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INVESTIGATION OF PHARMACOGNOSTICAL CHARACTERISTICS, PHYTOCHEMICAL ANALYSIS AND ASSESSMENT OF ANTIOXIDANT ACTIVITY OF *MAMMILLARIA BENECKEI* C. EHRENB STEM UTILIZING *IN-VITRO* TECHNIQUES

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Antioxidant, Bioactive Compounds, Cactaceae, FRAP, Pharmacognostical Parameters, Phytochemical Screening

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ABSTRACT: Background: *Mammillaria beneckei* C. Ehrenb, a species of cactus native to regions of Mexico, has been recognized for its medicinal potential due to its rich phytochemical composition. It belongs to the family Cactaceae and the genus *Mammillaria*, which is known for its globular or cylindrical shape. This study aimed to perform comprehensive phytochemical profiling, pharmacognostical parameters and evaluate the antioxidant properties of stem of cactus to understand its therapeutic potential. The present research involves the extraction and identification of bioactive compounds from the plant using various solvent systems. **Methods:** The preliminary phytochemical screening was performed as per standard protocols to standardize the aim, and physicochemical investigations were established. The TPC, TFC, DPPH (2, 2-diphenyl-1-picrylhydrazyl) and FRAP (Ferric reducing antioxidant power) methods were performed to evaluate antioxidant activities using various extracts. **Results & Discussion:** In the preliminary phytochemical screening bioactive compounds like Alkaloids, flavonoids, terpenoids, saponins and phenolic compounds were found. The results show significant inhibitory effects. **Conclusion:** The main objective of the present study is to conduct a phytochemical analysis of pharmacognostical parameters of the stem of this plant to identify its constituent compounds and evaluate its antioxidant activity. Some bioactive compounds (mentioned in the below, result portion) are responsible for the plant's therapeutic action. It also shows potential pharmacological effects for treating oxidative stress, supporting its traditional use in herbal medicine. Future studies are due to explore its phytochemical compounds and their mechanisms of action.

INTRODUCTION: *Mammillaria beneckei* C. Ehnerb is a species of cactus that is known for its medicinal properties, which have been used in traditional practice.

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This species of cactus is native to the central region of Mexico City and this plant also found in Rajasthan, India, particularly arid and semi-arid regions.

It belongs to the family Cactaceae & the genus *Mammillaria*, which is known for its shape, mainly globular or cylindrical, and its distinctive tubercles arranged in spiral patterns. *Mammillaria beneckei* C. Ehnerb, commonly known as a type of cactus, has gained attention not only for its ecological adaptations but also for its potential medicinal

properties. Traditional medicine in many cultures has long been utilizing various parts of cacti for their specific therapeutic effects, which include anti-inflammatory, antioxidant, antimicrobial, anti-diabetic, and analgesic activities. Despite their widespread use in herbal medicine, a limited amount of scientific research on the specific pharmacological activities and bioactive compounds found in *M. beneckei* C. Ehnerb. The stem, which is the most prominent part of the cactus, contains bioactive substances that could offer valuable health benefits to us. This cactus is a generally small, slow-growing plant, usually characterized by a rounded body with dense clusters of spines, often pale or white in colour, which give it a soft, attractive appearance.

A unique feature of these plants is that, produce the vibrant flowers, typically blooming in a ring around the crown of the cactus. These flowers are usually white or pinkish. When the plant is in bloom mode, it adds a touch of beauty; like many cacti, this plant is easily grow up in dry place, arid environments and is drought-tolerant once established in any dry place or any desert area. It's mainly an ornamental plant due to its manageable size and aesthetic flowers. In terms of care, this plant needs a well-draining system and good soil and requires direct sunlight. It requires infrequent watering, mainly during the winter months, as overwatering can lead to rot. Like other cacti, it should be protected from frost. Having its eye-catching appearance and low maintenance requirements, this cactus may be a pleasant addition to any collection of succulents or cacti [1, 2].

The study of its pharmacognostical parameters, phytochemical profiling, and antioxidant activity is of significant interest in modern pharmacology. Phytochemical profiling involves the identification and quantification of phytochemical compounds which is present in the plant's part, such as Alkaloids, Flavonoids, Glycosides, Carbohydrates, Tannins & Phenolic compounds which proof the presence of its therapeutic effects. The antioxidant activity may be present in the stem of the *Mammillaria beneckei* C. Ehnerb plant, which is particularly important because oxidative stress is linked with various diseases, like cancer, cardiovascular disease, and neurodegenerative illness.

