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Full Length Research Paper

Analgesic activity of *Ruta graveolens* L. (Rue) extracts

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The knowledge of pharmacological activity is mainly derived from the use that the population makes of plants. Rue (*Ruta graveolens* L.) is popularly used with a degree of mysticism due to its anti-inflammatory, healing, sedative and antispasmodic characteristics. In an attempt to confirm the well-known analgesic effects, the present study evaluated Rue extracts in animal models. Two organic extracts were produced and orally administered to mice, approximately 30 min before the tail immersion test in warm water ($55 \pm 0.5^\circ\text{C}$). The results reveal a small chronic analgesic effect and a high effect in acute assay (62.1% of pain inhibition) especially observed in the hexane extract, supporting the hypothesis of the use of this plant as having analgesic activity. However, the reports of the population about its analgesic activity should be caused also by its anti-inflammatory characteristics and the presence of compounds related in literature as analgesics. Further experiments should be done to better determine the dose-response analgesic effect of *R. graveolens* extracts.

Key words: *Ruta graveolens*, analgesic effect, tail immersion, rue.

INTRODUCTION

The *Ruta graveolens*, popularly known as Rue, from the Rutaceae family, aroused the interest of many researchers after reports on the traditional use of the plant by the population. The effects described were: diuretic, healing (Mendes et al., 2008), antiulcer (Raghav et al., 2006), antispasmodic, sedative and used in treatment of pain like toothache, pain in joints, headache and fever (Conway and Slocumb, 1979; San Miguel, 2003; Rodrigues et al., 2010; Khouri and El-Akawi, 2005; Raghav et al., 2006), and also antirheumatic. Studies have shown that Rue has potent anti-inflammatory activity due to the presence of the flavonoid rutin, which decreases nitric oxide production in macrophages inoculated without showing cytotoxicity (Raghav et al., 2006). Recent surveys show that the *R. graveolens* has abortifacient activity, (Roehsig et al., 2011);

antiandrogenic activity (Khouri and El-Akawi, 2005); potent antimicrobial activity (Mendes et al., 2008; Ivanova et al., 2005), antifungal activity (Meepagala et al., 2005; Ivanova et al., 2005), and antitumor activity (Hale et al., 2004; Preethi et al., 2006).

The Brazilian market is composed of a wide range of drugs, including anti-inflammatory and analgesic drugs from synthetic origin. However, herbal medicines have gained headway (Carvalho et al., 2008) after deployment policies for their use were implemented in National Health System by the Ministry of Health, provided that their production are ensured for safety, efficacy and quality.

With *R. graveolens* known as a common plant in Brazil with anti-inflammatory activity proven, this experimental study brings with it the possibility of developing new effective drugs in treating acute and chronic pain. Rue

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Table 1. Effect of oral administration of extracts of *Ruta graveolens* on response time of the animals in the tail immersion model.

Treatment (100 mg/kg)	Pain inhibition of extracts of <i>Ruta graveolens</i> in tail immersion test	
	Time of response (s)	Percentage of inhibition (%)
CR	1.3 ± 0.2	3.9
HR	3.6 ± 0.3*	62.1*
ASA	1.9 ± 0.5	17.6

These values represents pain inhibition in mean ± SEM and percentage (n= 4 animals). CR= chloroform extract of *Ruta graveolens*; HR= hexane extract of *Ruta graveolens*; ASA= acetylsalicylic acid. *p ≤ 0.05 compared with the control group (ANOVA, Dunn's test).

will contribute to the development of new drugs and increases the possibility of therapeutic treatment of pain. Thus, this study aimed to evaluate the analgesic efficacy of *R. graveolens* through animal testing after oral administration of this plant extracts.

MATERIALS AND METHODS

Plant material and extraction

The sample of *R. graveolens* was taken from a planting backyard in the city of Cotia, São Paulo, in August, 2012. Two excisicates were deposited in the herbarium of the Mackenzie Presbyterian University identified with the number 1259 and 1260. The collected fresh plant (800 g) was submitted to an exhaustive extraction with the solvent methanol for 24 h at room temperature. After filtration, the residual was extracted twice in the same conditions. Subsequently, the ethanol extract was gathered and was subjected to a solvent partition in hexane and later in chloroform. The solvents were removed by evaporation at room temperature under a laminar flow. The resulting hydroalcoholic extract was discarded in this study due to the proliferation of a kind of yeast after drying. Finally, the dried extract was obtained: chloroform (CR; 730 mg) and hexane (HR; 255 mg). For the essays, the extracts were prepared at 2.5 mg/ml (100 mg/kg) in dimethylsulphoxide (DMSO; 2,0 ml/kg body weight).

Animals

Thirty-five *Mus musculus* species, lineage BALB/c mice were purchased from Federal University of São Paulo (female, 6 week-old) These animals were placed in the vivarium of the Mackenzie Presbyterian University under a 12 h light/dark cycle, controlled temperature (22 ± 2°C) and *ad libitum* feeding. On the day of treatment, food was withdrawn for about an hour before. All experimental animal procedures adopted for this study met the standards established by the Research and Ethics Committee of Mackenzie Presbyterian University and were previously submitted to that committee, with the number CEUA 046/05/2009.

Tail immersion test

This method was used for many author like Nogueira et al. (2005), Sharma et al. (2011) and Sathesh et al. (2010). The tail immersion test was performed by immersing, gently, the tail of the mice in warm water (55 ± 0.5°C). The animals were divided into five groups of four animals established as: saline group, negative control

(DMSO), positive control (acetylsalicylic acid), chloroform extract of *R. graveolens* and hexane extract of *R. graveolens*. For these extracts, an amount corresponding to the concentration of 100 mg/kg of their body weight was orally administered 30 min prior to the tail immersion test. The acetylsalicylic acid group received the same concentration (100 mg/kg) to it analgesic effect which could be comparable with the other groups. The cutoff time was set as 5 s to avoid damage to the animal as observed by Sathesh et al. (2010). Each animal had its time of tail removal timed and recorded with a stop watch. In this study, a single treatment was performed as acute essay, and a five day treatment as a subchronic essay (five-day treatment). Through the equation that relates the time of tail removal and the baseline value with the cutoff value, the pain inhibition (PI, percentage inhibition) could be calculated (Nogueira et al., 2005):

$$PI = \frac{\text{tail removal} - \text{baseline value}}{\text{cutoff value} - \text{baseline value}} \times 100$$

In this study, the baseline value was based on the time average of the saline group of each test day.

Statistical analysis

Results were expressed as mean ± standard error of the mean (SEM) and were analyzed by analysis of variance (ANOVA), followed by Dunn's test and p < 0.05 was used as the significance level. Statistical analyzes were conducted using GraphPad Prism® software (version 6.01 GraphPad Software, Inc.). Dunn's test established significant correlation (p < 0.05) between the mean of all data presented.

