Academic Sciences Asian Journal of Pharmaceutical and Clinical Research

Vol 5, Issue 3, 2012 ISSN - 0974-2441

Research Article

EVALUATION OF IN VITRO ANTIOXIDANT POTENTIAL OF ETHANOLIC EXTRACT FROM THE LEAVES OF *ACHYRANTHES ASPERA*

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Received: 26 March 2012, Revised and Accepted: 27 May 2012

ABSTRACT

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their uses in traditional medicine. The aim of the present study was to investigate the antioxidant property of ethanolic extract from the leaves of *Achyranthes aspera.*In vitro antioxidant properties of the extract was analysed by DPPH, OH radical scavenging and reducing power assays.The extract showed radical scavenging and reducing property.
The present study showed that the *A.aspera* leaves ethanolic extract possess notable antioxidant property.

Keywords: *Achyranthes aspera,*antioxidant, ethanolic extract.

INTRODUCTION

Recently there has been an upsurge of interest in the therapeutic potential of medicinal plants as antioxidants in reducing such free radical-induced tissue injury [1]. Besides, well known and traditionally used natural antioxidants from teas, wines, fruits,vegetables and spices, some natural antioxidants (e.g.rosemary and sage) are already exploited commercially either as antioxidant additives or as nutritional supplements [2]. The major constituents of biological membranes are lipids and proteins. The number of functions of membranes increases as the protein amount increases. Reactive oxygen species can easily initiate the lipids causing damage of the cell membrane constituent i.e. phospholipids, ipoproteins by propagating a reaction cycle [3]. It has been mentioned that antioxidant activity of plants might be due to their phenolic compounds [4]. Flavonoids are a group of polyphenolic compounds with known properties which include free radical scavenging, inhibition of hydrolytic oxidative enzymes and anti-inflammatory action [5].

Achyranthes aspera belong to family Amaranthaceae , commonly known Rough chaff flower in English, is an annual herb that grows throughout India, consists of 160 genera and approximately 2400 species of shrubs, herbs, climbers[6]. Different parts of the plant are ingredients in many native prescriptions in combination with more active remedies. In traditional medicinal system, *A. aspera* is known for diuretic and hepatoprotective properties and used to cure several diseases viz., malarial fever, dysentery, asthma, hypertension and diabetics. Most recently, *A. aspera* is widely studied for its medicinal properties and reported to have immunostimulatory properties[7], wound healing activity[8], antioxidant activity, h**e**molytic activity[9], anti-inflammatory[10], antibacterial activity[11] and antifungal activity [12].The present study was designed to investigate the *A. aspera* leaves ethanolic extract for its antioxidant potential by using in vitro assays such as DPPH radical scavenging, Hydroxyl radical scavenging and Reducing power assays.

MATERIALS AND METHODS

Collection of plant materials

The plant material was collected in the month of November 2011 from the local area of Mangalore and was taxonomically identified at the Department of Botany,Mangalore University.

Extraction and fractionation

The leaves of the plant were separated and washed with distilled water and shade dried for about ten days. The dried leaves were ground to a fine powder using mixer grinder and subjected to soxhlet extraction with 99% ethanol for 24 hours. The mixture was evaporated to dryness in a rotary flash evaporator and stored in refrigerator. The condensed extract was used for analysis.

Preliminary Phytochemical Analysis

Extracts were tested for the presence of active principles such as Triterpenoids, Steroids, Glycosides, Saponins, Alkaloids, Flavonoids, Tannins, Proteins, Free Amino Acids, Carbohydrate and Vitamin C following standard procedures .[13-14]

Antioxidant Activity

DPPH radical scavenging assay

Antioxidants react with DPPH, a stable free radical which is reduced to DPPH-H and as a consequence the absorbance is decreased from the DPPH radical to the DPPH-H form. The degree of discolouration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability.

The free radical scavenging capacity of the *A. aspera* leaves ethanolic extract was determined using DPPH according to the method of Blois[15]. DPPH solution (0.004% w/v) was prepared freshly in 99% ethanol and was added to sample solutions (100µg/ml) in ethanol.The mixture was allowed to stand at room temperature in dark for 20 mins.Then the mixture was vortexed and the absorbance was read at 517nm using a spectrophotometer. Ellagic acid was used as a reference standard. Control sample was prepared containing the same volume without any extract and 99% ethanol was used as blank. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. All tests were performed in duplicates. Percentage scavenging of the DPPH free radical was measured using the following equation, DPPH radical scavenging activity(%) = (Acontrol-ATest)/Acontrol X 100. Where A control is the absorbance of the control reaction and A test is the absorbance in the presence of the extracts or standard.

