Jurnal Teknologi

Full paper

Utilization and Evaluation of Betalain Pigment from Red Dragon Fruit (*Hylocereus Polyrhizus*) as a Natural Colorant for Lipstick

N. N. Azwanida*, Normasarah, Asrul Afandi

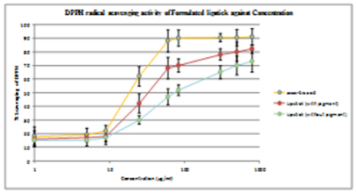
Faculty of Agro Based Industry, Universiti Malaysia Kelantan, Jeli Campus, Locked Bag No. 100, 17600 Jeli, Kelantan, Malaysia

*Corresponding author: azwanida@umk.edu.my

Article history

Received :1 January 2014
Received in revised form :
15 February 2014
Accepted :18 March 2014

Graphical abstract



Abstract

Lipstick formulations are most widely used to enhance the beauty of lips. However, current lipcare products are often caused irritation (dry lips, chapped lips and wrinkled lips) to the consumers. The use of synthetic ingredients has also raised safety concern due to adverse health effects in the long term use. The objective of this study was to formulate natural lipsticks by using betalain pigments extracted from *Hylocereuspolyrhizus* and the lipsticks were evaluated for their organoleptic properties (spreading, hardness, shine and gloss), antimicrobial and antioxidant potential. The organoleptic properties of the formulated lipsticks were found to be satisfactory in order to give attractive beauty to the lips. The antimicrobial activities of the formulated lipstick showed significant inhibition compared to commercial lipsticks and the betalain pigment was proved to have antimicrobial effect. The formulated lipstick showed to have significant antioxidant activities compared to the reference ascorbic acid. In DPPH radical scavenging assay, the IC_{50} value of the formulated lipstick was 54.29 µg/mL, whereas IC_{50} value for the reference ascorbic acid was 14.56 µg/mL. As the lipsticks were prepared using natural ingredients like dragon fruit, olive oil and vegetable fat, additional medicinal values were added to the product. Adverse health risk effects as concerned by the consumers was minimized and the product can be used without hesitation and confidence. The lipsticks with the natural ingredients like vegetable fat and olive oil is an alternative to synthetic product and serves as an economical and effective cosmoseutical product.

Keywords: Cosmetics; cosmeceutical; lipstick formulation; dragon fruit; vegetable fat; natural product

1.0 INTRODUCTION

Nowadays, the whole world is turning towards the use of traditional products and adopting more natural way of life through increased use of herbal products in their daily life. People are more interested in natural food, herbal medicines and traditional practices for healthy life. Higher demands were seen for the natural products cultivated through biological/organic farming without any used of chemicals and pesticides. Recently, herbal cosmetics are pushing through the cosmetics industry in the world and the demand for the herbal cosmetics is rising and quite significant [1].

Herb is known as a symbol of safety in contrast to synthetic products which portray negativity especially on human health. Many herbal preparations such as herbal medicines, herbal tonics, herbal pastes, herbal shampoos, herbal contraceptives and herbal lipsticks have seen great demand among the consumers. Herbal medicines in particular have become the fastest growing segment in herbal products as many positive testimonials from the consumers support the ability of the herbal medicines to heal various ailments. Currently, consumers not only care for their outer appearances but they are also aware of the importance of their health. The herbal users give priority to their health care needs, making them opt for herbal products than synthetic products [2, 3].

Lip coloring practice originated from the ancient prehistoric time. The coloring pigments came from natural resources such as crushed carmine beetle, henna and ants. Today, the practice continued and is widely accepted as an important cosmetic practice. The use of lipstick has increased with the available choice of color shades, textures, fragrance and other extra properties of today's lipstick. This can be observed from the fact that lipstick has steadily increased in market value in order to satisfy the demand from the customers particularly women [4].Recently, lipsticks have been under the attention of many health regulators because of the safety issues. A microscopic look at the ingredients that go in to the lipstick should be done because lipsticks are also unknowingly consumed by the users. Usually, the synthetic dyes used to form the color of the lipsticks are dangerous to humans for consumption. Coal tars used to form the synthetic dyes can cause allergy, nausea, dermatitis and drying of the lips. Worse, they can be carcinogenic and even fatal. Thus, this work was done to formulate herbal lipstick as an alternative

for the synthetic to minimize the side effects and help consumers to use the product with confidence.