RESULTS

Acute assay

This acute assay indicated an effect especially found in the HR extract, with a high value of 62.1% (Table 1). Despite the positive control (ASA) not being significant, it showed to be an analgesic agent with 17.6% of pain inhibition. In Figure 1 was clearly observed the effect of hexane extract of *R. graveolens* compared with the control group (DMSO) and the effect of the standard

Table 2. Effect of oral administration of extracts of *Ruta graveolens* on response time of the animals in the sub-chronic tail immersion model. Pain inhibition of extracts of *Ruta graveolens* in tail immersion test.

Days of treatment	Treatment (100 mg/kg)			
	DMSO	CR	HR	ASA
1	0.74 ± 0.08	1.16 ± 0.14	0.85 ± 0.08	1.54 ± 0.20 ^b
2	1.00 ± 0.07	1.36 ± 0.08	1.08 ± 0.12	1.96 ± 0.17 ^b
3	0.71 ± 0.05	0.97 ± 0.19	1.12 ± 0.21	1.17 ± 0.16 ^a
4	0.62 ± 0.04	1.08 ± 0.11 ^a	0.80 ± 0.07	0.73 ± 0.04
5	0.93 ± 0.11	0.82 ± 0.04	0.93 ± 0.02	1.33 ± 0.20

These values represents the time of removal (s) of tail in mean ± SEM (n= 4 animals). CR= chloroform extract of *Ruta graveolens*; HR= hexane extract of *Ruta graveolens*; ASA= acetylsalicylic acid. ^ap ≤ 0.05; ^bp≤0.0001 compared with the control group (DMSO) of each day of treatment (ANOVA, Dunnett's test).

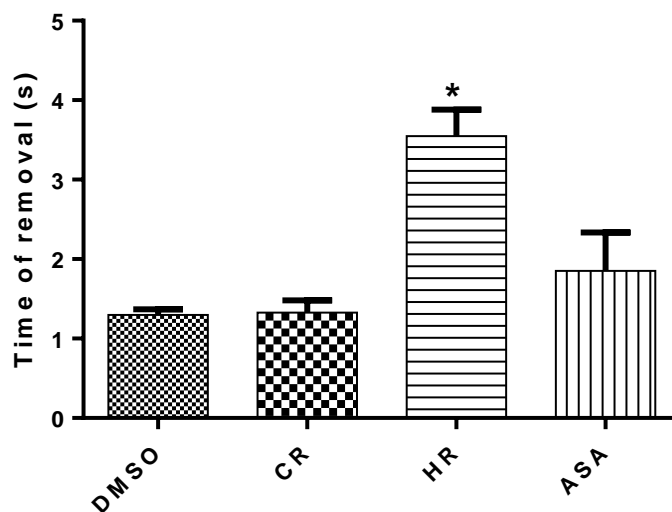


Figure 1. This Figure represent pain inhibition in mean ± SEM (n= 4 animals). DMSO= dimethylsulphoxide (2,0 ml/kg body weight); CR= chloroform extract of *Ruta graveolens* (100 mg/kg); HR= hexane extract of *Ruta graveolens* (100 mg/kg); ASA= acetylsalicylic acid (100 mg/kg). *p ≤ 0.05 compared with the DMSO group (ANOVA, Dunn's test).

(ASA).

second and third days of treatment, 17.8, 26.6 and 13.7% of pain in this model.

Sub-chronic assay

The sub-chronic assay was evaluated at the same model used in the acute. The animals receive daily (for 5 days) the extracts 30 min prior to the tail test. Although the acute assay demonstrated a high analgesic effect in the extracts of Rua, the results of sub-chronic assay showed lower results (Table 2). At the second day of treatment the CR extract showed maximum effect (12.1% of pain inhibition) and in third the HR extract (12.6% of pain inhibition). The standard group also demonstrated to be an effective analgesic drug inhibiting, respectively at first,

DISCUSSION

Our laboratory works with standard operating procedures (SOP), and the doses are standardized to 100 mg/kg when using plant extracts. Studies with other doses must occur to understand the dose-response effect. The solvent DMSO at 5% was used for its high capacity of dilution and low toxic effects (Jacob and Wood, 1967). Brown et al. (1963) observed that rats tolerate up to 10 daily doses of 10 ml/kg weight, and no effects was found. On this study was used only 2.0 ml/kg body weight.

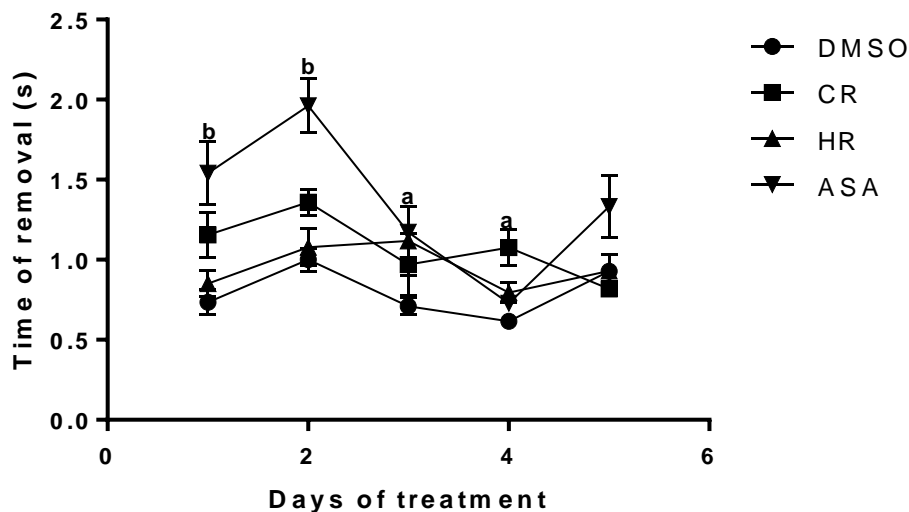


Figure 2. These values represents the time of removal (s) of tail in mean \pm SEM (n= 4 animals) along the five days of treatment. CR= chloroform extract of *Ruta graveolens*; HR= hexane extract of *Ruta graveolens*; ASA= acetylsalicylic acid. ^ap \leq 0.05; ^bp \leq 0.0001 compared with the control group of each day of treatment (ANOVA, Dunnett's test).

Furthermore, Wilson et al. (1965) also demonstrated reduced side effects at 25% solution of DMSO, suggesting concentrations below 10%. Thus, Worthley and Schott (1969) showed no mortality in mice at dose of 10% and dose of 10.1 g/kg, this study used only 2.2 g/kg body weight. This procedure was first used as an experimental model seeking to understand the dynamics of the methodology. The Dunn's and Dunnett's test proved, with moderate evidence, the hypothesis that the extracts of *R. graveolens* increase the time of removal of the tail when compared to the negative control, at both essay (acute and sub-chronic), being responsible for the analgesia.

