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PHYTOCHEMICAL AND PHARMACOLOGICAL STUDIES ON *HYLOCEREUS UNDATUS* SEEDS: AN *IN VITRO* APPROACH

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1. INTRODUCTION

The term "plant" implies the possession of multi cellular traits, whose cell walls contain cellulose and which have the ability to carry out photosynthesis. Green plants provide a substantial amount of world's molecular oxygen.[1] Plant cells are characterised by their large water-filled vacuole, chloroplasts and rigid cell walls made up of cellulose, hemicelluloses and pectin. Cell division includes the development of a phragmoplast used for the construction of the cell plate in the later stages of cytokinesis. Like in animal cells, plants cells also differentiate and develop into multiple cell types. Meristematic cells that are totipotent can differentiate into protective, vascular, storage or reproductive tissues.[2] Specialized structures called xylem and phloem are used in the transport of nutrients between different parts of the plants. They also have roots for taking up water and minerals. The xylem is involved with the transport of water and minerals to the rest of the plant from the roots and the phloem is involved with the transport of sugars from the leaves to the roots.[2]

1.1. Medicinal plants

Medicinal plants are generally used to treat specific conditions to maintain health in both modern and traditional medicine.[3] Medicinal plants provide three main benefits: health

benefits; financial benefits to people who sell them; and society-wide benefits.[4] However, development of plants or extracts having potential medicinal uses is blunted by weak scientific evidence, poor practices in the process of drug development and insufficient financing.[3]

1.2. Pitaya

Pitaya, commonly known as 'Dragon Fruit' in English and 'Buah Naga' in Malay belongs to the *Cactacae* family.[4] It holds the generic name *Hylocereus.* Three species of dragon fruits that are commonly used are present with varying peel colour and varying pulp colour. The fruit with red peel and white pulp has the scientific name *Hylocereus undatus;* the fruit with red peel and red pulp is named as *Hylocereus polyrhizus;* and the third species with yellow peel and white pulp is *Hylocereus megalanthus. Hylocereus megalanthus* is the hybrid of *Hylocereus costaricensis* and *Selenicereus inermis*. All these fruits have small black seeds interspersed in the pulp.[5]

1.3. Taxonomy

Kingdom	**:**	Plantae
Clade	**:**	Angiosperms
Clade	**:**	Eudicots
Order	**:**	Caryophyllales
Family	**:**	Cactacea
Subfamily	**:**	Cactoidea
Genus	**:**	*Hylocereus*
Botanical name	**:**	*Hylocereus undatus*

1.4. Angiosperms

Angiosperms are flowering and fruiting plants. The number of species range from 250,000 to 450,000.[6] Apomixis, reproduction through asexually formed seeds, is found in the 2.2% of the angiospermic genera.[7]

1.5. Eudicots

Eudicots comprise of about 75% of the angiosperm species.[8] Eudiots are the most diverse three major clades of angiosperms.[9] Eudicots are divided into two groups: the basal Eudicots and the core Eudicots.[10] Eudiots are monophyletic group. Tricolpates are a different name for Eudicots. This name was given by some botanists to avoid confusions with dicots.

1.6. Caryophyllales

Caryophyllales are an order of flowering plants. They comprise of about 6% of the Eudicots. Caryophyllales contain 33 families, 692 genera and 11,155 species. Caryophyllales are divided into two sub-orders: Caryophyllineae and Polygonineae.[11]

1.7. Morphology

Dragon fruit stems are of climbing, creeping and sprawling habit. They also branch profusely. There can be 4-7 fruits, which can be between 4 and 10 meter long and 10-12 cm thick. The distance between areoles is 2-5 cm. Spines on the adult branches are 1-4 mm long, being acicular to almost conical. Colour ranges from grayish –brown to blue-green.[12] The fruit is oblong to oval in shape. It is 4-10 cm long and 3-8 cm thick. It is red in colour with large bracteoles. These fruits have a white pulp and contain many small, black interspersed seeds that are edible.[12]

1.8. Location

The dragon fruit plant is a vine, epiphytic cacti which is believed to be the native of Mexico and was transplanted to Central America by Europeans.[5] It has been brought to Southeast Asian countries including Malaysia, Indonesia, Taiwan, Thailand, Sri Lanka, Bangladesh and Vietnam. Pitaya is now widely cultivated in the United States, the Canary Islands, Australia, Cyprus, Israel and Southeast Asia.

1.9. Secondary metabolites

Plants produce a wide variety of organic molecules which do not directly take part in the growth and development of the plant. These molecules are called secondary metabolites. Plant secondary metabolites have been used as sources for food additives, flavour, pharmaceuticals and other industrial materials. Unlike primary metabolites, the absence of secondary metabolite do not cause the death of the plant, instead result in the long-term effects. Secondary metabolites play an important role in defense mechanism in the plants. A large amount of secondary metabolites act as anti-microbial molecules.[13] Secondary metabolites are important for various aspects. They act against the microbes and hence act as a potent anti-microbial agent. An interesting aspect of secondary metabolite is that, it can be used as metal transporters. Some of these metabolites also act as sex hormones. Hence, secondary metabolites are the natural products obtained from the plants.[13] The aim of our study is to identify the possible phytochemical compounds along with its functional groups

present in the methanolic extract of *hylocereus undatus* seeds along with its pharmacological effects in gram-negative and gram-positive bacterial species.

2. MATERIALS AND METHODOLOGY

2.1. Collection of Sample

The *Hylocereus undatus* fruits were purchased from the market and authenticated by a Botanist, from the Department of Botany, Bangalore University, Bangalore.

