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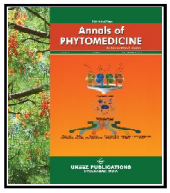

Original Article : Open Access

Plant chemical analysis and antioxidant commotion of lactobacillus contrived hydroethanolic extracts of *Opuntia dillenii* Haw.

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Abstract

The work is aimed at hydroalcoholic extraction of *Opuntia dillenii* Haw. cladodes (ODC), followed by its treatment with lactic acid bacteria to increase the beneficial secondary metabolites in them. Additionally, total phenolics, flavonoids and different *in vitro* antioxidant activities are determined (2, 2-diphenyl-1-picrylhydrazyl). In the current study, phytochemical assays were utilized to characterize dry matter plants, including sterols and terpenes, tannins, anthraquinones, alkaloids and saponins qualitatively and polyphenols and flavonoids quantitatively. The colorimetric method was employed to determine the number of active antioxidants, phenolic content and flavonoid content. The phytochemical analysis of several extracts from ODC revealed the presence of phenolic compounds and flavonoids. The total phenolic content (TPC), given as mg of gallic acid equivalent (GAE) per 100 g of dry matter, was found to be good in the ethanolic extract and was reported to be 88.16 ± 9.51 mg GAE/100g DW. Later, the extract modified by lactobacillus (LAB) increased the TPC in the ethanolic extract, measuring 126.25 ± 9.85mg GAE/100 g DW. The total flavonoid content (TFC) was also discovered to be high in ethanol (80%) extracts (54.55 ± 0.32 mg GAE/100 g DW) and the LAB-managed extract increased the TPC in the ethanolic extract, resulting in 72.64 ± 1.24 mg GAE/100 g DW. Using the IC_{50} method, it was discovered that the radical scavenging activity was 44.95 ± 1.2 g/ml and 37.16 ± 0.7 g/ml (LAB maneuvered), respectively. However, ascorbic acid's IC_{50} value was 53.52 ± 0.2 g/ml, showing a bigger difference in antioxidant activity. When employing the ODC and LAB procedures, the findings for all of the aqueous ethanolic extracts were remarkable. According to this study, the hydroalcoholic extract of ODC, which has strong antioxidant activity, may be used as a LAB contrived to reduce oxidative stress.

1. Introduction

"Oxidative stress" refers to an oxidant-antioxidant imbalance that favours oxidants. Free radicals and reactive oxygen species (ROS) are extremely reactive chemicals in cells that can damage nucleic acids, carbohydrates, lipids and proteins (Forman *et al.*, 2021). Many diseases, including atherosclerosis, cancer and neurological disorders, are caused by oxidative stress. There has been a lot of interest in antioxidant research in vegetable products and efforts to valorize novel natural resources for active antioxidant molecules, especially phenolic chemicals, have been made (Jimenez *et al.*, 2020). The antioxidants in the plant matrix aid in the prevention of oxidative damage in the body (Raja *et al.*, 2021; Suresh *et al.*, 2021; Chandra *et al.*, 2019). *Opuntia dillenii* (Family: Cactaceae) grows in wild regions of south India and other dried lands globally. It has a long season of fruiting and flowering. They have cladodes, which are modified flat stems with leaves that are either completely lacking in leaves or converted into spines. In place of leaves, elongated structures called cladodes carry out photosynthetic activity. The exploration aimed to see if there were any phytochemicals in the extracts from *O. dillenii* cladodes (ODC) and then analyse their antioxidant activity. Furthermore, the inquiry was improved by contriving the best extract with a lactic acid bacillus (LAB) to see if the activities improved. The authors promise to lay the groundwork for future uses of LAB-managed ODC extracts as natural antioxidants in the treatment of oxidative stress.

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2. Materials and Methods

2.1 Plant material

Young *O. dillenii* cladodes (2 × 5 cm) were gathered from plants growing in the dry hilly areas near Anantapur, Andhra Pradesh, India. The sample was submitted to the Department of Botany at S.K. University in Anantapur, which identified and authenticated the plant and cladodes. The Herbarium received a voucher specimen (Number: SKBD/17/084).

