Indian Journal of Pharmacy and Pharmacology 2022;9(2):81–86

Content available at: https://www.ipinnovative.com/open-access-journals

Indian Journal of Pharmacy and Pharmacology

Journal homepage: https://www.ijpp.org.in/

Original Research Article

Estimation of phytochemicals contents and in-vitro antioxidant activity of Opuntia ficus Indica fruit extract

Krati Sharma[1],*, Rajeev Kumar Malviya[1], Vishal Gupta[1]

[1] *Dept. of Pharmacology, Mansarovar Global University, Ratnakhedi, Madhya Pradesh, India*

ARTICLE INFO

Article history:
Received 05-05-2022
Accepted 20-05-2022
Available online 12-07-2022

Keywords:
Opuntia ficus indica
Antioxidant assay
DPPH
TPC
TFC

ABSTRACT

The plants isolated bioactive constituents are utilized as complementary and alternative medicine in various disorders. The present study was designed to evaluate chief photochemical constituents of *opuntia ficus indica* and its anti-oxidative aptitude against free radicals. The extract of the plant was subjected to screened for phytochemicals classes, and measurement of anti-oxidant activity. Different in vitro anti-oxidant assay parameters include DPPH assay, reducing power assay, hydrogen peroxide scavenging assay, total phenolic content estimation, and total flavanoid content estimation. Our results indicated the presence of various flavanoids, alkaloids etc. Various antioxidant assay exhibited significant correlation with TPC and TFC and renders both plants are with therapeutic potential against free- radical-associated oxidative damages.

1. Introduction

Today the world appears to be increasingly interested in the health benefits of food and has begun to look beyond the basic nutrional benefits of foodstuffs to disease prevention. It is generally accepted that the beneficial effects of herbal remedies can be obtain from active constituent present in whole plant, part of the plant (e.g. flowers, fruits, roots, stem, leaves, or herbs), or plant materials or combinations thereof, whether in crude or processed state.[1] Free radicals' which are delivered as a consequence of typical biochemical responses in the body are involved in cancer, ischemic heart disease, inflammation, diabetes, aging, arthrosclerosis, immunosuppressant and neurodegenerative disorders.[2] The human body has complex antioxidant defense systems that use antioxidant enzymes (eg.-catalase, glutathione peroxidase, superoxide dismutase) and non-enzymatic antioxidants (e.g. glutathion, melatonin carotenoids thiol antioxidants and vitamins E & C). Almost all organisms' posses' antioxidants and repair systems to protect themselves against oxidative damage, but these systems cannot completely prevent damage.[2] This deficiency may result in oxidative stress.[3] Antioxidants can be broadly defined as any substance that delays or inhibits oxidative damage to target molecules. The main characteristic of antioxidants is its ability to trap free radicals.[4]

Opuntia ficus-indica was screened for the phytochemical and antioxidant evaluation. The fruit part of the plant was selected for the present study.

Opuntia ficus-indica is a species of cactus that has long been a domesticated crop plant important in agricultural economies throughout arid and semiarid parts of the world. It is thought to possibly be native to Mexico. Some of the common English names for the plant and its fruit are Indian fig opuntia, barbary fig, and prickly pear, although this last name has also been applied to other less common Opuntia species.[5] The fruit ripen from August through October. The fruit are typically eaten, minus the thick outer skin, after chilling in a refrigerator for a few hours. They have a taste

* *Corresponding author*.
E-mail address: krati_sharma980@yahoo.com (K. Sharma).

https://doi.org/10.18231/j.ijpp.2022.015

similar to a juicy, extra sweet watermelon.Table 1 The bright red/purple or white/yellowish flesh contains many tiny hard seeds that are usually swallowed, but should be avoided by those who have problems digesting seeds.[6]

2. Material and Methods

Table 1: Detail of plant extract

Plant Name	Family	Species	Drug
Opuntia Ficus Indica	Cactaceae	Opuntia Ficus Indica	Fruits

2.1. Qualitative phytochemicals analysis

Qualitative analysis for various phytochemicals viz. alkaloids, carbohydrates, flavonoids, glycosides and saponin glycoside, tannins, phenol, steroids, amino acid, proteins was carried out for opuntia ficus indica. Test method & result shown in Table 2 .

