

IN VITRO ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITY OF THE FLOWER EXTRACTS OF *OPUNTIA STRICTA*

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ABSTRACT

Objective: The objective of this study was to evaluate the antioxidant and anti-inflammatory activities of the solid powder obtained from the ethyl acetate fraction from the flower *Opuntia stricta*.

Methods: The flower extract was evaluated for antioxidant activity by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay and reducing power assay was carried out by ferric-reducing capacity (FRC) assay method. The *in vitro* anti-inflammatory activity was evaluated using human peripheral blood mononuclear cells (PBMCs) stimulated by lipopolysaccharide (LPS) to evaluate nitric oxide (NO) production method.

Results: The solid powder obtained from the ethyl acetate fraction from the flower *O. stricta* showed a good antioxidant activity in scavenging DPPH radical and FRC assay with compared standard sample. This solid powder also showed good anti-inflammatory activity in cell viability (LPS-induced PBMCs) assay and NO assay.

Conclusion: These results suggest that the solid powder obtained from the ethyl acetate fraction from the flower *O. stricta* has significant antioxidant and anti-inflammatory activities.

Keywords: Antioxidant, Peripheral blood mononuclear cells, Lipopolysaccharide, *Opuntia stricta*, Ferric-reducing capacity.

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INTRODUCTION

Numerous higher plants are real wellsprings of regular items utilized as pharmaceuticals, agrochemicals, flavor and scent fixings, sustenance added substances, and pesticides [1]. The look for new plant metabolites ought to be a need in the present and future endeavors toward manageable protection and usage of biodiversity. Restorative plants are generally utilized as option helpful apparatuses for the counteractive action of numerous ailments [2,3]. Herbal-based drugs remain an important source due to the availability, relatively cheaper cost, and no side effects when compared to modern medicine [4].

The field of free radical science is increasing more consideration nowadays. Free radicals are responsive oxygen and nitrogen species which are produced by different physiological procedures in the body. Uncontrolled era of free radicals prompts assault on layer lipids, proteins, catalysts, and DNA causing oxidative anxiety and at last cell demise. These reactive oxygen species (ROS) are dependable for some degenerative human illnesses such as diabetes mellitus, tumor, neurodegenerative scatters, Alzheimer's malady, Parkinson's ailment, atherosclerosis, maturing, and provocative diseases [5]. Assurance against free radicals can be upgraded by taking adequate measures of exogenous cell reinforcements. Cell reinforcement is a steady atom which gives an electron to a rampaging free radical and ends the chain response before essential particles are harmed. Free radical rummaging property of cancer prevention agents delays or hinders cell damage [6]. To forestall free radical harm, the body has a barrier arrangement of antioxidants. In human body, there are different chemical frameworks with the expectation of complimentary radical rummaging yet micronutrients such as Vitamin E, beta-carotene, and Vitamin C are the significant antioxidant. These must be given in consume less calories as body cannot deliver these nutrients [7]. Plants are potential wellsprings of natural antioxidants, which ensure the cells against the harming

impacts of ROS. Antioxidants from plant materials diminish the activity of free radicals and shield our body from different infection [8,9].

Inflammation is the central feature of many physiopathological conditions in response to tissue injury and as part of host defense against microorganisms [10]. In inflammatory processes, macrophages have a key part in providing a prompt guard against outside operators. On enactment with an inflammatory jolt, for example, lipopolysaccharide (LPS), macrophages produce pro-inflammatory go-between, including nitric oxide (NO). Therefore, the search of effective non-toxic natural compounds with antioxidant activity has been identified in recent years [11]. Plants containing polysaccharides are the most potent in curing inflammatory diseases [12].

Opuntia stricta (Haw Syn. *O. Dillenii* Haw) is a fence having a place with Cactaceae family. This species is adjacent to the Caribbean district, tropical and subtropical bank of eastern North America, and interfacing South America. *O. stricta* is a tropical-subtropical animal social events and it happens in natural locales, ruderal, scraped zones, clean, bush grounds, tundra, urban regions, overwhelm prairie and woody field, dry sclerophyll timberland and forest, unforgiving slants, and riparian conditions close watercourses. The sprouts are overall yellow in shading and are found all through India, yet most normally in South India [13,14]. Ordinary names of *O. stricta* are Erect Prickly Pear, prickly cactus pear, sweet prickly pear (English), and Nopal Estricto (Spanish). In South India are called as Nagajemudu, Nagadali (Andhra Pradesh), Nagathali, and Sappathikalli (Tamil Nadu) [14,15]. *O. stricta* organic product juice was observed to be a potential wellspring of betacyanin shades and could be utilized as a characteristic red-purple nourishment colorant. This desert plant is utilized as a part of people solution as an antidiabetic and anti-inflammatory, antihyperglycemic movement, hypotensive action, neuroprotective action, and antispermatogenic action [15-19]. It is clear that *Argemone mexicana* L is rich in biological

activities but there are no reports so far on the subtleties of the possible role of this biological activities as antioxidants and anti-inflammatory activity of this flower.

