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PHYTOCHEMICAL SCREENING AND INVITRO ANTIBACTERIAL ACTIVITY OF *HYLOCEREUS UNDATUS* FRUIT PEEL EXTRACT

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ABSTRACT

Dragon fruit belongs to the genus Hylocereus of the Cactaceae family. There are two species that are commonly cultivated; *Hylocereus polytheizes* and *Hylocereus undatus* that have the same red skin but different flesh colours, red and white respectively. Although from the same genus, the phytochemical contents and bioactivities of both fruits may not be the same. This study aims to compare the phytochemical contents, and antibacterial activities of *H. undatus* to help consumers better choose nutritional fruits and to explore potential natural preservatives. The fruit samples were extracted using methanol and later were subjected to phytochemical, antibacterial assays. The phytochemical contents were determined using Folin Ciolcalteu and aluminium chloride methods for total phenolic content . Disk diffusion method was performed to evaluate antibacterial activities against *Escherichia coli*.

the most commonly cultivated vine cactus in the cactaceae family is normally *Hylocereus undatus*, which would be native to Mexico and America. it is commonly referred to as “dragon fruit” or “pitaya.” additionally, it goes by the name “buahnaga,” which means “dragon fruit.” in addition to their attractive colour, *Hylocereus undatus* fruits are popular around the world due to their abundance of polyphenolic components and their antioxidant activity. due to the characteristics, degree of production, and financial benefits of the pitahaya fruit, a member of the *Hylocereus* genus, more land has been planted in agriculture during the past two decades. stalks, fruits, and flowers from the plant are used in food and film.

Keywords: *Hylocereus undatus*, Disc diffusion method, Antibacterial activity, Phytochemical content, Total phenolic content.

Introduction

HYLOCEREUS UNDATUS, has high value as an edible fruit and ornamental plant, and the fruit can be consumed raw or transformed into wine, juice, jelly, yogurt, jam, preserves, and other desserts. It is majorly present in Mexico and Central America, whereas countries like, Israel, Malaysia, and Thailand use advanced technologies for growing them resulting in high yields . Mayan civilization used *H. undatus* fruits as hypoglycaemic, diuretic, cardio protectant, wound disinfectant, anti-tumorigenic (stem sap), dysentery cure and other digestive system disorders.The leaves, flowers and fruits of *H. undatus* exhibits wound healing properties The fruit peel has potential as an antibacterial , and antioxidant agent . It could reduce hypertension, and diabetes, apart from mediating carbohydrate metabolism, heart tissue formation, fortification of teeth and bones, improving the function of kidneys, sharpness of eyes, strengthening the brain function, and prevents colon and prostate cancer . Taken together, the extracts of *H. undatus* can impart antioxidant, anti-inflammatory, anti-obesity, anti-diabetic, anti-cancer, and anti-proliferative activities. Apart from the nutritional benefits of mature fruit, the young stem, and fresh flower buds are also edible and can be used as a vegetable .The dehydrated dragon fruit flowers were

used for making antioxidant rich tea.oligosaccride from *H. undatus* promotes *lactobacillus* and *bifidobacterias* as well as shows complete and partial resistance to acidic conditions in the stomach, and human α-amylase respectively, indicating pre-biotic characteristics .

Fig – 1: Dragon Fruit(*H. undatus*)

In the past decade interest on the topic of antibacterial property of plant extracts has been growing. Food processors and agencies are very concerned with the high and growing number of food-borne outbreaks and illnesses associated with microorganisms especially bacteria. Bacteria also have become far more resistant to many antibacterial agents. For instance, of two million people who acquired bacterial infection in United States of America (USA) hospitals annually, 70% of the cases involved the strains that are resistant to at least one antibacterial agent The emergence of antibiotic-resistant microorganisms had swiftly reversed the advances of previous fifty years of research on antibiotics. Consumers too have questioning the safety of foods containing the synthetic antibacterial agent as preservatives. Therefore, there has been increasing interest in developing new types of highly effective and non-toxic antibacterial agents from natural sources .Over the past two decades, scientists have turned back to traditional folk medicines or natural products to uncover the scientific basis of remedial effects such as antibacterial agents.

