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Original Article

Inhibition of Oxidative Stress Induced Free Radicals Damage and Cytoprotective Potential on *Saccharomyces cerevisiae* by Aqueous Extract of *Apium graveolens* (Celery)

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ABSTRACT

In this study free radical scavenging activity of the aqueous extract of Apium graveolens and cytoprotection of Saccharomyces cerevisiae (yeast cells) was studied. Antioxidant activity was studied in different in vitro models, viz., Diphenyl picrylhydrazyl (DPPH) radical, and inhibition of reactive oxygen species (ROS), reducing power, and total phenolic content and metal chelating activity. Cytoprotective assays performed were cell viability, ROS, TBARs, LDH. From the results obtained it was observed that the DPPH radical activity showed IC 50 of 14 µg, ROS and metal chelating activity with an IC 50 of 60 and 20 µg respectively. The extract showed good reducing activity, which is an index of hydrogen donor. Also total phenolic content was recorded 61.2 mg/ g of the extract. Further the extract showed cytoprotective effect on xenobiotic induced (CCl4) oxidative stress in yeast cells, From the obtained results it shows that the aqueous extract of Apium graveolens is having potent free radical scavenging and cytoprotection activity suggesting that the aqueous extracts of Apium graveolens may contain numerous antioxidant molecules, which can effectively scavenge various ROS/free radicals under in vitro conditions. The broad range of activity of the extract suggests that Apium graveolens is a source of natural antioxidants, which could be considered as nutraceutical with health promoting properties in the prevention, and amelioration of degenerative diseases.

Key word: Apium graveolens, CCl4, antioxidant, cytoprotection.

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1. INTRODUCTION

A molecule which inhibits the oxidation of other molecules is an antioxidant. In Oxidation there is transfer of electrons from a one molecule to another, this reaction can produce free radicals. These free radicals are generated in living systems during electron transport chain which is part of the

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normal metabolic process. During infection, physical exercise and fasting there is chemical mobilization of biomolecules (fat, protein starch) which result in increased free radical activity and damage the cell [1]. The antioxidants are radical scavengers, which protect the human body against free radicals. The reactive oxygen species, including superoxide, hydroxyl radical and hydrogen peroxide are generated in specific organelles in the cell under normal physiological conditions [1]. Excessive production of these ROS, beyond antioxidant defense capacity of the body can cause oxidative stress [2]. The reactive oxygen species ROS and free radicals mediated reactions are involved in various pathological conditions such as anemia, asthma, arthritis, inflammation, neurodegeneration against ageing process and perhaps dementia [3]. General consensus has been reached during the last few years that diet has a major role in the development of chronic diseases, such as, cancer, coronary heart disease, diabetic's type2, hypertension and cataract. Studies have also shown that consumption of food and beverages rich in phenolic content is correlated with reduced incidence of heart diseases, anemia, asthma, arthritis, inflammation, neurodegeneration[4]

Apium graveolens is a plant species in the family *Apiaceae* commonly known as celery their leaves, petioles and roots are commonly consumed. *Apium graveolens* grows to about 1 M tall; India is one of major producer of celery, exported to different countries. It requires high moisture with low temperature for its growth [5]. Good qualities are produced only in temperate regions [6]. The main parts of the plants used are leaves, stalks, seeds and their essential oils[7]. The main phytochemical present in celery is polyphenols viz., flavanoids, alkaloids and steroids [8]. Several molecules such as limonene, selinene, frocoumarin glycosides, flavonoids, and vitamins A and C are present and it is traditionally used as medicine[9]. It has been reported that the celery can prevent cardiovascular diseases,[10] jaundice, liver and lien diseases,[11] urinary tract obstruction,[7] and rheumatic disorders[12] *In vivo* studies on animal models have shown that that ethanol extracts of celery leaves increases spermatogenesis[13] and also improves their fertility. Studies have show that celery has antifungal anti-inflammatory and antibacterial effects. Its seeds are useful in the treatment of bronchitis, asthenopia, asthma, fever, chronic skin disorders, including psoriasis, [11-14]. The root of the celery is diuretic and it is used for the treatment of colic [15] Plants are an important source of natural active products, which vary, based on mechanism and biological properties. Since the plant is having the potent molecules, study was conducted to evaluate its free radical scavenging activity in different *in vitro* models and cytoprotection study was also performed to check that the aqueous extract of celery in protecting the oxidative stress induced yeast cells by ameliorating the level of free radicals.

