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Research Article

Free Radical Scavenging Activity of Ethanolic Extract of *Citrus paradisi* and Naringin -An *In vitro* Study

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ABSTRACT

The present study attempts to find naturally-occurring antioxidants of fruit-based which give efficacy by additive activities. *Citrus paradisi*, known as Grape fruit contains significant bioactive components such as Naringin. The present study examines the free radical scavenging activity of ethanolic extract of *Citrus paradisi* and Naringin. The study was carried out with different radical scavenging assays like hydroxyl, DPPH, hydrogen peroxide, nitric oxide, super oxide. *Citrus paradisi* extracts showed lower radical scavenging activities in assays such as DPPH, superoxide and hydroxyl when compared with Naringin. Naringin showed the higher radical scavenging effect with nitric oxide, and hydrogen peroxide in comparison with *citrus paradisi* extract. However, both were analysed by using ascorbic acid as standard. The current study gives evidence that both showed potential free radical scavenging activity.

Keywords: Ascorbic acid, *Citrus paradisi***,** Hydroxyl radical, Naringin, DPPH.

INTRODUCTION

Reactive oxygen species (ROS) or free radicals are produced from both exogenous and endogenous sources. The best instance for that sources are electron's leakage from the mitochondrial electron transport chain (ETC), auto-oxidation (dopamine, adrenaline etc.), activities of enzymes (NADPH oxidase, xanthine oxidase etc.), exposure to specific chemicals (cigarettes, doxorubicin etc.), catalytic action of free transition metals (Cu+, Fe2+ etc.) and environmental radiation (UV, radon etc.)[1]. It has been evaluated that one free radical is generated for every -25 molecules of oxygen which is decreased by normal respiration.

Increasing ROS shows oxidative stress, as a result, oxidative DNA damage that is incriminated in the pathogenesis of various disorders like reperfusion injury, an inflammatory disorder, diabetes, cancer, atherosclerosis, neurodegenerative and cardiovascular diseases[2,3]. Many antioxidants have progressed with defensive roles in opposition to those damages[4]. Numerous synthetic antioxidants like propyl gallate (PG), butylated hydroxyl toluene (BHT) and butylated hydroxy anisole (BHA) have been utilised to detain the process of oxidation however, the synthetic antioxidants use should be under uncompromising regulation in order to promote health hazards. The search for natural antioxidants as alternatives is high interest among academicians. The consumption of a diet rich in vegetables and fresh fruits has been related to various benefits for health which include the chronic diseases prevention. Those health benefits are chiefly because of phytochemicals present which include vitamin C and E, carotenoids, and polyphenols [5], those phytochemicals that scavenging OH. Out of them, the flavonoids in plants exhibit a broad range of biological activities, basically, flavonoids have been described as higher potential antibacterial and antioxidant activity in opposing the ROS species[6,7].

Naringin(4´,5,7-trihydroxyflavanone-7-β-Lrhamnoglucoside-(1,2)-α-D-glucopyranoside) is chief flavanone that is found in the peel and juice of *Citrus paradisi.* It is noticed that the Naringin shows ability to scavenge free radicals, higher antioxidant properties and acts as good metal chelating agent[8]. Further, it is identified as anti-mutagenic,anti-lipid peroxidation and Parkinson disease, reduce the radiation-induced the formation of micronuclei andchromosomal breakdown etc[9].

Scientific information with respect to radical scavenging activity of ethanolic extract of *Citrus paradisi* and Naringin is scare because the availability is limited to certain regions and only known by local populations. The analysis of properties of such plant and Naringin is still useful and interesting task specifically to examine promising sources from nutraceuticals and functional foods. Hence the present research attempts to carry out this gap.

MATERIALS AND METHODS

Plant materials collection

The raw *Citrus paradisi* was collected from Supermarket and authenticated by Dr. P. Jayaraman, Director of National Institute of Herbal Science, Plant Anatomy Research Centre, Tambaram. The fruits were cautiously chosen due to get a uniform batch in respect to their degree of maturity and size. Naringin was purchased from Sigma-Aldrich Co.

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Table 1: Comparison of free radical scavenging activity of *Citrus paradisi* extract, Naringinand Ascorbic acid.

