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ISOLATION OF TRITERPENES FROM *DISDICHIA MAJOR* AND ESTABLISHMENT OF β-AMYRIN AND LUPEOL AS REFERNCE SUBSTANCES

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Summary

Isolation of Triterpenes from *Dischidia major* and Establishment of β-Amyrin and Lupeol as Reference Substances

In this study, we reported the isolation of three main triterpenes from alcohol extract of *Dischidia major* (DM) including β-amyrin, α-amyrin and lupeol. The structures of isolated compounds were identified by UV, IR, NMR and MS spectrometry. β-Amyrin and lupeol were used for establishing reference compounds according to ISO GUIDE 35:2017 and ISO 13528:2022. The purity of β-amyrin and lupeol was determined as 99.81% and 99.80% on the basis of dry weight by HPLC-PDA, respectively.

Keywords: *Dischidia major, α-Amyrin, β-Amyrin, Lupeol, Reference substance.*

1. Introduction

Dischidia major (Vahl) Merr. (DM) has been traditionally used in Vietnam for the treatment of rheumatism, pain, inflammation, snake bites and liver dysfunction [1]. In Phu Quoc island, the leaves in pitcher shape of this plant are used to make the tincture which becomes the local specialties. The previous studies reported the main constituents of DM are triterpenes including six identified compounds: 3β-taraxeryl *trans*-cinnamate, 3β-taraxeryl acetate, friedelin, 3β-friedelinol, β-amyrin and 11α, 12α-oxidotaraxerol [2]. The alcohol extract of this plant showed good anti-inflammatory effects [3] and an anti-inflammatory cream product from DM has been developed [4]. Therefore, DM is a potential medicinal material and its quality needed to be controlled. This paper presents the extraction and isolation of three main contents from DM namely α-amyrin, β-amyrin and lupeol. β-amyrin and lupeol were proved to have anti-inflammatory properties [5],[6], were obtained with high amount. They are suitable to be selected as marker substances for quality control of DM. The β-amyrin and lupeol extraction procedure is also presented in this paper.

2. Material and methods

2.1. Plant material

Leaves of DM were collected in Phu Quoc island, Kien Giang province in March 2020 and authenticated by Dr. Tran Thi Van Anh (Department of Pharmacognosy, Faculty of Pharmacy, University of Medicine and Pharmacy at Ho Chi Minh city). Voucher specimen of the plant (KG0320) was deposited at the Department of Pharmacognosy, Faculty of Pharmacy, University of Medicine and Pharmacy at Ho Chi Minh city, Vietnam. Plant material was air-dried and ground to powder before further processing.

2.2. General experimental procedures

IR spectrum was recorded by FTIR IRAffinity-1S (Shimadzu, Japan). The NMR spectra were recorded by a Bruker Avance NEO 600 MHz using tetramethylsilane as an internal standard. The electrospray ionization mass spectrometry (ESI-MS) was obtained on a UPLC-ACQUITY Arc QDa system (Waters, USA). Column chromatography was performed with *silica gel* (40-63 μm, HiMedia, India) and RP-18 (75 μm, YMC-Gel, Japan). Thin-layer chromatography (TLC) was carried out on *silica gel* 60 F_{254} plates (Merck, Germanny). Spots were visualized by spraying with the vanillin - sulfuric acid reagent followed by heating to 105°C until maximum color formation. The fractions of RP-18 column were monitored by HPLC.

HPLC-PDA condition: An Alliance 2695 HPLC system (Waters, USA) was employed with a PDA 2998 detector; The Shimadzu C_{18} column (4.6 × 250 mm; 5 μm) was used for separation at 30°C, an injection volume of 10 μl, a detection wavelength of 207 nm. The mobile phase was MeOH 100%.

Chemicals: Acetonitrile, methanol, and formic acid (HPLC grade; purchased from Merck), double distilled water (Stuart A4000D, England) for LC-MS.

2.3. Extraction and isolation

The powder of DM leaves (1.7 kg) was percolated with 96% EtOH at room temperature (extraction ratio of 1:20). The extract was

evaporated under reduced pressure to get 1.5 L concentrated extract. Adding the same volume of water into the crude extract to get the precipitate (127 g); the precipitate (40 g) was then subjected to a *silica gel* flash column chromatography using gradient solvent system of *petroleum ether - ethyl acetate* (10:0 to 7:3, v/v) to obtain 8 fractions (T.1 - T.8). The fraction T6 (3.3 g) and T7 (3.0 g) were combined and then separated by a RP-C_{18} column (1 g x 6 times) eluted with MeOH (100%) to obtain 11 subfractions (T67.1 - T67.11). Fraction T67.7 was recrystallized in methanol to get compound **1** (1.38 g); compound **3** (65.8 mg) was obtained from fraction T67.11. Fraction T67.3 (899 mg) was purified through an RP-18 column with MeOH (100%) to obtain compound **2** (583 mg).

