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Comparative study of antioxidant activity of fruits of *Lycoperiscon esculentum* cultivars

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ABSTRACT

Antioxidant activity of *Lycoperiscon esculentum* fruit varieties in two different solvent fractions has been checked by different *in vitro* models. The methanolic fraction of S-22 variety showed significant antioxidant activity as well as it contained relatively higher amount of total phenolic and flavonoid compounds over Samrudhi variety. S-22 variety showed IC_{50} values as 115.92 µg/ml and 142.90 µg/ml to scavange DPPH; OH- and O_2.- generation respectively. 1 gm of S-22 extract in methanolic fraction contained 35.27 mg phenolic compound equivalent to gallic acid and 36.83 mg flavonoid equivalent to quercetin respectively. Methanolic fraction in all the cases showed promising activity over aqueous fraction and may be due to the fact that antioxidant compounds were extracted more in methanol.

Key words: Antioxidants, *Lycoperiscon esculentum*, DPPH radical, Superoxide radical, Nitric oxide radical, Hydroxyl radical, reducing power ability, total phenolic and flavonoid contents.

INTRODUCTION

Molecular oxygen is the primary need for the survival of aerobic life process. But, due to univalent reduction of molecular oxygen, there is generation of free radicals or reactive oxygen species (ROS) such as superoxide, hydroxyl radical etc. inside body system. After generation, ROS interact with important biomolecules such as lipid, protein, DNA, carbohydrates etc. and affecting the cellular functions[1].Human cells possess potent defence mechanisms to neutralise harmful effects of free radicals. But, when oxidant-antioxidant balances get disturbed, several disorders including cancer, neurodegenerative diseases, atherosclerosis etc. results[2].

Some natural compounds obtained mainly from fruits, vegetables, beverages, spices etc. such as ascorbic acid, α-tocopherol etc. exert radical detoxification. Major active antioxidant compounds include flavonoid, anthocyanins, catechin, lignans, catechol etc. A positive correlation between antioxidant activity and total phenolic content of plant extracts has been reported[3,4].

Lycoperiscon esculentum is used in Indian routine cuisine. Lycopene is the major dietary antioxidant found in *Lycoperiscon esculentum*. Lycopene possesses greatest quenching capability to scavenge singlet oxygen[5]. Tomato also contains flavonoids which are important free radical scavenger and their consumption is associated with a reduced risk of cancer[6]. Tomato varieties have already been screened for presence of total lycopene, ascorbic acid[7]. Tomato is available year-round and is part of daily diet in India. Hence, it was thought to check and do a comparative study on the presence of total phenolic and flavonoid content and free radical scavenging activities of two different tomato varieties mainly grown in Khandesh region of Maharashtra, India.

MATERIAL AND METHODS

1,1 – Diphenyl -2- picryl hydrazyl (DPPH) and quercetin were purchased from Sigma Chemical Co. (St., Louis, USA). 2- deoxy-d- ribose, nitroblue tetrazolium (NBT), naphthyl-ethylene-diamine-dihydrochloride, sodium nitropruside, butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT), thiobarbituric acid (TBA), sulphanilamide and curcumin were purchased from S.d. Fine-Chem Ltd., Mumbai, India. Ferric chloride, L-ascorbic acid sodium carbonate, phenazine methosulphate (PMS), ethylene diamine tetra acetic acid (EDTA) disodium salts, β- nicotinamide adenine dinucleotide (NADH) were purchased from Hi- media, Mumbai, India. Organic solvent used in the extraction process was of AR grade. All other chemicals and reagents used in the present study were of analytical grade.

Collection of Vegetable

The two varieties of *Lycoperiscon esculentum* namely S-22 and Samrudhi were collected from farmers at their full ripening stage (red) during April, 2008. The S-22 variety (rounded shaped) is characterized by a large - medium size fruit with three to four prominent lobes and has an average fruit weight between 120-130 g and Samrudhi variety (high-pigment hybrid) is a small-size fruit with three to four lobes and pointed end and has an average fruit weight between 40-60 g.

Preparation of Crude Extracts

Freshly harvested, healthy fruits at uniformly ripened (red ripen) stage were used for analysis. About hundred g fruit of each cultivar was extracted in 400 ml. of 99.99% methanol by maceration (96 h). The final extract was passed through No. 1 Whatman filter paper (Whatman Ltd., England). The filtrate obtained was concentrated under vacuum at 40^0 C and the extracts were freeze dried. The freeze dried extracts were stored at – 20^0 C for further use. The crude extract was obtained by dissolving a known amount of dry extract in 99.99% methanol and double distilled water to obtain a stock solution of 200µg/ml. and 2000 µg/ml. concentration respectively.

