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Evaluation of Nutritional and Medicinal Properties of *Opuntia elatior* Mill

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Abstract

Medicinal plant *Opuntia elatior* Mill., family Cactaceae, was studied for its nutritional value and health benefited properties from fruit. The fruit of the plants was extracted in sequential manner using methanol, hexane and distilled water. Out of these, maximum extract yield present in the methanolic extract was 36.84%. Nutritional value present in the 100 g of methanolic extracts of fruit was 1.02, 0.60, 63.26, and 0.11 mg of carbohydrates, protein, vitamin C and fat, respectively. Methanolic extract exhibits the highest antioxidant activity that is 54.10% and the lowest antioxidant activity is exhibited by the hexanoic extract at 45.66% and the distilled water at 50.40% of antioxidant activity. The anti-inflammation activity, the ability of protein denaturation in different fruit extracts of the maximum percentage of inhibition of 37.49% was observed from methanol extract followed by distilled water at 34.15% and then hexane at 30.38%. Phytochemical constituents present in the methanolic extract are alkaloids and phytosterols compound. High-performance thin-layer chromatography (HPTLC) analysis of methanolic extract showed the presence of three bands. Gas Chromatography-Mass Spectroscopy (GC-MS) analysis of methanolic extracts 19 characterization of bioactive compound. The methanolic extracts of fruits containing high content of protein, vitamin-C and carbohydrates provide good nutritional potential value and antioxidant activity and antiinflammation activity that may be possibly contribute to the treatment of arthritic disease.

Keywords: *Opuntia elatior*, nutritive elements, antioxidant activity, anti-inflammation activity, phytochemical constituents, bioactive compounds, HPTLC, GC-MS

1. Introduction

Traditionally useful medicinally important plants and health benefits foods related material last few years have more focused by common human population. The prevention of disease

and medical professionals very great demand for improving overall life. In this line, all types of wild fruits and vegetables have been recognized as valuable sources of nutraceuticals. The large number of natural product presents the chemically useful active compound and their multifunctional properties present. Opuntia *sp.* fruits and cladodes perfect member of traditionally useful for the health-prevention. The present research data show the high content of natural chemical constituents, which can give more added values to this fruit. High levels of some of chemical compounds like betalains, taurine, calcium, magnesium, and antioxidants present are very useful [1].

1.1. Nutrition

Human body requires the proper nutrition for growth, maintenance of body, reproduction, and health of organism is the science that interprets the interaction of nutrients and other substances [2]. It includes food intake, absorption, assimilation, biosynthesis, catabolism and excretion. Essential nutrient includes protein, carbohydrate, fat, vitamins, minerals and electrolytes. Human body normally requires 85% of energy for daily use from fat and carbohydrates and 15% from protein. In humans, nutrition is mainly achieved by the process of taking foods into our mouths, chewing, swallowing and digesting it. Nutrition is an essential component for maintaining the immune system, proper growth and development of cell, tissue and organ of human body. Eating a nutritional diet contributes to prevent the future disease, improves the quality of life and provides long lasting life. Your nutritional status is the state of your health determined by what you eat. Some of the minerals necessary for health are as follows: (1) Calcium: Calcium is a very important mineral in the diet. The major function of calcium is to build and help maintain strong bones. It is involved in blood clotting. (2) Iron: Iron in food exists as haem and nonhaem iron. In red meat, the haem iron is found, and 20–30% of this is relatively well absorbed. In cereals, pulses, certain vegetables and eggs, nonhaem iron is mostly found and is generally less well absorbed. (3) Zinc: It is essential for synthesizing protein, DNA and RNA in human body. Only 0.003% of zinc is present in the human body. It is required for growth in all stages of life. (4) Sodium: Sodium helps to maintain fluid volume outside of the cells and helps cells to function normally. (5) Potassium: Potassium maintains fluid volume inside and outside of cells and prevents the excess rise of blood pressure with increased sodium intake.

1.2. Essential nutrient requirements

In human body, essential element is not synthesized in the adequate amount and body cannot synthesize on its own and must be provided by the nutritional diet. The chemical components present in the food are classified into six major groups like protein, fats, carbohydrates, minerals, vitamins and water. Utilization of nutrients as an essential component requires water. In our body, nutrients are required for the various functions like respiration, digestion, growth and development. The amounts of the essential nutrients required differ by age and the state of the body [3].

1.3. Use of fruits as nutritional and medicinal source

Fruits are considered in dietary guidance because of their high concentration of dietary fibers, vitamins, minerals, electrolytes, phytochemicals, and especially antioxidants. Various reviews

have been associated with the low intake of fruits include chronic diseases such as cardiovascular diseases, blood pressure, hypercholesterolemia, many cancers, respiratory problems as well as mental health. Traditionally many fruits reported have been useful in many non-communicable diseases and reduce the risk of epidemiologically. Nowadays, people are more interested in the prevention of health-related diseases which is that vital role of antioxidants. This fruit of bright color act as scavengers clean up free radicals before they cause any health effects.

In this fruit, more amount of the fibers are present and are helpful in reducing intestinal passage rates resulting in a more amount of nutrient absorption and hence prevent the constipation. It increases the concentration of short chain fatty acids because of fermented in colon that having maintained gut health and anti-carcinogenic properties. Recent report shows that fruits containing high number of anthocyanins, flavanols, and procyanidins, such as berries, grapes, and pomegranate are effective at decreasing cardiovascular risks while citrus fruit and apples had a moderate effect on blood pressure and blood lipid level [4].

Fruits have also been suggested to prevent osteoporosis in adults mainly due to their rich source of calcium and other vitamins present in them, which are vital for bone health. The high fiber content of fruit may play a major role in the reduction of acid load of the diet and in enhancing bone formation through calcium absorption. Interestingly, phytochemicals in fruits have been found to act as antiobesity agents because they also play a role in suppressing growth of adipose tissue. Fruits have been suggested to prevent obesity since they add up to dietary variety.

