Research Article

การพัฒนายาเม็ดสอดช่องคลอดจากสารสกัดสมุนไพรไทยสำหรับผู้ที่ติดเชื้อแคนดิดา

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อาจารย์ประจำหลักสูตรสุขภาพความงาม วิทยาลัยการแพทย์แผนไทย มหาวิทยาลัยเทคโนโลยีราชมงคลธัญบุรี
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บทคัดย่อ

การพัฒนายาเม็ดสอดช่องคลอดจากสารสกัดสมุนไพรไทยสำหรับผู้ที่ติดเชื้อแคนดิดา
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โรคติดเชื้อแคนดิดาในช่องคลอดมีผลกระทบต่อสุขภาพของผู้หญิงทั่วโลก โดยองค์การอาหารและยาประเทศสหรัฐอเมริกาอนุมัติให้ใช้ยาโคลไตรมาโซลสอดช่องคลอดในการรักษาผู้ที่ติดเชื้อแคนดิดา ปัจจุบันอุบัติการณ์การดื้อยาโคลไตรมาโซลเพิ่มสูงขึ้น และร้านค้าออนไลน์ในหลายๆ ประเทศมีการขายสมุนไพรเพื่อรักษาการติดเชื้อแคนดิดาในช่องคลอด ลดกลิ่นอันไม่ประสงค์ของตกขาว ซึ่งสินค้าเหล่านี้ไม่ถูกต้องตามกฎหมายและไม่ปลอดภัยต่อผู้ใช้ งานวิจัยนี้จึงมีวัตถุประสงค์เพื่อศึกษาฤทธิ์ในการยับยั้งเชื้อแคนดิดา ตั้งตำรับและประเมินยาเม็ดสอดช่องคลอดจากสมุนไพรไทยบางชนิดในการรักษาโรคติดเชื้อแคนดิดาและเป็นไปตามข้อกำหนดของ USP35/NF30 **วัสดุและวิธีการทดลอง:** คัดเลือกสมุนไพรไทย 6 ชนิดที่มีประวัติการใช้เป็นยาพื้นบ้านมาอย่างยาวนานในการยับยั้งเชื้อแคนดิดา ได้แก่ กระเทียม ขิง ข่า กระทือ ไพล และว่านชักมดลูก ในรูปแบบสารสกัดสมุนไพรเดี่ยวและสารสกัดสมุนไพร 2 ชนิดผสมกันในอัตราส่วน 1 ต่อ 1 ระหว่างกระเทียมกับสมุนไพรชนิดอื่นๆ โดยทำการทดสอบฤทธิ์ในการยับยั้งเชื้อแคนดิดา (*Candida albicans* ATCC 10231) ด้วยวิธี agar disc diffusion และทดสอบหาค่าความเข้มข้นน้อยที่สุดที่สามารถยับยั้ง (MIC) และทำลายเชื้อแคนดิดา (MFC) ด้วยวิธี broth microdilution method แล้วจึงตั้งตำรับยาเม็ดสอดช่องคลอดจากสารสกัดสมุนไพรไทยที่มีฤทธิ์ในการยับยั้งเชื้อแคนดิดา **ผลการศึกษา:** สารสกัดผสมกระเทียมและข่ามีค่าบริเวณยับยั้งเชื้อ (11.5 mm) MIC (0.78 mg/mL) และ MFC (1.56 mg/mL) ดีที่สุด จึงพัฒนายาเม็ดสอดช่องคลอดจากสารสกัดกระเทียมและข่าจำนวน 6 ตำรับด้วยวิธีการทำแกรนูลเปียกและทดสอบค่าน้ำหนักแปรปรวน ความหนา ความแข็ง ความกร่อนและค่าละลายของเม็ดยา โดยประเมินตามข้อกำหนดของ USP35/NF30 สูตรตำรับที่ประกอบด้วย microcrystalline cellulose PH102 และแลคโตสเป็นสารเจือจาง และอาคาเชียเป็นสารยึดเกาะเหมาะสมในการใช้ผลิตยาเม็ดสอดช่องคลอด **สรุปผล:** มีความเป็นไปได้ในการพัฒนายาเม็ดสอดช่องคลอดจากสารสกัดกระเทียมและข่าเป็นผลิตภัณฑ์เพื่อยับยั้งเชื้อแคนดิดา

