

Original Article

Comparative analysis of free radical scavenging potential of several fruit peel extracts by *in vitro* methods

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ABSTRACT: We studied the radical scavenging potential of several fruit peel extracts using various standard chemical and biochemical *in vitro* methods. Peel extracts of *C. sinensis* (CS), *P. granatum* (PG), *M. paradisiaca* (MP), *C. vulgaris* (CV), *C. melo* (CM), *M. indica* (MI), and *C. papaya* (CP) were used in the present study and butylated hydroxyl anisole (BHA) was used as a standard. Marked 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity was observed with 25 µg/mL of CS and MP and 50 µg/mL of PG, while all the studied doses of CP were found to be pro-oxidative. β-Carotene bleaching revealed a higher singlet oxygen scavenging potential of all the peel extracts except MI. High NO-radical scavenging activity was observed at 25 µg/mL of PG and MP. Inhibition test of H₂O₂-induced LPO in erythrocytes or in liver tissue showed that all the peels tested were effective on peroxy-radicals at one or other doses. The present study revealed the radical scavenging activity of the test peel extracts in a manner that was dose- and radical/method-specific. Therefore, evaluation of the efficacy of herbal extracts should be carried out using different methods and not merely a single *in vitro* method.

Keywords: NO-radical scavenging, β-carotene bleaching, free radicals, peroxidation, *in vitro* study

1. Introduction

Dietary antioxidants including polyphenolic compounds, vitamin C, and carotenoids are believed to act as effective supplements in the prevention of several oxidative stress-related diseases (1,2). Various epidemiological studies have established an inverse correlation between the intake of fruits and

the occurrence of health-related problems such as cardiovascular diseases, cancer, diabetes, and aging (3-5). In addition, fruit peels are gradually emerging as a potential source of antioxidants that possess a rich amount of flavonoids, polyphenolics, ascorbic acid, dietary fibers, and dopamine (3,6,7). Recently, the current authors reported various biological/pharmacological properties of several fruit peel extracts, and their antiperoxidative properties were found to be one of the major mechanisms protecting against various diseases including diabetes, cardiovascular problems, and thyroid abnormalities (8-14). However, no detailed study of radicals scavenged by the fruit peel extracts of *Citrus sinensis*, *Punica granatum*, *Musa paradisiaca*, *Mangifera indica*, *Citrullus vulgaris*, *Cucumis melo*, and *Carica papaya* has been conducted to date. Therefore, the present study attempted to determine the radical scavenging potential of these peel extracts using various recommended *in vitro* methods (3,9,15).

2. Materials and Methods

2.1. Materials

1,1-Diphenyl-2-picrylhydrazyl (DPPH) was purchased from Sigma-Aldrich, St. Louis, MO, USA; while β-carotene, Greiss reagent, sodium nitroprusside, butylated hydroxyl anisole (BHA) and thio-barbituric acid (TBA) were supplied by Hi Media Laboratories Ltd., Mumbai, India. Malondialdehyde (MDA), sodium dodecyl sulphate (SDS), and acetic acid, and all other reagents were purchased from E-Merck (India) Ltd., Mumbai, India.

2.2. Plant materials

Sweet orange (*C. sinensis*, CS), pomegranate (*P. granatum*, PG), banana (*M. paradisiaca*, MP), mango (*M. indica*, MI), watermelon (*C. vulgaris*, CV), melon (*C. melo*, CM), and papaya (*C. papaya*, CP) were purchased from a local market in Indore, India. They were identified by the departmental taxonomist and voucher specimens (CS-16/05, PG-11/14, MP-11/07, MI-16/04, CV-14/04, CM-17/04, and CP-19/02,

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respectively) were deposited in the School of Life Sciences, D.A. University, Indore, India.

2.3. Preparation of fruit peel extracts

Fruit skins were removed mechanically and usable peels were air-dried in the shade and then ground into a fine powder. The dried powder of *C. sinensis* and *M. paradisiaca* was extracted in 10 volumes of cold water and stored at 4°C for three days. The extract was centrifuged at $1,500 \times g$ for 10 min and the supernatant was dried at 40°C and stored until use (7). In contrast, methanolic extraction was utilized for *P. granatum*, *M. indica*, *C. vulgaris*, *C. melo*, and *C. papaya* given its use by other works (16). In brief, 100 g of powdered peel were collected and extracted with 600 mL of methyl alcohol (100%) at 30°C; the solution was stirred continuously with a magnetic stirrer for 4 h and then filtered (16). The filtrate was then dried and stored until use.

