EVALUATION OF FREE RADICAL SCAVENGING ACTIVITY OF *Lycoperiscon esculentum* FRUIT VARIETY IN ETHANOLIC FRACTION

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Summary:

Ethanolic fractions of *Lycoperiscon esculentum* fruit varieties namely S-22 and Samrudhi were evaluated for free radical scavenging property in four different assay model. Among the two local tomato varieties tested, S-22 fruit variety found superior over Samrudhi fruit variety possessing higher ability to scavenge $DPPH^{\cdot}$, $NO^{\cdot}$, $OH^{\cdot}$, $O_2^{\cdot -}$ in reaction mixture. The free radical scavenging property of both types of fruits is due to their polyphenolic content. It was observed that different radical scavenging action of fruit extracts was directly related to their respective phenolic and flavonoid components. Total phenolic and flavonoid content in S-22 extract was found 29.13 and 33.03mg/g respectively. The S-22 extract also possessed more activity in ferric to ferrous transformation. Thus, tomato fruit could be considered as a cheap, easily available natural source of antioxidant.

Key words: Antioxidants, *Lycoperiscon esculentum*, radical scavenging activity.

Introduction

Free radicals are reactive chemical species with a very short life span. They are categorized as reactive oxygen species (ROS) and reactive nitrogen species (RNS) on the basis of their center molecule. Reactive species are continuously generated inside the body by some endogenous factors as the byproducts of different biological reactions or exogenously. These ROS and RNS cause great damage to DNA, protein, carbohydrate etc. and affect normal cellular functions (1). Due to their highly reactive capability they induce lipid peroxidation, protein oxidation, DNA base modifications etc. and this leads to neurodegenerative disorders, diabetes, cancer etc. (2).

Antioxidants are the molecules which neutralizes the damaging effects of free radicals. Mammalian cells possess some endogenous antioxidant enzymes to protect body from free radical attack but still sometimes the equilibrium between free radical generation and its detoxification get disturbed due to decreased level of endogenous antioxidants. Fruits, vegetables are the prime source of natural antioxidants. Mainly vitamin A, vitamin E, ascorbic acid, phenolic component, anthocyanin, flavonoid etc. are major active antioxidant compounds. The positive correlation between antioxidant activity and polyphenolic component has already been proved. (3, 4).

Lycoperiscon esculentum is a very common vegetable which come under fruit and flower vegetable subclass. It is having a year round availability and in India commonly used in many types of cuisines. The fruit rich in lycopene and ascorbic acid and therefore it can act as singlet oxygen scavenger (5). Tomato fruit is a part of daily diet among Indian people and hence it was thought to check the free radical scavenging property and polyphenolic contents of ethanol extract of two commonly cultivated tomato varieties in Khandesh region of Maharashtra, India.

Materials

Two tomato variety namely S-22 and Samrudhi were collected from farmers at their full ripen stage during March –April 2009. The fruit of S-22 variety is of completely round shape with average weight of 120-130g whereas Samrudhi variety fruit is of small size, highly pigmented with an average weight of 40-60g. All the chemicals and reagents used in this study were of AR grade and purchased from Hi – Media, Mumbai, India.

Methods

Preparation of crude fruit extracts:

Uniformly red ripen, healthy fruit of both the varieties were used for extraction purpose. A hundred g fruit of each variety was extracted in four volumes of ethanol (99.9%) by maceration (96 h). Final extract was passed through Whatman filter paper No. 1 (Whatman Ltd., England). The filtrate was concentrated under vacuum at 40^0 C and extract was freeze dried and stored at - 20^0 C for further use. A known amount of crude extract was dissolved in ethanol (99%) to obtain a stock concentration of 200µg/ml.

Free radical scavenging assay:

$DPPH^{\cdot}$, $NO^{\cdot}$, $OH^{\cdot}$ and $O_2^{\cdot -}$ scavenging activity of crude tomato extract were measured according to the established methods (2, 6-8). The percentage of radical scavenging for sample extracts and standard compound were calculated using following formula.