1.4. *Opuntia elatior* Mill. Fruit

1.4.1. Classification

Kingdom: plantae; Division: Magnoliophyta (Angiosperms); Class: Magnoliopsida (Dicotyledons); Subclass: Archichlamydeae; Order: Caryophyllales (cactales); Family: Cactaceae; Subfamily: Opuntioideae; Tribe: Opuntieae; Genus: *Opuntia*; Species: *elatior* Mill.

Opuntia elatior plant is scrubby, 3–4 m height (**Figure 1**). Leaves 6–7 mm long, recurved and reddish at the tops. Joints are 18–30 cm size in height by 10–18 cm in width, obovate, thin and dull bluish green color. Small area spike bearing is about 4–5 cm and increase up to 10 cm, rather slender straight prickles which are, grey and opaque except when quite young, the largest of around 3–5 cm long. Flower is 5 cm across, yellow or orange in color. The perianth rotate, with the outer segments short, ovate, acute, they are red in the center, yellow at the edges, and the inner spatulate is acute. Stamens are a little shorter than the perianth. Style exceeds the stamens; stigmas six in numbers. Berry pyriform are, bearing tufts of glochidia and a few prickles, reddish purple color when ripe [5].

1.5. Medicinal properties

Cactus have been used in treating several diseases, such as rheumatic disease, hypertension, diabetes, asthma and gastric mucosa diseases traditionally use as medicine in many countries over the world. This plant contains bioactive molecules that are well known for their health-related properties present in the cactus fruits and cladodes that is the reason for the

more focus of many studies. It has been shown that there is a positive correlation between a nutritional rich in prickly pear cactus and a reduced risk of diseases associated with such as diabetes, cancer, cardiovascular and neurodegenerative diseases [6].

Opuntia traditionally used as a valuable health supporting nutrient, the vegetative parts of *Opuntia* spp. is scarcely used in modern nutrition and medicine. It is suggested that *Opuntia* spp. could be considered as a new approach in treating noninsulin dependent diabetes mellitus. Prickly pear is widely used as folk medicine for burned wound and indigestion and it is found that the effect of fruit extract is better than those of stem extract. Fruits are recommended as an expectorant and remedy for whooping cough, asthma, gonorrhoea, ulcers, tumors, treatment of diarrhea and syphilis [7].

The presence of potentially active nutrients and their multifunctional properties make fruits and cladodes of *Opuntia* spp. ideal candidates to produce nutraceutical products. In India, traditionally acceptable for its pharmacological properties of prickly pear, but insufficient scientific information and knowledge on these plants is still rarely available. *Opuntia* species in the last several years have been used as antidiabetic, antihyperlipidemic, antioxidant, anti-ulcer, antiviral, diuretic, immunomodulatory, analgesic and anti-inflammatory, anticancer and neuroprotective. It is also used to improve platelet function, promote wound healing and is nutritionally important [8–11]. A novel food product which is a mixture of both soluble and insoluble fibers made from dehydrated leaves of the cactus *Opuntia* is found to have hypolipemic properties and hence useful for patients with lipid metabolism disorders.

Opuntia elatior (prickly pear) uses as a highly nutritive food. These people are interested in healthy, prevention disease, natural life-style often promote prickly pear fruit used as a nutritional fruit. The fruit reported the anticlotting, anti-inflammatory and antiviral properties. In India, ethno-medicine, the cactus pulp and juice are used to treat the urinary tract infection, skin wounds, digestive problem and stomach swelling. The natural extract is a useful for alcohol hangovers, and the plant's gel-like sap is often used as a hair conditioner.

This fruit is used commonly by tribal people of the Kachchh region of Gujarat, India. This fruit has high medicinal properties. But information about this fruit regarding the chemical constituents, nutritional value of fruit and medicinal properties, primary and secondary metabolites unknown is lacking. Thus, this fruit was analyzed for its nutritional value and medicinal properties.

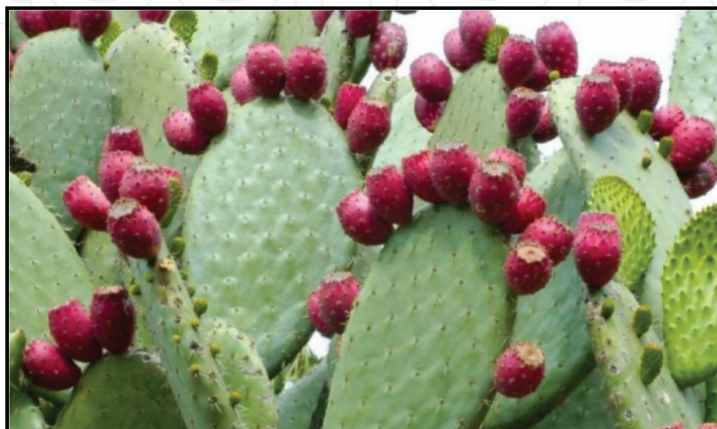


Figure 1. *Opuntia elatior* Mill. plant.

2. Materials and Methods

2.1. Collection of raw material

Opuntia elatior Mill plant species fruits were selected and collected between May and June 2017, from the surrounding areas Jamnagar city of Gujarat (**Figure 2**). These fruits were healthy and disease free and were used to check nutritional value and medicinal properties.

2.2. Drying and grinding the plant material

The fruits were collected and sliced into small pieces and distributed evenly for homogeneous drying. They were kept to dry at ambient temperature with adequate ventilation. Dry condition is required to prevent microbial contamination and subsequent degradation of metabolites. These fruits were kept away from direct sunlight to minimize chemical reaction which is caused by ultraviolet rays. After drying the fruits, they were grounded into a fine powder and passed through 60 mm mesh, this is then stored in an air tight container, in a dry and cool place. Grinding the fruits into a fine powder, for the extraction procedure, helps increase the surface area thus making it more homogeneous, and therefore making it easy for the solvent to penetrate the cells.