Preliminary Phytochemical Analysis

Preliminary phytochemical analysis of extracts was done using standard qualitative methods (colour test) to check presence of major phytoconstituents[8].

In vitro Antioxidant Study

DPPH Radical scavenging Activity

The free radical scavenging activity of methanol extract of tomato was measured by using stable radical DPPH according to the standard method[11]. The percentage of scavenging DPPH radical by sample extracts and butylated hydroxyl toluene (BHT) as positive control was calculated using the following equation:

$$\text{DPPH. Scavenged (\%)} = [(A_{cont} - A_{test}) / \text{Acont}] \times 100$$

Where, A_{cont} is the absorbance of control reaction and A_{test} is the absorbance in presence of sample of the extracts and standard compound.

Nitric Oxide Radical Scavenging Activity

The scavenging effects of methanol extracts from fruits and curcumin as positive control on NO were determined according to the method of Sreejayan and Rao[12]. The percentage of nitric oxide radical scavenging for sample extracts and curcumin was calculated using the following equation:

$$\text{NO Radical Scavenged (\%)} = [(A_{cont} - A_{test}) / A_{cont}] \times 100$$

Where, A_{cont} is the absorbance of control reaction and A_{test} is the absorbance in presence of sample of the extracts and reference compound.

Hydroxyl Radical Scavenging Activity

Hydroxyl radical scavenging activity of sample extracts were measured according to the method of Halliwell[13]. The hydroxyl radical scavenging activity of extracts reported as % inhibition of deoxyribose degradation and was calculated as

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

Determination of Superoxide Anion Radical Scavenging Activity

Superoxide anion radical scavenging activity of extracts was measured according to the established method of Robak with slight modification[14]. The percentage inhibition of superoxide anion generation was calculated using the following formula:

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

Measurement of Reductive Ability

Reductive ability of sample extracts and reference compound was measured according to the established method of Oyaizu[15]. Reductive ability of sample extracts was expressed as ascorbic acid equivalent (AAE) in mg per g of dry material.

Determination of Total Phenolics

The amount of total phenolics in the selected tomato varieties was determined with Folin – Ciocalteu reagents using the established method of Singleton and Rossi[9]. The amount of total phenolic content was expressed in terms of gallic acid equivalents (GAE) (mg/g of dry material).

Determination of Total Flavonoids

For the determination of total flavonoid content of sample extracts, the aluminium chloride colorimetric method was used[10]. Total flavonoid contents of the extracts were expressed in terms of mg quercetin equivalent per g of dry material.

Statistical Analysis

Data were generated by performing each assay in two replicates and values are expressed as mean ± standard deviation. The IC_{50} values were determined from linear regression analysis.

RESULTS AND DISCUSSION

Preliminary phytochemical analysis

Preliminary phytochemical analysis of crude methanolic extract of *Lycoperiscon esculentum* indicated the presence of alkaloids, flavonoids, lycopene, Vitamin C, which are potent free radical scavenger.

Antioxidant activity

Hydroxyl, superoxide, nitric oxide radicals are generated mainly by reduction of oxygen and some intermediate products. Inhibition of DPPH, superoxide, hydroxyl, nitric oxide radical generation was determined by decrease in its absorbance in presence of antioxidants. With increase in concentration of crude methanolic and aqueous tomato extracts, quenching of several radicals in reaction mixture also increased. Between the two tomato varieties, S-22 has higher capacity to scavenge several free radical generations in methanolic fraction as compared to its aqueous fraction. Inhibition of several free radical generations occurs in a dose dependent manner but after optimum concentration of crude extract, with further increase in dose, no change in scavenging radical generation has observed. Minimum concentration of crude methanolic tomato extract is required to scavenge 50% generation of DPPH radical than other free radicals. In both the solvent fractions

Superoxide Anion Radical Scavenging (%) = $[(A_{cont} - A_{test}) / A_{cont}] \times 100$

Table 1: Comparative account of antioxidant profile [IC_{50} values (µg/ml)] of two tested varieties of *Lycoperiscon esculentum* in two different solvent fractions

