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Research Article

EVALUATION OF *INVITRO* FREE RADICAL SCAVENGING ACTIVITY OF DIFFERENT ORGANIC EXTRACTS OF *PARTHENIUM HYSTEROPHORUS* LEAVES

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ABSTRACT

Synthetic drugs are potentially toxic and are not free of side effects on the host, an attempt has been made to study the anti-oxidant activity on plants. As plants and plant-based drugs are less toxic and have acceptable side effects. The crude extracts of leaves of *Parthenium hysterophorus* were selected for studying anti-oxidant activity. The plant extracts were prepared by using solvents (methanol, acetone and ethanol) and the activity was determined by DPPH (2, 2-diphenyl-1-picrylhydrazyl radical) scavenging assay with vitamin E as standard. By DPPH assay it was shown that acetone extract has higher anti-oxidant activity than methanol and chloroform extracts. The medicinal plants with anti-oxidant properties leading to possess good anti-cancer activities. The anti-oxidant phytochemicals protect the cells from oxidative damage caused by free radicals. Thus, consuming a diet rich in anti-oxidant foods (e.g. fruits and vegetables) will provide health-protective effects. It is a significance to exploit novel anti-oxidant drugs from the medicinal plants.

Keywords: Anti-oxidant activity, DPPH radical scavenging assay, *Parthenium hysterophorus,* Vitamin E.

INTRODUCTION

Plants and plant-based drugs are less toxic and have acceptable side effect. It is therefore essential to bring the use of these remedies into an existing frame work of rational scientific use of medicine based on the strong traditional knowledge. A rational approach is being developed to use medicinal plants as a lead for the discovery of active molecule, which act as one of the largest reservoirs. Many of the plants that were discovered by ancient civilizations are still in use today. The world health organization (WHO) estimated that 80% of the populations of developing countries rely on traditional medicines, mostly plant drugs, for their primary health care needs.

An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions by being oxidized themselves. As a result, antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols.

Low levels of antioxidants, or inhibition of the antioxidant enzymes, causes oxidative stress and may damage or kill cells. As oxidative stress might be an important part of many human diseases, the use of antioxidants in pharmacology is intensively studied, particularly as treatments for stroke and neurodegenerative diseases. Antioxidants are also widely used as ingredients in dietary supplements in the hope of maintaining health and preventing diseases such as cancer and coronary heart disease[1].

The amount of antioxidant principles present under normal physiological conditions may be insufficient to neutralize free radicals generated. Therefore, it is obvious to enrich our diet with antioxidants to protect against harmful diseases. Hence there has been an increased interest in the food industry and in preventive medicine in the development of "Natural antioxidants" from plant materials. That is why plants with antioxidant properties are becoming more and more popular all over the world. One such plant weed with anti-oxidant activity is *Parthenium hysterophorus.*

MATERIALS AND METHODS

Plant Material

The plant was identified based on the leaves which are lobed with fine soft hairs; the flowers on the top are small creamy color with black colored seeds. And based on the features of the plant it was confirmed as *Parthenium hysterophorus***.** The identification was confirmed by plant taxonomist. The plant was collected from Indira Park and Public Gardens, Nampally, Hyderabad. The leaves were separated and shade dried. The separated leaves were powdered in a mixer and fine powder was collected by passing through sieve no: 40.

Preparation of extracts

100g of dried and powdered plant material (leaves) was extracted at room temperature with 500 ml of methanol under constant shaking for 24 h. After filtration, the methanolic (MeOH) solutions were evaporated to dryness in a rotary evaporator for the biological assays, and then followed by extraction using solvents acetone and ethanol with same procedure.

Chemicals

2, 2 diphenyl-1picrylhydrazylhydrate (DPPH) and naphthylethylene diamine dihydrochloride (NEDA) were obtained from Sigma aldrich. Other chemicals used in these experiments were also of analytical grade.

