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Original Article

Uncover Itchy Leaves Ethnomedicine Usage: A Preliminary Study on Characterization and Bioactivity of Laportea Spp

Lukman La Basy[1,2], Djoko Santosa[3], Retno Murwanti[4], Triana Hertiani[3,*]

ABSTRACT

Background: *Laportea decumana* (Roxb.) Wedd. and *Laportea stimulans* (L.f.) Miq. (Urticaceae) or itchy leaves are endogenous plants from The Maluku Islands, Indonesia, which are used topically as analgesics by local people. **Objective:** As part of a preliminary investigation on the itchy leaves, this study was conducted to provide information on their macroscopic and microscopic characteristics, phytochemical profiles, and bioactivities according to the ethnomedicinal usages. **Materials and Methods:** The macroscopic examination was carried out on fresh leaves. The microscopic examination was carried out on leaf powder under a light microscope. The phytochemical analyses using TLC were conducted on those crude extracts and fractions. The bioactivity assays were conducted *in vitro* as Cox-2 inhibitory and DPPH radical scavenging activities. **Results:** reveal similar characteristics in the macroscopic and microscopic properties of both Laportea spp. leaves. Calcium oxalate crystals were observed in *L. decumana* but not found in *L. stimulans*. Both species have stomata with anisocytic type. More trichomes are found in the leaves of *L. decumana*, yet smaller and easily detachable. TLC analyses exhibited slightly different profiles. The crude extracts and fractions at 10 µg/ml showed similar inhibitory percentages on Cox-2. The DPPH scavenging activities of the crude extracts and fractions of *L. decumana* showed active moderate activity with an IC_{50} value < 250 µg/ml, while those of *L. stimulans* showed moderate to weak potency with an IC_{50} value < 500 µg/ml. **Conclusion:** Further exploration on *Laportea* spp. pharmacological activity is recommended to provide stronger evidence for its ethnomedicinal usage.

Key words: *Laportea decumana* (Roxb.) Wedd, *Laportea stimulans* (L.f.) Gaud, Itchy leaves, Characterization, Phytochemical profiles, Cox-2 inhibitor, DPPH radical scavenging activity.

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INTRODUCTION

With a vast biodiversity of 20,000 types of plants,[1] Indonesia provides various indigenous medicinal plants used for generations[2-4] based on indigenous knowledge and local wisdom.[5] Despite broader usage by the community, lack of scientific data is a significant issue for traditional medicine development, worldwide.[6]

Itchy leaves are plants used traditionally by local people in eastern Indonesia, mainly in the Maluku Islands, to relieve muscle aches following hard work in the field. Records of the ethnobotanical use of itchy leaves are to reduce pain, fatigue, headaches, stomachaches, and muscle pain.[7,8] At least two main species named itchy leaves were identified later as two different species.[8] *Laportea decumana* and *Laportea stimulans*[9] belong to family of Urticaceae, which are typical plants that grow in Maluku[10] dan Papua.[7]

Laportea spp. is widely distributed in Maluku and Papua.[11] The community cultivates those plants in the house yard and sells the leaves in traditional markets.[10,12] The *L. decumana*[13] is used more often by the local people since it is relatively easier to be found and more convenient to apply on skin.[10]

In an effort to widen the itchy leaves utilization as herbal products, a thorough investigation of the plants' pharmacological effects should be accompanied with a proper quality assurance process.[14] Considering there are plants with similar local name, it is of importance to provide a source identification as the initial stage in determining the bioactive components of a plant and ensuring the correctness, quality, and proper use of the simplicial.[15] This purpose can be achieved through a macroscopic, microscopic, and phytochemical comparison of the leaves of these similar plants, *Laportea decumana* and *Laportea stimulans*.[7,9,10,16]

This study aims to characterize the leaves of *L. decumana* and *L. stimulants* macroscopically and microscopically, as well as to conduct a phytochemical screening of these plants by using Thin Layer Chromatography. A preliminary investigation of the bioactivities was conducted by evaluating their Cox-2 inhibitory assay and DPPH radical scavenging activity by *in vitro* methods.

MATERIALS AND METHODS

Materials

Materials include the following: Cox-2 (human) inhibitor screening assay kit (Cayman Chemical); n-hexane, ethyl acetate, methanol, formic acid, 96% ethanol, all solvents are pro analyses grade (Merck, Darmstadt, Germany); distilled water; DPPH radical (2,2-diphenyl-1-phycrilhydrazyl) (Sigma Adrich); standard used for the TLC are quercetin, gallic acid, quinine, stigmasterol (Merck, Germany); spraying reagents used are $FeCl_3$, $AlCl_3$, Dragendorff, annisaldehyde, H_2SO_4 (Merck, Germany).

Equipments

Linomat® (Camag Linomat 5), Chamber (Durant), Microscope binocular XSG, Spectrophotometer (Geneys 10 UV Scanning, 335903).

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Plant taxonomy determination

Plants' taxonomy determination was conducted in the Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta Indonesia under specimen Number 05 with certificate number 10.25.03/UN1/FFA/BF/PT/2021. The plants were identified as *Laportea decumana* (Roxb.) Wedd. and *Laportea stimulans* (L.f.) Miq. (Urticaceae).

