

EFFECT OF ALOE VERA AND WAX GOURD EXTRACTS IN REDUCING BURNING SENSATION OF CAPSICUM GEL

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ABSTRACT: The aim of this research was to develop a capsicum analgesic gel with a reduced burning sensation. The study started by determining a suitable vehicle with optimal proportions. The soothing substances, aqueous extracts of aloe vera and wax gourd, were used with a formulary concentration of 0.0125% capsaicin. A clinical trial was conducted with 30 healthy volunteers to test the product's efficacy. It was observed that after applying the capsicum analgesic gel the burning sensation started with itching, then flushing followed by burning. The maximum burning sensation was experienced at 30 min., after which time it gradually declined and finally dissipated at 480 min. The burning sensation when using capsicum gel with aloe vera and wax gourd was reduced by 21.49% and 36.25%, respectively. The concentration of soothing vehicle was correlated with the burning sensation while the burning sensation was related to the skin erythema with a correlation coefficient (R^2) of 0.9584.

Keywords: capsicum gel, burning, aloe vera, wax gourd

INTRODUCTION: Capsicum is an annual crop which is found all over tropical countries such as Africa, Mexico, India and Asia. In Thailand, it is commonly found in home vegetable gardens. The pungent substances called "capsaicinoids" consist of capsaicin, dihydrocapsaicin, nordihydrocapsaicin, homocapsaicin and homodihydrocapsaicin^{1,2}. Capsaicin ($C_{18}H_{27}NO_3$) is a phenolic amide whose chemical name is 8-methyl-N-vanillyl-6-nonenamide or (*E*)-8-Methyl-*N*-vanillyl-6-nonenamide. The colorless crystal has a molecular weight of 305.46 and a melting point of 65°C, and a boiling point of 210-220 °C³. It is insoluble in cold water, but soluble in ethanol, ether and acetone. Capsaicinoids are located in the inner wall of the fruit and the septum of the placenta. The maximum amount of 89% capsaicin is found at the dissepiments⁴. Capsaicin is an analgesic due to selective action on the small diameter afferent nerve fibers, or C fibers of nocicepters which are believed to mediate muscle pain. It is believed that substance P is released and it is this which causes the burning sensation. After repeatedly applying capsaicin, substance P is depleted and then the pain is relieved⁵⁻⁷. Capsicum products such as creams and gels contain 0.0125-0.075% capsaicin. They are used to relieve pain from osteoarthritis, rheumatoid

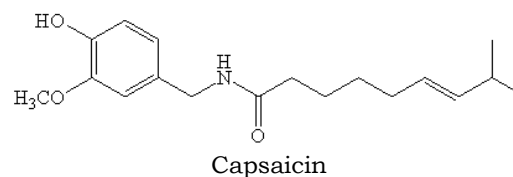
arthritis, and neuropathic pain. However, these products may cause irritation and burning skin even though they are potent pain relievers⁸.

In our laboratory, an analgesic gel containing capsicum extract with soothing vehicles was formulated. In order to assure the efficacy of this product, the reduction in the burning sensation was evaluated in this study.

MATERIALS AND METHODS:

Materials

Fresh plant materials (Capsicum Yod Son, Aloe Vera, and Wax Gourd) were purchased from a local market, Pathumthani Province. Chemicals purchased from PC drug center, Thailand were carbopol 934, carbopol 940, polysorbate 20 (tween20), methyl paraben, propyl paraben, propylene glycol, and ethyl alcohol. Chemicals imported from BASF, Germany were neutrol TE[®] (N,N,N',N'-Tetrakis (2-hydroxypropyl) ethylenediamine and uvinul MS 40[®] 3-(4-methylbenzylidene)-camphor. EDTA sodium was purchased from Carlo ERBA, France. Solvents purchased from



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Merck, Germany were acetonitrile and acetic acid. Acetone was purchased from LAB-SCAN, Thailand. The other instruments were a blender (Otto[®] PRO) and Cutometer MPA580 (CK electronics).

Chromatographic system

HPLC-UV (Thermo Separation Product[®]) consisted of Spectra system sn4000, Spectra system p1500, Spectra system uv1000, and Spectra system as3000. HPLC column (Phenomenex[®]) containing C18 with particle size 5 micron and diameter 4.6×250 mm. Mobile phase was acetonitrile : water : acetic acid (60 : 40 : 1). Flow rate was 1 ml/min. Injection volume was 20 µl. UV detector was set at wavelength 280 nm.