2.4. Thermal analysis

Thermo analytical curves were obtained by a TG-55 and DSC-25 equipment, TA - Instruments, operating under the following conditions: heating rate of 5°C/min in a nitrogen atmosphere with flow of 25 ml/min using an alumina crucible ($-Al_2O_3$) containing approximately 5 - 10 mg of sample at a temperature range of 25 - 500°C.

2.5. Reference standard establishment

Determination content of β-amyrin and lupeol by HPLD-PDA:

+ Test solution: the solution of 1 mg/mL of *β*-amyrin and lupeol in MeOH

+ Impurity testing of the isolated amyrin and lupeol were performed by HPLC with condition: Column RP C_{18}, 5 µm (25 cm x 4.6 mm), PDA detector at 207 nm, Mobile phase: 100% methanol (0-60 min), injection volume: 10 µl, temperature of column: 30°C flowrate: 1.0 mL/min

The method was validated following ICH guideline [7] and AOAC [8] for systematic suitability, specificity, linearity, limit of detection, and limit of quantification, repeatability, intermediate precision, and precision. The content of the isolated compound was determined by a validated HPLC-PDA method and calculated by the percentage of peak areas.

Packing and the batch uniformity evaluation

10 mg of *β*-amyrin and lupeol were packaged in brown glass vials and sealed with Teflon buffer layer. Package procedure processed in Glove-Box equipped with nitrogen 99.99% and relative humidity 10%. To evaluate the batch uniformity during the packaging, the content of compound in 10 vials were determined by validated HPLC-PDA method. The data were evaluated according to ISO Guide 35 [9].

Inter-laboratory evaluation of reference compounds

Three laboratories participated in the quantitation of the *β*-amyrin and lupeol. Six vials for each compound were randomly sampled and quantified in each laboratory. ANOVA test was employed for statistical analysis (n=18)

Lab 1: Department of Standardization and Reference Substances, Ho Chi Minh City Institute of Drug Testing

Lab 2: Department of Cosmetic Testing, Ho Chi Minh City Institute of Drug Testing

Lab 3: Department of Research and Development, Ho Chi Minh City Institute of Drug Testing

Evaluating the quantitative results, determining the declared value and measurement uncertainty were in accordance with ISO 13528:2022 [10].

3. Result and discussions

3.1. Structural elucidation

Compound 1: Colorless needle; mp 190-191°C, UV/MeOH λ_{max} 201.5 nm; IR (KBr) ν_{max} (cm^{-1}) 3271.27 (OH), 2945.30 (CH), 1035.77(>C-O; APCI-MS (*m/z*): 425.15; ^1H (600 MHz) and ^{13}C NMR (150 MHz) data, see Table 1.

Compound **1** was obtained as colorless needles. The APCI-MS of **1** revealed a *quasi*-molecular peak (*m/z* 425.42 $[M-H]^-$) which suggested its molecular weight as 426 corresponding with its molecular formula as $C_{30}H_{50}O$. The ^1H-NMR of **1** showed one signal of an olefinic proton at δ_H 5.18 (1H, t, J = 3.7 Hz), one carbinolic carbon at δ_H 3.22 (1H, dd, J = 4.7, 11.0 Hz) and δ_H seven singlet signals of eight methyl group at δ_H 0.79, 0.83, 0.87 (two signals), 0.94, 0.97, 1.00, 1.14. The ^{13}C-NMR spectra displayed 30 signals with two signals of olefinic carbons at δ_H 121.7 and 145.2. The NMR data suggested that 1 was an oleanane-type triterpene and was identified as *β*-amyrin, The NMR data of **1** agreed with the published data [6].

Compound 2: Colorless needle, mp 214 - 215°C UV/MeOH λ_{max} 200.5 nm; IR (KBr) ν_{max} (cm^{-1}) 3267.41 (OH), 2945.30 (CH), 1037.70 (>C-O); APCI-MS (*m/z*): 425.42; ^1H (600 MHz) and ^{13}C NMR (150 MHz) data, see Table 1.

