Research Article



International Journal of Pharma and Bio Sciences

ISSN 0975-6299

IN VITRO SCREENING AND IDENTIFICATION OF ANTIOXIDANT ACTIVITIES OF ORANGE (*CITRUS SINENSIS*) PEEL EXTRACT IN DIFFERENT SOLVENTS

S. SWAPNA REKHA AND M. BHASKAR*

Division of Animal Biotechnology, Department of Zoology, Sri Venkateswara University

ABSTRACT

Citrus plants are the rich source of secondary metabolites which includes alkaloids, steroids, tanins, flavonoids, phenols and polyphenols. Having the rich source of polyphenols and natural flavonoids draw attention to screen the phytochemical compounds and their beneficial activities from different solvents. The present investigation explore the *in vitro* antioxidant activity of orange (*Citrus sinensis*) peel extract from different solvents gives reliable information and best extraction procedures to screen the phytochemicals. Among the different solvent extracts methonolic extract exhibits the highest *in vitro* scavenging activity followed by other solvent extracts which includes Ethanolic, Hexane, Benzene, Ethyl acetate, and chloroform with IC_{50} values 65.44,120, 138.45, 151.34, 170.34, 185.35 with 55.6 % inhibitory concentration of ascorbic acid. The screening and scavenging activities of secondary metabolites form different solvents may improve the best extraction procedures and functional activities of beneficial compounds. In this regard to reach a conclusion that methonolic orange peel extract followed by ethonolic extract showed their high potential effectiveness as antioxidant activity when compared to the other solvent extracts.

KEYWORDS: *Citrus sinensis (L)* orange peel, phytochemicals, *in vitro* antioxidants, solvents.



M. BHASKAR Division of Animal Biotechnology, Department of Zoology, Sri Venkateswara *University*

INTRODUCTION

Citrus is one of the most important commercial fruit crops grown in all continents of the world ¹. Citrus family had rich source of phytochemicals such as flavanones, polyphenols, anthocyanins and hydroxycinnamic acids which are beneficial to most pathological conditions which includes, high cholesterol and antiinflammation; complications related to diabetes and cancer prevention. Having rich source of secondary metabolites like natural flavonoids, polyphenols, steroids, saponins etc, in *citrus* sinensis here pinpointing to screening the phytochemicals from different solvents and investigating on in vitro antioxidant activities of orange peel extract. The peel of Citrus fruit having abundant source of flavanones and many polymethoxylated flavones, which are very rare in other plants². These citrus fruit having high natural antioxidant by helping prevent free radicals from damaging the DNA of cells and causing cancer. The chief flavonoids found in citrus species are limonene, hesperidin, narirutin, naringin and eriocitrin etc³. Recent studies explore that the beneficial activities of citrus on dietary citrus flavonoids reduce the risk of coronary heart disease ^{4, 5}. The important aspects of these classe of compounds is due to their pharmacological activity as radical scavengers has been reported⁶. Flavonoids of citrus have been shown to be powerful antioxidant and free radical scavengers 7. There is lack of information regarding the inhibitory effects of orange peel extract on lipid oxidation. Therefore, the purpose of the present study was to evaluate the effect of using some different solvents (such as Hexane, Benzene, Chloroform, Ethylacetate. Methanol. and Ethanol) on the extraction efficiency of effective compounds (such as polyphenolic and flavonoid compounds) from the orange peel extracts.

MATERIALS AND METHODS

Plant material

The orange peels (*Citrus sinensis*) were soaked with different solvents (Hexane, Benzene, Ethylacetate, Chloroform, Methanol, and Ethanol) at 7 days. The solutions were filtered through Whatman No.1 filter paper. The filtrate was concentrated under reduced pressure at 50° C using rotary evaporator. Different solvents of orange peel extracts were subjected into preliminary phytochemical screening. The crude extracts of orange peel in different solvents subjected to finding the best solvent extract used for *In vitro* antioxidant studies.