2.2. Sample Preparation

Dragon fruit was washed and cut using a knife into two pieces. The pulp along with the seeds was soaked in water overnight. The seeds were then separated manually. The obtained seeds were dried in oven at 50°C for 24 h. The dried seeds were then crushed using motor and pestle to a fine powder and stored at room temperature for further analysis. Approximately 8.5 g of seeds were obtained from 300 g fruit.

2.3. Extraction

The seed powder was extracted using distilled water and methanol. It was then filtered using Whatmann No.1 filter paper to obtain a clear filtrate. The resulting filtrate was used for phytochemical analysis. The methanolic extract of seed powder was done using soxhlate apparatus. The resulting extract was used for GC/MS and FTIR analysis. 1 g of seed powder was dissolved in 20 ml of water in test tube. Tube was shaken vigorously for about 10 min. The contents were then filtered using Whatmann No.1 filter paper. The resulting filtrate was then diluted in 1:10, 1:20, 1:30, 1:40 and 1:50 ratio. These extracts were then used for quantitative analysis for carbohydrates, proteins and phenols.

2.4. Phytochemical analysis

Small amounts of both the aqueous and methanolic extract were used for the phytochemical analysis. The phytochemical tests include test for alkaloids, flavonoids, tannins and phenols, saponins, oils, anthraquinone, coumarins, terpenoids, steroids, carbohydrates, amino acids and proteins were performed qualitatively.

2.5. Preparation of the *Hylocereus undatus* seed methanolic extract for GCMS analysis

10 g of the dried *Hylocereus undatus* seed material was powdered and placed in Soxhlet extractor along with 150 ml of methanol, refluxed at 60^{o}C for 8h and filtered through Whatmann No. 1 filter. The filtrate was evaporated to dryness at 80^{o}C and stored until further

analysis. For analysis, the dried material was reconstituted in methanol in 1 ml methanol, and the reconstituted material was subjected for GCMS analysis as described below.[14]

2.6. Identification of Compounds

Interpretation of mass spectrum GC-MS was done using the database of National Institute of Standard and technology (NIST). The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library.[14]

2.7. Estimation of Carbohydrates by Ortho-Toluidine Method

Glucose condenses with ortho-toludine in glacial acetic acid when heated to 100^{o}C. The product formed is N-Glycosylamine which was blue green in colour, the absorbance which is measured at 630 nm.

2.8. Estimation of Protein by Lowry's Method

Protein reacts with Folin-Ciocalteau reagent to give a coloured complex which was due to the reaction of alkaline copper protein and the reduction of phosphomolybdate by tyrosine and tryptophan present in the protein. The intensity of the colour depends on the amount of these aromatic amino acids.

2.9. Estimation of Total Phenols by F C Method

The colorimetric method is the most widely used method for the estimation of total phenolic content. The reagent used for this estimation is the Folin-Ciocalteu reagent, which is a mixture of phosphomolybdate and phosphotungstate. This method consists of calibration using the standard phenolic compounds. FC reagent reacts with the nitrogen-containing compounds to form a blue coloured complex. The intensity of the color was read at 650 nm.

2.10. Estimation of antioxidant property by FRAP assay

Ferric reducing antioxidant power (FRAP) is a widely used method to determine the antioxidant capacity of the samples. This method uses antioxidants as reductants in a redox-linked colorimetric reaction in which ferric (Fe^{3+}) is reduced to ferrous (Fe^{2+}). The reduction of ferric to ferrous at low pH leads to the formation of a coloured ferrous-probe complex from a colourless ferric-probe complex.

2.11. Fourier Transform Infrared Spectrophotometer (FTIR) Analysis

Fourier Transform Infrared Spectrophotometer (FTIR) is the most important and powerful tool for identifying the functional groups present in the sample. The wavelength of light

observed is the characteristic of the chemical bond. The chemical bonds in a molecule can be determined by interpreting the infrared absorption spectrum.

2.12. Antibiotic Sensitivity Test

Micro titer plate method (96 wells) to check the Minimum Inhibition Concentration (MIC). The dragon fruit seed sample was assessed for its MIC property against organisms (*Staphylococcus aureus and E.coli*). 100 µl of culture was inoculated to serially diluted sample wells. *Staphylococcus aureus* culture was inoculated to wells from C4 –C11 and *E.coli* was inoculated to wells from D4 –D11 and 30 µl of 0.1% of resazurin was added to all the test sample wells. The plates were incubated at 37°C for 24 h. Presence of blue colour indicates no growth of the organism whereas appearance of pink colour indicates growth.

3. RESULTS

3.1. Phytochemical analysis

Table – 1: Qualitative analysis for phytochemicals present in methanolic extract of *Hylocereus undatus* seed.

Phytochemicals	Test	Observations	Inference
Alkaloids	Wagner's Test	Reddish-brown coloured precipitate.	Presence of Alkaloids
Flavonoids	2 M HCl + Aqueous NaOH	Yellow colour is observed.	Presence of Flavonoids
Tannins and Phenols	10% Lead Acetate + $FeCl_3$	Brick red colour is observed at top layer of the test tube White colour is observed at the bottom of the test tube.	Presence of Tannins and Phenols
Saponins	Foam Test	Absence of Foam is observed.	Absence of Saponins
Oils	Spot test	Oil stains are observed.	Presence of oil
Anthraquinone	Borntrager's test	Pink colour is observed	Absence of anthraquinone
Coumarins	NaOH test	Yellow colour change is observed.	Presence of coumarins
Steroids	Acetic Anhydride	Violet to Blue or Green is observed.	Absence of Steroids
Terpenoids	Salkowski's Test	Reddish Brown colour is observed.	Presence of Terpenoids
Carbohydrates	Benedict's test	Red colour precipitate is observed	Presence of Carbohydrates
Amino Acids	Ninhydrin Test	Purle Colour is not observed	Absence of Amino Acids
Proteins	FC Reagent	Green Colour is observed.	Presence of Proteins

Table – 2: Qualitative analysis for phytochemicals present in water extract of *Hylocereus undatus* seed.

