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# ANTIBACTERIAL PROPERTIES OF CRUDE AQUEOUS *Hylocereus polyrhizus* PEEL EXTRACTS IN LIPSTICK FORMULATION AGAINST GRAM-POSITIVE AND NEGATIVE BACTERIA

ASRUL AFANDI[1], AZWAN MAT LAZIM[1*], AZWANIDA, N.N.[2,3], MUMTAZ ABU BAKAR[1], OTHMAN B. AIRIANAH[4] and SHAZRUL FAZRY[4]

[1]*School of Chemical Sciences and Food Technology, Faculty of Science and Technology, University Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia*
[2]*Faculty of Agro Based Industry, Universiti Malaysia Kelantan, Jeli Campus, Locked Bag No. 100, 17600 Jeli, Kelantan, Malaysia*
[3]*School of Agriculture, Food and Rural Development (AFRD), Newcastle University, Newcastle Upon Tyne, United Kingdom*
[4]*School of Biosciences and Technology, Faculty of Science and Technology, University Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia*
[*]*Email: azwanlazim@ukm.edu.my*

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## ABSTRACT

Development of natural formulated lipstick using natural bio resources namely plant pigments and essential oils was carried out as an alternative to chemical-based ingredients due to their side effects and damages to health. *Hylocereus polyrhizus* (Costa Rican Pitahaya, also more popularly known among Malaysian as "Dragon Fruit") peels are normally treated as wastes and will be discarded during processing. In this study, the peel extracts were used in the lipstick formulation as a natural colorant. The antibacterial activity of the formulation against six pathogens was evaluated using disc diffusion method and broth micro-dilution method. The observation from disc diffusion method showed that extracts and formulated lipstick from *H. polyrhizus* were able to inhibit the growth of *Escherichia coli, Klebsiella pneumonia, Salmonella typhimurium, Staphylococcus aureus,* and *Enterococcus faecalis*. The observation from the minimum inhibitory concentration (MIC) showed that all extracts inhibit the growth of bacteria in the range of 1.00-7.00 mg/mL for all bacteria while their minimum bactericidal concentrations (MBC) indicated double of the MIC concentration except for *B. cereus*, which shows very high resistance toward the extract. Even though there is no clear trend indicating which bacteria were sensitive most to the extract, it can be concluded that the water extract of *H. polyrhizus* peel showed potent antibacterial activity. Essential oils from *H. polyrhizus* flower and the combination of virgin coconut and olive oil that were added to the products for flavors and aroma have the potential as preservative due to their antibacterial properties. With the incorporation of natural ingredients, the lipstick is considered as a safe and attractive product, with multi-functional uses namely to prevent chapped lips, freshen the breath, reduces mouth odor and contributes in improving general health quality.

**Key words:** *Hylocereus polyrhizus,* pigment, antibacterial, disc diffusion test and minimum inhibitory concentration

## INTRODUCTION

Natural cosmetics have become a major trend in recent years as global awareness on the long term harmful effects of synthetic material is increasing among consumers. Of the many cosmetic products available in the market, lip care products are essential daily cosmetic used by consumers. Lipstick is one of the leading lip care products available in variety of colors, designs and textures. It is composed mainly of an oil-wax base, stiff enough to form a stick with a staining dye dissolved or dispersed in oil, flavored, molded and enclosed in a case. According to Dwivedi (2009), lipstick formulations are most widely used to enhance the beauty of lips and to add a glamorous touch to the

* To whom correspondence should be addressed.

cosmetics. However, current demand for lip care product not only focuses on the aesthetic value but preferably having added medicinal value to the lip. As a consequence, medicated lipstick with active medicinal ingredients emerged in the market. Other than providing moisture and emollient action to prevent cracking and chapping of the lips, medicated lipstick may provide protection against bacterial infections as a result of the active ingredient in the formulation (Aher *et al.*, 2012). Hence, the main objective of this project is to determine the antibacterial properties of lipstick formulated from *H. polyrhizus* extracts as colorant.