Phytochemical screening

Different solvents (Hexane, Benzene, Ethyl acetate, Chloroform, Methanol, and Ethanol) of orange peel extracts were obtained subjected to preliminary phytochemical screening and the following tests were done to check the presence of phytoconstituents ⁸. Tests for Alkaloids (Wagner's & Dragendorff's test), Flavonoids (Shinoda's & Zn-Hcl test). Glycosides (Keller Kilani test), Saponins, Oils, Quinones. Tannins (Gelatin test). Monoterpenoid (Trim-Hill reagent test), steroids (Salkowski & Liebermann Burchad test). Lignins, Phenolsand Cardiac alvcosides (Raymond's test) were carried out.

In vitro antioxidant properties

Determination of DPPH (1-1-diphenyl 2picrylhydrazyl) radical scavenging activity

The DPPH free radical scavenging activity of different solvents(Hexane, Benzene, Ethyl acetate, Chloroform, Methanol, and Ethanol) of orange peel extracts were measured by the method of Bilos (1958)⁹. 0.1 mM of DPPH solution was prepared in different solvents and 1.0 ml of this solution was added to 1.0 ml different concentrations (10-250 µg/ml) of different solventsof orange peel extract. Thirty minutes later, the absorbance was measured at 517 nm in a spectrophotometer (Shimadzu UV-1201; Shimadzu, Kyoto, Japan) against blank. Vitamin C was used as the reference standard. Radical scavenging activity was expressed as percentage of inhibition and was calculated using the following formula

% inhibition = $[(A_0 - A_t)/A_0] \times 100$

Here, A_o was the absorbance of the blank (without plant extract) and A_t was the absorbance of different solvents of orange peel extract. All the tests were performed in triplicate.

Determination of Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity was measured using modified method of Halliwell et al. (1987)⁹. Stock solutions of EDTA (1 mM), FeCl₃ (10 mM), Ascorbic acid (1 mM), H₂O₂ (10 mM) and deoxyribose (10 mM) were prepared in distilled water. The assay was performed by adding 0.1 ml EDTA, 0.01 ml of FeCl₃, 0.1 ml of H₂O₂, 0.36 ml of deoxyribose, 1.0 ml of different concentrations of (10-150 µg/ml) different solvents of orange peel extract dissolved in different solvents, 0.33 ml of phosphate buffer (50 mM, pH 7.4) and 0.1 ml of ascorbic acid in sequence. The mixture was then incubated at 37°C for 1 h. About 1.0 ml portion of the incubated mixture was mixed with 1.0 ml of (10%) TCA and 1.0 ml of (0.5%) TBA to develop the pink colour measured at 532 nm in a spectrophotometer (Shimadzu UV-1201; Shimadzu, Kyoto, Japan) against blank. The hydroxyl radical scavenging activity of different solvents of orange peel extracts was reported as the percentage of inhibition of deoxyribose degradation and was calculated according to the following equation

% inhibition = $[(A_0 - A_t)/A_0] \times 100$

Where, A_o was the absorbance of blank (without plant extract) and A_t was the absorbance in the presence of different solvents orange peel extract. All the tests were performed in triplicate. Vitamin E was used as a positive control.

Determination of Superoxide radical scavenging activity

The superoxide radical scavenging activity was measured using NBT (nitrobluetetrazolium reagent) method as described by (Nishikimi, 1972).¹⁰Different concentrations of (10-150 μ g/ml) different solventsof orange peel extract was dissolved in different solvents and taken into test tube. To this, reaction mixture consisting of 1 ml of (50 mM) sodium

carbonate, 0.4 ml of (24 mM) NBT and 0.2 ml of 0.1 mM EDTA solutions were added to the test tube and immediate reading was taken at 560 nm in a spectrophotometer (Shimadzu UV-1201; Shimadzu, Kyoto, Japan). Vitamin C was used as the standard control. The absorbance was recorded and the percentage of inhibition was calculated according to the following equation

% inhibition = $[(A_0 - A_t)/A_0] \times 100$

Where, A_o was the absorbance of the blank (without extract) and A_t was the absorbance different solvents of orange peel extract. All the tests were performed in triplicate.

