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ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY OF SEED METHANOLIC EXTRACT OF APIUM GRAVEOLENS IN VITRO

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ABSTRACT

Natural metabolites especially those extracted from plants are useful for curing human cancer through their cytotoxicity and antioxidant activities as well as for the control of bacterial infection. Celery (Apium graveolens) is a medicinal herb used as a food, and also in traditional medicine. The extract of Apium gravelens seeds was prepared using methanol. The antioxidant activity of Apium gravelens seeds alcoholic extract (0.5, 1, 1.5, 2 and 2.5 mg/ml) were tested using DPPH method. The antimicrobial activity was assessed using agar diffusion method against Staphylococcus mutans, Streptococcus pyogenes, Klebsiella pneumonia, Pseudomonas aeruginosa, and Escherichia coli. Results indicated that the extract showed a scavenging activity of the DPPH in which the IC_{50} value for alcoholic extract was 2.31 mg/ml which is comparatively higher than the IC_{50} (1.10 mg/ml) of ascorbic acid. The extract exhibited antimicrobial activity on almost microorganisms tested, in which an inhibition zone recorded by using 2.5 mg/ml on Pseudomonas aeruginosa and Streptococcus pyogenes, however no inhibition zone showed using the same concentration on Klebsiella pneumonia, Staphylococcus mutans and Escherichia coli. Increasing the concentration of the extract, resulted in increasing the inhibitory activity against the sensitive microbial isolates and started to affect Klebsiella pneumonia and Staphylococcus mutans, though still no inhibitory activity against E. coli at 5 mg/ml. The antimicrobial effect reached its higher activity by using 10 mg/ml from the alcoholic extract, in which all the microbial isolates were affected.

KEYWORD: Klebsiella pneumonia, Staphylococcus mutans and Escherichia coli.

INTRODUCTION

The biological activities of different plant spices and aromatic herbs have been tested in many studies; including their role as natural food preservatives, anti-oxidants, and anti-tumor as well as anti-microbial agents. Celery (Apium graveolens) is a medicinal herb used as a food, and also in traditional medicine. Studies have documented that A. graveolens possess broad range of biological activities including antioxidant, antimicrobial, anticancer, hypoglyceamic, anti-inflammatory and analgesic effects (Asif et al., 2011).

It contains aromatic substances in the roots, stem and leaves. Biological properties of different anatomical parts of Celery and its extracts were reported in various literature sources (Bedin et al., 1999). Celery showed significant antimicrobial activity against a wide variety of both Gram positive and Gram negative bacteria as well as some yeast species (Krishna and Banerjee, 1999).

The healing properties of Celery are due to the essential oil and flavonoids, mostly apiin and apigenin. Flavonoids and essential oils have attracted the interest of researchers because they show promises of being powerful anti-oxidants that can protect the body from free radicals and against oxidative stress (Bors et al., 1996). Flavonoids and essential oils cannot be produced by the human body and are taken in through the daily diet. The evidence reported that flavonoids play a vital biological role, including the function of scavenging reactive oxygen species (Pietta and Simonetti, 1998). On the other hand, it has been shown that phenolic compounds from edible fruits and vegetables are also effective antioxidants. The anti-oxidative properties of phenolics arose from their high reactivity as hydrogen or electron donors and from the ability of polyphenol-derived radicals to stabilize and delocalize the unpaired electron or from their ability to chelate transition metal ions (Rice-Evans et al., 1997). Hence, there is a strong interest to search for potential antioxidant agents derived from natural products.

Celery has been found to have significant antioxidant activity. Essential oils from the leaves of celery were found to have significant toxic effects against the larvae of A. aegypti and also showed potential antioxidant activity. The ethanol extract of celery significantly protected the gastric mucosa and suppressed the basal gastric secretion in rats, possibly through its antioxidant potential (Yao et al., 2010).

The compound dl-3-n butylphthalide (NBP) extracted from the seeds of Celery was found to reduce the cytotoxicity of MPP (+) by suppressing the mitochondrial permeability transition, reducing oxidative stress, and increasing the cellular GSH content (Huang et al., 2010). Luteolin found in celery has been shown to possess pharmacological activities, including antioxidant, anti-inflammatory, and anticancer activities. Luteolin inhibits angiogenesis, induces apoptosis, prevents carcinogenesis in animal models, reduces tumor growth in vivo, and sensitizes tumor cells to the cytotoxic effects of some anticancer drugs, suggesting it has cancer chemopreventive and chemotherapeutic potential (Lopez-Lazaro 2009).

