International Journal of Current Pharmaceutical Research

ISSN- 0975-7066 Vol 9, Issue 5, 2017

Original Article

EVALUATION OF ETHANOLIC ROOT EXTRACT OF PARTHENIUM HYSTEROPHORUS LINN FOR ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITY

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Received: 20 May 2017, Revised and Accepted: 22 Jul 2017

ABSTRACT

Objective: The work is aimed to draw out the health beneficial properties of a weed (*Parthenium hysterophorus* Linn). The present work is organized to evaluate the antioxidant and anti-inflammatory activity of the ethanolic root extract of *Parthenium hysterophorus* Linn.

Methods: In the present work the ethanolic extract was determined by using soxhlet apparatus. The antioxidant scavenging activity of this extract was determined by applying three different assay methods: (1) DPPH (1, 1-diphenyl-2-picryl hydrazyl) free radical method. (2) Nitric oxide scavenging assay and (3) Reducing power method. The anti-inflammatory activity was determined by *in vivo* method i.e. Carrageenan induced rat paw edema.

Results: DPPH radical scavenging activities of the standard antioxidant and extracts were found to be increased in dose dependent manner. The percentage inhibition increases from 4.19% to 97.09% within the concentration range of 10µg/ml to 160µg/ml. *Parthenium hysterophorus* Linn effectively reduced the generation of nitric oxide radicals from sodium nitroprusside solution in a concentration dependent manner and percentage inhibition increases from 3.53% to 55.21% within the concentration range 10µg/ml to 160µg/ml. All the concentrations of extract significantly showed higher absorbance than the absorbance of control reaction (0.9705) in reducing power assay. A Higher absorbance indicates high reducing power due to the formation of reduced intermediates. *Parthenium hysterophorus* Linn showed highly significant anti-inflammatory activity at a dose of 200 mg/kg and the lesser effect was observed at 100 mg/kg with the percentage change in paw volume at 0 min, 30 min, 60 min, 90 min and 120 min.

Conclusion: Thus, from above experimental observations, it can be stated that *Parthenium* is a natural antioxidant and bearing anti-inflammatory activity.

Keywords: *Parthenium*, Ethanolic extract, Antioxidant, Anti-inflammatory activity

DOI: http://dx.doi.org/10.22159/ijcpr.2017v9i5.22327

INTRODUCTION

Oxygen is an essential and basic component for all living organisms which helps in the process of oxidation and maintenance of biological system [1]. Production of energy by the reduction of dioxygen (O_2) leads to the formation of reactive oxygen species. Free radicals are chemically unstable molecule and damage to cells, resulting imbalance between the generation of reactive oxygen species (ROS) and the antioxidant system collectively known as oxidative stress [2]. In the past decades, free radicals have aroused interest among the scientists. Their broad range of effects in biological systems has drawn the attention of many experimental works. Oxidative damage plays a significant pathological role in human diseases like arthritis, atherosclerosis, cancer etc [3]. Almost all organisms are well protected against free radical damage. There are many antioxidant defence systems in the body such as superoxide dismutase, catalase, α-tocopherol, ascorbic acid, glutathione, polyphenolic compounds, carotenoids etc. played an important role to maintain its integrity [4]. An antioxidant is a substance when present in low concentrations relative to the oxidizable substrate, significantly reduces oxidation process [5]. They scavenge the free radicals and prevent the tissue from damage. They can highly prevent the damage caused by free radicals by neutralizing the free radicals before they attack the cell. Flavonoids, ligans, phenolic acids and phenolic diterpenes are an example of phenolic compounds with the character of quenching oxygen-derived free radicals [6].

Inflammation is a protecting attempt by means of the organism to cast off the injurious stimuli and to initiate the healing manner. Although infection is resulting from a microorganism, inflammation is one of the response of an organism to the pathogen. However, for each pathogen, inflammation is a stereotyped response, and therefore it is considered as a mechanism of innate immunity, as compared to adaptive immunity [7]. Inflammation is a marker of the majority of diseases and number of people affected with inflammation, about 11% of adult men seen in urology clinics and 3% of children in globally balanitis. Chronic wounds counts 0.78% of population and the prevalence ranges from 0.18-0.32%. Globally the incidence of epiglottis was 15 per 1, 00,000 people [8].

