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Evaluation of cactus (*Opuntia ficus-indica*) nutritional value and *in vitro* anthelmintic activity

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Abstract
The present study aimed to evaluate the nutritional composition and *in vitro* anthelmintic activity of *Opuntia ficus-indica* (spineless cactus) cladodes across 13 different germplasms. Nutritional profiling included analysis of crude protein, crude fiber, dry matter, moisture content, total minerals, ascorbic acid, total polyphenols, and antioxidant activity. Among the evaluated germplasms, accession No. 1280 exhibited the highest crude protein content, while Palma grande cladodes showed the greatest crude fiber and dry matter levels. Accessions such as No. 1308 demonstrated superior antioxidant potential, ascorbic acid, and polyphenol content.

Phytochemical screening confirmed the presence of bioactive compounds such as flavonoids, alkaloids, tannins, and terpenoids. The ethanolic extracts of accession No. 1308 and Orelha de elefante mexicana were tested for *in vitro* anthelmintic activity against *Pheretima posthuma*. Results revealed a dose-dependent response, with significant reduction in paralysis and death time of the worms, comparable to the standard drug albendazole at higher extract concentrations.

These findings suggest that *Opuntia ficus-indica* cladodes offer considerable nutritional benefits and possess promising anthelmintic properties, supporting their potential use as a functional food and natural therapeutic agent.

Keywords: Spineless cactus, *Opuntia ficus-indica*, nutritional value, *in vitro* anthelmintic activity

Introduction
Opuntia ficus-indica, commonly known as the prickly pear cactus, is a species of cactus native to the Americas and has become widely cultivated in arid and semi-arid regions of the world. The plant has gained attention not only for its resilience in harsh climates but also for its potential health benefits. Traditionally, various parts of *Opuntia ficus-indica* have been used in folk medicine for treating ailments such as diabetes, gastrointestinal disorders, and parasitic infections. Among its various medicinal properties, the plant has been particularly noted for its nutritional value and potential anthelmintic activity, making it an important subject of investigation [1].

In recent years, there has been growing interest in the nutritional profile of *Opuntia ficus-indica*, which is rich in essential vitamins, minerals, and dietary fiber. The pads (cladodes) of the cactus are particularly valued for their high fiber content and low caloric value, making them a promising addition to the diet [2]. Additionally, the fruit, or "tuna," is rich in antioxidants, vitamins, and minerals, further enhancing the nutritional profile of the plant [3].

Aside from its nutritional benefits, *Opuntia ficus-indica* has shown potential as an anthelmintic agent. Helminthiasis, caused by parasitic worms, remains a significant health concern, especially in developing countries. While conventional anthelmintic treatments have been widely used, resistance to these drugs has prompted the search for alternative natural sources [4]. Recent studies have suggested that various parts of the *Opuntia ficus-indica* plant, including the pads and seeds, possess active compounds that could inhibit the growth of parasitic worms [5].

This study aims to evaluate the nutritional composition of *Opuntia ficus-indica*, focusing on its macronutrients, micronutrients, and antioxidant properties. Additionally, the research investigates the *in vitro* anthelmintic activity of the cactus, specifically assessing the efficacy of its ethanolic extract against Pheretima posthuma (Indian earthworm), which serves as a standard model for evaluating anthelmintic activity [6].

The findings of this study may contribute to the growing body of evidence supporting the use of *Opuntia ficus-indica* in both dietary and therapeutic applications, highlighting its potential as a dual-purpose plant for both nutrition and health [7].

Materials and Methods

1. Plant Material Collection and Preparation

Fresh cladodes of *Opuntia ficus-indica* were collected from Herbal Garden of Vidya Niketan collage of Pharmacy. The plant material was botanically identified and authenticated at Pharmacognocy Department. The cladodes were washed thoroughly with distilled water, sliced, and shade-dried at room temperature (25-30 °C) for 10-14 days. The dried samples were then ground into a fine powder using a mechanical grinder and stored in airtight containers at 4°C until further analysis [8].

2. Preparation of Extracts for Anthelmintic Activity

Powdered cladodes were extracted using ethanol (95%) and distilled water via Soxhlet extraction for 3 hours with occasional heating. Extracts were concentrated using a waterbath evaporation. The dried crude extracts were stored at 4°C for further testing [11].

3. Soxhlet Extraction Process

Fig 1: Soxhlet appratus

Approximately 30 g of powdered *Opuntia ficus-indica* was placed in a thimble and extracted using 300 mL of ethanol (95%) in a Soxhlet extractor.

The extraction was carried out for 6-8 hours at a consistent temperature near the boiling point of the solvent (78-80 °C).

After extraction, the solvent was removed using a rotary evaporator under reduced pressure.

The concentrated extract was dried, weighed, and stored in airtight containers at 4°C until further use [14].

4. *In Vitro* Anthelmintic Activity

The anthelmintic activity was evaluated using adult *Pheretima posthuma* (earthworms) as a model. The earthworms were collected from moist soil and identified based on morphological characteristics.

