STUDY ON PREPARING HARD CAPSULES FROM *PREMMA INTERGRIFOLIA L.* TO SUPPORT THE TREATMENT OF LIVER DISEASES

NGHIÊN CỨU BÀO CHẾ VIÊN NANG CỨNG HỖ TRỢ ĐIỀU TRỊ CÁC BỆNH LÝ VỀ GAN TỪ CÂY CÁCH NÚI (*PREMNA INTEGRIFOLIA* L.)

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DOI: http://doi.org/10.57001/huih5804.2024.112

ABSTRACT

Premma intergrifolia L. has been proven to have valuable biological activities such as anti-inflammatory, anti-cirrhosis, anti-steatohepatitis, helps cool the liver, detoxifies, clears heat and supports the treatment of liver diseases. This study has developed a process for preparing a hard capsule preparation to support the treatment of hepatitis with the main ingredient from the plant *Premma intergrifolia L.,* combined with *Schefflera heptaphylla L.* The hard capsule preparation has been evaluated with Physicochemical indicators such as uniformity; ash level; humidity according to the regulations of the Ministry of Health and Vietnamese Pharmacopoeia V. This opens up new research directions for using *Premma intergrifolia L.* in preparations and drugs to support the treatment of liver diseases and other diseases.

Keywords: *Premma intergrifolia L., hard capsule, hepatitis.*

TÓM TẮT

Cây cách núi đã được chứng minh có hoạt tính sinh học quý giá như chống viêm, xơ gan, chống viêm gan nhiễm mỡ, giúp mát gan, thải độc, thanh nhiệt và hỗ trợ điều trị các bệnh lý về gan. Nghiên cứu này đã xây dựng quy trình bào chế chế phẩm viên nang cứng hỗ trợ điều trị bệnh viêm gan với thành phần chính từ cây cách núi, kết hợp với cây ngũ chỉ thông. Chế phẩm viên nang cứng đã được đánh giá với các chỉ tiêu hóa lý như độ đồng đều; độ tro; độ ẩm theo quy định của Bộ Y tế và Dược điển Việt Nam V. Từ đó mở ra các hướng nghiên cứu mới sử dụng cây cách núi trong các chế phẩm và thuốc hỗ trợ điều trị các bệnh lý về gan và các bệnh lý khác.

Từ khóa: *Cây cách núi, viên nang cứng, viêm gan.*

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Received: 25/12/2023
Revised: 20/02/2024
Accepted: 25/3/2024

1. INTRODUCTION

Hepatitis is a condition in which the liver is damaged by infection, toxicity, or an autoimmune process of the body. Hepatitis can seriously impair liver function, form cirrhosis, and even cause liver cancer leading to death [1]. According to statistics from the World Health Organization, liver disease causes about 2 million deaths each year worldwide, and 1 million due to cirrhosis complications and 1 million due to viral hepatitis and hepatocellular carcinoma. Currently, cirrhosis is the 11th most common cause of death globally and liver cancer is the 16th leading cause of death; combined, they account for 3.5% of all deaths worldwide. Cirrhosis is among the top 20 causes of disability-adjusted life years and lost life years, accounting for 1.6% and 2.1% of the global burden. Approximately 2 billion people consume alcohol worldwide, and up to 75 million have been diagnosed with an alcohol use disorder and are at risk of alcohol-related liver disease. [2]. There are many causes of hepatitis such as viral hepatitis, parasitic hepatitis, auto-infectious hepatitis, and toxic hepatitis [3].

Many studies around the world have been published demonstrating the potential and use of *Premma intergrifolia* L. and *Schefflera heptaphylla* L. in the treatment of liver pathologies. *Premma intergrifolia* L. belongs to the Verbenaceae family, containing mainly flavonoids, steroids, terpenoids**,** and essential oils. According to Traditional Medicine, *Premma intergrifolia* L. aids with urination, lowers blood pressure supports aches, sedation and reduces anxiety and irritability. In 2009, R. Vadivu *et al*. studied the liver-protective potential of the alcohol extract from *Premma intergrifolia* L. The results showed that the alcohol extract at a dose of 250mg/kg was able to protect the liver through reduced activity of serum enzymes, bilirubin, and lipid peroxidation comparable to standard silymarine drugs [4]. The hepatoprotective effect has been demonstrated based on the presence of flavonoids compounds (neohesperidin, apigenin-7-*O*-glycoside, catechin hydrate, cyanidine chloride, quercetin-3-galactoside, diosmin, genistein, malvin chloride, 4-hydroxy-3-methoxycinnamic

acid, kaempferol-3-*O*-alpha-L-arabinoside, myricitrin, poncirin, vitexin and tiliroside [5].

