

1 Article

## 2 Standard Sunscreen from Local Thai Herb

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11 **Abstract:** A novel sunscreen containing *Acacia catechu*, remarkable local herb, was investigated  
12 for efficacy and standardized in this research. The developed sunscreens were evaluated Sun  
13 Protection Factor (SPF), stability, skin allergy or irritation and the satisfaction. The highest SPF of  
14 *A.catechu* sunscreen with synthetic sunscreen agents was 30.344. While, the highest SPF of the  
15 *A.catechu* sunscreen without synthetic sunscreen agents was 24.381. The stability testing with the  
16 different conditions was found that the sunscreen products in the usual storage, had been stabilized  
17 even without preservative. For irritation testing, there was no skin allergy and irritation from 20  
18 volunteers. Besides, results of the satisfaction evaluation for texture, consistency, sensory and odor  
19 after using the sunscreens, were high for all of the evaluation aspects. Obviously, the  
20 results in this research revealed that *A.catechu* was applicable for being a great candidate in a  
21 sunscreen product. Additionally, *A.catechu* sunscreen could prevent the adverse effects of UV  
22 radiation and artificial preservatives due to the UV absorption properties and antimicrobial  
23 properties of *A.catechu*.

24 **Keywords:** *Acacia catechu*, Stability, Preservative

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### 26 1. Introduction

27 Broad-spectrum ultraviolet radiation (UVR) is known to be a human carcinogen based on  
28 sufficient evidence from numerous studies. UV radiation can be divided into three bands: UVA  
29 (320–400 nm), UVB (290–320 nm), and UVC (200–290 nm) [1-2]. UVA radiation reaches the deeper  
30 layers of the epidermis and dermis and provokes the premature aging of the skin. UVB radiation is  
31 not completely filtered out by the ozone layer and is responsible for the damages due to sunburn  
32 [2]. UVC radiation is filtered by the atmosphere before reaching to the earth. Organic filters are  
33 molecules that interfere with incident radiation through the mechanism of absorption, when the  
34 filter acts as an exogenous chromophore by absorbing a photon of energy and evolving to the excited  
35 state of the molecule. Upon returning to the stable state (unexcited), the release of energy occurs at  
36 a longer wavelength. The process can be repeated numerous times by a mechanism called  
37 resonance. Depending on their capacity to absorb shorter or longer wavelengths, organic filters can  
38 be subclassified into UVA filters, UVB filters and filters for broad-spectrum protection (UVA and  
39 UVB). Natural substances extracted from plants have recently been considered as potential  
40 sunscreen resources because of their ultraviolet ray absorption in the UVA and B region and their  
41 antioxidant activity [1]. Thus, there are more interestig in the use of natural sources in sunscreens

42 to provide supplemental photoprotective action to discover products that can increase the sun  
43 protection factor (SPF) and stability [3]. Besides, several natural preservatives obtained from plants,  
44 animals, microbes and minerals contain antioxidant, antimicrobial and anti-enzymatic properties.  
45 Therefore, natural preservatives might be useful to prevent the adverse effects from the artificial  
46 preservatives such as hypersensitivity, allergy, asthma, neurological damage and cancer [4].  
47 *A.catechu* is a Thai herb belonging to the Fabaceae family [5]. This plant is a thorny tree which grows  
48 up to 15 m (50 ft.) in height. Flowers are pale yellow, fruits show flat brown pods, with triangular  
49 beak at the apex, shiny, narrowed at base. There are 3-10 seeds per pod [6]. It is utilized with betel  
50 leaves for chewing, one of the safety reason to select this herb. *A.catechu* presented several  
51 medicinal properties such as immune-modulatory, sore throat, anti-inflammatory, anti-viral, anti-  
52 microbial activities and wound healing [7,8]. Leaf methanol extract of *A.catechu* provided  
53 antioxidant, DNA protective and antiproliferative properties [9]. A 90-day oral safety study was  
54 conducted and presented the safety on a combination *S. baicalensis* and *A. catechu* product in rats  
55 [10]. A dose of 1000 mg/kg/ day was identified as the no-observed-adverse-effect level. and tissue-  
56 protective effects of heartwood extracts have been well documented, contributing to the overall  
57 safety. Furthermore, no adverse effects of *A. catechu* heartwood extracts have been reported in  
58 human subjects and animals [11- 15]. The major components of the *A.catechu* heartwood are  
59 catechin. Catechu (or cutch), a hot water extract of red heartwood of *A.catechu* is brown material  
60 with bitter taste. Catechins are also a group of important sunscreen from natural sources [16]. The  
61 ultimate goal of this research was to ensure *A.catechu* sunscreen as a good alternative to produce fine  
62 sunscreens.