2.2. In vitro anti-oxidants activities

2.2.1. DPPH Assay

DPPH (1, 1-Diphenyl-2-picrylhydrazyl) is a stable free radial with red colour (absorbed at 517nm). If free radicals have been scavenged, DPPH will generate its colour to yellow. This assay uses this character to show herbs free radical scavenging activity.[7]

2.2.2. Procedure

Scavenging activity on DPPH free radicals by the extract was assessed according to the method Reported by Gyamfi et al.[7] Briefly, 50 íL of the ethanol extract Containing varied amounts of powdered ethanol extract (1, 5, 10, and 50 μg/mL distilled water, respectively, in each reaction) was mixed With 1 mL of 0.1 mM DPPH-ethanol solution and 450 íL of 50 mM Tris-HCl buffer (pH 7.4). After the solution incubated for 30 min incubation at room temperature, reduction of DPPH free radicals was measured by reading the absorbance at 517 nm. The assays were carried out in triplicate and the results were expressed as mean values ± standard deviations.[8]

In the experiment, prepare control using solvent of dilution & 0.5ml of DPPH.

2.3. Percentage inhibition (I %) was calculated

Using the formula, $I\ \% = \frac{(Ac-As)}{Ac} \times 100$

Where Ac is the absorbance of the control and as is the absorbance of the sample

2.3.1. Reducing power assay

In this assay, the yellow color of the test Solution changes to various shades of green and blue, Depending on the reducing power of each compound. Presence of reducers causes the conversion of the Fe3+/ferricyanide complex used in this method to the ferrous form.[9]

2.4. Procedure

Reducing power ability the reducing power was assayed as described in kuda et al.2005 with some modifications. Different Deore S. L...et al /Int.J. ChemTech Res.2009, 1(2) 175 Concentrations of ethanolic extracts (1.0 ml) were mixed With 2.5 ml of phosphate buffer (0.2 molar, pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was then incubated at 50 ^{o}C for 20 min. After, 2.5 ml of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. Finally, 1.25 ml from the supernatant was mixed with 1.25 ml of distilled water and 0.25 ml $FeCl_3$ solution (0.1%, w/v). The absorbance was measured at 700 nm. The assays were carried out in triplicate and the results were expressed as mean values ± standard deviations. Increased absorbance values indicate a higher reducing power.[10]

2.5. Scavenging of hydrogen peroxide

The ability of the extracts to scavenge hydrogen peroxide was determined.

2.5.1. Procedure

Prepared a dilution series of extract (10, 20, 40, 60, 80, 100 μg/ml). Prepared hydrogen peroxide solution (2 mM/L) with standard phosphate buffer (pH 7.4). Different concentration of the extracts in distilled water was added to 0.6 ml of hydrogen peroxide solution. Absorbance was determined at 230 nm after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage inhibition of different concentrations of the extracts was determined and compared with the standard, Gallic acid.[11]

2.6. Determination of total phenol content

Total phenolic compound contents were determined by the Folin-Ciocalteau method. The extract samples (0.5 ml of different dilutions) were mixed with Folin Ciocalteu Reagent (5 ml, 1:10 diluted with distilled water) for 5 min and aqueous Na2CO3 (4 ml, 1 M) were then added.[12] The Mixture was allowed to stand for 15 min and the phenols were determined by spectrophotometry method at 765 nm. The standard curve was prepared by 20, 40, 60, 80 and 100 μg/ml solutions of Gallic acid in methanol: water (50:50, v/v). Total phenol values are expressed in terms of Gallic acid equivalent (mg/ g of dry mass), which is a Common reference compound.[13]

2.7. Determination of total flavonoid content

Flavonoids have been shown to play a number of biologically important roles such as antioxidant activity. Antioxidants may serve the task of reducing oxidative damage in humans induced by free radicals and reactive oxygen species under 'oxidative stress' conditions.[14] The aim of this work is to develop a sensitive, diversely applicable and simple indirect spectrophotometric and fluorometric method for the determination of total flavonoids content in several plants. The method is based on the oxidation of flavonoids with Ce(IV) salts at room temperature. The spectrophotometric determination of the remaining Ce(IV) or fluoro metric determination of the formed Ce(III) was performed after reaction with flavonoids. Quercetic was used as flavonoid standard. The procedure was successfully applied to the assay of total flavonoids in the area parts of Urtica Dioica L. The obtained results were statistically compared with those obtained by known methods.[15]

2.7.1. Procedure

Take 0.5 ml of sample (1mg /ml)diluted with 2.5 ml distilled water,add0.150ml of 0.5 percent sodium nitrite stand for 6 min and then add 0.150 ml of 10%aluminium chloride after 5 minute add 1 ml of sodium hydroxide and measure the absorbance 510 nm. The standard curve was prepared by 1mg/ml 100 ,80,60 40,20 μg/ml, and solutions of Rutin in water Total Flavonoid values are expressed in terms of Rutin equivalent (mg/g of dry mass), which is a common reference compound.