By using *in-vitro* methods (such as DPPH and FRAP assays), researchers can select the plant's, those having oxidative stress relief and the capacity to scavenge free radicals. The evaluation of these properties through scientific methods can contribute to the discovery of new natural Antioxidants and validate the plant's potential as a source of therapeutic compounds and bioactive compounds [3].

The extraction process focuses on obtaining the active compounds from the plant material for further evaluation of their biological activities, particularly antioxidant activity. Here, we follow the Successive Extraction process, where first we take the mature stem of *Mammillaria beneckei* C. Ehnerb, then dried under shade to protect it from direct sunlight and crush it in a grinder to prepare fine powder of mesh 40. The powder was then successively extracted with solvents from low polarity towards high polarity. Likewise, Pet. Ether, Chloroform, Methanol, Hydro-alcohol, and Water by using Funnel & Filter Paper. Then, the resulting extracts were filtered and dried through a rotary vacuum evaporator, after evaporating it transfer it on water bath at their respective temperature in the respective solvents. The first step in this direction is the characterization of the herb or a thorough pharmacognostic examination that provides the physical characteristics, exterior appearance, texture, and microscopy of the crude drug [4].

The present study comprises the analyses of the stem anatomy and structure as well as evaluations of their physicochemical parameters (loss on drying, foreign matter, ash values, extractive values), fluorescence analysis, preliminary phytochemical properties, total phenolic contents, total flavonoid contents and evaluation of antioxidant activity. In the end of this research, we can says that it will contribute to a better knowledge of the medicinal potential of cacti by bridging the gap between traditional applications and contemporary pharmacological validation [5].

As per our survey, the stem of *Mammillaria beneckei* C. Ehnerb has no well-recorded direct medicinal applicationin traditional systems in Mexican folk medicine [6].

FIG. 1: FLOWER OF CACTUS (*MAMMILLARIA BENECKEI* C. EHRENB)

MATERIALS AND METHODS:

Materials:

Plant materials: Stem of Cactus (*Mammillaria beneckei* C. Ehrenb)

Selection & Collection: *Mammillaria beneckei* C. Ehrenb, Cactus stems were selected based on traditional uses and literature survey. Then, the plants were collected and further proceed.

Identification & Authentication: The selected plant was collected in the flowering condition and was then transformed in the form of a herbarium as per guidelines and submitted to the Botanical Survey of India (Howrah, Shivpur), and authentication of the submitted plant was carried out by the authorized person [7].

Plant Processing: After collecting the plant parts, wash it clearly with water to remove if any unwanted materials present on them. Then, it was cut into small pieces to easily dry and dried in the shade for a few days. After complete drying, dried plant materials were prepared in coarsely powdered in the mechanical grinder, passed through a sieve, and stored in tightly closed containers [8].

Preparation of Extracts: The stem of *Mammillaria beneckei* C. Ehrenb was shade-dried below 40 °C and then powdered. Powdered crude drug (150 gm.) was taken for successive extraction through non-polar to polar solvents, such as Petroleum Ether (PE), Chloroform (CH), Methanol (MeOH), Hydro Alcohol (HA) (Alcohol: Water = 70:30), and Water (AQ), respectively, after 5-6 days. The extracts were filtered, followed by evaporation to dryness under a rotary evaporator, and finally transferred into a water bath at their respective solvent temperature [9].

Chemicals and Reagents: The major analytical grade chemicals were used as per the requirements like Pet. Ether, Chloroform, Methanol, HCl, sulphuric acid, α- naphthol, Benedict's reagent, Ninhydrin soln., Dragendroffs' reagent, Mayers' reagent, Hagers' reagent [10].

Equipment's: High-grade analytical equipment was used to perform this research work, such as a hot air oven, UV, Ultrasound-assisted sonicates, Water bath, Mettler, Microscope, Soxlet apparatus, pH meter, etc.

Glass Apparatus: Exquisite analytical grade glass apparatus was utilized in this research, including a beaker, funnel, glass rod, measuring cylinder, Petri dish, conical flask, test tube, test tube stand, test tube holder, spatula, glass slide, round bottom flask, spatula, cover slip, and other laboratory apparatus.

Methods:

Pharmacognostical Studies:

Macroscopic Evaluation: The macroscopical observation was carried out using standard methods to determine the shape, size, color, taste, and odor.

