STABILITY ASSESSMENT OF *Areca catechu* L. EXTRACT FOR APPLICATION IN COSMETIC

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Abstract

Stability of raw betel nut seed was investigated for its application in cosmetic products. The betel nut seed extract was obtained by using 95% ethanol extraction with microwave assistance. The extract was diluted in various buffer pH ranges between 2-11 before storage at 4°C, room temperature (27-32°C), and 50°C conditions for 84 days. Physiochemical and biological properties including pH, color, extractable phenolic content (EPC), ABTS radical scavenging capacity and tyrosinase inhibitory activity of the extract were determined at 7 days intervals during storage. Darker brown color of the extract was observed when the pH and temperature storages increased comparing to the light brown of the initial extract solution. Higher temperature at 50°C also induced precipitation of the extract composition and, consequently, decreases the EPC, and antioxidant capacity. The pH of all extracts tends to be decrease after 56 days storage, while the color change, ΔE*, slightly increased. It has been found that the EPC and antioxidant capacity of the extract were most stable at pH 4-6 and 8-10, especially when storing at low temperature. This study showed the high stability of betel nut seed extract.

Keywords: Antioxidant, *Areca catechu* L., physiochemical, stability, tyrosinase inhibition,

Introduction

Aging and darkening of the skin results from two main factors; programming and permanent actinic damage due to environment stress, UV, detergents, pollution, smoke [1,2]. Plant sources have been evaluated for developing natural antioxidants and melanogenesis inhibitors that are to be involved in anti-aging and skin whitening in cosmetic products [3]. Interest in finding naturally occurring antioxidants in cosmetics, foods or medicines to replace synthetic antioxidants has increased considerably, given that synthetic antioxidants are being restricted due to their side effect. Moreover, natural antioxidants were considered with more safety, stability, and better antioxidant effects [3,4].

Areca catechu L. (betel nut) is commonly known as betel nut and widely distributed in south-east Asia. The major constituents of the nut are carbohydrates, fats, proteins, mineral matter, crude fiber, polyphenols, and alkaloids. Polyphenols which are the main constituent in betel nut including simple phenolics, catechin, caffeic acid, ferulic acid, non-tannin flavans, hydrolysable and condensed tannins, alkaloids and flavonoids [5]. There have been reported that areca nut extract has potential anti-inflammatory, anti-elastase, anti-hyaluronidase activity as well as antimicrobial activity [1,6]. Therefore, the betel nut is attractive to be a potential source for cosmetic active ingredient extraction.

However, plant extract stability is affected by several factors such as pH, storage, temperature, chemical structure, concentration, light, oxygen, solvents, metallic ions, the presence of enzymes [7], basic pH condition, redox-active solvents, and formulation additive in finished product [8]. Since our previous studies show high yield of extractable phenolics content and bioactivities of raw betel nut seed extract [9,10]. However, the physiochemical stability of areca nut extract has not been documented. Hence, this study aims to evaluate the stability of the raw betel nut seed extract under the artificially conditions of various pH and temperature for its further application in cosmetics and health supplement products.

Methodology

Raw betel nut seed extract preparation
The raw betel seed was dried, ground, and sieved into 500 µm size. The extract was prepared by microwave-assisted method at 900 watts for 30min. The 95% ethanol was used as solvent at the sample: solvent ratio of 1:10 (w/v). The crude extract was completely dried by vacuum rotary evaporator.

Sample solution preparation
Fifty millimolar of buffer solutions were prepared from sodium phosphate (pH 2, 7, and 11), sodium citrate (pH 3, 6), sodium acetate (pH 4, 5), tris-HCl (pH 8), and sodium borate (pH 9, 10). The extract solutions were diluted to 1% (w/v) with each pH buffer for physical stability tests and 2 mg/ml (w/v) for stability determination of biological activities.

Physical stability determinations
The sample solutions were kept at 4°C, room temperature (approximately 27-32°C), and 50°C for 84 days. The pH and color of extract solutions were measured at 7 days intervals using pH meter and chromameter with CIE L*a*b* parameters system, respectively. The ΔE* values were calculated and expressed as color change result.

