

Research Article

Preparation and Properties of Anti-Nail-Biting Lacquers Containing Shellac and Bitter Herbal Extract

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The purpose of the present investigation was to formulate and evaluate anti-nail-biting lacquers consisting of bitter herbal extracts. The hydroalcoholic extracts obtained from *Andrographis paniculata* and *Tinospora crispa* were determined for phytochemical constituents, total phenolic contents, antioxidant activities, anti-inflammatory activities, and cytotoxicities. Anti-nail-biting lacquers were prepared by using herbal extracts (bittering agent), shellac (film forming polymer), ethanol (volatile solvent), and other indispensable additives with continuous stirring. Thus, attempts to enhance the film property and bitterness are accomplished by using polyvinylpyrrolidone (PVP K30) as a copolymer and varying concentrations of herbal extracts. Good accepted formulations were established for drying time, pH, viscosity, smoothness of film, film strength, water resistant, and solubility in simulated saliva and evaluated their bitterness in human volunteers. The results revealed that phytochemical constituents including tannins, glycosides, reducing sugars, alkaloids, terpenoids, and flavonoids were found present in both extracts while saponins were only detected in *A. paniculata* extract. Although *T. crispa* extract exhibited a significantly higher ($p < 0.05$) total phenolic content and antioxidant activity than *A. paniculata* extract, it showed lower protein denaturation inhibition property than *A. paniculata* extract. Because of the potentials of both extracts without cytotoxicity, anti-nail-biting lacquers containing either *A. paniculata* extract or *T. crispa* extract were developed and evaluated. Drying time of formulations was 6–11 min with visually seen glossiness of formulation. Formulations of the nail lacquer showed good pH, viscosity, smoothness of film, film strength, water resistant, and solubility in simulated saliva. The formulations displaying no significant cytotoxicity effect on CRL-2076 cells were assessed on healthy human volunteers to compare bitterness and film characteristics. The results revealed that the optimized formulation containing *A. paniculata* extract could successfully achieve good film forming property and bitterness release which is considered promising for stopping nail biting.

1. Introduction

Onychophagia (nail biting) means putting one or several fingers in the mouth and biting on nail with teeth. It is a chronic behavioral disorder in children and adults that commonly cooccur with thumb and finger sucking [1]. These behaviors probably associated with psychiatric disorders can cause paronychia, onycholysis, onychomycosis,

gingivitis, temporomandibular dysfunction, oral carriage of Enterobacteriaceae, parasitic infection, and some negative social impacts [2–5].

There are several approaches to cease from nail biting and finger sucking, such as painting a bitter tasting lacquer containing denatonium benzoate and sucrose octa-acetate onto the nails [2] and utilizing a nonremovable reminder, e.g., wristband, finger guard, and glove [6]. However, bitter

nail lacquers containing denatonium benzoate or sucrose octa-acetate for utilize as nail-biting and thumb-sucking deterrents cannot be generally recognized as safe and effective because there is lack of information acquired from adequate and well-controlled, double-blind studies [7]. As in the previous study, although the method of applying a distasteful lacquer exhibited higher drop-out rate than the method of wearing a nonremovable reminder, it was more effective to quit the nail-biting habit by examining only nondropouts [6]. The study indicated that the bitter nail lacquer was a potential alternative to prevent the nail-biting habit due to its long-lasting efficacy of coatings, patient compliance, and success with treatment.

As in earlier studies, the pitfalls of classical anti-nail-biting lacquers were unclear toxicity of synthetic polymers and bitter substances, apply several times daily due to easy to wash off, and cross contamination of nail lacquers during cooking and eating [6]. Consequently, the innovative anti-nail-biting lacquers mainly composed of natural edible ingredients have been developed in order to reduce the toxicity, enhance bitterness, improve water resistance and dissolution in simulated saliva, and also increase other biological properties, especially antioxidant and anti-inflammatory activities.

A chronic habit of nail biting or finger sucking causes dry and peeling skin and leads to inflammation of the skin surrounding the nail [8]. Plenty of unpalatable herbs grown throughout Southern and Southeastern Asia exhibited various biological effects, especially antioxidant and anti-inflammatory activities. *Andrographis paniculata*, commonly known as king of bitters, was found to possess antioxidant activities associated with an increase in the activity of antioxidant enzymes including catalase, glutathione S-transferase, and superoxide dismutase [9]. It could also safeguard cutaneous cells from inflammation [10]. *Tinospora crispa* was reported to have antioxidant activity through radical scavenging and metal chelating mechanisms and anti-inflammatory activity via increasing intracellular expressions of cytokine, INF-g, IL-6, and IL-8 [11]. Antioxidant and anti-inflammatory activities of extracts from *A. paniculata* and *T. crispa* were associated with the contents of phenolics and other phytochemicals [9, 11]. In this study, herbal extracts obtained from *A. paniculata* and *T. crispa* were selected as active ingredients in anti-nail-biting lacquers because of their bitterness and biological activities. One purpose of this study was to determine antioxidant and anti-inflammatory activities of selected herbal extracts.

A coating of nail lacquer can protect thin, friable, vulnerable, and irregular nails along with giving a sustainable exterior layer in order to make them look stronger and more beautiful [12]. The chemical properties of nail lacquers are established on polymerization, evaporation, and adhesion [13]. Monomers form strong bonds with other molecules through polymerization reactions resulting cross-linking of polymer films. After applying a nail lacquer, a solid polymer film is formed by solvent evaporation. A film forming polymer can adhere to the nail plate.

Nail lacquers generally consist of film-forming polymers, volatile solvents, plasticizing agents, and dyes [14, 15]. Coat-

ing formulas often contain pliable resins so as to amplify adhesion and offer glossy appearance. Because of optimum drying time, solvents including ethyl acetate, butyl acetate, and isopropyl alcohol are normally employed for dissolving various resins and other components of nail lacquers [15, 16]. Plasticizers such as camphor, triphenyl phosphate, trimethyl pentanyl diisobutyrate, and acetyl tributyl citrate can enhance flexibility and durability to the films. Dyes or colorants are coloring substances dissolved in nail lacquers and adsorbed onto the nail plates to which they are applied [14, 15].

With regard to help people safely utilize nail lacquers, it is important to study the materials engaged in the production of nail lacquers. Synthetic resins (e.g., toluenesulfonamide-formaldehyde, polyvinyl butyral, and polyester resins) may lead to paronychia, onycholysis, and onychodystrophy [17]. Toxic plasticizers (e.g., phthalate and organophosphate) and harmful solvents (e.g., toluene and formaldehyde) have been prohibited or restricted from use in nail lacquers because of their adverse effects on skin, reproductive system, embryonic development, thyroid gland, and central nervous system [18]. People may have a considerable risk of being exposed to detrimental polymers, plasticizers, and solvents in nail lacquers especially if they are swallowed. Therefore, this study was carefully selected the safe ingredients, aiming for the safety and quality of nail lacquers.

