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Original Research Article

Extractions, Standardizations, and *In-Vivo* Toxicological Investigations of The Vietnamese Fish Mint (*Houttuynia cordata* Thunb.)

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ABSTRACT

Houttuynia cordata Thunb. (HC), a perennial plant distributed mainly in the tropics and subtropics regions, has been widely used as a folk medicine in Asian countries such as Vietnam. Nevertheless, limited studies have reported the pharmacognostical standardization and toxicity of the Vietnamese HC. Therefore, this study collected, identified, extracted, and *in-vivo* toxicological tested various HC samples at four locations, representing the whole Vietnam, including Hanoi (the Northern area), Dak Lak (the Western area), Bien Hoa (the Middle area), and Long An (the Southern area). All plant samples satisfied the quality requirements according to the standards of the Vietnamese Pharmacopoeia V and Hong Kong Pharmacopoeia. Next, a standard extraction and preparation process of HC was developed with an extraction solvent of 70% ethanol, concentrated under reduced pressure to a density of 1.16 g/mL, and spray-dried with excipients of Syloid$_{244FP}$:lactose (1:2 w/w) to obtain the crude ethanolic extract of HC with optimal recovery efficiency (71.35%). Finally, the crude HC extract was evaluated its acute toxicity in rats at a dose of 50 g/kg body weight, and sub-acute toxicity in rabbits at a dose of up to 1.5 g/kg/day. No potential toxicity was noted in both settings. Conclusively, the Vietnamese HC extract, which was standardized and possessed safeness in both rat and rabbit animal models, could be further investigated to become a pharmaceutical agent in the future.

Keywords: *Houttuynia cordata* Thunb., pharmacognosy, acute toxicity, sub-acute toxicity, standardization

Introduction

Recently, research on herbal/traditional medicine and their relevant products have gained increasingly interests in numerous biomedical areas.[1–8] One of the potential herbal plant is *Houttuynia cordata* Thunb. (HC, fish mint), a perennial herb with heart-like leaf and stoloniferous rhizome native to Japan, Southeast Asia, and the Himalayas. HC is considered as one of the potential edible and medicinal wild plant resources in China, Korea, India, Vietnam, and Thailand. Various studies have focused on the bioactive substances presented in HC, namely essential oils, flavonoids, and alkaloids. Specifically, the essential oil components in HC have anti-inflammatory, antibacterial, and antiviral effects.[9,10] Flavonoid components possess anti-cancer, antioxidant, anti-mutagenic, and anti-free-radical properties.[11] Likewise, the alkaloid components demonstrate remarkably potent antiplatelet and cytotoxic effects.[12] Therefore, studies on extracting and isolating biological activities in HC have gradually become popular in Vietnam and around the world.[13–16]

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Nevertheless, the preparation of HC extract and its chemical constituents are strongly affected by numerous factors related to raw materials, solvents, extraction methods, and environmental factors such as pH, water, light, and temperature.[17,18] Thus, it is necessary to study an optimal extraction process of HC extract and standardize the process. Moreover, limited research have focused on the morphological characteristics of the HC plants collected from different areas, as well as their in-vivo cytotoxicity.

Therefore, this work studied the differences in HC samples collected from four specific regions of Vietnam, ranging from the North to the South areas, identified and standardized the samples according to the Vietnamese Pharmacopoeia V and Hong Kong Pharmacopoeia. Then, the plants were extracted and spray-dried using the experimental optimal conditions,[19] and the products were physicochemically characterized. Finally, HC extract was evaluated for acute and sub-acute toxicity in rats and rabbit model, respectively, to conclude on the safety of the extract, creating a premise for future product formulation.

Materials and Methods

Chemicals and reagents

The whole HC plant was collected in four regions of Hanoi, Dak Lak, Bien Hoa, and Long An (Vietnam) from January to March, 2018. Samples were stored in styrofoam during transportation. Then, the samples were identified and authenticated according to the classification system of A. Takhtajan (1997) and the database of the Vietnam Plant Data Center. The HC voucher specimen (No. CTUMP-199) were kept at the Can Tho University of Medicine and Pharmacy, Vietnam. The collected samples were dried until constant weights at ambient temperature, finely ground to powder size, and the powder was stored at 25ºC for further experiments.

Standard hyperoside (99.16%) and quercitrin (98.48%) were imported from TRC-Canada. Absolute ethanol was bought from VWR (France). Toluene, formic acid, sulfuric acid (98%), and phosphoric acid (85%) were purchased from Merck (Germany). Acetonitrile, methanol, and chloroform were bought from J. T. Baker (America). All other chemicals were of analytical grades.

Quality tests of plant material

To identify and determine the qualities of the collected HC, pharmacognostical studies, physicochemical evaluations, and qualitative analyses, were evaluated following instructions described in the Vietnamese Pharmacopoeia V.[20] The pharmacognostical studies included morphological, histochemical, and powder investigations, with staining reagents of chloramine T, chloral hydrate, iodine, and carmine solutions. Photographs of different magnifications were taken with Nikon microscopy (Eclipse 80i, Japan). The plant physicochemical constants of humidity, foreign matter, total ash, crumbling rate, extraction efficiency, and essential oils quantitation were carried out on based on the Vietnamese Pharmacopoeia V.

