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RESEARCH ARTICLE

In vitro evaluation of antioxidant activity in fruit and cladode extracts of *Opuntia ficus-indica* (L.) Mill.

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Abstract

Reactive oxygen species (ROS) are implicated in various degenerative diseases and the search for potent natural antioxidants remains a major focus of current research. The present study evaluates the *in vitro* antioxidant potential of *Opuntia ficus-indica* (L.) Mill. fruit and cladode extracts prepared using methanol, ethyl acetate and n-butanol. The antioxidant activity was assessed using four complementary assays- 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, reducing power, nitric oxide (NO) radical inhibition and hydrogen peroxide (H_2O_2) scavenging with gallic acid and ascorbic acid as standards. The methanolic extract of *O. ficus-indica* fruit exhibited the highest DPPH radical scavenging activity (70.69 ± 1.93 %) with an IC_{50} value of 13.72 µg mL^{-1}, which was comparable to that of gallic acid (IC_{50} = 9.39 µg mL^{-1}). In reducing power assays, the methanol extract showed the strongest Fe^{3+}-reducing ability, indicating the presence of potent electron-donating antioxidants. Methanol extracts also demonstrated superior NO (IC_{50} = 68.44 µg mL^{-1}) and H_2O_2 (IC_{50} = 62.94 µg mL^{-1}) scavenging capacities compared to other solvent fractions. These findings suggest that *O. ficus-indica* is a promising source of natural antioxidants and represents a potential natural source of antioxidant compounds, which warrants further investigation.

Keywords: DPPH assay; hydrogen peroxide scavenging; IC_{50}; nitric oxide scavenging; *Opuntia ficus-indica*; reducing power

Introduction

Reactive oxygen species (ROS) and free radicals are continuously generated in living systems as by-products of normal cellular metabolism. Under physiological conditions, they play essential roles in signalling and homeostasis (1). However, excessive production of ROS can disrupt the antioxidant defence balance, leading to oxidative stress and damage to biomolecules such as lipids, proteins and nucleic acids. This imbalance has been implicated in several chronic and degenerative diseases and cancer, cardiovascular disorders, neurodegenerative diseases, diabetes and premature aging (2, 3).

The human body counteracts oxidative stress through enzymatic antioxidants such as superoxide dismutase, catalase and glutathione peroxidase and non-enzymatic antioxidants like vitamins C and E, carotenoids and phenolic compounds. However, under increased oxidative load, endogenous defences may become inadequate, necessitating supplementation through exogenous, plant-derived antioxidants (4, 5). Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been widely used in food and pharmaceutical industries, but their potential toxicological effects have driven interest toward safer, natural alternatives (6–8).

Plant-based antioxidants, particularly polyphenols and flavonoids, have demonstrated excellent redox properties and health-promoting effects, making them suitable for functional food and nutraceutical applications (9, 10). Among these, *Opuntia ficus-indica* (L.) Mill. (Cactaceae), commonly known as the prickly pear cactus, has emerged as a valuable natural source of bioactive metabolites. The plant is abundant in arid and semi-arid regions and has long been utilised in traditional medicine for its anti-inflammatory, anti-diabetic and wound-healing properties (11).

Recent research has shown that *O. ficus-indica* fruit, cladodes and peel contain diverse bioactive compounds including phenolic acids, flavonoids, betalains, vitamins and polysaccharides, which collectively contribute to its antioxidant potential (12, 13). Studies have revealed that peel and cladode extracts exhibit strong radical-scavenging and enzyme-inhibitory activities (14), while metabolic profiling using liquid chromatography-mass spectrometry (LC-MS) has confirmed the presence of isorhamnetin glycosides and indicaxanthin as key antioxidant constituents (12). Furthermore, the plant's extracts have been shown to possess anti-steatotic, anti-inflammatory and cytoprotective effects in various *in vitro* models (15).

