

Color Stability Evaluation of Pigment Extracted from *Hylocereus polyrhizus*, *Clitorea ternatae* and *Pandanus amaryllifolius* as Cosmetic Colorants and Premarket Survey on Customer Acceptance on Natural Cosmetic Product

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Available online 4 May 2015

Keywords:

Hylocereus polyrhizus, *Clitorea ternatae*, *Pandanus amaryllifolius*, natural cosmetic, colorant.

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Abstract

As public concern towards the long term effect of synthetic materials used in cosmetic formulations, such as the synthetic colorant, therefore natural ingredients are getting more preferred. In this research, evaluation of potential natural colorant extracted from *H.polyrhizus*, *C.ternatae* and *P.amaryllifolius* were investigated in terms of pigments stability against heat exposure. Pigments from each sample were exposed at different temperatures (30°C, 37°C, 40°C, 50°C, 60°C, 70°C, 80°C). Color intensities were measured at 517 nm and compared with control over a week to observe color changes and absorbance readings. Heat treatments were done to investigate the suitability of pigments to be incorporated as colorant in cosmetics products (lipstick) because the production of lipstick required high temperature to melt the ingredients. Pigments from *H.polyrhizus* showed to be most stable after heat treatment in comparison to *C.ternatae* and *P.amaryllifolius*. A premarket survey was done to investigate the customer acceptance on natural vs. synthetic products. Extracts from *H. Polyrhizus* (red), *C.ternatae* (blue) and *P.amaryllifolius* (green) were used to produce natural lipstick where matching synthetic colorant were used to produce another set of lipstick. An online survey was sent to 50 respondents all over the world from the age of 16-52 years old to investigate acceptance on the natural vs. synthetic product. Ranking test (Likert Scale) and hedonistic test were used to compare the best accepted product, color and pH. The consumer acceptance was investigated using the Ranking Test (Likert Scale) and Hedonic Scale test. At the end of the investigation, natural red lipstick was being chosen as the best preferred lipstick at 80% regarding its color and pH. All statistical analysis was done by SPSS and p value <0.05 was considered as significant. The finding suggested that natural colorant (red) was more preferred in comparison to synthetic colorant in cosmetic product based on its color and pH preferences. Thus, focus should be directed towards use of *H.polyrhizus* in cosmetic products due to higher stability and customer preferences.

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1. Introduction

As far as people concern about their beauty, the product had been produced may not completely relatively safe for the consumer's health. Some side effect to the skin caused by additives and unsuitable ingredients may be seen after long term usage. Colorants or pigments are elements that play a vital role in lipstick manufacturing as it determine the aesthetic value of the lipstick. Colorants can be from either natural or synthetic sources. Natural colorants are extracted from natural sources such as plant, insects,

algae, etc, while the synthetic colorants are manufactured chemically. Synthetic colorants have been widely used in the food, pharmaceuticals and cosmetic industries. Dyes can be included by about 4-5% to 15-20% in lipstick depending on the formulation and fashion trends. Same amount of specific colorant may give different shade in different formulation as many external factors may affect the stability and activity of colorants, for example, in terms of pH value [1].

However, the safety use of synthetic colorants has been questioned hence lead to reduction in number of permitted colorants by Food and Drugs Administration (FDA). This limitation thus leads the world towards the uses of natural ingredients in production of consumer products and increased the worldwide interest in the uses of natural colorants [2]. The ancient Mesopotamian women used crushed semi-precious jewels to decorate their lips and eyes area. Meanwhile, the ancient Egyptian women too used a purplish-red dye they took from seaweed to color their lips. The famous ancient Egyptian, Cleopatra, also made her lips red by using the red color extracted from crushed carmine beetles and ants. Queen Elizabeth I also invented and popularized the black color lips. In the '30s, lipstick producers in the US produced a range of colors like light pink, dark lilac and bright red [3].