Compound **2** was obtained as colorless needles. The APCI-MS of **2** revealed a *quasi*-molecular peak at (*m/z* 425.47 $[M-H]^-$) which suggested the molecular formula of **2** as $C_{30}H_{50}O$. The ^1H-NMR spectrum of **2** showed signals of seven methyl singlets at δ_H 1.68, 1.03 0.97, 0.94, 0.83, 0.79 x 2,

two olefinic methylene protons at δ_H 4.57 (1H, dd J =2.5, 1.5 Hz) and 4.69 (1H, d, J = 2.0 Hz). The ^{13}C-NMR spectra showed 30 carbon signals including 2 olefinic carbons at δ_C 150.9 and 109.3, an oxymethine group at δ_C 79.0 and seven methyl groups. The HMBC showed the correlation of the olefinic protons at δ_H 4.57 and 4.69 with the methyl group at δ_C 19.3 and a methine carbon at δ_C 48.0. The NMR data identified **2** as lupeol by comparison with the published data [11].

Compound 3: White amorphous powder, UV/MeOH λ_{max} 202.00 nm; IR (KBr) ν_{max} (cm^{-1}) 3352.28 (OH); 2941.44 (CH); 1637.56 (C=C); 1043.49 (>C-O); APCI-MS (*m/z*): 425.47 ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) data, see Table 1.

Compound **3** was obtained as a white amorphous power. The APCI-MS of **3** revealed a *quasi*-molecular peak at (*m/z* 425. 42 [M-H]$^-$) which suggested the molecular formula of **2** as $C_{30}H_{50}O$ The ^{1}H-NMR spectrum of compound **3** resembled the spectrum of compound **1** with 8 methyl group except there was two doublet signal of methyl groups at δ_H 0.78 (d, J = 6.0 Hz, 3H) and 0.92 (d, J = 6.0 Hz, 3H). The ^{13}C-NMR spectra also showed 30 carbon signals with two olefinic carbons at δ_C 124.4 and 139.6. The characteristics of NMR data suggested compound **3** belonged to the ursane type triterpene. Analysis NMR data and comparison with the published spectral data [6], compound **3** identified as α-amyrin.

Table 1. ^{1}H (600 MHz) and 13C-NMR (150 MHz) spectroscopic data of compounds **1-3**

C	Compound 1 ($CDCl_3$)		Compound 2 ($CDCl_3$)		Compound 3 ($CDCl_3$)	
	^{13}C	^{1}H	^{13}C	^{1}H	13C	1H
1	38.8		38.7		38.7	
2	27.2		27.4		28.1	
3	79.0	3.22 dd (11.0, 4.7), 1H	79.0	3.19 dd (11.5, 4.8) 1H	79.0	3.23 dd (11.2, 5.0) 1H
4	38.6		38.8		38.8	
5	55.2		55.3		55.2	
6	18.4		18.3		18.3	
7	32.5		34.3		32.9	
8	39.8		40.8		40.0	
9	47.6		50.4		47.7	
10	36.9		37.1		36.9	
11	23.5		20.9		23.3	
12	121.7	5.18 t (3.7), 1H	25.1		124.4	5.13 t (3.6), 1H
13	145.2		38.0		139.6	
14	41.7		42.8		42.1	
15	26.9		27.4		27.3	
16	26.2		35.6		26.6	
17	32.7		43.0		33.7	
18	47.2		48.3		59.1	
19	46.8		48.0		39.7	
20	31.1		150.9		39.6	
21	34.7		29.8		31.2	
22	37.1		40.0		41.5	
23	28.1	1.00 s, 3H	28.0	0.97 s, 3H	28.1	1.00 s, 3H
24	15.6	0.79 s, 3H	15.3	0.79 s, 3H	15.7	0.80 s, 3H
25	15.5	0.94 s, 3H	16.0	0.83 s, 3H	15.6	0.96 s, 3H
26	16.8	0.97 s, 3H	16.1	1.03 s, 3H	16.8	1.01 s, 3H
27	26.0	1.14 s, 3H	14.5	0.94 s, 3H	23.2	1.07 s, 3H
28	28.4	0.83 s, 3H	18.0	0.79 s, 3H	28.8	0.79 s, 3H
29	33.3	0.87 s, 3H	109.3	4.69 d (2.4), 1H 4.57 dd (2.5, 1.5), 1H	17.4	0.78 d (6.0), 3H
30	23.7	0.87 s, 3H	19.3	1.68 s, 3H	21.3	0.92 d (6.0), 3H