Therefore, the present study aims to evaluate and compare the *in vitro* antioxidant activities of methanolic, ethyl acetate and n-butanol extracts of *O. ficus-indica* fruit and cladodes using DPPH radical-scavenging, reducing-power, nitric-oxide inhibition and hydrogen-peroxide scavenging assays. This comparative analysis seeks to identify the most potent extract and emphasise the role of *O. ficus-indica* as a promising source of natural antioxidants for functional food, nutraceutical and pharmaceutical applications.

Materials and Methods

Plant material and preparation of extracts

Fresh fruits and cladodes of *O. ficus-indica* were collected from local in and around Kalaburagi region (arid) during February to April. The plant material was identified and authenticated by Dr. Sidanand V. Kambhar, a renowned taxonomist and a voucher specimen was preserved for future reference (Herbarium number SUK0981). Approximately 2.5 kg of fresh plant material was collected used for the study. The collected plant materials were washed, sliced and shade-dried at room temperature (25 ± 2 °C) for 10–12 days, which yielded about 320 g of dried powder after shade drying.

The powdered fruit and cladode samples were extracted successively using methanol, ethyl acetate and n-butanol. For extraction, 100 g of powdered sample was soaked in 500 mL of the respective solvent and kept for maceration for 72 hr at room temperature with occasional shaking followed by filtration and concentration under reduced pressure using a rotary evaporator at 40 °C. The dried crude extracts were weighed, labelled and stored at 4 °C until further analysis.

Chemicals and reagents

All chemicals used in this study were of analytical grade. 1,1-Diphenyl-2-picrylhydrazyl (DPPH), ascorbic acid, gallic acid, potassium ferricyanide, trichloroacetic acid (TCA), ferric chloride ($FeCl_3$), sodium nitroprusside, sulfanilic acid, naphthyl-ethylenediamine dihydrochloride (NEDD) and hydrogen peroxide (H_2O_2) were purchased from Hi-Media Laboratories (Mumbai, India). All solutions were prepared using distilled water.

In vitro antioxidant assays

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity

The DPPH free-radical assay was performed following Blois method with slight modification (16). A 0.659 mM DPPH solution was prepared in methanol. Test extracts (25–100 µg mL^{-1}) and the standard gallic acid (1000 µg mL^{-1} stock) were mixed with DPPH solution (2 mL + 1 mL extract). The mixtures were incubated in the dark for 30 min and absorbance was measured at 517 nm using a UV-Vis spectrophotometer (Systronics UV-2201). The percentage of DPPH scavenging was calculated as:

$$\text{Initiation (\%)} = \frac{\text{A control - A sample}}{\text{A control}} \times 100$$

The IC_{50} value (concentration providing 50 % inhibition) was determined from the concentration-response curve.

Reducing-power assay

Reducing power was evaluated as described in a previous study (17). Various concentrations (10–100 µg mL^{-1}) of extracts were mixed with 2.5 mL phosphate buffer (0.2 M, pH 6.6) and 2.5 mL 1 % potassium ferricyanide. After incubation at 50 °C for 20 min, 2.5 mL of 10 % TCA was added and the mixture was centrifuged at 3000 rpm for 10 min. The supernatant (2.5 mL) was combined with 2.5 mL distilled water and 0.5 mL of 0.1 % $FeCl_3$. Absorbance was measured at 700 nm. Increased absorbance indicated higher reducing power. Ascorbic acid served as a reference standard.

