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Original Article

DETERMINATION OF (E)-4-(3', 4'-DIMETHOXYPHENYL)-BUT-3-EN-1-OL CONTENT IN ZINGIBER CASSUMUNAR ROXB. (PLAI) PATCHES

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ABSTRACT

Objective: The (*E*)-4-(3', 4'-dimethoxyphenyl)-but-3-en-1-ol is the main active compound in *Zingiber cassumunar* Roxb. (Plai) used for the treatment of asthma, for muscle and joint pain. The crude Plai oil was mixed in polymer blends between chitosan/hydroxypropylmethyl cellulose and chitosan/polyvinyl alcohol, and used glycerine as plasticizer. The main objective aimed to determine the (*E*)-4-(3', 4'-dimethoxyphenyl)-but-3-en-1-ol content in Plai patches which was extracted by sonication method.

Methods: Plai was extracted with ethanol and evaporated until obtain the dry crude Plai oil. All polymer blends were mixed homogeneously. The crude Plai oil was dissolved in absolute ethanol and mixed together in polymer blends solution by traditional beaker method until homogenious in appearance. They were transferred into Petri-dish and dried in hot air oven at 70±2ºC for 5 hours. These patches were photographed the surface and cross section by scanning electron microscopy (SEM). The Plai patches were extracted in phosphate buffer pH 7.4: ethanol = 8:2 under sonication for 30 minutes. Then, they were analyzed by HPLC method to determine the (*E*)-4-(3', 4'-dimethoxyphenyl)-but-3-en-1-ol content.

Results: The SEM photography showed the smooth surface and dense cross section of blank patched and Plai patches. The determination of (*E*)-4-(3', 4'-dimethoxyphenyl)-but-3-en-1-ol content was 5.33±1.05 and 8.75±0.63 mg in 2 cm ×2 cm for chitosan/hydroxypropylmethyl cellulose/glycerine patches and chitosan/polyvinyl alcohol/glycerine patches, respectively.

Conclusions: We successfully determined the (*E*)-4-(3', 4'-dimethoxyphenyl)-but-3-en-1-ol content in Plai patches. This method might be use further study such as patched preparation and *in vitro* study including release and permeation studies.

Keyword: crude Plai oil, Plai patches, (*E*)-4-(3', 4'-dimethoxyphenyl)-but-3-en-1-ol, *Zingiber cassumunar* Roxb.

INTRODUCTION

Zingiber cassumunar Roxb (Plai) is used for the relief of pain and inflammation in a great number of conditions involving the joints and muscles. It has a wonderful, uplifting peppery-green eucalyptus aroma, and is highly regarded for its therapeutic properties in massage. Also similar to ginger are the anti-inflammatory and analgesic actions, though it has an overall cooling, rather than warming effect.

Plai may be blended with other essential oils: helichrysum, ginger, marjoram, nutmeg, black pepper, soothing oils such as lavender and neroli, or bergamot for increasing a synergistic effect. Plai essential oil is considered non-toxic, non-sensitizing and non-irritating [1, 2].

The volatile oil of (*E*)-4-(3', 4'-dimethoxyphenyl)-but-3-en-1-ol was extracted from the rhizomes of Plai, which are very similar in appearance to ginger. Analgesic and antipyretic properties of (*E*)-4-(3', 4'-dimethoxyphenyl)-but-3-en-1-ol were also reported [3-5]. It was found to exhibit inhibitory and anti-inflammation activity by using various experimental models of inflammation [4, 6]. It is also used as topical treatment for sprains, contusions, joint inflammations, muscular pain, abscesses, and similar inflammation-related disorders.

The Plai patches composed the two formulas of polymer blends between chitosan/hydroxypropylmethyl cellulose and chitosan/polyvinyl alcohol, and used glycerine as plasticizer. Then, these patches were photographed the surface and cross section by scanning electron microscopy (SEM).

The Plai patches were extracted in phosphate buffer pH 7.4: ethanol = 8:2 under sonication for 30 minutes. The main objective aimed to determine the (*E*)-4-(3', 4'-dimethoxyphenyl)-but-3-en-1-ol content in Plai patches which was analyzed by HPLC method.