Natural lac is a resin secreted by lac insects (e.g., *Laccifer lacca* Kerr and *Kerria lacca*) [19]. Seed lac derived from stick lac is processed into shellac, a natural gum resin, by hand-crafted, heat, or solvent methods. The special properties of shellac include energetically favorable adhesion to the surface, water protection, and shimmering appearance. Furthermore, shellac has been accepted by US Food and Drug Administration (US FDA) and European Food Safety Authority (EFSA) to utilize in pharmaceuticals and food [19, 20]. According to the consumer product safety, synthetic resins have been superseded by durable natural polymers, especially shellac, which could be developed to improve drying time, adhesiveness, and flexibility of nail lacquers [15]. In case of solvent selection, ethanol could be employed as a solvent in nail lacquers because of its rather low toxicity compared with other organic solvents used for nail lacquers and efficacy to dissolve various active constituents in herbal extracts.

The objective of this study was to develop nontoxic anti-nail-biting lacquers containing bitter herbal extracts obtained from *A. paniculata* and *T. crispa*. Not only the selection of ingredients but also the evaluation of products is essential for product safety and quality. Therefore, this study was invented to determine the quality control parameters (e.g., drying time, pH, viscosity, smoothness of film, film strength, water resistant, and solubility in simulated saliva) of formulated nail lacquers and then evaluate the bitterness in human volunteers.

2. Materials and Methods

2.1. Materials. Ethanol was supplied by Thai Food and Chemical Co., Ltd., Bangkok, Thailand. All chemicals

utilized in phytochemical screening were of analytical grade purchased from Sigma-Aldrich Corporation (Missouri, USA). Potassium bromide, FTIR grade, attained from Thermo Fisher Scientific (Massachusetts, USA). Folin-Ciocalteu was purchased from Loba Chemie (Mumbai, India). Gallic acid, sodium carbonate, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), acetate buffer, ferric chloride hexahydrate, and bovine serum albumin were procured from Sigma-Aldrich Corporation (Missouri, USA). L-Ascorbic acid was obtained from Chem-Supply Pty Ltd. (Gillman, South Australia). Ultrapure water generated by GenPure equipment (TKA Wasseraufbereitungssysteme GmbH, Niederelbert, Germany) and ICP multielement standard solution XIII obtained from Agilent Technologies (Santa Clara, USA) were utilized to determine heavy metal contents. Diclofenac diethylamine and microbiological media were acquired from Merck KGaA (Darmstadt, Germany). Iscove's Modified Dulbecco's Media (IMDM), fetal bovine serum (FBS), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich Corporation (Missouri, USA). Dewaxed bleached shellac and polyvinylpyrrolidone (PVP K30) USP were procured from Union Shellac Part., Ltd. (Bangkok, Thailand) and Shanghai Yuking Chemtech Co., Ltd. established by China Functional Polymer Industry Committee (Shanghai, China), respectively.

2.2. Extraction of Plant Materials. The dried aerial parts of *A. paniculata* and stems of *T. crispa* (Figure 1) were collected from gardens in Nakhon Pathom, Thailand, in April 2020. Both plant specimens were substantiated using the key to species and description in the Botanical Garden Organization (BGO) plant database, Ministry of Natural Resource and Environment, Thailand [21]. Voucher specimens were deposited in the Faculty of Pharmacy, Silpakorn University, Thailand. Samples were separately ground into fine particles and sieved to obtain the particle size < 0.149 mm. Each sample was then macerated two times in 95% v/v ethanol at dried sample-to-solvent ratio of 1:5 g/ml, maceration time of 3 d, and maceration temperature of $25 \pm 1^\circ\text{C}$ with periodic agitation. The mixtures were independently filtered using Whatman no. 1 filter paper to collect the filtrates for subsequent evaporation of solvents using a rotary evaporator (R-100, Buchi, Japan) under reduced pressure at 45°C to obtain *A. paniculata* and *T. crispa* ethanolic extracts. The extracts were dried to constant weight in a hot air oven (Heraeus, Hanau, Germany) at $50 \pm 5^\circ\text{C}$ and kept at -20°C until used.

2.3. Phytochemical Investigation. Preliminary phytochemical screening tests in the 10 mg/ml ethanolic solutions of *A. paniculata* and *T. crispa* extracts were accomplished using the standard methods [22–25]. All chemical tests were executed in triplicate.

2.3.1. Tests for Tannins [22, 23, 25]. For ferric chloride test, several drops of 10% w/v ferric chloride solution were added to the sample solution. A brownish green color indicates the existence of tannins.

For lead acetate test, a few drops of 10% w/v lead acetate were added to the sample solution. The white precipitate was formed designating the presence of tannins.

2.3.2. Tests for Glycosides [22]. 2 ml of glacial acetic acid and 1 ml of ferric chloride were transferred into 1 ml of sample solution, and then, 1 ml of concentrated sulfuric acid was added. The appearance of blue-green color represents the presence of glycosides.

2.3.3. Tests of Reducing Sugars [22, 24, 25]. Ten drops of each solution A and B were added to a test tube containing 2 ml of sample solution. After heating for 15 min at $60 \pm 0.5^\circ\text{C}$, orange red precipitate or green suspension was formed stipulating the existence of reducing sugars.

2.3.4. Tests of Alkaloids [22–25]. For Dragendorff's test, the sample solution was acidified with diluted hydrochloric acid. The mixture was heated on a water bath and then filtered through a Whatman no. 1 filter paper. Equal volumes of the resulting solution and Dragendorff's reagent were reacted. The formation of an orange red precipitate indicates the existence of alkaloids.

For Mayer's test, equal volumes of the resulting solution and Meyer's reagent were mixed. The turbidity or a yellow precipitate indicates the presence of alkaloids.

2.3.5. Tests of Saponins [22–25]. For frothing test, 5 ml of distilled water was added to a test tube containing 2 ml of sample solution. The mixture was shaken for 5 min to observe the formation of 1 cm thick layer of stable liquid foams.

2.3.6. Tests of Terpenoids [22, 23, 25]. 1.5 ml of sample solution was mixed with 1 ml of chloroform, and then, 1 ml of concentrated sulfuric acid was slowly added to form a reddish-brown layer at the junction specifying the presence of terpenoids.

2.3.7. Tests for Flavonoids [22–25]. For ferric chloride test, 2 ml of sample solution was treated with 1 ml of 10% w/v ferric chloride solution. Formation of a wooly brownish precipitate indicates the presence of flavonoids.

For Shinoda's test, 1.5 ml of sample solution was treated with 1 ml of methanol. The solution was warmed, and magnesium ribbons were added. 5 drops of concentrated hydrochloric acid were carefully added, and orange or red color was observed for flavonoids.

2.3.8. Tests for Steroids [22, 25]. 1.5 ml of chloroform was mixed with 1.5 ml of sample solution. 0.5 ml of acetic anhydride and 1 ml of 10% w/v sodium hydroxide solution were added. After mixing and standing for 10 min, the appearance of a blue-green ring indicates the presence of steroids.

2.4. Fourier-Transform Infrared Spectroscopy (FTIR) Analysis. With regard to produce potassium bromide (KBr) pellets of *A. paniculata* and *T. crispa* extracts, approximately 2 to 3 mg of each dried extract was amalgamated with 100 mg of dried KBr using a mortar and pestle, and then, the KBr/extract mixture was compressed into a thin transparent disc under a hydraulic press. The characteristic



FIGURE 1: Dried aerial parts of *A. paniculata* (a) and stems of *T. crispa* (b).

functional groups of both extracts were analyzed using a FTIR spectrometer (Thermo Electron Scientific Instruments Corporation, Madison, WI, USA) at the frequency region of $4000\text{--}400\text{ cm}^{-1}$.