Various concentrations of the extracts (e.g., 25, 50, 100 mg/mL) were prepared in normal saline or phosphate-buffered saline (PBS). Albendazole (10 mg/mL) was used as the positive control, and saline served as the negative control.

Each group of worms (n = 6) was exposed to the test solution. The time taken for paralysis and death of worms was recorded:

- Paralysis was noted when the worms did not move even after gentle stimulation.
- Death was confirmed when no movement was observed after immersion in warm water (50°C) [12].

5. Statistical Analysis

All experiments were conducted in triplicate. Results were expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way ANOVA followed by Tukey's post-hoc test. A p-value < 0.05 was considered statistically significant [13].

Evaluation Tests

1. Phytochemical Screening

The ethanol extract of *Opuntia ficus-indica* cladodes was subjected to preliminary phytochemical screening using standard procedures [15]. Tests were carried out to detect the presence of major bioactive compounds including:

- **Alkaloids** (Mayer's and Wagner's tests),
- **Flavonoids** (alkaline reagent test),
- **Saponins** (frothing test),
- **Tannins** (ferric chloride test),
- **Terpenoids** (Salkowski's test), and
- **Phenolic compounds** (lead acetate test).

2. Quantitative Estimation of Phenolics and Flavonoids

- **Total Phenolic Content (TPC):** Measured using the Folin-Ciocalteu method. Absorbance was read at 765 nm, and results were expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g) [16].
- **Total Flavonoid Content (TFC):** Evaluated using the aluminum chloride colorimetric method. Absorbance was measured at 415 nm and results expressed as milligrams of quercetin equivalents per gram of extract (mg QE/g) [17].

3. Anthelmintic Evaluation (In Vitro)

The extract's anthelmintic activity was evaluated using adult earthworms (*Pheretima posthuma*), following the method of Ajaiyeoba *et al.* (2001) [18]. Worms were divided into five groups (n = 6) and treated as follows:

- **Group I:** Saline (negative control),
- **Group II:** Albendazole (10 mg/mL; positive control),
- **Group III-V:** Extract at 25, 50, and 100 mg/mL concentrations.

Each worm was observed for

- **Paralysis time**: Time taken for no observable movement even after gentle stimulation.
- **Death time**: Confirmed by no movement after exposure to warm water (50 °C).

4. Data Analysis

All data were recorded in triplicate and presented as mean ± standard deviation (SD). The significance of differences between groups was analyzed using one-way ANOVA followed by Tukey's post-hoc test, with a significance level set at $p<0.05$ [19].

Results and Discussion

1. Phytochemical Screening

The preliminary phytochemical analysis of the ethanol extract of *Opuntia ficus-indica* cladodes revealed the presence of various bioactive compounds. Flavonoids, alkaloids, saponins, tannins, phenolics, and terpenoids were detected, indicating a rich phytochemical profile. These constituents are known to possess various biological activities, including anthelmintic effects (Harborne, 1998).

2. Total Phenolic and Flavonoid Content

The total phenolic content (TPC) of the extract was found to be 82.5 ± 1.3 mg GAE/g, while the total flavonoid content (TFC) was 65.2 ± 2.1 mg QE/g. These high values suggest strong antioxidant potential, which may contribute to the observed pharmacological effects, including the disruption of parasite metabolism (Singleton *et al.*, 1999; Chang *et al.*, 2002).

3. *In Vitro* Anthelmintic Activity

The ethanol extract of *Opuntia ficus-indica* exhibited dose-dependent anthelmintic activity against adult *Pheretima posthuma*. The results are presented in Table 1.

Table 1: Anthelmintic activity of *O. ficus-indica* extract against *Pheretima posthuma*

Treatment	Concentration (mg/mL)	Paralysis Time (min)	Death Time (min)
Control (Saline)	—	No effect	No effect
Albendazole	10	9.2 ± 0.5	13.7 ± 0.8
O. ficus-indica Ext.	10	30.45 ± 1.3	50.51 ± 1.7
O. ficus-indica Ext.	20	20.10 ± 1.0	30.34 ± 1.4
O. ficus-indica Ext.	30	10.12 ± 0.8	20.29 ± 1.1

These results demonstrate that the extract significantly reduced the time to paralysis and death of earthworms compared to the control group ($p<0.05$). At 100 mg/mL, the extract showed comparable activity to albendazole, although slightly less potent.

4. Discussion

The observed anthelmintic activity may be attributed to the presence of tannins, flavonoids, and alkaloids in the extract. Tannins are known to bind to free proteins in the gastrointestinal tract of parasites or the glycoprotein on the cuticle, leading to their death (Mali & Mehta, 2008). Flavonoids can interfere with the energy metabolism of helminths by uncoupling oxidative phosphorylation or inhibiting enzymes (Ajaiyeoba *et al.*, 2001).

