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RESEARCH ARTICLE

Determination of Free Radical Scavenging Potential in *Cucumis melo* (L). Fruit Extract

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ABSTRACT:
The human body has an elaborate antioxidant defence system. Antioxidants are manufactured within the body and may even be extracted from the food like fruits, vegetables, seeds, nuts, meats and oil. Fresh fruits and vegetables are a rich source of antioxidant vitamins such as A, C and E, that prevent cellular damage associated with many diseases. This present investigation was designed to evaluate the radical scavenging activity of ethanolic extract of *Cucumis melo* fruit. The *in vitro* free radical scavenging effect of the *Cucumis melo* (L) fruit extract was assessed by using 1,1 – diphenyl 2-picryl hydrazyl radical (DPPH), ABTS (2, 2'-Azinobis (3-ethyl Benz Thiazoline-6-Sulfonic acid) hydroxyl radical, reducing power and nitric oxide scavenging assay. The scavenging activity of the fruit extract was tested at different concentrations. *C.melo* fruit extract showed concentration dependant free radical scavenging activity. Ethanol extract of *Cucumis melo* fruit (EECMF) exhibited maximum scavenging activity against DPPH (64.46%), ABTS (70.52%) hydroxyl radical (62.57%), and nitric oxide (61.18%) in comparison with standard antioxidants. The reducing power of ethanol extract of *C.melo* fruit was found to be 0.11 at 0.2mg/ml and increased to 0.49 at 1mg/ml. These results clearly indicate that ethanolic extract of *C.melo* fruit has great potential of free radical scavenging activity and it may be used as a bioactive source of natural antioxidants for pharmacology of functional foods.

KEYWORDS: Antioxidants, *Cucumis melo* (L), Free radicals, DPPH, ABTS, Reducing power, IC 50, EECMF- Ethanolic extract of *Cucumis melo* fruit.

INTRODUCTION:
Plants are a source of phytochemical compounds and secondary metabolites that play a major role in their medicinal properties. In olden civilization, plants were used in the treatment of various diseases. Since, plants are a source of natural remedies, due to the presence of potential of bioactive compounds or extract which provide new and novel products for disease treatment and prevention is still enormous[1,2].

In healthy individuals, there is equilibrium between the natural antioxidative defense system and the reactive oxygen species (ROS), generated from both living organisms and exogenous sources[3].

When the equilibrium is disrupted, the ROS can induce oxidative damage to various biomolecules, including; protein, lipid, DNA and RNA in the human body associated with lipid and protein peroxidation, cell structural damage, tissue injury or gene mutation. This oxidative damage is considered to play a causative role in aging, also several diseases such as cardiovascular diseases, atherosclerosis, neural disorders, skin irritations, inflammations, hypertension, cognitive dysfunction and cancer[4,5].

Cucumis melo (Musk melon) a short duration vegetable crop belonging to family Cucurbitaceae. Musk melon is a beautiful, juicy, tasty and delicious fruit and popular for its nutritive and medicinal properties. Traditionally, it is used for treatment of Kidney stones, cancer, cardiovascular disorders and stroke. The fruit pulp is used as a lotion for chronic and acute eczema. The fruit are often used as a cooling agent, cleanser or moisturizer for the skin[6]. Hence this study was aimed to evaluate the

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free radical scavenging potential of the fruit extracts of *Cucumis melo*.

MATERIALS AND METHODS:

Plant Material:

Cucumis melo (L.) (Family - Cucurbitaceae) fruits were collected from the local markets of Coimbatore district, Tamilnadu, India. The specimen sample was identified and authenticated by Dr. M. Palanisamy, Scientist-C, Botanical Survey of India, Southern Regional Centre, Coimbatore, Tamilnadu, India. The identification No. BSI/SRC/5/23/2014-15/Tech/482. The pulp of fresh fruits of *C. melo* was chopped into pieces and dried at room temperature for 24 hours. The air-dried pulps were kept at 40℃ in hot air oven for 24 hours to get rid of moisture content. The completely dried fruits were ground into powder by using a mixer grinder and stored. 10g of the edible fruit powder was successively extracted with 100ml of ethanol using Soxhlet apparatus and filtered through Whatmann No 1 paper. The filtrate was concentrated and dried under reduced pressure and controlled temperature. The concentrated extracts of fruit were stored in small vials at 20ºC and used for further analysis.