Microscopic Evaluation: To perform microscopic evaluation, fresh stem parts were collected and washed with distilled Water, then cut using the blade and razor method; the thinnest feasible transverse sections were prepared, PHLOROGLUCINOL: HCl = 1:1. Microscopic evaluation of this transverse section of the stem was carried out. Stains like safranin and fast green dye were used to stain the section.

The Crude powdered drug was mounted on a clean glass slide with the aid of reagents like phloroglucinol and conc. HCl, iodine solution & sulphuric acid to check for the presence of substances like fibres, oil glands, and calcium oxalate crystals [11].

Physicochemical Analysis: Physicochemical parameters such as loss on drying, foaming index, determination of volatile oil, total ash, water-soluble ash, acid insoluble ash, water-soluble extractive values, and alcohol soluble extractive values of the powdered samples were determined based on the WHO guidelines (WHO 1998) [12].

Preliminary Phytochemical Assessment: The presence of several kinds of phytochemicals, such as carbohydrates, alkaloids, phenolic and flavonoidal compounds, saponins, lipids, tannins, and steroids, was identified using a variety of chromophoric reagents. Its primary purpose was to find the active secondary metabolites. The qualitative results were expressed as (++) strongly positive results, (+) positive results, and (-) negative results for bioactive phytochemicals [13].

Total Phenolic Content: At first, 0.4 ml of the sample was withdrawn into the test tube and then, 0.4 ml of the Folin-Ciocalteu reagent and 4 ml of distilled Water were added to the sample, which was incubated for 5 minutes in a dark place. After this, 4 ml of 7% Na_2CO_3 solution was added to each tube, and volume makeup was done up to 10 ml with distilled Water, which was further incubated in a dark place for 90 minutes at room temperature. Absorbance value were taken by using the UV-vis spectrophotometer at 730 nm against a blank; same methods are repeatedly occurred for all standard solutions. The total phenolic content in the extract expressed in Gallic acid equivalents (GAE) was calculated by the following formula:

$$T = C \times V / M$$

Where,

T = Total phenolic contents, mg g-1 plant extract, in Gallic Acid Equivalent (GAE).

C = Concentration (mg ml) of Gallic acid obtained from calibration curve.

V = Volume of extract (ml).

M = Weight (mg) of plant extract

The result was expressed as mg/g of Gallic acid equivalents in milligrams Gallic acid equivalent per gram (mg GAE/g) of dry extracts [14, 15].

Total Flavonoid Content: 2 mg of Quercetin was dissolved in 2 ml of Methanol to prepare a concentration of 1 mg/ml and finally diluted to 100 µg/ml, 200 µg/ml, 300 µg/ml, 400 µg/ml, and 500 µg/ml for standard. Take 1 ml of each different concentration of standard/ sample. Then, it was mixed with 0.2 ml of 10% Aluminium Chloride and 0.2 ml of 1M potassium acetate, and then, 5.6 ml of double distilled Water was added to each concentration. Then, samples were incubated for 30 minutes at room temperature. Absorbance was measured through UV at 415 nm against the control [16]. The total flavonoid content was determined as µg quercetin equivalent by using the standard (quercetin) graph and this following formula is below:

$$T = C \times V / M$$

Where,

T = Total flavonoid content, mg g-1 plant extract, in Quercetin Equivalent (QE).

C = Concentration (mg ml-1) of Quercetin obtained from calibration curve.

V = Volume of extract (ml).

M = Weight (mg) of plant extract.

***In-vitro* Antioxidant Activity:** By using some models like DPPH and FRAP, we can see whether or not the antioxidant activity is present in the plant. These are discussed below in brief.

Determination of Ferric Reducing Antioxidant Power (FRAP) Assay: 2 mg of plant extract was dissolved in 2 ml of Methanol to prepare a concentration of 1 mg/ml finally diluted for serial dilution, like as 100 µg/ml, 150 µg/ml, 200 µg/ml, 250 µg/ml, and 300µg/ml. For the preparation of the standard sample, 2 mg of Ascorbic acid was dissolved in 2 ml of Methanol to prepare a concentration of 1 mg/ml and finally diluted to 100 µg/ml, 150 µg/ml, 200 µg/ml, 250 µg/ml, and 300 µg/ml. At first, different concentrations of plant extracts or standards were added to2.5 ml of phosphate buffer sol. (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide. Then needed 30 minutes of incubation at 55°C, shake the mixtures and cool them. Then, 2.5 ml of trichloro acetic acid was added through micropipette. From the mixture, 2.5 ml was taken and again mixed with 2.5 ml of Water and 0.5 ml of 1% $FeCl_3$. The Absorbance of the mixture soln. was measured at700 nm using a UV-Vis spectrophotometer after 30 min of allowing the solution to stand. The results were expressed as mg/ml. Here, some preparations should be made carefully to get good results.