Hydroxyl radical scavenging assay

This was assayed as described by Elizabeth and Rao [16] with a slight modification. The assay is based on quantification of the degradation product of 2-deoxyribose by condensation with TBA. Hydroxyl radical was generated by the Fe^{3+}-ascorbate-EDTA-H_2O_2 system (the Fenton reaction). The reaction mixture contained, in a final volume of 1 ml, 2-deoxy-2-ribose (2.8 mM); KH_2PO_4-KOH buffer (20 mM, pH 7.4); $FeCl_3$ (100 µM); EDTA (100 µM); H2O2 (1.0 mM); ascorbic acid (100 µM) and various concentrations (0–200 µg/ml) of the test sample or reference compound. After incubation for 1 h at 37°C, 0.5 ml of the reaction mixture was added to 1 ml 2.8% TCA, then 1 ml 1% aqueous TBA was added and the mixture was incubated at 90°C for 15 min to develop the color. After cooling, the absorbance was measured at 532 nm against an appropriate blank solution.Reaction mixture without test substances/extracts was used as control. All tests were performed in duplicates. Ellagic acid, a classical OH scavenger, was used as a positive control.Lower absorbance of the

reaction mixture indicated higher OH radical scavenging activity. Percentage inhibition was evaluated by comparing the test and blank solutions. Percentage scavenging of the OH radical was measured using the following equation, OH radical scavenging activity(%) = $(A_{control}-A_{test})/A_{control}$ X 100. Where $A_{control}$ is the absorbance of the control reaction and A_{test} is the absorbance in the presence of the sample or the extract.

Reducing power(FRAP) assay

The Fe3+-reducing power of the extract was determined by the method of Oyaizu [17] with a slight modification. Different concentrations (0 - 400µg/ml) of the extract (0.5ml) were mixed with 0.5 ml phosphate buffer (0.2 M, pH6.6) and 0.5 ml potassium hexacyanoferrate (1%), followed by incubation at 50°C in a water bath for 20 min.After incubation, 0.5 ml of TCA (10%) was added to terminate the reaction. The upper portion of the solution (1ml) was mixed with 1 ml distilled water, and 0.1 ml FeCl3 solution (0.1%) was added. The reaction mixture was left for 10 min at room temperature and the absorbance was measured at 700 nm against an appropriate blank solution. All tests were performed in duplicates. A higher absorbance of the reaction mixture indicated greater reducing power. Ellagic acid was used as a positive control.

Statistical Analysis

All values were expressed as Mean±SD of two measurements. Comparison between standard and extracts were performed by using Students't' test. In all these test criterion for statistical significance was P<0.05.

RESULTS

Phytochemical Analysis

The preliminary phytochemical screening showed the presence of flavonoids and reducing suigar for the compounds tested.

DPPH radical scavenging assay

DPPH test provides simplified version to detect the antioxidant properties of various molecules present in the extracts. A DPPH solution is decolourized when the odd electron becomes paired off in the presence of a free radical scavenger. The colour becomes light yellow from deep violet. The results of the assay are given in the table1. The percentage of radical scavenging property of the extract and standard in this assay were 57.53±1.71% and 93.70± 0.70% at 100µg/ml respectively.

Hydroxyl radical scavenging assay

This assay shows the abilities of the extract and standard Ellagic acid to inhibit hydroxyl radical-mediated deoxyribose degradation in an Fe^{3+}-EDTA-ascorbic acid and H2O2 reaction mixture. The results are shown in Table 1. The percentage of radical scavenging property of the extract and standard in this assay were 36.12±0.91% and 88.79±0.70% at 200µg/ml respectively.

Reducing power assay

The extract showed potent antioxidant power by reducing power ability. Results of reducing power assay are shown in table 1. The EC50 value of the extract and standard were found to be 753.90±1.13µg/ml and 104.88±0.92 µg/ml respectively.

