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# Qualitative and Quantitative Phytochemical Screening and Antioxidant Potential of different extracts of *Opuntia ficus indica* Fruits

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**ABSTRACT: The fruits of *Opuntia ficus indica* or prickly pear belongs to the family Cactaceae. The fruit is high in vitamin C, as well as other nutrients like magnesium, calcium, and potassium. In addition to its culinary uses, prickly pear fruit is also used in traditional medicine to treat a variety of ailments, such as wounds, inflammation, and high blood sugar. In a world facing various health problems and ailments, there is a growing need for research to uncover the potential benefits of natural resources. Oxidative stress-related disorders, such as cardiovascular diseases, neurodegenerative disorders, and cancer, pose significant health challenges worldwide. However, it is important to note that more research is needed to fully understand the potential health benefits and vital bioactive content of prickly pear fruit. Therefore, the current study aimed at investigating the quantitative phytochemical analysis of phenols, flavonoids, alkaloids, and glycosides in different extracts of fruits from the *Opuntia ficus indica* plant. The results showed that the aqueous extract had the highest phenolic content, while the hexane extract had the highest flavonoid content. The fruit was found to have a low alkaloid content. The glycoside content varied depending on the extract used, with the aqueous and hexane extracts having the highest values. Research on natural compounds like the phenols, flavonoids, and glycosides found in prickly pear fruit can provide insights into their antioxidant properties and their potential to mitigate oxidative stress, offering promising avenues for developing preventive and therapeutic interventions for these disorders.**

**Keywords:** *Opuntia ficus indica*, fruits, antioxidant, phenol, flavonoid, glycosides, cactus.

## INTRODUCTION

High value-added food items have been much more in demand over the past few years. Functional foods enhance the health by supplying more minerals, vitamins and antioxidants (Cha *et al.*, 2013). Plant-based products are crucial sources of secondary metabolites in addition to macronutrients like carbohydrates, proteins, lipids, sugars (Osuna-Martínez *et al.,* 2014). Secondary metabolites have the benefit of having a wide range of chemical structural variation, thus providing significant usage in the pharmaceutical, medical, agro-food and cosmetic industries (Bouaouich *et al.*, 2023). Bioactive compounds are gaining popularity due to their positive benefits on consumer health, such as through supplying antioxidants. Epidemiology research suggests that a diet richer in natural antioxidants may shield us from a range of deadly diseases, such as cancer, ageing, and hepatitis. Such conditions develop because of the occurrence reactive oxygen species (ROS) in the body (Zeghad *et al.,* 2019). There are several types of activated oxygen or reactive oxygen species (ROS) including free radicals like hydroxyl radicals (-OH), superoxide anion radicals ($O_2$) as well as non-free radical species such as single oxygen and $H_2O_2$ (Iftikhar *et al.,* 2023). Antioxidants can aid in protecting cellular components from oxidative damage brought on by free-radical species by promoting and maintaining cellular defence systems. Thus, antioxidants from food sources must therefore be present in adequate quantities to combat reactive oxygen species (Zeghad *et al*., 2019). Since many of these antioxidants are synthetic, there are significant health hazards. For instance, synthetic antioxidants have been linked to allergies, cancer, nausea, abnormal DNA and sperm development. Moreover, synthetic antioxidants could not provide any significant nutritional advantages. Due to safety issues associated with the use of synthetic antioxidants, the need for natural antioxidants has grown globally. Natural antioxidants, on the other hand, show minimal to no negative effects and are easily absorbed by the body (Aruwa *et al.,* 2019). Due to these reasons, out of all the various pharmacological activities of plant extracts, antioxidant activity is the one that is most frequently studied. Natural antioxidants, particularly those derived from plants like fruits and vegetables, have received a lot of attention in recent years.

According to several experts, the existence of polyphenolic chemicals is responsible for the antioxidant properties of vegetables or fruits (Zeghad *et al.,* 2019).

