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Development of Wound Gauze from *Hylocereus undatus* Dragon Fruit (Pigment) Peel Extract

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ABSTRACT: The dragon fruit peels (*Hylocereus undatus*) are typically thrown away as agricultural waste. The disposal of bio-wastes, like peels from dragon fruit and microbiological contamination during their mass breakdown, all contribute to environmental degradation. Peel can be used to extract naturally occurring colour for pigments. The objective of this study is to investigate the feasibility of using the peel of *Hylocereus undatus* as a coating material for wound healing. The extraction method and potential uses of the pigments derived from the peel will be explored. The study aimed to determine the phytochemical components of *Hylocereus undatus* (peel) extract and the peel can be utilized as a raw material for pigment extraction due to the betalain content. With the use of a magnetic stirrer extraction, the peel of Hylocereus undatus was extracted with aqueous extraction. Thin layer chromatographic techniques are used to identify the pigment components. In the present study, we recorded both UV-VIS and FT-IR profile of peel extract to known the various phytoconstituents and determine the functional group present in *Hylocereus undatus* (white dragon fruit). The pigment needs to be applied to the wound gauze, and the stain resistance should be evaluated by determining how well the wound gauze can withstand contamination from the outside coating. Furthermore, the pharmaceutical and nutraceutical sectors are interested in *Hylocereus undatus peel* due to its components, which may have potential health applications such as anti-inflammatory, antioxidant, antibacterial, and anti-cancer characteristics.

KEYWORDS: anti-inflammatory, antioxidant, *Hylocereus undatus* peel, wound gauze.

INTRODUCTION

Dragon fruit(*Hylocereus undatus)*, is a tropical cactus vine cultivated in Southeast Asia. Varieties include those *Hylocereus undatus* with pink or red peel, black seeds, and white flesh; *Hylocereus costaricensis* with pink or red peel, black seeds, and red flesh; *Hylocereus megalanthus* with yellow peel, black seeds, and white flesh; and *Hylocereus polyrhizus* with red peel and red flesh. Dragon fruit is rich in nutrients like fiber and magnesium, with low calories. The fruit peel, particularly from *Hylocereus undatus*, is gaining attention for its antioxidants and potential health benefits, including anti-cancer properties. Studies suggest dragon fruit consumption may aid in diabetes prevention and cardiovascular health, while neutralizing toxins. Its bioactive compounds show various health-promoting properties, including anti-inflammatory and anticancer effects (1). Improper disposal of dragon fruit wastes, like the peel and rind, can harm the environment. These wastes account for 30 to 45 percent of the fruit's bulk and are often discarded as agricultural industry waste. Slow decomposition of these wastes leads to environmental contamination, attracting pests and posing health risks. Incineration of dragon fruit peels produces air pollutants and eliminates potentially valuable chemicals. Repurposing dragon fruit peel into a valuable by-product can generate revenue and provide a sustainable solution to this ecological issue. Researchers are exploring the wound-healing properties of compounds derived from dragon fruit peels, aiming to utilize this agricultural waste effectively (2).

Cotton gauze is widely used in wound care for its absorbency, clot-promoting ability, and capacity to maintain a moist healing environment. It acts as a protective barrier against contaminants, aiding wound cleanliness and healing. This gauze plays a crucial role in wound care by providing protection, moisture balance, tissue regeneration support, medication delivery, and patient comfort. Combining white dragon fruit peel with wound gauze enhances wound healing benefits, offering a protective barrier and reducing infection risk. It's advisable to use biodegradable, eco-friendly cotton gauze for wound dressings.

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Dragon fruit peels yield extracts used in wound healing. The fruit's skin, often discarded, contains pigments called betalains, providing red colour. Betalains include betaxanthin (for yellow-orange) and betacyanine (for purple-red). Water extraction maintains stability, crucial due to sensitivity to factors like temperature and light. *Hylocereus undatus* peel also holds flavonoids and polyphenols, with antibacterial and antioxidant properties, along with betalains (3).

The pigment from *Hylocereus undatus* peel, a type of dragon fruit, Is a biocompatible natural colorant for wound gauze, potentially with antimicrobial properties. Studies show antibacterial qualities and bioactive components in the peel extract, suitable for wound treatment. This natural colorant meets demand for sustainable wound care, replacing synthetic dyes that may cause allergies. Using agricultural waste like dragon fruit peel supports circular economy and sustainable resource management, promoting wound healing. (4).

