RESEARCH ON FORMULATION OF HARD CAPSULES CONTAINING EXTRACTS OF *HOUTTUYNIA CORDATA* THUMB (SAURURACEAE), *MORUS ALBA* L. (MORACEAE), AND *CARICA PAPAYA* L. (CARICACEAE)

Huynh Thi My Duyen*, Nguyen Thanh Sil, Ta Kien Tuong, Le Thi Minh Ngoc
Can Tho University of Medicine and Pharmacy
**Corresponding author: htmduyen@ctump.edu.vn*
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ABSTRACT

Background: *previous studies have demonstrated that Houttuynia cordata Thumb (Saururaceae), Morus alba L. (Moraceae), and Carica papaya L. (Caricaceae) are effective against various viruses, especially the SARS-CoV-2 virus. In Vietnam, even though the three of these plant species are grown quite popularly and are pretty commonly consumed by residents regularly, there have not been any applications to the dosage forms to promote their full potential effects and make these dosage forms easy to use and preserve. Furthermore, no research has been conducted on a capsule form that combines all three of these medicinal herbs*. ***Objectives:*** *the research focuses on the composition of a hard capsule formula containing the extracts of Houttuynia cordata, Morus alba, and Carica papaya as well as the development of testing standards for prepared hard capsules.* ***Materials and methods:*** *the ratio of adsorbents and fillers in the formula was investigated so that the granules had low moisture, a suitable flow rate, and the appropriate apparent density for packaging; standards for testing capsules were developed according to current regulations.* ***Results:*** *the selected adsorbent was light $MgCO_3$, with a ratio of plant extract: filler: adsorbent of 10: 5: 0.5 and a ratio of Avicel: Starch 1500 of 1: 3.* ***Conclusions:*** *a hard capsule containing medicinal plant extract was formulated with a moisture content of < 5% and well-flowing granules that had the suitable apparent density for packing the capsule size of 0. Capsules met standards in terms of appearance, weight uniformity, disintegration, heavy metal and microorganisms, with a quercetin content of 74.45 ± 0.23 mg. The result of the study is considered one of the most essential bases for further research on determining the effectiveness of medicines derived from medicinal plants and applied to support the treatment of COVID-19, which is still happening in a quite complicated way at the moment.*

Keywords: *Capsules, Houttuynia cordata, Morus alba, Carica papaya, SARS-CoV-2.*

I. INTRODUCTION

Houttuynia cordata and *Morus alba* have the main chemical composition of flavonoids, of which quercetin has been shown to have anti-inflammatory, antioxidant, and antiviral effects [1], [2], [3], [4]. *Houttuynia cordata* extract has immunomodulatory and anti-severe acute respiratory syndrome (SARS) effects. *Houttuynia cordata* water extract could stimulate the proliferation of mouse splenic lymphocytes significantly and dose-dependently. Flow cytometry revealed that *Houttuynia cordata* increased the proportion of CD4+ and CD8+ T cells. In addition, it caused a significant increase in the secretion of interleukin IL-2 and IL-10 by mouse splenic lymphocytes. In the anti-viral aspect, *Houttuynia cordata* exhibited significant inhibitory effects on SARS-CoV 3C-like protease and RNA-dependent RNA polymerase [3], [5]. Santana et al. (2021) presented a list of

different bioactive compounds against SARS-CoV-2 for many medicinal plant species, of which the active ingredient in *Morus alba* was considered to have great potential [6]. A study by Arm Adel et al. (2022) showed that papaya extract had significant effects in the treatment of dengue virus with antiviral, immunomodulatory, and cytokine storm-reducing properties, so it would reduce inflammatory conditions caused by COVID-19 [7], [8].

SARS-CoV-2 is a new strain of the virus that has never appeared in humans, first identified amid an outbreak of respiratory illnesses in Wuhan City, Hubei Province, China. This outbreak was first reported to the World Health Organization (WHO) on December 31, 2019. On January 30, 2020, WHO declared that the COVID-19 outbreak was a global health emergency; the virus has spread around the world. The epidemic has caused great harm to people, the economy, etc. Right now, the epidemic is in danger of resurfacing. There are many methods of treating COVID-19, and traditional medicine is an integral part of the Vietnamese health system, always playing an important role in preventing and supporting the treatment of acute respiratory infections caused by the SARS-CoV-2 virus [9].