Besides, *Schefflera heptaphylla* L. is used for sympathy, sore throat, rheumatism, bone pain, and painful swollen wounds [6]. Some studies indicate that *Schefflera heptaphylla* L. extract has anti-inflammatory, and anticancer effects [4].

The compounds *β*-sitosterol, Betulin**,** and Luteolin contained in *Premma intergrifolia* L. have antioxidant, and anti-inflammatory abilities and disrupt their molecular mechanisms. *β*-sitosterol has a hepatoprotective effect on liver damage caused by carbon tetrachloride [7]. Betulin's ability to support the treatment of liver diseases shows that: Carbon tetrachloride (CCl4) is a popular animal model to study the hepatoprotective activity of a biologically active molecule [8]. CCl_4 causes hepatocyte degeneration, altering liver enzyme functions [9, 10]. Betulin is a potent free radical cleaner and provides hepatoprotective activity by increasing levels of catalase, superoxide dismutase, glutathione peroxidase and by reducing levels of malondialdehyde and ROS in animal livers [11-13]. Besides, Luteolin protects against liver dysfunction. It may also protect the liver from damage caused by liver toxins [14].

In this study, we conducted research on the preparation of hard capsules from Premna integrifolia L. and proved the effect of capsules through the isolation of pharmaceutical substances in *Premma intergrifolia* L., the effect of supporting the treatment of hepatitis. The physico-chemical chemical indicators and content of the main compounds in the capsule preparation are determined according to the methods specified in Vietnamese Pharmacopoeia V.

2. MATERIALS AND METHOD

2.1. Material, Equipment, Chemical

Premma intergrifolia L. and *Schefflera heptaphylla* L. were collected in Hoa Binh and Nghe An province, respectively in November 2022. Samples were separated from seeds and pods, transferred to a sealed chamber of a vacuum oven at 40°C, 10mbar, and dried until the humidity was below 10%. Then, the sample was crushed by hammer mill with a sieve of 60 Mesh and samples of *Premma intergrifolia* L. powder and *Schefflera heptaphylla* L.. powder. After that, samples were stored in a sealed bag to avoid hygroscopic phenomena during the research.

Chemicals: *n*-hexane (China, 99%), dichloromethane (China, 99%), ethyl acetate (China, 99%), H_2SO_4 (China, 98%), ethanol (China, 99%). Silicagel 60: 0.04 - 0.06mm (Merck), thin plate TLC Silicagel 60 F_{254} (Merck), alcohol 70^0.

Equipment: NMR spectroscopy was measured in solvent DMSO-d_6 on the Bruker Avance machine (Brucker, Berlin, Germany) at frequencies of 600 MHz for ^{1}H-NMR and 150 MHz for ^{13}C-NMR at the Institute of Chemistry, Vietnam Academy of Science and Technology.

2.2. Isolation by column chromatography

300 (g) samples of *Premma intergrifolia* L. powder were extracted by using alcohol at 80° for 24 hours (3L), collecting the extracts of the soaks, filtration**,** and rotovap distillation to recover the solvent under reduced pressure the result obtained was the EtOH extract (58g). Add 150 mL of distilled water and extract the distribution with *n*-hexane and EtOAc, respectively, to obtain two extracts of *n*-hexane and ethyl acetate. Distillation of the solvent under reduced pressure yields *n*-hexane extract (10.3g) and EtOAc extract (**6**g).