คำสำคัญ: ติดเชื้อแคนดิดาในช่องคลอด, กระเทียม, ข่า, ยาเม็ดสอดช่องคลอด

Development of Vaginal Tablets from Thai Herb Extracts for Candidiasis

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Abstract

Development of Vaginal Tablets from Thai Herb Extracts for Candidiasis

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Vulvovaginal candidiasis (VVC) affects women health worldwide. The United States Food and Drug Administration (U.S.FDA) has approved clotrimazole vaginal tablet in vaginal candida infection treatment. The incidence of *Candida spp* resistance to clotrimazole vaginal tablets has recently been increasing and the herbs for vaginal used for different treatments such as treatment vaginal candidiasis and unpleasant smell of vaginal discharge are available at online shopping in many countries. Many of those products available online are illegal trade and unsafe to use. The objectives of this study were to determine anticandidal activity, to formulate, and to evaluate vaginal tablets from some Thai herbs to treat VVC according to the requirements of USP35/NF30. **Materials and Method:** Selecting 6 Thai medicinal herbs with a long history of folk medicine which have proved effective treatment for anticandidal activity. The 6 Thai herbs included garlic, ginger, galangal, champoo ginger, cassumunar ginger and java ginger. The extracts of these herbs were used alone as well as a combination of two extracts between garlic and other herbs, with ratio of 1:1. Anticandidal (*Candida albicans* ATCC 10231) activities were tested by agar disc diffusion method. Minimal inhibitory concentrations (MIC) and minimal fungicidal concentrations (MFC) were determined by broth microdilution method. Furthermore, vaginal tablet was formulated and evaluated from the best anticandidal Thai herb. **Results:** The mixture of garlic and galangal extracts had the strong inhibition zone (11.5 mm) and great MIC (0.78 mg/mL) and MFC (1.56 mg/mL). Six vaginal tablets formulations were prepared using wet granulation and then weight variation, thickness, hardness, friability, and dissolution were evaluated based on the USP35/NF30. Formulation consisting of microcrystalline cellulose PH102 and lactose as a diluent and acacia as a binder were appropriate of vaginal tablet manufacturing. **Conclusion:** It is possible to bring an extract of garlic and galangal vaginal tablets to develop a product that inhibits *Candida albicans* infections.

Keywords: Vagina candidiasis, Garlic, Galangal, Vaginal tablets

Introduction

Vulvovaginal candidiasis (VVC) affects approximately 138 million women worldwide annually and more than a half of American women aged over 25 years old have experienced at least one time of being diagnosed to VVC (Sobel, 2016). Seventy-two percent for other populations have been reported a history of at least one time of VVC (Sobel, 1988). *Candida albicans* (*C. albicans*) is a major cause of VVC. *C. albicans* is an opportunistic infection and can also be found naturally in vaginal mucosa, intestinal tract, buccal mucosa and skin. This infection is caused by the imbalance of normal flora. This imbalance occurs when the body is weak, and it finally leads to *C. albicans* infection (Rattanasook, 2014). The different causes of vaginal candidiasis are easily found in pregnant women, diabetics, patients with other diseases that cause immune deficiency disease such as acquired immunodeficiency syndrome (AIDS), hormone imbalance, use of broad spectrum antibiotics or oral contraceptive, nutritional disorders, and poor hygiene. VVC symptoms include vaginal discharge, unpleasant vaginal odor, vaginal pruritus, irritation, soreness, and dyspareunia. The first line dosage regimen over-the-counter for treatment VVC is clotrimazole vaginal tablet (Sobel, 2016). Previous studies reported that there has been increasing incidence of *Candida spp* resistance to clotrimazole vaginal tablets. Clotrimazole resistance may be due to the treatment of VVC for a long time and most patients do not often follow the advice of the doctors and pharmacists, making the discontinuous medication (Preechasuth, 2008) and the overuse of antifungal by physicians for prophylaxis and/or treatment lead to an increase in resistance to azole in *Candida spp*, too (Vandeputte, 2012). Hence the appropriate use of antifungals medicines is important factors in fighting to drug resistance (Vandeputte, 2012) and the new generation of triazoles medicines include fluconazole, itraconazole, ravuconazole and voriconazole have extended antifungal spectrums may be used in first generation azole resistance. However, these azole antifungals medicines have renal and liver toxicity (Crowley & Gallagher, 2014). Therefore, nutrition therapy and/or alternative medicines in fungal infection are needed.