2.5. Total Phenolic Contents. Total phenolic contents of *A. paniculata* and *T. crispa* extracts were ascertained as mg of gallic acid equivalents per g of dried extract (mg GAE/g dried extract), in consonance with an improved Folin-Ciocalteu method [26]. A standard curve was created using gallic acid solutions which dissolved in methanol at concentrations between 20 and $100\text{ }\mu\text{g/ml}$. For sample estimation, $50\text{ }\mu\text{l}$ of 1 mg/ml extract solution was completely mixed with $50\text{ }\mu\text{l}$ of 50% v/v Folin-Ciocalteu reagent at $25 \pm 1^\circ\text{C}$ for 5 min. The solution was combined with $100\text{ }\mu\text{l}$ of 7.5% w/v sodium carbonate solution and then incubated in the dark for 90 min at the same temperature. The absorbance at 765 nm wavelength was measured using a UV-visible spectrophotometer (Model U-2990, Hitachi, Japan).

2.6. Antioxidant Activities

2.6.1. DPPH Assay. Free radical scavenging activities of samples were assessed by a modified DPPH method [26]. L-Ascorbic acid in methanol was employed as a positive control, and scavenging activities of samples were evaluated using their calibration curves and expressed as SC_{50} values ($\mu\text{g/ml}$), the concentration required to reduce the initial DPPH radical concentration by 50%. A volume of $300\text{ }\mu\text{l}$ of each sample solution at various concentrations from 1.25 to 10 mg/ml in methanol was pipetted into 2.7 ml of 0.5 mM DPPH methanolic solution. After mixing, the solutions were allowed to stand in the dark for 30 min at $25 \pm 1^\circ\text{C}$. Absorbance values of solutions were read against a methanol blank at 515 nm using a UV-visible spectrophotometer (Model U-2990, Hitachi, Japan). The measurements were conducted in triplicate.

2.6.2. FRAP Assay. Ferric reducing antioxidant power (FRAP) assay was carried out in keeping with our foregoing report [26]. In short, the FRAP reagent consisted of 10 mM TPTZ in 40 mM hydrochloric acid, 20 mM ferric chloride hexahydrate in ultrapure water, and 0.3 M acetate buffer (pH 3.6) in the volume ratio of 1:1:10. A volume of $90\text{ }\mu\text{l}$ of FRAP reagent was incubated with $30\text{ }\mu\text{l}$ of 1 mg/ml sample solution in 96-well plates in the dark at $37 \pm 1^\circ\text{C}$ for 30 min.

The absorbance values determined at 593 nm using a multi-mode microplate reader (VICTOR® Nivo™, Perkin Elmer, UK) were calculated by subtracting a reagent blank value. L-Ascorbic acid dissolved in methanol was used as a positive control, and all determinations were attained in triplicate. FRAP values were expressed as micrograms of ascorbic acid equivalent per gram of a dried extract ($\mu\text{g AAE/g}$ dried extract).

2.7. Anti-inflammatory Activities. Inhibition of protein denaturation was determined according to our previous procedure [27], and the inhibitory activities were expressed as IC_{50} (mg/ml), the concentration of the extract producing 50% inhibition of the protein denaturation. Briefly, the reaction mixture was comprised of 0.2 ml of fresh egg albumin, 2.8 ml of phosphate buffered saline (pH 7.4), and 2 ml of sample solution with a concentration range varying between 0.2 and 4 mg/ml . All mixtures were incubated at $37^\circ\text{C} \pm 1^\circ\text{C}$ for 15 min and then heated at $70 \pm 1^\circ\text{C}$ for 5 min. After cooling to room temperature, the absorbance values were determined at 660 nm using a UV-visible spectrophotometer (Model U-2990, Hitachi, Japan). Ultrapure water and diclofenac diethylamine were performed as negative and positive controls, respectively.

2.8. Quantification of Heavy Metals. After nitric acid assisted closed vessel microwave digestion, the concentrations of arsenic (As), cadmium (Cd), mercury (Hg), and lead (Pb) in samples were analyzed by inductively coupled plasma-mass spectrometry (ICP-MS) as described in our previous report [26]. The standard solutions at five different concentrations used for establishing the calibration curves for heavy metals were prepared by diluting an ICP multielement standard solution XIII with 5% v/v nitric acid solution. All samples were digested by a microwave digester (Model ETHOS ONE, Milestone Corporation, Sorisole, Italy) and determined by an ICP-MS spectrometer (Model 7500ce, Agilent Technologies, Santa Clara, USA) in triplicate.

2.9. Microbial Limit Test. The microbiological examination including total aerobic mesophilic microorganisms (bacteria, yeast, and molds), *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Candida albicans*, and *Clostridium* spp. was done in accordance with the microbial enumeration test of the United States Pharmacopeia (USP) 41 [28].

2.10. Evaluation of the Cytotoxicities of Bitter Extracts and Nail Lacquers Containing Bitter Extract on Human Dermal Fibroblasts. The cellular viability of human dermal fibroblasts was evaluated upon treatment with either bitter extract or bitter nail lacquer using MTT colorimetric assay [29]. ATCC® CRL-2076 cells (Manassas, VA, USA) were seeded at density of 1×10^4 cells/well in a 96 well plate and incubated with 100 μ l supplemented IMDM until the confluency reached 80–90%. Samples were separately serially diluted with IMDM to obtain the appropriate concentration ranges for testing. After treatments, the cells were incubated for 24 h at 37°C, 5% CO₂. 10 μ l of MTT solution (5 mg/ml) was put into each well and incubated at 37°C for 2 h. The solution was then removed, and 100 μ l of DMSO was subsequently added to dissolve the formazan crystals, which are generated by mitochondria of viable cells. The absorbance was measured at 550 nm using a fusion universal microplate analyzer (Model A153601, Packard BioScience Company, Connecticut, USA), and the percentages of cell viability were calculated compared with untreated controls.

2.11. Formulation of Nail Lacquers

2.11.1. Preparation of Extract-Free Nail Lacquers. Film-forming solutions with various concentrations (10, 15, 20, 25, and 30% w/w) of shellac were prepared by dissolving different weights of dewaxed bleached shellac in the required amount of 95% v/v ethanol using a magnetic stirrer (Stuart Overhead Stirrer Model SS20, Staffordshire, UK) with rotational speed at 60 rpm. To allow for a comparison of drying time and weight gain (Table 1), three replicates (samples) were produced.

2.11.2. Preparation of Nail Lacquers Containing Bitter Extract. The formulations containing either *A. paniculata* extract or *T. crispa* extract were performed as per formula delineated in Table 2. The mixture of shellac and bitter extract was dissolved in 95% v/v ethanol using a magnetic stirrer at a constant speed 60 rpm for at least 6 h until well combined. After mixing, the total volume of mixture was adjusted to the final desired amount by adding 95% v/v ethanol. The homogeneities, viscosities, and bitterness intensities of formulations containing bitter extract are shown in Table 2.

