

# Phytochemical Screening, Antioxidant, Antibacterial Activities And GC-MS Analysis Of Methanolic Extract Of *Opuntia ficus-indica* (L.) Mill. Fruit

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## Abstract

**Background:** *Opuntia ficus-indica* (L.) Mill., often known as Indian fig opuntia a drought-tolerant Cactaceae species that's grown in semi-arid and desert environments all over the world.

**Objectives:** The purpose of the research is to investigate the antioxidant, anti-inflammatory, antidiabetic, and antibacterial effects of *O. ficus-indica* fruit extracted in methanol.

**Methods:** The antioxidant activity of methanolic fruit extract of *O. ficus-indica* was assessed by DPPH (1,1-diphenyl-2-picrylhydrazyl), phosphomolybdenum assay, superoxide radical scavenging test, and ferric reduction power assay using ascorbic acid as the reference.

**Results:** Antibacterial activity was observed in gram-positive pathogens such as *Staphylococcus aureus* and *Enterococcus faecalis*, as well as gram-negative pathogens such as *Escherichia coli* and *Pseudomonas aeruginosa*. A membrane stabilization experiment was carried out utilizing blood saline to examine anti-inflammatory effects. Amylase inhibition, which employs iodine as a marker, is used to assess anti-diabetic activity.

**Interpretation:** Based on the GC-MS analysis, the presence of 4H-1-Benzopyran-4-one, 7-hydroxy-2-phenyl compound can be the reason for and this study demonstrated that future therapeutic development for a range of illnesses has promise.

**Keywords:** *Opuntia ficus-indica*, Antidiabetic, Anti-inflammatory, Antioxidant, Antibacterial

## INTRODUCTION

Many therapeutic plants have been recognized and used by people throughout history. Despite the fact that the world has become more industrialized, most people still prefer medicinal herbs for the treatment of many diseases because they are less expensive than modern medicine. According to the World Health Organization, 80 percent of the world's population uses traditional medicine, particularly African and Asian people. In modern science, about 120 active chemicals derived from higher plants are frequently employed. It has a link to both traditional and modern therapeutic medicine (Rachuonyo, Ogola, Arika, Wambani, & Gatheri, 2016)

Many therapeutic plants are widely available and regularly consumed as food. Individuals grow these plants as a result of their widespread use. Modern synthetic medicines are extremely effective at healing ailments, but they also have a slew of negative side effects. Crude medicine is less cost-effective in terms of curing ailments, but it is relatively devoid of side effects. For millennia, parasites have been a source of concern in medicine, with helminths thought to be a major source of difficulties for both humans and animals (Karimi, Majlesi, & Rafieian-Kopaei, 2015).

*Opuntia ficus-indica* (L.) Mill. is a drought-tolerant Cactaceae species cultivated in semi-arid and dry climates around the world. Its fruit and cladodes are employed in the culinary and pharmaceutical sectors as a source of minerals and phytochemicals (Galati, Tripodo, Trovato, Miceli, & Monforte, 2002). Despite the fact that various plant-derived nutraceuticals have been identified, only a few have been successfully incorporated into everyday foods for routine intake. The eligibility of a bioactive component to be employed as a functional ingredient in food is influenced by a variety of factors. Nutraceuticals generated from plants can alter food sensory properties by imparting an unpleasant

texture, odour, or taste. The food matrices utilized to deliver the bioactive component may eventually impair the nutraceuticals stability and/or bioavailability, lowering the desired effect on disease risk. Mucilage is a water-retaining hydrocolloid that, when combined with Crassulacean Acid Metabolism (CAM), permits *O. ficus-indica* to survive in semi-arid and arid environments (Stintzing & Carle, 2005).

*Opuntia* cladodes is a beneficial nutrient that has been used for centuries, although it is rarely used in modern food and pharmaceutical sectors. The vegetative parts of *Opuntia* spp. are rarely employed in modern nutrition and medicine, despite its historic use as a vital health-supporting nutrient (Zhang, Lin, & Ye, 2018).

It can also be used in the pharmaceutical industry. Prickly pear is extensively used as a folk remedy for burned wounds, oedema, and indigestion, and fruit extract has been proven to be more effective than stem extract. Vitamin C and radical scavenging capabilities are found in cactus pear fruit. Consumption of cactus pear fruit enhances antioxidant status, lowers oxidative damage to lipids, and improves body redox equilibrium in healthy adults, making it advantageous for people with lipid metabolism disorders (Wang, Barrow, Dunshea, & Suleria, 2021).

The aim of the present study is to investigate the pharmacological properties of methanol fruit extract of *Opuntia ficus-indica* and to evaluate the active compounds obtained from GC-MS analysis. The active compounds are further analyzed against cancer cells to evaluate the anti-cancer properties.