2.2 Extraction of mucilage

Juvenile cladodes from *O. dillenii* were collected and cleaned for mucilage extraction. With a knife, the medullar parenchyma was manually separated and shattered. For 100 g of parenchyma, 250 ml of water was included (Ahad *et al.*, 2021; Babu *et al.*, 2021). As a result, the material was frantic (100 rpm) for 6 h at 40-60°C for 60-90 min before being filtered (first filtrate); the process was then repeated with an equal volume of water, resting and filtering to obtain the second, third and fourth filtrates. The mucilage was later extracted from the filtrate by using ethanol to precipitate it (80%). We mixed 100 ml of filtrate with 300 ml of ethanol. After 3 h of drying at 50°C, powder mucilage was achieved. All extractions were carried out three times (Rex *et al.*, 2019).

The same route was accomplished with absolute methanol, absolute ethanol, ethyl acetate, chloroform, hexane, distilled water and methanol (80%) (Mathew *et al.*, 2019).

2.3 Phytochemical characterization

In this present study, the authors adopted traditional approaches to conduct qualitative testing on ODC to screen for distinct phytochemical components (Aruwa *et al.*, 2019; Salehi *et al.*, 2019).

2.4 Hydroalcoholic extraction

The hydroalcohol extraction (Lakshana *et al.*, 2020) value was resolute as per the equation:

$$\% \text{ yield} = \frac{M1 - M0}{M2} \times 10C$$

where M0- is the weight of the empty flask (g); M1- is the weight of the flask after evaporation (g) and M2- the weight of the cladodes (g). The formed extract was maintained at a low temperature and kept away from light.

2.5 Total phenolic contents (TPC)

To regulate the TPC in various extracts, the Folin-Ciocalteu process was used with minimum revisions. With 1.25 ml of Folin-Ciocalteu reagent, the 0.25 ml sample was diluted 10 times. Then 1 ml of 7.5% sodium carbonate was included. For 30 min, the mixture was left in the dark. The absorbance was premeditated at 765 nm in contradiction to a blank. TC was used to quantify gallic acid equivalents (GAE) per 100 g of dry material (Bujor *et al.*, 2019).

2.6 The fortitude of total flavonoids content (TFC)

Chougui's method (Zolghadri *et al.*, 2019) was used to resolve the TFC. To summarise, 1.5 ml of extract was mixed with 1.5 ml of $AlCl_3$ reagent (2%). The absorbance was premeditated at 430 nm alongside a blank after 30 min of incubation in darkness (Salih *et al.*, 2021). Quercetin was employed as the calibration curve's standard. The results are given in milligrams of quercetin equivalent (QE) per 100 g of dry material (Mahindrakar *et al.*, 2020; Aryal *et al.*, 2019).

2.7 Judging antioxidant activity by DPPH scavenging assay

The DPPH test is often used to evaluate the radical scavenging capacity (RSC) of plant extracts. When hydrogen-donating antioxidants (2, 2-diphenyl-1-picrylhydrazine) are present, the purple-colored DPPH radical (2, 2-diphenyl-1-picrylhydrazyl) is converted to the yellow-colored non-radical form of DPPH (Yeligar *et al.*, 2021). Antioxidants easily diminish the DPPH radical's original purple colour to make reduced DPPH, a yellow-colored species that can be premeditated using a UV Visible spectrophotometer at 517 nm (Benajiba and Khojah, 2021; Wołosiak *et al.*, 2021). Different concentrations of extracts (20, 40, 60, 80, 120 g/ml) were made. 1 ml of DPPH methanolic solution is mixed with 4 ml of test solution (0.2 mM). Absorbance at 517 nm was detected after 30 min of vigorous shaking. All trials were done in triplicate with ascorbic acid as the control (Table 1 and Figure 1).