2.11.3. Development of Nail Lacquers Containing Bitter Extract. The nail lacquers containing 20% w/w bitter extract and 20% w/w shellac in 95% v/v ethanol (formulation Nos. 7 and 19) were chosen for this study pursuant to their expected homogeneity, viscosity, and bitterness (Table 2). A study of a similar nail lacquer with added polypropylene glycol (PPG) in two concentrations (5% w/w and 10% w/w) showed that PG significantly reduced bitterness (data not shown). Nevertheless, PVP (polyvinylpyrrolidone) K-30 was used as a copolymer and cosolvent for enhancing the bitterness of nail lacquers and improving the solubility of bitter extracts. The formulation trials were carried out as per the formula described in Table 3. The mixture of shellac and PVP K-30 and the bitter extract were separately dissolved in 95% v/v ethanol in the required quantity using a

TABLE 1: Evaluation of drying time and weight gain of extract-free nail lacquers.

Film-forming solution no.	Concentrations of shellac (% w/w)	Drying time (min)	Weight gain (g)
1	10	15	0.0090 \pm 0.0010
2	15	16	0.0153 \pm 0.0006
3	20	17	0.0217 \pm 0.0025
4	25	21	0.0347 \pm 0.0067
5	30	26	0.0387 \pm 0.0064

magnetic stirrer with adjustable speed range of 50–60 rpm. Two resulting solutions were thoroughly mixed to ensure homogeneity and then made up to 100 g with 95% v/v ethanol. The developed nail lacquer was stirred until all parts of the solution were homogeneous and transferred to a tightly closed amber glass bottle with narrow mouth and plastic screw cap.

2.11.4. Physicochemical and Mechanical Evaluation of Developed Nail Lacquers [30]. Each prepared formulation was gently applied in the same direction on an acrylic fake nail with a brush. After hardening of a film at $25 \pm 1^\circ\text{C}$ without any materials adhering to the finger, drying time and weight gain were determined. Drying time or dry-to-touch time was measured using a stopwatch. Weight gain measurements were carried out by weighing the samples to the nearest 0.0001 g with an analytical balance (Sartorius BP210S Electronic Balance, Sartorius Group, Göttingen, Germany) before and after applying formulations to each fake nail. The nail lacquer formulations were determined for their physical characteristics including homogeneity and color by visual inspection and pH values using a pH meter (SevenEasy, Mettler-Toledo, Switzerland). The viscosities of formulations were measured by using a Brookfield DV-III Ultra Programmable rheometer (Model RVDV-III Ultra, Brookfield engineering laboratories, Inc., Massachusetts, USA). All measurements were done in triplicate. A dial thickness gauge (Model G (0.01–10 mm), Peacock, Ozaki MFG. Co., Ltd., Tokyo, Japan) was used to measure dry film thickness. Tensile testing using a texture analyzer (TA.XT.-plus, Stable Micro Systems Ltd., Surrey, UK) was performed to determine stress values of films formed by nail lacquers. The viscosity, pH, drying time, weight gain, film thickness, and stress of each developed formulation are provided in Table 4.

The water resistance test was done by applying a tested nail lacquer onto a Teflon tray, leaving it to dry, peeling it off, cutting it to the same size, weighing each piece of film (known dry weight, W_o), placing the lacquer films in each testing basket, then immersing the baskets in distilled water at $25 \pm 1^\circ\text{C}$ using a disintegration tester (ZTx20 series, Erweka GmbH, Heusenstamm, Germany). The higher the percentage of the remaining weight in distilled water, the better the water resistance. *In vitro* bitterness release test was performed in simulated saliva (pH 6.8) at $37 \pm 1^\circ\text{C}$ according to the above method. The lower the percentage

TABLE 2: Composition, homogeneity, viscosity, and bitterness of nail lacquers containing bitter extract.

Formulation no.	Concentrations (% w/w)		Shellac	Homogeneity*	Viscosity*	Bitterness*
	<i>A. paniculata</i> extract	<i>T. crispa</i> extract				
1	10		10	+++	+	+
2	10		15	++	++	++
3	10		20	++++	+++	++
4	10		25	+	+++	+
5	20		10	+++	+	+
6	20		15	++	++	+++
7	20		20	++++	+++	++++
8	20		25	+	++++	++
9	30		10	Precipitation	+	+
10	30		15	Precipitation	++	+
11	30		20	Precipitation	+++	+
12	30		25	Precipitation	++++	+
13		10	10	+++	+	+
14		10	15	+++	++	++
15		10	20	++	+++	++
16		10	25	+	+++	+
17		20	10	+++	+	+
18		20	15	+++	++	+++
19		20	20	+++	+++	++++
20		20	25	+	++++	+
21		30	10	Precipitation	+	+
22		30	15	Precipitation	++	+
23		30	20	Precipitation	+++	+
24		30	25	Precipitation	++++	+

*Grade offers four levels of quality: high (++++), moderate (+++), low (++), and very low (+).

TABLE 3: Composition of developed nail lacquers containing bitter extract and copolymer.

Formulation no.	Concentrations (% w/w)			PVP K-30
	<i>A. paniculata</i> extract	<i>T. crispa</i> extract	Shellac	
7A	20		10	10
7B	20		15	5
19A		20	10	10
19B		20	15	5

of the remaining weight in simulated saliva, the greater the bitterness release. The dried lacquer film was weighed and dedicated as dry weight after testing (W_t) in distilled water or simulated saliva, and then, the percentage of the remaining weight was calculated as illustrated in equation (1).

$$\% \text{remaining weight} = \left(\frac{W_t}{W_o} \right) \times 100. \quad (1)$$

The percentages of the remaining weight obtained from water resistance and bitterness release tests are illustrated in Table 5.

2.11.5. Evaluation of Film Appearances and Bitterness Intensities in Human Volunteers. A total of 20 healthy participants (10 males and 10 females) ranging in age from 18 to 30 years volunteered to participate in the study. All of them were non-Muslims and had no prior history of allergic reactions to alcohol, food, medicines, natural extracts, and cosmetic ingredients. In addition, they were advised to steer clear of drinking (except water) and eating for a time no less than 1 h before starting the test. Each formulated nail lacquer was applied on participants' thumb nails once a day. After the nail lacquer had dried, participants evaluated the film appearances and bitterness intensities of nail lacquer films by finger touching, visualizing, and sucking, in line with their own perceptions and then answered questionnaires. Sensory assessments were performed in triplicate. The experimental protocol (REC 62.0912-038-4567) was approved by the Human Research Ethics Committee, Silpakorn University, Thailand.

2.11.6. Determination of Stability. The developed nail lacquers were stored individually in tightly closed amber glass containers. The physical stability of samples was evaluated by heat-cool cycling for six cycles between temperature of $4 \pm 1^\circ\text{C}$ and $45 \pm 1^\circ\text{C}/75 \pm 2\%$ RH (relative humidity) with storage at each temperature for 24 h. The samples were then analyzed for their pH values, viscosities, phase separation,

TABLE 4: Physicochemical and mechanical properties of developed nail lacquers containing bitter extract and copolymer.

