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IN VITRO EVALUATION OF ANTI-OXIDANT PROPERTIES OF CUCUMIS MELO L. EXTRACTS OF LEAVES AND FRUIT

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ABSTRACT

The main objective of this study is to evaluate the anti-oxidant properties in methanolic extract of Leaves and fruit of cucumies melo () The methanol leaf extract noticed significant scavenging activity on DPPH, superoxide, nitric oxide , hydrogen peroxide and hydroxyl radical. The total phenolic compounds were found to be high with leaves rather than the flesh of fruit.

KEYWORDS: **Anti-oxidant, Cucumis melo, Scavenging activity**

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INTRODUCTION

Anti-oxidants neutralize free radicals by accepting or donating an electron to become unpaired. The anti-oxidant molecule itself becomes free radical during the process of neutralizing a free radical molecule to a non-free-radical molecule. Usually the anti-oxidant molecule is much less reactive free radical than that of free radical which is neutralized. The free radicals of anti-oxidant molecule which is generated by the process of neutralization, is readily neutralized by another anti-oxidant or it may exhibit another mechanisms to inhibit its own free radical property. An anti-oxidant can neutralize a free radical by donating one of its electrons without jeopardizing its own chemical stability. The ROS (Reactive Oxygen Species) and other free radicals which are continuously produced in the human body are neutralized either by enzymatic or non-enzymatic processes. In recent, year's majority of the population owes to depend on the traditional medicine to minimize the hazardous effects generated by the ROS. Generally, medicinal plants, are considered as factories or major source of natural drugs.[1] These natural drugs are very much potent in preventing many human diseases produced due to oxidative stress, including atherosclerosis, ischemic heart disease, ageing, heaptotoxicity, inflammation, diabetes, immunosupression, neurodegenerative conditions [2,3]

Cucumies melo belongs to family cucurbitaceae commonly called as "Kachri" in Rajasthan (India) is one such medicinal plant which possesses several pharmacological properties useful in the treatment of irritable bowel syndrome (IBS) which is a gastrointestinal disorder and bilious disorder, such as liver dysfunction due to excess secretion of bile. The other useful properties of Cucumies melo which play vital role in stomach pain, vomiting and constipation [4,5 6,7] With the reference to above literature cited, in the present investigation, few fractions of methanolic extracts of Cucumis melo leaves and fruits were evaluated to carry out the anti-oxidant properties.

MATERIALS AND METHODS

Chemicals

Sulphanilamide, Folin- Ciocalteu reagent, Sodium nitroprusside, Nitro blue tetrazolium (NBT), tannic acid were procured from SRL Mumbai. Pyrocatechol, 1,1-diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma-Aldrich Chemical Co,. All the other chemicals and reagents were purchased are of research grade.

Plant material

Leaves and fruits of Cucumis melo were collected during August to November, from the fields nearer to Rampet, district Warangal, Andhra Pradesh, India. Species authentification was done by Prof. V. S. Raju Taxonomist, Department of Botany, Kakatiya University, Warangal.

Extraction procedure

Leaves are shade dried and grinded in homogenizer in to coarse powder. Whereas, the fruits are peeled off and seeds removed, shade dried and grinded in homogenizer in to coarse powder. The 100 grams of each powdered material is extracted with methanol and concentrated under rotavapour at 40-50^{o} C.

Reagents preparation

Preparation of Nash Reagent: 75.0 g of ammonium acetate, 3 ml of glacial acetic acid and 2 ml of acetyl acetone were mixed and distilled water was added to total volume of 1 L.Preparation of Griess Reagent: 1% sulphanilamide, 2% Phosphoric acid and 0.1% N-1- napthylethylenediamine dihydrochloride in distilled H_2O.Preparation of Ferrous EDTA: 0.13% ferrous ammonium sulfate and 0.26% EDTA in distilled H_2O.

Determination of total phenol content

The amount of total phenolics in both extracts was determined with Folin–Ciocalteu reagent by following described method with slight modifications[8]. Separately, 1 ml of each methanolic extract of Cucumis melo leaves and fruit of different concentrations (50,150 and 250 µg/ml) and standard solution of pyrocatechol (10 µg/ml) was added separately to 100 ml volumetric flask separately, that contained about 60 ml distilled water and followed by the addition of 5 ml of Folin–Ciocalteu reagent. The content was mixed thoroughly and kept constant for about 10 min. To this, add 15 ml Na_2CO_3 (20 %) and make up to 100 ml using distilled water. The mixture was allowed to stand for 2 h with intermittent shaking. The absorbance was measured at 760 nm using a UV-visible spectrophotometer. The content was expressed as equivalent of pyrocatechol (µg) by using the following equation, which was obtained from a standard pyrocatechol graph.