Beside plants, fruits also have become the main subject for researchers to be investigated since their bioactive compounds close related with herbs, commonly referred as phytochemicals such as carotenoids, polyphenols and

anthocyanins that are abundantly present in fruits and vegetables such as tomatoes, grapes, pomegranates and strawberries are gaining lot of interest due to their functional property.

Furthermore, natural compounds in fruits and vegetables such as polyphenols such as flavonoids and tannins have shown very promising results in combating bacteria, fungus and viral.

Hylocereus species or better known as dragon fruit or pitaya from the Cactaceae family had become interest subject to many researchers mainly due to its unique taste, shape and the flesh colour. Many studies have been conducted to investigate the chemistry of betalains, the major bioactive compounds in *H. polyrhizus*. Furthermore, *H. polyrhizus* and *H. undatus* seeds have been reported to contain high level of essential fatty acid, namely linoleic acid and linolenic acids. The functionality of *H. polyrhizus* seed are including antioxidant propertyand the uses of different part of *H. undatus* in promoting wound healing in diabetic rats also have been reported. However, investigations on the antibacterial activity on the fruit peels are lacking due to less popular in commercial application. Therefore, the present study was aimed to evaluate the antibacterial properties of ethanol, chloroform and methanol extracts from *H. undatus* peel using disc diffusion and broth macro dilution methods in determining minimum inhibitory concentration (MIC) and minimum bacteriacidal concentration (MBC). The findings obtained in the study could support the potential application of pitaya peels as a natural source of antibacterial agent rather than being discarded as domestic waste as currently practiced.

Material and methods

Chemicals and reagents:

Methanol, α naphthol, Fehling's Solution A and B, Benedict's reagent, Mayer's reagent, Dragondroff's reagent, Hager's reagent, Wagner's reagent, Dil. HCL, Ethanol, Ammonia, Conc. H_2SO_4, NaOH, NaCl Lead Acetate, Aq. Bromine solution, Acetic anhydride, β- sitosterol, Ferric chloride, Quercetin, Potassium Acetate, Aluminium chloride, Mueller—Hinton agar, E.coli(ATCC 25922)

Fig 2 – Drying of Dragon Fruit

sample preparation:

H. undatus the white flesh pitaya fruits *(H. undatus)* were purchased from local supermarket **in** Hyderabad, India . The fruits were rinsed

distilled water and hand peeled and cut it down into pieces using knife. Then the pieces were kept in tray for shear drying. was ground into powder using a

PANASONIC blender (MX-GM1011H) and weighted the powder.

Extraction of red dragon fruit peel extract:

Fresh red dragon fruit was peeled and the peels were collected in a clean container and cut into pieces, dried, and turned into powder. Extraction was conducted by maceration method using methanol as the solvent. In the maceration jar, 500 grams of red dragon fruit peel powder was mixed with 1.5 litres of methanol. This mix was then stirred every 30 minutes for 6 hours and kept in a dark chamber for three days. After three days, the mix was filtered using filter paper, the filtrate was kept in a separate container, while the residue was returned to the maceration jar, and into it added another 1.5 litres of methanol, and kept in the dark chamber for another three days (re- maceration). This re-maceration process was conducted twice. In the end, about 4.5 litres of the filtrate were produced. This filtrate then evaporated in a rotary evaporator until most of the solvent evaporate. This concentrated filtrate was then reduced further in a water bath until it produced a thick consistent extract.

Phytochemicals screening:

Determination of phytochemical compounds in the dragon fruit peel and characterization of the phytochemical compounds (*H. undatus*) were performed at Deccan school of pharmacy, Hyderabad, Telangana from November 2024 to June 2025. Descriptive research was used to properly determine and characterize the presence of bioactive compounds in the two varieties.

The nutrients and phytochemicals found in dragon fruit are beneficial to the body. Numerous investigations have revealed that dragon fruit may be able to shield people from disease. The value of the fruits of the Hylocereus cactus has increased lately. Certain phytochemicals found in dragon fruit or pitaya include carbohydrates, proteins, saponin phenolic compounds, terpenoids, oils, flavonoids, tannins, phenols, coumarin, and steroids. Nutritional analyses show that the fruit contains all the nutrients required for a balanced diet, including minerals, fat, vitamins, and crude fibre. An overview of the current bioactive substances discovered in dragon fruit (Hylocereus spp.) stems and their potential medicinal uses.