Phytochemicals	Test	Observations	Inference
Alkaloids	Wagner's Test	Reddish-brown coloured precipitate.	Presence of Alkaloids
Flavonoids	2 M HCl + Aqueous NaOH	Yellow colour is observed.	Presence of Flavonoids
Tannins and Phenols	10% Lead Acetate + $FeCl_3$	Brick red colour is observed at top layer of the test tube White colour is observed at the bottom of the test tube.	Presence of Tannins and Phenols
Saponins	Foam Test	Appearance of Foam is observed.	Presence of Saponins
Oils	Spot test	Oil stains are observed.	Absence of oil
Anthraquinone	Borntrager's test	Pink colour is observed.	Absence of anthraquinone
Coumarins	NaOH test	Yellow colour change is observed.	Presence of coumarins
Steroids	Acetic Anhydride	Violet to Blue or Green is observed.	Absence of Steroids
Terpenoids	Salkowski's Test	Absence of Reddish Brown colour.	Absence of Terpenoids
Carbohydrates	Benedict's test	Red colour precipitate is observed.	Presence of Carbohydrates
Amino Acids	Ninhydrin Test	Purle Colour is not observed.	Absence of Amino Acids
Proteins	FC Reagent	Green Colour is observed.	Presence of Proteins

3.2. Gas Chromatography Mass Spectrometry (GCMS) Analysis

The GCMS chromatogram for the methanolic extract of *Hylocereus undatus* seed is shown in the Figure – 1 and the interpretations are given in Table – 3.

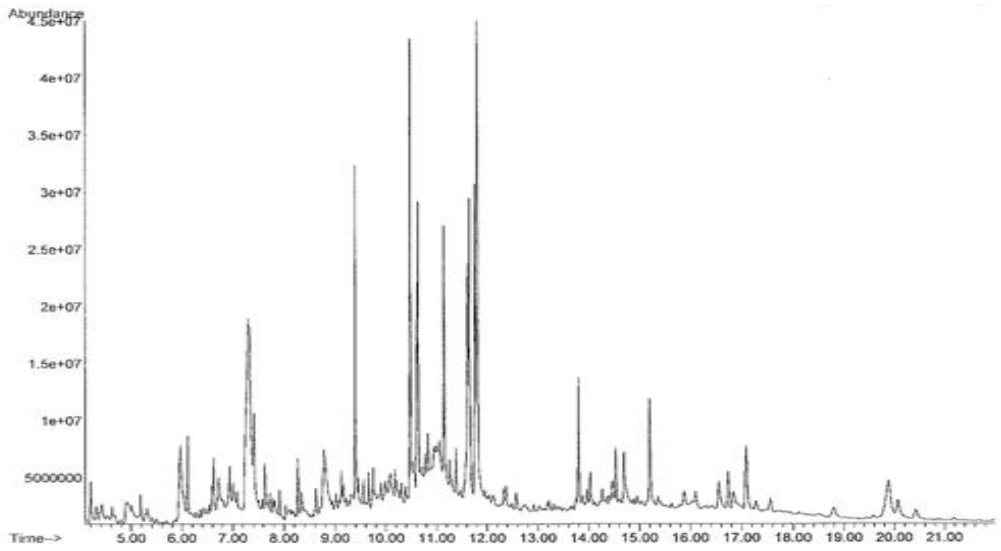

Figure – 1: GC- MS chromatogram of methanolic extract of *Hylocereus undatus* seed.

Table – 3: GC- MS chromatogram of methanolic extract of *Hylocereus undatus* seed.

Retention Time	Name of the Compounds	Peak area	Activity
7.303	S-(-)-1,2,4-butanetriol 2-acetate	11.20	Used for drug delivery, Anti-bacterial
	Propanoic acid		Anti-viral, Non-nucleoside reverse transcriptase inhibitor, Treatment of Flavivirus infection, Anti-bacterial
			Anti-viral, Cyclic protein tyrosine kinase inhibitor
9.454	Tetradecanoic acid	0.33	Anti-constipation, Protein kinase inhibitor, Used in the treatment of mycosis, neoplastic diseases, inflammatory, immune diseases
	Nonanoic acid		Anti-bacterial, Inhibition of blood platelet aggregation, Immunosuppressant Immunomodulators, Used in the treatment of obesity, schizophrenia and bipolar disease, HIV integrase inhibitor
	Octadecanoic acid		
10.484	Octadecanoic acid	6.92	Immunomodulators, Used in the treatment of obesity, schizophrenia and bipolar disease, HIV integrase inhibitor
	n-hexadecanoic acid		Anti-oxidant, Hypocholesterolemic, Anti-androgenic, Hemolytic, Lubricant, 5-Alpha reductase inhibitor, antipsychotic.
10.610	9,12,15-octadecatrienoic acid	2.39	Antimicrobial, Anticancer, Hepatoprotective, Anti-arthritic, anti-asthama, diuretic.
	Methyl-8,11,14-heptadecatrienoate		Anti-malarial, anti-dengue, anti-filariasis.
	9,12,15-octadecatrienoic acid		Antimicrobial, Anticancer, Hepatoprotective, Anti-arthritic, anti-asthama, diuretic.
11.143	Phytol	3.66	Antimicrobial, Anti-inflammatory, Anticancer, Diuretic, Antifungal against S. typhi, resistant gonorrhea, joint dislocation, headache, hernia, stimulant and anti-malarial
11.382	9,12,15-octadecatrienoic acid	0.59	Antimicrobial, Anticancer, Hepatoprotective, Anti-arthritic, anti-asthama, diuretic.
	Methyl ester		Antimicrobial, Anticancer, Hepatoprotective, Anti-arthritic, anti-asthama, diuretic.
	7,10,13-hexadecatrienoic acid		Anti-depressant, Used in the treatment of psychiatric and neurodegenerative diseases
	Methyl ester		No function reported