Vietnam is a tropical monsoon country with vegetation diversity, especially the three types of medicinal plants mentioned above that are widely grown. A successful formulation of a hard capsule containing extracts of *Houttuynia cordata, Morus alba, and Carica papaya* will be the basis for further research in the evaluation of the therapeutic effect of the product on patients with SARS-CoV-2, thereby helping to bring safe products with ingredients extracted from medicinal plants capable of supporting treatment in patients with mild and moderate disease conditions. The study was carried out with the following two objectives:

1. Research on the formulation of hard capsules containing *Houttuynia cordata, Morus alba,* and *Carica papaya extracts*.
2. Development of testing standards for prepared hard capsules in the form of healthy food.

II. MATERIALS AND METHODS

2.1. Materials

Extracts of *Houttuynia cordata (*extracted with 70% alcohol*), Morus alba (*extracted with 70% alcohol*)* and *Carica papaya* (extracted with 95% alcohol) with moistures of 5.69%, 5.45%, and 5.34%, respectively, were prepared at Department of Pharmaceutical and Pharmaceutical Technology, Faculty of Pharmacy, Can Tho University of Medicine and Pharmacy by the hot extraction method in the water bath at a temperature of 40°C, then through the rotary evaporation process at 60°C, and stored in the refrigerator to avoid termites and mold.

Light $MgCO_3$ was purchased from France. Florite-R was from Japan. Starch 1500 was bought in the US. Avicel PH102 was purchased from Taiwan. Colloidal Silicon Dioxide (CSD) and calcium stearate were made in India. $FeCl_3$, chloroform, ethyl acetate, toluene, acetic acid, formic acid, methanol, dichloromethane, NH_4OH, and HCl were bought from China. Ethanol (96%) and distilled water were made in Vietnam. All chemicals and solvents were of pharmaceutical grade or higher.

The standard substance, quercetin, with a $C_{15}H_{10}O_7$ purity of 99.5%, was calculated according to the anhydrous substance, moisture 8.44% provided by the National Institute of Drug Quality Control, SKS E0319322.03.

2.2. Methods

2.2.1. Research on the formulation of hard capsules containing extracts of Houttuynia cordata, Morus alba and Carica papaya

In the study of Lau K.-M et al. (2018) [6], an oral dose of 250 mg/kg/day of *Houttuynia cordata* leaf extract could be considered an unobserved side effect level in rats for 28 days. According to Vietnamese Pharmacopeia V [10], *Morus alba* leaf doses range from 5 g to 12 g in the decoction dosage form. Ekong M. et al. (2011) [11] showed the safety of *Carica papaya* leaf extract through evaluation in pregnant Wistar rats at 12-18 days' gestation with a dose of 60 or 120 mg/kg. The results showed a decrease in the number of viable fetuses, the weight of the fetuses, and the length of the crest, head, and tail.

From the above data, the total amount of extracts in a capsule was 308.2 mg, including 132 mg of *Houttuynia cordata*, 100 mg of *Morus alba* and 60 mg of *Carica papaya*.

Survey of adsorbents

Adsorbents were Florite-R and light $MgCO_3$, both of which were surveyed with percentages of 1%, 3%, 5%, and 7% compared to the amounts of the extracts.

Table 1. Survey of adsorbents

Ingredients/ Formulas	CT1	CT2	CT3	CT4	CT5	CT6	CT7	CT8
The mixture of extracts (mg)	308.2							
Light $MgCO_3$ (mg)	3.08	9.25	15.41	21.57	-	-	-	-
Florite-R (mg)	-	-	-	-	3.08	9.25	15.41	21.57

After mixing the ingredients, their appearance and moisture were evaluated. Requirements: the powder was dry with a moisture of < 5%, and the formula selected had to meet the requirements with the minimum amount of adsorbent.

Survey of fillers

From the above data, such as the selected adsorbents, the apparent density of the granules, and the apparent density of the used fillers, the weight of the fillers used was estimated at 150 mg.

The ratio of fillers Avicel PH102: Starch 1500 (x: y) was surveyed at 1:2, 1:3, 2:1, and 3:1 **(CT9-CT12)**, and the ratio of the remaining ingredients was similar to the survey of adsorbents.

The way of mixing powder was similar to the survey of adsorbents. Requirements: the powder was dry with a moisture of < 5%; the granules flew well; the apparent density of the powder was so high that its volume was close to the volume of the smallest capsule size.

