Chemical composition analysis and antibacterial-antiinflammatoryactivity tests of tamanu seed oil extracted by supercritcial fluid technology

- **Nguyen Huu Hieu**
- **Tran Thi Minh Thuy**

Ho Chi Minh city University of Technology, VNU-HCM.

(Manuscript Received on July 08th, 2016, Manuscript Revised September 03rd, 2016)

ABSTRACT

The natural product of tamanu oil is valuable in medicine and cosmetic. In this study, the tamanu oil was extracted by supercritical CO2 (sCO2) and Soxhlet (using hexane solvent) methods. The fatty acids and coumarins compound of the obtained tamanu oil were analyzed by gas chromatography–mass spectrometry (GC-MS). The antibacterial and anti-inflammatory activities were also investigated by the diffusion method and performed on the white mice ears (Swiss albino). Content of fatty acid in tamanu oil extracted using sCO_2 (72.62 %) was higher than that using Soxhlet method. The compound of coumaron (2H-1-bezopyran-2-one, $C_9H_6O_2$) was identified belonging the simple coumarin group and its content in the tamanu oil extracted using sCO_2 was the highest (169.69 µg/g), 1.79 and 2.04 times as much as that using Soxhlet method and the commercial oil, respectively. Besides, the results of investigation of the antibacterial activity suggested that the tamanu oil extracted using sCO_2 was able to against some of strains namely: Staphylococcus aureus (S. aureus) ATCC 25923, S. aureus ATCC 29213, methycilin-resistant S. aureus (MRSA) ATCC 43300, and Pseudomonas aeruginosa (P. aeruginosa) ATCC 277853.

In term of the anti-inflammatory activity, the tamanu oil extracted using sCO_2 was anti-inflammatory effectively with reducing flammatory: 21.41 – 25.39 % as for the thickness of mice ears and 33.88 – 39.40 % as for the weight of mice ears.

Keywords: *Tamanu oil, coumarin, antibacterial, anti-inflammatory, extraction, supercritical CO_2.*

1. INTRODUCTION

Tamanu with scientific name of *Calophyllum inophyllum L* is widely dispersed throughout the tropics including: Thailand, Myanmar, Malaysia, South India, Sri Lanka, Melanesia, and Polynesia…. In Vietnam, tamanu is mainly planted in coastal areas of Hai Phong, Ba Ria-Vung Tau, and Mekong Delta [1,2].

The oil is tinted green, thick, and woodsy or nutty smelling and is easily absorbed into the skin [3]. Tamanu oil is mainly extracted from seeds containing roughly 50.3-73% of tamanu oil. The extracted tamanu oil has high amount of many actives like: calophyllic acid and lactone with the antibacterial, anti-inflammatory activities, and promote healing the scars. This was discovered by the French researcher-professor of Lederer [4].

All species of tamanu tree have various pharmacological uses. The tamanu oil has been used in medicine, cosmetic, energy, and some other industries [5,6]. The tamanu oil provides plenty of valuable compounds namely: fatty acid, coumarin, calophyllolid, and calophyllic acid.

Omega is a mixture of essential fatty acids, it is one of the most important compositions in tamanu oil helping to treat various skin injuries, cell regeneration. Therefore, it plays an important role in cosmetic, omega usually includes 3 main categories [7,8]:

+ Omega 3: α-linolenic, eicosapentaenoic acid and docosahexaenoic acid; are necessary for skin texture, reduce the dehydration of skin so that the skin could become softer.

+ Omega 6: linoleic acid, γ-linolenic acid and arachidonic acid; have anti-inflammatory and analgesic properties, heal the injuries and treat the skin diseases.

+ Omega 9: oleic acid, eicosenoic acid, and erucic; help to reduce the cholesterol content, sugar steady and prevent the cardiovascular deseases.