Hydrogen peroxide scavenging activity

The ability of the different solvents of orange peel extracts to hydrogen peroxide scavenging activity was determined according to the method of (Yen and Chen, 1995).¹¹ A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Different concentrations (10-200 µg/ml) different solvents of orange peel prepared in different solvents were added to a hydrogen peroxide solution (0.6 ml, 40 mM). Absorbance of hydrogen peroxide was determined spectrophotometrically from absorption at 230 nm in a spectrophotometer (Shimadzu UV-1201; Shimadzu, Kyoto, Japan) against a blank solution containing phosphate buffer without peroxide. hvdroaen The percentage of scavenging of hydrogen peroxide of different solvents of orange peel extract and Vitamin C was calculated using the following equation

% inhibition = $[(A_0 - A_t)/A_0] \times 100$

Where A_0 was the absorbance of the control, and A_1 was the absorbance in the presence of the sample of different solvents of orange peel and standards.

Anti-lipid peroxidation assay (ALP)

The anti-lipid peroxidation assay was measured using liver homogenate of mice by the standard method (Kikuzaki et al., 1991).¹²followed by slight modification. 2.8 ml of 10% mice liver homogenate, 0.1 ml of 50

different mΜ FeSO₄ and 0.1 ml of concentration of different solvents of orange peel was mixed. The reaction mixture was incubated for 30 min. at 37°C. 1 ml of reaction mixture was taken with 2 ml 10% TCA-0.67% TBA in acetic acid (50%) for stopped the reaction. Then the mixture was boiled for 1 hour at 100°C and centrifuged at 10,000 rpm for 5 min. Absorbance supernatant was read at 535 nm in a spectrophotometer (Shimadzu UV-1201; Shimadzu, Kyoto, Japan) against a blank

(expect liver homogenate and different solvents of orange peel extract). Identical experiments were performed to determine the control (without extract and $FeSO_4$) and induced (without extract). Vitamin E was used for standard. Anti-lipid peroxidation percentage was calculated using the following formula. IC₅₀ values of all the experiments were calculated using different concentration of different solvents of orange peel extract.

```
\% \text{ ALP} = \frac{\text{Absorbance of Fe2} + \text{ induced peroxidation} - \text{Absorbance of sample}}{\text{Absorbance of Fe2} + \text{ induced peroxidation} - \text{Absorbance of control}} X 100
```

RESULTS

Phytochemical analysis of different solvents of orange peel extract

Phytochemical screening and analysis carryout with standard procedures of different solvent systems, orange peel extracted and evaluated the presence of phytochemicals such as saponins, terpenoids, flavonoids, tannins, steroids, alkaloidsand glycosides are present¹³. The cardiac glycosides and lignins were complete absent in orange peel extracts of all solvents systems (Table 1).

Phytochemicals	Hexane	Benzene	Ethyl acetate	Chloroform	Ethanol	Methanol
Alkaloids					+	+
Flavonoids		+	+	+		+
Glycosides	+	+		+		
Saponins	+	+	+	+	+	
Tannins					+	+
Terpenoids	+	+	+			
Steroids	+	+		+	+	+
Lignins						
Cardiacglycosides						
Phenols					+	+
Oil	+	+	+	+	+	
Quinones				+		+

Table 1Phytochemical constituents of orange peel extract in different solvents

DPPH Radical-Scavenging activity

There is significant decrease in the concentration of DPPH due to scavenging activity of orange peel extracts. Maximum difference among the extracts was observed at 6 min of the reaction. The remaining % of DPPH radical at 5 min after initiation of reaction was 65.44, 120,138.45, 151.34, 170.34 and 185.35% for extracts of methanol, ethanol,hexane,benzene,hexane, ethyl acetate, chloroform respectively as shown in (Table 2).

