RESEARCH AND EVALUATE THE ANALGESIC EFFECTS OF THE TINCTURE FROM THE *MOMORDICA COCHINCHINENSIS* SEEDS - *RAMULUS CINNAMOMI*

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SUMMARY

Objective: To develop and evaluate the analgesic effects of the tincture from the Momordica cochinchinensis seeds - Ramulus cinnamomi. Methods: The Momordica cochinchinensis seeds were extracted by the Soxhlet method. Total saponins were determined by UV-Vis spectrophotometry after extracts had been colored with perchloric acid, vanillin in glacial acetic acid. Ramulus cinnamomi were extracted by the percolation. The tincture was made by combining the extract of the Momordica cochinchinensis seeds and Ramulus cinnamomi. The skin irritation of tincture was evaluated in rabbits. The analgesic effects of tincture were evaluated in rats in hot plate model with the use of pain agent carragenaan. Results: The Soxhlet extraction in 16 hours by 70% ethanol solvent was the highest total saponins content (about 13.53%). The low rate of Ramulus cinnamomi in the tincture made it very unpleasant. Their skin irritation was proportional to the amount of Ramulus cinnamomi. Evaluation of analgesic effect showed that the tincture was capable of reducing pain on the hot-plate model. Conclusion: The tincture that ratio 16.7% of Ramulus cinnamomi had good quality and unirritated the skin and had analgesic effect on the experimental model.

* Keywords: *Momordica cochinchinensis seed*; *Ramulus cinnamomi; Tincture; Irritation, Analgesic*.

INTRODUCTION

Momordica cochinchinensis (MC) seeds are one kind of the precious medicinal herbs, which is used in traditional medicine for treatment in case of falling, injuries, poisoning, breasts swelling, hemorrhoids and especially injuries with hematomas. MC seeds use almost like the bear's bile because it is also called "the bear bile tree". Since ancient times, the Vietnamese have made wine from MC seeds by the manual method. They could be used for bruises, blood clots, pimples, mumps, injuries with hematomas by applying to the trauma. The modern studies shown that the saponins of MC seeds extracts have performed the analgesic and anti-inflammatory effect in the experimental model.

For thousands of years, *Ramulus cinnamomi* (RC) had been used in the traditional Oriental medicine. It could reduce the toothache, the bad breath, the gingivitis, the headache, the migraine, the muscle and osteoarthritis pain. Currently, RC is also used a lot of anti-arthritis folk remedies.

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Both MC seeds and RC had the analgesic effect. The combination of RC with MC seeds can increase the analgesic and anti-inflammatory effects of each medicinal ingredient. Moreover, this combination may also increase the absorption of the drug into the skin. The study was designed to develop and evaluate the analgesic effect of the tincture from the MC seeds - RC, therefore we can choose the optimal formula.

MATERIALS AND METHODS

1. Research subjects and study time.

** Material:*

Momordica cochinchinensis (Lour.) Spreng. seeds were collected in Thainguyen province. *Ramulus cinnamomi* were collected in Langson province. They met the standard of Vietnam Pharmacopoeia V.

Swiss rats weighing 20 - 30 g and rabbits weighing 2,000 - 2,200 g were used in this study. They were provided by the National Institute of Hygiene and Epidemiology. Before conducting this research, rats were raised in a laboratory of Pharmacology 5 to 7 days. In this study, they were fed by the standard food (provided by the National Institute of Hygiene and Epidemiology) and drunk water free.

This study time from 02 - 2018 to 10 - 2018.

2. Methods.

** Extraction:*

MC seeds were prepared and ground into powder sizes from 0.3 to 0.8 mm. Then, they were removed fatty oil and extracted the saponins with 70% ethanol solvent. The extracts were concentrated under rotary evaporator to the ratio of medicinal herbs and solvents by 1:10 [4].

RC were prepared and ground into powder sizes from 0.3 to 0.8 mm. They were extracted with 80% ethanol solvent [1]. The extracts were concentrated under rotary evaporator to the ratio of herb and solvent by 1:5.