## 63 2. Materials and Methods

### 64 2.1 Sunscreen formulation

65 The formulation strategy of *A.catechu* sunscreen was to use a combination of silicone, synthetic  
66 UV filters (ethylhexyl methoxycinnamate and titanium dioxide) and natural UV-filter (ethanolic  
67 extract of *A.catechu* heartwood). Sunscreens were formulated by mixing Part A (Aqueous Phase;  
68 Distillated Water, Propylene Glycol, Sodium Chloride) and B (Silicone Phase; Phenoxyethanol,  
69 Cyclopentasiloxane and Dimethicone/Vinyl Dimethicone Crosspolymer, Cyclopentasiloxane (and)  
70 Cyclohexasiloxane, Cyclopentasiloxane (and) PEG/PPG-18/18 Dimethicone, Ethylhexyl  
71 Methoxycinnamate, Titanium Dioxide, Talcum) separately. The aqueous phase was gently added  
72 to part B, while it was homogenizing at 2000 rpm. Then, Part C (*A.catechu*) was added to formulate  
73 the sunscreens. Part C (*A.catechu*), the ethanolic extract of *A.catechu* was the extract under the  
74 optimal extraction conditions reported by Oraphan [17]. When the sunscreens were formed and  
75 cooled, fragrance was gently added at room temperature and mixed until it was homogenous.  
76 Fourteen sunscreens were prepared. The sunscreens were prepared under the different conditions  
77 (Table. 1) to compare the resultants between adding preservative and sunscreen without  
78 preservative, different concentrations of the *A.catechu* heartwood extract and adding synthetic UV  
79 filters and without synthetic UV filters.

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82 **Table 1.** Ingredients for each sunscreen.

	Sample No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14
	Ingredients														
1	Distillated Water	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
2	Propylene Glycol	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
3	Sodium Chloride	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
4	Phenoxyethanol (preservative)	✓	✓	✓	✓	✓	✓	✓	-	-	-	-	-	-	-
5	Cylopentasiloxane (and) Dimethicone/Vinyl Dimethicone Crosspolymer	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
6	Cyclopentasiloxane (and) Cyclohexasiloxane	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
7	Cyclopentasiloxane (and) PEG/PPG- 18/18 Dimethicone	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
8	Ethylhexyl Methoxycinnamate (synthetic UV filters)	✓	✓	✓	✓	✓	✓	-	✓	✓	✓	✓	✓	✓	-
9	Titanium Dioxide (synthetic UV filters)	✓	✓	✓	✓	✓	✓	-	✓	✓	✓	✓	✓	✓	-
10	Talcum	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
11	Fragrance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
12	<i>Acacia catechu</i> heartwood Extract	-	+++++	++++	+++	++	+	+++	-	+++++	++++	+++	++	+	+++

83 Remark: + represented followed the concentrations

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## 89 2.2 Determination of the *in vitro* sun protection factor

90 Determination of the *in vitro* sun protection factor was slightly modified by Siliva [18].  
 91 The investigated sunscreens were dissolved in methanol: water (6:4) and diluted to 150 µg/mL.  
 92 Scanning spectra of six commercial sunscreens and samples in the solution were obtained by running  
 93 from 320 to 290 nm (at 5 nm intervals). The UV-absorbance of the commercial sunscreens was used  
 94 as standard for calculation of the correction factor (CF). Equation was proposed by Mansur [19] and  
 95 used to calculate the SPF values for sunscreen samples.

$$96 \text{ SPF} = \text{CF} \times \text{EE}(\lambda) \times I(\lambda) \times \text{abs}(\lambda) \dots\dots\dots(1)$$

98 Where CF is correction factor, determined by six commercial sunscreens which known SPF;  
 99 EE ( $\lambda$ ) the erythemal efficiency spectrum;  $I(\lambda)$  the solar simulator spectrum as measured with a  
 100 calibrated spectrometer;  $\sum_{290}^{320} \text{EE}(\lambda) \times I(\lambda) = 290\text{-}320$  nm in 5 nm increments;  $\text{abs}(\lambda)$  is the spectrometer  
 101 measure of the sunscreen absorbance. Readings of the absorbance were taken in triplicate at each  
 102 point. Table 2 shows the normalized values of the product function used in this research.