Nitric-oxide (NO) radical-inhibition assay

The nitric-oxide scavenging activity was determined using the modified Griess-Illosvay method (18). A reaction mixture containing 1 mL sodium nitroprusside (10 mM in 0.1 M phosphate buffer, pH 7.4) and different extract concentrations (10–100 µg mL^{-1}) was incubated at 25 °C for 2 hr. After incubation, 1.5 mL of the mixture was reacted with 1.5 mL Griess reagent (1 % sulfanilamide, 2 % o-phosphoric acid and 0.1 % NEDD). The absorbance of the resulting azo dye was read at 546 nm. Ascorbic acid and gallic acid were used as standards.

$$\text{NO inhibition (\%)} = \frac{\text{A control - A sample}}{\text{A control}} \times 100$$

Hydrogen-peroxide (H_2O_2) scavenging activity

Hydrogen-peroxide scavenging was assessed by a method described in a previous study with modifications (19) . The reaction mixture contained 0.5 mL of H_2O_2 solution (30 % H_2O_2 diluted to 1:45 with distilled water), 1 mL phosphate buffer (0.2 M, pH 7.4), 0.4 mL distilled water and 0.1 mL extract (25–100 µg mL^{-1}). After 1 min, 2 mL of dichromate-acetic acid reagent (5 % potassium dichromate in glacial acetic acid, 1:3 v/v) was added to terminate the reaction. Tubes were heated for 10 min, cooled and absorbance was measured at 240 nm. Ascorbic acid served as a reference standard.

$$H_2O_2 \text{ scavenging activity (\%)} = \frac{\text{A control - A sample}}{\text{A control}} \times 100$$

Statistical analysis

All assays were carried out in duplicate (n = 2) and results are expressed as mean ± standard deviation (SD). IC_{50} values were determined using linear regression analysis in Microsoft Excel.

Results

The results of each assay revealed distinct activity profiles depending on the solvent system and plant part used.

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

All extracts showed concentration-dependent scavenging of DPPH radicals. The methanolic fruit extract exhibited the highest activity (70.69 ± 1.93 %) with an IC_{50} of 13.72 µg mL^{-1}, followed by ethyl acetate (IC_{50} = 46.55 µg mL^{-1}) and n-butanol (IC_{50} = 49.96 µg mL^{-1}) extracts. Among cladodes, the ethyl acetate extract demonstrated the strongest activity (66.58 ± 3.87 %; IC_{50} = 28.17 µg mL^{-1}), while the methanolic extract showed moderate activity (54.86 ± 1.05 %; IC_{50} = 34.72 µg mL^{-1}). Gallic acid served as the reference standard (IC_{50} = 9.39 µg mL^{-1}) (Table 1).

Reducing power assay

A progressive increase in reducing power was observed with rising extract concentrations. The methanolic fruit extract showed the highest absorbance (0.368 ± 0.006 at 100 µg mL^{-1}), approaching the reducing power of ascorbic acid (0.162 ± 0.001). n-Butanol fruit extract also exhibited significant reducing ability (0.411 ± 0.003). For cladodes, the methanolic extract displayed the greatest absorbance (0.135 ± 0.003) (Table 2).

Nitric oxide radical inhibition

All extracts demonstrated nitric oxide radical-scavenging activity in a concentration-dependent manner. The methanolic fruit extract was most effective, with an IC_{50} of 68.44 µg mL^{-1}, followed by n-butanol (73.08 µg mL^{-1}) and ethyl acetate (107.59 µg mL^{-1}) extracts. In the cladode samples, the methanolic fraction exhibited 67.35 % inhibition (IC_{50} = 54.83 µg mL^{-1}), outperforming the ethyl acetate (40.12 %) and n-butanol (60.98 %) extracts. Ascorbic acid (IC_{50} = 12.59 µg mL^{-1}) served as the standard (Table 3).

Table 1. 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity of *O. ficus-indica*

Extract source	Solvent	Inhibition at 100 μgmL^{-1} (Mean ± SD) (%)	IC_{50} (μgmL^{-1})	Reference standard (Gallic acid IC_{50} = 9.39 μgmL^{-1})
Fruit	Methanol	70.69 ± 1.93	13.72	-
Fruit	Ethyl acetate	61.88 ± 2.41	46.55	-
Fruit	n-Butanol	59.12 ± 1.82	49.96	-
Cladode	Methanol	54.86 ± 1.05	34.72	-
Cladode	Ethyl acetate	66.58 ± 3.87	28.17	-
Cladode	n-Butanol	48.27 ± 2.26	52.41	-

