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Original Article

Phytochemical Screening and Pharmacognostic Standardization of Red Algae *Gracilaria verrucosa* Simplicia for Phytopharmaceutical Development

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Abstract

Indonesian coastal waters harbor abundant macroalgae resources, including the red algae *Gracilaria verrucosa* (Hudson) Papenfuss, a species known to accumulate diverse secondary metabolites of pharmaceutical interest. This study was conducted to identify the secondary metabolite profile through phytochemical screening and to evaluate the quality of *G. verrucosa* simplicia in accordance with the specific and non-specific parameters of the Indonesian Herbal Pharmacopoeia. Fresh thalli (20,000 g) were collected from the coastal waters of Nongsa, Batam City, processed into simplicia, and extracted by Soxhletation using 96% ethanol. Phytochemical screening covered alkaloids, flavonoids, terpenoids, saponins, tannins, and phenols. Quality characterization encompassed organoleptic, microscopic, water- and ethanol-soluble extractive values, moisture content, loss on drying, ash content, heavy metal contamination (Cd, Pb, Hg, Sn, As) determined by ICP-MS, and microbial contamination (total plate count, coliforms, molds). The simplicia yield was 5.5% and the ethanolic extract yield was 4%. The extract contained alkaloids, flavonoids, terpenoids, tannins, and phenols, whereas saponins were not detected. Water- and ethanol-soluble extractive values were 35% and 17%, respectively. Moisture content (13.3%) met the pharmacopoeial limit, while loss on drying and ash content reached 15% and 39%. Cadmium (0.55 mg/kg), lead (0.47 mg/kg), and arsenic (13.46 mg/kg) exceeded the regulatory thresholds, and microbial counts surpassed the standard except for molds. *G. verrucosa* from Nongsa shows promising bioactive potential, but post-harvest refinement, arsenic detoxification, and microbial control are required before its application as a phytopharmaceutical raw material.

Keywords: *Gracilaria verrucosa; Phytochemical Screening; Pharmacognostic Standardization; Phytopharmaceuticals; Red Algae*

Introduction

Indonesia, an archipelagic country bordered by extensive coastal ecosystems, is recognized as one of the largest reservoirs of macroalgae diversity in the world, accounting for approximately 6.42% of the global macroalgal genetic resources (Sarita *et al.*, 2021). Within this richness, the red algae group (Rhodophyta) represents the most dominant taxon, comprising more than 560 species across the archipelago. Despite such abundance, the strategic utilization of macroalgae in industrial and health-oriented sectors remains underexplored, and a substantial fraction of these biological resources is still left untapped along the shoreline (Pradana *et al.*, 2020).

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Among the commercially valuable red algae, *Gracilaria verrucosa* (Hudson) Papenfuss is widely cultivated as a primary source of agar, yet recent investigations have highlighted its broader pharmacological relevance. The thalli contain phenolic compounds, flavonoids, sulfated polysaccharides, alkaloids, terpenoids, and tannins, all of which contribute to antioxidant, antimicrobial, immunomodulatory, and anti-inflammatory activities (Kim *et al.*, 2023; Hakim *et al.*, 2024). These constituents underscore the candidacy of *G. verrucosa* as a sustainable raw material for the development of phytopharmaceuticals, defined by the Indonesian regulatory framework as evidence-based herbal preparations supported by preclinical and clinical evaluations (BPOM RI, 2023).

The translation of any natural source into a phytopharmaceutical raw material relies fundamentally on the preparation of standardized simplicia. According to the Indonesian Herbal Pharmacopoeia, simplicia must comply with specific and non-specific quality parameters encompassing organoleptic features, soluble extractive content, moisture, ash, loss on drying, microbial contamination, and heavy metal residues (Pambudi *et al.*, 2023; Sutomo *et al.*, 2021). Such standardization is critical to ensure consistency of the active constituents and to protect consumers from biological and chemical contaminants.