2.4. Sample preparation for in vitro assays

For the analysis of radical scavenging activity (RSA), β -carotene bleaching assay, and NO-radical scavenging assay, the aliquots of 25, 50, and 100 $\mu\text{g}/\text{mL}$ concentrations of the peel extracts were prepared in methanol and used, while the actual concentrations of the peel extracts at 0.25, 0.50, 1.0, and 2.0 $\mu\text{g}/\text{mL}$ in reactions mixtures were used in H_2O_2 -induced lipid peroxidation (LPO) study in erythrocytes and liver tissues (9).

2.5. Radical scavenging potential

A few methods are available for total antioxidant determination and each has its limitations (15,17). Several *in vitro* methods are thought to be nonspecific (18), while others are more specific for the determination of antioxidant potential, particularly in fruits and peels (3,9,15). Therefore, different methods were used for each peel to understand trends in radical specificity in terms of scavenging activity as described below.

2.6. Radical scavenging activity (RSA) using the DPPH method

DPPH is a synthetic, relatively stable nitrogen radical and this assay system is based on electron transfer, where an antioxidant compound reduces the oxidant by donating an electron, resulting in a change of color and subsequent change in absorbance (19,20).

Different concentrations of the peel extracts (25, 50, and 100 $\mu\text{g}/\text{mL}$) were prepared in separate test tubes. The volume of each was adjusted to 100 μL by adding methanol. Five milliliters of a 0.1 mM ethanolic solution of DPPH were added to all. Tubes were then allowed to stand at 27°C for 20 min. Control tubes had

all of the components mentioned above except for the peel extract. Methanol was used for baseline correction. Changes in the absorbance of the samples were measured at 517 nm. RSA was calculated as the percent inhibition [%RSA = $100 \times (\text{control OD} - \text{sample OD}) / \text{control OD}$] as described earlier (3). BHA was used as a standard antioxidant and antiperoxidative activity was expressed in % in relation to BHA.

2.7. Antioxidant assay using β -carotene

Singlet oxygen produced during photosensitized oxidations can cause cellular damage by reacting with DNA and proteins or by inducing lipid peroxidation (21), and β -carotene commonly protects against photosensitized tissue injury by scavenging free radicals or quenching singlet oxygen. Because of this, β -carotene is often used clinically to prevent photosensitized tissue damage in humans with erythropoietic porphyria (22). Because of the significance of singlet oxygen radicals in biological systems, a β -carotene bleaching assay is considered an elegant model in which to evaluate the singlet oxygen scavenging potential of various natural products (19,21).

β -Carotene (0.2 mg) dissolved in 0.2 mL of chloroform and linoleic acid (20 mg) was mixed with 200 mg of Tween-40 (polyoxyethylene sorbitan monopalmitate). Chloroform was removed at 40°C under a vacuum. The resulting mixture was diluted with 10 mL of distilled water, to which 40 mL of oxygenated water was added. Aliquots of this emulsion (4 mL) were mixed with 0.2 mL of the extract at the concentrations of 25, 50, and 100 $\mu\text{g}/\text{mL}$. A control was prepared by adding 0.2 mL of ethanol instead of the extract. The reaction mixture was initially incubated at 50°C for 5 min and absorbance was determined at 470 nm at the starting time ($t = 0$). Measurement of absorbance continued for 180 min at an interval of 15 min. A mixture prepared as above without β -carotene served as the blank.

The antioxidative activity (AA) of each peel extract was evaluated in terms of potential in bleaching the fixed quantity of β -carotene: $\text{AA} = 100 \times [1 - (A_0 - A_t) / (A_0^0 - A_t^0)]$, where A_0 and A_0^0 are the absorbance values measured at the start of incubation for the test sample and control, respectively, and A_t and A_t^0 are those measured in the test sample and control, respectively, after incubation for 180 min (23).

2.8. NO scavenging activity

NO radicals are known to be involved in cytotoxicity and can also interact with superoxide anions to result in the formation of peroxynitrite (ONOO^-), which is the most reactive nitrogen species (RNS) (24).

