Evaluation of *In Vitro* Anti-inflammatory Activity, Cytotoxic Activity and Physicochemical Properties of Activated Carbon - Treated Sahasthara Ethanolic Extract

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Background: *Sahasthara (SHT) is a Thai traditional remedy for relieving muscles and bones pain. Presently, topical dosage forms of SHT extract (SHT_E) are used instead of oral dosage forms of SHT to avoid gastrointestinal side effects such as nausea and vomiting. However, topical products of SHT_E have not been accepted by some patients because of unpleasant odor and color of SHT_E used in formulations. Therefore, to increase patient's acceptance, the unpleasant odor and color of the crude extract should be removed before use as an active ingredient in the products.*
Objective: *To determine an optimum dose of activated carbon for improving physical appearance of SHT_E and to evaluate its anti-inflammatory activity, chance of being skin irritant and physicochemical properties of SHT_E after being treated with activated carbon.*
Material and Method: *SHT_E was treated with various amounts of activated carbon and investigated for its inhibitory activity on nitric oxide production in RAW 264.7 cells and potential for being a skin irritant by MTT assay on skin fibroblasts. Measurement of lightness values, piperine contents by HPLC and pH values of treated SHT_E were also performed.*
Results: *SHT_E treated with 0%, 10% and 20% activated carbon showed an anti-inflammatory activity with IC_{50} values of 4.15 ± 0.58, 7.27 ± 0.53, 7.92 ± 0.23 μg/mL, respectively. None of them was toxic to skin fibroblasts. Lightness of SHT_E and piperine contents of such extracts decreased with increase in doses of activated carbon. On the other hand, the higher amount of activated carbon used, the higher is the pH value.*
Conclusion: *SHT_E treated with 20 % w/w activated carbon resulted in better physical appearance of SHT_E than that of either untreated- or 10% activated carbon-treated SHT_E. Yet, it retained an anti-inflammatory activity with no potential for being a skin irritant. Therefore, treating SHT_E with 20 % w/w activated carbon can be further used for preparation of SHT topical formulations.*

Keywords: *Activated carbon, Anti-inflammatory activity, Physicochemical properties, Sahasthara remedy, Thai traditional medicine*

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Non-steroidal anti-inflammatory drugs (NSAIDs) are common drugs used for muscle pain treatment. In the last decade, number of prescriptions of NSAIDs increased continuously in Thailand leading to increase in amount of imported drugs. In 2009, 97.4% of NSAIDs were prescribed for patients by doctors in public hospitals[1]. In 2010, the total expenditure for imported musculo-skeletal drugs was 7,427.40 million baht[2]. Consequently, the Ministry of Public Health has implemented a policy to decrease the cost of

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imported drugs by promoting the use of Thai traditional medicines and assigned in Thailand National List of Essential Medicines (NLEM).

Sahasthara remedy (SHT) is one of the official Thai traditional medicines in NLEM. It has been recommended for relieving muscle and bone pain due to its anti-inflammation activity[3]. According to broad spectrum side effects of NSAIDs, i.e., gastrointestinal and cardiovascular events, renal toxicity, increased blood pressure, and worsening congestive heart failure[4], SHT may be more beneficial than NSAIDs because it possesses less systemic side effects. The results of our previous studies showed that SHT ethanolic extract (SHT_E) exhibited an anti-inflammatory activity by inhibiting release of nitric oxide (NO) from lipopolysaccharide (LPS)-induced RAW 264.7 cell line. Interestingly, it could inhibit cyclo-oxygenase-2 activity (COX-2) with a comparable IC_{50} value to that of indomethacin[5]. The results from a clinical study revealed that oral administration of SHT_E capsules for 14 days could relieve knee pain in patients suffering from primary osteoarthritis. Its efficacy was insignificantly different from diclofenac sodium tablets[6].

SHT is composed of pepper as a major constituent and other 20 herb species. Therefore, its biological activity mostly comes from an anti-inflammatory activity of piperine containing in pepper[5]. It was found that piperine significantly inhibited nitric oxide (NO), prostaglandin E_2 (PGE_2), tumor necrosis factor (TNF-α) production in LPS-stimulated RAW264.7 cells, and inhibition transcription level via suppression of inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2) and TNF-α gene and protein expression[7]. Black pepper oil could reduce acute inflammation induced by carrageenan and dextran. It also reduced inflammation of formaldehyde-induced chronic inflammation in rat model[8]. Consequently, the treatment of pain and inflammation of muscles and bones using SHT can reduce the amount of prescribed NSAIDs and the cost of imported drugs.