Table 1: DPPH scavenging activity of ODC extract

Conc. (µg/ml)	% Inhibition									
	Absolute methanol	Absolute ethanol	Ethyl acetate	Chloroform	Hexane	Distilled water	Methanol (80%)	Ethanol (80%)	Ethanolic extract (80%) LAB treated	Ascorbic acid
20	16.58 ± 0.9	23.65 ± 0.5	24.68 ± 0.2	22.35 ± 0.7	19.84 ± 0.2	24.11 ± 0.6	27.15 ± 0.7	29.84 ± 0.5	32.68 ± 0.2	30.21 ± 0.2
40	27.05 ± 0.8	20.86 ± 0.7	29.68 ± 0.6	28.84 ± 0.9	20.54 ± 0.2	29.68 ± 0.4	35.62 ± 0.8	36.54 ± 0.5	45.25 ± 0.3	42.35 ± 0.3
60	38.26 ± 0.7	40.01 ± 0.8	39.65 ± 0.8	32.67 ± 0.5	30.75 ± 0.3	33.67 ± 0.6	40.15 ± 0.2	45.68 ± 0.6	53.28 ± 0.4	50.32 ± 0.2
80	50.12 ± 0.7	50.32 ± 0.6	45.61 ± 1.2	40.91 ± 0.8	40.00 ± 0.6	41.25 ± 0.7	48.91 ± 0.1	53.62 ± 1.2	58.92 ± 0.3	55.61 ± 0.2
100	58.11 ± 0.9	59.69 ± 0.2	50.27 ± 2.3	47.99 ± 1.7	45.85 ± 0.9	44.17 ± 0.8	55.62 ± 0.6	60.28 ± 0.8	70.32 ± 0.9	66.39 ± 0.3
120	60.23 ± 0.8	61.35 ± 1.2	53.26 ± 0.8	50.22 ± 2.5	49.68 ± 1.2	47.71 ± 2.1	60.34 ± 0.3	64.98 ± 0.5	76.43 ± 0.8	74.65 ± 0.1
IC_{50}	81.40 ± 1.1	58.28 ± 0.8	119.11 ± 1.5	166.08 ± 1.5	174.56 ± 1.5	99.65 ± 1.5	62.08 ± 0.8	44.95 ± 1.2	37.16 ± 0.7	53.52 ± 0.2

