# Comparing the content of hydro ethanolic *Opuntia ficus indica* cladode extracts following lactobacillus treatment using quantitative phytochemical screening

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**ABSTRACT**

The objective of the study is to investigate the quantitative assessment of phytochemicals from *Opuntia ficus indica* cladodes (OFIC) and to determine whether adding lactobacillus to the mixture improves the components. The beneficial components found in plants and herbs are being investigated by researchers because they are abundant, renewable, and inexpensive. The components, antioxidants, and other assets of *O. ficus indica* plant sections have been sufficiently studied. However, no attempts were made to investigate how the treatment with lactobacillus might affect any increase in the components. In this work, the phytoconstituents of OFIC, including phenolics, flavonoids, sugars, minerals, vitamins, and others, were quantitatively assessed after being extracted with ethanol (90 %). These phytoconstituents are responsible for the antioxidant activity to relieve oxidative stress, cardiovascular issues, atherosclerosis, cancer, and diabetes. The study revealed that the phytoconstituents present in OFIC can be enhanced by treating lactobacillus.

**Keywords:** constituents, extract, lactobacillus, *Opuntia*, quantitative

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## INTRODUCTION

Since many years ago, natural plant alternatives have been thoroughly researched as an excipient in the pharmaceutical sector[1,2]. The food, cosmetic, pharmaceutical, and nutraceutical industries are the main consumers of herbal products. Both plant extracts and partially completed herbal products are in high demand. Among the cactuses, desert-adapted plants are distinct. Since ancient times, the cactus has been utilized as a medicine[3,4].

*Opuntia ficus-indica* thrives in hot, arid climates all over the world. This plant, sometimes known as the Barbary fig, has tall blooms and fruits[5,6]. As a result, it is highly suited to storing a lot of rain during unpredictable downpours in arid regions.

It is especially suited for dry places because the plant is made to swiftly absorb and store rainwater from erratic showers. The vegetative components of these plants are commonly referred to as pads, joints, and cladodes[7]. The modified stems, which have an ovoid or elongated shape and measure 18–25 cm in length, take the place of leaves in the photosynthetic process. Photosynthetic processes are carried out by the chlorenchyma. The inner section, which is composed of white medullar parenchyma, is where water is primarily kept. The inner section, which is composed of white medullar parenchyma, is where water is primarily kept. Areoles are tiny bristles that are formed as leaves change into spines. On these rocks, thorny areole-producing plants flourish. The bigger ones are 2 cm long and have sharp edges. Each internode contains 35 areoles[8].

There has been a great deal of research done on the extraction, phytochemical screening, and biological screening of *Opuntia Ficus-indica* cladodes (OFIC). The lactobacillus treatment of an ethanolic (90%) extract of OFIC was not attempted to quantitatively assess the components.

## MATERIAL AND METHODS

### Plant material



*Opuntia Ficus-indica* young cladodes (4-5 weeks) (2X5 size) were collected from plants growing in the dry hills surrounding Anantapur, Andhra Pradesh, India. These were identified by the department of Botany of SK University, Anantapur, and authenticated. An Exemplar (SKBD/17/081) was deposited in the Herbarium.

The young cladodes (figure 1) were cleaned under running water and dried in the air. Later the outer skin was trimmed with a knife.

**Fig. 1:** Young cladodes of OFIC

**Extraction of Mucilage**

The OFIC were prepared and cleaned. A sharp knife was used to trim, slice, and then crush the outer layer. The substance was heated, refluxed, and combined (1:2 ratio) with a solvent (table 1). Later, Acetone was added to the filtrate to precipitate the mucilage out of it (1:3 ratio). Centrifuged for 10 minutes afterwards. The mucilage was then dried for 3 hours at 50°C. Three duplicate extractions of each sample were made [9-12].

**Fermentation of OFIC extract**

**Bacterial strains and culture conditions**

Bacterial strains used in this study were obtained from the food microbiology laboratory, at the Indian Institute of Science, Bengaluru, India. Lactic acid bacteria were activated successively three times in MRS broth (SRL, India) at 37°C for 18 h before use. All stock cultures were maintained at −80°C with sterile 50% (v/v) glycerol as a cryoprotectant. The strains were subcultured three times before use [13-15].