Briefly, the plant humidity (water content) should be <13% and was determined by distillation method. The percentage of water in the test sample was calculated according to equation (1), with V_1 (mL) and V_2 (mL) is the volume of water in the receiver after the first and second distillation, respectively, and m is the sample weight.

$$Water\ content\ (\%) = \frac{100\ x\ (V_2 - V_1)}{m}\ (1)$$

The foreign matter (i.e., impurities) should not be >2% w/w, and was determined by observation with 50 g of the sample, spreading evenly on a sheet of paper. The percentage of impurities was calculated based on equation (2), where a and p is the weight of the impurities/ash/powder-under-the-sieve and the sample, respectively. The total ash should not be >14% w/w, which was determined by heating the plant powder (2 g) at 400℃ until no carbon remains, following by ash cooling and weighing. The percentage of total ash was calculated based on formula (2). The crumbling rate should not be >5% w/w, and was measured by sieving method, in which 100 g of the sample was sieved through a 3.15-mm sieve. The crumbling rate (%) was calculated using equation (2).

$$Foreign\ matter/Total\ ash/Crumbling\ rate\ (\%) = \frac{a}{p} \times 100\ (2)$$

For the extraction efficiency, 2 g of the plant powder was hot macerated at 90ºC for 1 h with 96% ethanol. Then, the extract was filtered and the filtrate was dried at 105℃ for 3 h, cooled, and weighed. The extraction efficiency (%) was calculate based on the amount of the dried extract and the plant powder weight, and should not be <11% w/w.

The essential oils quantitation was conducted by steam distillation in essential oil distillers. Distillate was collected into a graduated tube using xylene to retain the essential oil. The results were calculated as percentages (mL of essential oil in 100 g of the test sample), and should be >0.08%.

Finally, the HC chemical qualitative analysis was performed using colorimetric assays, in which (1) the HC powder fluorescence under UV light at 254 nm should reveal a dark brown color, (2) the HC powder reaction with decolorized fuchsine solution should yield pink or purple-red color, and (3) the HC ethanolic extract (1 g HC powder + 10 mL ethanol) reaction with magnesium powder in HCl should reveal a red color.

Extract preparation and standardization

Investigation of the extraction solvent

To find the optimal extraction solvent, the HC powder was extracted with various solvents of absolute ethanol, ethanol 96%, ethanol 70%, ethanol 50%, ethanol 30%, and water. Specifically, 1 g of sample was ultrasound-assisted extracted twice, each time with 20 mL solvent for 30 min. Then, the solvents were evaporated, and the products were evaluated their properties of color, dryness, and mass.

The extract main chemical constituent (quercitrin) was determined by thin layer chromatography (TLC) according to Hong Kong Pharmacopoeia. To this end, 3 µL of standard quercitrin 0.045% (w/v) in 70% methanol and 10 µL of 3.3% (w/v) HC sample solution in 70% methanol were dotted onto activated GF_{254} silica gel TLC plates. The TLC plate was run using a mobile phase of ethyl acetate - butan-2-one - formic acid - water (24:3.6:1.5:0.9 v/v/v/v), followed by air drying and observed under UV light at 336 nm. The test solution should possess dots of the same color and R_f as the standard solution.

Investigation of spray-drying conditions

The optimal extracts were further spray-dried to obtain the dry powder that was ready for the in-vivo tests. For this, the crude HC extract was spray-dried under the varied conditions, with desired evaluation criteria, presented in Table 1.

Evaluation of acute and sub-acute toxicity of Houttuynia cordata Thunb. extract

All in-vivo animal experiments were conducted based on the international regulations for the usage and welfare of laboratory animals. The study protocol and other relevant documentations were ethically approved by the IDQC HCMC approval research document No. 03/2022/TB-IDQC_AEC and approval research certification No. 03/2022/GCN-IDQC_AEC.

In-vivo acute toxicity in rats

All rats were fasted for at least 12 h before the experiment. Then, the exploratory test (i.e., to determine the maximum dose) was conducted with 04 rats. The rats were administered the HC optimal extract (reconstituted from the HC spray-dried powder, 0.2 mL/10 g of body weight) via oral gavage, and observed for 72 h.[21–24] In case all rats die, the dose was reduced until a dose in which a maximum of one rat dies. This dose was selected for the formal test.

Table 1: Spray-drying varied conditions and required product properties for the *Houttuynia cordata* Thunb. extract

Spray-drying excipients			
$Syloid_{244FP}$: Lactose (1:2 w/w)	Aerosil : $Syloid_{244FP}$: Maltodextrin (1:1:2 w/w/w)	Aerosil : Maltodextrin (1:2 w/w)	Aerosil : Florite : Lactose (1:1:2 w/w/w)
Requirements: The product has the least hygroscopicity, dryness, and high flowability			
Spray-drying conditions			
Temperature: 55ºC, 60ºC, 65ºC			
Pump speed: 35 rpm, 40 rpm, 45 rpm			
Fan speed: 500 rpm, 1000 rpm, 1400 rpm			
Spray waiting time: 3 min, 4 min, 5 min			
Spray time: 0.1 min, 0.15 min, 0.2 min			
Requirements: The product has the highest recovery efficiency of the extract			

For the formal test, the rats (50% male, 50% female) were randomly allocated into groups consisting of at least six rats per group. The groups were given exponential doses ranging from LD_0 to LD_{100} (based on the exploratory test) once daily for 14 days. At doses close to LD_{50}, the number of rats was increased for more accurate measurements. To evaluate the acute toxicity, any indications of clinical toxicity were closely monitored during the test period (movement, behavior, fur state, eating habit, urination, and mortality). LD_{50} was calculated according to the Behrens method.