## MATERIALS AND METHODS

### Collection of plant material and preparation of the extract

The fresh fruits of *O. ficus-indica* were collected from Masinagudi village, Ooty, The Nilgiris, Tamilnadu, India. The fruits were washed, cut into very small pieces and about 10 g was soaked in methanol for 48 h. The dark pinkish supernatant liquid was filtered by filter paper and condensed in a hot plate at 50 °C, which yields gummy extract (El-Hawary et al., 2020).

### Qualitative phytochemical analysis

The fruit extract of *O. ficus-indica* was subjected to a preliminary phytochemical investigation for numerous types of phytoconstituents using specialised reagents.

## QUANTITATIVE PHYTOCHEMICAL ANALYSIS

### Estimation of total phenols

The total phenolic compounds were determined using the Folin-Ciocalteu reagent methodology with minor modifications. 100 µL of methanol fruit extract (1 mg/ml) of *O. ficus-indica* was added with 900 µL of methanol and 1 mL of Folin-Ciocalteu reagent (1:10 diluted with distilled water). Then, 1 mL of 20% (w/v) Na<sub>2</sub>CO<sub>3</sub> solution was added and thoroughly mixed. The mixture was then incubated and allowed to stand for 30 min in dark at room temperature. The absorbance was observed at 765 nm. The total phenolic content was expressed in terms of gallic acid equivalent (µg/mg of extract), which is a common reference compound (M. Ziaul Amin, 2020).

### Estimation of total flavonoids

With minimal modifications, the total flavonoid content of methanol fruit extract of *O. ficus-indica* was measured using the aluminium chloride reagent method (Peskin & Winterbourn, 2017). 500 µL of extract (1 mg/ml) was mixed with 500 µL of methanol and 0.5 mL of 5% (w/v) sodium nitrite solution. Then, 0.5 mL 10% (w/v) aluminium chloride solution was added followed by 50 µL of 1 M NaOH solution was added and thoroughly mixed. The absorbance was observed at 570 nm using a spectrophotometer and the result was expressed as quercetin equivalent (µg/mg of extract), which is a common reference compound.

### *In vitro* antioxidant activity

#### DPPH<sup>•</sup> radical scavenging activity

On the basis of stable 1, 1-diphenyl 2-picrylhydrazyl (DPPH) radical scavenging activity, the antioxidant activity of methanol fruit extract of *O. ficus-indica* was determined (Umdale et al., 2021). 1 mL of 0.1 mM DPPH solution in methanol was mixed with 1 mL of various concentrations (50-300 µg/ml) of fruit extract. The mixture was incubated and allowed to stand for 30 min in dark. Ascorbic acid was used as the reference standard. 1 mL methanol mixed with 1 mL DPPH solution was used as the control. The decrease in absorbance was observed at 517 nm. Ascorbic acid was used as the standard reference. The percentage of inhibition was calculated as:

$$\% \text{ of DPPH inhibition} = \frac{\text{Control} - \text{Sample}}{\text{Control}} * 100$$

#### Superoxide radical scavenging assay

The potential of the fruit extract of *O. ficus-indica* is to reduce blue formazan production by scavenging superoxide radicals generated in the riboflavin-light-NBT system was used as the basis for the superoxide radical scavenging activity assay (Suktham, Jones, Soliven, Dennis, & Shalliker, 2019). The reaction mixture contained various concentrations of (20-120 µg/ml) of fruit extract, 1.5 mM of riboflavin (200 µL), 12 mM of EDTA (100 µL) and 50

mM of NBT (50 µL) and added in that sequence. The reagents should be prepared in 50 mM of phosphate buffer (pH 7.6) solution. The reaction was initiated by illuminating the reaction mixture for 5 min. The absorbance was measured at 590 nm immediately after illumination. Ascorbic acid was used as the standard reference. The percentage of inhibition was calculated as:

$$\% \text{ of Superoxide radical inhibition} = \frac{\text{Control} - \text{Sample}}{\text{Control}} * 100$$

#### Phosphomolybdenum reduction assay

The antioxidant capacity of *O. ficus-indica* methanol fruit extract was determined using the method developed (Md Ziaul Amin et al., 2021). The fruit extract was mixed with 1 mL of reagent solution comprising ammonium molybdate (4 mM), sodium phosphate (28 mM), and sulphuric acid at varied doses (20-120 µg/ml) (600 mM). For 90 minutes, the reaction mixture was incubated in a water bath at 95 °C. The coloured complex's absorbance was measured at 695 nm. The standard reference was ascorbic acid. The percentage of reduction was calculated as:

$$\% \text{ of phosphomolybdenum reduction} = \frac{\text{Control} - \text{Sample}}{\text{Control}} * 100$$