MATERIALS AND METHODS

Materials

Plai was purchased from Charoensuk Osod, Thailand. Chitosan was purchased from Seafresh Industry Public Co., Ltd, Thailand. Hydroxypropylmethyl cellulose was purchased from Onimax, Thailand. Polyvinyl alcohol (*Mw* ~195,000 g/mol) and glycerine were purchased from Sigma-Aldrich, USA. All organic solvents were analytical grade obtained from Merck KGaA, Germany.

Plai patches preparation

The chitosan was dissolved in 1% acetic acid in distilled water in concentration of 3.5%w/v. The hydroxypropylmethyl cellulose or polyvinyl alcohol was dissolved in distilled water in concentration of 20%w/v. Then, 2 g of 3.5%w/v chitosan was mixed together with 5 g of 20%w/v of hydroxypropylmethyl cellulose or polyvinyl alcohol, and mixed homogeneously mixed with glycerine as plasticizer. After that, the crude Plai oil was dissolved in absolute ethanol and continuously mixed in polymer blends solution. They were transferred into Petri-dish and dried in hot air oven at 70±2ºC for 5 hours. The appearances of blank patches and Plai patches after drying were photographed by digital camera.

SEM photography

The Plai patches surface and cross section were photographed by SEM (model: Quanta 400, FEI, Czech Republic) with high vacuum and high voltage of 20 kV condition, and using everhart thornley detector (ETD).

Preparation of isotonic phosphate buffer pH 7.4

Isotonic phosphate buffer pH 7.4 was prepared by mixing two stock solutions, 200 mL of a stock solution containing 8 g of monobasic

sodium phosphate (NaH_2PO_4) per liter and 800 mL of a stock solution containing 9.47 g of dibasic sodium phosphate (Na_2HPO_4) per liter, the weights being on an anhydrous basic. Then, the obtained solution was adjusted with respect to tonicity by adding 4.4 g of sodium chloride (NaCl). The obtained isotonic phosphate buffer pH 7.4 was filtered through a 0.45 µm of polyamide membrane and degassed by sonication before use [7].

Preparation of Plai patches sample

The Plai patches were cut into 2 cm × 2 cm from different site Fig. 1. Each Plai patches sample were soaked with isotonic phosphate buffer pH 7.4: ethanol = 8:2 in 10 mL volumetric flask, and sonicated at 25ºC for 30 minutes. Then, the solution was sampled for 0.5 mL and then transferred into 100 mL volumetric flask and adjusted the volume to 100 mL with isotonic phosphate buffer pH 7.4: ethanol = 8:2. The solution was filtered through a 0.45 µm and analyzed with HPLC method

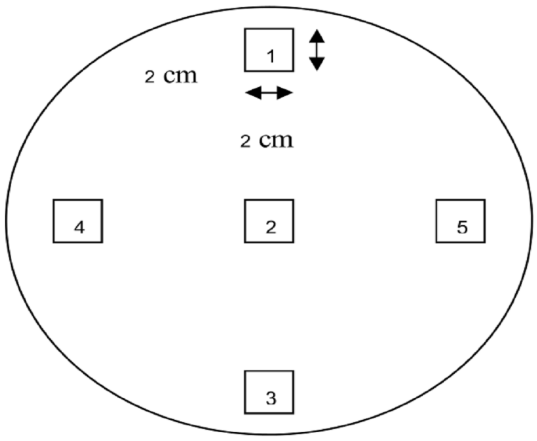

Fig. 1: The preparation of Plai patches samples from different place on the Plai patches