Inflammation is a biochemical and cellular response that takes place in all vascularised tissue whose fitness and vitality or energy is threatened via either an internal or an external source. The important components of the inflammatory response can be observed within the blood, and mediators of inflammation function to increase the motion of plasma and infection fighting blood cells from the capillary bed into or across the injured tissue. The local inflammatory response is later observed via prominent systemic response called acute phase response {APR}. This response is marked by the induction of fever, anorexia, increased synthesis of hormones such as adrenocorticotropic hormone {ACTH}, increased leukocytosis and changed production of proteins in the liver. Proteins whose level changes during inflammation are known as acute phase protein {APP} [9].

Macrophages play principle roles within the immune and inflammatory responses involved in host protection. A number of different inflammatory mediators, including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6) reactive oxygen species (ROS), prostaglandin E_2 (PGE2), nitric oxide (NO) are secreted by activated macrophages [10].

MATERIALS AND METHODS

Plant material

Congress grass (*Parthenium hysterophorus*) roots were collected from Haldwani, Uttarakhand, India and identified in the IIIM Jammu. The roots were cleaned and cut into small pieces and were subjected to dry at room temperature. The dried roots were grinded and powdered.

Preparation of extracts

The 30g of dried and powdered plant material was extracted with 300 ml of solvent ethanol by using soxhlet apparatus for 48h at a temperature not exceeding the boiling point of the solvents. The extract was filtered by using Whatman No. 1 paper. The extract was concentrated by using rotary evaporator then dried and was used for further investigations.

Antioxidant activity

DPPH radical scavenging activity assay

The stable 1, 1-diphenyl-2-picryl hydrazyl radical (DPPH) was used for determination of free radical-scavenging activities of the extracts, as per the method described by Hatano *et al.* A different concentration of each extract was added, at an equal volume, to an ethanolic solution of DPPH (0.1 mmol). After 30 minutes, at room temperature, the absorbance was recorded at 517 nm. The experiment was repeated for three times. Ascorbic acid was used as standard antioxidants. IC50 value represents the inhibitory concentration of the sample, which is required to scavenge 50% of DPPH free radicals [11].

Percentage inhibition was calculated as,

$$\% \text{ scavenged DPPH radical} = [(\text{Abs control}-\text{Abs sample})]/(\text{Abs control})] \times 100$$

Where, Abs control is the absorbance of DPPH radical+methanol; Abs sample is the absorbance of DPPH radical+sample extract/ standard.

Reducing power method

Reductive ability of the extract was measured according to the method of Oyaizu (Oyaizu, 1986) [12]. Different concentrations (10-320µg/ml) of extract were mixed with 2.5 ml of sodium phosphate buffer (pH 6.6) and 2.5 ml of potassium ferricyanide (1%). The mixture was incubated at 50C for 20 min. Trichloroacetic acid (2.5 ml of 10%) was added to it, the mixture was mixed and centrifuged at 650 rpm for 10 min. The upper layer (5 ml) was mixed with 5 ml of deionized water and 1 ml of ferric chloride (1%) and absorbance was measured at 700 nm. Control reaction contains all the reagents except test compound. Higher absorbance indicated higher reducing power.

Nitric oxide scavenging activity

Sodium nitroprusside (5 mmol) in phosphate buffer saline was mixed with different concentrations of ethanolic extract (10-320 µg/ml) dissolved in DMSO and incubated at 25˚C for 30 min. After 30 min, 1.5 ml incubated solution was removed and diluted with 1.5 ml of Griess reagent (1% suphanilamide, 2% orthophosphoric acid and 0.1% napthylethylene diamine dihydrocholride). The absorbance of the chromophore formed during diazotization of the nitride with sulphanilamide and subsequent coupling with napthyethylene diamine was measured at 546 nm along with a control [13]. The percentage inhibition of nitric oxide generated was measured by comparing the absorbance values of control and test samples using following formula.

$$\text{Percent inhibition (\%)} = [((A_{control}-A_{sample})/A_{control})]*100$$

Where, $A_{control}$ is the absorbance of the control reaction (containing all reagents except test compound), and A_{sample} is the absorbance of test compound. IC_{50} value (concentration of sample required to scavenge 50% of free radicals) were calculated from the regression equation, prepared from the concentration of the samples and percentage inhibition of free radical. Ascorbic acid was used as positive control and all tests were carried out in duplicate.

