



# PHYTOCHEMICAL AND PHARMACOLOGICAL SCREENING OF FRUIT OF OPUNTIA ELATIOR MILL.FOR HAEMATINIC ACTION

Sheetal Pal<sup>1</sup>, Swati Tiwari<sup>2</sup>, Shubhi Khare<sup>2</sup>, Parveen Nisha<sup>2</sup>, Vaibhav Rajorya<sup>2</sup>, Durga Vishwakarma<sup>3</sup>

<sup>1</sup>M.Pharm. Vedic Institute of Pharmaceutical Education and Research, Sagar (M.P), India

<sup>2</sup>Associate Professor, Vedic Institute of Pharmaceutical Education and Research, Sagar (M.P), India

<sup>3</sup> Assistant professor, Vedic Institute of Pharmaceutical Education and Research, Sagar (M.P), India

Corresponding Author: Shubhi Khare<sup>2</sup> Assistant professor, Vedic Institute of Pharmaceutical Education and Research, Sagar (M.P), India

**Abstract :** Opuntia elatior Mill. (OE) (Family Cactaceae), commonly recognized as Red Prickly pear, is a plant of varied nutritional and medicinal benefits. The species has been scrutinized for the composition and wide array of pharmacological activities. The fruits of Opuntia elatior Mill. are known as prickly pear and folkloric use as hematinic, anti-inflammatory and antiasthmatic action. Previously, the fruit juice of prickly pear was evaluated in reversed anemia induced by phenyl-hydrazine in a dose dependant manner and present study revealed about its effect in acute hemolytic anemia.

Keywords: Opuntia elatior Mil, Red Prickly pear, anemia, hematinic, phenyl-hydrazine.

## INTRODUCTION

The haematological disorders include various types of anaemia where the patient having deficiency or reduction of haemoglobin content. Anaemia is a global public health problem affecting both developing and developed countries with major consequences for human health as well as social and economic development. It occurs at all stages of the life cycle, but is more prevalent in pregnant women and young children.<sup>1</sup>

## Herbal drugs

Plant-based products used to treat diseases or to maintain health, are called herbal products, botanical products, or phytomedicines. A product made from plant sources and used only for internal use is called an herbal supplement. Many prescription medicines and over-the-counter medicines are also made from plant products. But these products contain only purified ingredients and are regulated by the FDA. Herbal supplements may contain entire plants or plant parts. Herbal supplements come in all forms. They may be dried, chopped, powdered, or in capsule or liquid form.<sup>2</sup>

## Haematopoiesis

Haematopoiesis (from Ancient Greek: haima blood; poiesis to make) is the formation of blood cells. All cellular blood components are derived from haematopoietic stem cells. In developing embryos, blood formation occurs in aggregation of red blood cells in the yolk sac, called blood islands. As development progresses, blood formation occurs in the spleen, liver and lymph nodes.<sup>3</sup>

## Anaemia

Anaemia is defined as a reduction of haemoglobin concentration in the blood. It may give rise to fatigue but, especially if it is chronic, is often surprisingly asymptomatic. The commonest cause is blood loss related to menstruation and child bearing, but there are several different types of anaemia, and several different diagnostic levels.<sup>4</sup>

## Aim and objective

This study aimed to evaluate the haematinic effect of Opuntia elatior Mill. fruit on PHZ – induced anaemia. The aim of studying Opuntia elatior Mill. (also known as prickly pear) in relation to its haematinic (blood-building) activity is to evaluate its potential in treating anemia and related conditions. The objective is to assess how the fruit of this plant affects blood parameters like hemoglobin, red blood cell count, and other indicators of anemia in both healthy and anemic subjects.<sup>5</sup>

The study aims to demonstrate whether *Opuntia elatior* can improve the hematological profile of anemic individuals and potentially offer a natural alternative or complement to conventional treatments for anemia.

The primary aim is to investigate whether the fruit of *Opuntia elatior* possesses properties that can effectively address anemia.

To determine the phytochemical composition of the fruit, and to evaluate the effects of the fruit on hematological parameters (e.g., Hb, RBC count, etc.) in anemic subjects. To assess any potential benefits or adverse effects of the treatment on various organs and to explore the potential mechanisms by which the plant might exert its haematinic effects (e.g., increased erythropoiesis or antioxidant activity).