S.No	Free radical scavenging methods	IC50 Values(µg/ml)		
		Citrus paradisi extract	Naringin	Standard (Ascorbic acid)
1.	DPPH radical	382 ± 2.00	80 ± 2.00	212 ± 2.00
2.	Superoxide radical	194 ± 2.00	104±2.00	114 ±2.00
3.	Nitric oxide radical	138 ± 2.00	58 ±2.00	72 ± 2.00
5.	Hydrogen peroxide radical	124 ± 2.00	70 ±2.00	50 ± 2.00
6.	Hydroxyl radical	376 ± 2.00	70 ±2.00	188 ± 2.00

The chemicals used such as 2,2-diphenyl,1-picrylhydrazyl (DPPH) were obtained. In addition to this, some of the major chemicals were used in this experiment such as NADH, NBT, EDTA, TCA, and PMS, Tris HCL, Hydrogen peroxide, Deoxy ribose, TBA and ascorbic acid were of analytical grade.

Sample extraction

The skin of fruits *Citrus paradisi* was peeled off and seeds were removed. The pulp of the fruit was taken and cut into small pieces, dried and later made into powder form.Later the dried powder was extracted with water, ethanol and ethyl acetate by utilizing soxhlet apparatus. Further, the extract was preserved in refrigerator via glass bottle throughout the experimental period.

DPPH radical scavengingactivity

In order to examine the effect of scavenging activity of ethanolic extract of *Citrus paradisi* and Naringin on DPPH radicals was examined as per the procedure of Koleva el at.[10]for *citrus paradisi* extract and Prieto et al.[11]for Naringin. The procedure of an aliquot of sample solution (100 - 500µg/ml) was mixed with the solution of reagent (0.6 M Sulfuric acid, 28 mM Sodium Phosphate and 4 mM Ammonium molybdate). Ethanol was considered as a standard instead of a sample. The respective tubes were closed and incubated in boiling water at 950˚C for 60-90 mins. Later the samples were cooled to room temperature and the absorbance rate was noted at 695nm along with the standard solution in as pectrophotometer.

The scavenging effect of ethanolic extract of citrus paradisi was carried out by Koleva et al.[10] 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) was used and various concentrations of (50, 100, 150, 250, 500 µg/ml) ethanolic extract of *citrus paradisi* was added with an equal volume of DPPH ethanolic solution (100 µM). Later the samples were cooled at room temperature (15 mins), the rate of absorbance was measured at 517 nm. Ascorbic acid was used as control. DPPH scavenging activity was determined and IC_{50} values were calculated.

(%) inhibition

$$= \frac{\text{Abs (control)} - \text{Abs (sample)} \times 100}{\text{Abs (control)}}$$

Where Abs (control): Absorbance of the control and Abs (test): Absorbance of the extracts/standard.

Superoxide free radical scavenging activity

The Naringin scavenging effect on superoxide free radical activity was measured as by the procedure developed by Hyland *et al.*[12]. The reaction mixture contains various concentrations of Naringin(100-500µg/ml), alkaline DMSO and nitro blue tetrazolium. The standard was taken as DMSO. The absorbance was read at 560 nm by utilizing a UV-Visible double beam spectrophotometer. While for *citrus paradisi* extract, the method of Liu et al. (1997) was used. Radicals of superoxide are produced in Nicotinamide adenine dinucleotide (NADH)- phenazinemethosulphate (PMS) systems by NADH oxidation and determined by the Nitro Blue Tetrazolium (NBT) reduction. Various concentrations of (50, 100, 150, 250, 500 µg/ml) *citrus paradisi* extract were added in different test tubes. Radicals of Superoxide were produced by 1 ml of PMS (10 µM), 1 ml of NBT (50 µM), 1 ml of Tris-HCl buffer (16 mM, pH-8) and 1 ml of NADH (78 µM) solution. The reaction mixture was incubated for 5 min at 25°C and the absorbance was recorded at 560 nm. Various concentration of ascorbic acid was used as a standard. The percentage inhibition of superoxide free radical scavenging activity was calculated as follows:

Nitric oxide radical scavenging activity

Nitric oxide radical scavenging activity of *citrus paradisi* extract and Naringin was measured by Green *et al.*[13]. 3ml of the reaction mixture (CA) containing sodium nitroprusside in PBS and sample was incubated at 25°C for 150 minutes. Controls were kept without test compound in an identical manner. After incubation, 0.5 ml of Griess reagent was added. The absorbance of the chromophore formed was read at 546nm. The percentage inhibition of nitric oxide generation was measured by comparing the absorbance values of control and those of test compounds.