Determination of extractable phenolic content, antioxidant and tyrosinase inhibitory activities
The extractable phenolic content was determined by Folin-ciocalteu method [6]. The result was expressed as gallic acid equivalent per gram extract (mg GAE/g). The $ABTS^{+}$ radical scavenging and tyrosinase inhibitory activity of the extract solutions were determined as the method described in Thaipong et al. [11 and Onar et al. 12], respectively. The antioxidant capacity was exhibited as mg trolox equivalent antioxidant capacity per gram extract (mg TEAC/g) and the tyrosinase inhibitory activity was shown in mg kojic acid equivalent per gram extract (mg KAE/g). Stability of the extract was calculated into residual EPC, TEAC, and KAE comparing with those at day 0 for the phenolic content, antioxidant and tyrosinase inhibition activity, respectively.

Results

pH and color stability
Figure 1A shows high pH stability of extract in all pH solution during 56 days storages and gradually decrease in the last 4 weeks. Noticeably that the pH of extract solution was not exactly the same with the buffer pH because of the extract itself was weak acidic (about pH 4 in DI water). The storage temperatures seem to be not affected the pH stability.

The color of extracts was expressed as ΔE* which is the color change detected by human eyes. The more value of ΔE*, the more color changes. For the stability of extract color in

Figure 1B, thermal is rather considered as the significant factor than pH condition. At 4°C and room temperature the color seems to be stable at every pH. In contrast at the high temperature, the color of extracts, especially at pH 2-4, was obviously changed due to the precipitation of the extract under accelerated condition of 50°C.

For color stability at day 84, as showed in Figure 2B, high temperature storage was greatly effected to the color changing, expressed as ΔE* value. When temperature raised, more change in extraction color was observed in acidic condition. The pH profile of all extract solution after 84 days storage is depicted in Figure 2A. Some change in pH was observed when storage in the buffer pH between 6-7 and 11.

