	Bitter
	n-Hexane Fraction	Distinctive taste
	Ethyl acetate Fraction	Distinctive taste
	n-Butanol Fraction	Distinctive taste
5.	Level of soluble compounds in water	14,5976 ± 0,850 %
6.	Level of soluble compounds in ethanolic extract	13,7896 ± 0,1699 %

Phytochemical Constituents of Extracts/Fractions

The ethanolic extract of the aerial parts of E. fluctuans contains saponins, phenolics, terpenoids, steroids, and coumarins. The n-hexane fraction was found to contain terpenoids and steroids. The ethyl acetate fraction tested positive for saponins, terpenoids, steroids, and alkaloids, while the n-butanol fraction contained saponins, phenolics, and coumarins.

Table 4. Phytochemical constituents of the ethanol extract and fractions of the aerial parts of Cikarau (*E. fluctuans*).

No	Phytochemical constituents	Extract/Fraction (n=3)			
		Ethanolic extract	n-Hexane fraction	Etyl acetae fraction	n-Butanol fraction
1.	Flavonoids	-	-	-	-
2.	Saponins	+	-	+	+
3.	Phenolic	+	-	-	+
4.	Terpenoids	+	+	+	-
5.	Steroids	+	+	+	-
6.	Alkaloids	-	-	+	+
7.	Coumarins	+	-	-	-

Legend: + = present, − = absent.

Non-Specific Parameters

The non-specific parameters of the aerial parts of Cikarau (*E. fluctuans*) include total ash content, acid-insoluble ash, moisture content, and heavy metal contamination (Cd and Pb). The aerial parts of E. fluctuans exhibited a total ash value of 14.12%, an acid-insoluble ash value of 0.44%, and a moisture content of 13.35%. The levels of heavy metal contamination were 0.0659 mg/kg for Cd and 0.09308 mg/kg for Pb.

CONCLUSION

This study provides the first pharmacognostic characterization and standardization profile of the aerial parts of *E. fluctuans* Lour. collected from West Sumatra, Indonesia. Detailed morphological and anatomical evaluations such as the presence of bicollateral vascular bundles, paracytic–actinocytic stomata, and diagnostic microscopic features including glandular trichomes and prismatic calcium oxalate crystals establish clear anatomical markers for authenticating the species. Physicochemical standardization revealed characteristic organoleptic properties, defined extractive values, and a distinct phytochemical profile dominated by phenolics, terpenoids, steroids, saponins, and coumarins. Although the total ash and moisture content slightly exceeds pharmacopeial limits, heavy metal levels remain within acceptable safety thresholds, indicating minimal contamination and supporting the suitability of the material for further use. Overall, the pharmacognostic and standardization data generated in this study provide robust reference parameters for the identification, authentication, and quality control of *E. fluctuans*. These findings strengthen its potential application as a safe and reliable herbal raw material for future pharmacological investigations and herbal product development.

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