This assay is based on the principle that scavengers of nitric oxide compete with oxygen, leading to a reduced production of nitrite. Samples at various

concentrations (0.5 mL each) were diluted with 0.5 mL of 10 mM sodium nitroprusside solution and incubated at 25°C for 150 min. At the end of incubation, 1 mL of Greiss reagent was added to each sample and absorbance was determined at 542 nm. The nitrite concentration was calculated in relation to the absorbance of a standard solution of sodium nitrite. Results were expressed as percentage nitrite produced with respect to the control, which was devoid of peel extract (25).

2.9. *In vitro* LPO estimation in red blood cells (RBCs) and in liver slices

Reactive oxygen species (ROS) are known to be involved in the pathogenesis of many diseases, including thyroid and heart problems, diabetes, and cancer (4), so a crucial step in this study was to evaluate the ROS scavenging potential of the test peel extracts. In fact, H₂O₂-induced lipid peroxidation is believed to be the result of ROS formation. Therefore, RBCs and chopped liver tissues were found to be a suitable biochemical *in vitro* model in which to evaluate the antiperoxidative potential of herbal extracts (26-29). Erythrocytes are considered suitable because they contain high concentration of polyunsaturated fatty acids (PUFA), ferrous ion, and molecular oxygen, which make them vulnerable to oxidative stress (27), while the liver is considered suitable as it is a major drug target site (30).

About 50 mL of rat blood were collected in 2% citrated vials after the animals were decapitated. Erythrocytes were washed thrice with 0.1 M phosphate buffered saline (PBS, pH 7.4) and the volume was adjusted to 5% with PBS (pH 7.4). LPO in the erythrocytes was determined after the addition of 2 mL of 5% RBCs and 2 mM sodium azide to a test tube (9,26,28). The erythrocyte suspension was exposed to a 100 mM H₂O₂ solution and different concentrations of the extracts (0.25, 0.5, 1.0, and 2.0 µg/mL of CS/PG/MP/MI/CV/CM/CP). The total volume of each tube was brought to 4 mL with 0.1 M PBS (pH 7.4). Samples were incubated at 37°C for 1 h followed by the addition of 2 mL of 28% TCA solution. The solution was then centrifuged at 1,000 × g for 5 min. One mL of 1% TBA was added to 4 mL of supernatant and this was then placed in a boiling water bath for 1 h, cooled, and absorbance was measured at 532 nm. A control sample was prepared without the H₂O₂ solution, while the blank was prepared without tissue. In both of these tubes the volume was adjusted by 0.1 M PBS, pH 7.4. For liver slices, a similar method was used in which 200 mg of the tissues were incubated with 2 mL PBS and different concentrations of the extracts along with 100 mM H₂O₂. Simultaneously a standard curve for malondialdehyde (MDA) was determined based on its different concentrations (1-10 nM prepared in 0.1 M

PBS). A blank was prepared without the MDA solution.

2.10. Determination of polyphenols

Total polyphenolic contents of the test peel extracts were estimated following the protocol of Leontowicz *et al.* (3). In brief, 0.125 mL of each peel extract of known concentration (100 mg/mL) were diluted with 0.5 mL distilled water and then 0.125 mL of Folin-Ciocalteu reagent were added to the mixture and incubated at room temperature for 6 min. After incubation, 1.25 mL of 7% sodium carbonate were added to the mixture. The final volume was adjusted to 3.0 mL with distilled water and incubated at room temperature for 90 min. The absorbance was measured against the prepared blank at 765 nm in comparison to a standard of known concentrations of gallic acid. The mean of three readings was used, and results were expressed in mg gallic acid equivalent/100 g dry weight of the extract. The coefficient of determination was $R^2 = 0.9748$.

2.11. Determination of total flavonoids

Total flavonoids were determined colorimetrically by following the protocol of Leontowicz *et al.* (3). An amount of the peel extracts (0.25 mL) in 100 mg/mL concentration was diluted with 1.25 mL of distilled water. Then, 75 µL of 5% sodium nitrite (NaNO₂) solution were added to the mixture followed by 150 µL of 10% aluminium chloride (AlCl₃·6H₂O) solution. After incubation for 5 min, 0.5 mL of 1 M NaOH were added. The total volume was brought to 2.5 mL with distilled water. Finally, the absorbance was measured with respect to the prepared blank at 510 nm in comparison to standards prepared similarly with known concentrations of quercetin. The mean of three readings was used, and results were expressed in mg quercetin equivalent/100 g dry weight of the peel extract. The coefficient of determination was $R^2 = 0.9008$.