Although a number of studies have characterized different species of Gracilaria in various Indonesian waters (Insani *et al.*, 2022; Purwaningsih & Deskawati, 2021; Syafaat *et al.*, 2024), data on *G. verrucosa* collected from the coastal waters of Batam, particularly Nongsa, remains scarce. Considering the geographical position of Batam adjacent to dense maritime traffic and industrial activity, the local thallus may exhibit a distinctive metabolite profile as well as contamination patterns that diverge from previously documented populations. Therefore, an integrated study that simultaneously investigates the secondary metabolite content and the pharmacopoeial quality of *G. verrucosa* from Nongsa is needed to support its prospective use as a phytopharmaceutical raw material. This research was undertaken to identify the secondary metabolites of *G. verrucosa* simplicia through phytochemical screening and to evaluate its quality based on the specific and non-specific parameters of the Indonesian Herbal Pharmacopoeia.

Research Methods

Study Design, Time, and Location

This study applied an experimental laboratory approach. Sample preparation and analyses were carried out from June to August 2025 at the Laboratory of Natural Product Chemistry, Bachelor of Pharmacy Study Program, Faculty of Health Sciences, Universitas Batam, Kepulauan Riau. Heavy metal and microbiological analyses were performed at the accredited Saraswati Indo Genetech (SIG) Laboratory.

Materials and Instruments

The principal material was fresh red algae *Gracilaria verrucosa* (Hudson) Papenfuss obtained from the coastal waters of Nongsa, Batam City. Reagents used included 96% ethanol, distilled water, chloroform, ethyl acetate, n-hexane, Dragendorff's reagent, Mayer's reagent, magnesium powder, hydrochloric acid, ferric chloride ($FeCl_3$), sulfuric acid (H_2SO_4), and sodium hydroxide (NaOH). Instruments comprised a Soxhlet apparatus, rotary evaporator, oven, autoclave, analytical balance, 60-mesh sieve, desiccator, TLC plates, and standard glassware.

Plant Determination

Botanical determination was conducted at the Animal Ecology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Andalas, Padang, to confirm the identity of the collected specimen as *Gracilaria verrucosa* (Hudson) Papenfuss.

Simplicia Preparation

Fresh samples were weighed and subjected to wet sorting to remove foreign matter such as sand, debris, and other macroalgae (Diharmi *et al.*, 2020). The thalli were then rinsed under running water and soaked for 24 hours to reduce salt residues (Numberi *et al.*, 2020). Drying was carried out under direct sunlight from 08:00 to 16:00 local time for three to four days; this approach was selected because it is economical, accessible, and has been shown to preserve the bioactivity of Gracilaria spp. (Shakeel *et al.*, 2024). The dried thalli were ground in a blender and sieved through a 60-mesh sieve to obtain a uniform powder, which was stored in a tightly sealed, light-protected container until further analysis.

Extraction

A 100-g portion of the simplicia powder was extracted by Soxhletation with 1,000 mL of 96% ethanol at a sample-to-solvent ratio of 1:10 at 70 °C (Virginia & Ariyanti, 2024). The extraction was continued until the returning solvent appeared nearly colorless. The combined extract was filtered and concentrated using a rotary evaporator at 40 °C to remove the solvent, followed by evaporation in a porcelain dish over a water bath until a viscous extract was obtained (Ndahawali *et al.*, 2021). The extraction yield was calculated using the equation: % yield = (weight of viscous extract / weight of simplicia) × 100%.

Phytochemical Screening

Phytochemical analysis was performed to detect alkaloids, flavonoids, terpenoids, saponins, tannins, and phenols. Alkaloid screening employed Dragendorff's and Mayer's reagents, with an orange and a yellowish precipitate, respectively, indicating a positive reaction (Kurniasari *et al.*, 2018). Flavonoids were identified by the appearance of a yellow color after the addition of NaOH (Gajalakshmi *et al.*, 2018). Terpenoid detection used the Liebermann–Burchard reaction with chloroform and concentrated H_2SO_4, where a reddish-brown ring at the interface confirmed a positive result (Mubarak *et al.*, 2018). Saponins were investigated through foam stability after vigorous shaking with hot water for 15 minutes. Tannins were detected with 1% $FeCl_3$, while phenols were confirmed with 10% $FeCl_3$, both yielding bluish to dark green coloration as positive indications (Möwes *et al.*, 2025).