11.610	9,17-octadecadienal 8-hexadecyne 2-chloroethyl linoleate	4.09	Anti-depressant, Antioxidant activity, Used in the treatment of neurodegenerative diseases Antianrogenic agent, Anti-cancerous No function reported
11.646	9,12-octadecadienoic acid Cis-7-dodecen-1-yl acetate	4.52	Hepatoprotective, antihistaminic, hypocholesterolemic, anti-eczemic Used in the neurodegenerative diseases
11.760	9,12-octadecadienoic acid 9,12,15-octadecatrienoic acid Methyl ester	4.24	Hepatoprotective, antihistaminic, hypocholesterolemic, anti-eczemic Antimicrobial, Anticancer, Hepatoprotective, Anti-arthritic, anti-asthama, diuretic. Antimicrobial, Anticancer, Hepatoprotective, Anti-arthritic, anti-asthama, diuretic.
11.808	9,12,15-octadecatrienoic acid Methyl-8,11,14-heptadecatrienoate	8.27	Antimicrobial, Anticancer, Hepatoprotective, Anti-arthritic, anti-asthama, diuretic Anti-malarial, anti-dengue, anti-filariasis

3.3. Estimation of Carbohydrates by Ortho-Toluidine Method

The total amount of carbohydrates present in the water extract of *Hylocereus undatus* seed was found to be 0.71 mg/ml.

3.4. Estimation of Protein by Lowry's Method

The total amount of proteins present in the water extract of *Hylocereus undatus* seed was found to be 2.48 mg / ml.

3.5. Estimation of total phenols by F C Method

The total amount of phenols present in the water extract of *Hylocereus undatus* seed was found to be 0.35 mg /ml.

3.6. Estimation of antioxidant property by FRAP assay

The total amount of antioxidants present in the methanolic extract of *Hylocereus undatus* seed was found to be 0.08 mg / ml.

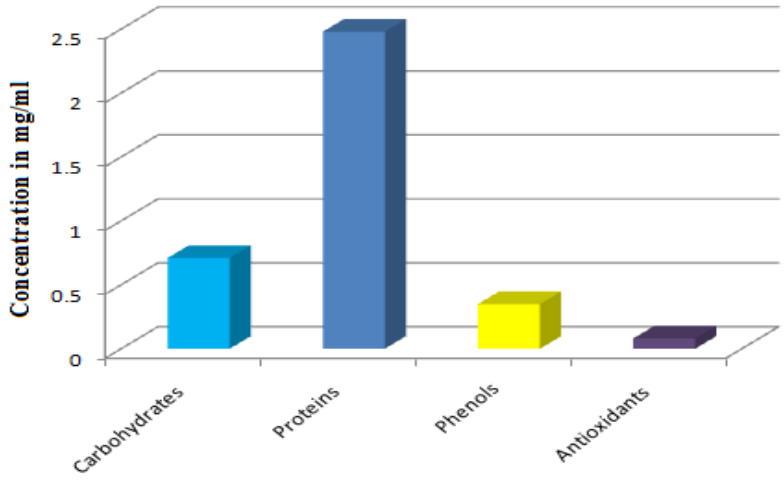

Figure – 2: Comparison of concentration of Carbohydrates, proteins, phenols and antioxidants present in methanolic extract of *Hylocereus undatus* seed.

3.7. Fourier Transform Infrared Spectrophotometer (FTIR) Analysis

Infrared spectroscopy spectrum and its table for *Hylocereus undatus* seed dry powder and methanolic extract are given in Figure – 3, Figure – 4, Table – 5 and Table – 6.

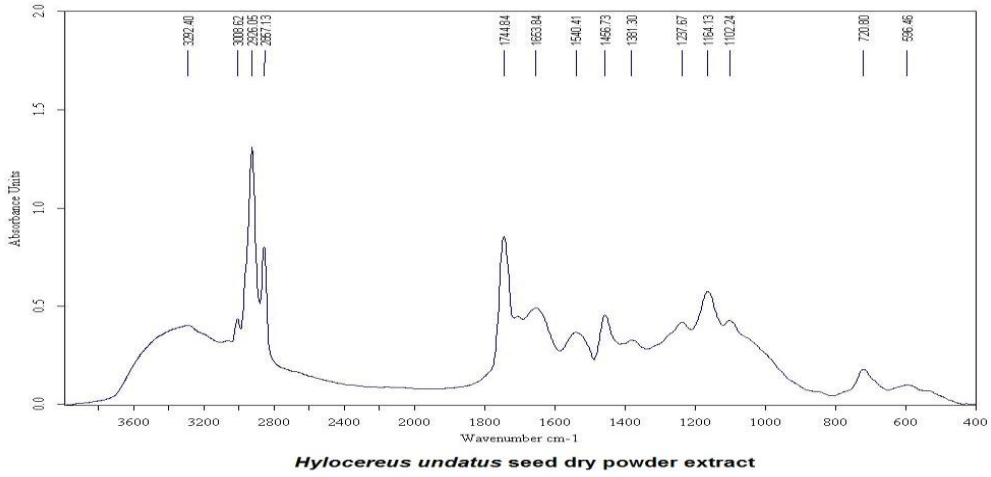

Figure – 3: Infrared spectroscopy spectrum for *Hylocereus undatus* seed dry powder.