Determination of Free Radical Scavenging Activity:

The radical scavenging activity such as DPPH, ABTS+ and hydroxyl radical scavenging, reducing power assay, and nitric oxide scavenging assay was determined. All the analyses were done in triplicates and average values were taken.

DPPH Radical Scavenging Activity[7]:

DPPH radical is scavenged by antioxidants through the donation of a proton forming the reduced DPPH. The colour change from purple to yellow after reduction can be quantified by its decrease in absorbance at wavelength 517nm. Various concentrations (200 - 1000 µg/ml) of fruit extract (4.0ml) were mixed with 1.0ml of methanolic solution containing DPPH radicals, resulting in the final concentration of DPPH being 0.2mM. The mixture was shaken vigorously and left for 30 minutes at room temperature and the absorbance was measured at 517nm. Ascorbic acid was used as a control.

The DPPH radical scavenging activity was calculated as follows;

Inhibition = Ao – A1 / Ao x 100,

Where, Ao is absorbance of control reaction, A1 is absorbance of test compound.

$ABTS^+$ Radical Scavenging Activity[8]:

$ABTS^+$ decolourisation assay involves the generation of the $ABTS^+$ chromophore by the oxidation of $ABTS^+$ with potassium per sulphate. It is applicable for both hydrophilic and lipophilic compounds. The scavenging activity of the fruit extracts on $ABTS^+$ radical cation was measured at 734nm. The reaction was initiated by the addition of 1.0ml of diluted ABTS+ to 10µl of different concentrations (100 - 500µg/ml) of fruit extract and also to 10µl of ethanol as control. Ascorbic acid was used as positive control. The absorbance was read at 734nm after 6 minutes and the percentage inhibitions were calculated.

The inhibition was calculated according to the equation,

Inhibition = Ao – A1/Ao x 100,

Where, Ao is absorbance of control reaction, A1 is absorbance of test compound.

Hydroxyl Radical Scavenging Assay[9]:

Hydroxyl radicals were generated from $FeSO_4$ and hydrogen peroxide and detected by their ability to hydroxylate salicylate and the hydroxylated salicylate complex is measured at 562nm. A reaction mixture of 3.0ml volume contained, 1.0ml of 1.5mM FeSO4, 0.7ml of 6 mM hydrogen peroxide, 0.3 ml of 20 mM sodium salicylate and 1.0ml of different concentrations (100 - 500mg/ml) of fruit extract. After incubation for an hour at 37°C, the absorbance of the hydroxylated salicylate complex was measured at 562nm. Vitamin E was used as positive control.

The percentage scavenging effect was calculated as,

Scavenging activity= [1-(A1-A2) /A0] X 100

Where, Ao is absorbance of the control, A1 is absorbance in the presence of the extract, A2 is absorbance without sodium salicylate.

Reducing Power Assay[10]:

The reaction mixture contained 2.5ml of various concentrations (200-1000µg/ml) of fruit extract, 2.5ml of 1% potassium ferricyanide and 2.5ml of 0.2M sodium phosphate buffer. The control contained all the reagents except the sample. The mixture was incubated at 50°C for 20 minutes and was terminated by the addition of 2.5 ml of 10% (w/v) of trichloroacetic acid, followed by centrifugation at 3000rpm for 10 minutes. 2.5ml of an aliquot of supernatant was mixed with 2.5ml of deionized water and 0.5ml of 0.1% ferric chloride. After 10 minutes of incubation, the absorbance was measured at 700nm against blank that contained distilled water and phosphate buffer. BHT (butylated hydroxytoluene) used as control. Increased absorbance indicates increased reducing power of the sample.

Nitric Oxide Scavenging Assay[11]:

The interaction of ethanolic extract of *Cucumis melo* with nitric oxide was assessed by the nitride detection method. Nitric oxide was generated from sodium nitroprusside and measured by Griess illosvoy reaction.