Specific and Non-Specific Quality Parameters

Specific parameters included organoleptic evaluation (color, odor, taste, shape), microscopic examination, and determination of water- and ethanol-soluble extractives. Five grams of 28implicial were macerated separately in 100 mL of chloroform-saturated water and 100 mL of 95% ethanol for 24 hours under intermittent shaking; 20 mL of each filtrate was evaporated to a constant weight to calculate the soluble extractives.

Non-specific parameters comprised moisture content (gravimetric, 105 °C until constant weight), loss on drying, total ash (AOAC, 550 °C for 4–6 hours), microbial contamination, and heavy metal contamination. Total plate count (ISO 4833-1:2013/Amd 1:2022), coliforms (SNI ISO 4831:2012), and molds (SNI ISO 21527-2:2012) were determined at the SIG Laboratory. Heavy metals (As, Cd, Pb, Hg, Sn) were quantified by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) after microwave-assisted nitric acid digestion (Pertiwi *et al.*, 2025).

Thin-Layer Chromatography (TLC)

The ethanolic extract was spotted onto a 10 × 1 cm silica gel TLC plate, eluted with an appropriate mobile phase, and visualized under UV light at 254 nm. Retention factors (Rf) values were recorded for each separated spot.

Data Analysis

All data were analyzed descriptively and presented in tabular form. The results were benchmarked against the relevant Indonesian National Standards (SNI) for traditional medicine raw materials and the Indonesian Herbal Pharmacopoeia.

Results and Discussion

Plant Determination and Simplicia Preparation

Plant determination at Universitas Andalas confirmed the specimen as *Gracilaria verrucosa* (Hudson) Papenfuss, belonging to the family Gracilariaceae. Of 20,000 g of fresh thalli, 1,100 g of dried implicial was obtained, corresponding to a implicial yield of 5.5%. The implicial appeared as a fine green powder with a characteristic odor and a bitter taste. The substantial weight reduction reflects the inherently high-water content of *G. verrucosa*, which is consistent with the report of Yanuarti *et al.* (2017) indicating that macroalgae generally contain 80–90% water in their fresh state. Sun drying was used because it has been shown to preserve bioactive compounds of Gracilaria spp (Shakeel *et al.*, 2024).

Extraction Yield

The Soxhletation of 100 g of *G. verrucosa* implicial using 96% ethanol resulted in 4 g of viscous extract, equivalent to a 4% yield. This value is higher than the 2–3% range previously reported for ethanolic extraction of macroalgae (Hidayati *et al.*, 2017) and aligns with the report that polarity of the solvent dictates the type and quantity of metabolites recovered (Azzahra *et al.*, 2022). The relatively low yield, however, is typical for ethanol extraction of macroalgae and reflects the selective recovery of secondary metabolites rather than bulk hydrophilic polysaccharides (Arianto & Dalimunthe, 2022). The summary of implicial and extract yield is presented in Table 1.

Table 1: *Simplicia and Ethanolic Extract Yield of G. Verrucosa*

Parameter	Result
Fresh sample weight	20,000 g
Dry simplicia weight	1,100 g
Simplicia yield	5.5%
Sample extracted	100 g
Viscous extract obtained	4 g
Extract yield	4%

Phytochemical Screening

Qualitative screening of the ethanolic extract revealed the presence of alkaloids, flavonoids, terpenoids, tannins, and phenols, while saponins were not detected (Table 2). The presence of alkaloids was confirmed by orange and yellow precipitates with Dragendorff's and Mayer's reagents, respectively, suggesting that *G. verrucosa* possesses nitrogen-containing bioactive constituents that may contribute to antioxidant and antibacterial activities (Momuat *et al.*, 2024). The positive flavonoid and phenol responses are consistent with the antioxidant capacity previously reported for *G. verrucosa* extracts, in which both groups act as effective radical scavengers (Kim *et al.*, 2023). Detection of terpenoids and tannins further supports the anti-inflammatory and cytotoxic potential of the species, in agreement with the multi-target pharmacological profile attributed to Gracilaria spp. (Hakim *et al.*, 2024; Möwes *et al.*, 2025).

