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Research Paper

Phytochemical Screening and Potential on Antioxidative Efficacy of Five Varieties of Citrus Fruit Peels in Bangladesh

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ABSTRACT
Citrus fruits play the potential role in diverse clinical complications although the mechanism is not clarified well. Therefore, the current study has been done to assess the antioxidative effects of five varieties of citrus fruits (C. assamensis (Ada), C. maxima (Ba), C. meyeri (Chi), C. sinensis (Ma) and C. aurantium (Ka) through petroleum ether and methanol extraction. Among the five varieties of peels, C. sinensis (absorbance at 695 nm: 0.081, 0.142, 0.212, 0.263 and 0.293 respectively) and C. assamensis (absorbance at 695 nm: 0.082, 0.141, 0.211, 0.262 and 0.292 respectively) were found to be potential showing higher antioxidant capacity when compared to catechin. Other varieties of peels also showed increased absorbance indicating higher antioxidative effects. During methanol extraction, C. aurantium and C. maxima showed the potent effect (C. aurantium: absorbance at 695 nm: 0.091, 0.171, 0.231, 0.279, 0.330; C. maxima: absorbance at 695 nm: 0.056, 0.130, 0.171, 0.211 and 0.262 respectively). Other varieties of peels were shown to have increased antioxidative effects because of their increasing absorbance. The higher reducing power capacity of C. maxima (absorbance at 700 nm: 1.473, 2.113, 2.370, 2.513 and 2.676 respectively) and C. assamensis (absorbance at 700 nm: 1.108, 1.523, 1.883, 2.133 and 2.353 respectively) were observed because of their higher absorbance in peel extract. The other three varieties also showed potent reducing power capacity when compared to ascorbic acid. C. aurantium (absorbance at 700 nm: 1.530, 2.360, 2.620, 2.760 and 2.840 respectively) and C. meyeri (absorbance at 700 nm: 1.376, 2.130, 2.350, 2.420 and 2.480 respectively) showed the higher effect on reducing power capacity when compared to control while the other varieties also showed enhanced effect during different solvent extractions. Therefore, five varieties of fruits are major sources of phytochemicals and play the critical role on antioxidative effects during adverse environmental circumstances.

KEYWORDS: *citrus peel, petroleum ether extract, methanol extract, TAC, RPC, phytonutrients*

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I. INTRODUCTION

Fruits and vegetables are considered as an important part of a good diet. Besides their delicious taste and flavor, they are known to reduce risk of several chronic diseases. Fruits and vegetables contain significant amounts of phytoconstituents which are negatively associated with the morbidity and mortality from cerebrovascular, cardiovascular and certain types of cancers [1][2]. Foods are generally consumed for their nutritive value and bioactive compounds [3]. Fruits and vegetable wastes and their by-products are formed in great amounts during industrial processing and hence represent a serious problem, as they exert harmful impact on environment. It is well known that citrus fruits have been shown to play the potential role on the prevention of oxidative stress caused by the environmental stress either biotic or abiotic. Therefore, it is assumed that some of the compounds are present in the citrus fruits and play the critical role regarding this phenomenon although the mechanism is not clarified well.

Citrus fruits are rich sources of useful phytochemicals, such as vitamins A, C and E, mineral elements, flavonoids, coumarins, limonoids, carotenoids, pectins, and other compounds [4]. For the prevention of oxidative stress induced by environmental stimuli either biotic or abiotic, phytochemicals play the critical role because of their potent antioxidative effects. These phytochemicals, consumed through fresh fruits or their derived products, have been suggested to have a wide variety of biological functions including antioxidant, antiinflammation, antimutagenicity, anticarcinogenicity and anti-aging to human health [5][6]. Therefore, it is generally accepted and reasonable that extraction of phytochemicals from the fruit peel is of great importance in the prevention of diverse clinical complications.

Citrus byproducts, if utilized fully, could be major sources of phenolic compounds. The peels, in particular, are an abundant source of natural flavonoids and contain higher amount of phenolics compared to the edible portions. It has been reported that the contents of total phenolics in peels of lemons, oranges, and grapefruit were 15% higher than those in the peeled fruits [7]. Flavonoids in citrus are a major class of secondary metabolites. The peel contains the higher amount of flavonoids than other parts of the fruits and is involved in playing the vital biological and biochemical functions [7]. Because of the presence of large amount of phytonutrients in citrus peel, they are believed to play a vital role on the prevention of diverse complications caused by the environmental adverse stimuli either biotic or abiotic. These adverse stimuli or stresses cause severe effects in the biological system and produce cellular damage and impairment of biological and biochemical functions.

