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ANTI-AGEING ACTIVITIES OF THAI HIGHLAND *PETROSELINUM CRISPUM* AND *ROSMARINUS OFFICINALIS* OILS

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Natnaree Laothaweerungsawat[1], Wantida Chaiyana[2]

[1]Research Scholar, Master's Degree Program in Cosmetic Science, Department of Pharmaceutical Science, Faculty of Pharmacy, Chiang Mai University, Chiang Mai 50200, Thailand

[2]Associate Professor, Department of Pharmaceutical Science, Faculty of Pharmacy,Chiang Mai University, Chiang Mai 50200, Thailand

Address for Correspondence: serviceheb@gmail.com

ABSTRACT:

The purposes of this study were to investigate the chemical compositions, antioxidant, and anti-hyaluronidase activities of highland parsley and rosemary essential oils. Highland parsley and rosemary oils, obtained from Thai highland area, were extracted by hydro-distillation. Then their chemical compositions were evaluated using gas chromatography-mass spectrometry (GC-MS) and compared with the commercial oils. Antioxidant activity was determined by lipid peroxidation inhibition using ferric thiocyanate assay, whereas, anti-hyaluronidase, was determined by in vitro spectrophotometric methods. Highland rosemary essential oil had higher yield (0.47%v/w) than that of parsley (0.03%v/w). The physical appearances of the highland and commercial oils were exactly similar in both parsley and rosemary oils. All essential oils were yellowish liquid with the unique odor that can easily vapour at the ambient temperature. Additionally, both relative density and refractive index of highland essential oils were comparable to that of commercial oils. GC-MS analysis revealed that the major chemical constituents of highland and commercial oils were similar in both parsley and rosemary. L-camphor was noted as a major component of rosemary essential oils (24.6% and 12.1% in highland and commercial oil, respectively), whereas, myristicin was a major component of parsley essential oils (60.9% and 23.7% in highland and commercial oil, respectively). Highland parsley oil possessed significantly higher inhibition against lipid peroxidation reaction comparing to highland rosemary oil with inhibition of 68.5±11.62% and 26.2±23.7%, respectively. The anti-hyaluronidase activity of highland parsley oil was also higher than that of rosemary oil. Therefore, it could be concluded that highland parsley oil had a potential for using as active compound in anti-ageing cosmetic products.

INTRODUCTION

Both intrinsic and extrinsic factors can cause skin ageing [1]. Hyaluronic acid or hyaluronan, the polysaccharide molecule that can hold water over one thousand times of its dried weight, possess

variety functions to the skin, e.g. instance reduction of fine lines and wrinkles, soften and smoothen the skin surface, reconstruct the connective tissues, etc. Degradation of hyaluronic acid due to the passing time by hyaluronidase has been known as intrinsic factors that lead to skin ageing. Moreover, free radicals are another major cause of skin wrinkle since they set off the chain reactions that lead to the oxidation process which could destroy the cell membrane and cell functions. Therefore, prohibition of the oxidation process or lipid peroxidation can slow down the aging process. In the cosmetic market, there are several compounds used in the cosmetic products as antioxidant and anti-ageing agents. However, most of them are chemicals which could lead to skin reaction, irritation, or other adverse events. Hence, natural compounds are recently popular due to their lower side effects. Essential oils, which are natural oils obtained from aromatic plants, has been reported for various health benefits. However, the anti-ageing activity of the essential oil, especially from highland area of Thailand, has not been reported before. Therefore, this study purposed to extract essential oil from highland aromatic plants in Thailand and investigated their chemical compositions. Additionally, the inhibitory activities against lipid peroxidation and hyaluronidase of essential oils from highland parsley and rosemary were also investigated in a comparison with the commercial oils.

MATERIALS AND METHODS

1) Plant materials

Rosmarinus officinalis was cultivated by the Royal Project Foundation, Thailand in highland area of Mae Chaem district, Chiang Mai, Thailand, whereas, *Petroselinum crispum* was cultivated by the Royal Project Foundation, Thailand in highland area of Chom Thong district, Chiang Mai, Thailand. The plant materials of *R. officinalis* and *P. crispum* obtained during January 2018.

