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IN VITRO EVALUATION OF ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITY OF CELERY

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ABSTRACT

Celery (*Apium graveolens*) is an indigenous plant belongs to family Apiaceae. The present study was designed to evaluate the *in vitro* antioxidant and anti-inflammatory activity of aqueous leaf extract of *Apium graveolens* (ALAG). The scavenging activity of ALAG was screened for superoxide, hydroxyl, hydrogen peroxide, nitric oxide and DPPH radicals using ascorbic acid as standard. The anti-inflammatory activity was evaluated by using HRBC membrane stabilization assay and albumin denaturation assay using diclofenac as standard. The ALAG showed concentration dependent activity in superoxide, hydroxyl, hydrogen peroxide, nitric oxide and DPPH radical scavenging activities. The ALAG showed anti-inflammatory activity by stabilizing erythrocyte membrane and significant effect on denaturation of protein at a concentration range of 100-500µg/ml. The antioxidant and anti-inflammatory activity of *Apium graveolens* might be due to the flavanoids, tannins and phenols.

KEYWORDS: *Apium graveolens*, Antioxidant, Anti-inflammatory activity.

INTRODUCTION

Oxidative stress is known to be associated with tissue inflammation, damaged cell membranes, autoimmunity, and cell death due to premature aging of cells. Free radicals are generated as a result of both exogenous and endogenous oxidative stress. There are also various free radical dependent systems involved in the formation of cytokines, inflammatory mediators, and cellular functions (Vajdovich P., 2008). Oxidants are generated as a result of normal intracellular metabolism in mitochondria and peroxisomes, as well as from a variety of cytosolic enzyme systems. The inflammatory process is complex and the role of free radicals and antioxidants can be found in hemodynamic and permeability changes and the function of the cells. Antioxidant enzyme SOD, CAT and GPX prevents inflammation and chemotaxis of neutrophils (McCord JM., 1980). Several antioxidants have anti-inflammatory properties and antiinflammatory agents are having antioxidant property. Aspirin and salicylic acid act as both antioxidant and anti-inflammatory agents (Taubert D., 2004).

Now a day researchers looking on plant based medicine for preventive and curative treatment. Herbal medicines are used in the treatment of many inflammation related ailments such as pain, edema, rheumatism, muscle swelling, cut wound and bone fracture (Namsa ND., 2009). Celery (Umbelliferae), reported to have antidiabetic, antifungal, antihypertensive, antibacterial, anticoagulant, anticancer and antiulcer activities. The present study was designed to evaluate the antioxidant and antiinflammatory effect of aqueous leaf extract of *Apium graveolens* (ALAG) in *in vitro*.

MATERIALS

Plant Material

The Celery (*Apium graveolens*) was obtained from local market. The leaves were manually separated and shade dried. The leaves were powdered in a grinder to get 40-mesh size powder. The moisture content of the dried powder was found to be 13.5%. The extract was prepared with methanol using Soxhlet apparatus. The extract was suspended in 2% gum acacia and used in the experimental studies.

Methods

Superoxide radical scavenging activity

The riboflavin-light- NBT system contained 58 mM phosphate buffer, pH 7.6, 20µM riboflavin, 6mM EDTA, and 50µM NBT, final volume made up to 3 ml, added in that sequence. Reaction was started by illuminating 40 volts. Immediately after illumination, the absorbance was measured at 560 nm. The potency of ALAG measured in terms of NBT photo reduction capacity (Beauchamp C., 1971).

Hydroxyl radical scavenging activity

The Fenton reaction ($Fe3^{+}$-ascorbate-EDTA-H_2O_2 system) reaction mixture contained 2-deoxy-2-ribose (2.8 mM), phosphate buffer (0.1 mM, pH 7.4), ferric chloride (20 µM), EDTA (100 µM), hydrogen peroxide (500 µM), ascorbic acid (100 µM) and various concentrations of the test sample in a final volume of 1 ml. The mixture was incubated for 1 h at 37 °C. After the incubation an aliquot of the reaction mixture (0.8 ml) was added to 2.8% TCA solution (1.5 ml), followed by TBA solution (1% in 50 mM sodium hydroxide, 1 ml) and sodium dodecyl sulphate (0.2ml). The mixture was then heated (20 min at 90 °C) to develop the colour. After cooling, the absorbance was measured at 532 nm against an appropriate blank solution. The percentage of inhibition of ALAG was expressed deoxyribose degradation capacity (Halliwell, B., 1989).

