THE DEVELOPMENT OF INNOVATIVE CLAY MASK PRODUCT MIXED WITH THEACANTHUS EBRACTEATUS VAHL EXTRACT

ABSTRACT

*This research is a study on the development of innovative clay mask mixed with the Acanthus ebracteatusVahl extract product. The purposes of this research are as follows. Firstly, the research is todevelop a clay mask product mixed with natural extracts from the Acanthus ebracteatusVahl. Secondly, it is aimed to study on the antioxidant activity, anti-inflammatory effect, antimicrobial effect and total Phenolic contents of the Acanthus ebracteatusVahl extracts.Thirdly, it is tostudy stability of the product. Finally, the research is to study on the calculation of suitable product formulations for clay products mixed with Acanthus ebracteatusVahl extracts. By starting from the selection of raw materials, the extracts are prepared to determine the total phenolic content. Later, DPPH Assay method is conducted for antioxidant activity test, followed by anti-inflammatory effect test (Nitric oxide production inhibitory activity test, inhibition of LPS-induced TNF-α secretion test and Anti-inflammatory activity test (COX-2). Afterwards, a suitable formulation is developed. The test for the safety and physical properties of the product is then carried out followed by the skin irritation test in a group of 10 volunteers in order to ensure that theclay mask product containing Acanthus ebracteatusVahl extracts is effective and safe.From the results of the product stability study, the findings indicated that the clay mask product containing Acanthus ebracteatusVahl extracts did not show any phase separation. The irritation test in 10 volunteers after 12 hours of using the product showed no sign of allergic reaction, itching or irritation. Toxicity test results found no cytotoxicity.The anti-inflammatory effect test was f*ound to have *potent* anti-inflammatory properties, *when tested in the* RAW 264.7 macrophage cell. *This study is only for the development of an innovative product of skin clay mask mixed with the Acanthus ebracteatusVahl extracts.*

Keywords:Clay mask, nature, elasticity

INTRODUCTION

Clay has been used for many purposes. Clay therapy has played a major role in the treatment and prevention of diseases or enhancement the beauty of the body since ancient times. Numerous studies have demonstrated its clinical benefits and its effects on inflammatory mediators. Interleukins, the immune system, respiratory factors,collagen productionboost (Meier et al., 2012; Valenti et al., 2012). In addition, it is beneficial to reduce excess oil, absorbs unwanted toxins and impurities in the skin (Robles Velasco et al., 2016). In the area of Na Yung District, Udon Thani Province, a source of clay is found along the Phu Phan Mountains, which is rich in various minerals. However, there are no studies and use in promoting, preserving, and preventing disease in accordance with the government's policy that supports the use and reliance on nature, create a better quality of life andimprove the level of sustainable living.The *Acanthus ebracteatus*Vahlis aplant that iscommonlyseen, often up along rivers and canals (The Botanical Garden Organization, n.d.) It is also a plant that has many medicinal properties such as helping to reduce inflammation, reduce inflammation of acne containing antioxidants that are good for skin. The *Acanthus ebracteatus*Vahlhas also been used to make shower gel products (Wongsarn, 2000). Therefore, in regard to the aforementioned properties, the researcher is interested in using ihe*Acanthus ebracteatus*Vahlin this researchto improve the efficacy of the products. In addition, this research will be a contribution to society which is in line with the sufficiency economy. The research will also generate income, expand tourist attractions and further improve community potential.

MATERIALS AND METHODS

Collecting clay samples, soak in clean water, then filter out thesubstances which are dissolved in water, dry in an incubator at 60 °C, weigh, calculate the percentage of the extracts (%Yield), then take

the extracts in the experiment to make products with various formulations, perform product stability test by the method of freeze and thaw cycle, perform product irritation test on humans using the International Contact Dermatitis Research Group (ICDRG) method.Buy *Acanthus ebracteatus*Vahlleaves and find them from natural sources, clean them up, cut *Acanthus ebracteatus*Vahl leaves into small pieces. Later, dry in an incubator at 50 °C., weigh *Acanthus ebracteatus*Vahl leaves as specified, coarsely ground with a plate grinder. Then,ferment with 95% ethanol for 3 days, filterby filter paper. The remaining pulp is fermented and filtered 2 more times, the 3 times filtered out extracts are mixed and then concentrated with the Rotary Evaporator. Finally, put it into the incubator to get the concentrated extracts.