According to alternative medicines and oral yogurt containing *Lactobacillus acidophilus* are more attractive to use in VVC treatment. At the present online shopping in many countries have the herb for treatment vaginal candidiasis, bad smell vaginal discharge (Mintkyshop, 2017; Liverstrong, 2017). Some products are not approved by Food and Drug Administration (FDA) and illegal trade. These products are unsafe to use. According to Thailand's National List of Essential Herbal Medicines (NLEM) 2016, it recommended Leudngam and Faipralaikul recipe for reduce of leucorrhea in women. These regimens compose of many herbal medicines such as cassumunar ginger (*Zingiber cassumunar* Roxb.), champoo ginger (*Zingiber zerumbet* (L.) Roscoe ex Sm. subsp. zerumbet), garlic (*Allium sativum* Linn.) and ginger (*Zingiber officinale* Roscoe.) (NLEM, 2016). The medical use of garlic has been a history of use for centuries in antifungal activity, anticandida, antibacterial, antiviral, antiseptic and anti-inflammatory of garlic are well known (Onyechi, 2011). The 1'- acetoxychavicol and acetate 1'- acetoxyeugenol acetate are active components from galanga (*Alpinia galangal* (L.) Willd.), that have the anticandidal effect (Plant Genetic Conservation Project under the Royal Initiative of Her Royal Highness Princess Maha Chakri Sirindhorn, 2017). The effect of inhibiting *C. albicans* infection from java ginger extracted by distillation method with hot water revealed that MIC of xanthorrhizol was 8-32 µg/mL, which is the main substance in java ginger that can inhibit the *C. albicans*. (Sodachan, 2015). Moreover, Anankabundit (2017) showed the anticandidal activity of *Zingiberaceae* family extracts such as champoo ginger, ginger, galangal, cassumunar ginger, and java ginger. Galangal with cassumunar ginger showed the lowest minimal inhibitory concentrations value (MIC) (1.17 mg/mL) and minimal fungicidal concentration (MFC) was 3.12 mg/mL. In

addition many herbs have anticandidal activity example grape seed extract, tea tree oil and probiotics (Ignacio & Thai, 2005) but there are expensive herbs, unusual and hard to find in Thailand. The purpose of this study was determined anticandidal activity of mixing between garlic and *Zingiberaceae* family, that there are easy to find, and low cost. Then formulate and evaluate vaginal tablets include weigh variation, friability, thickness, hardness, and disintegration time that there are meet the requirements of universal standard agreement (USP35/NF30).

Materials and Methods

1. Materials

In this research, the dried power of garlic were acquired from V.P Pharmacy (Thailand). Pharmaceutical grade of microcrystalline cellulose PH 102, corn starch, lactose, magnesium stearate, talcum, and 95% ethanol were supplied by TTK Sciences (Thailand). Clotrimazole reference standard was obtained from Sigma Aldrich (USA.). All other reagents and solvents were analytical grade and were used as supplied.