Values in mean ± SD; n = 3

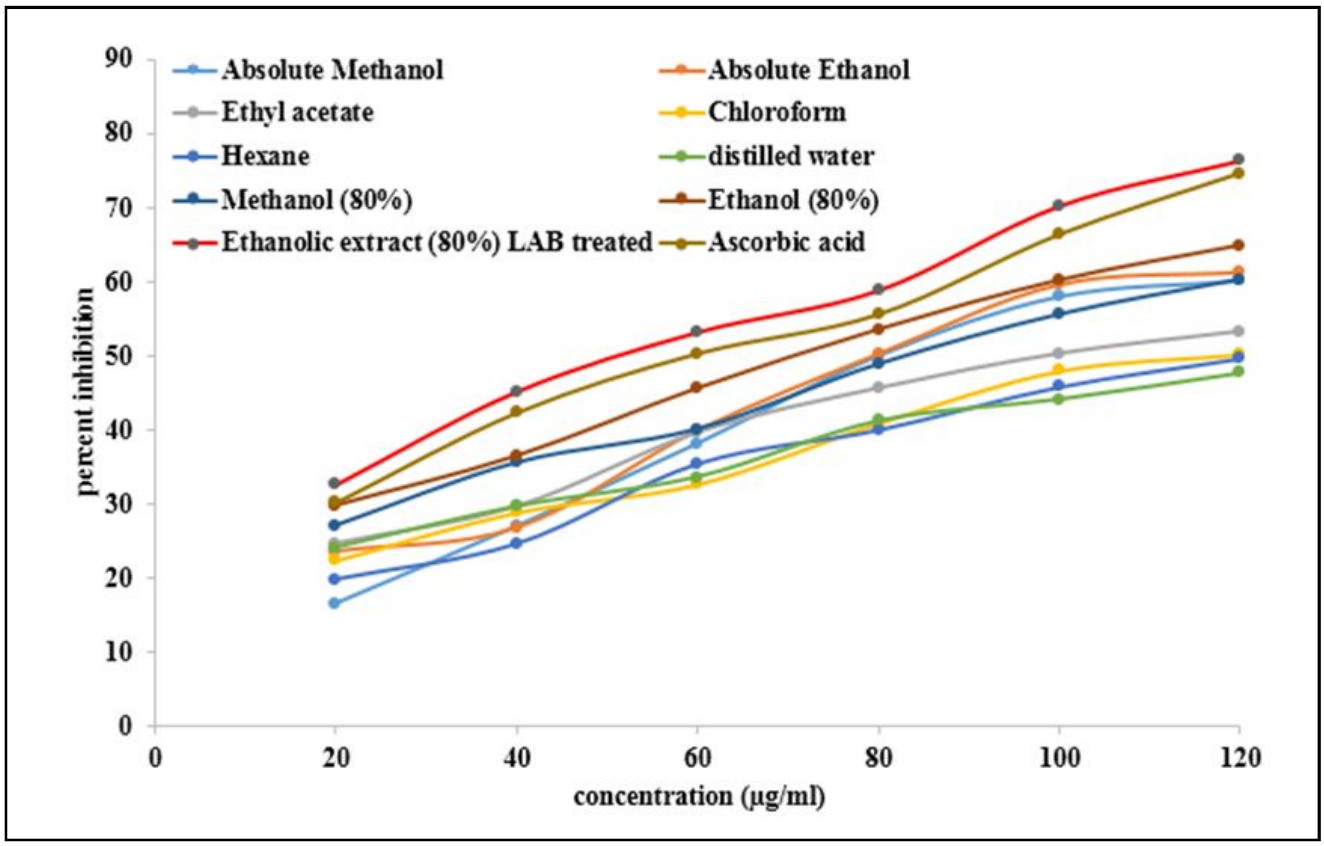

Figure 1: DPPH scavenging activity of ODC extract.

The inhibition of free radicals resulted in a % of DPPH inhibition using the formula:

$$\% \text{ of inhibition} = \frac{Ac - Ae}{Ac} \times 100$$

where, Ac- absorbance of the control; Ae- absorbance of the aqueous ethanolic extract sample. The IC_{50}, or the concentration that inhibited 50% of the DPPH radical, was used to premeditate RSC. The graph of DPPH inhibition % alongside extract concentration was used to compute the IC_{50}.

2.8 Statistical analysis

Three different observations were used to obtain the mean and standard deviation. A one-way ANOVA test was used to examine the significance of the difference between the extracts tested (p = 0.05) for *in vitro* antioxidant (DPPH) and TPC assays (Annepogu *et al.*, 2021). A linear regression approach was used to obtain the IC_{50}.

3. Results

3.1 Phytochemical characterization

All the extracts from ODC showed the existence of alkaloids, flavonoids and terpenes. The ODC extract was digested with 2 M HCl and amyl alcohol was added. A pink colour was observed in the alcoholic layer, indicating the presence of alkaloids. Chougui's method was adopted for flavonoids and terpenes were tested by adding 5 ml of chloroform to ODC and heating. The chloroform solution was treated with concentrated sulphuric acid and a red colour indicates the presence of triterpenes.

3.2 Total phenolic contents

The TPC was rich in ethanol (80%) extracts, *i.e.*, 88.16 ± 9.51 mg GAE/100 g DW. Later, the LAB contrived extract enhanced the TPC in the ethanolic extract, *i.e.*, 126.25 ± 9.85 mg GAE/100 g DW (Table 2 and Figure 2).

3.3 Total flavonoid content

The TFC was rich in ethanol (80%) extracts, *i.e.*, 54.55 ± 0.32 mg GAE/100 g DW. Later, the LAB contrived extract enhanced the TPC in the ethanolic extract, *i.e.*, 72.64 ± 1.24 mg GAE/100 g DW (Table 2 and Figure 3).

