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STANDARDIZATION OF VIETNAMESE *Peperomia pellucida* (L.) KUNTH ETHANOLIC EXTRACT

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ABSTRACT

Medicinal plants, due to their potential therapeutic properties, have been employed in the national healthcare systems of various countries, including Vietnam. In Vietnam, at least 1,863 plant species have been officially used. However, limited information is reported on the standardizations of these plant extracts in the most up-to-date Vietnamese Pharmacopoeia 5.0, which only covers 19 monographs of plant extracts. Thus, this work presents the standardization of the ethanolic extract of the crab claw ["Càng cua" in Vietnamese] *Peperomia pellucida* (L.) Kunth, Piperaceae family, a wild-growing short-day vegetable plant commonly found in the South of Vietnam. The research described all the required characteristics of the *Peperomia pellucida* extract, their experimental processes, and the proposed acceptable criteria. These parameters included appearances, identification (apiol, thin layer chromatography, Rf = 0.214), loss on drying (6.38 ± 0.04%), total ash (23.9 ± 0.5%), heavy metals (≤ 20 ppm), microbial limits, and assay (total phenolic content of 677.97 ± 0.17 µg gallic acid equivalent/g powder). The complete standard data could be considered to be incorporated into the Vietnamese Pharmacopoeia in the future.

Keywords: *Peperomia pellucida* (L.) Kunth, Extract, Standardization, Vietnam, Pharmacopoeia

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INTRODUCTION

Recently, medicinal plants have been increasingly exploited for their medicinal properties to meet human healthcare needs.[1–5] More importantly, they are employed in the national healthcare systems (i.e., traditional medicine hospitals and the Department of Traditional Medicine in general hospitals) of numerous countries, including Vietnam.[6] Specifically, as of 2021, at least 1,863 plant species belonging to 238 families and nearly 1,000 traditional remedy formulations, have been officially utilized in Vietnam.[7] However, limited information is reported on the standardizations of these plants, as well as their respective extracts. In fact, the most up-to-date Vietnamese Pharmacopoeia, version 5.0, only covers 330 monographs of different plant species and/or usage parts, 19 plant extracts, and 23 traditional remedy formulations.[8] Thus, owing to the wide variety of Vietnamese medicinal plants and their high potential in disease treatments, it is crucial to standardize additional plant extracts. Amongst numerous plants, the crab claw ["Càng cua" in Vietnamese], scientifically known as *Peperomia pellucida* (L.) Kunth (PP), Piperaceae family, is a wild-growing short-day vegetable plant that is commonly found in the South of Vietnam. The PP's main chemical compositions include alkaloids, flavonoids, phenolics, saponins, terpenoids, steroids, and glycosides.[9] PP rhizomes, leaves, and the entire plants have been ethnopharmacologically utilized in the forms of infusions, powders, and liquids, as folklore medicines to treat various disorders and symptoms such as fever, colds, cough, insect bites, infectious hepatitis, gastrointestinal inflammation, viral/bacterial infection, high blood pressure, urinary diseases, arthritis, asthma, and hormonal disorders.[9–13] Moreover, for similar purposes, PP has also been used in other countries, namely Nigeria,[14] Laos,[15] Indonesia,[16] and the Philippines.[17] Most of these activities have been scientifically proven *in vitro* and *in vivo* experiments.[9,10] Although popularly utilized, the Vietnamese PP extracts have not been extensively standardized and indicated in the Vietnamese Pharmacopoeia. Therefore, this work extracted the PP using the simple maceration technique with ethanol and standardized the PP extract following all requirements stated in the Vietnamese Pharmacopoeia V. For

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this, the PP concentrated extract was determined its properties of appearances, identification (apiol), loss on drying, total ash, heavy metals, microbial limits, and assay (total phenolic content).