Hydrogen peroxide radical scavenging activity

The hydrogen peroxide scavenging assay was carried out following the procedure of. The principle of this method is that there is a decrease in absorbance of H_2O_2 upon oxidation of H_2O_2. A solution of 43mM H_2O_2 was prepared in 0.1Mphosphate buffer (pH 7.4). ALAG at concentration in 3.4mL phosphate buffer was added to 0.6mL of H_2O_2 solution (43mM) and absorbance of the reaction mixture was recorded at 230 nm. A blank solution contained the sodium phosphate buffer without H_2O_2.(Ruch RJ., 1989)

DPPH radical scavenging activity

The potential AA and ALAG was determined on the basis of the scavenging activity of the stable 1,1 -diphenyl-2-picrylhydrazyl (DPPH) free radical. Aliquots of 1ml of a methanolic solution containing each pure compound were added to 3ml of 0.004% MeOH solution of DPPH. Absorbance at 517 nm, against a blank of methanol without DPPH, was determined after 30 min (UV, Perkin-Elmer-Lambda 11 spectrophotometer) and the percent inhibition activity was calculated. IC50 values denote the concentration of sample required to scavenge 50% DPPH free radicals. All tests were run in triplicate and averaged (Braca A., 2001).

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 Griess reaction. The reaction mixture (3 ml) containing sodium nitroprusside (10 mM) in phosphate buffered saline (PBS) and ALAG in different concentrations were incubated at 25°C for 150 min. After incubation 1.5ml of the Griess reagent (1% sulphanilamide, 0.1% naphthyl ethylene diamine dihydrochloride in 2% H3PO4) was added. The absorbance of the chromophore formed was measured at 546nm. The percentage inhibition of nitric oxide generated was measured by comparing the absorbance values of control and test samples (Ebrahimzadeh MA., 2001).

HRBC membrane stabilization assay

The HRBC membrane stabilization has been used as method to study the anti-inflammatory activity. Blood was collected from healthy volunteer and was mixed with equal volume of sterilized Alsever solution (2%dextrose,0.8% sodium citrate,0.5% citric acid and 0.42% sodium chloride in water). The mixer was centrifuged at 3000 rpm and packed cell were washed with isosaline (0.85%.pH 7.2) and a 10 %(v/v) suspension was made with isosaline. The assay mixture contained the drug, 1 ml of phosphate buffer (0.15M, pH 7.4), 2 ml of hyposaline (0.36%) and 0.5ml of HRBC suspension. Diclofenac was used as reference drug. All the assay mixture were incubated at 370C for 30 min and centrifuged. The hemoglobin content in the supernatant solution was estimated using spectrophotometer at 560 nm (Gandhisan R., 1991)

Albumin denaturation inhibition assay

Different concentrations of ALAG (1mL) was mixed with 1 mL of 1 mM albumin solution in phosphate buffer and incubated at 27° ± 1° C in BOD incubator for 15 min. Denaturation was induced by keeping the reaction mixture at 60° ± 10° C in water bath for 10 min. After cooling, the turbidity was measured at 660 nm (UV-Visible Spectrophotometer SL-159, Elico India Ltd.). The diclofenac sodium was used as standard drug. Percentage of inhibition of denaturation was calculated from control where no drug was added. Each experiment was done in triplicate and average was taken (Perumal R., 2008).

RESULTS AND DISCUSSION

Superoxide is the first, a highly toxic radical is superoxide produced from the reduction product of molecular oxygen several enzymatic and non-enzymatic pathways in all aerobic cells. It acts by scavenging the biological molecules and leads to molecular alterations including DNA (Waris & Alam, 2004). Photochemical reduction of flavins generates superoxide radical, which reduces NBT, resulting in the formation of blue formazan (Beauchamp & Fridovich, 1971). The ALAG showed notable scavenging activity against superoxide radical (figure 1).

Hydrogen peroxide is a weak oxidizing agent usually by oxidation of essential thiol (-SH) groups and inactivates a few enzymes directly. It can able to cross cell membranes easily and react with Fe2+ and possibly Cu2+ ions to form hydroxyl radicals (Miller MJ., 1993). The ALAG had both hydroxyl and hydrogen peroxide radical scavenging activity compare with standard ascorbic acid in a concentration dependent manner (figure 2 and 3).