2. Preparation and extraction of Thai medicinal herbs

Maceration garlic, cassumunar ginger, champoo ginger, galangal, and ginger in 500 g with 95% ethanol. Shaker at a temperature of 37 °C for 3 days, then filtered extracts filter paper No. 1. The solution was evaporated with a rotary evaporator at 100 rpm, temperature 50 °C pressure of 80 psi until the extracts had weight of 25 g equal 5% yield, except the java ginger which was prepared by water-steam distillation. Java ginger was cut into small square pieces then the steam was distilled until the %yield of java ginger was 5. All herb extracts are prepared at 1 mg/mL by 25 mg of herb extracts are dissolved in 25 mL of 10% DMSO and stored in refrigerator until used.

3. Inhibitory and extraction of Thai medicinal herbs

C. albicans ATCC 10231 culture on Sabouraud's dextrose agar (SDA, Diffco, USA) and incubated at 30 °C for 48 hours. *C. albicans* were suspended 1 loop in 0.85% sterile normal saline. Then the inoculum was standardized to equal the turbidity standard solution 0.5 McFarland (1.5×10^6 CFU/mL) (Anankabundit, 2017; Sodachan 2015).

4. Agar disc diffusion

Inoculate the agar by streaking with the cotton swab containing the inoculum. Then load single garlic extracts and combine garlic with java ginger, cassumunar ginger, champoo ginger, galangal, and ginger extracts at 1 mg/mL, 30 µL of herb extracts sample (Table 1) in sterile paper disc (6 mm diameter). Then press the sterile paper disc down lightly to ensure complete contact between the disc and the agar surface by using sterile forceps. Negative control was dimethyl sulfoxide (DMSO) and positive control was clotrimazole at 1 mg/mL incubated at 30 ° C for 48 hours. Measure the diameter of inhibition zone (mm). All tests were done in triplicate (Anankabundit, 2017; Sodachan 2015).

Table 1. Volume of herb extracts sample in the concentration of 1 mg/mL

Extracts (µL)	E1	E2	E3	E4	E5	E6
Garlic	30	15	15	15	15	15
Cassumunar ginger	-	15	-	-	-	-
Champoo ginger	-	-	15	-	-	-
Galangal	-	-	-	15	-	-
Ginger	-	-	-	-	15	-
Java ginger	-	-	-	-	-	15
Total	30	30	30	30	30	30

5. Broth microdilution

Test minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of *C. albicans* were conducted in microdilution method, 96 well plate. The MIC was defined at the lowest concentration that inhibited growth of *C. albicans* by light microscope. MFC were evaluated by transferring 0.1 mL from all clear MIC wells (no growth seen in microdilution trays) streak plate in Sabouraud's dextrose agar (SDA). The MFC was the lowest sample extracts concentration that killed $\geq$ 99.99% of cells (Anankabundit, 2017; Lima, 2012; Sodachan, 2015).

6. Wet granulation method for medicinal herb extracts vaginal tablets preparation

The previous study reported that when herb extracts were developed to a new product, the effective concentration ranged from 2-16 times of the MIC. Therefore, the active ingredients in vaginal tablets were 16 times of the MIC which was the highest in the range for the most effectiveness ingredient (Termrangsee, 2011; Macé *et al.*, 2017). First, mixed the extracts of garlic 6.25 mg and galangal 6.25 mg. This study mixing crude extracts in 1:1 ratio because this is the lowest ratio that are effective when developing commercial products which will lowered cost. Then mixed active pharmaceutical ingredients with the diluents i.e. microcrystalline cellulose PH 102, corn starch, and lactose were mixed together by geometric dilution method in mortar. The powder mixtures were moistened with the appropriate amount of binder solution i.e. acacia or gelatin. The wet mass was then screened through a 20-mesh sieve to produce granules. The wet granules were dried at 60 °C for 0.5-1 hour. in a hot air oven. After that, the dried granules were screened all over again through a 20-mesh sieve. The granule were mixed with talcum and magnesium stearate for 1 min, and the tablets were prepared by single punch tableting machine. The ingredients ratio was presented in Table 2. After that, the vaginal tablets were evaluated the physical properties.

