A STUDY ON SEPARATION, ANTIBACTERIAL, ANTIOXIDANT AND PHYTOCHEMICAL ANALYSIS OF BETALAIN PIGMENT FROM RED DRAGON AND PRICKLY PEAR FRUIT AND USED AS A NATURAL COLORANT FOR LIPSTICK

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ABSTRACT :

Synthetic dyes were mostly utilised in the production of commercial lipsticks. However, the usage of synthetic dye has been associated with many health problems. Natural colorant might offer a promising solution to this problem. The betalains which are found in Red dragon fruits (*Hylocereus polyrhizus*) and Prickly Pear (*Opuntia stricta*) have drawn attention as a potential source of natural colorant to synthetic dye. The Phytochemical and Antioxidant activity were performed for the extracts of *Hylocereus polyrhizus* and *Opuntia stricta*. The Antibacterial activity was done against *E. coli, Pseudomonas aeruginosa* and *Staphylococcus aureus*. The purpose of this study was to formulate lipsticks with betalains. In this research, Betalains was extracted from flesh of red dragon fruit and Prickly bear fruit using ethanol at ratio of 1: 3 and the concentrated betalains extract were used for the production of Lipstick. The betalains pigment were then used to formulate lipsticks with basic lipstick formula and the formulated lipsticks were store under light or dark condition. The physico-chemical properties of the synthesised lipstick such as spreadability, skin irritation test, surface anomalies, melting point and colour were determined. This study has proven that betalains could replace synthetic dye in cosmetics industry for lipstick application.

KEYWORDS : Lipstick, Betalains, *Hylocereus polyrhizus, Opuntia stricta* .

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 .

Herb is known as a symbol of safety in contrast to synthetic products which portray negativity especially on human health. Currently, consumers not only care for their outer appearances but they are also aware of the their health. The herbal users give priority to their health careneeds, making them opt for herbal products than synthetic lipsticks. (N. N. Azwanida*et.al.*,2014).

Health Defects from Commercial lipsticks :

In this modern time and age, lipstick has diversified to moisturising, glossy, matte, sheer, satin, frosted, andpearl, just to mention but a few. The characteristic sign of lipsticks is mainly their colour, which is a result of the pigmentation drawn from minerals or organics, for instance, from lead, cadmium, chromium, cobalt, and nickel, among others. Lipsticks that contain dyes are obtained from lead, suggests that the metallic properties in these lipsticks could also be attributable to their process of manufacture by the machines used. Lipsticks present in the market sometimes contain the heavy metals mentioned above, which are harmfulto consumers in the long run. The average woman issaid to ingest up to 4.5 pounds of lipstick in a life time since wearing lipstick; there are possibilities of licking, drinking or eating the lipstick ; hence accumulation of heavy metals in the body. Lead accumulation causes disorders of the centralnervous system, reproductive system (infertility and miscarriage), hepatic and renal system, and decreasedlearning and hearing. Furthermore, cadmium is associated withatherosclerosis, hypertension, and cardiovascular diseases. In addition, cadmiumand lead are carcinogenic in nature (WHO, 2004). (Rose Obat*et.al.*,2022).

Betalains as Alternate Dye for Lipstick Production :

Betalains are the plant pigments. Its purple-red colour is due to the presence of betalain pigments. Betalains are water-soluble nitrogenous pigments and are divided into two classes: red-violet betacyanin and yellow betaxanthin, which provide beet roots with their typical colour. The chemical structure betalains have no toxic effects on the human body and are seen as a natural and safe alternative to synthetic red dyes.Natural pigments such as betalains may therefore become increasingly used in food products. Betalains are pigments of about 17 families of plants belonging to the order Caryophyllales. Dietary betalains may play an important role in

maintaining human health because of their many beneficial activities. There are numerous data on the health effects of betalains.

For natural source, we selected the betalain contained *Hylocereus polyrhizus* fruits and *Opuntia stricta* fruits.