Table 2DPPH radical scavenging activity of the different solvents of
orange peel extracts and standard (Vitamin C)

Concentration (µg/ml)	% Inhibition of methonolic peel extract	% inhibition of Ethanolic peel extract	% inhibition of Hexane peel extract	% inhibition of Benzene peel extract	% inhibition of Ethyl acetatepeel extract	% inhibition of Chloroform peel extract	% inhibition of Ascorbic acid
10	30.23 ± 0.42	29.45 ± 0.12	28.32 ± 0.32	26.76 ± 0.21	26.62 ± 0.13	25.32 ± 0.41	23.12 ± 0.25
20	32.57 ± 0.35	27.65 ± 0.23	27.43 ± 0.22	23.23 ± 0.32	23.41 ± 0.32	20.16 ± 0.32	28.34 ± 0.22
30	39.65 ± 0.26	32.45 ± 0.23	30.14 ± 0.17	27.34 ± 0.54	26.40 ± 0.35	25.13 ± 0.41	39.6 4± 0.32
40	45.67 ± 0.41	37.32 ± 0.34	34.76 ± 0.34	32.65 ± 0.91	30.31 ± 0.22	30.42 ± 0.23	51.45 ± 0.37
50	56.12 ± 0.32	40.53 ± 0.32	39.65 ± 0.31	38.32 ± 0.65	35.87 ± 0.87	34.16 ± 0.73	59.25 ± 0.39
100	64.67 ± 0.13	45.56 ± 0.21	43.54 ± 0.23	43.13 ± 0.91	43.63 ± 0.31	40.54 ± 0.32	70.44 ± 0.27
150	72.73 ± 0.32	53.45 ± 0.76	52.54 ± 0.31	51.21 ± 0.31	49.42 ± 0.54	45.52 ± 0.43	84.45 ± 0.44
200	79.87 ± 0.24	65.46 ± 0.56	58.71 ± 0.12	57.31 ± 0.67	50.31 ± 0.23	49.72 ± 0.17	89.43 ± 0.22
IC 50	65.44	120	138.45	151.34	170.34	185.35	55.67

Values are mean ± S.D three replicates.

The free radical scavenging activity

The free radical scavenging activity of the different solvent systems of orange peel extract against hydroxyl radical is shown in (Table 3). The % of inhibition of methanolic orange peel extract (10-200 μ g/ml) on hydroxyl radical scavenging was found to be higher 77.33.Than the other solvent extracts,85.687, 90.78, 91.34,95.67, and96.78 respectively. A 200 μ g/ml of methanolic orange peel extract and Vitamin E exhibited64.76 % and 82.72 % inhibition, respectively and IC₅₀value was found to be 77.33 μ g/ml and 58.88 μ g/ml respectively. All results showed that antioxidant activity in dose dependent manner.

Table 3
Hydroxyl radical scavenging activity of the different solvents of
orange peel extracts and standard (Vitamin E)

Concentration (µg/ml)	% inhibition of Methanolic grape seed extract	% inhibition of Ethanolic peel extract	% inhibition of Hexane peel extract	% inhibition of Benzene peel extract	% inhibition of Ethyl acetatepeel extract	% inhibition of Chloroform peel extract	% inhibition of Vitamin E
10	15.35 ± 0.44	14.54 ± 0.14	13.43 ± 0.16	12.54 ± 0.12	12.44 ± 0.72	10.82 ± 0.23	23.45 ± 0.34
20	23.57 ± 0.56	22.52 ± 0.32	21.87 ± 0.31	20.41 ± 0.14	18.21 ± 0.41	16.32 ± 0.43	38.58 ± 0.65
30	35.71 ± 0.64	32.53 ± 0.41	31.51 ± 0.23	30.23 ± 0.12	28.35 ± 0.43	26.42 ± 0.71	42.53 ± 0.64
40	40.23 ± 0.46	38.41 ± 0.31	37.23 ± 0.25	38.31 ± 0.43	36.19 ± 0.23	35.78 ± 0.16	50.38 ± 0.32
50	54.62 ± 0.75	50.43 ± 0.45	48.35 ± 0.31	47.28 ± 0.52	46.61 ± 0.14	48.23 ± 0.18	63.75 ± 0.26
100	67.87 ± 0.45	62.31 ± 0.32	61.22 ± 0.21	62.32 ± 0.32	59.52 ± 0.45	62.82 ± 0.31	70.87 ± 0.75
150	74.35 ± 0.28	73.51 ± 0.28	71.82 ± 0.52	70.45 ± 0.54	68.43 ± 0.67	69.92 ± 0.12	81.67 ± 0.43
200	82.46 ± 0.86	82.72 ± 0.42	81.62 ± 0.51	78.54 ± 0.79	77.82 ± 0.14	74.76 ± 0.41	85.65 ± 0.23
IC ₅₀	77.33 ± 0.25	85.687	90.78	91.34	95.67	96.78	50.88 ± 0.19