2) Chemical materials

Commercial *R. officinalis* essential oil (CRO) was purchased from United Chemical & Trading Co., Ltd., (Chiang Mai, Thailand), whereas, commercial *P. crispum* essential oil (CPO) was purchased from Plant Guru, (New Jersey, USA). Hyaluronidase from bovine testes, sodium chloride (NaCl), sodium phosphate monobasic dihydrate (H2NaO4P.2H2O), sodium phosphate dibasic dihydrate (HNa2O4P.2H2O), and linoleic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). Alpha-ascorbic acid was purchased from Asia Pacific Specialty Chemicals Limited (New South Wales, Australia). Anhydrous sodium sulfate (Na2SO4), ferrous sulphate heptahydrate (FeSO4.7H2O), and ammonium thiocyanate (NH4SCN) were purchased from Loba Chemie (Boisar, Tarapur, India). Bovine serum albumin was purchased from Merck (Darmstadt, Germany). Sodium acetate trihydrate (CH3COONa.3H2O), sodium hydroxide (NaOH), dimethyl sulfoxide (DMSO), dichloromethane, methanol, and ethanol were analytical grade purchased from RCI Labscan Co., Ltd. (Bangkok, Thailand).

3) Extraction of essential oils by hydrodistillation

Plant materials of R. *officinalis* and *P. crispum* were cut into small pieces and subjected to the hydrodistillation for the essential oil extraction for 2 h. After that the essential oils from highland *R. officinalis* (HRO) and highland *P. crispum* (HPO) were then kept in a glass bottle after their temperature was cooling back to the ambient temperature. The water was then removed using anhydrous sodium sulfate. Yield of each essential oil was calculated by using the following equation; %Yield = (a/b) × 100, where a is a volume of the essential oil and b is the dry weight of plant materials used in the hydrodistillation. The essential oil was stored at 4ºC in the light protecting container until further use.

4) Characterization of essential oils

The characteristics of both highland and commercial essential oils, including physical appearance, color, and scent were investigated by organoleptic inspections. Furthermore, relative density and refractive index of each oils were also determined using pycnometer and refractometer (Altago Co., Ltd., Tokyo Japan), respectively.

5) Chemical compositions determination of essential oils

The chemical components of each essential oils were investigated by gas chromatography-mass spectrometry (GC-MS) using fused-silica HP-5 ms and hydrogen gas was used as the mobile phase. The flow rate of mobile phase was set at 1 ml/min, injection temperature set at 260°C, and column temperature set at 100-280°C. The temperature was held at 100°C for 3 min. Then, the mobile phase was flown at the rate of 3°C/min until 280°C and held again for 3 min. The chromatogram was used to identify the chemical compositions of each essential oil by using the differential of the retention time compared with Wiley, NIST and NBS libraries.

6) Determination of lipid peroxidation inhibition by ferric thiocyanate (FTC) assay

Essential oils were evaluated for the inhibition against lipid peroxidation by using FTC assay [2]. The occurrence of ferric thiocyanate complex was detected at 500 nm using a multimode detector (BMG Labtech, Offenburg, Germany). The experiments were performed in triplicate. The inhibition of lipid peroxidation was calculated using the following equation; % Inhibition = [1 – (a / b)] × 100, where a is the absorbance of the mixtures of the sample, linoleic acid, NH4SCN, and FeCl2 solution, whereas, b is the absorbance of the mixtures of linoleic acid, NH4SCN, and FeCl2 solution. Ascorbic acid was used as a positive control.

7) Determination of hyaluronidase inhibitory activity by spectrophotometric method

The inhibition against hyaluronidase of each essential oils was determined by spectrophotometric method [3]. The absorbance of the final mixture was measured at 600 nm using a multimode detector

(BMG Labtech, Offenburg, Germany). All experiments were performed in triplicate. The inhibition of hyaluronidase was calculated using the following equation; % Inhibition = [1 - (a/b)] × 100, where a is the absorbance of the mixtures containing sample, hyaluronidase, hyaluronic acid and the bovine serum albumin solution, whereas, b is the absorbance of the mixtures containing hyaluronidase, hyaluronic acid, and the bovine serum albumin solution. Ascorbic acid was used as a positive control.