Carrageenan induced rat paw edema [14]

- Initially weigh the rats in each group.
- Make a mark on both the hind paws (right and left) just beyond tibio-tarsal junction, so that every time the paw is dipped in the mercury column upto the fixed mark to ensure constant paw volume.
- Note the initial paw volume (both right and left) of each rat by mercury displacement method.
- Divide the group in four groups i.e. standard, control, test higher dose and test lower dose.
- To control group, inject saline and to standard group inject indomethacin subcutaneously.
- After 30 min inject 0.1 ml (w/v) carrageenan in the plantar region of the left paw of control as well as standard. The right paw will serve as reference non-inflammed paw for comparison.
- Note the paw volume of both legs of control and standard treated rats at 30, 60, 90, 120 min after carrageenan challenge.
- Calculate the percent difference in the right and left paw volume of each animal of control, standard, tests group. Compare the mean per cent change in paw volume in all groups and express as percent edema inhibition by the drug.

RESULTS AND DISCUSSION

DPPH free radical scavenging activity assay

DPPH radical scavenging activities of the standard antioxidants and extracts were found to be increased in dose dependent manner. The IC50 values of the standard antioxidant i.e. ascorbic acid was evaluated as 7.84 µg/ml. While the IC50 of the extract was calculated in various as 3.53 µg/ml. Therefore, it is concluded that *Parthenium hysterophorus* Linn is having excellent antioxidant activity against DPPH radical.

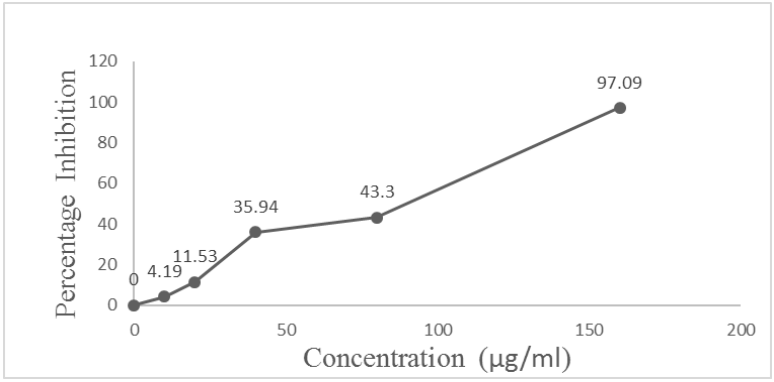

Fig. 1: DPPH scavenging activity of ethanol extract of *Parthenium hysterophorus* root with different concentration (µg/ml)

Reducing power method

The extract exhibited concentration dependent increase in absorbance. Absorbance indicated by all the concentrations of extract was significantly higher than the absorbance of control reaction (0.9705). Higher absorbance indicates high reducing power due to formation of reduced intermediates. Ascorbic acid has much high reducing ability than the ethanolic extract of *Parthenium hysterophorus* Linn.

Nitric oxide scavenging effect

The ethanolic extract of *Parthenium hysterophorus* Linn effectively reduced the generation of nitric oxide radicals from sodium nitroprusside solution in a concentration dependent manner. This showed significant nitric oxide scavenging activity of the extract (IC_{50} of ascorbic acid was found to be 7.84µg/ml.)

Carrageenan induced rat paw edema

The *in vivo* anti-inflammatory activity was performed by the carrageen an induced rat paw edema. *Parthenium hysterophorus* Linn showed significant anti-inflammatory activity at a dose of 200 mg/kg and lesser effect was observed at a dose of 100 mg/kg.

