การพัฒนาผลิตภัณฑ์สปา ตำบลบางระกำ อำเภอบางเลน จังหวัดนครปฐม: กรณีศึกษา เบื้องต้นเถาวัลย์เปรียง เถาเอ็นอ่อน และว่านนางคำในลูกประคบสมุนไพร Development of the Spa Products in Bangrakam Subdistrict, Banglen District, Nakhon Pathom Province: Primary Case Study of Derris scandens, Cryptolepis buchanani and Curcuma aromatica in Herbal Compress

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บทคัดย่อ

การวิเคราะห์องค์ประกอบทางเคมีเบื้องต้นของสารสกัดน้ำเถาวัลย์เปรียง เถาเอ็นอ่อนและว่านนางคำ พบว่า สมุนไพรทั้งสามมี สารต้านอนุมูลอิสระ และเฉพาะเถาวัลย์เปรียงเท่านั้นที่พบฟลาโวนอยด์ การศึกษาฤทธิ์ผ่อนคลายกล้ามเนื้อของลูกประคบสมุนไพร กับกลุ่มผู้ทดสอบเฉพาะโดยใช้แบบสอบถามความพึงพอใจ 5 ระดับ และมีข้อเสนอแนะปลายเปิด พบว่า ผู้ทดสอบรู้สึกผ่อนคลาย กล้ามเนื้อหลังใช้ลูกประคบสมุนไพรสูตรที่มีเถาวัลย์เปรียงและเถาเอ็นอ่อน ซึ่งมีความแตกต่างอย่างมีนัยสำคัญทางสถิติที่ 0.05 (p<0.05) ในขณะที่สูตรที่มีว่านนางคำผสมเถาวัลย์เปรียงและเถาเอ็นอ่อน ตามลำดับ มีแนวโน้มช่วยเสริมฤทธิ์ผ่อนคลายกล้ามเนื้อได้ รวมทั้งให้ความชุ่มชื้นและความนุ่มเนียนกับผิวหลังการประคบอีกด้วย สำหรับความพึงพอใจคุณภาพโดยรวมของสูตรลูกประคบ สมุนไพรทั้งหมด จะเห็นว่า ผู้ทดสอบพึงพอใจสูตรที่มีเถาวัลย์เปรียง เถาเอ็นอ่อนและว่านนางค่ำเป็นส่วนประกอบทั้งหม[ิ]ด อย่างไร ก็ตาม ควรใช้เครื่องมืออิเล็กโทรไมโอแกรม (electromyogram) วัดการทำงานการหดเกร็งของกล้ามเนื้อด้วย เพื่อยืนยันผล การทดสอบการออกฤทธิ์ผ่อนคลายกล้ามเนื้อให้น่าเชื่อถือยิ่งขึ้น

คำสำคัญ: ลูกประคบ เถาวัลย์เปรียง เถาเอ็นอ่อน ว่านนางคำ ตำบลบางระกำ อำเภอบางเลน จังหวัดนครปฐม

Abstract

The preliminary phytochemical analysis of Derris scandens, Cryptolepis buchanani and Curcuma aromatica aqueous extracts showed free radical scavenging activity and only D. scandens was found that containing flavonoids. The herbal compress, comprising of D. scandens, C. buchanani and C. aromatica as an active ingredient, was studied on relieving of muscular pains and aches in the purposive sampling using a 5-Heidonic scale satisfactory questionnaire with an opening comment. For the results, it was found that the volunteers perceived to relieve a muscular tension after treatment of the herbal compress formula that containing D. scandens and C. buchanani with a statistical difference at 0.05 (p<0.05). While the formulae containing C. aromatica / D. scandens and C. aromatica / C. buchanani trended to be a synergism as a tendolysis activity. Moreover, C. aromatica also exhibited as a skin softener and a moisturizer. Generally, all volunteers pleased with the quality of the herbal compress formula that comprising entirely D. scandens,

C. buchanani and C. aromatica. However, this activity should be further confirmed by using an electromyogram as the scientific equipment for determination of muscular contractility.