These are 0.2M phosphate buffer, 1% Potassium ferricyanide, 10% Trichloro acetic acid (TCA), and 1% Ferric chloride solution ($FeCl_3$) [17, 18].

DPPH Radical Scavenging Activity: It is the most commonly documented assay to determine the antioxidant activity of different compounds present in plant extracts. It is evaluated according to the method: different concentrations of methanolic and aqueous extract were added to 0.4 mM DPPH solution, respectively, the mixture was vigorously shaken, and it was placed in a black area for 15 min at 37 °C, and the Absorbance was checked at 517 nm. Ascorbic acid was taken as the control/ standard. Decolorization of the purple-colored to yellowish DPPH solution indicates the scavenging activity of the extracts. The antioxidant scavenging activity of the extract was expressed as IC_{50}. That IC_{50} values were calculated by linear regression of plots [19, 20].

The IC_{50} value is defined as the concentration (in µg/ml) of extract that inhibits the formation of DPPH soln. by 50%. Radical scavenging is expressed as a percentage of inhibition; the formula is;

$$\% \text{ Scavenging of DPPH} = [(A_0 - A_1) / A_0] \times 100$$

Where,

A_0 = Absorbance of the control

A_1 = Absorbance of the test extracts

RESULTS AND DISCUSSION:

Extraction Technique: Here we followed the Successive extraction technique, at first we take the 100 gm of powder and transfer into the 500 ml conical flask, add Pet. Ether 300 ml, keep it on a normal temperature and wait for 5-7 days, after that we start the filtration process & collect the extract which is 900.4 mg, again we are start drying the powdered drug then add Chloroform 350 ml wait 5-6 days, sample collect 1420.8 mg, as followed by another solvent Methanol 400 ml and collect 7748.5 mg extract, add Hydro alcohol (70:30) water : methanol & collect extract 8489.1 mg, and finally water 500 ml and add 1 drop alcohol because, it will be help to protect from the grow of microbial contamination, and collect the extract 4117.5 mg extract.

Plant Selection & Collection: *Mammillaria beneckei* C. Ehrenb, Cactus's stems were selected based on traditional uses and literature survey. The main thing is that, firstly, it was found in our garden corner, and these plants were collected in bulk quantity from the native area (Sundarbans), West Bengal, in August 2024.

Plant Identification & Authentication: The selected plant was collected in the flowering condition and was then transformed in the form of a herbarium as per guidelines and submitted to the Botanical Survey of India, (Central National Herbarium) Howrah, Shivpur with specimen no – UNB/RD-01 on 24.10.2024 [21].

FIG. 2: HERBARIUM SPECIMEN COPY OF *MAMMILLARIA BENECKEI* C. EHRENB

Macroscopic Evaluation:

TABLE 1: MACROSCOPIC EVALUATION OF STEM OF *MAMMILLARIA BENECKEI* C. EHRENB

Sl. no.	Characters	Observation of the stem
1	Shape	Spherical
2	Size	4” tall, 3.5” wide
3	Colour	Green
4	Surface	Rough
5	Texture	Smooth
6	Odour	Odourless
7	Appearance	Thorn outside

Microscopic Evaluation: In the microscopic evaluation, we have performed the following;

- Transverse Section (T.S) & Longitudinal Section (L.S.) of stem of *Mammillaria beneckei* C. Ehrenb **Fig. 3** [23].