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104 **Table 2.** The normalized product function used in the calculation of SPF data

105 $\lambda$ (nm)	EE×I (normalized)
106 290	0.0150
107 295	0.0817
108 300	0.2874
109 305	0.3278
110 310	0.1864
111 315	0.0839
112 320	0.0180
113 Total	=1.000

114 EE: erythemal efficiency spectrum;  $I$ : solar simulator intensity spectrum

## 115 2.3 Stability testing

116 The samples which had the highest concentration of *A.catechu* with and without preservative,  
 117 were selected to test the stability. Stability testing of the developed *A.catechu* sunscreen was  
 118 evaluated and monitored the appearance (the color, odor / fragrance, pH value) under the different  
 119 temperature (kept in the refrigerator, room temperature and outdoor for 96 hours). The dispersed  
 120 phase had a good test method to predict creaming was centrifugation. The sunscreens were  
 121 centrifuged for thirty minutes at 3000 rpm. Additionally, the accelerated stability test was  
 122 conducted in this work by kept the products in difference temperature ( $5\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$ ,  $25\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$  and  
 123  $40\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$ ) and centrifuged for ten minutes at 5000 rpm then monitored and reported the appearance  
 124 of the sunscreens.

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## 128 2.4 Patch testing

129 Patch testing was used for *the irritation evaluation*. The researchers recruited 20 healthy, adult  
130 volunteers with no underlying skin disease or skin lesion on the test area. All subjects were older  
131 than 18 years old, non-pregnant, and not breastfeeding. All volunteers were informed of objectives,  
132 test procedures, and possible adverse effects, and were rewarded for their time of participation. The  
133 Ethics Committee of Phranakorn Rajabhat University, Bangkok, Thailand, approved the present  
134 protocols. All participants gave consent before entering the study. Five grams of the developed  
135 sunscreen was kept at the inside of the arm in contact with the skin for 48 hours under  $2 \times 2$  cm<sup>2</sup>  
136 occlusive patch. After the required periods of skin contact, the patches were opened and observed  
137 for any signs of skin irritation. The clinical observation of the effects was carried out after  
138 withdrawal of the patch. The ranking of the irritant potential is assessed according to a determined  
139 numerical scale depending on the irritation observed; ranking varies between: Not irritant, Very  
140 Slightly Irritant, Slightly Irritant, Moderately Irritant, Irritant and Very Irritant.

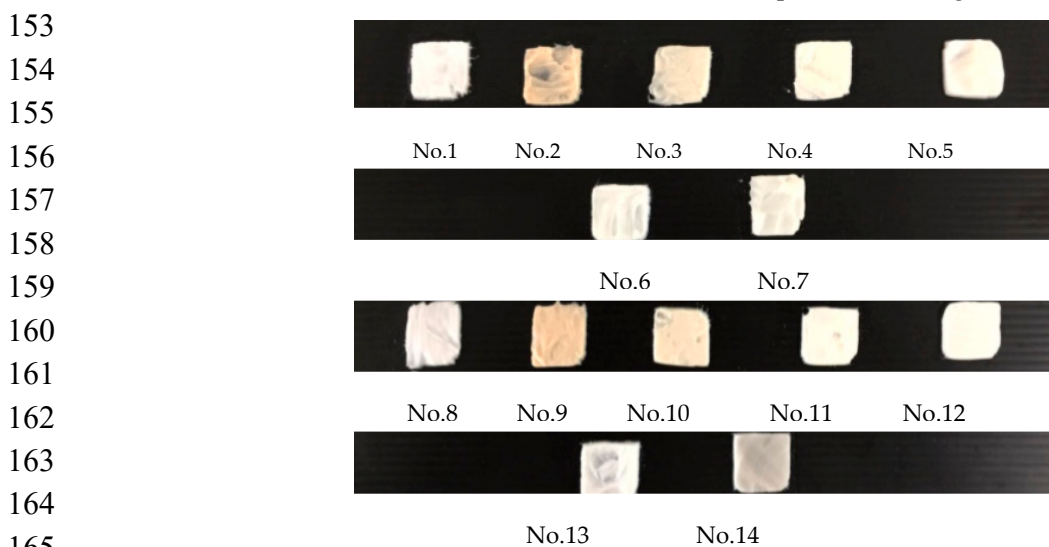
## 141 2.5 Testing of Satisfaction

142 In order to investigate the satisfaction of the products. All volunteers from irritation testing  
143 shown their satisfaction using questionnaires. The five-rating scale questionnaire consisted of two  
144 parts; personal data and product data. Questions in personal data were about gender, age, and type  
145 of skin. While product questions were the sensorial evaluation of the sunscreen (texture, colour, odor,  
146 consistency), improvement in the skin upon use, skin nourishment, and satisfaction with the  
147 sunscreen. Additionally, the developed product was compared satisfaction with the commercial  
148 product by the blind testing.