3.4 DPPH scavenging activities

Various extracts were tested for free RSC with quercetin as a reference in the DPPH free RSC. The concentrations of 1-120 g/ml were evaluated. The DPPH technique was used to assess the free RSC of aqueous ethanolic extracts of ODC. The crude extracts can be classified into three categories based on their IC_{50} values (Yap *et al.*, 2019): high antioxidant capacity (IC_{50} value less than 50 g/ml), moderate antioxidant capacity (IC_{50} value 50 g/ml to 100 g/ml) and low antioxidant capacity (IC_{50} value greater than 100 g/ml). The extracts of ODC had IC_{50} values of 44.95 ± 1.2 g/ml and 37.16 ± 0.7g/ml (LAB maneuvered), but ascorbic acid's IC_{50} value was 53.52 ± 0.2 g/ml, indicating a greater difference in antioxidant activity (Figure 4). The reports were impressive with ethanol (80%)

extracts.This suggests that the ethanol extract could operate as a DPPH free radical scavenger (Almarshad *et al.*, 2019). Later, the LAB-treated extract reduced the IC_{50} values, indicating improved antioxidant possessions.

Table 2: Total phenolic and flavonoid content of ODC

ODC	Total phenolic content (mg gallic acid equivalent /100 g DW)	Total flavonoid content (mg quercetin equivalent/ 100/g DW)
Absolute methanol	54.15 ± 7.18	32.56 ± 0.03
Absolute ethanol	65.52 ± 9.52	49.25 ± 0.03
Ethyl acetate	25.28 ± 4.52	31.28 ± 0.03
Chloroform	30.27 ± 2.31	26.52 ± 2.30
Hexane	27.65 ± 0.35	17.28 ± 0.58
Distilled water	45.25 ± 9.37	42.18 ± 0.34
Methanol (80%)	75.64 ± 9.85	36.77 ± 0.36
Ethanol (80%)	88.16 ± 9.51	54.55 ± 0.32
Ethanolic extract (80%) LAB treated	126.25 ± 9.85	72.64 ± 1.24

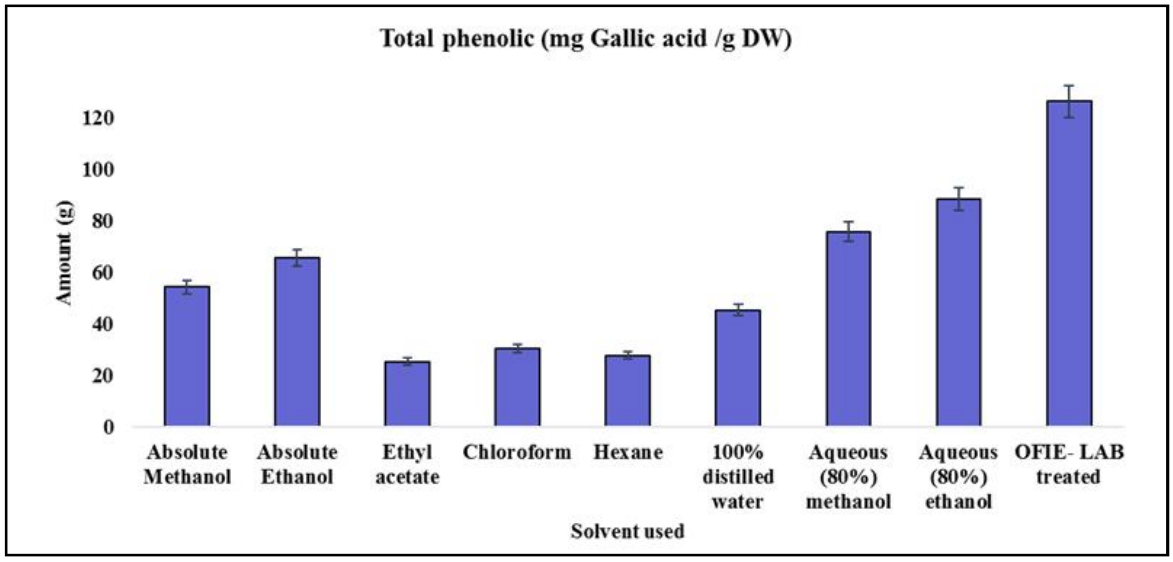

Figure 2: Histogram of total phenolic content in *O.dillenii* cladodes in various solvents.