Coumarins are classified as a member of the benzopyrone family. All of which consist of a benzene ring joined to a pyrone ring. The benzopyrones can be subdivided into the 5,6-benzo-alpha-pyrone to which the coumarins belong and the benzo-gamma-pyrone. Courmarins were isolated in 1820 and identified the structure. There are four main coumarin sub-types: the simple coumarins, furanocoumarins, pyranocoumarins, and the pyrone-substituted coumarin. The coumarins are great important due to their biological properties such as: the antibacterial, anti-inflammatory, anti-fungal, antioxidant properties that make these compounds more attractive [9].

In addition, the tamanu oil was used as massage oil and a skin moisturizer, tamanu oil has been traditionally used for treating various skin injuries such as scrapes, burns, insect bites, sunburn, and diseases such as dry skin, psoriasis, eczema, ringworm, and even diaper rash. Besides, the results of some studies have even shown that *Calophyllum inophyllums* extracted from tamanu oil, could inhibit HIV reverse transcriptase in a novel way, which indicates that some day they may be used as part of a combination therapy for AIDS [10].

Mechanical pressing is the traditional and popular method for extraction of tamanu oil. Accordingly, tamanu seeds were dried to a certain humidity before being entered the presses. Although, this method is simple and convenient, the crude tamanu oil is not pure and content of oil is not high. Besides, tamanu oil is also separated by Soxhlet method using organic solvents. The crushed tamanu seeds were extracted with various solvents: ethanol, n-hexane, petroleum ether,… However, this method is toxic, consume a large amount of solvents, last for a long time and have the impurities of solvent in product [11]. On the other hand, the extraction using supercritical CO_2 (sCO_2) has been attractive the attention of many researchers as for the separation of essential oil and flavonoids from natural materials. CO_2 is generally the most desirable solvent for supercritical fluid extraction. The critical temperature of CO_2 is only 304 K, which makes it attractive for the extraction of heat sensitive compounds. In addition, it is an inert, non-flammable, non-explosive, inexpensive, odorless, colorless, and clean solvent those leave no solvent residue in the product, it is also non-toxic and is generally accepted as a harmless [12].

The supercritical CO_2 solvent has been utilized to extract essential oils from different plants as: red pepper, cinnamon, Melaleuca, Momordica cochinchinensis…. which were reported in the previous literatures [10-12]. In this study, the tamanu oil was extracted from seeds using supercritical CO_2 and the obtained oil was analyzed by gas chromatography–mass spectrometry (GC-MS) to determine the content and composition of oil. The properties of tamanu oil extracted using supercritical CO_2 were also compared with the commercial oil and the oil extracted using Soxhlet method. Finally, the antibacterial property was investigated using the diffusion method on agar plate and investigation of anti-inflammatory was performed on ear skin of white mice.

2. EXPERIMENTAL

2.1. Materials

Tamanu seeds and commercial tamanu oil were provided by production facility at 25/21 Hau Giang, Ward 4, Tan Binh District, HCM city with resources collected from the Mekong Delta.

Chemicals: ICL, $Na_2S_2O_3$, KI, n-hexane, coumarin, acetic acid, chloroform, and starch indicator were purchased from Merck, Germany.

2.2. Extraction of tamanu oil

2.2.1 Using solvent of n-hexane

The tamanu seeds were dried at 40°C and crushed to certain particle size about 1-2 mm. These crushed tamanu seeds were placed in the thimble of Soxhlet extractor and the extraction was carried out for 6 hours at 20°C. The obtained product was concentrated using rotary evaporator (Buchi R-215) at 37°C.

2.2.2 Using supercritical CO_2

Table 1. Operating parameters of experiment

Pressure (bar)	Temperature (^oC)	Flow rate (g/min)	Time (min)
280	40	18	180

The crushed tamanu seeds, average particle size of 1-2 mm, were charged into a 100 mL extraction vessel (20 cm in height). Then liquid CO_2 was pumped into the extractor at a flow rate

of 18 gCO_2/min and the parameters were established at Table I [13].

2.3. Composition analysis of tamnanu oil

The fatty acid composition of the tamanu seed oil was analyzed by GC-MS Agilent technology DB5-MS system coupled with a flame ionization detector (FID) with a capillary column (HP-INNOWax, 30m x 0.25µm x 0.25 mm).

