

***In vitro* Evaluation of Free Radical Scavenging Activity of *Solanum lycopersicum* (Tomato) Fruit Extract**

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Abstract: The present study aimed to evaluate the *In vitro* antioxidant and free radical scavenging potential of methanolic extracts of *Solanum lycopersicum* (tomato) fruit extract. The antioxidant activity determinate by hydroxyl radical scavenging method. The *Solanum lycopersicum* fruit extract have dose depended antioxidant activity. Methanol extract was found to be good solvent for extraction and having good antioxidant activity. IC₅₀ value of *Solanum lycopersicum* is 20.0 µg/ml. The reducing power of extract was carried out with ascorbic acid as a standard reducing agent. All the analysis was made with the use of UV-Visible Spectrophotometer (UV -1601 SHIMADZU). In this plant *Solanum lycopersicum* fruit extract there was a remarkable concentration dependent free radical scavenging and reducing power was exhibited. These findings demonstrated that *Solanum lycopersicum* possess free radical and hydroxyl radical scavenging activity as well as antioxidant activity *in vitro*. In conclusion the present study indicates that *Solanum lycopersicum* may be a potential source of natural antioxidant.

Key words: Antioxidant activity • Hydroxyl radical • Ascorbic acid • *Solanum lycopersicum* • TBARS

INTRODUCTION

Solanum lycopersicum (tomato) is the second most produced and consumed vegetable nationwide and it is a rich source of lycopene, beta-carotene, potassium, vitamin C, flavonoids and vitamin E [1,2]. Over 80% of the lycopene in the American diets come from tomato itself and tomato-derived products such as ketchup, tomato paste and sauce [3]. Some epidemiological and experimental data suggest an inverse relation between intake of tomato and risk of cancer at various anatomical sites, especially prostate and colon [4, 5, 6]. Besides its potential role in preventing and treating cancer, tomato intake has also been studied for use in the prevention of atherosclerosis [7, 8], reduction of asthma symptoms [9, 10] as hypolipidemic effect [11], inhibitor of the

angiotensin converting enzyme [12], present spasmolytic activity [13], hypoglycemic activity [14] and decrease of DNA strand breakages of cells of the immune system [15, 16]. The importance of these plants has promoted their inclusion in Brazilian Pharmacopoeia [12]. The tomato effects may be related mainly to lycopene which acts on biological mechanisms altering the oxidant status and could be responsible for its positive protective actions [3, 2]. A proposed mechanism of lycopene action includes both inhibition of tumor growth by decrease or loss in junctional cell communication [17]. However, the most widely accepted theory is the antioxidant effects of lycopene acting as a scavenger for singlet oxygen, hydrogen peroxide and nitrogen dioxide that are associated with DNA damage and the development of cancer [18, 2].

In recent years, the role of the diet in preventing the occurrence of cancer has been a popular and important area of research. The examination of the role of other carotenoids, specifically beta carotene, in preventing cancer began in the 1920s. However, research in lycopene began from the late 1980s when it was found that the antioxidant activity of lycopene was twice that of beta carotene [19, 20]. After that several studies suggest that diets rich in tomato intake may account for a reduction in the risk of several different types of cancer. The strongest evidence is for a protective effect against cancers of the lung, stomach and prostate gland [21]. People who have diets rich in tomatoes, which contain lycopene, appear to have a lower risk of certain types of cancer, of the prostate, lung and stomach. Oxidative stress is recognized as one of the major contributors of increased risk of cancer and in chemical assays, lycopene is the most potent antioxidant among various common β -carotenoids [22]. Lycopene can trap singlet oxygen and reduce mutagenesis in the Ames test. The antioxidant activity of β -carotenoids in multilamellar liposomes has been assayed by inhibition of formation of thiobarbituric acidreactive substances [23]. The purpose of this study was to evaluate the antioxidant activity of *Solanum lycopersicum* fruit extract as new potential sources of natural antioxidants. The antioxidant activities were determined by *in vitro* assays: inhibition of hydroxyl radicals by TBRAS system.

MATERIALS AND METHODS

Plant Material: Aerial parts of *Solanum lycopersicum* fruit was collected in the early stages of vegetation from the Bhopal (M.P.), India. The identification of the plant *Solanum lycopersicum* (family: *Solanaceae*) was done by botanist Dr. S.S. Khan (Voucher Specimen No: WR/101/LGOB/2006), Department of Botany, Safia Science College, Bhopal, Madhya Pradesh India.

Reagent and Authentic Sample: The reagents used were of highest purity (>99.95%) and were purchased from Sigma Chemical Co. (Germany). Sample absorbance was read using a Lambda 532 nm, UV - 1601 Spectrometer Shimadzu (Japan).