2. MATERIALS AND METHODS

2.1 SAMPLE COLLECTION OF FRUITS

Dragon fruit (*Hylocereus undatus*) was purchased from the local market of Coimbatore, Tamil Nadu, India (Latitude 11.0113834, Longitude 76.9966748). The fruits were cleaned and first stripped of their peel and were diced into small pieces. The peel pieces were collected and dried under direct exposure to sunlight and oven-drying methods. The dried peel was taken and stored. Then the dried peel was ground into powder using the blenders.

2.2 EXTRACTION OF PIGMENT

26 grams of powdered extract was taken and mixed with 1000ml of distilled water. All these were kept in a magnetic stirrer at 50°C. The obtained samples were filtered by using what's man filter paper and a funnel. The pigment was extracted and stored in the refrigerator at 4°C. (5).

2.3 QUALITATIVE ANALYSIS OF PHYTOCHEMICALS

Aqueous extract of Hylocereus undatus was analyzed for the presence of various phytoconstituents like Tannins, Flavonoids, Alkaloids, Saponins, Terpenoids, Glycosides, Protein, steroids, phenol, quinone and carbohydrates was tested. (6)

2.3.1 TEST FOR TANNINS:

To identify the presence of tannins about 1ml of Dragon fruit peel extract and about 3 ml of bromine water are added, the disappearance of color indicates the presence of tannins

2.3.2 TEST FOR ALKALOIDS:

To identify the presence of alkaloids about 1ml of Dragon fruit peel extract and 1ml of Sodium hydroxide are added, the formation of a yellow color indicates the presence of alkaloids.

2.3.3 TEST FOR SAPONINS:

To identify the presence of saponins about 1 ml of extract and 3 ml of water are added and shaken well, the formation of foam after 10 minutes indicates the presence of saponins.

2.3.4 TEST FOR TERPENOIDS:

In this test with 1 ml of extract 2 ml of chloroform 1 ml of acetic acid then 2 ml of sulfuric acid, the presence of red, pink, and violet, indicates the presence of Terpenoids.

2.3.5 TEST FOR GLYCOSIDE:

To identify the presence of glycoside about 1 ml of the extract is added to 1 ml of acetic acid, 1 ml of ferric chloride, and 1 ml of sulfuric acid, the presence of blue or green color indicates the presence of glycoside.

2.3.6 TEST FOR PHENOL:

To 1ml of extract acid and 1ml of ferric chloride, observance of black or green color indicates the presence of phenol

2.3.7 TEST FOR ALKALOIDS:

To 1 ml of extract added 1ml of iodine solution, observance of blue color indicates the presence of alkaloids.

2.3.8 TEST FOR QUINONE:

To identify the presence of quinone about 1ml of the extract is added to 1ml of hydrochloric acid, observance of green, red, and pink indicates the presence of quinone.

2.3.9 TEST FOR PROTEIN:

About 1 ml of plant extract and 1 ml of nitric acid (Ninhydrin test), the observance of golden yellow indicates protein presence.

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2.3.10 TEST FOR CARBOHYDRATES:

To identify the presence of carbohydrates about 1ml extract is added to 2 ml of benedict's solution, the observance of blue, green, and orange colors indicates the presence of carbohydrates

2.3.11 TEST FOR FLAVONOIDS

To identify the presence of flavonoids about 1ml of the extract is added to 1ml of sulphuric acid, the observance of yellow color indicates the presence of flavonoids.

2.4 THIN LAYER CHROMATOGRAPHY

Thin Layer Chromatography (TLC) is a method for separating non-volatile mixtures. It involves using a sheet coated with silica gel as the adsorbent material. After separation, components appear as vertical spots, each with a retention factor (Rf) expressed as:

Rf =distance traveled by the solute/distance traveled by the solvent

Thin-layer chromatography was performed to check the presence of the Betalain pigment

The TLC eluent, prepared with a methanol and acetic acid ratio of 12:8 (v/v), was conditioned in the chamber until it reached 0.5 cm high. After 1 hour of closed saturation, the extract was spotted on the TLC plate using a capillary pipe up to 2 cm. The plate was then inserted into the chamber until the eluent reached the upper edge, and then removed and dried. Separation results appeared as spots and irregular areas, marked with a pencil, followed by Rf value calculation using the formula.