*H. polyrhizus* or known as dragon fruit is from the Cactaceae family and has become a subject of interest to many researchers mainly due to its unique taste, shape and color which can be utilized as a natural colorant. Initially, scarce research was done on this plant until the last decade, in which ample amount of articles were published on this plant. Originated from Latin America, this cactus like plant has acclimatized and is widely cultivated in Malaysia, Vietnam, China, Taiwan, Okinawa and Southern China (Mizrahi, 2014). There are three genus of this Cactaceae depending on the number of its ribbed shoots. *Hylocereus* is a three-ribbed shoot, while *Epiphyllum* and *Selenicereus* genus are the two-ribbed and four to five-ribbed shoot respectively. Other than being eaten as a whole fruit, *H. polyrhizus* is utilized as a food colorant agent due to its aesthetic red color. The red color in *H. polyrhizus* is contributed by the betalain compounds (Azeredo, 2009).

Hydrophillic betalain consists of two sub-groups; betacyanins (red-violet) and betaxanthins (yellow-orange) that have nitrogen in the structure (Strack *et al.*, 2003). It was once thought that betalains were related to anthocyanins (i.e. a flavonoid derivative) as the color hues were quite alike. However, anthocyanins and betalains are structurally and chemically different. Betalains are more relatively stable to heat and over broad pH from 3-7 compared to anthocyanins (Azeredo, 2009; Stintzing & Carle, 2004). Nevertheless, both compounds have the ability to act as a screen against UV light (Stintzing & Carle, 2004). Additionally, betalains compounds not only have antimicrobial and antiviral properties but possess the ability to inhibit lipid peroxidation, cyclooxygenase (COX-1 and COX-2) enzymes and human tumor cells proliferation (Reddy *et al.*, 2005; Strack *et al.*, 2003).

With an optimal extraction temperature of 100°C (Harivaindaran *et al.*, 2008), the crude extract of betalain pigments are potentially suitable to be incorporated in lipstick formulation that requires temperature of more than 60°C during the production process (Aher *et al.*, 2012; Bhise & Shaikh, 2008). Our research group had previously demonstrated the ability of lipstick formulation using *H. polyrhizus* flesh extracts to inhibit the growth of *Salmonella aureus, Pseudomonas aeraginosa* and *Klebseilla* (Azwanida *et al.*, 2014). In this present study, *H. polyrhiruz* peel was used instead of the flesh in the formulation. The formulated lipstick was evaluated against six human pathogenic bacteria from gram-negative (*Escherichia coli, Klebsiella pneumonia* and *Salmonella typhimurium*) and gram-positive (*Bacillus cereus, Staphylococcus aureus* and *Enterococcus faecalis*) bacteria using disc diffusion and broth macrodilution methods. Positive outcomes from the study could maximize the utilization of *H. polyhizus* peel rather than being discarded as domestic waste.

## MATERIALS AND METHODS

### Sample preparation

*H. polyrhizus* sample were obtained from Malaysian Research and Development Institute (MARDI) farm located in Bachok, Kelantan and deposited in Faculty of Agro-based Industry (FIAT), Universiti Malaysia Kelantan, where all experiments were conducted. The fruits were rinsed with distilled water, dried with paper towel and hand peeled. The peel was then freeze-dried using a freeze dryer (LabGear, Australia) and was ground into powder using a domestic blender (Khind, Malaysia). The sample was stored at -20°C until further use.

### Extraction process

The extraction of the *H. polyrhizus* peel powder was conducted according previous method with some modifications (Siddhuraju & Manian 2007). 50 grams of *H. polyrhizus* powder was extracted using distilled water at a ratio of (2:3 w/v). The extractions were carried out in an Innova 4000 incubator shaker (New Brunswick Scientific, New Jersey, USA) at 30°C for 2 hours. Then the solution was filtered using Whatman No. 4 cellulose filter paper. The filtrates were then concentrated by rotary-evaporator (Büchi, Flawil, Switzerland) at 40°C. For the antibacterial study, the dried extracts were dissolved in 5% dimethyl sulfoxide (DMSO).