Antioxidant Activity

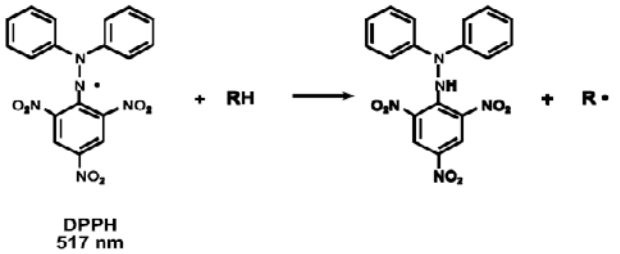
Several methods have been developed to evaluate the total antioxidant activity of fruits or other plants and animal tissues. Among them, trolox equivalent antioxidant capacity, total radical absorption potentials, oxygen radical absorption capacity assays and the ferric reducing ability of plasma (FRAP) assay are commonly used.

DPPH free radical scavenging activity

DPPH assay can accommodate a large number of samples in a short period of time and is sensitive enough to detect natural compounds at low concentrations so it was used in the present study for screening of antioxidant activity. The free radical scavenging activity of leaf crude extracts of *Parthenium hysterophorous* was measured in terms of hydrogen donating or radical scavenging ability using the stable radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH). Antioxidants react with DPPH which is a stable free radical, and convert it to 1, 1-diphenyl-2-picryl hydrazine. The DPPH solution was also prepared in methanol, ethanol and acetone independently. 3.96 mg of DPPH was dissolved in 20 ml of each solvent to get stock solution. With 0.5 ml of sample solution was added to 1 ml of DPPH solution separately. These solution mixtures were kept in dark for 30 min (incubation period) at room temperature. After which the absorbance was measured at 517 nm[2]. All tests were carried out in triplicate. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. Finally the radical scavenging activity was calculated as percentage of DPPH discoloration using the equation;

$$\% \text{ scavenging DPPH free radical} = 100 \times (1\text{-}AE/AD)$$

Where AE is absorbance of the solution, when extract has been added at a particular level and AD is the absorbance of the blank DPPH solution.



Nitric oxide radical scavenging activity

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions, which were measured by the method of Garrat (1964)[3].The reaction mixture (3ml) containing 2 ml of sodium nitroprusside (10mM), 0.5 ml of phosphate buffer saline (1M) were incubated at 25^{0}C for 150 min. After incubation, 0.5 ml of the reaction mixture containing nitrite was pipetted and mixed with 1 ml of sulphanilic acid reagent (0.33 %) and allowed to stand for 5 min for completing diazotization. Then 1 ml of naphthylethylene diamine dihydrochloride (1% NEDA) was added, mixed and allowed to stand for 30 mins. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions which can be estimated by the use of Griess illosvery reaction at 540 nm[4].

RESULTS

The anti-oxidant activity of different crude extracts of *P.hysterophorus* (leaf) was evaluated by DPPH and Nitric oxide radical scavenging assay. Vitamin E was used as positive control for investigation of anti-oxidant activity for both the assays. From the results, it was confirmed that acetone extract of *P. hysterophorus* (leaf) has shown potent anti-oxidant activity i.e., 63.09% for 100 µg/ml by DPPH assay and 63.45% for 100 µg/ml by Nitric oxide radical scavenging assay.