The nitric oxide is produced through aerobic respirations and increases the risk of chronic diseases. The antioxidants may help block the chain of reactions initiated by excess generation of NO that are detrimental to human health (Moncada, A., 1991). The ALAG

showed significant antioxidant activity by scavenging the nitric oxide radical in concentration dependent manner (figure 4). The reduction of methanolic DPPH solution in the presence of hydrogen donating constituent leads to the formation of the non-radical form DPPH-H (Oyaizu, 1986). The ALAG was able to reduce the stable radical DPPH to the yellow-coloured diphenylpicrylhydrazine (figure 5).

Different haemolytic stimuli like heat, osmotic shock and free radicals may affect the erythrocytes membrane [Takebayashi J., 2007]. Membrane stabilization assay is a method to assess the integrity of erythrocyte membrane and lysosomal membrane [Mizushima Y., 1970]. In the present study the ALAG and standard diclofenac was tested for stabilizing effect on certain proteins in the erythrocyte membrane. The ALAG showed significant membrane stabilizing effect on erythrocytes compare with diclofenac (figure 6). The denaturation of proteins is one of the causes of inflammation. The reason might be due to the production of auto antigens involved in denaturation of protein and membrane lysis action. The ALAG showed notable denaturation capability along with standard diclofenac (figure 7).

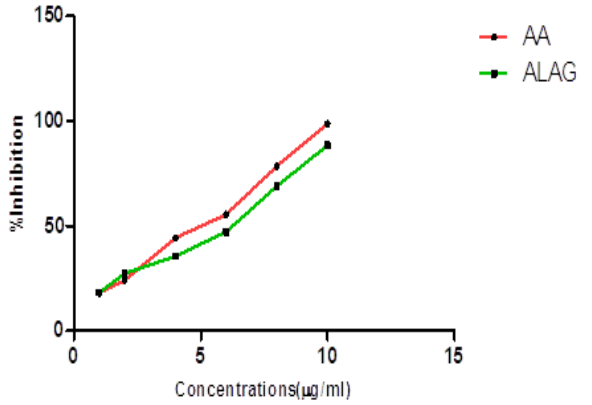

Figure 1: Effect of ALAG on superoxide radical scavenging activity.

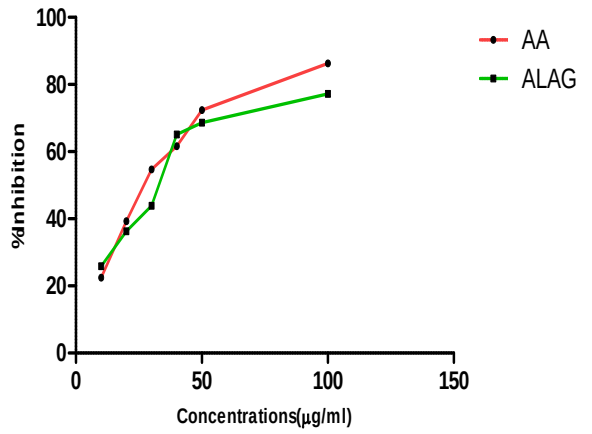

Figure 2: Effect of ALAG on hydroxyl radical scavenging activity.

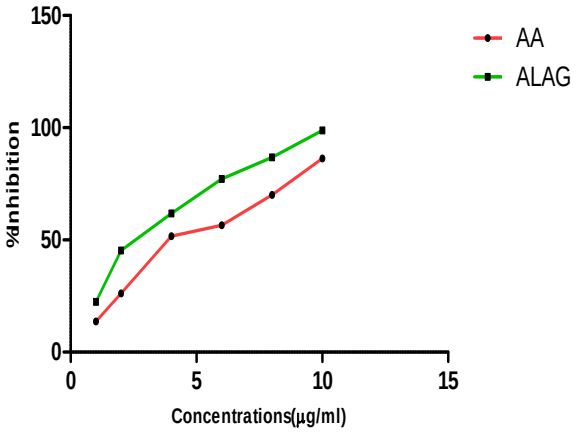

Figure 3: Effect of ALAG on hydrogen peroxide radical scavenging activity.