Table 2. Composition of vaginal tablets from garlic and galangal extracts (mg)

Ingredients	Categories	T1	T2	T3	T4	T5	T6
Garlic extract	Active ingredient	6.25	6.25	6.25	6.25	6.25	6.25
Galangal extract		6.25	6.25	6.25	6.25	6.25	6.25
Gelatin	Binder	15	15	15	15	15	15
Acacia	Binder	-	13	-	-	-	-
Talcum	Glidant	1	1	1	1	1	1
Magnesium stearate	Lubricant	3	3	3	3	3	3
Microcrystalline cellulose PH102 qs	Diluent	450	450	-	-	-	-
Corn starch qs	Diluent	-	-	450	-	225	97.5 (intragranular)#
Corn starch qs	Diluent	-	-	-	-	-	127.5 (extragranular)#
Lactose qs	Diluent	-	-	-	450	225	225

#corn starch-intragranular mean the corn starch was added to active ingredient prior to the addition of the binding for made the granules; corn starch-extragranular mean the corn starch was added to the granules after drying.

7. Physical properties evaluations

Weight variation: The value of weight variation test is calculated by the following formulae

$$\text{Weight variation} = \frac{(\text{individual weight of tablet} - \text{average weight of tablet})}{\text{average weight of tablet}} \times 100\%$$

The weight variation is expressed in percentage. Twenty tablets were randomly weighed individually using an analytical balance. The average weights were calculated and compared the individual tablet weights to the average. The test accepted value by the USP35 if it was not more than 2 tablets of the individual weights deviate from the average weight by more than the percentage deviation (±5%) and none deviates by more than twice that percentage (Monton, 2014).

Friability: Dust were removed from twenty tablets before testing. Ten tablets were randomly weighed together. Friability was tested by Auto-friability tester for 5 min of rotation at 25 rpm, any loose dust from the tablets was removed and weighed again. If friability was not more than 1%, it was considered meeting the requirement.

$$\text{Friability} = \frac{(\text{weight before} - \text{weight after})}{\text{weight before}} \times 100\%$$

weight before = weight of vaginal tablets before test (mg)

weight after = weight of vaginal tablets after test (mg)

Thickness and Hardness: Ten tablets were evaluated using hardness tester (Phama Test Operating Manual Type PTB 311E). Results were reported as mean ± SD.

Disintegration time: Six tablets were measured by a disintegration tester (AUTO EZ Series Tablet Disintegration Testing Instrument Version 2.0) following the USP method, and simulated vaginal fluid (pH 4.2) was used as the disintegration medium at 37±2 °C (Kumar, 2016).

8. Statistical analysis

Data were analyzed by one-way ANOVA analysis of variance. $p < 0.05$ was considered statistically significant.

Results and discussion

The physical appearance of a vaginal tablets finished products was brown, and had smooth surface. Formulation T1, T2, and T4 produced vaginal tablets in the acceptable limits of USP35/NF30. Weight variation of these 3 formulae had deviation of < 5%, which was required in USP for tablets weighing more than 324 mg. (Kumar, 2016), friability less than 1%, hardness 2.7-4.5 kp, and disintegration time less than 20 min, in accordance with USP standard of vaginal tablets. T3 and T5 did not compress to tablets due to corn starch was used as a diluent for these formulae. This finding is consistent with previous studies of a high proportion of corn starch resulted in reducing the powder flowability and decreasing tablet hardness. The corn starch has a poor binding ability (Leesawat, 2004). Odeku and Akinwande (2012) found that tablets containing corn starch incorporated intragranularly showed slower disintegration but higher tensile strength than those containing starches incorporated extragranularly. Therefore T6 formulae using a corn starch extragranular showed a faster disintegration time, high friability and all of formulae used corn starch did not meet the USP standard. Thus, T3 and T5 used corn starch as a diluent don't suite for a tablet production because they had high percentage of friability. Friability properties is

important during packaging, shipping and dispensing to patients (Onyechi, 2011).

