Validation of a headspace gas chromatography-flame ionization detector (HS GC-FID) method for determination of terpinen-4-ol in volatile oil from *Zingiber montanum* rhizome

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Abstract

This study aimed to develop a headspace GC-FID method for the analysis of terpinen-4-ol in *Zingiber montanum* (Plai) essential oil. The volatile oil was extracted from fresh *Z. montanum* rhizomes by steam distillation. The amount of terpinen-4-ol was determined by a headspace gas chromatography-flame ionization detector (HS GC-FID) method using benzyl alcohol as an internal standard. The developed method was validated according to the parameters recommended by the International Conference on Harmonization (ICH). Linearity was accessed across the concentration range of 2.5 – 15 µL/mL. The plot of the peak area ratio versus the concentration provided a good linear of this method with a correlation coefficient (r^2) of 0.9989. The LOD and LOQ were found to be 0.20 and 2.5 µg/mL, respectively, which indicated a high sensitivity of the method. The instrument (n = 6), intra-day (n = 9), and inter-day (n = 3) precision as indicated by %RSD were 1.77, 1.73, and 1.77 %, respectively. The recoveries at 3 different levels of terpinen-4-ol were between 100.23 to 100.85%. The content of terpinen-4-ol in Plai essential oil was 15.53 ± 0.27 % v/v. The developed method can be used in routine analysis of Plai essential oil and its products in both cosmetic and pharmaceutical industries.

Keywords: *Terpinen-4-ol, Zingiber montanum, Plai, Gas chromatography, Validation*

1. Introduction

There has been a rising interest in using herbal medicinal products for the treatment of many diseases and symptoms in the primary healthcare system in Thailand because they are considered harmless and inexpensive. The problem that arises regarding the use of herbal medicines is mainly related to the lack of suitable quality control of the raw plant material and its derived products. Standardization of herbal products is essential for the evaluation of the quality of drugs, based on the content of their active constituents. The development of an analytical method for quantitative analysis of bioactive compounds or markers within the herbal preparations is an important step for the herbal products to display a consistent biological activity. For that, the validation of analytical methods is established to ensure that the methods are suitable for their intended purpose.

Zingiber montanum (Koenig) Link ex Dietr. or previously named as *Zingiber cassumunar* Roxb. (Zingiberaceae), locally known in Thai as "Plai" is a perennial herb with underground rhizomes with camphoraceous aroma. Plai rhizome has been usually found in Thai Traditional remedies for the treatment of various diseases, namely gastrointestinal disorders, dysmenorrhea, wound healing, muscle pain & swelling, rheumatoid arthritis, dermatitis, cough, and asthma (The Institute of Thai Traditional Medicine, 1995). Several studies have reported that biologically active compounds in Plai rhizome possessing anti-inflammatory action includes compound D [(*E*)-4-(3',4'-dimethoxyphenyl)but-3-en-2-ol] (Panthong et al., 1997), (*E*)-1-(3,4-dimethoxyphenyl)but-1-ene) (Ozaki, Kawahara & Harada, 1991), DMPBD [(*E*)-1-(3,4-dimethoxyphenyl) butadiene)] (Jeenapongsa et al., 2003), TMPBD [(*E*)-4-(2,4,5-triimethoxyphenyl)but-1,3-ene)], cassumunaquinones, and cassumunins (Nakamura et al., 2009). Plai oil obtained from frying with vegetable oil (hot oil extract) has been widely used as a rubbing oil in Thai folk medicine to relieve musculoskeletal pain. A randomized placebo-controlled clinical study of Plai oil extracted by frying with hot palm oil revealed a similar analgesic effect as 1% diclofenac gel (Wisuitiprot et al., 2019).