**OFIC extract fermentation**

For the fermentation of the extract, a 45 ml screw-capped bottle with a hermetic seal was utilized. Each container contained 40 ml of ethanolic OFIC extract. The fermentation of the extract used a single starter, Lactobacillus plantarum S-811. To inoculate pasteurized OFIC extract (2% v/v; initial cell density in the extract: 5.5X104 CFU), 18h (overnight) cells were grown in MRS broth at 37°C. The cells were harvested by centrifugation (12g, 5 min), washed twice with sterile saline solution, and re-suspended to the original volume in sterile saline solution. To achieve a pH of 3.7 and a cell count of 1.2X109 CFU ml1, OFIC extract was fermented. Pasteurized OFIC extract that wasn't inoculated with a lactic acid bacillus (LAB) served as the control and wasn't given any treatment. They further extracted with hydro-ethanolic solvents for OFIC separately because the extractive yield of relevant active principles was high in methanol, ethanol, and water extractives. Later, aqueous ethanol was abundant in the extraction yield of important active ingredients. So, LAB was used to further treat this extract. The phytochemical content of OFIC was examined using the techniques described in the preceding literature [14,16,17].



**Quantitative estimation of phytochemical constituents**

**Phenolic extraction**

The phenolic chemicals were extracted from powdered defatted skin and cladode seeds using the solid-liquid extraction technique described by Sardar *et al*[18]. To extract 30 g of powdered materials, 100 ml of aqueous ethanol (ethanol: water, 70:30 v/v) was used. The solution was stirred for an hour at room temperature and in complete darkness before being filtered. The extraction process was carried out twice under the same circumstances. All filtrates were mixed and evaporated using a Buchi 461 rotary evaporator at 40°C under vacuum[19,20]. The value of hydro-alcohol extraction was calculated as eq.1.

$$\% \text{ yield} = [(M_1-M_0)/M_2] \times 100 \text{---- (1)}$$

Where $M_0$ is the weight of the empty flask (g), $M_1$ is the weight of the flask after evaporation (g) and $M_2$ is the weight of

the seeds powder (g). The obtained extract was kept away from light at low temperatures.

**Determination of total phenolic contents**

With a few minor modifications, the Folin Ciocalteu technique was used to ascertain the total phenolic contents of various extracts. 1.25 ml of the ten-fold diluted Folin-Ciocalteu reagent was added to the 0.25 ml aliquot. 1 ml of sodium carbonate (7.5%) was then added. For 30 min, the mixture was incubated in complete darkness. At 765 nm, the absorbance was calculated in comparison to a blank. The total phenolic content was calculated as mg of gallic acid equivalents (GAE) per 100 g of dry material[21,22].

**Determination of flavonoids contents**

The flavonoid content was calculated by Chougui's description. In conclusion, 1.5 ml of extract and 1.5 ml of $AlCl_3$ reagent were combined (2%). The absorbance at 430 nm was measured in the absence of light after 30 min of incubation. The calibration curve was calibrated using quercetin as the reference. For each 100 g of dry matter, the results are reported as mg equivalent of quercetin (QE)[23].

**RESULTS AND DISCUSSION**

**Phytochemical characterization**

Table 1 summarizes the qualitative analysis of various extracts. Alkaloids, Flavonoids, 5-hydroxy flavones, Saponins, Tannins, and Terpenes are a few different types of phytochemicals. The % of proteins, lipids, total fibres, and ash was shown. Fructose, Glucose, and Sucrose were all represented in g/100g. The inorganic components, including calcium, phosphorus, sodium, potassium, magnesium, iron, manganese, zinc, and copper are in mg/Kg. The total anthocyanin and water-soluble vitamins (B-complex and C) were quantified (table 3).



**Total phenolic contents and flavonoids contents**

Using the standard curve formulae for gallic acid equivalent and quercetin equivalent, respectively, y = 0.0107x + 0.0049, $R^2$ = 0.9989 and y = 0.0015x + 0.0256, $R^2$ = 0.9965 were used to represent these contents (figure 2).