Determination of phenolic content :

Preparation of Standard Gallic Acid for Calibration Curve:

Total phenolic contents (TPC) was determined by Folin–Ciocalteu colorimetric method with some modifications. Standard gallic acid solution was prepared by dissolving 10mg of it in 10mL of methanol (1 mg/mL). Various concentrations of gallic acid solutions in methanol (25, 50, 75, and 100µg/mL) were prepared from the standard solution. To each concentration, 5mL of 10% Folin–Ciocalteu reagent (FCR) and 4mL of 7% Na2CO3 were added making a final volume of 10mL. thus, the obtained blue coloured mixture was shaken well and incubated for 30min at 40°C in a water bath. then, the absorbance was measured at 760nm against blank. the FCR reagent oxidizes phenols in plant extracts and changes into the dark blue colour, which is then measured by UV-visible spectrophotometer. All the experiments were carried out in triplicates, and the average absorbance values obtained at different concentrations of gallic acid were used to plot the calibration curve.

Preparation of samples for total phenolic content

Various concentrations of the extracts (2,4,8,16&32µg/mL) were prepared. The procedure as described for standard gallic acid was followed, and absorbance for each concentration of the extracts was recorded. The samples were prepared in triplicate for each analysis, and the average value of absorbance was used to plot the calibration curve to determine the level of phenolics in the extracts. Total phenolic content of the extracts was expressed as mg gallic acid equivalents (GAE) per gram of sample in dry weight (mg/g). The total phenolic contents in all the samples were calculated by the using the formula:

$C=c\ V/m$

where C=total phenolic content mg GAE/g dry extract, c =concentration of gallic acid obtained from calibration curve in mg/mL, V=volume of extract in mL, and m=mass of extract in gram.

ANTIBACTERIAL ACTIVITY (IN VITRO):

Preparation of concentrations:

HYLOCEREUS UNDATUS peel extract were stratified into three concentrations of 10, 20, 30, 40µg/ml for anti-bacterial activity,

Culture media:

Mueller--Hinton agar (himedia, Lot 0000333943, Code M173), BHI (LOK 190) (Brain heart infusion-himedialabs) center of Indo-American Cancer Hospital and Research Center.

Table 1: showing the composition of Mueller Hinton agar

S.NO	INGREDIENTS	in/gm litre
1.	Beef extract	2.0g
2.	Acid hydrolysate of casein	17.5g
3.	Starch	1.5g
4.	Agar	17.0g
5.	Distilled water	1000ml
Final pH 7.3 ± 0.1 at 25ºC		

ANTIBACTERIAL TESTING BY AGAR WELL DIFFUSION METHOD:

Assessment of antibacterial activity *HYLOCEREUS UNDATUS* peel extract was determined by agar well diffusion method. Inoculum of *E.*coli bacterial strain were plated using sterile swabs into petri dishes containing approximately 25ml of MHA media, where 6mm wells were made and filled with different concentrations of 10, 20, 30, and 40 µg/ml of extract. A 0.01 mg/mL ciprofloxacin was used as positive control. An extraction solvent (distilled water) was used as negative control. Then, the petri dishes were then, incubated at 37±1ºC for 24 h. Further antibacterial activity of the above samples was determined by measuring the zone of inhibition in millimetres [mm] according to the standard clinical parameters.