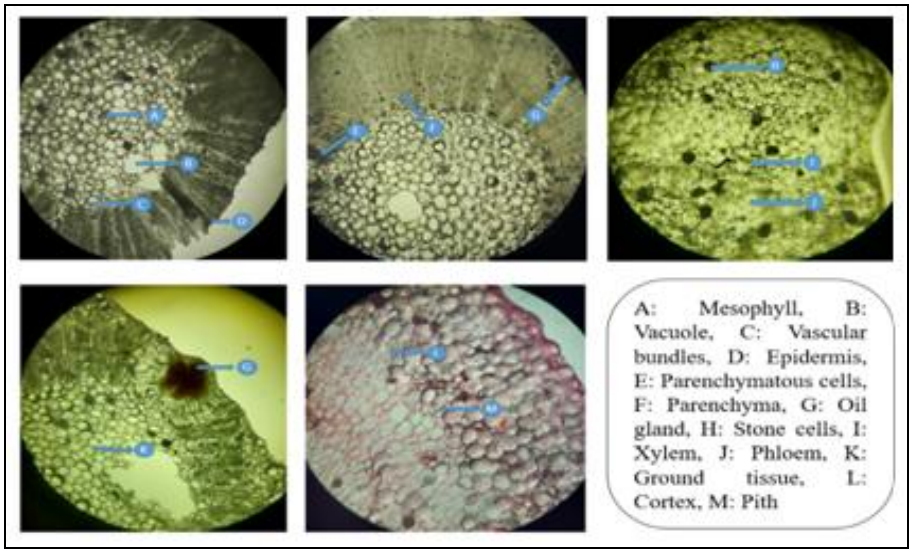


FIG. 3: DIFFERENT IMAGES OF TRANSVERSE SECTION &LONGITUDINAL SECTION (10X, 40X) OF STEM OF *MAMMILLARIA BENECKEI* C. EHRENB

- Powder Microscopy of stem of *Mammillaria beneckei* C. Ehrenb **Fig. 4** [24].

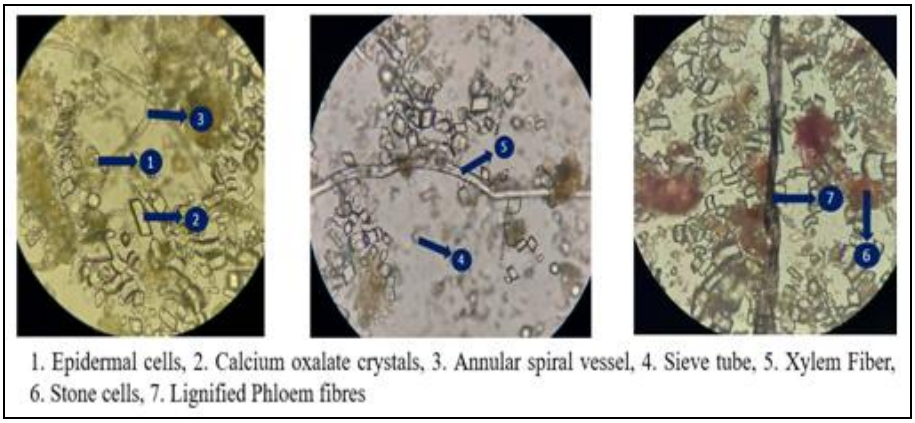


FIG. 4: DIFFERENT IMAGES OF POWDER MICROSCOPY (10X, 40X) OF STEM OF *MAMMILLARIA BENECKEI* C. EHRENB

Physicochemical Analysis: In this physicochemical analysis, different values obtained from the present drugs are recorded below, Physicochemical parameters such as swelling index, foaming index, determination of volatile oil, total ash, water-soluble ash, acid insoluble ash, water-soluble extractive values and alcohol soluble extractive values of the powdered samples of *Mammillaria beneckei* C. Ehrenb were determined based on the WHO guidelines [25, 26].

TABLE 2: PHYSICOCHEMICAL PARAMETERS OF *MAMMILLARIA BENECKEI* C. EHRENB (VALUES ARE PRESENTED AS MEAN ± STANDARD DEVIATION)

Sl. no.	Physicochemical parameters	Results
1.	Swelling index	6.85 ± 0.324
2.	Total ash	15.5 ± 0.90
3.	Water soluble ash	9.4 ± 0.61
4.	Acid insoluble ash	3.4 ± 0.034
5.	Water soluble extractive values	10.40 ± 0.08
6.	Alcohol soluble extractive values	13.61 ± 0.13
7.	Moisture content (LOD)	9.66 ± 0.29

Determination of Fluorescence Analysis: The results of the fluorescence analysis of the entire plant powder of *Mammillaria beneckei* C. Ehrenb are presented in **Table 3**; the fluorescence test is done through Daylight UV light (254 nm and 365nm) [27].