2.4. Investigation of antibacterial and anti-inflammatory activities of tamnanu oil

Investigation of antibacterial activity of tamanu oil using the agar diffusion method [14-16] were carried out by Pasteur Institute of HCM City, Vietnam, and Microbiology Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Medicine and Pharmacy.

The anti-inflammatory activity was tested on mice ears-Swiss albino by Microbiology Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Medicine and Pharmacy, HCMC.

3. RESULTS AND DISCUSSION

3.1. The composition of fatty acid

From Table II, there are total 18 fatty acids including: 9 saturated fatty acids (caprylic, capric, lauric, myristic, palmitic, stearic, arachidic, behenic, and lignoceric) and 9 unsaturated fatty acids (OA, LA, ALA, GLA, eicosenoic, ARA, EPA, erucic DHA) which were identified in tamanu oil.

Table 2. The fatty composition in tamanu oil

Name			**Content of fatty acid (%)**		
			Extraction method		
			Soxhlet	***sCO₂***	***Cold press***
(1)			***(2)***	***(3)***	***(4)***
Saturated fatty acid	Caprylic acid	C8:0	-	-	-
	Capric acid	C10:0	-	-	-
	Lauric acid	C12:0	-	-	-
	Myristic acid	C14:0	0.02	0.02	0.02
	Palmitic acid	C16:0	13.09	13.49	12.89
	Stearic acid	C18:0	13.25	12.42	14.03
	Arachidic acid	C20:0	0.02	0.77	0.8
	Behenic acid	C21:0	12.97	0.25	0.27
	Lignoceric acid	C24:0	13.41	0.06	0.07
Unsaturated fatty acid	Oleic acid (OA)	C18:1	37.66	38.66	38.86
	Linoleic acid (LA)	C18:2	33.83	33.32	31.82
	α-linolenic (ALA)	C18:3	0.29	0.29	0.27
	γ-linolenic (GLA)	C18:3	-	-	-
	Eicosenoic	C20:1	-	0.29	0.21

	Arachidonic (ARA)	C20:4	-	0.03	0.03
	Eicosapentaenoic (EPA)	C20:5	-	-	0.05
	erucic	C22:1	-	0.03	0.08
	Docosahexaenoic (DHA)	C22:6	-	-	0.05
Omega	Omega 3 (ALA + EPA + DHA)		0.29	0.29	0.37
	Omega 6 (LA + GLA + ARA)		33.23	33.35	31.85
	Omega 9 (OA + C20:1 + C22:1)		37.66	38.98	39.16
Total			71.18	72.62	71.38

According to these results, the tamanu oil extracted using supercritical CO_2 has the total content of omega (3, 6, and 9) of 72.62%, being higher than that of tamanu oil extracted by Soxhlet (71.18%) and the commercial oil (71.38%). It was explained that the fatty acids were able to dissolve and easily diffused into the supercritical CO_2 fluid. Therefore, using supercritical CO_2 fluid technology is one of the advance method for extraction of natural products.

3.2. Identification and quantification of coumarin

The compound of 2H-1-bezopyran-2-one (coumaron, $C_9H_6O_2$) belong to the simple coumarin group and its chemical structure was represented at Figure 1.

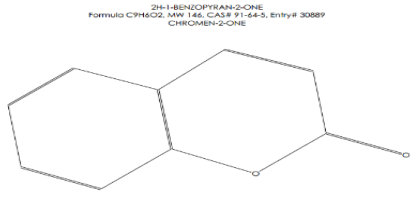

Figure 1. The chemical structure of coumaron [5]

Table 3. The quantification of courmarin in tamanu oil

Oil samples	Mm (g)	Volume (ml)	(areas)	Results (µg/g)
sCO_2	2.5265	50	1428694251	169.68
Soxhlet	2.6854	50	1255148434	95.01
Comerci-al	3.1685	50	1263521548	83.17

From Table 3, the amount of coumarin in tamanu oil extracted by supercritical CO_2 was higest (169.68 µg/g), being 1.79 and 2.04 times as much as that of tamanu oil extracted by Soxhlet and commercial oil, respectively. This suggested that the supercritical CO_2 fluid technology could be utilized to extract the precious compounds more effective in comparison with the other traditional methods.