HPLC method

The solution from above method was analyzed by the RP-HPLC system using an Agilent 1260 Infinity system (Agilent Technologies, USA.) with detection at 254 nm. A 4.6 mm × 250 mm diameter, 5 µm particle size C18 column (ACE 5, DV12-7219, USA.), a flow rate of 1 mL/min, and injection volume of 10 µL were used for this experiment. The mobile phase was a gradient elution of 2% acetic acid in ultrapure water (A) and methanol (B) of 60 to 50% of A, 50 to 30% of A, 30 to 20% of A, 20 to 50% of A, 50 to 60% of A, and 60% of A for 0 – 5 min, 5 – 15 min, 15 – 25 min, 25 – 30 min, 30 – 32 min, and 32 – 40 min, respectively. The (*E*)-4-(3', 4'-dimethoxyphenyl)-but-3-en-1-ol content was calculated comparing with the validated calibration curve in the required concentration range of 2 – 40 µg/mL (r^2 > 0.9999). A limit of detection was 0.2 µg/mL, limit of quantitation was 0.8 µg/mL, the intraday and interday precision of injection results demonstrated relative standard deviation of less than 2% and 5%, respectively, the mean recovery values were 95.38-104.76% and 88.94-102.43% for intraday and interday accuracy, respectively [8].

RESULTS AND DISCUSSION

The appearances of blank patches and Plai patches after drying were photographed by digital camera Fig. 2. The blank patches were transparency patches in both two formulas of polymer blends of chitosan/hydroxyl propylmethyl cellulose/glycerine and chitosan/polyvinyl alcohol/glycerine. In addition, when mixed the crude Plai oil in their blank patches, they became dark yellow patches due to the individual appearance of crude Plai oil.

SEM photography (Fig. 3), the blank patches showed the smooth surface and dense cross section in both two formulas (chitosan/hydroxypropylmethyl cellulose/glycerine, Fig. 3A and chitosan/polyvinyl alcohol/glycerine, Fig. 3B). However, after crude Plai oil was mixed in their patches, the surface became the roughness, unevenness, and inconstancy, and increased dense cross section of crude Plai oil/chitosan/hydroxypropylmethyl cellulose/glycerine (Fig. 3C) and crude Plai oil/chitosan/polyvinyl alcohol/glycerine (Fig. 3D).

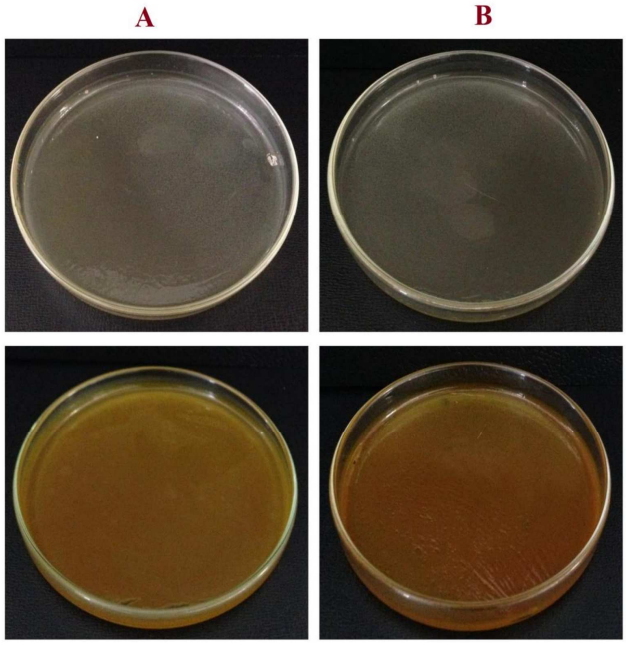

Fig. 2: The appearances of blank patches (top) and Plai patches (below) in different polymer blends between chitosan/hydroxypropylmethyl cellulose/glycerine (A) and chitosan/polyvinyl alcohol/glycerine (B) after drying were photographed by digital camera