METHODS

Collection of flowers

The new flowers of *O. stricta* were gathered from Z. Suthamalli, Ariyalur (Dt.), Tamil Nadu, India. This plant was legitimately recognized by Dr. S. John Britto, Director, The Rapinat Herbarium and Center for Molecular Systematics (Authentication No. DP005 dated: January 22, 2016). St. Joseph's College (Campus), Tiruchirappalli, Tamil Nadu, India.

Extraction and fractionation

Crisp flowers (3 kg) of *O. stricta* were removed with 90% ethanol (5×500 ml). The consolidated alcoholic concentrate was amassed in vacuum and the aqueous concentrate was progressively fractionated with petroleum ether (60–80°C) (6×250 ml), peroxide free diethyl ether (4×250 ml), and ethyl acetate (8×250 ml). Petroleum ether division and diethyl ether division did not yield any isolable substance. Ethyl acetate fraction on focus yielded a dry powder which was broken up in dimethyl sulfoxide (DMSO) to get different fixations and was utilized for additionally examines.

1,1-Diphenyl-2-picrylhydrazyl (DPPH) scavenging assay

DPPH radical searching action of the sample was decided as indicated by the technique detailed by Blois (1958). An aliquot of 0.5 ml of test arrangement in methanol was blended with 2.5 ml of 0.5 mM methanolic arrangement of DPPH. The blend was shaken energetically and hatched for 30 min oblivious at room temperature. The absorbance was estimated at 517 nm utilizing UV spectrophotometer. Ascorbic acid was utilized as a positive control. DPPH free radical rummaging capacity (%) was ascertained by utilizing the recipe [20-22].

$$\% \text{ Inhibition} = \left[\frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \right] \times 100$$

Where, $\text{Abs}_{\text{control}}$ is the absorbance of the DPPH radical+ethanol and $\text{Abs}_{\text{sample}}$ is the absorbance of DPPH radical+test separate/standard.

Ferric-reducing capacity (FRC) assay

FRC assay is like the FRAP assay with the exception of that it utilizes o-phenanthroline rather than TPTZ. Phenanthroline shapes a Fe^{III} - $(\text{Phen})_3$ complex that is diminished to an orange-red-shaded Fe^{II} - $(\text{Phen})_3$ complex. The response blend containing 1 ml of 0.05% o-phenanthroline in methanol, 2 ml ferric chloride (200 μM), 20 ml of acetate buffer, and 2 ml of different focuses extending from 10 to 250 μg was brooded at room temperature for 10 min and the absorbance of the same was estimated at 510 nm. Newly arranged FRC reagent clear perusing was taken at 510 nm. The samples tried and compared and that of ascorbic acid (standard). All estimations were done in triplicate and the average was taken [23-25].

$$\% \text{ Reduction} = \frac{A_{510 \text{ sample}}}{A_{510 \text{ blank reagent}}}$$

Isolation of human peripheral blood mononuclear cells (PBMCs) and culture

PBMCs from sound givers were disengaged from EDTA tube blood by Ficoll-Hypaque angle centrifugation. To put it plainly, peripheral blood from the givers was weakened with sterile phosphate buffer saline and overlaid on the Ficoll-Hypaque arrangement, and centrifugation was performed at 350×g for 10 min at room temperature. The recuperated PBMCs were refreshed in RPMI-1640 and brooded at 37°C 95% O_2 +5% CO_2 for 30 min before playing out the investigations [26].