8) Statistical analysis

The statistical data, mean ± standard deviation (SD) were analyzed by the Student t-test using SPSS 17.0 for Windows (SPSS Inc., Chicago, IL, USA). The probability values of $*P < 0.05$ were considered significant.

EXPERIMENT RESULT

1) Yield and appearances of essential oils

HRO and HPO are yellowish liquid with characteristic odor. The external appearance of HRO was exactly the same as CRO (**Fig. 1A**). Similarly, the external appearance of HPO and CPO were exactly the same (**Fig. 1B).** HRO had the higher yield than that of HCO which were 0.47±0.02%v/w and 0.03±0.01, respectively. The relative density and refractive index of each oils are shown in **Table I**. The outcomes implied that essential oils extracted from highland area of Thailand had the same external appearance and the same quality as the commercial oils imported from foreign countries.

(A)

(B)

Fig. 1: Physical appearance of plant material (left figure), distillated essential oil from highland plants (middle figure), and commercial oil (right figure)

of R. *officinalis* (A) and *P. crispum* (B)

Table I. Relative density (RD) and refractive index (RI) of essential oils

Sample	Source	RD (g/ml)	RI
Parsley	Highland Commercial	1.042 0.978	1.523 1.507
Rosemary	Highland Commercial	0.847 0.921	1.691 1.465

2) Chemical components of essential oils

The GC chromatograms of essential oils are shown in Fig**. 2** and **Fig.3.** L-camphor was noted as the major component of HRO and CRO with the amount of 24.6% and 12.1%, respectively. On the other hand, myristicin was noted as the major component of HPO and CPO with the amount of 60.9% and 23.7%, respectively. These findings were similar with the previous studies reported that myristicin was the major compound in parsley essential oil extracted by hydrodistillation [4].

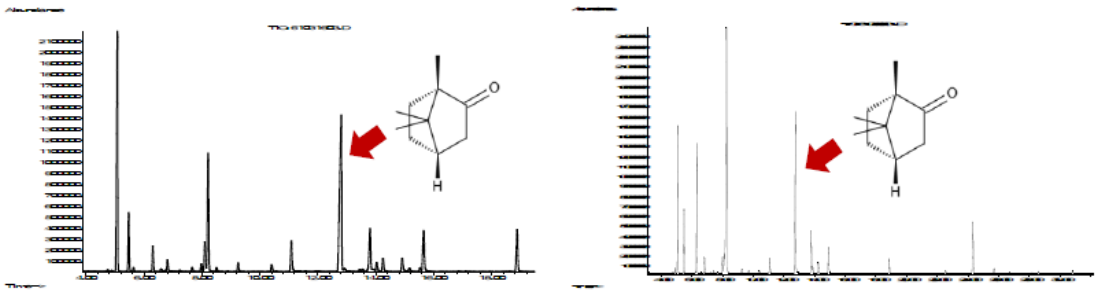

Fig. 2: GC chromatograms of HRO (A) and CRO (B)

Red arrow denoted the peak of L-camphor

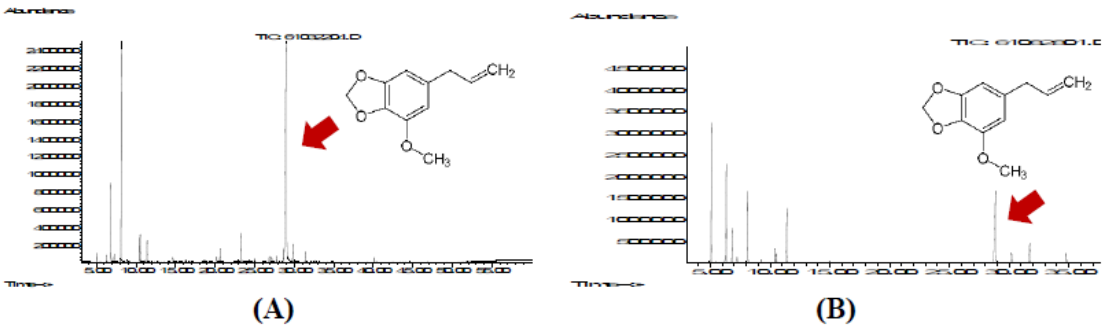

Fig. 3: GC chromatograms of HPO (A) and CPO (B)