Absorbance = 0.001 × pyrocatechol(µg)+0.0033

Anti-oxidant assay

DPPH radical scavenging activity

Free radical scavenging capacity of methanolic leaves and fruit extract of Cucumis melo was determined by using DPPH as described elsewhere[9]. DPPH radical scavenging activity was done by serial dilution by taking diluted methanol (1:20) as standard. 10 ml of various diluted methanolic extracts of various concentrations (50,150 and 250 µg/ml) were added to 1 ml DPPH solution (0.004%) and incubated for 10 min at room temperature. Absorbance of test and reference standard, ascorbic acid was measured at 517 nm. The amount of DPPH scavenging was calculated by using the formula:

% DPPH radical scavenging = [(Absorbance of control – Absorbance of test sample)/ (Absorbance of control)] × 100

Super oxide radical scavenging activity

The super oxide radical scavenging activity was measured by the following described method by[10-11]. 1ml of each methanolic leaves and fruit extracts of Cucumis melo at various concentrations (50,150 and 250 µg/ml) were mixed with 1 ml of nitro blue tetrazolium (NBT) solution (156 mM NBT in phosphate buffer of pH 7.4) and 1 ml NADH in phosphate buffer of pH 7.4. The reaction was initiated by adding 100 ml of phenazine methosulfate (PMS) solution (60 mM PMS in phosphate buffer, pH 7.4) to the mixture. The reaction mixture was incubated at 25^0C for 5 min and the absorbance was measured at 560 nm against blank sample and compared with reference standard ascorbic acid. Decreased absorbance of reaction mixture indicated increased superoxide anion scavenging activity. The percentage of inhibition of superoxide anion generation was calculated using the following formula:

% inhibition = [(Absorbance of control – Absorbance of test sample)/ (Absorbance of control)] × 100

Nitric oxide scavenging activity

Nitric oxide scavenging activity was determined by the following method described by[12]. Briefly, 5 mM sodium nitroprusside was prepared in phosphate buffered saline and mixed with different concentrations of methanolic leaves and fruit extracts of Cucumis melo at (50,150 and 250 µg/ml) followed by incubation at 25^0C for 30 min. A control without the extracts but with equivalent amounts of methanol was taken. After 30 min, 1.5 ml of incubated solution was removed and diluted with 1.5 ml of Griess reagent. The absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with N-1- napthylethylenediamine dihydrochloride was measured at 546 nm and percentage scavenging activity was measured with reference standard ascorbic acid.

Scavenging of hydrogen peroxide

Scavenging of hydrogen peroxide was measured by the following described method elsewhere[13]. A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH

7.4). 1 ml of each methanolic leaves and fruit extract of Cucumis melo at different concentrations (50,150 and 250 µg/ml) were added to 0.6 ml of 40 mM hydrogen peroxide solution. Absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank containing phosphate buffer without hydrogen peroxide. The percentage scavenging of hydrogen peroxide of plant extract and reference standard ascorbic acid was calculated using the following formula:

% scavenged [H_2O_2] = [(Absorbance of control – Absorbance of test sample)/ (Absorbance of control)] × 100

Hydroxyl radical activity

Hydroxyl radical activity was measured by the following described method elsewhere[14]. 1 ml of each methanolic leaves and fruit extracts of Cucumis melo at various concentrations (50,150 and 250 µg/ml) were placed in tubes and evaporated to dryness. 1 ml of ferrous-EDTA (0.13% ferrous ammonium sulfate and 0.26% EDTA), 0.5ml of 0.018% EDTA, 1 ml of DMSO (0.85% v/v in 0.1 M phosphate buffer, pH 7.4) and 0.5 ml of freshly prepared 0.22% ascorbic acid were added to each tube. The tubes were capped tightly and heated in a water bath at 80-90^0 C for 15 min. The reaction was terminated by adding 1 ml of ice cold TCA (17.5% w/v). Latter 3 ml of Nash reagent was added to each tube and left at room temperature for 15 min for color development. The intensity of color formed was measured at 412 nm against the reagent blank. The percentage inhibition was compared with reference standard ascorbic acid and test compounds.