Formulation no.	pH	Viscosity (cP)	Drying time (min)	Weight gain (g)	Film thickness (mm)	Stress (N/mm ²)
7A	5.09 ± 0.02	96.59 ± 6.88	8.00 ± 1.00	0.0234 ± 0.0022	0.9467 ± 0.2210	0.079 ± 0.013
7B	4.96 ± 0.01	45.34 ± 4.72	6.33 ± 0.58	0.0183 ± 0.0022	0.6483 ± 0.1179	0.136 ± 0.047
19A	4.66 ± 0.02	215.8 ± 6.34	10.67 ± 1.15	0.0488 ± 0.0012	0.7963 ± 0.0132	0.233 ± 0.050
19B	4.52 ± 0.10	101.04 ± 3.64	7.67 ± 0.58	0.0124 ± 0.0007	0.6417 ± 0.0534	0.553 ± 0.255

TABLE 5: Percentages of the remaining weight of lacquer films after testing in distilled water (at 25 ± 1°C) and simulated saliva (at 37 ± 1°C).

Formulation no.	Water resistance test (in distilled water)		Bitterness release test (in simulated saliva)	
	Percentages of the remaining weight (%)	Time (min)	Percentages of the remaining weight (%)	Time (min)
7A	0.00 ± 0.00	180	0.00 ± 0.00	60
7B	58.60 ± 12.37	180	0.00 ± 0.00	40
19A	10.86 ± 11.88	180	0.00 ± 0.00	60
19B	74.20 ± 2.31	180	0.00 ± 0.00	40

and colors (Table 6). DPPH free radical scavenging and protein denaturation inhibitory activities of developed formulations were determined, before and after the stability test (Table 7). In addition, heavy metal concentrations and microbial loads in developed formulations were also examined.

2.12. Statistical Analysis. Experimental data is shown as mean ± standard deviation (SD) of three independent experiments. Statistical significance ($p < 0.05$) was estimated by one-way analysis of variance (ANOVA) using SPSS 16.0. The Duncan's test was employed to measure specific differences between pairs of means.

3. Results and Discussion

3.1. *A. paniculata* and *T. crispa* Ethanolic Extracts

3.1.1. Extraction Yields and Chemical Constituents. The extraction yields of *A. paniculata* and *T. crispa* ethanolic extracts were $7.75 \pm 0.32\%w/w$ and $3.50 \pm 0.23\%w/w$, respectively. Appearances of *A. paniculata* and *T. crispa* ethanolic extracts were of dark greenish brown mass, as illustrated in Figure 2. Both extracts showed the presence of tannins, glycosides, reducing sugars, alkaloids, terpenoids, and flavonoids except steroids. However, saponins were found in *A. paniculata* extract but absent in *T. crispa* extract.

The structures of compounds in *A. paniculata* and *T. crispa* extracts were identified by comparing their FTIR spectra with previously reported data. The FTIR spectra of both extracts (Figure 3) showed broad bands (3418 and 3413 cm^{-1}) in harmony with hydroxyl groups and medium bands (2928 and 2925 cm^{-1}) and pairs of absorption bands (1631 and 1384 and 1644 and 1460 cm^{-1}) in congruence with aromatic rings, thus suggesting that phytochemicals in both

extracts were flavonoids. The FTIR spectrum of *A. paniculata* extract (Figure 3(a)) exhibiting peaks at 3418 , 2928 and 2851 , 1750 , 1460 , and 1219 cm^{-1} may account for the presence of O-H, C-H, C=O, and C=C stretching of an exo-methylene double bond and C-O-C stretching of a lactone ring, respectively, suggesting the presence of andrographolide (terpenoids) [31]. The FTIR spectrum of *T. crispa* extract (Figure 3(b)) displayed hydroxyl (3413 cm^{-1} with strong intensity and width), aromatic (2925 and 1460 cm^{-1}), carbonyl (1711 cm^{-1}), and amide carbonyl (1644 cm^{-1}) absorptions, characteristic of aporphine alkaloids [32]. The results of this study were consistent with several earlier investigations showing that terpenoids and alkaloids are the most common bitter compounds found in *A. paniculata* and *T. crispa*, respectively [9–11].

3.1.2. Total Phenolic Contents and Biological Activities of Bitter Extracts. The total phenolic contents of *A. paniculata* and *T. crispa* extracts were evaluated based on the standard curve of gallic acid $y = 0.0296x + 0.0796$; $R^2 = 0.9997$. The total phenolic content of *T. crispa* extract ($64.43 \pm 3.77\text{ mg GAE/g}$ dried extract) was found to be significantly higher ($p < 0.05$) than that of *A. paniculata* extract ($21.05 \pm 1.94\text{ mg GAE/g}$ dried extract). *A. paniculata* and *T. crispa* extracts obtained with 95% v/v ethanol contained greater amounts of phenolic compounds than previously reported extracts prepared with 70% v/v methanol (7.78 mg GAE/g dried extract) [33] and 80% v/v ethanol ($29.83 \pm 2.14\text{ mg GAE/g}$ dried extract) [34], respectively. Therefore, 95% v/v ethanol seemed to be an appropriate solvent for extraction of natural phenolics from *A. paniculata* and *T. crispa*.

The lower the SC_{50} value (concentration of extract required to scavenge 50% of DPPH radicals) and the higher FRAP value (capacity of extract to reduce ferric (III) ion to ferrous (II) ion) imply the higher the antioxidant activity. The results revealed that *T. crispa* extract with SC_{50} value of $116.15 \pm 2.40\text{ }\mu\text{g/ml}$ and FRAP value of $15.03 \pm 1.87\text{ }\mu\text{g AAE/g}$ extract showed significantly stronger ($p < 0.05$) antioxidant activity than *A. paniculata* extract with SC_{50} value of $276.15 \pm 16.70\text{ }\mu\text{g/ml}$ and FRAP value of $4.05 \pm 0.35\text{ }\mu\text{g AAE/g}$ extract, while ascorbic acid exhibited DPPH scavenging activity with SC_{50} value of $4.30 \pm 0.06\text{ }\mu\text{g/ml}$.

The lower value of albumin denaturation IC_{50} indicates the higher anti-inflammatory activity. It was observed that *T. crispa* extract with IC_{50} value of $0.90 \pm 0.01\text{ mg/ml}$ displayed significantly stronger ($p < 0.05$) anti-inflammatory activity than *A. paniculata* extract with IC_{50} value of $1.03 \pm 0.16\text{ mg/ml}$, while diclofenac diethylamine is $0.68 \pm 0.00\text{ mg/ml}$ as a comparison.