The aim and objective of the present study were to formulate herbal lipsticks with vegetable fat, olive oil and virgin coconut oil as natural excipients that replaced conventional synthetic vehicles of lipsticks. Olive oil and virgin coconut oil help to promote moisturizing and have antibacterial effects. Olive oil and virgin coconut oil will also act as preservatives to the lipstick produced.

2.0 EXPERIMENTAL

2.1 Preparation of Lipstick

Dragon fruits were obtained from MARDI, Bachok, Kelantan. They provided the matured red *Hylocereuspolyrhizus* for our research works and all experimental works was done at the Faculty of Agro Based Industry Laboratory, University Malaysia Kelantan (UMK), Jeli campus, Kelantan.

Extraction of Colour (Betalain Pigment)

The red-fleshed dragon fruit (*Hylocereuspolyrhius*) was cut into similarly sized small cubes using a knife and blended in the laboratory blender for 30 s. The homogenised sample was firstly freeze dried in order to reduce moisture content of the sample for a more efficient extraction process [5]. The blended dragon fruit flesh was mixed with distilled water in a 500 mL flask at the ratio of 2:3 (w/v). Then, the flask was covered with aluminium foil and placed into a shaking water bath for 60 minutes at 60ºC. After that, the filtrate was concentrated by rotary evaporator for 4 hours at 40ºC to remove the solvent and followed by lyophilisation to remove the remaining water inside the extracts. The dried extract was packed in air tight glass container and stored at 4ºC for further studies.

Formulation of Lipstick

The lipstick was prepared as the method described in the literature [1, 2]. The ingredients used was tabulated in Table 1.

Table 1 Ingredients with their prescribed quantity in the formulation of lipstick

Ingredient	Quality percentage (%)	Importance
Bees wax	15	Glossy & hardness
Vegetable Fat	75	Blending properties/antioxidant
Olive oil + virgin coconut oil	5	Moisturizing/ antimicrobial
Glycerin	1	Surfactant
Colour Extract	4	Colouring agent/antioxidant
Rose essence	s.q	Fragrance

2.2 Evaluation of Lipstick

Preliminary Stability Study

The formulation developed was evaluated in the Preliminary Stability Tests which include organoleptic characteristics (color, odor and appearance) and spreadability over a minimum of three days at room temperature (24.0 ± 3.0ºC) and oven temperature (40.0 ± 2.0ºC). The oven condition was used in highest temperature for stability study as lipstick usually undergoes softening and deformation at temperatures over 50ºC [6]. From this formulation, no organoleptic or spreadability changes were observed.

Normal Stability Study

The Stability Test of a lipstick usually begins 48 hours after formulation [6]. A quantity of 350 g of the formulation was prepared for the Normal Stability Test, in which the organoleptic characteristics (color, odor, appearance), spreadability and melting point were evaluated for 60 days under the conditions of Room Temperature (RT, 24.0 ± 3.0ºC) and Oven (O, 40.0 ± 2.0ºC) [6]. The samples were stored as indicated in duplicates. The formulation was stored at room temperature (24.0 ± 3.0ºC) for 48 hours and then evaluated at baseline (t_0). It was then stored under different conditions in accordance to the Normal Stability Study, and characteristics was assessed on the 3rd, 7th, 15th, 30th and 60th days. Assessments at t_0 were considered as a reference to compare the results.

Melting Point

To determine the melting point, the material was made molten to fill the capillaries (duplicate). The capillaries were coupled to a system with a thermometer and immersed in a vial with water at a controlled temperature. The melting point was assumed at the temperature at which melting of the lipstick sample was first observed [6].

Organoleptic Characteristics

Color and appearance were characterized visually with a loupe of 10x magnification while the odour was compared by the evaluator. The characteristic described below were developed by the evaluator to determine organoleptic criteria. Samples were analysed twice during the predetermined time for each condition, and compared with a freshly prepared formulation (t_o):

N – Normal;
M – Modified;
IM - Intensely Modified.

Test of Spreadability

The test of spreadability consist of applying the product (at room temperature of (24.0 ± 3.0ºC) repeatedly onto a glass slide to visually observe the uniformity in the formation of the protective layer and whether the stick fragmented, deformed or broke during application. For this test, the following criteria below were developed by the analyst:

G - Good: uniform, does not leave fragments; perfect application, without deformation of the lipstick;
I - Intermediate: uniform; leaves few fragments; appropriate application; little deformation of the lipstick;
B - Bad: not uniform; leaves many fragments; difficult or inappropriate application, intense deformation of the lipstick.