Keywords: herbal compress; Derris scandens; Cryptolepis buchanani; Curcuma aromatica; Bangrakam subdistrict, Banglen district, Nakhon Pathom Province

1. INTRODUCTION

Bangrakam subdistrict is located in Banglen district, Nakhon Pathom Province and also has 15 villages which cover about 30 square kilometers. The landscape is a low plan area and there is the Ta Jeen River flowing through. The principal career of people who inhabit in the community is an agriculturist, and also an employee and an owner are subordination [1]. Some housewife and older have gotten together the united groups, such as a spa group, following their attention and aptitude to propose for main or supply earning. In addition, major customers of the spa group are inhabitants who have pains and aches after routine. After our group has organized the spa group's workshop, we found that many products which were used for massage and distributions have been mostly available from elsewhere. This affect gets them to have a little income. Thus, the members have required the manual preparation of the spa products and the herbal compress is the first choice product.

The herbal compress is a spa product containing a variety of fresh or dried herbs that are known to remedy deformed tendon, ached and a tired muscle, to relieve a swell and inflammation, and also to treat dermatitis. All herbs are mixed and packed in a calico as a ball, after steamed, and it's taken to compress on the organ. The action of remedy is the releasing of active compounds from herbs via a stored heat inside a ball [2]. Probably, three herbs, Derris scandens, Cryptolepis buchanani and Curcuma aromatica, could be interestingly used as an important ingredients in the herbal compress.

Derris scandens (Roxb.) Bent. (family: Legumminosae), knew as Thao Wan Priang, is one of the alternative medicinal plants commonly used in Thailand [3], [4]. D. scandens is a woody vine growing throughout Southeastern Asia. Its natural leaves are similarly sorted as a feather. The flower is a pale pink likely as a bean flower. The fruit of D. scandens is a sheath. There are two kinds of D. scandens, red and white, that show similar properties. Normally, the white kind is popular used because the red one is difficultly available. In Thai traditional medicine [5], D. scandens stem was widely used instead of tea leaves for drinking with treatment of expectorant, antitussive, diuretic, antidysentery, ache, beriberi and cachexia. Biologically, D. scandens aqueous extract displayed anti-inflammatory activity both in vivo and in vitro experiments [6], anti-free radical scavenging activity [7], [8], anticancer [9] and diabetes [10]. In addition, it was found that a 400 mg/day dosing of D. scandens extract was used for immunostimulating activity [11]. Recently, the Department of Medicinal Sciences has successfully research of the D. scandens extract to cure back and joint pains [12]. Moreover, it would be further promoted to be an inflammatory medicine instead of a steroidal drug from the foreign countries. Most chemical constituents, which had been reported, were free and glycoside isoflavones [13], [14], [15], such as chadalone (1), derriscandenoside A-E (2-6), derriscannoside A-B (7-8) and etc (Figure 1).

Cryptolepis buchanani Roam. & Schult (family: Asclepiadaceae) are commonly called in Thai name as Thao En On. It is distributed throughout hot deciduous forests of Southeastern Asia including Thailand. Its botany is a woody vine with inner white latex, a yellow flower, a double sheath and a single leaf [3]. Ethnologically, C. buchanani was used as a medicine [16] for anti-diarrhoeal, anticulcerative, anti-inflammation, cough treatment and antibacterial activity, by drinking of the stems or leaves aqueous extracts. Its pharmacology had been reported such as anti-inflammatory activity [17], [18], whose cause was muscular and joint pains and arthritis, immune stimulating activity [16], [19], [20], antifungal activity against a human dermatophytic fungi [21], antibacterial [22], [23] and cardiac [24] activities. The major chemical constituents [22], [25], [26], [27] were found in this plant, were cardenolide glycosides i.e. cryptosin (9) and cryptanosides A (10), and alkaloids such as buchananine (11) together with keto fatty acids [28] (Figure 2).

Curcuma aromatica Salisb, commonly known as Wann Naang Kham (or wild tumeric), belongs to the family Zingiberaceae. It is an erect perennial species herb scattering throughout the tropical and subtropical regions of the world and is widely cultivated in Asia countries including Thailand. Its rhizome is a light yellow and possesses a camphoraceous odour [3]. Historically, C. aromatica rhizome was used as an antibiotic against various microbial infections, tonic, carminative, rash, infected and blued skin and improvement of complexion. The root of C. aromatica has been also used as expectoration and chronic gonorrhea. The bioactive potential activities were reported to exert various activities such as antibacterial and antifungal activities [29], healing for wound, inflammation [30] and muscular contraction [31]. The active compounds were found in C. aromatica rhizome contained largely curcuminoids, curcumin (12), demethoxycurcumin (13) and bis-demethoxycurcumin (14) (Figure 2) as antioxidant and whitening agents [32]. Mainly, volatile oil composed of camphor, borneol, caryophyllene oxide, camphene, curcumadione and curcumol [33], [34], [35], [36].