#### Ferric (Fe<sup>3+</sup>) reducing power assay

The reducing activity of methanol fruit extract of *O. ficus-indica* was determined by slight modification of the potassium ferricyanide method (Gunathilake, Ranaweera, & Rupasinghe, 2018). Fruit extract of different concentrations (20 - 120 µg/ml) was mixed with 1 mL of phosphate buffer (0.2 M, pH 6.6) and 1 mL of potassium ferricyanide [K<sub>3</sub>Fe(CN)<sub>6</sub>] (1%, w/v) solution. The mixture was then incubated at 50°C in a water bath for 20 min. 500 µL of trichloroacetic acid (TCA) (10% w/v) was added to each test tube. Then 100 µL of freshly prepared FeCl<sub>3</sub> (0.1%, w/v) solution was added, mixed well and the absorbance was measured at 700 nm. Ascorbic acid was used as the standard reference. The percentage of reduction was calculated as:

$$\% \text{ of Fe}^{3+} \text{ reduction} = \frac{\text{Control} - \text{Sample}}{\text{Control}} * 100$$

#### Antimicrobial Activity

The microorganisms used for determination of antibacterial activity is of gram-positive strains such as *Enterococcus faecalis* and *Staphylococcus aureus* as well as gram-negative strains such as *Escherichia coli*, *Pseudomonas aeruginosa* were taken for antibacterial activity. The agar well diffusion method (Khan et al., 2021) was used to test the antibacterial activity of methanol fruit extract of *O. ficus-indica*. The microorganism was inoculated in muller hinton agar (MHA) medium for the antibacterial activity and was incubated at 37 °C for 24 hours. Each plate had five wells made with a sterile well-borer with an 8 mm diameter. The fruit extract was then added into each well with different concentrations of 250, 375, and 500 µg/ml. To assess the diameter of the inhibition zone formed around the well, all plates with fruit extract-loaded wells were incubated at 37 °C for 24 hours and the zone of inhibition was observed.

#### Anti-inflammatory activity

The blood was cleansed three times in a 10 mM sodium phosphate buffer with an isotonic buffer solution (154 mM NaCl) (pH 7.4). Each time, the blood was centrifuged for 10 minutes at 3000 rpm. The reaction combination (2 mL) contained 1 mL fruit extract as a test sample at various concentrations (20-120 µg/ml) and 1 mL 10% RBC suspension, while the reference test tube only contained saline. For 30 minutes, all centrifuge tubes containing reaction mixture were immersed in a water bath at 56 °C. and the supernatant's absorbance was measured at 560 nm. Aspirin was the most usual treatment (Sivaraj et al., 2020).

The following formula was used to compute the percentage inhibition of haemolysis:

$$\% \text{ of inhibition} = \frac{\text{Control} - \text{Sample}}{\text{Control}} * 100$$

#### Alpha amylase enzyme inhibition assay

The amylase enzyme inhibition experiment was carried out using the starch-iodine test (H Dib, Belarbi, Beghdad, & Seladji, 2014). The -amylase inhibitory activity of antioxidative substances and extracts is underestimated by the starch-iodine assay method. The entire test combination was incubated at 37 °C for 10 minutes and contained varying quantities (20-120 µg/ml) of prickly pear fruit extract and 10 mL of 1 percent (w/v) alpha amylase enzyme solution generated in 0.02 M sodium phosphate buffer (pH 6.9 containing 6 mM sodium chloride). Following that, each reaction set was given 1 percent soluble starch (w/v) and incubated for 60 minutes at 37 °C. 200 litres of 1 M HCl were added, followed by 200 litres of iodine reagent, to stop the enzymatic process (5 mM I<sub>2</sub> and 5 mM KI). The colour change was detected after the absorbance was measured at 595 nm.

$$\% \text{ of inhibition} = \frac{\text{Control} - \text{Sample}}{\text{Control}} * 100$$

#### Gas chromatography-Mass Spectrometry (GC-MS)

The chromatographic parameters were set to use helium as the carrier gas at a flow rate of 1 mL/min, with the injector set to 200 °C and the column oven set to 50-250 °C with a 10 °C/min injection mode, with the injector set to 200 °C and the column oven set to 50-250 °C with a 10 °C/min injection mode. The mass spectrum parameters were 70eV

ionisation voltage, 250 °C ion source temperature, 250 °C interface temperature, and a mass range of 50-600 mass units (HANANE Dib, Beghdad, Belarbi, Seladji, & Ghalem, 2013).