Retardation Factor Value (Rf)-Distance traveled by solute/Distance traveled by solvent (7)

2.5 FTIR SPECTROSCOPY

FTIR-4600typeA is a technique used to obtain infrared spectra of solids, liquids, or gases, revealing their composition. It's commonly used for identifying unknown materials and confirming production materials.

To use the FTIR, turn it on and wait for 3 beeps. Calibrate and run a background scan. Clean the sample holder, then apply a drop or small portion (15-20 mg) of the sample. Avoid scratching the crystal. Press and secure the sample, then select "Monitor Sample" from the Measure menu to read and save the spectra. Power off the equipment after measurement.

2.6 UV-SPECTROSCOPY

Different colored pigments absorb light at varying wavelengths due to their chemical structure, often characterized by conjugated compounds with alternating double and single bonds. This conjugation can range from short (low degree) to long (high degree) depending on the number of these alternating bonds.

The extracts were analyzed using UV-visible spectrophotometer, scanning wavelengths from 200-1100 nm with a Systronic Spectrophotometer. Distilled water served as the blank, and the sample was added to a curette for absorbance readings. The peak absorbance value in the UV-visible spectrum was recorded. (8).

2.7 DETERMINATION OF INVITRO ANTIOXIDANT ACTIVITY

2.7.1 DPPH free radical scavenging activity

Antioxidants play crucial roles in preventing damage from free radicals, which are implicated in various chronic diseases like cardiovascular diseases, aging, cancer, and inflammation.

Antioxidant activity was measured using aqueous extracts of Dragon fruit peel to calculate Radical scavenging % and IC50 via DPPH assay.

DPPH solution (2,2-diphenyl-1-picrylhydrazyl)

Methanol

Preparation of DPPH-2,2-diphenyl-1-picrylhydrazyl

IM DPPH 4g DPPH and 100 ml of 99% methanol and keep it in cool conditions (Tris HCI buffer (pH-7.4)).

The free radical scavenging capacity of Dragon fruit extracts was measured using the stable DPPH radical (absorption maximum at 517 nm). A solution of DPPH was prepared by dissolving 4 mg in 100 ml methanol. Different volumes (100 µl, 200 µl, 300 µl, 400 µl, and 500 µl) of the sample were added to 3 ml of methanolic DPPH, then incubated in darkness at room temperature for 30 minutes. The percentage of free radical inhibition activity was calculated using a specific formula.

- DPPH Scavenged (%) =((AB-AA))/AB×100
- Where AB is the absorbance of blank at t = 0 min

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- AA is the absorbance of the antioxidant at t = 30 min.
- A calibration curve was plotted with the 5% DIPIT scavenged versus the concentration of standard antioxidants.

The percentage of inhibition obtained was then plotted in a linear regression curve, with the equation y = ax + b.
Where the x-axis was the concentration and the y-axis was the Percent of inhibition. The value of IC50 (inhibition concentration 50) was obtained by entering the number 50 into the Equation, as y (9).

2.8 IN-VITRO ANTI-INFLAMMATORY

Extracts of Dragon fruit peel was prepared and evaluated for the presence and quantification of phytochemicals. In vitro, anti-inflammatory assays, such as protein denaturation activity were conducted. (10)

2.8.1 PROTEIN DENATURATION

Standard solutions of Bovine Serum Albumin (BSA) were prepared in ten test tubes, with concentrations ranging from 0.1 ml to 1 ml. Each test tube received 4.78 ml of Phosphate Buffer Solution (PBS). Another set of ten test tubes was prepared for the samples, with varying concentrations of the sample added (0.1 ml to 1 ml). To each sample test tube, 0.2 ml of BSA and 4.78 ml of PBS were added. The test tubes were incubated in a water bath at 37ºC for 15 minutes, then heated at 70ºC for 5 minutes. After cooling, turbidity was measured at 660 nm using a colorimeter.