TABLE 3: FLUORESCENCE ANALYSIS OF THE POWDER OF *MAMMILLARIA BENECKEI* C. EHRENB (STEM)

Sl. no.	Powder + Reagent	Day light	UV light (254 nm)	UV light (365 nm)
1.	Powder as such	Light yellow	Light yellow	Yellow
2.	Powder + Water	Whitish yellow	Yellowish green	Light yellow
3.	Powder + n-hexane	Whitish yellow	Bluish brown	Light yellow
4.	Powder + chloroform	Whitish yellow	Bluish yellow	Golden yellow
5.	Powder + Methanol	Fade Yellow	Dark green	Bluish green
6.	Powder + H_2SO_4	Greenish brown	Light brown	Dip yellow
7.	Powder + HCl	Light brown	Black	Faded yellow
8.	Powder + Ammonia sol.	Green	Brownish green	Gold yellow
9.	Powder + Acetic acid	Light yellow	Whitish green	Whitish blue
10.	Powder + %5 $FeCl_3$	Dark green	Green	Dark green
11.	Powder + 1N NaOH	Greenish yellow	Brown	Greenish black
12.	Powder + 5% KOH	Brown	Dip brown	Black

Phytochemical Analysis: The preliminary phytochemical analysis of the crude powder of *Mammillaria beneckei* C. Ehrenb Stem results are shown below in **Table 4**.

TABLE 4: PHYTOCHEMICAL ANALYSIS OF VARIOUS EXTRACTS PREPARED FROM THE POWDER OF *MAMMILLARIA BENECKEI* C. EHRENB (STEM)

Sl. no.	Test	Pet. Ether extracts	Chloroform extracts	Methanol extracts	Hydro alcohol extracts (70:30)	Water extracts
1.	Alkaloids	-	+	+	+	-
2.	Flavonoids	-	++	+	+	-
3.	Glycosides	+	+	+	-	-
4.	Carbohydrates	-	+	-	-	-
5.	Tannins & Phenolic compounds	-	+	++	+	-
6.	Saponins	-	-	+	-	-
7.	Steroids	-	-	-	-	-
8.	Amino acid	-	-	-	-	-
9.	Protein	-	+	-	-	-

(++) strongly positive results, (+) positive results, (-) negative results [28]

Determination of Total Phenolic Content: The total phenolic content of different extracts of *Mammillaria beneckei* C. Ehrenb stem was assessed by the oxidation of phenolic groups by using Folin–Ciocalteu's reagent, which can be easily detected at 730 nm in a UV-Vis spectrophotometer. Here, Gallic acid was used as a standard, and the calibration curve was established in **Fig. 5**.

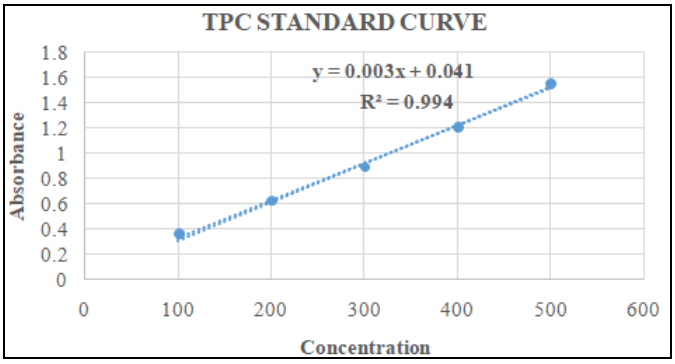


FIG. 5: STANDARD CURVE OF GALLIC ACID

By using the linear equation A = 0.003C + 0.0411, R2 = 0.9943, the phenolic component concentration was estimated as Gallic acid equivalents based on the calibration curve. A is the Absorbance, and C is Gallic acid equivalents (µg/ml) [29].

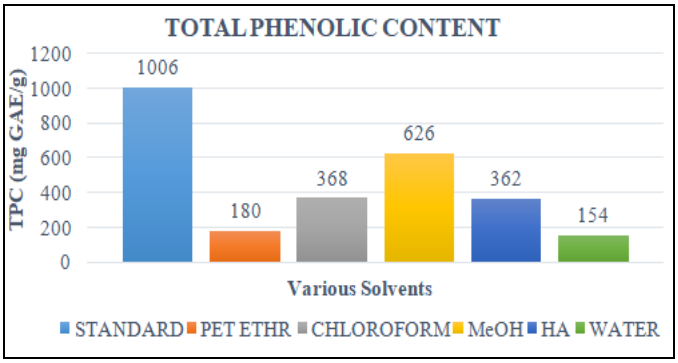


FIG. 6: STANDARD, PE, CH, MeOH, HA, AQ

The total phenolic content of Gallic acid, which was used as a standard, was found (1006 mg) and the different extract was as follows: Pet. ether (180 mg), Chloroform (368 mg), Methanol (626 mg), Hydro-alcohol (362 mg) and Water (154 mg) of Gallic acid equivalent per gm. of the dry extract [30, 31].

