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ORIGINAL RESEARCH ARTICLE

In vitro Free Radical Scavenging Activity of Ethanolic Extract of *Cucumis trigonus* Roxburxii fruit

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ABSTRACT

Cucumis trigonus Roxburghii of family Curcurbitaceae is a perennial scabrid monoecious tendrillar herb commonly used in Indian folklore medicine. The present study investigates the antioxidant potential of ethanolic extract of the fruits of *Cucumis trigonus*. They were investigated for 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical, Superoxide radical, Nitric oxide radical, hydroxyl radical scavenging activity and reducing power assay. The extract showed strong antioxidant capabilities, which were subjected for their dose dependent activity at different concentration to calculate IC_{50} values.

Key words: Antioxidant, *Cucumis trigonus*, DPPH free radical, Superoxide radical and reducing power assay

INTRODUCTION

Free radicals are chemical entities characterized by a high reactivity, varying reactivity's notwithstanding, free radicals inclusives have been known to be generally less stable than non-radicals. Free radical formation during the metabolism of xenobiotics is therefore an important mechanism employed by toxic agents in causing cellular damage. Reactive oxygen species (ROS) capable of damaging DNA, proteins, carbohydrates and lipids are generated in aerobic organisms. These ROS include superoxide anion radical, hydrogen peroxide, hydroxyl radical, and single molecular oxygen. The effects of these ROS are controlled by a system of enzymic and non- enzymic antioxidants. These antioxidants eliminate pro-oxidants and scavenge free radicals [1].

Antioxidants which scavenge active oxygen species are found in a variety of foodstuffs and are commonly referred to as scavengers. An antioxidant is a substance that prevents oxidative damage caused by free radicals. Antioxidants hold promise in preventing diseases like cancer and heart diseases[2].The protective action of an antioxidant would probably be due to an inhibition of free radical induced chain reaction with prevention of peroxidative deterioration of structural lipids in membranous organ cells[3].

Many antioxidants are plant based and play an important role in protecting plants that are exposed to strong sunlight and live under severe oxygen stress[4]. *Cucumis trigonus* Roxburghii of family Curcurbitaceae is a perennial scabrid monoecious tendrillar herb with slender angled stem, leaves deep palmately five lobed, hispid on the nerves beneath and rounded at the apex. Male flowers are small and are found in clusters where as female flowers are solitary. Fruits are ellipsoid or sub-global, yellow or yellow with green stripes, seeds are white and ellipsoid. The title plant is reported to possess analgesic, anti-inflammatory and diuretic activity.

MATERIALS AND METHODS

Collection of the plant material:

Cucumis trigonus Roxb. fruits were collected from Kovanur area of Coimbatore district, Tamil Nadu, India during the month of September to November,2009. The plant was identified and authenticated by taxonomist Dr.K. Arumugasamy, Assistant Professor, Department of Botany, Kongunadu Arts and Science College, Coimbatore, Tamilnadu, India. Voucher specimen was deposited herbarium centre, Department of Botany, Kongunadu Arts and Science College, Coimbatore.

In vitro antioxidant studies of the ethanolic fruit extract of *Cucumis trigonus* Roxb:

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DPPH radical scavenging activity:

The DPPH scavenging effect was assayed according to the method of Sreejayan and Rao (1996). DPPH scavenging activity was measured by the spectrophotometric method. To a methanolic solution of DPPH (20µm), 0.05 ml of the test compound dissolved in ethanol was added at different concentration (100 - 500µg). An equal amount of ethanol was added to the control. After 20 min. the decrease in the absorbance of test mixure (due to quenching of DPPH free radicals) was read at 517nm and the percentage inhibition calculated by using the formula.

$$\text{Inhibition (\%)} = \frac{(\text{Control} - \text{Test})}{\text{Control}} \times 100$$

Superoxide radical scavenging activity:

The superoxide radical scavenging effect was assayed according to the method of Liu *et al*. (1997). Superoxide radical was generated from the photo reduction of riboflavin and was detected by NBT reduction method. The reaction mixture contained EDTA (6µm), with 3µg NaCN, riboflavin (2µM), NBT (2µM), KH_2PO_4 - Na_2HPO_4 buffer (67mM, pH 7.8) and various concentrations of the extracts in a final volume of 3.0ml. The tubes were illuminated under incandescent lamp for 15min. The optical density at 530nm was measured before and after illumination the inhibition of superoxide radical was determined by comparing the absorbance values of the control with those of treatments. Ascorbic acid was used as standard.

