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Original Article

FORMULATION STRATEGY, STABILITY ISSUES, SAFETY AND EFFICACY EVALUATIONS OF *ACACIA CATECHU* WHITENING CREAM

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ABSTRACT

Objective: To investigate among formulation strategy, stability issues, safety and efficacy (tyrosinase inhibitory activity and sun protection**)** of a novel whitening cream containing *Acacia catechu* heartwood extract**.**

Methods: The tyrosinase inhibitory activity of the *A. catechu* extract was investigated using L-DOPA as a substrate**.** A combination of silicone and three photo-protective filters; natural UV filter **(***A. catechu* extract**)**, chemical and physical filters, was formulated and evaluated**.** The Sun Protection Factor **(**SPF**)**, stability, skin allergy or irritation, and the satisfaction of the developed whitening creams, were investigated**.**

Results: The *A. catechu* extract showed the strong tyrosinase inhibitory activity with the percentage of inhibition of 61.58% at the concentration of 120 µg/ml compared to a positive control of kojic acid (98.73 % inhibition) at the same concentration of 120 µg/ml. impressively, the highest SPF of *A. catechu* whitening cream with physical and chemical UV filters was 30. While, the highest SPF of the *A. catechu* whitening cream without those filters, was 24. No changes in color, liquefaction and phase separation were observed for the developed whitening cream when stored in the refrigerator. Among the twenty volunteers, eighteen volunteers had no skin irritation, while one of them had redness (Transient) and then another one had erythema or very slight irritation to the developed whitening creams. Besides, there was no statistically significant difference for the satisfaction between *A. catechu* whitening creams and commercial whitening creams ($p>0.05$).

Conclusion: Obviously, *A. catechu* whitening creams had very high efficacy, stability, and safety for being a commercial product in a near future.

Keywords: *Acacia catechu*, Tyrosinase inhibitory activity, Stability, Efficacy

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INTRODUCTION

Broad-spectrum ultraviolet radiation (UVR) is known to be a human carcinogen based on sufficient evidence from numerous studies. The UV radiation is divided into three bands: UVA (320–400 nm), UVB (290–320 nm), and UVC (200–290 nm) [1-2]. UVA radiation reaches the deeper layers of the epidermis and dermis and provokes the premature aging of the skin. UVB radiation is not completely filtered out by the ozone layer and is responsible for the damages due to sunburn [2]. UVC radiation is filtered by the atmosphere before reaching to the earth. Organic filters are molecules that interfere with incident radiation through the mechanism of absorption. Upon returning to the stable state (unexcited), the release of energy occurs at a longer wavelength. The process can be repeated numerous times by a mechanism called resonance. Depending on their capacity to absorb shorter or longer wavelengths, organic filters subclassified into UVA filters, UVB filters and filters for broad-spectrum protection (UVA and UVB). Natural substances extracted from plants have recently been considered, as potential whitening cream resources because of their ultraviolet ray absorption in the UVA and B region and their antioxidant activity [1].

Thus, there are more interesting in the use of natural sources in whitening creams to provide supplemental photoprotective action to discover products that can increase the sun protection factor (SPF) and stability [3]. Besides, the cosmetic and personal care market has been driven, toward natural ingredients for safer cosmetics or free of harmful chemicals. Several natural resources provide antioxidant, antimicrobial and anti-enzymatic properties. Therefore, natural UV filters and natural preservatives might be useful to prevent the adverse effects of artificial preservatives such as hypersensitivity, allergy, asthma, neurological damage, and cancer [4]. *A. catechu* is a Thai herb belonging to the Fabaceae family [5]. This plant is a thorny tree which grows up to 15 m (50 ft.) in height. Flowers are pale yellow, fruits show flat brown pods, with the triangular beak at the apex, shiny, narrowed at base. There are 3-10 seeds per pod [6]. The major components of the *A. catechu* heartwood are catechin. Catechins are also a group of natural UV filters [7]. Catechu (or cutch), a hot water extract of red heartwood of *A. catechu* is the brown powder with the bitter taste. The *A. catechu* heartwood utilized with betel leaves for chewing. Additionally, *A. catechu* has several medicinal properties such as immune-modulatory, sore throat, anti-inflammatory, anti-viral, anti-microbial activities and wound healing [8, 9]. A Leaf methanol extract of *A. catechu* provided antioxidant, DNA protective and antiproliferative properties [10].