Hylocereus Polyrhizus :

The red pitaya is known as a red dragon fruit *(Hylocereus polyrhizus*). It is one of the tropical fruits in the cactus family, Cactaceae. Dragon fruit plants were first discovered in Mexico and then have been developed mostly in tropical countries such as Malaysia, the Philippines, and Indonesia. In general, there are four types commonly found in Indonesian markets, i.e., red dragon fruit (*H. polyrhizus*) and white dragon fruit (*Hylocereus undatus*).

Opuntia Stricta :

Opuntia stricta is a species of large cactus that is endemic to the subtropical and tropical coastal areas of the America, especially around the Caribbean. Common names include erect prickly pear and nopal estricto .It producing lemon yellow flowers in the spring and summer, followed by purplish-red fruits. It is quick to colonize hot, open environments with sandy soils.

MATERIALS AND METHODS :

Sample Collection :

The *Hylocereus polyrhizus* fruits (Red dragon fruits) were purchased from the markets of Pondichery. The *Opuntia stricta* fruits (Prickly Pear) were collected from the region of Kalakad, Tirunelveli district. The Fruits were taken to the laboratory as soon as they were collected.

Extraction and Purification of Betalains from *Hylocereus polyrhizus* and *Opuntia stricta* :

Fresh Red dragon fruits (*Hylocereus polyrhizus*) and fresh Prickly pear fruits (*Opuntia stricta*) were collected and washed with tap water to remove the dirt and dust on the surface of the fruits. Then, both the fruits were cut and the flesh was blended for 30 seconds in a blender.After that, the blended flesh was homogenized at a ratio of 1:3 with ethanol (95 % v/v). The solution was kept for 15 min for betalains to leach out from the red dragon fruit juice and prickly pear fruit juice. After that, the fruit juices were sieved separately to remove the seeds that found in the fruits. The solutions obtained were stored in two separate containers in a dark place.

Phytochemical Activity :

To identify the chemical constituents of fruit extracts by standard procedures have been followed. The crude extracts were qualitatively tested for the presence of chemical constituents using the following reagents and chemicals.

Test for Terpenoids :

Chloroform test:To 5 ml of the extract few drops of chloroform and concentrated H2SO4 was added carefully along the sides of the test tube. Formation of brown color at interface was a positive indicator.

Test for Anthraquinones :

0.5 ml of extracts were boiled in 10% hydrochloric acid and filtered hot. To this, 2 ml chloroform and 10% ammonia solution each were added .Formation of pink color in the aqueous layer indicated presence of anthraquinones.

Test for Saponins :

Foam test:To 1 ml of the extracts 5 ml distilled water was added and shaken vigorously. Formation of foam indicated presence of saponins.

Test for Tannins :

Lead acetate test:The sample were treated with 10% lead acetate solution; appearance of white precipitate indicated the presence of tannins. When the extract was treated with aqueous bromine solution, appearance of white precipitate indicated the presence of tannins.

Test for Phenols :

Ferric chloride test:A fraction of the extracts were treated with 5% ferric chloride solution and observed for the formation of deep blue or black colour.

Test for Flavanoids :

Shinoda test:Sample extracts were treated with 5 ml of 95% ethanol; few drops of concentrated Hydrochloric acid and 0.5g of magnesium turnings were also added. Pink colour was observed.

Test for Steroids :

Salkowski's test:Sample (2 ml) was mixed with 2 ml of concentrated Sulphuric acid, it was well shaken then chloroform layer appeared red and acid layer shown greenish yellow fluorescence.

Test for Alkaloids :

Mayer's test:Sample (2-3 ml) was treated with few drops of Mayer's reagent. Appearance of white precipitate indicated the presence of alkaloids.