2.12. Determination of ascorbic acid content

Ascorbic acid content was measured by a modified method of Omaye *et al.* (31). One mL of the sample including ascorbic acid was mixed with 1 mL of ice cold 10% metaphosphoric acid and 2 mM EDTA for deproteinization and stabilization of ascorbic acid. Six hundred µL of the 50 mM citrate/acetate buffer (pH 3.5) were added to the mixture, followed by 0.3 mL of 2,6-dichlorophenolindophenol (DCPIP) solution (0.1 mg/mL); the solution was then vortexed. One min later, the absorbance of the mixture was measured at 520 nm. Standard ascorbic acids were dissolved in 5% metaphosphoric acid at 2.5, 5, 10, 20, and 50 µg/mL. A standard curve of ascorbic acid was determined using the assay method described above. The coefficient of determination was $R^2 = 0.9095$.

2.13. Statistical analysis

Data are expressed as mean \pm SEM and were analyzed by the analysis of variance (ANOVA) followed by a post hoc Newman-Keuls Multiple Comparison Test using a trial version of Prism 4 software for Windows (GraphPad Software, Inc., La Jolla, CA, USA) (8).

3. Results

Although peel extracts with more than 50% free radical scavenging activity were considered to be effective, almost all peels were found to be effective according to one or the other method.

3.1. DPPH radical scavenging activity

The DPPH radical scavenging assay revealed that the 25 μ g/mL concentration of CS and MP showed a marked inhibition in DPPH radicals (95.65 and 61.37%, respectively), while PG showed the highest radical scavenging potential at 50 μ g/mL (152.07%) (Table 1). In addition, a marked inhibition was also observed with a 25 and 100 μ g/mL concentration of PG. Interestingly, all the studied doses of CP were found to be pro-oxidative, as evidenced by increased DPPH absorbance, whereas MI, CV, and CM showed less inhibition in the DPPH radical scavenging assay.

3.2. β -Carotene bleaching activity

Results of β -carotene bleaching revealed the highest activity at 50 μ g/mL of CV (165.19%), while all the studied doses of MI and 100 μ g/mL of CS were found to be not as effective since their inhibition was less than 50% (Table 2).

3.3. NO-radical scavenging activity

A NO radical scavenging assay revealed the highest scavenging potential at 25 μ g/mL of PG (52.06%) and almost similar inhibition by 25 μ g/mL of MP (51.38%)

Table 1. Radical scavenging activity in various fruit peel extracts observed with a DPPH assay system

Peel extracts ¹	Radical scavenging activity (%) ²		
	25 μ g/mL	50 μ g/mL	100 μ g/mL
CS	95.65 \pm 1.56 ^a	41.95 \pm 4.83 ^a	9.86 \pm 0.85 ^a
PG	116.25 \pm 8.52 ^b	152.07 \pm 3.30 ^b	90.41 \pm 4.62 ^b
MP	61.37 \pm 4.30 ^c	8.67 \pm 0.72 ^c	7.03 \pm 1.16 ^c
MI	12.61 \pm 2.01 ^d	17.96 \pm 1.69 ^c	11.33 \pm 1.34 ^d
CV	18.02 \pm 2.54 ^d	11.95 \pm 2.23 ^c	7.14 \pm 1.72 ^c
CM	7.19 \pm 2.18 ^d	19.31 \pm 3.05 ^c	3.83 \pm 0.48 ^c
CP	102.52 \pm 1.75 ^e \uparrow	105.72 \pm 1.40 ^d \uparrow	115.12 \pm 3.31 ^d \uparrow

¹ CS, *C. sinensis*; PG, *P. granatum*; MP, *M. paradisiaca*; MI, *M. indica*; CV, *C. vulgaris*; CM, *C. melo*; CP, *C. papaya*; ² Data are expressed in % inhibition in comparison to that of BHA (mean \pm SE of five measurements). Means in columns without letters in common differ significantly ($P \leq 0.05$). \uparrow indicates an increase in radicals.

(Table 3). The remaining test peel extracts at all the studied doses were found to be less effective.