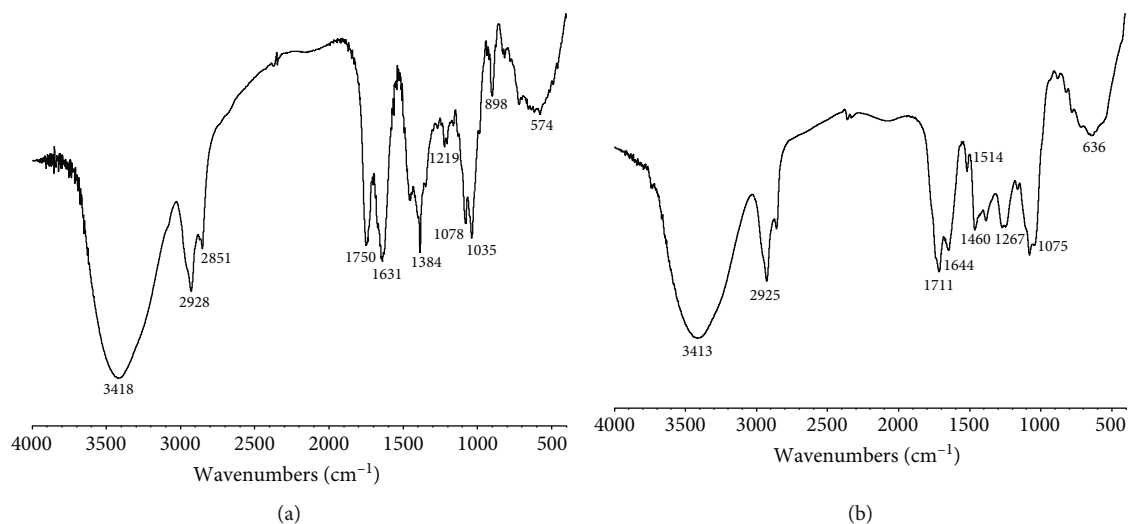
TABLE 6: pH values, viscosities, phase separation, sediment volumes, and colors of developed nail lacquers after six cycles of heating/cooling treatment.

Formulation no.	pH	Viscosity (cP)	Phase separation	Color
7A	5.07 ± 0.01	85.90 ± 9.41	No separation	Dark green
7B	4.96 ± 0.01	31.07 ± 9.20	No separation	Dark green
19A	4.57 ± 0.01	179.97 ± 12.44	No separation	Dark brown
19B	4.52 ± 0.01	65.84 ± 9.37	No separation	Dark brown

TABLE 7: DPPH free radical scavenging and protein denaturation inhibitory activities of developed nail lacquers before and after six cycles of heating/cooling treatment.

Formulation no.	DPPH radical scavenging SC_{50}		Albumin denaturation IC_{50}	
	Before	After	Before	After
7A	1.46 ± 0.06 mg/ml	2.11 ± 0.07 mg/ml	4.94 ± 0.18 mg/ml	5.03 ± 0.20 mg/ml
7B	1.54 ± 0.08 mg/ml	2.34 ± 0.10 mg/ml	5.09 ± 0.24 mg/ml	5.18 ± 0.27 mg/ml
19A	0.82 ± 0.07 mg/ml	1.92 ± 0.04 mg/ml	2.95 ± 0.12 mg/ml	5.13 ± 0.24 mg/ml
19B	0.89 ± 0.09 mg/ml	1.99 ± 0.08 mg/ml	3.06 ± 0.17 mg/ml	5.34 ± 0.29 mg/ml
Ascorbic acid	4.47 ± 0.08 μ g/ml		ND	
Diclofenac diethylamine	ND		0.69 ± 0.01 mg/ml	

ND: not determined.

FIGURE 2: Ethanolic extracts of *A. paniculata* (a) and *T. crispa* (b).FIGURE 3: FTIR spectra of *A. paniculata* (a) and *T. crispa* extracts (b) in potassium bromide disc.

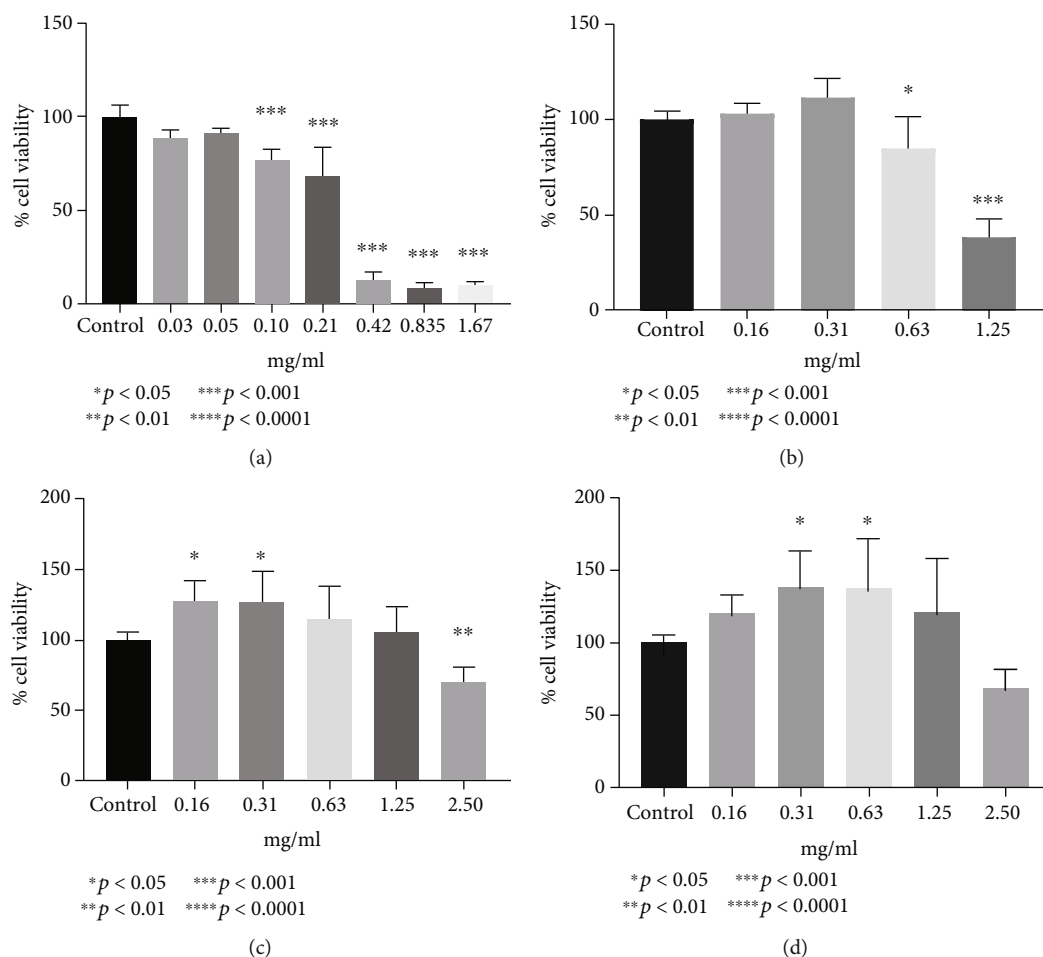


FIGURE 4: Percentages of CRL-2076 viability after 24 h incubation with *A. paniculata* extract (a), *T. crispa* extract (b), formulation No. 7A (c), and formulation No. 19A (d). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Many earlier studies showed the positive correlation between total phenolic content, antioxidant activity, and anti-inflammatory property [33–35]. In consistent with previous studies, the present study revealed that *T. crispa* extract with higher total phenolic content exhibited stronger antioxidant and anti-inflammatory activities than *A. paniculata* extract with lower total phenolic content.