### Formulation of Lipstick

Formulation for lipstick was based on herbal lipstick formulation by Aher *et al.* (2012). The ingredients used are tabulated in Table 1.

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

| Ingredient | Quality percentage (%) | Importance |
|---|---|---|
| Beeswax | 15 | Glossiness & hardness |
| Vegetable Fat | 75 | Blending properties/antioxidant |
| Olive oil + virgin coconut oil | 5 | Moisturizing/ antimicrobial |
| Glycerin | 1 | Surfactant |
| Colour Extract | 4 | Colouring agent/antioxidant |
| Essential oil from flower | s.q | Fragrance |

**Antibacterial activity**

**Disc diffusion assay**

Kirby-Bauer disc diffusion susceptibility test suggests the susceptibility or resistance of the pathogenic bacteria against antimicrobial compounds and the absence of growth around the disk is an indirect measure of the inhibitory ability of the compounds (Parekh & Chanda, 2010; Hudzicki, 2013). The disc diffusion assay was employed according to Clinical and Laboratory Standards Institute Protocol (CLSI 2009) to observe the inhibitory spectrum of the extracts against six pathogenic bacteria namely *Escherichia coli, Klebsiella pneumonia* and *Salmonella typhimurium* for gram-negative bacteria and *Bacillus cereus, Staphylococcus aureus* and *Enterococcus faecalis* for gram-positive bacteria. Five well-isolated colonies of each bacterial strain were picked from an overnight plate culture and inoculated into saline suspension at room temperature. The turbidity was then adjusted to 0.5 McFarland standards (108 CFU/mL) and streaked onto Mueller-Hinton agar plates using sterile cotton swabs. Whatman No. 1 filter paper discs of 6mm diameter were impregnated with 25 µL of the samples (20 mg/mL). Chloramphenicol disc (30µg/disc) was used as positive control. Extraction solvents (distilled water) were used as negative controls. The plates were incubated at 37°C for 24 hours. The screening of antibacterial activity was assessed based on the diameter of the clear zone surrounding the paper disc (including the disc diameter) in millimeter (mm). The tests were conducted in triplicates and repeated for three times.

**Minimum inhibitory concentration (MIC)**

The determination of MIC and minimum bactericidal concentration (MBC) values for each sample were done by using broth microdilution method recommended by the Clinical and Laboratory Standards Institute Protocol (CLSI 2009). A sterile 96-wells flat-bottomed microplate was filled with 100 µL of inoculum and 100 µL sample of different concentrations. It was then transferred to each microplate well. Serial dilutions of the extract were carried out in 0.1% dimethyl sulfoxide (DMSO) which had no inhibitory activity against test microorganism. The final volume in the well was adjusted at 100 µL with the concentration 100 mg/mL. Serial dilutions were made to obtain concentration ranging from 0.1 mg/mL to 100 mg/mL. The control used included blank containing bacteria, chloramphenicol as the positive control while 0.1% dimethyl sulfoxide as the negative controls. The microplates were incubated at 37°C from 18 h to 24 h. Bacterial growth was indicated by the presence of broth turbidity in the microplate wells. MTT [3-(4.5-dimethylthiazol-2-yl)-2.5-diphenyltetrazolium bromide] was used as color indicator in order to ensure the occurrence of color changes (color being changed from yellow to dark blue). The MIC values were taken as the lowest concentration of the extract in the well of the microplate that showed no turbidity after incubation (Sule *et al.*, 2012).

**Minimum bactericidal concentration (MBC)**

The incubation of bacteria after being treating with samples from the MIC studies which did not show any growth of bacteria after incubation period were sub-cultured on the surface of the fresh agar plates and incubated at 37°C for 24 h. The MBC values were recorded as the lowest concentration of samples that did not permit any visible bacteria colony growth on the agar plate after the period of incubation (Rodríguez-Tudela *et al.*, 2001).

**Statistical analysis**

All analyses were performed in triplicate and data reported as mean±standard deviation (SD). Data were subjected to analysis of variance (ANOVA). Results were processed by Excel (Microsoft Office 2007) and SPSS Version 17.0 (SPSS Inc., Chicago, IL, USA).