Preparation of capsules

After selecting the ratio of fillers, we calculated the capsule size and the amount of filler to fit the volume (if necessary), and prepared hard capsules with a batch size of 1,000 capsules through the following steps: (1) medicinal plant extracts, fillers, and adsorbents were mixed; (2) the mixture was dried in an oven at 60^oC; (3) the powder was sieved through a 0.5-mm sieve; (4) powder was mixed with lubricants; (5) a semi-finished product test was conducted; (6) the product was packaged; (7) finished hard capsule product was tested.

Table 2. Ingredients of a hard capsule

Ingredients	Weight of 1 capsule (mg)
Mixture of extracts	308.2
Colloidal Silicon Dioxide	Corresponds to 1.5% of capsule weight
Calcium stearate	Corresponds to 1.5% of capsule weight

Ingredients	Weight of 1 capsule (mg)
Adsorbent	z
Avicel PH102 : Starch 1500 (x : y)	150
Additional filters (if needed)	ad. 1 capsule

2.2.2. Development of testing standards for prepared hard capsules

Appearance: we visually observed and described capsules.

Moisture content: by Infrared Moisture Analyzer with 3 g drug powder, temperature of 105°C and Resolution 0,01%, 1mg. Requirements: moisture content ≤ 5%.

Mass uniformity: according to Vietnamese Pharmacopeia V [10], Appendix 11.3. Requirements: a mass difference within ±5% range of the average capsule weight.

Disintegration: according to Vietnamese Pharmacopeia V, Appendix 11.6. Requirements: the test sample would be satisfactory if all 6 capsules disintegrated within ≤ 30 minutes. If 1 or 2 capsules did not disintegrate, we would repeat the test with 12 others. The test sample would pass if not less than 16 of the 18 test capsules disintegrated.

Determination: thin-layer chromatography.

Plate: silica gel F254, size 3 x 10 cm.

Trace detection: normal light; UV 254 nm and 366 nm; reagent of $FeCl_3$ 5% in alcohol 96%.

Test sample: an amount of finely ground drug powder was weighed corresponding to about 29 mg of medicinal plant extract, then 30 mL of 96 % ethanol was added, sonicated for 20 minutes, and filtered. Then, the filtrate was evaporated to dryness. It was dissolved in 1 mL of methanol to obtain the test solution.

Standard sample: standard quercetin in MeOH.

Solvent: Toluene: Ethyl acetate: Methanol: Formic acid = 10: 1: 2: 0.1

Quantification: the total flavonoid was calculated by quercetin content using UV-Vis spectroscopy [12].

Preparation of the test solution: we weighed exactly 10 times more powder than the mean capsule weight, placed it in a 250-mL conical flask, and then added 150 mL of a solution (methanol: water 1:1, v/v). The extract was then sonicated at 50°C for 60 minutes and filtered to remove tiny debris. The filtrate was depressurized to a half volume, then hydrolyzed with 100 mL of HCl 20% at 85°C for 3 hours. The hydrolyzed solution was cooled and shaken three times with 15 mL of ethyl acetate. Ethyl acetate extract was placed in a 50 mL volumetric flask, and then we added it to fit the volume. Quantification of total flavonoids was carried out based on quercetin content.

The preparation of the photometric sample is as follows:

Table 3. Preparation of the photometric sample

Sample	Standard	Blank standard	Test	Blank test
Reference solution (mL)	2	2	0	0
Test solution (mL)	0	0	2	2
$AlCl_3$ 5% (mL)	2	0	2	0
Sodium citrate 1% (mL)	0.5	0.5	0.5	0.5
MeOH / 5% acetic acid	ad. 25 mL			

After adding all the ingredients, we shook it well, kept it still for 45 minutes, and then assayed. The total flavonoid content in the finished powder, when assayed by UV-Vis spectroscopy, was calculated according to the following equation:

$$X(mg) = \frac{At+b}{a(100-h)} \times 125.10^3 \qquad (1)$$

X (mg) was the total flavonoid content in the finished powder; a and b were the coefficients in the linear standard linear equation, y = ax + b; At was the absorption at 425 nm; h was the moisture of the finished powder.

Setup of the linear regression equation between the concentration and absorption of the quercetin standard with the form y = ax + b. We prepared the quercetin standard solution with a mass of 0.05 to 0.8 mg and measured the absorption to derive the standard curve.