Values are mean ± S.D three replicates

The superoxide scavenging activity of orange peel extracts in different solvents was shown in (Table 4). Different concentrations of methanolic orange peel extract (10-200 μ g/ml) has strong superoxide scavenging activity exhibiting 19.45 %, 25.32 %, 36.44 %, 42.74%, 51.89 %,54.14 %,62.52% 55.61 % and 73.42 % inhibition, respectively. The IC₅₀ Value of the methanolic orange peel extract was 92.43 μ g/ml compared with that of standard Vitamin C which was 50.17 μ g/ml respectively. All results showed scavenging activity in dose dependent manner.

Table 4
Super oxide scavenging activity of the different solvents of
orange peel extracts and standard (Vitamin C)

Concentration (µg/ml)	% inhibition of ethanolic grape seed extract	% inhibition of Ethanolic peel extract	% inhibition of Hexane peel extract	% inhibition of Benzene peel extract	% inhibition of Ethyl acetatepeel extract	% inhibition of Chloroform peel extract	% inhibition of Vitamin C
10	19.45 ± 0.25	17.32 ± 0.13	18.14 ± 0.32	15.43 ± 0.28	14.21 ± 0.32	15.36 ± 0.18	28.44 ± 0.33
20	25.32 ± 0.26	23.42 ± 0.32	21.32 ± 0.25	20.43 ± 0.35	19.24 ± 0.32	18.26 ± 0.49	37.76 ± 0.42
30	36.44 ± 0.36	35.32 ± 0.38	34.51 ± 0.31	31.27 ± 0.61	29.71 ± 0.41	28.63 ± 0.35	48.23 ± 0.26
40	42.74 ±0.32	40.21 ± 0.31	39.72 ± 0.43	37.62 ± 0.43	32.42 ± 0.38	31.42 ± 0.42	54.53 ± 0.19
50	51.89 ± 0.25	52.45 ± 0.51	49.52 ± 0.16	48.82 ± 0.63	47.92 ± 0.41	45.92 ± 0,91	60.34 ± 0.23
100	54.14 ± 0.23	52.82 ± 0.34	51.89 ± 0.45	49.34 ± 0.24	48.23 ± 0.23	47.13 ± 0.25	63.15 ± 0.32
150	62.52 ± 0.45	59.42 ± 0.17	59.13 ± 0.13	58.51 ± 0.63	57.26 ± 0.52	55.61 ± 0.67	73.82 ± 0.29
200	73.42 ± 0.51	67.54 ± 0.18	64.43 ± 0.18	63.56 ± 0.15	66.54 ± 0.41	60.52 ± 0.53	79.34 ± 0.37
IC ₅₀	92.43	105.56	104.67	115.56	114.67	130.56	50.17

Values are mean ± S.D three replicates

The scavenging activity of the methanolic orange peel extract against Hydroxyl radical scavenging activity is shown in (Table 5). The various solvent systems used to screen the scavenging activity of orange peels. The methanolic orange peel extract(10-200 μ g/ml) showed best hydroxyl scavenging activity with 21.25 %,28.56 %,36.67 %,43.76 %49.33 %55.62 %63.45 %,75.67 % than the other solvent extracts and 90% inhibition, respectively. Results was shown that the percentage of inhibition in a dose dependent manner. A 200 μ g/ml of methanolic orange peel extract and Vitamin C exhibiting 145.21% and 95.66% inhibition, respectively and the concentration of ethanolic orange peel extract needed of 50 % of inhibition was found to be 98.15 μ g/ml and 54.51 μ g/ml was needed for Vitamin C.