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity of ethanolic extract of *citrus paradisi* and Naringin was determined by the method of Halliwell*et al.*[14]. Ascorbic acid (1mM), EDTA Stock solutions (1mM), deoxyribose (10mM), H2O2 (10mM) and FeCl3 (10 mM) were prepared in deionized distilled water. The assay was performed by adding 0.36 ml of deoxyribose, 0.1 ml of EDTA,0.1 ml of H_20_2, Naringin concentration of(100-500µg/ml), 0.01 ml of FeCl3, 1.0 ml of *citrus paradisi* extract concentrations of (50, 100, 150, 250, 500 µg/ml) 0.1ml of ascorbic acid and 0.33 ml of phosphate buffer (50 mM, pH 7.4) in sequence. The mixture was later incubated at 37°C for 1hr. 1.0 ml portion of the reaction mixture was mixed with 1.0 ml of 0.5% TBA and 10% TCA to produce the chromogen in pink and read at 532 nm.

Radical scavenging activity of Hydrogen peroxide

The radical scavenging activity of hydrogen peroxide of ethanolic extract of *citrus paradisi* and Naringin was carried out by adopting the method recommended by Ruch *et al.*[15] Different concentration *citrus paradise* (50, 100, 150, 250, 500 µg/ml) and Naringin of (100-500 µg/ml) was dissolved in 0.1M phosphate buffer at 3.4 ml of pH 7.4 and

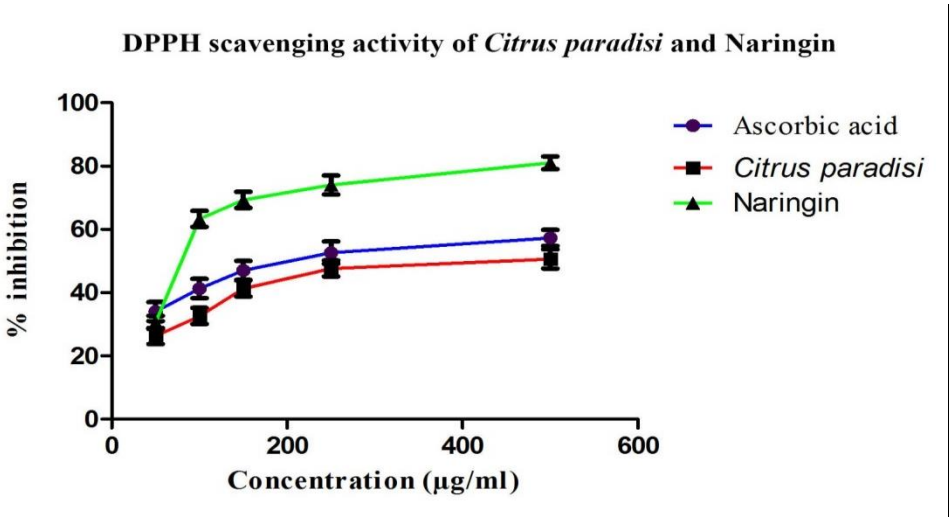

Figure 1: DPPH scavenging activity of *Citrus paradisi* and Naringin with Ascorbic acid.