A 90-day oral safety study was conducted and showed the safety on a combination of S. baicalensis and *A. catechu* product in rats [11]. Moreover, a dose of 1000 mg/kg/day was studied. There was no adverse-effect observed. Furthermore, no adverse effects of *A. catechu* heartwood extracts have been reported in human subjects and animals [12-16]. Therefore, *A. catechu* should be a good candidate for inspiration and innovation of an efficacy whitening cream. The ultimate goal of this research was to ensure and present the efficacy of *A. catechu* whitening cream as being a premium standard whitening cream.

MATERIALS AND METHODS

Plant material

A. catechu heartwood was gathered from *A. catechu* plantation in Wang Nam Khiao District, Nakhon Ratchasima Province, Thailand, in June 2016 in which the plant grows widely under natural condition. The heartwoods authenticated with a voucher specimen number of PNU/P/023 at the Department of Biological, Phranakorn Rajabhat University. The ethanolic extract was the extract under the optimal extraction conditions reported by Oraphan [17].

Whitening cream formulation

The formulation strategy of *A. catechu* whitening cream was to use a combination of silicone, chemical UV filter; ethylhexyl

methoxycinnamate and physical UV filter; titanium dioxide and natural UV-filter ethanolic extract of *A. catechu* heartwood.

Whitening creams were formulated by mixing Part A (Aqueous Phase; Distilled Water, Propylene Glycol, Sodium Chloride) and B (Silicone Phase; Phenoxyethanol, Cyclopentasiloxane and Dimethicone/Vinyl Dimethicone Crosspolymer, Cyclopentasiloxane, and Cyclohexasiloxane, Cyclopentasiloxane and PEG/PPG-18/18 Dimethicone, Ethylhexyl Methoxycinnamate, Titanium Dioxide, and Talcum) separately. The aqueous phase was gently added to part B, while it was homogenizing at 2000 rpm. Then, Part C (*A. catechu* extract) was added to formulate the whitening creams. When the whitening creams were formed and cooled, the fragrance was gently added at room temperature and mixed until it was homogenous.

Fourteen whitening creams were prepared under the different conditions (table 1) to compare the stability resultants between adding preservative and whitening cream without preservative, different concentrations of the *A. catechu* heartwood extract and adding chemical and physical UV filters and without the UV filters.

Table 1: Ingredients for each formulation

No.	Ingredients	Formulation code													
		F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**	**F10**	**F11**	**F12**	**F13**	**F14**
1	Distilled Water	69.54	69.30	69.00	69.40	69.30	69.40	69.20	69.22	69.11	71.00	71.00	69.11	71.00	69.20
2	Propylene Glycol	1.51	1.95	1.55	1.73	1.95	1.73	1.52	1.52	1.53	1.69	1.59	1.53	1.59	1.52
3	Sodium Chloride	0.76	0.75	0.78	0.78	0.75	0.78	0.75	0.75	0.75	0.78	0.78	0.75	0.78	0.75
4	Phenoxyethanol (preservative)	0.76	0.81	0.80	0.94	0.81	0.94	2.58	-	-	-	-	-	-	-
5	Cylopentasiloxane (and) Dimethicone/Vinyl Dimethicone cross polymer	11.25	11.28	11.30	11.14	11.28	11.14	11.29	11.18	11.26	11.32	11.25	11.26	11.25	11.29
6	Cyclopentasiloxane (and) Cyclohexasiloxane	7.55	7.60	7.50	7.68	7.60	7.68	7.55	7.65	7.53	7.62	7.56	7.53	7.56	7.55
7	Cyclopentasiloxane (and) PEG/PPG-18/18 Dimethicone	5.28	5.25	5.25	5.37	5.25	5.37	5.29	5.29	5.43	5.48	5.26	5.43	5.26	5.29
8	Ethylhexyl Methoxycinnamate (Chemical UV filters)	3.75	3.75	3.75	3.75	3.75	3.75	-	3.75	3.76	3.76	3.75	3.76	3.75	-
9	Titanium Dioxide (Physical UV filters)	0.08	0.08	0.08	0.08	0.08	0.08	-	0.08	0.08	0.08	0.08	0.08	0.08	-
10	Talcum	0.08	0.07	0.08	0.08	0.07	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
11	Fragrance	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
12	*Acacia catechu* heartwood Extract	-	0.13	0.09	0.08	0.06	0.05	0.08	-	0.13	0.09	0.08	0.06	0.05	0.08