Although the effect was evidenced by the results analyzed, it was noticed as a sharp decline in the response of animals to the treatment with extracts of *R. graveolens*, a fact seen in the reduction of the time of removal of tail in the tested groups (Figure 2). This variation may be related to operant conditioning, where the animal realizes that removing the tail causes a relief from the pain induced, and removes it faster (Skinner, 1974; Desse and Hulse, 1975; Barbosa et al., 2007).

This study with organic extracts of *R. graveolens* confirmed the analgesic effect of Rue despite its low response in the sub-chronic model. The better extract analyzed was the hexane extract that showed to be effective on both essays. Kostova et al. (1999) and Sinshemko et al. (2000) describes the presence of a large amount of substances in the Rue as flavonoids (rutin), alkaloids (quinolines, furanocoumarins and furoquinolines), coumarins (furocoumarin and pyranocoumarin) and essential oils (2-nananone and 2-undecyl acetate). According to Atta and Alkofahi (1998), some of these components could be responsible for the

analgesic effect observed, among them the volatile oils, flavenoids and resins. Although it has analgesic effects, the Rue is known as an abortive plant, and many times is reported about its toxicity at high doses and in some cases even of death (De Freitas et al., 2005). The indiscriminate use by the population should be known by government health programs to teach the people about rational phytotherapy (Schultz et al., 1998) and to reduce the incidence of adverse effects related to this plant. Thus, this study support the results obtained by Atta and Alkofahi (1998) which concluded that Rue is an analgesic plant with dose-dependent response against thermal stimuli.

Conclusion

Despite the methodology only proving a small chronic analgesic effect of Rue, in the studied extracts, a high effect was observed in acute essay, supporting the hypothesis of the use of this plant as having analgesic activity. However, the authors believe that the reports of the population about its analgesic activity should be caused also by its anti-inflammatory characteristics extensively studied and it is the compounds related in literature as analgesics. In view of this, it is suggested that further research should be done to determine the best dose-response effect of extracts of *R. graveolens*.

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Full Length Research Paper

Semantic validation of subtitles and analysis of understanding of pictograms taken from the United States Pharmacopeia Dispensing Information (USP-DI)

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The aim of this study was to validate pictogram subtitles and analyze the understanding of pictograms taken from the United States Pharmacopeia Dispensing Information (USP-DI). The subtitles of 25 pictograms from the USP-DI were translated and retro-translated, and the generated subtitles were compared by two committees of judges to evaluate semantic and cultural equivalence. Semantic validity was analyzed by submitting the translated subtitles to a convenience sample of 23 elderly people in Aracaju-SE (Brazil). Additionally, 15 of the 81 USP-DI pictograms were presented to participants individually, without subtitles and in random order, to analyze participants' understanding of them. The process of cross-cultural translation and validation resulted in a Portuguese version of each pictogram's subtitle. Changes in grammatical structure were applied to some items. Twelve subtitles showed less than 80% concordance of interpretation between judges, and were modified. The semantic validation phase indicated that four participants had difficulty understanding one particular subtitle. The analysis of understanding phase indicated that only one pictogram met the criterion for acceptable understanding established by ISO 3864. The Brazilian Portuguese version of pictogram subtitles from the USP-DI presents cross-cultural equivalency with the original English version. Most of the USP-DI pictograms assessed in the present study were not well understood by participants. The pictograms that did not meet the comprehension criteria are being redrawn in ways specific to the local culture.

Key words: Cross-cultural adaptation, pictograms, validation, comprehension, elderly.

INTRODUCTION

Cognitive ability and memory function characteristically decline with age. This hampers comprehension of basic

health-related information, increasing the risk of treatment regimen non-adherence (Liu et al., 2009).

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Hence, poorer comprehension and retention of health-related information expose elderly patients to an increased risk of therapeutic failure (Cornélio et al., 2009). Low patient literacy is one of the factors that most influences non-adherence to treatments (Braich et al., 2011). A low-literacy or illiterate patient is more likely to have difficulty with reading related to health services (e.g., prescriptions, package inserts, educational materials), and thus can be considered to have low “health literacy” (Maragno, 2009). Health literacy is the capacity of individuals to obtain, process, and understand the basic health-related information needed to make appropriate decisions about their health. It involves the ability to understand and interpret text, documents, and numbers effectively, skills that may seem distinct but are highly correlated with one another (Weiss et al., 2005).

The incorporation of visual guides is an alternative means of transmitting information to patients that facilitates the understanding of prescribed pharmacotherapy (Vaillancourt and Grenier, 2011). Among the different types of these guides are pictograms, which are graphic symbols displaying and signaling information relevant to the treatment. Pictograms associate images with concepts, and can be used to convey information in a clear, fast, and simple manner (Mansoor and Dowse, 2006).

In clinical practice, studies have shown that complementing pictograms with written information may increase the degree to which patients comprehend, recall and adhere to their treatment regimen (Mansoor and Dowse, 2003; Dowse and Ehlers, 2005; Mwingira and Dowse, 2007). Corroborating these data, Korenevsky et al. (2013) showed that participants preferred pictograms which were presented along with simple text. Thus, it is important that pictograms be accompanied by simple text in order to maximize treatment regimen comprehension and minimize medication errors.

As shown earlier, the use of simple language along with pictographic resources, which communicate a culturally appropriate message, can minimize the impact of communication barriers (Moreira et al., 2003). Furthermore, according to Dowse and Ehlers (2011), text not written in the reader’s first language represents a significant barrier to information access and diminishes the use and accessibility of leaflets containing pictograms. Therefore, there is a need to validate native-language subtitles that are intended to be used as a supplementary system of verbal communication.

Accordingly, the translation and validation of guides for use in a different culture requires the careful preservation of semantic equivalence between the original and translated versions. With regard to the translation of the subtitles in question, this equivalence may be said to be achieved fully when all items have the same significance to individuals belonging to the culture for which the translation is intended as they did to members of the culture for which the original items were intended. The

present study aimed to validate translated pictogram subtitles and analyze people’s understanding of the pictograms taken from the United States Pharmacopeia-Dispensing Information (USP-DI).

MATERIALS AND METHODS

Translation and cross-cultural adaptation

Translation and cross-cultural adaptation was carried out from March to May 2012, in accordance with international recommendations (Guillemin et al., 1993). Twenty-five pictogram subtitles out of the 81 total pictograms from the USP-DI were studied (Table 1). Subtitles were selected by the researchers involved in the present study, with consideration for the greater need for pharmaceutical services among the elderly.

Subtitles were first translated separately by two researchers whose first language is Portuguese, and who are graduate students in pharmacy, natives of Aracaju, capital of Sergipe, and fluent in English. These researchers were aware of the objectives and concepts of the present study. The two translations were compared, and ambiguities or discrepancies resolved by generating a consensus translation. Then, back-translation was performed by a Brazilian professor from the University of Sydney, Australia, who has resided in that country for 31 years, and a Brazilian professor from the University of Minnesota, USA, who has resided in that country for four and a half years. These two professors did not participate in the previous translation steps and were unaware of the objectives of the present study.