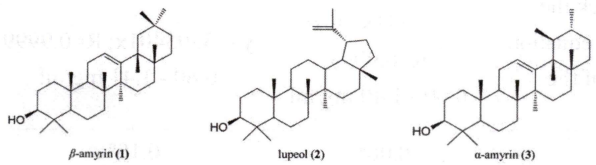

Fig.1. Chemical structures of isolated compounds **1-3**

β-amyrin were previously isolated from DM but lupeol and α-amyrin were identified from *D.major* for the first time. α-amyrin and β-amyrin were usually found as mixture in various plants, notably plant resin of *Bursera* or *Protium* species of the Burseraceae family [6]. These compounds have been shown to have various pharmacological properties such as anti-microbial, anti-fungal, anti-inflammation, analgesic activity, gastroprotective and hepatoprotective effects, antihyperglycemic

and hypolipidemic effects [6] [12]. Lupeol was considered as an anti-inflammatory and anti-cancer dietary triterpene with many mechanisms of action proved [13]. Therefore, *D.major* with high content of α-amyrin, β-amyrin and lupeol could be considered as the pharmaceutical materials. Standard references are necessary in quality control of herbal medicine. With the result of isolation, we chose β-amyrin and lupeol to establish the reference compounds for further development of quantification triterpenoid in *D.major*.

3.2. Thermal analysis

The β-amyrin weight loss was initiated at 178.19°C, ended at 188.77°C and 1.612% of the first loss was observed (closed to its melting point 189 - 191°C in the ref. [6]). The second loss continued until the temperature of 288.43°C (Fig.2A). Its DSC curve appeared only one sharp valley from 194.19°C to 198.08°C and have the decreased tendency until more than 300°C (Fig.2B). This could be explained by its impurities such as α-amyrin which have melting point at 184 - 186°C [6] and others.

The TGA curve of lupeol (Fig.2C) has two mass loss steps, from 69.32°C to 84.02°C and from 249.49°C to 288.02°C, while the DSC curve (Fig.2D) has 2 peaks, from 69.67°C to approximately 84°C and from 215.35°C to 216.66°C. The first loss on TGA result is consistent with the boiling point of methanol (64.7°C), which is the solvent used for crystallization of lupeol. These curves show that the compound is stable up to 249.49°C, and then is decomposed by high temperature. The second peak in the DSC curve is totally suitable with the melting point of lupeol in ref. [13]. Thus, the dry loss of the lupeol is 1.98%.

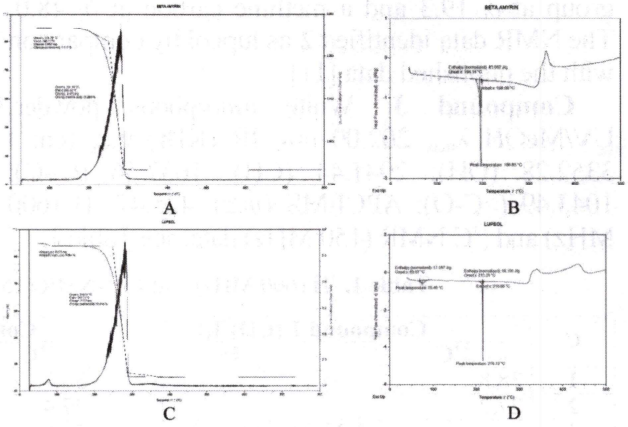

Fig.2. The result of TGA and DSC of β-amyrin (A and B, respectively) and lupeol (C and D, respectively)

3.2. Establishment of reference standard

The results of method validation for the quantification of β-amyrin and lupeol were satisfactory in accordance with the ICH [7] and AOAC guideline [8] (Table 2). After being validated, the HPLC-PDA method was applied to determine the β-amyrin and lupeol content by calculating the percentage of the peak area.