The best examples of plants adapted to dry terrain and various climatic conditions are cacti species. Approx. 130 genera and around 1500 species are present in the family Cactaceae, which has naturalised in many regions across the world, like the Middle East, Mediterranean basin, South Africa, India and Australia (Osuna-Martínez *et al*., 2014). Cactaceae plants are often regarded as an essential part of arid and semi-arid ecosystems and has a substantial impact on human societies and culture (García- Morales *et al*., 2022). In addition to being extensively consumed as food, the *Opuntia* genus has also been studied for its potential use in treating and preventing a wide range of human ailments, including cancer, microbial infections, and atherosclerotic cardiovascular disorders (Bouaouich *et al*., 2023). *Opuntia ficus-indica* (L.) Mill, often known as Indian fig, nopal or prickly pear is a crassulacean acid metabolism (CAM) plant in the Cactaceae family (Giraldo-Silva *et al*., 2023). Although in some nations different parts of this plant are used in the cosmetic and food industries, this species is also grown in South Africa, South America and the Mediterranean region for its sweet, nutrient rich edible fruit known as prickly pear. The soft cladodes of *Opuntia ficus indica* are utilised as fresh green vegetables and salad (Osuna-Martínez *et al*., 2014). The fruits of *Opuntia ficus-indica* have a long history of usage in folk medicine for a variety of therapeutic purposes across a number of nations. Consumption of cactus pear fruit has gained popularity in recent years due to its beneficial impacts on human health as well as its functional and nutritional properties (Chbani *et al*., 2020).

The pulp of prickly pear fruit comprises 87.5% of water with energy value of 170 kJ/100 g, of which 94% energy is obtained from its fructose and glucose carbohydrate content. Compared to pineapples, oranges and bananas, prickly pear fruit has lower titratable acidity of 1.83 g citric acid/ kg (Iftikhar *et al.,* 2023). Prickly pear fruits can have significant colour variations amongst cultivars, ranging from yellow to orange, green to white and red to purple. The betalain type of pigments are responsible for these differences. In addition to having a strong flavour, fruits contain a variety of interesting and beneficial components, including polyphenols, carotenoids, dietary fibres, minerals, vitamins, polysaccharides and amino acids (Cha *et al.,* 2013; Giraldo-Silva *et al.,* 2023). The pulp of prickly pears includes many polyphenolic metabolites, notably gallic acid, rutin and catechin being the most prominent. The antioxidant potential of prickly pear juice is boosted by vitamin C. This fruit is known to possess higher antioxidant properties than vitamins because of its significant polyphenolic content (Palmeri *et al*., 2020). Prickly pear syrup concentrates contain polyphenols that can inhibit the growth of tumor-causing fibroblast and neuroblastoma cell lines, while fermented juice may lessen UV-B damage to fibroblasts (Giraldo-Silva *et al.,* 2023). However, because of its richness in bioactive metabolites such as polyphenols, betalains pigments, vitamins, flavonoids, phenols and others, the fruit exhibits crucial health benefits and protection against several chronic diseases like antiproliferative, anti-inflammatory, neuroprotective, antidiabetic activities. In addition, prickly pear offers a wide variety of uses beyond fresh fruit consumption, including the production of processed goods like jams and juices, animal feeding and the generation of biogas and bioethanol. Moreover, prickly pear can be employed in the pharmaceutical, nutraceutical and cosmetic industries because it contains a substantial number of bioactive components, particularly polyphenolic molecules (Hernández *et al.,* 2022).

The current work focuses on the comparative study of qualitative and quantitative profile of important secondary metabolites such as phenolics, flavonoids, alkaloids, tannins, glycosides content in different solvent polarity extracts of fruits of *Opuntia ficus indica.* The antioxidant potential of the obtained extracts was conducted using DPPH and FRAP assays and compared among the different extracts.

## MATERIAL AND METHOD

**Plant Collection.** The fruits of *Opuntia ficus indica* were collected from Chotila town of Surendranagar district located near Rajkot, Gujarat, India. The collected fruits were sorted and the fresh ones were washed with the distilled water properly so as to remove dust and small spines. The large spines present on the fruits were removed with the help of sterile blade. Later, the fruits were cut into pieces and oven dried for 30 hours at 45° temperature. Further, the dried fruits were grinded using mixer and converted into fine powder form and stored at room temperature for further analysis.