3.3. Antibacterial activity

Table 4 indicated that the extracted oil against *S. aureus* ATCC 25923, *P. aeruginosa* ATCC 277853 at the concentration of 10^0, 10^{-1}, and 10^{-2}; when diluting further to concentration of 10^{-3}, the oil just has the inhibition for *P. aeruginosa* ATCC 277853. As for *C. albicans*

ATCC 10231 do not have the zone of inhibition for all values of concentration.

Table 4. Results of investigation of antibacterial activity of tamanu oil extracted by sCO2

Strains	Diluted concentration			
	10^0	10^{-1}	10^{-2}	10^{-3}
S. aureus ATCC 25923	D = 12.5	D = 12	D = 11	D = 6
P. aeruginosa ATCC 277853	D = 18	D = 17.5	D = 17	D = 12
C. albicans ATCC 10231	D = 6	D = 6	D = 6	D = 6

where D: diameter of zone of inhibition (mm); Diameter of antibiotic disk = 6 mm; 10^0: the initial concentration.

Table 5. Results of investigation of antibacterial activity of tamanu oil extracted by Soxhlet method

Strains	Diluted concentration			
	10^0	10^{-1}	10^{-2}	10^{-3}
S. aureus ATCC 25923	D = 15	D = 13	D = 11.5	D = 9.5
P. aeruginosa ATCC 277853	D = 18	D = 17.5	D = 15.5	D = 13.5
C. albicans ATCC 10231	D = 6	D = 6	D = 6	D = 6

The oil that separated by Soxhlet method appeared the inhibition on *S. aureus* ATCC 25923, *P. aeruginosa* ATCC 277853 and did not against *C. albicans* ATCC 10231.

Table 6. Results of investigation of antibacterial activity of commercial oil

Strains	Diluted concentration			
	10^0	10^{-1}	10^{-2}	10^{-3}
S. aureus ATCC 25923	D = 11	D = 11	D = 9	D = 6
P. aeruginosa ATCC 277853	D = 6	D = 6	D = 6	D = 6
C. albicans ATCC 10231	D = 6	D = 6	D = 6	D = 6

The Table 6 shows that the commercial oil just against *S. aureus* ATCC 25923 at high concentration and was inactive as for the others.

Table 7. Results of investigation of antibacterial activity of oil at low concentration 10-4

Oil samples	*P. aeruginosa*	*S. aureus*	MRSA
sCO_2	-	9	7
Soxhlet	-	7	7
Commercial	-	9	7
Control	-	-	-

Antibacterial activities of oil samples extracted by supercritical CO_2, mechanical pressing, and Soxhlet at low concentrations are presented in Table 7. Results show that the zone of inhibition on *S. aureus* ATCC 29213, MRSA ATCC 43300 at concentrations of 10^{-4}; *P. aeruginosa* ATCC strains 277853 did not appear the inhibition.

3.4. The anti-inflammatory activity on mice ears

Tables 8 and 9 indicated that the tamanu oil was extracted by supercritical CO_2 is anti-inflammatory effectively (reducing flammatory: 21.41 % as for the thickness of mice ears and 33.88 % as for the weight of mice ears).

Table 8. Weight and thickness of mice ears

Group	Intervention	Thickness (µm)	Weight (mg)
Solvent	Just using actone	20.40 ± 0.16	20.64 ± 0.50
Desease	Using croton oil	27.56 ± 0,69	38.10 ± 1.81
Compari-son	Clobetason, croton oil	23.00 ± 0.42	25.18 ± 0.98
The tamanu oil extracted using sCO_2	The tamanu oil extracted using sCO_2, croton oil	25.80 ± 0.51	31.06 ± 1.33
The tamanu oil extracted using Soxhlet	The tamanu oil extracted using Soxhlet, croton oil	28.45 ± 0.34	34.24 ± 1.08
Commercial oil	Commercial oil, croton oil	25.60 ± 0.22	33.95 ± 1.39