2.3. Preparation of fruit extract

2.3.1. Soxhlet extraction method

An extract is prepared using the soxhlet extraction method. In this method, “thimble” made up of cellulose or cloth placed put up the material to be extracted is placed in a central compartment, lower compartment connected with a siphon device and side-arm both. The solvent is placed in the lower compartment, and a reflux condenser is attached above the central sample compartment. It is made sure that all the components of the setup are assembled together with appropriate contents to complete the apparatus [12]. For the extraction procedure, three different solvents were used, one after another, they were methanol, hexane and distilled water, respectively. Each extraction procedure took around 6 h. For each extraction, 230 ml of the solvent was placed in the lower compartment. A sample of 25 g of the fruit

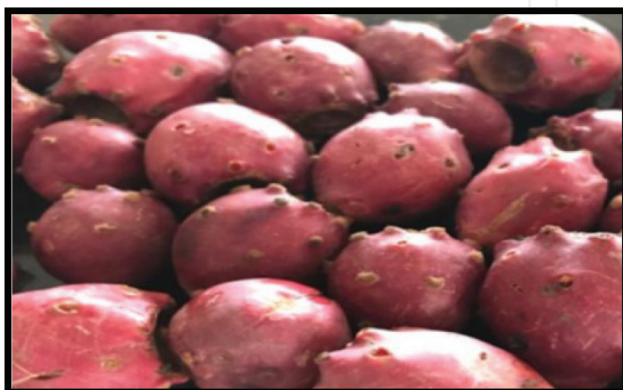


Figure 2. *Opuntia elatior* Mill. fruits.

powder was placed in a porous thimble and kept in the middle compartment. For the procedure, the solvent in the lower container is heated to its boiling temperature (different solvents have different boiling temperature to maintain), and reflux condenser vapor passes through the side arm up. The thimble containing the material to be extracted using the vapor liquefies and drips. Here, central compartment extract gradually collects from warm water percolates through the material and the wall of the thimble. The entire liquid in the central compartment flows through the side tube and back into the lower solvent container of the height of the extract reaches to the top of the siphon. The extract removed in a petri dish and left aside to evaporate. After evaporation of solvent, the leftover extract was filled in the eppendorf tube. This process is then repeated with the other solvents.

2.4. Analysis of nutritional value

2.4.1. Sample preparation and nutritive analysis

Stock solution is prepared by dissolving 30 mg of methanolic, hexanoic and distilled water extract in to 30 ml of methanol, hexane and distilled water respectively to give the concentration of 1 mg/1 ml [13].

2.4.2. Estimation of moisture

(1) Ten grams of fruit sample were taken in a Petri dish and kept in a hot air oven at 90–100°C for 5–6 h. (2) Weight of the fruit was measured after it was completely dry. (3) Loss in weight was regarded as a measure of moisture content.

2.4.3. Estimation of fat content

(1) A mixture of 50 ml of methanol and diethyl ether were taken in 1:1 concentration. (2) From 30 ml of stock solution, 1 ml of test sample was added in the mixture and few drops of phenolphthalein as an indicator. (3) The solution was titrated with 0.1 N NaOH. (4) The change in color was observed from light yellow to a brownish coffee color.

Calculation

$$\text{Acid value (mg/L)} = \frac{(\text{Test} - \text{Blank}) \times 0.1 \times 254}{\text{Sampled used (ml)}}$$

2.4.4. Estimation of crude protein

The biuret test is a chemical test used to detect the presence of peptide bonds. In the presence of peptides (–CO–NH– groups), a copper (II) ion forms violate coordination complexes in an alkaline solution. Copper (II) reduces to copper (I). The intensity of the color complex is measured by colorimetrically at 520 nm.

Reagents: (1) Biuret reagent: Dissolve 3 g of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and 9 g of sodium potassium tartrate in 500 ml of 0.2 N NaOH solutions. To this solution, add 5 g of potassium iodide and make about 1 L with 0.2 N NaOH. (2) Standard protein solution: 5 mg of bovine serum albumin/ml water. Prepare fresh.

Method: (1) From 30 ml of stock solution, take 1 ml of sample and make volume of up to 4 ml with distilled water. (2) Add 6 ml of biuret reagent to it and mix well. (3) Heat the mixture at 37°C for 10 min. (4) Cool the tubes and read the absorbance at 520 nm against a reagent blank. (Prepare similarly with 4 ml of distilled water). (5) Draw the standard graph by pipetting out 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 ml, of standard protein solution into a series of test tubes and make volume of up to 4 ml with distilled water in each. Carry out steps 2 to 4. (6) Calculate the protein content in the sample using a standard graph.

2.4.5. Estimation of carbohydrate by Anthrone method

Carbohydrates are first hydrolyzed into simple sugars using hydrochloric acid to form furfural. This compound condenses with anthrone to form a green colored complex which can be measured by using colorimetrically at 620 nm.

Procedure: (1) From 30 ml of stock solution, take 0.5 ml of test sample in a test tube. (2) Make up volume of 1 ml with distilled water then add 4 ml of Anthrone reagent. (3) Heat the tubes in a boiling water bath for 10 min, the color changes from blue to green after boiling. (4) Prepare the working standards by taking 0, 0.2, 0.4, 0.6, 0.8 and 1 ml, where '0' serves as blank. (5) All the tubes including the sample tubes by adding distilled water and make up the total volume to 1 ml. (6) After adding the 4 ml of anthrone reagent. (7). Ten minutes of heat is provided in a boiling water bath. (8) After the tube is cooled rapidly and read the absorbance at 620 nm the green to dark green color. (9) The plotting concentration of the standard on the X-axis versus absorbance on the Y-axis and draw the standard graph. (10) The amount of carbohydrates present in the sample tube find out from the graph calculate.

2.4.6. Determination of vitamin-C

Preparation of standard solution: 5 mg of ascorbic acid was taken and dilute in 5 ml of distilled water to give concentration of 1 mg/1 ml.