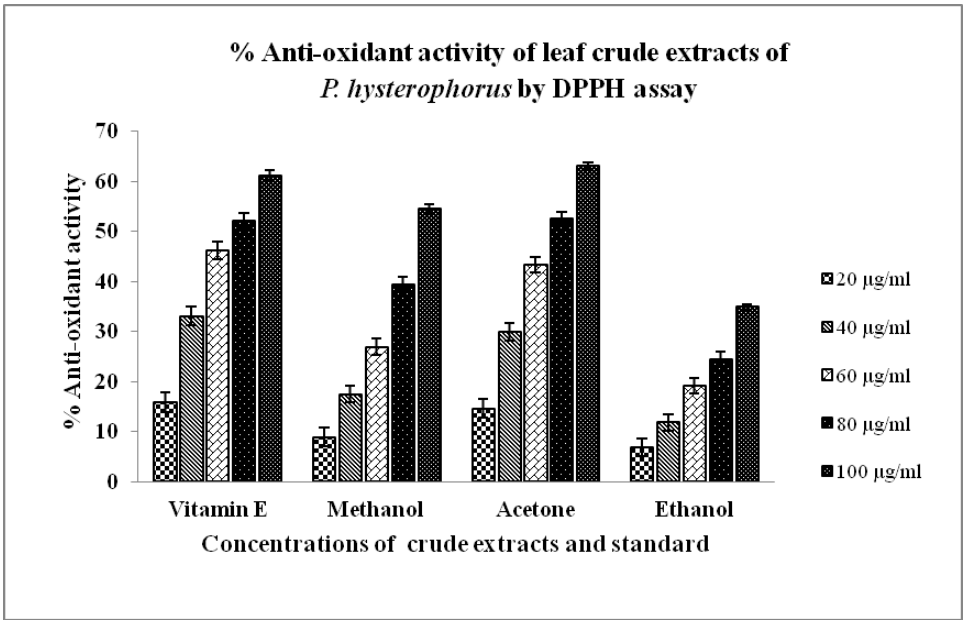
The results of both the DPPH assay and Nitric oxide radical scavenging assay of different extracts of *P. hysterophorus* (leaf) were represented in table 1 & 2 and graphically represented in graph 1& 2 respectively.

Table 1: Percent Anti-oxidant activity of crude extracts of Leaves of *P. hysterophorous* by DPPH assay.

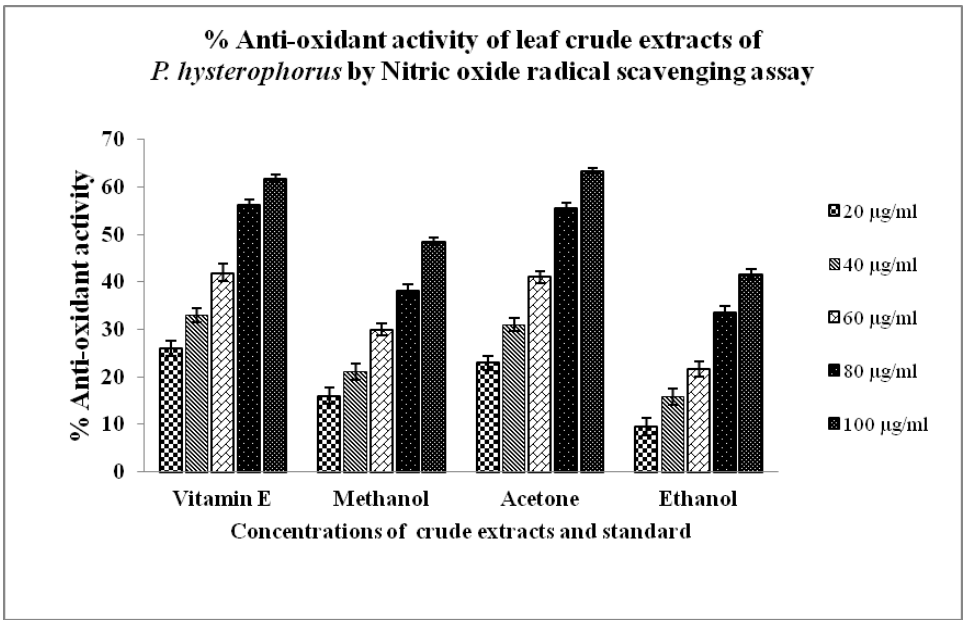
S. No	Conc. µg/ml	% Scavenging (Mean ± SD) of Triplicates			
		Vitamin E	Methanol	Acetone	Ethanol
1	20	15.97±1.98	8.92±1.8	14.6±1.88	6.9±1.76
2	40	33.12±1.84	17.5±1.72	29.97±1.74	11.87±1.63
3	60	46.24±1.7	26.95±1.63	43.42±1.54	19.09±1.52
4	80	52.09±1.66	39.42±1.42	52.63±1.23	24.53±1.44
5	100	61.25±0.94	54.62±0.87	63.09±0.65	34.92±0.54

Table 2: Percent Anti-oxidant activity of crude extracts of Leaves of *P. hysterophorous* by Nitric oxide radical scavenging assay.