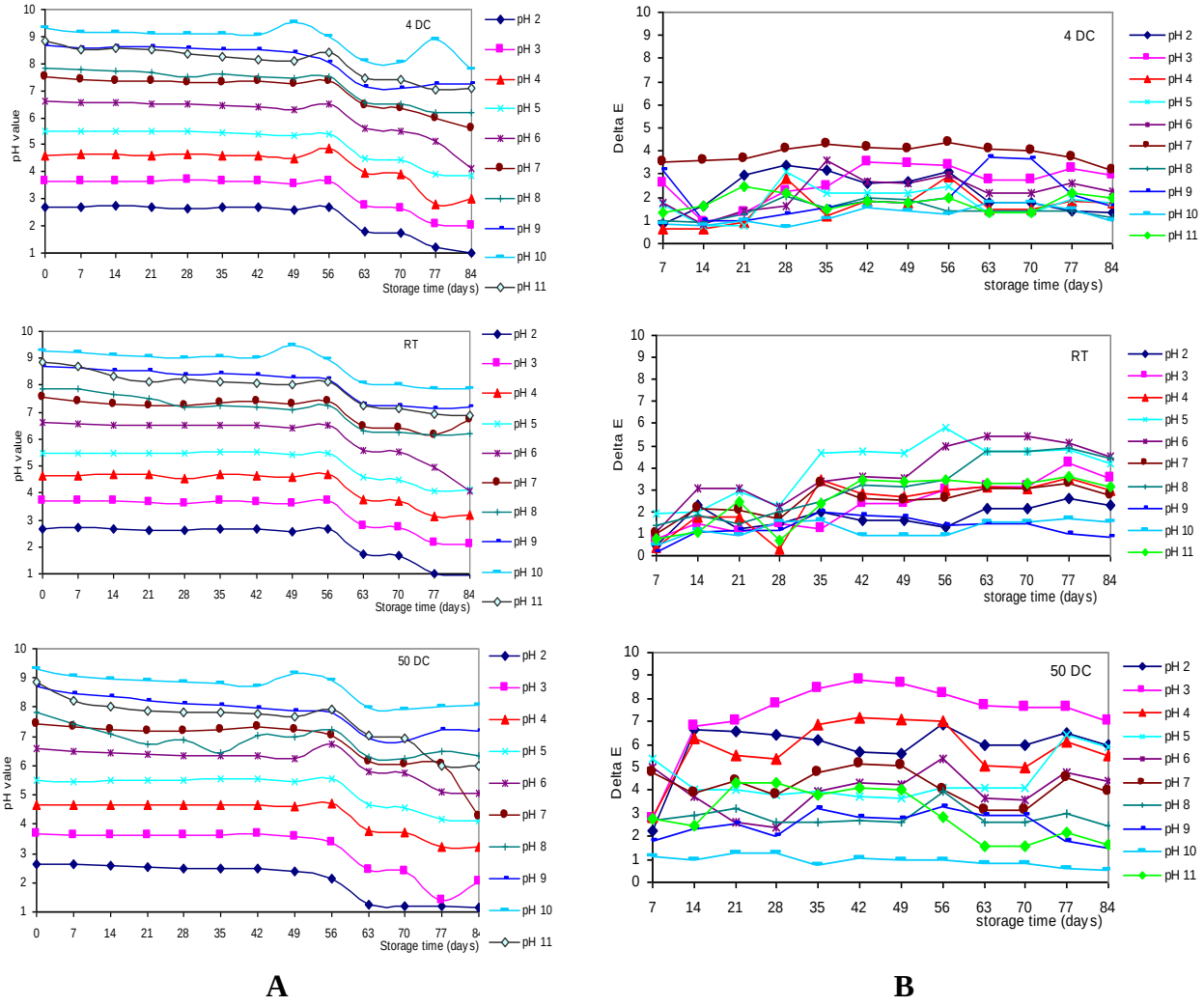

Figure 1 Stability of pH (A) and ΔE* (B) of raw betel nut seed extract during storage at 4 °C, room temperature, and 50 °C for 84 days

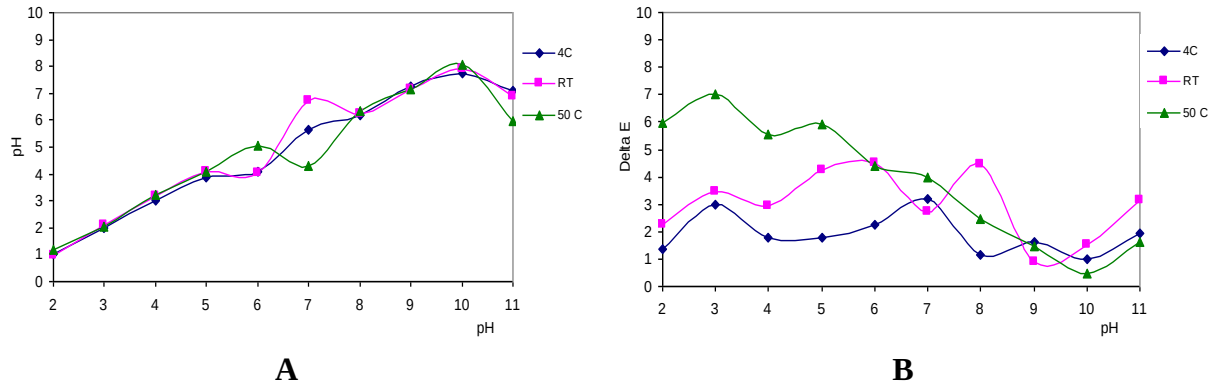

Figure 2 pH (A) and ΔE* (B) values of raw betel seed nut extract after 84 days storage at 4°C, room temperature, and 50 °C at day 84