Antioxidant Potential

The DPPH radical scavenging activity was calculated according to literature [7]. 1 mL of a DPPH solution (100 mM in methanol: water (50% v/v) was mixed with 1 mL of diluted 1mg/ml sample. The reaction mixture was incubated in the dark for 20 min, and the optical density of the samples was recorded at 517 nm against

the blank (methanol: water, (50% v/v)). For the control, 1 mL of the DPPH solution was mixed with 1 mL of methanol: water (50% v/v) and the absorbance of the solution were recorded after 20 min. The ability to scavenge DPPH radical was calculated using the following equation:

$$\%IP = [\frac{(Ao-A1)}{Ao}]x100(1)$$

Where A1 = absorbance of the test sample and Ao = absorbance of control. Each assay was carried out in triplicates.

Antibacterial Activity

The formulated lipstick with dragon fruit colour extract added was investigated for its antimicrobial activity as well as formulated lipstick without colour extract. The water soluble antibiotic, gentamicin sulfate (10 µg) was used as positive control. Sterile nutrient agar was inoculated with the test organism under sterile condition and then poured into sterile petri dishes. A sterile cork borer was used to remove four plugs from each agar plates to produce 8 mm diameter wells. A 100 µl of formulated lipstick was added to each well and allowed to diffuse at room temperature for 20 minutes and the plates were incubated overnight at 37ºC. Test sample was tested against each organism in triplicate. The calculated of agar diffusion technique was used as zone of inhibition. The recorded diameter of inhibition zones of growth measured in millimetres is presented in Table 3.

■3.0 RESULTS AND DISCUSSION

Preliminary Study of Stability

The formulated lipstick showed appropriate organoleptic characteristics (color red, rose fragrance, uniform aspect) and solidified at the temperature of 45°C. It was observed that there were no sweating, bleeding, streaking and blooming after three days of observation when the product was stored in room temperature (24ºC) and oven temperature (40ºC).

Normal Stability Test

The results for Normal stability test were summarized in Table 2. Color, odor, appearance, spreadability and melting point of the formulation were evaluated for 60 days. Melting point remained stable throughout the stability test and had a mean of 66.0± 0.5ºC.

The color of the formulation was considered stable under room temperature, but there were changes under the oven condition, from red to darker red after one month of storage, classified as modified in Table 2. Therefore, it was evident that this formulation of lipstick was not stable after being subjected to a temperature of 40.0 ± 2.0ºC as the base mostly consist of vegetable fat.

The odor characteristic of rose essence remained stable throughout the 60 days of testing under all conditions evaluated. The visual aspect was considered uniform under the room temperature, while under oven conditions, the presence of some white spots (fat bloom) was observed on the surface of the lipstick from the third evaluation day onwards. The fat bloom phenomenon resulted in a whitish appearance on the surface of the product. This phenomenon is related to the crystalline formation and a phase separation of the triglycerides within the crystalline structure of the vegetable fat. The liquid fraction of fat within the matrix migrated to the surface and recrystallized into a whitish spots [8].

The spreadability of the formulation at room temperature was considered good, as they showed smooth uniformity upon application without deforming of the lipstick. Under the oven condition, the lipstick material showed inconsistency such as rough application, impaired spreadability and deforming the product during the test. Furthermore, storage in the oven (40.0 ± 2.0°C) caused loss of product functionality. However, the formulations stored under this condition were found to return to baseline characteristics after a few hours at room temperature.

Table 2 Evaluation of organoleptic characteristics, spreadability and melting point of lipstick formulation

		Storage condition									
		Room temperature					Oven temperature				
		Time (days)									
Parameters*	t_0	3^0	7^0	15^0	30^0	60^0	3^0	7^0	15^0	30^0	60^0
Aspect	Uniform	N	N	N	N	N	N	IM	IM	IM	IM
Colour	Red	N	N	N	N	N	N	N	N	M	M
Odor	Rose	N	N	N	N	N	N	N	N	N	N
Spreadability	G	G	G	G	G	G	G	I	I	I	I
MP**(^{0}C) ±	65±0.0	65.5	68.8	64.8	64.8	66.1	69.0	63.8	63.5	67.0	65.5
CV***		0.5	0.3	0.3	0.7	0.7	0.5	1.8	0.5	0.1	0.5