Preparation of Extract: Dried powdered of *S. lycopersicum* (10 g) were extracted by continuous mixing in 100 ml 50% methanol, 24 h at room temperature. After filtration, methanol was evaporated

until only water remained through evaporation on water bath at 60-70°C temperature. The dried powder was kept in air tied box.

Deoxyribose Assay to Assess OH⁻ Radical Scavenging

Activity: The OH⁻ radical scavenging activity of *Solanum lycopersicum* (tomato) fruit extract (10–100 μ g/ml) was determined according to the deoxyribose method of Halliwell *et al.* [24] in the presence of 100 IM EDTA, FeCl₃, H₂O and ascorbic acid were prepared in degassed H₂O prior to use. The reaction tube contained (final concentrations) 3.6 mM deoxyribose, 100 IM EDTA, 1 mM H₂O₂, 100 IM L- ascorbic acid, 100 IM FeCl₃, H₂O in 25 mM phosphate buffer, pH 7.4 in 1.0 ml total volume. Follow in incubation at 38°C, 1 hrs, 1.0 ml 1.0% TBA in 0.05 M NaOH and 1.0 ml 10% TCA were added to the reaction mixture which was then heated in a boiling water bath for 15 min. Once samples were cooled, the absorbances were read at 532 nm. The IC₅₀ value of the crude extract was compared with that of ascorbic acid, which was used as the standard. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The percent inhibition of hydroxyl radical was calculated as follows:

$$\% \text{ Inhibition} = \frac{\text{Abs: 532 nm Control Abs.} - \text{532 nm sample Abs.} \times 100}{532 \text{ nm Control Abs}}$$

Antioxidant capacity of test compounds was expressed as IC₅₀, the concentration necessary for 50% inhibition concentration of TBARS.

RESULT

Our study shows that antioxidant activity of *S. lycopersicum* extract using fenton reaction. The antioxidant activities of *S. lycopersicum* (tomato) extract scavenge OH⁻ radical was assessed using the fenton reaction assay. Extent of hydroxyl radical scavenged was determined by the decrease in intensity of pink coloured chromophore in the form of IC₅₀ values which was determined at 532 nm. Lower IC₅₀ value represents higher antioxidant activity. The dose dependent inhibition of TBARS formation at the different concentration of *S. lycopersicum* extract. ranging from 10 to 100 μ g/ml. The antioxidant activity was compared with ascorbic acid as positive control. The IC₅₀ values of ascorbic acid and *S. lycopersicum* extract were found to be 19.0, 20.0 μ g/ml, respectively. The results are summarized to Table 1.

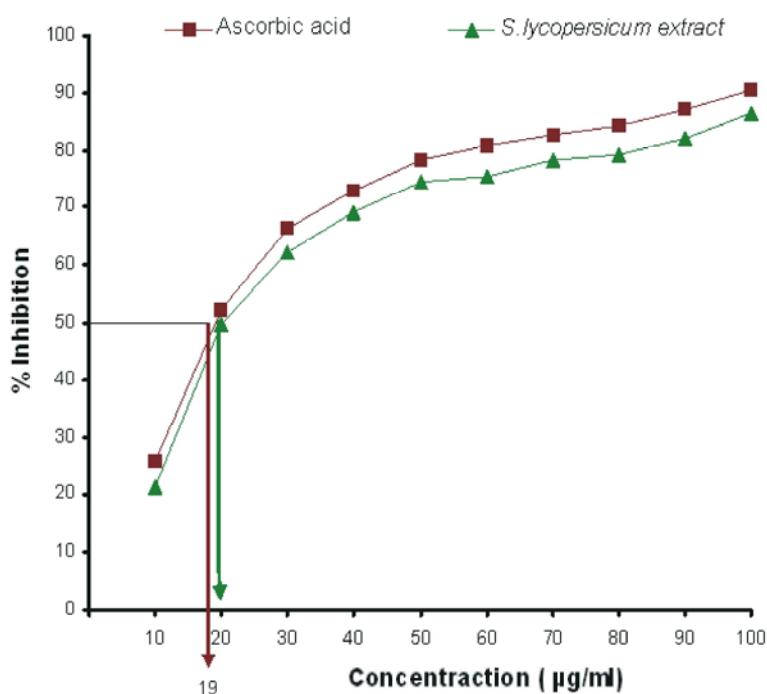
Table 1: Antioxidant activity of *S. lycopersicum* (tomato) extract