Formulations for calculating the % yield of plant extracts

This is a comparison of the extracted amount with the amount of the reactants. Hence, the production cost can be calculated each time (Phrompittayarat et al., 2007).

% yield = weight of plant extracts / dry weight of medicinal plants × 100

Test on the safety and physical properties of the product (Chuayprom,2010)

Physical stability: observing the texture of the gel, separation by sedimentation and odor. Test the pH using the product's pH meter when freshly prepared, and after 1 week, store at room temperature.

Assess the accelerated stability test of the product:conducting 5 cycles of freeze and thaw cycles method. That means the product isstored in a temperature and humidity-controlled cabinet which has been set at 4 °C for 24 hours. After 24 hours, the temperature is set to 45. °c for another 24 hours.This can count as 1 cycle.Repeat it 5 cycles in total.

Skin irritation test

Testing of skin irritation and allergic reactions on human skin for extracts and clay mask products mixed with *Acanthus ebracteatus*Vahlextracts is performedat a concentration of 5% by means of a closed patch test under occlusion for observing irritant reaction and allergic reaction. The test results will be evaluated according to the scoring system recommended by International Contact Dermatitis ResearchGroup (ICDRG) (Traisut et al., 2016).

Inclusion criteria: 10 normal healthy volunteers aged 20-35 years, 5 males and 5 females. The volunteers do not undergo other research studies, and consent to participate in the study. They have no symptom associated with itching from severe infection, no abscess and no cellulitis including itching from skin disease caused by infection or immune system disease. There were no lesions or skin lesions on the subjects' upper back.

Skin irritation testing method: The test area is the upper back, which includes 3 points in the size of 2x2 cm^2 with 3 cm apart. At the beginning of the test, wipe the skin on the upper back with saline solution, wait until dry. Use a gauze pad soaked with the 3 substancesfor testing Including saline solution,clay mask base and claymask mixed with *Acanthus ebracteatus*Vahlextracts, 0.3 ml each, put on the points specified above, 3 samples each. Irritation symptoms are assessed by the researcher including irritation, itching, blisters, and rashes immediately after use and 12 hours after that.

Cytotoxicity test

Dissolve *Acanthus ebracteatus*Vahlextracts with 10% DMSO in cell culture medium. It is sterilized by filtering through a membrane of 0.2 micron porous. The sample substances are diluted to the desired concentration with 10% DMSO in the sterilized cell culture medium. Taken to test for cytotoxicity by sulforhodamine B, SRBassay (VichaiandKirtikara, 2006) and percentage cell survival is calculated compared to the control group.

Test for anti-inflammatory activity in macrophage cell cultures (RAW 264.7)

Dissolve 10% (v/v) DMSO of *Acanthus ebracteatus*Vahlextracts in a colorless cell culture medium, then sterilize by filtering through a membrane with a porous size of 0.2 microns and diluting the sample substances to the desired concentration. Later test for inhibition of nitric oxide generation from LPS-induced macrophages (Torres-Rodríguez et al, 2016). Nitric oxide content is determined with Griess′s reagent while calculation is carried out for the percentage of inhibition of nitric oxide formation compared to the control group.