2.9 COATING THE PIGMENT ON THE COTTON GAUZE

The pigments of *Hylocereus undatus* peel extract were coated on the cotton gauze. The cotton gauze was taken and cut into pieces with 5*1(length*breadth). Later the cotton gauze was dried under the sun for 24 hours. (11)

2.10 ANTIBACTERIAL ACTIVITY

The qualitative determination of antibacterial activity was done based on the protocol of the AATCC test method 147. For the test culture, nutrient agar was prepared and Staphylococcus aureus & E. coli were inoculated. The test tubes were incubated at 37°C for 24 hours. (12)

2.11 SWATCHES TEST

The antibacterial activity test compared dragon fruit extract coated and uncoated pigments on cotton gauze against pathogens like Staphylococcus aureus and Escherichia coli. Nutrient agar plates were prepared and swabbed with the organisms. Coated and uncoated gauze pieces (5*1 cm) were placed on each plate side and incubated at 37°C for 24 hours. The observed zone of inhibition was recorded.

2.12 STAIN RESISTANCE

Stain resistance (AATCC, 175-2005 & AATCC TM 130) evaluates cotton gauze's ability to resist stains.
The test involved using citric acid with coated gauze. Citric acid concentrations ranged from 1% to 5%. Coated gauze pieces (5*1 cm) were placed in the center of Petri plates and immersed in citric acid solution with distilled water. The observation time was recorded.

RESULTS AND DISCUSSION

3.1 SAMPLE COLLECTION OF FRUITS

Dragon fruit (*Hylocereus undatus)* purchased from a market in Coimbatore, Tamil Nadu, India, was cleaned, peeled, diced, and dried using both sunlight and oven methods. The resulting dried peel was ground into a powder using blenders for storage and further use.

Fig1.Collection of Dragon fruit (*Hylocereus undatus*).

Fig2.Dragon fruit peel

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Fig3.Fine powder of peel
Sunlight exposure method

Fig 4. Fine powder of peel
oven dry method

EXTRACTION OF PIGMENT FROMA QUEOUS EXTRACT

(a) Fig 5. A) Aqueous extract by using a magnetic stirrer

Fig6.B) Filtered extract of pigment *Hylocereus undatus*

1) 3.3QUALITATIVE ANALYSIS OF PHYTOCHEMICALS

The *H. undatus* peel extract was screened for major secondary metabolites by observing color changes or precipitation formation with specific reagents. Results in Table 7 showed positive for tannins, saponins, terpenoids, flavonoids, and carbohydrates, indicating the peel as a rich source of phytonutrients.

Photochemical analysis of *Hylocereus undatus*

Similar results are by (Ashima Beatrice Wany *et al.*, 2020)

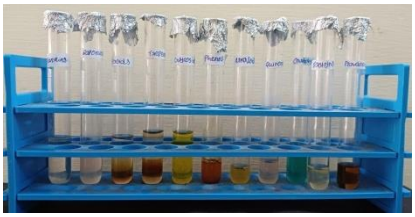

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Table1. Phytochemical analysis of *Hylocereus undatus*

Phytochemical constituents	Extract of peel of *Hylocereus undatus*
Tannins	Positive
Saponins	Positive
Steroids	Negative
Terpenoids	Positive
Glycoside	Positive
Phenol	Negative
Alkaloids	Negative
Quinones	Negative
Proteins	Negative
Flavanoids	Positive
Carbohydrates	Negative

3.4 THIN LAYER CHROMATOGRAPHIC TECHNIQUE

Thin-layer chromatography is carried out for the identification of pigment.

The Rf value is calculated by using the formula.

Each other a retention factor R) expressed as:

RF-DISTANCE TRAVELED BY THE SOLUTE /DISTANCE TRAVELED BY THE SOLVENT RF VALUE OF BETA LAIN = 43-0.52

Rf value=0.4

The pigment presents Betalain.

