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Phytochemical, anti-oxidant, antiviral and cytotoxic evaluation of *Opuntia dillenii* flowers

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Abstract

Opuntia dillenii used in Asian traditional medicine especially in China. We here report on the investigation of the phytochemical content, anti-oxidant, cytotoxicity and antiviral activity of methanolic extract of *O. dillenii* flowers. The anti-oxidant activity was measured with the DPPH, hydrogen peroxide and hydroxyl radicals scavenging method. In the antiviral and cytotoxic assay we used different viruses in different cell lines. In anti-oxidant assay, the DPPH assay exhibited potent anti-oxidant abilities with IC_{50} of 58.7 µg/mL. In antiviral assay, the extract possess strongest antiviral activity against herpes simplex 1 (EC_{50}= 25 µg/mL) and 2 (EC_{50}= 20 µg/mL), vaccinia (EC_{50}= 100 µg/mL) and moderate activity for remaining viruses (EC_{50}= >100 µg/mL). The cytotoxicity effect was evaluated using MTT assay and the results revealed that the extracts exhibited cytotoxicity above the range of 100 µg/mL. Our present reports confirmed that the *O. dillenii* could be a potential anti-oxidant and antimicrobial agent in near future.

Introduction

In the past 10 years, more interest has been taken to study about free radical scavengers or anti-oxidants to reduce the risk of various diseases. Although the availability of some synthetic anti-oxidants in markets such as, propyl gallate and butylated hydroxyl toluene, it is not predominantly used due to its toxicity (Carocho and Ferreira, 2013). The use of traditional herbal medicine (80% worldwide) is well-known and plants still present a huge source of natural anti-oxidants that might leads for the development of novel drugs. Several anti-inflammatory, digestive, antinecrotic, neuroprotective, and hepatoprotective drugs have recently been shown to have an anti-oxidant and/or antiradical scavenging mechanism as part of their activity (Perry et al., 1999; Repetto and Llesuy, 2002; Lin and Huang, 2002). Currently, there is an urgent need to develop a new antimicrobial drug due to panic increase of new infectious diseases (Cowan, 1999). Therefore, actions required replacing the use of antibiotics and the best alternative is natural products.

Opuntia dillenii (Cactaceae) is commonly known as pear bush, prickly pear, mal rachette or tuna, is a succulent shrub growing in semi-desert regions in the tropics and subtropics regions (Ahmed et al., 2005). The various parts of this plant were used for the treatment of diabetes (Perez de Paz and Medina Medina, 1988), gastric ulcers, anti-inflammatory (Park et al., 2011), analgesics (Loro et al., 1999), and antihyperglycemic (Perfumi and Tacconi, 1996). The purpose of the present study was to evaluate the anti-oxidant, antiviral and cytotoxic activity of *O. dillenii* extract.

Materials and Methods

Plant materials

The plant materials were collected from the tropical

areas of Western Ghat regions of Erode, shade dried at room temperature and a voucher specimen (SC 23/559) was deposited in Herbarium of Laboratory of Botany, Coimbatore, Tamilnadu, India.

Extraction of plant material

To the 100 g of coarsely powdered flowers were mixed and boiled with 1 L of methanol using automatic non-pressure pot. The extract of the herb was centrifuged for 15 min at 150 × g, and the supernatant was lyophilized using vacuum freeze drying system and stored at - 20°C for further use. The extraction yield was 3.5%.

Preliminary phytochemical screening

The various solvent extracts of *O. dillenii* were screened for the presence of various phytoconstituents such as steroids, alkaloids, terpenoids, glycosides, flavonoids and carbohydrates (Evans, 1996).

DPPH assay

The scavenging activity of DPPH was assessed by scavenging of 2,2-diphenyl-1-picrylhydrazyl radicals (Brand Williams, 1995). A stock solution of 0.3 mM DPPH was made by dissolving the DPPH in methanol. DPPH solution of 100 µL was added to 100 µL of the extract at varying concentrations (10-500 µg/mL) and vibrated vigorously. Absorbance was recorded at 517 nm after 15 min of incubation in room temperature. Water and DPPH was used as control. The experiment was conducted in three times and averaged. The DPPH scavenging activity of various extracts was calculated by the following equation

Percentage inhibition (%) = [(Control absorbance – Sample absorbance)/(Control absorbance)] x 100