For centuries, there are many natural dyes and pigments have been used for cosmetic. The annatto (*Bixa orellana*) is commonly known as the lipstick plant due to the production of beautiful orange red carotenoid pigment from the plant. It could stain a temporary red color on human lips. Natural indigo from *Indigofera tinctoria* was used in eyeshadow and body painting. *C.ternatea*, or commonly known as butterfly pea, is a native plant in equatorial Asia regions and is a potential source of anthocyanins, which contribute to its deep blue color of the flowers [4]. The high amount of anthocyanins of the butterfly pea flowers are derived from the dephinidin [5]. It has been used as food colorant in Asia countries. For example, in Malaysia, the Malays used the flower extract to make a blue rice meal called nasi kerabu. *H.polyrhizus*, or commonly known as dragon fruit, is a member in the Cactaceae family from Latin America [6]. The deep purple color of the pulp is contributed by a set of pigments known as the betalains which are nitrogen-containing pigments [7,8], made up of the red-violet betacyanins and yellow betaxanthins [9]. *P.amaryllifolius*, or more commonly known as the pandan leaves, are an important raw material used as a green food colorant among South-East Asian countries. The green color can be produced by the pandan leaves is due to the presence of large amount of pigment known as chlorophyll. The origin of *P.amaryllifolius* is uncertain but its occurrence is widely found in cultivated ground and

household gardens across Malaysia, Sri Lanka, India and Hawaii [10]. The natural colorants *C.ternatea*, *P.Amaryllifolius* and *H.polyrhizus* have always been used as traditional food coloring agents. Therefore, this research attempted to use them as colorants for lipstick production as by evaluating their stability towards heat and light treatment for cosmetic applications.

The sales for entire worldwide cosmetic industry reached about RM533 billion ringgit Malaysia a year. It's distributed relatively even around the world with approximately RM125 billion in the Americas, RM188 billion in Europe, RM188 billion in Australia and Asia, and another RM31 billion in Africa. The make-up industry made up 20% of the total industries and the color cosmetic market represents about 15% of the cosmetic industry and that includes anything from lipstick to nail polish [11]. To maintain healthy skin, achieving a slightly acidic pH of around 5.5 is critical. Skin care products with acidic pH value, typically pH 3-6 that could help to restore natural defense mechanism in faster way as recent report of hydroxy acids (HAs) has shown to contribute an important role in cosmetic and dermatologic application [12]. Research on the nutritional analysis and stability studies of some natural and synthetic food colorant showed that samples of natural colorants has the most acidic and basic pH [13]. However, the study only focused on the comparison of natural and synthetic colorants but did not refer to the comparison of pH of the colorant in lipstick formulation. This research will therefore further investigate on the comparison of pH values of three natural colorants and three similar color-ranged synthetic colorants especially when they are incorporated into the lipstick formulation.

2. Materials and Methods

2.1. Pigment extraction

***C.ternatae*:** The fully bloomed *C.Ternatea* was harvested from Kampung Ayer Lanas, Kelantan by plucking off the sepal from the plant to get the whole flowers. The green part of the sepal were then cut and removed as the chlorophyll content of that particular part may affect the blue anthocyanins intended to be extracted from the flowers. The flower parts of *C. Ternatae* were then grinded. The grinded petals were put in a beaker and distilled water was added at ratio 1:10 (w/v) [14]. The sample was soaked and settled for

10 minutes. After that, the extract was filtered using gauze. The initial pH of the crude extract was measured using a pH meter to investigate the influence of pigment on the formulated lipstick. The filtrate was then transferred into amber bottles and clear bottles to be stored at 4°C. Amber bottles were to minimize degradation caused by the effect of UV light. Meanwhile, clear bottles were as control of the storage stability test.

H.polyrhizus: *H.polyrhizus* was obtained from the Pantai Timur Hypermarket at Tanah Merah, Kelantan and Pengkalan Chepa, Kelantan. The peel of *H.polyrhizus* was removed and only the flesh was taken for use. The flesh was cut into similarly sized small cubes using a knife and blend in the laboratory blender for 10s. 20 g of *H.polyrhizus* flesh was mixed with 30 ml distilled water into a flask and had final weight of 50 g, which was at the ratio of 2:3 (w/v) [15]. Then, the blended flesh was filtered through gauze to remove the residue. Three times of filtering was required to ensure the residues were fully removed. Initial pH was measured and the same storage conditioned was prepared as *C.ternatae*.

P.amaryllfolius: The leaves of *P.amaryllfolius* were obtained from the nearby villages at Jeli, Kelantan. The leaves were washed and chopped into small pieces and blended with distilled water at the ratio 1:4 (w/v) [16] in order to obtain the colour pigment from the plant. The leaves were chopped into small pieces and homogenized in the blender for 2 minutes. The blended juice was filtered through gauze to remove the residue. Three times of filtering required ensuring the residues were fully removed. Initial pH was measured and the same storage conditioned was prepared as *C.ternatae* and *H.polyrhizus*.