Preparation of capsicum extract

Capsicum materials were collected and dried at 50°C for 4 hr. The dry plants were then blended using the electric blender and passed through a no.60 sieve. 50 grams of dry capsicum powder was extracted with ethanol with the proportions of 1:5 using the sonicator for 30 min. The crude extract was then filtered under vacuum and passed through a no.1 paper filter and washed with 50 ml ethanol 3 times. The ethanol extract was concentrated using the rotary evaporator. The capsaicin in the capsicum extract was then analyzed using the HPLC technique.

Preparation of aloe vera extract

Two year-old aloe vera plants were selected and the leaves were minced. The leaves were peeled and washed with water. The leaves were cut and blended into small pieces. The pieces were heated in water at 70-80 °C for 30 min. The water extracts were then filtered through cotton cloth and kept in a tightly sealed container in the refrigerator at 4-8 °C.

Preparation of wax gourd extract

Three month-old wax gourd fruit were selected. The outer peels and inner seeds were separated and thrown away. The fruits were cut and blended into small pieces. The pieces were heated in water at 70-80 °C for 30 min. The water extracts were then filtered through cotton cloth and kept in a tightly sealed container in the refrigerator at 4-8 °C.

Preparation of capsicum gel

Eleven formulations of capsicum gel containing 0.0125% capsaicin were prepared using water, aloe vera extract and wax gourd extract at 0%, 10%, 25%, 50%, 75% and 100% as the soothing vehicle as shown in Table 1. First the capsicum extract was dissolved in ethanol. Then, Carbopol 940 was ground in the mortar and dispersed with uvinul MS 40[®], EDTA was added to the soothing vehicle and then mixed. Next, TWEEN[®], Paraben conc., and Neutral TE[®] were dissolved in water and mixed. Finally all the ingredients were mixed together in the mortar to form a smooth gel. Finally, the capsicum extract was added to gel to make the capsicum gel.

The evaluation of burning sensation level

The burning sensation level was measured after applying the capsicum products in order to compare them with the commercial product. The study was approved by the Institutional Ethics Committee at Faculty of Pharmacy, Srinakharinwirot University on January 2008. A clinical trial was conducted on 30 healthy volunteers, aged 20-23 yr. The subjects were instructed to apply 200 mg of each of the test products to the lower arm to an area of 5×10 cm. The burning sensation was recorded after applying the test product at 5, 15, 30, 120, 240, 360 and 480 min on a work sheet to record the burning sensation as shown in Table 2. The burning sensation was rated on a 9-point severity scale (0 – 9). The degree of burning sensation for each formulation was compared with the commercial product.

The evaluation of erythema after applying capsicum gel

The erythema value was measured by Cutometer[®] in 30 healthy volunteers. The changes in the erythema value were determined in terms of the difference between before and after applying the capsicum gel. The maximum burning sensation was at 30 min and then it declined to nil 480 min after applying the product. The difference of the erythema value from the base line was then analyzed for four test products; capsicum gel with three different vehicles; water, aloe vera extract and wax gourd extract and these

figures were compared with the commercial product.

RESULTS:

Physicochemical properties of capsicum gel containing the soothing vehicles

The capsicum gel containing water as a vehicle was dark orange in color, had a very strong odor, a high viscosity and pH > 7. The capsicum gel containing 100% aloe vera extract as a vehicle was orange in color, had a strong odor, medium viscosity and pH < 7. The capsicum gel containing 100% wax gourd extract as a vehicle was pale orange in color, had a less strong odor, lower viscosity and pH < 7. The results showed that among the three soothing vehicles, the capsicum gel containing 100% aloe vera had the best physicochemical properties as shown in Table 3.

The effect of the different of vehicles on reducing burning sensation of capsicum gel

The burning sensation experienced by healthy subjects after applying the four test products was similar. The level of the burning sensation showed a typical bell shaped curve. The observed bell shaped curve was located in the time range (0-480 min). The peak of the bell shape curve was at 30 min, then the burning sensation gradually reduced at 120, 240 and 360 min and then reached a minimum at 480 min as shown in Figure 1. The maximum burning sensation was experienced with the capsicum gel in the commercial product, followed by the formulation with water vehicle, aloe vera extract and the wax gourd extract. The reductions in the burning sensation with aloe vera and wax gourd were 21.49% and 36.25% respectively.

