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RESEARCH ARTICLE

IN VITRO ANTIOXIDANT ACTIVITIES OF *AVENA SATIVA* (OATS) ALCOHOLIC SEED EXTRACTS

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ABSTRACT

The research envisaged was carried out to explore the antioxidant potential of alcoholic seed extract of Avena Sativa (Oats) invitro. The seeds ofAvenasativawere collected and air dried seed sample rinsed with water and extracted with ethanol in a soxhlet apparatus. The suspension of extract was prepared freshly in normal saline with the help of 0.5% w/v carboxymethylcellulose (CMC). Varying concentration of extract (10, 25, 50 and 100 µg/ml) was tested for invitro antioxidant properties. Antioxidant activity was determined by DPPH assay, reducing power ability, hydrogen peroxide scavenging assay and hydroxyl radical (OH˙) scavenging activity. The ethanolic seed extract of Oats demonstrated antioxidant potential dose dependently with best activity at100 µg/ml. Based on the outcomes of the present research, anti-oxidative potential could be attributed for traditionally claimed potencies of seeds of Avena Sativa (Oats). However, further studies to be carried out on animal models using their biological tissues before exploiting for its clinical benefits.

Keywords: *Avena Sativa, antioxidant, Invitro, DPPH.*

INTRODUCTION

Large numbers of diseases are caused by oxidative stress. Augmented oxidation of cell contributes tocardiovascular disease, tumor growth, wrinkled skin, cancer, Alzheimer's disease, and even adecline in energy and performance [1-4]. Antioxidants are substances that delay or prevent theoxidation of cellular oxidizable substrates. The antioxidant potential is exhibited by scavenging oxidative free radicals, activating a buttery of detoxifying proteins or preventing the generation of reactive oxygenspecies [5-7]. In recent years, there has been an increasing interest in finding natural antioxidants,which can protect the human body from free radicals and retard the progress of many chronicdiseases [8, 9]. Natural antioxidants such as α-tocopherol and ascorbic acid are widely used becausethey are regarded as safer and causing fewer adverse reactions but their antioxidant activities arelower than the synthetic antioxidants such as butylatedhydroxyanisol (BHA) and butylatedhydroxytoluene (BHT) which have been restricted by legislative rules because they are suspected tohave some toxic effects and as possible carcinogens [10-12]. Hence it is imperative to promote credible research for exploring newer safe antioxidants from natural sources to replace these syntheticantioxidants [13, 14].

Recently, natural plants have received much attention as sources of biological active substancesincluding antioxidants. Numerous studies have been carried out on some plants, vegetables andfruits because they are rich sources of antioxidants, such as vitamin A, vitamin C, Vitamin E, carotenoids, polyphenolic compounds and flavonoids [15] which prevent free radical damage,reducing risk of chronic diseases. Thus, the consumption of dietary antioxidants from these sourcesis beneficial in preventing cardiovascular disease [16]. The search for newer natural antioxidants,especially of plant origin has ever since increased.

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Oats (*Avena sativa*) is a species of cereal grain, rich in dietaryfibers, and also contain relatively high levels of protein and unsaturated fat compared with other cereals [17-19]. Besides the nutritional value, the higher fat content makes oats rich in lipid-soluble phytochemicals with antioxidative and possibly other physiologically active properties [20]. The fiber content of oats include cellulose, arabinoxylans, and the soluble fibers, mixedlinked (1→3), (1→4)--D-glucan [19]. Various components in the oat grain protect its lipids from oxidation. Such antioxidative components include vitamin E (tocopherols and tocotrienols), phenolic compounds (such as the oat-specific polyphenols avenanthramides), phytic acids, sterols, andflavonoids [20]. The potential of plant antioxidants to scavenge free radicals and protect the plant from oxidation is transferred to the human body when oats or other antioxidant-rich foods are consumed [18]. Antioxidant potential of oats are exhibited by number of studies, however, scientific confirmation of anti-oxidative property of alcoholic extract of *Avena sativa* seeds have not been carried out. Thus the present study was an attempt in the direction of elucidation antioxidant profile of alcoholic extract of oats seeds in-vitro.

MATERIALS AND METHODS

Extraction procedures

The seeds of *Avenasatvia*were collected from natural habitats Bhuna village, DistFatheabad, Haryana during April 2011, flowering season. The collected seeds were recognized and authenticated by Dr. ShaliniKapoor Mehta, Department of Pharmacognosy, Krupanidhi College of Pharmacy, Bangalore. Air dried plant sample rinsed with water and dried. After evaporation of the solvent, the residue (250 g) was extracted with 500 ml, 70% ethanol in a soxhlet apparatus and the extract was evaporated to dryness by a rotary evaporator [21]. The suspension of extract was prepared freshly 30 minutes prior to experiment/administration in normal saline with the help of 0.5% w/v carboxymethylcellulose (CMC).