Macroscopic characterization

Macroscopic examination was carried out by observing the morphology and size of the fresh leaves and carrying out organoleptic tests including the taste, flavor, and color of the dried powder.[17]

Microscopic characterization

Microscopic examination, the observed part includes, the upper epidermis section of the section used is leaf slices from the upper leaf surface measuring 1x1 cm. For examination of leaf powder used powder from the leaves. Prepare a glass object and drop it with 1-2 drops of chloral hydrate, then heat it over a spirit lamp and then cover it with a glass cup. Then observed under a microscope with revolver objective lens of 40 × magnification.[17]

Sample preparation

Leaves were collected from village Nuanea districts center of the Maluku Islands, Seram Island, Indonesia on December 2021. Leaves samples were immediately processed to avoid damage of the secondary metabolites contained therein;[18] washed, chopped, and dried. The drying process was done at room temperature protected from direct sunlight. Afterward the dried samples were powdered.[19]

Extraction

Dried powder was macerated by using 96% ethanol p.a as a solvent in a ratio of 1:10.[19] The macerate was filtered by using a Buchner funnel, followed by evaporation with a rotary vacuum evaporator at a temperature of 60 °C at a speed of 80-110 rpm to obtain a thick extract.[20,21]

Fractionation

The fractionation process is carried out using the liquid-liquid partition method using a separating funnel. The crude extract was successively partitioned to gain the n-hexane and ethyl acetate fractions.[20,21]

Phytochemical screening

The screening was carried out semi-quantitatively with thin layer chromatography technique.[20,21] The plate was activated by heating it in an oven at 50^{0}C, approximately10 minutes prior sample application.[1] The TLC system for the crude extracts, hexane and ethyl acetate fractions used a precoated silica G_{60} GF_{254} plate as the stationary phase, while the mobile phase used were as follows n-hexane: ethyl acetate (7:3) v/v. The system used for the water fraction was a precoated silica G_{60} GF_{254} plate as the stationary phase and the mobile phase used were as follows methanol: ethyl acetate: formic acid (7:3:0,2) v/v.

Detection for flavonoid compound

Extracts from *L. decumana* and *L. stimulans* were separately dissolved in methanol and then each was applied 1 mg/mL using Linomat® on to a precoated silica gel plate F254 and eluted using the aforementioned mobile phase. Afterwards, dried plate was sprayed by $AlCl_3$ reagent. The plate was observed under UV lamps of 254 nm and 366 nm before and after spraying. Quercetin was used as a reference standard.

Detection for phenolic compounds

Extracts from *L. decumana* and *L. stimulans* were dissolved methanol with a concentration of 1 mg/mL and then each was applied 3 µL using Linomat® on to a precoated silica gel plate F_{254} and eluted using the aforementioned mobile phase. Afterwards, dried plate was sprayed by a $FeCl_3$ reagent. The plate was observed under UV lamps of 254 nm and 366 nm before and after spraying. Gallic acid was used as a reference standard.

Detection for alkaloids compounds

Extracts from *L. decumana* and *L. stimulans* were dissolved methanol with a concentration of 1 mg/mL and then each was applied 3 µL using Linomat® on to a precoated silica gel plate F_{254} and eluted using the aforementioned mobile phase. The plate was observed under UV lamps of 254 nm and 366 nm and sprayed by a dragendroff reagent. Quinine was used as a reference standard.

Detection for terpenoids compounds

Extracts from *L. decumana* and *L. stimulans* were dissolved in methanol with a concentration of 1 mg/mL and then each was applied 3 µL Linomat onto a precoated silica gel plate F_{254} and eluted using the aforementioned mobile phase. Then the plate was dried and sprayed with annisaldehyde H_2SO_4 and observed under UV lamps at 245 nm and 366 nm. Stigmasterol was used as a reference standard.

Cox-2 inhibitory assay

The assay was carried out *in vitro* using a Cox-2 (human) inhibitor screening assay kit (Cayman Chemical) in accordance with the standard procedures listed on the kit and was dissolved methanol with a concentration of extract and fraction used 10 µg/ml.[22,23] The assay was done in triplicates. The percentage of inhibition was calculated by the equation as follows:

$$\% \text{ Inhibition} = \frac{\text{Abs control - (Abs extract - Abs blanko)}}{\text{Abs control}} \times 100\%$$

DPPH radical scavenging activity

The extracts and fractions were separately dissolved in methanol p.a followed by a serial dilution. To each solution was added 0.1 mM DPPH solution in a ratio of 2:1, followed by homogenization.[24] The solution was then incubated for 30 min in the dark, and the absorbance was measured at a wavelength of 516 nm.[25] The blank solution used methanol p.a. The assay was done in triplicates. The ability to inhibit DPPH radicals was calculated by the equation as follows:

$$\% \text{ radical scavenging activity} = \frac{\text{Abs control - Abs sample}}{\text{Abs control}} \times 100\%$$

Statistical analysis

The data obtained from the Cox-2 inhibitory and the DPPH radical scavenging activity were analyzed statistically by one-way ANOVA or Kruskal-Walli's test with 95% significance level, which were processed by SPSS 24.