*All parameters were evaluated in duplicate
t_0: Preparation of the formulation after 48h
Aspect, Color and Odor: **N** – Normal; **M** – Modified; **IM** – Intensely modified;
Spreadability: **G** – Good; **I** – Intermediate; **B** – Bad;
**MP - Melting Point: mean of two determinations.
***CV – Coefficient of variation

DPPH Radical Scavenging Activity Assay

In DPPH radical scavenging assay, as shown in Figure 1, the formulated lipstick solution exhibited a concentration dependent antiradical activity. The formulated lipstick showed significant antioxidant potential because of the properties of olive oil and virgin coconut oil, which are known for their high antioxidant properties. However, the antioxidant potential was increased after the addition of the betalian pigment. The result suggested that the pigment itself also exhibit the DPPH radical scavenging activity and has antioxidant properties.

The IC_{50} value of the formulated lipstick (with pigment added) was 54.29 ± 1.9 µg/ml and IC_{50} value of the formulated lipstick (without pigment added) was 76.31±0.43µg/mLwhile the IC_{50} value for the reference ascorbic acid was 14.56± 0.24 µg/mL. The DPPH antioxidant assay is based on the ability of 1, 1-diphenyl-2-picryl-hydrazyl (DPPH), a stable free radical, todecolorize in the presence of antioxidants [9]. The method is basedon the reduction of $DPPH^-$ solution by ahydrogen donating antioxidant forming a non-radicalform of DPPH-H. The samples were able to reduce DPPH radical (visible deep purple color) to the yellow-coloured diphenyl picryl hydrazine. It has been found that cysteine, glutathione, ascorbic acid, α-tocopherol, polyhydroxyaromatic compounds (e.g. hydroquinone, pyrogallol, gallic acid), and aromaticamines (e.g. p-phenylenediamine, p-aminophenol) reduce and decolorize 1,1-diphenyl-2-picrylhydrazyl through their hydrogen donating ability [10]. Therefore, one of the possible mechanism the formulated lipstick (with pigment) has better antioxidant capacity might be attributed to good amount of phenolic compounds in the pigment extract of dragon fruit. The pigments play an important role in absorbing and neutralizing free radicals by redox reaction.

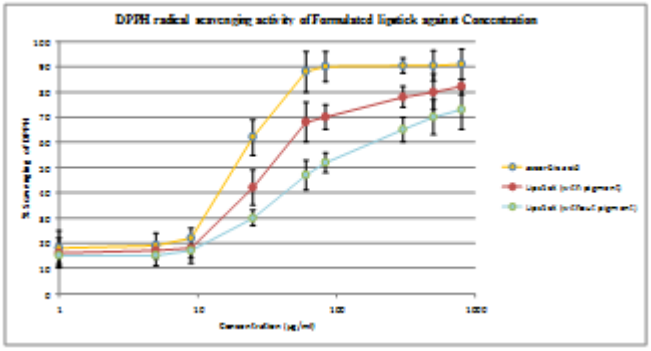


Figure 1 DPPH radical scavenging activity of Formulated lipstick (without pigment), Formulated Lipstick (with pigment) along with standard Ascorbic acid (Mean ± SD,n=3). Concentrations are displayed on logarithmic scales.

Antimicrobial Activity Test

For the antimicrobial test, the results indicate that the formulated lipstick has significantly comparable antimicrobial activity against the well-known microorganisms that causes skin diseases namely *Salmonella aureus, Pseudomonas aeraginosa and Klebseilla*. The data also showed that there are significant antimicrobial effect of pigment when the formulated lipstick (without pigment) were compared with the formulated lipstick (with pigment) ($p<0.05$).

The pigment extracts containedphenolic compounds [11] which possess high levels ofantimicrobial activity [12], e.g.carvacrol, oxygenated derivatives (thymol methylether) and its precursors;p-cymene andγ –terpinene[13].The mechanism of the phenolic compounds focused onthe bacterial cellular membranes, altering theirfunction and in some instances their structure,causing swelling and increase their permeability.The increase in cytoplasmic membrane permeability appeared to be a consequence of the loss ofthe cellular pH gradient, decreased ATP levels,and the loss of the proton motive force, causing death to the microorganisms.