Antibacterial Activity - Well Diffusion Method :

Well diffusion assay was carried out to check the antibacterial activity of *Hylocereus polyrhizus* extract and *Opuntia stricta* extract against *Staphylococcus aureus*, *Escherichia coli*,*Pseudomonas aeruginosa*. The sterile cotton swab was then dipped into the inoculum and the surface of the agar plate was swabbed. 5mm diameter hollow tube was used to prepare the wells on each plate, and then the fruit extracts were added into the respective wells. Four wells were made, one is for *Hylocereus polyrhizus* fruit extract, second well is for *Opuntia stricta* fruit extract, the third well is for positive control (Streptomycin antibiotic) and the last well is for negative control (DMSO). The plates were then incubated for 18-24 hr at 37℃ in an incubator.

Antioxidant Activity :

Chemicals and reagents:

2,2-Diphenyl-1-picrylhydrazyl (DPPH) and ascorbic acid was purchased from Sigma-Aldrich.Phosphate buffer (pH- 7.4) and Methanol. **A**ll the chemicals and solvents used were of analytical grade.

DPPH free radical scavenging activity:

DPPH solution (0.004 %), sample extracts and standard (vitamin C) was prepared in methanol. Sample extract and standard (vitamin C) solution were prepared in different concentrations 20, 40, 60, 80 and 100 µg/ml. 0.5ml of different concentrations of standard solution or sample extracts was taken in different test tubes and then 0.5 ml of DPPH (0.004 %) solution was added and kept in dark for 30 min. and absorbance was recorded at 517 nm. The decrease in absorbance of the DPPH radical caused by antioxidant was due to the scavenging of the radical by hydrogen donation. It was visually noticeable as a colour change from purple to yellow. (Majo ., 2008) The percentage inhibition activity was calculated using the formulae below:

$$\% \text{ DPPH free radical scavenging} = \frac{(\text{Absorbance of control} - \text{Absorbance of test})}{\text{Absorbance of control}} \times 100$$

Preparation Of Lipstick :

The formulation of *Hylocereus polyrhizus* and *Opuntia stricta* lipsticks with betalains pigment was based on the formula informed below.

Table 1: Ingredients for Lipstick Formulation

Ingredients	Composition Per Lipstick	Importance
Bees wax	0.32 g	Provide hardness and glossy.
Carnauba wax	0.15 g	Provide hardness.
Candelilla wax	0.38 g	Provide hardness.
Shea Butter	1 g	Used as emollient.
Castor oil	1.4 g	Aid in blending.
Jojoba oil	0.5 g	Used as moisturizer.
Sweet Almond Oil	0.5 g	Used as moisturizer.
Fragrance	Few Drops	Used to provide fragrance.
Vit E	Few Drops	Used as anti – oxidant.
Betalains Extract	5 ml	Used as natural colorant.

Formulation Of Lipstick :

The betalains extracts were mixed with swemond oil and jojoba oil in a small beaker (beaker A) and stir continuously to ensure the pigment well dispersed in the mixture. All waxes, shea butter, and castor oil were measured in another small beaker (beaker B) and heated in water bath at 80°C with gentle stirring until beeswax melts completely.Both mixtures were then mixed together with spatula in water bath until uniform colour was achieved. Then, the mixture was

remove from heat source. A few drops of vitamin E and fragrance oil were added to the mixture and the mixture was stirred again to ensure evenly blending of all ingredients.

A little amount of oil was applied on the surface of the lipstick mould to ease the process of removing lipstick from the mould. After blending vitamin E and fragrance oil into the lipstick formula, the mixture was poured into lipstick mould. The mould was chilled immediately in the fridge for 15 minutes to solidify the lipstick mixture. When demoulding, the lipstick were lift upwards carefully. At this point, the lipstick was done. The betalains lipsticks were stored at room temperature with the storage concept under light or dark condition.

Evaluation of Lipstick :

The formulated lipsticks were tested with its spreadability, melting point, surface anomalies and skin irritation test.

Spreadability Test :

Spreadability test were conducted for the lipsticks at room temperature. The lipsticks were applied at least 3 cm onto a paper in order to visually observe the smoothness and uniformity of a protective layer formation from the lipsticks.