2. MATERIALS AND METHODS

2.1 Extraction

Celery leaves were washed thoroughly with tap water for two to three times and allowed to dry at room temperature. Further the dried leaves was broken into small pieces and grounded into fine powder using grinder/mixer. Powdered material was extracted with luke warm distill water in temperature controlled orbital shaker for 12-14 hr. The extractant was filtered using muslin cloth and further filtered in whatman No 1 paper, the extract obtained was concentrated using lyophilizer and stored at 4^0C till use.

2.2 Inhibition of Lipid Peroxidation

Microsomes were isolated by the method described by Kamat and Rubin [16.] To 100 µl of liver microsomal suspension, 1 mmol/l each of FeSo4 and ascorbic acid were added, with or without extracts in a total volume of 1 ml in 0.1 mol/l phosphate buffer (pH 7.4) and incubated at 37 °C for 1 h. After incubation, the reaction mixture was added with 2 ml each of 20% TCA and 1% TBA, followed by heating in a water bath for 10 min, cooled and centrifuged. Malondialdehyde (MDA), which is the byproduct of the reaction mixture, was measured at 535 nm.

2.3 DPPH radical scavenging assay

The DPPH assay was carried out as described by Guohua et al [17] with some modifications. Different concentrations of both aqueous and ethanolic extracts were mixed with of 1 ml DPPH solution (0.1 mmol/l, in 95% ethanol (v/v)), and the reaction mixture incubated for 30 min at room temperature. The optical density was measured spectrophotometrically at 517 nm against a blank. BHA was used as a positive control. Decrease in the absorbance of DPPH indicates a higher radical scavenging activity.

2.4 Superoxide radical scavenging assay

Superoxide anion was generated by the reaction of NADH and phenazine methosulphate (PMS)

Coupled with a reduction of Nitro Blue Tetrazolium chloride (NBT) [18]. The reaction mixture contained NBT (100 lM), NADH (300 lM) with or without extract in a total volume of 1 ml Tris buffer (0.02 M, pH 8.3). The reaction was measured spectrophotometrically at 560 nm every 30 sec for 1 min by adding PMS to the mixture

2.5 Reducing power

The reducing power of the extracts was measured according to the method described by Oyaizu [19]. 1 ml of reaction mixture containing extracts in phosphate buffer 0.2 mol/l, pH 6.6 was incubated with 3 ml of 1% potassium ferricyanide at 50 °C for 20 min. After incubation, the reaction was stopped by adding 1 ml of 10% TCA solution and the mixture was centrifuged at 3,000 rpm for 10 min. The supernatant was mixed with distilled water (2.5 ml) and ferric chloride solution (0.1 g/ 100 ml), and the absorbance was measured at 700 nm in a spectrophotometer. Higher absorbance indicated increased reducing power.

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2.6 Cytoprotection

Cell viability

CCl_4 was used as toxicant, to check the cytoprotection of the extract. The concentration of CCl_4 was used at 50 percent lethality (LC 50). Cytoprotection investigations were performed by incubating 1.0 ml of yeast cells (10 X 10^6) suspended in YEPD with CCl_4, (dissolved in DMSO) at LC50 concentration 1mM with/without the extract for 1 hr in a shaking water shower at 37^0C. After the incubation period, an aliquot of cells was taken for viability test by the trypan blue exclusion method [20].

Lactate dehydrogenase leakage

The supernatant obtained after incubation followed by centrifugation from the reaction mixture of yeast cells in the presence of xenobiotics with/without extract was assayed for LDH with sodium lactate as the substrate [21].

Lipid peroxidation of yeast cell

The cells were centrifuged after incubation, with CCl_4 along with the extract and the cell pellet was washed in saline and the pellet was boiled in TCA (5.5%) and TBA (0.34%) for 15 min, cooled and centrifuged. The supernatant was measured in a spectrophotometer at wavelength of 535 nm [22].

Reactive oxygen species (superoxide anion)

The cells (10 X 10^6) suspended in 1.0 ml YEPD were incubated with NBT (0.2 mM) with or without xenobiotics (in DMSO) and extracts in a shaking water bath at 37^0C. The generation of ROS by cells (respiratory burst) was measured by the formation of coloured formazan due to reduction of NBT [23].

2.7 Statistical analysis: Data are expressed as mean ± S.E. of three separate experiments

3. RESULTS AND DISCUSSION

3.1 DPPH Radical Scavenging Activity

DPPH is a stable free radical which has the tendency to accept electrons to become stable molecules. Basically, it is used to screen the antioxidant activity of various samples. DPPH radical can be measured at absorbance maxima at 517 nm. Decrease in the absorbance indicates the acceptance of electrons which is induced by the antioxidants. The results are shown in Table. 1 with IC_{50} of 14µg/ml. The antioxidant activity of the extracts is credited to their hydrogen donating ability [24]. Results suggest extracts have strong potential in scavenging the free radical, which could be attributable to its hydrogen donating ability.