RESULTS

Macroscopic characterization

The macroscopic observation was conducted to differentiate the morphology, size, colour of the leaves of *L. decumana* and *L. stimulans*. Prior to the macroscopic examination, organoleptic tests were carried out, including taste, flavour and colour (Table 1).

Table 1: Organoleptic observation of samples.

Plant	Sample	Organoleptic		
		Color	Taste	Flavor
L. decumana	Fresh leaves	Green	typical	Bland taste
	Dried powder	Slightly brownish green	typical	Bland taste
L. stimulans	Fresh leaves	green	typical	Bland taste
	Dried powder	Slightly brownish green	typical	Bland taste

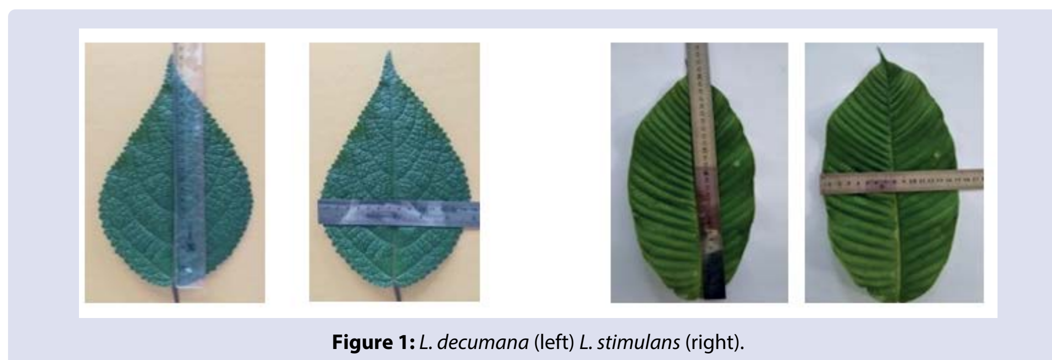

Figure 1: *L. decumana* (left) *L. stimulans* (right).

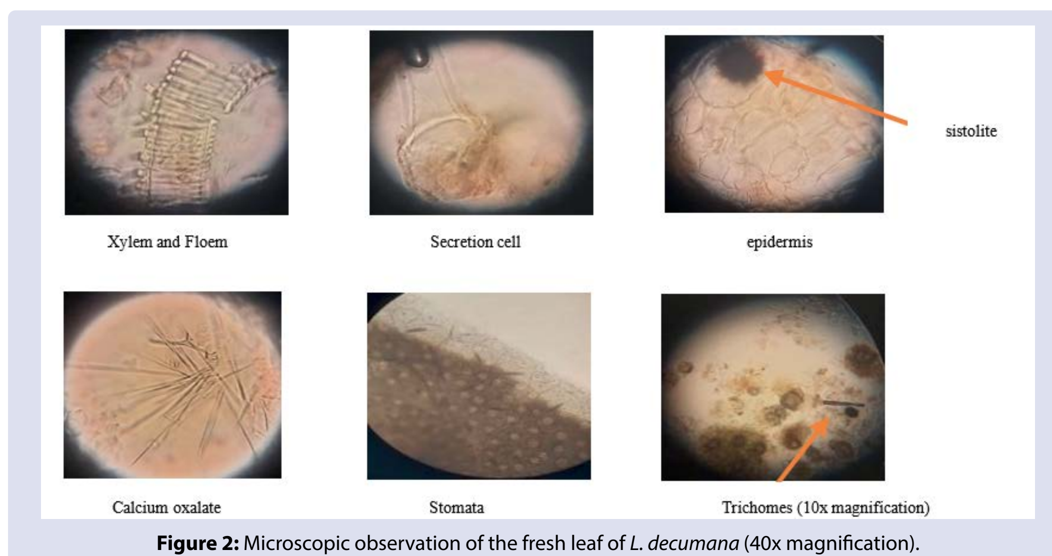

Figure 2: Microscopic observation of the fresh leaf of *L. decumana* (40x magnification).