Statistical analysis

The data from the experiments are presented as mean_±S.D. (n=3). Student's t-test was used for statistical analyses (SAS software 9.0). Values were considered statistically significant when $p<0.001$.

RESULT AND DISCUSSION

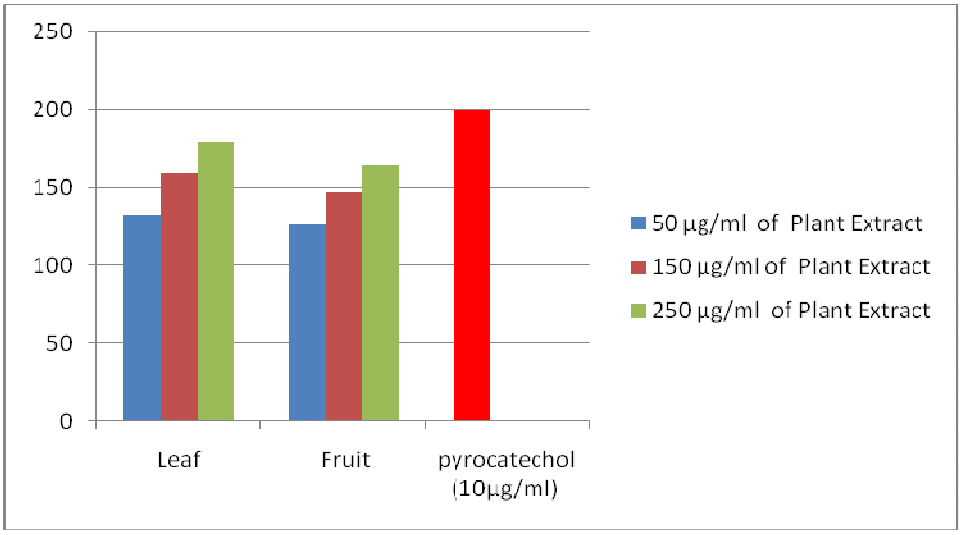
The estimation of total phenolic compounds of Cucumis melo leaf and fruit methanolic extract at all tested concentrations showed that of contains 179.2, 163.8, µg which is equivalent to pyrocatechol (Fig. 1). Use of synthetic antioxidants which are associated with several hazardous effects, was restricted, and paid more attention to replace these with natural drugs which are capable of acting as good antioxidants[15]. In this pertinent, we investigated few fractions of Cucumis melo for the evaluation of antioxidant properties. The antioxidant activity of Cucumis melo methanolic leaf and fruit extract exhibited concentration dependent scavenging activity.1,1-diphenyl-2-picrylhydrazyl (DPPH) is extensively used as a free radical for the evaluation of reducing substances. Generally, the scavenging activity of DPPH radical was determined by the decrease in its absorbance due to the change in color from purple to yellow. In the present studies, the methanolic leaves extract showed significant scavenging activity rather than methanolic fruit extract. The highest values 21.8±0.2 and 20.1±0.2 which was noticed at 250 µg/ml of methanolic leaf and fruit extract respectively (Table. 1).

Table 1
Scavenging activity of various plant extracts of Cucumies melo var. leaves and fruit methanolic extract

	DPPH			SUPER OXIDE			NITRIC OXIDE			HYDROGEN PEROXIDE			HYDROXYL		
Con µg/ml 50	150	250	50	150	250	50	150	250	50	150	250	50	150	250	
LEAF	19.5 ± 1.2*	19.8 ± 0.1*	21.8 ± 0.2	17.4 ± 0.2*	17.8 ± 0.2*	18.7 ± 0.1	16.9 ± 0.1	17.1 ± 0.2	17.7 ± 0.2	17.2 ± 0.2	17.4 ± 0.1*	17.8 ± 0.1*	15.6 ± 0.2*	16.1 ± 0.1*	16.8 ± 0.2*
FRUIT	18.8 ± 0.2*	19.5 ± 0.1*	20.1 ± 0.2*	17.1 ± 0.2*	17.5 ± 0.05*	17.8 ± 0.1*	16.0 ± 0.1*	16.2 ± 0.1*	16.7 ± 0.1*	16.8 ± 0.3*	17.4 ± 0.1*	17.7 ± 0.1*	16.9 ± 0.1*	17.0 ± 0.1*	17.0 ± 0.1*