The material translated into Portuguese back-translated subtitles were as submitted for the purposes of evaluation and adaptation to a expert committee consisting of four pharmacists and three graphic artists (designers), who have basic training in communication, all of whom were natives of Brazil, bilingual, and informed of the purpose of the present study. The purpose of this committee was to compare, evaluate semantic and idiomatic equivalencies between the original version and the Portuguese version. The subtitles were assessed by the expert committee by Likert scale with semantic and idiomatic equivalence (-1: not equivalent, 0: undecided, +1: equivalent). For both a comparative and descriptive analysis, between scales of judges was held.

After the evaluation of semantic and idiomatic equivalence, the subtitles were presented to another committee of judges, composed of five pharmacists, each one being a native resident of one of the five regions of Brazil (North, Northeast, Southeast, Midwest, and South), in order to evaluate the cultural equivalence of the subtitles. The purpose of this committee was to verify that the back-translation used expressions that could be understood in all regions of Brazil.

Semantic validation

Semantic validation (Fegadolli et al., 2010) was subsequently conducted between June and July, 2012 to identify problems related to the understanding, acceptance, and relevance of subtitles, and to evaluate the need for any adaptation of the subtitles. Participants were 23 elderly patients living in an aged care facility in Aracaju-SE, Brazil. Subtitles were evaluated for attributes such as pictogram–subtitle equivalence, understandability, and appropriateness. The following questions were asked concerning all subtitle–pictogram pairs: Do you think the sentence accords with the image? Do you have difficulty understanding the sentence? How would you say or express it? Could you tell me, in your words, what this phrase

Table 1. Pictogram subtitles from the USP-DI.

S/N	Original USP-DI subtitles
1	Take 1 h before meals
2	Take 1 h after meals
3	Take 2 times a Day
4	Take 4 times a Day
5	Take 3 times a Day
6	Take by mouth
7	Do not store near heat or in sunlight
8	Take with meals
9	Do not take with meals
10	Wash hands/place drops in ear/wash hands again
11	Wash hands/place drops in nose/wash hands again
12	Wash hands/insert into vagina/wash hands again
13	Wash hands/place drops in lower eyelid/wash hands again
14	Do not store medicine where children can get in
15	Do not drink alcohol while taking this medicine
16	This medicine may take you drowsy
17	Store in refrigerator
18	Use this medicine as a gargle
19	Chew
20	Dissolve under the tongue
21	For headaches
22	Do not take if pregnant
23	Do not take if breast-feeding
24	Inhaler
25	Shake well

means to you?

Analysis of understanding of USP-DI pictograms

The participants were shown 15 of the 81 USP-DI pictograms. Pictograms were selected based on their expected relevance to the participants, and their expected usefulness in participants' everyday lives.

Pictograms were printed in monochrome, each with a size of 28 mm ($\pm 5\%$) \times 28 mm ($\pm 5\%$), as recommended by the International Organization for Standardization [ISO] 9186. Pictograms were presented without subtitles and in random order. The ISO specifies methods for testing the comprehensibility of graphical symbols, including methods to be used in testing the extent to which a variant of a graphical symbol communicates its intended message, and the methods to be used in testing which is a variant of a graphical symbol, is the most comprehensible (ISO, 2007).

Two researchers, one undergraduate research student and one master's student in pharmacy, presented pictograms to participants individually. Both followed the same presentation protocol. Before administering the pictograms, the researchers came to a consensus about what kinds of responses would be regarded as correct or incorrect.

Responses were coded as "correct" when they matched the specific subtitles given for each pictogram by the USP-DI, as shown in Table 1. Responses that did not match these legends were coded as "incorrect." Participants who said they did not understand the images and reported no meaning for them had their responses

coded as "do not know."

Participants were informed that the pictograms related to the use of prescription drugs, but did not receive any explanation of the significance of individual pictograms. Pictograms were presented without their accompanying subtitles. The respondents were asked to verbally report how they interpreted each image by answering the question, "If you had to take a prescription drug and the usage information was represented by this figure, what would you understand?" Responses were transcribed for further evaluation.

After this process, the two researchers independently assessed the participants' interpretations as either correct or incorrect. Discrepancies in coding were resolved by consensus after discussion. The ISO 3864 provides guidelines regarding the minimum acceptable rate of understanding of a pictogram (ISO, 1984). According to ISO 3864, pictograms are considered understandable when at least 67% of the sample's answers concerning these images are correct.

This study was submitted to the Research Ethics Committee from the University Hospital at the Federal University of Sergipe. All study participants were informed of the objectives and nature of the study and signed a volunteer informed consent form, as per the National Health Council Resolution n° 196/96.

RESULTS

During the steps of translation, changes were made to the grammatical structure of a few subtitles to obtain

Table 2. Description of the modifications carried out in the subtitles of pictograms, in Aracaju (SE), 2010.

Before	After
Do not store near heat or in sunlight	Do not save near heat or in sunlight
Store in refrigerator	Save in refrigerator
Do not store medicine where children can get in	Do not save medicine where children can get in
Take with meals	Take with food
Do not take with meals	Do not take with food
Dissolve under the tongue	Let dissolve under the tongue

(breakfast, lunch, and dinner). Subtitle 20 “dissolve under your tongue” was changed by the judges to “let dissolve under the tongue” to convey more clearly the intention that the pill be allowed to dissolve under the tongue by itself.

Of the 23 participants assessing subtitles’ semantic validity, three (13%) had completed a college education, five (21.7%) had completed high school, twelve (52.1%) had not completed high school, one had completed middle school (4.3%), and two (8.6%) were illiterate. Four participants had difficulty understanding the subtitle of pictogram 6; of these, one had completed a college education, two had not completed high school, and one was illiterate. Additionally, participants suggested modifications to two subtitles. For subtitle 3, 17 (74%) participants suggested changing “take twice a day” to “take in the morning and in the evening”; for subtitle 4, 19 (82%) participants suggested it be changed to “take in the morning, at noon, in the afternoon, and in the evening”. However, the researchers chose not to apply these changes to the final versions of these subtitles, because the times suggested by the participants cannot be considered standard for all treatments.

In the image/subtitle equivalency evaluation, 23 (100%) participants did not understand the images associated with subtitles 7 and 16, and could not see the relationship between those pictograms and their subtitles. Eighteen (78.2%) participants incorrectly understood pictograms 8 and 9, reporting that the meaning was “take the pill before eating food”.