The effective doses of oral SHT are 3 to 4.5 g/day. However, the most common side effects of SHT found in patients using this remedy are GI disturbances i.e. abdominal pain, nausea, vomiting and skin rash. In rare cases, SHT may be toxic to liver and kidney. Furthermore, more careful attention should be paid to patients with heart disease, high blood pressure, peptic ulcer and gastro-esophageal reflux disease. Although topical products containing SHT_E have been recently developed and used instead of oral SHT to avoid severe side effects, some patients have still not accepted these products because of strongly unpleasant odor and color of SHT_E. Consequently, improvement of physical appearance of SHT_E is crucial for development of SHT_E topical products.

Activated carbon is an adsorbent widely used for remediation of polluted water by removing organic pollutants. Due to its high surface area (>1,000 m^2/g), activated carbon can adsorb large amount of pollutants or contaminants[9]. It has been extensively applied to remove color from several types of juice, coffee and tea infusion, sugar beet vinasse, vinegar, syrups and liquor[10]. In this study, we used activated carbon as an adsorbent to adsorb the unpleasant odor and color of SHT_E before further use of SHT_E as an ingredient in topical formulations. The aim of this study was to determine an optimum dose of activated carbon for improving physical appearance of SHT_E and to evaluate anti-inflammatory activity, its potential for being skin irritant and its physicochemical properties after being treated with activated carbon.

Material and Method

Materials

Dimethyl sulfoxide (DMSO) was purchased from RCI Labscan, Thailand. Fetal bovine serum (FBS) was purchased from Biochem, Germany. Hydrochloric acid and sodium hydroxide were purchased from Univar, Australia. Penicillin-Streptomycin (P/S), RPMI medium 1640, Trypan blue stain 0.4% and Trypsin-EDTA were purchased from Gibco, USA. Phosphate buffered saline (PBS) was purchased from Amresco, USA. Sodium bicarbonate was purchased from BHD, England. Lipopolysaccharide (LPS, from *Escherichia coli*), 3-(4, 5-dimethy-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide (MTT), N-(1-Naphthy) ethylenediamine dihydrochloride, Sulfanilamide, Phosphoric acid solution were purchased from Sigma, USA.

Preparation of SHT_E

All ingredients of SHT were listed and purchased from Kanokkangsadal, 2013. They were weighed according to the formulation published in the Thailand NLEM[3] and mixed thoroughly. The mixture was macerated in 95% ethanol for three days to obtain SHT_E. The residue of the first extraction was further extracted with the same procedure for another two cycles of the process. All three extracts were pooled and dried by using an evaporator under reduced pressure at 45°C (Rotavapor R-205, Germany).

Treatment of SHT_E with activated carbon

SHT_E was accurately weighed and dissolved in 65% ethanol to make two flasks of 100 g of 5% w/w of SHT_E solution. Then, 10 g or 20 g of AC (P&N Hightec Chemical, Thailand) were added into each SHT solution. AC in SHT_E solution was dispersed by shaking manually for 1 min and filtered through filter papers (Whatman No. 1, USA). In this study, 100 g of 5% w/w of SHT_E solution without AC treatment was also prepared for determination of the effect of AC on properties of SHT_E.

The filtrates of SHT_E solution after treated with AC (treated SHT_E) and the filtrate obtained from SHT_E solution without treatment with AC (untreated SHT_E) were determine for their anti-inflammatory activity and physicochemical properties as follows: piperine contents, pH and lightness values. The untreated- and treated SHT_E solutions were dried using an evaporator. Their extraction yields were calculated using the following equation:

$$\text{Extraction yield (\%)} = \frac{\text{weight of dried treated } SHT_E}{\text{weight of dried untreated } SHT_E} \times 100$$

Measurement of pH of SHT_E

pH values of untreated- and treated SHT_E were measured using a pH meter (Inolab WTW, Mexico) at a room temperature in triplicate. The data were shown as a mean $\pm$ SD.

Measurement of Luminance of SHT_E

Luminance (L*) of AC-untreated- and AC-treated SHT_E were investigated via a spectrophotometer (ColorFlex EZ Spectrophotometer, Hunter Associates Laboratory, Inc., USA) with scales of 0 to 100 from ranging black to white. Each sample was measured in triplicate and the obtained data were presented as mean $\pm$ SD.

Determination of Piperine content

Contents of piperine in SHT_E was analyzed by using a High Performance Liquid Chromatography (HPLC) method according to Itharat and Sakpakdeejaroen[11] with some modifications. The HPLC system (Agilent® 1200) composed of a solvent degasser (G1322A), a quaternary solvent pump (G1311A), an autosampler (G1329A), a column oven (G1316A) and a photodiode array detector (G1315D). The chromatographic data were processed by the Chemstation software revision B.04.01 SP1.

Chromatographic system was carried out along a C18 column (Phenomenex® Luna, 4.6x250 mm, 5 micron) connected with a C18 guard column. The sample volume of 10 µL was injected into the HPLC system and the samples were eluted using gradient mobile phase composing of water (A) and acetonitrile (B) with various ratios as follows: 0 min, 60: 40 A:B; 30 min, 50: 50 A:B; 60 min, 0: 100 A:B at flow rate of 1.0 mL/min. The diode array detector was set at wavelength of 340 nm.