Values are mean±S.D. n=3
***significance is set at p<0.001(Student's t-test).**

Figure.1
.***Yield percentage of total phenolic content (100 g) in various parts at various concentrations of Cucumis melo L. compared to reference standard pyrocatechol 10 µg/ml.***



Generally, superoxide anion radicals produced by the enzymes like xanthine oxidase are converted to hypoxanthine and to uric acid[16]. The decrease in absorbance at 560nm indicates the utilization of superoxide anion in the reaction mixture and thereby exhibiting a concentration dependent increase in superoxide scavenging activity. In the present investigation the methanolic leaf and fruit extracts noticed significant scavenging activity. The values which are obtained at various concentrations (50,150, 250 µg/ml) of methanolic leaf and fruit extracts are 17.4±0.2, 17.8±0.2, 18.7±0.1 and 17.1±0.2, 17.5±0.05, 17.8±0.1 respectively however the values remain below ascorbic acid (Table.1).

Nitric oxide (NO), being a potent pleiotropic mediator in several physiological processes reacts with superoxide anion to form a potentially cytotoxic molecule, peroxynitrous acid (ONOOH) which is a very strong oxidant and undergoes the nitration of aromatic compounds such as tyrosine[17]. Peroxynitrite ($ONOO^-$) with the combination of carbon dioxide which is dissolved in body fluid is more responsible for oxidative damage of proteins in living systems[18-19]. The present investigation of the scavenging activity of Cucumis melo methanolic leaf and fruit extracts produced satisfactory results. However, it was noticed that the methanolic leaf extract possessed better scavenging activity rather than methanolic fruit extract. The values which were obtained at various concentrations (50,150, 250 µg/ml) of methanolic leaf and fruit extracts are 16.9±0.1, 17.1±0.2, 17.7±0.2 and 16.0±0.1, 16.2±0.1, 16.7±0.1 respectively (Table.1).

Biologically, H_2O_2 is a weaker oxidizing and reducing compound but it acts as a good toxicant, by interaction with metal ions and superoxide anion and thus converting itself into hydroxyl radical and thus leads to produce singlet oxygen. Hydrogen peroxide has immense potency to break down heamoglobin, to release Fe ions[20]. Therefore, it is very important to measure the scavenging of H_2O_2 of the test samples. The present studies revealed that methanolic extract of both leaf and fruit extracts possessed noticeable scavenging activity. The values which were obtained at various concentrations (50,150, 250 µg/ml) of methanolic leaf and fruit extracts are 17.2±0.2, 17.4±0.1, 17.8±0.1 and 16.8±0.3 17.4±0.1 17.7±0.1 respectively (Table.1).

Hydroxyl radical produced during the Fenton's reaction causes loss of base pairs from DNA due to fragmentation of sugar molecule of DNA[21]. The methanolic fruit extract, here holds little bit more scavenging activity rather than the methanolic leaf extract. This might because of nature generated free radical and physical and chemical differences in the naturally occurring antioxidant by the both plant extracts[22-23]. However, there is a large difference in the reactivity of stable free radicals and non-stable free radicals with antioxidants, for example, the stable free radical DPPH react stoichometrically with the antioxidants which are good hydrogen donors but whereas antioxidant which are very effective chelators of transition metal ions involve in different manner in the hydroxyl radical scavenging assay when compared to assays with stable free radical. The values which were obtained at various concentrations (50,150, 250 µg/ml) of methanolic leaf and fruit extracts were 15.6±0.2, 16.1±0.1, 16.8±0.2 and 16.9±0.1, 17.0±0.1, 17.0±0.1 respectively (Table. 1).

CONCLUSION

Depending up on the results obtained in the present study it is concluded that the methanolic leaf and fruit extract of Cucumis melo L. possesses the significant anti-oxidant activity. This investigation suggest that this plant is a potential source of natural anti-oxidant and could play a vital role as therapeutic agents in preventing oxidative stress related disorders. Further studies are being carried out for the isolation and characterization of anti-oxidant components.

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