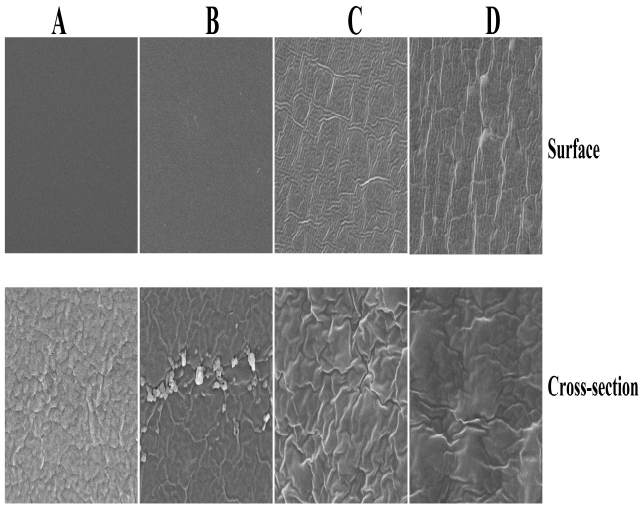

Fig. 3: The surface (1,500×, top) and cross section (5,000×, below) of blank patches of chitosan/hydroxypropylmethyl cellulose/glycerine (A), blank patches of chitosan/polyvinyl alcohol/glycerine (B), Plai patches of crude Plai oil/chitosan/hydroxypropylmethyl cellulose/glycerine (C), and Plai patches of crude Plai oil/chitosan/polyvinyl alcohol/glycerine (D)

The content of (*E*)-4-(3', 4'-dimethoxyphenyl)-but-3-en-1-ol in Plai patches were soaked with isotonic phosphate buffer pH 7.4: ethanol = 8:2, and sonicated at 25ºC for 30 minutes. Then, they were analyzed by HPLC method (Table 1). We found the (*E*)-4-(3', 4'-

dimethoxyphenyl)-but-3-en-1-ol content of 5.3400±1.0476 and 8.7468±0.6298 per 2 cm × 2 cm for crude Plai oil/chitosan/ hydroxypropylmethyl cellulose/glycerine patches and crude Plai oil/chitosan/polyvinyl alcohol/glycerine patches, respectively. Generally, hydroxypropylmethyl cellulose is the water-swelling and high eroded cellulose polymer, and low mechanical properties in term of ultimate tensile strength and elongation at break [9-13].

But polyvinyl alcohol is the water-soluble synthetic polymer, and high tensile strength and flexibility [13-16].

Thus, the patches comprised of polyvinyl alcohol in formula could highly incorporated and entrapped the crude Plai oil more than the patches comprised of hydroxypropylmethyl cellulose in formula that showed high (*E*)-4-(3', 4'-dimethoxyphenyl)-but-3-en-1-ol content.

Table 1: The determination of (*E*)-4-(3', 4'-dimethoxyphenyl)-but-3-en-1-ol content in Plai patches from two polymer blends formulas of crude Plai oil/chitosan/hydroxypropylmethyl cellulose/glycerine patches and crude Plai oil/chitosan/polyvinyl alcohol/glycerine patches

Site on the Plai patches	(*E*)-4-(3', 4'-dimethoxyphenyl)-but-3-en-1-ol content in Plai patches (mg per 2x2cm²)	Average	SD
crude Plai oil/chitosan/hydroxypropylmethyl cellulose/glycerine patches			
1	4.0959	5.3400	1.0476
2	4.5128		
3	6.5742		
4	6.1356		
5	5.3816		
crude Plai oil/chitosan/polyvinyl alcohol/glycerine patches			
1	9.2769	8.7468	0.6298
2	8.7989		
3	7.6799		
4	9.1428		
5	8.8355		

CONCLUSION

The Plai patches become the dark yellow patched after mixed crude Plai oil into blank patches that photographed by digital camera. In addition, the Plai patches were found the roughness, unevenness, and inconstancy on their surface, and high density on their cross section under SEM photography. Finally, we successfully determined the (*E*)-4-(3', 4'-dimethoxyphenyl)-but-3-en-1-ol content in ranged of 5.34 – 8.75 mg per 2 cm × 2 cm of Plai patches. This method might be use further study such as patched preparation and *in vitro* study including release and permeation studies.