### PLANT PROFILE

- Family: CACTACEAE (Cactus Family)
- Family (Hindi name): NAG PHANI FAMILY / CACTUS FAMILY
- Family (as per The APG System III) : CACTACEAE
- Species Name (as per The Plant List): *Opuntia elatior* Mill.
- Common name: Common Prickly-pear, *Opuntia*
- Vernacular name: Shappathi kalli (Tamil); Jamudu (Telugu); Nagphani (Oria); Pathikalli
- Habit: Shrub Comments / notes: Cultivated as ornamental plant
- Flower, Fruit: April-August
- Distribution:
- Kerala: Kozhikode district, Alappuzha district, Kollam district



*Opuntia elatior* plant is scrubby, 3–4 m height. 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.<sup>6</sup>

### Traditional Uses of *Opuntia* species

The plant is bitter, hot; laxative, stomachic, carminative, diuretic, antipyretic, alexiteric; cures biliousness, burning, leucoderma, “vata”, urinary complaints, tumors, ascites, loss of consciousness, piles, inflammations, vesicular calculi, anaemia, ulcers, cures bronchitis of children, ophthalmia, liver complaints lumbago and enlargement of the spleen.<sup>7</sup>

### RESEARCH METHODOLOGY

#### Collection and authentication of plant

The plant material *Opuntia elatior* Mill of was collected and authenticated.

#### Preparation of fruit juice of *Opuntia elatior* Mill. (OFJ)

Mature fruits of *Opuntia elatior* Mill. were collected and immediately taken to the laboratory. Spines and glochides were removed from fruits by just heating on the burner and then washed with water. The peel of the fruits was removed manually and pulp subjected to homogenization for 5 minute using boss portable blender. After homogenization, fruits juice was filtered through muslin cloth and filtered juice was used for various estimation and biological studies.<sup>8</sup>

**Determination of pH of OFJ**-The pH of OFJ was determined five times using pH meter

**Preparation of fruit peel extracts**-Manually removed peel of fruits was subjected to air drying at room temperature. Air dried peel was pulverized and passed through 10 # sieve. A finely peel powder was extracted successively with petroleum ether (60-80°C), benzene, chloroform, methanol and water in Soxhlet extractor for 24 hours.<sup>9</sup>

### Phytochemical studies

- **Test for carbohydrate**-Small quantity of extract was dissolved in 5ml of water and filtered.  
**Molisch test**-The filtrate was treated with a few drops of  $\alpha$ - naphthol (20% in ethyl alcohol). Then 1 ml of concentrated H<sub>2</sub>SO<sub>4</sub> was added along the sides of inclined test tube and observed for formation of violet coloured ring at the interface.

- **Test for glycosides and anthroquinones**

**Borntrager's test**-A small amount of ethanolic extract was hydrolysed with hydrochloric acid for few hours on water bath and the hydrosylate was extracted with benzene. The benzene layer was treated with dilute ammonia solution and observed for the formation of reddish pink colour.

**Legal test**-The extract was dissolved in pyridine and made alkaline with few drops of 10% NaOH and freshly prepared sodium nitroprusside was added and observed for formation of blue colour.<sup>10</sup>

- **Test for flavonoids**

**Ammonia test**

Filter paper strips were dipped in the dilute solution of the extract, ammoniated and observed for colour change from white to yellow.

- **Test for Proteins and Amino acids**

Small amount of extract was dissolved in distilled water and filtered.

**Biuret's test**

To the ammoniated alkaline filtrate add 2-3 drops of 0.002% copper sulphate and observed for appearance of red or violet colour.

**Millon's test**

To 2 ml of filtrate 5-6 drops of millons reagent (1 g of mercury + 9 ml of fuming nitric acid solution) was added and observed for red precipitates.

## Pharmacological studies

### Animals

Albino rats weighing 150- 200 gm were used for this study. The animals were obtained from animal house, Vedic Institute of Pharmaceutical Education and Research Sagar (M.P). On arrival, the animals were placed randomly and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2°C and relative humidity of 30-70%. A12: 12 light: day cycle was followed. All animals were allowed to free access to water and bed with standard commercial pelleted chow.