Hydrogen peroxide scavenging assay

Hydrogen peroxide scavenging assay was carried out according to the previous method with some modifications (Muller, 1985). 80 µL of different concentrations (10-500 µg/mL) of methanol extracts were mixed with 20 µL of H_2O_2 (10 mM) in micro titer plate. Then, 100 µL of phosphate buffer pH 5 (0.1M) was introduced in to wells. The plate was incubated at 37°C for 5 min. Finally 60 µL of ABTS (1.25 mM) prepared with 1 IU/mL of peroxidase was mixed and the plates kept for incubation at 37°C for 10 min. The absorbance was measured at 405 nm. Percentage scavenging activity of various extracts was calculated by the following equation

Percentage inhibition (%) = [(Control Absorbance – Sample Absorbance)/(Control Absorbance)] x 100

Hydroxyl radical scavenging assay

Hydroxyl radical scavenging activity was analyzed as previously described with some minor modifications (Smirnoff and Cumbes, 1989). To 1 mL of different concentrations of extract solution, 300 µL of $FeSO_4$ (8 mM) solution, 250 µL of H_2O_2 (20 mM) were mixed. To initiate the reaction 250 µL of salicylic acid in ethanol (3 mM) was added. The reaction mixture was allowed to stand for 30 minutes in water bath at 37°C, after which, 450 µL of distilled water was added and the mixture was centrifuged at 10,000 rpm for 10 min. The supernatant was collected and the absorbance was measured at 510 nm. Extracting solvent was used as control instead of sample. Percentage scavenging activity of various extracts was calculated by the following equation

Percentage inhibition (%) = [(Control Absorbance – Sample Absorbance)/(Control Absorbance)] x 100

Viruses and cell lines

Herpes simplex virus-1 and 2, vaccinia virus, vesicular stomatitis virus, coxsackie virus, respiratory syncytical virus, feline corona virus, feline herpes virus, para influ -enza virus, reo virus-1, sindbis virus and puntatoro virus. The cell lines used were human embryonic lung [HEL] cells, human epithelial [HeLa] cells, crandell reus feline kidney [CRFK] cells and monkey kidney epithelial cells (Vero).

Cell lines and growth conditions

Dulbecco's modified eagle medium (DMEM) media was used to maintain the cell cultures supplemented with sodium bicarbonate, 3.7 g/L, glucose, 4.5 g/L, hydroxyethylpiperazine ethane sulfonic acid buffer, 15 mM, glutamine, 2 mM, gentamicin, 16 µg/mL, penicillin, 12 µg/mL and fetal calf serum. Cells were grown in humidified atmosphere with 5%CO_2 at 37°C.

Antiviral assays

The microlitre plates was used and inoculated with the confluent cell cultures with virus stock dilution (Taylor et al., 1996). After 1 hour of virus adsorption to the cells, residual virus were removed and replaced by eagle minimal essential medium containing 3% fetal calf serum and various concentrations of the methanolic extracts ranging from 2 to 200 µg/mL. Viral cytopathogenicity was recorded as soon as it reached completion in the untreated virus-infected cell cultures. Antiviral activity was expressed as minimal inhibitory concentration (MIC_{50}) required reducing virus induced cytopathogenicity by 50%.

Cytotoxicity

The 4-fold dilutions of the methanol extract incubated with confluent cell monolayers in 96-well plates and were observed microscopically for changes in cell morphology and viability at 24, 48 and 72 hours of incubation (Chiang et al., 2002). The cytopathic effect was scored under an inverted microscope. The dilution causing microscopically detectable alteration of normal cell morphology of the confluent cell cultures were estimated as 50% cytopathogenic effect with respect to cell control.

Table I

Results of preliminary phytochemical screening of *opuntia dillenii*

Plant names	Solvents used	Flavonoids	Tannins	Alkaloids	Anthraquinone glycosides	Steroids
Opuntia dillenii	Petroleum ether	+	+	+	+	+
	Chloroform	+	+	+	+	+
	Ethyl acetate	++	+	++	++	+
	Methanol	+++	++	++	++	++
	Ethanol	++	+	+	++	+
	Water	++	+	+	+	+