Preparation of standard piperine and SHT_E

One milligram of standard piperine (Merck, Germany) was weighed accurately and dissolved in acetonitrile (Labscan, Thailand) to make concentrations of 50, 100, 200, 400, 600 and 800 µg/ml. These solutions were analyzed for piperine by HPLC technique following the method described in the previous section. The standard curve of piperine showing linear relationship between concentrations of piperine and area under curve of chromatogram were constructed.

SHT_E was dissolved with acetonitrile, sonicated for 5 minutes, and filtered through a 0.45 µm membrane filter before use. Concentrations of SHT sample were prepared at 10 mg/ml. Piperine content of each sample was calculated using linear equation of standard curve.

Determination of anti-inflammatory activity

An inhibitory effect on nitric oxide produced by murine macrophage like RAW 264.7 cells is evaluated according to the method of Tewtrakul and Subhadhirasakul[12] with some modification. Briefly, cells were seeded in 96-well plates to have a density of $1x10^5$ cells/well/100 µl and incubated for 24 hours in a CO_2 incubator at 37°C. Then, medium was removed and replaced with 100 µl RPMI medium containing 4 ng/mL of lipopolysaccharide (LPS). One hundred microliters of each SHT_E at various concentrations was added to each well, to give final concentrations of 50, 30, 10, 1 and 0.1 µg/ml. The treated cells were incubated for 24 hours. Finally, supernatant was removed to another 96-well plate and mixed with Griess reagent. The color of reaction was detected and presented as a value of optical density (OD) using microplate reader at a wavelength of 570 nm. Percentage of inhibition was calculated using the following equation and IC_{50} values were determined using GraphPad Prism 5.

$$\text{Inhibition (\%)} = \frac{OD_{control} - OD_{sample}}{OD_{control}} \times 100$$

Where $OD_{control}$ is the difference between a

mean OD of control media with LPS and a mean OD of control media without LPS; OD_{sample} is the difference between a mean OD of sample with LPS and a mean OD of sample without LPS.

In this study, cytotoxicity of each SHT_E at various concentrations on RAW 264.7 cells was investigated simultaneously by the MTT colorimetric method. After cells were incubated with SHT_E at various concentrations, the supernatant was removed. Then, MTT solution was added to each wells (10 µl/well) and incubated for 2 hours in a CO_2 incubator at 37°C. The medium was then removed, and 100 µl/well of isopropanol containing 0.04 M HCl was added to dissolve the formazan crystal. The OD was measured at 570 nm. The test sample was considered to be cytotoxic when % cytotoxicity of the sample-treated group was more than 30% compared to control group.

$$\text{Cytotoxicity (\%)} = \frac{OD_{control} - OD_{sample}}{OD_{control}} \times 100$$

Where $OD_{control}$ is a mean OD of control media without LPS and OD_{sample} is a mean OD of sample without LPS.

Cytotoxicity test by MTT assay

Neonatal Human Dermal Fibroblasts (HDFn) were purchased from the Thermo Fisher Scientific Incorporation (USA) Catalog number C0045C. DMEM, fetal bovine serum, L-glutamine, trypsin-EDTA solution, and phosphate buffer were purchased from Invitrogen (USA). Methylthiazolydiphenyltetrazolium bromide (MTT) was purchased from Sigma-Aldrich (USA).

Potential for being a skin irritant of SHT_E was determined by the MTT colorimetric method on HDFn cells, skin fibroblast cells. This study was performed according to the method described previously[13] with some modifications. Briefly, cells were seeded in 96-well plates with a density of $5x10^4$ cells/well and incubated for 24 hours in a CO_2 incubator at 37°C. Then, SHT_E in DMSO solution was added to the wells, to reach final concentrations of 20, 10, 5 and 0.5 µg/ml. The treated cells were incubated for 24 hours. Finally, MTT solution was added to each wells and incubated for 4 hours. The medium was removed, and isopropanol containing 0.04 M HCl was added to dissolve the formazan crystal (100 µl/well). The OD of sample and control were measured at 570 nm. The percentage of cell viability was calculated by the following equation:

$$\text{Cell viability (\%)} = \frac{OD_{sample}}{OD_{control}} \times 100$$

The test sample was considered to be skin irritant when the percentage of cell viability was less than 70% compared to control group.

Statistical analysis

IC_{50} for anti-inflammatory activity, piperine content, pH and L* values of SHT_E treated with various doses of AC were compared by using a one-way ANOVA with Fisher's LSD method for multiple comparisons at a significant level of 0.05.