In-vivo sub-acute toxicity in rabbits

The experiments were conducted in accordance with the guidelines issued by the Vietnam Ministry of Health, OECD, and World Health Organization. For this, the rabbits were randomly allocated into three groups consisting of six rabbits per group. Group I (control) received distilled water once daily. Group II was given a single dose (0.3 g/kg body weight/day) of the HC extract (equivalent to the intended human dose, calculated by a factor of 3). Group III was given a single dose (1.5 g/kg body weight/day) of the HC extract (5-fold increment in dosing compared to that of group II). All doses were administered at 9 AM once daily for 28 days.

To evaluate the sub-acute toxicity, the rabbit body weights, hematological parameters, biochemical markers, and histopathology were examined. To this end, the weights of control and experimental rabbits were recorded using Mettler Toledo XP86 analytical balance (± 0.002 mg, Switzerland) on the first day of the study (prior to the administration of test extracts), at day 14^{th}, and day 28^{th}. Similarly, the rabbit blood were taken from the rabbit ear vein on the first day, at day 14^{th}, and day 28^{th}, to evaluate the hematological markers using an Automated Hematology Analyzer (Drew Scientific - Excell 2280), the biochemical markers (i.e., liver enzymes of aspartate aminotransferase (AST) and alanine aminotransferase (ALT)) by Clinical Chemistry Auto-analyzer TC-MATRIX. Then, at the end of the experimental period (i.e., day 28^{th}), the rabbits were sacrificed according to the MT/WI-DL-13 Laboratory Animal Anesthesia and Humane Slaughter procedure, and histopathologically examined the liver and kidney tissues. The tissues were fixed with 10% formalin solution, macroscopically observed for the gross pathological changes (i.e., developed lesions), and microscopically examined.

Statistical analysis

The results were presented as mean ± standard error (SD) and statistically significant were evaluated by SPSS software version 25, using Student's t-test. Specifically, the t-test was used to confirm the significant differences between the experimental groups or between the time before and after treatment of each group. A p-value of <0.05 was considered as statistically significant.

Results and Discussions

Although HC has been critically investigated and researched regarding its phytochemical constituents and therapeutic actions, limited research have focused on the morphological characteristics of the HC plants collected from different areas, as well as its extract standardization and toxicity. Thus, this study determined the Vietnamese HC plants collected from 4 representative areas of Hanoi (the Northern area), Dak Lak (the Western area), Bien Hoa (the Middle area), and Long An (the Southern area), standardized the HC extract, and determined its acute and sub-acute toxicity in in-vivo settings.

Quality tests of plant material

To identify and determine the qualities of the collected HC, pharmacognostical studies, physicochemical evaluations, and qualitative analyses were performed.

Pharmacognostical studies

The morphological characteristics of HC plants collected in 4 areas of Bien Hoa, Dak Lak, Hanoi, and Long An are presented in Table 2 and Figure 1.

Histologically, the transverse section of the HC stem is nearly round, composed of two regions, the cortex and the dermis. The outermost part of the cortex is the epidermis consisting of a rectangular cell layer and a serrated cuticle. Under the epidermis is the lower epidermis, consisting of a layer of polygonal cells. The chollenchyma cells are polygonal/oval with irregular sizes. The innermost part of the cortex is the casparian strip. The dermis is limited from the pericycle to the pith, with its cell wall hardened into a continuous ring of 1-4 polygonal cells. Under the dermis are vascular bundles, composing of a primary structure of phloem-xylem ring (Figure 2A).

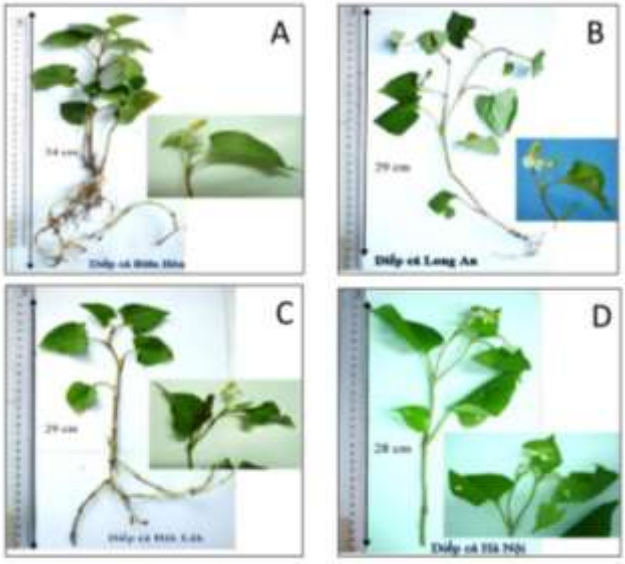

Figure 1. Morphological characteristics of *Houttuynia cordata* Thunb. collected in (A) Bien Hoa, (B) Long An, (C) Dak Lak, and (D) Hanoi.