Fig 8. TLC of *Hylocereus undatus*

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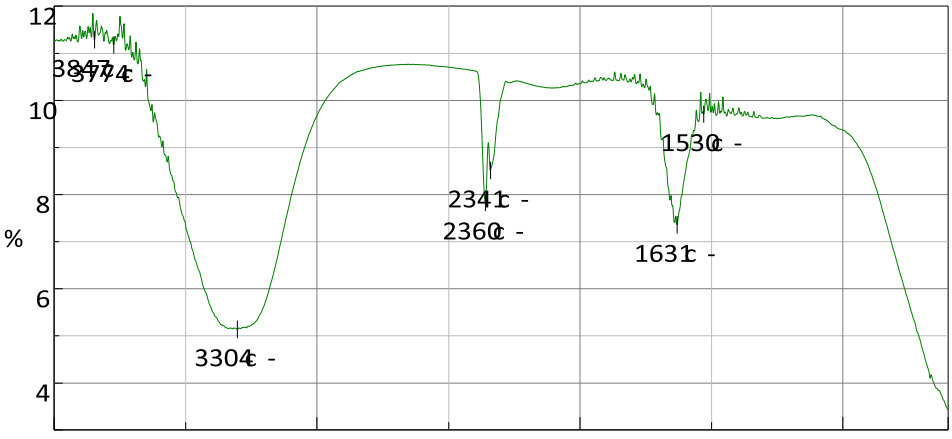
3.5 CHARACTERISATION OF PIGMENT FROM DRAGON FRUIT PEEL EXTRACT

3.5.1FTIR –SPECTRUM ANALYSIS OF SAMPLE

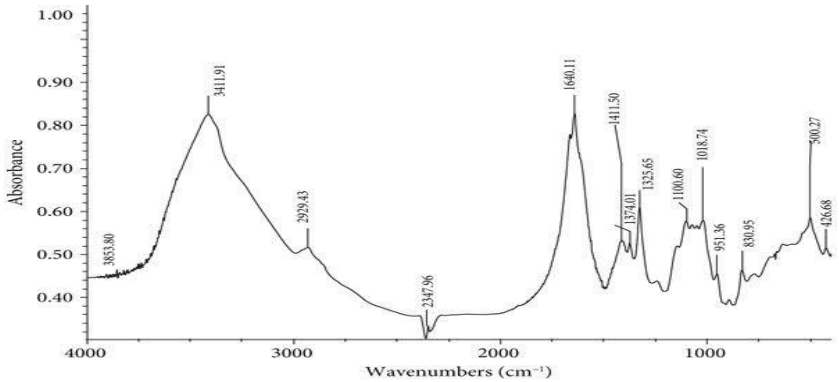
FT-IR analysis determined the functional groups of active components by analyzing peak values in the infrared spectrum. Results are shown in Graph 1 and Table 2. The analysis confirmed the presence of Hydroxyl, Amines, Alkanes, Carbonyl, methine, Alkene, and Aromatic compounds, identified by their corresponding peaks at 3304, 2360, 2341, 1631, 1530, respectively.

Table 2. Functional group identification

Peak	Standard Peak	Sample Peak	Appearance	Functional group	Compound
1	3411	3304	Medium	O-H, NH	Hydroxyl group, amino group
2	2929	2360	Strong	C-H Stretch	Alkanes
3	2347	2341	Medium	O-H, NH	Hydroxyl group, amino group
4	1640	1631	Strong	C=O, C-H, C=C	Carbonyl group, methine group, alkene
5	1411	1530	Medium	C-C stretch	Aromatics



Graph 1. FTIR analysis sample graph



Graph 2. FTIR analysis of standard graph

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Peak 1 (3304) in Graph 1 indicates O-H and NH groups (Hydroxyl and amino), similar to the standard peak in Graph 2. Peak 2 (2360) suggests C-H stretching (Alkanes), also observed in Graph 2. Peak 3 (2341) shows O-H and NH groups, consistent with the standard peak in Graph 2. Peak 4 (1631) indicates C=O, C-H, and C=C (Carbonyl, Methine, and alkene), similar to the standard peak in Graph 2. Peak 5 (1530) shows C-C stretch (Aromatics), also observed in Graph 2.*(*R syafinar*et al.*, 2015)
Dragon fruit peel pigment shows active components with a C=O peak at 1631 (carbonyl) and an O-H peak at 3304 (hydroxyl group), typically found in carboxylic acids. The presence of the Carboxyl group in Betalains pigment (Graph 1) indicates strong hydrogen bonding, with a shift to lower frequencies.