EXPERIMENTAL

The PP whole plants were gathered in Can Tho, Vietnam, in 2022. The samples were recognized based on the morphology following the report in the book "An Illustrated Flora of Vietnam",[18] and the gene analysis following the internal transcribed spacer (ITS) region and matK gene sequencing.[19] In brief, the PP total DNA was extracted by the cetyltrimethylammonium bromide (CTAB) procedure,[20] followed by PCR reactions with primer sequences of ITS1 (5'-TCCGTAGGTGAACCTGCGG-3'), ITS4 (5'-TCCTCCGCTTATTGATATC-3'), matK-390F (5'-CGATCTATTATTCATATTTC-3'), and matK-1326R (5'-TCTAGCACACGAAAGTCGAGT-3'), with 35 thermo-cycles of 95°C (denaturation temperature), 55°C (primer annealing), and 72°C (primer extension). The PCR products were then purified (Invitrogen kit), and the DNA sequence was checked using agarose gel electrophoresis with a 2% agarose gel stained with Safeview dye, using a 100-bp DNA ladder as a standard. The DNA patterns were compared with the PP NCBI gene bank through the BLAST program to confirm the PP, with a similarity of 97.9% to 98.8%. The PP voucher specimens were preserved at the Can Tho University, Vietnam. All chemicals, solvents, and reagents were of at least reagent grade or better. The PP whole plants were thoroughly washed, and damaged, infested, or diseased samples were removed. Subsequently, the plants were dried at 50°C until a moisture content of less than 13%, measured by an infrared moisture balance, and finely ground using a grinder. Then, the powder was extracted using the simple maceration method at room temperature with ethanol 90% v/v for 24 h at a powder: solvent ratio of 1:10 w/v. The solvent in the container is poured out, and fresh solvent is added to the sample container. Next, the extract was filtered and the solid residue was subjected to the same process three more times. This optimal condition was thoroughly investigated in our previous report.[21] The resulting extracts were combined and the solvent was evaporated by a rotavapor until an ethanol content of ≤ 20% to obtain the concentrated extract. The product was characterized and standardized using the parameters and respective experimental procedures described in the Vietnamese Pharmacopoeia V,[8] and are briefly presented in the following sections. All experiments were conducted in triplicate. Using the standard physical observation method, the PP concentrated extract was homogenous, dark brown to black with an herbal smell, and did not precipitate or contain any solid residues (Fig.-1).

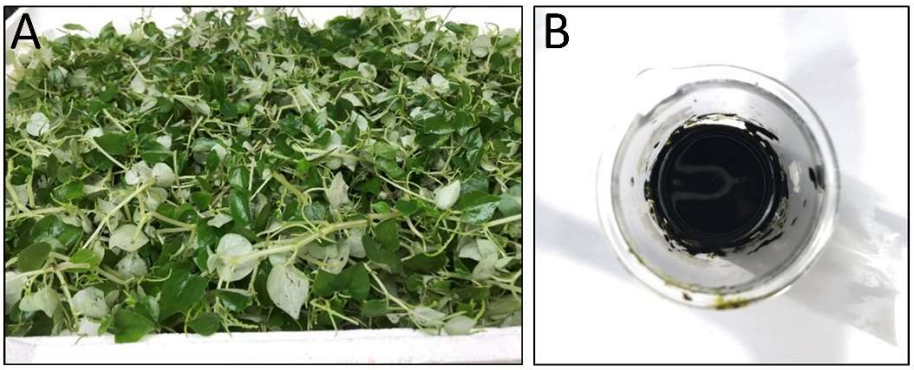

Fig.-1: Physical Appearances of (A) Vietnamese *Peperomia pellucida* (L.) Kunth and (B) its Respective Ethanolic Concentrated Extract

The PP extract identification was based on its main compound, apiol, employing the thin-layer chromatography method (Appendix 5.4, Vietnamese Pharmacopoeia V[8]). For this, 20 mg of the PP concentrated extract was re-dissolved in 2 mL of ethanol 90% to obtain the test sample. The reference sample was pure apiol (> 99%) dissolved in acetone at a concentration of 1 mg/mL. Apiol was selected as the biomarker since it is the main component in the PP extract, with the highest content.[22] The test and reference samples were separately spotted (20 µL) onto the thin silica gel 60 GF254 with a mean particle size of 15 µm that had been activated at 100°C for 30 min. The test was run using the mobile phase of ethyl acetate: hexane (10:90 v/v) until the solvent reached the top of the plate. Then, the plate was dried at room temperature, and spot visualized with sulfuric acid solution in ethanol (10% v/v) at 105°C. The plate was

observed under both normal light and ultraviolet light at a wavelength of 366 nm, and the R_f values were determined using equation (1).

$$R_f = \frac{Running\ distance\ of\ the\ sample\ (cm)}{Running\ distance\ of\ the\ solvent\ (cm)} \quad (1)$$

The results clearly showed that the PP concentrated extract contained apiol, with a similar spot shape and retention factor (R_f) of 0.214 compared to the reference sample (Fig.-2). Another issue to notice is that although the extract contains various compounds, a limited number of spots was observed, possibly due to the small amounts of other substances.