Concentration ($\mu\text{g/ml}$)	O.D. at 532 nm		% inhibition (TBRAS)	
	Ascorbic acid	<i>S. lycopersicum</i> (tomato)	Ascorbic acid	<i>S. lycopersicum</i> (tomato)
10	0.208	0.221	25.71	21.42
20	0.134	0.141	52.14	49.64
30	0.094	0.106	66.42	62.14
40	0.076	0.086	72.85	69.28
50	0.061	0.071	78.21	74.64
60	0.054	0.069	80.71	75.35
70	0.048	0.061	82.85	78.21
80	0.044	0.058	84.28	79.28
90	0.036	0.050	87.14	82.14
100	0.026	0.038	90.71	86.42

Control OD at 532 nm – 0.280

IC₅₀ values:

S. No.	Group	IC ₅₀ Values
1.	Ascorbic acid	19.0 ($\mu\text{g/ml}$)
2.	<i>S. lycopersicum</i> extract	20.0 ($\mu\text{g/ml}$)

Fig. 1: Antioxidant activity of *S. lycopersicum* fruit extract with IC₅₀ values

The OH⁻ radical scavenging activity of *S. lycopersicum* is given in Fig. 1. This activity was increased by increasing the concentration of the sample extract. OH⁻ radical antioxidant assay is based on the ability of OH⁻ radical, a stable free radical, to decolorize in the presence of antioxidants. The OH radical contains an odd electron, which is responsible for the absorbance at 532 nm and also for a visible deep pink color. When OH accepts an electron donated by an antioxidant compound, the OH is decolorized, which can be

quantitatively measured from the changes in absorbance. The IC₅₀ value of the extract was 19.00 $\mu\text{g/ml}$, as opposed to that of ascorbic acid (IC₅₀ 20.00 $\mu\text{g/ml}$), which is a well known antioxidant.

DISCUSSION

The antioxidative action is one of the important physiological functions of foods, which is supposed to protect living organisms from oxidative damages, resulting

in the prevention of various diseases such as cancer, cardiovascular diseases and diabetes. Food plants including fruits, vegetables and spices are the primary sources of naturally occurring antioxidants for humans [25]. Reactive oxygen species (ROS) contribute to a great variety of diseases. ROS including hydrogen peroxide, super oxide radical anion, nitric oxide and singlet oxygen react with biological molecules leading to cell and tissue injury [26]. Plants exhibit efficient antioxidant activity owing to their phenolic constituent [27]. It is believed that lycopene is a powerful antioxidant, a compound that blocks the action of activated oxygen molecules, known as free radicals that can damage cells [20, 19]. It is reported in the literature the antioxidant activity of lycopene is at least twice as great as beta carotene, another carotenoid that is also thought to be an effective cancer preventing nutrient [20, 19]. Lycopene is considered one of the more effective antioxidants because it is not converted to vitamin A when ingested. Conversion to vitamin A weakens the antioxidant properties of carotenoids like beta carotene. Antioxidant activity of *S. lycopersicum* (tomato) extract and the possible mechanism involved was investigated by us using fenton reaction.

The ability of *S. lycopersicum* extracts to scavenge OH[•] radical was assessed using the Fenton reaction assay. The *S. lycopersicum* (tomato) extract was found to have antioxidant activity and it was compared with ascorbic acid as a positive control. The IC₅₀ values of ascorbic acid and *S. lycopersicum* extract were found to be 19.0 and 20.0 µg/ml, respectively. Lower IC₅₀ value represents higher antioxidant activity.

Studies have been reported that several naturally occurring compound exhibited antioxidant activity. These include *Soyimida febrifuga* extract using from DPPH scavenging and Nitric oxide scavenging method, shows that methanol extract has greater antioxidant activity and is comparable with standard drug ascorbic acid [28], the *B. variegata* was reported the antioxidant activity using deoxyribose assay to assess OH[•]-radical scavenging activity [29]. Antioxidant activity was also reported *Jasminum sambac* extract using DPPH radical scavenging, Nitric oxide scavenging and fenton reaction, shows that extract has higher antioxidant activity [30]. The exact mechanism of protection is however unknown but *S. lycopersicum* (tomato) extract an active principal lycopene which have been shown to be able to participate in various mechanism of the chemoprevention virtue are acting as a neutrophills an antioxidant. Several mechanisms may contribute to protection such as scavenging of potentially toxic

electrophills and free radicals and modification of enzyme profile to inhibit that enhance the detoxification pathway.

In conclusion the results of this study demonstrated that using *in vitro* model *S. lycopersicum* (tomato) was found to have antioxidant activity. This activity was found due to presence of lycopene, beta-carotene, folate, potassium, vitamin C, flavonoids and vitamin E [1, 2]. Overall *S. lycopersicum* (tomato) can be considered as a model compound for the experimental studies including free radical induced disorders like cancer, diabetes, atherosclerosis etc. Further studies are required to establish its *in vivo* antioxidant activity using different animal models. These results are important because *S. lycopersicum* (tomato) is one of the important vegetable used in Indian diets.

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