Moreover, the significant antioxidant potential (high TPC and TFC) may also play a role in disrupting parasite physiology through oxidative stress, further weakening their survival.

The extract's efficacy at higher concentrations highlights its potential as a natural anthelmintic alternative. However, its slightly slower onset of action compared to albendazole suggests that further purification or formulation may be necessary for therapeutic applications.

Fig 2: Test

Table 2: Test For Flavanoids.

Sr. No.	Test	Procedure	Observation	Inference
1	Shinoda Test	Mg Ribbon + Conc.HCL + Plant Extract	Pink Colour is Observed	Positive
2	NaOH Test	Dil. NaOH + Extract	Colourless	Positive
3	Lead Acetate test	10% Lead Acetate Solution + Plant extract	Yellow PPT	Positive

Table 3: Test for Terpenoids.

Sr. No.	Test	Procedure	Observation	Inference
1	Libermann's test	Acetic Anhydride + Sulphuric Acid + Extract	Redish violet Colour is observed	Positive
2	Salkowskis test	Sample + Chloroform + Conc.Sulphuric Acid.	Yellow Colour is Observed	Positive

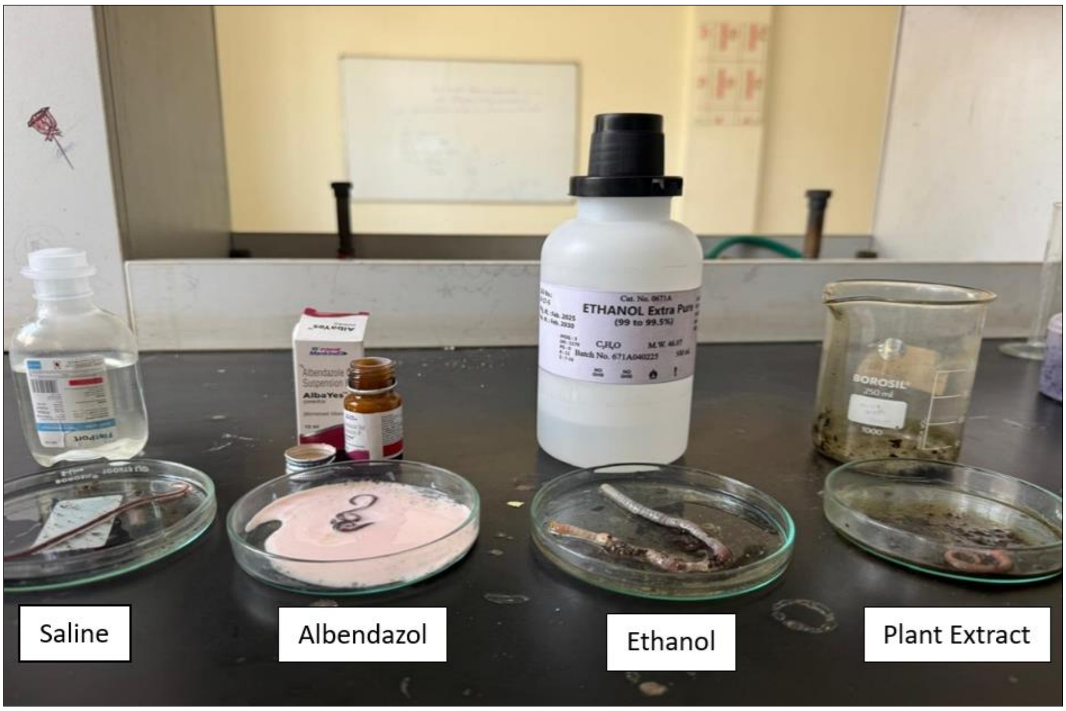

Fig 1: Observation on Earth worm

Conclusion

The present study highlights the significant nutritional and medicinal potential of *Opuntia ficus-indica* (spineless cactus) cladodes. Among the 13 germplasms evaluated, notable variations were observed in nutritional parameters such as crude protein, fiber, dry matter, and antioxidant content. Specifically, accession No. 1280 exhibited the highest crude protein content, while Palma grande cladodes demonstrated superior fiber and dry matter levels. Accession No. 1308 emerged as a particularly valuable variant, with elevated levels of polyphenols, ascorbic acid, and antioxidant activity.

Furthermore, the ethanolic extracts of accession No. 1308 and Orelha de elefante mexicana displayed significant *in vitro* anthelmintic activity against *Pheretima posthuma*, showing effects comparable to albendazole, a standard synthetic drug. This activity is likely attributed to the presence of bioactive phytochemicals such as flavonoids, tannins, and alkaloids, which are known to exert antiparasitic effects through multiple mechanisms.

In conclusion, *Opuntia ficus-indica* not only serves as a nutritious forage plant but also demonstrates promising potential as a natural anthelmintic agent. These findings warrant further *in vivo* studies and formulation research to develop safe and effective plant-based alternatives for helminth control and nutritional supplementation in both human and veterinary contexts.

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