+ = Trace amounts; ++ = Moderate amount; +++ = High amount

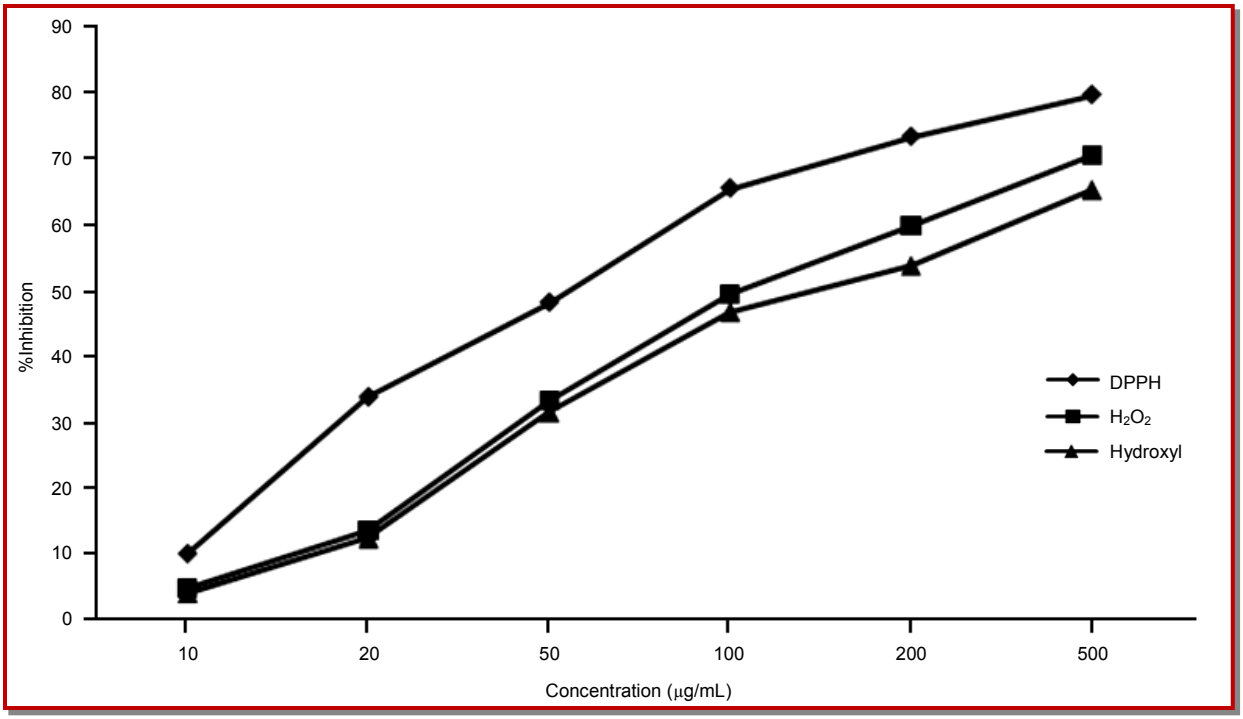

Figure 1: Percentage inhibition of free radical scavenging activity

Results and Discussion

To the detection of various phytoconstituents in, the preliminary phytochemical screening was carried out. The following solvent extracts were used for the study, petroleum ether, chloroform, ethyl acetate, methanol, ethanol and water. The chemical tests used for the study were shinoda test (flavonoid), phlonatanins test (tannins), wagners test (alkaloids), and salkowskii test (glycosides). Among these the methanolic extract was found to contain high amount of flavonoids as shown in Table I. The preliminary phytochemical screening of methanolic extract reveals the presence of alkaloids, flavonoids, tannins, triterpenes.

DPPH is a stable organic nitrogen free radical has been widely used to studying the anti-oxidant capacity of plant extracts or compounds. In this assay, picryl hydrazyl radical (purple color) was reduced to picryl hydrazine (pale yellow color) by plant extracts or anti-oxidant compounds (Blois, 1958). The color change or discoloration indicates free radical scavenging activity of tested sample. The capability of DPPH reduction was determined by the decrease in its absorbance at 517 nm, which is roused by anti-oxidants. In the DPPH assay, the extract had significant radical scavenging effect with increasing concentration. The investigated extract was demonstrated with higher percentage inhibition (Figure 1) and lowest IC_{50} value of 58.7 ± 0.0 µg/mL. Ascorbic acid was used as standard and it showed IC_{50} value of 1.2 ± 0.0 µg/mL.