## 149 3. Results

### 150 3.1 Sunscreen formulation

151 Fourteen sunscreens were prepared using silicone-based. The fresh, smooth and soft textures  
152 of the sunscreens from the different formulations were represented in Figure.1.



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**Figure 1.** An illustration of the textures of the sunscreens.

168 3.2 Determination of the *in vitro* sun protection factor

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170 The SPF values were represented in Table 3. Table 3 represented that an increasing of *A.catechu*  
 171 causes SPF to increase. The highest SPF value was 30.344 from sample 2, that corresponds to  
 172 a 97 percent UVB-protection [20]. While, the lowest SPF value was 24.143 from sample 7.  
 173 The average SPF values of the samples with and without preservative were 28.247 and 28.549,  
 174 respectively. Moreover, the results show a high SPF value (24) even no synthetic UV-filters.  
 175 Impressively, the results of this research reveal high SPF values compared with other natural  
 176 sunscreens. SPFs of methanol solutions of flowers of *Calendula officinale* and flowering tops of  
 177 *Hypericum perforatum* were 12.01 and 12.21 respectively [21]. SPF values of the products containing  
 178 *Mentha piperita* (Leaves), *Azadirachta indica* (Leaves), *Oscimum sanctum* (Leaves), *Aloe vera* (Leaves),  
 179 *Lycopersicon esculantum* (fruits), and *Carica papaya* (fruits) were 8.184, 4.368, 2.904, 5.437, 6.083, and  
 180 2.310, respectively [22]. Their SPF values of the *Zanthoxylum rhetsa* sunscreen were  $3.60 \pm 0.28$  (F1)  
 181 and  $6.90 \pm 0.57$  (F2) [23]. *Moringa oleifera* sun care demonstrated SPF 2 [24]. The carrot and coconut  
 182 cream containing 2% of coconut ethyl acetate extract showed the lowest SPF value of 0.64 [25].

183 **Table 3.** Sun Protection Factor of the sunscreen samples using Equation (1) (n=3).

Sunscreen with preservative	SPF	Sunscreen without preservative	SPF
1. Control sunscreen without <i>A.catechu</i>	$27.797 \pm 0.009$	8. Control sunscreen without <i>A.catechu</i>	$28.278 \pm 0.133$
2. +++++ <i>A.catechu</i> sunscreen	$30.344 \pm 0.011$	9. +++++ <i>A.catechu</i> sunscreen	$30.341 \pm 0.005$
3. ++++ <i>A.catechu</i> sunscreen	$29.597 \pm 0.010$	10. ++++ <i>A.catechu</i> sunscreen	$29.797 \pm 0.014$
4. +++ <i>A.catechu</i> sunscreen	$29.259 \pm 0.015$	11. +++ <i>A.catechu</i> sunscreen	$29.110 \pm 0.014$
5. ++ <i>A.catechu</i> sunscreen	$28.945 \pm 0.054$	12. ++ <i>A.catechu</i> sunscreen	$29.079 \pm 0.023$
6. + <i>A.catechu</i> sunscreen	$28.344 \pm 0.006$	13. + <i>A.catechu</i> sunscreen	$28.858 \pm 0.029$
7. +++ <i>A.catechu</i> sunscreen without synthetic UV filters	$24.143 \pm 0.009$	14. +++ <i>A.catechu</i> sunscreen without synthetic UV filters	$24.381 \pm 0.020$
<b>Average</b>	<b><math>28.247 \pm 2.205</math></b>	<b>Average</b>	<b><math>28.549 \pm 1.954</math></b>

184 **Remark:** + *A.catechu* sunscreen represented followed the concentrations

## 185 3.3 Stability testing

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187 After testing the stability for 96 hours, all selected samples were inspected centrifuged and  
 188 reported. The sunscreen products were stabilized. The colour, odor / fragrance, pH value and  
 189 texture of the samples were not changed, (all sample had the same pH, 6.0). Except the colour of  
 190 outdoor samples, which had slightly darker colour after 96 hours as shown in Figure 2. Besides,  
 191 accelerated stability results with different temperatures and faster centrifuged, had no physical  
 192 changes were found in all tested sunscreen. The results were probably due to the silicone and  
 193 *A.catechu*. The use of silicone elastomers as delivery systems for active ingredients in a sunscreen  
 194 had been explored. Silicone elastomers contain polar functionality within their cross-linked  
 195 structure. Therefore, silicone elastomers were suitable vehicles to the formulation or stability of  
 196 sunscreens might be prolonged by silicone. Moreover, *A.catechu* extract could presented several  
 medicinal properties especially anti-inflammatory activity, according to previous research reports.