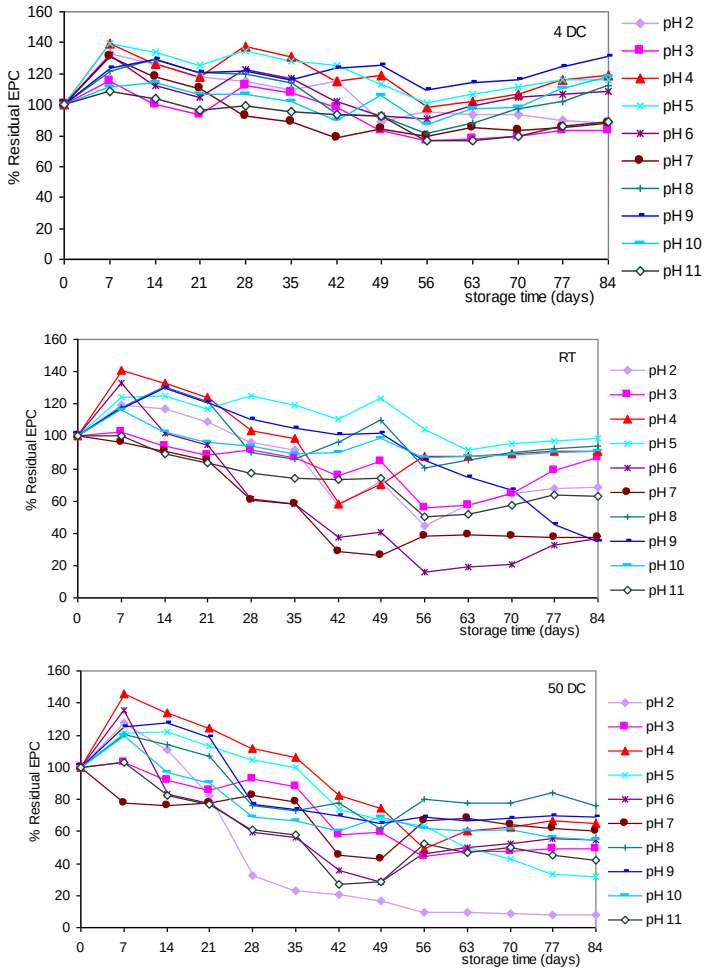

Figure 3 Percentage of residual extractable phenolic content of raw betel nut extract solutions 4 °C, room temperature, and 50 °C at 7 days interval for 84 days

Stability of extractable phenolic content (EPC)
The EPC in the extract was weekly evaluated and calculated into gallic acid equivalent then expressed as percentage of residual EPC (Figure 3). At the first 7 days storage, increment of the EPC in every condition was remarked. The EPC was the most stable at 4°C and its stability decrease when the temperature raising. After 7 days, the EPC in every pH storages were gradually decreased.

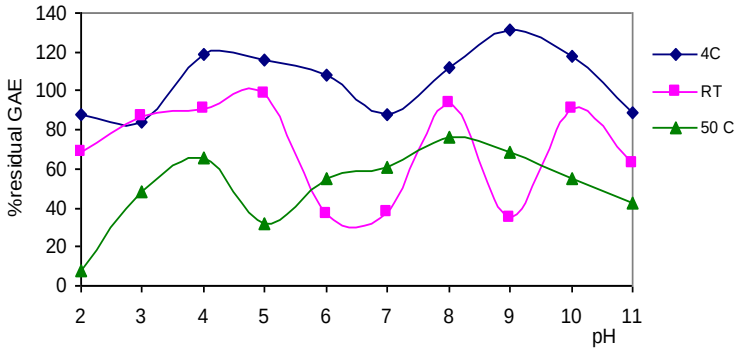

Figure 4 Percentage of residual extractable phenolic content of raw betel nut extract solutions at 4 °C, room temperature, and 50 °C at day 84

After 84 days storage, Figure 4 showed that the most stable EPC at 4°C was found in pH ranges 4-6 and 8-10. Moreover, the phenolic residual was not less than 80% in all pH solution. At room temperature, neutral pH of 6, 7, and 9 tend to possess the high degradation rate, while the rest solution retained the phenolic content more than 60%. The high temperature of 50°C resulting in the residual EPC decreased. At the most acidic condition of pH 2, phenolic content was dramatically degraded into lower than 20%. This result verified the observation from Figure 3 that the higher temperature increased the oxidation and degradation of the phenolic compounds in the extract.

Stability of cosmetic biological activities
The residual ABTS radical scavenging and tyrosinase inhibitory activity of extract at the tested conditions were showed in Figure 5. The antioxidant (Figure 5A) and tyrosinase inhibition (Figure 5B) activities of the extract were obviously found in the same trends as residual EPC implying relation between of phenolic content and radical scavenging as well as tyrosinase inhibitory activities.