Table – 4: Infrared spectroscopy table for *Hylocereus undatus* seed dry powder.

S. No	Frequency	Group	Intensity
1	3373.86	Alcohols, phenols (hydrogen bonded)	Broad, small
2	2926.23	Carboxylic acids (hydrogen bonded)	Broad, variable
3	2858.41	Alkyl (C-H [stretching])	Medium-small
4	1727.80	Carboxylic acids	Small
5	1657.54	C=O (stretching)	Small
6	1510.83	Aromatic (C=C [stretching])	Medium
7	1451.83	Aromatic (C=C [stretching])	Medium

8	1278.19	C-N	
9	1057.23	Primary alcohol (C-O [stretching])	
10	915.77	Alkenyl (out-of-plane C-H bending)	Small
11	868.84	p-substituted benzene (out-of-plane bending)	
12	818.99	Aromatic (p-disubstituted)	Very small
13	775.65	Aromatic (m-disubstituted)	Very small
14	708.75	Aromatic (o-disubstituted)	Small
15	627.32	Alkyl halide (C-Cl)	Small

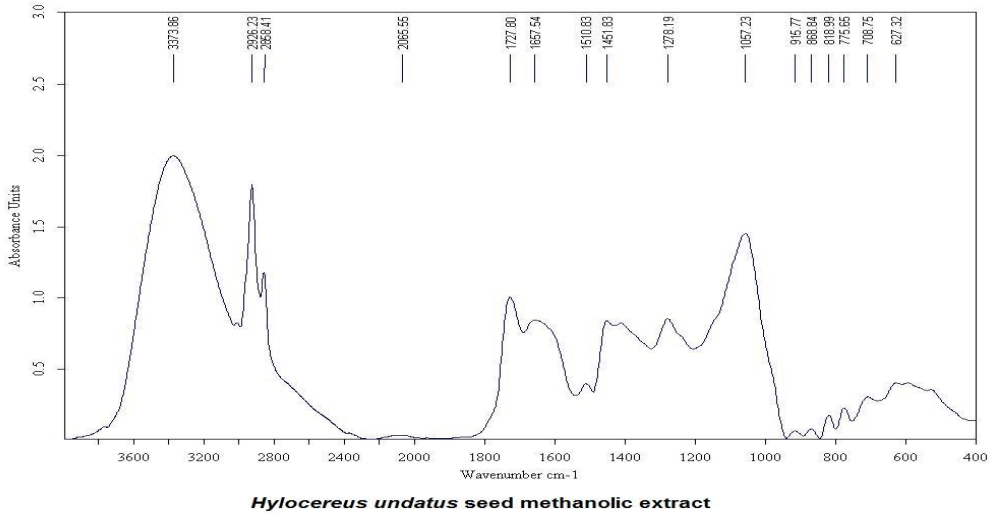

Figure – 4: Infrared spectroscopy spectrum for *Hylocereus undatus* seed methanolic extract.

Table – 5: Infrared spectroscopy table for *Hylocereus undatus* seed methanolic extract.

S. No	Frequency	Group	Intensity
1	3292.40	Alcohols, phenols (hydrogen bonded)	Broad, small
2	3008.62	Alkene (C-H)	Medium-small
3	2926.05	Alkyl (C-H[stretching])	Medium-small
4	2867.13	Alkyl (C-H[stretching])	Medium-small
5	1744.84	Esters	Small
6	1653.84	C=O (stretching)	Small
7	1540.41	Aromatic (C=C [stretching])	Medium
8	1456.73	Aromatic (C=C [stretching])	Medium
9	1381.30	Isopropyl (-CH(CH_3))	Small
10	1237.67	Ethers and alcohols (C-O-C [stretching])	Small
11	1164.13	Ethers and alcohols (C-O-C [stretching])	Small
12	1102.24	Ethers and alcohols (C-O-C [stretching])	Small
13	720.80	Alkenyl (cis-RCH=CHR)	Small
14	596.46	Alkyl halide (C-Br)	Small

3.8. Minimum Inhibitory concentration

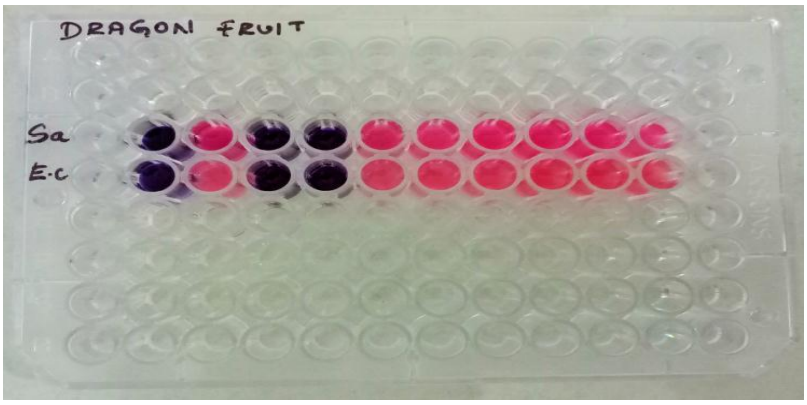

Figure – 5: Micro titer plate showing the Minimum Inhibitory concentration (MIC).