Table 9. Reducing anti-inflammatory (%) on mice ears

Group	Reducing the flammatory, %	
	Thickness	***Weight***
Compare	63.93 ± 6.56	73.20 ± 7.02
sCO_2	21.41 ± 8.66	33.88 ± 14.92
Soxhlet	-7.90 ± 2.61	13.48 ± 5.85
Comercial-oil	28.27 ± 3.01	30.37 ± 4.79

4. CONCLUSIONS

In this study, the application of supercritical fluid technology for the extraction of tamanu oil from seeds and the chemical composition analysis of the oil were carried out successfully. Simultaneously, the antibacterial and anti-inflammatory activities of tamanu oil extracted by sCO_2 were also compared with those of by Soxhlet method and commercial oil. Accordingly, using the sCO_2 could obtain the higher amount of omega (3, 6, 9) and coumarin than those of the others. In term of the antibacterial activity, the tamanu oil has ability to against some kind of strains as: *S. aureus* ATCC 25923, *S. aureus* ATCC 29213, MRSA ATCC 43300, and *P. aeruginosa* ATCC 277853. Besides, the investigation of anti-inflammatory was also conducted on the mice ears. As the result, all of the tests demonstrated that the product oil extracted by sCO_2 was the most biological effect in comparison with the other methods. In conclusion, the supercritical fluid technology is the advance method that could be applied to enhance the yield of extraction of tamanu oil and contribute to the development of pharmaceutical, medical, and cosmetic industries of Vietnam.

Phân tích thành phần và thử nghiệm hoạt tính kháng khuẩn-kháng viêm của dầu hạt mù u được trích ly bằng kỹ thuật lưu chất siêu tới hạn

- **Nguyễn Hữu Hiếu**
- **Trần Thị Minh Thùy**

Trường Đại học Bách Khoa, ĐHQG-HCM

TÓM TẮT

Dầu mù u được sử dụng nhiều trong y học như là một dược liệu. Trong nghiên cứu này, dầu mù u được trích ly từ hạt bằng kỹ thuật lưu chất CO_2 siêu tới hạn (sCO_2) và phương pháp Soxhlet với dung môi n-hexan. Phương pháp pháp phân tích sắc ký khí ghép khối phổ (GC-MS) được sử dụng để xác định thành phần axit béo và hợp chất coumarin trong các sản phẩm dầu trích được. Đồng thời, hoạt tính kháng khuẩn và kháng viêm của dầu được khảo sát thử nghiệm bằng phương pháp khuếch tán dầu trong thạch và trên chuột nhắt trắng-chủng Swiss albino. Kết quả phân tích cho thấy thành phần axit béo (hỗn hợp ba loại omega: 3, 6 và 9) trong dầu mù u trích ly bằng sCO_2 là cao nhất (72,62%) so với dầu trích bằng Soxhlet. Đồng thời, đã định danh được chất có tên là coumaron (2H-1-bezopyran-2-one, C9H6O2) thuộc nhóm coumarin đơn giản và hàm lượng chất này trong dầu trích bằng sCO_2 là cao nhất (169,69 µg/g) gấp 1,79 lần dầu trích bằng Soxhlet và gấp 2,04 lần dầu thị trường. Bên cạnh đó, kết quả thử nghiệm hoạt tính kháng khuẩn cho thấy dầu trích bằng sCO_2 có khả năng kháng khuẩn với bốn chủng phổ biến: Staphylococcus aureus (S. aureus) ATCC 25923, S. aureus ATCC 29213, S. aureus đề kháng methycilin (MRSA) ATCC 43300 và Pseudomonas aeruginosa (P. aeruginosa) ATCC 277853. Kết quả khảo sát hoạt tính kháng viêm của dầu trích bằng sCO_2 mạnh hơn so với dầu trích bằng Soxhlet ở mức độ giảm viêm từ 21,41 – 25,39 % theo độ dày tai chuột và 33,88 – 39,40 % theo khối lượng.