Table 5Hydrogen peroxide scavenging activity of the different solvents of
orange peel extracts and standard (Vitamin C)

Concentration (µg/ml)	% inhibition of ethanolic grape seed extract	% inhibition of Ethanolic peel extract	% inhibition of Hexane peel extract	% inhibition of Benzene peel extract	% inhibition of Ethyl acetatepeel extract	% inhibition of Chloroform peel extract	% inhibition of Vitamin C
10	21.25 ± 0.12	20.32 ± 0.35	19.63 ± 0.17	19.52 ± 0.42	17.82 ± 0.52	16.62 ± 0.42	26.69 ± 0.15
20	28.56 ± 0.22	27.24 ± 0.25	20.34 ± 0.73	19.67 ± 0.28	18.87 ± 0.35	17.41 ± 0.48	34.62 ± 0.12
30	36.67 ± 0.35	37.93 ± 0.18	35.89 ± 0.32	32.79 ± 0.31	30.48 ± 0.71	29.23 ± 0.51	43.67 ± 0.23
40	43.76 ± 0.22	42.94 ± 0.43	39.32 ± 0.42	38.23 ± 0.42	39.54 ± 0.83	37.34 ± 0.43	48.33 ± 0.32
50	49.33 ± 0.19	38.23 ± 0.45	37.82 ± 0.41	36.78 ± 0.54	34.69 ± 0.14	23.82 ± 0.72	54.54 ± 0.42
100	55.62 ± 0.21	54.73 ± 0,73	54.71 ± 0.93	52.78 ± 0.36	51.93 ± 0.62	50.78 ± 0.43	64.46 ± 0.24
150	63.45 ± 0.48	59.93 ± 0.89	56.89 ± 0.42	55.78 ± 0.64	55.34 ± 0.48	52.72 ± 0.82	74.35 ± 0.43
200	75.67 ± 0.24	73.10 ± 0.13	70.18 ± 0.43	72.78 ± 0.43	69.67 ± 0.15	55.67 ± 0.43	79.27 ± 0.33
IC ₅₀	88.15	95.66	108.56	110.23	115.67	145.67	62.51

Values are mean ± S.D three replicates

DISCUSSION

In vitro phytochemical screening of orange peel extracts from different solvents which includes (Hexane, Benzene, Ethylacetate, Chloroform, Methanol and Ethanol) given the worthy information for best solvent extraction procedure to screen the beneficial compounds from the citrus peel. Above observations and results clearly indicating that the methonolic extract of orange peel exhibits high *in viro* antioxidant activity when compared to the other solvent extracts. By the presence of flavo compounds in orange peels where the chief constituents of citrus involving in reducing the peroxide formation in free radical activity^{14,15}. In addition to the flavanones, flavones and flavonols are the three types of flavonoids that occur in citrus peel and polymethoxylated flavones are the unique to citrus family involving in reducing the free radicals¹⁶. The total extracted amount and the