3.2 Superoxide Radical Scavenging

Inhibitory effects of aqueous extract of Celery on superoxide radicals are shown in Table.1. Scavenging of superoxide radicals with IC_{50} of 60 µg/ml was observed in aqueous extract. Superoxide radicals are generated during the normal physiological process, mainly in mitochondria. It is well-known that superoxide anion is a weak oxidant and further undergoes oxidation to give hydroxyl radical and singlet oxygen, which are harmful, leading to oxidative stress [25]. Therefore, superoxide radical scavenging by antioxidants has physiological implications.

3.3 Lipid Peroxidation

Inhibition of lipid peroxidation was observed in extracts of Celery with an IC_{50} of 26 µg/ml (Table.1). Oxidation of polyunsaturated fatty acids in the cell membrane produces Malondialdehyde (MDA), which is the index of lipid peroxidation and marker of cell injury. Cell damage can occur in any internal organ by free radicals, leading to various disorders, viz., atherosclerosis, hepato and nephro damage [26-27]. Our results showed inhibition of lipid peroxidation with increase in concentration of the extracts, indicating aqueous extract has certain antioxidant molecules which are able to repair the damage caused by the free radicals. The mechanism in inhibiting the lipid peroxidation by the extract could be by preventing the chain initiation of polyunsaturated fatty acid chain by donating the hydrogen atom to the damaged lipid bilayer.

3.4 Reducing Power

The reducing power of the extract values are shown in Table.1. The extracts showed increasing activity with increase in the concentration of extracts. Reducing capacity of the extracts could be considered an indicator towards its potential antioxidant properties. Total potential of the antioxidant activity has been attributed to various mechanisms viz., prevention of chain initiation, binding of transition metal ion, inhibition of hydrogen abstraction, radical scavenging and preventing lipid bilayer damage [28,29] has shown that reducing properties are associated with the presence of reductones. Reductones play a major role in exerting antioxidant action by donating hydrogen atom and preventing the free radical chain damage. Results suggest that the Celery extract have potential to scavenge free radical damage by donating hydrogen atom thereby preventing oxidative stress.

3.5 Phenolic Content

Phenolic content in the aqueous extract of Celery was (61.2±1.4 mg gallic acid equivalent per gram, respectively). Phenols play a major role in radical scavenging because of their hydroxyl groups. Total phenolic content present in the extract is directly related to antioxidant activity [29]. In our study, there is a correlation between antioxidant activity and phenol content. The various antioxidant activities of aqueous extracts of Celery demonstrated in this study clearly indicates the potential application value of Celery.

3.6 Cytoprotection of Celery on yeast cells against CCl_4, ***induced damage***

In vitro cell culture play a model role in evaluating the phytochemical in ameliorating the level of oxidative stress induced by xenobiotic in cells, which is measured by cell viability. Earlier studies on photochemical have shown cytoprotection in both *in vitro* and *in vivo* models [20]. In this test we have demonstrated the celery extract in protecting xenobiotic induced cell death in yeast cells. CCl_4, inducer of oxidative stress in cells is utilized as toxicant. Our outcomes

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indicated restraint of xenobiotic induced lipid peroxidation, inhibition of ROS by preventing cell death and reduction in level of LDL leakage with increase in concentration of extract. Cytoprotection observed as cell viability, was observed for cells co treatment with 50–200µg/ml of extract and 1mM CCl_4, Fig.1. LDH leakage in the cells was altogether decreased when cells co treated with increasing concentration of extract, compared with CCl_4, treated Fig.2. Lipid peroxidation was depleted in the cells treated with high convergence of extracts, in which the development of Malondialdehyde was measured as marker record of lipid bilayer damage Fig 3. Reactive oxygen species (ROS), level increases when cells exposed to stress condition. The level of ROS was reduced when cells co treated with the extracts Fig 4. These outcomes demonstrate that the unrefined extract might contain cocktail of phytochemicals, which improve the level of oxidative stress instigated by the CCl_4, by protecting the cell from undergoing death. Further work needs to investigate the photochemical responsible from protecting cell death.