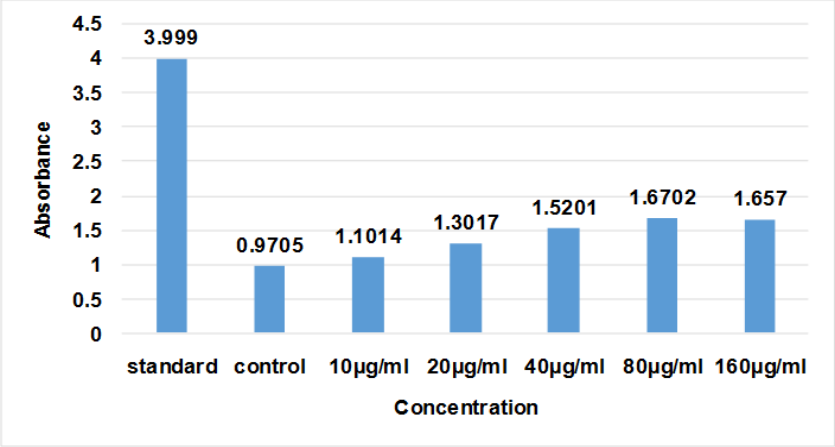

Fig. 2: Effect of different samples i.e. standard, control and test samples for reducing power assay

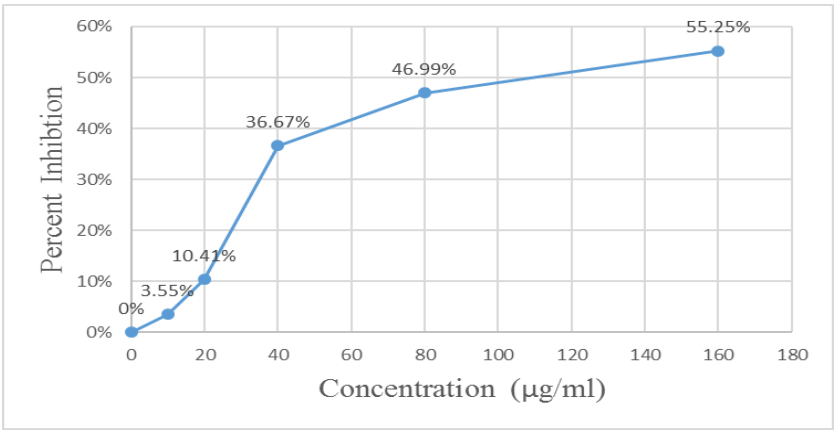

Fig. 3: Effect of different concentrations of ethanolic extract of *Parthenium hysterophorus* Linn on nitric oxide scavenging assay

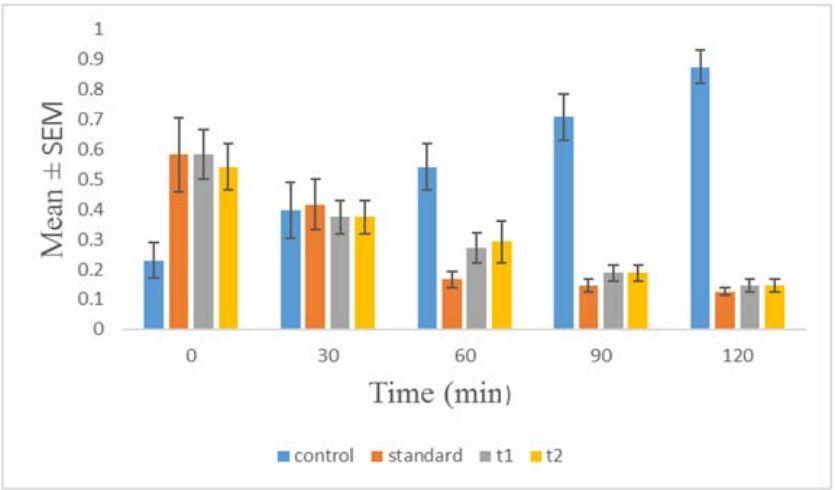

Fig. 4: Effect of different samples (Control, Standard, T1 higher dose and T2 lower dose on Carrageenan induced rat paw edema)