EtOAc extract (6.0g) was taken to silica gel column chromatography, eluted with a solvent system with increasing polarity from *n*-hexane to CH_2Cl_2 and MeOH obtained 6 fractions (PI1~PI6). The PI2 fraction was placed on a silica gel column with the eluent system increasing gradually from *n*-hexane to MeOH to obtain 2 fractions (PI2.1 and PI2.2). The PI2.2 fraction was crystallized and washed in MeOH, obtaining the compound **PIE1** (130mg). The PI3 fraction was placed on a silica gel column with increasing elution solvent system from *n*-hexane to (CH_2Cl_2-MeOH 9:1, *v/v*) obtaining 3 fractions (PI3.1 to PI3.3). The PI3.2 fraction was crystallized and washed in MeOH obtaining the compound **PIE2** (97.5mg). The PI5 fraction was placed on a silica gel column with increasing elution solvent system from *n*-hexane to (CH_2Cl_2-MeOH 8:2, *v/v*) obtaining 4 fractions (PI5.1 to PI5.4). The fraction PI5.2 continued to be put on the silica gel and eluted with CH_2Cl_2-MeOH 7:3 (*v/v)* to get 3 fractions with symbols from PI5.2.1 to PI5.2.3. The PI5.2.2 fraction was crystallized and washed in MeOH obtaining the compound **PIE3** (72.5mg).

2.3. Preparation of hard capsules

Premma intergrifolia L. extract after freeze drying at -105°C, pressure reaching 0.1mbar for 48 hours, is finely ground into powder. Mix medicinal herb powder with *Schefflera heptaphylla* L. powder with the ratio shown in Table 1 according to the uniform mixing technique in a homogenous mixer to ensure the active ingredients are distributed evenly, mixing time is 30 minutes. After mixing, the medicinal powder was dried at 50°C for 1 hour. Then, the mixture of ingredients was crushed, ground**,** and sifted through a 60 Mesh sieve. Pack hard capsules No. 0, blue and white, using a semi-automatic capsule packaging machine. The machine must be cleaned with 70° alcohol 30 minutes before packaging. The jar is cleaned by washing it with alcohol. The powder after grinding finely, is encapsulated and packaged according to Figure 1.

Table 1. The ratio of material mixture [15]

No.	Materials	Unit	Weight
1	*Premma intergrifolia* L. extract	mg	285
2	*Schefflera heptaphylla* L. powder	mg	215
3	Gelatin	mg	80

Determining the basic criteria for hard capsule from *Premma intergrifolia* L.

Weight uniformity: Criteria tested according to Vietnamese Pharmacopoeia V, Appendix 11.3 met the requirement of ± 7.5%.

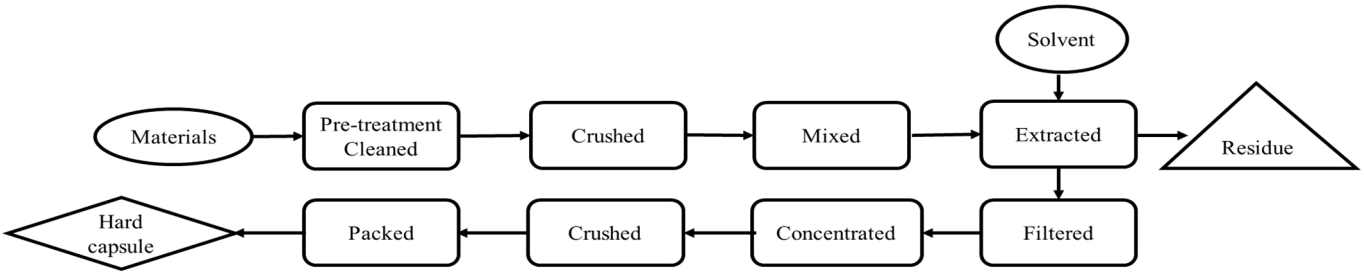

Figure 1. Capsule preparation process

Moisture content was determined by drying to constant weight.

Ash content was determined according to TCVN 5611-1991.

Disintegration: according to Vietnamese Pharmacopoeia V, Appendix 11.6 by Disintegration Tester, requirement: No more than 30 minutes.

Microbial indicators such as total aerobic microorganisms, *E. coli*, *Coliform* and total yeast and mold spores were determined according to TCVN 4884-1:2015 (ISO 4833-1:2013); TCVN 7924-2:2008; TCVN 6848:2007 and TCVN 8275-2:2010, respectively.

3. RESULTS AND DISCUSSION

3.1. The compound was isolated by column chromatography

3.1.1. PIE1 compound

^{1}H-NMR (600Hz, DMSO-d_6): δ_H = 3.53 (1H, H-3); 5.35ppm (1H, t, H-6); 0.93ppm (3H, d, J = 6.6Hz, H-19); 0.84ppm (3H, t, H-24); 0.83ppm (3H, d, J = 6.6Hz, H-26); 0.81ppm (3H, d, J = 6.6Hz, H-27); 0.68ppm (3H, s, H-28); 1.00ppm (3H, s, H-29).