When using acacia as a binder in T2, it showed highest hardness significantly (p<0.05) when compared to T1 and T4 and fastest disintegration time that correlated to previous studies that acacia had a fastness of disintegration time more than gelatin and sodium carboxymethylcellulose. When increasing concentration or proportion of binders give increasingly tablets hardness and disintegration time. Therefore, acacia prefer to use in tablet binder more than gelatin in Thai herb extracts vaginal tablet. Vaginal tablets in all formulations were prepared by wet granulation method and all physical properties were shown in Table 3. There properties of T1, T2 and T4 formulae were followed by The USP 35/NF 30.

The anticandidal activity of Thai medicinal herb extracts by disc diffusion and broth microdilution method on MIC were shown in Table 4. The MIC of E4 showed the lowest significant (p<0.05) when compared among herb extracts. The MFC of combine extracts between garlic and galangal extracts (E4) was the lowest in all herb extracts (1.56 mg/mL) which the MFC value was better than the previous study of Anankabundit (2017) reporting that the galangal and cassumunar ginger extract in 6.25 mg of each herbs had the MFC was 3.12 mg/mL. The active ingredient in vaginal tablets developed from the extracts of garlic and galangal in the same concentration in this study showed higher anticandidal activity than previous study. The results support Thai traditional wisdom that garlic and galangal have been used for a long time in antifungal activity.

Table 3. Physical properties of garlic and galangal vaginal tablets

Ingredient	T1	T2	T3	T4	T5	T6
Average weight (mg)	431±0.01	452±0.01	NA	479±0.01	454±0.02	511±0.01
Friability (%)	0.45	0.31	NA	0.04	NA	1.8
Thickness (mm)	8.4±0.02	9.1±0.03	NA	8.9±0.05	NA	9.2± 0.01
Hardness (kP)	3.9±0.32	4.5±0.2*	NA	2.7±0.55	NA	2.6±0.71
Disintegration time (min)	6.5	3.0	NA	3.2	2.2	2

NA: not available

*P < 0.05 indicates statistically significant

Table 4. Anticandidal activity of E1-E6 and clotrimazole

Extracts	Inhibition zone (mm.)	MIC (mg/mL)
E1	0.8±0.00	4.685
E2	0.6±0.00	6.25
E3	No activity	
E4	11.5±2.12	0.78*
E5	0.75±0.07	3.12
E6	0.6±0.00	12.5
Clotrimazole (1 mg/mL)	37.3± 3.23	0.0125

*P < 0.05 indicates statistically significant

Conclusions

The results indicated that T2 formulation comprised of microcrystalline cellulose pH102 as a diluent and acacia as a binder were the most appropriate formulation for vaginal tablets and complied with the requirement specified in the USP35/NF30. The vaginal tablet was developed from garlic and galangal extracts because this combined extract showed the high potency of anticandidal. It is possible to bring an extract of garlic and galangal vaginal tablets to develop a product that inhibits *C. albicans* infections. Further study will investigate clinical efficacy, topical toxicity and stability test.

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References

Anankabundit K, Juthaporn J, Songsummanus A, *et al*. Anticandidal activity of Zingiberaceae family extracts. The 5th Academic Science and Technology Conference (ASTC) 2017; 1535-1541.

Crowley PD, and Gallagher HC. Clotrimazole as a pharmaceutical: past, present and future. Journal of Applied Microbiology 2014; 117: 611-617.

Ignacio C, and Thai D. Comparative analysis of antifungal activity of natural remedies versus miconazole nitrate salt against *Candida albicans.* Biological Sciences Department, San Luis Obispo 2005; 1-25.

Kumar D, Singh J, Antil M, et al. Quality control of tablets: A review. International Journal of Universal Pharmacy and Bio Sciences 2016; 5(4): 53-167.