Heavy metal: test method by AOAC 999.11. Requirements: Cd ≤ 1 ppm; Pb ≤ 3 ppm.

Microorganisms: test method by ISO 16649-3:2025 and ISO 21527-2:2008. Requirements: *E. coli* ≤ 3; Total fungi ≤ 10^2 Cfu/g.

III. RESULTS

3.1. Research on the formulation of hard capsules containing extracts of Houttuynia cordata, Morus alba and Carica papaya

3.1.1. Survey of adsorbents

Table 4. Survey results of adsorbents

Formulas	Appearance	Moisture (%)
CT1	Damp and lumpy powder	7.47
CT2	Damp and lumpy powder	6.56
CT3	**Dry and loose powder**	**4.34**
CT4	Dry and loose powder	4.37
CT5	Damp and lumpy powder	8.63
CT6	Damp and lumpy powder	6.34
CT7	Dry and lumpy powder	5.62
CT8	Dry and loose powder	4.35

CT3 (the percentage of $MgCO_3$ was 5% compared to the amount of extract) was selected for the next research.

3.1.2. Survey of fillers

Table 5. Survey results on fillers

Formulas	Moisture (%)	Flow rate (s/100 g)	Apparent density (g/mL)
CT9	4.31	3.1	0.63
CT10	**4.32**	**3.6**	**0.72**
CT11	4.35	2.9	0.74
CT12	4.37	2.5	0.83

The results showed that all formulas had suitable moisture and flow rates. The capsule formula contained 308.2 mg of the extract. The total weight of the extract, the fillers, and the adsorbent was 487.6 mg. According to the apparent density results in *Table 5*, the suitable capsule size for packaging was size 0 (0.68 mL). Thus, CT10 was the most suitable formula for packaging.

3.1.3. Ingredients of the hard capsule formula

The mass of powder packed into the capsule was 0.68 x 0.72 = 490 mg. The ingredients of the capsule formula are presented in Table 6.

Table 6. Ingredients of one hard capsule

Ingredients	Weight of one capsule (mg)
Mixture of extracts	308.2
Colloidal Silicon Dioxide	7.4
Calcium stearate	7.4
Light $MgCO_3$	15.4
Avicel PH102: Starch 1500 (1:3)	150

The average result of testing semi-finished powder products before packaging: the powder had a moisture content of 4.33% with an apparent density of 0.72 g/mL and a flow rate of 3.2 s/100 g.

3.2. Development of testing standards for prepared hard capsules

The average results of the testing criteria for the three batches are presented in Table 7.

Table 7. Results of testing capsules

No.	Parameters	Acceptable criteria	Results
1	Appearance	Capsules with one end of blue, one end of orange, no dent, and no open	Pass
2	Moisture content	Moisture content of powder ≤ 5%	Pass (4,35%)
3	Capsule mass uniformity	All units within ±5% range of the weight stated on the label	Pass (489 ± 12.36 mg)
4	Disintegration	Disintegration time ≤ 30 minutes	Pass (5.16 minutes)
5	Determination	The test spot has the same R_f value as the reference spot	Pass
6	Heavy metal	Cd ≤ 1 ppm Pb ≤ 3 ppm	Not detected Not detected
7	Microorganisms	*E. coli* ≤ 3 Total fungi ≤ 10^2 Cfu/g	Pass (0) Pass (50 Cfu/g)

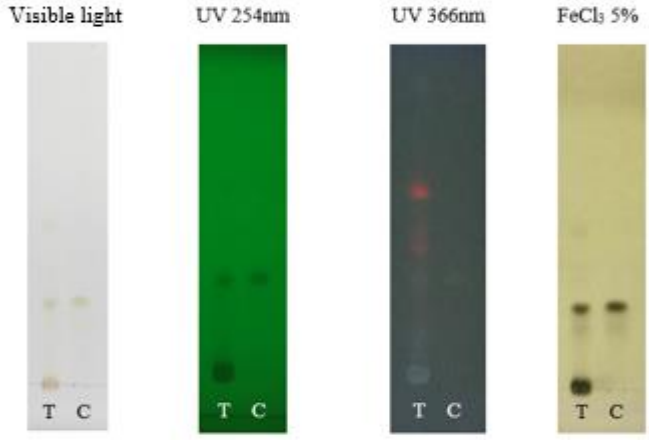

Figure 1. Determination of finished powder containing extracts by thin layer chromatography (*T: test; C: standard)*

Capsules met the criteria of appearance, mass uniformity, disintegration, and determination.