3. Result & Discussion

Phytochemical screening of fruit extract of Opuntia ficus indica

3.1. In vitro antioxidant assay

3.1.1. DPPH Assay

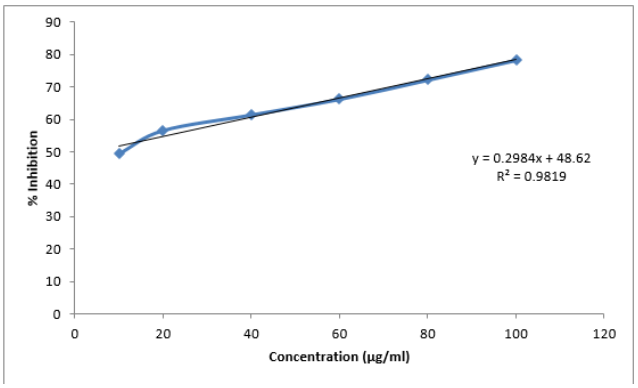

Fig. 1: Concentration vs. % inhibition

3.1.1.1. Reducing power assay. Absorbance of blank = 3.080. The result is the mean of three separate experiments.

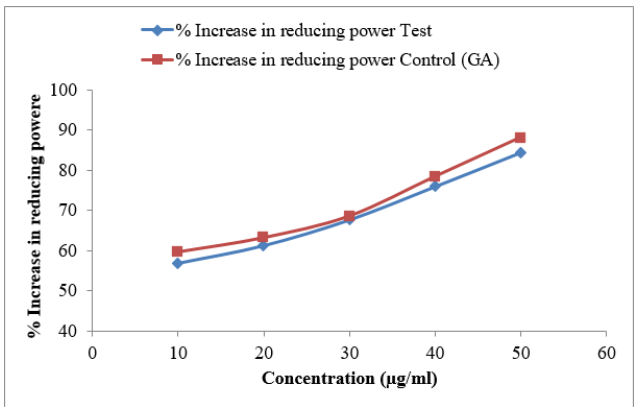

Fig. 2: Concentration vs. % increase in reducing power

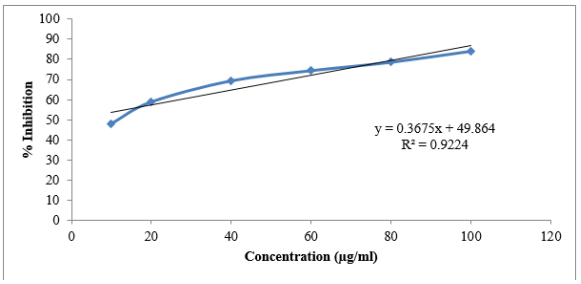

Fig. 3: Concentration vs. % inhibition in hydrogen peroxide scavenging assay

3.1.1.2. Scavenging of hydrogen peroxide. Extract showed good hydrogen peroxide scavenging activity in range of 10 to 100 μg/ml. I_C50 of extract for H_2O_2 was also found to be 0.381μg/ml.

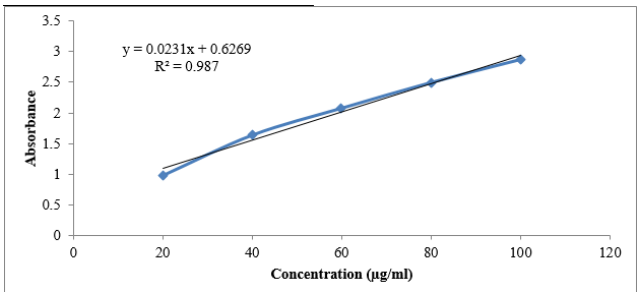

Fig. 4: Concentration vs. absorbance at 765nm in TPC

3.1.1.3. Determination of total phenol content.

3.1.1.4. Determination of total flavanoids content. In total flavonoid content estiofItion it was observed that extract was having 6 μg/mg extract of flavonoid (Rutin equivalent).