**Plant Extract Preparation.** The extracts of *Opuntia ficus indica* fruits were prepared using Soxhlet (hot) extraction method. The extraction thimble was stuffed with 20 g of dry fruit powder and allowed to heat using 200 ml of solvent. Three different solvents- methanol, aqueous and hexane were used separately to prepare three different polarity fruit extracts. Further, the extracts were filtered with the help of Whatman filter paper no. 1 and the remaining solvents were evaporated using rotary evaporator. The obtained extracts were stored at 4° for future use. The yield of all the three extracts were calculated with the help of formula,

Yield (%) = Weight of fruit crude extract × 100/ Weight of fruit powder used

**Preliminary phytochemical screening.** Preliminary phytochemical analysis is an important step in the identification and characterization of plant constituents. It involves the qualitative detection of various chemical groups present in plant extracts. Commonly used tests for preliminary phytochemical analysis include tests for alkaloids, flavonoids, tannins, saponins, glycosides, terpenoids, and phenolic compounds. The specific reagents used for testing depend on the class of phytochemicals being screened. The following

protocols were followed as described by Shah *et al*. (2021).

**Tests for Alkaloids.** The filtered extract is tested for the presence of alkaloids using Dragendorff's reagent or Mayer's reagent.

For Dragendorff's reagent, a few drops of the reagent are added to the extract, and the formation of an orange-red precipitate indicates the presence of alkaloids.

For Mayer's reagent, a few drops of the reagent are added to the extract, and the formation of a creamy white precipitate indicates the presence of alkaloids.

**Tests for Phenols.** To test for the presence of phenols using the Folin Ciocalteu test, 1 ml of the plant extract is mixed with 1 ml of Folin Ciocalteu reagent. The formation of a blue-green color indicates the presence of phenolic compounds:

To the filtrate, 1-2 drops of 1% ferric chloride solution is added. The appearance of a blue or green color indicates the presence of phenols.

1 ml of the plant extract is added to 0.5 ml of lead acetate solution. The mixture is observed for the formation of a white precipitate, which indicates the presence of phenolic compounds.

**Tests for Flavonoids.** The sodium hydroxide test involves adding 1 ml of the plant extract to 3 ml of 2% NaOH, resulting in a yellow color. Then, adding 1 ml of dilute $H_2SO_4$ will cause the yellow color to disappear, indicating the presence of flavonoids.

The lead acetate test, on the other hand, involves treating 1 ml of the plant extract with a few drops of 10% lead acetate solution, and the formation of yellow precipitates confirms the presence of flavonoids.

**Tests for Glycosides.** To test for the presence of cardiac glycosides using the sodium nitroprusside test, 2 ml of the plant extract is mixed with 1 ml of 20% sodium nitroprusside and 1 ml of pyridine. The mixture is observed for the formation of a pink or red color, which indicates the presence of cardiac glycosides.

**Tests for Terpenoids.** To test for the presence of terpenoids using the copper acetate test, 1 ml of the plant extract is treated with 1-2 drops of copper acetate solution. The formation of an emerald green precipitate indicates the occurrence of terpenoids.

To test for the presence of terpenoids using the chloroform test, 1 ml of the plant extract is treated with 2 ml of chloroform and 3 ml of concentrated $H_2SO_4$. A layer is formed, and the presence of terpenoids is confirmed by the formation of a red-brown colored ring.

**Tests for Saponins.** To test for the presence of saponins using the foaming test, 1 ml of the plant extract is added to 20 ml of distilled water, and the mixture is shaken vigorously. The appearance of foam indicates the presence of saponins.

**Quantitative phytochemical analysis**

**Total Phenol Content (TPC).** The total phenolic content of fruits of *Opuntia ficus indica* were measured using Folin- Ciocalteau method doing several changes in the protocol as described by Rutuba *et al*. (2021). The standard- gallic acid or the sample (500 µl) with concentration 1 mg/ml was used with the dilution of 10 ml with the distilled water. To this, 500 µl of Folin-ciocalteau reagent was added and incubated for 5 minutes. Later, 2 ml of 20% sodium carbonate was added and the total volume was made up to 25 ml using distilled water. The reaction mixture was then mixed thoroughly and left to incubate for 30 minutes in dark. Further, the absorbance was taken at 765nm using spectrophotometer. The total phenolic content was calculated from the regression equation derived from standard calibration curve and is denoted as milligrams of gallic acid equivalent per gram of sample (mg GAE/ g of sample).