2.2. Stability test

Heat treatment: The crude extracts of *C.ternatae*, *H.polyrhizus* and *P.amaryllfolius* were exposed to different temperatures at 30°C, 37°C, 40°C, 50°C, 60°C, 70°C and 80°C respectively. Effect of different temperatures on the color intensity of the extracts was measured by reading the absorbance at 520 nm. All samples were prepared in triplicate.

Light treatment: Effect of light treatment on the crude extracts was measured by reading the absorbance at 520 nm over 7 days. Both samples exposed to light (clear bottle) vs unexposed (wrapped bottle) were kept at room temperature. All samples were prepared in triplicate.

Lipstick formulation: Both natural and synthetic lipstick produced for the pre-market survey in this study was using the formulation as in Table 1. For natural lipstick, extracts from *C.ternatae*, *H.polyrhizus* and *P.amaryllfolius* were included as the colorants and best matching synthetic colorants for the extracts, in which blue no.1 (CI 42090.2), red no.7(CI15850) and green (CI 77288) were chosen respectively to be included in the formulation. The synthetic colorants were chosen based on its best match of the naturals and may not be suitable for actual lipstick production. Raw ingredients were heated up to 80°C before mixing with the colorants. The mixture was homogenized using the WiseTis® Homogenizer until the colorant was fully dispersed. The mixture was poured into 8 ml lipstick container and it was left to cool into shape for consumer acceptance investigation. The rest of the fluid lipstick was kept for pH tests. Samples were made triplicate for all types of colorants used in each formulation to ensure accuracy and consistency in pH testing.

Table 1: Lipstick formulation

Lipstick formulation Material	Weight in gram (g)	Composition (%)
Castor Oil	33.60 g	56 %
Natural / synthetic colour	2.40 g	4 %
Beeswax	21.00 g	35 %
Glycerol	3.00 g	5 %
Total weight	60.00 g	100 %

2.3. pH test

The fluid lipstick pH was measured immediately after production. The test was carried out for 14 days at 7 days intervals by melting the lipsticks each day. The methods were repeated for all types of lipsticks produced using different colorants: *C.ternatae*, *H.polyrhizus*, *P.amaryllfolius*, synthetic blue, synthetic red and synthetic green.

2.4. Pre-market research - Consumer acceptance investigation

The method used for investigation of consumer acceptance was the Ranking test and Hedonic Scale test [17]. In order to obtain the best pigment accepted in lipstick formulation, the analysis of the Ranking test and Hedonic Scale test data were

conducted using the Scientific Package for Social Science (SPSS) software and p value of <0.05 was considered as significant.

2.5. Ranking test

A total of 6 lipstick samples were prepared. The samples were using the parameter known as P1, P2, P3, P4, P5 and P6 for pH parameter; C1, C2, C3, C4, C5 and C6 for color parameter. The Ranking test was conducted by using 50 respondents for one parameter. The best 3 color of choice was obtained for each parameter tested. Likert scales below were used for each parameter respectively

pH: 1	2	3	4	5
Well below average	Below average	Average	Above average	Well above average

Colour: 1	2	3	4	5
Not interested	Somewhat uninterested	Neutral	Somewhat interested	Interested

2.6. Hedonic Scale test

The overall consumer acceptance was examined using the Hedonic Scale test. 50 respondents were inquired in selecting the best colorant from the 3 best samples obtained in the previous Ranking test. Hedonic scale below was used:

Preferred Colour : 1	2	3	4	5
Dislike	Somewhat dislike	Neutral	Somewhat like	Like

3. Results and Discussion

Stability test (heat treatment-data was not shown as too many): Significant changes in the blue color of the heated *C. Ternatea* extracts were observed after the 4th day. The blue color eventually turned into dull color. The *C. ternatae* solution heated at low temperature showed higher color stability than that heated at higher temperature. Temperature hence affected the color stability as the aglycones are less stable and lead to the loss of anthocyanin colour due to increasing temperature that induces the loss of glycosyl moieties of anthocyanin by the hydrolysis of glycosidic bond [18]. However, loss of color for *H.polyrhizus* was observed at day 5 at all temperatures may be due