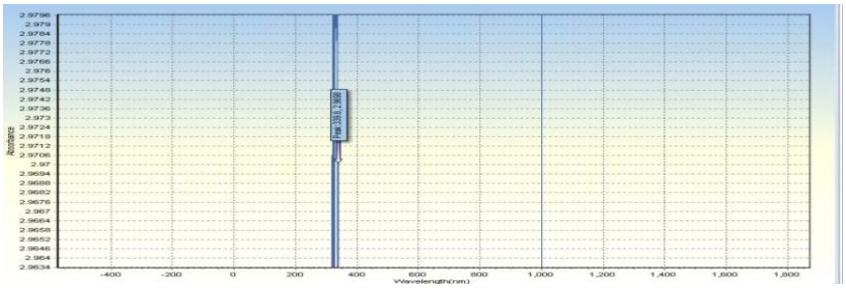
3.5.2 UV SPECTROSCOPY

Spectroscopic method has become a powerful tool for secondary metabolite profiling as Well as for qualitative and quantitative analysis of the pharmaceutical and biological material.
The UV Spectroscopy was characterized for the betalain pigment. The peaks were observed in the wavelength range of 200-1100 nm. (Graph 3) The peak absorbance for the betalain pigment at 2.9698 in wavelength of 339.8 nm. (Table 3).

Table 3. Absorbance in UV Spectroscopy

Wavelength (nm)		Absorbance
339.8		2.9698

Similar results are by *R syafinar et al., (2015)et al., (2020)*



Graph 3. Graph of UV spectroscopy of peel extract

The present study of UV-visible spectrophotometer revealed that the presence of betalain pigment and also the phenolic Like tannin and flavonoids compound which indicates the medicinal properties of this peel.Phenolic compound tannin used as antioxidant, anti-inflammatory and anticancer and Flavonoid compound used as antioxidant activity,anti-inflammatory activity of this peel extract also observed.

3.6 ANTIOXIDANT ACTIVITY

The antioxidant activity is carried out by DPPH Assay as shown in Fig 10

Table 4. Antioxidant activity of white dragon fruit peel extract

Concentration	Control absorbance at 517nm	Sample absorbance at 517nm	%RSA	IC 50
100	1.025	0.44	57.07	740.829
200	1.025	0.32	68.78	2366.846
300	1.025	0.24	76.58	3992.862
400	1.025	0.21	79.51	5618.878
500	1.025	0.18	82.43	7244.894

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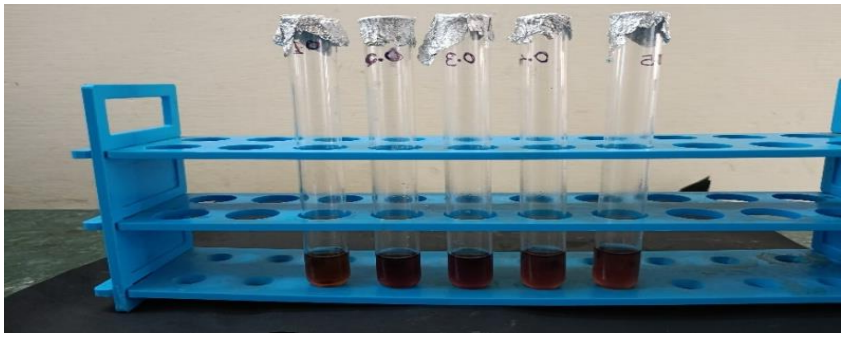
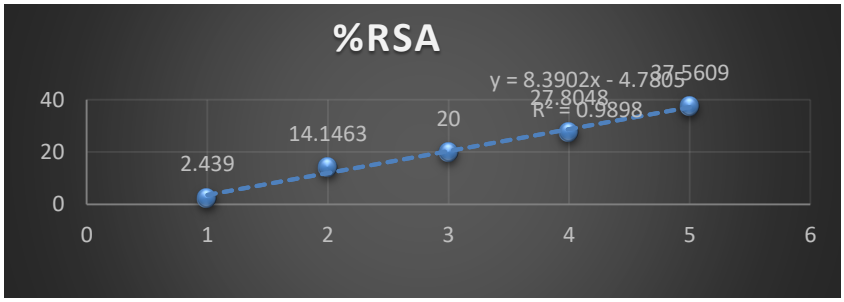


Fig 10. Antioxidant activity of dragon fruit peel extract



Graph 4. Antioxidant activity graph of dragon fruit peel

3.7 ANTI-INFLAMMATORY ACTIVITY

The protein denaturation method is utilized to measure the anti-inflammatory properties of the extract obtained from dragon fruit peel.