The minimum inhibitory concentration (MIC) of the sample is as follows:

Table – 6: Minimum inhibitory concentration (MIC) in µl against organism.

Test organism	Minimum Inhibitory Conc. (MIC) (µl)
Staphylococcus aureus	50
E.coli	50

From the above table, it is observed that the minimum inhibitory concentration (MIC) is 50 µl for both the bacterial species.

4. DISCUSSION

4.1. Phytochemical analysis in the water and methanolic extract of *Hylocereus undatus* seed

From the Table – 1 and Table – 2, the phytochemical components present in the water and methanolic extract of *Hylocereus undatus* seed are found to have alkaloids, flavonoids, tannins, phenols, carbohydrates, coumarins and proteins. The methanolic extract was found to have oils and terpenoids which were absent in the water extract. The water extract was found to have saponins which was absent in methanolic extracts. Steroids, anthroquinones and amino acids were absent in both the extracts.

Alkaloids like donepezil, tacrine, rivastigmine, velnacrine are cholinesterase inhibitors which are used in the treatment of Alzheimer's disease. Another set of alkaloids which belong to steroidal, triterpenoidal and *lycopodium* class like sarcodine, saracocine, epipachysamine-D, buxamine B and C, conessimin, lycoparin have anticholinesterase activity.[15]

The aqueous and methanolic extract was found to contain flavonoids. Flavonoids are the secondary metabolites present in the plants. Flavonoids such as isoflavones, quercetin,

isoflavonoids, naringenin, kaempferol, chalcones, flavans and many other flavonoids have been found to have anti-microbial activites.[16] Flavonoids such as kaempferol, quercetin and isorhamnetin were found to be present in higher amounts in the peel. These flavonoids possess radical scavenging and metal chelating properties.[4]

Tannins are a class of polyphenolic groups having the molecular weight ranging from 500 to 3000. Both aqueous and methanolic extract of the seed powder was found to contain tannins and phenols. Condensed tannins are found to lower the blood glucose levels in the body. Both condensed tannins and hydrolyzable tannins are found to have anti-bacterial, anti-viral, anti-inflammatory, anti-cancerous properties and also various other properties.[17] Condensed tannins include proanthocyanidins and anthocyanidins and hydrolyzable tannins include gallotannins, ellagitannins.[18] Phenolic compounds like tyrosol and hydroxytyrosol were found to have anti-microbial activity, anti-inflammatory activity and anti-cholestrimic effect.[19] The previous studies on the pulp and peel of *Hylocereus undatus* showed the presence of betacyanin, a red coloured pigment was responsible for the antioxidant property.[20] Polyphenols present in the pulp and peel were also found to show antibacterial properties, anti-thrombotic effect.[4]

Saponins were found to be present in the aqueous extract but not in the methanolic extract. These are glycosides of steroids with a distinctive foaming property. Saponins provide a waxy layer on the plants which are important for the plant protection. Saponins like oleanolic acid, betulininc acid, lupane aglycone are responsible for the anti-dermatophytic activity, anti-fungal activity, anti-cancerous activity and anti-inflammatory activity. They are also used to treat chronic kidney diseases and type-2 diabetes.[21]

Coumarins were found to be present in both the aqueous and methanolic extract of the seed powder. Coumarins such as phenylpropanoids have anti-bacterial, anti-tubercular, anti-fungal, anti-viral, antioxidant, anti-inflammatory properties.[22]

Upon testing for the presence of oils, only the methanolic extract gave positive results. Functional lipids which include conjugated linoleic acids, omega-3 fatty acids, phytosterols and medium chain triglycedrides have beneficial effects on the bone health and obesity. They are also used in the treatment of depression, blood pressure and cardiovascular health [23] Previous studies on the seed extract showed to contain higher amounts of linolenic acid which is used to keep the skin moist.[24]

Carbohydrates present in the aqueous and methanolic extract were tested in the presence of Benedict's reagent. Both the extracts showed the presence of sugars. Methanolic extract contained high amounts of sugars as compared to aqueous extracts. Proteins are a major part of the plants which are made up of amino acids. Proteins were found to be present in both the aqueous and methanolic extract of seed powder. Terpenoids such as limonene, carvone, retinoids, lutein, and lycopene have effects on carcinogensis, cardiovascular diseases, and oxidative stress.[25] β-amyrin, a triterpenoid, was found in the peel which was effective against cancer cell lines.[4]

4.2. Gas Chromatography Mass Spectrometry (GCMS) Analysis

From the Figure – 1 and Table – 3 the GC-MS chromatogram of a methanolic extract of *Hylocereus undatus* seed are showed nearly 25 compounds. Most of the compounds which were reported from seeds were found to be rich in S-(-)-1,2,4-butanetriol, 2 acetate, Propanoic acid, Tetradecanoic acid, Nonanoic acid, Octadecanoic acid, n-hexadecanoic acid, 9,12,15-octadecatrienoic acid, Methyl-8,11,14-heptadecatrienoate, Phytol, 7,10,13-hexadecatrienoic acid, 9,17 – octadecadienal, 8 – hexadecyne, 2 – chloroethyl linoleate, 9,12–octadecadienoic acid, Cis-7-dodecen-1-yl acetate, Methyl-8,11,14-heptadecatrienoate at retention time 7.303, 9.454, 10.484, 10.610, 11.143, 11.382, 11.610, 11.646, 11.760 and 11.808 respectively.