Table 2. Morphological characteristics of *Houttuynia cordata* Thunb. whole plants, collected in 04 areas of Vietnam (Bien Hoa, Dak Lak, Hanoi, and Long An)

Part \ Area	Bien Hoa	Dak Lak	Long An	Hanoi
Stem	Cylindrical, about 25-35 cm high, green color	Cylindrical, about 25-35 cm high, green color, slightly purplish at the petiole corner	Cylindrical, about 25-30 cm high, purplish green color	Cylindrical, about 25-28 cm high, green color
Leaves	Alternating, heart-shaped, sheathed, possess characteristics odor and slight pungent in taste			
Flowers	Surrounded by 4 white bracts, containing numerous small yellow flowers			
Roots	Roots grow underground, small roots grow at the nodes			

Table 3: Physicochemical evaluation of *Houttuynia cordara* Thunb. plants collected in 04 areas of Vietnam (Bien Hoa, Dak Lak, Hanoi, and Long An)

Areas Property	Bien Hoa	Long An	Hanoi	Dak Lak
Humidity	- (15.99%)	- (17.89%)	+ (11.94%)	- (14.95%)
Total ash	+ (8.51%)	+ (12.37%)	+ (10.27%)	+ (12.62%)
Foreign matter	+ (None)	+ (None)	+ (None)	+ (None)
Crumbling rate	+ (0%)	+ (0%)	+ (0%)	+ (0%)
Extraction efficiency	+ (20.51%)	+ (18.34%)	+ (20.31%)	- (9.90%)
Essential oils quantitation	+ (0.23%)	+ (0.24%)	+ (0.22%)	+ (0.11%)

Note: (+) qualified, (-) unqualified.

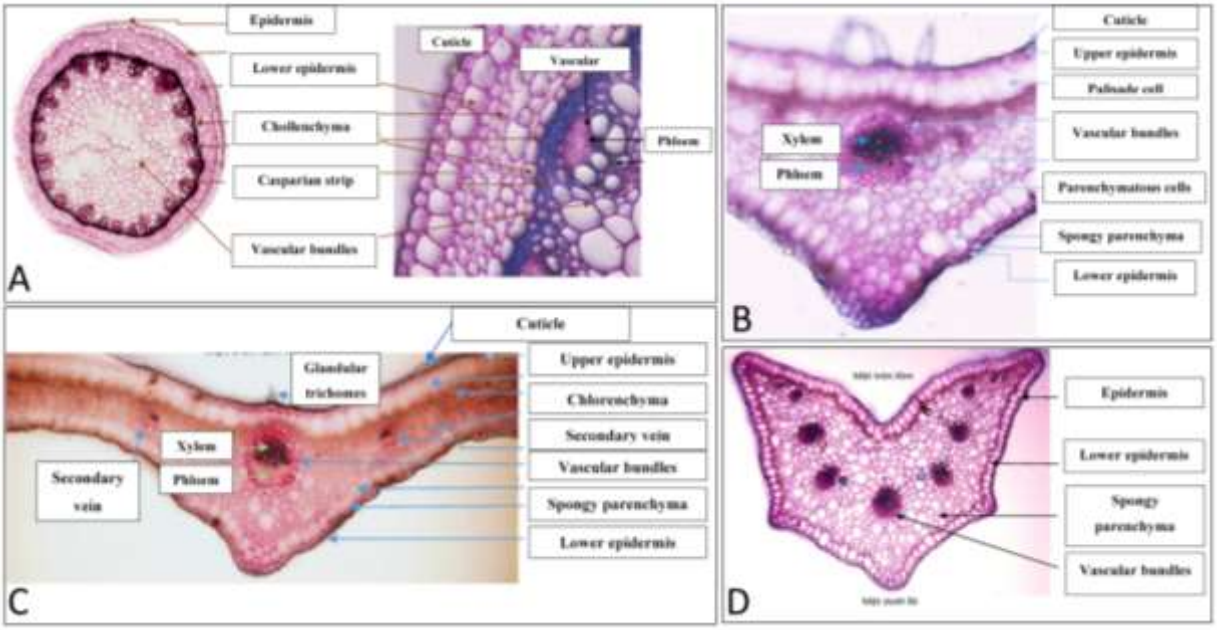

Figure 2: *Houttuynia cordara* Thunb. histological images of (A) Stem transverse section, (B) Midrib portion of leaf veins, (C) Leaf blade, and (D) Petiole.

The transverse section of the HC leaf veins showed the epidermal layer (upper and lower) covered with a serrated cuticle, with very little glandular trichomes. The phloem and the xylem are arranged in an arc in the middle. The parenchymatous cells consist of irregular cells, round, oval or polygonal (Figure 2B). The leaf blade has an asymmetrical heterostructure, with epidermal cells covered with a thin layer of cuticle. The lower epidermis has secretory cells and stomata, secretory hairs, and the glandular trichomes located on the leaf surface. The chlorenchyma has 3-4 elongated cell layers and the parenchymatous cells consist of irregular cells, scattered with vascular bundles of the secondary leaf veins (Figure 2C).