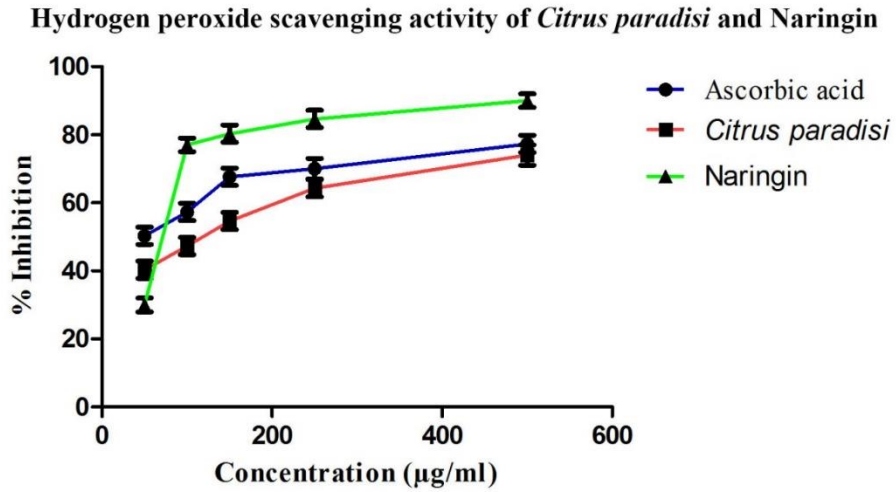

Figure 2: Hydrogen peroxide scavenging activity of *Citrus paradisi* and Naringin with Ascorbic acid.

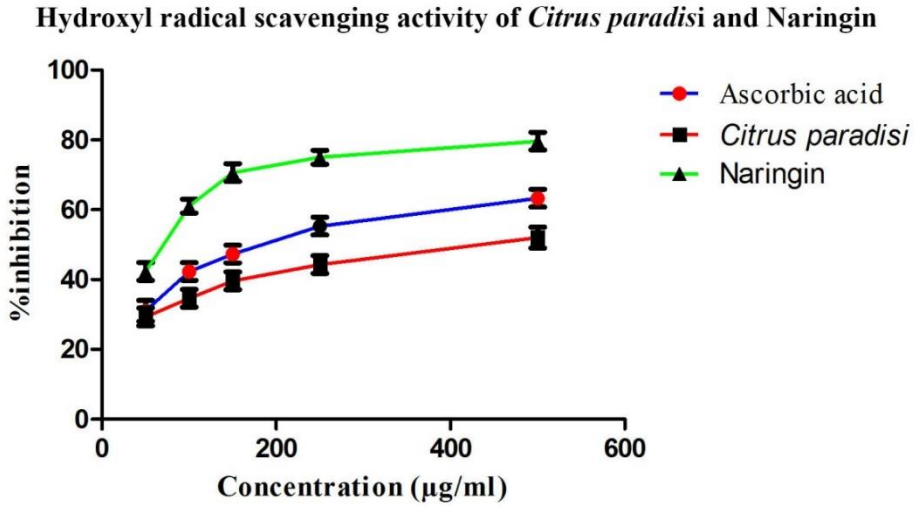

Figure 3: Hydroxyl radical scavenging activity of *Citrus paradisi* and Naringin with Ascorbic acid.

stirred with 600µl of 43 mM solution of hydrogen peroxide. The absorbance was recorded at 570nm for a separate standard was utilised for background subtraction. For statistical analysis, data were examined by using Sigma Plot (version 11.0). The findings were expressed as standard deviation (mean ± SD) and the IC_{50} values were obtained from the plots of linear regression. Measurements