Table 5. Anti-inflammatory activity of standard and sample

Concentration (µl)	Absorbance (660nm) of standard	Absorbance(660nm)of Sample
0.1	0.1	0.2
0.2	0.4	0.3
0.3	0.88	0.65
0.4	0.89	0.6
0.5	0.91	0.64
0.6	0.89	0.59
0.7	0.88	0.57
0.8	0.86	0.55
0.9	0.84	0.53
1.0	0.83	0.49

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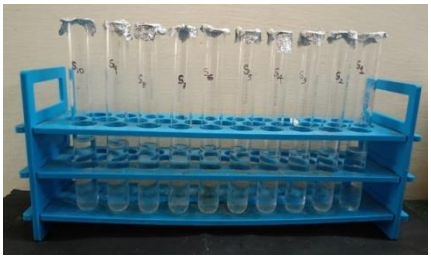


Fig 11. Standard dilution

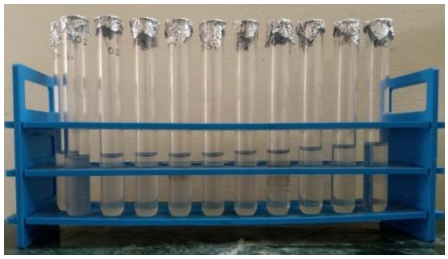
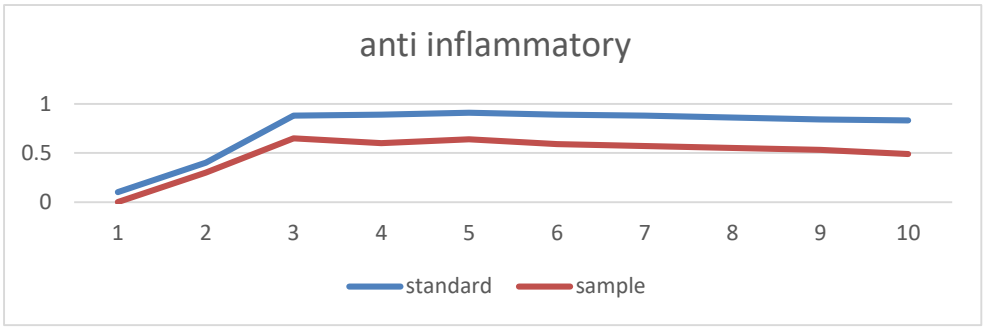


Fig 12. Sample dilution



Graph 4. Graph of anti-inflammatory activity

The graph of Standard solution regarding anti-inflammatory studies is plotted by concentration (at the x-axis) and absorbance (at the y-axis) and shows the highest absorbance values of 0.91 and 0.65 when the concentration is 0.5 and 0.3 µl.

3.8 COATING ON COTTON GAUZE (DIP DRY METHOD)

The pigments of *Hylocereus undatus* peel were coated on the cotton gauze. The cotton gauze was taken and cut into pieces with 5*1(length*breadth). Later the cotton gauze was dried under the sun for 24 hours

Fig 13. wound gauze soaked in dragon fruit peel extract

Fig 14. Extract coated in wound gauze

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3.9 ANTIBACTERIAL ACTIVITY

3..9.1 SWATCHES TEST

The antimicrobial activity test was carried out for the development of coated pigment and uncoated pigment (control) in cotton gauze against *Staphylococcus aureus* and *Escherichia coli.*

Fig 15 A). Anti-bacterial activity of *S. aureus*

Fig 16 B). Anti-bacterial activity of *E. coli*

Table 6. Antimicrobial activity of coated cotton gauze

Pathogen	Zone of inhibition
Staphylococcus aureus	3 cm
Escherichia coli	2cm

The zone of inhibition is shown in Fig 15 and Fig 16. The coated cotton gauze is more sensitive to *Staphylococcus aureus* than *E.coli*

3.10 STRAIN RESISTANT TEST

The stain resistance of cotton gauze is evaluated using citric acid to retain stains. This assessment, known as AATCC 175-2005 & AATCC TM 130, determines the effectiveness of the gauze in withstanding permanent discoloration caused by stains. The chemical nature of the gauze plays a role in this property, but it can be improved by specific treatments. The results of the stain resistance assessment are presented in a table.