Radical scavenge (%) = $[(A_{cont.} - A_{test}) / A_{cont.}] X 100$,

where $A_{cont.}$ is the absorbance of control and A_{test} is the absorbance in presence of sample extracts and standard.

Reducing power ability:

The reducing power ability of crude extracts was determined according to the method of Oyaizu (9). The capability of extracts to reduce Fe^{3+} to Fe^{2+}, which turns the reaction mixture yellow to green appearance with absorbance maxima at 700nm, was measured.

Total phenolic content:

The amount of total phenolic compounds was determined according to Folin-Ciocalteu procedure (10). The test was performed by introducing samples (0.2 ml.) and Folin-Ciocalteu reagent and the mixture was incubated for 3 minutes. To the incubated mixture, sodium carbonate solution was added and incubated for 30 minutes at room temperature followed by measurement of absorbance at 760nm (UV-1601, UV- Visible Spectrophotometer, Shimadzu, Japan). Gallic acid was used for preparation of standard curve.

Total flavonoid content:

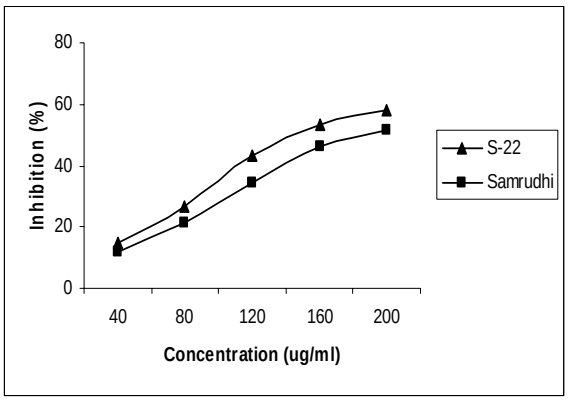
Total flavonoid content of the sample extracts was determined by $AlCl_3$ method (11). 1.5 ml. of sample extracts were added to the equal volume of $AlCl_3.6H_2O$ solution. After incubation, the absorbance was measured at 367nm (UV-1601, UV- Visible Spectrophotometer, Shimadzu, Japan). Quercetin was used as a standard for calibration.

Results

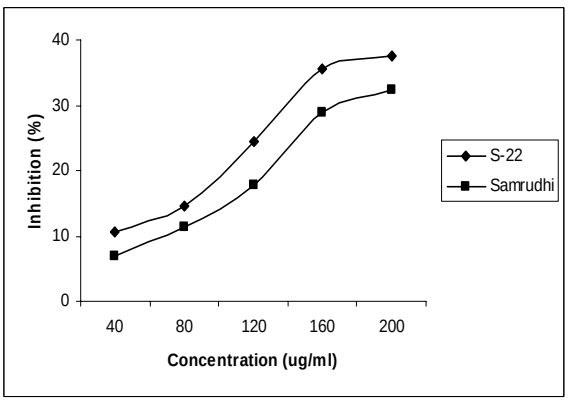
Free radical scavenging assay:

The ethanolic extracts of the two *Lycoperiscon esculentum* fruit varieties at different concentrations (40-200µg/ml) were tested for their ability to scavenge different free radicals *in vitro* models. The scavenging ability of crude extracts were determined by decrease in absorbance at respective absorbance maxima in presence of antioxidant compounds. At every concentration of crude fruit extracts, percentage inhibition of each radical generated were calculated. With increasing concentration of crude ethanolic extract, more quenching of free radical generation in reaction mixture was observed.

In all radical scavenging assays S-22 fruit extract was found more superior in response to scavenging free radicals than Samrudhi extract (Figure 1). For both the fruit extracts, it was observed that, by increasing concentration, inhibition of generation of different radical increasesd but at higher concentration around 200µg/ml, $NO^{\cdot}$ and $O_2^{\cdot -}$ scavenging activity became saturated. In case of DPPH $^{\cdot}$ and $OH^{\cdot}$ scavenging assay, the quenching property increased steadily up to 160µg/ml concentration for both the crude extracts but with further increase the concentration, the radical scavenging capacity increased slowly.