Red arrow denoted the peak of myristicin

3) Lipid peroxidation inhibitory activities of essential oils

T h e lipid peroxidation inhibitory activities o f each essential oils are shown in **Table II.** The results noted that highland essential oils possessed comparable lipid peroxidation inhibitory activities to the commercial oils in both *R. officinalis* and *P. crispum.* On the other hand, *P. crispum* essential oils possessed significantly higher lipid peroxidation inhibitory activities than that of *R. officinalis.* The likely explanations might be due to myristicin which was the major component of *P. crispum* essential oil. Since it has been reported that myristicin from the organic extraction possessed high lipid peroxidation inhibition [5].

Table II. Lipid peroxidation inhibitory activities of essential oils and ascorbic acid

Sample	Source	% Lipid peroxidation inhibition
Parsley	Highland	68.5±11.6
	Commercial	68.0±4.6
Rosemary	Highland	26.2±23.7
	Commercial	20.1±17.1
Ascorbic acid		IC50 = 0.29 mg/mL

4) Antihyaluronidase activities of essential oils

The inhibitory activity against hyaluronidase of each essential oils are shown in **Fig. 4.** Ascorbic acid was used as a positive control since it has been reported to inhibit hyaluronidase [6]. In the present study, ascorbic acid possessed low antihyaluronidase activity (12.8±0.7%) at the final concentration of 4 µg/ mL. Surprisingly, HPO possessed comparable anti hyaluronidase activity to ascorbic acid with the inhibition of 11. 2±0. 7% . Additionally, HPO possessed significantly higher antihyaluronidase activity than CPO ($P < 0.05$). Therefore, the essential oil from highland area of Thailand had more beneficial for using in ageing skin than the commercial oil. Since hyaluronic acid is known as natural moisturizing factor in the skin that could keep the skin youthful by increasing the levels of skin moisture. However, hyaluronic acid is degraded by hyaluronidase which would lead to lower amount of hyaluronic acid and also lower amount of skin moisture. The skin wrinkles are then

observed due to the lower levels of skin hydration. Therefore, inhibition of hyaluronidase would slow down the above process and keep the skin look younger [7].

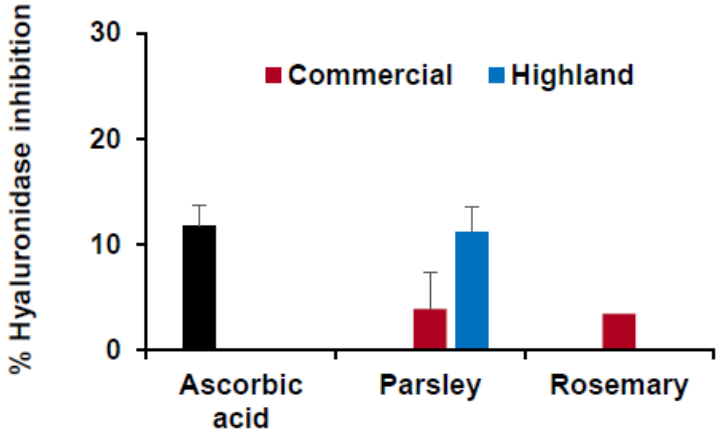

Fig. 4: Hyaluronidase inhibitory activity of highland essential oils and ascorbic acid

CONCLUSION

Essential oils from *R. officinalis* and *P. crispum* cultivated in highland area of Thailand had the same external appearance and quality as the commercial oils. Therefore, it could be used instead of the commercial oils that need to be imported oversea. Additionally, HRO possessed the significantly highest inhibitory activities against lipid peroxidation and hyaluroidase. Therefore, it was suggested to be used as anti-ageing compounds in further cosmetic products development.

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CONFLICT OF INTEREST

There are no conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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