The extracts of Celery seed and root were good scavengers of OH and DPPH radicals and reduced liposomal peroxidation intensity in liposomes. The phenyl-sulfotransferase-P activity was significantly reduced by celery and was ascribed to the phenolic acids present. The essential oil of Celery seed was shown to have good antiradical activity and thus could be used as natural antioxidants in food applications (Kiralan et al. 2012). This study was aimed to determine the antioxidant and antimicrobial activities alcoholic extract of Celery seed.

MATERIALS AND METHODS

Microorganisms

Two groups of microorganisms were used throughout this study, Gram positive microorganisms including: Staphylococcus mutans and Streptococcus pyogenes. The second group was Gram negative bacteria including: Klebsiella pneumonia, Pseudomonas aeruginosa and Escherichia coli. The above microorganisms were obtained from Biotechnology Department/ Al-Nahrain University.

Plant Seeds

The seeds of Celery A. graveolens were purchased from the local markets, and were kept in dry place until use.

Culture Media

Nutrient broth (Oxiod-England) and Mueller Hinton agar (Himedia-India) were prepared as recommended by the manufacturing company. Both media were sterilized by autoclaving at 121°C for 15 min.

Methods

Measurement of Bacterial Inoculum

Cells of bacterial isolates were grown in nutrient broth until mid-exponential phase (OD_{600nm} = 0.5- 0.6) before used in experiments. The optical densities of liquid cultures were measured using spectrophotometer at 600nm.

Alcoholic Extraction of Apium graveolens Seeds.

Methanol Extraction of A. graveolens seeds was done according to Anesini and Perez (1993). The seeds were grinded to obtain fine powder and extracted with methanol (BDH-England) (95%) by mixing 50 g of Celery seeds with 500ml of the solvent. The mixture was left for 48 hours with stirring using magnetic stirrer at room temperature. Then the mixture was filtrated using filter paper Whatman No. 1 and centrifuged at 3,000 rpm for 15 min. The pellet was discarded and the supernatant (solvent) was dried and concentrated using vacuum rotary evaporator at 40 °C.

The evaporation was done until a solid state of extracted liquid was obtained. Extract from this method was then weighed and stored in closed container at 4 °C until further use.

Different concentrations (1.25, 2.5, 5 and 10 mg/ml) were prepared from these extracts by dissolving the dry extract in methanol-DMSO (Merck-Germany) solution (9:1).

Antimicrobial Assay

The antibacterial activity of the extracts was determined using the agar disk diffusion method (Davis and Stout, 1971), using Muller Hinton agar. The prepared culture media were inoculated with strain of bacteria. Filter paper disks were immersed in different A. graveolens extract concentrations (2.5, 5 and 10 mg/ml) were embedded onto the surface of Muller Hinton agar.

The plates were then maintained at room temperature for 1h allowing for diffusion of the solution. All plates were then incubated at 37 °C for 24 hours and the zones of inhibition were calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter. The readings were taken in three different fixed directions and the average values were tabulated. Plates containing antibiotic Gentamicin Disks (Sigma-USA) were used as positive control. Statistical Analysis Mean ±SE was used to describe variables.

DPPH Radical Scavenging Activity

The antioxidant activity of plant seed methanolic extract and standard (vitamin C) were assessed on the basis of the radical scavenging effect of the stable DPPH free radical, and the method of Sanja et al. (2009) was followed. An aliquot of 0.1 ml of the seed extract or standard (0.5, 1, 1.5, 2 and 2.5 mg/ml) was added to 3.9 ml of DPPH solution in a test tube. After incubation at 37°C for 30 minutes, the absorbance of each solution was determined at 517nm using spectrophotometer. All measurements were made in triplicates. The ability to scavenge DPPH radical was calculated by the following equation:

$$\text{DPPH radical scavenging activity } (\%) = \left(1 - \frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}}\right) \times 100$$