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions which were measured at 540nm. The reaction mixture (6.0ml) containing sodium nitroprusside (4.0ml), phosphate buffer saline (PBS, 1.0 ml) and different concentrations (50 - 250µg/ml) of fruit extract (1.0ml) in DMSO was incubated at 25°C for 15 minutes after incubation, 0.5ml of the reaction mixture containing nitrite was removed, 1.0ml of sulphanilic acid reagent was added, mixed well and allowed to stand for 5 minutes for completion of diazotization and 1.0ml of naphthyl ethylene diamine dihydrochloride was added, mixed well and allowed to stand for 30 minutes in diffused light. A pink coloured chromophore was formed. The absorbance of these solutions was measured at 540 nm against corresponding blank solutions. Rutin was used as a standard.

The inhibition was calculated according to the equation,

Inhibition = Ao – A1/Ao x 100,

Where, Ao is absorbance of control reaction, A1 is absorbance of test compound.

RESULTS AND DISCUSSION:

DPPH Radical Scavenging Activity:

The scavenging effect of the ethanolic extract of *Cucumis melo* fruit on the DPPH radical was increased from 13.14% at 200µg/ml to 64.46% at a concentration of 1000µg/ml. The standard antioxidant ascorbic acid showed the maximum value of 70.79% at a concentration range from 200 to 1000µg/ml, the scavenging effect also increased in a dose dependent manner. The IC50 value of *Cucumis melo* fruit extract was found to be 748.92µg/ml comparable to reference standard 626.94µg/ml and the inhibition value was found to be high for standard ascorbic acid when compared to *Cucumis melo* fruit extract. *Cucumis melo* fruit extract exerted dose dependent DPPH radical quenching ability which is due to the presence of antioxidants in the fruit extract through hydrogen donation[12].

The model of scavenging the stable DPPH radical is a widely employed method to evaluate the scavenging potential of natural antioxidants present in the fruits and herbal leaves. The DPPH assay constitutes a rapid and low-cost method and it needs relatively short time compared with other methods. The effect of antioxidants on DPPH radical was thought to be due to their hydrogen donating ability[13,14]. The DPPH radical can accept an electron or hydrogen radical to become a stable diamagnetic molecule. During this process, the plant extract donates a hydrogen atom to the DPPH free radical, thus reducing DPPH to the stable, non-radical compound 1, 1-diphenyl-2-picril-hydrazine. This transition is characterized by a color change from purple to pale yellow or colorless and the reduction capability of the DPPH radical is determined by its decreased absorbance at 517nm in the presence of natural antioxidants[15].

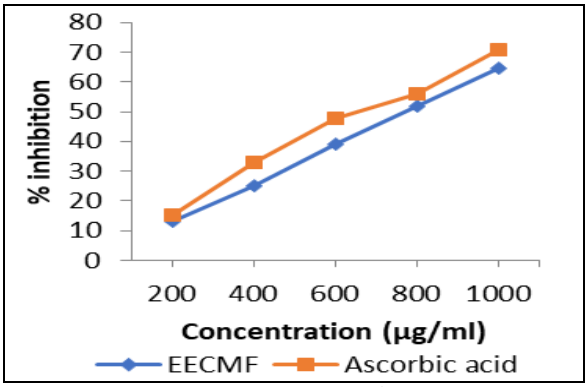

Fig. 1: DPPH radical scavenging activity of the ethanolic extract of *Cucumis melo* fruit

ABTS$^+$ Radical Scavenging Activity:

The ABTS$^+$ radical scavenging property of *Cucumis melo* increased from 21.82% at 100µg/ml concentration to 70.52% at 500µg/ml concentration which was compared to the standard ascorbic acid. The activity was found to be increased in a dose-dependent manner and the extract exhibited an IC50 value of *Cucumis melo* fruit extract was 287.07µg/ml and 247.56µg/ml for the standard antioxidant ascorbic acid. Therefore, the ABTS$^+$ radical scavenging activity of ethanolic extract of *Cucumis melo* fruit indicates its ability to scavenge free radicals, thereby preventing lipid oxidation via a chain-breaking reaction[16].

The reduction of the 2,2'azinobis [3-ethylbenzothiazoline sulphonate] radical cation [ABTS$^+$] has been widely used to measure the antioxidant capacity of natural extracts and is an excellent tool for determining the antioxidant capacity of hydrogen-donating antioxidants[17]. The presence of bioactive chemical compounds in the fruit extract that inhibit the potassium persulfate activity may reduce the production of ABTS$^+$[18].

