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THEP-TA-RO ESSENTIAL OIL IN SOLUTION AND EMULGEL DOSAGE FORMS

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Abstract

Cinnamomum porrectum Kosterm (Thep-ta-ro) is nominated as a provincial plant of Phang-nga. Essential oil extracted from Thep-ta-ro leaves has been reported to have antimicrobial activity against *Candida albicans*. Safrole is a major component in the oil. To make use of the oil for pharmaceutical purposes, it was prepared in solution and emulgel topical dosage forms at various concentrations (1, 2, and 5% w/w). Cosolvents and micellar solubilization were utilized in the formulations. Clear solutions containing the oil in micellar systems with or without cosolvents were prepared. Suitable polymers were added for preparing emulgel formulations. Oil preparations, 1% and 2% w/w, were tested for activity against *Candida albicans*, the inhibition zones were 9 and 12 mm, respectively. At 5% w/w Thep-ta-ro oil, the activity is not significantly different from that of Mycostatin® with an inhibition zone of 12-18 mm. Viscosity enhancing agents and surfactants in the preparations have no effect on the activity of the thep-ta-ro oil. Thep-ta-ro oil is likely to be developed for the treatment of topical candidiasis and 5% w/w oil concentration is recommended.

Key words: Essential oil, *Cinnamomum porrectum* Kosterm, *Candida albicans*, Solutions, Emulgels

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น้ำมันระเหยเทพธาโรในรูปแบบสารละลายและอิมัลเจล

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บทคัดย่อ

เทพธาโร ถูกจัดเป็นต้นไม้ประจำจังหวัดพังงา ได้มีรายงานถึงความสามารถในการยับยั้งเชื้อแคนดิดาของน้ำมันระเหยที่สกัดได้จากใบเทพธาโร แซฟรอลเป็นสารหลักที่พบในน้ำมันระเหย เพื่อนำน้ำมันนี้มาใช้ประโยชน์ทางเภสัชกรรม น้ำมันเทพธาโรได้ถูกเตรียมที่ความเข้มข้นต่าง ๆ (ร้อยละ 1, 2 และ 5 โดยน้ำหนัก) ในรูปของยาสารละลาย และ อิมัลเจลสำหรับใช้ภายนอก ใช้เทคนิคตัวทำละลายร่วม และการละลายด้วยไมเซลล์ ทำให้ได้สารละลายใสที่มีอนุภาคของน้ำมันกระจายตัวอยู่ในรูปของไมเซลล์ที่มีและไม่มีตัวทำละลายร่วม โดยเลือกสารโพลิเมอร์ที่เหมาะสมเพื่อเตรียมให้ได้อิมัลเจลที่ต้องการ น้ำมันระเหยเทพธาโร ความเข้มข้นร้อยละ 1 และ 2 โดยน้ำหนักของตำรับถูกทดสอบฤทธิ์ในการยับยั้งเชื้อแคนดิดา พบว่าได้โซนใสที่เชื้อถูกยับยั้ง 9 และ 11 มิลลิเมตรตามลำดับ ที่ความเข้มข้นร้อยละ 5 โดยน้ำหนักของน้ำมันเทพธาโรมีฤทธิ์ต้านเชื้อแคนดิดาได้ไม่ต่างกันอย่างมีนัยสำคัญกับไมโครสตาติน โดยมีโซนใสที่เชื้อถูกยับยั้ง 12-18 มิลลิเมตร สารเพิ่มความหนืดไม่มีผลต่อการออกฤทธิ์ของน้ำมันเทพธาโร ดังนั้นน้ำมันเทพธาโรสามารถนำมาพัฒนาเป็นตำรับสารละลาย และ อิมัลเจล และที่ความเข้มข้นร้อยละ 5 โดยน้ำหนักของน้ำมันเป็นความเข้มข้นที่แนะนำ