Antioxidant activity (DPPH free radical scavenging activity) of extract

The antioxidant activity of the *Avenasatvia* (AS) was assessed on the basis of the radical scavenging effect of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH)-free radical activity by modified method [22]. 1, 1-Diphenyl-2-picryl-hydrazyl (DPPH) was obtained from Sigma Aldrich Co., St. Louis, USA. The diluted working solutions of the test extracts were prepared in methanol. Ascorbic acid was used as standard in 1-100 µg/ml solution. 0.002% of DPPH was prepared in methanol and 1 ml of this solution was mixed with 1 ml of sample solution and standard solution separately. These solution mixtures were kept in dark for 30 min and optical density was measured at 517 nm using UV Spectrophotometer. Methanol (1 ml) with DPPH solution (0.002%, 1 ml) was used as blank. The optical density was recorded and % inhibition was calculated using the formula given below [23]:

Percent (%) inhibition of DPPH activity = (A-B/A) 100

Where A = optical density of the blank and B = optical density of the sample.

Reducing power ability

The extracts of *Avenasatvia* (AS) was determined for their reducing power modifying the method of Oyaizu (1986) [24]. Reaction mixtures were prepared by adding 2.5 ml of phosphate buffer (0.2 M, pH 6.6), 2.5 ml Potassium Ferricyanide (1%) and varying concentrations of extracts (40-200 mg). After, the reaction mixtures were incubated at 50°C in water bath for 30 min, allowed to cool at room temperature (28°C), and 2.5 ml of 10% TCA (Trichloroacetic acid) were added to each reaction mixture, and then centrifuged at 2000 rpm for 10 min. The supernatant (2.5 ml) was separated in the test tube and added with 2.5 ml of distilled water and 0.5 ml FeCl3 (1.0%), and allowed to react for 10 min at room temperature and the absorbance was measured at 700 nm. Ascorbic acid solution (ASA) was used as standard.

Hydrogen peroxide scavenging assay

Hydrogen peroxide scavenging strengths of extracts of *Avenasatvia*(AS)was determined by the method described by Ruch *et al.* (1989) [25]. A solution of H_2O_2 (10 mM) was prepared in phosphate buffer (pH 7.4). Reaction mixtures contained 10mM of H_2O_2 and different concentrations of test samples, and absorbance values were measured at 0 min and after 60 min using wavelength of 240nm. Ascorbic acid was used as the standard.

Hydroxyl radical (OH^-) scavenging activity **(**Halliwell *et al.,* 1987) [26]

Hydroxyl radical were generated by phenyl hydrazine in solution which was measured by appearance of pink colour (TBA) – MDA chromogen (due to OH- mediated decomposition of 2-Deoxyribose) (Halliwell *et al.,* 1987). The reaction was performed in incubation mixture containing 50 mm phosphate buffer (PH 7.4), 1 mm deoxyribose, 0.2 mm phenylhydrazine hydrochloride and *Avenasatvia* (AS)extract (10, 25, 50 and 100 µg/ml) or (10 and 20) mm mannitol. The final reaction value was made up to 2ml. incubation was terminated after 1hr or 4 hrs with 2.8% trichloro acetic acid (1ml). Thiobarbituric acid (1%w/v) was then added to the reaction mixture followed by fitting for 10 min on a boiling water bath. The tubes were than cooled briefly and absorbance was taken at 532 nm. A decreased in absorbance indicated hydroxyl radical scavenging activity.

RESULTS AND DISCUSSION

This study was carried out to determine the phytochemical and invitro antioxidant potential of alcoholic seed extract of *Avenasatvia* (AS). Antioxidant compounds in food and diet play an important role as a healthprotectingfactor. Scientific evidence suggests that antioxidantsreduce the risk for chronic diseases including cancer and heartdisease. Primary sources of naturally occurring antioxidantsare whole grains, fruits and vegetables. Plant sourced foodantioxidants like vitamin C, vitamin E, carotenes, phenolic acids, phytate and phytoestrogens have been recognized as havingthe potential to reduce disease risk. Most of the antioxidantcompounds in a typical diet are derived from plant sources andbelong to various classes of compounds with a wide variety ofphysical and chemical properties. Some compounds, such asgallates, have strong antioxidant activity, while others, such asthe mono-phenols are weak antioxidants.The main characteristic of an antioxidant is its ability to trapfree radicals. Highly reactive free radicals and oxygen speciesare present in biological systems from a wide variety of sources.These free radicals may oxidize nucleic acids, proteins, lipidsor DNA and can initiate degenerative disease. Antioxidantcompounds like phenolic acids, polyphenols and flavonoidsscavenge free radicals such as peroxide, hydroperoxide or lipidperoxyl and thus inhibit the oxidative mechanisms that lead todegenerative diseases.