The ROS is very powerful chemicals causing diverse biochemical and biological adverse effect in the organisms. Molecular oxygen receives electrons from high energy level to produce ROS which are deleterious to plant cells at high concentration. Under severe stress conditions, rate of ROS generation exceeds the scavenging potential of cellular defence system resulting in oxidative stress. Oxidative stress damages cellular components resulting in their dysfunction and ultimately cell death. ROS includes H_2O_2, O_2^- as well as free radicals i.e., $^1O_2$, O_2^{2-}, O_2H, OH^-, RO^- etc., which are toxic to plant metabolism [8][9] affecting every cellular macromolecule including DNA. Phytochemicals present in the plant kingdom plays the critical role through the antioxidative functions. It is assumed that fruit peel having active ingredients and causes antioxidative effects in the biological system and scavenge and prevents ROS. Therefore, the current study has been undertaken regarding the antioxidative effects of citrus fruit peels. In this study, five varieties of citrus fruit peels were examined to find the antioxidative effects and were screened accordingly.

II. MATERIALS AND METHOD

Plant Materials

The fresh unriped five citrus fruits, *C. assamensis* (Ada), *C. maxima* (Ba), *C. meyeri* (Chi), *C. sinensis* (Ma) and *C. aurantium* (Ka) were collected from citrus research centre, Jointiapur, Sylhet and the garden of local farmer to get chemically treated free sample. All the samples were peeled off, dried, grinded into coarse powder and were extracted with petroleum ether for fat free and methanol under sonication bath for highest yield of extracts.

Local names of citrus species: Ada = Adalebu; Ba = Batabilebu; Chi = Chinalebu.; Ma = Malta and Ka = Karuljamir.

Extraction Procedure

Dried peel powder of different citrus samples were taken in glass bottle containing plastic cap and extracted initially with petroleum ether under sonication bath (Trans sonicator, T-60) to remove the fatty constituents of peel. The sample was extracted by three times to get the maximum extract. The mixture (sample + solvent) was filtered through Whatman No.1 filter papers. The filtrate was then concentrated with a rotary evaporator under reduced pressure at 50 °C to obtain brownish mass of peels. After getting the petroleum ether treated peel extract, residue peel powders were allowed to dry and were dissolved in methanol for further extraction with the same process as mentioned above.

***In vitro* Assay of Total Antioxidant Capacity**

The total antioxidant capacity of different citrus peel extracts, *C. assamensis* (Ada), *C. maxima* (Ba), *C. meyeri* (Chi), *C. sinensis* (Ma) and *C. aurantium* (Ka) was determined according to the procedure of [10] with some modifications. Briefly, 0.3 mL solutions of different extracts or standard (catechin) at different concentrations (100, 200, 300, 400 and 500 µg/mL) were taken in the test tubes. 3.0 mL of reaction mixtures containing 0.6 M sulphuric acid, 28 mM sodium phosphate and 1% ammonium molybdate were added into each of the test tubes. The test tubes were incubated at 95 ^{0}C for 10 min to complete the reaction. The absorbance of the solutions was measured at 695 nm using spectrophotometer against blank after cooling at room temperature. A typical blank solution contained 3.0 mL of reaction mixtures and the appropriate volume (300 µL) of the same solvent used for the sample and it was incubated at 95 ^{0}C for 10 min and the absorbance was measured similarly at 695 nm.