Results

Physicochemical properties of AC-untreated SHT_E and AC-treated SHT_E

The obtained SHT_E without AC treatment had dark green color, malodor and some sediment. However, its physical appearances were improved after it was treated with AC. It had lighter color, softer odor and looked clearer. These sensory perceptions were consistent with physicochemical properties of SHT_E measured after AC treatment i.e. % yield, pH and L* value as shown in Table 1.

The percentage yield of SHT_E was decreased significantly with increase in AC concentrations. In addition, the more AC content led to the more pH values and the more luminance of SHT_E color.

Determination of Piperine content

Because pepper is a major constituent of SHT, therefore, piperine which is an alkaloid found in pepper possessing anti-inflammatory activity, was used as a chemical marker for quality control of the extract. Fig. 1 shows chromatograms of piperine from HPLC analysis. It was found that the peak of standard piperine appeared around 21 minutes was consistent with chromatograms of AC-untreated and AC-treated SHT_E (Fig. 3-5). However, area under curve of the chromatograms of AC-treated SHT_E were lower than that of AC-untreated SHT_E. This suggested that piperine content in AC-treated SHT_E was lower than that of AC-untreated SHT_E. In this study, the content of piperine in SHT_E was calculated from the following regression equation; $y = 72.378x+203.2$ ($R^2 = 0.9998$) obtained from the standard curve of piperine shown in Fig. 2. The results in Table 1 show that piperine contents of SHT_E were reversely proportional to amount of AC used for improving physical characters of the extract.

Determination of anti-inflammatory activity

Anti-inflammatory activity of SHT_E was

Table 1. Physicochemical properties and IC_{50} for inhibition of NO production of AC-untreated- and AC-treated SHT_E

SHT_E	AC content (% w/w)	% Yield	pH	L*	Piperine content (mg/g)	IC_{50} (µg/ml)
Untreated-SHT_E	0	83.4	4.24±0.05	1.37±0.03	118.41±1.16	4.15±0.58
10% AC treated-SHT_E	10	54.4[a]	5.41±0.01[a]	1.87±0.14[a]	96.80±0.36[a]	7.27±0.53[a]
20% AC treated-SHT_E	20	38.4[a]	5.41±0.00[a]	1.98±0.1[a]	85.08±1.03[a]	7.92±0.23[a]

Data of physicochemical properties are presented as Mean ± SD and IC_{50} are presented as Mean ± SEM
[a] Indicates significant difference at *p*-value <0.05, compared to untreated-SHT_E

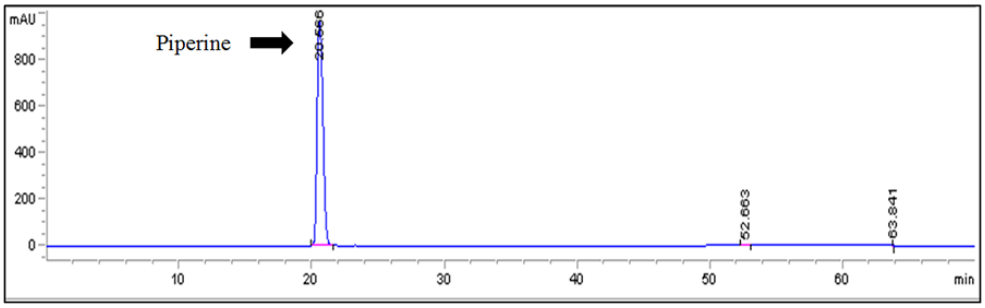

Fig. 1 Chromatograms of piperine (concentration 400 µg/ml) at wavelength of 340 nm by using HPLC.

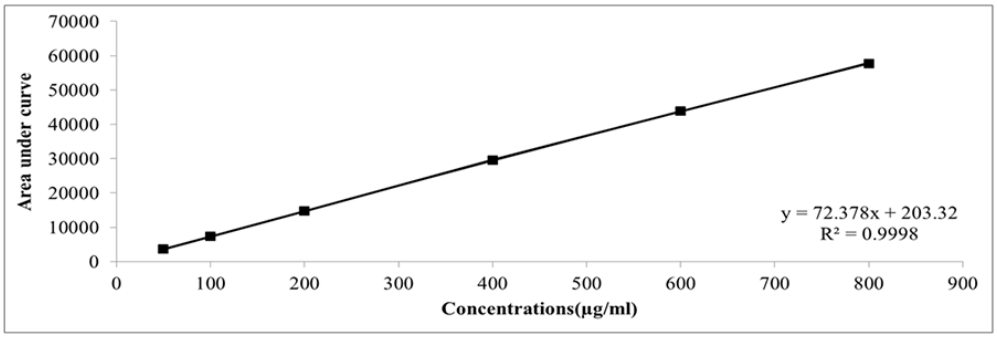

Fig. 2 Calibration curve of standard piperine, concentrations range from 50 to 800 µg/ml.

determined and presented as mean of IC_{50} values of SHT_E for inhibition of NO production by RAW 264.7 cells in Table 1. The results indicated that treatment of SHT_E with AC led to decrease in anti-inflammatory activity compared to that of untreated AC. Furthermore, the more AC content, the higher IC_{50}. This tendency coincided with the decline of piperine content in SHT_E treated with higher AC content.

