

Chemical Marker Identification of Mixed Essential Oil Formulation

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ABSTRACT

The objective of this study was to identify chemical markers of mixed essential oil formulation from medicinal plants used in herbal compressed ball. The chemical markers serve as standard substances for preliminary quality control of the formulations containing mixed essential oils. In this study, the formulation contained 40.28% plai oil, 5.56% turmeric oil, 1.39% lemongrass oil and 8.33 % kaffir lime oil. All the essential oils used in the formulation were extracted from medicinal plants commonly used in traditional herbal compressed ball. Camphor, borneol and menthol: 13.89%, 8.33% and 22.22%, respectively, were added to the formulation in order to get more aroma and cool feeling when applied onto skin. The chemical components of the formulation were analyzed by gas chromatographic method. The main chemical components that showed sharp and clear peaks on gas chromatogram were sabinene, limonene, camphor and menthol. The correlation between the concentrations of mixed essential oils in the formulation and the detector responses of main chemical components was determined. The correlation coefficients (R^2) of sabinene, limonene, camphor and menthol were all higher than 0.995 in the concentration ranging from 0.20%w/v to 2.6%w/v which indicated the linear relationship between the detector responses and the concentrations of the formulation. Therefore, sabinene, limonene, camphor and menthol can be used as the chemical markers of the mixed essential oil formulation from medicinal plants used in herbal compressed ball.

Keywords: Chemical marker, Essential oil, Herbal compressed ball, Mixed essential oil formulation

INTRODUCTION

The herbal compressed ball is traditionally used to treat muscle and joint pain in Thai traditional medicine. The medicinal plants which are commonly used in herbal compressed ball are plai (rhizome of *Zingiber cassumunar* Roxb.), turmeric (rhizome of *Curcuma longa* Linn.), lemongrass (leaves of *Cymbopogon citratus* Stapf.) and kaffir lime (fruit of *Citrus hystrix* DC.), all these crude drugs

contain essential oils which have been proved to possess anti-inflammatory (Iyengar et al., 1994; Jeenapongsa et al., 2003) analgesic (Viana et al., 2000) and muscle relaxant activity. However, the traditional herbal compressed ball has to be steamed to let the essential oils evaporate from crude drugs before use, making it somewhat inconvenient. Moreover, it usually lacks the quality control so it may harbor some microorganisms and may get fungal contamination after 2-3 times of reuse. It is also difficult to control the proportion of essential oils released from each time of usage. These cause inconsistency for the quality control of the traditional herbal compressed ball. In order to improve the quality of the herbal compressed ball, we developed the mixed essential oil formulation from the same crude drugs used in traditional herbal compressed ball to be an alternative. We also tried to identify the chemical markers of the mixed essential oil formulation which served as standard substances for quality evaluation. The chemical markers have to be main chemical components of the mixed essential oil formulation that can be identified and measured with appropriate analytical methods.

MATERIALS AND METHODS

Materials

Plai oil, turmeric oil, lemongrass oil and kaffir lime oil were purchased from Thai-China Flavors and Fragrances Industry Co., Ltd. Ethanol, camphor, borneol and menthol were purchased from Union Science Co., Ltd. Gas chromatography with flame ionization detector (GC/FID) was performed on Shimazu 14-B, equipped with DB-1 capillary column (30 m 30.53 mm ID, 1.5 μ m film thickness). Gas chromatography coupled with mass spectrometer (GC/MS) was performed on Agilent6850 gas chromatography instrument attached to an Agilent5973 mass spectrometer and HP-1MS capillary column (30 m 30.25 mm ID, 0.25 μ m film thickness).

Methods

Preparation of mixed essential oil formulation

Firstly, the initial mixed essential oil formulation was formulated. The amount of each essential oil in the initial mixed essential oil formulation was calculated by using the amount of crude drugs used in the traditional herbal compressed ball and the percentage of essential oils contain in the crude drugs. Then, the amount of each essential oil was varied to get the formulations of which the odor was similar to the traditional herbal compressed ball and gave cool feeling when applied onto the skin. The satisfaction of the mixed essential oil formulations was rated by the volunteers to get the most suitable mixed essential oil formulation.

Identification of chemical markers

Each essential oil and the selected mixed essential oil formulation were diluted with ethanol and analyzed by GC/MS to identify the main chemical components that can be used as chemical markers. The column temperature was

started at 75°C held for 10 min., then programmed at 5°C/min to 230°C and held for 5 min. Split injection (1 µl) was conducted with a split ratio of 10:1 and helium was used as carrier gas at 1.0 ml/min flow rate. The mass spectrometer was operated in electron-impact (EI) mode; the ionization energy was 70 eV. The inlet and ionization source temperatures were 250 and 230°C, respectively. The main chemical components that showed clear and sharp peaks on chromatogram could be the chemical markers of the formulation.

