6. Summary
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Pharmacognostic analysis of fruits of *Opuntia elatior* Mill. confirms the authenticity of the plant as per previous reports. Phylloclade is an aerial modification of stem, broadly ovate to oblong in shape and greenish in color. Flowers are arising from areoles and contain perianth pink color in centre with yellow at the edge. Fruits are berry pyriform, reddish purple when ripe and greenish when unripe. It is indehiscent, depressed at the apex and many seeded pulpy fruit.

The phytochemical analysis was carried out for standardization of fruit juice contained carbohydrates, flavonoids, phenolics and betalains. The fruits were preliminary evaluated by estimation of proximate analysis. The average weight of fruit was 24.568 ± 7.134 g/unit and among percentage of peel and seed was very low compared to the edible portion. A comparatively low ash value indicates presence of less inorganic matter. The fruits are very good source of watery content as high amount of moisture content and low amount of solid content. The fruit juice can resist microbial growth as low pH and acidic in nature. The density of fruit juice was comparatively near to the water as high moisture content. Viscosity of juice was 53.62±0.402 centipoise at 100 rpm.

Results of extraction of fruits peel indicate maximum extractive values attained with polar solvents like methanol and water whereas with non polar solvents like petroleum ether, benzene and chloroform were comparatively less. A comparison of *R*<sub>f</sub> values and spot color obtained in TLC of carbohydrates, it can be concluded that the fruit juice contained reducing hexose monosaccharides like galactose and glucose. TLC of betalain indicates presence of betacyanins type of color pigments.

Visible spectrum of betacyanin in methanol and water extracts of fruit demonstrated maximum absorbance at 535 nm indicate presence of betacyanin. HPLC chromatogram exhibited peak 1 with retention time 21.76 min at 484 nm was identified as indicaxanthin which present in very low amount and peak 2 with retention time 22.76 min at 535 nm as betanin, present in high amount in fruits of *Opuntia elatior* Mill. LC – MS suggested the presence of betanin confirmed by its identical spectral properties and the
presence of their protonated molecular ions \([M+H]^+\) with \(m/z\) 551, while the prominent secondary ion at \(m/z\) 390 due to the presence of protonated aglycones \([\text{betanidine} + \text{H}]^+\).

The total sugar content (11.41 g/100 ml) equivalent to glucose, total phenolic content (49.82 mg/100 ml) equivalent to gallic acid and titratable acidity (0.94%) equivalent to citric acid obtained in fruits of *Opuntia elatior* Mill. The total betacyanin content (47.10 mg/100 ml) equivalent to betanin obtained from fruits of *Opuntia elatior* Mill. was higher compare to *Opuntia ficus-indica* and *Opuntia undulata* Griff. while lower compare to *Opuntia stricta* Haw. The mineral composition is characterized by high amounts of potassium followed by calcium while other minerals are in the normal range of fruits.

The fruit juice of *Opuntia elatior* Mill. reversed anaemia induced by HgCl$_2$ and phenylhydrazine in dose dependant manner. It was observed that there was a remarkable increase in body weight in animals treated with fruit juice at dose 10 ml/kg (212.2 g) and 15 ml/kg (227.7 g) after continuous treatment for 30 days. At the dose of 10 ml/kg and 15 ml/kg of fruit juice showed good percentage of recovering in haemoglobin, 32.99 % and 38.18 %, respectively, which was higher than standard treated group (29.8 %) indicating correction of anaemia. fruit juice of *Opuntia elatior* Mill. improved the total and differential WBC with platelet functions and the results was dose dependant. The increment of blood sugar indicated that the fruit juice was a source of energy due to presence of high amount of carbohydrates. The fruit juice has not major effect on serum cholesterol and triglyceride levels. The kidney, liver and spleen functions were restored by fruit juice of *Opuntia elatior* Mill. The antioxidant phenolics and betanin constituents and mineral compositions appear most likely as the active ingredients responsible for haematinic effect of *Opuntia elatior* Mill. fruits. This results support at least partially the traditional use of fruits in the treatment of anaemia.