## RESULTS AND DISCUSSION

*Opuntia* spp. appears to have been subjected to intensive exploitation, according to the data presented. This secret wisdom has to be uncovered and re-evaluated in today's world. In the present study the methanolic fruit extract of *O. ficus-indica* was investigated with the antioxidant, anti-inflammatory and antidiabetic activity. Plants contain a wide range of phenolic compounds, including phenolics, flavonoids, and derivatives. In the present study, qualitative analysis of phytochemicals such as Alkaloids, terpenoids, steroids, phenolic compounds, flavonoids, tannins, glycosides, and saponins were observed in the methanol fruit extract of *O. ficus-indica*. In Quantitative analysis the total phenol and flavonoid content of methanolic extract of *O. ficus indica* were 445.85 µg/ml GAE and 11.37 µg/ml QE respectively. Total antioxidant capacity of phenolic extracts from *Opuntia* fruit, expressed in ascorbic acid equivalents. Because the extracts have electron-donating properties, they could operate as radical chain terminators, converting reactive free radical species into more stable non-reactive products. The extracts were found to have some action in a dose-dependent way.

Polyphenols had antioxidant activity similar to tannins, as did flavonoid extracts (ethyl ether and n-butanol) from *Opuntia* flowers. Furthermore, the antioxidant activity of ethyl acetate flavonoids extract is higher ( $0.406 \pm 0.05$  mgAEE/g extract), followed by tannins ( $0.14 \pm 0.005$  mgAEE/g extract) and polyphenols ( $0.121 \pm 0.004$  mgAEE/g extract) (Aruwa, Amoo, & Kudanga, 2019). The antioxidant activity of flavonoids extracts is characterised as acetate ethyl, n-butanol, and ethyl ether. The presence of phenolic acids, flavonoid derivatives, and other unidentified chemicals was discovered in LC/MS extract profiles. For the first time, isovitexin 7-O-xyloside-2''-O-glucoside, polyhydroxypregnane glycoside, and neohancoside C were discovered in *Opuntia* cladode extracts (Hikal, Said-Al Ahl, & Kačániová, 2021). The antioxidant activity of plant materials is closely connected with their phenolic content, according to research. Antioxidant assay such as DPPH, phosphomolybdenum assay, superoxide radical scavenging assay, ferric reduction power assay was performed using ascorbic acid as the standard. At a concentration of 300 µg/ml, the highest DPPH radical scavenging activity was  $31.28 \pm 0.33\%$ . At 300 µg/ml concentrations, the maximal phosphomolybdenum reduction was  $72.09 \pm 0.30$  percent, and the RC50 was 112.25 µg/ml concentrations. At 300 µg/ml concentrations, the maximal phosphomolybdenum reduction was  $72.09 \pm 0.30$  percent, and the RC50 was 112.25 µg/ml concentrations.

The antibacterial ability of nopal extracts was determined using the agar well diffusion technique. Each standardised bacteria (0.5 McFarland turbidity standard) was disseminated aseptically on Mueller Hinton agar solidified Petri dishes. The inoculated agar media was then divided into 6 mm wells using a sterile cork borer. Methanol extract (20 mm) also had a considerable inhibitory effect on *Enterococcus faecalis*. MRSA and *Enterococcus faecalis* were significantly suppressed, with the biggest zones measuring 34 and 35.1 mm, respectively (Nieto-Maldonado et al., 2022). The antibacterial activity of the oils derived from the two kinds of cactus pear seeds was tested using six distinct bacteria and two microscopic fungus species. Because the oil extracted with ethanol had the highest antioxidant activity, it was used to test the antibacterial and antifungal activity. The extracted oils suppressed *Saccharomyces cerevisiae* growth (38–40 mm), but growth was seen only in the presence of antimicrobial drugs (Brahmi et al., 2022). Methanol fruit extract of *O. ficus-indica* was tested for antibacterial activity against bacteria with thick cell walls, such as *Enterococcus faecalis* and *Staphylococcus aureus*, as well as bacteria with thin cell walls, such as *Escherichia coli* and *Pseudomonas aeruginosa*. The maximum zone of inhibition showed for *Enterococcus faecalis*, which was 23 mm at 500 µg/ml concentration. The maximal zone of inhibition for *Enterococcus faecalis*, which was 23 mm at 500 µg/ml concentration. NPDP had an IC<sub>50</sub> of 86.68 µg/ml, whereas NPWE had an IC<sub>50</sub> of 67.33 µg/ml, both having lower inhibitory activity than the conventional medicine acarbose (IC<sub>50</sub> of 38.05 µg/ml). The glucosidase inhibitory activity of each extract fraction was determined using a modified version of the standard procedure. When compared to Acarbose, the aqueous and acetone fractions had higher activity, with IC<sub>50</sub> values of  $16.98 \pm 0.77$  and  $25.11 \pm 0.89$ , respectively. While the other fractions were lesser, the methanol fraction, with an IC<sub>50</sub> of  $309 \pm 0.7$ , was regarded as quite weak (Przeor, 2022). At a concentration of 120 µg/ml, *O. ficus-indica* had a maximal anti-diabetic activity of  $63.69 \pm 0.81$  percent, with an IC<sub>50</sub> of 292.68 µg/ml. Still, further research is needed, including well-designed pharmacological tests and randomised clinical trials, to unlock the plant's secret medicinal potential, perhaps leading to new *O. ficus indica* medication discoveries.