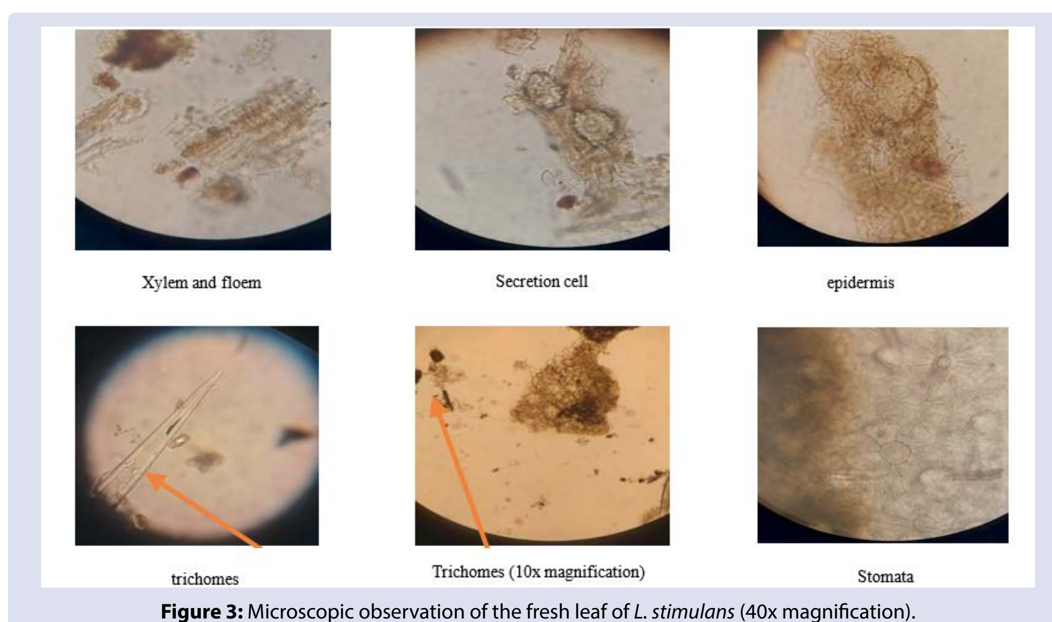

Figure 3: Microscopic observation of the fresh leaf of *L. stimulans* (40x magnification).

L. decumana leaf is a green heart-shaped which tip is pointed, and the leaf edge is serrated and oval. It has trichomes on the leaf surface, behind the leaves and bones, and the petiole is red and has a length of 24.5 cm and a width of 14.7 cm. *L. stimulans* leaf color is green, oval shape, pointed tip, and elliptical leaf edge. There are trichomes on the surface of the leaves, behind the leaves, and the bones and stalks. The leave has a length of 32 cm and a width of 15 cm (Figure 1).

L. decumana leaf has a light green color on its upperside, while on the other side is purplish red. *L. stimulans* leaf has a dark green upperside, and the other side of the leaves was green. *L. decumana* has an oval leaf shape and tapered leaf tips in all parts of the leaf. There are many tight trichomes. While *L. stimulans* have an oval leaf shape and pointed leaf tip, the shape is larger, and the trichomes are rare but firm on the upperside and and the other side of the leaves.

Microscopic characterization

Result of the microscopic observation of *L. decumana* fresh leaf as described on Figure 2, exhibited a phloem and xylem ladder form, secretory cells located on the upper epidermis and there are 3-7 palisade in the upper epidermis (Figure 2).

Result of the microscopic observation of *L. stimulans* fresh leaf as described on Figure 3, exhibited carrier bundles as ladder-shaped, secretory cells located on the upper epidermis and stomata of an anisocytic type.

Phytochemical screening

Phytochemical screening exhibits similar TLC profiles of both crude extracts having similar Rx for the spots reacted to the flavonoids, phenolics and terpenoids reagents sprayed while exhibited different Rx for the spots reacted to $AlCl_3$ and $FeCl_3$ (Table 2 and 3). Both crude extracts showed similar Rx and colour change following the annisaldehyde H_2SO_4 spraying (Table 2, 3 and 4). DPPH spraying showed similar positive spots of both Laportea however those of *L. decumana* exhibited additional positive spots. Table 4 summarize the results while also exhibited the positive result of the alkaloids detection by using dragendorff as the spraying agent.

DISCUSSION

Research on medicinal plants and herbs ("Ristoja") in 2017 reported Indonesia biodiversity covers 11,218 medicinal plants, of which 9,516 plant species used as part of traditional remedy.[26] The 2018 Basic Health Research Report ("Riskesdas") revealed traditional medicine usage by the Mollucas as 39.03% of which 78.22% are self-made.

Itchy leaves are typical plants found in the eastern Indonesia which was identified as *Laportea decumana* (Roxb.) Wedd. and *Laportea stimulans* (L.f.) Miq. (Family: Urticaceae). The people of Mollucas and Papua have used itchy leaves[10] to overcome various health complaints.[8] Itchy leaves are used topically to relieve aches, fatigue, headaches, stomach aches, joint and muscle aches, and bruises.[7] The local people usually rub the leaves on the painful area, and afterwards there will be a stinging sensation followed by numb and anesthetic sensation.[8]

L. decumana widely grows and well distributed in the Maluku Islands and Papua. It can be found in humid areas, and most of these plants grow side by side with Sago palms, next to a small river.[11] The plant is also cultivated in the houseyard, and easily found also in the traditional markets.[10,12]

On the other hand, the *L. stimulans* is also named by the local people as itchy leaves, while it also can cause itching when is applied on skin.

Table 2: TLC profiles of *L. decumana* Crude extract and fractions.