Determination of Total Flavonoid Content: The total flavonoid content of different extracts of *Mammillaria beneckei* C. Ehrenbstem was assessed by utilizing the aluminium chloride colorimetric method, where this reagent forms a complex with flavones and flavanol, which can be easily detected at 415 nm in a UV spectrophotometer.

Here, Quercetin was used as a standard, and the calibration curve was established in **Fig. 7**.

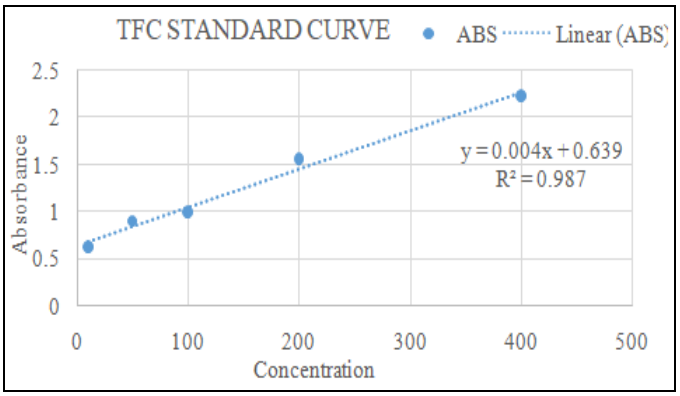


FIG. 7: STANDARD CURVE OF QUERCETIN

The linear equation is based on the calibration curve which we can see in this above graph, that; A = 0.0041c, R2 = 0.9879. A is the Absorbance, and C is Quercetin equivalents (µg/ml) [32, 33].

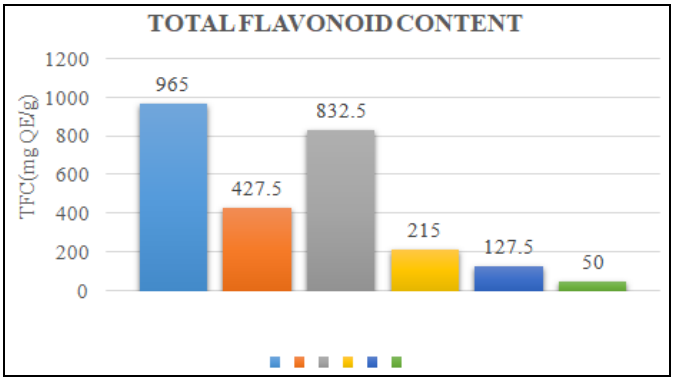


FIG. 8: STANDARD, PE, CH, MeOH, HA, AQ

The total flavonoid content of Quercetin, which was used as a standard found (965 mg) and the different extracts were as follows: Pet. ether (427.5 mg), Chloroform (832.5 mg), Methanol (215 mg), Hydro-alcohol (127.5 mg) and Water (50 mg) of Quercetin equivalent per gram of the dry extract [34, 35].

***In-vitro* Antioxidant Assay:**

DPPH Radical Scavenging Activity: DPPH radical scavenging activity assay helps in the detection of the reduction of DPPH, where we can see this reaction color changes from purple to yellowish, and DPPH is reduced to DPPH2 because the presence of an antioxidant compound in this extract, which can be easily detected in Absorbance at 517 nm. The IC_{50} values of Ascorbic acid were found to be (79.038), and IC_{50} values of different extracts of stem of *Mammillaria beneckei* C. Ehrenb was found as follows – Pet. ether (64.808), Chloroform – (112.18), Methanol - (126.8), Hydro alcohol – (83.61) & Water (266.21). *Fig. 9* represents the graph of the standard drug with different solvent extracts [36, 37, 8].