After 84 days storage, the residual radical scavenging activity is illustrated in Figure 6A. It is mentioned again that higher temperature caused more instability of the antioxidant. However, it is obviously seen that the pH 4 and 9 could provide the high radical scavenging activity at all temperature tests. The low temperature of 4 °C can prolong the activity more than 80% at all pH. In contrast to tyrosinase inhibitory activity (Fig. 6B), its stability could not be prolonged even at low temperature of 4 °C.

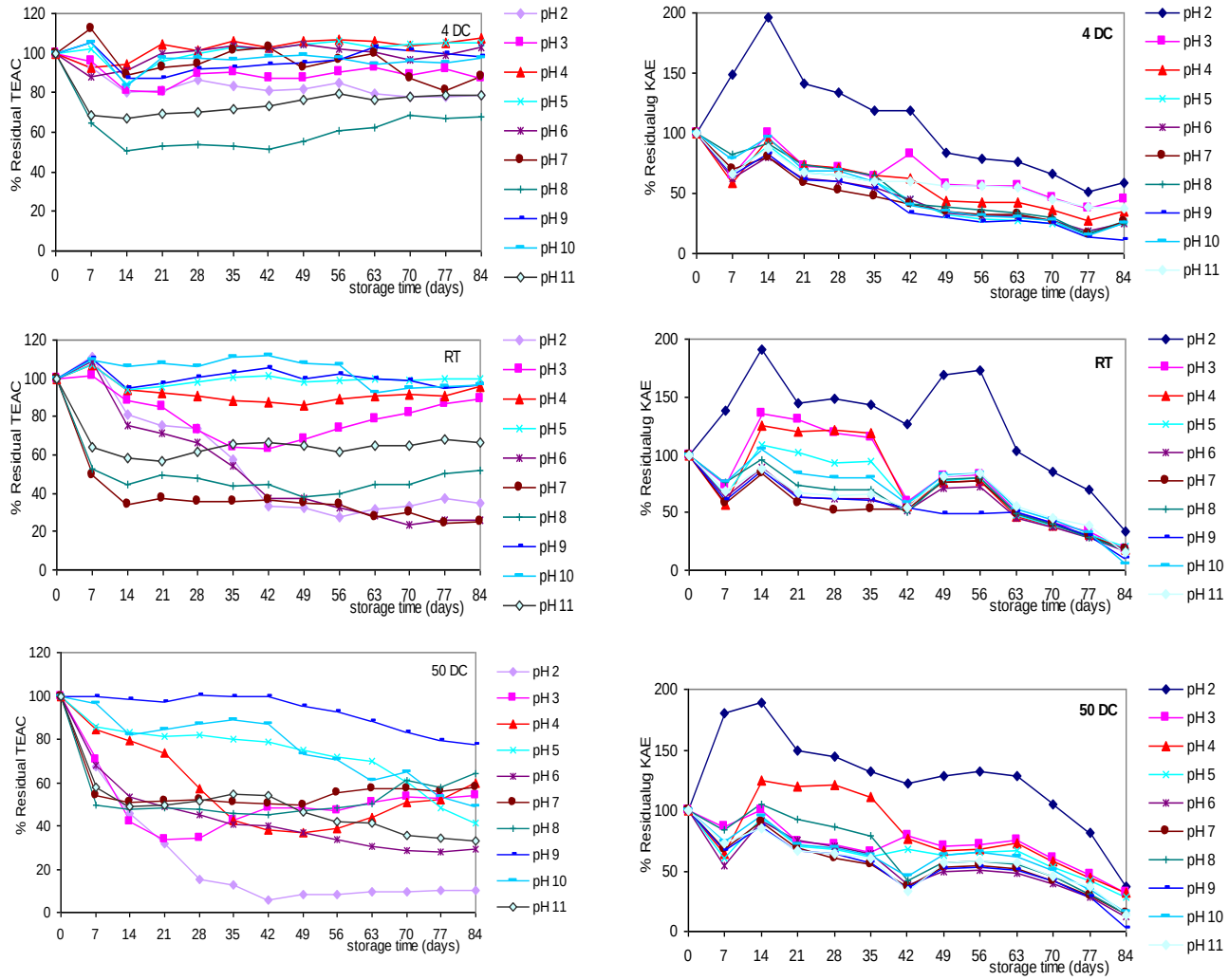

Figure 5 Residual of ABTS radical scavenging capacity (A) and tyrosinase inhibition activity (B) of raw betel nut seed extract during storage at 4 °C, room temperature and 50 °C for 84 days