Pictogram understanding

One hundred and sixteen individuals were interviewed regarding their understanding of the pictograms. Most (88.7%) of the participants were female. Their ages ranged from 60 to 90 years, with 68% of respondents between 60 and 75 years old. The education levels were as follows: 24% of participants had completed secondary education (that is, they had up to 12 years of education); 3.4% had not completed high school; 12% had completed higher education; 12.9% had completed primary education; 21.5% had not completed primary education;

7.7% had had only early childhood education; and 13.7% had not had any schooling. Regarding income, 60.3% of participants reported earning from zero to three times the minimum wage.

Most participants did not understand most of the USP-DI pictograms selected for the present study (Table 3). Only pictogram 10 gave a comprehension level greater than 67% (68% understanding), indicating that that pictogram is comprehensible for the target population of the municipality, according to the ISO 3864 standards.

DISCUSSION

The result of this study showed that the USP-DI pictograms selected for the present study tend to be poorly understood by elderly people in Brazil, as only one pictogram reached the understanding criterion of ISO 3864. Complexity in a prescription reduces elderly people’s ability to correctly interpret the instructions of a pharmacotherapeutic regimen. Consequently, adherence to the treatment regimen is likely to be reduced, the disease diagnosed is likely to worsen, and patient morbidity and mortality is likely to increase, leading to increased rates of hospitalization and increased health care costs (Korenevsky et al., 2013). This problem is exacerbated by the characteristic lower literacy and diminished memory function and cognitive ability of elderly patients, since these factors increase the difficulty of understanding essential information.

With respect to the translation of subtitles, due to the absence of previous studies of pictogram subtitles, the results of the present study cannot be compared to those of other studies. The lack of extant similar studies emphasizes the importance of the adaptation and validation of subtitles intended for use as a supplementary verbal means of information transmission in the Brazilian pharmaceutical practice.

The translated versions generated by the two translators matched in all subtitles. Silva and Thuler (2008) stress that it is important for translation to be performed by professionals whose mother tongue and culture are the same as those of the target audience. In addition, literal equivalence in translation may be insufficient to

Table 3. Understanding USP-DI pictograms. Aracaju, 2012 (n = 116).

S/N	Subtitle (USP-DI)	Correct [n (%)]	Wrong [n (%)]	Do not know [n (%)]
1	Take 1 h before meals	29 (25)	79 (68.2)	8 (6.8)
2	Take 1 h after meals	32 (27.5)	74 (63.9)	10 (8.6)
3	Take 2 times a Day	41 (35.3)	66 (56.8)	9 (7.7)
4	Take 4 times a Day	7 (6)	93 (80.3)	16 (13.7)
5	Take 3 times a Day	12 (10.3)	86 (74.2)	18 (15.5)
6	Take by mouth	49 (42.2)	53 (45.8)	14 (12)
7	Do not store near heat or in sunlight	3 (2.5)	82 (70.8)	31 (26.7)
8	Take with meals	16 (13.7)	87 (75.1)	13 (11.2)
9	Wash hands/place drops in ear/wash hands again	34 (29.3)	70 (60.4)	12 (10.3)
10	Wash hands/place drops in nose/wash hands again	79 (68)	33 (28.6)	4 (3.4)
11	Wash hands/insert into vagina/wash hands again	34 (29.3)	58 (50.1)	24 (20.6)
12	Do not store medicine where children can get in	52 (44.8)	53 (45.8)	11 (9.4)
13	Do not drink alcohol while taking this medicine	38 (32.7)	63 (54.4)	15 (12.9)
14	This medicine may take you drowsy	0 (0)	73 (63)	43 (37)
15	Store in refrigerator	25 (21.5)	62 (53.5)	29 (25)

maintain the intention of an item between different cultures (Malloy-Diniz et al., 2010). Therefore, the present study not only performed a literal translation, but also considered relevant features of the culture of the target population, as advocated by the literature (Louis and Parker, 2000).

Some changes were made to certain subtitles by the committee evaluating semantic, idiomatic, and cultural equivalence, in order to enhance understanding. Amaral et al. (2011) pointed out that the committee of judges should critically evaluate an instrument and verify that it has not been translated literally. The committee should instead capture the intent of the original instrument, even if a number of words need to be changed. In short, after evaluation by the committee of judges, the translated versions must show semantic, idiomatic, conceptual, and cultural equivalence along with retention of the original meaning.

Some participants had difficulties understanding subtitles in the semantic validation phase. Conti et al. (2012) reported that the translation of an instrument can only be finalized after its evaluation by the target population. Moreover, the instrument should be translated and validated by members of the target population to ensure that distortions have not occurred during the translation process (Cha et al., 2007). Thus, the submission of translated versions to target populations allows the detection and correction of discrepancies that might have occurred during the translation, and evaluation of its clarity and appropriateness.

Conclusion

Most of the USP-DI pictograms assessed were not well

understood by elderly people. Of the pictograms selected for the comprehension test, only one met the comprehension criterion established by ISO 3864. Thus there is a need to design and validate new, culturally adapted pictograms for use in Brazil. Therefore, subtitles of pictograms validated in this study, along with the pictograms which are being redesigned, will be used to increase elderly people's understanding and recall of information relevant to the use of prescription medicine.

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Full Length Research Paper

Extraction of resveratrol and emondin from *Polygonum cuspidatum* by supercritical CO₂ with different solubilizers

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In order to improve the extraction yields of resveratrol and emondin obtained by supercritical CO₂ extraction (SCE), different solubilizers were added in the supercritical CO₂ extraction process. Resveratrol and emondin were extracted from *Polygonum cuspidatum* by SCE with different solubilizers, including bis(2-ethylhexyl) sodium sulfosuccinate (AOT), povidone k-90 (PVP k-90), Poloxamer 188 and Tween 80, at extraction pressure 25 MPa and extraction temperature 50°C. The results show that resveratrol was hardly extracted by SCE with ethanol as modifier, but AOT, PVP k-90, Poloxamer 188 and Tween 80 could enhance the extraction yield of resveratrol significantly, while the yield of emondin was influenced gently by using these solubilizers. Based on the comparison of SCE and heat reflux extraction, the advantage of SCE is less extraction time at lower operating temperature without using large amount of organic solvent. The yields of resveratrol and emondin extraction from *P. cuspidatum* by SCE can be improved by introducing AOT, PVP k-90 and Poloxamer 188 into supercritical CO₂.

Key words: *Polygonum cuspidatum*, resveratrol, emondin, supercritical CO₂ extraction, solubilizer.

INTRODUCTION

Polygonum cuspidatum (*P. povidone*) is a member of the Polygonaceae family, which is widely distributed in Asia and North America. The dried root of *P. povidone* is a well-known Traditional Chinese Medicine (called Huzhang) officially listed in the Chinese Pharmacopoeia (The State Pharmacopoeia Committee of the People's 2010) and also used as folk medicine remedies for the treatment of cuts, burns and abscesses in Korea and Japan (Bralley et al., 2008; Wu et al., 2012). Resveratrol and emondin were the major polyphenols in *P. povidone*

extracts, having antibacterial, antioxidant and antimutagenic properties (Shan et al., 2008; Pandit et al., 2012). Several studies have evaluated the anti-tumor effect of *P. povidone* extracts (Lin et al., 2010; Shin et al., 2011).