The HC petiole is concave on the upper surface and convex on the lower surface. The epidermis consists of a single layer of living cells, covered with a serrated cuticle dotted with stomata. The lower epidermis composes of polygonal cells and the parenchymatous cells consists of irregular, oval or polygonal cells. The vascular bundles in the middle are arranged in a continuous arc, the xylem is above and the phloem is below (Figure 2D).

Regarding the HC powder analyses, it possesses a yellow-green color with a slightly fishy odor. Powder microscopy showed stomatal cells, parenchymatous cells, secretory cells, and starch grains (sizes of 5-15 µm and various shapes of round, ovoid, and oval beads) (Figure 3).

Conclusively, these properties were in well agreements with the described HC characteristics stated in the Vietnamese Pharmacopoeia V,[20] indicating the collected plants were authentic.

Physicochemical evaluations

The physicochemical properties of HC (humidity, foreign matter, total ash, crumbling rate, extraction efficiency, and essential oils quantitation) were determined according to the Vietnamese Pharmacopoeia V and the results are presented in Table 3. The total ash index of HC collected in Bien Hoa area was the lowest (8.5%), whereas this number was the highest in Dak Lak (12.6%). At the same time, the Dak Lak HC yielded the lowest quantitative essential oils amount (0.11%). These data indicated that the geographical areas significantly affect the HC plant impurities and compositions. Since Dak Lak located in the mountainous region in the West highland of Vietnam, most of its soil is red basaltic with limited nutrients, thus, the plants grown in this area possess more inorganic impurities and less active ingredients.

Qualitative analyses

Qualitatively, all HC from 04 areas demonstrated colorimetric assays/reactions according to the Vietnamese Pharmacopoeia V (Figure 4). Conclusively, all data, including pharmacognostical studies, physicochemical evaluations, and qualitative analyses were in accordance with the Vietnamese Pharmacopoeia V, consequently confirmed that the collected HC were authentic plants and could be used in extraction standardization.

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Table 4. Effects of the spray-drying excipients and operating conditions on the *Houttuynia cordara* Thunb. powder properties (n = 3)

Excipient / Parameters	Syloid$_{244FP}$: Lactose (1:2 w/w)	Aerosil : Maltodextrin (1:2 w/w)	Aerosil : Syloid$_{244FP}$: Maltodextrin (1:1:2 w/w/w)	Aerosil : Florite : Lactose (1:1:2 w/w/w)
Humidity (%)	0.99 ± 0.05	0.81 ± 0.04	0.75 ± 0.06	0.70 ± 0.05
Compression coefficient	14.5 ± 1.20	17.1 ± 1.00	16.2 ± 1.10	16.8 ± 1.00

Level / Parameters	Level 1	Level 2	Level 3
Spray drying temperature	55ºC	60ºC	65ºC
Pump speed	35 rpm	40 rpm	45 rpm
Fan speed	500 rpm	1000 rpm	1400 rpm
Spray waiting time	3 min	4 min	5 min
Spray time	0.1 min	0.15 min	0.2 min
Appearance	Green dry powder with characteristic aroma		
Recovery efficiency (%)	70.27 ± 1.15	71.73 ± 1.04	72.04 ± 1.28