Membrane stabilization assay performed using blood saline to evaluate anti-inflammatory property. Stabilizing the lysosome membrane is critical for limiting the inflammatory response because it limits the extracellular release of lysosomal components of activated neutrophils, such as bactericidal enzymes and proteases, which cause more tissue inflammation and damage. *O. ficus-indica* fruit extract displayed strong anti-inflammatory activities at various dosages, according to the findings. The prevention of heat-induced HRBC membrane lysis was utilised to examine the anti-inflammatory activities of different plant extracts since the membranes of human red blood cells (HRBCs) are similar to those of lysosomes (Kola et al., 2022). The results showed that *O. ficus-indica* fruit extract had strong anti-inflammatory properties at varied doses. IC<sub>50</sub> = 401.92 µg/ml compared with the positive control Aspirin.

## Qualitative Phytochemical Analysis

Alkaloids, terpenoids, steroids, phenolic compounds, flavonoids, tannins, glycosides, and saponins were observed in the methanol fruit extract of *O. ficus-indica*.

**Table 1:** Phytochemical analysis of methanol fruit extract of *O. ficus-indica*.

Phytochemical	Test	Result
Alkaloids	Hager's test: To the extract, a saturated aqueous solution of picric acid was added and shaken well.	+
	Dragendorff's test: To the extract, a saturated aqueous solution of Dragendorff's reagent was added and shaken well.	+
Terpenoids	Salkowski test: To the extract, chloroform was added and mixed well. Then, a few drops of Conc.H <sub>2</sub> SO <sub>4</sub> were added along the sides of the test tube.	+
Steroids	Liebermann-Burchard's test: To the extract, 1 mL of acetic anhydride was added and shaken well. To this, a few drops of Conc.H <sub>2</sub> SO <sub>4</sub> were added along the sides of the test tube.	-
Flavonoids	Alkaline Reagent test: To the extract, a few drops of 2% NaOH solution was added and shaken well.	+
Tannins	Lead acetate test: To the extract, a few drops of 5% Pb(CH <sub>3</sub> COO) <sub>2</sub> solution was added and shaken well.	+
Glycosides	Legal's test: To the extract, few drops of pyridine and few drops of alkaline sodium nitroprusside solution was added and shaken well.	+
Phenolic compounds	FeCl <sub>3</sub> test: To the extract, 200µl of neutral ferric chloride solution was added and shaken well.	+
Saponins	Foam test: To the extract, 3 mL of distilled water was added and shaken vigorously.	+

## Total Phenol and Flavonoid content

Quercetin, myricetin, caffeic acid, gallic acid, chlorogenic acid, coumaric acid, ferulic acid, and ellagic acid are examples of flavonoids and phenolic acids found in food that have been shown to have both antioxidant and prooxidant properties. The total phenol and flavonoid content of methanol fruit extract of *O. ficus indica* were 445.85 µg/ml GAE and 11.37 µg/ml QE respectively.

**Table 2:** Quantitative estimation of methanol fruit extract of *O. ficus-indica*.

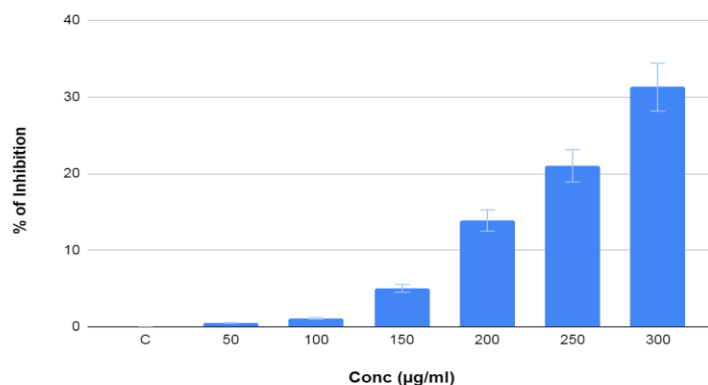
Phytochemicals	Amount (µg/ml)
Phenols	445.85 µg/ml GAE
Flavonoids	11.37 µg/ml QE

## DPPH<sup>•</sup> radical scavenging activity

At a concentration of 300 µg/ml, the highest DPPH radical scavenging activity was 31.280.33%. Methanol fruit extract of *O. ficus-indica* has good capacity to scavenge DPPH free radicals and the IC<sub>50</sub> was 486.69 µg/ml concentrations. It was compared with the standard ascorbic acid (IC<sub>50</sub> = 121.98 µg/ml concentration).