Table 2. Result of method validation for β-amyrin and lupeol quantitative analyses

Criterion	Requirement	Result	
		β-amyrin	**lupeol**
System compatibility	Apparent theoretical disk number (≥ 2000)	10807	10281
	Tail drag coefficient (T) is in the range of 0.8-2.0	1.01	1.03
	The RSD of the retention time and the peak area ≤ 2%	0.54	1.20
Specificity	Blank sample, sample degraded in acid, alkali, and peroxide do not affect the analysis results of the test sample	Complied (Fig.3)	Complied (Fig.4)
Linearity range	Use regression analysis with t-test to check the significance of coefficients in the regression equation and F-test to check the appropriateness of the regression equation. R > 0.98.	$\hat{y}$ = 2538190x; R=0.9993 0.60 - 1.40 mg/ml	$\hat{y}$ = 3395401x; R=0.9999 0.60 - 1.41 mg/ml
Precision	Repeatability (n=6) RSD ≤ 2%.	0.06%	0.10%
	Intermediate Precision RSD (n= 12) ≤ 2%	0,10%	0.70%
Accuracy	The recovery range in 3 levels 80, 100, 120% is 98 - 102%. RSD ≤ 2%.	100.57% %RSD 1.30%	99.95% %RSD 1.07%

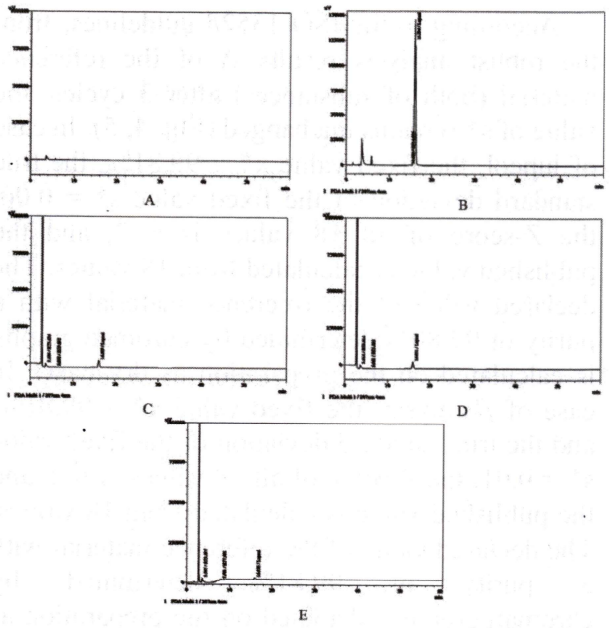

Fig.3. The chromatogram obtained in the specificity test of the purity determination
(A): Blank; (B): Lupeol; (C): Sample in 0.5N HCl; (D): Sample in 0.5N NaOH; (E): Sample in 30% H_2O_2.

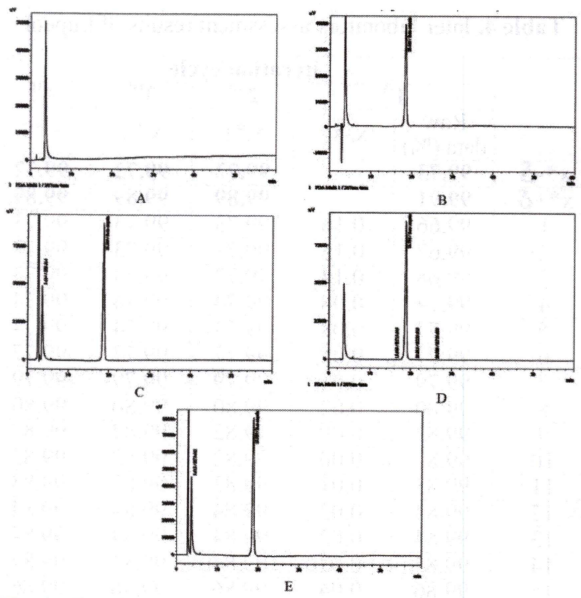

Fig.4. The chromatogram obtained in the specificity test of the purity determination (A): Blank; (B): β-amyrin; (C): Sample in 0.5N HCl; (D): Sample in 0.5N NaOH; (E): Sample in 30% H_2O_2.

Evaluation of materials for establishment of reference standard

Table 3. Result of Evaluation of materials for establishment of reference standard

Criterion		Requirement		Result	
		Lupeol	**β-amyrin**	**Lupeol**	**β-amyrin**
Identification	Meltpoint	215 - 216°C	189 - 191°C	Confirmed (251 - 216°C)	Confirmed (190 - 191°C)
	IR	3235 and 1640	3360 and 1650	3352 and 1637	3271 and 1463
	UV-Vis	λ_{max}=205 ± 2 nm	λ_{max}=205 ± 2 nm	Confirmed (205 nm)	Confirmed (201,5 nm)
	MS	475.24	475.26	Complied	Complied
	NMR	corresponding to ref. [11]	corresponding to ref. [6]	Complied	Complied
Loss of drying (TGA)		≤ 5.00%	≤ 5.00%	1.98%	0%
Purity	TLC	Appear only one dot on the chromatography of 3 mobile phase systems	Appear only one dot on the chromatography of 3 mobile phase systems	Complied	Complied
	HPLC (as dry basis)	% peak area >95% Purity angle> Purity Threshold	% peak area >95% Purity angle> Purity Threshold	Complied (99.8%)	Complied (99.8%)

