



## *In vivo* and *In vitro* Evaluation of Antimicrobial Activity of Peel Extracts of Red Dragon Fruit (*Hylocereus polyrhizus*)

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### Abstract

This study aimed to characterization and evaluates the effectiveness of red dragon fruit (*Hylocereus polyrhizus*) peel extracts as an antimicrobial agent. Red dragon fruit peel extracts were obtained by maceration using solvent at pH 5. Phytochemical characteristics, total phenols, antioxidant, and antimicrobial activity of the peel extracts were observed. Antioxidant and antimicrobial activities of the extracts were associated with high phytochemical compounds and total phenols contained in the extracts. Red dragon fruit peel extracts with various percentages prepared and their physicochemical characteristics, nutrients, antioxidant activity, and microbiological profile were analyzed. The current study is to evaluate the susceptible antimicrobial activity of crude 50% hydro alcoholic Peel Extracts of Red Dragon Fruit (*Hylocereus polyrhizus*) against different clinical isolates (*Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *A. niger* and *Candida albican*) of microbial disease so as to trip up on the other alternatives and overcome the upcoming era of increasing microbial resistance. Furthermore, the results of the *In vivo* assay on thirty mice model showed that the treatment of mice with the 100mg/ml of aqueous extract and Chloramphenicol have more effectiveness than other concentrations and the number of bacteria *P. aeruginosa* was reduced significantly ( $P \leq 0.001$ ) during the treatment days. Results showed that the red dragon fruit peel extracts have an Antimicrobial Activity.

**Keywords:** antimicrobial, red dragon fruit peel extract, pitaya etc.

### Introduction

**Dragon fruits:** Pitaya usually refers to fruit of the genus *Stenocereus*, while pitaya or dragon fruit refers to fruit of the genus *Hylocereus*, both in the Cactaceae family. Sweet pitayas come in three types, all with leathery, slightly leafy skin.

- *Hylocereus undatus* (*Pitaya blanca* or white-fleshed pitaya) has pink-skinned fruit with white flesh. This is the most commonly seen "dragon fruit".
- *Hylocereus costaricensis* (*Pitaya roja* or red-fleshed pitaya, also known as *Hylocereus polyrhizus*) has red-skinned fruit with red flesh.
- *Hylocereus megalanthus* (*Pitaya amarilla* or yellow pitaya, also known as *Selenicereus megalanthus*) has yellow-skinned fruit with white flesh.

Early imports from Colombia to Australia were designated *Hylocereus ocampensis* (supposedly, the red fruit) and *Cereus triangularis* (supposedly, the yellow fruit). It is not quite certain to which species these taxa refer, though the former is probably the red pitaya.

The fruit normally weighs from 150 to 600 grams (5.3 to 21.2 oz); some may reach 1 kilogram (2.2 lb).



Fig 1

### Experimental

**Reagent and Materials:** All the reagents in these experiments along with triple distilled water were of analytical grade. All the chemicals were purchased from Merck Research Chemicals pvt. Ltd. Red Dragon Fruit (*Hylocereus polyrhizus*)

were purchased from local market. For *In vivo* activity required mice supply by Savitribai Phule Pune University, Animal Experiments Department, Pune.

**Apparatus**

Automatic B.O.D. INCUBATOR, Model No.TC141 and Antibiotic Zone Reader Model No.FG 456 made by LABGO are also used this research work.

**Antibacterial activity (*In vitro*)**

In radial or 2-D technique petri-dishes of agar are prepared by pouring melted agar media previously inoculated with selected microorganism after the solidification of agar cups are made with the help of borer and cups are filled with solution of suitable conc. of sample and standards respectively and are inoculated at 37°C for 24 hr. the anti-microbial agent diffuses through the agar around its cup and produces a characteristic zone of inhibition of the microorganism sensitive to the

sample, the diameter of which can be measured.