Inhibition of tyrosinase activity

The tyrosinase inhibitory activity used a modified method of that performed by Batubara [18]. Inhibition of tyrosinase activity measurements performed on a Microplate Reader (Biotek Synergy HT, USA). The test samples, L-DOPA, and tyrosinase enzyme solutions were dissolved in 20 mmol phosphate buffer solution of pH 6.8. The activity of mushroom tyrosinase performed in 96-well plates. The reaction mixture consisted of 150 µl of 20 mmol phosphate buffer at pH 6.8, 50 µl of a test sample solution, and 50 µl of mushroom tyrosinase (100 unit/ml, E. C. 1.14.18.1, Sigma). The mixture was pre-incubated at 25 °C for 10 min. Subsequently, 20 µl of 2.5 mmol L-DOPA added, and the mixture incubated for 60 min at 37 °C. During the reaction, L-DOPA converted to dopachrome, which resulted in a change in color from colorless to be orange. This change measured, through absorbance at 490 nm. After incubation at 37 °C for 60 min, the absorbance at 490 nm was determined using a microplate reader. The tyrosinase inhibitory percentages were calculated at the concentration of 120 µg/ml by Kojic acid used as a positive control. Percentages of tyrosinase inhibitory activity calculated as following the equation:

$$\% \text{ Tyrosinase inhibitory activity} = \left(\frac{A_0 - A_1}{A_0}\right) \times 100$$

Where A_0 refers to the absorbance of the control solution, and A_1 represents the absorbance of the sample

Determination of the *in vitro* sun protection factor

Siliva report was slightly modified to determine the in vitro sun protection factor [19]. The investigated whitening creams were dissolved in methanol: water (6:4) and diluted to 150 µg/ml. SPF measurements performed on a Microplate Reader (Biotek Synergy HT, USA). The measurements were made by switching to xenon flash lamp and a monochromator for wavelength selection. Scanning spectra of six whitening creams which SPF standardized and samples in the solution were obtained by running from 320 to 290 nm (at 5 nm intervals). The UV-absorbance of the whitening creams which SPF standardized, used as standard whitening creams for calculation of the correction factor (CF). The equation was proposed by Mansur [20] and used to calculate the SPF values for whitening cream samples. Mansur's method is simple and easily reproducible.

$$SPF = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times abs(\lambda)$$

Where CF is correction factor, determined by six whitening creams which SPF standardized; EE (λ) the erythemal efficiency spectrum; *I* (λ) the solar simulator spectrum as measured with a calibrated spectrometer; $\sum_{290}^{320} EE(\lambda) \times I(\lambda)$ = 290-320 nm in 5 nm increments; abs (λ) is the spectrometer measure of the whitening cream absorbance. The absorbance has taken in triplicate at each point. Table 2 shows the normalized values of the product function used in this research.

Table 2: The normalized product function used in the calculation of SPF data

Wavelength (nm)	EE×*I* (normalized)*
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180
Total	=1.000

*EE: erythemal efficiency spectrum; *I*: solar simulator intensity spectrum

Stability test

Accelerated stability test; whitening creams which had the highest concentration of *A. catechu* with and without preservative (F2, F9), were prepared in replicates (n = 3) and tested the stability under the different temperature (5°C ± 2 °C, 25 °C ± 2 °C and 40 °C ± 2 °C) for 28 days. Color, liquefaction and phase separation changes using centrifuged at 5000 rpm for ten minutes, were investigated.

Normal stability test; stability testing of the developed *A. catechu* whitening cream was evaluated and monitored the appearance (color, liquefaction, and phase separation changes), then centrifuged at 3000 rpm for thirty minutes. The stability was investigated, under the different temperature (kept in the refrigerator, ambient temperature and outdoor for 3 months).