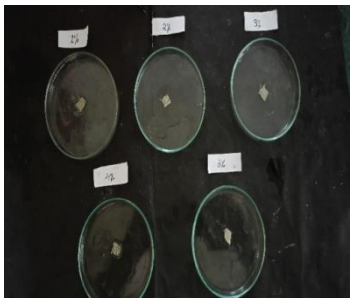

Fig 16. Day 1 citric acid test

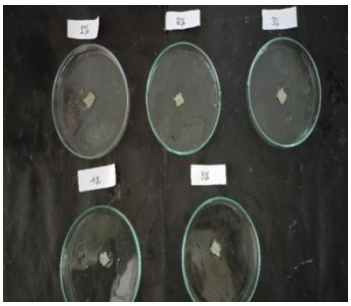

Fig 17.Day 2 citric acid test

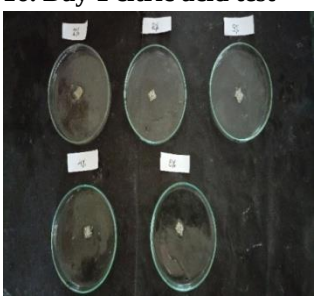

Fig 18. Day 3 citric acid test

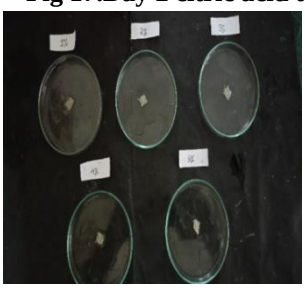

Fig 19. Day 4 citric acid test

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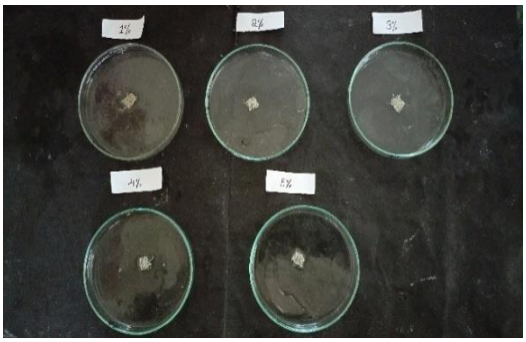

Fig 20. Day 5 citric acid test

Table 7. Citric acid test of coated cotton gauze

	CONCENTRATION				
DAY	1%	2%	3%	4%	5%
DAY 1	No change	No change	No change	No change	No change
DAY 2	No change	No change	No change	No change	No change
DAY 3	No change	No change	No change	No change	No change
DAY 4	No change	No change	No change	No change	No change
DAY 5	No change	No change	No change	No change	Mild change

Table 7 shows a mild change for a 5% concentration. The wound gauze retains stain, indicating high resistance.

4. SUMMARY AND CONCLUSION

Natural pigments, like those from white dragon fruit (*Hylocereus undatus*), are gaining popularity due to their eco-friendliness and safety. The peel extract contains betalain, useful for pigment extraction. Techniques like thin-layer chromatography, UV-VIS, and FT-IR can identify its components. This pigment can be applied to wound gauze, with its stain resistance indicating durability against contamination. Hylocereus undatus peel offers health benefits, appealing to pharmaceutical and nutraceutical sectors for its anti-inflammatory, antioxidant, antibacterial, and anti-cancer properties.

Dragon fruit peels, readily available as agricultural waste, are utilized in developing wound gauze with antimicrobial, anti-inflammatory, and antioxidant properties. This gauze aids wound healing by providing protection, maintaining moisture, promoting tissue regeneration, and ensuring patient comfort. Combining dragon fruit peel with gauze offers dual benefits, acting as a protective barrier against contaminants and promoting wound healing. Biodegradable cotton gauze is recommended. White dragon fruit peel, a potential source of betalains, can enhance wound healing by reducing inflammation and oxidative stress, promoting tissue regeneration, and preventing infection. Further research is needed to assess the cytotoxicity and pharmacological properties of this gauze.

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