Procedure: (1) Take 25 ml of test sample and add 25 ml of 20% Meta phosphoric acid. (2) Dilute it to 100 ml with distilled water. (3) Take 10 ml of aliquot from the above solution and add 2.5 ml of acetone. (4) Titrate it with 0.05% dye solution till a pink color persist for 15 s (V1). (5) For the standard readings take 0.05 g of vitamin C (ascorbic acid) (A). (6) Add 60 ml of 20% Metaphosphoric acid and dilute it to 50 ml with distilled water. (7) Titrate the known volume of the above solution with 0.05% dye solution till a pink color persist for 15 s (V2).

Calculation:

$$\text{Amount of ascorbic acid (mg/100ml)} = \frac{(A \text{ mg}) \times (V2) \times 250 \times 100}{(V1 \text{ ml}) \times (B) \times (\text{wt of Sampled})}$$

A mg = Std. vitamin C taken; V1 ml = Vol. of dye taken to titrate the sample; V2 ml = Vol. of dye taken to titrate std. vitamin C; B = Total vol. of std. solution taken which is to be titrated against 0.05% dye; 250 = Total vol. of std. solution made after dilution.

2.5. Antioxidant activity by DPPH method

This method is simple and sensitive. The assay is based on the theory of hydrogen donor is an antioxidant. It measures compounds that are total radicle scavengers. DPPH accept hydrogen from an antioxidant. The antioxidant effect is proportional to disappearance of DPPH in the test sample. These methods involve measurement of decrease in absorbance of DPPH at its absorption maxima of 516 nm, which is proportional to concentration of free radicle scavenger added to DPPH reagent solution [14].

2.5.1. Preparation of standard solution, test sample and DPPH solution

Standard solution is prepared by dissolving 10 mg of ascorbic acid in 10 ml of methanol to give the concentration of 1 mg/1 ml. Stock solution is prepared by dissolving 5 mg of methanolic, hexanoic and distilled water extract in to 5 ml of methanol, hexane and distilled water respectively to give the concentration of 1 mg/1 ml. 0.1 mM DPPH is prepared by dissolving 11 mg of DPPH in 8.46 ml of distilled water. It is protected from light by covering the tubes by aluminum foil. **Procedure:** (1) In 3 ml of methanol, 150 μ l DPPH is added and reading is taken at 516 nm as control. (2) 0.2, 0.4, 0.6, 0.8, and 1.0 ml aliquots are taken from the test sample. (3) The test sample is diluted by adding methanol up to 3 ml. (4) 150 μ l DPPH is added to each of the tubes. (5) Absorbance is taken by UV-visible spectrophotometer at 516 nm. (6) The % of antiradical activity is calculated by using the following equation.

Calculation

$$\% \text{ of antiradical activity} = \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \times 100$$

2.6. Anti-inflammatory activity by antidenaturation activity

The anti-inflammatory activity of *Opuntia elatior* was studied by using inhibition of albumin denaturation technique [15].

2.6.1. Preparation of test sample and standard solution

Stock solution is prepared by dissolving 5 mg of methanolic, hexanoic and distilled water extract in to 5 ml of methanol, hexane and distilled water respectively to give the concentration of 1 mg/ 1 ml. From this stock solution 100, 200, 300, 400, 500 μ l of each solution was taken and added in to 900, 800, 700, 600, 500 μ l of their respective solvents, to make 1 ml of working standard solution. 5 mg of Diclofen tablet was dissolved in 5 ml of methanol water to make 1 mg/ 1 ml of concentration. From this stock solution 100, 200, 300, 400, 500 μ l of solution was taken and added in to 900, 800, 700, 600, 500 μ l of methanol respectively to make 1 ml of working standard. **Procedure:** 0.05 μ l of test samples were taken from working stand and 0.45 ml of 5% BSA solution was added to it. The tubes were incubated for 30 min at 37°C in an incubator. A 2.5 ml of PBS buffer was then added and the O.D was taken at 660 nm. Distilled water was served as a blank. Distilled water and BSA solution was taken as a control.

Calculation

$$\% \text{ of anti - denaruration activity} = \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \times 100$$

2.7. Qualitative phytochemical analysis

The qualitative phytochemical analysis of test for alkaloids, test for flavonoids, test for phytoosterols, test for saponin, test for phenol and test for tannin perform by standard method reported by Parekh and Chanda (2008) [16].

2.8. Thin Layer chromatography

The separate chemical mixtures using the thin layer chromatography (TLC). TLC is performed on a sheet of aluminum foil, thin layer coated with of adsorbent material use the silica gel. This thin layer of adsorbent is known as the stationary phase. The solvent mixture (mobile phase) is drawn up the plate via capillary action and after the sample has been applied on the plate. Separation is achieved based on the different ascends the TLC plate at different rates [17]. **Procedure:** 10 ml of Methanol: chloroform (6: 4) was taken in glass beaker as a stationary phase. Test sample was applied on thin layer of absorbent material using capillary tube. This absorbent sheet was placed in beaker containing methanol: chloroform solvents and allowing it to run. After running of sample with solvent mixture absorbent sheet was removed from the beaker and was allowed it to dry. After drying of sheet, it was observed in UV light at high and short wavelength. Spray of concentrated sulfuric acid was applied to it to detect presence of alkaloids in the sample.

Calculation

$$R_f \text{ value} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

2.9. HPTLC analysis

HPTLC analysis based on the principles of adsorption the separation of compound. The mobile phase solvent flows based upon the principle of capillary action. The chemical components separation according to their affinities toward the adsorbent. The stationary phase travels slower because component with more affinity. Travels faster the chemical molecule component with lesser chemical charge toward the stationary phase. Thus, the component separated on a chromatographic plate. A 10 mg of methanolic extract was dissolved in 1 ml of methanol. This test solution was used for HPTC analysis. HPTLC aluminum sheet pre-coated with silica gel was used as the adsorbent. The chromatographic development chamber was saturated with mobile phase to place the plates. The plates were run and derivatized. The derivatized plates were heated, bands were observed and scanned at 254 nm and photographs were taken for record.