deterioration of betalain stability when it was exposed to the light. The loss of red purple tone observed during 5th day of storage explained that betacyanin in red dragon fruit is sensitive towards light and temperature. The degradation of betacyanin in red dragon fruit might follow the mechanism explained by Von Elbe [19]. This research has found that the degradation of red color after heated at 80°C has shown a more stable trend. Hence, it has potential for use in manufacturing of lipstick which requires a heat till 80°C. Significant changes in heated *P.Amaryllifolius* extracts were observed at day 5. The green color eventually turned into dull-yellowish colour afterwards. The intensity of green color from the extract has found to be influenced by pH, as lower pH will make the value of absorbance higher. Therefore, the sudden rising of absorbance value can be seen after day 5 which correspondent to the extract that has found to be decreased in pH value on day 7 onwards. Storage conditions may also influence the stability of the green extract, intensity of color and value absorbance were found decreased at room temperature, but an appreciation absorbance has been found in those stored in cold condition which, in refrigerator. The absorbance values exhibited by three colorants after heated at 37°C shown that *P.amaryllifolius* has the lowest stability towards heat. The samples were subjected to such temperature as to determine its stability towards normal human body temperature while lipstick is a product to be worn on human. At all temperatures, *H.polyrhizus* extracts showed to be the most stable.

Stability test (light treatment): The color intensity of the natural colorants has been observed for 7 days. Results revealed the *H.polyrhizus* or dragon fruit extract (DFE) has the strongest color intensity compared to *P. Amaryllifolius* or pandan extract (PDE) and *C.ternatae* or butterfly pea extract (BPE). However, the trend of the color intensities of each colorant (Figure 1) shows that the colorants stored in dark bottles has higher absorbance than that of clear bottles. Hence, light was found to accelerate color loss. Three types of colorants also had shown the tendency to degrade its intensity after day 4. *P.amaryllifolius* extract changed its color to a more yellowish color at day 5 hence the abnormal peaks observed. Meanwhile *H.polyrhizus* extracts maintained as the most stable colorant. *C.ternatae* extracts showed its constant increment of absorbance as the color gradually get darker but started to turn grey after day 5.

pH test (data not shown as too many) : The results of pH values exhibited by both types of

colorants has indicated that the pH values of that of synthetic were relatively lower than that of natural ones. The red and blue natural colorants based lipsticks exhibited relatively neutral pH in values even after weeks. The natural green colorants and its corresponding lipstick produced shown the incapability of usage in lipstick production due to its low performance in storage stability and pH stability. The relative low value of synthetic colorant based lipstick indicates the possible negative effect that may be the cause of occurrences of chapped lips and dry lips among lipstick users nowadays.

Mean Absorbance of Natural Colorants in Dark and Clear Bottles over Days

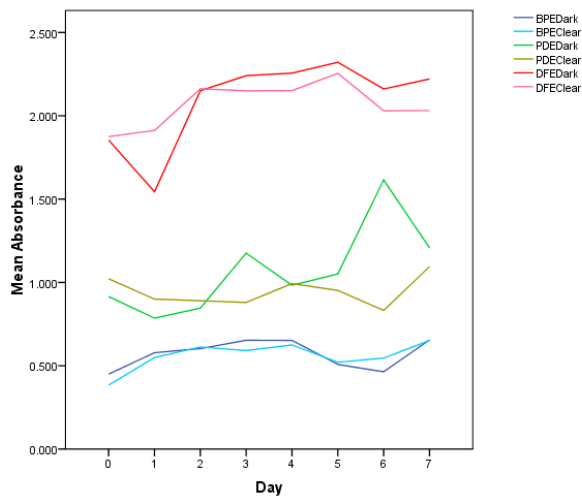


Figure 1 : Graph showing the trend of color intensity exhibited by *C. Ternatae* or butterfly pea extract (BPE), *P.amaryllifolius* or pandan extract (PDE) and *H.polyrhizus* or dragon fruit extract (DFE), stored at dark and clear bottles to observe the storage stability over a week.

Consumer Acceptance Survey: The survey was conducted via online questionnaire method. Lipstick samples were not provided to the respondents but pictures of the lipsticks produced using different types of colorants had been attached to the questionnaire for reference purpose regarding the colour preferences and pH attributed by each colorant. The respondents were all females and the age is ranged between 16 to 52 years old. Respondents of different races and from worldwide country participated in the survey. A question regarding irritating effects caused

by the lipsticks currently in-use by the consumer has been raised to investigate the effect of the lipsticks offered by the current markets to consumers. The results obtained found that there were more than 50% cases of irritation after applying the lipstick bought from current market. The findings also found that there were less than 15% of consumers concerned on the pH of the lipsticks they are using. There were more than 90% of people preferred natural lipstick rather than synthetic lipstick (Figure 2).