Hydrogen peroxide (H_2O_2) generated in large quantity during inflammatory process, may be due to the activation of mast cells, macrophages, eosinophils, and neutrophils, which generate superoxide radical, predominantly via NADPH oxidase (Barnes, 1990). The super-oxide is then rapidly converted into H_2O_2 by super-oxide dismutase (SOD). H_2O_2 can easily enter the membranes of surrounding cells, whereas superoxide

Table II

Antiviral activities[1] of methanolic extract of *Opuntia dillenii* in different cell lines

Viruses (strain)	Cells	Extract (µg/mL)	Brivudin (µg/mL)	Ganciclovir (µg/mL)	Ribavirin (µg/mL)
Herpes simplex 1	HEL	25	0.1	0.1	NA
Herpes simplex 2	HEL	20	50	0.1	NA
Vaccinia	HEL	100	10	>100	NA
Vesicular stomatitis	HEL	>100	>250	>100	NA
Feline corona	CRFK	>100	NA	>100	NA
Feline herpes	CRFK	>100	NA	6.1	NA
Coxsackie B4	HeLa	>100	NA	NA	143
Respiratory syncytical	HeLa	>100	NA	NA	12
Parainfluenza- 3	Vero	>100	NA	NA	110
Reo- 1	Vero	>100	NA	NA	148
Sindbis	Vero	>100	NA	NA	>250
Cox sackie	Vero	>100	NA	NA	>250
Punta toro	Vero	>100	NA	NA	50

[1]Minimum inhibitory concentration (µg/mL) required to reduce virus-induced cytopathogenicity by 50%; HEL - Human embryonic lung cells; CRFK - Crandell reus feline kidney cells; HeLa - Human epithelial cells; Vero - Monkey kidney epithelial cells; NA - Not Applicable

Table III

Cytotoxic activities of methanolic extract of *Opuntia dillenii*

Cell lines	Extract (µg/mL)	Brivudin (µg/mL)	Ganciclovir (µg/mL)	Ribavarin (µg/mL)
Hel[a]	>250	>250	>100	>250
CRFK[a]	>100	NA	>250	NA
HeLa[a]	>100	NA	NA	>250
Vero[a]	>100	NA	NA	>250

[a]Microscopically detectable alteration of normal cell morphology; Hel - Human embryonic lung cells; CRFK—Crandell reus feline kidney cells; HeLa - Human epithelial cells; Vero - Monkey kidney epithelial cells; NA - Not Applicable

usually cannot. In our study, the extract was confirmed for its scavenging property and it exhibits the IC_{50} value of 131.1 ± 1.1 µg/mL. The percentage inhibition was shown in Figure 1. Ascorbic acid was used as standard and no IC_{50} value was observed.

The hydroxyl radicals is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging species in free radical pathology, capable of damaging almost every molecule found in living cells (Hochestein and Atallah 1988). This radical has the capacity to join nucleotides in DNA and can cause strand breakage which contributes to carcino-genesis, mutagenesis and cytotoxicity (Manian et al., 2008). The Fenton reaction generates hydroxyl radicals which degrade DNA deoxyribose, using Fe^{2+} salts as an important catalytic component. Oxygen radicals may attack DNA either at the sugar or the base, giving rise to a large number of products (Rajeshwar et al., 2005). The lowest IC_{50}value with highest scavenging capacity was found to be 159.3 ± 3.5 µg/mL. The percentage inhibition was shown in Figure 1. Ascorbic acid was used as standard and no IC_{50} value was obser-ved. Recently, one report demonstrated that phenolic compounds present in the methanolic seed extract had marked anti-oxidant activity in ORAC, TEAC and lipid peroxidation *in vitro* (Chang et al., 2008). Our findings also suggest that the same phenolic compounds and flavonoids may play a potential role for anti-oxidant activity.

Estimation of antiviral activity in this study was based on the cytopathic effect (CPE) of the virus infected monolayer of the different cells. The results were shown in Table II and III. We can conclude that, the extract showed significant anti-herpes simplex type 1 and 2 with lowest EC_{50} of 25 and 20 µg/mL respectively. The extract showed moderate activity against vaccinia virus with EC_{50} value of 100 µg/mL. The extract was not toxic against all viruses used (EC_{50}= >100 µg/mL). The reference drugs (brivudin, ganciclovir and ribavirin) possess antiviral activity in the concentration range

between 0.05 and 148 µg/mL. The cytotoxic effects produced by the reference drugs (brivudin and ribavirin) were above 250 µg/mL in HeLa, CRFK and Vero cell lines and above 100 and 250 µg/mL (ganciclovir) in Hel and CRFK cell lines.

Conclusion

Our study on the *in vitro* anti-oxidant and antiviral properties of extract prepared from *O. dillenii* flowers showed effective anti-oxidant properties by DPPH assay. DPPH assay showed lower IC_{50} values compared with hydrogen peroxide and hydroxyl scavenging assay. We also found that methanol extract of *O. dillenii* flowers exhibit significant antiviral activity against vaccinia and herpes simplex virus type 1 and 2.

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