Linearity study

Linearity of chemical markers was determined by preparing the selected mixed essential oil formulation and diluted in ethanol to obtain a series of concentrations ranging from 0.20%w/v to 2.60%w/v; linalool was added to serve as an internal standard and then analyzed by GC/FID. The injection temperature and detector temperature were 250°C. The column temperature started at 75°C, held for 10 min, then programmed at 5°C / min to 230°C and held for 5 min. The correlation between peak area ratios of chemical markers to internal standard and concentrations of mixed essential oil formulation was determined.

RESULTS AND DISCUSSION

Preparation of mixed essential oil formulation

The herbal compressed balls usually consist of several medicinal plants which are the indigenous plants in the local area and the amount of each medicinal plant in the formulation is different. Most of the herbal compressed balls contain 500 g of plai (*Z. cassumunar* Roxb.), 200 g of turmeric (*C. longa* Linn.), 200 g of lemongrass (*C. citratus* Stapf.) and 100 g of kaffir lime (*C. hystrix* DC.). In this study, the amount of each essential oil used in the initial formulation was calculated based on the amount of crude drugs used in the traditional herbal compressed ball formulation and the percentage of essential oil contain in crude drugs as shown in Table 1.

Table 1. The amount of essential oils in the initial mixed essential oil formulation.

The essential oil	Amount of crude drugs used in traditional formulation (gram)	% essential oil in crude drugs*	The calculated amount of essential oil (gram)
Plai oil	500	1.00	5.00
Turmeric oil	200	0.40	0.80
Lemongrass oil	200	0.50	1.00
Kaffir lime oil	100	1.20	1.20

*Data from Thailand Institute of Scientific and Technological Research (TISTR) (2005)

From the initial formulation, the amount of each essential oil was varied to obtain several mixed essential oil formulations. The odor of each formulation

depended on the composition of each essential oil. The four mixed essential oil formulations as shown in Table 2 were selected to rate the satisfaction by thirty volunteers. The most suitable formulation of mixed essential oils contained 40.28% of plai oil, 5.56% of turmeric oil, 1.39% of lemongrass oil and 8.33% of kaffir lime oil; 13.89% camphor, 3.33% borneol and 22.22% menthol. The last three compounds were added in order to get more aroma and cool feeling when applied onto the skin. The odor of the mixed essential oil formulation was similar to the herbal compressed ball and the color of the formulation was pale- yellow.

Table 2. The four mixed essential oil formulations which were rated for satisfaction by the volunteers.

Essential oil	The amount of essential oil (gram)			
	PT7 (%)	LK8 (%)	TL7 (%)	*PL9 (%)
Plai oil	38.89	34.72	34.72	40.28
Turmeric oil	1.39	5.56	11.11	5.56
Lemongrass oil	6.94	2.78	1.39	1.39
Kaffir lime oil	8.33	12.50	8.33	8.33
Camphor	13.89	13.89	13.89	13.89
Borneol	8.33	8.33	8.33	8.33
Menthol	22.22	22.22	22.22	22.22

*PL9 was the selected mixed essential oil formulation.

Identification of chemical markers

Each essential oil used in the formulation and the selected mixed essential oil formulation were analyzed by GC/MS to identify the main chemical components of each essential oil and the main chemical components of the mixed essential oil formulation. Sabinene and terpinen-4-ol were the main chemical components of plai oil while turmerone was the main chemical component of turmeric oil. The main chemical component of lemongrass oil was citral and those of kaffir lime oil were sabinene and limonene. Although, the main chemical components of each essential oil could be used as chemical markers of the mixed essential oil formulation, the peak distinction of each chemical component depended on the quantity of each essential oil in the formulation. When the formulation was analyzed, the chemical components that showed clear and sharp peaks on chromatogram were sabinene, limonene, camphor and menthol which appeared at 5.71, 7.77, 12.95 and 14.90 min, respectively, as shown in Figure 1. While the citral a and citral b peaks were too small to be the markers compared to the others. Therefore, sabinene, limonene, camphor and menthol were selected to be the chemical markers of the mixed essential oil formulation.