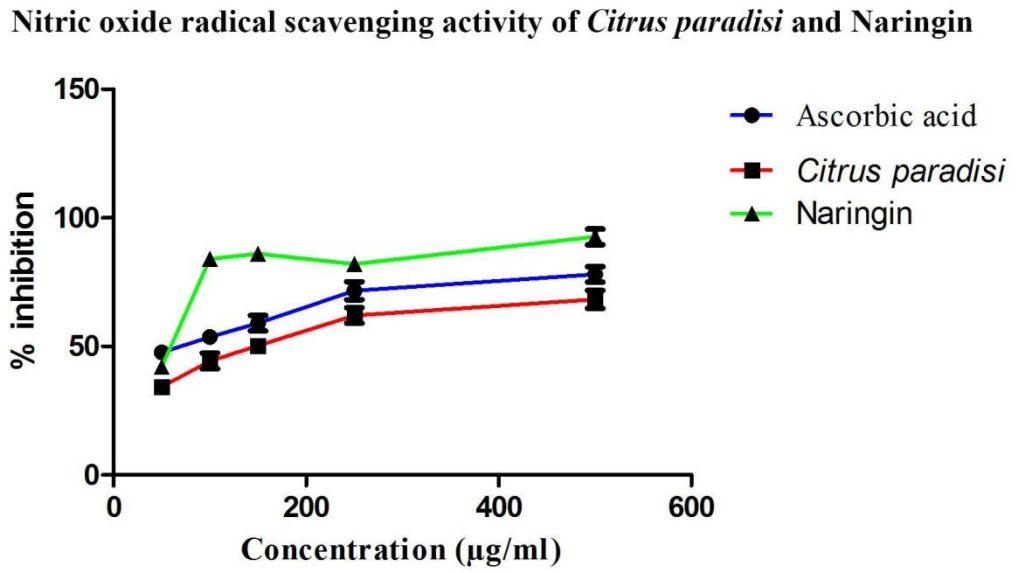

Figure 4: Nitric oxide radical scavenging activity of *Citrus paradisi* and Naringin with Ascorbic acid.

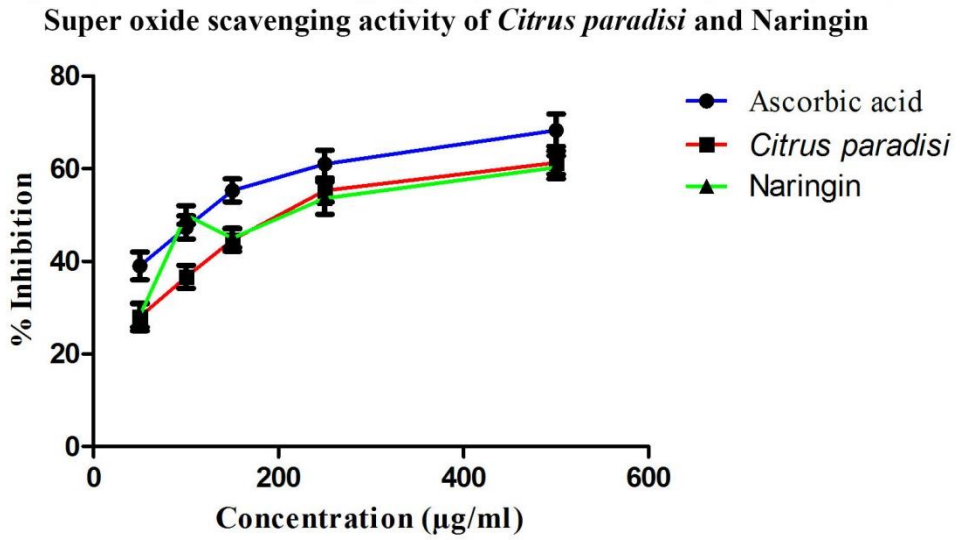

Figure 5: Super oxide scavenging activity of *Citrus paradisi* and Naringin with ascorbic acid.

of IC50 Values and graphs were carried out by utilising Graph Pad Prism Software 6.0.

RESULTS AND DISCUSSION

DPPH radical scavenging activity

In the free radical scavenging assays, DPPH possesses a distinctive absorbance at 517 nm, which significantly decreases on exposure to radical scavengers by donating a hydrogen atom to become a stable diamagnetic molecule[16]. The principle of the reduction of DPPH free radical is that the antioxidant reacts with the stable free radical DPPH and converts it to 1, 1- diphenyl-2-picryl hydrazine[17]. DPPH free radical has been widely used to test the free radical scavenging ability of *citrus paradisi* and Naringin. The antioxidant present neutralizes the DPPH by the transfer of an electron or hydrogen atom. The reduction capacity of DPPH could be determined by colour changes from purple to yellow by reading at 517 nm. The ethanolic extract of *citrus paradisi* and Naringin demonstrated H-donor activity in our study. The DPPH radical scavenging activity of extracted material of *citrus paradisi* and Naringin was detected and compared with standard ascorbic acid. The extract and Naringin tested against DPPH stable radical revealed that the radical scavenging activity of *Citrus paradisi* extract and Naringin possess good antioxidant capacity which was shown in (figure 1). The IC_{50} values of ethanolic extract of *Citrus paradisi* were found at the concentration of 382 µg/ml and Naringin exhibited80 µg/ml concentrations while the standard ascorbic acid at 212µg/ml (Table 1).