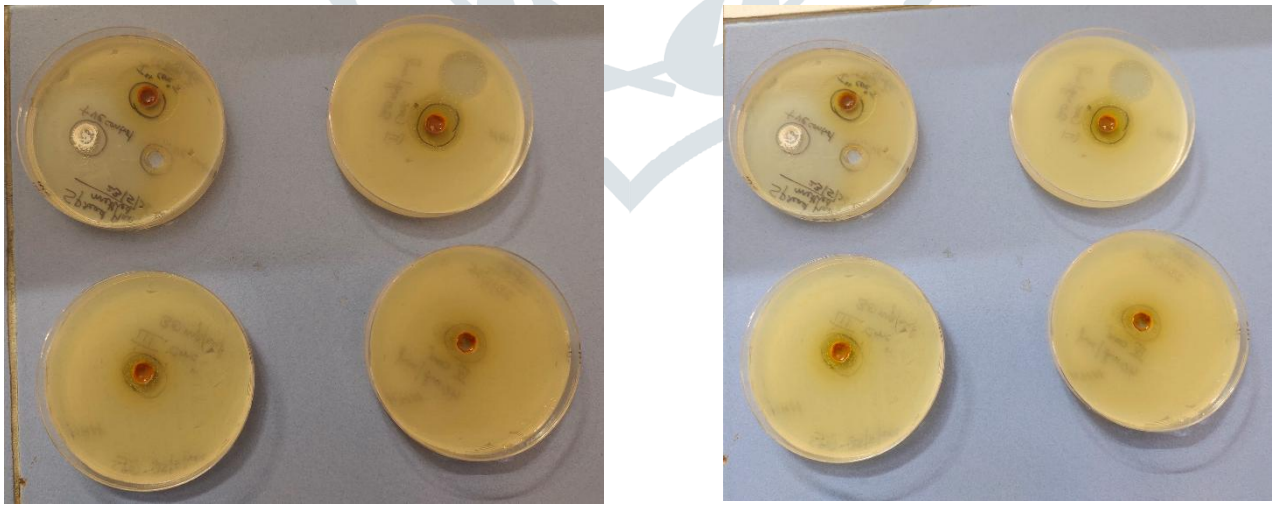


fig – 3: photographs of zone of inhibition

Statistical Analysis:

All the in vitro experimental data were presented as mean ± S.E [M=standard error] of three parallel measurements and data were evaluated by graph pad statistical software [one way], p-values <0.05 were regarded as significant followed by post hoc.

RESULTS AND DISCUSSION

PHYTOCHEMICAL SCREENING OF *HYLOCEREUS UNDATUS* PEEL EXTRACT:

The methanolic extract of *HYLOCEREUS UNDATUS* peel was subjected to preliminary phytochemical screening, to identify the presence of primary and secondary metabolites are mentioned in table no 2.

Table 2: **Phytochemical screening of *HYLOCEREUS UNDATUS* peel extract.**

TEST	RESULTS
Carbohydrates	+
Proteins	+
Amino acids	+
Steroids	+
Glycosides	-
Saponins	+
Flavonoids	+
Alkaloids	+
Phenolic compounds	+
Tannins	+
Cholesterol	-
Resins	+
Volatile oils	-
Terpenoids	+
Triterpenoids	+

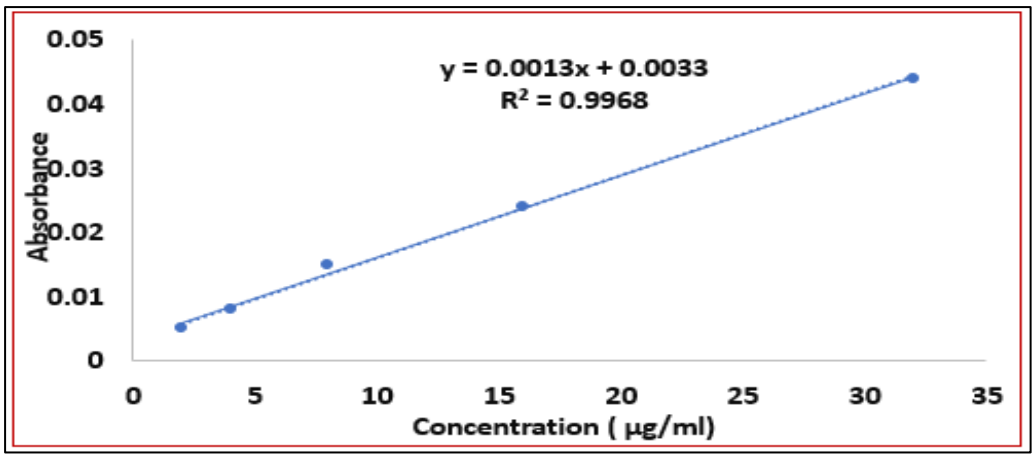
TOTAL PHENOLIC CONTENT:

Total phenolic content in methanolic extract of plant material was determined by Folin–Ciocalteu (F–C) method using gallic acid as the standard.

Total phenolic content of the extract was calculated from the regression equation of calibration curve ($Y = 0.0013x + 0.0033$; $R^2 = 0.9968$) and expressed as mg gallic acid equivalents (GAE) per gram of sample in dry weight (mg/g).

Total phenolic content value of the plant extract was found to be 65.16± 1.52mg GAE/g .