Cytotoxicity test by MTT assay

Skin irritation test was conducted to evaluate the potential of SHT_E for being a skin irritant. However, to avoid using laboratory animals, this test was performed in human skin fibroblasts. The results shown in Table 2 as percentage cell viability (% CV) indicated that the cells could tolerate AC-untreated- and AC-treated SHT_E at various concentrations. Because % CV of human skin fibroblasts exposed to all extracts were higher than 70%, AC-untreated- and AC-treated SHT_E could be accepted as safe for being an active ingredient of topical products.

Discussion

Nowadays, topical products containing SHT_E for treatment of muscle and bone pain have been marketed in Thailand. Due to unpleasant odor and color of the extract, these products have not been accepted by some patients. Consequently, removal of unpleasant odor and color usually causes loss in potency of activity of the extract because some ingredients containing

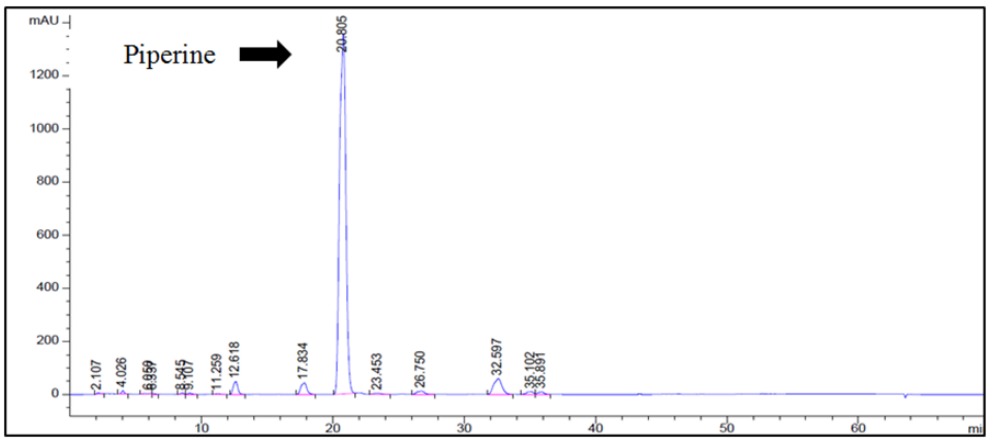

Fig. 3 Chromatograms of AC-untreated SHT_E (0%) at wavelength of 340 nm by using HPLC.

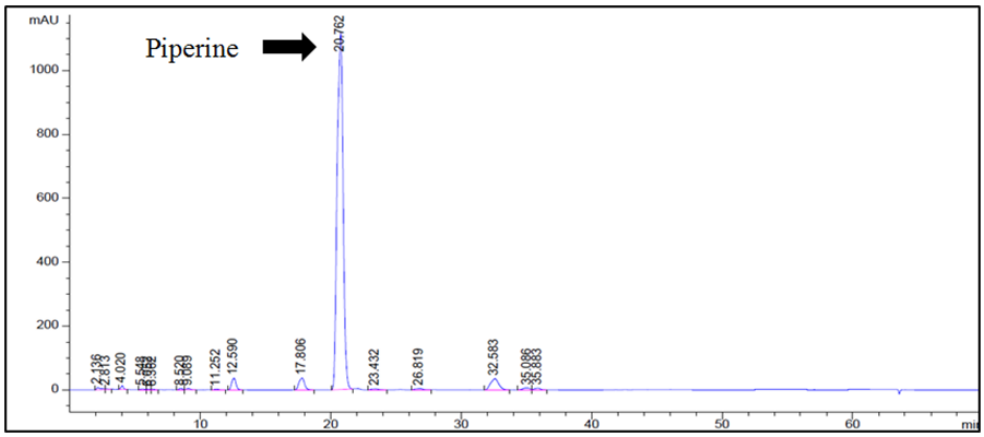

Fig. 4 Chromatograms of AC-treated SHT_E (10%) at wavelength of 340 nm by using HPLC.