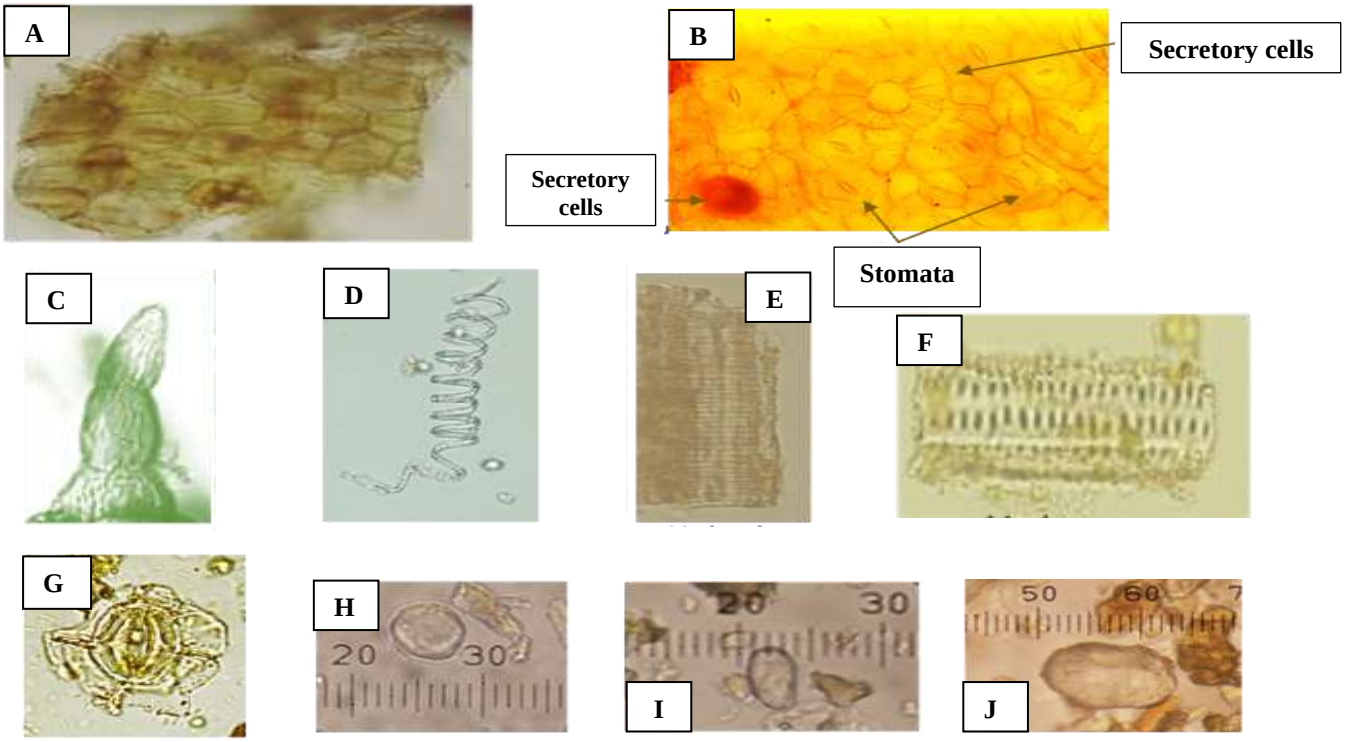

Figure 3: Powder microscopic characteristics of *Houttuynia cordara* Thunb. (A) Stem fragment, (B) Stomata and secretory cells, (C) Glandular trichomes, (D-F) Vascular bundles, (G) Stomata, and (H-J) Starch grains.

Extract preparation and standardization

Prior to the extract standardization, the optimal extraction solvent and spray-drying condition were investigated. For this, the optimal solvent was ethanol 70%, with a plant powder:solvent ratio of 1:16 w/v. The optimal extract possessed a density of 1.16 g/mL. Regarding the spray-drying optimal condition, the excipient system of Syloid$_{244FP}$:lactose (1:2 w/w) was selected, with a spraying temperature of 65ºC, a pump speed of 45 rpm, a fan speed of 1400 rpm, a spray waiting time of 5 min, and a spray time of 0.2 min (Table 4). The process of using solvents and excipients is routine and with controlled parameters, the spray-drying product of HC extract has a dry texture, good compression index, and good fluidity, making it easy to encapsulate the finished product.

Then, the standardized extract was evaluated its main chemical compositions using TLC technique based on the Hong Kong Pharmacopoeia (Figure 5). Obviously, both the standardized extract and the extracts obtained from HC of different areas possessed the clear quercitrin signals, which was considered the main component in HC.

Acute and sub-acute toxicity of Houttuynia cordara Thunb.

Acute toxicity

The exploratory test showed that the HC extract did not show mortality in all 04 investigated rats at a dose of up to 50 g/kg body weight (D_{max}). Thus, the formal tests were conducted at this dose on 12 rats (6 male and 6 female). After 30 min, the rats showed mild agitation, nervousness, fear, increased abdominal muscle contraction, and fatigue. Nevertheless, after 1 h, all rats were active normal and no mortality was recorded in the next 72 h. Follow-up continued for 14 days, the rats were living normally and no mortality was recorded. The rat weights were monitored twice a week and no significant changes were noted, compared with the control group. Conclusively, at the highest dose of 50 g/kg, the HC extract, administered orally, was safe and caused no observable acute toxicity in experimental rats.

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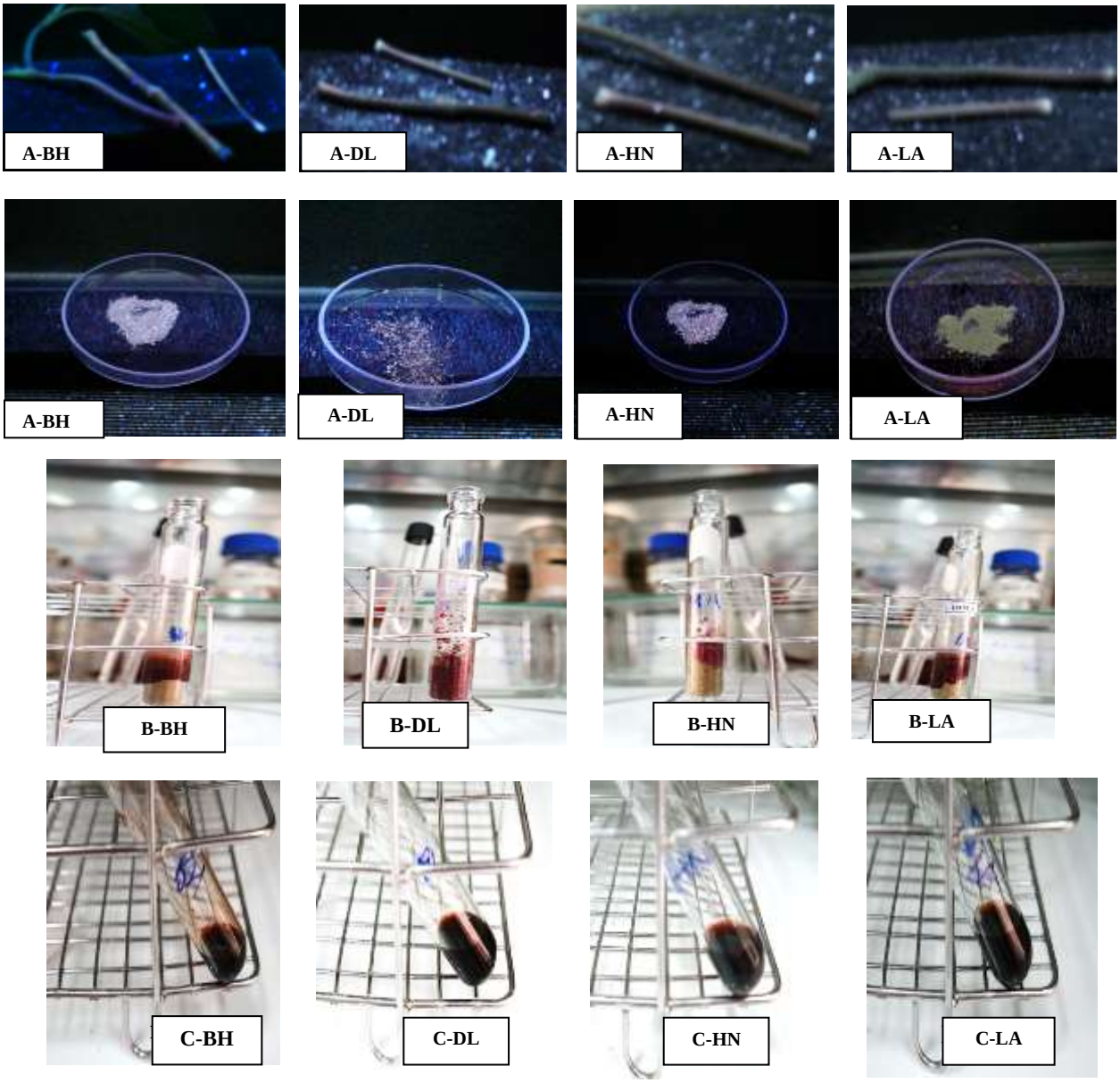