Nitric oxide radical scavenging activity:

The nitric oxide radical scavenging effect was assayed according to the method of Madan *et al.* (2005). Nitric oxide was generated from sodium nitroprusside and measured by Greiss reaction. Sodium nitroprusside in standard phosphate buffer solution was incubated with different concentration (100 - 500µg) of the ethanol extract dissolved in phosphate buffer (0.025M, pH 7.4) and the tubes were incubated at 25°C for 5hr. Control experiments without the test compounds but with equivalent amounts of buffer were conducted in an identical manner. After 5hr., 0.5ml of incubation solution was removed and diluted with 0.5 ml of griess reagent (1% sulphanilmide, 2% orthophoshoric acid and 0.1% naphthylethylene diamine dihydrochloride). The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and its subsequent coupling with naphthylethylene diamine was read at 546 nm. The experiment was repeated in triplicates and percentage inhibition calculated.

Hydroxy radical scavenging activity:

The hydroxy radical scavenging effect was assayed according to the method of Rajeshwari *et al*. (2005). The reaction mixture contains deoxyribose (2.8mM), $Fecl_3$ (0.1mM), EDTA (0.1mM), H_2O_2(1mM), ascorbate (0.1mM), KH_2PO_4 - KOH buffer (20mM pH 7.4) and various concentration of sample extracts in final volume of 1.0ml. The reaction mixture was incubated for 1 hr. at 37 ºC. The extent of deoxyribose degradation was measured by TBA method. 1.0ml of TBA 1% (w/v) were added to the mixture and heated in a water bath for 100ºC for 20min. the absorbance of resulting solution was measured spectrophotometrically at 530nm. The inhibition of degradation was calculated according to the equation $I = A_0\text{-}A_1/A_0 x100$, where A_0 is the absorbance of the control reaction, A_1 is the absorbance of test compound.

Reducing power radical scavenging activity:

The reducing power radical scavenging effect was assayed according to the method of Oyaizu (1986). Different concentrations of the extracts in 1.0ml of ethanol were mixed with phosphate buffer (2.5 ml, 0.2M, pH 6.6) and potassium ferrocyanide (2.5ml, 1%). The mixture was incubated at 50ºC for 20min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000rpm for 10 min. The upper layer of the solution (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%) and the absorbance was measured at 700nm. Increased absorbance of the reaction mixture indicates increased reducing power.

RESULTS AND DISCUSSION

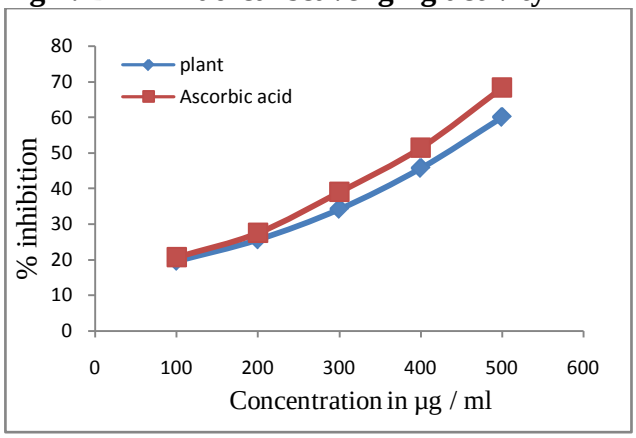
DPPH radical scavenging activity:

DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. DPPH radical was usually used as a substrate to evaluate antioxidant activity of antioxidants. It involves reactions of specific antioxidant with stable free radical 2,2-diphenyl-1-picryl-hydrazyl (DPPH). As a result, there is reduction of DPPH concentration by the antioxidant, which decreases the optical absorbance of DPPH, and it is detected at 517nm[10].

The radical scavenging activity of the selected fruit extract estimated by DPPH is shown in (**Fig 1**). The scavenging effect of the fruit extract of *C. trigonus on* the DPPH radical was 60.09 % at a concentration of 500µg/ml and the scavenging activity also increased in a dose dependent

manner. These results indicate that the extract has noticeable effects on scavenging the free radicals. The standard antioxidant ascorbic acid showed the maximum value of 68.37 % at a concentration range from 100 to 500 µg / ml, the scavenging effect also increased in a dose dependent manner. The inhibition value was found to be high in standard ascorbic acid when compared to the *C. trigonus*.