Table 1: Antioxidant activity of aqueous extract of *Apium graveolens*

Aqueous extract	Free radical Scavenging activity IC50 µg/ml				Phenol mg/g
	DPPH	Metal chelating	ROS	LPO	
	14	20	60	26	61.2±1.4

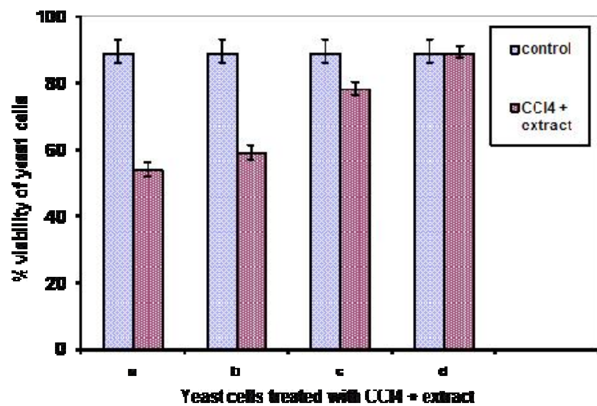

Fig 1: Cell viability of yeast cell co treated with different concentration of extracts (a-50µg, b-100µg, c-150µg, d-200µg) and CCl_4 (1mM)

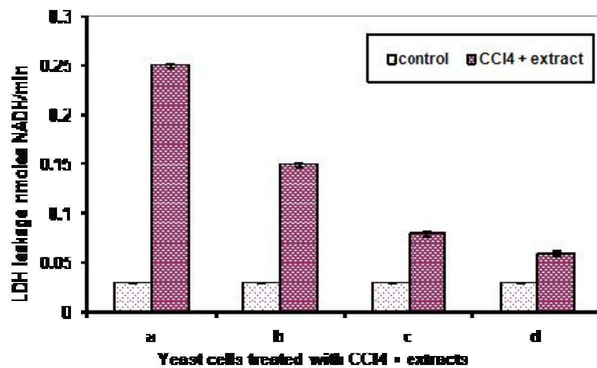

Fig 2: LDH leakage of yeast cell co treated with different concentration of extracts (a-50µg, b-100µg, c-150µg, d-200µg) and CCl_4 (1mM)

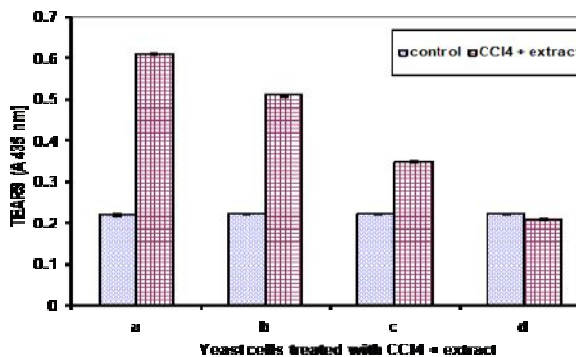

Fig 3: Lipid peroxidation of yeast cell co treated with different concentration of extracts (a-50µg, b-100µg, c-150µg, d-200µg) and CCl_4 (1mM)

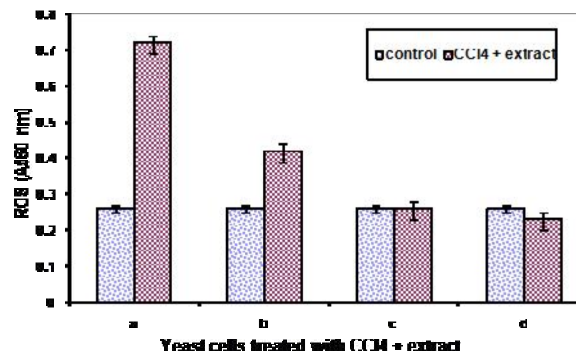

Fig 4: ROS of yeast cell cotreated with different concentration of extracts (a-50µg, b-100µg, c-150µg, d-200µg) and CCl_4 (1mM)

4. CONCLUSION

The results obtained in the present study clearly suggest that the aqueous extract of Celery may contain numerous antioxidant compounds which can effectively scavenge various ROS/free radicals under *in vitro* conditions. Further the extracts have show the cytoprotective action on xenobiotic induced toxicity in yeast cells, caused by the toxicant and preventing the cell death The broad range of activity of the extract suggests that celery is a source of natural antioxidants which could be considered as nutraceutical with health promoting properties in the prevention and amelioration of degenerative diseases. Although we have not isolated and characterized the antioxidant molecules responsible for the antioxidant activity and cytoprotection, we speculate that it could be related to the phenolic and nonphenolic compounds present in the extract. Therefore, further work will be carried out to isolate and identify the effective cytoprotective molecules.

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