คำสำคัญ: น้ำมันระเหย, เทพธาโร, ฤทธิ์ต้านแคนดิดา, สารละลาย, อิมัลเจล

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Introduction

Cinnamomum porrectum Kosterm is a native plant grown in Southeast Asia.[1-3] This plant is found mostly in the southern part of Thailand and nominated as a provincial plant of Phang-nga.[2] Its local names are Thep-ta-ro, Chuang, Chuang-hom, Cha-kai-hom, Phluu-ton-khaao, and Mue-dae-ka-maa-nging.[1] Thep-ta-ro is classified in the Lauraceae family, Laurales order.[2] There is evidence that Thep-ta-ro has been used in Thai indigenous medicines since 1659 to improve body immunity and as anti-flatulent.[2] Essential oil extracted from Thep-ta-ro leaves has been reported to have antimicrobial activity against *Candida albicans*.[4] *Candida* species can infect many areas of the body not only confined to skin, vagina, penis, mouth, nails but can be systemic and infect multiple internal organs. Candida infections are reported to be the second most common cause of vaginitis in women and are largely opportunistic ones made possible by diminished host defenses. Oral candidiasis is a common, early, and often initial manifestation of infection with HIV. Nystatin along with other antifungal agents, such as clotrimazole, are usually used for the treatment. Hydrocortisone, such as triamcinolone acetonide, may be used to relieve the pain and itching that accompany skin infections.[5]

Safrole is a major compound found in Thep-ta-ro oil. Activity of Thep-ta-ro oil against *Candida albicans* was studied and its minimal inhibitory concentration was determined to be 0.063 % v/v.[6] To make use of the oil, it should be prepared in a suitable pharmaceutical dosage form. In this work, Thep-ta-ro oil was formulated in solution and emulgel topical dosage forms at various concentrations (1, 2, and 5% w/w). Cosolvents and micellar solubilization were utilized in both dosage forms and polymers were added for preparing emulgels. Activity of the formulations was evaluated using *Candida albicans* cell culture.

Materials and Methods

All chemicals, obtained from S. Tong Chemical Co Ltd. unless otherwise specified, were pharmaceutical grade and were used as received. Essential oil was extracted from Thep-ta-ro leaves (Khao Hin Sorn Royal Development Study Centre, Chachoengsao) using a simple distillation method.

Cosolvent solubilization

One percent oil (w/w) was prepared in ethanol and propylene glycol (PG) aqueous solutions. PG concentration in the aqueous solutions was varied from 5 to 40 % w/w

and ethanol was used at concentrations of 10 or 20 % w/w. The solutions were then observed.

Micellar solubilization

Micellar solubilization was utilized to dissolve Thep-ta-ro oil. Three different types of surfactants were used for preparing 1 % w/w Thep-ta-ro oil solutions. Firstly; an anionic surfactant, sodium lauryl sulfate (SLS), was used at 2.5, 5, and 10 % w/w. Secondly; a cationic surfactant, cetyltrimethyl ammonium bromide (CTAB, Sigma Co. Ltd,), was prepared at 1, 2, and 5 % w/w. Thirdly; three nonionic surfactants, tween 80 (Srichand United Dispensory Co. Ltd), tween 20 (Srichand United Dispensory Co. Ltd), and cremophore RH 40, were employed at 5 and 10 % w/w. Combinations of the nonionic surfactants were also utilized. Thep-ta-ro oil was dissolved in these micellar solutions. The solutions were then observed.

Combinations of cosolvents and surfactants

Thep-ta-ro solutions were also prepared in mixed systems of proper surfactants, which were chosen from the micellar solubilization studies, ethanol; and PG. The solutions contained 2 or 5 % w/w of the oil.