P. povidone is used to produce resveratrol supplements because it grows easily and quickly. Because of the wide range of possible health benefits of resveratrol and emondin, a huge effort has been made towards the development of extraction, isolation, purification and

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Table 1. The gradient elution time for determining the contents of resveratrol and emodin in extracts.

T/min	Water % (v/v)	Acetonitrile % (v/v)
0	82	18
5	68	32
7	50	50
12	28	72
14	28	72
20	82	18

quantification methodologies (Wang et al., 2008; Mantegna et al., 2012).

The conventional extraction method is heat reflux extraction with ethanol, followed by filtration, concentration and purification. This procedure is time consuming and requires a large amount of solvent (Xiang et al., 2005; Liu et al., 2007). Due to its environmental friendliness and unique physical and chemical properties, SCE has attracted much attention (Melo et al., 2014; Ruttarattanamongkol et al., 2014). However, supercritical CO₂ (SC) is not a good solvent for polar molecules such as saponins, flavones and alkaloids. Therefore improvements in polar compound solubility and mass transfer are required for SCE. By introducing polar solvent into SC can improve CO₂ dissolution of polar molecules. Ethanol and acetonitrile are always added in SC as modifier to improve CO₂ dissolution of polar molecules. By introducing surfactants or solubilizers into SC to form reverse micelles or microemulsion can improve CO₂ dissolution of polar molecules too. The aim of this paper is to explore the effects of different solubilizers on the extraction yields of resveratrol and emodin from *P. povidone* by SCE.

MATERIALS AND METHODS

Chemicals and reagents

P. povidone was purchased from Guangzhou Qingping Market for Traditional Chinese Medicine. Resveratrol and emodin standard (pharmaceutical grade) were purchased from National Institute for Control of Pharmaceutical and Biological Products. Bis(2-ethylhexyl) sodium sulfosuccinate (AOT) of analytical reagent grade was purchased from Aladdin Chemistry Co. Ltd. PVP k-90 and Poloxamer 188 (pharmaceutical grade) was provided by BASF (Germany). Tween-80 (analytical grade) was obtained from Guangzhou Chemicals Company. Ethanol (HPLC grade) was purchased from Tianjin Chemicals Company.

Supercritical CO₂ extraction

The SCE equipment (capacity 100 ml) was manufactured by Guangzhou Hanwei Co. Ltd. 30 g of the milled powder of dried *P. povidone* roots, particle size 0.5 to 0.2 mm, was placed in the extractor. The solubilizer (30 ml) was added via the high-pressure pump. The powder was statically extracted for 45 min followed by circularly extraction for 15 min at extraction pressure 25 MPa, extraction temperature 50°C, separation pressure 2 MPa, separation

temperature 25°C. The process was repeated 2 times. The extracts were collected and analyzed.

Heat reflux extraction

The heat reflux extraction was performed as follows (Xiang et al., 2005): 30 g of the milled powder of dried *P. povidone* roots was mixed with 150 ml of 70% ethanol in water, boiled at boiling point for 4 h. Then, the mixture was filtrated through filter paper. The extracts were collected. The residue was mixed with 150 ml 70% ethanol in water, boiled at boiling point for 4 h. This extraction process was repeated 2 times. The total extraction yields of resveratrol and emodin were calculated.

HPLC analysis

The high performance liquid chromatography (HPLC) system consisted of a pump, a column (Hypersil ODS, 5 μm, 4.6 mm × 150 mm), an auto-injector and a UV-detector (Agilent 1100, USA). The gradient elution HPLC method was used for determining the contents of resveratrol and emodin in extracts. The gradient elution time was shown in Table 1. HPLC was performed at a flow-rate of 1.0 ml/min at 30, using a mobile phase of acetonitrile and water, and a UV detector at 306 nm. The SCE sample was put into a 100 ml volumetric flask and reached the scale accurately with methanol. 5 ml sample solution was pipetted into a 25 ml volumetric flask and diluted to the scale accurately with methanol. 20 μl diluted solution was detected by HPLC. The chromatograms of the reference substances and samples are shown in Figure 1.

RESULTS

Optimization of supercritical fluid extraction

In this study, parameters of SCE such as type of solubilizer, concentration of solubilizer, pressure and extraction times were optimized. Each experiment was performed with 30 g of dried and milled *P. povidone* roots.

Effect of solubilizer

Type of solubilizer was selected at the beginning of the whole optimization process. Tested solubilizers were as follows: Ethanol, AOT, PVP k-90, Poloxamer 188 and Tween 80. The samples obtained by SCE with these solubilizers were analyzed and the results are shown in Figure 2. The highest extraction yield of resveratrol was 0.304 mg/g when AOT was used. The highest extraction yield of emodin was 1.008 mg/g when PVP k-90 was used. The lowest extraction yield of resveratrol was 0.015 mg/g when ethanol was used. The highest extraction yield of resveratrol was 20 times more than the lowest yield.

Effect of AOT concentration

Five different concentrations of AOT in 95% ethanol (that is, 0.02, 0.05, 0.1, 0.2 and 0.5 mol/l) were investigated