The common oat (*Avena sativa*) is a species of cereal graingrown for its seed and used traditionally for lowering lipid profile. Its consumption is believed to lower LDL ("bad") cholesterol, and possibly to reduce the risk of heart disease [27]. Moreover, traditional claims are available for myocardial potency and hypoglycemic property [28]. In the present study, an effort was made to elucidate antioxidant potential of alcoholic seed extract of oats which might be used as foundation stone for our advance studies towards scientific confirmation of its hypoglycemia and myocardial protective potentials.

A freshly prepared DPPH solution exhibited a deep purple color with a maximum absorption at 517 nm. This purple color disappears when an antioxidant is present in the medium. Thus, antioxidants molecules can quench DPPH free radicals and convert them to a colorless product, resulting in a decrease in absorbance at 517 nm. The RSA values of *Avenasatvia* (AS) are presented in Table 1; results are expressed as IC50 values (concentration of sample required to scavenge 50% of free radicals) of *Avenasatvia* (AS) and ascorbic acid are indicated Table 1. Alcoholic seed extract of AS dose dependently demonstrated antioxidant potentials by scavenging DPPH radical scavenging activity. The DPPH scavenging potential of extract might be due to its reducing actions, which might donate hydrogen to a free radical, reducing it to nonreactive species (Wang *et al*., 2008) [29]. Higher DPPH scavenging potential of *Avenasatvia*might be due to the higher reducing potential.

The reducing power of ethanolic extract of *Avenasatvia*(AS) was found to be correlated with increasing absorbance (at 700 nm) as compared with ASA, a known antioxidant (Table 1). Similar observations were also reported earlier (Duh, 1998) [30]. The presence of reductones are responsible for reducing capacity, which involved in prevention of chain initiation, binding of metal ions, decomposition of peroxides and radical scavenging (Yildirm *et al*., 2001) [31].

The scavenging ability of species of ethanolic extract of *Avenasatvia*(AS)with H_2O_2 is compared with the ascorbic acid and is depicted in Table 1. Though H_2O_2 itself not very reactive, it generates highly reactive molecule such as OH^- by reacting with metals (Fe^{2+} or Cu^{2+}), and superoxide anions in the Haber-Weiss reaction. Therefore, removing of H_2O_2 is very essential from the cell or food systems. A significant dose dependent H_2O_2 scavenging potential of ethanolic extract of *Avenasatvia*(AS)was observed during the present study. Electronic donors might accelerate the conversion of H_2O_2 to H_2O (Ruch *et al*, 1984) [32], which could possible to scavenge H_2O_2 in the ethanolic extract of *Avenasatvia*(AS).

The hydroxyl radical (generated secondarily by the reaction of superoxide and hydrogen peroxide) actually brings about depolymerisation of hyaluromic acid as a OH^- scavenger can return the viscosity of hyaluromic acid solution. Hydroxyl radical (OH-) is closely associated with inflammatory disorder like arthritis where a progressive loss of hyaluromic acid in joint important feature of disease. In our study *Avenasatvia*extracts in concentration 10, 25, 50 &100 µg/ml produces a dose dependent scavenging of OH- radical. The activity of extract was most effective in the first hour of the study.

In conclusion, ethanolic extract of *Avenasatvia* (AS) showed dose dependent antioxidant properties in different invitro evaluations. These revelations are significantly noticeable as *Avena sativa* induced amelioration of numerous metabolic disorders and functional defects might be attributed to its antioxidant potential. However, scientific confirmation of traditional claims is necessary for exploiting the therapeutic benefits of this wonder grain.

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Table - 1: In vitro antioxidant activity of *Avenasatvia*(AS)

Drug	**Dose μg/ml**	IC50 (μg/ml) DPPH (Mean ± SD)	Reducing power * (absorbance at 700 nm) (Mean ± SD)	H_2O_2 scavenging assay
Vitamin C	100	5.94 ± 0.11	1.98 ± 0.23	24.32 ± 1.46
	50	9.90 ± 0.12	1.04 ± 0.17	14.54 ± 1.33
Avenasatvia	100	3.71 ± 0.12	0.78 ± 0.32	24.58 ± 1.09
	50	5.62 ± 0.15	0.68 ± 0.42	18.49 ± 1.09
	25	7.90 ± 0.14	0.59 ± 0.21	15.29 ± 1.32
	10	8.94 ± 0.12	0.48 ± 0.18	07.58 ± 1.09

*Higher absorbance indicates higher reducing power

Table - 2: Effect of *Avenasatvia* (AS) extract on Hydroxyl radical scavenging activity

Drug	**Dose μg/ml**	**Hydroxyl radical scavenging activity**	**% ofInhibition**
Control	-	0.390 + 0.0035	
Quercetin	10	0.114 + 0.029*	70.77
	25	0.131 + 0.026*	67.10
Vitamin C	50	0.078 + 0.026*	79.8
	100	0.068 + 0.058*	81.6
Avenasatvia	10	0.121 + 0.049*	53.2
	25	0.163 + 0.0058*	66.77
	50	0.151 + 0.058*	62.79
	100	0.142 + 0.018*	58.31

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