**Table 3:** DPPH<sup>•</sup> radical activity of ethanol fruit extract of *O. ficus-indica*

S. No	Conc (µg/ml)	% of inhibition
1	C	0
2	50	0.51±0.11
3	100	1.10±0.06
4	150	5.01±0.08
5	200	13.87±0.12
6	250	21.01±0.58
7	300	31.28±0.33



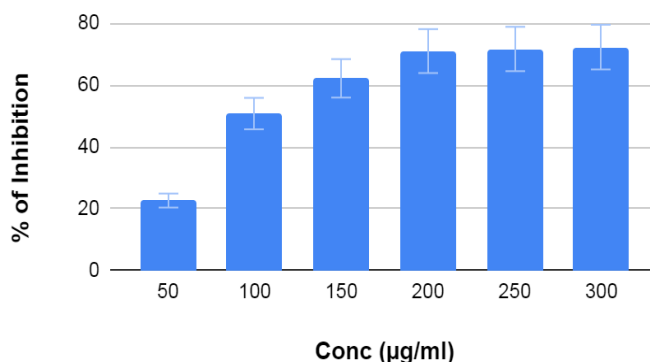
**Fig 1:** DPPH<sup>•</sup> radical activity of methanol fruit extract of *O. ficus-indica*

#### Superoxide radical scavenging activity

The IC<sub>50</sub> of *O. ficus-indica* was 97.80 µg/ml and the highest superoxide radical scavenging activity was 72.270.31 percent at 300 µg/ml concentration. It was compared to an ascorbic acid standard (IC<sub>50</sub> = 39.65 µg/ml concentration).

**Table 4:** Superoxide radical scavenging activity of methanol fruit extract of *O. ficus-indica*

S. No	Conc (µg/ml)	% of inhibition
1	C	0
2	50	22.66±0.55
3	100	50.77±0.24
4	150	62.16±0.58
5	200	70.97±0.11
6	250	71.65±0.06
7	300	72.27±0.31



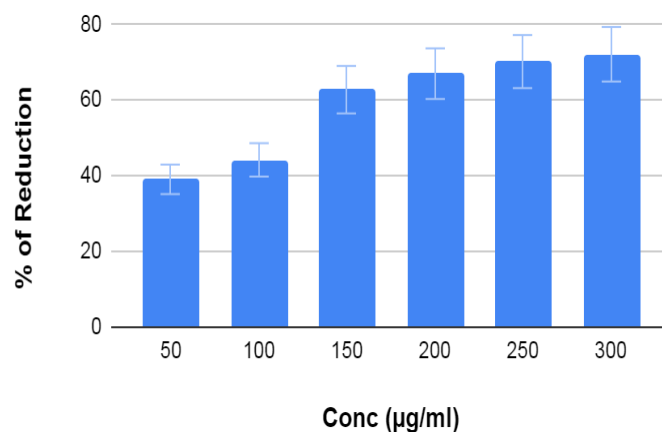
**Fig 2:** Superoxide radical scavenging activity of ethanol fruit extract of *O. ficus-indica*

#### Phosphomolybdenum reduction activity

The maximum phosphomolybdenum reduction was 72.09±0.30 % at 300 µg/ml concentration and the RC<sub>50</sub> was 112.25 µg/ml concentrations. It was compared with the standard ascorbic acid (RC<sub>50</sub> = 86.34 µg/ml concentration).

**Table 5:** Phosphomolybdenum reduction activity of methanol fruit extract of *O. ficus-indica*

S. No	Conc (µg/ml)	% of reduction
1	C	0
2	50	39.05±0.41
3	100	44.16±0.27
4	150	62.72±0.53
5	200	66.95±0.66
6	250	70.15±0.56
7	300	72.09±0.30



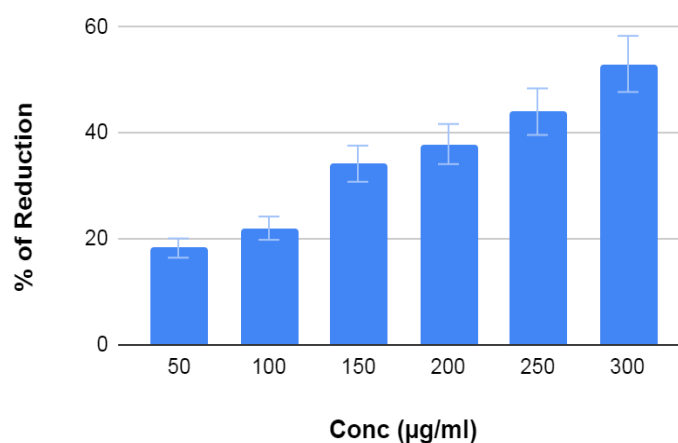
**Fig 3:** Phosphomolybdenum reduction activity of methanol fruit extract of *O. ficus-indica*