***In vitro* Assay of Reducing Power Capacity**

The reducing power capacity of different extracts, *C. assamensis* (Ada), *C. maxima* (Ba), *C. meyeri* (Chi), *C. sinensis* (Ma) and *C. aurantium* (Ka) was evaluated by the method described by Oyaizu [11]. Briefly, 0.25 mL solutions of different extracts or standard (ascorbic acid) at different concentrations of solution (20, 40, 60, 80 and 100 µg/mL) were taken into the test tubes. 0.625 mL of potassium phosphate buffer (0.2 M) (pH 6.6) and 0.625 mL of potassium ferricyanide [$K_3Fe(CN)_6$] (1%) solution were added into each of the test tubes. The reaction mixtures were incubated for 20 min at 50 ^{0}C to complete the reaction. 0.625 mL solution of 10% trichloro-acetic acid (TCA) was added into each of the test tubes. The total mixtures were centrifuged at 3000 rpm for 10 min. 1.8 mL supernatant was withdrawn from the mixture and mixed with 1.8 mL of distilled water. 0.36 mL solution of 0.1% ferric chloride ($FeCl_3$) was added to the diluted reaction mixtures. Then the absorbance of the solutions was measured at 700 nm using a spectrophotometer against blank. A typical blank solution contained the same solution mixture without plant extract or standard and it was incubated under the similar conditions as done for the sample solution. The absorbance of the blank solution was measured at 700 nm against the solvent used in solution preparation. The increased absorbance of the reaction mixture indicated the increase reducing power capacity of the sample extract.

III. RESULTS

Effects of different concentrations of peel extracts treated with petroleum ether on total antioxidant capacity

Total antioxidant capacity of peel extract of different varieties of fruits was determined spectrophotometrically and the results were compared with standard catechin. Different concentrations of peel extracts (100, 200, 300, 400 and 500 µg/mL) of different varieties of fruits were used in the assay and the absorbance were recorded. Table 1 shows the total antioxidant capacity of petroleum ether extract of citrus peel, *C. assamensis* (Ada), *C. maxima* (Ba), *C. meyeri* (Chi), *C. sinensis* (Ma) and *C. aurantium* (Ka). For *C. assamensis* (Ada), the absorbance of the extracts of different doses (100, 200, 300, 400 and 500 µg/mL) were recorded as 0.082, 0.141, 0.211, 0.262 and 0.292 respectively while for *C. maxima* (Ba) variety, the following absorbance were recorded as 0.073, 0.134, 0.183, 0.231 and 0.262 respectively for the above concentrations of the test extract. Similarly, the absorbances for *C. meyeri* (Chi) variety were found as 0.123, 0.173, 0.213, 0.242 and 0.292 respectively for different concentrations of extract. The effects of different concentrations (100, 200, 300, 400 and 500 µg/mL) of standard catechin on total antioxidant capacity were demonstrated in Table 1. The absorbance was increased dose dependently and the values were recorded as 0.13, 0.23, 0.26, 0.33 and 0.37 respectively. The results showed that the total antioxidant capacity had increased gradually in presence of the increased concentrations of the peel extracts. Among the three different varieties, the higher absorbance was recorded for *C. assamensis* (Ada) and *C. meyeri* (Chi) when compared to the control catechin however the values were lower than catechin. Other variety of extracts of traits also showed the potent antioxidant capacity because of their increasing absorbance when compared to standard catechin. The total antioxidant capacity were examined for another two varieties, *C. sinensis* (Ma) and *C. aurantium* (Ka) where the absorbance were found as 0.081, 0.142, 0.212, 0.263 and 0.293 respectively for *C. sinensis* (Ma) variety for the similar doses of extracts of peels. For *C. aurantium* (Ka) variety of peel, the following absorbances were noted as: 0.038, 0.077, 0.101, 0.145 and 0.192 respectively for the above five concentrations. The experimental findings indicated that the total antioxidant capacity had enhanced dose dependently and the effects for *C. sinensis* (Ma) were much higher than *C. aurantium* (Ka) variety when compared to the control. The results suggest that the peel may have some essential phytochemicals causing the antioxidative effects. Among the five varieties of peels, the higher potency on total antioxidant capacity was found for *C. sinensis* (Ma) (Table 1). Therefore, the different peel extracts were essential constituents extracted from petroleum ether and showed antioxidative effects however; *C. sinensis* (Ma) and *C. assamensis* (Ada) varieties of peel exhibited the potent effects regarding this

phenomenon. The peels might be involved in prevention of pathogenic syndromes caused by microorganisms and other abiotic stresses.