The absence of saponins differs from observations on *G. salicornia* and *G. corticata*, in which foaming reactions have been documented (Momuat *et al.*, 2024). Such variability is plausibly driven by interspecific differences, environmental conditions of the collection site, harvesting time, and the polarity of the extraction solvent (Ndahawali *et al.*, 2021). The qualitative profile obtained in the present work confirms that *G. verrucosa* from Nongsa is a promising matrix for the recovery of multiple classes of phytochemicals.

Table 2: *Phytochemical Screening of the Ethanolic Extract of G. Verrucosa*

Metabolite	Reagent	Result
Alkaloid	Mayer	+
	Dragendorff	+
Flavonoid	NaOH	+
Terpenoid	$CHCl_3$ + H_2SO_4	+
Saponin	Hot water	−
Tannin	$FeCl_3$ 1%	+
Phenol	Distilled water + $FeCl_3$ 10%	+

Specific Quality Parameters

Organoleptic analysis showed that the simplicia was a fine green powder with a characteristic odor and a bitter taste, while the viscous extract was dark brown, with the same odor profile and bitter taste. Color changes during drying are commonly attributed to enzymatic browning of polyphenols and to non-enzymatic Maillard-type reactions when biomass is exposed to high temperatures (Ismail & Gögüs, 2023). Microscopic observation revealed irregular tissue fragments together with reddish granules consistent with phycobilin residues, the photosynthetic pigments that are a hallmark of Rhodophyta and are known to bind to water-soluble phycobiliproteins (Pagels *et al.*, 2019).

The water-soluble extractive value (35%) was approximately twice the ethanol-soluble extractive value (17%). This trend mirrors the report of Hossain *et al.* (2024) for red algae simplicia and reflects the abundance of hydrophilic sulfated polysaccharides such as agar and carrageenan in *G. verrucosa* (Reboleira *et al.*, 2020). Although the ethanol-soluble fraction was lower, it is enriched in secondary metabolites of pharmacological interest, particularly phenolics and flavonoids (Arianto & Dalimunthe, 2022). All specific parameter findings are summarized in Table 3.

Non-Specific Quality Parameters and TLC Profile

The moisture content (13.3%) was below the maximum threshold of 16% set by the Indonesian National Standard (SNI 2690:2018) for dried seaweed (Nasional, 2018), supporting the long-term storage stability of the simplicia. Loss on drying reached 15%, slightly higher than moisture, an outcome that is typical when volatile constituents are present in addition to water (Sutomo *et al.*, 2021).

Ash content was 39%, comparable to other reports on *Gracilaria* spp. and largely attributable to the natural mineral richness of macroalgae as well as to potential residual sediment from incomplete post-harvest washing (Andiska *et al.*, 2019; Insani *et al.*, 2022). Heavy metal analysis showed that cadmium (0.55 mg/kg), lead (0.47 mg/kg), and especially arsenic (13.46 mg/kg) exceeded the permissible limits of the Indonesian National Standard (Nasional, 2015) of 0.1, 0.3, and 1.0 mg/kg respectively, while mercury and tin were not detected. The high arsenic level is consistent with the well-documented ability of macroalgae to bioaccumulate metalloids from seawater (Supriyantini *et al.*, 2018; Syafaat *et al.*, 2024).