Từ khóa: *Dầu mù u, coumarin, kháng khuẩn, kháng viêm, trích ly, CO_2 siêu tới hạn.*

REFERENCES

[1]. Friday J.B and Dana Okano. *Calophyllum inophyllum* (kamani). Species profiles for Parcific Island Agroforestry, ver.2 (April 2006).

[2]. By James Friday B and Richard Ogoshi. Forestry Producstion and Marketing Profile for Tamanu (*Calophyllum*

inophyllum). Farm and, Specialty crops for Pacific Island Agroforestry (2011).

[3]. Tamanu oil Material safety data sheet, Natural sourcing specialists in Cosmeceutial İngredient.

[4]. Subhash Hathurusingha. Periodic variation in kernel oil content and fatty acid profiles of *Calophyllum inophyllum* L: A medicinal plant in northern Australia. Industrial Crops and Products, 33, 775–778 (2011).

[5]. Fresdreric Laure and et al. Screening of anti-HIV-1 inophyllums by HPLC-ĐA of *Calophyllum inlophyllum* leaf extracts from French Polynesia Islands. Analytica chimica acta, 624, I 45–I53 (2008).

[6]. Dweck A. C and Meadows T. Tamanu (*Calophyllum inophyllum*) the African, Asian, Polynesian and Pacific Panacea. International Journal of Cosmetic Science, 24, 1–8 (2002).

[7]. Venkanna B.K, Venkatamana Reddy C. Biodiesel production and optimization from *Calophyllum inophyllum* linn oil (honne oil)–A three stage method. Bioresourcs Technology, 100, 512–5125 (2009).

[8]. Leitner W. Supercritical carbon dioxide as green reaction for catalysis. Accounts of chemiscal research, 35, 746 (2002).

[9]. Edgar Uquiche, Jose M. del Valle, Jaime Ortiz. Supercritical CO_2 extraction of red pepper (Capsicum annuum L.) oleoresin. J. Food Engineering, 65, 55–66 (2004).

[10]. Andres M. Hurtado, Benavides, Francisco J. Senx-orasns, Elena Ibasnez, Guillermo Reglero. *Counter-current packed column supercritical CO_2 extraction of olive oil, Mass transfer evaluation*. J. of Supercritical Fluids, 28, 29–35 (2004).

[11]. Catchpole O.J, Grey J.B, Noermark K.A. Fractionation of fish oils using supercritical CO_2 and CO_2+ethanol mixture. J. Supercritical Fluids, 19, 25–37 (2000).

[12]. AOCS Official Method Cd. Peroxide value. 8-53 (1997).

[13]. Nguyen Huu Hieu, Huynh Ky Phuong Ha, Trinh Thi Cat Ha, Nguyen Trong Huy. Ứng dụng kĩ thuật lưu chất siêu tới hạn để trích ly dầu mù u. Tạp chí Khoa học và Công nghệ 52 (5B), 441 – 447 (2014).

[14]. Minh Hien Ha, Van Thi Nguyen, Khac Quynh Cu Nguyen, Emily LC Cheah, Paul WS Heng. Antimicrobial activity of Calophyllum inophyllum crude extracts obtained by pressurized liquid extraction. Antimicrobial activity of *Calophyllum inophyllum* crude extraction-Asian Journal of Traditional Medicine, 4 (2009).

[15]. Minh Hien Ha, Van Thi Nguyen, Khac Quynh Cu Nguyen, Emily LC Cheah, Paul WS Heng. Antimicrobial activity of Calophyllum inophyllum crude extracts obtained by pressurized lyquid extraction. Antimicrobial activity of *Calophyllum inophyllum* crude extraction, Asian Journal of Traditional Medicines 4 (2009).

[16]. Saravanna R, Dhachinamoorthi D, Senthilkumar K and Thamizhvanan K. Antimicrobial activity of variuos extracts from various pardts of *Calophyllum inophyllum* L. J. Applyed Pharmaceutical Science 01 (03), 102 – 106 (2011).