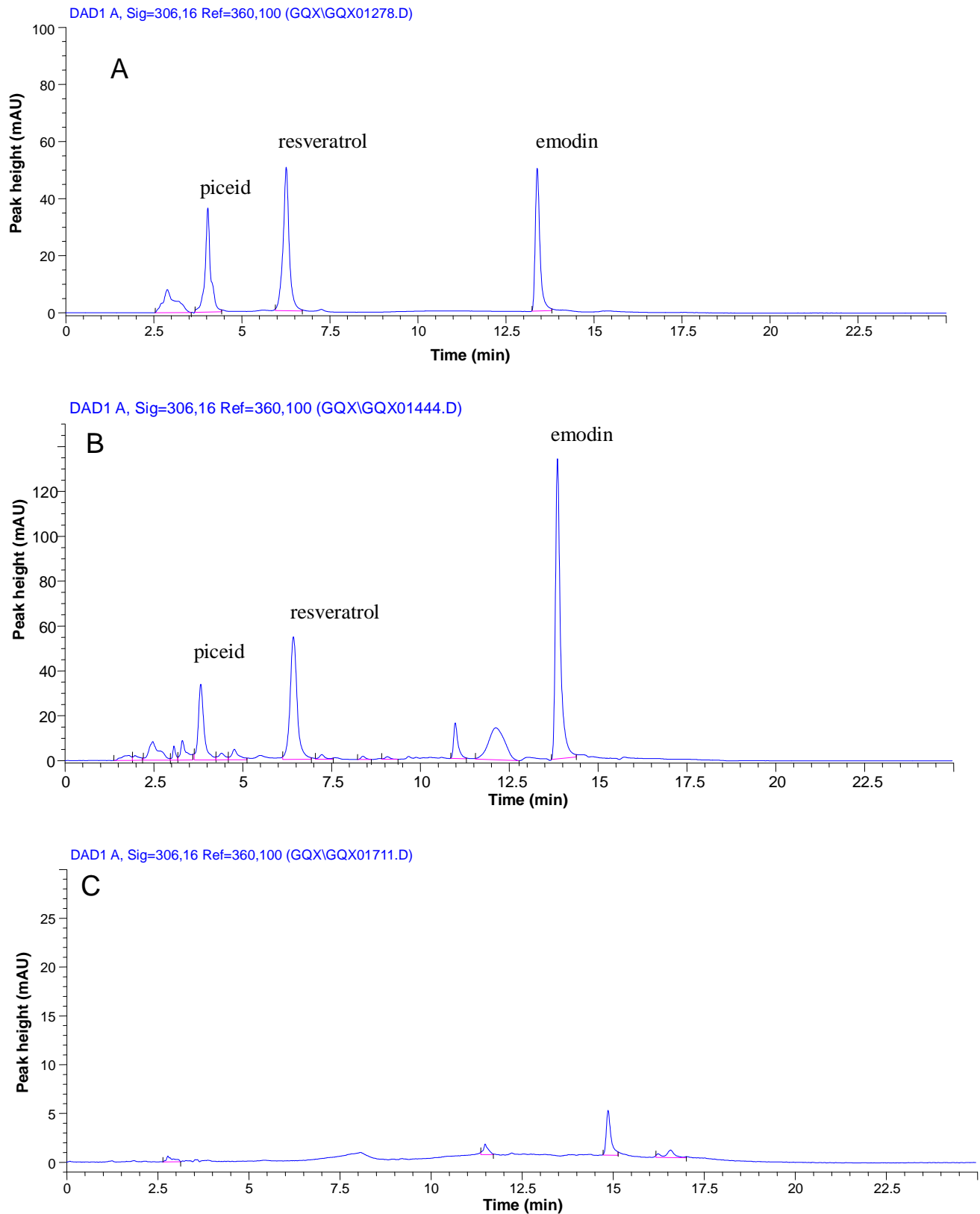


Figure 1. Chromatogram of standard solution (A), typical chromatogram of SC extracts of *P. povidone* (B) and blank sample (C). The blank sample solution contained 0.5 mg/ml PVP K-90, Poloxamer 188, Tween 80 and AOT. As shown in chromatograms, the determination of target compounds (resveratrol and emodin) was not interfered by solubilizers or other compounds in the sample.

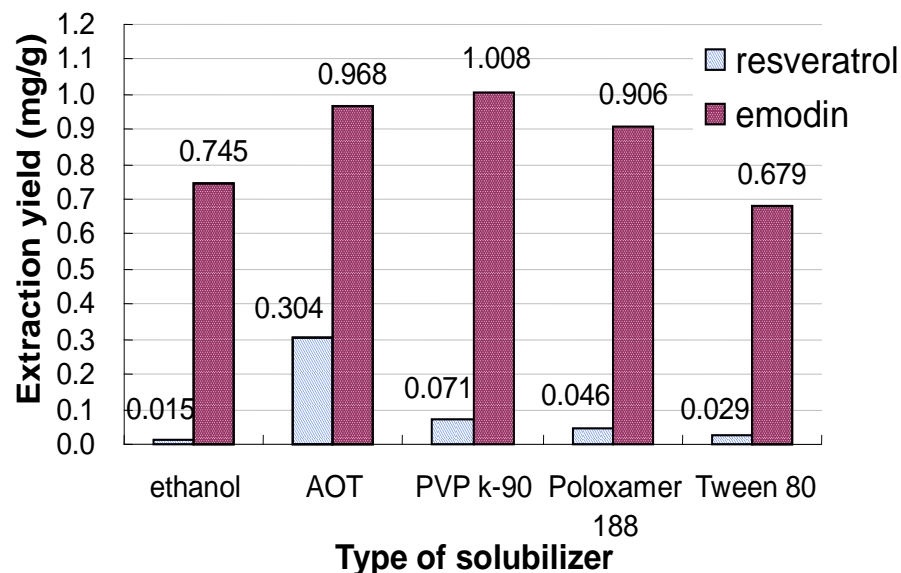


Figure 2. Effect of solubilizer on extraction yields of resveratrol and emodin. Extraction condition: 25 MPa, 50°C, 1 h, extraction 3 times.

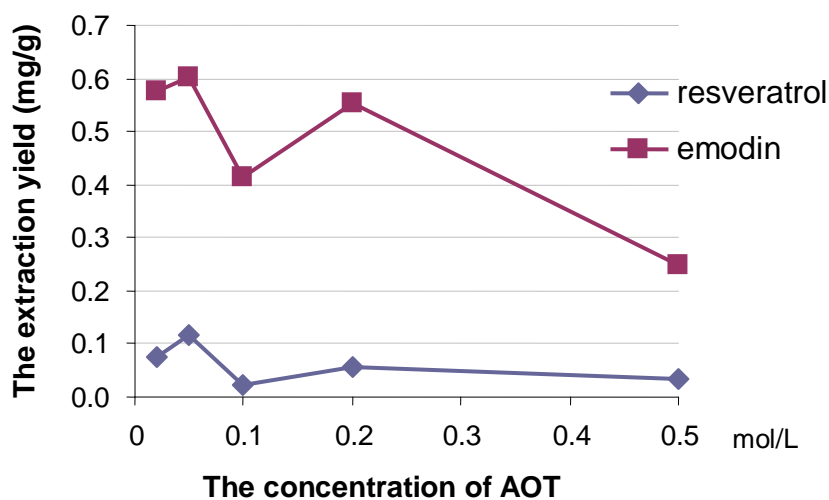


Figure 3. Effect of concentration of AOT on extraction yields of resveratrol and emodin. Extraction condition: 20MPa, 50°C, 1h, extraction 1 time

while the other parameters were fixed: 50 L, extraction 1 time and 20 MPa. As seen in Figure 3, the extraction yields of resveratrol and emodin were greatly influenced by the AOT concentration in 95% ethanol from 0.02 through 0.5 mol/L. The best results were achieved at the concentration 0.05 mol/L. The extraction yield was increased as AOT concentration increased to 0.05 mol/L. When the concentration was higher than 0.05 mol/L, the extraction yield decreased with the increase in AOT concentration. This is because the concentration of AOT is too high to completely dissolve in the SC phase, so excess AOT deposits on the material surface and limits

mass transfer.