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REFERENCES

1. Pongprayoon U, Soontornsaratune P, Jarikasem S, Sematong T, Wasuwat S, Claeson P. Topical antiinflammatory activity of the major lipophilic constituents of the rhizome of *Zingiber cassumunar*. Part I: The essential oil. Phytomedicine 1997; 3(4): 319-22.
2. Pongprayoon U, Tuchinda P, Claeson P, Sematong T, Reutrakul V, Soontornsaratune P. Topical antiinflammatory activity of the major lipophilic constituents of the rhizome of *Zingiber cassumunar*. Part II: Hexane extractives. Phytomedicine 1997; 3(4): 323-26.
3. Ozaki Y, Kawahara N, Harada M. Anti-inflammatory effect of *Zingiber cassumunar* Roxb. and its active principles. Chem Pharm Bull (Tokyo) 1991; 39(9): 2353-59.
4. Panthong A, Kanjanapothi D, Niwatananant W, Tuntiwachwuttikul P, Reutrakul V. Anti-inflammatory activity of compound D {(*E*)-4-(3′,4′-dimethoxyphenyl)but-3-en-2-ol} isolated from *Zingiber cassumunar* Roxb. Phytomedicine 1997; 4(3): 207-12.
5. Panthong A, Kanjanapothi D, Niwatananun V, Tuntiwachwuttikul P, Reutrakul V. Anti-inflammatory activity of compounds isolated from *Zingiber cassumunar*. Planta Med 1990; 56(6): 655.
6. Kanjanapothi D, Soparat P, Panthong A, Tuntiwachwuttikul P, Reutrakul V. A uterine relaxant compound from *Zingiber cassumunar*. Planta Med 1987; 53: 329 - 32.
7. Arun RR. Formulation evaluation and *in vitro* permeation studies of transdermal nifedipine from matrix types patches. Int J Pharm Pharm Sci 2014; 6(1): 185-88.
8. Suksaeree J, Madaka F, Monton C, Sakunpak A, Chusut T, Charoonratana T. Method validation of (*E*)-4-(3',4'-dimethoxyphenyl)-but-3-en-1-ol in *Zingiber cassumunar* Roxb. with different extraction techniques. Int J Pharm Pharm Sci 2014; 6(3): 295-98.
9. Barba AA, d'Amore M, Chirico S, Lamberti G, Titomanlio G. Swelling of cellulose derivative (HPMC) matrix systems for drug delivery. Carbo Polym 2009; 78(3): 469-74.
10. Kavanagh N, Corrigan OI. Swelling and erosion properties of hydroxypropylmethylcellulose (Hypromellose) matrices - Influence of agitation rate and dissolution medium composition. Int J Pharm 2004; 279(1-2): 141-52.
11. Miranda A, Millán M, Caraballo I. Study of the critical points of HPMC hydrophilic matrices for controlled drug delivery. Int J Pharm 2006; 311(1-2): 75-81.
12. Nyamweya N, Hoag SW. Assessment of polymer-polymer interactions in blends of HPMC and film forming polymers by modulated temperature differential scanning calorimetry. Pharm Res 2000; 17(5): 625-31.
13. Pichayakorn W, Suksaeree J, Boonme P, Taweepreda W, Ritthidej GC. Preparation of deproteinized natural rubber latex and properties of films formed by itself and several adhesive polymer blends. Ind Eng Chem Res 2012; 51(41): 13393-404.
14. Kumar HMPN, Prabhakar MN, Prasad CV, Rao KM, Reddy TVAK, Rao KC, et al. Compatibility studies of chitosan/PVA blend in 2% aqueous acetic acid solution at 30 °C. Carbo Polym 2010; 82(2): 251-55.
15. Saettone MF, Chiellini G, Giannaccini B, Chetoni P, Montanari ML. Release of miconazole from topical PVA matrices: preliminary *in vitro* and *in vivo* data. J Controlled Release 1991; 16(1-2): 197-202.
16. Stammen JA, Williams S, Ku DN, Guldberg RE. Mechanical properties of a novel PVA hydrogel in shear and unconfined compression. Biomaterials 2001; 22(8): 799-806.