3.4. Inhibition of H₂O₂-induced LPO in erythrocytes

With respect to the inhibition of H₂O₂-induced LPO in erythrocytes, all the test peels were found to be effective at one or other doses (Table 4). The highest inhibition was observed with 0.25 μ g/mL of MP (147.31%).

3.5. Inhibition of H₂O₂-induced LPO in liver tissue

H₂O₂-induced LPO in chopped liver tissue was inhibited by all the peel extracts at one or other doses. The maximum activity was observed at 0.5 μ g/mL of MP (Table 5).

3.6. Total amounts of the deduced radical scavenging compounds in peel extracts

As shown in Table 6, the highest amount of total polyphenolic and flavonoid compounds were present in PG (1,799 mg gallic acid equivalent/100 g dry weight of peel extract and 741.71 mg quercetin equivalent/100 g dry weight of the extract, respectively), while the highest amount of ascorbic acid was present in CV (879.12 mg/kg dry weight of peel extract).

Table 2. Radical scavenging activity in various fruit peel extracts observed with a β -carotene bleaching assay system

Peel extracts ¹	Radical scavenging activity (%) ²		
	25 μ g/mL	50 μ g/mL	100 μ g/mL
CS	67.68 \pm 1.50 ^a	57.52 \pm 9.47 ^a	37.95 \pm 2.08 ^a
PG	62.91 \pm 4.14 ^a	59.25 \pm 2.81 ^a	120.21 \pm 1.69 ^b
MP	105.01 \pm 3.40 ^b	121.48 \pm 2.38 ^b	99.72 \pm 7.99 ^c
MI	12.61 \pm 2.01 ^c	17.96 \pm 1.69 ^c	11.33 \pm 1.34 ^d
CV	133.86 \pm 7.48 ^d	165.19 \pm 4.82 ^d	159.36 \pm 7.91 ^e
CM	122.79 \pm 4.45 ^d	123.95 \pm 12.11 ^e	149.53 \pm 11.92 ^e
CP	147.49 \pm 5.52 ^e	145.69 \pm 4.57 ^f	148.39 \pm 4.42 ^e

¹ CS, *C. sinensis*; PG, *P. granatum*; MP, *M. paradisiaca*; MI, *M. indica*; CV, *C. vulgaris*; CM, *C. melo*; CP, *C. papaya*; ² Data are expressed in % inhibition in comparison to that of BHA (mean \pm SE of five measurements). Means in columns without letters in common differ significantly ($P \leq 0.05$).

Table 3. NO radical scavenging (% inhibition) by various fruit peel extracts

Peel extracts ¹	NO radical scavenging (% inhibition) ²		
	25 μ g/mL	50 μ g/mL	100 μ g/mL
CS	33.47 \pm 1.81 ^a	31.38 \pm 1.84 ^a	8.10 \pm 1.87 ^a
PG	52.06 \pm 3.62 ^b	9.90 \pm 0.36 ^b	19.13 \pm 2.50 ^b
MP	51.38 \pm 1.37 ^b	12.43 \pm 1.24 ^b	5.32 \pm 1.17 ^c
MI	17.04 \pm 1.00 ^c	17.11 \pm 1.20 ^b	13.00 \pm 1.25 ^d
CV	31.38 \pm 1.84 ^d	8.49 \pm 0.68 ^c	3.63 \pm 0.89 ^e
CM	12.21 \pm 1.83 ^e	20.31 \pm 2.50 ^d	15.42 \pm 3.37 ^d
CP	24.46 \pm 2.46 ^f	38.98 \pm 3.84 ^e	21.07 \pm 2.44 ^f

¹ CS, *C. sinensis*; PG, *P. granatum*; MP, *M. paradisiaca*; MI, *M. indica*; CV, *C. vulgaris*; CM, *C. melo*; CP, *C. papaya*; ² Values are the mean \pm SE of five measurements. Means in columns without letters in common differ significantly ($P \leq 0.05$).