### Acute toxicity study

Acute toxicity studies were performed for fruit juice according to the acute toxic classic method as per guidelines 423 prescribed by OECD (2001). Albino wistar rats were used for acute toxicity study. The animals were kept fasting for overnight providing only water. These were divided into two groups of each containing six animals. Each of these groups was then administered with water and fruit juice of *Opuntia elatior* Mill. at the dose of 20 ml/kg p.o. The animals were observed for 30 min and then periodically for first 24 h special attention during first 4 h and thereafter daily for 14 days. The observations like sedation, convulsions, tremors, lethargy, death etc were systemically recorded with individual records of each animal.<sup>11</sup>

## Estimation of haematinic action Phenylhydrazine-induced anaemia

### Experimental design-

Anemia was induced by intraperitoneal (i.p.) injection of phenylhydrazine hydrochloride (PHZ) at 40 mg/kg for 2 days. The dose of OFJ was selected as per our previous study. Animals were divided in seven groups (n=6) as per followings and treated accordingly.

Group A: Negative control (saline solution for 15 days)

Group B: Positive control (PHZ, 40 mg/kg, i.p., for 2 days)

Group C: PHZ + Standard ferrous sulphate containing drug (0.0214 mg/kg, p.o., treatment started on day 3 upto day 15)

Group D5: PHZ +OFJ (5 ml/kg, p.o., treatment started on day 3 upto day 15)

Group D10: PHZ+OFJ (10 ml/kg, p.o., treatment started on day 3 upto day 15)

Group D15: PHZ+OFJ (15 ml/kg, p.o., treatment started on day 3 upto day 15)

Group E: OFJ (15 ml/kg, p.o., treatment started on day 3 upto day 15)

Phenylhydrazine hydrochloride solution was prepared in dimethyl sulfoxide and injected intraperitoneally for 2 days except group A and group E. The vehicle, standard drug and fruit juice were administered orally from day 2 to day 15 after phenylhydrazine administration. Group B animals were allowed to recover naturally. Group E animals treated only 15 ml/kg fruit juice as a high dose to check the effect of fruit juice in normal condition.<sup>12</sup>

## Measurement of Haematological Parameters

Haematological parameters were estimated on day 0, 3, 7, 10 and 15 in phenylhydrazine – induced anaemia. Blood samples were withdrawn from retro-orbital plexus under light ether anesthesia, collected in heparinized capillary tubes and analyzed for haematological parameters<sup>13</sup>. Haematological parameters like haemoglobin (gm%), total red blood cells (RBC), total white blood cells (WBC), differential white blood cells, haematocrit, mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), platelet count, mean platelet volume (MPV), platelet distribution width (PDW) and red blood cell distribution width (RDW) were estimated using methods.<sup>14</sup>

## RESULTS

### Macroscopic examinations

*Opuntia elatior* Mill. is xerophytic subarborescent or shrubby cacti, 3 meter high or more with ovate – oblong limbs. Herbarium was prepared in 1 % v/v formalin and preserved in the museum. <sup>15</sup>Phylloclade is an aerial modification (metamorphoses) of stem, having dimension (Length: 20 – 25 cm; Width: 12 – 15 cm; Thickness: 1 – 1.5 cm), broadly ovate to oblong in shape and greenish in color<sup>16</sup>. Areoles bearing about 4-5 increasing up to 10, rather slender straight prickles which are grey and yellow when young, the largest was 3-5 cm long. Mature prickles are 3 – 5 cm long and young are 0.5 to 1.5 cm long; glochidia inconspicuous, almost hidden amongst woolly hairs, rusty-brown. Flowers are 4 – 6 cm long arise from areoles initially yellow coloured, turns pink upon maturation. Mature flowers comprises of perianth with pink color in centre and yellowish at the edge.<sup>17</sup>

**Proximate analysis**-The results obtained from various determinations of proximate analysis. The average weight of fruit, percentage of peel, pulp and seed were found  $24.568 \pm 7.134$  g/unit,  $5.344 \pm 2.150\%$ ,  $94.655 \pm 2.150\%$ ,  $3.835 \pm 0.828\%$  (mean  $\pm$  SD), respective.<sup>18</sup>