Patch testing

The irritation evaluation was studied using patch testing. The researchers recruited 20 healthy adult volunteers with no underlying skin disease or skin lesion in the test area. Inclusion criteria were participants had normal skin and not used any anti-allergic or steroid medication, older than 18 years old, non-pregnant, and not breastfeeding. Exclusion criteria were the use of a topical medication containing steroids to treat skin diseases, subjects with severe skin-related pathologies and abnormalities, such as erythema, eczema. All volunteers were informed of objectives, test procedures, and possible adverse effects, and rewarded for their participation. The Ethics Committee of Phranakorn Rajabhat University, Bangkok, Thailand, approved the present method (AF05-06 study code: 60-23). All volunteers have given consent before entering the study. Volunteers have the right to withdraw from the study at any time without consequence or penalty.

Testing of satisfaction

In order to investigate the satisfaction of the products, all volunteers form irritation testing shown their satisfaction using questionnaires. The five-rating scale questionnaire has consisted of two parts, personal data, and product data. Questions in personal data were about gender, age, and type of skin. While, product questions were the sensorial evaluation of the whitening cream (texture, color, odor, consistency), improvement in the skin upon use, skin nourishment, and satisfaction with the whitening creams. The five-rating scale used a scale that ranged from 1 (Strongly Dissatisfied) to 5 (Strongly Satisfied). Additionally, the developed product was compared satisfaction with the commercial product by the blinded experiment.

RESULTS AND DISCUSSION

Whitening cream formulation

Fourteen whitening creams were prepared using silicone-based. The fresh, smooth and soft textures of the whitening creams from the different formulations were represented in fig.1.

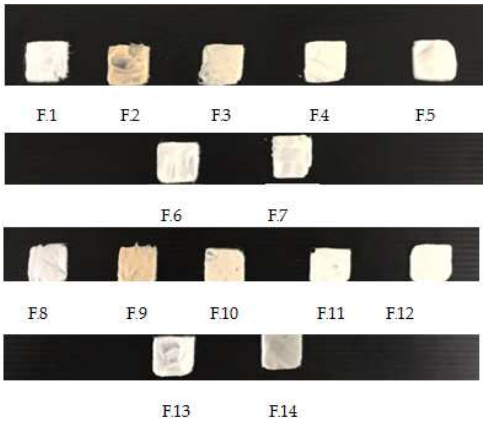

Fig. 1: Appearance of the whitening creams

Table 3: The percentages of tyrosinase inhibition at a concentration of 120 µg/ml

No.	% Tyrosinase inhibition at a concentration of 120 µg/ml	
	A. catechu extract	**Kojic acid**
1	62.03	98.73
2	59.74	98.70
3	62.96	98.77
Average	61.58	98.73
SD	1.66	0.04
%RSD	2.69	0.04

Note: Number of experiments: 3

Inhibition of tyrosinase activity

The present study aimed to determine the potential of *A. catechu* whitening cream. The tyrosinase inhibition of the extract was studied in triplicate.

The *A. catechu* extract showed the strong tyrosinase inhibitory activity with the percentage of inhibition of 61.58 at a concentration of 120 µg/ml compared to a positive control of kojic acid (98.73 % inhibition) at the same concentration of 120 µg/ml (table 3). Interestingly, the inhibitory activity of *A. catechu* extract was about two times weaker than kojic acid compared an anti-tyrosinase activity of ellagic acid-rich pomegranate peel extract which was about seven times weaker than kojic acid [21]. In another study, the percentages of tyrosinase inhibitory activity of kojic acid and curcumin at a concentration of120 µg/ml were about 84 and 72, respectively [22]. The tyrosinase inhibitory ability of the phenolic compounds strongly influenced by the structure of the phenolic compounds. The inhibition percentage of the ethanol extract of

Matoa leaves and stem bark with a final concentration of 200 µg/ml, was 24.54±0.22% and 21.93±0.57%, respectively. The value of the inhibition percentage showed that the inhibitory ability of the tyrosinase of the ethanol extract of Matoa leaves and stem bark was much weaker than that of kojic acid (6.63 µg/ml) [23].