Fig 1: DPPH radical scavenging activity



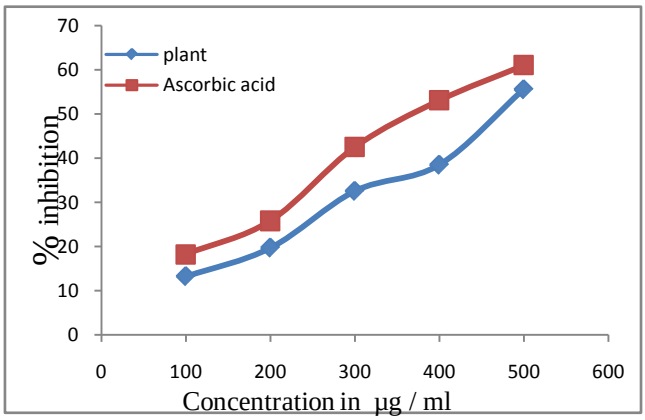
DPPH is a free radical, stable at room temperature, which produces a purple color solution in methanol. It is reduced in the presence of an antioxidant molecule giving rise to uncolored methanol solution. There is a decrease in the concentration of DPPH radical due to the scavenging ability of the ethanolic fruit extract and vitamin C. The vitamin C obtained is comparable to the reported value of Ghasemzadeh *et al*. (2011).

The results were expressed as IC_{50} (the concentration required to scavenge 50% of free radicals) value. The IC_{50} of the extract was found to be 430 for *P. murex* fruit extract and for the standard antioxidant ascorbic acid was found to be 380.

Superoxide radical scavenging activity:

Free radicals have been implicated in many disease conditions, the important one being superoxide radical. Herbal drugs containing free radical scavengers are gaining importance in treating various diseases. The superoxide anion derived from dissolved oxygen by phenazine methosulphate/NADH coupling reaction reduces nitro blue tetrazolium. The decrease in the absorbance at 560nm with the fruit extract thus indicates the consumption of superoxide anion in the reaction mixture. The superoxide radical scavenging activities of fruit extract and ascorbic acid are represented above in (**Fig 2**).

Fig 2: Superoxide radical scavenging activity



The scavenging effects of the *C.trigonus* fruit extract on the superoxide radical are 55.62 % in *C.trigonus* at 500 µg/ml. Standard ascorbic acid showed the inhibition value of 61.09 % at 500µg/ml concentration increasing the sample concentration range from 100 to 500 µg/ml, the scavenging effect also increased in the dose dependent manner.

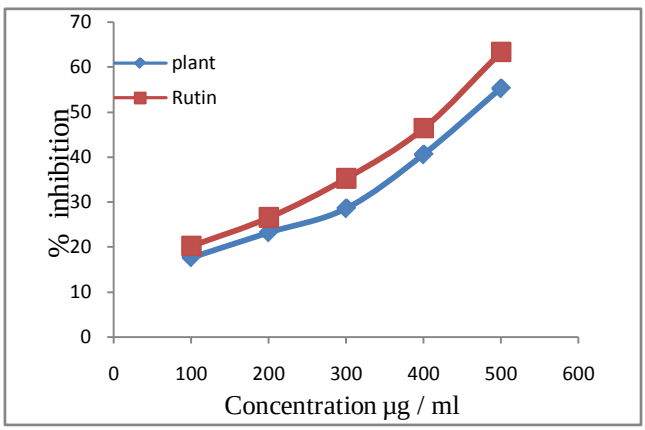
The inhibition value of standard BHT was high when compared to the *C.trigonus*. The IC_{50} value of the fruit extract was found to be 470 in *C.trigonus*. For the superoxide radical test, ascorbic acid was used as a standard were the IC_{50} values was found to be 360.

Nitric oxide radical scavenging activity:

Nitric oxide radical inhibition study 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. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide[12].

The scavenging of nitric oxide by fruit extract increased in a dose dependent manner as illustrated in (**Fig 3**). The results were expressed as IC_{50} values.

Fig3:Nitric oxide radical scavenging activity

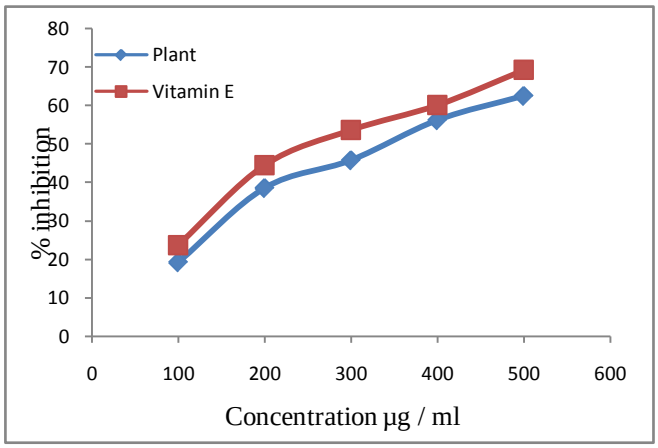


All the tested extracts showed nitric oxide scavenging activity. *C.trigonus* exhibited inhibition of 55.29 % at a concentration of 500 µg/ml. Rutin, a standard nitric oxide scavenging agent showed an inhibition of 58.29 % at the concentration of 500 µg/ml. The inhibition value of rutin was found to be high when compared to the *C. trigonus.*

IC_{50} value of the extract was found to be a 460 for *C. trigonus*. For the nitric oxide test, rutin was used as a standard were the IC_{50} values was found to be 410. The inhibition value of the rutin is compared to the reported values of Badami *et al*. (2003) and found to be similar. **Hydroxy radical scavenging activity:**

Hydroxyl radicals were generated from the substrate deoxyribose by the reaction of ferric-EDTA together with H_2O_2 and ascorbic acid. When the fruit extract was incubated with the above reaction mixture, it can prevent the damage against sugar. The results are shown in the (**Fig 4**).