Table 1: Comparison of radical scavenging property and reducing property of *Achyranthes aspera* leaves ethanolic extract with Ellagic Acid

	DPPH radical scavenging capacity	**OH radical scavenging capacity**	**FRAP Assay**
	% of radical scavenging property/100µg of extract	% of radical scavenging property/200µg of extract	EC50 conc.µg/ml
***A.aspera* Extract**	57.53±1.71%	36.12±0.91%	753.90±1.13
Ellagic Acid	93.70± 0.70%	88.79±0.70%	104.88±0.92

P<0.05, P<0.05 is statistically significant.Data are expressed as mean±SD.

DISCUSSION

Biological and chemical research in Life Science evidenced that free radical and reactive oxygen species can be involved in a high number of diseases[18]. Numerous physiological and biochemical processes in the human body may produce oxygen centered free radical and other reactive oxygen species and byproducts. Overproduction of such free radical cause oxidative damage to biomolecules leading to many chronic diseases[19]. Plants are the important source for free radical scavenging molecules. Intake of natural antioxidant has been associated with reduced risk of cancer,cardiovascular diseases, diabetes and other diseases associated ageing. Antioxidant is one of the most essential ingredient of today's menu/therapy because the antioxidative system protects the animal against reactive oxygen species (H2O2, superoxide, OH, singlet oxygen & nitrogen species) induced oxidative damage.Various synthetic antioxidants (BHT) are on the use, but they are suspected to be carcinogenic[20].

The preliminary phytochemical screening tests may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development. Further, these tests facilitate their quantitative estimation and qualitative separation of pharmacologically active chemical compounds. [21]

Natural antioxidants, therefore, have gained importance. Ethanolic extract of *A. aspera* leaves has been studied for its antioxidant properties using different invitro antioxidant methods. Potent antioxidant activity of *A. aspera* leaves ethanolic extract was analysed by making use of three different methods. However, the efficiency of the extract differed against various free radicals depending on the specific assay methodology, which reflects the complexity of the mechanisms and diversity of the chemical nature of the plant material.

The effect of antioxidants on DPPH is thought to be due to their hydrogen donating activity[22]. As DPPH is considered as the lipophilic radical, it readily accept electron from the antioxidant compound and converts its colour from violet to yellow which is detected at 517nm. In this study, free radical scavenging activities of A.aspera leaves ethanolic extract and standard ellagic acid were determined by using DPPH method. The result obtained in the study indicates that the extract exhibited good radical savenging activity but was to a lesser extent compared to standard ellagic acid.

Hydroxyl radicals are the major active oxygen species causing lipid peroxidation and enormous biological damage [23]. They were produced in this study by incubating ferric-EDTA with ascorbic acid and H_2O_2 at pH 7.4, and reacted with 2-deoxy-2-ribose to generate a malondialdehyde (MDA)-like product. This compound forms a pink chromogen upon heating with TBA at low pH [24]. When *A. aspera* extract was added to the reaction mixture, it removed the hydroxyl radicals from the sugar and prevented the reaction. The result obtained in the study indicates that the extract showed moderate OH radical scavenging activity compared to standard ellagic acid.

Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity[25].Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants .Presence of reducers causes the conversion of the Fe^{3+}/ferricyanide complex used in this method Antioxidant to the ferrous form. By measuring the formation of Pearl's Prussian blue at 700nm, it is possible to determine the concentration of Fe^{3+} ion. In this study the extract showed high absorbance than blank indicating the ability of the extract to take part in electron transfer reaction. This shows that the extract exhibited reductive activity.

The phytochemical screening showed the presence of only flavonoids and reducing sugar of the compounds tested. Also the antioxidant activity exhibited was less compared to previous studies. This difference might be attributed to the fact that concentrations of nutrients and bioactive substances(flavonols, anthocyanins, ellagic acid, etc.) are known to be strongly influenced by extrinsic factors such as variations in plant type and growth, climate, season, temperature, as well as degree of ripeness .[26]

CONCLUSION

In conclusion, the data obtained in the present study show that *A.aspera* leaves ethanolic extract possess antioxidant activity but to a lesser extent compared to previous experiments. This difference in antioxidant activity, confirms the fact that the type of cultivation is important for antioxidant capacity and is greatly influenced by variations in plant type and growth, climate, season, temperature and soil conditions.

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