Thep-ta-ro emulgel preparation

Preliminary studies showed that the use of neutralized carbomer (Carbopol 934® neutralized with triethanolamine) resulted in thick and clear gels while the use of hydroxypropylmethyl cellulose 4000 (HPMC) resulted in gels with good texture and spreadability. The ratio and concentration of the polymers were varied to obtain a suitable base. The chosen gel base contained 0.5 % w/w carbomer and 0.5 % w/w HPMC. Thep-ta-ro emulgels (1, 2, and 5 % w/w) were prepared using the aforementioned base.

Determination of anticadidiasis activity

Preparation of *Candida albicans*

Candida albicans ATCC 10230 (Department of Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University) was cultivated on sabouraud dextrose agar (SDA). The microorganism was washed from surface agar slant with sterile normal saline (NSS). The culture was then adjusted to match the turbidity of standard Mcfarland no. 0.5 before used.

Preparation of test plates and test procedure

SDA medium was utilized for agar diffusion susceptibility test described in the standard guideline technique.[7] Plates with internal diameter of 100 mm containing 25 ml of enriched agar media were inoculated with 1 % microorganism suspension by seed layer method.[7] Sterile stainless steel cylinders (6 mm internal diameter and 10 mm height) were placed on the inoculated agar surface. The samples were then filled into the cylinders (300µl/cylinder). After pre-diffusion at room temperature for 1 hour the plates were incubated at 37°C overnight. Results were obtained by measuring the diameters of inhibition zones. Purified water filled in a cylinder was used as negative control and commercial products (Kenacomb®, Bristol-Myers Squibb; Canesten®, Bayer; Mycostatin®, Bristol-Myers Squibb) were used as positive controls. The individual base of each formulation was prepared in the same manner as the formulation without the addition of Thep-ta-ro oil. The activity of the bases against *Candida albicans* was then tested to serve as another control. The experiments were carried out in triplicate. ANOVA and student *t*-test were employed for statistic test.

Results and Discussion

Cosolvent solubilization

PG and ethanol are widely used cosolvents.[8] Due to the dehydrating properties of PG, and the skin normal moisturizing factor removing properties of ethanol, both PG and ethanol can cause skin dryness at high concentrations. However, PG and ethanol can provide moisturizing effect and cooling effects at low concentrations.[9] Therefore, the amount of cosolvents was limited in these preparations.

Table 1 Thep-ta-ro (1% w/w) in cosolvent aqueous solutions

Ingredients, g	control	Formulation							
		#1	#2	#3	#4	#5	#6	#7	#8
Thep-ta-ro oil	1	1	1	1	1	1	1	1	1
PG	-	5	5	10	10	20	20	40	40
Ethanol	-	10	20	10	20	10	20	10	20
Water qs	100	100	100	100	100	100	100	100	100
Turbidity*	+++, oil droplet	++, oil droplet	++	++, oil droplet	++	++	+	++	+

* +++, ++, and + = turbidity (high to low)

Turbid solutions were observed when 1% w/w oil was added to the cosolvent aqueous solutions at the studied concentrations of PG and ethanol (Table 1). High concentrations of cosolvents (40% w/w PG and 20 % w/w ethanol) could not completely dissolve 1 % w/w oil and the solution became very viscous due to the high concentration of PG. This implied that the use of cosolvents alone is not enough for dissolving Thep-ta-ro oil.

Micellar solubilization

Clear solutions of the oil were obtained using 10 % w/w SLS, 5 % w/w CTAB, and 10 % w/w cremophore RH40 (Table 2). Since concentrations of the surfactants were greater than their reported critical micelle concentrations ($2.3x10^{-3}$ g/ml SLS, $3.4x10^{-4}$ g/ml CTAB, $1.3x10^{-4}$ g/ml tween 20, $1.3x10^{-4}$ g/ml tween 80, and $3.9x10^{-4}$ g/ml cremophore RH40)[10,11], micellar solutions were expected. The results show that CTAB is a more powerful solubilizer for the oil than the other surfactants. However, cationic surfactants have long been known to be more toxic than anionic or non-ionic surfactants. SLS LD_{50} (rat, oral) is 1.3 g/kg, CTAB LD_{50} (rat, oral) is 0.41 g/kg, tween 20 LD_{50} (rat, oral) is 37 g/kg, tween 80 LD_{50} (mouse, oral) is 25 g/kg, and cremophore RH40 LD_{50} (rat, oral) is >16 g/kg.[11] Of the three classes of surfactants, nonionic surfactants have been recognized to be the safest and the least irritating to skin.[12] In this study, 1% w/w Thep-ta-ro oil could be dissolved in 10 % w/w SLS or 10 % w/w cremophore RH40 solutions. Since the LD_{50} for cremophore RH40 is much greater than that for SLS, 10% w/w cremophore RH40 solution was selected for further studies.