RESULTS AND DISCUSSION

The Anti-oxidant Activity of Apium graveolens Alcoholic Extracts

The antioxidant activity of A. graveolens alcoholic extract was evaluated by using DPPH method. The samples were able to reduce the stable violet DPPH radical to the yellow DPPH-H. Apium graveolens alcoholic extract **IC_{50} mg/ml** 2.31 ± 0.63 Vitamin C (Ascorbic acid) 1.10 ± 0.21

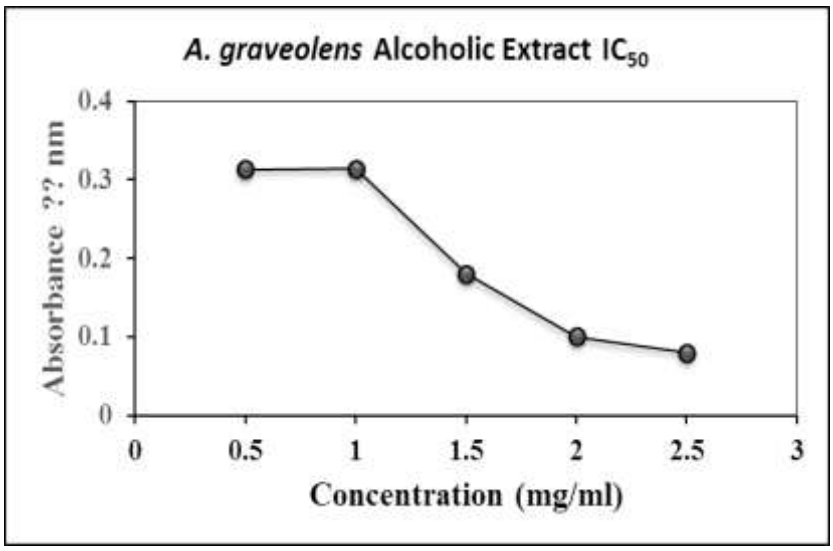

Figure (1): IC_{50} for Apium graveolens alcoholic extract

The results illustrated in Figure (1) showed that the higher concentrations of crude extracts from the seeds of A. graveolens showed the better antioxidant activity in which concentrations (0.5, 1, 1.5, 2 and 2.5 mg/ml) were evaluated for their free radical scavenging activity with ascorbic acid as standard compound.

The IC_{50} was calculated for the extract as well as ascorbic acid as standard and summarized in Table (1). The scavenging effect increased with the increasing concentrations of test samples. The IC_{50} value for alcoholic extract was 2.31 mg/ml which is comparatively higher than the IC_{50} (1.10 mg/ml) of ascorbic acid. From the results of DPPH scavenging, it showed that the alcoholic extract of A. graveolens is effective as antioxidant compared to ascorbic acid. DPPH is relatively stable nitrogen centered free radical that easily accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Jain et al., 2012).

Different methodologies used to determine the antioxidant activities of plant extracts have led to diverse results, difficult to compare and often conflicting. This method was chosen because of its simplicity, rapidity, sensitivity and reproducibility (Prieto et al., 1999). They are also very convenient for screening large numbers of samples with different polarity.

Therapeutic activities of celery depend mainly on the presence of lutein and the flavones, luteolin and apigenin. It was found that luteolin possesses a variety of pharmacological activities, including antioxidant and antiinflammatory activities. Different Celery leaf extracts are scavengers of OH and DPHH radicals and reduce liposomal peroxidation, which points to their antioxidant activity. The antioxidant activity of Celery leaf and seed extracts may be due to the presence of flavonoids (Lopez-Lazaro, 2009).

Enhancement of plasma and hepatic antioxidant status (glutathione GSH, pyrogallol peroxidase PPx, glutathione-S-transferase GST,glutathione reductase GR,SOD, CAT, Vitamin C, Vitamin A and ß-carotene) in rats with 1,2-dimethylhydrazine induced colon cancer upon intragastric administration of 0.2 mg/kg luteolin (which is a flavone contained in celery) (Kolarovic et al., 2010).

Moreover, reports showed that co-administration of methanolic extract of A. graveolens seeds along with Di-(2- ethylhexyl) phthalate (DEHP) induced hepatotoxicity in rats, significantly prevented the rise in TBARS level with a concomitant elevation in the concentration of hepatic glutathione and ascorbic acid suggesting alleviation of oxidative stress and restoration of antioxidant defense system resulting in membrane stabilization (Osman, 2013).