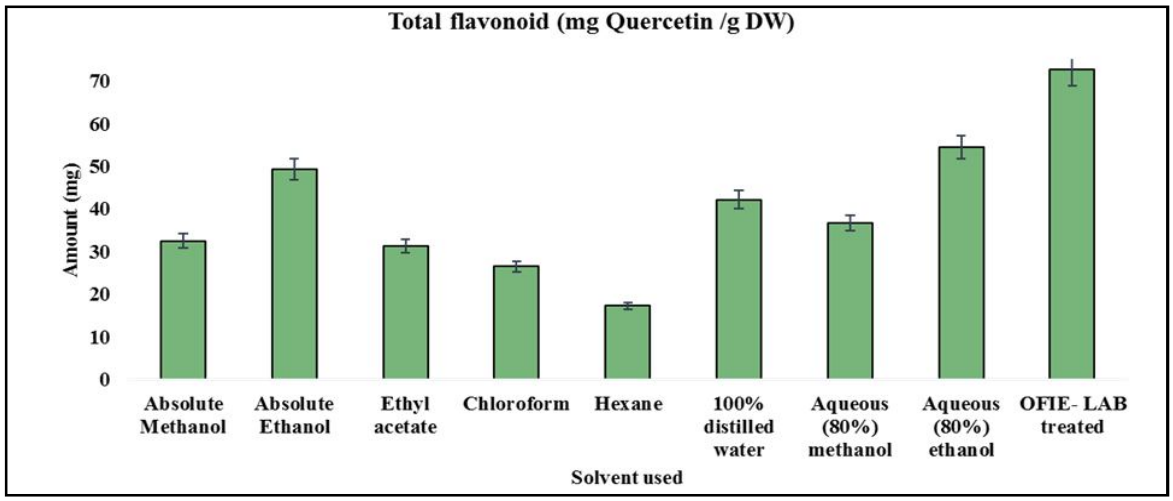

Figure 3: Histogram of total flavonoids in *O.dillenii* cladodes in various solvents.