S. No	Conc. µg/ml	% Scavenging (Mean ± SD) of Triplicates			
		Vitamin E	Methanol	Acetone	Ethanol
1	20	26.01±1.64	15.98±1.72	22.99±1.52	9.45±1.84
2	40	32.96±1.56	21.07±1.65	31.06±1.46	15.87±1.74
3	60	41.94±1.82	29.98±1.2	41.09±1.26	21.66±1.56
4	80	56.24±1.22	38.25±1.34	55.55±1.09	33.47±1.38
5	100	61.85±0.85	48.56±0.72	63.45±0.56	41.56±1.09



Graph 1: Percent Anti-oxidant activity of crude extracts of Leaves of *P. hysterophorous* by DPPH assay.



Graph 2: Percent Anti-oxidant activity of crude extracts of Leaves of *P. hysterophorous* by Nitric oxide radical scavenging assay

DISCUSSION

The consumption of foods containing considerable amounts of polyunsaturated fatty acids makes the use of antioxidants to prevent their oxidation more significant. Adding antioxidants is a way of prolonging the storage of lipids and foods containing lipids. Besides, the addition of antioxidants to foods would help the human body reduce the losses from oxidation disturbances related to ageing and diseases, such as atherosclerosis, cancer, and cirrhosis. Although almost all organisms have an antioxidant protection and systems for correction and prevention of oxidation damages, these systems are insufficient to wholly prevent the damages[5].

The commonly used antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are included in various foods, but their safety, however, can be doubted [6,7]. For this reason, importance and demand for natural antioxidants has grown over the recent years. These antioxidants occur in all plants and in all parts of the plants[8]. It is already well known that the antioxidant activity shown by plants is mainly due to the presence of polyphenol components, which are secondary metabolites of plant[9,10]. The initiation of lipid peroxidation is induced by the superoxide radical or by hydroxyl radicals. Therefore, antioxidation is an extremely significant action, which can be used as a preventive agent against a number of diseases[11-15]. Polyphenols capture the free radicals by giving hydrogen atoms or electrons. Furthermore, their bioactivity may be related to the ability to chelate metals and inhibit lipooxygenases[16]. Consequently, polyphenols can be used as natural preservatives, inhibiting the onset of lipid peroxidation and hydroperoxidation in foodstuffs and in living tissues.

The scavenging the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples[17]. It was found that the radical- scavenging activities of all the extracts increased with increasing concentrations.

DPPH assay is a widely used method to evaluate the free radical scavenging activity, which can accommodate a large number of samples in a short period of time and is sensitive enough to detect natural compounds at low concentrations so it was used in the present study for screening of antioxidant activity.

CONCLUSION

The anti-oxidant activity of various extracts of *P.hysterophorus* was evaluated by DPPH free radical scavenging assay. The results obtained in the course of this study, showed that the acetone extract has higher anti-oxidant activity than methanol and ethanol extracts. It was also found that the radical- scavenging activities of all the extracts increased with increasing concentrations. The medicinal plants with anti-oxidant properties leading to possess good anti-cancer activities. The anti-oxidant phytochemicals protect the cells from oxidative damage caused by free radicals. Thus, consuming a diet rich in anti-oxidant foods (e.g. fruits and vegetables) will provide health-protective effects. It is a significance to exploit novel anti-oxidant drugs from the medicinal plants.

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