The effect of the different concentration of vehicles on reducing the burning sensation of capsicum gel

The different concentrations of vehicles on reducing the burning sensation of capsicum gel were observed in the concentration range of 0-100. The results in Table 4 showed that the ability to reduce the burning sensation was correlated with the concentration of the vehicle, especially for the wax gourd extract. The reduction in burning

sensation achieved with the 75% and 100% aloe vera extracts were similar. The burning sensation level showed a typical bell shaped curve which gave the highest figures at 30 min, and decreased gradually at 120, 240 and 360 min reaching a minimum at 480 min as shown in Figure 2.

The evaluation of erythema value

The erythema value measures the redness of the skin when stimulated or irritated. In this experiment, the erythema value was measured

Table 1 The Composition of preliminary batch of capsicum gel

Ingredients	M.F.
Capsaicin	0.0125%
Carbopol 940	1%
Neutral TE®	1.5%
Polysorbate 20 (Tween 20®)	0.2%
Uinul MS 40®	0.01%
Paraben conc.	1%
EDTA	0.02%
Ethanol	38%
Vehicle q.s. to	100%

Table 2 The work sheet for recording the burning sensation

Time (min)	Rating scale of burning sensation									
	0	1	2	3	4	5	6	7	8	9
5										
15										
30										
60										
120										
240										
360										
480										

No symptoms; 0 = none

Mild symptoms; 1= itch, 2 = red, 3 = hot

Moderate symptoms; 4 = less burning, 5 = burning, 6 = more burning

Severe symptoms; 7 = a lot of burning 8 = burning, but tolerable 9 = burning, not tolerable

Table 3 Physicochemical properties of capsicum gel containing three soothing vehicles

Vehicle	pH	Color	Odor	Viscosity
Water	7.38	Dark orange	Very strong	High
Aloe vera extract 100%	6.52	Orange	Strong	Medium
Wax gourd extract 100%	6.53	Pale orange	Less strong	Less

with a Cutometer® and studied for the relationship with the burning sensation after applying four capsicum gel products to 30 healthy products. The maximum erythema value was reached at 30 min while the minimum value occurred at 480 min. The average erythema value (Δ erythema value) was the value subtracted from the base line as shown in Table 5. The results showed that the burning sensation was similar among the four products and it was extinguished at 480 min.

The average erythema values of healthy volunteers after 30 min applying four products were different. Capsicum gel containing wax gourd extract as the vehicle gave the lowest average erythema value of 80.91 ± 12.98 . The average burning sensation level was correlated with the average difference of erythema value 30 min after applying the capsicum gel with a correlation coefficient (R^2) of 0.9584 as shown in Figure 3. The degree of redness of the skin was obviously related to the burning sensation level.

DISCUSSION AND CONCLUSION: The formulation of capsicum gel containing soothing vehicles was studied in order to reduce the burning sensation of capsaicin. The physicochemical properties of capsicum gel were enhanced in terms of pH, color, order and viscosity. The burning sensation level showed a typical bell shaped curve that was located in the time range (0- 480 min). The peak burning sensation was experienced at 30 min, then it gradually declined at 120, 240 and 360 min and was extinguished at 480 min. The maximum burning sensation at 30 min was similar for two test products; the commercial product and the formulated product containing the water vehicle. The reductions in the burning sensation achieved with capsicum gel with aloe vera and wax gourd were 21.49% and 36.25%, respectively. The higher soothing vehicle concentrations were correlated with a lower burning sensation level. In addition, the burning sensation was related to the erythema value with a correlation coefficient (R^2) of 0.9584. The best formulation in this study was the analgesic gel containing 0.0125% capsaicin with 100% wax gourd extract which gave the lowest erythema value and the lowest burning sensation level.