Sample	hRf	Before spraying		After spraying				
		UV 254	UV 366	$AlCl_3$	$FeCl_3$	Dragendorff	annisaldehyde H_2S0_4	DPPH
	27	+	+	-	-	-	-	-
	37	+	+	Dark yellow	-	-	-	+
L. decumana extract	42	+	+	Dark yellow	-	-	-	+
	61	+	+	Dark yellow	Dark blue	-	Brick red	+
	71	-	+	-	-	-	-	-
n-Hexane fraction	12	-	+	-	-	-	-	+
	18	-	+	-	-	-	-	-
	27	+	+	-	-	-	-	-
	37	-	+	-	-	-	-	-
	42	+	+	Dark yellow	-	-	-	-
	59	+	+	Dark yellow	Dark blue	-	Brick red	+
	61	+	+	-	Dark blue	-	-	+
	80	-	+	-	-	-	-	-
	6	+	-	-	-	-	-	-
	12	+	Blue	-	-	-	-	+
	21	+	Blue	-	-	-	-	+
Ethyl acetate fraction	31	-	+	-	-	-	-	-
	43	-	+	Dark yellow	-	-	-	-
	51	-	+	Dark yellow	Dark blue	-	-	-
	60	+	+	-	Dark blue	-	Brick red	+
	75	-	+	-	-	-	-	-
Water fraction	81	+	Blue	Light yellow	Light blue	-	Brick red	+

Notes: + : probably contains the respective compound group, - : no compound group detect

samples were spotted on to the silica gel TLC plate and analyzed by using different spraying reagents. The mobile phases use was a mixture of n-hexane: ethyl acetate (7:3) v/v (for crude extract, n-hexane, and ethyl acetate fractions); while the water fraction was eluted by using the mobile phase metanol: ethyl acetate: formic acid (7:3:0.2) v/v.

Table 3: TLC profiles of *L. stimulans* crude extract and fractions.

Sample	hRf	Before spraying		After spraying				
		UV 254	UV 366	$AlCl_3$	$FeCl_3$	Dragendorff	annisaldehyde H_2SO_4	DPPH
L. stimulans extract	27	-	+	Dark yellow	-	-	-	-
	53	-	+	-	-	-	-	-
	60	+	+	Dark yellow	Dark blue	-	Brick red	+
	70	-	+	-	-	-	-	-
	80	-	+	-	-	-	-	-
n-hexane fraction	26	-	+	-	-	-	-	-
	40	-	+	-	-	-	-	-
	53	-	+	Dark yellow	-	-	-	-
	55	-	+	-	-	-	-	-
	60	+	+	Dark yellow	Dark blue	-	Brick red	+
	68	-	+	-	-	-	-	-
	80	-	+	-	-	-	-	-
Ethyl acetate fraction	6	-	+	-	-	-	-	-
	26	-	+	-	-	-	-	-
	36	-	+	Dark yellow	-	-	-	-
	43	-	+	Dark yellow	Dark blue	-	-	-
	60	+	+	-	Dark blue	-	Brick red	+
	71	-	+	-	-	-	-	-
Water fraction	75	+	Blue	Light yellow	Light blue	-	Brick red	+

Notes: + : probably contains the respective compound group, - : no compound group detect

Samples were spotted on to the silica gel TLC plate and analyzed by using different spraying reagents. The mobile phases use was a mixture of n-hexane: ethyl acetate (7:3) v/v (for crude extract, n-hexane, and ethyl acetate fractions); while the water fraction was eluted by using the mobile phase metanol: ethyl acetate: formic acid (7:3:0.2) v/v.

Table 4: Summary of the phytochemical screening results.

Group of compounds	Reference standards		L. decumana		L. stimulans		Group of compound Identifications/ DPPH	
			hRf	Rx	hRf	Rx	L. decumana	L. stimulans
Flavonoids	Quercetin	Crude extract	37	3.08	27	2.25	+	-
Phenolics	Gallic acid		42	5.25	53	6.62	+	-
Terpenoids	Stigmasterol		61	1	60	1	+	+
Alkaloids	Quinine		0	1	0	1	-	-
Flavonoids	Quercetin	n-hexane fraction	42	3.5	53	4.4	-	-
Phenolics	Gallic acid		59	7.3	55	6.8	+	-
Terpenoids	Stigmasterol		61	1	60	1	+	+
Alkaloids	Quinine		0	1	0	1	-	-
Unidentified			12	-	-	-	+	-
Flavonoids	Quercetin	Ethyl acetate fraction	43	3.5	36	3	-	-
Phenolics	Gallic acid		51	6.3	43	5.3	-	-
Terpenoids	Stigmasterol		60	1	60	1	+	+
Alkaloids	Quinine		0	1	0	1	-	-
Unidentified			12	-	-	-	+	-
			21	-	-	-	+	-
Flavonoids	Quercetin	Water fraction	81	1.10	-	-	+	-
Phenolics	Gallic acid		81	1.27	75	0.10	+	+
Terpenoids	Stigmasterol		81	0.90	75	0.83	+	+
Alkaloids	Quinine		-	-	-	-	-	-

Notes: + : probably contains the respective compound group, - : no compound group detec.

Samples were spotted on to the silica gel TLC plate and analyzed by using different spraying reagents. The mobile phases use was a mixture of n-hexane: ethyl acetate (7:3) v/v (for crude extract, n-hexane and ethyl acetate fractions); while the water fraction was eluted by using the mobile phase metanol: ethyl acetate: formic acid (7:3:0.2) v/v.

Table 5: Cox-2 inhibition following samples application at 10 µg/ml.