#### Proximate analysis of fruit of *Opuntia elatior* Mill.

Parameters	Mean $\pm$ SD
Total ash	$0.579 \pm 0.164$ % w/w
Acid insoluble ash	$11.9 \pm 3.4$ % w/w
Water soluble ash	$23.1 \pm 1.43$ % w/w
Moisture content	$86.086 \pm 1.23$ % w/w
Total solids	$13.913 \pm 1.23$ % w/w
pH	$4.583 \pm 0.0411$
Density	$1.10063816 \pm 0.007565$ g/cm <sup>3</sup>
Viscosity	53.62 0.402 centipoise

**Fruit peel extracts**-The dried peel of fruits was subjected to successive solvent extraction using solvents in the order of increasing polarity. The average percentage yield and physical properties of the peel extracts so obtained.<sup>19</sup> Maximum percentage yield was obtained in methanolic peel extract ( $23.37 \pm 4.58$  %).<sup>20</sup>

#### Percentage yield and physical properties of the peel extracts.

Peel Extracts	Color & Consistency	Percentage Yield (% w/w)
		(Mean $\pm$ SD, n=5)
Petroleum ether(60 – 80 °C)	Yellow sticky	$4.42 \pm 2.07$
Benzene	Brown sticky	$1.98 \pm 0.90$
Chloroform	Light brown sticky	$2.13 \pm 0.72$
Methanol	Brown solid	$23.37 \pm 4.58$
Water	Brown solid	$\pm 7.41$

### Qualitative Chemical test

Presence of phytoconstituents in peel extracts and OFJ. Methanol and water extract of peel and OFJ contained carbohydrate, flavonoids and betalains in abundant amount.<sup>21</sup>

- : Absent, +: Present

#### Presence of phytoconstituents in peel extracts and OFJ.

Class of compounds	Peel Extracts					OFJ
	Pet. Ether(60– 80C)	Benzene	Chloroform	Methanol	Water	
Alkaloids	-	-	-	-	-	-
Carbohydrate	-	-	-	+	+	+
Fats & fixedoil	+	-	+	-	-	-
Flavonoids	-	+	+	+	+	+
Glycosides	-	-	-	-	-	-
Phenolics/Tannins	-	-	-	+	+	+
Steroids/Terpenoids	+	+	-	+	-	-
Waxes	+	+	+	-	-	-
Saponins	-	-	-	+	+	-
Betalains	-	-	-	+	+	+

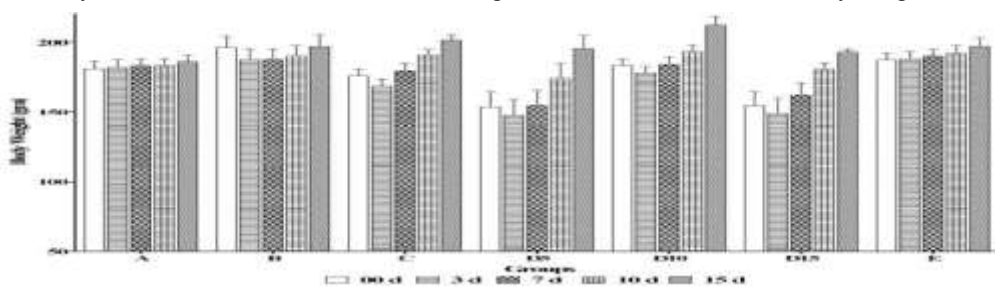
### Pharmacological studies of fruit juice

#### Acute toxicity study

The OFJ (20 ml/kg) showed no significant change in the various autonomic and behavioral responses of rat compared to the control animals. No mortality was recorded till 48 h in the animals treated with OFJ up to 20 ml/kg oral dose and therefore considered to be safe. Fruit juice (OFJ) was investigated for haematinic, activity using different models.<sup>22</sup>

## Haematinic action

**Phenylhydrazine –induced anaemia Effect on Body weight**-The mean body weight (g) of the albino rats in all groups were recorded on the day 0, 3, 7, 10, 15. There was not found significant difference in mean body weight of different treatment groups.<sup>23</sup>



**Effect of OFJ on body weight in PHZ –induced anaemia.**

Values are Mean  $\pm$  SEM (n = 6), analyzed by one way ANOVA followed by Turkey's multiple comparison test