Fig 4: Hydroxy radical scavenging activity



The scavenging activity of the fruit extract of *Cucumis trigonus* against hydroxyl radical was found to be 62.53%. Standard vitamin E for hydroxyl radical showed the inhibition value of 69.24 % at 500µg/ml concentration. The inhibition value of standard vitamin E was high when compared to the *C. trigonus.*

IC_{50} value of the extract was found to be 325 for *C. trigonus*. For the hydroxyl radical test, vitamin E was used as a standard were the IC_{50} values was found to be 270.

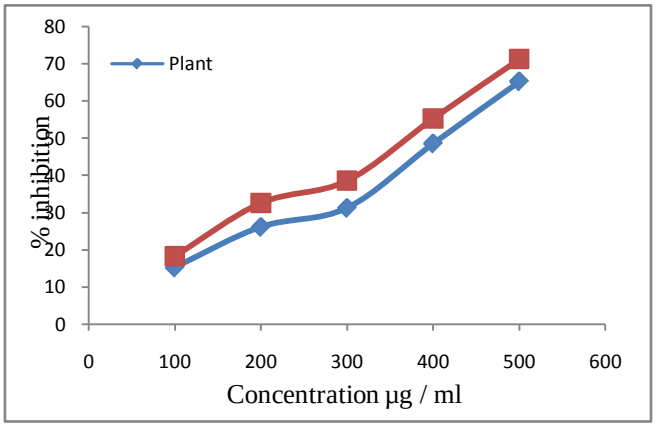
The extract and vitamin E exhibited strong scavenging effects for hydroxyl radicals which could inhibit lipid damage at different concentrations. Thus scavenging effect of vitamin E is in accordance with the reported value of Jadav and Bhutani (2002).

Reducing power assay:

Reducing power was measured by the direct reduction of $Fe^{3+}(CN^-)_6$ to $Fe^{2+}(CN^-)_6$ and it was determined by measuring absorbance resulting from the formation of the Perl's Prussian blue complex followed by the addition of excess ferric ions (Fe^{3+}). This method is based on the reduction of Fe^{3+} ferricyanide in stoichiometric excess relative to the antioxidants[15].

(**Fig 5**) represents the reductive capabilities of the *C. trigonus* fruit extract, which was compared with butylated hydroxyl toluene (BHT) standard. The reducing power of the extract was potent and it increased with the concentration of the samples. The fruit extract could reduce most Fe^{3+} ions, which had lesser reactive activity than the standard BHT.

Fig 5: Reducing power assay



The reducing power increased with increasing the concentration of the extract. The reducing capacity of the compound may serve as a significant indicator of its potent antioxidant activity [16].The absorbance values of the extract at different concentrations was found to be less than that of the reference (BHT) compound and this is in accordance with the report of Gulcin *et al*. (2002).

The scavenging effects of the *C.trigonus* fruit extract on the reducing power assay are 62.53% in *C.trigonus* at 500 µg/ml. Standard BHT showed the inhibition value of 71.24 % at 500µg/ml concentration.Increasing the sample concentration range from 100 to 500 µg/ml, the scavenging effect also increased in the dose dependent manner.

The inhibition value of the standard BHT was high compared to the *C. trigonus.* The IC_{50} value of the fruit extract was found to be 415. For the reducing power assay, BHT was used as a standard were the IC_{50} values was found to be 380.

CONCLUSION

The ethanolic extract of *Cucumis trigonus* was subjected to dose dependent studies to calculate IC_{50} values. Free radical scavenging activity was estimated using DPPH, Superoxide, Nitric oxide, Hydroxyl radical and Reducing power assay. 50% reduction of DPPH free radicals were obtained at 430 µg / ml, for superoxide radical the extract concentration was found to be 470 µg / ml, 460 µg / ml of fruit extract was required for 50% reduction of nitric oxide free radicals and hydroxyl radical was scavenged using 325 µg / ml of *C.trigonus* extract. The results of reducing power assay shows the 415 µg / ml of extract was required to reduce 50% of the free radicals. From the studies, it could be concluded the ethanolic extract of *Cucumis trigonus* fruit has a good potential in retarding the activity of the free radicals, thus possessing a good composition of antioxidants.

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