3.1.3. Heavy Metals and Microbial Loads of Bitter Extracts. Maximum limits of Hg (1 mg/kg), Pb (20 mg/kg), As (5 mg/kg), and Cd (5 mg/kg) in cosmetics have been set by Association of South East Asian Nations (ASEAN) guidelines [36]. The concentrations of Pb and Cd in *A. paniculata* extract were 0.010 ± 0.001 mg/kg and 0.002 ± 0.002 mg/kg, respectively, but Hg and As were not detected. The heavy metal concentrations found in *T. crispa* extract were Hg = 0.002 ± 0.002 mg/kg, Pb = 0.010 ± 0.009 mg/kg, and Cd = 0.001 ± 0.003 mg/kg, but As was not found. The concentrations of heavy metals in both extracts were below the permissible values.

ASEAN microbiological limits in products for children under 3 years; eye area and mucous membranes were total aerobic mesophilic microorganisms not more than 500 cfu/g and specified pathogens including *P. aeruginosa*, *S. aureus*,

and *C. albicans* absent in 0.1 g of test sample [36]. Owing to Thailand local concerns, the extracts performed that the additional test for *Clostridium* spp., *A. paniculata*, and *T. crispa* extracts showed total aerobic mesophilic microorganisms below 10 cfu/g, but all specified pathogens including *P. aeruginosa*, *S. aureus*, *C. albicans*, and *Clostridium* spp. were not detected in any of the samples analyzed. The results were consistent with the microbiological requirements. In conclusion, both extracts were safe from harmful heavy metals and pathogens.

3.1.4. Cytotoxicity of Bitter Extracts. This experiment was performed to assess the cytotoxic activities of bitter extracts at various concentrations on CRL-2076 human dermal fibroblasts. The percentages of cell viability upon 24 h treatments with *A. paniculata* and *T. crispa* extracts are illustrated in Figures 4(a) and 4(b), respectively. The viability of cells treated with *A. paniculata* (0.05 mg/ml) and *T. crispa* (0.63 mg/ml) extracts was not significantly different from the control. Their % survival values were 92% and 83%, respectively. Thus, the results showed that *A. paniculata* and *T. crispa* extracts at the concentration ranges of 0.03–0.05 mg/ml and 0.16–0.63 mg/ml, respectively, exhibited low levels of cytotoxicity upon 24 h incubation.

3.2. Developed Nail Lacquers

3.2.1. Formulation Development of Nail Lacquers. The extract-free nail lacquers were prepared by dissolving shellac in 95% v/v ethanol to obtain film-forming solutions in the concentration range of 10–30% w/w. As delineated in Table 1, the shellac concentration affects the drying time and weight gain. The results revealed that the higher the shellac concentration, the longer the drying time, the greater the weight gain. Film-forming solution No. 5 composed of shellac concentration up to 30% w/w produced a very thick film with the highest weight gain that was prone to peeling. Furthermore, its drying time was too long, and it was too viscous to apply. Therefore, film-forming solution No. 5 was cut off from the next experiment.

The nail lacquers containing *A. paniculata* extract (formulation Nos. 1–12) and *T. crispa* extract (formulation Nos. 13–24) were performed by a simple mixing technique, and their homogeneities, viscosities, and bitterness intensities were evaluated at room temperature. The results are depicted in Table 2 which shows that the formulation Nos. 9–12 and Nos. 21–24 containing 30% w/w extract were precipitated after observing at $25 \pm 1^\circ\text{C}$ for one night. In addition, the other formulations (Nos. 1–6 and 8 and Nos. 13–18 and 20) exhibited unfavorable homogeneity, viscosity, or bitterness. However, the results also showed that the formulation No. 7 and No. 19 containing 20% w/w bitter extract and 20% w/w shellac were the most bitter nail lacquers with good homogeneity and suitable viscosity. Consequently, the formulation No. 7 and No. 19 were selected for further studies by adding (+10% w/w or +5% w/w PVP K-30) and subtracting (–10% w/w or –5% w/w shellac) of the nail lacquer composition (Table 3) to obtain the formulation Nos. 7A and 7B and 19A and 19B, respectively. Formulations contained PVP K-30 which acts as a copolymer and solubilizer to improve mechanical property of shellac film and enhance the solubility of bitter extract, respectively.

3.2.2. Physicochemical and Mechanical Evaluation of Developed Nail Lacquers. Table 4 shows physicochemical and mechanical properties of formulation Nos. 7A and 7B and 19A and 19B which were developed from formulation Nos. 7 and 19, respectively. All formulations had pH values between 4.52 ± 0.10 and 5.09 ± 0.02 ($p < 0.05$), and their pH values were not affected by the type of extract and the ratio of shellac: PVP K-30. The viscosities of all developed formulations except No. 7B ranged approximately between 100 and 220 cPs, indicating a highly glossy film likely owing to a smooth surface [37]. Additionally, this viscosity range came up with good adherence and flow property. The formulation No. 7A and No. 19A containing 10% w/w shellac and 10% w/w PVP K-30 possessed higher viscosity values (96.59 ± 6.88 cPs and 215.8 ± 6.34 cPs) than the formulation No. 7B and No. 19B containing 15% w/w shellac and 5% w/w PVP K-30 (45.34 ± 4.72 cPs and 101.04 ± 3.64 cPs), respectively. It indicates the impact of polymer ratio (shellac: PVP K-30) on the viscosity of nail lacquers.

Furthermore, the average values of drying time, weight gain, and film thickness of formulation No. 7A and No. 19A were greater than those of formulation No. 7B and No. 19B, respectively (Table 4). Drying times of nail lacquers should be less than 10 min and vary depending on the volatility characteristics of their solvent systems used in the formulations [38]. The drying times for all developed nail lacquers were between 6.33 ± 0.58 and 10.67 ± 1.15 min. Nevertheless, the formulation No. 19A had a slightly longer drying time (10.67 ± 1.15 min) than the criterion. Weight gain, the weight of the residual film after solvent evaporation, indicates the nonvolatile content [38]. It was seen that as the PVP K-30 concentration increases from 5% w/w to 10% w/w and the shellac concentration decreases from 15% w/w to 10% w/w the weight gain increases (Tables 3 and 4). The results revealed that nonvolatile content of nail lacquers varied depending on the type and concentration of polymer used. The thicknesses of the films obtained from all developed formulations were varied in the range between 0.6417 ± 0.0534 and 0.9467 ± 0.2210 mm (Table 4). The thickness of film was found to be consistent for all formulations. It can be concluded that the variation of film thicknesses was solely affected by the ratio of shellac: PVP K-30 and the amount of herbal extract employed in nail lacquers. The results also revealed that film thickness decreased as the percentage of shellac increased, perhaps because of the lower solid contents of shellac [19] and *T. crispa* extract compared to PVP K-30 and *A. paniculata* extract, respectively. The film obtained from the formulation No. 7A (20% w/w *A. paniculata* extract, 10% w/w shellac, and 10% w/w PVP K-30) had the greatest thickness value (0.9467 ± 0.2210 mm).