Mixed nonionic surfactants were then employed for preparing oil solutions in order to decrease the toxicity of each surfactant (Table 3). A clear 1 % w/w oil solution was obtained in a mixed surfactant solution of 6 % w/w cremophore RH40 and 5 % w/w tween 20. It is possible that mixed-micelles, containing cremophore RH40 and tween 20, were formed in this solution.

Combinations of cosolvents and surfactants

Both cosolvency and micellar solubilization were then employed in order to increase the ability of the system to dissolve the oil.[13] Clear solutions of 2% w/w oil were achieved using Formulations #26 - #28 and a clear solution of 5 % w/w oil was obtained using Formation #31 (Table 4). The cosolvents themselves could not completely dissolve the oil but in combination with cremophore RH40 clear solutions were achieved as shown in

Formulations #27 and #31. Cosolvents. are known to lower the polarity and dielectric constant of water[8], consequently, improving solubility of non polar compounds. Surfactants solubilize non polar compounds in micelles and also lower the polarity of water.[10] In this work, cosolvents and nonionic surfactants provided a synergistic effect to dissolve Thep-ta-ro oil.

Table 2 Thep-ta-ro solutions containing surfactants

Ingredients, g	Formulation											
	#9	**#10**	**#11**	**#12**	**#13**	**#14**	**#15**	**#16**	**#17**	**#18**	**#19**	**#20**
Thep-ta-ro oil	1	1	1	1	1	1	1	1	1	1	1	1
SLS	2.5	5	10	-	-	-	-	-	-	-	-	-
CTAB	-	-	-	1	2	5	-	-	-	-	-	-
Cremophore RH40	-	-	-	-			5	10	-	-	-	-
Tween 20	-	-	-	-	-	-	-	-	5	10	-	-
Tween 80	-	-	-	-	-	-	-	-	-	-	5	10
Water qs.	100	100	100	100	100	100	100	100	100	100	100	100
Turbidity*	++	+	clear	++	+	clear	+	clear	++	+	++	+

* +++, ++, and + = turbidity (high to low)

Table 3 Thep-ta-ro solutions containing mixed nonionic surfactants

Ingredients, g	Formulation			
	#21	**#22**	**#23**	**#24**
Thep-ta-ro oil	1	1	1	1
Cremophore RH40	5	6	5	6
Tween 20	5	5	-	-
Tween 80	-	-	5	5
Water qs	100	100	100	100
Turbidity*	+	clear	+	+

* +++, ++, and + = turbidity (high to low)

Thep-ta-ro emulgel preparation

Emulgels of 1, 2, and 5% w/w Thep-ta-ro oil with good texture and appearance were obtained using formulations containing 0.5% w/w HPMC and 0.5% w/w carbomer (Table 5). The oil and other ingredients were compatible with both carbomer, an anionic polymer; and HPMC, a nonionic polymer.

Table 4 Thep-ta-ro solutions containing cosolvents and surfactants

Ingredients, g	Formulation						
	#25	#26	#27	#28	#29	#30	#31
Thep-ta-ro oil	2	2	2	2	5	5	5
Cremophore RH40	10	20	10	10	10	10	15
Ethanol	-	-	10	20	10	20	20
Propylene glycol	5	5	5	5	5	5	5
Water qs	100	100	100	100	100	100	100
Turbidity*	++	clear	clear	clear	++	+	clear

* +++, ++, and + = turbidity (high to low).