The tincture formulations were prepared by combining two extracts at different proportions, mixing, settling and refining if needed.

** Assay saponins:*

Total saponins in MC seeds were purified by liquid-liquid extraction with n-butanol solvent. They were determined by UV-Vis spectrophotometry at wavelength of 555 nm after being colored with perchloric acid and vanillin in glacial acetic acid [2, 3, 8, 9].

** Assay trans-cinnamic acid:*

Trans-cinnamic acid in RC extracts was quantified by high performance liquid chromatography method. The chromatographic conditions comprised: A reversed-phase RP-8 column, flow rate 1 mL/min and the mobile phase that was a mixture of aceonitrile and 0.2% acetic acid solution (65:35 v/v). The detection was carried out at 280 nm [7].

** Test skin irritation:*

Test skin irritation was determined by the Ministry of Health Guidelines [5].

Before the experiment date, rabbits were shaved off the hairs at both sides of the back. Only rabbits had the same color and healthy skin which could be used in the experiment.

Each sample was tested on three rabbits. The dose of reagent on each rabbit was 0.5 mL. The gauze (2.5 x 2.5m) containing samples were covered on the specified positions of rabbit skin. The gauze adhesive was fixed in four hours. Then, the gauze was removed, the remaining reagent was cleaned with a suitable solvent without causing irritation. The reaction site on the skin was observed and recorded at 1, 24, 48 and 72 hours after cleaning the sample. Skin reactions at levels of erythema and edema were assessed.

** The analgesic effect:*

The analgesic effect was determined by the heating method (hot plate) with using carrageenan pain agent. We selected 60 Swiss rats at random and shaved all the hairs at their hind legs. Rats were divided randomly into 6 lots.

- Lot of control: Apply 0.1 mL - 10 g of NaCl 0.9% solution.

- Lot of comparison: Apply of 0.01 g - 10 g voltaren.

- Lot 1: Apply a dose of 0.1 mL - 10 g the sample M5.

- Lot 2: Apply a dose of 0.2 mL - 10 g the sample M5.

- Lot 3: Apply a dose of 0.1 mL - 10 g the sample M3.

- Lot 4: Apply a dose of 0.2 mL - 10 g the sample M3.

Rats were applied reagent samples in the morning for three consecutive days. Measure the reaction time with the temperature of the rat before and after one hour when they were last used. Rats were placed on the hot plate that has been maintained at a temperature of 56^0C by the thermostatic system. Calculate the time when the rats were placed on the hot plate until the rats lick their back leg. Record the pain response time of each rat. Rats with a pain threshold of 8 - 30 seconds were selected to continue this study. Each rat was injected carrageenan 0.5% pain agent in physiological saline (0.025 mL/foot) into its back legs and applied the sample. After 3 hours, the rat were placed on a hot plate to determine the duration of each rat's pain response. Evaluate the analgesic effect by extending the pain response of rat after they were placed on the hot plate. Comparing the response time before and after applying the reagent of a different batch of rats, calculated percentage of response time.

RESULTS AND DISCUSSION

1. Extraction and formulation of tincture.

About 25 g of MC seeds powder which were removed fatty oil by diethyl ether and plated in the extraction flask. They were extracted by the immersion, the ultrasound, the percolation, the Soxhlet method with 70% ethanol solvent. The extracts were concentrated under rotary evaporator to 250 mL. Total saponins content in extracts were determined by UV-Vis spectrophotometry. The results of the evaluation efficiency of extraction methods and extraction intervals were shown in figures 1 and 2.