Table 3 Antimicrobial activity of formulated lipstick

Microorganism	Zone of inhibition*			
	Formulated Lipstick(with pigment added)	Formulated Lipstick (without pigment added)	Commercial product	Gentamicin (Antibiotic)
Salmonella aureus	16.0 ± 0.1	11.0 ± 0.1	0.0	22.0 +0.1
Pseudomonas aeraginosa	16.1 ± 0.2	11.1 ± 0.2	0.0	22.1 + 0.2
Klebseilla	14.2 ± 0.2	9.2 ± 0.2	0.0	21.2 +0.2

* Mean diameter ± SD (mm), (n=3), $p<0.05$ between column.

■4.0 CONCLUSION

The combination of natural ingredients such as vegetable fat, oliveoil and virgin coconut oil as blending agent and vehicle produces a good uniform product. This formulation would increase customer acceptance because of the alternative natural ingredients in the formulation substituting chemical ingredients like isopropyl myristate and lanolin that may cause darkening of lips. The formulated lipstick also offers excellent properties of spreadability, smoothness, covering and stable when stored under room temperature. Additional properties of antioxidant and antimicrobials properties added extra market values to the product as a cosmoseutical product.

Acknowledgement

The authors would like to thank Ministry of Higher Education, Malaysia and Research Acculturation Grants (RAGs) for funding this research activity.

References

[1] Abhijeet, A. A., Shripad, M. B., Preeti, T. K., Swapnil, S. D.and Pradeep, K. N. 2012. Formulation and Evaluation of Herbal Lipstick from Colour Pigment of Bixa Orellana (Bixaceae) Seeds. *International Journal of Pharmacy and Pharmaceutical Sciences*. 4(5): 357–359.

[2] Dwivedi, S., Dwivedi, S. N. and Patel, P. C. 2009. Formulation Evaluation and Antimicrobial Activity of Herbal Lipstick. *Recent Adv in Prosp and Potent of Medi Plants*. 4(1): 39–43.

[3] Muanda, N. F., Dicko, A. and Soulimani, R. 2010. Chemical Composition and Biological Activities of Ficus Capensis Leaves Extracts. *Journal of Natural Products.* 3(1): 147–160.

[4] Romanowski, P. 2011. A Cosmetic Industry Overview for Cosmetic Chemists. Retrieved March 10, 2013 from http://chemistscorner.com/a-cosmetic-market-overview-for cosmetic-chemists.

[5] Lim, Y. Y., Lim, T. T. and Tee, J. J. 2007. Antioxidant Properties of Several Tropical Fruits: A Comparative Study. *Food Chemistry.* 103: 1003–1008.

[6] Alessandra, R. F., Michelli, F. D., Claudinéia, A. S. O., Telma, M .K., André, R. B. and Maria, V. R. V. 2013. Stability Evaluation of Organic Lip Balm. *Brazilian Journal of Pharmaceutical Sciences.* 49(2): 293–299.

[7] Muanda, N. F., Dicko, A. and Soulimani, R. 2010. Chemical Composition and Biological Activities of Ficus Capensis Leaves Extracts. *Journal of Natural Products*. 3: 147–160.

[8] Foubert, I., Cauteren, I.V., Dewettinck, K. and Huyghebaert, A. Differential Scanning Calorimetry as a Means Of Predicting Chocolate Fat-Blooming. In: Widlak, N., Hartel, R., Narine, S. (Eds.). Crystallization and Solidification Properties of Lipids. United States of American: AOCS Press, 2001. 6: 79–86.

[9] Kumarasamy, Y., Byres, M., Cox, P.J., Jaspars, M., Nahar, L. and Sarker, S. D. 2007. Screening Seeds of Some Scottish Plants for Free-Radical Scavenging Activity. *Phytother Res.* 21: 615–21.

[10] Blois, M. S. 1958. Antioxidant Determinations by the Use of a Stable Free Radical. *Nature.* 181: 1199–1200.

[11] Wu, L., Hsu, H. W., Chen, Y. C., Chiu, C. C., Lin, Y. I. and Ho, J. A. 2006. Antioxidant and Antiproliferative Activities of Red Pitaya. *Food Chem.* 95: 319–327.

[12] Baydar, H., Sagdic, O., Ozkan, G. andKaradogan, T. 2004. Antibacterial Activity and Composition of Essential Oils from Origanum, *Thymbra* And *Satureja* Species With Commercial Importance In Turkey. *Food Control.* 15: 169–172.

[13] Skočibušić, M., Bezić, N. and Dunkić V. 2006. Phytochemical Composition and Antimicrobial Activities of the Essential Oils from *Satureja subspicata* Vis. growing in Croatia. *Food Chemistry.* 96: 20–28.