^{13}C-NMR (150MHz, DMSO-d_6): δ_C = 37.28 (C-1); 31.69 (C-2); 71.82 (C-3); 42.35 (C-4); 140.79 (C-5); 121.72 (C-6); 31.93 (C-7); 31.93 (C-8); 50.17 (C-9); 36.53 (C-10); 21.11 (C-11); 39.81 (C-12); 42.35 (C-14); 26.14 (C-15); 28.26 (C-16); 56.10 (C-17); 36.16 (C-18);19.06 (C-19); 33.98 (C-20); 26.14 (C- 21); 45.88 (C-22); 23.10 (C-23); 12.00 (C-24); 29.20 (C-25); 19.82 (C-26); 19.46 (C-27); 18.80 (C-28); 12.0 (C-29).

On spectral data ^{1}H-NMR appears the signal of the double splicing at δ_H = 5.35 (1H, t, H-6), there are 6 methyl groups (CH_3) resonating at δ_H = 0.68ppm (3H, s, H-28) and δ_H = 1.00 ppm (3H, s, H-29); 0,81ppm (3H, d, J = 6.6Hz, H-27); 0,831ppm (3H, d, J = 6.6Hz, H-26); δ_H = 0,842ppm (3H, t, H-24), δ_H = 0,93ppm (3H, d, J = 6.6Hz, H-21). The proton of the CH group bound to the multiplete OH (H-3) group is attributed to the resonant signal at δ_H = 3.53ppm. On the spectrum ^{13}C-NMR indicates that compound PIE1 has 29 C atoms in the molecule, of which 1 double bond (δ_C 121.72 and 140.79 correspond to C-6 and C-5), 6 methyl groups CH_3 (δ_C 21.11); 12.0 (C-29); 12.00 (C-24); 19.82 (C-26); 19.06 (C-19); 19.46 (C-27), 9 CH methine groups, 11 methylene groups CH_2 and 2 C of the 4th order. Combining the above spectral data and comparing with the data published by Chaturvedula *et al.* [16] the result allows to confirm the structure of PIE1 as β-sitosterol.

3.1.2. PIE2 compound

^{1}H-NMR (600Hz, DMSO-d_6): δ_H = 0.86ppm - 1.63ppm (1H, m, H-1); 1.59ppm - 1.53ppm (1H, m, H-2); 3.18ppm (1H, dd, J = 10.8, 4.2Hz - J = 10,8 - 5,4Hz, H-3); 0.67ppm (1H, s, H-5); 1.51ppm - 1.38ppm (1H, m, H-6); 1.37ppm - 1.36ppm (1H, m, H-7); 1.27ppm (1H, m, H-9); 1.41ppm - 1.23ppm (1H, m, C-11); 1.01ppm - 1.63ppm (1H, m, H-12); 1.63ppm (1H, m, H-13); 1.67ppm - 1.03ppm (1H, m, H-15); 1.92ppm - 1.20ppm (1H, m, H-16); 1.56ppm (1H, m, H-18); 2.37ppm (1H, ddd, J = 6,0 - 11,4Hz); 1.92ppm - 1.40ppm (1H, m, C-21); 1,02ppm - 1,68 ppm (1H, m, H-22); 0.95ppm (3H, s, H-23); 0.74ppm (3H, s, H-24); 0.80ppm (3H, s, H-25); 1,01 (3H, s, H- 26); 0,96 (3H, s, H-27); 3.79ppm - 3.33ppm (2H, dd, J = 10.7 – 3.7Hz; H-28); 4.66ppm (1H, s, H-29), 4.56ppm (1H, s, H-29); 1.66 (3H, s, H-30).

^{13}C-NMR (150 MHz, DMSO-d_6): δ_C = 38.74 (C-1); 27.47 (C-2); 77.00 (C-3); 38.87 (C-4); 55.34 (C-5); 18.33 (C-6); 34.32 (C-7); 40.84 (C-8); 50.47 (C-9); 37.2 (C-10); 20.95 (C-11); 25.18 (C-12); 37.29 (C-13); 27.03 (C-15); 29.18 (C-16); 47.76 (C-17); 48.34 (C-18); 47.83 (C-19); 150.97 (C-20); 29.69 (C-21); 60.55 (C-28); 109.31 (C-29); 19.06 (C-30).