The cell viability of PBMCs with LPS stimulation

After the PBMC isolation and pre-incubation period, 1×10^5 cells/ml PBMC were cultured in a 96-well polypropylene plate in serum-free RPMI-1640 medium with LPS at a final concentration of 100 ng/ml and various concentrations of the sample in DMSO. Cells in all conditions were incubated at 37°C, 95% O_2 +5% CO_2 for 24 h. Supernatants were

removed from the treated cells to investigate nitrous oxide (NO) scavenging activity. The viability of the cells was performed to evaluate the cytotoxicity of the sample using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. In the control wells, cells were incubated with DMSO vehicle and without LPS. In the positive control wells, cells were incubated with LPS and DMSO vehicle. The cell viability was determined by measuring the absorbance at 570 nm by a microplate reader [26].

$$\% \text{ Cell viability} = (\text{OD of the sample}/\text{OD of the control}) \times 100$$

NO inhibition assay

NO discharged in the supernatants was explored utilizing Griess reagent. The supernatants (100 μl) were blended with 20 μl of 1% sulfanilamide in 5% phosphoric acid and hatched for 10 min at room temperature, 20 μl of 0.1% naphthyl-ethylenediamine dihydrochloride were included and brooded for 10 min at room temperature. After that, the absorbance of the response blend was perused at 540 nm. Sodium nitrite was utilized for the adjustment bend [27-29].

Statistical analysis

All the data were reported as the mean \pm standard deviation (SD). All statistical analyses were performed by methods for one-way analysis of variance (ANOVA) and Student's t-test utilizing GraphPad Prism statistical software package version 7.02. The IC_{50} esteeme was processed from nonlinear backslide examination using the GraphPad Prism programming with the condition: $Y = 100 [1 + 10^{(X - \text{LogIC}_{50})}]^{-1}$. Only a value of $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Antioxidant activity

DPPH assay is quick, simple, and practical strategy to quantify an antioxidant effect, which includes the utilization of the free radical [30], DPPH. DPPH is usually used to test the capacity of mixes to go about as free extreme foragers or hydrogen contributors and it depends on the capacity of DPPH to decolorize within the sight of antioxidant [31,32]. The inhibition of the DPPH radical by the solid powder obtained from the ethyl acetate fraction from the flower *O. stricta* was concentration dependent (Fig. 1). The inhibition percentage of the solid powder at different concentrations (10, 50, 100, 200, and 250 $\mu\text{g}/\text{ml}$) ranged between $55.92 \pm 0.29\%$ and $78.48 \pm 0.22\%$. This solid fraction was capable of reducing the DPPH radical 50% with IC_{50} of $< 10 \mu\text{g}/\text{ml}$ compared to standard ascorbic acid which also has an $\text{IC}_{50} < 10 \mu\text{g}/\text{ml}$. Hence, the solid obtained from the ethyl acetate fraction from the flower *O. stricta* showed a very good antiradical activity in scavenging DPPH radical and showed a maximum percentage inhibition of $78.48 \pm 0.22\%$ at 250 $\mu\text{g}/\text{ml}$ concentration. DPPH scavenging activity of *O. stricta* is shown in Table 1.

Values are shown as for means \pm SD of triplicate. * $p < 0.05$ compared with control (one-way ANOVA and t-test).

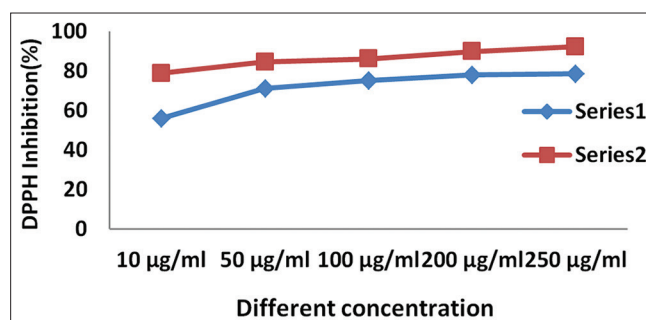
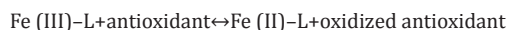
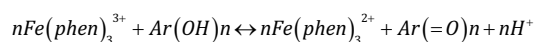


Fig. 1: 1,1-Diphenyl-2-picrylhydrazyl inhibition (%) activity of the solid powder (*Opuntia stricta* flower fraction) and standard (ascorbic acid) at different concentrations

The decreasing furthest reaches of a compound may fill in as a basic pointer of its potential antioxidant activity. The science of iron-based assays might be stuffed with the response condition:



Where, L is the ferrous-specific chromogenic ligand delivering the shaded species Fe(II)-L due to the concerned redox response. Longer wavelengths quite often constitute an imperative preferred standpoint in spectrophotometric strategy determination, on the grounds that most plant pigments, and additionally, a few antioxidants demonstrate huge assimilation at shorter wavelengths near UV scope of the unmistakable range. This reasoning applies for L: O-Phenanthroline instead of TPTZ ligand for modern FRAP assay. The redox response with tris(phen) Fe(III) of a polyphenolic compound Ar(OH)_n is given as