**Total Flavonoid Content (TFC).** Aluminium chloride colorimetric assay was used to determine the total flavonoid content of fruits of *Opuntia ficus indica* with some modifications in the procedure as given by Shah *et al.* (2021). 500 µl of sample along with 50 µl each of 10% aluminium chloride and 1 M potassium acetate was added to test tubes and diluted with 10 ml of distilled water. The reaction mixture was stirred properly and incubated for 30 minutes. Later, the readings were noted at 415 nm using spectrophotometer. Quercetin with different concentration gradient series was used as a standard with the same protocol. A regression equation was obtained from the standard curve which was used to calculate the total flavonoid content of the sample and the results were expressed as milligrams of quercetin equivalent per gram of sample (mg QE/g of sample).

**Total Alkaloid Content (TAC).** The fruit powder (2 g) was mixed with 20% of acetic acid (80 ml) in a beaker and covered and stand still for 4 hours. After incubation, the mixture was filtered and reduced to one-fourth by heating in water bath. Further, few drops of concentrated ammonium hydroxide were added to the solution until the precipitates were obtained. This solution mixture was filtered and the precipitates obtained were weighed (Senguttuvan *et al.,* 2014). However, using below formula, the total alkaloid content (%) of sample can be known,

Total alkaloid content (%) = Weight of precipitates x 100/ weight of sample powder taken

**Total Glycoside Content (TGC).** Total glycoside content of *Opuntia ficus indica* fruits was determined using Baljet method (Snehalatha and Rasmi 2021). The baljet's reagent was prepared by mixing 1% picric acid (95 ml) and 10% NaOH (5 ml). Equal amount of sample and baljet's reagent (500 µl) was mixed and incubated for 1 hour and diluted with distilled water (9 ml). Later, the readings were noted using spectrophotometer at 495 nm. Digoxin was selected as standard reference and different concentration series with same protocol was followed to obtain the regression equation. The total glycoside content of samples was expressed as milligrams of digoxin equivalent per gram of sample.

**Total Tannin Content (TTC).** To estimate the total tannin content, Folin-ciocalteau method was followed with several modifications in the procedure as described by CI and Indira (2016). 100 µl of sample was diluted with 5 ml of distilled water and 0.5 ml of Folin-ciocalteau reagent and 1 ml of 35% sodium

carbonate was added. The total volume of mixture solution was made up to 10 ml by distilled water and left for incubation for 30 minutes. Using spectrophotometer, the readings were taken at 700 nm. Tannic acid was used as standard at different concentration gradients and regression equation was derived to calculate the total tannin content denoted by milligrams of tannic acid equivalent per gram of sample.

**Antioxidant activity**

**DPPH assay.** The DPPH assay is a commonly used method to measure the antioxidant activity of plant extracts. The DPPH molecule is a stable free radical that accepts an electron or hydrogen radical to become a stable molecule, and this change is detected by spectrophotometry. A lower absorbance at 517 nm indicates a higher scavenging activity of free radicals, which is indicative of a stronger antioxidant capacity of the plant extract. The protocol typically involves the addition of 2 ml of DPPH solution to the series of concentrations of standard or the plant extract. The solution was kept in the dark at room temperature. Further, the absorbance of the solution was measured at 517 nm using a spectrophotometer. The percentage of DPPH radical scavenging activity was calculated using the following formula:

DPPH radical scavenging activity (%) = $[(A_{control}-A_{sample})/A_{control}] \times 100$

Where $A_{control}$ is the absorbance of the control (DPPH solution without sample),

$A_{sample}$ is the absorbance of the sample extract and DPPH solution.

A higher percentage of DPPH radical scavenging activity indicates a stronger antioxidant activity of the plant extract. The percentage of inhibition against the concentration of the plant extract was plotted to obtain a standard curve. The antioxidant activity of the plant extract can be evaluated by calculating the $IC_{50}$ value, which is the concentration of the extract required to inhibit 50% of the DPPH radicals. The lower the $IC_{50}$ value, the higher the antioxidant activity of the plant extract.