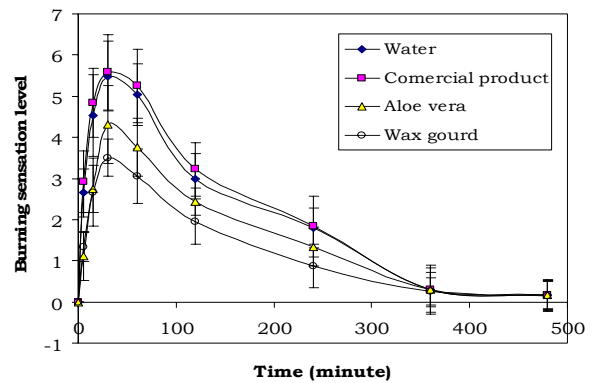


Figure 1 The burning sensation level experienced by subjects after applying capsicum gel with the different vehicles; water (♦), aloe vera extract (Δ) and wax gourd extract (○) compared with the commercial product (◆)

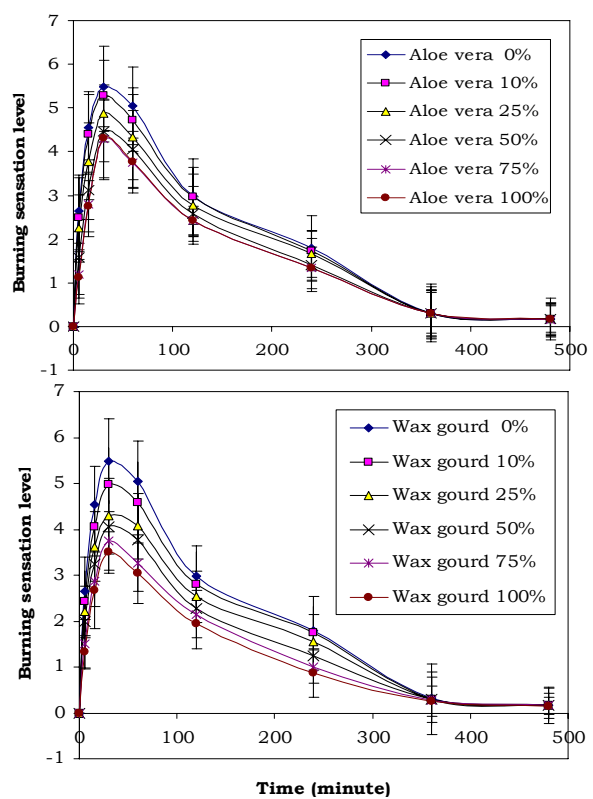


Figure 2 The burning sensation level experienced by subjects after applying capsicum gel containing different concentrations of aloe vera and wax gourd extract

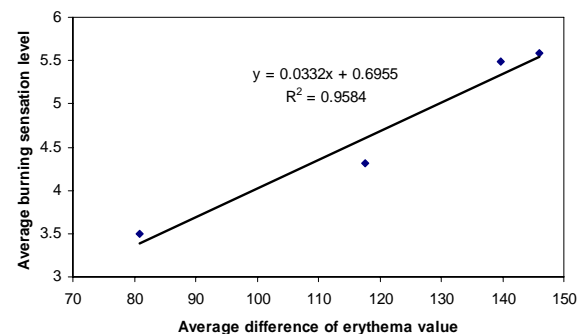


Figure 3: The linear regression between the average burning sensation level and the average difference of erythema value 30 min after applying the capsicum gel

Table 4 The average burning sensation in healthy volunteers (n=30) after applying the capsicum gel formulations containing different vehicles: water, aloe vera extract and wax gourd extract