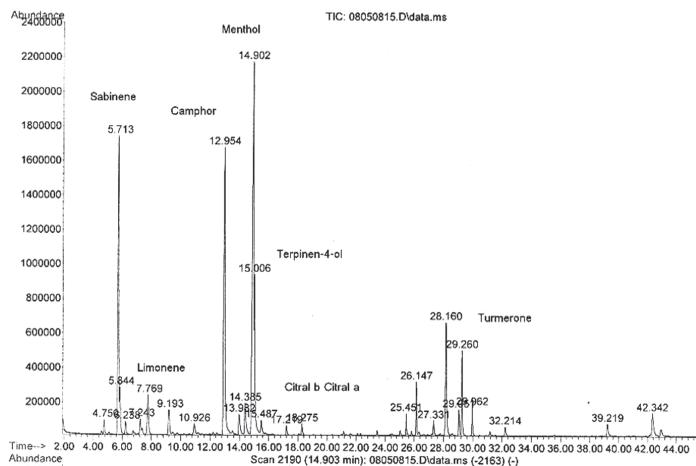


Figure 1. GC/MS chromatogram of mixed essential oil formulation.

Linearity study

The selected mixed essential oil formulation was analyzed by GC/FID, using the same gas chromatographic conditions as GC/MS. Sabinene, limonene, camphor and menthol showed distinct peaks at 4.61, 6.54, 11.99 and 14.06 min, respectively, while other chemical components such as terpinen-4-ol, citral and turmerone did not show distinct peaks on the chromatogram as shown in Figure 2. The four chemical markers were used to determine the correlation between their peak responses and the concentrations of mixed essential oil formulation, ranging from 0.20%w/v to 2.60%w/v.

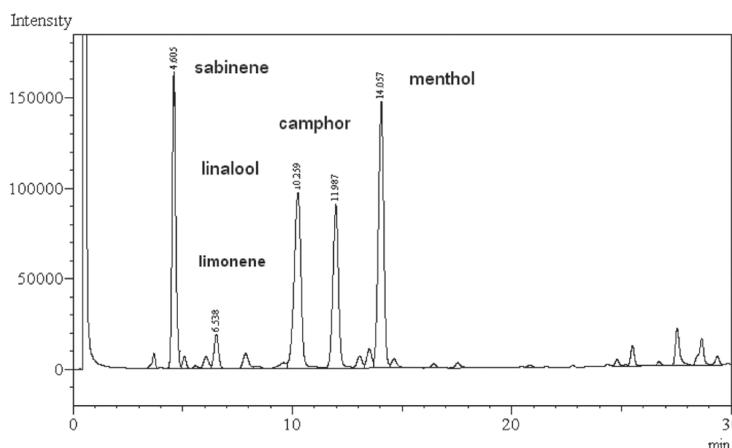


Figure 2. GC/FID chromatogram of mixed essential oil formulation.

The result of each chemical marker showed the correlation coefficients (R^2) higher than 0.995 which indicated linear relationship between the peak responses of the chemical markers and the concentration of mixed essential oil formulation as shown in Figure 3.

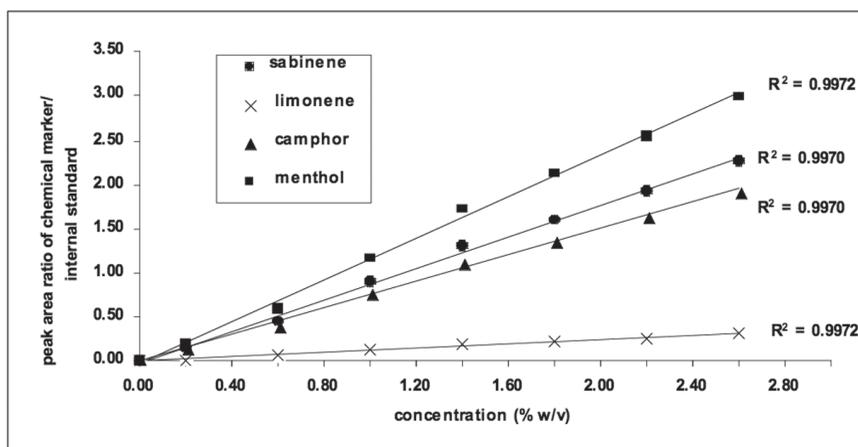


Figure 3. The correlation between peak area ratios of chemical markers to internal standard and the concentrations of mixed essential oil formulation.

CONCLUSION

The mixed essential oil formulation prepared from essential oils of medicinal plants commonly used in traditional herbal compressed ball is an alternative way to use instead of traditional herbal compressed ball because of its convenience and low risk of the fungal contamination. For the quality evaluation of the formulation, the chemical markers of the mixed essential oil formulation must be identified to serve as standard substances in assuring the quality of the formulation.

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