Table 2: Phytochemical screening of fruit of Opuntia ficus indica

S. No.	Plant constituent	Test applied	Opuntia ficus indica fruit extract
1.	Carbohydrates and reducing sugar	Molish's test	+ve
		Fehling's test	+ve
		Benedipn's test	+ve
2.	Flavonoids	Shinoda test	+ve
		Sodium hydroxide test	
3.	Cardiac glycosides a	Legal's test	-ve
4.	Anthraquinone glycosides	Borntrager's test	
5.	Saponin glycosides	Foam test	-ve
6.	Tannins	Lead acetate solution	-ve
7.	Polyphenol	Polyphenol test	+ve
8.	Steroids	Salkowaski test	+ve
9.	Inulin	Inulin	-ve
10	Alkaloid	Hager's test	-ve
		Ofiyer's test	-ve
		Dragendroff's test	-ve
		Wagner's test	-ve
11.	Amino acid	Ninhydrine test	-ve
12.	Protein	A) biuret test: B)millon test	-ve

Table 3: Absorbance of test solution at 517nm. The results are the means of three separate experiments.

S. no	Concentration μg/ml	Absorbance	
		Without DPPH	With DPPH
1)	10	0.002	0.039
2)	20	0.004	0.034
3)	40	0.005	0.030
4)	60	0.007	0.023
5)	80	0.010	0.020
6)	100	0.12	0.015

Table 4: Concentration vs. absorbance & calculation of % inhibition in DPPH

S. no.	Concentration nm	Absorbance AT 517nm	% Inhibition
1)	10	0.042	49.39
2)	20	0.036	56.62
3)	40	0.032	61.44
4)	60	0.028	66.26
5)	80	0.023	72.2
6)	100	0.018	78.31

Table 5: Concentration vs. absorbance & Calculation of % reducing power

S. no.	Concentration	Absorbance AT 700 nm		% increase in reducing power	
	μg/ml	Test	control	Test	Control
1)	10	2.750	2.840	56.8	59.7
2)	20	2.886	2.948	61.2	63.24
3)	30	3.064	3.113	67.6	68.6
4)	40	3.340	3.415	75.9	78.4
5)	50	3.602	3.715	84.4	88.1

Table 6: Concentration vs. absorbance without H_2O_2 & with H_2O_2

S.no	Concentration (μg/ml)	Absorbance of Test Solution At 517 nm	
		Without H_2O_2	With H_2O_2
1)	10	0.011	0.470
2)	20	0.013	0.415
3)	40	0.015	0.375
4)	60	0.016	0.350
5)	80	0.018	0.245
6)	100	0.020	0.200

Table 7: Concentration vs. absorbance &calculation of % inhibition in hydrogen peroxide scavenging assay.

S. no.	Concentration μg/ml	Absorbance AT 517nm	% Inhibition
1)	10	0.61	48
2)	20	0.49	58.82
3)	40	0.365	69.33
4)	60	0.305	74.37
5)	80	0.255	78.57
6)	100	0.19	84.03

Table 8: Concentration vs. absorbance at 765nm.

Drug	Concentration μg/ml	Absorbance (at 765 nm)
	20	0.985
	40	1.64
Standard (Gallic Acid)	60	2.078
	80	2.495
	100	2.868
Extract	1000	0.993

Table 9: Concentration vs. absorbance at 515 nm

Drug	Concentration μg/ml	Absorbance (at 515 nm)
	20	0.08
	40	0.19
Rutin	60	0.29
	80	0.37
	100	0.46
Extract	1000	0.020

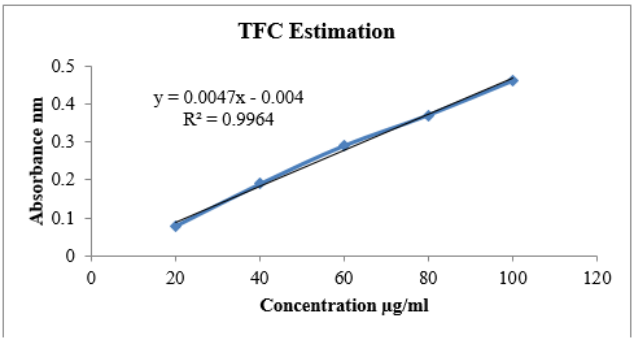

Fig. 5: Concentration vs. absorbance at 515 nm.