Figure 4. Qualitative analyses (based on Vietnamese Pharmacopoeia V) of *Houttuynia cordara* Thunb. plants, collected from Bien Hoa (BH), Dak Lak (DL), Hanoi (HN), and Long An (LA) areas. (A) Fluorescence analysis under UV light, (B) Reaction with fuchsine, and (C) Reaction with magnesium.

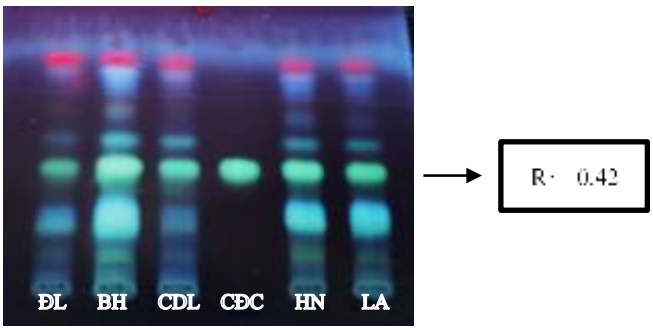

Figure 5. Thin layer chromatography analysis of the standardized *Houttuynia cordara* Thunb. extract. (CDL) Standard *Houttuynia cordara* Thunb. extract, (CĐC) Quercitrin standard solution, (ĐL), (BH), (HN), (LA) *Houttuynia cordara* Thunb. collected from Dak Lak, Bien Hoa, Hanoi, and Long An areas, respectively.

Sub-acute toxicity

During the testing period, rabbits in all 3 groups functioned normally. The results revealed no significant weight changes in rats treated with HC extract at experimental dose, compared to the control group (Figure 6A). Similarly, the HC extract did not yield negative effects on the rabbit hematological parameters of the red blood cell number (Figure 6B), the white blood cell number (Figure 6C), the platelet count (Figure 6D), and the hematocrit (Figure 6E). In terms of the biochemical markers of liver toxicity, no significant differences on the ALT and AST levels were noted between the HC-treated group and the control group (Figure 6F and 6G). Moreover, the extract showed no kidney toxicity on the rabbits, with no alterations in the urea and creatinine levels compared with the control group (Figure 6H and 6I). Finally, after the treatment durations, histopathological examinations on the experimental rabbit organs of heart, liver, kidney, lung, stomach, and intestines showed that there were no abnormalities in the organ shapes and colors in the test group compared to those of the control group (Figure 7).

In summary, both the acute and sub-acute tests resulted in no potential toxicity of the HC extract in the in-vivo settings, with a dose of up to 50 g/kg in rats and 1.5 g/kg/day in rabbits.