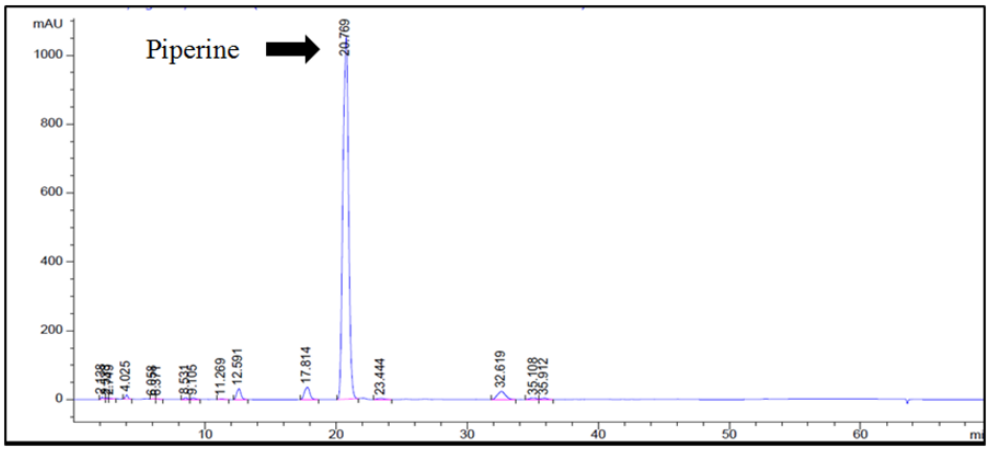

Fig. 5 Chromatograms of AC-treated SHT_E (20%) at wavelength of 340 nm by using HPLC.

in the extract were removed as well. Therefore, the treatment process of extract should be optimized.

AC has an excellent adsorb ability property[9]. It has been widely applied to remove organic pollutants

Table 2. Percentage cell viability of HDFn cells exposed to AC-untreated SHT_E 10% AC treated-SHT_E and 20% AC treated-SHT_E (n = 3)

Sample	% cell viability			
	0.5 µg/ml	5 µg/ml	10 µg/ml	20 µg/ml
Untreated-SHT_E	104.79±3.23	112.15±7.32	118.74±6.22	129.73±5.93
10% AC treated-SHT_E	95.98±3.07	117.57±8.73	121.25±5.69	121.48±11.05
20% AC treated-SHT_E	92.98±3.85	99.70±1.86	105.57±4.26	103.99±1.66[a]

Data are presented as Mean ± SEM

[a] Indicates significant difference at *p*-value <0.05, compared to untreated-SHT_E

including sources of odor and color from wastewater, drinking water, herb extracts, juices and syrup. Furthermore, it could also improve separation yield at one-step process leading to reduced processing cost. In this study, AC was used as a filtering aid in treatment process of SHT_E. It not only removed sediments in SHT_E, but also improved odor and color of the extract. Therefore, % yield and luminance of AC-treated SHT_E were lower than that of AC-untreated SHT_E.

The pH values of SHT_E tended to increase in direct proportion to amount of AC, while, the contents of piperine in SHT_E was declined with the increase amount of AC. These findings could be explained that AC adsorbed some ingredients including piperine in the extract and finally, they were removed from the extracts via a filtration process.

The previous report on ethanolic extract of Sahasthara recipe showed that potent inhibitory activity on lipopolysaccharide (LPS) induced nitric oxide (NO) production in RAW 264.7 cells, with IC_{50} values of 2.81±0.13 µg/ml[5]. SHT_E was already approved that it could inhibit inflammatory process through inhibition of NO production. Consequently, it could relieve muscle and bone pain effectively. SHT composes of 21 herbs species of which pepper is the important one. Several studies showed that for anti-inflammatory activity of pepper; piperine, a major alkaloid in pepper, is an alkaloid that inhibits the production of PGE_2 and nitric oxide[7]. Nitric oxide is a mediator generated from the nitric oxide synthase pathway. It's well known to produce in tissues affected by the inflammatory processes i.e. rheumatic diseases, chronic degenerative disorders, central neurodegenerative processes associated with brain ischemia[14].

Loss in piperine content led to loss in the potency of anti-inflammatory activity of AC-treated SHT_E as well. The results showed that IC_{50} of SHT_E for inhibition of LPS-induced NO released from RAW264.7 cells was increased after it was treated with AC. This suggested that the concentration of AC-treated SHT_E used in topical products should be higher than that of AC-untreated SHT_E for the effective pain relief.

The potential of cytotoxicity observed with AC-untreated SHT_E and AC-treated SHT_E indicated that they were not toxic to human skin fibroblasts. Cytotoxicity activity showed that percentages of cell viability were more than 70% at every concentration. However, higher concentration of SHT_E showed increased proliferation of HDFn cells (percentage of cell viability was more than 100% with compared to control group). SHT_E through 0% AC showed the highest proliferation, followed by 10% AC-treated SHT_E, and 20% AC-treated SHT_E respectively. Adsorption of compounds in SHT_E by activated carbon may cause change in the percentage of cell viability. This led to the reducing amount of active ingredients which otherwise activates proliferation of HDFn cells.