Tensile strength designated as a stress is estimated as units of applied force per area (N/mm^2). Measured stress values of films derived from developed nail lacquers ranged from 0.079 ± 0.013 to 0.553 ± 0.255 N/mm^2 (Table 4). The film stress increased with increasing the proportion of shellac (Tables 3 and 4). If film stress is too high, it can lead to film cracking [39]. Accordingly, lower stress value might possibly lead to higher mechanical flexibility of films. The formulation No. 7A and No. 19A having a lower percentage of shellac and higher percentage of PVP K-30 as compared with the formulation No. 7B and No. 19B, respectively, exhibited significantly lower stress values (Table 4). The result indicated that PVP K-30 added to compensate for the lower percentage of shellac could improve the flexibility of films by reducing stress values.

Water resistance test was performed in distilled water. It could be seen that an increase in the concentration of shellac and a decrease in the concentration of PVP K-30 would lead to an increase in the water resistance as depicted in Tables 3 and 5. Formulation No. 7B and No. 19B displayed higher water resistance as compared with formulation No. 7A and No. 19A, respectively. The nail lacquer films disclosed a slow deterioration during 180 min, except for formulation No. 7A which was quickly eroded in distilled water. It seems reasonable to assume that the formulation No. 7B and No. 19B (20% w/w bitter extract, 15% w/w shellac, and 5% w/w PVP K-30) showed much better resistance to water wash out than formulation No. 7A and No. 19A (20% w/w bitter

extract, 10% *w/w* shellac, and 10% *w/w* PVP K-30), respectively. Although the formulation No. 7A was the least water-resistant nail lacquer (remaining weight $0.00 \pm 0.00\%$), it was able to withstand distilled water for up to 180 min that fell within acceptable criteria.

Bitterness release test was conducted in simulated salivary fluid pH 6.8, and the results are illustrated in Table 5 which shows that the rapid disintegration and fast release of bitter extract were observed in the case of formulation No. 7B and No. 19B (remaining weight $0.00 \pm 0.00\%$ for 40 min). The extract contained in the films composed of 15% *w/w* shellac and 5% *w/w* PVP K-30 took about 40 min for the complete dissolution, resulting in a faster dissolution as compared with those composed of 10% *w/w* shellac and 10% *w/w* PVP K-30 (remaining weight $0.00 \pm 0.00\%$ for 60 min). The results revealed that formulation No. 7A and No. 19A were gradually released the bitter substance for a prolonged period.

The results of the physicochemical and mechanical evaluation indicated that nail lacquer formulation No. 19A and No. 19B were not suitable for further evaluation by volunteers, for the following reasons. The nail lacquer formulation No. 19A was too viscous with the highest viscosity (215.8 ± 6.34 cPs) and took too long to dry with the longest drying time (10.67 ± 1.15 min). Furthermore, the film derived from the formulation No. 19A had too much solid content with the greatest weight gain (0.0488 ± 0.0012 g). The highest film stress value obtained from the formulation No. 19B was high enough to cause cracking (0.553 ± 0.255 N/mm²). Accordingly, the nail lacquer formulation Nos. 7A and 7B were selected for volunteer evaluation.

3.2.3. Evaluation of Film Appearances and Bitterness Intensities in Human Volunteers. The selected nail lacquers including formulation Nos. 7A and 7B were assessed in 20 healthy volunteers by applying on the nail plates of the left and right thumbs, respectively. After the nail lacquer had dried, volunteers evaluated the film characteristics including smoothness, glossy, color, and adhesiveness by finger touching and visualizing. After that, they sucked their thumbs and maintained the bitter solutions in their mouths for 5 s to estimate the bitterness intensity. All volunteers completed this study and reported no side effects caused by nail lacquers. Eighty-five percent of the volunteers reported that the formulation No. 7A was more bitter than the formulation No. 7B while seventy-five percent of the volunteers found that the film of formulation No. 7B presented better film characteristic than that of formulation No. 7A.

3.2.4. Cytotoxicity of Developed Nail Lacquers. Because of their greater bitterness, the cytotoxicity assay was performed for formulation No. 7A and No. 19A. These developed nail lacquers were screened for their cytotoxic activities against CRL-2076 human dermal fibroblasts at different concentrations to determine the viability by MTT assay. The cell survival upon treatments of the formulations are depicted in Figures 4(c) and 4(d). Formulation No. 7A and No. 19A at the concentration up to 1.25 mg/ml did not show toxicity

to the cells within 24 h of exposure. These results indicate the nontoxic nature of formulation Nos. 7A and 19A.

3.2.5. Heavy Metals and Microbial Loads of Developed Nail Lacquers. The contents of Pb (0.001 ± 0.004 mg/kg) and Cd (0.001 ± 0.005 mg/kg) in developed formulations were lower than the permissible limits of ASEAN guidelines for cosmetics [36] while Hg and As were not detected. Total aerobic mesophilic microorganisms and specified pathogens of developed formulations were within ASEAN microbiological limits. Therefore, all developed nail lacquers were found to be safe and acceptable, especially in children.

3.2.6. Stability Studies. The stability studies were obtained to find out the alteration of pH values, viscosities, phase separation, colors, and biological activities. The results are shown in Tables 6 which indicates that there were no major changes in physical appearances and pH values except for a significant decrease in viscosities. After six cycles of heating/cooling stability testing, the developed nail lacquers clumped into precipitates, but they were more uniformly distributed after hand-shaking. Therefore, all developed formulations might require gentle shaking before use.

DPPH free radical scavenging and protein denaturation inhibitory activities of developed formulations subjected to accelerated stability were investigated and compared with freshly prepared nail lacquers. The results obtained are detailed in Table 7. All developed formulations showed significant increase ($p < 0.05$) in DPPH radical scavenging SC₅₀ and albumin denaturation IC₅₀ without exhibiting microbial growth on the investigation. Even though their biological activities and viscosities were reduced, they were within the acceptable ranges. The results of stability studies showed that there were no serious stability problems of all developed formulations. The recommended storage condition for the developed formulations was 4°C in order to preserve their biological activities.

4. Conclusion

Anti-nail-biting lacquers containing bitter herbal extracts were successfully prepared using shellac and PVP K-30 as film formers. Formulation No. 7A containing 20% *w/w* *A. paniculata* extract, 10% *w/w* shellac, and 10% *w/w* PVP K-30 was the most appropriate formulation in relation to preventing nail biting. *A. paniculata* and *T. crispa* extracts loaded in formulations at 20% *w/w* possessed antioxidant and anti-inflammatory properties, harmonizing the traditional medicine practice. After six cycles of heating/cooling treatment, the analysis of all developed formulations has revealed the existence of biological activities. Moreover, all developed formulations had gratifying appearances with consistency and without the tendency of separation. The innovative anti-nail-biting lacquer mainly composed of herbal extract and shellac was not only without toxic chemicals but also with antioxidant and anti-inflammatory substances.

Data Availability

The data employed to support the findings of this study are contained in the article. The other data are available from the corresponding author upon request.

Disclosure

A preprint has previously been published [40].

Conflicts of Interest

All researchers state that there are no conflicts of interest.

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Supplementary Materials

Figure S1: formulation No. 7A containing *A. paniculata* extract at 1% w/w (A), 10% w/w (B), and 20% w/w (C). Figure S2: formulation No. 19A containing *T. crispa* extract at 1% w/w (A), 10% w/w (B), and 20% w/w (C). Figure S3: sensory evaluation form (Supplementary Materials). (Supplementary Materials)

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