The results of quercetin quantification show that there was a linear correlation between the content and the absorption of quercetin according to the equation y = 3.1039x – 0.0169 with R^2 = 0.9992 (the quercetin content range was from 0.05 to 0.8 mg).

Figure 2. The linear curve between the content and the absorption of quercetin

Table 8. Survey results of repeatability of quercetin content of capsules

Samples	Weight of finished powder (g)	Powder moisture (%)	Absorption at 425 nm	Total flavonoid content from quercetin (mg)	Results
1	4.9102	4.03	0.835	744.850	$\overline{X}$= 744.534 SD = 2.191 RSD% = 0.616 e = ± 2.3 μ = 744.53 ± 2.3
2	4.8960	4.13	0.833	746.610	
3	4,9103	4.07	0.823	740.390	
4	4.9104	4.05	0.837	747.151	
5	4.8971	4.10	0.830	743.350	
6	4.8930	4.04	0.827	744.853	

Quantification results of total flavonoids had an RSD value of 0.616% (< 2%). Hence the quantification results of total flavonoids calculated according to the quercetin content in the finished capsule powder passed the repeatability criterion.

IV. DISCUSSION

Medicinal plant extracts are extremely hygroscopic, so using adsorbents in drug formulations is essential. The adsorbents are usually inorganic salts such as $CaCO_3$, $Ca(H_2PO_4)_2$, MgO, $MgCO_3$, etc... However, these adsorbents have low hygroscopicity, which may affect the dissolution of extracts. Light $MgCO_3$ and Florite-R are two new types of adsorbents with more advantages. Light $MgCO_3$ is a kind of magnesium with a high purity and a low density (0.15 g/cc), containing 40-45% MgO, so using a small amount can create a good hygroscopic property [13]. Experimental results show that when the amount of $MgCO_3$ is higher, the moisture of the powder is lower. With 5% and 7% (compared to the amount of extract) of this adsorbent, the moisture of the extract powder of Houttuynia cordata and Morus alba, respectively, was both < 5%. As for formulas containing Florite-R, only granules in Formula 8 were satisfactory. To save costs as well as limit the influence on the dissolution of the product, the study chose $MgCO_3$ with a percentage of 5% compared to the amount of extract for further investigation. In addition, fillers were also used in the formula to increase hygroscopicity, facilitating the packaging process. Starch 1500 has the

advantage of being suitable for active ingredients that are less resistant to moisture [14]. It has good compression resistance, good disintegration, and good adhesion. Still, it also has the drawback of poor flowability, so it is necessary to combine it with Avicel 102 to increase the smoothness of the powder. The ratio between Starch 1500 and Avicel 102 is chosen so that the granules have good flow, low moisture, and an apparent density suitable for the smallest capsule size, making it easy for patients to get. *Table 5* shows that formulas of CT10, CT11, and CT12 could contain whole medicinal plant granules, but the capsules for CT11 and CT12 would have big sizes and be difficult to swallow.

The test results of the prepared hard capsules showed that the capsules met all the criteria in terms of appearance, mass uniformity, disintegration, determination, heavy metal, and microorganisms. In addition, the quantification method of total flavonoids based on quercetin content also determined that the quercetin content in the capsule was 74.45 ± 0.23 mg.

The process of this study was limited for the following reasons. Firstly, research on medicinal plants, especially the combination of many types of them, so there were few references, so the comparison of results with previous studies was limited. Secondly, it was hard to find a standard for each medicinal herb's active ingredients, so the *Caruca papaya* extract could not be determined and quantified. Despite these difficulties, the research also initially formulated a hard capsule containing medicinal plant extracts with low moisture, a good flow rate, and a suitable size. The capsule met the basic criteria of the capsule dosage form, in which the quantification results of quercetin, which was the main active ingredient, were compared with the proven effects of *Houttuynia cordata* and *Morus alba***.**

V. CONCLUSIONS

The study selected the type and ratio of adsorbents and fillers to help produce powder containing the extracts of *Houttuynia cordata*, *Morus alba* and *Carica papaya* with the lowest moisture, good flowability, and a density suitable for common capsule sizes. The product achieved some testing standards to meet the basic requirements of capsules used as a healthy food.

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