Hydrogen peroxide radical scavenging assay

The ethanolic extract of *Citrus paradisi* effectively reduced the generation of hydrogen peroxide. IC_{50} value of this extract showed hydrogen peroxide scavenging activity at the concentration of 124µg/ml and for Naringin at concentration of 70 µg/ml, while the standard ascorbic acid

at 50 µg/ml (refer Table 1). The findings showed that *citrus paradisi* extract exhibited moderate radical scavenging activity of hydrogen peroxide and Naringin (figure 2).

Hydroxyl radical scavenging assay

The hydroxyl radical is the most reactive of the ROS which induces severe damage in adjacent biomolecules[18]. The hydroxyl radical can cause oxidative damage to DNA, lipids, and proteins[19]. Iron can stimulate lipid peroxidation by the Fenton reaction and accelerate peroxidation by decomposing lipid peroxide into peroxyl and alcoxyl radical that can themselves abstract hydrogen and perpetuate the chain reaction of lipid peroxidation[4]. Hydroxyl radicals (OH) generated in the human body may play an important role in tissue injury at sites of inflammation in oxidative stress-originated diseases. Hydroxyl radicals were formed in free solution and were detected by their ability to degrade 2-deoxy-2- ribose into fragments formed a pink chromogen upon heating with TBA at low pH. Ferric – EDTA was incubated with H2O2 and ascorbic acid at pH 7.4. The *citrus paradisi* extract and Naringin showed hydroxyl radical scavenging activity. The ethanolic extract of *Citrus paradisi* and Naringin inhibited hydroxyl radical-mediated deoxyribose damage which was assessed by means of the iron dependent DNA damage assay. In the present study, the hydroxyl radical-scavenging effect of the *Citrus paradisi* extract was at concentration of 376 µg/ml and Naringin exhibits at concentration of 70 µg/ml (Table 1) and ascorbic acid was used as a standard for both samples and it is reported to be significantly effective in inhibition of hydroxyl radicals with the concentration of 188 µg/ml (Table 1 and figure 3).

Nitric oxide radical scavenging assay

Nitric oxide is an unstable free radical involved in many biological processes which are associated with several diseases. It reacts with oxygen to produce stable product nitrate and nitrite through intermediates and high concentration of nitric oxide can be toxic and inhibition of over production is an important goal[20]. The ethanolic extract of *Citrus paradisi* effectively reduced the generation of nitric oxide from sodium nitroprusside. IC_{50} value of this extract showed nitric oxide scavenging activity at the concentration of 138µg/ml and for Naringin at the concentration of 58µg/ml while the standard ascorbic acid at 72 µg/ml (Table 1and figure 4). Scavenging of nitric oxide radical is based on the generation of nitric oxide from sodium nitroprusside in buffered saline, which reacts with oxygen to produce nitrite ions that can be measured by using Griess reagent.

Super oxide radical scavenging activity

Superoxide radical is a highly toxic species, which is generated by numerous biological and photochemical reactions[21]. Superoxide radical can further interact with other molecules to generate secondary ROS (e.g., hydroxyl radical, hydrogen peroxide and singlet oxygen), either directly through enzyme or metal catalyzed processes[22]. The result of superoxide radical (SO) scavenging activity obtained for the *citrus paradisi* extract and Naringin showed a dose dependent free radical scavenging activity and the percentage inhibition was shown in (Figure 5). In the present study, the *Citrus paradisi* extract and Naringin was found to be a notable scavenger of superoxide radicals generated in the riboflavin-NBT light system. The scavenging activity of *Citrus paradisi* extract (IC50 value is 194µg/ml) and for Naringin the IC50 values noted at 104 µg/ml was compared with the standard ascorbic acid (114 µg/ml).

CONCLUSION

From the overall findings of this study, both the samples that is Ethanolic extract of *Citrus paradisi* and Naringin show antioxidant activity, though, in comparison with *Citrus paradisi*, Naringin has high activity. These in-vitro analyses demonstrate that *Citrus paradisi* extract and Naringin could serve as a significant natural rich antioxidant and easily obtainable product that may increase the immunity against oxidative damage and other health issues or it may act as a possible source of therapeutic agent.

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