Effect of pressure

Four different pressures (that is, 10, 15, 20 and 25 MP) were investigated while the other parameters were fixed: 50 L, extraction 1 time and 0.05 mol/L AOT as solubilizer. As shown in Figure 4, it is evident that the extraction yield of resveratrol was increased gently with increasing pressure, while the increase of extraction yield of emodin is more significant. The extraction yield of resveratrol and emodin is highest when applying the pressure 25 MPa.

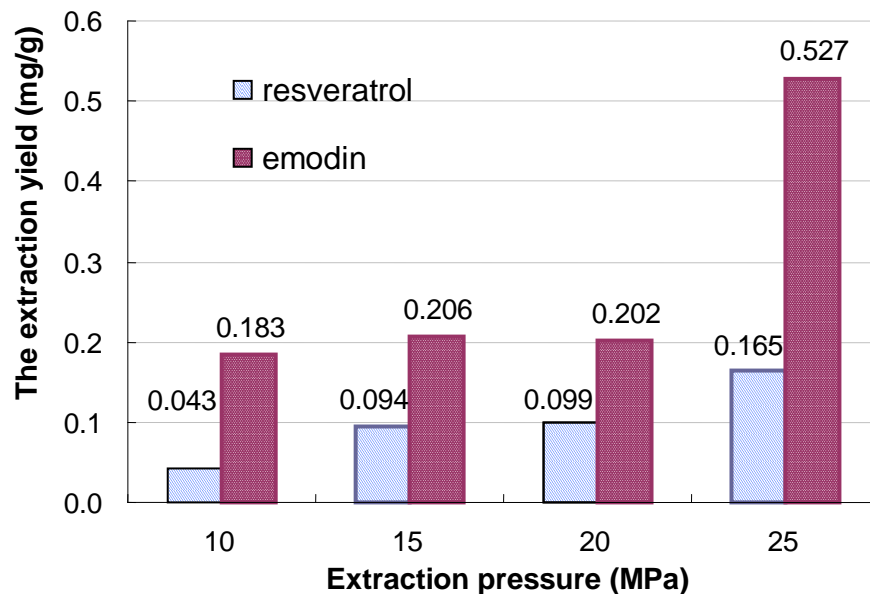


Figure 4. Effect of pressure on extraction yields of resveratrol and emodin. Extraction condition: 50°C, 1h, 0.05mol/l AOT, extraction 1 time.

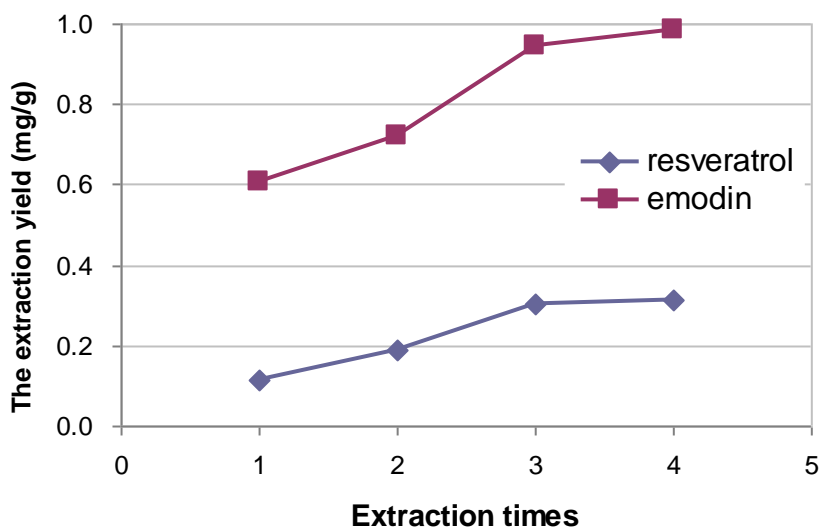


Figure 5. Effect of extraction times on extraction yields of resveratrol and emodin. Extraction condition: 50°C, 25MPa, 1h, 0.05mol/l AOT.

Effect of extraction times

Four different extraction times (that is, 1, 2, 3 and 4 times) were investigated while the other parameters were fixed: 50 L, 25 MPa and 0.05 mol/L AOT as solubilizer. As shown in Figure 5, the extraction yields of resveratrol and emodin increased gently with the increase of extraction times. The increase of extraction yield is noticeable to the extraction 3 times: the extraction yield stays at the same

level even the extraction more than 3 times. The results demonstrated that 3 times was enough to extract resveratrol and emodin from *P. cuspidatum* by SCE.

Comparison of SCE and Heat reflux extraction

Heat reflux extraction is a conventional extraction technique, but this procedure is time consuming and requires

Table 2. Comparison of the extraction yields of resveratrol and emodin by SCE and heat reflux extraction.

Extraction method	Extraction condition	The extraction yield (mg/g)	
		Resveratrol	Emodin
SCE	30 g sample, 50 L, 1 h, 25 MPa, AOT (0.05 mol/L) as solubilizer, three replicate extraction	0.31±0.03	0.97±0.06
Heat reflux extraction	30 g sample, 90 L, 4 h, 150 ml 70% ethanol as extraction solvent, three replicate extraction	1.68±0.07	0.52±0.05

Standard deviations are given from three replicate experiments.

a large amount of organic solvent. SCE is a new extraction technique, which primarily uses CO₂ as its extraction medium, has been widely used in food preparation and natural herbs extraction at low operating temperature without using large amount organic solvent. These two techniques were compared as shown in the Table 2. The extraction yield of resveratrol by heat reflux extraction was approximately 5.5 times higher than by SCE method, while the extraction yield of emodin by heat reflux extraction was approximately 2 times lower than by SCE.

DISCUSSION

CO₂ is a non-polar solvent, it is hardly polarised by ethanol. Resveratrol is hardly extracted by SC under normal condition. However, AOT, PVP k-90, Poloxamer 188, and Tween 80 could increase the extraction yield of resveratrol significantly, because they could induce to form micelles or microemulsion in SC. The extraction yield of resveratrol obtained by SCE could be improved significantly by introducing AOT, PVP k-90 and Poloxamer 188 into SC, while the extraction yield of emodin was influenced gently by using these solubilizers. SCE for 3 h gave higher extraction yield of emodin than the heat reflux extraction for 12 h at 90 L using 450 ml 70% ethanol. From time point of view the SCE is advantageous than heat reflux extraction and SCE also do not require large amount of solvent. Based on the comparison of SCE and heat reflux extraction, SCE is more suitable for emodin than for resveratrol. These results are compatible with Blanka B's research (Beňová et al., 2010). But in Blanka B's research the extraction yield of resveratrol is 0.1 mg/g by SCE with ethanol as modifier while the extraction yield is only 0.015 mg/g in our research. This phenomenon may be due to the difference of medicinal materials breeds.

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Conflict of interest

The author(s) have not declared any conflict of interests.

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