Figure 1: Active constituents from D. scandens

Figure 2: Active constituents from C. buchanani and C. aromatica

As according to, people have currently preferred to use the herbal compress for relieving muscular aches and pains. Also, many formulae have been prepared depend on herbs in each area [37]. However, its specification must contain at least three herbs, Zingiber cassumunar (or Plai), Curcuma longa (or Kha Min Chan) and Cymbopogon citratus (or Ta Krai), that has been assigned by the Thai Community Product Standard (TCPS) no. 176/2003 [38]. Therefore, the aim of this research is to study and develop the herbal compress formulae, containing D. scandens, C. buchanani and C. aromatica that could be used in following their properties. The useful of this research, we hope that it would be increasing of their efficacy and added product value. Additionally, the local people would have more earn corresponding to the local development planning of Bangrakam subdistrict in 1998 [39] that has been assigned to inhabitants for learning of a research methodology by themselves and to live on one's own toil for sustainable.

2. MATERIAL AND METHODS

2.1 Plant materials

All fresh herbs, D. scandens stems, C. buchanani stems, C. aromatica rhizomes, Z. cassumunar rhizomes, C. longa rhizomes, C. citratus aerial part, Citrus hystrix peels, Tamarindus indica old leaves and Acorus calamus rhizomes, were collected surrounding Nakhon Pathom Province.

2.2 General procedures

The dried and ground D. scandens or C. buchanani or C. aromatica, 20 g, was macerated with water at 60-70 °C during 6 hrs. Then, it was filtered to afford the aqueous extracts of *D. scandens* or *C. buchanani or* C. aromatica, respectively.

The R_f value was carried out on TLC aluminum sheet as Kiesel gel 60 (SiO₂), F_{254} (230-400 mesh) of Merck.

2.3 Phytochemical screening methods

1. Flavonoids screening as follows: [40]

• Flavones, Flavonols and Flavonones:

A testing method was usually called as a cyanidin reaction. Three or four small pieces of magnesium metal following by 10 drops of conc. HCl were added into the extract. The color of solution was observed in order to classify the presence of flavonoids that would specifically display their color, a yellow to a red as flavones, a crimson as flavonols, a green or a blue as a flavonones and a magenta as flavonone glycosides.

Anthocyanidins:

1% HCl was added into the extract and then heated to appear an orange to a bluish red solution as a positive test.

• Leucoanthocyanidins:

2 M HCl and 1-propanol were added into an extract and then heated for 15-30 min to afford a red or purple solution as a positive test.

2. Anti-free radical scavenging screening as follows:

Each extract was spotted on TLC and then developed in a chamber containing a suitable mobile phase of each aqueous extract, 75% ethanol/ethyl acetate for D. scandens extract, 75% ethanol/H₂O for C. buchanani and 75% methanol/H₂O for C. aromatica, until the mobile phase reached to the solvent front. Then, it was carried off to give a chromatogram and the position of the spot was marked after detection with $UV_{254 \text{ and } 366 \text{ nm}}$ lamps and further sprayed over with 1.2×10^{-4} M DPPH. A positive test showed an off-purple spot whose R_f value was calculated as follow:

 R_f = A distance of compound spot / A distance of solvent front

2.4 Preparation of the herbal compress

All herbs were cleaned and then sliced into the smaller pieces before incubating at 60-70 °C until dry. Each ingredient of herbal compress formula was weighed following the ratio (Table 1) and then mixed together. A mixture was filled into a calico and tightly packed with a rope yarn to afford the herbal compress as a ball with a diameter of 9 cm.

The efficacy of the tested herbal compress, was steamed for 5 min before using, was compared to a control that were performed by a 5-Heidonic scale satisfactory questionnaire with an opening comment. Statistical data analysis was evaluated by Microsoft office Excel 2007. The sampling method was a purposive sampling [41] for using 10-15 volunteers/formula who had aches and pains.