2.10. Gas chromatography-mass spectroscopy

The GC-MS method separates different chemical compound and correct identifies the component of chemical constitute. It is one of the most accurate tools for analyzing environmental samples. The GC works on the principle that a mixture will separate in to individual substances when heated. Mass spectroscopy identifies the compound by the mass of the analyte compounds is stored on a computer. A 1 ml of test sample of methanolic extract of *Opuntia elatior* fruit was analyzed by GC-MS. Concentration of the test sample was 10 mg/ml. The GC-MS analysis was done by electron impact ionization (EI) method on Auto system XL gas chromatography, coupled to a Turbo Mass Spectrophotometer at, Sophisticated and Instrumentation Centre for Applied Research and Training (SICART), Vallabh Vidyanagar, Gujarat. The column was a fused silica capillary column.

3. Result and discussion

Medicinal plants due to therapeutic values have long been used to address human diseases. From as early as 800 Before Common Era, plants and herbs were used for their medicinal properties. In ancient Indochina culture, herbal remedies were part of the day to day usage. India has a long history of safe and continuous usage of plant originated drugs. Officially recognize systems of medicine such as Ayurveda, unani, homeopathy and so on. Constantly use herbal drugs to cure illnesses. Today, these ancient practices of Southern Asia are widely growing and highly valued field of the medical industry.

Opuntia elatior (prickly pear) uses as a highly nutritional rich food. These people are interested in healthy body and illness from disease often uses the prickly pear fruit. This fruit present the many medicinal properties in the fruit. In Mexican and India folk medicine, the cactus pulp and juice are used to treat many diseases like skin wounds, stomach swelling and digestive problems. The natural extract is a useful for alcohol hangovers, and the plant's gel-like sap is often used as a hair conditioner.

This fruit is widely used in America, Mexico and many other counties. This fruit is part of their daily diet as it is highly nutritious but, not many people are aware about this fruit in India. It grows best in hot and dry regions and therefore can be easily cultivated in the north-western regions of India. Thus, this fruit was used to analyze its nutritional and medicinal properties.

3.1. Extract yield (%) of *Opuntia elatior* Mill

Pharmaceutical industries prefer plants that yield higher extract and are rich in their potency. Therefore, the work was carried out with yield calculation. We have selected hexane, methanol and distilled water solvent for extracting the plant constituents. A 36.84% of extract was produced by methanol, 15.79% extract was produced by distilled water and 3.40% of extract was produced by hexane from 25 g of powder by using 230 ml of solvents shown in **Figure 3**. Maximum extract was produced by methanol and minimum extract was produced by hexane.

3.2. Nutritive value of *Opuntia elatior* fruit extract

Appropriate knowledge about the nutritive value of fruit may enhance utility of the fruit. With the aim of increasing the utility, we have selected this fruit based on the ethno-botanical information and medicinal properties. For determination of nutritive value, various parameters were studied using fruit extract. *Opuntia elatior* fruit contained 84% moisture, as compared to *Opuntia ficus-indica* which had 87.07% moisture [18]. This shows that both the fruits have close moisture content and therefore are both great sources for reducing dehydration. This plant an indication of the stems adapted the storing water nature of the fruit pericarp. The small variation may result from difference in season whether wet or dry. Sample was collected during the driest month of the region when temperatures were over 35°C during May and June.

The percentage of various nutritional parameters that are analyzed in methanolic extract, distilled water extract and hexanoic extract of fruit are summarized in **Table 1**. Nutritive value of methanolic extract is higher than distilled water and hexanoic extract. As compared to Feugang *et al.* report *Opuntia spp.* contained 0.21–1.6% of protein, 0.09–0.7% of fat and 12–17% of carbohydrates whereas *Opuntia elatior* has 0.60%, 0.11% and 1.02% of protein, fat and carbohydrates respectively [19]. This shows that *Opuntia elatior* has an average protein and fat content and very low carbohydrate content.

Opuntia elatior fruit extract showed highest presence of vitamin C in distilled water extract that is 63.29 mg/ml. Hydrophilic extract of purple cactus pear fruit contains 36.6 mg/100 g of vitamin C [20]. From this we can say that *Opuntia elatior* is a rich source of vitamin C; this can help to reduce the oxidative stress in the human body. From the abovementioned, it can be seen that *Opuntia elatior* is a good source of nutrients.

3.3. Antioxidant activity of different extracts of *Opuntia elatior* fruit

3.3.1. DPPH Method (1, 1diphenyl 2, picryl hydrazyl)

In a living organism, free radicals are constantly generated; few amongst those remain as the unregulated radicals, which can cause extensive damage to tissue and biomolecules leading to various disease conditions, especially degenerative diseases and extensive lysis. This is the most widely reported method for screening of antioxidant activity. The lipophilic radical uses the model of DPPH radical. The lipid autoxidation was initiated by chain of lipophilic radicals. The stable free radical of DPPH at room temperature and stable diamagnetic molecule accepts an electron or hydrogen radical. The DPPH reducing capacity was observed by the decrease in its absorbance at 516 nm, which is induced by antioxidants. The suggested that the samples were free radical scavengers the DPPH test positive.