The Anti-microbial Activity of Apium graveolens Extracts

The effect of different concentrations of A. graveolens alcoholic extracts were examined against two groups of microorganisms, Gram positive microorganisms including: Staphylococcus mutans and Streptococcus pyogenes and Gram negative bacteria including: Klebsiella pneumonia, Pseudomonas aeruginosa and Escherichia coli.

The results illustrated in Table (1) showed that the higher concentrations of crude extracts from the seeds of Apium graveolens had antibacterial activity against most of bacterial isolates.

Table 1: The antibacterial activity of alcoholic extracts of A. gravelens against the growth of S. mutans, S. pyogenes, K. pneumonia, P. aeruginosa and E. coli.

Bacterial Isolate	Extract concentration (2.5 mg/ml)	Extract concentration (5 mg/ml)	Extract concentration (10 mg/ml)	Gentamicin
S. mutans	NIZ*[1]	13.83 ± 1.59	17.01 ± 1.49	18.05 ± 0.27
S. pyogenes	15.2 ± 0.12*[2]	21.06 ± 1.03	22.37 ± 1.86	17.39 ± 0.58
K. pneumonia	NIZ	14.84 ± 2.91	15.05 ±2.10	12.08 ± 0.16
P. aeroginosa	15.32 ± 0.46	16.62 ± 0.95	19.59 ± 1.37	14.43 ± 0.60
E. coli	NIZ	NIZ	21.36 ± 1.93	23.88 ± 0.11

*[1]NIZ: No Inhibition Zone.

*[2]Mean inhibition zone (mm) ± Standard deviation.

In vitro results indicated that bacterial strains showed a degree of sensitivity towards higher concentrations of alcoholic A. graveolens seeds extracts. The extract was highly effective against S. pyogenes and P. aeroginosa when inhibition zones reached to 15.2 ± 0.12, 21.06 ± 1.03 and 22.37 ± 1.86 mm inhibitory against S. pyogenes and 15.32 ± 0.46, 16.62 ± 0.95and 19.59 ± 1.37 mm inhibitory against P. aeruginosa for the concentrations 2.5, 5 and 10 mg/ml respectively (Figure 2).

Figure (2): The antibacterial activity of alcoholic extracts of A. gravelens against P. aeroginosa (left) and S. pyogenes (right).

On the other hand no inhibitory activity obtained by using 2.5 mg/ml from the extract against S. mutans, K. pneumonia and E. coli. At concentration 5 mg/ml a mild inhibitory activity 13.83 ± 1.59 and 14.84 ± 2.91 mm for S. mutans, and K. pneumonia, respectively was recorded as compared with that obtained against S. pyogenes and P. aeruginosa, while no inhibitory against E. coli was showed. Growth sensitivity for S. mutans, K. pneumonia and E. coli (18.05 ± 0.27, 12.08 ± 0.16 and 23.88 ± 0.11 mm, respectively) using 10 mg/ml concentration was clearly obtained (Figure 3).

Figure (3): The antibacterial activity of alcoholic extracts of A. gravelens against K. pneumonia (left), E. coli (right) and S. mutans (down).

Apium graveolens is known as a mild diuretic and urinary antiseptic and has been used in the treatment of urinary calculi, in addition to other biological activities like hypoglycemic, regulates blood pressure, reduces cholesterol and as such is helpful in diabetes. The volatile oil in Apium has been shown to have antifungal activity, and it is active against many bacteria (Sipailiene et al. 2005).The main constituents in the oil of A. gravelens were limonene, carvone and 3n-butylphthalide, phthalides, beta selinene, giaveobisides and fatty oil (Amin and Saleem, 2007).

The effect of the extract was increased with high concentrations indicating that increasing the active chemical composition of Apium gravelens affecting the growth of the microorganisms. Antimicrobial activity may be due to numerous free hydroxyls, limonene, beta selinene and other compounds that have the capability to combine with the carbohydrates and proteins in the bacterial cell wall. They may get attached to enzyme sites rendering them inactive (Harborne and Baxter, 1995).

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