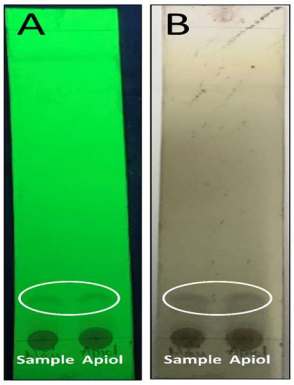

Fig.-2: Identification (apiol) of the *Peperomia pellucida* Extract using the Thin Layer Chromatography Method Under (A) the UV Light at 366 nm and (B) the Normal Light

The extract moisture content (loss on drying) was determined using the drying method, according to Appendix 9.6, Vietnamese Pharmacopoeia V.[8] For this, 1 g of the PP concentrated extract was put into a ceramic bowl and dried in an oven at 70°C to a constant weight (i.e., the weight differences before and after drying was ≤ 5 mg). The moisture content (X%) of the sample was calculated by equation (2).

$$X(\%) = \frac{Initial\ weight\ (g) - Weigh\ \ after\ drying\ (g)}{Initial\ weigh\ \ (g)}\ x\ 100\% \quad (2)$$

The moisture content of the extract was 6.38 ± 0.04%, suggesting that the extract satisfied these parameters according to the Vietnamese Pharmacopoeia V standard requirements for all concentrated extracts, which should not be more than 20% for the moisture content. The total ash of the PP concentrated extract was measured using method 1, appendix 9.8, Vietnamese Pharmacopoeia V.[8] For this, 2 g of the extract was put in a ceramic bowl, heated at 400°C until no carbon remains (approximately 3 h), cooled, and weighed. The percentage of total ash (T%) of the sample was calculated based on equation (3).

$$T(\%) = \frac{Initial\ weigh\ \ (g) - Weigh\ \ after\ carbon\ removal\ (g)}{Initial\ weight\ (g)}\ x\ 100\% \quad (3)$$

The extract total ash was 23.9 ± 0.5%, not more than 35% as a threshold indicated in the Vietnamese Pharmacopoeia V. Thus, the extract satisfied this parameter. The heavy metals content in the PP concentrated extract was evaluated using method 3, appendix 9.4.8, Vietnamese Pharmacopoeia V.[8] Briefly, the test solution was made by the process of (1) mixing 1 g extract in 4 mL of magnesium sulfate solution (25% w/v) in 1 M sulfuric acid, (2) heating the mixture at 700°C in an incinerator until white/light gray powder, (3) dissolving the powder by 1 M sulfuric acid, (4) heating the solution at 600°C for 30 min, (5) dissolving the powder in 10 mL of 2 M hydrochloric acid, (6) adding 0.1 mL of phenolphthalein ethanolic solution into the mixture, (7) adding ammonia until the mixture color turns pink, (8) adding glacial acetic acid until the solution become colorless, and (9) filtering and diluting the solution with water to 20 mL. The reference solution was fabricated similarly to the test solution, but using 2 mL of 10 ppm Pb solution instead of the extract. The obtained solution (10 mL) was then added to 2 mL of the test solution

to make the final reference solution. The blank solution was made by mixing 10 mL of water and 2 mL of the test solution. To all solutions, 2 mL of acetate buffer pH 3.5 and 0.1 mL of sodium sulfide were added, and shaken for 2 min, and the solutions were observed their color under normal light. The result shows that the heavy metals content of the extract was < 20 ppm since the color of the test solution was lighter than that of the reference solution (Pb 20 ppm) (Fig.-3).

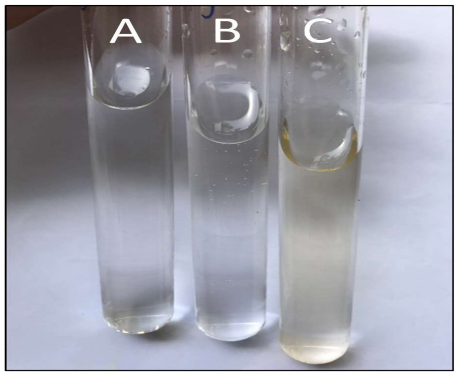

Fig.-3: The Heavy Metals Limit Test of the *Peperomia pellucida* Extract. (A) The Test Solution, (B) the Blank Solution, and (C) the Reference Solution (Pb 20 ppm)