Table 4. Inhibition of H₂O₂-induced LPO in erythrocytes by various fruit peels

Peel extracts ¹	Inhibition (%) ²			
	0.25 µg/mL	0.5 µg/mL	1.0 µg/mL	2.0 µg/mL
CS	72.31 ± 4.42 ^a	119.67 ± 6.26 ^a	94.68 ± 2.76 ^a	76.60 ± 3.87 ^a
PG	107.12 ± 5.77 ^b	140.33 ± 4.74 ^b	109.98 ± 6.10 ^a	73.09 ± 2.33 ^a
MP	147.31 ± 8.31 ^c	89.95 ± 4.01 ^c	99.43 ± 4.25 ^a	35.76 ± 5.25 ^b
MI	86.87 ± 6.58 ^d	95.12 ± 6.46 ^c	81.84 ± 5.50 ^a	82.48 ± 1.54 ^c
CV	111.18 ± 10.66 ^d	102.33 ± 9.06 ^c	100.54 ± 6.58 ^a	85.99 ± 4.09 ^c
CM	89.45 ± 6.84 ^d	24.49 ± 8.37 ^d	55.65 ± 3.12 ^b	12.27 ± 1.87 ^d
CP	45.41 ± 8.05 ^e	82.62 ± 5.10 ^e	40.67 ± 7.79 ^b	46.09 ± 4.34 ^e

¹ CS, *C. sinensis*; PG, *P. granatum*; MP, *M. paradisiaca*; MI, *M. indica*; CV, *C. vulgaris*; CM, *C. melo*; CP, *C. papaya*; ² Data are expressed in % inhibition in comparison to that of BHA (mean ± SE of five measurements). Means in columns without letters in common differ significantly ($P \leq 0.05$).

Table 5. Inhibition of H₂O₂-induced LPO in liver slices by various fruit peels

Peel extracts ¹	Inhibition (%) ²			
	0.25 µg/mL	0.5 µg/mL	1.0 µg/mL	2.0 µg/mL
CS	65.84 ± 7.75 ^a	123.12 ± 11.74 ^a	63.76 ± 4.43 ^a	20.87 ± 0.92 ^a
PG	44.12 ± 5.77 ^a	72.02 ± 2.12 ^b	121.78 ± 3.07 ^b	99.99 ± 6.84 ^b
MP	44.49 ± 6.88 ^a	160.19 ± 7.01 ^c	51.56 ± 2.97 ^c	99.64 ± 2.43 ^b
MI	65.35 ± 5.58 ^a	77.96 ± 3.42 ^d	90.94 ± 5.99 ^d	89.11 ± 1.77 ^b
CV	131.00 ± 3.96 ^b	104.19 ± 4.49 ^e	79.33 ± 1.99 ^e	55.08 ± 3.87 ^c
CM	24.89 ± 6.53 ^c	107.91 ± 7.13 ^e	74.01 ± 2.55 ^e	81.74 ± 2.73 ^d
CP	111.78 ± 7.86 ^d	57.89 ± 2.45 ^f	60.42 ± 1.09 ^f	72.62 ± 7.30 ^d

¹ CS, *C. sinensis*; PG, *P. granatum*; MP, *M. paradisiaca*; MI, *M. indica*; CV, *C. vulgaris*; CM, *C. melo*; CP, *C. papaya*; ² Data are expressed in % inhibition in comparison to that of BHA (mean ± SE of five measurements). Means in columns without letters in common differ significantly ($P \leq 0.05$).

Table 6. Total amounts of radical scavenging compounds deduced from peel extracts

Peel extracts ¹	Polyphenols ²	Flavonoids ³	Ascorbic acid ⁴
CS	1,407 ± 4.15	311.80 ± 6.17	780.30 ± 11.3
PG	1,799 ± 7.29	741.71 ± 8.08	733.40 ± 11.32
MP	89 ± 2.12	47.85 ± 3.75	756.45 ± 10.89
MI	1,161 ± 6.88	558.09 ± 7.28	811.17 ± 11.12
CV	1,147 ± 4.94	105.23 ± 5.0	879.12 ± 13.21
CM	1,139 ± 4.77	136.47 ± 5.19	826.15 ± 11.89
CP	272 ± 2.03	49.70 ± 4.09	810.19 ± 12.88

¹ CS, *C. sinensis*; PG, *P. granatum*; MP, *M. paradisiaca*; MI, *M. indica*; CV, *C. vulgaris*; CM, *C. melo*; CP, *C. papaya*; ² Values are presented as mg gallic acid equivalent/100 gm dry weight of peel extract (mean ± SE); ³ Values are presented as mg quercetin equivalent/100 g dry weight of the extract (mean ± SE); ⁴ Values are presented as mg/100 g of dry weight of the extract (mean ± SE).