## RESULTS AND DISCUSSION

**Disc diffusion assay**

Results for disc diffusion assay were depicted in Table 2. Among those selected bacteria, gram-negative *E. coli* and gram-positive *S. aureus* showed

**Table 2.** Antibacterial properties of *H. polyrhizus* peel extracts and formulated lipstick using disc diffusion method

| Bacterial strain | Sample | Mean diameter of inhibition zone (mm) | | |
|---|---|---|---|---|
| | | Sample concentration (0.5mg/disc) | Positive control (Chloramphenicol) 30µg/disc | Negative control |
| **Gram-negative bacteria** | | | | |
| *Escherichia coli* | PE | 7.0±0.12 | 8.0±0.33 | NA |
| | PE+FL | 9.0±0.16 | | NA |
| *Klebsiella pneumoniae* | PE | 10.0±0.27 | 28.0±0.27 | NA |
| | PE+FL | 13.0±0.32 | | NA |
| *Salmonella typhimurium* | PE | 9.0±0.09 | 28.0±0.25 | NA |
| | PE+FL | 12.0±0.18 | | NA |
| **Gram-positive bacteria** | | | | |
| *Bacillus cereus* | PE | NA | 28.0±0.34 | NA |
| | PE+FL | 12.0±0.11 | | NA |
| *Staphylococcus aureus* | PE | 16.0±0.14 | 9.0±0.25 | NA |
| | PE+FL | 19.0±0.22 | | NA |
| *Enterococcus faecalis* | PE | 13.0±0.07 | 28.0±0.22 | NA |
| | PE+FL | 15.0±0.25 | | NA |

NA=Non Active, PE=*H. polyrhizus* peel extract, FL=formulated lipstick.
Numbers indicate the mean diameters of inhibition of triplicate experiments ±SD in millimeters.

to be less sensitive chloramphenicol with 8.0mm and 9.0mm inhibition zone respectively compared to other strains with 28.0mm inhibition zone. Inhibition zones were observed for *H. polyrhizus* peel extract against *E. coli, K. pneumonia, S. typhimurium, S. aureus,* and *E. faecalis*, which are 7.0±0.12mm, 10.0±0.27mm, 9.0±0.09mm, 16.0±0.14mm, 13.0±0.07mm, respectively. Interestingly, *B. cereus* is not inhibited by *H. polyrhizus* peel extract. The lipstick formulation was observed to inhibit all bacteria that were tested (*E. coli* (9.0±0.16mm)*, K. pneumonia* (13.0±0.32mm)*, S. typhimurium* (12.0±0.18mm)*, S. aureus* (19.0±0.22mm) *and E. faecalis* (15.0±0.25mm)) including *B. cereus* (12.0±0.11mm).

Overall antimicrobial properties for *H. polyrhizus* peel were higher when in formulation than in crude aqueous alone. This might due to the used of beeswax in the formulation, which has been known to very effective as antimicrobe; especially against *S. aureus, Salmonella enterica*, *Candida albicans* and *Aspergillus niger* (Fratini *et al.*, 2016).

**Minimum inhibitory concentration (MIC)**

MIC is defined as the lowest concentration of the extract to inhibit the growth of the bacteria at the end of 24 hours incubation (Preuss *et al.*, 2005). The broth micro-dilution method using the 96 well microplates was conducted to determine the minimum inhibitory concentration of *H. polyrhizus* peel extracts (PE) and within lipstick formulation (PE + FL). MIC data as in Table 3 were coherent with disc diffusion assay. *B. cereus* indeed needed higher concentration in order to exhibit inhibitory activity and MIC *H. polyrhizus* peel extracts against *E. coli, S. typhimurium* and *E. faecalis* were lower than in disc diffusion assay, suggesting the possible high potency of the extracts. In the present study, there is no definite trend being observed on the inhibition between gram-negative bacteria or gram-positive bacteria.