The best antioxidant activity was exhibited by the methanolic extract compared to hexanoic and distilled water extract. Methanolic extract exhibit the highest antioxidant activity that is 54.10% and the lowest antioxidant activity was exhibit by the hexanoic extract at 45.66% and the distilled water at 50.40% of antioxidant activity. The data obtained for different solvent

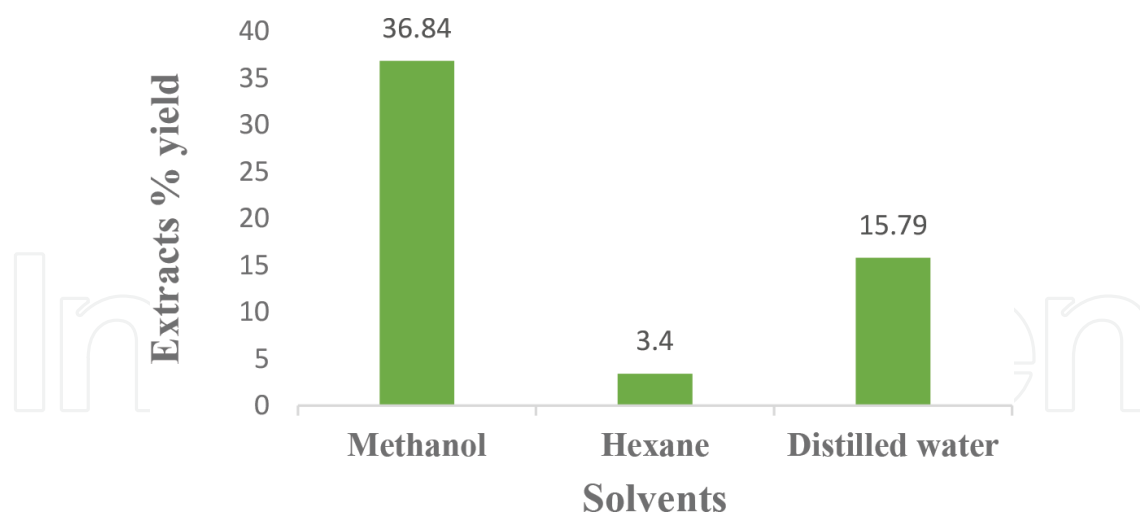


Figure 3. Extract yielded (%) of *Opuntia elatior* fruit.

Nutrients	Methanolic extract	Hexanoic extract	Distilled water extract
Protein (%)	0.60	0.42	0.40
Carbohydrate (%)	1.02	0.66	0.91
Fat (%)	0.11	0.08	0.05
Vitamin C (mg/100 g)	63.29	31.64	45.21

Table 1. Nutritional value of *Opuntia elatior* fruit extract.

extracts using DPPH method are shown in **Figure 4**. DPPH radical scavenging activity of methanolic extract of *Cantaloupe* (muskmelon) pulp shows 48.55% [18] (Ibrahim *et al.*, 2016), and *Opuntia* shows 57.37% activity. This shows that *Opuntia* has a good antioxidant potential.

Itankar *et al.* reported that *Opuntia elatior* fruit has 64.14% of activity which is close to methanolic extract of *Opuntia elatior* [21]. *Opuntia* is a good source of vitamin C this helps increase antioxidant properties and reduces the risk of diseases such as atherosclerosis and cancer. Vitamin C is an electron donor. As an electron donor, it helps stabilize unpaired electrons in the body and reduces oxidative stress.

3.4. Anti-inflammatory properties of *Opuntia elatior* extract

Inflammation in the body acts as a defense mechanism. When a foreign substance enters in the body, it causes infection and injury. To protect our body from this, the infected area swells. The purpose of inflammation is to localize and eliminate the foreign substances so that the body can heal itself. Inflammation prevents excess blood flow to rich the site of damage.

The anti-inflammatory activity of *Opuntia elatior* was studied using inhibition of protein denaturation method. Protein denaturation means the loss of biological properties of protein molecules. Denaturation of proteins is responsible for the cause of inflammation and its conditions like rheumatoid, arthritis, diabetes, cancer, and so on. Hence, prevention of protein denaturation may also help in preventing inflammatory conditions.

As a part of the investigation on the mechanism of the anti-inflammation activity, the ability of protein denaturation in different fruit extracts with different solvents was studied. The maximum percentage of inhibition of 37.49% was observed from methanol extract followed by distilled water at 34.15% and then hexane at 30.38%. All the solvent extract inhibited the albumin denaturation. The methanol extract shows the highest inhibition and distilled water extract shows the lowest inhibition. Diclofen, a standard anti-inflammatory drug, showed the maximum inhibition of 63.33%.

Several anti-inflammatory drugs have shown dose-dependent ability to inhibit protein denaturation. *Opuntia* fruit has more than 50% of protein denaturation inhibition properties present in anti-inflammation drugs. This shows that *Opuntia* can be used as a natural source of anti-inflammatory activities. The ability of *Opuntia elatior* extract to bring down denaturation of protein is a contributing factor for its anti-inflammatory activity. The data of this study suggest that *Opuntia elatior* shows significant anti-inflammatory activity with the tested in vitro methods. The data collected by protein denaturation method for inflammation are given in **Figure 5**.

3.5. Qualitative phytochemical analysis of *Opuntia elatior* fruit extract

The phytochemical constituents present in the methanolic extract of *Opuntia elatior* are phytosterols and alkaloid compounds. The presence of yellow color precipitates of test sample by Mayer's reagent shows the presence of alkaloids. The presence of golden yellow color of test sample by adding conc. H_2SO_4 shows the presence of phytosterols. Flavanoids, saponin, phenol and tannin are absent in methanolic extract of *Opuntia elatior*. Phytochemical constituents of the methanolic extract of *Opuntia elatior* were qualitatively tested for their presence as depicted in **Table 2**.

The presence of these phytochemicals in the plants extract enhances their pharmaceutical and therapeutic potentials. Alkaloid is reported to carry antimicrobial activities [22]. The presence of alkaloids in the investigated plants of the wild cucurbits indicates that they have medicinal values. Alkaloids have a powerful effect on the physiology of animals [23]. Sterols in modern clinical studies have shown that they play an important role as anti-inflammatory and analgesic agents [24].