All values are mean ± SD (n = 2)

Table 2. Reducing-power activity of *Opuntia ficus-indica* extracts

Extract source	Solvent	Absorbance at 700 nm (Mean ± SD, 100 μgmL^{-1})	Comparative reference (Ascorbic acid = 0.162 ± 0.001)
Fruit	Methanol	0.368 ± 0.006	-
Fruit	Ethyl acetate	0.255 ± 0.004	-
Fruit	n-Butanol	0.411 ± 0.003	-
Cladode	Methanol	0.135 ± 0.003	-
Cladode	Ethyl acetate	0.092 ± 0.002	-
Cladode	n-Butanol	0.101 ± 0.002	-

All values are mean ± SD (n = 2)

Table 3. Nitric-oxide radical-inhibition activity of *Opuntia ficus-indica* extracts

Extract source	Solvent	Inhibition (Mean ± SD)(%)	IC_{50} (μgmL^{-1})	Reference (Ascorbic acid IC_{50} = 12.59 μg mL^{-1})
Fruit	Methanol	74.26 ± 2.11	68.44	-
Fruit	Ethyl acetate	61.37 ± 1.65	107.59	-
Fruit	n-Butanol	69.42 ± 1.93	73.08	-
Cladode	Methanol	67.35 ± 2.07	54.83	-
Cladode	Ethyl acetate	40.12 ± 1.26	122.45	-
Cladode	n-Butanol	60.98 ± 1.48	89.31	-

All values are mean ± SD (n = 2)

Hydrogen peroxide scavenging activity

The methanolic fruit extract showed the strongest hydrogen peroxide scavenging activity (IC_{50} = 62.94 μg mL^{-1}), followed by n-butanol (92.19 μg mL^{-1}) and ethyl acetate (124.49 μg mL^{-1}) extracts. Among cladode extracts, the methanolic fraction again displayed the highest activity (IC_{50} = 141.6 μg mL^{-1}), whereas the ethyl acetate and n-butanol fractions showed IC_{50} values of 156 μg mL^{-1} and 180.6 μg mL^{-1}, respectively. Ascorbic acid exhibited an IC_{50} of 18.20 μg mL^{-1} (Table 4).

Overall, the methanolic extracts, particularly those derived from the fruit, consistently exhibited stronger antioxidant activity across all assays compared to the other solvent fractions.

Discussions

The findings of this study confirm that *O. ficus-indica* possesses strong antioxidant activity, particularly in its methanolic fruit extract, across all tested assays. The superior performance of the methanolic fraction indicates that solvent polarity significantly influences the extraction efficiency of phenolic compounds, which are primarily responsible for the plant's antioxidant potential (20).

The DPPH radical scavenging activity observed in the present study is comparable with earlier reports on *O. ficus-indica*. The methanolic fruit extract exhibited an IC_{50} value of 13.72 μg mL^{-1}, which is in same to near range reported in earlier studies for methanolic or hydroalcoholic extracts of fruits. Where IC_{50} values between 10 and 30 μg mL^{-1} have been documented (12, 21) and variations in IC_{50} values reported in many studies may be attributed on the basis of geographical origin of the plant material, season of collection, extraction method and solvent polarity, which are known to influence the recovery of phenolic antioxidants (22, 23). Thus, the DPPH IC_{50} value obtained in the present study indicates a slightly stronger antioxidant potential of the methanolic fruit extract in comparison to previously reported extracts of *O. ficus-indica.*

The DPPH and reducing-power assays demonstrated that methanolic fruit extracts exhibited greater electron-donating and free-radical-quenching capacity than ethyl acetate or n-butanol extracts. This result aligns with previous studies showing that methanol efficiently extracts polar phenolic constituents, including flavonoids and betalains, which are potent antioxidants (12, 21). The antioxidant activity observed in the present study may be associated with phenolic and betalain compounds that have been previously reported in *O. ficus-indica* (22).