#### Ferric (Fe<sup>3+</sup>) reducing power assay

At 300 µg/ml concentrations, the maximal phosphomolybdenum reduction was 72.090.30 percent, and the RC50 was 112.25 µg/ml concentrations. It was compared to a conventional ascorbic acid concentration (RC50 = 86.34 µg/ml).

**Table 6:** Fe<sup>3+</sup> reducing power activity of methanol fruit extract of *O. ficus-indica*

S. No	Conc (µg/ml)	% of reduction
1	C	0
2	50	18.22±0.17
3	100	21.98±0.31
4	150	34.16±0.65
5	200	37.87±0.19
6	250	43.99±0.50
7	300	53.01±0.66



**Fig 4:** Fe<sup>3+</sup> reducing power activity of methanol fruit extract of *O. ficus-indica*

#### Antibacterial activity

Gram-positive bacteria like *Enterococcus faecalis* and *Staphylococcus aureus*, as well as Gram-negative bacteria like *Escherichia coli* and *Pseudomonas aeruginosa*, were tested for antibacterial activity of methanol fruit extract of *O. ficus-indica*. At a dose of 500 µg/ml, the greatest zone of inhibition for *Enterococcus faecalis* was 23 mm.

**Table 7:** Antibacterial activity of ethanol fruit extract of *O. ficus-indica*

Organism	Zone of inhibition			
	250 µg	375 µg	500 µg	Standard (Tetracycline)
<i>Enterococcus faecalis</i>	13	15	16	23
<i>Pseudomonas aeruginosa</i>	13	14	15	22
<i>Escherichia coli</i>	16	18	19	22
<i>Staphylococcus aureus</i>	14	15	16	17

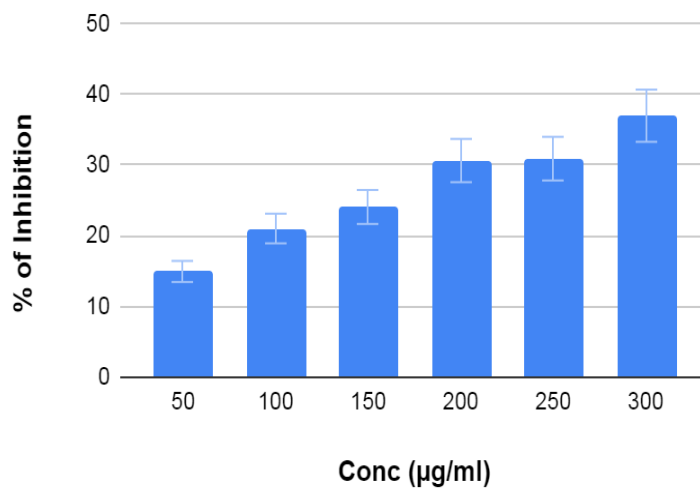


**Fig 5:** Antibacterial activity of methanol fruit extract of *O. ficus-indica*

**Anti-inflammatory activity**

**Table 8:** Anti-inflammatory activity of methanol extract of *O. ficus indica*

S. No	Conc (µg/ml)	% of inhibition
1	C	0
2	50	14.92±0.45
3	100	21.01±0.21
4	150	24.06±0.16
5	200	30.63±0.46
6	250	30.90±0.4
7	300	36.98±0.24

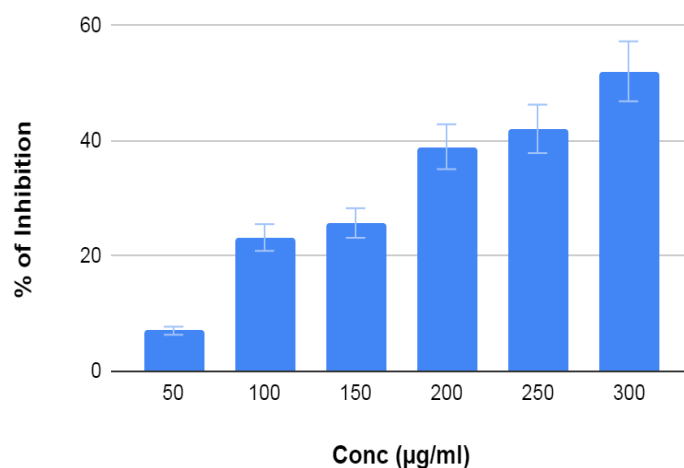


**Fig 6:** Anti-inflammatory activity of methanol extract of *O. ficus indica*

**Alpha amylase enzyme inhibition assay**

**Table 9:** Anti diabetic activity from the methanol extract of *O. ficus indica*

S. No	Conc (µg/ml)	% of inhibition
1	C	0
2	50	7±0.07
3	100	23.175±0.51
4	150	25.69±0.83
5	200	38.93±0.30
6	250	42.025±0.41
7	300	52.015±0.54