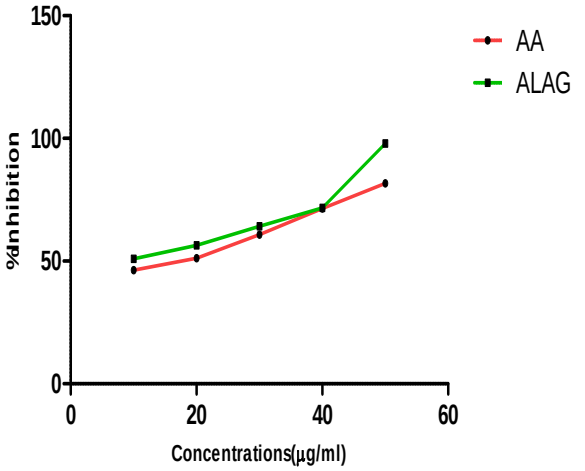

Figure 4: Effect of ALAG on nitric oxide radical scavenging activity.

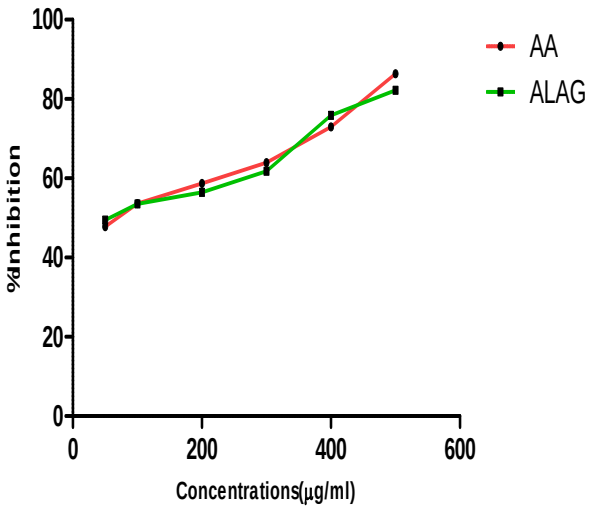

Figure 5: Effect of ALAG on DPPH radical scavenging activity.

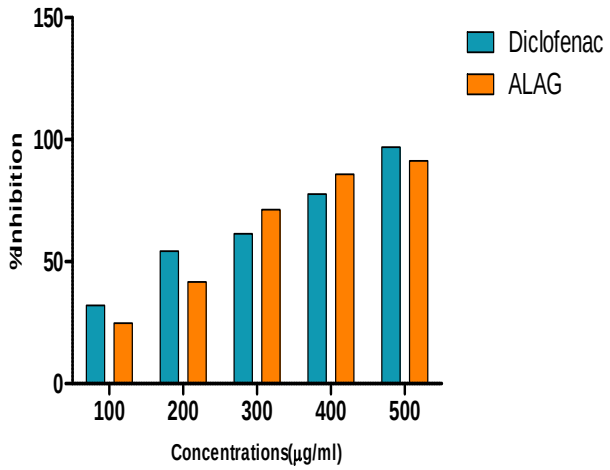

Figure 6: Effect of ALAG on HRBC membrane stabilization assay.

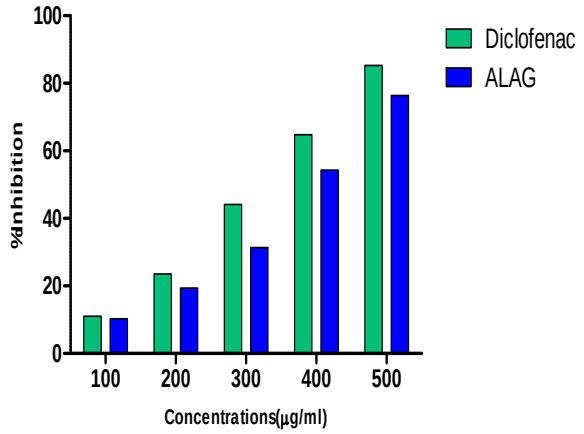

Figure 7: Effect of ALAG on albumin denaturation assay.

CONCLUSIONS

The antioxidant activity of ALAG might be due the presence of phenols and phenolics are act as antioxidants by reducing agents, hydrogen donators, singlet oxygen quenchers and metal chelater (Rice-Evans., 1995). The *in vitro* antioxidant and anti-inflammatory activities of *Apium graveolens* might be due to the presence of flavanoids and polyphenols present in the leaf.

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