4. Discussion

This study has shown that different fruit peel extracts exhibit varying degrees of free radical scavenging/antiperoxidative potential depending on their concentrations and on the type of assay system/radicals used. In particular, the DPPH assay method revealed a different trend, where, unlike other test peels, CP extract was found to be pro-oxidative. In fact, marked inhibition (more than 50%) of DPPH radicals was observed only with CS, PG, and MP peel extracts. These findings are similar to those for several other fruit peels reported previously (3,7,23,32). Like CP, toxic or pro-oxidative effects of several other herbal extracts have also been reported (32-34).

DPPH radicals are believed to act as a suitable model compound for free radicals originating in lipids and have been used in several studies of other natural

products (35-37). The present study revealed that the test peel extracts, and particularly CS, PG, and MP, contain a considerable amount of compounds scavenging for nitrogen radicals and that these compounds might be mediated *via* direct electron donation and subsequently reduce DPPH radicals. However, only CP appeared to be negatively involved in the redox reaction, as it increased the color intensity/absorbance of the solution, suggesting the presence of several compounds similar to DPPH radicals or with an absorption maxima (517 nm) like that of DPPH. The other three test peels (MI, CV, and CM) did not substantially quench DPPH radicals. Therefore, these peels may not scavenge nitrogen radicals or may not work *via* direct electron donation.

In the present study, the β -carotene bleaching assay showed that all of the test peel extracts except MI exhibited marked antiperoxidative or radical scavenging activity, suggesting their potential for scavenging singlet oxygen evolved in the reaction (19). Similar findings have been obtained by other authors using the β -carotene bleaching assay (38,39). However, only limited studies of peel extracts have been conducted (3,23,32). The present findings, therefore, indicate that most of the test peels have the potential to inhibit singlet oxygen produced during photosensitized oxidations (21).

When the NO radical scavenging activity of the experimental peels was evaluated, results revealed that only 25 µg/mL of PG and MP peel extracts are able to reduce NO radicals substantially. However, CS, CV, and CP peel extracts did not substantially inhibit NO radical generation, which further tallied with the results

of DPPH scavenging, *i.e.* that these peels may not work *via* nitrogen radical inhibition. These results are also in accordance with earlier reports on fruit peels (3,32) and on other natural products (24,25,39).

In the present study, H₂O₂-induced lipid peroxidation in RBCs and in liver slices was inhibited by all of the test peel extracts at one or other concentrations, suggesting their potential to scavenge peroxyradicals or other reactive oxygen species (ROS) induced by H₂O₂ (19) as reported by several earlier works (24,28,40). Similar findings were also obtained for several of the test peel extracts by using RBCs (9) and other herbal extracts using liver slices (40).

Reviewing all of these findings suggest that the antiperoxidative activity of test peel extracts tends to be method-specific, as was thought earlier (18). This could be due to the differences in the mechanism of action(s) and the radical specific scavenging activity of the peel extracts studied (19,24). The radical scavenging activity of the test peel extracts might have been mediated by the presence of total polyphenolic components, flavonoids, and ascorbic acid, as posited by earlier works (3,7,41). This possibility is further supported by the high content of total phenolic compounds, total flavonoids, and ascorbic acid (Table 6). Interestingly, the present study found that the test peels have a dose-specific radical scavenging potential, which is also in accordance with earlier reports on fruit peel extracts based on *in vivo* studies (8-14). This is explained by the fact that polyphenolic compounds and/or flavonoids present in herbal extracts usually lead to various polymerization reactions and changes in chemical nature and spatial conformations that can modify the reactivity of molecules in a concentration-dependent manner (41-43). In fact, naturally occurring polyphenolic compounds are known to exhibit dual behavior in a dose-dependent manner as their antiperoxidative and/or pro-oxidative nature depends upon their dose (8-16,41,44-47). In conclusion, the present findings clearly suggest that the test peel extracts have a varying degree of radical scavenging potential for different radicals in a dose-specific manner. Therefore, evaluation of the efficacy of herbal extracts should be carried out using different methods and not merely a single *in vitro* method. Further study of this point may lead to the development of specific antioxidant formulations for different peel extracts in accordance with the radicals to be scavenged.

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