### Effect on Haemoglobin content

The mean Hb content of rats at day 3 was highly significant ( $p < 0.001$ ) decrease compare to negative control (group A) after intraperitoneal administration of PHZ.<sup>24</sup> PHZ treated positive control (group B) rats demonstrated significant decrease ( $p < 0.001$ ) in Hb content compared to non-treated negative group (group A) at day 3. The percentage reduction in Hb was found  $27.82 \pm 2.18$ ,  $29 \pm 2.94$ ,  $26.35 \pm 2.84$ ,  $23.7 \pm 4.88$  and  $25.57 \pm 2.86$  in group B, C, D5, D10 and D15 on day 3 after PHZ administration. The percentage recovery in Hb content at the day 7, 10 and 15 were presented in figure 15. In group D10 and D15, the Hb content (gm %) was significantly increase  $12.75 \pm 0.4$  ( $p < 0.05$ ) and  $12.70 \pm 0.17$  ( $p < 0.05$ ) at day 7 respectively which was better improvement compared to group C and statistically compare with group B values at the day 3.<sup>25</sup>

### Estimation of haemoglobin content (gm %) of rats in PHZ – induced anaemia.

Groups	Day				
	0	3	7	10	15
A	15.32 $\pm$ 0.37	15.38 $\pm$ 0.42	15.78 $\pm$ 0.24	15.3 $\pm$ 0.21	15.12 $\pm$ 0.36
B	15.23 $\pm$ 0.49	11.02 $\pm$ 0.11+++	11.27 $\pm$ 0.061	11.58 $\pm$ 0.07	12.53 $\pm$ 0.30
C	15.63 $\pm$ 0.22	10.68 $\pm$ 0.25+++	11.97 $\pm$ 0.21	12.98 $\pm$ 0.20**	14.02 $\pm$ 0.29***
D5	15.8 $\pm$ 0.20	11.23 $\pm$ 0.23+++	11.73 $\pm$ 0.13	12.57 $\pm$ 0.14	14.98 $\pm$ 0.19***
D10	15.72 $\pm$ 0.27	11.6 $\pm$ 0.42+++	12.75 $\pm$ 0.40*	13.85 $\pm$ 0.33***	15.63 $\pm$ 0.34***
D15	15.88 $\pm$ 0.27	11.35 $\pm$ 0.19+++	12.7 $\pm$ 0.17 *	14.02 $\pm$ 0.31***	15.68 $\pm$ 0.37***
E	15.48 $\pm$ 0.23	15.63 $\pm$ 0.18	15.93 $\pm$ 0.20	16.13 $\pm$ 0.21	16.83 $\pm$ 0.42

**DISCUSSION** - 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 middle administrated dose of 10 ml/kg reduced the recovery time of the blood parameters from 15 days in the anaemic control to 10 days. Also the recovery was progressive such that after 15 days of continuous treatment, the haemoglobin concentration was higher in group D10, D15 and E treated rats than in the negative control group. It was also observed that the recovery of the treated groups was dose dependant with the highest dose of 15 ml/kg effecting the highest change. Giving the highest dose 15 ml/kg fruit juice to normal rats did not significant alter the haematological parameters.

**SUMMARY** - The fruit juice of *Opuntia elatior* Mill. reversed anaemia induced by 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.

## REFERENCE

1. Anonymous, 2003. The Ayurvedic Formulary of India Vol. I and II. Controller of Publications, Ministry of Health & Family Welfare, Government of India.
2. Rang, H.P. and Dale, M.M., 2003. Pharmacology, 6th edition, Churchill Livingstone Elsevier, Philadelphia, USA, 338-350.
3. Bruno de Benoist, Erin McLean, Ines Egli, Mary Cogswell, editor, 2008. Worldwide prevalence of anaemia 1993 – 2005: WHO global database on anaemia. WHO, Geneva, ISBN 9789241596657.
4. Vaidya, A.D.B. and Devasagayam, T.P.A., 2007. Current status of herbal drugs in India: an overview. Journal of Clinical