Ranking Test: Ranking Test showed three types of lipstick, the natural blue, natural red and synthetic red, have been selected. The mentioned three types of lipstick were chosen due to their highest score among all six samples for both pH desirability and color preference. The absence of synthetic blue in the pH chart presented (Figure 3) was due to it has not been given any votes by the involved respondents. Similar case happened to natural green, synthetic blue and synthetic green colored lipstick when it came to the preference of consumer towards the lipstick’s color (Figure 4). From that it can be seen that red was ranked higher as it is a common lipstick color known by society since long ago. Meanwhile, the green and blue colors were less preferred.

Hedonic Scale Test: From the analysis done for the Hedonic Scale Test regarding the customer acceptance of the color chosen from previous Ranking Test, where only natural blue, natural red and synthetic red colored lipsticks were being shortlisted as the options available in the Hedonic Scale test, consumers have shown their highest interest towards the preferences on the natural red lipstick (Figure 5). From the correlation drawn from the most preferred color and the most desirable pH value, the natural red colorant has shown its capability to meet the interests of consumers. However, the outcome of this study did not take account on customer’s personal or favorite color into consideration which may influence their choice. Overall, this study pointed out the most preferred color is natural red.

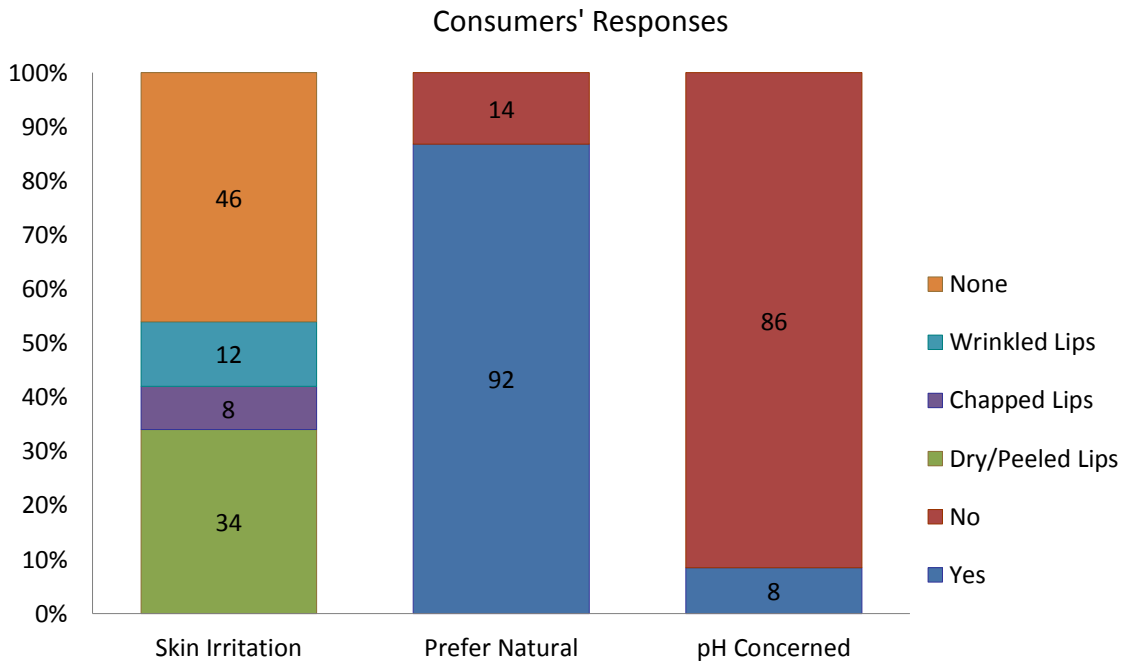


Figure 2 Consumer Acceptance Survey : (Bar chart showed the percentages of cases according to consumers' responses where the skin irritation bar inclusive of different irritation cases as indicated in the legend, while both the preference towards natural lipstick and concerns towards lipstick's pH only inclusive of the responses in terms of agreements)