**FRAP assay.** Ferric reducing antioxidant power (FRAP) assay was used to evaluate the antioxidant potential of fruit extracts of *Opuntia ficus indica*. The FRAP reagent was prepared using acetate buffer, TPTZ solution and $FeCl_3$ solution in the ratio of 10:1:1. Following protocol was followed with minor modifications i.e. 500 µl of sample was diluted with 10 ml of distilled water and 4 ml of FRAP reagent (Wu *et al*., 2022). The readings were noted at 593 nm in spectrophotometer and $FeSO_4.7H_2O$ was used as standard at different concentration series to obtain the standard curve. The findings were reported as milligrams ferrous equivalent Fe (II)/ gram of sample.

**Statistical analysis.** Using the Graph Pad Prism Software, Version 8, all statistical analysis was performed. The experiments were conducted thrice to avoid any error and the findings of each experiment were represented as mean± standard deviation. Significant differences in the means were determined using the ANOVA method and the linear correlation between the variables in the test was measured using Pearson's correlation coefficient.

## RESULTS AND DISCUSSION

**Plant extraction yield.** A variety of solvents are commonly employed to extract a wide range of secondary metabolites, including ethanol, methanol, water, hexane, propane, acetone and many ionic liquids (Wani and Uppaluri 2022). In the present study, three different solvents such as methanol, aqueous and hexane based on different polarities were selected and subjected to Soxhlet extraction method for preparing different extracts of fruits of *Opuntia ficus indica*. Polar solvents were used to extract polar phytochemicals while non-polar solvent was used to extract the non-polar phytoconstituents present in the sample. Using the standard formula of plant yield, the extraction yield of aqueous extract of *Opuntia ficus indica* fruit was highest (63.02%) followed by the methanol extract (57.57%) and lowest in hexane extract (2.76%) as shown in Fig. 1. This is because of the strong polarity of aqueous and methanol solvent that draws large spectrum of plant secondary metabolites than other solvents (Senguttuvan *et al.,* 2014). The type of solvent employed, the length of the extraction process, the sample to solvent ratio and the extraction method used, all have a significant impact on the quality and quantity of the extract (Nuzul *et al.*, 2022).

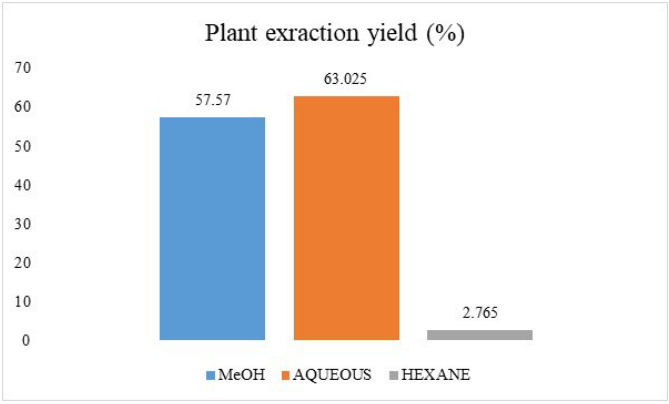


**Fig. 1.** Extraction Yield (%) of different fruit extracts of *Opuntia ficus indica.*

**Results of Preliminary phytochemical screening.** Preliminary phytochemical analysis is a qualitative screening method used to identify the presence of various phytochemicals in plant samples. Phytochemicals are naturally occurring compounds found in plants that play a vital role in the plant's growth, development, and protection against environmental stressors such as UV radiation, pathogens, and herbivores. Each class of phytochemicals has a unique structure and properties, and many have been shown to have anti-inflammatory, antioxidant, anti-cancer, anti-microbial, and other health-promoting effects. The concentration and type of phytochemicals can vary depending on the plant species, environmental conditions, and stage of growth. However, the following phytochemicals are found to be present in the respective solvent extracts of *Opuntia ficus indica* fruits (Table 1).

**Table 1: Preliminary Phytochemical screening of fruits of *Opuntia ficus indica.***

| Phytochemical | Test | Solvent | | |
|---|---|---|---|---|
| | | Methanol | Aqueous | Hexane |
| Alkaloids | Mayer's test | + | - | + |
| | Dragendorff's test | + | + | - |
| Glycosides | Acetic acid test | - | + | - |
| | Ammonia test | - | - | + |
| | Ferric chloride test | + | + | + |
| Phenols | Ferric chloride test | - | - | + |
| | Lead acetate test | + | + | + |
| | Folin-Ciocalteu test | + | + | + |
| Flavonoids | Sodium hydroxide test | + | - | + |
| | Lead acetate test | - | + | + |
| Saponins | Foaming test | - | - | - |
| Terpenoids | Copper acetate test | + | - | + |
| | Chloroform test | - | + | - |
| Cardiac glycosides | Sodium nitroprusside test | - | + | + |