S-(-)-1, 2, 4 –butanetriol having the retention time of 7.303 and measuring the peak area of 11.20 is used for drug delivery and anti-bacterial agent. 2 acetate having the same retention time and peak area is used as anti-viral agent, non-nucleoside reverse transcriptase inhibitor, in treatment of Flavivirus infection and as anti-bacterial agent. Propanoic acid is used as anti-viral agent and cyclic protein tyrosine kinase inhibitor. At the retention time of 9.454 and measuring the peak area of 0.33, the major compounds found are -Tetradecanoic acid, used as anti - constipation agent, protein kinase inhibitor, used in the treatment of mycosis, neoplastic diseases, inflammatory and immune diseases. Nonanoic acid is used as anti-bacterial agent, in inhibition of blood platelet aggregation and as immunosuppressant. Octadecanoic acid is used as immunomodulators, in the treatment of obesity, schizophrenia and bipolar disease and as HIV integrase inhibitor.[5]

n-hexadecanoic acid having the retention time of 10.484 and measuring the peak area of 6.92 is used as anti-oxidant, hypocholesterolemic agent, as anti-androgenic agent, as hemolytic,agent, as lubricant, 5-Alpha reductase inhibitor and antipsychotic agent. 9,12,15-

octadecatrienoic acid and Methyl-8,11,14 heptadecatrienoate having the retention time of 10.610 and measuring the peak area of 2.39 is used as antimicrobial, anticancer, hepatoprotective, anti-arthritic, anti-asthama, diuretic and anti-malarial, anti-dengue, anti-filariasis agent respectively. Phytol having retention time of 11.143 and measuring the peak area of 3.66 is used as antimicrobial, anti-inflammatory, anticancer, diuretic, antifungal against *S. typhi*, resistant to gonorrhe, ,helps in joint dislocation, reduce headache, hernia, and used as anti-malarial agent. 7, 10, 13-hexadecatrienoic acid having retention time at 11.382 and measuring the peak area of 0.59 is used as anti-depressant and in the treatment of psychiatric, neurodegenerative diseases. 9,12-octadecadienoic acid and Cis-7-dodecen-1-yl acetate having retention time of 11.610 and measuring the peak area of 4.09 used as hepatoprotective, antihistaminic, hypocholesterolemic, anti-eczemic and used in the neurodegenerative diseases respectively. Methyl-8, 11, 14-heptadecatrienoate having retention time at 11.808 and measuring the peak area of 8.27 is used as anti-malarial, anti-dengue and anti-filariasis agent.[26]

4.3. Carbohydrates concentration in the water extract of *Hylocereus undatus* seeds

From the Figure – 2, the total amount of carbohydrates present in the water extract of *Hylocereus undatus* seed was found to be 0.71 mg/ml. Carbohydrates have been effective in the treating chronic diseases like cardiovascular diseases and cancer. They are also found to treat gastrointestinal diseases.[27] As per the previous studies, it has been proved that the pitaya peel contains soluble fibers which have positive effects in the digestive process and also in neutralizing the toxic substances.[28]

4.4. Protein concentration in the water extract of *Hylocereus undatus* seeds

From the Figure – 2, total amount of proteins present in the water extract of *Hylocereus undatus* seed was found to be 2.48 mg/ml. Previous studies on the peel extract showed to contain 0.95± 0.15 of protein, which is lower as compared to other fruits.[29] Proteins are shown to have anti-proliferative, antioxidant and anti-microtubule activities.[30]

4.5. Phenols concentration in the water extract of *Hylocereus undatus* seeds

From the Figure – 2, the amount of polyphenols present in water extract of *Hylocereus undatus* seed is 0.35 mg/ml. Polyphenols in plants are mostly in the form of flavonoids that acts as an antioxidants. In addition, the other classes of polyphenols include tannins and lignins. Some of the other phenolic components include Betalains, gallic acid and betacyanins.[18]

4.6. Antioxidant property in the water extract of *Hylocereus undatus* seeds

From the Figure – 2, the total amount of antioxidants present in the water extract of *Hylocereus undatus* seeds was found to be 0.08 mg/ml. FRAP assay estimates the reducing ability of antioxidants against free radical effect of reactive oxygen species. Total antioxidant power directly refers to the total reducing power. The radical scavenging and inhibition of lipid peroxidation by the extract was due to the quenching free radicals or reduction of Fe^{3+} to Fe^{2+}, which can be attributed to the presence of the number of polyphenolics such as flavonoids, anthocyanins etc.[31]

4.7. Fourier Transform Infrared Spectrophotometer (FTIR) Analysis

FT-IR spectroscopy is used to identify some qualitative aspects regarding the organic compounds in the *Hylocereus undatus* seeds. Several indicator bands that are pertained to functional groups represent chemical components or metabolic products. The FT-IR spectrum exhibits the characteristic fingerprint band features. The infrared spectrum can identify not only the major components in organic materials but also to find some differences among them.