Phenylhydrazine produces both aryl and hydroxyl radicals when incubated with rat liver microsomes and oxidised by hydrogen peroxide. PHZ-induced haemolytic injury seems to be derived from oxidative alterations to red blood cell proteins rather than to membrane lipids. In this study, PHZ altered the haematological parameters by haemolysis characterized by
decrease in haemoglobin concentration, total RBC counts and PCV on day 3. However, the haematological parameters were restored to normal range after treatment with fruit juice of *Opuntia elatior* Mill. The speedy and progressive recovery of anaemic rats responding to treatment of *Opuntia elatior* Mill. fruits may be due to increased erythropoiesis. The improvement in the haematological indices exhibited by fruit juice might be connected with the minerals, phenolics and betacyanin content of the fruits of *Opuntia elatior* Mill. The phenolics and betacyanin have remarkable antioxidant activity. These constituents might have direct influence on the protection of haemolysis by reactive oxygen species generated by PHZ.

The potential antinociceptive as central analgesic by using tail immersion test and peripheral analgesic by using acetic acid induced writhing test of the fruits of *Opuntia elatior* Mill. was investigated. According to antinociceptive tests, we can concluded that the fruits of *Opuntia elatior* Mill. is endowed with central and peripheral analgesic properties might be due to presence of phenolics and betain content.

Bronchial asthma is characterized by increased airway reactivity to spasmogens, mediator releases and inflammation. Various animal models and experimental protocols were used in the present study to evaluate anti-asthmatic activity of fruit of *Opuntia elatior* Mill. In present study, significant increase in preconvulsion time was observed due to pretreatment with fruit juice of *Opuntia elatior* Mill., when the guinea pigs were exposed to either acetylcholine or histamine aerosol. The non-parallel rightward shift in acetylcholine and histamine log dose-response curves obtained in the presence of the fruit juice, with lowered maximum contraction effect to acetylcholine and histamine would indicate a non-competitive or an irreversible antagonistic effect of *Opuntia elatior* Mill. fruits at muscarinic and histamine H₁ receptors.

Mast cell degranulation is important in the initiation of immediate responses following exposure to allergens. In the present study, the fruit juice of *Opuntia elatior* Mill. was found to inhibit the degranulation of mast cells induced by an immunological (egg albumin) and a non-immunological (compound 48/80) stimulus. A significant protection of rat peritoneal mast cells from disruption by antigen and compound 48/80 by fruit juice of *Opuntia*


elatior Mill. points towards its ability to interfere the release and/or synthesis of mediators of inflammation, indicating its mast cell stabilizing activity. Hence it may be assumed that the cytoprotective effect induced by fruit juice of Opuntia elatior Mill. on mast cell surface could be due to its ability to alter the influx of calcium ions.

Fruit juice of Opuntia elatior Mill. was found effective against carrageenan-induced inflammation and also reduced the neutrophil adhesion which suggested the anti-inflammatory action.

The peel of various fruits serves the protective action against microbial invasion. The peel extracts of Opuntia elatior Mill. fruits exhibited antimicrobial actions in a dose dependant manner against both test bacteria and fungi. Antimicrobial activity of the peel extracts is directly concerning with the components that they contain. Petroleum ether, benzene and methanol extracts showed maximum inhibitory action against gram positive bacteria, gram negative bacteria and fungi, respectively.

In conclusion, our pharmacognostical data authenticate the plant and phytochemical results indicate that the flavonoids betacyanin equivalent to betanin is the active principle in the fruits of Opuntia elatior Mill. The fruits are exhibiting haematinic effect against anaemia induced by mercuric chloride and phenylhydrazine while antinociceptive effect against thermal and chemical stimuli. Fruits also appear anti-asthmatic property due to its bronchodilator, mast cell stabilization, anti-inflammatory and reduction of neutrophil adhesion property. Furthermore, peel extracts of fruit appear antimicrobial property against bacteria and fungi. These findings on haematinic, analgesic and anti-asthmatic effects of fruit juice, further add value to the nutritional characteristics of the fruits of Opuntia elatior Mill.