(+ sign indicates the presence; - sign indicates the absence of respective phytochemicals)

**Results of Quantitative phytochemical analysis.** Phenols are a class of organic compounds that contain a hydroxyl group (-OH) attached to an aromatic ring. They are widely distributed in the plant kingdom and play important roles in plant growth, development, and defense against biotic and abiotic stresses. Phenols are present in various plant parts, including leaves, stems, roots, flowers, and fruits. The total phenolic content (TPC) of methanol, aqueous and hexane extracts of fruits of *Opuntia ficus indica* was estimated using Folin-Ciocalteau method. In the present study, the aqueous extract demonstrated highest total phenolic content of 19.50 ± 0.50 mg GAE/ g of sample followed by hexane extract (14.50 ± 0.50 mg GAE/ g of sample) and methanol extract (11.17 ± 0.29 mg GAE/ g of sample) of *Opuntia ficus indica* fruits. All the extracts displayed significant variation ($P<0.05$) in their TPC values. Mabrouki *et al.* (2015) reported in their study that the extract obtained from the methanolic pulp of *O. ficus-indica* and *O. streptacantha* demonstrated total phenolic content to be 54.33 ± 2.51 mg GAE/100 g extract and 104.66 ± 1.52 mg GAE/100g respectively while Medina *et al.,* (2007) stated comparatively lower phenolic content in fruits of *Opuntia ficus indica* (45.2 ± 7.4 mg GAE/100g). Numerous studies have found a strong correlation between total phenolic content and antioxidant activity and this is in consistent with the current study. Due to their reactivity as hydrogen- or electron-donating compounds and their ability to chelate metal ions, phenolic compounds display significant free radical scavenging capabilities. Thus, it is proved that the phenolic compounds attribute for the antioxidant activity of fruits of *Opuntia ficus indica* plant.

Flavonoids are a type of polyphenol that contains multiple phenol groups in their structure. Flavonoids are present in many common foods such as citrus fruits, berries, cocoa, tea, and wine. In plants, flavonoids play a crucial role in protecting them from UV radiation, pests, and disease. In humans, flavonoids are believed to have many health benefits, including antioxidant, anti-inflammatory, and anti-cancer properties. Flavonoids may also have cardiovascular and neuroprotective effects (Harborne, 1998). The maximum flavonoid content (TFC) of *Opuntia ficus indica* fruits was found in the hexane extract as compared to the methanol and aqueous extracts to be 17.83 ± 0.76 mg QE/ g of sample, 6.33 ± 0.29 mg QE/ g of sample and 4.33 mg QE/ g of sample respectively. The results of total flavonoid content of all three extracts showed remarkable differences ($P<0.05$) in their values. Many research work has suggested that fruits of *Opuntia ficus indica* possesses comparatively lower flavonoid content as compared to the phenolic compounds. Saravanakumar *et al.* (2015) in their study revealed the highest flavonoid content in methanol extract (21.24±1.73 mg $100g^{-1}$ of QE) as compared to the aqueous extract (15.77±2.16 mg100 $g^{-1}$ of QE) which is comparatively higher as reported in the present study. However, in the present study, the total flavonoid content showed positive correlation with the total glycoside content. Galati *et al*. (2003) reported that the fruits of *O. ficus indica* displayed the total flavonol glycosides content to be 652.5 (38 µg/mL) with the help of HPLC.

Alkaloids are a diverse group of naturally occurring chemical compounds that are typically found in plants. They contain a basic nitrogen atom and often have potent physiological effects on humans and other animals. Due to their pharmacological properties, alkaloids have been extensively studied for their potential therapeutic applications. Many pharmaceutical

drugs, such as painkillers and anti-cancer agents, are derived from alkaloids or are synthetic versions of alkaloids found in plants (Raffauf, 1996). The current study reported the total alkaloid content of prickly pear fruit powder to be 3.7%. However, it should be noted that *Opuntia ficus indica* is not known to be a significant source of alkaloids compared to other plants. The main bioactive compounds found in *Opuntia ficus indica* are betalains, which are water-soluble pigments responsible for the fruit's characteristic red and yellow colors.