Table 1: Weight of active ingredients in a variety of herbal compress

Ingredients	Weight (g) / formula						
	control	1	2	3	4	5	6
Z. cassumunar rhizomes	37.5	37.5	37.5	37.5	37.5	37.5	37.5
C. longa rhizomes	15.0	15.0	15.0	15.0	15.0	15.0	15.0
C. citratus aerial part	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Citrus hystrix peels	3.75	3.75	3.75	3.75	3.75	3.75	3.75
Tamarindus indica old leaves	7.5	7.5	7.5	7.5	7.5	7.5	7.5
Acorus calamus rhizomes	7.5	7.5	7.5	7.5	7.5	7.5	7.5
camphor	3.75	3.75	3.75	3.75	3.75	3.75	3.75
D. scandens stems	-	7.5	-	-	7.5	-	7.5
C. buchanani stems	-	-	7.5	-	-	7.5	7.5
C. aromatica rhizomes	-	-	-	7.5	7.5	7.5	7.5

3. RESULTS AND DISCUSSION

The qualities of D. scandens, C. buchanani and C. aromatica were identified by the preliminary phytochemical testing for flavonoids and free radical scavenging activity. Consequently, it was found that the flavonoids screening was only appeared in D. scandens aqueous extract [21], [42], while all extracts showed the free radical scavenger constituents (Table 2). We expected that they contained flavonoids and/or phenolic compounds. Mechanically, these compounds (Ar-OH) were reacted with DPPH radical to give the most stable radical (Ar-O'). In addition, the radical on the oxygen atom could be delocalized, commonly called as a resonance effect (Figure 3).

Table 2: Preliminary phytochemical analysis of D. scandens, C. buchanani and C. aromatica aqueous extracts

A ativa a constituents	Tested results					
Active constituents	D. scandens	C. buchanani	C. aromatica			
Flavones, Flavonols and Flavonones	+	-	-			
Anthocyanidins	+	-	-			
Leucoanthocyanidins	-	-	-			
Anti-free radical scavengers	$+ (R_f = 0.45 - 0.55)$	$+ (R_f = 0.7-0.85)$	$+ (R_f = 0.6-0.8)$			

Figure 3: Reaction between DPPH radical and anti-free radical compound

Comparatively, the herbal compress (formulae 1-6) consisting of D. scandens, C. buchanani and C. aromatica were tested for their effects with the control formula containing rhizomes of Z. cassumunar [43] and C. longa [44] with anti-inflammation and relieving for aches and pains, C. citrus peels for treatment of nausea [45], T. indica leaves for anti-itching [46] and camphor [44], A. calamus rhizomes and C. citratus aerial part [47] for an aroma. The effects were tested with the specified volunteers and then evaluated by using the 5-Heidonic scale satisfactory questionnaire with an opening opinion. For the results (Table 3), it was found that both formulae 1 (3.50 score) and 2 (3.48 score) comprising D. scandens and C. buchanani, respectively, showed to relieve muscular aches and pains with a statistical difference at 0.05 (p<0.05). The volunteers preferred the formulae 4 (3.10 score) and 5 (3.40 score), containing C. aromatica/D. scandens and C. aromatica/ C. buchanani, respectively, by comparison with the formula 3 (3.07 score) that included only C. aromatica. This means that C. aromatica could be inclined to a synergism of D. scandens and C. buchanani. In addition, C. aromatica also provided a good skin softening and moisturizing effects. In general, the herbal compress composing of D. scandens, C. buchanani and C. aromatica especially formula 6 (p<0.05), that had three herbal components, which showed higher quality than control.

Table 3: Satisfactions of the herbal compress formulae using a 5-Heidonic scale questionnaire

ltems —	Satisfactions / the herbal compress formula						
	control	1	2	3	4	5	6
to relieve aches and pains	3.00 ^{a, b}	3.50 ^a	3.48 ^b	3.07	3.10	3.40	3.33
aroma	3.07 ^c	3.10	3.07	3.83 ^c	3.33	3.40	3.40
skin moisturizer	3.26	3.47	3.10	3.33	3.40	3.27	3.54
skin softener	3.38 ^d	3.47	3.69	3.26	3.50	3.20	3.83 ^d
average	3.18	3.39	3.34	3.37	3.33	3.32	3.53

^{a, b, c, d} were a statistical difference of 0.05 (p<0.05)

4. CONCLUSION

D. scandens and C. buchanani in herbal compress should be used for a muscular relaxation, while C. aromatica trended to be a synergism including skin moisturizer and softener. Unfortunately, these results were evaluated from small amounts of the available volunteers. Thus, it should be accurately confirmed by using an electromyogram which is the scientific equipment. Furthermore, we have continually attempted for research to develope the herbal compress which leading to its standard in the future.

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