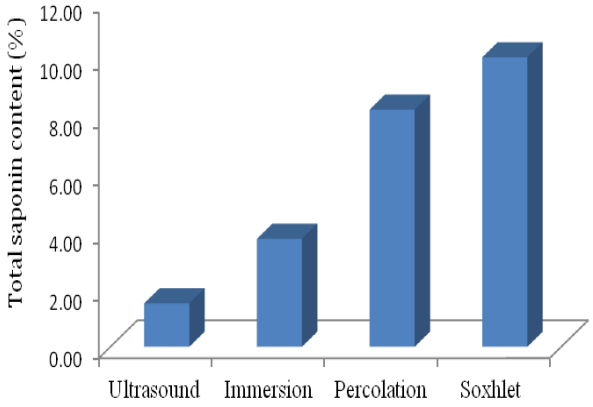

Figure 1: Total saponin content corresponding to different extraction methods.

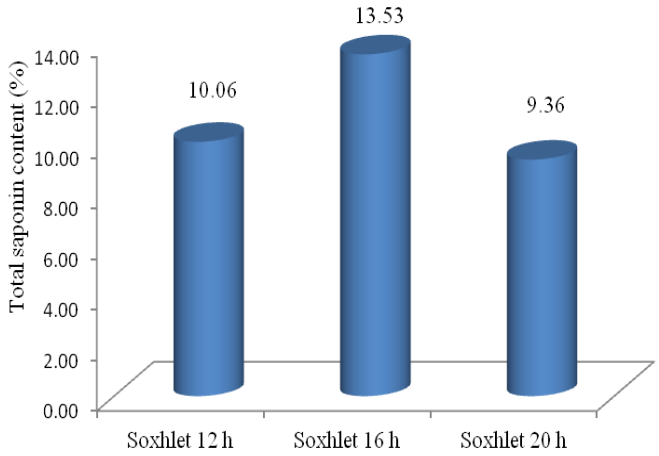

Figure 2: Survey of the effect of extraction time on total saponin content.

At the laboratory scale, total saponins of MC seeds were extracted by Soxhlet method for 16 hours for the highest extraction efficiency (about 13.53%). It could be explained that the herbs were always exposed to new solvents in Soxhlet method. There is a large concentration difference in the extraction process so that the active substances easily diffuse into the solvent. Moreover, the increase of temperature in Soxhlet extraction could speed up the reaction.

RC were extracted by the immersion method with 800C ethanol solvent. The extracts were concentrated under rotary evaporator to the ratio of medicinal herbs and solvents by 1:5 [4]. Trans-cinnamic acid content of extracts was quantified as 1.45% by HPLC method.

Tinctures were prepared by combining two extracts with ratio 2 - 50% of RC. The formulas were evaluated for the form, the density, the residue after the evaporation and skin irritation.

Table 1: Results of the quality evaluation of the samples.

Number	The samples	The form	The density	The residue after evaporation (g/l)	The irritationmedium
1	M1 (RC: 2.0%)	The solution is yellow, transparent, unpleasant odor	0.90	45.5	0
2	M2 (RC: 9.1%)	The solution is yellow, transparent, unpleasant odor	0.85	44.6	0
3	M3 (RC: 16.7%)	The solution is yellow, transparent, aromatic	0.88	45.1	0
4	M4 (RC: 28.6%)	The solution is yellow, transparent, aromatic	0,91	48.3	0.2
5	M5 (RC: 37.5%)	The solution is yellow, transparent, aromatic	0.99	45.0	0.3
6	M6 (RC: 44.4%)	The solution is yellow brown, with precipitate and aromatic	0.98	48.9	1.1
7	M7 (RC: 50%)	The solution is yellow brown, with precipitate and aromatic	0.99	47.5	1.4

The results showed that the samples with low ratio of RC (M1 and M2) had an unpleasant odor. The skin irritation was proportional to the amount of RC in the recipe. Samples with a high percentage of RC (M6 and M7) had mild skin irritation. The main reason was the hot and spicy of RC tincture so it could cause skin irritation. Among the samples, the samples (M3, M4 and M5) were achieved the required form, negligible skin irritation. The samples M3 and M5 were selected to continue to assess the analgesic effect.