The total glycoside content in *Opuntia ficus-indica* fruits can vary depending on various factors such as the ripeness of the fruit, the part of the fruit analyzed, and the analytical method used. In the current work, the aqueous extract (76.33 ± 1.04 mg digoxin equivalent/ g of sample) and hexane extract (72 ± 5.77 mg digoxin equivalent/ g of sample) of prickly pear fruits exhibited highest glycosides content while methanol extract showed poor glycosides content (9.83 ± 0.76 mg digoxin equivalent/ g of sample). There was prominent variation ($P<0.05$) seen in the TGC values of all the extracts of prickly pear fruit. According to a study published in the Journal of Agricultural and Food Chemistry, the total glycoside content in *Opuntia ficus-indica* fruits ranges from 18.52 to 35.56 mg/g of dry weight (Souad *et al.,* 2011) which is comparatively low as reported in the present work. Betalains are the main glycosides found in the fruit, and they are responsible for its characteristic red and yellow colors.

Tannins are a group of compounds that have varying chemical structures and properties. In the present study, total tannin content (TTC) of methanol, aqueous and hexane extracts of prickly pear fruit does not show any differences ($P>0.05$) in their tannin content. The total tannin content of methanol, aqueous and hexane extracts of prickly pear fruit was noted to be 10.37 ± 0.55, 10.93 ± 0.29 and 9.97 ± 0.38 mg of tannic acid equivalent/ g of sample respectively in this study. The total tannin content in the fruits of *Opuntia ficus-indica* can vary depending on factors such as the variety of the fruit, the ripeness of the fruit, and the method of extraction used to measure the tannin content. There have been several studies conducted to determine the tannin content of *Opuntia ficus-indica* fruits. One study published in the Journal of Food Composition and Analysis in 2015 found that the total tannin content of the fruits ranged from 17.3 to 45.2 mg GAE (gallic acid equivalents) per 100 g of fresh weight. The methanolic extract of fruits of *O. ficus indica* exhibited higher level of tannin content than the methanolic cladode extract of *O. ficus indica* (7.80 ± 0.24 mg GAE/g of dry samples) which proves the bioactive richness of fruits as compared to the cladodes of *O. ficus indica*.

Among the quantitative phytochemical profiling in this study, the aqueous extract of prickly pear fruit was found to be the most active in having highest secondary metabolites contents such as phenolic, glycosides and tannin content and has low flavonoid content as compared to the methanol and hexane extracts. The hexane extract showed good amount of phenolic, flavonoid, glycosides content than the methanolic extract while tannin content was recorded more in methanol extract than hexane extract (Fig. 2).

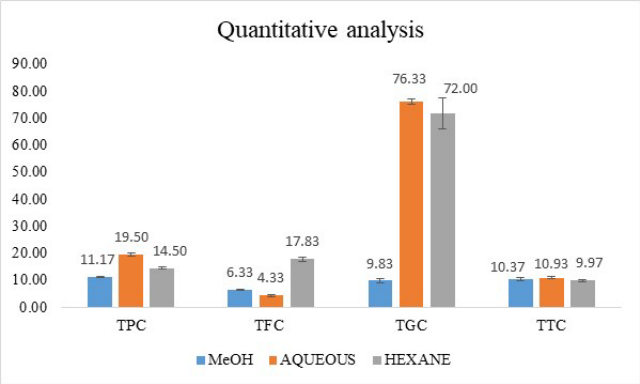


**Fig. 2.** Quantitative phytochemical analysis of total phenols (TPC), flavonoids (TFC), glycosides (TGC) and tannin content (TTC) in methanol (MeOH), aqueous and hexane extracts of *Opuntia ficus indica* fruits.