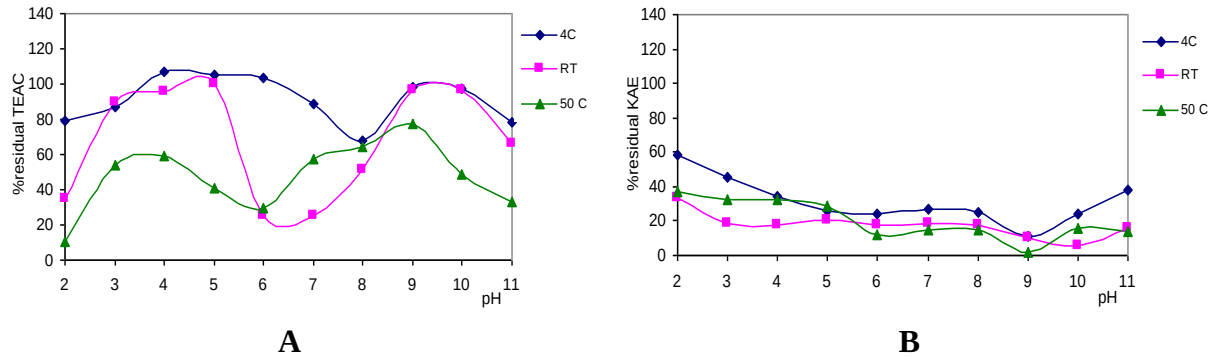

Figure 6 Remaining trolox equivalent antioxidant capacity (A) and kojic acid equivalent tyrosinase inhibition activity (B) of raw betel nut seed extract after 84 days storage at various temperatures

Discussion and Conclusion

The storage conditions of various pH and temperatures influence the maintenance of color, phenolics and biological activities of the extract. Betel nut seed extract contained high amount of phenolic compounds [5]. High temperature accelerated oxidation of these substances and subsequently darker color was observed. There has been reported that the synthetic phenolics stability found the basic-induced degradation with the irreversible structural deformation by heat [14). In contrast with the color, temperature was not influenced to the extract pH. Therefore, the pH of extract was stabilized even the temperature increase.

For the phenolic stability test, increment of the EPC in every condition was remarked at the first 7 days storage. This might be due to liberation of small phenolic compounds from complex phenolic substances in the betel nut seed extract. However, gradual decrease of the EPC after 7 days can be explained that high temperature enhanced oxidation and degradation of the phenolic compounds in the extract [14].

The different in rate of antioxidant activity degradation depended on the thermal and pH tolerance of phenolic compounds. The phenolics in the betel nut extract mainly exhibited the biological activity. Loss of the phenolics at high temperature resulted as decrease of the antioxidant and tyrosinase inhibition activities. The degradation of phenolic compounds such gallic acid and its ester in plant extract under high pH was reported to be irreversible by the possible formation of unstable quinone [14). The result in the antioxidant activity decrease was obviously related to the phenolic degradation, implying the attribution of phenolic compound to major possess the antioxidant activity. The unrelated result of tyrosinase inhibitory activity to the antioxidant activity might be due to different substances in the extract attributing for different biological activities.

The comparison of acid and base catalyzed hydrolysis on phenolics and antioxidant activities of rice extract showed the higher phenolics and flavonoids content in acidic than basic and least in neutral condition, similar with the DPPH radical scavenging activity. However, the highest ABTS and FRAP activities were found in basic condition. The results were suggested to deal with the structural hydrolysis and releasing of bound phenolic compound by either acidic or basic condition [13].

The recent study shows the stability of phenolics content and antioxidant activity in betel nut extract not only in acidic but also basic condition at any storage condition. Furthermore, the physical stabilities were acceptable with minor changes in pH and color which used in the application in the product. The results from this study are meaningful for using betel nut seed extract as the natural active ingredient in wide range cosmetics, food, and health supplement process.

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