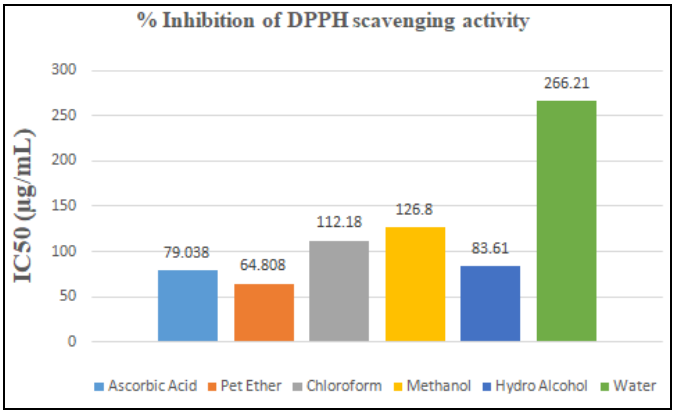


FIG. 9: AA, PE, CH, MeOH, HA, AQ

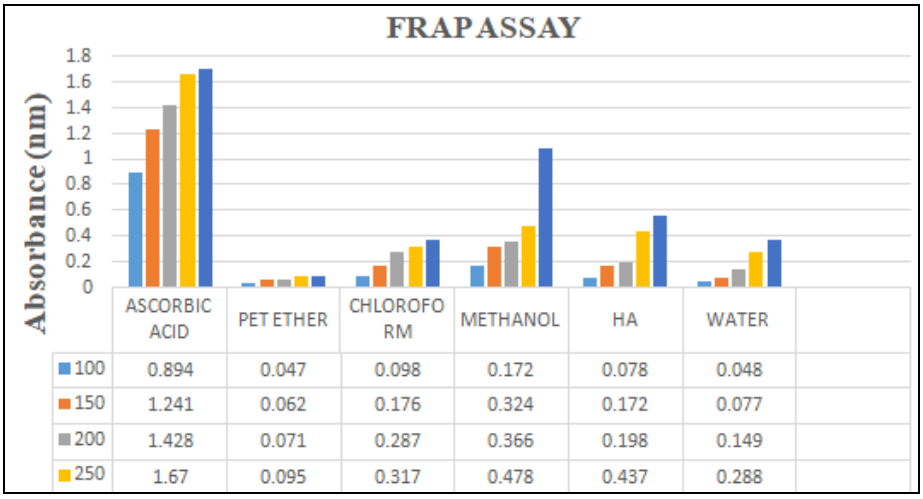
Determination of Ferric Reducing Antioxidant Power (FRAP) Assay: In this experiment, the extracts/ standard changes color from pale yellow/ white to greenish blue depending on the concentration.

The color change is due to the presence of an antioxidant group present in the plant, which reduces ferricyanide (Fe2+) to ferrocyanide (Fe3+).

When the ferric chloride was added, the ferric-ferrous complex was formed, which will be measured at 700 nm. The reducing power assay of plant extracts / standard increases with concentration.

Fig. 10 represents the reducing power assay of both the extract and the standard. The extract was as follows: Pet. Ether, Chloroform, Methanol, Hydro-alcohol & Water, with standard Ascorbic acid.

We can see that methanol extracts showed good results, followed by petroleum ether, Chloroform, and hydro-alcohol [38, 39, 40].



	ASCORBIC ACID	PET ETHER	CHLOROFORM	METHANOL	HA	WATER
100	0.894	0.047	0.098	0.172	0.078	0.048
150	1.241	0.062	0.176	0.324	0.172	0.077
200	1.428	0.071	0.287	0.366	0.198	0.149
250	1.67	0.095	0.317	0.478	0.437	0.288

FIG. 10: AA, PE, CH, MeOH, HA, AQ

CONCLUSION: The pharmacognostic, phytochemical, and antioxidant activity evaluation of the *Mammillaria beneckei* C. Ehrenb stem has provided significant insights into its medicinal potential. Through detailed pharmacognostic analysis, the morphological and anatomical characteristics of the stem were observed, which can aid in the standardization and identification of this species for medicinal use. In this experiment, phenols and the flavonoid group of compounds were found in the stem of *Mammillaria beneckei* C. Ehrenb. The presence of these compounds is responsible for the entrapment of free radicals and acts as a natural antioxidant. Phytochemical investigation confirmed the presence of flavonoids, alkaloids, carbohydrates, terpenoids, tannins, and glycosides. Total phenolic content showed the highest potency in methanolic extract, and flavored content showed the highest potency in chloroform extract, followed by pet ether, hydro-alcohol, and Water. Pet ether extract of the stem of *Mammillaria beneckei* C. Ehrenb showed good antioxidant activity by inhibition of DPPH. The ferric reducing power assay of the extract increased with the increasing amount of sample. It should be concluded, the stem of *Mammillaria beneckei* C. Ehrenb demonstrates the considerable pharmacological value, particularly its phytochemical profile and good antioxidant activity. Further research, including *in-vivo* studies and clinical trials, are recommended to explore its therapeutic potential and possible applications in modern medicine.

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