**Minimum bactericidal concentration (MBC)**

Both diffusion assay and MIC portrayed the ability of *H. polyrhizus* peel extracts to inhibit microbial growth. In contrast, MBC is defined as the lowest concentration of an antibacterial agents needed to kill the microorganisms after 48 hours incubation (Preuss *et al.*, 2005). The MBC were found to be double of the MIC concentration except for *B. cereus*, where it requires more than 100.00 mg/mL for *H. polyrhizus* peel extract (PE) and 60 mg/mL for formulated lipstick (PE+FL) to be completely killed. According to the result obtained as in Table 4, formulated lipstick (PE +FL) was found to exhibit better activity as compared to *H. polyrhizus* peel extract. This could be observed on the inhibition of *S. aureus*, *K. pneumonia* and *B. cereus* where *H. polyrhizus* peel extract (PE), which required double doses to inhibit the bacteria.

**Table 3.** Minimum inhibitory concentration (MIC) of *H. polyrhizus* peel extracts alone and within the formulation

| Bacterial Strain | MIC (mg/mL) of sample | | Chloramphenicol (µg/mL) |
|---|---|---|---|
| | PE | PE + FL | |
| *Escherichia coli* | 2.0 | 1.5 | 30.0 |
| *Klebsiella pneumoniae* | 5.0 | 2.0 | 8.0 |
| *Salmonella typhimurium* | 2.0 | 1.5 | 8.0 |
| *Bacillus cereus* | 7.0 | 4.0 | 4.0 |
| *Staphylococcus aureus* | 5.0 | 2.0 | 30.0 |
| *Enterococcus faecalis* | 2.0 | 1.0 | 8.0 |

PE=dragon fruit peel extract, FL=formulated lipstick.
Numbers indicate the mean values of inhibition of triplicate experiments (n=3) at mg/mL.

**Table 4.** Minimum bactericidal concentration (MBC) of *H. polyrhizus* peel extracts and formulated lipstick

| Bacterial Strain | MBC (mg/mL) of sample | | Chloramphenicol (µg/mL) |
|---|---|---|---|
| | PE | PE +FL | |
| *Escherichia coli* | 3.0 | 2.0 | 40.0 |
| *Klebsiella pneumoniae* | 10.0 | 5.0 | 10.0 |
| *Salmonella typhimurium* | 3.0 | 2.0 | 10.0 |
| *Bacillus cereus* | 100.0 | 60.0 | 5.0 |
| *Staphylococcus aureus* | 20.0 | 10.0 | 40.0 |
| *Enterococcus faecalis* | 3.0 | 2.0 | 10.0 |

PE=dragon fruit peel extract, FL=formulated lipstick.
Numbers indicate the mean values of inhibition of triplicate experiments (n=3) at mg/mL.

Current use of *H. polyrhizus* peel extracts in the lipstick formulation is very promising. Antioxidant studies on pomegranate peel extracts confirmed that the total phenolic compounds of pomegranate peel extracts was found 10 times higher than the pulp extracts with 25 times higher metal chelating property of antioxidant (Li *et al.*, 2006). Hence, higher antioxidant property in *H. polyrhizus* might also provide protection against microbial growth. Negi *et al.* (2008) has suggested that phenolics might be one of the responsible compounds that contribute to its high antimicrobial activity. The antibacterial activity of plant extracts may be due to the capability of bioactive compounds to form a complex with extracellular and soluble proteins, inhibiting enzyme activity and also affecting bacteria cell walls (Cowan, 1999).

## CONCLUSION

The application of natural ingredients such as vegetable fat, olive oil and virgin coconut oil in normal lipstick shows a good uniform product with antimicrobial properties. In this present research, *H. polyrhizus* peel extracts formulation exhibited high antimicrobial properties against all gram-positive bacteria. Good antimicrobial activities were also seen against gram-negative bacteria when the extract was within the formulation. Hence, this present study believes that current natural formulation would increase customer acceptance as all the ingredients were natural with an additional property of antimicrobial activity. Utilization of *H. polyrhizus* peels would also be maximized as raw materials in cosmetic industry rather than being discarded as waste materials.

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