Packaging and evaluation of the batch homogeneity

1.3 g of β-amyrin was packed in 120 vials and 583 mg of lupeol were packed in 50 vials (10 mg/vial). The results of system compatibility determination of purity by UPLC-QDA in 3 laboratories were satisfactory with RSD% (n = 6) of all chromatography parameters are less than 2.00% (resolution ≥ 3.0, theoretical plates ≥ 2000, 0.8 ≤ peak symmetry ≥ 2.0). The relative repeatability of β-amyrin and lupeol purity between the vials during the packaging process was less than 1.00% (the average purity value (n=18) was 99.81% (RSD = 0.0113) and 99.79% (RSD = 0,0711), respectively. Thus, the packaging process is stable and consistent.

Interlaboratory vial homogeneity assessment

The content uniformity among the vials (n = 18) in a production batch has an RSD < 1.0%. Therefore, according to the ASEAN regulations [14], the finished vials in the production batch have uniform content for both lupeol and β-amyrin. From this, it can be concluded that the packaging conditions are stable and suitable (Table 4, 5)

Determined of assigned value

Based on the analysis results from 3 participating laboratories, statistical analysis was carried out according to ISO 13528.

Table 4. Inter-laboratory assessment results of Lupeol

	iteration/cycle				
	1st		2nd	3rd	4th
	Raw data (%)	x_i-x*	x_i*1	x_i*2	x_i*3
x*-δ	**99.73**		**99.72**	**99.72**	**99.72**
x*+δ	**99.91**		**99.89**	**99.89**	**99.89**
1	99.66	0.16	99.73	99.73	99.73
2	99.67	0.15	99.73	99.73	99.73
3	99.68	0.14	99.73	99.73	99.73
4	99.74	0.08	99.74	99.74	99.74
5	99.74	0.08	99.74	99.74	99.74
6	99.77	0.05	99.77	99.77	99.77
7	99.79	0.03	99.79	99.79	99.79
8	99.80	0.02	99.80	99.80	99.80
9	99.82	0.00	99.82	99.82	99.82
10	99.82	0.00	99.82	99.82	99.82
11	99.83	0.01	99.83	99.83	99.83
12	99.84	0.02	99.84	99.84	99.84
13	99.84	0.02	99.84	99.84	99.84
14	99.84	0.02	99.84	99.84	99.84
15	99.86	0.04	99.86	99.86	99.86
16	99.86	0.04	99.86	99.86	99.86
17	99.87	0.05	99.87	99.87	99.87
18	99.88	0.06	99.88	99.88	99.88
x*	**99.82**		**99.81**	**99.81**	**99.81**
s*	**0.06**		**0.06**	**0.06**	**0.06**
δ	**0.09**		**0.09**	**0.09**	**0.09**

Table 5. Inter-laboratory assessment results of β-amyrin

	iteration/cycle				
	1st		2nd	3rd	4th
	Raw data (%)	x_i-x*	x_i*1	x_i*2	x_i*3
x*-δ	**99.79**		**99.79**	**99.79**	**99.79**
x*+δ	**99.83**		**99.83**	**99.83**	**99.83**
1	99.79	0.02	99.79	99.79	99.79
2	99.80	0.01	99.80	99.80	99.80
3	99.80	0.01	99.80	99.80	99.80
4	99.80	0.01	99.80	99.80	99.80
5	99.80	0.01	99.80	99.80	99.80
6	99.80	0.01	99.80	99.80	99.80
7	99.80	0.01	99.80	99.80	99.80
8	99.81	0.00	99.81	99.81	99.81
9	99.81	0.00	99.81	99.81	99.81
10	99.81	0.00	99.81	99.81	99.81
11	99.82	0.01	99.82	99.82	99.82
12	99.82	0.01	99.82	99.82	99.82
13	99.82	0.01	99.82	99.82	99.82
14	99.82	0.01	99.82	99.82	99.82
15	99.82	0.01	99.82	99.82	99.82
16	99.82	0.01	99.82	99.82	99.82
17	99.83	0.02	99.83	99.83	99.83
18	99.83	0.02	99.83	99.83	99.83
x*	**99.81**		**99.81**	**99.81**	**99.81**
s*	**0.01**		**0.01**	**0.01**	**0.01**
δ	**0.02**		**0.02**	**0.02**	**0.02**