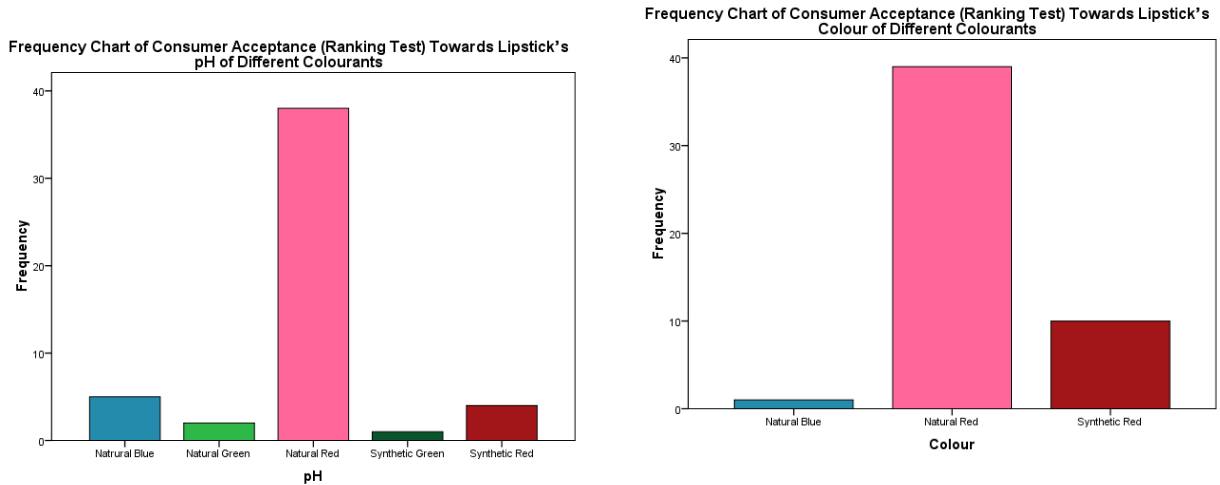


Figure 3 (Consumer Acceptance based on PH preferences): Bars showed the frequency of consumer acceptance towards Lipstick's pH of different colorants. Highest bar indicates highest ranked lipstick, where its pH is best accepted.

Figure 4 (Customer Acceptance based on Color): Bars showing mean scores of consumer acceptance towards Lipstick's colour of different colorants. Highest bar indicates highest ranked lipstick, where its colour is best accepted.

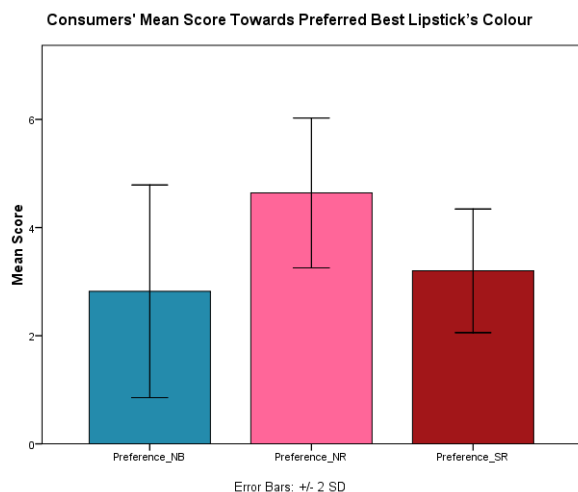


Figure 5 (Hedonistic Test): Bars show the mean score of consumer acceptance from Hedonic Scale Test towards best Lipstick's colour with natural blue (NB), natural red (NR) and synthetic red (SR) as selection options. Highest bar indicates highest ranked lipstick, where its colour is best accepted.

4. Conclusion

Light and temperature has been found to influence the degradation of the pigments. Of all three extracts, *H.polyrhizus* was shown to have high stability towards light and temperature treatment. *H.polyrhizus* extract was less affected at high temperature treatment particularly at the lipstick production temperature (80°C) and at human body temperature (37°C). Thus, *H.polyrhizus* was the most suitable extracts to be included in the formulation. The consumer acceptance revealed that the best preferred colorant with consideration of its pH for new lipstick formulation is the natural red colorant from *H.polyrhizus* extract. The synthetic colorant were less preferred based on the pH values as it is more acidic and may be the main reason of the occurrences of chapped lips and dry lips among lipstick users nowadays. Natural red also has been chosen as the most preferred color and pH of all types of lipsticks (natural and synthetic) produced in this study.

Acknowledgement

The authors would like to thank Ministry of Higher Education, Malaysia and Research Acculturation Grants (RAGs) for funding this research activity (R/RAGS/2013/00807A/00125).

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