However, Because of the physical appearances of 10% AC treated-SHT_E and 20% AC treated-SHT_E there is no difference. Therefore, the researchers tested by allowing the mixture to stand overnight and found that10% AC treated-SHT_E showed sediment but not in 20% AC treated-SHT_E. In addition, better physical appearances of 20% AC-treated SHT_E, it could provide better sensory perceptions of the topical product than that of AC-untreated and 10% AC-treated SHT_E, therefore, 20% AC-treated SHT_E was suitable for further use as an active ingredients of topical products of SHT_E.

Conclusion

The results indicated that AC could remove unpleasant odor, color and sediment from SHT_E properly and did not cause skin irritation. However, it also reduced piperine content in SHT_E leading to higher

IC_{50} for inhibition of NO production in RAW264.7 cells. Because 20% AC-treated SHT_E still had anti-inflammatory activity with the most well accepted appearance, 20% of AC could be accepted as an optimized dose for improving physical appearances of SHT_E. These results support the use of activated carbon for reduction of unpleasant odor and color of SHT_E. The product developments for external use is demonstrated for preparing product with good cosmetic appearance, and no skin irritation, yet retaining the anti-inflammatory activity. Therefore, this study will be very useful for development of SHT_E as anti-inflammatory drug for pain treatment.

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What is already known on this topic?

SHT is assigned in the NLEM 2554 to relieve pain and inflammation of muscles and bones. The results of previous studies showed that SHT_E had an anti-inflammatory activity[5]. Its mechanism of action is similar to that of NSAIDs which are inhibition of nitric oxide and COX-2. Nowadays, use of SHT in muscle and bone pain can decreased the use of imported NSAIDs drugs.

What this study adds?

This study investigated anti-inflammatory activity, toxicity in skin cells, and physicochemical properties of AC-treated SHT_E. The results indicated that AC could improve physical appearance of SHT_E. Therefore, it could be added into topical products without unpleasant odor and color. Furthermore, it still had an anti-inflammatory effect and was not toxic to skin fibroblasts.

Potential conflicts of interest

None.

References

1. Kittiboonyakun P, Ploylearmsang C, Somsaard P, Suttajit S, Leelathanaroek A, Srimongkhol P , et al. Study research efficacy and cost-effectiveness analysis of selective Cyclooxygenase inhibitors (COX-II inhibitors). Nonthaburi: Health Systems Research Institute; 2012.
2. Thai Drug Control Division. Bureau of Drug Control Ministry of Public Health. The value of medicine import in 2010 Sorted by pharmacological [Internet]. 2010 [cited 2015 Mar 17]. Available from: http://drug.fda.moph.go.th/zone_search/files/sea001_b22.asp.
3. The Traditional Medicine Knowledge Fund. List of herbal medicine products A.D 2011. Nonthaburi: Department for Development of Thai Traditional and Alternative Medicine, The Institute of Thai Traditional Medicine; 2011.
4. Sostres C, Gargallo CJ, Lanas A. Nonsteroidal anti-inflammatory drugs and upper and lower gastrointestinal mucosal damage. Arthritis Res Ther 2013; 15 (Suppl 3): S3.
5. Kakatum N, Jaiarree N, Makchucit S, Itharat A. Antioxidant and anti-inflammatory activities of Thai medicinal plants in Sahasthara remedy for muscle pain treatment. J Med Assoc Thai 2012; 95 (Suppl 1): S120-6.
6. Pinsornsak P, Kanokkangsadal P, Itharat A. The clinical efficacy and safety of the sahastara remedy versus diclofenac in the treatment of osteoarthritis of the knee: A double-blind, randomized, and controlled trial. Evid Based Complement Alternat Med 2015; 2015: 103046.
7. Ying X, Yu K, Chen X, Chen H, Hong J, Cheng S, et al. Piperine inhibits LPS induced expression of inflammatory mediators in RAW 264.7 cells. Cell Immunol 2013; 285: 49-54.
8. Jeena K, Liju VB, Umadevi NP, Kuttan R. Antioxidant, anti-inflammatory and antinociceptive properties of black pepper essential oil (*Piper nigrum* Linn). J Essent Oil Bear Pl 2014; 17: 1-12.
9. Bae W, Kim J, Chung J. Production of granular activated carbon from food-processing wastes (walnut shells and jujube seeds) and its adsorptive properties. J Air Waste Manag Assoc 2014; 64: 879-86.
10. Bernal M, Ruiz MO, Geanta RM, Benito JM, Escudero I. Colour removal from beet molasses by ultrafiltration with activated charcoal. Chem Eng J 2016; 283: 313-22.
11. Itharat A, Sakpakdeejaroen I. Determination of cytotoxic compounds of Thai traditional medicine called Benjakul using HPLC. J Med Assoc Thai