Melting Point :

The melting point of lipsticks were determined by heating lipsticks using double boiler method in water bath. A thermometer was immersed in the water bath to determine to melting point of the lipsticks. The melting point was determined by the thermometer at the moment when the lipsticks started to melt.

Surface Anomalies :

Surface anomalies analysis was used to determine the surface defect by visual observation. Surface defects such as crystal and fungi formation, wrinkles formation indicated that the lipsticks were contaminated.

Skin Irritation Test:

The lipsticks were applied on skin and left for 5 minutes. The lipsticks were tested with few respondents with skin conditions.

Colour : The colour of the prepared lipsticks were analysed.

RESULT AND DISCUSSION :

Table 2. Results for Phytochemical activity of *Hylocereus polyrhizus* and *Opuntia stricta*

PHYTOCHEMICAL ANALYSIS	*HYLOCEREUS POLYRHIZUS*	*OPUNTIA STRICTA*
TERPENOIDS	POSITIVE (Presence of Brown Colour)	POSITIVE (Presence of Brown Colour)
ANTHRAQUINONES	POSITIVE (Presence of Pink colour in aqueous layer)	POSITIVE (Presence of Pink colour in aqueous layer)
SAPONINS	POSITIVE (Presence of Foam)	POSITIVE (Presence of Foam)
TANNINS	POSITIVE (Presence of White Precipitate)	POSITIVE(Presence of White Precipitate)
PHENOLS	POSITIVE (Presence of Black colour)	POSITIVE (Presence of Black colour)
FLAVANOIDS	POSITIVE (Presence of Pink colour)	NEGATIVE (Absence of Pink colour)
STEROIDS	NEGATIVE (Absence of Greenish yellow fluroscence)	NEGATIVE (Absence of Greenish yellow fluroscence)
ALKALOIDS	NEGATIVE (Absence of White precipitate)	NEGATIVE (Absence of White precipitate)

In the present study, Both the extracts show positive results for Terpenoids, Anthraquinones, Tannins, and Phenols.For the test of Flavanoids, *Hylocereus polyrhizus* shows a positive result but *Opuntia stricta* shows a negative result. Both the extracts shows a negative result for Saponins, Steroids and Alkaloids.

Antibacterial activity – Well diffusion assay:

After incubation of Muller Hinton agar plate,zone of inhibition was shown by *Hylocereus polyrhizus* fruit extract and *Opuntia stricta* fruit extract against *E.coli,Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Table 3. Antibacterial activity of *Hylocereus polyrhizus* and *Opuntia stricta*

Test Bacterial Strains	**Zone of inhibition (mm)**			
	Hylocereus polyrhizus	***Opuntia stricta***	**Positive Control (Streptomycin)**	**Negative control (DMSO)**
Escherichia coli	2mm	nil	9mm	nil
Staphylococcus aureus	5mm	3mm	8mm	nil
Pseudomonas aeruginosa	4 mm	3mm	8mm	nil

In the present study, the antimicrobial activity was analysed in both the fruit extracts of *Hylocereus polyrhizus* and *Opuntia stricta.* The fruit extracts shows more inhibition against *Pseudomonas aeruginosa* and *Staphylococcus aureus*than *Escherichia coli.*

Antioxidant Activity :

Table 4.Antioxidant effect of sample-1(*Hylocerus polyrhizus*) on DPPH

Treatment	**Dose (µg/ml)**	**Absorbance @517 nm**	**% activity against DPPH radicals**
Control	---	DPPH control= 0.993	---
Vit C	100	0.188	80.36
Sample 1	20	0.645	35.04
Sample 1	40	0.482	51.46
Sample 1	60	0.320	67.07
Sample 1	80	0.252	73.92
Sample 1	100	0.196	80.26

Table 5. Antioxidant effect of sample-2 (*Opuntia stricta*) on DPPH

Treatment	**Dose(µg/ml)**	**Absorbance @ 517 nm**	**% activity against DPPH radicals**
Control	---	DPPH control = 0.993	---
Vit C	100	0.188	80.36