The reduction values of the solid powder obtained from the ethyl acetate fraction from the flower *O. stricta* at various concentrations (10, 50, 100, 200, and 250 µg/ml) ranged between 31.36±0.80% and 93.10±1.06% are depicted in Table 2. The IC₅₀ values of this solid powder were found to be 23.48±1.44 µg/ml compared to standard ascorbic acid which has a 32.88±1.06 µg/ml. In the concentration extend examined, all the solid powders exhibited reduced power that expanded straightly with concentration (Fig. 2). The diminishing power activity of the compound could fill in as an important pointer of the antioxidant potential. The reduction (%) was estimated by the reductive limit of antioxidant operator and it was evaluated by the change of Fe³⁺ to Fe²⁺ oxide within sight of the solid powder. Hence, the solid powder obtained from the ethyl acetate fraction from the flower *O. stricta* showed a very good antioxidant activity in FRC assay and showed a maximum percentage reduction 93.10±1.06% at 250 µg/ml.

Anti-inflammatory activity

The ideal condition for LPS-icited pro-inflammatory cytokines production was in PBMCs. LPS is a Gram-negative microbe which has an endotoxin and a constituent of the external layer. LPS stimulates innate immunity by regulating the production of inflammatory mediators such as tumor necrosis factor-α in PBMCs. The results showed (Fig. 3) that the solid powder obtained from the ethyl acetate fraction from the flower *O.*

stricta showed dose dependent. Examination of the cell viability of the fraction in PBMCs using the MTT assay had indicated that the fraction at 25–200 µg/ml did not affect the viability of PBMCs. Thus, the inhibition of LPS-induced mediator inflammation by the solid powder was not the result of a possible cytotoxic effect on these cells. Hence, these outcomes propose the anti-inflammatory impact of the solid powder (Table 3), focusing on pro-inflammatory cytokines generation, and using this solid powder possibly did not bring about unfavorable impacts.

Values are shown as for means ± SD of triplicate. *p<0.05 compared with control and positive control (one-way ANOVA and t-test).

NO is a pivotal biological messenger. As an inflammatory arbiter, NO assumes a part in a wide range of physiological and pathophysiological procedures, for example, macrophage-intervened cytotoxicity, vein dilatation, smooth muscle unwinding, and neurotransmission [33]. PBMCs were refined with LPS (100 ng/ml) and the substance of the proved NO was measured in the supernatants as a component of macrophage actuation. The impact of NO was seen in the cell culture medium of PBMC containing diverse concentration (25, 50, 100, and 200 µg/ml) of the solid powder from the ethyl acetate fraction from the flower *O. stricta* as appeared in Table 4. NO production percentage of the solid powder at different concentrations (25–200 µg/ml) ranged between 95.73±0.91% and 66.07±1.07% is depicted in Table 4. This study demonstrated that the solid powder ethyl acetate fraction from the flower *O. stricta* much reduced the production of NO. The data demonstrate a significant decrease in NO production in the solid powder as compared to control group (Fig. 4).

Hence, it is clear that the solid powder obtained from the ethyl acetate fraction of *O. stricta* flowers has anti-inflammatory activity in NO assay and showed a maximum reduced NO production 66.07±1.07% at 200 µg/ml.

Table 1: DPPH radical scavenging assay

Concentration (µg/ml)	% of inhibition	
	Sample	Standard (ascorbic acid)
10	55.92±0.29*	78.98±0.43
50	71.10±0.27*	84.68±0.04
100	75.15±0.34*	86.10±0.34
200	77.89±0.21*	89.82±0.82
250	78.48±0.22*	92.15±0.50
	IC ₅₀ <10 µg/ml	IC ₅₀ <10 µg/ml

DPPH: 1,1Diphenylpicrylhydrazyl

Table 2: FRC assay

Concentration (µg/ml)	% of reduction	
	Sample	Standard (ascorbic acid)
10	31.36±0.80*	25.07±0.98
50	66.96±1.11*	55.20±1.11
100	79.62±0.22*	78.00±0.48
200	82.06±0.42*	92.57±1.02
250	93.10±1.06*	95.73±0.91
	IC ₅₀ =23.48±1.44 µg/ml	IC ₅₀ =32.88±2.51 µg/ml