4. Conclusion

The phytochemical investigation of the ethanolic extract of, *Opuntia ficus-indica* fruits was carried out under the standard procedure. All the extracts showed the presence of carbohydrate, flavonoid, phenol, and steroid etc. The *in vitro* antioxidant study of all the plant extracts were carried out by DPPH,hydrogen peroxide radical scavenging, reducing power assay, phenolic content estimation and total flavanoid content estimation showed significant free radical scavenging activity. In DPPH assay it was observed that extract showed good antioxidant activity in range of 10 to 100 μg/ml. I_C50 of extract for DPPH was found to be 4.630 μg/ml. Extract showed good hydrogen peroxide scavenging activity in range of 10 to 100 μg/ml. I_C50 of extract for H_2O_2 was also found to be 0.381μg/ml. In total flavonoid content estiOFItion it was observed that extract was having 6 μg/mg extract of flavonoid (Rutin equivalent).

5. Source of Funding

None.

6. Conflict of Interest

None.

References

1. Soladoye MO, Amusa NA, Raji-Esan SO, Chukwuma EC, Taiwo AA. Ethnobotanical survey of anti-cancer plants in Ogun state. *Nigeria Ann Biol Res*. 2010;1(4):261–73.
2. National Cancer Institute. Cancer Facts. National Institute of Health; 1999. Available from: https://www.cancer.gov/about-cancer/understanding/statistics.
3. Fox AM, Gordon DR, Dusky JA, Tyson L, Stocker RK. IFAS Assessment of the Status of Non-Native Plants in Florida's Natural Areas. *EDIS*. 2005;2004(11):234. doi:10.32473/edis-ag234-2004.
4. Dardi MS, Telang RS, Simarjeet K. Anticancer drugs from plant origin. *Plant Arch*. 2012;6:6.
5. Gramont AD, Cutsem EV, Schmoll HJ, Tabernero J, Clarke S, Moore MJ, et al. Bevacizumab plus oxaliplatin-based chemotherapy as adjuvant treatment for colon cancer (AVANT): a phase 3 randomised controlled trial. *Lancet Oncol*. 2012;13(12):1225–33. doi:10.1016/S1470-2045(12)70509-0.
6. Bolarin DM. Cancer: Essentials and Basic Information. Nigeria: Babs-Olatunji Publisher; 2009.
7. Soladoye MO, Amusa NA, R-Esan SO, Chukwuma EC, Taiwo AA. Ethnobotanical survey of anti-cancer plants in Ogun state. *Nigeria Ann Biol Res*. 2010;1(4):261–73. doi:10.1002/ijc.25516.
8. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer*. 2008;127(12):2893–2917. doi:10.1002/ijc.25516.
9. Eckhouse S, Lewison G, Sullivan R. Trends in the global funding and activity of cancer research. *Mol oncol*. 2008;2(1):20–32. doi:10.1016/j.molonc.2008.03.007.
10. Gadhvi NP. Herbal therapy for advanced breast cancer: Personal experience with 100 patients. *East Cent Afr J Surg*. 2002;8(1):1–2.
11. Gadhvi NP. Herbal therapy for advanced breast cancer: Personal experience with 100 patients. P 2. *Sciences*. 2002;24(1):12.
12. Imbert TF. Discovery of podophyllotoxins. *Biochemistry*. 1998;80(3):207–12. doi:10.1016/s0300-9084(98)80004-7.
13. Konczak I. An evaluation of health enhancing compounds. *Rural Ind Res Dev Corp*. 2009;19(3):330–4.
14. Haridas V, Nishimura G, Xu ZX, Connolly F, Hanausek M, Walaszek Z. Avicin D: A protein reactive plant isoprenoid dephosphorylates Stat 3 by regulating both kinase and phosphatase activities. *PLoS ONE*. 2009;4(5):5578. doi:10.1371/journal.pone.0005578.
15. Nassr-Allah AA, Aboul-Enein AM, Aboul-Enein KM, Lightfoot DA, Cocchetto A, Shemmy E. Anti-cancer and antioxidant activity of some Egyptian medicinal plants. *J Med Plants Res*. 2009;3(10):799–808.

Author biography

Krati Sharma, Student

Rajeev Kumar Malviya, Professor

Vishal Gupta, Principal

Cite this article: Sharma K, Malviya RK, Gupta V. Estimation of phytochemicals contents and in-vitro antioxidant activity of Opuntia ficus Indica fruit extract. *Indian J Pharm Pharmacol* 2022;9(2):81-86.