Table 3: *Specific and Non-Specific Quality Parameters of G. Verrucosa Simplicia.*

Parameter	Sub-parameter	Result	Standard
Organoleptic (simplicia)	Color	Green	–
	Odor	Characteristic	–
	Taste	Bitter	–
	Shape	Fine powder	–
Organoleptic (extract)	Color	Dark brown	–
	Odor	Characteristic	–
	Taste	Bitter	–
	Shape	Viscous	–
Microscopic	Pigment	Phycobilin	–
	Other	Cell wall fragments	–
Specific	Water-soluble extractive	35%	–
	Ethanol-soluble extractive	17%	–
Non-specific	Moisture content	13.3%	Max 16%
	Loss on drying	15%	–
	Total ash	39%	–
Heavy metals	Cadmium (Cd)	0.55 mg/kg	Max 0.1
	Lead (Pb)	0.47 mg/kg	Max 0.3
	Mercury (Hg)	Not detected	Max 0.5
	Tin (Sn)	Not detected	Max 40.0
	Arsenic (As)	13.46 mg/kg	Max 1.0
Microbial	Total plate count	5.45×10^6 CFU/g	Max 3×10^3
	Coliform	1.75×10^6 MPN/g	< 3
	Mold	< 10 CFU/g	Max 5×10^2
TLC	Spot 1 (Rf)	0.25	–
	Spot 2 (Rf)	0.66	–
	Spot 3 (Rf)	0.80	–

Microbiological evaluation revealed a total plate count of 5.45×10^6 CFU/g and coliform contamination of 1.75×10^6 MPN/g, both exceeding the SNI 3836:2013 limits of 3×10^3 CFU/g and < 3 MPN/g. Molds were below 10 CFU/g, complying with the standard of $\leq 5 \times 10^2$. The microbial burden observed here is likely linked to the proximity of the harvesting area to residential and maritime activity (Bizzaro *et al.*, 2022) and to non-aseptic post-harvest handling (Løvdal *et al.*, 2021). The polysaccharide-rich matrix of the simplicia itself can also act as a substrate for microbial growth when drying and storage are not carefully controlled (Banach *et al.*, 2022). Thin-layer chromatography of the extract yielded three resolvable spots with Rf values of 0.25, 0.66, and 0.80, indicating that the ethanolic extract contains constituents with different polarities that warrant further isolation and identification (Evelyn, 2023; Nittya & Hafiludin, 2025). The combined specific and non-specific results are presented in Table 3.

Implications for Phytopharmaceutical Development

The findings indicate that *G. verrucosa* from the coastal waters of Nongsa contains an attractive secondary metabolite spectrum and complies with the moisture criterion of the Indonesian Herbal Pharmacopoeia. However, arsenic and microbial loads remain key limitations. Post-harvest detoxification with citric acid soaking has been reported to reduce inorganic arsenic by more than 80% in *Sargassum fusiforme*, suggesting a feasible strategy for *G. verrucosa* (Wang *et al.*, 2022). Likewise, careful selection of cleaner cultivation sites and stricter post-harvest sanitation are required to minimize microbial contamination (Banach *et al.*, 2022; Torres-Narváez *et al.*, 2025). With such corrective measures, *G. verrucosa* from Batam may serve as a competitive marine-derived raw material for the local phytopharmaceutical industry.

Conclusion

The ethanolic extract of *Gracilaria verrucosa* (Hudson) Papenfuss simplicia from Nongsa, Batam, contained alkaloids, flavonoids, terpenoids, tannins, and phenols, while saponins were not detected. The simplicia met the moisture limit of the Indonesian Herbal Pharmacopoeia (13.3%) and exhibited water- and ethanol-soluble extractive values of 35% and 17%, with loss on drying and ash content of 15% and 39%. Cadmium, lead, and particularly arsenic exceeded the permissible thresholds, and total plate count and coliform levels surpassed the SNI 3836:2013 standard, whereas molds remained acceptable. Taken together, *G. verrucosa* from Nongsa shows a promising phytochemical profile for phytopharmaceutical development, but post-harvest optimization, arsenic detoxification, and microbial control are required before it can be applied as a safe and standardized raw material. Future investigations should expand the geographical scope of sampling and quantify the major bioactive constituents.

Conflict of Interest

The authors declare that they have no competing interests.

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