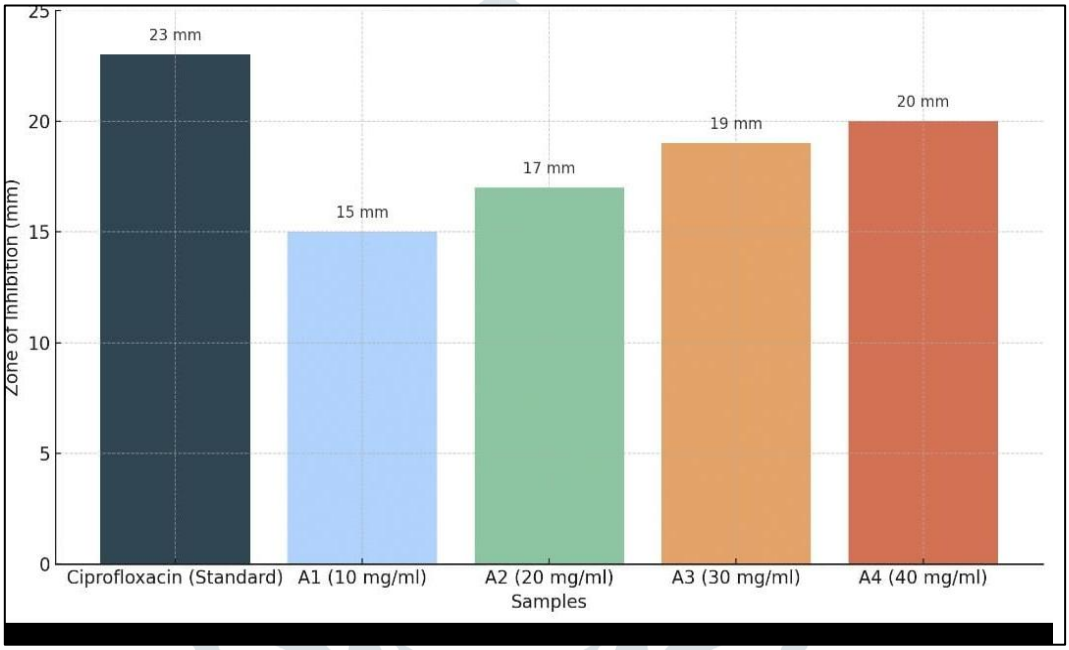
Discussion: The phenolic content of any plant is directly related to their antioxidant properties. Phenolic compounds act as reducing agents, hydrogen donors, acapable of scavenging free radicals. Presence of considerably good amount of phenolics in the fruit extract of Dragon fruit peel may contribute significantly to the antioxidant properties.



Graph-1: caliberation curve between absorbence and concentration is linear

Antibacterial Activity:

The antibacterial activity of extracts *H.Undatus* peel were studied in different concntrations (10,20,30,40 µg/ml) against *E.coli* pathogenic bacterial strains. The extract shows the varying degree of antimicrobial activity 40 µg/ml shows in the graph.



Graph-2: Showing anti-bacterial activity of *Hylocereus undata* fruit against E.coli

CONCLUSION:

Hylocereus undatus, a plant species known for its nutritional and medicinal properties, exhibits promising phytochemical and antibacterial activities. Phytochemical screening of *Hylocereus undatus* peel extract reveals the presence of various bioactive compounds, including flavonoids, phenolics, polyphenols, alkaloids, steroids, . These compounds contribute to its anti-bacterial properties, with studies indicating significant anti-bacterial activity in methanolic extracts. The phenolic content of *Hylocereus undatus* is notable, with potential health benefits associated with its consumption.

Regarding antibacterial activity, research on *Hylocereus undatus* peel, demonstrates potent antibacterial effects against Escherichia coli attributed to the presence of above mentioned phytoconstituents. Although specific studies on *Hylocereus undatus* are limited, its phytochemical profile suggests potential antibacterial properties.

In conclusion, *Hylocereus undatus* shows promising phytochemical and anti-bacterial activities, with potential antibacterial effects that warrant further investigation. Its rich composition of bioactive compounds makes it a valuable resource for food, pharmaceutical and cosmetic industries. As we have concluded that in the above activity that the anti-bacterial effect is shown more in 40mg/ml among other concentrations [10,20,30mg/ml].

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