Sample		% Inhibition			Average inhibition ± SD
		Replication I	Replication II	Replication III	
L. decumana	Crude extract	79	79	89	83 ± 0.05
	n-hexane fraction	67	72	75	72 ± 0,04
	Ethyl acetate fraction	82	86	75	81 ± 0.0
	Water fraction	78	77	67	74± 0.05
L. stimulans	Crude extract	78	76	73	76 ± 0.02
	n-hexane fraction	79	74	76	76 ± 0.02
	Ethyl acetate fraction	80	75	77	78 ± 0.02
	Water fraction	79	75	69	74 ± 0.07

Note: Statistical analysis with 95% significance level showed no significant difference between the extracts and the fractions of *L. decumana* with a value of 0.066 > 0.05; while those of *L. stimulans* showed a value 0.05.

Table 6: DPPH radical scavenging activity of *L. decumana* crude extract and fractions.

Sample	Concentration (ppm)	% Inhibition			Average % Inhibition ± SD		IC (μg/ml)	Antioxidant activity
		Replication I	Replication II	Replication III				
Crude extract	25	20.33	16.94	16.69	17.00±2.03			
L. decumana	50	25.72	26.10	24.59	25.47±0.78			
	75	32.24	31.99	33.00	39.36±0.69	y=0.26x+12.40 r = 0.99	146.28	Moderate
	100	38.64	39.40	40.02	39.36±0.69			
	125	39.65	45.04	44.79	43.16±3.04			
n-Hexane	25	24.61	20.37	19.63	21.53±2.68			
fraction	50	25.09	25.21	25.58	25.29±0.25			
	75	30.79	31.64	32.24	31.56±0.73	y=0.15x+18.26 r = 0.97	207.86	Moderate
	100	33.33	34.54	33.58	33.82±0.64			
	125	37.33	33.21	38.54	36.36±2.78			
Ethyl acetate	25	15.54	15.42	14.93	15.30±0.32			
fraction	50	22.64	23.62	23.26	23.17±0.49			
	75	27.17	29.25	29.38	28.60±1.24	y=0.23x+10.68 r = 0.99	170.75	Moderate
	100	33.05	43.64	34.27	33.97±0.08			
	125	37.94	38.07	40.02	38.68±1.17			
Water	25	30.63	23.80	25.67	26.80±3.53			
fraction	50	40.25	41.27	41.77	41.10±0.77			
	75	57.21	55.57	56.46	56.41±0.82	y=0.54x+13.93 r = 0.99	66.15	Active
	100	68.35	69.49	69.11	68.99±0.58			
	125	80.13	81.14	80.52	80.93±0.72			

Note: Statistical analyses on *L. decumana* showed significant difference between the extract and the fractions at various concentrations following the DPPH radical scavenging assay with a value 0.027 <0.05.

Table 7: DPPH radical scavenging activity of *L. stimulans* crude extract and fractions.

Sample	Concentration (ppm)	%Inhibition			Average % Inhibition ± SD		IC (μg/ml)	Antioxidant activity
		Replication I	Replication II	Replication III				
Crude extract	25	11.92	10.54	10.03	10.83±0.97			
L. stimulans	50	12.42	12.67	12.67	11.44±0.78			
	75	14.05	16.56	16.31	15.64±1.38	y=0.08x+9.09 r = 0.95	522.45	weak
	100	17.81	17.81	17.44	17.69±0.21			
	125	18.57	17.81	17.81	18.07±0.43			
n-Hexane	25	14.91	12.00	10.18	12.36±2.38			
fraction	50	16.36	13.18	13.81	14.66±1.47			
	75	16.72	15.27	15.75	15.92±0.74	y=0.09x+10.09 r = 0.99	453.94	weak
	100	19.64	18.30	18.54	18.83±0.71			
	125	21.70	21.58	20.61	21.29±0.60			
Ethyl acetate	25	8.93	8.08	7.59	8.20±0.68			
fraction	50	11.51	10.04	10.16	10.57±0.81			
	75	13.34	12.24	13.10	12.89±0.58	y=0.09x+5.96 r = 0.98	469.96	weak
	100	15.91	16.16	16.20	16.20±0.31			
	125	17.09	16.89	17.38	17.09±1.02			
Water	25	8.23	7.21	6.58	7.34±0.83			
fraction	50	9.49	8.99	7.72	8.73±0.91			
	75	9.87	9.62	9.75	9.75±0.13	y=0.04x+6.41 r = 0.91	105.55	Moderate
	100	10.63	9.87	8.61	9.70±1.02			
	125	12.15	11.90	12.02	12.02±0.13			

Note: Statistical analyses on *L. decumana* showed significant difference between the extract and the fractions at various concentrations following the DPPH radical scavenging assay with a value 0.00 <0.05.

It grows well in the Maluku area but not as much as *L. decumana*. It is rarely used topically since it induces pain following application.

The leaves of *L. decumana* and *L. stimulans* can be easily differentiated macroscopically (Figure 1). On the other hand, the major difference of the microscopic characteristics was observed on the absent of oxalate crystals in *L. stimulans*.