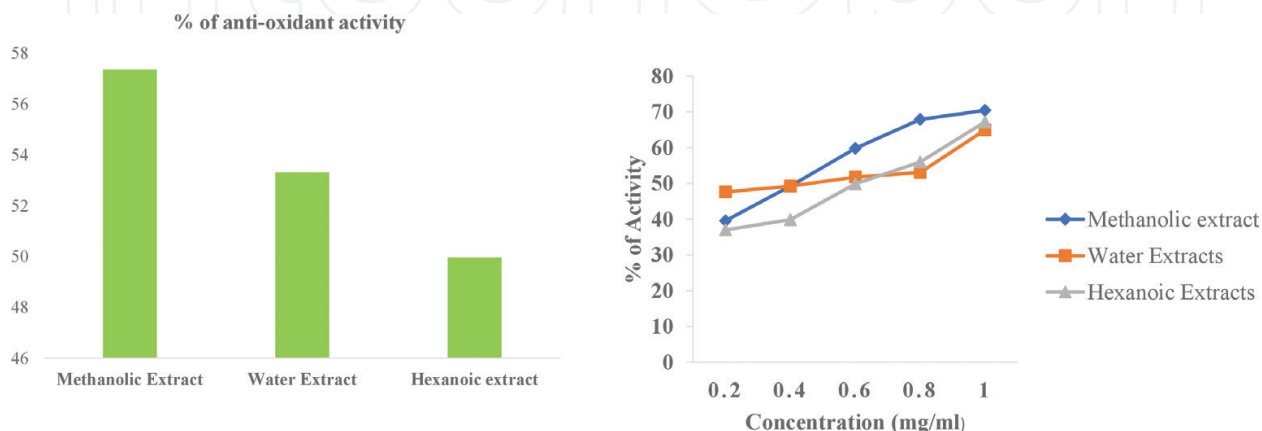


Figure 4. Antioxidant activity of different extract of *Opuntia elatior* fruit.

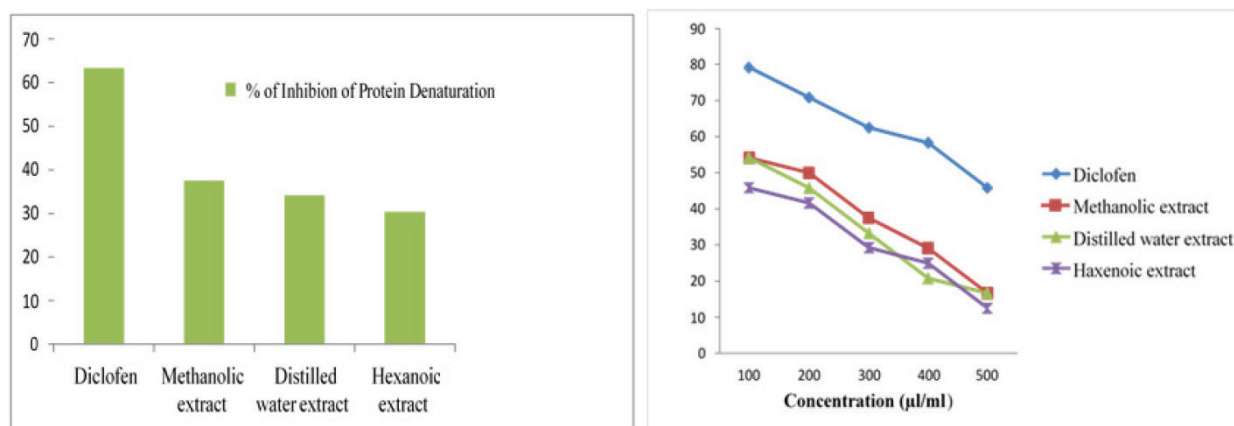


Figure 5. Inhibition of protein denaturation, of different extract of *Opuntia elatior* fruit.

Phytochemical constituent	Color observed	Methanolic extract	Distilled water extract	Hexanoic extract
Alkaloids	Yellow color ppt	+	—	+
Flavanoids	Purple to cherry red	—	—	—
Saponin	Presence of foam	—	—	—
Phytosterols	Golden yellow	+	+	+
Phenol	Blue green	—	—	—
Tannin	White ppt	—	—	—

Table 2. Phytochemical constituents of *Opuntia elatior* fruit extract.

3.6. Thin layer chromatography of *Opuntia elatior* fruit extract

Methanolic extract obtained from *Opuntia elatior* fruit was carried out to thin layer chromatography to establish the purity and composition of materials. The component present in methanolic fruit extract was identified by the concentrated H_2SO_4 test. After separation of methanolic fruit extract, one band was observed. The band observed under UV light is shown in figure-3.8. R_f value of the band was measured that is 0.85. After application of H_2SO_4 band, H_2SO_4 was applied to the band to check the presence of compound. Brownish color of the band was observed after applying of H_2SO_4 . This brownish color of the band shows the presence of alkaloid in the methanolic extract of *Opuntia elatior*.

3.7. HPTLC analysis of methanolic extract of *Opuntia elatior* fruit

For the HPTLC analysis the extract of *Opuntia elatior* was run in a mobile phase of methanol:chloroform (8:2). The plate was visualized at 254 nm. HPTLC profile of methanolic extract of *Opuntia elatior* shows three bands at 254 nm, it is shown in Figure 6. Maximum R_f values were obtained by track 1. Different peak spectral comparison of methanolic extract is shown in Figure 5. This shows the different compounds present in the sample. Each compound has its own unique pattern. Determination of the peak height of chromatographic peak