- Biochemistry and Nutrition 41, 1–11.
5. Patwardhan, B., 2005. Traditional Medicine: Modern Approach For Affordable Global Health. CIPIH Studies, WHO, Geneva. Through [http://www.who.int/intellectualproperty/studies/traditional\\_medicine/en/index.html](http://www.who.int/intellectualproperty/studies/traditional_medicine/en/index.html).
  6. Williams, D. A. and Lemake, T. L., 2002. Foye's Principles of Medicinal Chemistry, 5th edition, Lippincott Williams and Wilkins, New York, London, 892-901.
  7. Greenburg, A. G., 1996. Pathophysiology of Anemia. American Journal of Medicine 101 (suppl 2A), 7S-11S.
  8. Hall, G. W., 2007. Understanding haemolytic anaemia. Paediatrics and Child Health 17(8), 333-339.
  9. Karimi, M., Mirzaei, M., Dehghani, A., 2004. Prevalence of Anemia, Iron Deficiency and Iron Deficiency Anemia in 6-60 Month Old Children in Yazd's Rural Area. International Pediatrics, 19(3), 180-184.
  10. Pazdernik, T. L. and Kerecsen, L., 2009. Rapid review: Pharmacology. 2nd edition, published by Elsevier, a division of Reed Elsevier India Private Limited, Noida, U. P., India, 137-146.
  11. Seth, S. D. and Seth, V., 2009. Textbook of Pharmacology, 3rd edition published by Elsevier, a division of Reed Elsevier India Private Limited, Noida, U.P., India. pp VII.
  12. Aster, J. C., 2007. Red blood cell and bleeding disorders. In: Robbins and Cotran Pathologic Basis of Disease, Kumar, V., Abbas, A. K. and Fausto, N. (Ed), 7th edition, published by Elsevier, a division of Reed Elsevier India Private Limited, Noida, U. P. India, 619-627.
  13. Omolo, O.J., Chhabra, S.C., Nyagah, G., 1997. Determination of iron content in different parts of herbs used traditionally for anaemia treatment in East Africa. Journal of Ethnopharmacology, 58, 97-102.
  14. Silja, V.P., Verma, S.K. & Mohanan, K.V., 2008. Ethanomedicinal plant knowledge of the Mullu kuruma tribe of Wayanad district, Kerala. Indian Journal of Traditional Knowledge, 7(4), 604-612.
  15. Hong-Fang Ji, et al., 2009. Natural products and drug discovery. EMBO Reports 10(3), 194–200.
  16. Diallo A, Gbeassor M, Vovor A, Eklü-Gadegbeku K, Aklikokou K, Agbonon A, et al. Effect of Tectona grandis on phenylhydrazine-induced anaemia in rats. Fitoterapia 2008;79:332-6.
  17. Pandit S, Biswas TK, Debnath PK, Saha AV, Chowdhury U, Shaw BP, et al. Chemical and pharmacological evaluation of different ayurvedic preparations of iron. J Ethnopharmacol 1999;65:149-56.
  18. Chauhan SP, Sheth NR, Suhagia BN. Haematinic evaluation of fruit of Opuntia elatior Mill. On phenylhydrazine induced anemia in rats. Int J Res Ayurveda Pharm 2014;5:115-22.
  19. Feugang, J.M., Konarski, P., Zou, D., Stintzing, F.C., Zou, C., 2006. Nutritional and medicinal use of cactus pear (Opuntia spp.) cladodes and fruits. Frontiers in Bioscience 11, 2574-2589.
  20. Hodges, V. M., Rainey, S., Lappin, T. R., Maxwell, A. P., 2007. Pathophysiology of anemia and erythrocytosis. Critical Reviews in Oncology/Hematology 64, 139–158.
  21. Gurbachan singh, 2003. General Review of Opuntias in India. Journal of the Professional Association for Cactus Development, 30-46.
  22. Gurbachan S. & Felker, P., 1998. Cactus: new world foods. Indian Horticulture 43, 29– 31.
  23. Duke, J.A., and Vasquez, R., 1994. Amazonian Ethnobotanical Dictionary. Boca Raton, Florida: CRC Press.
  24. Kirtikar, K.R. and Basu, B.D., 1999. Indian Medicinal Plants, 2nd edition, International Book Distributors, Dehradun, India. II, 1173-1178.
  25. Kokate, C. K., 1996. Practical Pharmacognosy, 1st edition, Vallabh Prakashan, India, 110-114.