2010; 93 (Suppl 7): S198-203.
12. Tewtrakul S, Subhadhirasakul S. Effects of compounds from *Kaempferia parviflora* on nitric oxide, prostaglandin E2 and tumor necrosis factor-alpha productions in RAW264.7 macrophage cells. J Ethnopharmacol 2008; 120: 81-4.
13. Watts P, Kittakoop P, Veeranondha S, Wanasith S, Thongwichian R, Saisaha P, et al. Cytotoxicity against insect cells of entomopathogenic fungi of the genera Hypocrella (anamorph Aschersonia): possible agents for biological control. Mycol Res 2003; 107: 581-6.
14. Salvemini D, Kim SF, Mollace V. Reciprocal regulation of the nitric oxide and cyclooxygenase pathway in pathophysiology: relevance and clinical implications. Am J Physiol Regul Integr Comp Physiol 2013; 304: R473-87.

การประเมินฤทธิ์ต้านการอักเสบ ฤทธิ์ความเป็นพิษต่อเซลล์และคุณสมบัติทางเคมีกายภาพของสารสกัดสหัสธาราที่ถูกกรองด้วยผงคาร์บอน

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ภูมิหลัง: *ตำรับสหัสธาราเป็นตำรับยาไทยที่อยู่ในบัญชียาหลักแห่งชาติ พ.ศ. 2554 ใช้รับประทานเพื่อบรรเทาอาการปวดอักเสบของกล้ามเนื้อและกระดูก การใช้สารสกัดยาตำรับสหัสธาราในรูปแบบยาใช้ภายนอกเพื่อหลีกเลี่ยงอาการไม่พึงประสงค์กับผู้ป่วยจากการรับประทานยา เช่น ร้อนท้อง แสบท้อง คลื่นไส้ คอแห้ง ผื่นคัน อย่างไรก็ตาม สารสกัดสหัสธารามีสีและกลิ่นที่ไม่พึงประสงค์ ดังนั้นเพื่อให้ผู้ป่วยยอมรับ กลิ่นและสีดังกล่าวควรถูกกำจัดออกก่อนนำมาใช้ในการพัฒนาผลิตภัณฑ์*

วัตถุประสงค์: *เพื่อทดสอบปริมาณที่เหมาะสมของผงคาร์บอนและศึกษาฤทธิ์ต้านการอักเสบ ฤทธิ์ความเป็นพิษต่อเซลล์ผิวหนังและคุณสมบัติทางเคมีกายภาพของสารสกัดสหัสธาราที่ถูกนำมากรองผ่านผงคาร์บอน*

วัสดุและวิธีการ: *สารสกัดสหัสธาราจะถูกนำมากรองด้วยผงคาร์บอนด้วยปริมาณที่แตกต่างกัน หลังจากนั้นนำมาศึกษาฤทธิ์ต้านการอักเสบ โดยการยับยั้งการผลิตไนตริกออกไซด์ในเซลล์ RAW 264.7 และประเมินความเป็นพิษต่อ skin cells ด้วยเทคนิค MTT ในเซลล์ HDFn และการประเมินค่าความสว่าง การวิเคราะห์หาปริมาณ Piperine ด้วยเทคนิค HPLC และประเมินค่าความเป็นกรดด่าง*

ผลการศึกษา: *สารสกัดสหัสธาราที่กรองด้วยผงคาร์บอน 0, 10, 20% มีฤทธิ์ยับยั้งการสร้างไนตริกออกไซด์จากเซลล์ RAW264.7 ที่ถูกเหนี่ยวนำโดย LPS โดยมีฤทธิ์ในการยับยั้งการสร้างไนตริกออกไซด์มีค่า IC_{50} เท่ากับ 4.15±0.58, 7.27±0.53, 7.92±0.23 μg/ml ตามลำดับ และไม่มีความเป็นพิษต่อเซลล์ผิวหนังทุกความเข้มข้น มีค่าความสว่างเท่ากับ 1.37±0.03, 1.87±0.14, 1.98±0.1 มีปริมาณ Piperine 118.18±1.16, 97.01±0.36, 5.29±1.03 mg/g ตามลำดับ และค่าความเป็นกรดด่าง 4.24±0.05, 5.41±0.01, 5.41±0.00 ตามลำดับ*

สรุป: *การเลือกใช้สารสกัดสหัสธาราที่ผ่านการกรองด้วยผงคาร์บอน 20% มีลักษณะปรากฏทางเครื่องสำอางที่ดีที่สุด สามารถกำจัดกลิ่นและสีที่ไม่พึงประสงค์ได้มากกว่า 0% และ 10% และยังคงประสิทธิภาพในการต้านการอักเสบอีกทั้งไม่เป็นพิษต่อเซลล์ผิวหนังไม่ก่อให้เกิดการระคายเคืองผิวหนัง ซึ่งสามารถนำมาพัฒนาผลิตภัณฑ์ต่อไปได้*