Table 5 Thep-ta-to emulgel formula

Ingredients, g	Formulation		
	#32	#33	#34
Thep-ta-ro oil	1	2	5
Cremophore RH40	6	10	15
Tween 20	5	-	-
Ethanol	-	10	20
Carbomer	0.5	0.5	0.5
HPMC	0.5	0.5	0.5
Propylene glycol	5	5	5
Water qs	100	100	100

Determination of activity against *Candida albicans*

No activity against *Candida albicans* was observed when the bases of the formulations were tested. Although ethanol was used in some formulations, it is bactericidal in aqueous mixtures at concentrations of 60-95 % v/v. These ethanol concentrations are much higher than the ones used in the formulations in this work. Moreover, ethanol is inactivated in the presence of nonionic surfactants.[11] Formulations containing Thep-ta-ro oil provided activity against *Candida albicans* as shown in Table 6.

One-way ANOVA analyses were performed to determine whether the means of inhibition zones for each concentration are equal. The critical value of $F_{2, 6, 0.95} = 5.14$ is less than the value of the test statistic F = 12.24 and 18.12 in the solution preparations and the emulgel preparations, respectively. Thus, the means of inhibition zones for each concentration are not the same. The higher the oil concentration, the greater the activity against *Candida abicans*. The inhibition zone is significantly increase ($P < 0.05$) except for

Formulation #32 and #33 ($P > 0.05$). At the same concentrations of Thep-ta-ro oil, no significant difference is observed between solution and emulgel formulations ($P > 0.01$). The best activity is found in the 5% w/w Thep-ta-ro preparations.

Table 6 Activity against *Candida albicans* using agar diffusion susceptibility test

Formulation	Thep-ta-ro oil	Dosage form	Inhibition zone, mm			
			1	2	3	Average
Control (water)	-	-	no zone	no zone	no zone	no zone
#16	1%	solutions	10.0	9.8	9.9	9.9
#22	1%	solutions	10.0	9.8	9.9	9.9
#27	2%	solutions	12.0	11.8	11.8	11.9
#31	5%	solutions	16.7	17.8	12.9	15.8
#32	1%	emulgels	10.4	8.9	9.6	9.6
#33	2%	emulgels	11.1	9.8	9.8	10.2
#34	5%	emulgels	13.9	12.8	12.5	13.1
Kenacomb®*		creams	no zone	no zone	no zone	no zone
Mycostatin®*		suspensions	18.2	12.0	13.8	14.7
Canesten®*		creams	35.6	33.5	31.5	33.5

*active ingredient(s) of the commercial products, Kenacomb® (nystatin 100,000 u, neomycin sulfate 2.5 mg, gramicidin 250 µg, and triamcinolone acetonide 1 mg), Mycostatin® (nystatin 100,000 u/ml), Canesten® (clotrimazole 1%).

Canesten® provided the biggest inhibition zone among the tested commercial products. The activity of a 5% w/w Thep-ta-ro oil is not significantly different from that of Mycostatin® where the value of the test statistic $F = 0.98$ is less than the critical value of $F_{2,6,0.95} = 5.14$. No inhibition zone was observed around the cylinder of Kenacomb® possibly due to the inability of its active ingredient to diffuse in the agar medium.

Conclusion

Thep-ta-ro solutions were prepared using cosolvents and micellar solubilization at 1, 2, and 5% w/w oil. Carbomer and HPMC are suitable for preparing Thep-ta-ro emulgel formulations. Both solution and emulgel dosage forms provide activity against *Candida albicans*. At 5 % w/w oil, the activity of Thep-ta-ro is not significantly different from that of Mycostatin®.

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