Effects of different concentrations of peel extracts treated with methanol on total antioxidant capacity

Fruit peels are the major source of different phytochemicals responsible for the prevention of diverse complications caused by microorganisms and other environmental factors. The activity of fruit peels depends on the extraction procedure. To examine the effectiveness of purification of phytochemicals, petroleum ether and methanol extractions were performed in this study. As shown in Table 2, total antioxidant capacity of five varieties of fruit peels of different concentrations were shown. For *C. assamensis* (Ada) variety, the absorbance of different doses of peel extracts (100, 200, 300, 400 and 500 µg/mL) were recorded as 0.040, 0.068, 0.108, 0.142 and 0.172 respectively while for *C. maxima* (Ba) variety of peel, the absorbance 0.056, 0.130, 0.171, 0.211 and 0.262 respectively were found for the above concentrations. Similarly, the absorbances for *C. meyeri* (Chi) variety of peel were recorded as 0.073, 0.145, 0.187, 0.223 and 0.243 respectively for different concentrations of extracts. The results demonstrated that the total antioxidant capacity had increased gradually in presence of the increased concentrations. Among the different three varieties, the higher absorbances were recorded for *C. maxima* (Ba) variety when compared to the control catechin however other two varieties of peel also showed potent antioxidant capacity when compared to the control. The absorbance of catechin were found to 0.13, 0.23, 0.26, 0.33 and 0.37 respectively for different concentrations (100, 200, 300, 400 and 500 µg/mL) and increased dose dependently. Total antioxidant capacity were also examined for another two varieties, *C. sinensis* (Ma) and *C. aurantium* (Ka) where the following absorbance were recorded for *C. sinensis* (Ma) for the similar doses of extracts of peels: 0.06, 0.12, 0.17, 0.186 and 0.223 respectively. Similarly, the following absorbances for *C. aurantium* (Ka) variety of peel were determined respectively for the above five different concentrations: 0.091, 0.171, 0.231, 0.279 and 0.330. The findings indicated that the total antioxidant capacity of the two species of peel had enhanced dose dependently, however the effects were much pronounced for *C. aurantium* (Ka) extract when compared to the control. *C. sinensis* (Ma) also showed potent antioxidant capacity because of the increasing absorbance although the values were lower than catechin. The petroleum ether extraction of the variety of peel, *C. sinensis* (Ma) was assumed to be more effective than methanol extraction (Table 1 and 2). Among the five varieties of peels during methanol extraction, the higher potency on total antioxidant capacity was found for *C. aurantium* (Ka) extract (Table 2). The results would suggest that the peels may have some essential phytochemicals and cause the antioxidative effects.

Table 1. *In vitro* total antioxidant capacity of petroleum ether extract of *C. assamensis* (Ada), *C. maxima* (Ba), *C. meyeri* (Chi), *C. sinensis* (Ma) and *C. aurantium* (Ka) varieties of citrus fruit peel. Different concentrations of peel extracts or catechin (100, 200, 300, 400 and 500 µg/mL) were used in the assay and the absorbances were recorded.

	Total antioxidant capacity (absorbance at 695 nm)					
Concentrations of peel extracts (µg/mL)	Catechin	Ada	Ba	Chi	Ma	Ka
100	0.13 ± 0.005	0.082 ± 0.0010	0.073 ± 0.0005	0.123 ± 0.0005	0.081 ± 0.0005	0.038 ± 0.0005
200	0.23 ± 0.010	0.141 ± 0. 0005	0.134 ± 0.0020	0.173 ± 0.0010	0.142 ± 0.0005	0.077 ± 0.0050
300	0.26 ± 0.010	0.211 ± 0.0005	0.183 ± 0.0032	0.213 ± 0.0010	0.212 ± 0.0005	0.101 ± 0.0007
400	0.33 ± 0.005	0.262 ± 0.0005	0.231 ± 0.0005	0.242 ± 0.0005	0.263 ± 0.0010	0.145 ± 0.0010
500	0.37 ± 0.010	0.292 ± 0.0005	0.262 ± 0.0010	0.292 ± 0.0005	0.293 ± 0.0005	0.192 ± 0.0008

Table 2. *In vitro* total antioxidant capacity of methanol extract of *C. assamensis* (Ada), *C. maxima* (Ba), *C. meyeri* (Chi), *C. sinensis* (Ma) and *C. aurantium* (Ka) varieties of citrus fruit peel. Different concentrations of peel extracts or catechin (100, 200, 300, 400 and 500 µg/mL) were used in the assay and the absorbances were recorded.