**Results of Antioxidant activity.** Antioxidants are compounds that protect cells from oxidative damage caused by free radicals. Free radicals are highly reactive molecules that can cause damage to cells and DNA, leading to aging, chronic diseases, and cancer. Plants contain various antioxidants such as polyphenols, flavonoids, carotenoids, and vitamins that have been found to exhibit potent antioxidant activity. Studies have shown that antioxidant-rich plants have various health benefits, including reducing the risk of chronic diseases such as cancer, cardiovascular disease, and neurodegenerative disorders. The current study reports the antioxidant capacity of prickly pear fruits using two assays- DPPH and FRAP. DPPH assay is considered to be the most reliable in comparison to the other antioxidant assays. The findings of the present work report the notable free radical scavenging activity by the methanol extract ($IC_{50}$- 29.46 µg/ml) followed by the hexane ($IC_{50}$- 68.27 µg/ml) and aqueous extracts ($IC_{50}$- 107.34 µg/ml) of prickly pear fruits (Table 2). El Mannoubi, (2021) in their study found DPPH $IC_{50}$- 0.784 mg/ml value of *Opuntia ficus indica* fruits which is comparable with the findings of the current study. The methanolic extract of cladodes of *O. ficus indica* displayed DPPH free radical scavenging activity with the $IC_{50}$ value of 25.76, that is again comparatively less than the methanolic extract of fruits of *O. ficus indica*as reported in the current study.

The antioxidant potential of prickly pear fruit was evaluated using FRAP assay. The methanol extract displayed prominent antioxidant activity to be 23.17 ± 1.76 mg $FeSO_4$ equivalent/ g of sample followed by aqueous extract (18.50 ± 0.87 mg $FeSO_4$ equivalent/ g of sample) and hexane extract (7 ± 0.50 mg $FeSO_4$ equivalent/ g of sample) as given in Table 2. There was remarkable change ($P<0.05$) found in the FRAP results of methanol, aqueous, hexane extracts of prickly pear. Dasgupta and Khatarani (2021) gave FRAP value of 43.74 ± 0.36mg AAE/100gm which shows that prickly pear fruits are rich in antioxidant activity. For the purposes of evaluating the antioxidant activity, Alexandra Silva *et al.* (2019) gave 17.12 ± 0.71 mg/ml and 46.6 ± 1.99 mg Trolox eq./g for the DPPH• and FRAP tests respectively. However, the above results prove the remarkable antioxidant potency in the fruits of *Opuntia ficus indica* which can be utilized as a natural antioxidant source in the daily routine diet and several industries.

**Table 2: Antioxidant activity of different extracts of *O. ficus indica* fruits (mean ± s.d.).**

| Antioxidant activity of fruits of *O. ficus indica* | | |
|---|---|---|
| | **DPPH values ($IC_{50}$ µg/ml)** | **FRAP values (mg $FeSO_4$equivalent/ g)** |
| Methanol extract | 29.46 | 23.17 ± 1.76 |
| Aqueous extract | 107.34 | 18.50 ± 0.87 |
| Hexane extract | 68.27 | 7 ± 0.50 |

## CONCLUSIONS

The study analyzed the total phenolic, flavonoid, alkaloid, tannin and glycoside content of *Opuntia ficus-indica* fruit extracts. The results showed that the aqueous extract had the highest total phenolic content, while the hexane extract had the highest total flavonoid content. The total alkaloid content in *Opuntia ficus-indica* fruit powder was found to be 3.7%, which is comparatively lower than other plants. However, the fruit extracts showed high glycoside content. Phenolic compounds and flavonoids have strong antioxidant activity, while glycosides have therapeutic potential. Although the current study focused on phenols, flavonoids, alkaloids, and glycosides, there are many other bioactive compounds present in *Opuntia ficus indica* fruits that have not been fully characterized. Future studies could explore the presence and potential health benefits of other compounds such as betalains, carotenoids, and vitamins. While the present study provides evidence for the potential health benefits of *Opuntia ficus indica* fruits, more research is needed to fully understand the mechanisms behind these effects.

## FUTURE SCOPE

*In vitro* studies could be conducted to investigate the anti-diabetic, anti-inflammatory, and anti-cancer properties of the bioactive compounds present in *Opuntia ficus indica* fruits. Additionally, *in vivo* studies using animal models and human clinical trials could provide further evidence for the health benefits of consuming *Opuntia ficus indica* fruits. This study provides a basis for further research to explore the potential applications of *Opuntia ficus-indica* fruit extracts in the food and pharmaceutical industries. Further research could focus on exploring the practical applications of these compounds in the development of new drugs or dietary supplements.

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**Conflict of Interest.** None.

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