According to the ISO 13528 guidelines, from the robust analysis results A of the reference material (both of substances) after 3 cycles, the value of s* remains unchanged (Fig. 4, 5). In case of lupeol, the fixed value x* = 99.81%, the true standard deviation of the fixed value s* = 0.06, the Z-score of all 18 values is ≤ 2, and the published value is calculated from 18 values. The declared value of the reference material with a purity of 99.80% determined by chromatography is calculated on the preparation as dry basis. In case of β-amyrin, the fixed value x* = 99.81%, and the true standard deviation of the fixed value s* = 0.01, the Z-score of all 18 values is ≤ 2, and the published value is calculated from 18 values. The declared value of the reference material with a purity of 99.81% determined by chromatography calculated on the preparation as dry basis. The measurement uncertainty of lupeol and β-amyrin are 0.017 and 0.003, respectively, calculated by the formula $\mu = (1.25 \times s^*) / \sqrt{n}$.

Therefore, the products meet the requirements for registering as a national standard with a determined content of 99.80% lupeol and 99.81% β-amyrin calculated on the preparation as dry basis, the dry loss of lupeol of 1.98%. Proceed to make a standard substance profile, label the standard vial, store at 0-8°C, protected from light.

5. Conclusion

Dischidia major is a potential medicinal material with high content of triterpenes which have anti-inflammatory properties. In this phytochemical study, α-amyrin and lupeol were identified in DM for the first time. The isolation procedure obtained the high amount of β-amyrin and lupeol. Then, two compounds were used to establish the reference substances for DM. The reference substances establishment for the isolated β-amyrin and lupeol was conducted according to ISO Guide 35. 120 vials of β-amyrin RS (10 mg) (batch No.01012022) and 50 vials of lupeol (10 mg) (batch No.03012022) were produced with the purity 99.81% and 99.80%, respectively. β-amyrin and lupeol reference substances could be used for the quality control of DM and its products.

References

1. Do H. B., Dang Q. C., Bui X. C., Nguyen T. D., Do T. D., Pham V. H., Vu N. L., Pham D. M., Pham K. M., Doan T. N., Nguyen T., Tran T. (2006), *Medicinnal Plants and Animals in Vietnam*, Science and Technology Publisher, Vol II, 989-990. **2.** Tran D. H., Tran T. V. A. (2019), Triterpenes from *Dischidia major* (Vahl) Merr., *Journal of Medicinal Materials*, 24(5), 259-264. **3.** Doan T. H. D., Nguyen B. T., Pham T. H., Tran T. V. A., Do T. H. T. (2017), Study on oral acute toxicity, anti-

inflammatory and analgesic effects of extracts and traditional wine from *Dischidia major*, *Journal of Medicine Ho Chi Minh city*, 21(6), 93-100. **4.** Nguyen T. D., Nguyen B. T., Do T. H. T., Pham D. D. (2017), Study on gel emulsion formulation from the extract of *Dischidia rafflesiana* with anti-inflammatory effect, *Journal of Medicine Ho Chi Minh city*, 21(6), 203-211. **5.** Geetha T., Varalakshmi P. (2001), Anti-inflammatory activity of lupeol and lupeol linoleate in rats, *Journal of Ethnopharmacology*, 76(1), 77-80. **6.** Hernández Vázquez L., Palazón Barandela J., Navarro Ocaña A. (2012), The pentacylic triterpenes α, β-amyrin: A review of sources and biological activities, *Phytochemicals: A Global Perspective of Their Role in Nutrition and Health*, 23, 487-502. **7.** International Conference on Harmonisation of Technica Requirements for Registration of Pharmaceuticals for Human Use (ICH) (2022), *Validation of analitical procedures: Text and methodology* Q2(R1), 1-17. **8.** Association of Official Analytical Chemists (2023), Appendix K: Guidelines for Dietary Supplements and Botanicals, in *Offical Methods of Analysis of AOAC international (22)*, AK1-AK32. **9.** International Organization for Standardization (2017), ISO Guide 35:2017 Reference materials — Guidance for characterization and assessment of homogeneity and stability. **10.** International Organization for Standardization (2022), ISO 13528:2022 Statistical methods for use in proficiency testing by interlaboratory comparison, 14-31. **11.** Pereira Beserra F., Xue M., Maia G. L. A., Leite Rozza A., Helena Pellizzon C., Jackson C. J. (2018), Lupeol, a pentacyclic triterpene, promotes migration, wound closure, and contractile effect *in vitro*: possible onvolvement of PI3K/Akt and p38/ERK/MAPK Pathways, *Molecules*, PMID: 30380745. **12.** Nogueira, A. O., Oliveira Y. I. S., Adjafre B. L., De Moraes M. E. A. and Aragão G. F. (2019), Pharmacological effects of the isomeric mixture of alpha and beta amyrin from *Protium heptaphyllum*: a literature review. *Fundamental and Clinical Pharmacology*, 33, 4-12. **13.** Saleem M. (2009), Lupeol, a novel anti-inflammatory and anti-cancer dietary triterpene, *Cancer Letter*, PMID: 19464787. **14.** Committee on Asean Reference Substances (2005), Guidelines for the establishment, handling, storage and use of Asean Reference Substances, 3-12.