2. The analgesic effect.

The samples (M3 and M5) were evaluated the analgesic effect by the hot plate with using carrageenan pain agent. This model was applied to assess the central or peripheral pain relief effect of the sample by designing and selecting appropriate evaluation parameters. On this model, the first pain reaction of the rat was caused by sensation (heat acting on the sensory nerve endings). The duration of the rat's pain response reflects this effect, so it was used to assess the central pain relief ability of the test product. Next, the rat were painful due to inflammation. When rat were injected with carrageenan into their feet, the inflammatory reaction caused pain in the rat. When injecting carrageenan into mouse feet, the first stage of the rat pain due to stimulation, then they were painful due to inflammation. The prolongation of the pain response time of rat after injection of pain was shown to reduce the perception of pain from inflammation. If the sample increased the duration of pain response, the animal would decrease the pain of inflammation. The results of the evaluation of the analgesic effects of test samples are shown in table 2 and table 3.

Table 2: Effect on the rat's pain response time of the sample.

Number	Lot	n	Pain response time (s)		p
			Normal	3 hours after injection	
1	Control	10	20.5 ± 2.3	12.6 ± 2.3*	-
2	Voltaren	10	16.9 ± 1.2	20.8 ± 1.5	< 0.05
3	Samples M5 (dose 0.1 mL/10 g)	10	17.1 ± 2.2	25.9 ± 2.7*	< 0.01
4	Samples M5 (dose 0.2 mL/10 g)	9	18.9 ± 2.1	24.1 ± 2.3	< 0.01
5	Samples M3 (dose 0.1 mL/10 g)	10	13.9 ± 2.6	20.9 ± 2.1*	< 0.05
6	Samples M3 (dose 0.2 mL/10 g)	9	16.2 ± 2.0	26.6 ± 4.9	< 0.05

*(*p < 0.05 compared to normal time)*

The results showed that the control rat's duration of pain response was shortened to their normal time ($p < 0.05$). The M5 and M3 samples, the time of rats' pain response increased to normal time ($p < 0.05$). Both the samples and voltaren had a higher pain response time than the control rat ($p < 0.05$). The samples and voltaren antagonists found no difference ($p > 0.05$).

Table 3: Effect on the prolongation of rat's pain response time of the sample.

Number	Lot	n	Prolong the pain response time (s)	Inhibition compared to control (%)
1	Control	10	-7.9 ± 2.4	-
2	Voltaren	10	4.5 ± 1.0**	211.4
3	Samples M5 (dose 0.1 mL/10 g)	10	8.8 ± 1.3**	166.1
4	Samples M5 (dose 0.2 mL/10 g)	9	5.2 ± 2.6**	188.6
5	Samples M3 (dose 0.1 mL/10 g)	10	7.0 ± 1.5**	230.8
6	Samples M3 (dose 0.2 mL/10 g)	9	10.3 ± 3.7**	157.0

*(**: p < 0.001 compared to the control lot)*

The results showed that the prolongation of the pain response time at all samples increased after three hours of injecting carrageenan ($p < 0.001$). In addition, the duration of feeling pain in the samples significantly increased compared to the initial time. These results demonstrated that the samples were capable of reducing pain on the hot-plate model.

After three days of testing, there was edema phenomenon in rat legs which were applied the M5 sample (at about 20% of total rats), whereas, the M3 sample did not have this phenomenon. Because the amount of RC in the M5 sample was higher than the M3 sample. RC was hot and spicy, a large percentage of RC tincture which concentrated on the

skin surface could increase vascular permeability, peripheral vasodilation, therefore it caused edema. However, this phenomenon was decreased rapidly after stopping the sample application. The ratio of RC in the sample M3 was lower than the sample M5, so there was no edema phenomenon. This result was similar to the study on skin irritation.

CONCLUSIONS

- *Momordica cochincinensis* seeds were extracted by Soxhlet method with 70% ethanol solvent for 16 hours gave the best results (total saponin content of 13.53%) in the experimental scale.

- The tincture that had contents 16.7% of *Ramulus cinnamomi* met quality requirements: beautiful form (brown yellow, not opaque), pleasant aroma, no skin irritation and significant analgesic effect in the experimental assay.

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