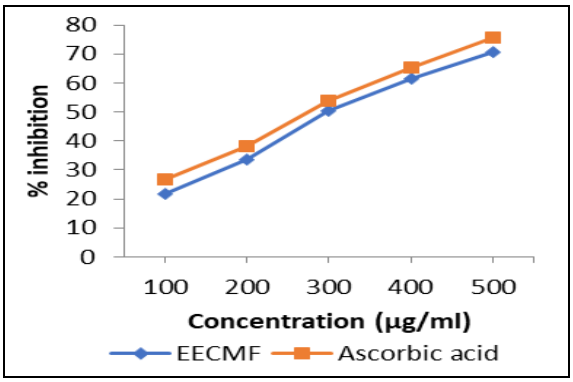

Fig. 2: ABTS+ radical scavenging activity of the ethanolic extract of *Cucumis melo* fruit

Hydroxyl Radical Scavenging Activity:

Hydroxyl radicals are highly potent oxidants, which can react with biomolecules in living cells and cause severe damage. In the present study, administration of fruit extract to the reaction mixture significantly inhibited the hydroxyl radical activity of 19.86% at 100mg/ml and increased to 62.57% at 500mg/ml, whereas the scavenging activity of standard vitamin E was 24.84% at 100mg/ml and increased to 71.25% at 500mg/ml.

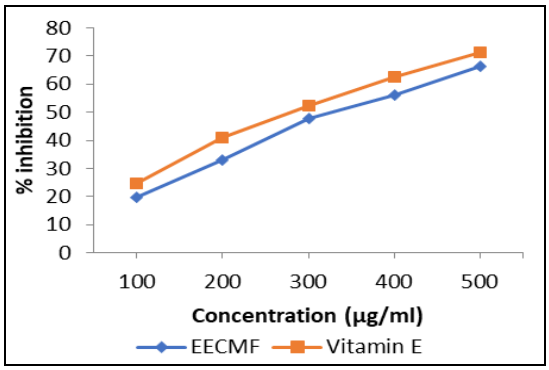

Fig. 3: Hydroxyl radical scavenging activity of the ethanolic extract of *Cucumis melo* fruit

The inhibition value of standard vitamin E was high when compared to *Cucumis melo* fruit. IC50 value was found to be 318.92mg/ml for *Cucumis melo* fruit extract. For the hydroxy radical test, vitamin E was used as a standard and the IC50 value was found to be 260.55mg/ml. The above result is in accordance with the study of were coincides with the study Sudha *et al*., (2011) who reported the *in vitro* free radical scavenging activity of raw pepino fruit that exhibited more than 60% inhibition. The results indicate the scavenging potential of *Cucumis melo* fruit against hydroxyl radicals[19,20].

Reducing Power Activity:

The reducing power increased with the increase in the extract concentrations. Reducing power of the *Cucumis melo* fruit extract was 0.112 at 200µg/ml and increased to 0.498 at 1000µg/ml, whereas the reducing power of the standard BHT (butylated hydroxytoluene) was increased from 0.173 to 0.529 at a concentration of 200-1000µg/ml. The reducing power of the extracts may serve as a significant indicator of its potential antioxidant activity. Reducing power, which was used to measure the reduced ability of antioxidants, was evaluated by the transformation of Fe (III) to Fe (II) in the presence of the fruit extracts[21]. Antioxidants reduce the Fe^{3+} ferricyanide complex to the ferrous form by donating an electron. The color of the test solution, then changes from yellow to different shades of green and blue. The ability to reduce Fe (III) may be attributed to the hydrogen donating effect of phenolic compounds[22].

Many studies have indicated that the antioxidant effect relates to the high amounts of rejection, which could react with radicals to stabilize and terminate radical chain reactions. This may be served as significant indicator of its potential antioxidant activity.