On spectrum ^{1}H-NMR, there are resonant signals of double coupling at δ_H = 4.66ppm and 4.56ppm (2H), there are 2H of methyl groups adjacent to hydroxyl groups at δ_H = 3.79ppm and 3.33ppm and many signals of H are present at high intensities. On spectrum ^{13}C-NMR there is a resonant signal of group 2 unsaturated carbon at δ_C = 109.31ppm, 150.97ppm. There is a signal of 18 carbons including 6 carbon methine, 6 carbon order 3. Combining the above spectral data and comparing with the data published by Noori *et al.* [17] the result allows to confirm the structure of PIE2 as Betulin.

3.1.3. PIE3 compound

^{1}H-NMR (600Hz, DMSO-d_6): δ_H = 6.67ppm (1H, s, H-3); 6.19ppm (1H; d; J = 2.1Hz; H-6); 6.45ppm (1H, d, J = 2.4Hz, H-8); 7.2ppm (1H, dd, J = 8.4 – 2.1Hz, H-6′, H-2′); 6.19 (1H, d, J = 8.4Hz, H-5′); 12.98ppm (1H, s, 5-OH), 10.83ppm (1H, s, 7-OH); 9.94ppm (1H, s, 3′-OH); 9.43ppm (1H, s, 4′-OH).

^{13}C-NMR (150MHz, DMSO-d_6): δ_C = 163.9 (C- 2); 102.9 (C-3)181.7 (C-4); 161.5 (C-5); 98 (C-6); 164.2 (C-7); 93.9 (C-8); 157.3 (C-9); 103.7 (C-10); 121.6 (C-1′); 113.4 (C-2′); 145.8 (C-3′); 149.7 (C-4′); 116.1 (C-5′); 119 (C-6′).

On spectrum ^{1}H-NMR resonant signals appear 4 hydroxyl groups at δ_H = 12.98ppm (1H, s, 5-OH), 10.83ppm (1H, s, 7-OH), 9.94ppm (1H, s, 3'-OH) and 9.43ppm (1H, s, 4'-OH). There are 4 protons in the aromatic ring at δ_H = 7.42ppm (1H, dd, J = 8.4

- 2.1Hz, H6'-H2'), 6.19 (1H, d, J = 8.4Hz, H-5'), 6.45ppm (1H, d, J = 2.4Hz; H-8) and 6.67ppm (1H, s, H-3). On the ^{13}C-NMR spectrum there is a resonance signal of the carbonyl group at δ_C 181.7ppm and there are 16 carbon atoms, of which 12 carbons belong to the benzene ring and 4 carbons belong to the oxepan ring. Combining the above spectral data and comparing with the data published by Lee *et al.* [18] the result allows to confirm the structure of PIE3 as Luteolin.

3.2. Preparation of hard capsule

In the preparation of hard capsules, the main ingredient is *Premna integrifolia* L. In oriental medicine, this is an acrid herb, that calms, supports urination, stimulates the digestive system, helps to sedate, and reduces anxiety, and irritability. In particular, the preparation shows the most obvious uses in supporting the treatment of dysentery, jaundice, colon diseases, and hepatitis.

In addition, to increase the therapeutic effect, the authors have a street with *Schefflera heptaphylla* L. The plant has an acrid bitter taste, light aroma, and coolness [19]. Schefflera heptaphylla L. has many pharmacological effects such as hypertonic, anti-cold, and hypoglycemia. In particular, Ethanol extract of *Schefflera heptaphylla* L. has anti-inflammatory and antitumor, dose-dependent activity [6].

The composition of the hard capsule product to support liver disease is a combination of 2 herbs: *Schefflera heptaphylla* L. powder and *Premna integrifolia* L. extract. The compound β-sitosterol has been shown to have anti-inflammatory, antibacterial, and cancer activity [20], stopping fatty liver caused by a high-fructose diet and stopping the progression of NAFLD to steatohepatitis [7]. Betulin has been shown to have anti-dyslipidemia effects, acting on neurodegeneration [21, 22]. Luteolin is a flavonoid shown to inhibit cancer enzymes and antioxidant, vascular, antiallergic, anti-osteoporosis**,** and anti-inflammatory activities [23, 24]. *Premna integrifolia* L. There have been many domestic and foreign studies demonstrating that the compounds contained in the plant have biological activity that supports liver disease.