Conclusion

This study successfully collected, identified, extracted, and in-vivo toxicological tested various HC samples at four locations representing the whole Vietnam, including Hanoi (the Northern area), Dak Lak (the Western area), Bien Hoa (the Middle area), and Long An (the Southern area). All plant samples and the optimal extract satisfied the quality requirements according to the standards of the Vietnamese Pharmacopoeia V and Hong Kong Pharmacopoeia. Moreover, the HC extract possessed no potential acute toxicity in rats at a dose of up to 50 g/kg body weight, and sub-acute toxicity in rabbits at a dose of up to 1.5 g/kg/day. In conclusion, the Vietnamese HC extract, which was standardized and possessed safeness in both rat and rabbit animal models, could be further explored and investigated, possibly in terms of therapeutic actions and drug delivery systems, to become a pharmaceutical agent in the future.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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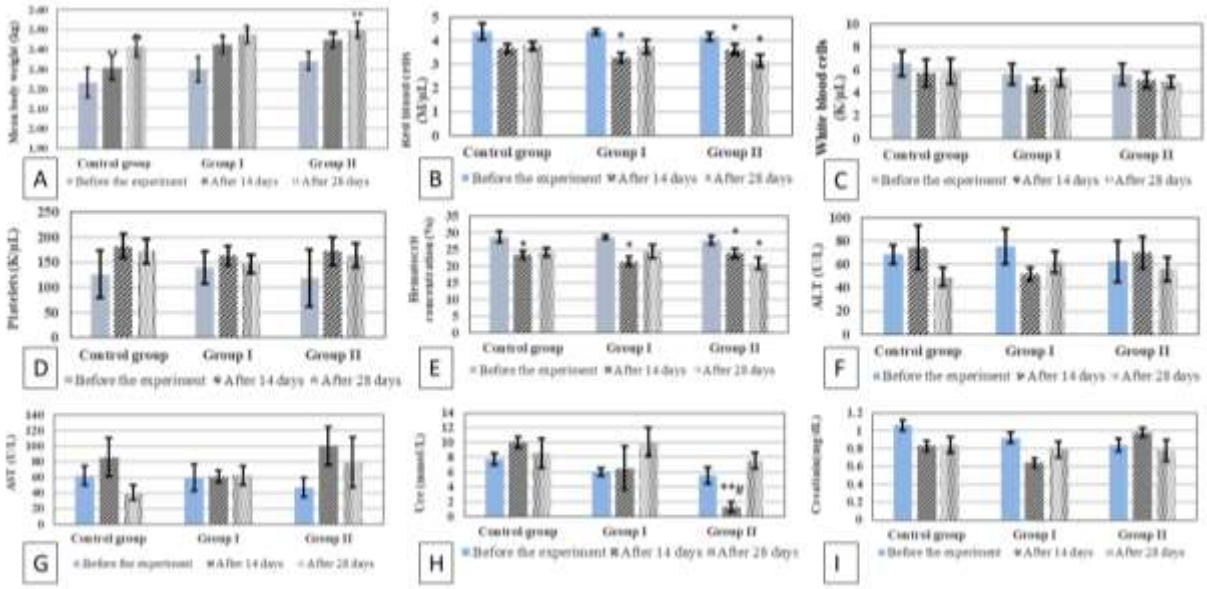

Figure 6. Effects of *Houttuynia cordara* Thunb. extract in the sub-acute toxicity test on the experimental rabbits' (A) body weights, (B) red blood cell amounts, (C) white blood cell amounts, (D) platelet amounts, (E) hematocrit, (F) ALT (alanine aminotransferase) levels, (G) AST (aspartate aminotransferase) levels, (H) urea levels, and (I) creatinine levels. Values are represented as mean ± SD (n = 6). *: $p<0.05$, **: $p<0.01$.

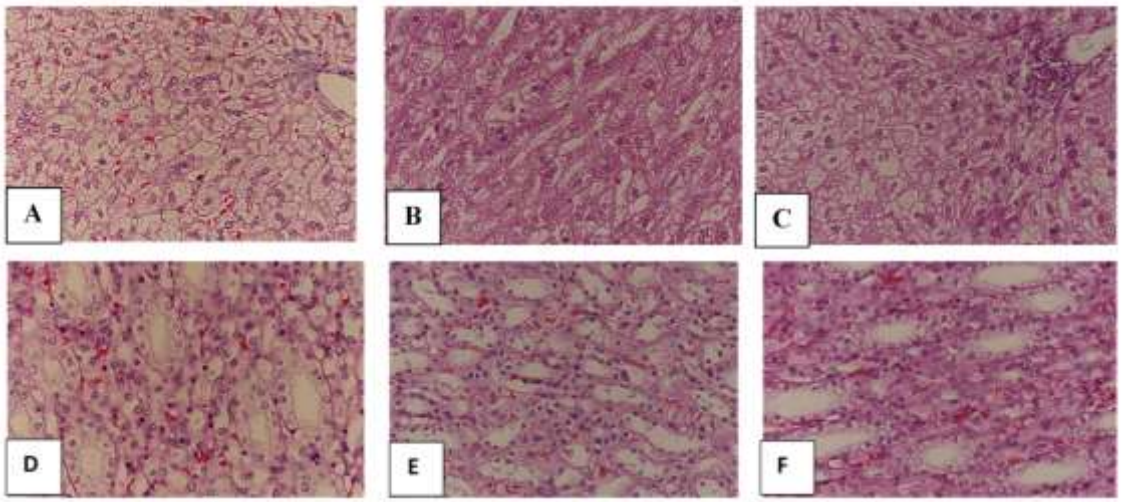

Figure 7: Microscopic histopathologic examinations of the rabbit organs subjected to the in-vivo sub-acute toxicity tests, after 28 days of testing. (A-C) liver: (A) control group, (B) group I, and (C) group II; (D-F) kidney: (D) control group, (E) group I, and (F) group II.

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