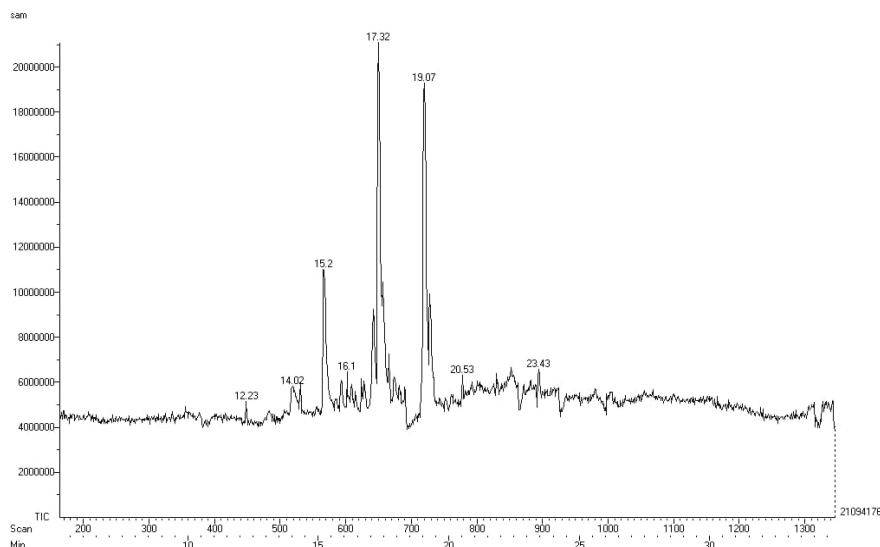
**Fig 7:** Antidiabetic activity of methanol extract of *O. ficus indica*

### GC-MS analysis

GC-MS analysis was carried out for the methanol extract of *O. ficus-indica* and the eluted compounds were shown in Table 10.

**Table 10:** Identification of compounds from methanolic extract of *O. ficus-indica* fruit using GC-MS analysis.

RT	Compound name	Compound structure	Molecular formula	Molecular weight
12.23	Benzene, 2,4-diethyl-1-methyl		C <sub>11</sub> H <sub>16</sub>	148.24µg/mol
14.02	2-Ethoxy-4-methylphenol		C <sub>9</sub> H <sub>12</sub> O <sub>2</sub>	152.19µg/mol
14.93	Flavone		C <sub>15</sub> H <sub>10</sub> O <sub>2</sub>	222.24µg/mol
15.2	[1,1'-Biphenyl]-2,2'-diol		C <sub>24</sub> H <sub>34</sub> O <sub>2</sub>	354.5µg/mol
16.1	Flavone		C <sub>15</sub> H <sub>10</sub> O <sub>2</sub>	222.24µg/mol
17.32	4H-1-Benzopyran-4-one, 7-hydroxy-2-phenyl		C <sub>9</sub> H <sub>8</sub> O <sub>2</sub>	148.16µg/mol
19.07	Oleic acid		C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	282.5µg/mol
20.53	Phytol		C <sub>20</sub> H <sub>40</sub> O	296.5µg/mol
23.43	Oxiraneoctanoic acid, 3-octyl-, methyl ester		C <sub>18</sub> H <sub>34</sub> O <sub>3</sub>	298.5µg/mol



**Fig (8):** Peak representing overall Bioactive compound identification

## CONCLUSION

When a drug is used after consulting with a professional, it is said to be safe. The indiscriminate use of plant medicine might result in long-term toxicity as well as short-term side effects. Traditional plants offer a wealth of prospects for exploration, as well as the opportunity to do the significant research required for their rational application. The rise in publications on the pharmacological potential of numerous traditionally claimed or recently discovered medicinal plants suggests a growing global interest in medicinal plants pharmacological potential. The therapeutic efficacy of *O. ficus indica* is exceptional, which aids in further and in-depth study. As a result, this plant was created in the hopes of identifying promising therapeutic leads for future development. Still, further research is needed, including well-designed pharmacological tests and randomised clinical trials, to unlock the plant's secret medicinal potential, perhaps leading to new *O. ficus indica* medication discoveries.

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