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CHEMICAL COMPOSITION, ANTI-MICROBIAL, ANTI-INFLAMMATORY, AND CYTOTOXIC ACTIVITIES OF ESSENTIAL OILS FROM *MELICOPE PTELEIFOLIA* (CHAMP. EX BENTH.) T.G.HARTLEY LEAVES AND STEMS COLLECTED IN LAM DONG PROVINCE

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Summary

Chemical Composition, Anti-Microbial, Anti-Inflammatory, and Cytotoxic Activities of Essential Oils from *Melicope pteleifolia* (Champ. ex Benth.) T.G.Hartley Leaves and Stems Collected in Lam Dong Province

The essential oils extracted by hydrodistillation from the leaves and stems of *Melicope pteleifolia* (MP) collected in Bidoup-Nui Ba National Park, Lam Dong province, Vietnam were investigated for their chemical composition and bioactivity. 29 compounds were identified in the leaf essential oil (MPL), of which *trans*-α-bergamotene (41.1%), *trans*-γ-bisabolene (13.0%), linalool (7.6%), and β-atlantol (7.3%) were the most abundant. The stem oil (MPS) contained 21 identified constituents, mainly *trans*-α-bergamotene (63.7%), limonene (7.4%), and linalool (7.1%). MPL exhibited inhibitory effects on *S. aureus, B. subtilis,* and *L. fermentum*, while MPS significantly inhibited the growth of *S. aureus, B. subtilis, E. coli,* and *C. albicans* and showed minimal inhibition against three other bacteria. MPL inhibited NO production in macrophage RAW 264.7 cells (IC_{50} 19.86 ± 0.16 μg/mL). Both MPL and MPS showed moderate cytotoxic effects on SK-LU-1, MCF-7, and HepG2 cell lines with the IC_{50} values of 20.80 ± 1.42, 25.90 ± 1.47, and 23.42 ± 1.78 μg/mL (for MPL) and 25.36 ± 2.41, 46.46 ± 3.13, and 47.41 ± 2.91 μg/mL (for MPS), respectively. Their antifungal, anti-inflammatory, and cytotoxic activities were evaluated and reported for the first time.

Keywords: *Melicope pteleifolia, Essential oil, GC-MS, Anti-microbial activity, NO inhibition, Cytotoxicity.*

1. Introduction

Melicope pteleifolia (Champ. ex Benth.) T.G. Hartley (MP), synonym *Euodia lepta* (Spreng.) Merr. belongs to the family Rutaceae and is mainly found in tropical areas of Asia such as South China and Southeast Asia countries including Cambodia, Laos, Myanmar, Thailand, and Vietnam [1]. It is a perennial herbaceous shrub or tree, and parts of the plant have traditionally been used to treat rheumatism, sore throat, dermatitis, eczema, and insect and snake bites [1]. Compounds that have been isolated from MP include chromenes, dichromenes, flavonoids, coumarins, and alkaloids [2],[4]. Previous studies indicated that MP possessed antioxidant, anti-inflammatory, hepatoprotective, analgesic, antimicrobial, and anti-tumor effects [1]. The essential oil of its plant parts collected from China, Vietnam (Nghe An, Ba Ria - Vung Tau, Da Nang provinces) has been investigated for its chemical composition, antibacterial, and insecticidal activities [5],[6],[7],[8],[9]. This