The amount increased to 126.25±9.85mg GAE/100g in LAB treated with 80% ethanolic extract. The total phenolic contents varied from 25.28±4.52mg GAE/100g (ethyl acetate extract) to 79.68±5.51mg GAE/100g (80% ethanolic extract).

The amount of quercetin increased to 72.64 mg GAE/100 g in LAB-treated 80 % ethanolic extract, ranging from 17.28±0.58 mg GAE/100 g in hexane extract to 57.07±0.04 mg GAE/100 g.

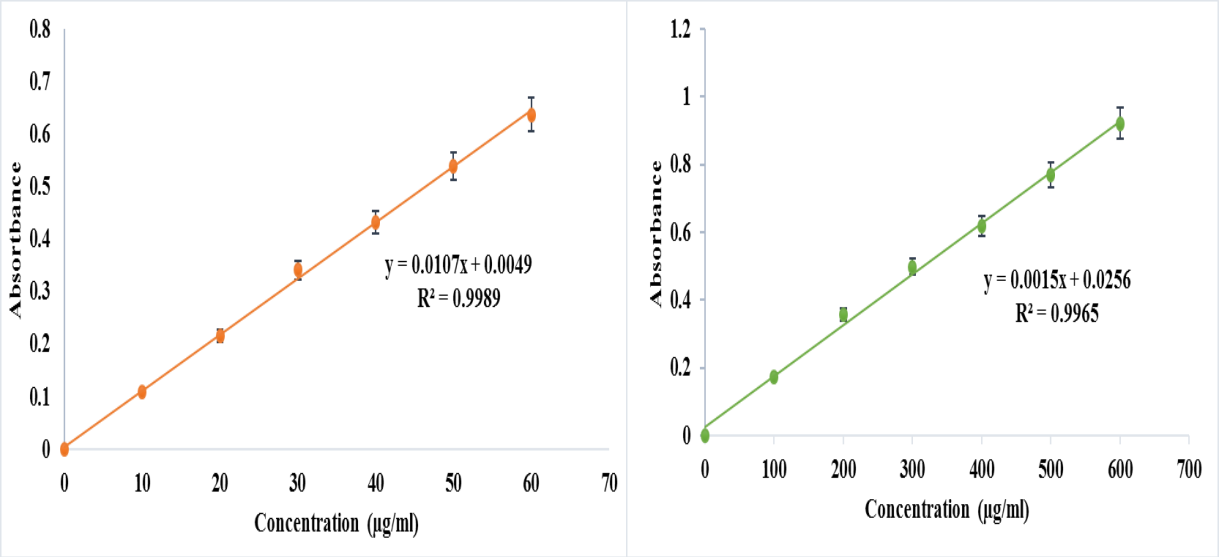




**Fig. 2:** Calibration curve of A) gallic acid and B) quercetin

Graphical representation of the Chemical composition of OFIC in various solvents (Figures 3, 4, 5)