From the Figure – 3 and Table – 4, the *Hylocereus undatus* seeds dry powder the very strong absorption bands at 3373.86 cm^{-1} due to hydrogen bond which corresponds to alcohol and phenols, 2926.23 cm^{-1} due to hydrogen bond which corresponds to carboxylic acids, 2858.41 cm^{-1} due to stretching of C-H bonds which corresponds to alkyl groups, 1727.80 cm^{-1} corresponds to carboxylic acids, 1657.54 cm^{-1} is due to stretching of C=O bonds which might correspond to carbonyl structure of ketones,[32] 1510.83 cm^{-1} due to stretching of C=C bonds which corresponds to aromatic compounds, 1451.83 cm^{-1} due to stretching of C=C which corresponds to aromatic compounds ,1278.19 cm^{-1} is due to C-N bonds, 1057.23 cm^{-1} is due to stretching of C-O bonds which corresponds to primary alcohols ,915.77 cm^{-1} due to bending of C-H bonds which corresponds to alkenyl compounds, 868.84 cm^{-1} due to out-of-plane bending which corresponds to p-substituted benzene, 818.99 cm^{-1}, 775.65 cm^{-1} and 708.75 cm^{-1} corresponds to para-, meta- and ortho-disubstituted aromatic compounds, and 627.32 cm^{-1} is due to C-Cl bond which indicates the presence of alkyl halides.

From the Figure – 4 and Table – 5, the *Hylocereus undatus* seeds methanolic extract the very strong absorption bands at 3292.40 cm^{-1} due to hydrogen bonds which corresponds to alcohols and phenols, 3008.62 cm^{-1} due to C-H bonds which corresponds to alkenes, 2926.05 cm^{-1} and 2867.13 cm^{-1} due to stretching of C-H bonds which correspond to alkyl groups,

1744.84 cm^{-1} corresponds to esters, 1653.84 cm^{-1} due to stretching of C=O bonds which might correspond to carbonyl structure of ketones [32], 1540.41 cm^{-1} and 1456.73 cm^{-1} due to stretching of C=C bonds which corresponds to aromatic compounds, 1381.30 cm^{-1} due to – $CH(CH_3)$ which corresponds to isopropyl group, 1237.67 cm^{-1}, 1164.13 cm^{-1} and 1102.24 cm^{-1} due to stretching of C-O-C bonds which corresponds to ethers and alcohols, 720.80 cm^{-1} due to cis-RCH=CHR which corresponds to alkenyl compounds and 596.46 cm^{-1} due to C-Br which indicates the presence of alkyl halides.

Previous studies on FTIR analysis of the *H. undatus* peel extract was reported to show maximum peaks at: 3420-3433, which was due to O-H stretching of phenols or carboxylic acids; 2934-2922, which was due to C-H stretching of alkane; 1600-1627 and 1409-1428, which was due to C=C stretching of alkene; and 1103-1022, which was due to C-OH of carboxylic acid. The studies on both the dry seed powder and methanolic extract of the seed extract showed maximum peaks at: 3373.86, which was due to alcohols or phenols (hydrogen bonded); 2926.23, which was due to carboxylic acids (hydrogen bonded) and 2926.05, which was due to C-H stretching of alkyl groups.

4.8. Minimum Inhibitory concentration against *E.Coli* and *Staphylococcus aureus*

There are two quantitative methods used to determine MIC values. They are microdilution and agar dilution.[33] Of this, the microdilution method is prefered due to its accuracy, standardization, less expensive, and easily carried out in normal laboratory. The addition of resazurin dye as a redox indicator enhanced the microdilution method from the problems associated with sparingly soluble test materials. Active bacterial cells reduce the non-fluorescent resazurin (blue) to the fluorescent resorufin (pink) which can be further reduced to hydroresorufin [34] as shown in Figure – 5, giving a direct quantifiable measure of bacterial metabolic activity. Anti-bacterial analysis was conducted using the micro titre method. From Figure – 5 and Table – 6, it was observed that the minimum inhibitory concentration (MIC) is 50 µl for the bacterial species, *Staphylococcus aureus* and *E.coli*.

5. CONCLUSION

On the basis of the results obtained in our study, it is observed that the seed contains high amounts of phytochemicals which have various activities like anti-cancerous activites, anti-microbial activities, antioxidant activities, etc.

GC/MS analysis for the methanolic extract was found to give 10 major peaks. The relatable compounds present in these peaks were octadecanoic acid, octadecenoic acid, tetradecanoic acid and octadecatrienoic acid. Also the quantitative analysis of the seed extract for carbohydrates, proteins and phenols showed that the amount of protein was higher as compared to carbohydrates and phenols. The amount of protein was found to be 2.48 mg, carbohydrates was found to be 0.71 mg and phenols was found to be 0.35 mg. Proteins have found to have antioxidant activities as well which will increase the antioxidant properties of the seed. FRAP assay was conducted to check the antioxidant activity of the seeds. 0.08 mg of antioxidants was found to be present in the seed. FTIR analysis was conducted to find out the functional groups present in the seed. As per the data analysis of FTIR for dry powder, it was found that alcoholic and phenolic groups at the frequency of 3373.86 and carboxylic acids at frequency of 2926.23 gave maximum peaks. Hence, we can conclude that the dry seed powder is rich in alcoholic, phenolic and carboxylic acid groups. As per the data analysis of FTIR for methanolic extract, it was found that alkyl group at the frequency of 2926.05 gave maximum peak. Hence, we can conclude that the methanolic extract is rich in alkyl group. Anti-bacterial analysis was conducted using the micro titre method. It was observed that the minimum inhibitory concentration (MIC) is 50 µl for the bacterial species, *Staphylococcus aureus* and *E.coli*.

5.1. Suggestion for further research

The present study on *Hylocereus undatus* seeds extract proved that the seeds confirm the existence of various biologically active molecules with its possible functional groups possessing Minimum Inhibitory concentration. Moreover, these fruits are already in use for a wide range of treatments traditionally and possess anticancer, antimicrobial activities etc. The present study may be an initiative for further phytochemical and pharmacological investigations required to separate the novel active compounds from the seeds to formulate new drugs to treat incurable diseases.

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