Solvent used	Variety	IC_{50} values (µg/ml) (Antiradical activity)			
		$DPPH^{-}$	NO^{-}	OH^{-}	O_2^{-}
1. Methanol	S-22	115.92±0.3	236.61±0.1	274.96±0.06	142.90±0.2
Samrudhi		123.70±0.2	249.59±0.1	341.52±0.07	172.09±0.1
2. Aqueous	S-22	1001.94±0.02	2835.02±0.09	738.55±0.06	670.25±0.16
Samrudhi		1009.66±0.05	3256.89±0.1	886.01±0.07	687.22±0.12

[Values are mean ± Standard deviation of two replicates]

S-22 variety showed significant activity to quench DPPH, hydroxyl and superoxide radical generation in reaction mixture over Samrudhi variety where Samrudhi variety exerted promising activity to inhibit nitric oxide radical generation (Table 1). S-22 variety in its methanolic solvent fraction requires 115.92µg/ml, 274.96 µg/ml and 142.90 µg/ml concentrations for 50% inhibition of DPPH, OH and superoxide radical respectively. But, 236.61 µg/ml of Samrudhi variety in methanol fraction is required for 50% inhibition of nitric oxide radical. Comparatively high concentration of fruit extracts were required to inhibit the different free radical generation in their respective aqueous fraction. 1001.94 µg/ml, 738.55 µg/ml and 670.25 µg/ml concentrations of S-22 variety in aqueous fraction were effective for scavenging 50% of DPPH, OH and superoxide radical respectively and 2835.02 µg/ml concentration of Samrudhi variety in aqueous solvent fraction was efficient in 50% inhibition of nitric oxide radical.

In comparison, metahnolic fraction of both the variety extracts possessed higher activity than aqueous extract. Several synthetic antioxidants such as BHA, BHT, ascorbic acid, curcumin etc. were used as reference standard in free radical scavenging assay and it was observed *Lycoperiscon esculentum* have less radical scavenging potential than several synthetic antioxidants.

In one report, author had mentioned, water extract of tomato at 200ìg/ml concentration inhibit almost 20% of nitric oxide and DPPH radical[16]. The values are quite similar to our observation in case of aqueous extract of tomato varieties but values are not in agreement in case of methanolic fraction of both the varieties, because of different solvent system used for extraction in respective studies.

Reducing power ability

Reducing power ability of two tomato extracts was evaluated as mg AAE/g of dry material. Transformation of ferric ion to ferrous ion in presence of antioxidant occurs in a dose dependent manner. S-22 variety showed greater reducing power ability than Samrudhi variety (Table 2).

Amounts of total phenolic and flavonoids contents

Phenolic and flavonoid compounds have free radical scavenging ability. Mainly phenolic compounds terminate free radical generating chain reactions and flavonoid compounds result in chelation through which they detoxify several free radicals. In the present study, S-22 variety possessed higher amount of total phenolic and total flavonoid content than Samrudhi in methanolic and aqueous fractions (Table 2). In the present study it was also observed that, almost equal amount of phenolic and flavonoid compounds are present in each extract in their methanolic fraction. In case of both the varieties, phenolic contents, extracted in methanolic fraction was in range of 32.60-35.27 mg of GAE/g of dry material and total flavonoid contents were in the range of 32.33-36.83 mg/g of dry material.

Table 2: Comparative account of two tomato fruit cultivars in two different solvent fractions in response to their reducing power ability, total phenolic and total flavonoid contents

Tomato variety (AAE mg/g)	Reducing power Ability (mg of GAE/g)	Total phenolic content (mg of QE/g)	Total flavonoid content
S-22			
Methanol	43.78±0.06	35.27±0.02	36.83±0.01
Water	6.3±0.01	30.37±0.04	28.50±0.02
Samrudhi			
Methanol	37.40±0.01	32.60±0.02	32.33±0.03
Water	1.65±0.01	19.93±0.01	22.33±0.01

[Values are mean ± Standard deviation of two replicates]

CONCLUSION

Plant polyphenolic compounds mainly terminate chain reaction of lipid peroxidation, scavenge several types of free radicals and possess antioxidant activity. Human being mainly consumed polyphenolic compounds from dietary sources including fruits and vegetables. Antioxidant in diet work best in combination of individual antioxidants[17]. Higher phenolic and flavonoid contents in tomato varieties may be responsible for antioxidant capacity on hydroxyl, superoxide, DPPH radical scavenging model.

Out of the two varieties selected for study and cultivated in India, S-22 variety showed more antioxidant potential than Samrudhi based upon various *in vitro* antioxidant assays. Methanolic extract has more antioxidant activity than aqueous, mainly because of more extraction of antioxidant compounds in methanol than water. Hence, it is advisable that, S-22 variety is highly rich in natural antioxidants and can be consumed safely.

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