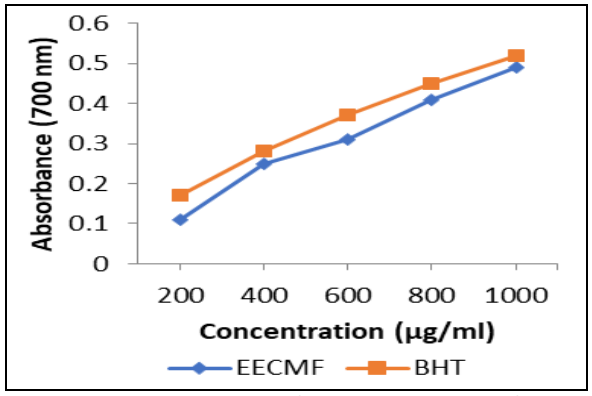

Fig. 4: Reducing power activity of the ethanolic extract of *Cucumis melo* fruit

Hence, it is presumed that the ethanolic extract of *Cucumis melo* fruit may have a high amount of reductones and hence the antioxidant property. The reducing power is highly related to the number of phenolic compounds, especially flavonoids that is present in the extract, which can serve as electron donors to terminate the radical chain reaction. Hence the flavonoids present in the fruit extract promises the antioxidant properties [23].

Nitric Oxide Scavenging Activity:

The nitric oxide scavenging activity of the ethanolic extract of the *Cucumis melo* fruit was found to be 15.77 % at 50 µg / ml and increases to 61.18 % at 250 µg / ml. This was compared with the standard antioxidant possessing 68.62 % for rutin respectively. The ability to scavenge 50 % of nitric oxide was found to be 177.56 µg / ml for *Cucumis melo* fruit extract and 142.11 µg / ml for the standard antioxidant Rutin. The results were expressed as IC50 values.

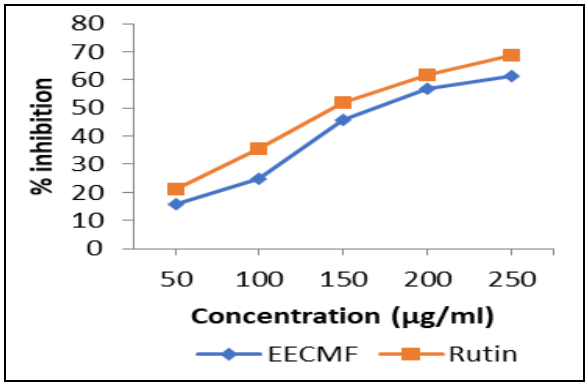

Fig. 5: Nitric oxide scavenging activity of the ethanolic extract of *Cucumis melo* fruit

Nitric oxide (NO) is an important chemical mediator generated by endothelial cells, macrophages, neurons, etc., and is involved in the regulation of various

physiological processes. Nitric oxides formed during their reduction with oxygen or with superoxides such as NO_2, N_2O_4, N_3O_4 are very reactive. The excess concentration of NO will cause diseases in human beings which can alter the structural and functional behavior of many cellular components. Nitric oxide inhibition studies proved that the fruit extract is a potent scavenger of nitric oxide generated from sodium nitroprusside which reacts with oxygen to form nitrite. The fruit extract inhibits nitrite formation by competing with oxygen to react with nitric oxide directly and also to inhibit its synthesis[24].

It has been reported that the antioxidative effect is mainly due to phenolic components, such as flavonoids, phenolic acids, and phenolic diterpenes. The nitric oxide scavenging of the fruit extract is due to presence of antioxidant principle in the extract which competes with oxygen to react with nitric oxide and thus inhibits the generation of nitrite molecules[25,26].

CONCLUSION:

The present study indicated that the *Cucumis melo* fruit contained more polyphenols and flavonoids and exhibited good antioxidant activity by effectively scavenging various free radicals, such as DPPH, ABTS+ and hydroxyl radicals. These *in vitro* assays indicate that *Cucumis melo* fruit extract could serve as free radical inhibitors or scavengers and significant source of natural antioxidants such as flavonoids and tannins. In order to reduce ROS-induced oxidative damage, both synthetic and natural antioxidants are used. *Cucumis melo* fruit possessed potential antioxidant activity. It may be due to the presence of respective secondary metabolites such as phenolics, flavonoids, tannins etc. in the plant species. Therefore, researchers have focused their studies on plant-derived natural antioxidants. New sources of natural antioxidant became very important for human health and received more attention for their potential role in prevention of human diseases.

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