Determination of the *in vitro* sun protection factor

The SPF values represented in table 4. Table 4 represented that an increasing of *A. catechu* causes SPF to increase. A good positive correlation found between SPF values and *A. catechu* contents. Pearson Correlation was 0.983 with the correlation coefficient of significant at the 0.01 level. The highest SPF value was 30.344 from sample 2, that corresponds to a 97 percent UVB-protection [24]. The lowest SPF value was 24.143 from sample 7. The average SPF values of the samples with and without preservative were 28.347 and 28.549, respectively. Moreover, the results show a high SPF value (24) even no synthetic UV-filters. Impressively, the results of this research reveal high SPF values compared with other natural whitening creams. SPFs of methanol solutions of flowers of Calendula officinale and flowering tops of Hypericumperforatum were 12.01 and 12.21, respectively [25]. SPF values of the products containing extracts from Menthapiperita (Leaves), Azadirachtaindica (Leaves) Oscimum sanctum (Leaves), Aloe vera (Leaves), Lycopersicon esculantum (fruits), and Carica papaya (fruits) were 8.184, 4.368, 2.904, 5.437, 6.083, and 2.310, respectively [26]. Their SPF values of the Zanthoxylumrhetsa whitening cream were 3.60±0.28 (F1) and 6.90±0.57 (F2) [27]. Moringaoleifera sun care demonstrated SPF 2 [28]. The carrot and coconut cream containing 2% of coconut ethyl acetate extract showed the lowest SPF value of 0.64 [29]. The experimental results performed in the triplicate.

The data recorded as mean+standard derivation. The analysis of variance performed by ANOVA. The significant differences between means of the concentrations of *A. catechu* vs. sun protection properties were determined. A good positive correlation found between SPF values and *A. catechu* contents. Pearson Correlation was 0.98 and the correlation coefficient was significant at the 0.01 level.

Table 4: Sun protection factor of the whitening cream samples

Whitening cream with preservative	SPF	Whitening cream without preservative	SPF
F1. Control whitening cream without *A. catechu*	27.797c±0.009	F8. Control whitening cream without *A. catechu*	28.278c±0.133
F2. *A. catechu* whitening cream	30.344a±0.011	F9. *A. catechu* whitening cream	30.341a±0.005
F3. *A. catechu* whitening cream	29.597b±0.010	F10. *A. catechu* whitening cream	29.797b±0.014
F4. *A. catechu* whitening cream	29.259b±0.015	F11. *A. catechu* whitening cream	29.110b±0.014
F5. *A. catechu* whitening cream	28.945b±0.054	F12. *A. catechu* whitening cream	29.079b±0.023
F6. *A. catechu* whitening cream	28.344c±0.006	F13. *A. catechu* whitening cream	28.858b±0.029
F7. *A. catechu* whitening cream without physical and chemical UV filters	24.143d±0.009	F14. *A. catechu* whitening cream without physical and chemical UV filters	24.381d±0.020
Average	28.347	Average	28.549

Note: $^{a-d}$Averages for different SPF values with different letters (P<0.05): Number of experiments: 3: Data given in mean+SD

Stability testing

For accelerated stability testing for 28 d, the liquefaction and color of the samples were slightly changed after 21 d as shown in table 5. Besides, the normal stability under the different conditions (room temperature, refrigerator and outdoor) was investigated. The color and liquefaction had slightly changed after 1 mo for sample F9 (sample without preservative), while the sample F9 was separated after 3 mo as represented in table 6. However, no changes in color, liquefaction and phase separation were observed for the developed whitening cream after stored in the refrigerator for three months**.**

Table 5: Physical characteristics of F2 and F9, formulations kept at 5±2 °C, 25±2 °C and 40±2 °C

		Fresh		24 h		3 d		7 d		14 d		21 d		28 d	
		F2	F9	F2	F9	F2	F9	F2	F9	F2	F9	F2	F9	F2	F9
Liquefaction	5 °C	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	25 °C	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	40 °C	N	N	N	N	N	N	N	N	N	N	N	SC	N	SC
Color	5 °C	PY	PY	PY	PY	PY	PY	PY	PY	PY	PY	PY	PY	PY	PY
	25 °C	PY	PY	PY	PY	PY	PY	PY	PY	PY	PY	PY	PY	PY	PY
	40 °C	PY	PY	PY	PY	PY	PY	PY	PY	PY	PY	PY	SWY	PY	SWY
Phase separation	5 °C	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	25 °C	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	40 °C	N	N	N	N	N	N	N	N	N	N	N	N	N	N

N = No change; SC = Slight change; SP= Separate; PY = Pale yellow; SYW = Soft yellowish white; YW = Yellowish white = Yellow; W = White.