List 1: Development clay mask product formulations with Acanthus ebracteatusVahl extracts

List of Ingredients/ Formulae	Volume(%w/w)				
	1	2	3	4	5
Deionized water	30.7%	28.98%	26.05%	24.93%	23.1%
Base cream	23%	23%	23%	23%	23%
Nature Clay Mask Powder	14%	15%	16.9%	17%	18%
Kaolin	11%	11%	11%	11%	11%
Butylene Glycol	0.3%	0.3%	0.3%	0.3%	0.3%
Bentonite	7%	7%	7%	7%	7%
French Green Clay	3%	3%	3%	3%	3%
Beta hydroxy acids	7%	7%	7%	7%	7%
Menthol	0.1%	0.12%	0.15%	0.17%	0.2%
Panthenol and Propylene Glycol	1%	1%	1%	1%	1%
Glycerin	0.8%	1%	1.5%	2%	2.3%
Fragrance	0.1%	0.1%	0.1%	0.1%	0.1%
*Acanthus ebracteatus*Vahl Leaf extract	2%	2.5%	3%	3.5%	4%

Results

Cay mask characteristic of 5 formulations were presented in Table 1 and most suitable formulation of this product was in Table 2.% Yield of crude extract was shown in Table 3. Accelerated product stability was evaluated by undertaking 5 freeze and thaw cycles method and presented in Table 4. No skin irritation when used of saline solution, clay mask base and clay mask mixed with *Acanthus ebracteatus*Vahl Leaf extracts (Table 5, 6 and 7).

Table 1 shows the results of the test of 5 formulations of clay mask formulations with *Acanthus ebracteatus*Vahlextracts

Characteristic	1	2	3	4	5
Color	Dark brown	Dark brown	Dark brown	Dark brown	Dark brown
Odor	Not rancid, no sour-smell	Not rancid, no sour-smell	Not rancid, no sour-smell	Not rancid, no sour-smell	Not rancid, no sour-smell
Clarity	cloudy	cloudy	cloudy	cloudy	cloudy
Sunscreen gel texture	Smooth, liquid	Smooth, slightly liquid	Smooth, slightly viscous	Smooth, sticky, viscous	Smooth, Sticky, highly viscous

Table 2 Formulationclay mask mixed with *Acanthus ebracteatus*Vahlextracts

No.	List of Ingredients	Volume(%w/w)
1.	Deionized water	26.05%
2.	Base cream	23%
3.	Nature Clay Mask Powder	16.9%
4.	Kaolin	11%
5.	Butylene Glycol	0.3%
6.	Bentonite	7%
7.	French Green Clay	3%
8.	Beta hydroxy acids	7%
9.	Menthol	0.15%
10.	Panthenol and Propylene Glycol	1%
11.	Glycerin	1.5%
12.	Fragrance	0.1%

13.	*Acanthus ebracteatus*VahlLeaf extract	3%

Table 3 Yield of crude extract from *Acanthus ebracteatus*Vahl Leaves

Extract	Weight before extraction (g)	Extract weight (g)	Percentage yield (% Yield)
Clay	1,350	77.42	5.735
*Acanthus ebracteatus*Vahl Leaves	800	22.9	2.8625

Table 4 Observations of phase separation

Circle	**1 (-2)**	**2 (-1)**	**3(0)**	**4 (+1)**	**5 (+2)**
1	Not separated	Not separated	Not separated	Not separated	Not separated
2	Not separated	Not separated	Not separated	Not separated	Not separated
3	Not separated	Not separated	Not separated	Not separated	Not separated
4	Not separated	Not separated	Not separated	Not separated	Not separated
5	Not separated	Not separated	Not separated	Not separated	Not separated

Table 5 Results of skin irritation test when used of saline solution

Subject	After 12 hours of saline solution testing				
	+?	**+**	**++**	**+++**	IR
1	-	-	-	-	-
2	-	-	-	-	-
3	-	-	-	-	-
4	-	-	-	-	-
5	-	-	-	-	-
6	-	-	-	-	-
7	-	-	-	-	-
8	-	-	-	-	-
9	-	-	-	-	-
10	-	-	-	-	-