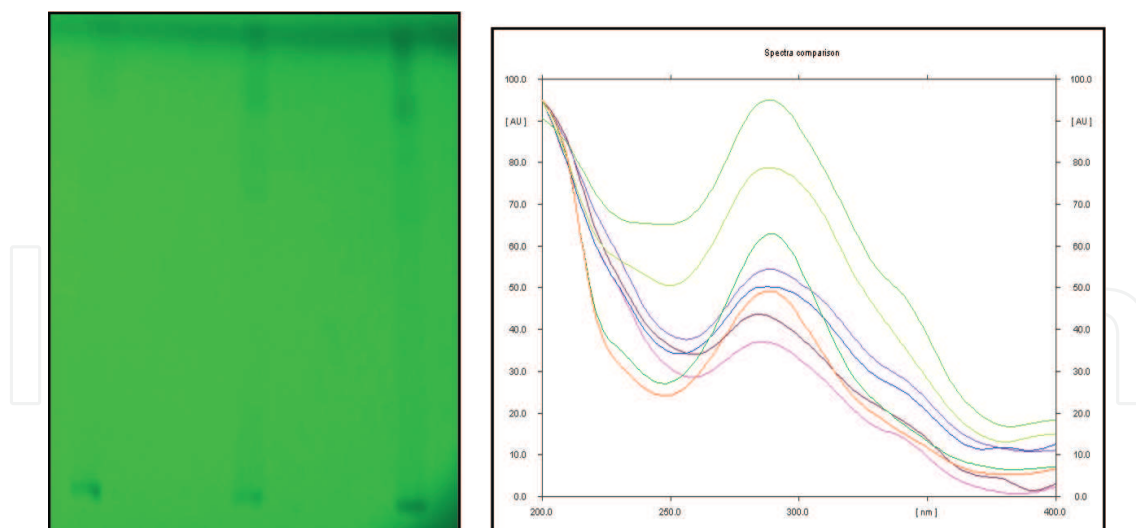


Figure 6. HPTLC profile and chromatogram of methanolic extract of *Opuntia elatior* fruit.

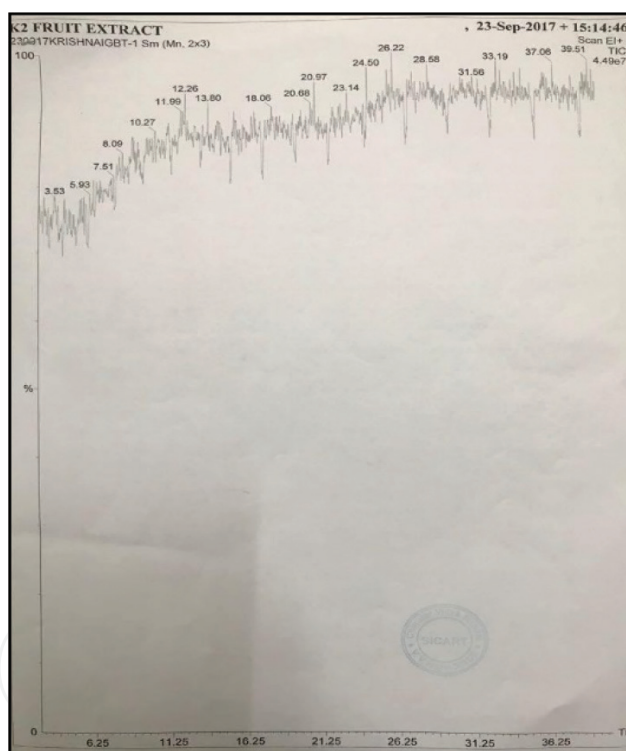


Figure 7. GC-MS chromatogram of methanolic extracts of *Opuntia elatior*.

gives quantitative assessment of a compound. Peak size of the area is nearly equal to the amount of the substance present in the mixture. For the identification of the chemical profile, the sample was subjected to further characterization and isolation of compound. In this study, total nine bands were obtained in the sample.

3.8. GC-MS analysis of methanolic extract of *Opuntia elatior* fruit

GC-MS analysis of methanolic extract of *Opuntia elatior* is shown in **Figure 7**. The separation techniques coupled with GC-MS allowed separation of constituents as shown in the GCMS

trace in **Figure 7**. Compounds are not separated properly because of its higher background value. Because of higher background peaks are not seen clearly. The peaks start forming at 70% and continued for minutes. The identifications of phytochemical compound were based on the peak area, retention time and molecular formula. The GC-MS data can be used to identify major bioactive, phytochemical constituent. The GC-MS analysis of the fruit sample revealed the presence of 19 compounds. Thus, the presence of unidentified compound initiated further investigation on the fruits of *Opuntia elatior*. Further analysis of the fruit extract is being done for the NMR studies for structural analysis of the compound. Thus, the presence of alkaloids and phytosterols present in the methanolic fruit extract of *Opuntia elatior* open ample scope for further investigations. Thus, chemical present of alkaloids and phytosterols present give activity of antioxidant and anti-inflammatory. The different author reported the ethanolic extracts present the 14 compounds compared to the methanolic extracts 19 chemical compound presents. This indication more support to the further analysis of the fruit extract is required for the NMR studies for structural analysis of the compound identification. It gives better idea of which chemical compound is responsible for the antioxidant and anti-inflammatory activity.

4. Conclusion

This study shows the analysis of nutritional and medicinal properties of *Opuntia elatior* fruit. From the study, it is seen that the *Opuntia* fruit was rich in nutrients. It also has antioxidant and anti-inflammatory properties that can be useful in the medical stream. The intake of proper nutrients helps increase your daily metabolism, improves the strength of your bones, increases blood flow to the brain and helps maintain a healthy life style. To date, around 25% of drugs that have alkaloid agent come from plant origin. Through the Qualitative phytochemical analysis of this fruit, alkaloids and phytosterols were found. TLC analysis of methanolic extract of *Opuntia elatior* exhibits the one band. The brownish color of band shows the presence of alkaloid. The alkaloid acts as a phytoprotective agent against invading microorganisms. These phytosterols play an important role in cell membrane function. This helps reduce blood cholesterol levels in the body. HPTLC analysis of methanolic extract showed the presence of three bands. From this result we can conclude that three different components are present in the *Opuntia elatior* fruit extract. By the analysis of GC-MS of *Opuntia*, fruit extract peaks were not shown clearly. It may be because of high background values that the peaks of the compounds were not seen.

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