Vehicle	Burning sensation level \pm SD (n=30)							
	5 min	15 min	30 min	60 min	120 min	240 min	360 min	480 min
Water	2.65 \pm 0.75	4.54 \pm 0.84	5.49 \pm 0.92	5.04 \pm 0.89	2.98 \pm 0.65	1.80 \pm 0.73	0.31 \pm 0.59	0.17 \pm 0.38
Aloe vera 10%	2.50 \pm 0.96	4.39 \pm 0.91	5.28 \pm 0.82	4.73 \pm 0.73	2.96 \pm 0.88	1.73 \pm 0.45	0.31 \pm 0.66	0.17 \pm 0.47
Aloe vera 25%	2.25 \pm 0.77	3.78 \pm 0.87	4.88 \pm 0.65	4.33 \pm 0.97	2.77 \pm 0.72	1.66 \pm 0.53	0.30 \pm 0.53	0.16 \pm 0.35
Aloe vera 50%	1.59 \pm 0.85	3.13 \pm 0.68	4.47 \pm 0.71	4.06 \pm 0.89	2.58 \pm 0.63	1.41 \pm 0.60	0.31 \pm 0.46	0.17 \pm 0.31
Aloe vera 75%	1.20 \pm 0.55	2.80 \pm 0.74	4.30 \pm 0.89	3.75 \pm 0.56	2.43 \pm 0.54	1.35 \pm 0.32	0.31 \pm 0.52	0.15 \pm 0.40
Aloe vera 100%	1.12 \pm 0.60	2.75 \pm 0.58	4.31 \pm 0.95	3.76 \pm 0.71	2.43 \pm 0.33	1.34 \pm 0.47	0.29 \pm 0.55	0.16 \pm 0.33
Wax gourd 10%	2.43 \pm 0.66	4.05 \pm 0.64	4.98 \pm 0.79	4.59 \pm 0.88	2.81 \pm 0.70	1.75 \pm 0.56	0.30 \pm 0.45	0.17 \pm 0.27
Wax gourd 25%	2.20 \pm 0.56	3.62 \pm 0.76	4.29 \pm 0.83	4.07 \pm 0.72	2.53 \pm 0.56	1.54 \pm 0.60	0.29 \pm 0.77	0.16 \pm 0.19
Wax gourd 50%	1.98 \pm 0.43	3.24 \pm 0.69	4.06 \pm 0.57	3.78 \pm 0.77	2.28 \pm 0.44	1.24 \pm 0.43	0.30 \pm 0.52	0.17 \pm 0.36
Wax gourd 75%	1.50 \pm 0.54	2.86 \pm 0.55	3.75 \pm 0.64	3.26 \pm 0.61	2.15 \pm 0.51	1.01 \pm 0.37	0.28 \pm 0.49	0.16 \pm 0.27
Wax gourd 100%	1.33 \pm 0.35	2.67 \pm 0.83	3.50 \pm 0.45	3.05 \pm 0.66	1.95 \pm 0.55	0.87 \pm 0.53	0.25 \pm 0.33	0.15 \pm 0.38

Table 5: The average erythema value of healthy volunteers after applying four products at 30 and 480 min compared with the base line

Test samples	Δ Erythema value \pm SD (n = 30)	
	30 min	480 min
Capsicum gel (commercial product)	145.96 \pm 17.62	10.51 \pm 8.43
Capsicum gel using water as the vehicle	139.72 \pm 20.13	12.98 \pm 9.17
Capsicum gel using aloe vera extract as the vehicle	117.64 \pm 15.27	13.92 \pm 7.82
Capsicum gel using wax gourd extract as the vehicle	80.91 \pm 12.98	11.79 \pm 9.96

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REFERENCES:

- Schweiggert U, Carle R and Schieber A. 2006. Characterization of major and minor capsaicinoids and related compounds in chili pods (*Capsicum frutescens* L.) by high-performance liquid chromatography/atmospheric pressure chemical ionization mass spectrometry. *Anal Chim Acta* 557(1-2): 236-44.
- Constant HL, Cordel GA, West DP, Johnson JH. 1995. Separation and quantification of capsaicinoids using complexation chromatography. *J Nat Prod* 58(12): 1925-8.
- Capsaicin-USP Monograph. Available from: [http://www. Pharmacopoeia.com.cn/v29240/usp29nf24s0_m12365.html](http://www.Pharmacopoeia.com.cn/v29240/usp29nf24s0_m12365.html) [Accessed Dec 10, 2008].
- Sosanuy S. Capsicums and the advantages of capsaicin. Available from: http://yalor.yru.ac.th/~dolah/notes/4902-1-48G13/SEMREP/Sb_404652078.doc [Accessed Jun 30, 2008].

th/~dolah/notes/4902-1-48G13/SEMREP/Sb_404652078.doc [Accessed Jun 30, 2008].

- Lynn B. 1990. Capsaicin: actions on nociceptive C-fibres and therapeutic potential. *Pain* 41(1): 61-9.
- Purkiss J, Welch M, Doward S, Foster K. 2000. Capsaicin-stimulated release of substance P from cultured dorsal root ganglion neurons: Involvement of two distinct mechanisms. *Biochem Pharmacol* 59: 1403-6.
- Erin N, Zik B, Sarıgül M, Tütüncü S. 2009. The effects of chronic low-dose capsaicin treatment on substance P levels. *Regul Pept* 153: 83-7.
- Keawnopparat N. 2000. Department of Pharmaceutical Technology, Faculty of Pharmacy, Prince Songkla University, Capsicums and capsicum extracts in medicinal used. Available from: <http://herbal.pharmacy.psu.ac.th/Article/03-45/Capsicum.htm> [Accessed Jun 30, 2008].