IR = irritation

Table 6 Results of skin irritation test when used of clay mask base

Volunteer	12 hours after testing with clay mask base				
	+?	**+**	**++**	**+++**	IR
1	-	-	-	-	-
2	-	-	-	-	-
3	-	-	-	-	-
4	-	-	-	-	-
5	-	-	-	-	-

6	-	-	-	-	-
7	-	-	-	-	-
8	-	-	-	-	-
9	-	-	-	-	-
10	-	-	-	-	-

Table 7 Results of skin irritation test when used of clay mask mixed with *Acanthus ebracteatus*Vahl Leaf extracts

Subject	12 hours after testing with clay mask				
	+?	+	++	+++	IR
1	-	-	-	-	-
2	-	-	-	-	-
3	-	-	-	-	-
4	-	-	-	-	-
5	-	-	-	-	-
6	-	-	-	-	-
7	-	-	-	-	-
8	-	-	-	-	-
9	-	-	-	-	-
10	-	-	-	-	-

At a concentration of 0.0001-1 mg/ml, *Acanthus ebracteatus*Vahl Leaf extractswere nontoxic to human skin fibroblasts and at 97.30-114.87 Percentage of cell viability. Whereas sodium lauryl sulfate was cytotoxic at 0.1 and 1mg/ml concentrations, percentage of cell viability was 12.03±1.82 and 9.13±0.23%, respectively (Table 8)**.**

Table 8 Percentage of human skin fibroblast cellsviability

Sample (mg/ml)	**Percentage of cell viability**				
	0.0001	**0.001**	**0.01**	**0.1**	**1**
*Acanthus ebracteatus*Vahl Leaf extract	97.30±0.89	102.90±2.57	107.42±5.09	108.98±30.6	114.87±3.50
Sodium lauryl sulfate	105.33±4.31	103.33±4.31	98.05±1.01	12.03±1.82	9.13±0.23

Remark: Values shown are the results of 4 repeated trials (mean ±SD).

*Acanthus ebracteatus*Vahl Leaf extracts showedanti-inflammatory activity when tested in macrophage cell cultures (RAW 264.7) with the ability to inhibit nitric oxide generation from LPS-induced cells up to 13.38±2.23% at 0.1 concentrations. mg/ml (1 and 10 mg/ml *Acanthus ebracteatus*Vahl Leaf extracts may cause interference in the absorbance measurement). Triamcinolone acetonide anti-inflammatory drugs inhibits nitric oxide generation up to 34.58±1.55 at a concentration of 1 mg/ml (Table 9).

From the experiment on the development of innovative products for clay mask mixed with *Acanthus ebracteatus*Vahl Leaf extracts for preventing oxidation and the establishing of this community spa, natural clay mask products were tested. The findings suggested that the clay and *Acanthus ebracteatus*Vahl Leaf had % yield of 5.735 and 2.8625 respectively, and the most suitable formulation for making clay mask products with *Acanthus ebracteatus*Vahl Leaf extract is formulation 3. Based on product stability test, it was found that the clay mask products containing the extracts of *Acanthus ebracteatus*Vahl Leaves were not separated. The irritation test in 10 volunteers after 12 hours of using the product, showed no allergic reaction, itching or irritation. Toxicity test results was found no cytotoxicity. Moreover, anti-inflammatory activity was found to have anti-inflammatory activity when tested in macrophages culture cells.

Table 9 inhibition of nitric oxide formation of the test samples at various concentrations.