Trichomes of the family of *Urticacae* easily fall off by touch; and have a sharp shape like syringes.[27] Syringes can penetrate the skin and release irritants.[28] In *L. decumana* the presence of more dense and dense trichomes are located on the leaf surface but easily detached, biomineralized, and less sharp in contrast to *L. stimulans* trichomes, which are rare on the leaf surface but have a more muscular shape and

are attached to the leaf base which can penetrate the skin and provide more pungent irritant.[29,30] The trichomes in *L. decumana* are easy to be detached. When the trichomes are separated from the base of the leaves, the injured plant cells cause the cells to secrete formic acid compounds, which provide an analgesic effect.[7] When *L. decumana* is applied to the skin surface, formic acid secretion occurs from the leaf trichomes, which will cause the skin pores to widen and further relieves aches, pains, and fatigue in the muscles and body.[7] The exact mechanism is not fully understood. At the same time, the trichomes of *L. stimulans* are fewer but larger in size to induce more dominant pain.

The phytochemical screening of both crude extracts detected the presence of flavonoids, phenolics, alkaloids, and terpenoids. Both leaves seem to have a similar chemical content based on the TLC profile focusing on phytochemical groups characterization; however different TLC profiles were observed amongst crude extracts and the respective fractions following the DPPH spraying. This finding is consistent with the results of the DPPH radical scavenging activity, showing that the *L. decumana* crude extracts and fractions have higher activity in comparison to those of *L. stimulans*.

The crude extracts and the fractions from both leaves exhibited similar Cox-2 inhibition assayed on 10 µg/ml (Table 5). Several phytochemicals have been reported elsewhere to exhibit analgesic and anti-inflammatory activities by inhibiting Cox enzymes[31,32] of which some refers to terpenoids, phenolics, and flavonoids as the responsible bioactive compounds.[33,34] Plant extract might serve as a pain reliever through a mechanism by inhibiting the Cox-2 enzyme.[31] By inhibiting the Cox-2 enzyme, prostaglandins as the pain mediators are not formed, thereby reducing pain.[35-37] However, the extract and fractions exhibited significant difference in the DPPH radical scavenging potency.[38] It is interesting to explore further whether or the radical scavenging activity correlated to the Cox-2 inhibitory mechanism and further exerts the analgesic action of the leaves.[31,32]

This measurement of the DPPH radical scavenging activity was carried out using the visible spectrophotometric method.[25,39] This method was selected due to the prediction of the possibility of the contribution of flavonoid and phenolic contents to the antioxidant activity. Those group of compounds is widely reported to contribute to the DPPH radical scavenging activity which is further responsible for anti-inflammatory activity.[25,39] Reactive oxygen species are caused by oxidative stress conditions, cellular damage occurs and causes various inflammatory diseases, through the activity of membrane phospholipase which catalyzes the biotransformation of arachidonic acid to prostaglandins and thromboxanes by the cyclooxygenase enzyme activity.[40] Therefore, much attention has been paid to correlating the radical scavenging activity of plant extracts as an initial screening for an anti-inflammatory and analgesic activity.[41]

The radical scavenging activity was indicated by a decrease in the absorbance of the DPPH solution to which the sample had been added.[42-45] Differences in the IC_{50} values of extracts and fractions of both leaves are in accordance with the difference in the TLC profiles following the DPPH spraying, which confirmed different chemical contents of both leaves.[46,47] The DPPH radical scavenging activity is classified into 5 groups, *i.e.*, highly active (<50 µg/ml), active (50-100 µg/ml), moderate (101-250 µg/ml), weak (250-500 µg/ml) and inactive (>500 µg/ml).[48] Based on the aforementioned classification, only the water fraction of *L. decumana* showed an active for radical scavenging activity, while crude extract, hexane and ethyl acetate fraction of *L. decumana* and the water fraction of *L. stimulans* exhibited moderate activity, while others are classified as inactive or weak.[48,49] Noteworthy, the water fraction of both Laportea showed higher DPPH radical scavenging activity in comparison to its crude extract, hexane and ethyl acetate fractions. A similar TLC profile was also observed but with a slight difference in polarity. Positive reaction to $AlCl_3$, $FeCl_3$ suggests the presence of a flavonol derivate, while the high polarity property as well as the positive reaction to the annisaldehyde H_2SO_4 spraying suggesting the glycoside form. Further structure elucidation following isolation of the respective compounds is needed to ensure the result.

The *L. decumana* extract showed a moderate DPPH radical scavenging activity with an IC_{50} value of 146.28 µg/ml; the n-hexane fraction had a moderate antioxidant activity with an IC_{50} value of 207.86 g/ml; the ethyl acetate fraction had a moderate antioxidant activity with an IC_{50} value of 170.75µg/ml, and the water fraction has weak antioxidant activity with an IC_{50} value of 66.15 µg/ml (Table 6). On the other hand, the *L. stimulans* extract showed a weak DPPH radical scavenging activity with an IC_{50} value of 522.45 µg/ml; the n-hexane fraction had weak antioxidant activity with an IC_{50} value of 453.94 µg/ml; the ethyl acetate fraction had weak antioxidant activity with an IC_{50} value of 469.95 µg/ml, and the water fraction has weak antioxidant activity with an IC_{50} value of 105.55 µg/ml (Table 7). The radical scavenging activity is probably related to the presence of phenolic and flavonoid compounds and their structural types.[50]

The fact that the crude extract and fractions are *L. decumana* are consistently exhibited a radical scavenging activity, supports the preference of its usage by the local community, besides its more convenient usage. Itchy leaves, *i.e.*, *L. decumana* and *L. stimulans* are both used topically as analgesic by the local people in the Mollucas and Papua. However, there are also other types of itchy leaves plants reported from the same ordo. *i.e.*, *Laportea bulbifera, Laportea interrupta*.[50-52] A further study on the potency of the itchy leaves extracts according to the ethnomedicinal usage are necessary to be conducted to support its development as a standardized and scientifically proofed herbal medicinal products.