The microbial limits of the PP concentrated extract was determined following the appendix 13.6, Vietnamese Pharmacopoeia V,[8] on different strains of (1) aerobic bacteria, (2) fungi and molds, (3) Enterobacteriaceae and other gram (-) bacteria, (4) *Escherichia coli*, (5) *Salmonella spp.*, (6) *Pseudomonas aeruginosa*, and (7) *Staphylococcus aureus*. For this, 1 g of the extract was dissolved in appropriate culture media, dependent on the tested bacteria/fungi, and subjected to the experimental procedures and calculations described in the Vietnamese Pharmacopoeia V. The results indicated that the PP concentrated extract possessed no observable colonies in all tested strains of aerobic bacteria, fungi and molds, Enterobacteriaceae and other gram (-) bacteria, *Escherichia coli*, *Salmonella spp.*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* (Table-1). This fact confirms that the extract was not infected with the microbes and satisfied the Vietnamese Pharmacopoeia V standard requirements for all concentrated extracts. The total phenolic content in the PP concentrated extract was measured using the Folin-Ciocalteu reagent by the well-defined process.[23] To this end, 100 mg of the extract was first dissolved in 10 mL of methanol. Then, 0.5 mL of the test solution was mixed with 2.5 mL of Folin-Ciocalteu reagent solution (10% v/v) and 2 mL of sodium carbonate aqueous solution (2% (w/v)). Next, the mixture was incubated for 45 min at room temperature, and the UV-Vis spectroscopic was measured at a wavelength of 721.5 nm. Finally, the total phenolic content was calculated based on the gallic acid standard curve (y = 0.0908x – 0.0121, R^2 = 0.9980, range: 0-12 µg/mL) and equation (4).

$$Total\ phenolic\ content\ (\mu g\ gallic\ acid\ equivalent/g) = a\ x\ \frac{V}{m} \qquad (4)$$

Where a is the total phenolic content values calculated from the gallic acid standard curve (µg/mL), V is the extract volume (mL), and m is the extract initial mass (g). The total phenolic content of the PP concentrated extract was 677.97 ± 0.17 µg gallic acid equivalent/g powder.

CONCLUSION

In conclusion, taken all parameters into consideration, the acceptable criteria for the standardized PP ethanolic extract were proposed and summarized in Table-1.

Table-1: Summary of the Characteristics and Their Respective Proposed Criteria of the Vietnamese *Peperomia pellucida* (L.) Kunth Ethanolic Concentrated Extract

No.	Characteristic	Proposed criteria	Results
1	Appearances	Homogenous, black color, herbal smell, and did not precipitate or contain any solid residues	Pass

No.	Characteristic	Proposed criteria	Results
2	Identification (Apiol)	Positive in thin layer chromatography (R_f = 0.2-0.4)	Pass (R_f = 0.214)
3	Loss on drying	Not more than 20%	Pass (6.38 ± 0.04%)
4	Total ash	Not more than 35%	Pass (23.9 ± 0.5%)
5	Heavy metals	Not more than 20 ppm	Pass
6	Microbial limits		
	Aerobic bacteria	Not more than 10,000 CFU/g	Pass (Negative)
	Fungi and molds	Not more than 100 CFU/g	Pass (Negative)
	Enterobacteriaceae and other gram (-) bacteria	Not more than 500 CFU/g	Pass (Negative)
	Escherichia coli	Negative	Pass (Negative)
	Salmonella spp.	Negative	Pass (Negative)
	Pseudomonas aeruginosa	Negative	Pass (Negative)
	Staphylococcus aureus	Negative	Pass (Negative)
7	Assay (total phenolic content)	At least 500 µg gallic acid equivalent/g powder	Pass (677.97 ± 0.17 µg gallic acid equivalent/g powder)

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID IDs, given below:

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REFERENCES

1. M. H. Le, H. S. Sam, D. T. Pham, N. C. L. Nguyen, N. D. Le and T. N. P. Dao, *Pharmacia*, **70(4)**, 943(2023), http://doi.org/10.3897/pharmacia.70.e108879
2. D. T. M. Huynh, M.-N. T. Le, V. D. Tran, V.-H. Tran and D. T. Pham, *Jordan Journal of Pharmaceutical Sciences*, **16(4)**, 830(2023), http://doi.org/10.35516/jjps.v16i4.1365
3. D. T. Pham, Q. C. Huynh, R. Lieu, V. B. Nguyen, V. D. Tran and B. T. P. Thuy, *Clinical, Cosmetic and Investigational Dermatology*, **16**, 1109(2023), http://doi.org/10.2147/CCID.S405464
4. T. V. Hung, P. N. T. Thang, N. T. T. Phuong, D. T. Pham, H. M. Hien and D. T. M. Huynh, *Journal of Applied Pharmaceutical Science*, **13(11)**, 178(2023), http://doi.org/10.7324/JAPS.2023.147199
5. T. V. Hung, P. N. T. Thang, N. T. T. Phuong, H. M. Hien, D. T. Pham and D. T. M. Huynh, *Tropical Journal of Natural Product Research*, **7(10)**, 4215(2023), http://doi.org/10.26538/tjnpr/v7i10.14
6. V. D. Tran, D. T. Pham, T. T. N. Cao, M. Bahlol, R. S. Dewey, M. H. Le and V. A. Nguyen, *Annali di Igiene Medicina Preventiva e di Comunita*, **34(5)**, 515(2022), http://doi.org/10.7416/ai.2021.2484
7. P. H. Nguyen, V. D. Tran, D. T. Pham, T. N. P. Dao and R. S. Dewey, *European Journal of Integrative Medicine*, **44**, 101328(2021), http://doi.org/10.1016/j.eujim.2021.101328

8. P. V. Ninh, *Dược điển Việt Nam V- Tập 2 (Vietnamese Pharmacopoeia V - Part 2)*, 2017.
9. I. Ahmad, B. D. Hikmawan, R. Sulistiarini and A. Mun'im, *Journal of Applied Pharmaceutical Science*, **13(1),** 1(2023), http://doi.org/10.7324/JAPS.2023.130201
10. N. S. F. Alves, W. N. Setzer and J. K. R. da Silva, *Journal of Ethnopharmacology*, **232**, 90(2019), http://doi.org/10.1016/j.jep.2018.12.021
11. K. L. Ho, P. H. Yong, C. W. Wang, U. R. Kuppusamy, C. T. Ngo, F. Massawe and Z. X. Ng, *Journal of Integrative Medicine*, **20(4)**, 292(2022), http://doi.org/10.1016/j.joim.2022.02.002
12. R. H. L and P. K. T. R, *International Journal of Pharmacy and Pharmaceutical Sciences*, **10(2)**, 1(2018), http://doi.org/10.22159/ijpps.2018v10i2.23417
13. F. C. Saputri, I. Hutahaean and A. Mun'im, *Saudi Journal of Biological Sciences*, **28(11)**, 6191(2021), http://doi.org/10.1016/j.sjbs.2021.06.075
14. L. Usman and I. Ridwan Olanrewaju, *Journal of Essential Oil Bearing Plants*, **23(3)**, 628(2020), http://doi.org/10.1080/0972060X.2020.1794983
15. A. Z. Wakhidah, C. Novianti and W. A. Mustaqim, in *Ethnobotany of the Mountain Regions of Southeast Asia*, ed. F. M. Franco, Springer International Publishing, Cham, 2020, pp. 1–8, http://doi.org/10.1007/978-3-030-14116-5
16. S. Hadi and J. Bremner, *Molecules*, **6(2)**, 117(2001), http://doi.org/10.3390/60100117
17. L. Olowa and C. Demayo, *Advances in Environmental Biology*, **9(27)**, 204(2015)
18. H. H. Phạm, *Cây cỏ Việt Nam = An illustrated flora of Vietnam*, Nhà xuất bản Trẻ, Thành phố Hồ Chí Minh, Tái bản có sửa chữa và bổ sung., 1999
19. Đ. T. Khang, P. T. Huynh, T. G. Huy, N. P. A. Thi, T. T. Mến and N. V. Ây, *CTU Journal of Science*, **57(4)**, 109(2021), http://doi.org/10.22144/ctu.jvn.2021.119
20. S. O. Rogers and A. J. Bendich, in *Plant Molecular Biology Manual*, eds. S. B. Gelvin and R. A. Schilperoort, Springer Netherlands, Dordrecht, 1994, pp. 183–190, http://doi.org/10.1007/978-94-011-0511-8_12
21. T. T. Men, L. T. K. Tu, N. T. K. Anh, H. H. Phien, N. T. B. Nhu, N. T. T. Uyen, N. T. A. Thu, T. N. Quy and D. T. Khang, *Veterinary Integrative Sciences*, **20(3)**, 683(2022), http://doi.org/10.12982/VIS.2022.052
22. A. Bakrudeen, T. Lydia, M. Musthafa, R. Taha and F. Marikar, *Modern Phytomorphology*, **15**, 6(2020)
23. R. Yadav and M. Agarwala, *Journal of Phytology*, **3(12)**, 10(2011)

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