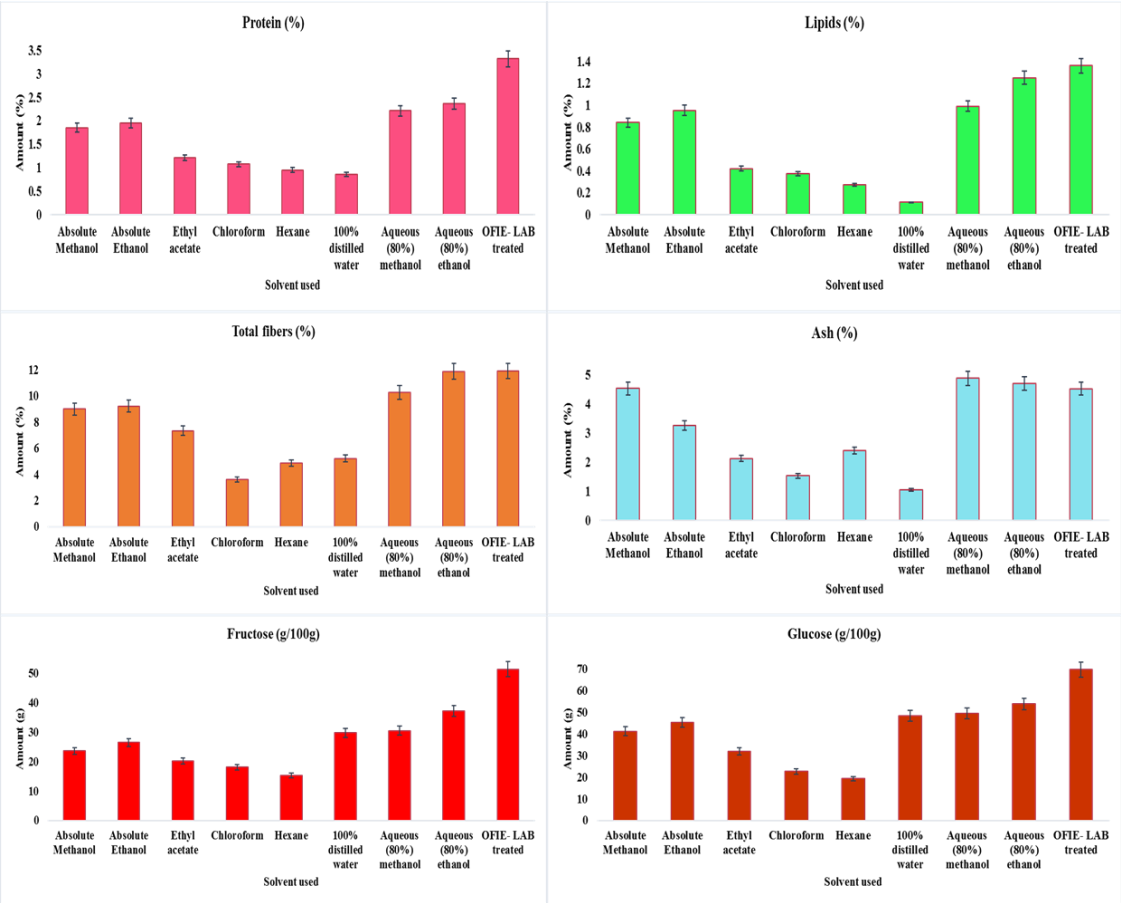


**Fig. 3:** Histogram of proteins, lipids, total fibres, ash, fructose and glucose content in OFIC in various solvents