Table 4. Hydrogen-peroxide scavenging activity of *Opuntia ficus-indica* extracts

Extract source	Solvent	Inhibition (Mean ± SD) (%)	IC_{50} (μg mL^{-1})	Reference (Ascorbic acid IC_{50} = 18.20 μgmL^{-1})
Fruit	Methanol	77.48 ± 2.38	62.94	-
Fruit	Ethyl acetate	63.19 ± 1.74	124.49	-
Fruit	n-Butanol	69.03 ± 1.89	92.19	-
Cladode	Methanol	61.84 ± 1.92	141.60	-
Cladode	Ethyl acetate	54.67 ± 1.68	156.00	-
Cladode	n-Butanol	52.11 ± 1.55	180.60	-

All values are mean ± SD (n = 2)

Nitric oxide scavenging by the methanolic extract further supports its potential role in mitigating reactive nitrogen species (RNS)-induced oxidative damage. Polyphenols are known to inhibit nitric oxide production and nitrosative stress, providing a biochemical basis for the anti-inflammatory and cytoprotective effects of *O. ficus-indica* (11, 23). Similar trends have been reported in hydro-soluble seed extracts exhibiting antioxidant and anti-inflammatory properties (24).

Hydrogen peroxide scavenging by methanolic extracts indicates the ability of *O. ficus-indica* to prevent hydroxyl radical generation via Fenton reactions. The activity may be associated with phenolic compounds and betalains found in the plant by earlier reports (4, 15). In agreement with our findings, recent studies have reported the anti-steatotic and redox-modulating activities of *O. ficus-indica* peel and pulp extracts, further substantiating the biological significance of its phytoconstituents (10, 22).

Although different antioxidant assays performed in the present study, yet all of them showed a similar trend in activity, but particularly from the methanolic fruit extract. The DPPH and reducing power assays mainly reflect electron-donating ability, while nitric oxide and hydrogen peroxide scavenging assays indicate the capacity to neutralise reactive nitrogen and oxygen species. The consistent performance across these assays suggests a broad-spectrum antioxidant potential of the methanolic extract.

The results highlight that *O. ficus-indica* extracts, especially methanolic fractions of the fruit, act through both electron-transfer and hydrogen-donation mechanisms. The strong correlation between solvent polarity and antioxidant activity reinforces the importance of optimising extraction protocols for maximum yield of bioactive constituents. With growing research and evidence highlighting the antioxidant potential of *O. ficus-indica*, further studies pointing on the bioavailability, metabolic pathways and standardisation are necessary.

The present study focused on the antioxidant assay of crude extract obtained from successive extraction method using *in vitro* methods. Therefore, further studies involving chromatographic profiling and *in vivo* models are required to better understand the antioxidant potential and biological relevance of *O. ficus-indica* extracts.

Conclusion

The present study shows that *O. ficus-indica* possess considerable *in vitro* antioxidant activity and the activity was found to vary depending on the plant part and solvent used. Among all the extracts tested, the methanolic fruit extract showed consistently higher antioxidant activity, with a DPPH radical scavenging IC_{50} value of 13.72 µg mL^{-1}, the same extract also exhibited better reducing power and effective scavenging of nitric oxide (IC_{50} = 68.44 µg mL^{-1}) and hydrogen peroxide (IC_{50} = 62.94 µg mL^{-1}) radicals when compared with other solvent fractions. However, since the present study is limited to *in vitro* assays and experiments were carried out on crude solvent extracts, further studies including phytochemical characterisation and *in vivo* validation are required before drawing any application-oriented conclusions.

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Authors' contributions

KP designed the study and reviewed the results. KP and DDJ wrote the first draft of the manuscript, contributed to the review and revision of the manuscript and participated in drafting the final version. Both authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used QuillBot only for paraphrasing. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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