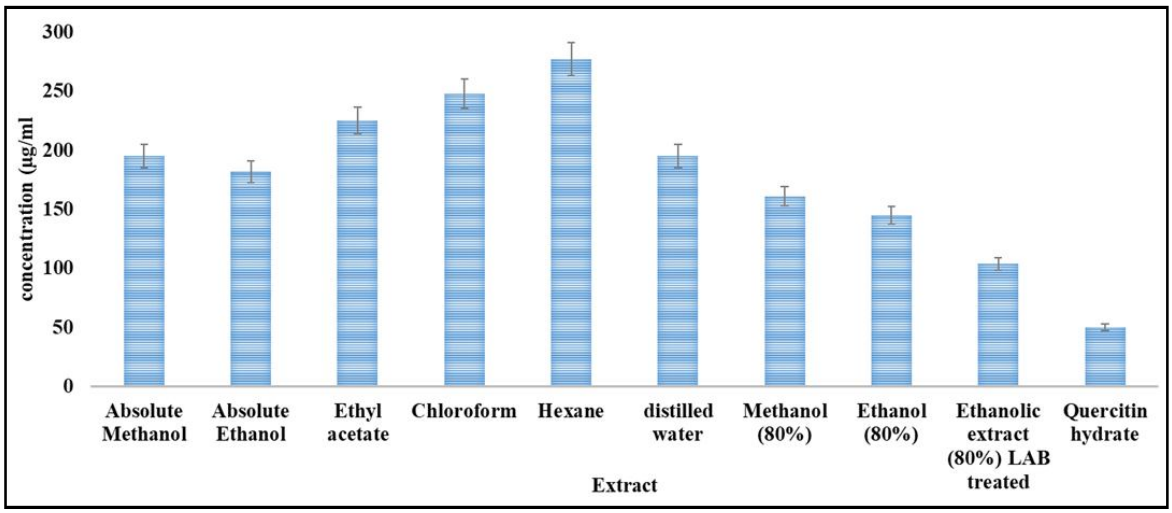

Figure 4: The DPPH radical scavenging capacity (IC_{50}) values of various extracts of ODC and quercetin.

4. Discussion

All population means (TPC, TFC and RSC of the DPPH assay) are substantially different (p = 0.01) at the 0.05 level. When comparing different extracts of ODC, phytochemical screening revealed the incidence of secondary metabolites that were rich in ethanolic solvent (80%). Such an observation was found by in methanolic and ethanolic extracts of *O. ficus indica* (Katanic *et al.*, 2019). The medicinal significance of ODC extract is well established. Studies confirmed antioxidant (Babitha *et al.*, 2019), antibacterial and antifungal activities with *O. dillenii* (Bouhrim *et al.*, 2019), antiparasitic activity with *O. dillenii* fruits (Moon *et al.*, 2020), and anti-inflammatory effects with *O. humifusa* fruits (Wolosiak *et al.*, 2021) were established. Among the biological constituents, phenolic compounds are rich in ODC extract. Phenolic compounds are secondary metabolic products found in plants that have a variety of biological and pharmacological actions that may protect against the development of chronic diseases. These compounds have a greater antioxidant effect. They can counteract the effects of reactive oxygen and oxidative free radicals (Garcia *et al.*, 2019; Wali *et al.*, 2019).The total polyphenol content (TPC) was calculated in ODC extracts, TPC is important for antioxidant activity and it is rich in cladodes. The LAB-mopped ethanolic (80%) extracts of ODC had the highest TFC, while hexane extracts had the lowest.

The high TFCs contribute to antioxidant capacity and have been intensively explored for potential health benefits. TFC and TPC in general are more antioxidants because they can postpone the prooxidative effects of proteins, DNA and lipids by creating stable radicals (Ali *et al.*, 2020).

The DPPH test is often used to define free RSC. At normal temperatures, DPPH is a stable free radical that gives a violet solution in methanol. DPPH has a significant absorption band at 517 nm in the visible spectrum (deep violet colour).

According to the DPPH findings, aqueous ethanol extracts exhibited the highest antioxidant activity when compared to extracts made with other solvents (Shirazinia *et al.*, 2021;Chbani *et al.*, 2020). The antioxidant activity of ODC in the DPPH system is comparable to that of restrained antioxidant activity using the DPPH antioxidant scavenging capability (Karabagias *et al.*, 2020). As the concentration of extract grew, the scavenging effect became stronger. Based on our observations, we assume the extracts' high activity is due to the component's accessible hydroxyl group (Nassrallah *et al.*, 2021).

5. Conclusion

The study is helpful in finding the antioxidant assets of *O. dillenii* cladodes (ODC) and it can be enhanced with lactobacillus treatment. The existence of tannins and flavonoids in ODC was explored. Total phenolic components and flavonoid levels were resolute and antioxidant properties were demonstrated in aqueous ethanolic extracts of ODC. The total polyphenol content (TPC) in ODC extracts was calculated. TPC was found to be significantly more distinct in cladodes. The maximum TFC was found in the ethanolic (80%) extracts of ODC which were treated with lactobacillus (5.5 X 104 CFU ODC ethanolic extract was fermented until it reached a pH of 3.7, which corresponds to a cell count of 1.2 × 109 CFU ml^{-1}), whereas the lowest was found in the hexane extracts. All of this suggests that this plant may have antioxidant qualities and that its polar extracts could be useful in the development of new chemicals for the prevention of diseases caused by oxidative stress.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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