CONCLUSION

Itchy leaves of *L. decumana* and *L. stimulans* can be differentiated easily according to its appearance in color, shapes of fresh leaves, as well as the typical trichomes and the absent of the oxalate crystals in *L. stimulans*. Despite having similar COX-2 inhibition activity, *L. decumana* crude extract and fractions showed higher potency as the DPPH radical scavenging activity in comparison to *L. stimulans*. TLC profiles suggesting different chemical contents which responsible for the pharmacological activity. It is noteworthy to further investigate the active ingredients of the leaves as well as to provide pharmacological evidence by an *in vivo* assay to support the ethnomedicinal usage.

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CONFLICTS OF INTEREST

None declared.

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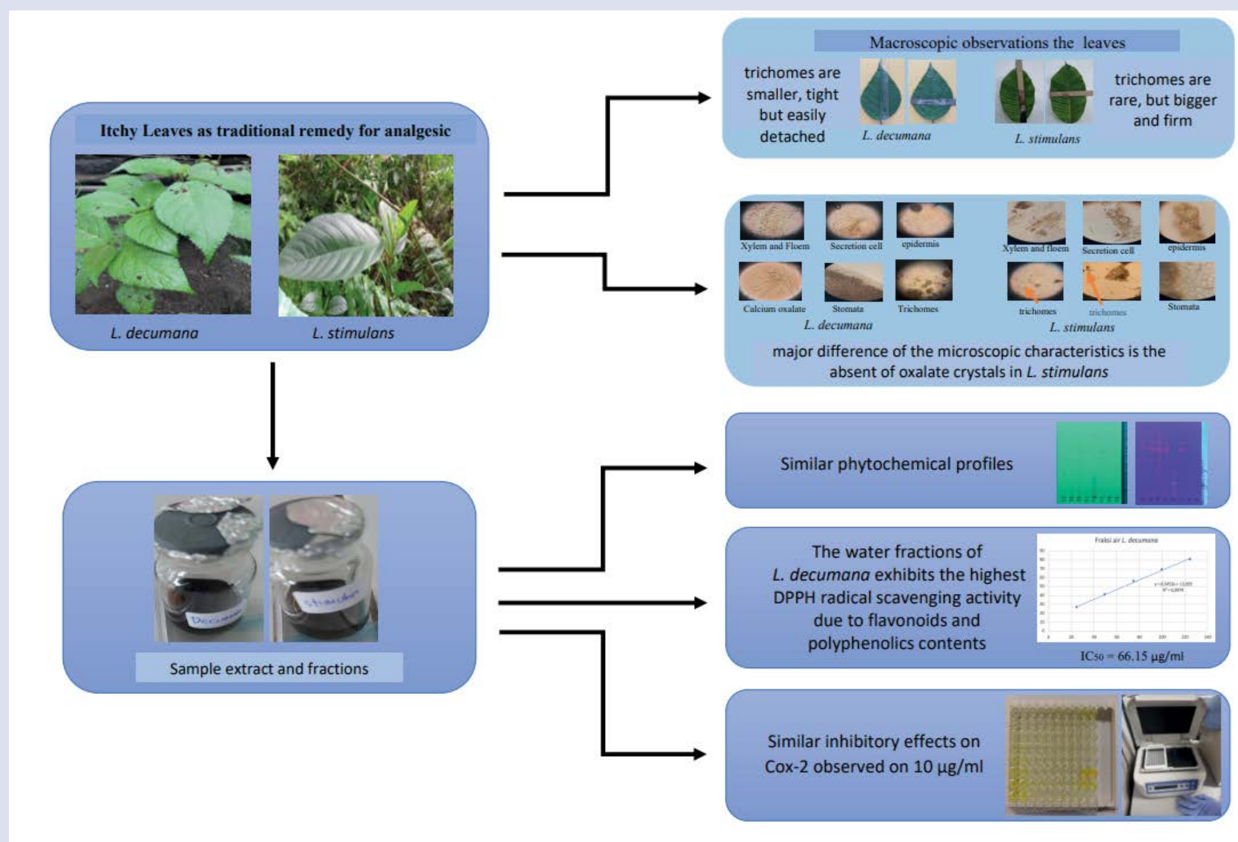
GRAPHICAL ABSTRACT



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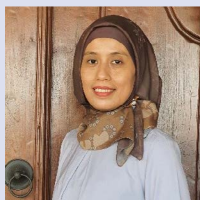

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