total polyphenols contents from different solvent extracts of orange peels show difference about 10% more in methonolic extract, than the other solvent extract of ethanol. These polyphenols of citrus act as natural antioxidants mainly in foods to prevent the rancidity and oxidation of lipids.^{17, 18, 19}. Among the different solvent extracts of orange peel, metholic extract exhibits the high levels of steroidal compounds followed by ethonolic extract. In citrus phytosterols are fats that are naturally present as minor components of various plants by presence of these chemical steroidal compounds which constituent's regulates the body cholesterols by lowering low-density lipoprotein cholesterol (LDL-C) levels. These phytosterols produce a wide spectrum of biological activities in animals and humans and are particularly considered an efficacious cholesterol-lowering agent ²⁰. The results of DPPH scavenging activity assay indicates that the methonolic peel extract of orange was potently active when compare with the other solvent extracts. These experiments was suggests that the methonolic extract is the best solvent system to screen the phytochemicals and exhibiting the in vitro antioxidant properties with IC₅₀ value 65.44 µg/ml for DPPH radical scavenging assay followed by 120 µg/ml in ethanolic extract compounds that are capable of donating hydrogen to a free radical in order to remove odd electron which is responsible for radical's reactivity. Methonolic extract of orange peels shows greater ability to inhibit the formation of ABTS⁺. The scavenging activity of ABTS⁺ radical by the peel extract of orange was found to be appreciable; this implies that the methanol solvent extract of orange peel may be useful for treating radical-related pathological damage especially at higher concentration. Superoxide anion radical is one of the strongest reactive oxygen species among the

REFERENCES

 Tao NG, Hu ZY, Liu Q, Xu J, Cheng YJ, Guo LL, Guo WW, Deng XX. Expression of phytoene synthase gene is enhanced during fruit ripening of navel orange (Citrus sinensis). Plant Cell Rep. 26: 837-843,(2007). free radicals that are generated ²¹. The scavenging activity of this radical by the methanol extract of orange peel exhibits the higher scavenging activity with IC₅₀ value 92.43 when compared with the other solvent extracts (Table 4) favorably with the standard reagents such as gallic acid suggesting that the methanol extract of orange peel having high potent scavenger of superoxide radical. The measurement of H_2O_2 scavenging activity is one of the useful methods for determining the ability of antioxidants to decrease the level of pro-oxidants, such as $H_2O_2^{22}$. The hydrogen peroxide scavenging activity of various extracts is shown in (Table 5). Comparably the methonolic solvent extract of orange peel exhibits higher scavenging activity inhibiting the peroxide molecules with IC₅₀ value 88.15 followed by ethanol, chloroform, ethyl acetate, benzene, and hexane is shown in (Table 5).

CONCLUSION

Methonolic extract of *citrus sinensis.(L)*Orange peel exhibits the inimitable inhibition of the free radical scavenging activity when compared with the other solvent extracts . Potentially we recommended that methonol solvent extract is the best solvent system to screen the phytochemicals and exhibiting the best beneficial activities for the respective phytochemical compounds.

ACKNOWLEDGEMENT

We are highly thankful to The Research Fellowship in Sciences for Meritorious Students (RFSMS) UGC BSR Division, Government of India, for supporting with UGC-BSR Research Fellowship in department of Zoology, Sri Venkateswara University, Tirupati.

 Ahmad, M.M., Salim-ur-Rehman, Z. Iqbal, F.M. Anjum and J.I. Sultan, Genetic variability to essential oil composition in four citrus fruit species. Pak. J. Bot., 38(2): pp.319-324,(2006).