**Table 1:** Antibacterial activity of crude 50% hydro alcoholic Peel Extracts of Red Dragon Fruit (*Hylocereus polyrhizus*)

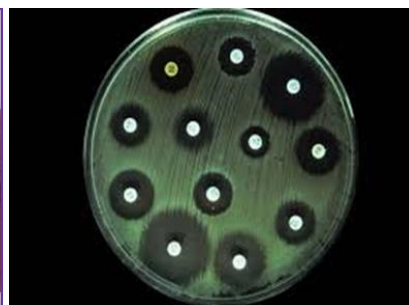
Extracts	Zone of inhibition at 200µg/MI (in mm.)				
	<i>E.coli</i>	<i>B. Subtilis</i>	<i>S. aureus</i>	<i>A. niger</i>	<i>C. albicans</i>
A1	24	25	26	15	22
A2	20	23	25	16	21
A3	20	24	25	19	22
A4	25	26	23	20	21
A5	24	23	26	21	22
A6	20	22	24	18	23
A7	21	23	22	20	21
A8	22	24	25	20	22
A9	21	23	22	20	21
Levofloxacin	27	26	28	-	-
Amphotericin B	-	-	-	23	24



**Fig 2:** Antibacterial Study (*In vitro*) in Lab



**Fig 3:** Antibiotic Zone Reader



**Fig 4:** Antibiotic Zone Development

Antibacterial activity (*In vivo*): (Inhibitory effect of Peel Extracts of Red Dragon Fruit on growth of *P. aeruginosa*) Thirty male Bal b/c mice (26-28 gm) aged between 12 and 16 weeks were used for all *In vivo* experiments. They were kept in a light, food and temperature-controlled room and all mice were acclimatized for at least one week prior to beginning the experiments. The dorsal back skins of the mice were shaved and ethanol (70%) was used as antiseptic for the shaved region and then burned by using inflamed knife and then contaminated with bacteria *P. aeruginosa* (1.5×10<sup>8</sup> bacteria/ml), which was the more common species in burn infection in present study. After two days of injury the inflammation, redness and suppuration region were observed and the experimental mice were randomly divided into the following groups (n = 30)

- Group 1:** untreated mice (control).
- Group 2:** burn mice treated with ointment composed of antibiotic (Cm).
- Group 3:** burn treated with ointment (concentration 50mg/ml)

consisting of 0.5g of aqueous extract added to 9.5g of Vaseline.

- Group 4:** burn treated with ointment (concentration 100mg/ml) consisting of 1g of aqueous extract added to 9 g of Vaseline.
- Group 5:** burn treated with ointment (concentration 200mg/ml) consisting of 2g of aqueous extract added to 8 g of Vaseline.
- Group 6:** burn treated synergistically with ointment Cm and 100mg/ml of aqueous crude extract of *P. granatum*.

The treatment continued twice each day during 15consecutive days and the numbers of bacteria was calculated.

**Statistical analysis**

Results were expressed in Mean± SD and were analyzed by one way analysis of variance (ANOVA) and post HOC analysis was done using Tukeys using SPSS package version 16. P value< 0.05 was considered to be significant.

**Table 2:** Numbers of *P. aeruginosa* between the Mice Groups.

Duration of treatment per day	Group(1) Control	Group(2) CM	Group(3) Aqueous extract 50mg/ml	Group(4) Aqueous extract 100mg/ml	Group(5) Aqueous extract 200mg/ml	Group(6) CM + Aqueous extract 100mg/ml
3	0	145	175	165	160	125**
6	0	115	80	70*	82	7*
9	0	32	20	10	35	7*
12	0	0	2	1	5	0
15	0	0	0	0	0	0

\* Significant at P ≤ 0.001

## Results & Discussion

Antibacterial activity (*In vitro*): The extract A1, A2, A3, A5, A8 has excellent Antibacterial activity *S. aureus*, the extract A1 have shown Antibacterial activity *B. subtilis*, while A4 show Antibacterial activity against *E. coil*, when compared with standard Levofloxacin.

Antibacterial activity (*In vivo*): The *In vivo* assay revealed that the treatment on mice with the different concentrations of aqueous extract of Red Dragon Fruit peels and Cm antibiotic during 15 days and it was found to have marked effects on the number of *P. aeruginosa* especially the group six which represent 100mg/ml of aqueous extract and Cm antibiotic which showed more effectiveness than other groups and the number of bacteria was reduced significantly ( $P \leq 0.001$ ) from 120 to  $5 \times 10^6$  cell/ml during the treatment days and this results due to Red Dragon Fruit Peel extract which have antibacterial activities and exhibited synergistic effects when used with antibiotic Cm as well as have wound healing properties. In addition, according to the results presented in table 3 it was noted that the group four (100mg/ml aqueous extract) significantly ( $P \leq 0.001$ ) was more effective than other groups and generally the skin of the mice was healed after nine days of treatments.

## Conclusions

The *In vivo* and *In vitro* study clearly demonstrated that the importance of Red Dragon Fruit Peel extracts in the control of burn infection resistant bacteria which are becoming a threat to human health and the aqueous extracts of peels are reported to have therapeutic properties.

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