(a)



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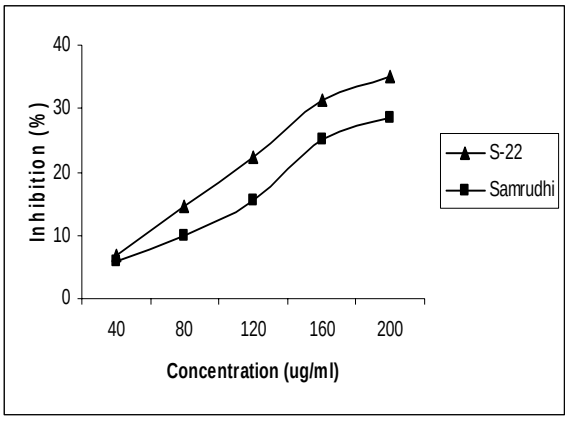

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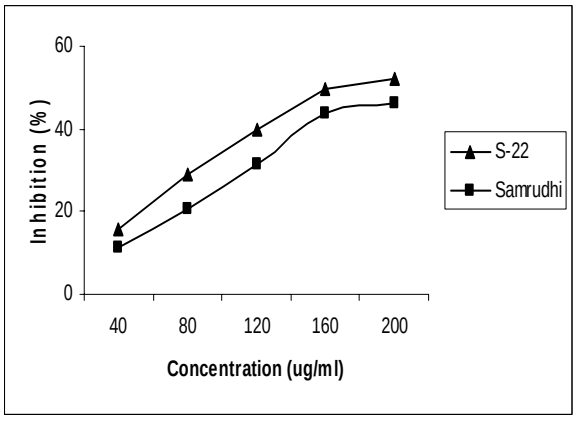

(d)

Figure 1: Scavenging effect of two tomato ethanolic extract (S-22 and samrudhi) in different free radicals (a) $DPPH^{\cdot}$, (b) $NO^{\cdot}$, (c) $OH^{\cdot}$, (d) $O_2^{\cdot-}$ generation.

S-22 fruit extract at a concentration of 160µg/ml, inhibited around 53%, 35%, 31% and 49% of $DPPH^{\cdot}$, $NO^{\cdot}$, $OH^{\cdot}$ and $O_2^{\cdot-}$ generation respectively. Whereas, the same concentration of Samrudhi extract inhibited 46%, 28%, 24% and 43% of $DPPH^{\cdot}$, $NO^{\cdot}$, $OH^{\cdot}$ and $O_2^{\cdot-}$ generation respectively.

Reducing power ability:

S-22 variety possessed superior reducing power ability over Samrudhi variety when compared with ascorbic acid equivalent. S-22 variety has almost nine times more activity to convert ferric ions to ferrous forms (Table 1).

Total phenolic and total flavonoid content:

S-22 variety fruit contained greater amount of total phenolic content (29.13mg/g) and total flavonoid content (33.03mg/g) than Samrudhi variety (Table 1). The higher content of phenolics and flavonoids of S-22 might be responsible for its higher free radical scavenging ability than Samrudhi variety.

Table 1: Reducing power ability, total phenolic and flavonoid contents of two tomato variety.

Tomato variety	Reducing power ability (mg of AAE/g)	Total phenolic content (mg of GAE/g)	Total flavonoid content (mg of QE/g)
S-22	37.21±0.17	29.13±0.09	33.03±0.12
Samrudhi	4.98±0.23	23.27±0.41	30.57±0.92

Values are mean ± standard deviation of three replicates

Conclusions

From this study it was clear that, though both fruit extracts in ethanolic fraction possessed a remarkable free radical scavenging property but, S-22 variety had higher potential than Samrudhi variety. In compositional analysis also it was observed that, S-22 variety has higher amount of total phenolic and flavonoid contents than Samrudhi variety. Hence, it can be correlated that, due to higher amount of polyphenolic content, S-22 variety expressed more radical scavenging action than Samrudhi variety. So, fruit of tomato can be considered as an easily available source of natural bioactive components with antioxidant potential for dietary and therapeutic applications.

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