- 3. Schieber, A., F.C. Stintzing and R. Carle, Byproducts of plant food processing as a source of functional compounds-recent developments. Trends in Food Science and Technology, 12: 401- 413,(2001).
- Hertog, M.G., E.J. Feskeens, C.H. Holmann, M.B. Katan and D. Kromhout, Dietary antioxidant flavonoids and risk of coronary heart disease: The Zutphen elderly study. Lancet, 342: 1007-11,(1993).
- 5. Di Majo, D., M. Giammanco, M. La Guardia and E. Finotti, Flavanones in citrus fruit: Structure- antioxidant activity relationships. J. Rese. Intern, 38: 1161-66,(2005).
- Cotelle, N., J.L. Bernier, J.P. Catteau, J. Pommery, J.C. Wallet and E.M. Gaydou,Antioxidant properties of hydroxylflavones. Free Radic Biol. Med., 20(1): 35-43.(1996).
- 7. Zia-ur-Rehman. Citrus peel extract- A natural source of antioxidant. Food Chem. 99, 450-454.(2006).
- 8. Kokate Ć.K, Purohit A.P and Gokhale S.B.Pharmacognosy (Text book), Appendices-A.1-A.5, (2009).
- Halliwell B, Gutteridge JMC, Arurma OI. The deoxyribose method: a simple "test-tube" assay for determination of rate constants for reactions of hydroxyl radicals. Anal Biochem 165: 215–219, (1987).
- 10. Nishikimi M *et al.* The occurrence of super oxide anion in the reaction of reduced Phenazine methosulphate and molecular oxygen. Biochem Biophys Res Commun; 46: 849-853, (1972).
- 11. Yen G, Chen H: Antioxidant activity of various tea extract in relation to their antimutagenicity. *J Agric Food Chem*, 43:7-32. (1995).
- 12. Kikuzaki H, Usuguchi J, Nakatani N: Constituents of Zingiberaceae I. Diarylheptanoid from the rhizomes of ginger (*Zingiber officinale* Roscoe). *Chem Pharm Bull*, 39:120. (1991).
- 13. Harborne J.B. Phytochemical Methods; A guide to modern techniques of plant Analysis.2nd Edition, London New York.(1973).
- Martýn, F.R., M.J. Frutos, J.A. Perez-Alvarez, F. Martýnez-Sanchez and J.A. Del Rýo, Flavonoids as nutraceuticals: structural related antioxidant properties and their role on ascorbic acid preservation. In: Atta- Ur-

Rahman (editor), Studies in natural products chemistry Elsevier Science. Amsterdam, pp: 324-389.(2002).

- Tripoli, E., M. La Guardia, S. Giammanco, D. Di Majo and M. Giammanco, Citrus flavonoids: molecular structure, biological activity and nutritional properties: A review. Food Chemistry, 104: 466-479,(2007).
- Calabro, M.L., V. Galtieri, P. Cutroneo, S. Tommasini and R. Ficarra, Study of the extraction procedure by experimental design and validation of a LC method for determination of flavonoids in Citrus bergamia juice. J. Pharm and Biomed Anal.,35: 349-363,(2004).
- 17. Muhammad Kamran KHAN. These. Polyphénols d'Agrumes (flavanones): extraction de glycosides de la peaud'orange, synthèse de métabolites chez l'homme (glucuronides) et étude physico-chimique de leur interaction avec la sérum albumine, Académie d'Aix-Marseille, Université d'Avignon et des Pays de Vaucluse, 18.(2010).
- Anagnostopoulou MA, Kefalas P, Papageorgiou VP, Assimopoulou AN, Boskou D. Radical scavenging activity of various extracts and fractions of sweet orange peel (Citrus sinensis), Food Chem., 94, 19-25.(2006).
- Peschel, W. Sànchez-Rabaneda, F. DiekmannW., Plescher, AGartzìa, I. Jiménez, D. Lamuela-RaventósR., Buxaderas, S. CodinaC., An industrial approach in the search of natural antioxidants from vegetable and fruit wastes, Food Chem., 97, 137-150.(2006).
- Martijn B. Katan, Scott M. Grundy, Peter Jones, Malcolm Law, Tatu Miettinen, Rodolfo Paoletti,Efficacy and Safety of Plant Stanols and Sterols in the Management of Blood Cholesterol Levels.*Mayo Clin Proc.*; 78(8):965-78,(2003).
- 21. Gulcin I, Oktay M, Kirecci E, Kufrevioglu OI: Screening of antioxidant and Antimicrobial activities of anise (*Pimpinella anisum* L) seed extracts. *Food Chem*, 83:371-382, (2003).
- 22. Czochra M.P. and Widensk A. Spectrometric determination of hydrogen peroxide scavenging activity. J.Anal. Chimica Acta 452: 177-184. (2002).