Sample **(mg/ml)**	**Percentage inhibition of nitric oxide**

	0.0001	0.001	0.01	0.1	1	10
*Acanthus ebracteatus*Vahl Leaf extract	ND	7.81±2.58	12.64±6.80	13.38±2.23	5.58±3.10*	-136.06±3.07*
Triamcinolone Acetonide	16.77±4.64	20.15±2.24	25.37±3.38	30.28±2.72	34.58±1.55	ND

Remark: Values shown are the results of 4 repeated trials (mean ±SD), - Percentage inhibition of nitric oxide, * sample color caused interference in the absorbance measurement, ND = not tested

DISCUSSION AND CONCLUSION

According to the experiment on the development of innovative products for clay mask mixed with *Acanthus ebracteatus*Vahl Leaf extracts to prevent Oxidation and hence to set up community spa,by including natural clay and Acanthus*ebracteatus*Vahl Leaf extracts to develop a formulation of clay mask products mixed with Acanthus*ebracteatus*Vahl Leaf extracts, the research revealed that result of the development for the best formulationfor skin masking clay containing *Acanthus ebracteatus*Vahl Leaf extract wasformulation 3. The amount of clay extract used for the preparation of clay products contained16.9 g natural clay powder, 3 g Acanthus ebracteatusVahl extract, 26.05 g Deionized water, 23 g cream Base, 11 g Kaolin, 7 g Bentonite, 3 g French Green Clay, 0.3 g Butylene Glycol, 7. g Beta hydroxy acids, 0.15 g Menthol, 1 g D-panthenol 50p, 1.5 g Glycerin, 0.1 g Fragrance. After the product was obtained and tested the stability of the product, it was found that the product was not separated. Furthermore, an irritation test was conducted on 10 volunteers and found that there was no allergic reaction. In toxicity tests, it was found no cytotoxicity, while has a good level of anti-inflammatory effect. Because this experiment is just an innovative product development of clay mask mixed with Acanthus ebracteatusVahl extract, further study and development of the product is highly recommended.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

REFERENCES

- Meier, L., Stange, R., Michalsen, A., &Uehleke, B. (2012). Clay jojoba oil facial mask for lesioned skin and mild acne – results of a prospective, observational pilot study. ForschKomplementmed, 19: 75-79.
- Phrompittayarat, W., Putalun, W., Tanaka, H., Jetiyanon, K., Wittaya-areekul, S., &Ingkaninan, K. (2007). Comparison of various extraction methods of Bacupamonnier. Naresuan University Journal, 15(1): 29-34.
- Robles Velasco, M., Zague, V., Dario, M., & Nishikawa, D. O. (2016). Characterization and Short-Term clinical study of clay facial mask. Revista de CienciasFarmaceuticasBasica e Aplicada , 37(1).
- Torres-Rodríguez, M., García-Chávez E, Berhow, M., & Gonzalez de Mejia, E. (2016). Anti-inflammatory and anti-oxidant effect of Caleaurticifolia lyophilized aqueous extract on lipopolysaccharide-stimulated RAW 264.7 macrophages. Journal of Ethnopharmacology, 188: 266-274.

- Valenti, D., Silva, J., Teodoro, W., Velosa, A., & Mello, B. (2012). Effect of topical clay application on the synthesis of collagen in skin: an experimental study. Clinical and Experimental Dermatology, 37(2): 164-168.
- Vichai, V., &Kirtikara. (2006). K. Sulforhodamine B colorimetric assay for cytotoxicity screening. Nature Protocols, 1(3): 1112-1116.
- Piyata, W., &Pinonong, P. (2000). The use of herbal extracts to develop cleansing products in gel form. Science Library: Project.
- Warangkana, T., Arunporn, I., Panlop, C., &Puritat, K.adan. (2016). Skin irritation of Diple alcohol
- extract in healthy volunteers. (Clinical research phase I). Thammasat Medical Journal,•16(4): 608-615.
- The Botanical Garden Organization, n.d.). Acanthus ebracteatusVahl. accessible from Plant
- Database Botanic Garden Organization: shorturl.asia/5SWEF
- Asawachai, C., Nasaphol, P., Malee, B., Thidarat, B., Piyawan, B., &Bunyanee, S. (2010).
- Development of the Cosmeceutical Formulation for combination skin care.
- Rajamangala University of Technology Srivijaya Research Journal, 2(2): 69-77.