Sample 2	20	0.680	30.82
Sample 2	40	0.545	44.41
Sample 2	60	0.452	53.78
Sample 2	80	0.368	62.24
Sample 2	100	0.295	69.59

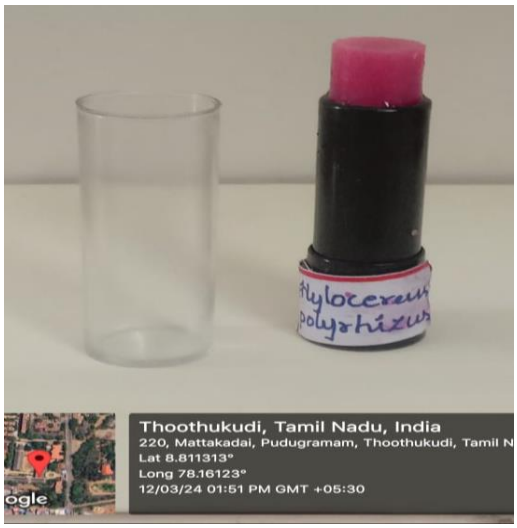
The antioxidant activity of *Hylocereus polyrhizus* and *Opuntia stricta*were analysed. *Hylocereus polyrhizus* shows high antioxidant activity than *Opuntia stricta.*

Evaluation Parameters :

Table 6: Evaluation of Lipstick

EVALUATION PARAMETERS	**RESULTS**
Spreadability	Excellent
Melting point (℃)	Above 60 ℃
Surface Anomalies	No
Skin Irritation	No
Colour	Dark Pinkish colour

In the present study, The spreadability test all lipsticks were excellent as no fragment upon spreading with no deformation of lipstick was observed. The melting point of a lipsticks indicates the safe limit of storage. The standard melting point of a commercial lipstick is above 50 ℃ in order to remain its rigid structure and do not melt in room temperature. For surface anomalies test, no fungi and crystallization were detected in the surface of lipsticks. Lastly, the synthesized lipsticks were applied on skin of the hands for 5 minutes to test for skin irritation. The results showing no skin irritation was observed as all ingredients used in the formulation are natural ingredients.



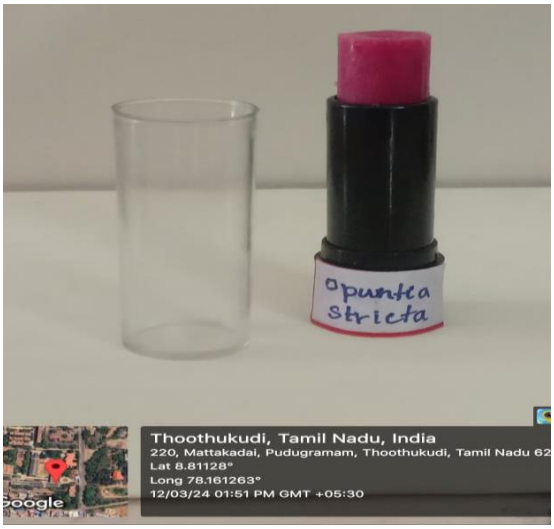


Figure1:*Hylocereus polyrhizus* Lipstick

Figure 2: *Opuntia stricta* Lipstick

CONCLUSION :

In the comparison of *Hylocereus polyrhizus* and *Opuntia stricta, H.polyrhizus* shows a great inhibitory action against microorganisms like E.coli, *Staphylococcus aureus* and *Pseudomonas aeruginosa. H.polyrhizus* also have a good antioxidant potential with various phytochemical constituents like terpenoids, anthroquinons, tannins, phenols, flavonoids and alkaloids. For the production of Lipstick, various tests were conducted like spreadability test, Melting point, Surface Anomalies and Skin Irritation Test. Thus, I conclude that both the *Hylocereus polyrhizus* and *Opuntia stricta* fruits are the good source for the production of lipsticks.

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