FRC: Ferric-reducing capacity

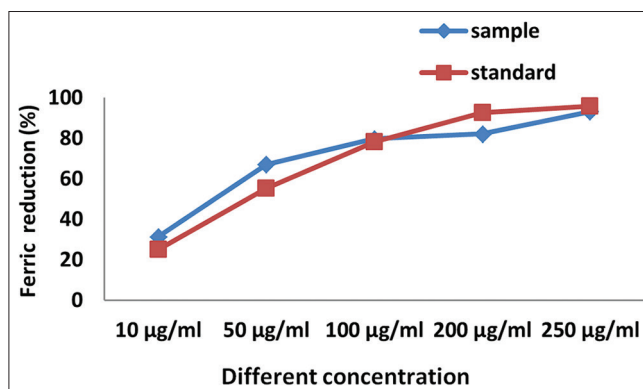


Fig. 2: Ferric reduction (%) of the solid powder (*Opuntia stricta* flower fraction) and standard (ascorbic acid) at different concentrations

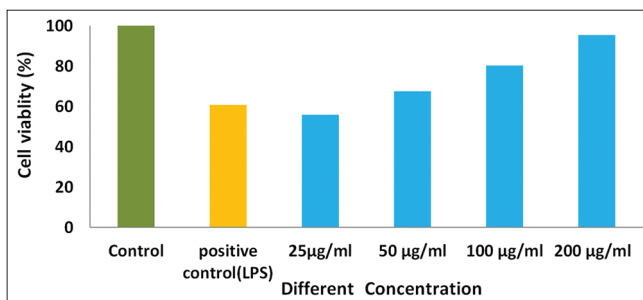


Fig. 3: The cell (peripheral blood mononuclear cells) viability percentage of without lipopolysaccharide (LPS) (control), with LPS (positive control), and the sold powder (*Opuntia stricta* flower fraction) at different concentrations

Table 3: PBMCs with LPS of cell viability

Concentration ($\mu\text{g/ml}$)	Cell viability (%)
Control	100
Positive control (LPS)	60.79 \pm 0.74*
25	55.91 \pm 0.87*
50	67.49 \pm 1.14*
100	80.23 \pm 0.62*
200	95.37 \pm 0.87*

PBMCs: Peripheral blood mononuclear cells, LPS: Lipopolysaccharide

Table 4: NO inhibition assay

Concentration ($\mu\text{g/ml}$)	NO production (%)
Control (LPS)	100
25	95.73 \pm 0.91*
50	82.89 \pm 1.04*
100	78.87 \pm 0.30*
200	66.07 \pm 1.07*

NO: Nitric oxide, LPS: Lipopolysaccharide

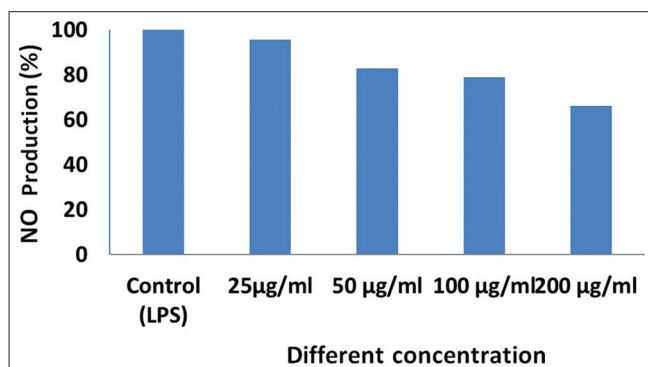


Fig. 4: The effect of solid powder (*Opuntia stricta* flower fraction) different concentrations on NO production by human peripheral blood mononuclear cells stimulated with lipopolysaccharide

Values are shown as for means \pm SD of triplicate. * $p < 0.05$ compared with control (one-way ANOVA and t-test).

CONCLUSION

To conclude, one might say that the present investigation had included methodical proof to the effectively accessible writing on the in vitro antioxidant and anti-inflammatory of the solid powder from the ethyl acetate fraction of the flower *O. stricta*. Moreover, work is required remembering the true objective to develop the character of the substance constituent responsible for antioxidant and anti-inflammatory activities. Studies are ahead of time in our examination office to outline the subnuclear structure of the compound. This contributes toward the progression of extreme antioxidant and anti-inflammatory pharmaceutical.

AUTHORS' CONTRIBUTIONS

All the authors give the same contributions to this research.

CONFLICTS OF INTEREST

The authors declare that no conflicts of interest exist among them.

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