197 Therefore, *A.catechu* sunscreen without preservative provided the same stability for the sunscreen  
 198 with preservative.

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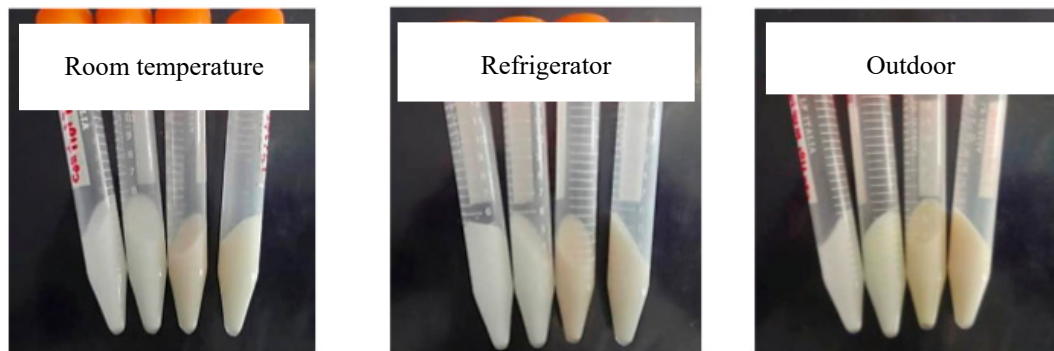


Figure 2. Appearance of sunscreens after testing stability for 96 hours.

### 212 3.4 Patch testing

213 For the irritation evaluation, patch testing under the clinical observation and approved protocol  
 214 by The Ethics Committee of Phranakorn Rajabhat University, all 20 volunteers were female  
 215 (18-22 years old). The highest *A.catechu* concentration in proper sunscreen product without  
 216 preservative and synthetic UV filters, was investigated. Among the volunteers, there was no allergic  
 217 reaction (Ranking: not irritant) to the developed sunscreens.

### 218 3.5 Testing of Satisfaction

219 *A.catechu* sunscreen with and without preservative and commercial sunscreen obtained high  
 220 satisfaction or good level for all aspects (texture, consistency, sensory and odor). For considering  
 221 each product, the developed sunscreen had slightly more satisfied than the commercial product as  
 222 shown in Table 4.

223 **Table 4.** Satisfaction evaluation

Sunscreen	Satisfaction score on each aspect (n=20)				Average
	Texture	Consistency	Sensory	Odor	
Commercial sunscreen	3.90	3.85	3.90	3.50	3.79
++++ <i>A.catechu</i> sunscreen with preservative	4.05	3.95	4.00	3.75	3.94
++++ <i>A.catechu</i> sunscreen without preservative	4.05	4.20	3.95	3.75	3.99

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#### 228 4. Discussion

229 High efficiency sunscreen was presented and compared the satisfaction with the commercial  
230 product. High SPF sunscreen under positive relationship with *A. catechu* concentration.  
231 The influence of preservative and *A. catechu* were evaluated and reported in this work as well.  
232 For storage as usual, *A. catechu* sunscreen without preservative provided good stability. Therefore,  
233 synthetic preservative might be substituted by the natural compound which has a lot of potential for  
234 being sun protection agent or *A. catechu* as well. Combination of natural and synthetic UV filters  
235 could provide synergistic efficacy for a novel cosmeceutical, *A. catechu* sunscreen. However,  
236 *A. catechu* sunscreen without synthetic UV filters provided high SPF (SPF 24) and no irritation in the  
237 volunteers. As the results of this work, there is now a solid body of scientific evidence to confirm  
238 high efficacy and satisfaction of *A. catechu* sunscreen before being a commercial product.

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240 the experiments; O.A. analyzed the data; O.A. wrote the manuscript; O.A. N.L. and R.B. acquired fund.

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