Table 6: Physical characteristics of F2 and F9, formulations kept in Room temperature, Refrigerator, and Outdoor

		Fresh		24 h		1 mo		2 mo		3 mo	
		F2	F9	F2	F9	F2	F9	F2	F9	F2	F9
Liquefaction	Room temperature	N	N	N	N	N	N	N	SC	SC	SC
	Refrigerator	N	N	N	N	N	N	N	N	N	N
	Outdoor	N	N	N	N	N	N	N	SC	SC	SC
Color	Room temperature	PY	PY	PY	PY	PY	PY	PY	PY	SYW	SYW
	Refrigerator	PY	PY	PY	PY	PY	PY	PY	PY	PY	PY
	Outdoor	PY	PY	PY	PY	PY	PY	SYW	SYW	SYW	YW
Phase separation	Room temperature	N	N	N	N	N	N	N	N	N	N
	Refrigerator	N	N	N	N	N	N	N	N	N	N
	Outdoor	N	N	N	N	N	N	N	N	N	SP

N = No change; SC = Slight change; SP= Separate; PY = Pale yellow; SYW = Soft yellowish white; YW = Yellowish white; Y = Yellow; W = White

Patch testing

For the irritation evaluation, patch testing under the clinical observation and approved protocol by The Ethics Committee of Phranakorn Rajabhat University. Twenty volunteers aged from 18 to 34 years old, were selected to participate the irritation evaluation.

The whitening cream with highest *A. catechu* concentration and without preservative and synthetic UV filters (F9), was used to investigate. Among the twenty volunteers, eighteen volunteers had no skin irritation, while one of them had redness (Transient) and another one had erythema or very slight irritation to the developed whitening creams.

Testing of satisfaction

The analysis of satisfaction toward the *A. catechu* whitening cream showed the high satisfaction level (3.94) in overall aspect (texture, consistency, sensory and odor). When considering in each aspect, the results indicated that the highest average value was the texture of the whitening cream (4.05) followed by the sensory (4.00), the consistency (3.95) and the least was the odor (3.75). Averages of satisfaction of the developed whitening and the commercial product were 3.94 and 3.78, respectively, as shown in table 7. Additionally, there was no statistically significant difference for the satisfaction, between *A. catechu* whitening creams and commercial whitening creams (p>0.05).

Table 7: Satisfaction evaluation from five-rating scale questionnaire

Whitening cream	Average of the satisfaction score on each aspect(n=20)				Average
	Texture	Consistency	Sensory	Odor	
Commercial whitening cream	3.90+0.55	3.85+0.49	3.90+0.64	3.50+0.61	3.78
A. catechu whitening cream with preservative (F2)	4.05+0.69	3.95+0.22	4.00+0.56	3.75+0.55	3.94

Note: Number of experiments: 20: Data given in mean+SD

CONCLUSION

This current study presented high-efficiency whitening cream. Combination of natural and synthetic UV filters could provide synergistic efficacy for a novel cosmeceutical of *A. catechu* whitening cream. *A. catechu* whitening cream without synthetic UV filters provided high SPF, the SPF value of 24. The *A. catechu* extract showed the strong tyrosinase inhibitory activity with the percentage of inhibition of 61.58 at a concentration of 120 µg/ml. *A. catechu* whitening cream without preservative provided good stability for three months. Moreover, patch testing and satisfaction of the developed whitening compared with the commercial product were studied. The developed whitening provided very good results. Therefore, the *A. catechu* whitening cream by this work shows the readiness to be a premium commercial product in the near future.

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AUTHORS CONTRIBUTIONS

O. A. conceived and designed the experiments; O. A.,N. L. and R. B. performed the experiments; O. A. analyzed the data; O. A. wrote the manuscript; O. A. N. L. and R. B. acquired fund.

CONFLICTS OF INTERESTS

All authors have none to declare

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