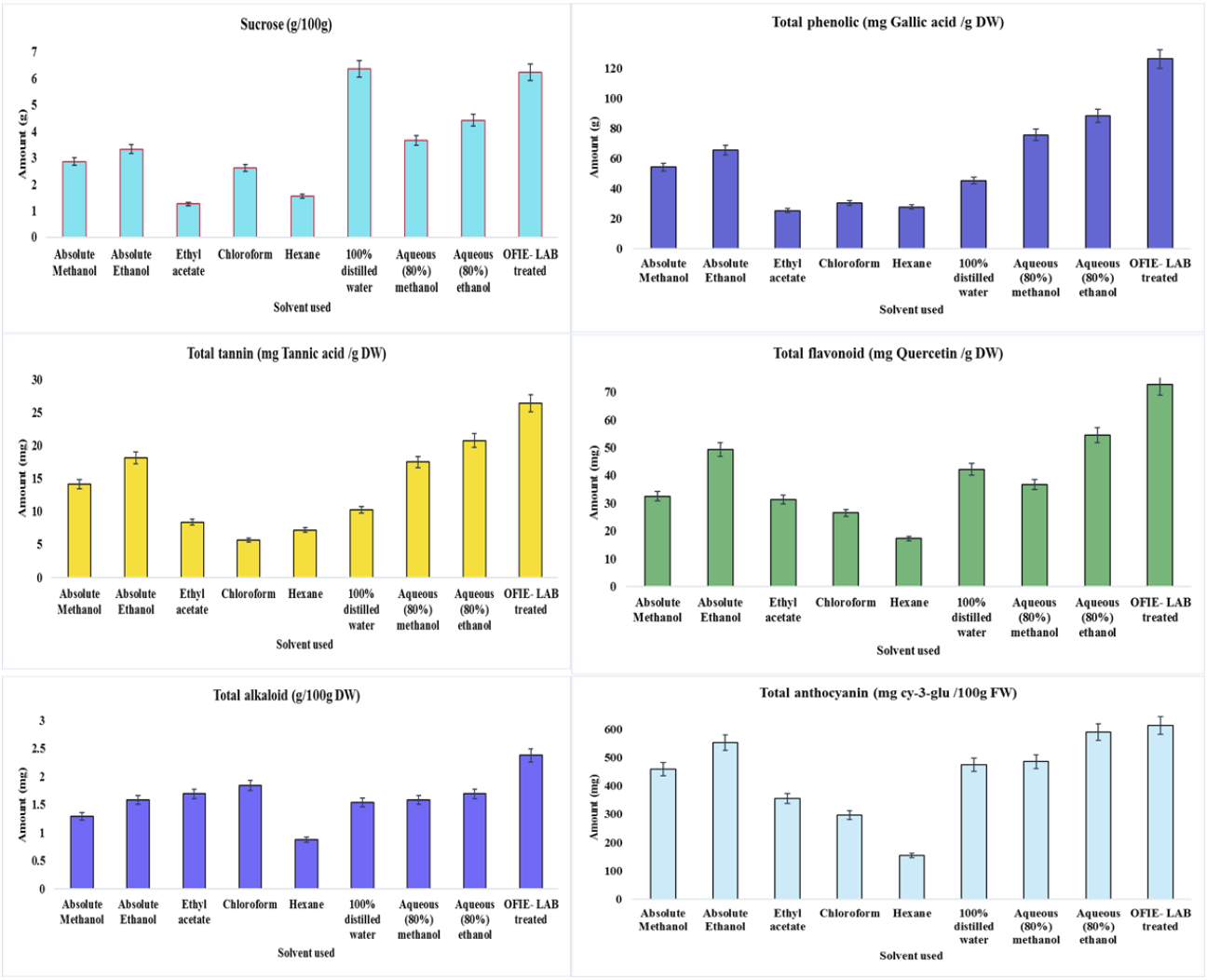


**Fig. 4:** Histogram representing the presence of sucrose, total phenolic, tannins, flavonoids, alkaloids and anthocyanin in OFIC in various solvents