Leesawat P, Laopongpaisan A, Sirithunyalug J. Optimization of direct compression aspirin tablet using statistical mixture design. CMU Journal 2004; 3(2): 97-112.

Lima IO, Nobrega FM, Oliveira WA, et al. Anti-*Candida albicans* effectiveness of citral and investigation of mode of action. Pharmaceutical Biology 2012; 50(12): 1536-1541.

Livestrong. Herbs for vaginal odor [Online]. 2016 July 1 [cited 2 Jan 2017] Available from: http://www.livestrong.com/article/249729-herbs-for-vaginal-odor/

Macé S, Hansen LT, Rupasinghe V. Anti-bacterial activity of phenolic compounds against *Streptococcus pyrogenes*. Medicine 2017; 4(25): 1-9.

Mintkyshop. Vaginal insert from herbs [Online]. 2017 Feb 2017 [cited 2017 July 1] Available from: http://www.white-qutashop.com

Monton C, Saingam W, Suksaeree J, *et al*. Formulation development and physical properties study of Thai traditional herbal tablets: original JIT-TRA-ROM recipe. International Journal of Pharmacy and Pharmaceutical Sciences 2014; 6(4): 611-614.

Odeku O, Akinwande BL. Effect of the mode of incorporation on the disintegrant properties of acid modified water and white yam starches. Saudi Pharmaceutical Journal 2012; 20: 171-175.

Onyechi JO, Chime SA, Onyishi IV, *et al*. Formulation and evaluation of *Allium Sativum* tablets for improved oral delivery. International Journal Pharmaceutical Sciences Review and Research 2011; 22(1): 6-10.

Plant Genetic Conservation Project under the Royal Initiative of Her Royal Highness Princess Maha Chakri Sirindhorn. Galangal [Online]. 2016 Feb 5 [cited 2017 Jun 1]. Available from: http://www.rspg.or.th/plants_data/herbs/herbs_02_1.html

Preechasuth K, Trakulpua K, Pawichai S, *et al*. Susceptibility of Yeast Isolated from Vaginal Swab to Antifungal Drugs in Chiang Mai. *J Med Tech Assoc Thailand* 2008; 36(2): 2387-2395.

Rattanasook S. Efficiency of local medicinal plant on growth inhibition of Candida albicans. Kamphaeng Phet Rajabhat. Journal of Sciences and Technology 2014; 1(2): 33-40.

Sobel JD, Faro S, Force RW, *et al*. Vulvovaginal candidiasis: Epidemiology, diagnostic, and therapeutic considerations. Am J Obstet Gynecol 1998; 178(2): 203-211.

Sobel JD. Recurrent vulvovaginal candidiasis. Am J Obstet Gynecol 2016; 214(1): 15-21.

Sodachan J, Sungthong B, Rattanakiat S. Chemical Profiling and Antimicrobial Activities against Oral Pathogens of Ocimum spp. Essential Oils. IJPS 2015; 11(Supplement): 304-310.

Temrangsee P. Antibacterial activity of herbal extracts against clinical isolates from wound infection. A Thesis for the Degree of Master of Sciences in Applied Thai Traditional Medicine, Faculty of Medicine, Thammasat University 2011.

The Subcommittee. Development national list of essential drugs. The National Committee with its medicine. The national list of essential drugs in 2016 (accounts. From the herb) Bangkok: rally cooperatives. Agriculture net operating Thailand; 2016; 34-36.

Thailand national list of essential medicine (NLEM). Thai Herbal Medicine development [Online]. 2016 Jan 1 [cited 2017 Aug 8]. Available from: http://drug.fda.moph.go.th:81/nlem.in.th/sites/default/files/ratchakitcha-nlem_ver_2_2559.pdf

The United States Pharmacopoeia Convention. The United States Pharmacopoeia 35/National Formulary 30, Maryland: United Book Press; 2013.

Vandeputte P, Ferrari S, Coste AT. Antifungal resistance and new strategies to control fungal infections. International Journal of Microbiology 2012; 1-27.