Results of evaluating the criteria of hard capsules

Figure 2. Hard capsule from *Premna integrifolia* L.

Greenish-white capsule, gray-brown medicinal powder, characteristic medicinal aroma, slightly bitter and astringent taste.

3.2.1. Physico-chemical indicators

Capsule uniformity

The results of surveying the uniformity of capsule weight are presented in Table 2.

Table 2. Weight uniformity test results

No.	Capsule weight	Empty cyst weight	Medicinal powder weight	% of average weight	% Difference from average weight
1	581.3	79.6	501.7	99.749	0.251
2	584.7	79.9	504.8	100.366	0.366
3	581.9	80.2	501.7	99.749	0.251
4	582.7	79.8	502.9	99.988	0.012
5	582.9	79.7	503.2	100.048	0.048
6	582.3	80.1	502.2	99.849	0.151
7	584.1	80.3	503.8	100.167	0.167
8	584.4	80.2	504.2	100.247	0.247
9	581.6	80.3	501.3	99.670	0.330
10	585	79.8	505.2	100.445	0.445
11	582.7	80.1	502.6	99.928	0.072
12	581.2	80.1	501.1	99.630	0.370
13	582.3	80.2	502.1	99.829	0.171
14	582.6	80.0	502.6	99.928	0.072
15	582.7	79.9	502.8	99.968	0.032
16	583.4	80.0	503.4	100.087	0.087
17	581.9	80.2	501.7	99.749	0.251
18	583.3	79.8	503.5	100.107	0.107
19	582.6	80.3	502.3	99.869	0.131
20	586.5	80.4	506.1	100.624	0.624
Average			**502.96**		
SD			**1.34**		
RSD (%)			**0.27**		

The results from Table 2 showed that the relative accuracy and repeatability in capsule making was RSD% < 4%. The capsule filling process does not vary too much, the use of capsules to support the treatment of liver diseases is not affected by the volume of the capsule

Moisture, ash, disintegration

Table 3. Results of measuring disintegration time, moisture, and ash

Parameters	Times			Average
	1	2	3	
Disintegrattion time (min)	22	22	22	22
Moiture (%)	3.15	3.19	3.17	3.17
Ash (%)	11.34	11.28	11.26	11.29

The results of disintegration time, moisture and ash are presented specifically in Table 3. The results showed that the hard capsule product prepared from Premna integrifolia L. has a disintegration time of < 30 minutes; the moiture and ash content were reached 3.17% (< 5%) and 11.29% (< 15%), respectively. They have met the requirements specified in TCVN and Vietnamese Pharmacopoeia V.

3.2.2. Microbiological indicators

Table 4. The results of microbial indicators

No.	Parameters	Method Analysis	Results	Unit
1	Total aerobic microorganisms	TCVN 4884-1:2015	$5x10^3$	CFU/g
2	*E. coli*	TCVN 7924-2:2008	0	CFU/g
3	*Coliforms*	TCVN 6848:2007	0	CFU/g
4	Total number of yeast spores, molds	TCVN 8275-5:2010	$< 2.5x10^2$	CFU/g

In Table 4, the results of the evaluation of microbiological criteria showed that the indicators of total aerobic microorganisms are $5x10^3$ CFU/g; the total number of yeast and mold spores is $2.5x10^2$ CFU/g; *Coliforms; E.coli* does not exceed the allowable limit specified in Decision No. 46/2007/QD-BYT on the maximum limit of biological and chemical contamination in food of the Ministry of Health Viet Nam.

4. CONCLUSION

Premma intergrifolia L. was collected in Hoa Binh province, we have successfully prepared hard capsules to support the treatment of hepatitis. The hard capsule preparation has been evaluated with physicochemical criteria, disintegration time (22 min), moisture (3.17%), ash (11.29%), and the microbial indicators are all within the allowable limits of the Ministry of Health. In addition, the presence of isolated compounds also demonstrated the possibility of anti-inflammation, cirrhosis, and anti-steatohepatitis, helping to cool the liver, detoxify, clear heat**,** and support the treatment of liver diseases. This opens up new research directions for using *Premma intergrifolia* L. in preparations and medicines to support the treatment of liver diseases and other diseases.

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