Table 1: Variations in paw edema for different groups with respect to time

Group	Dose	Increase in paw edema				
		0 min	**30 min**	**60 min**	**90 min**	**120 min**
Control	10 ml/kg	0.2292±0.05966	0.3958± 0.0936*	0.5417± 0.0768***	0.7083± 0.0768***	0.8750± 0.0559
Standard	10 mg/kg	0.5833±0.1236	0.4167± 0.08333**	0.1667±0.02635**	0.1458± 0.02083***	0.125± 0.00
Test T_1	200 mg/kg	0.5833±0.08333	0.375± 0.0559**	0.2708±0.05017***	0.1875± 0.02795***	0.1458± 0.02083
Test T_2	100 mg/kg	0.5417±0.07683	0.375± 0.0559*	0.2917±0.06972**	0.1875± 0.02795***	0.1458± 0.02083

All values are significant and are expressed in mean±SEM, $^{***}p \leq 0.001$, $^{**}p \leq 0.01$, $^{*}p \leq 0.05$ indicates the level of statistical significance as compared to control.

In the present work, after cumulating the results, it could be considered that the congress grass root extract might be a potent source of antioxidant and possesses anti-inflammatory potential.

CONCLUSION

From the above experimental observation, it can be clearly stated that the *Parthenium hysterophorus* is a promising source of natural antioxidant and anti-inflammatory agent and surely provides an alternative towards synthetic antioxidant and opens a new aspect of research trend for Parthenium as a natural antioxidant and anti-inflammatory agent.

ACKNOWLEDGEMENT

I would like to express my heartfelt appreciation to all those who provided me the possibility to complete my report. A special gratitude to my guide, Dr. Tirath Kumar, research supervisor whose contribution in encouragement helped me out to coordinate my work throughout the year.

CONFLICT OF INTERESTS

Declare none

REFERENCES

1. Parr A, Bolwell GP. Phenols in the plant and in man: the potential for possible nutritional enhancement of the diet by modifying the phenols content or profile. J Sci Food Agric 2000;80:985-1012.
2. Hussain SR, Cillard P. Hydroxyl radical scavenging activity of flavonoids. Phytochemistry 1987;26:2489-97.
3. Gupta M, Mazumdar UK, Gomathi P. *In vitro* antioxidant and free radical scavenging activities of Galena purpurea root. Pharmacogn Mag 2007;3:219-25.
4. Kaur IP, Geetha T. Screening methods for antioxidants-a review, mini reviews. Med Chem 2006;6:305-12.
5. Kehrer JP. Free radicals as mediators of tissue injury and diseases. Crit Rev Toxicol 1993;23:21-48.
6. Yener Z, Celik I, Ilhan F, Bal R. Effect of Urtica diocia L. seed on lipid peroxidation, antioxidant and liver pathology in aflatoxin-induced tissue injury in rats. Food Chem Toxicol 2009;47:418-24.
7. Abbas AB, Litcthman AH. In saunderes basic immunology functions and disorders of the immune system. ch 2. 3rd ed. innate immunity; 2009. p. 19-21.
8. Filomena C. *In vivo* anti-inflammatory and *in vitro* antioxidant activities of mediterranean dietry plants. J Ethnopharmacol 2008;116:144-51.
9. Pro Sono. Principles of the inflammatory response. Professional ultrasound services; 2011. p. 22-4.
10. Khan F, Khan M. Design and evaluation of levofloxacin hemihydrate floating tablets. Int J Appl Biol Pharm Technol 2010;1:25-7.
11. Hatano T, Edmatsu R, Mori A, Yasuhara T, Yoshida T, Okuda T. Effects of the interactions of tannins with co-existing substances on 1,1-diphenylpicrylhydrazyl radical. Chem Pharm Bull 1989;37:2016-21.
12. Oyaizu M. Studies on product of browning reaction prepared from glucose amine. Jpn J Nutr 1986;44:307-15.
13. Sreejayan N, Rao MNA. Free radical scavenging by curcuminoids. Drug Res 1996;46:169-71.
14. Kulkarni SK. Handbook of experimental pharmacology. Vallabh Prakashan. 3rd edition reprint; 2010. p. 128-31.

How to cite this article

- Pankaj Lohumi, Tirath Kumar, Lipi Nogai. Evaluation of ethanolic root extract of parthenium hysterophorus linn for antioxidant and anti-inflammatory activity. Int J Curr Pharm Res 2017;9(5):194-197.