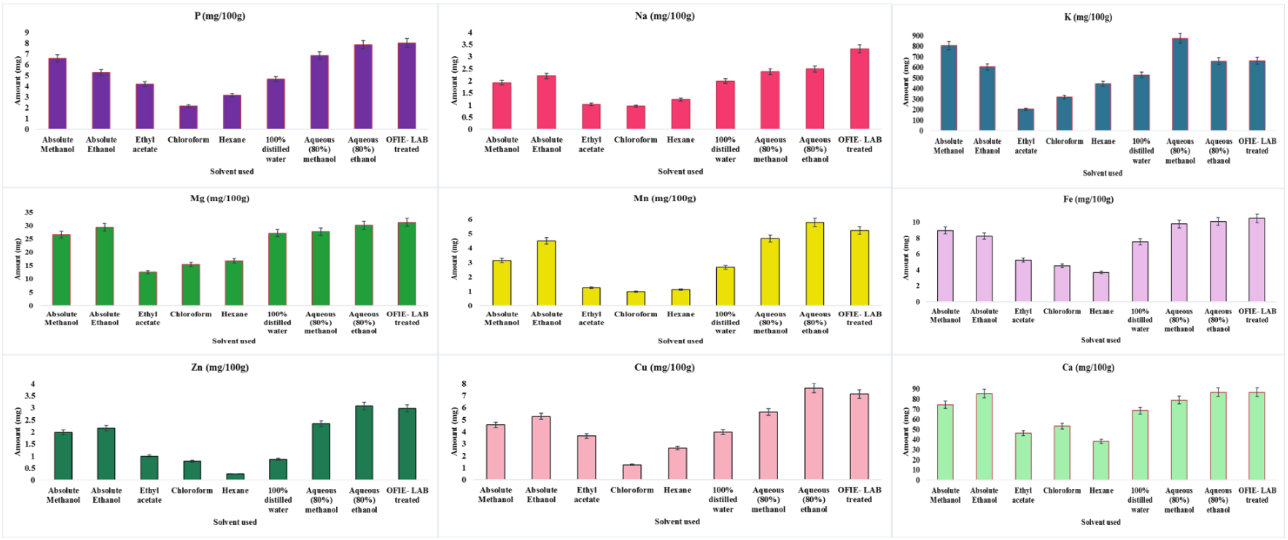

**Fig. 5:** Histogram of phosphorous, sodium, potassium, magnesium, manganese, iron, zinc, copper and calcium content in OFIC in various solvents

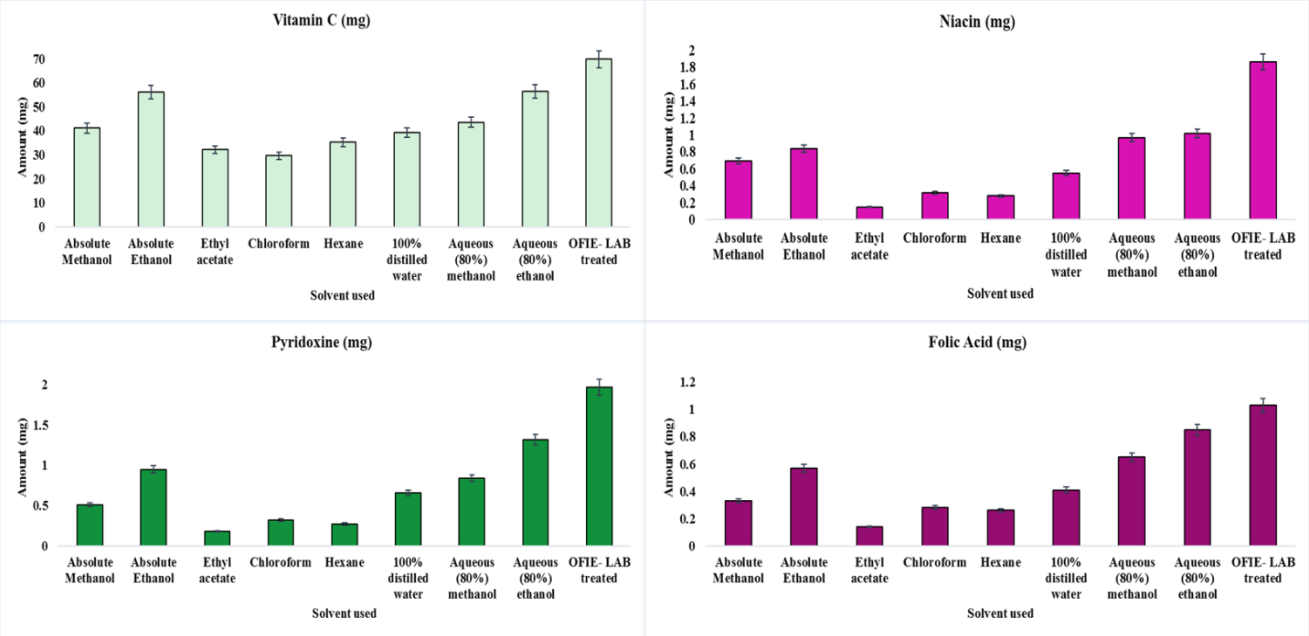


**Fig. 6:** Histogram of Vitamin-C, niacin, pyridoxine and folic acid content in OFIC in various solvents

## CONCLUSIONS

Components, antioxidants, and other resources. However, no attempts were made to investigate how the treatment with lactobacillus might affect any increase in the components of *Opuntia ficus indica* cladode (OFIC) ethanolic extract. In this work, the phytoconstituents of OFIC, including phenolics, flavonoids, sugars, minerals, vitamins, and others, were quantitatively assessed after being extracted with 90% ethanol. These phytochemicals are in charge of the antioxidant activity to treat diabetes, cancer, atherosclerosis, oxidative stress, and cardiovascular problems. The study found that lactobacillus treatment can improve the phytoconstituents found in OFIC.

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