	Total antioxidant capacity (absorbance at 695 nm)					
Concentrations of peel extracts (µg/mL)	Catechin	Ada	Ba	Chi	Ma	Ka
100	0.13 ± 0.001	0.040 ± 0.0005	0.056 ± 0.002	0.073 ± 0.0050	0.060 ± 0.005	0.091 ± 0.001
200	0.23 ± 0.010	0.068 ± 0.0010	0.130 ± 0.001	0.145 ± 0.0005	0.120 ± 0.010	0.171 ± 0.001
300	0.26 ± 0.010	0.108 ± 0.0005	0.171 ± 0.001	0.187 ± 0.0005	0.170 ± 0.010	0.231 ± 0.002
400	0.33 ± 0.005	0.142 ± 0.0010	0.211 ± 0.001	0.223 ± 0.0050	0.186 ± 0.005	0.279 ± 0.001
500	0.37 ± 0.010	0.172 ± 0.0010	0.262 ± 0.001	0.243 ± 0.0050	0.223 ± 0.005	0.330 ± 0.001

Effects of different concentrations of peel extracts treated with petroleum ether on reducing power capacity

To examine whether the peels of different varieties are involved in prevention of oxidative stress caused by biotic and abiotic environmental stimuli, reducing power capacity of each extract was examined and evaluated during petroleum ether and methanol extraction. As shown in Table 3, the reducing power capacity of petroleum ether extract of citrus peel, *C. assamensis* (Ada), *C. maxima* (Ba), *C. meyeri* (Chi), *C. sinensis* (Ma) and *C. aurantium* (Ka) has been demonstrated. For *C. assamensis* (Ada) variety, the absorbance of different concentrations (20, 40, 60, 80 and 100 µg/mL) were determined as 1.108, 1.523, 1.883, 2.133 and 2.353 respectively while for *C. maxima* (Ba) variety, the values for the above mentioned concentrations were found as 1.473, 2.113, 2.370, 2.513 and 2.676 respectively. The absorbances of the standard ascorbic acid for the above concentrations were found as 2.032, 2.826, 3.016, 3.144 and 3.173 respectively. Similarly, the absorbances of the different concentrations (20, 40, 60, 80 and 100 µg/mL) of the extract of *C. meyeri* (Chi) were noted as 0.853, 1.126, 1.310, 1.466 and 1.633 respectively. Among the three varieties of peel, the higher absorbances were found for *C. maxima* (Ba) against different concentrations however the values were lower when compared to the standard ascorbic acid. The other two varieties of peels also showed the potent reducing power capacity due to the increasing absorbance. Total reducing power capacity was enhanced for the above three varieties dose dependently. Similar increasing tendency was shown for the standard ascorbic acid at different concentrations (Table 3). The reducing power capacity of another two varieties of peel, *C. sinensis* (Ma) and *C. aurantium* (Ka) was also examined during petroleum ether extraction for different extract concentration (20, 40, 60, 80 and 100 µg/mL). The increased and pronounced reducing power capacity were demonstrated for *C. sinensis* (Ma) and *C. aurantium* (Ka) and the absorbance were increased dose dependently showing higher antioxidative effects of the peel. As shown in Table 3, *C. sinensis* (Ma) variety of peel showed higher potency on reducing power capacity (absorbance at 700 nm: 0.460, 0.826, 1.130, 1.243 and 1.276 respectively) against different doses of extract rather than *C. aurantium* (Ka) (absorbance at 700 nm: 0.351, 0.58, 0.716, 0.86 and 1.213 respectively). Therefore, all the varieties of peel extract produced the increased reducing power capacity when compared to standard ascorbic acid however; *C. maxima* (Ba) *and C. assamensis* (Ada) showed the potential role on antioxidative effects and might be involved in prevention of different pathogenic complications caused by microorganisms in response to adverse environmental stimuli either biotic or abiotic.

Effects of different concentrations of peel extracts treated with methanol on reducing power capacity

Table 4 shows the reducing power capacity of methanol extract of citrus peel varieties, *C. assamensis* (Ada), *C. maxima* (Ba), *C. meyeri* (Chi), *C. sinensis* (Ma) and *C. aurantium* (Ka). The absorbance were recorded in response to different concentrations of extracts of peel (20, 40, 60, 80 and 100 µg/mL) and compared to standard ascorbic acid for the similar concentrations. For *C. assamensis* (Ada), the absorbance in different concentrations (20, 40, 60, 80 and 100 µg/mL) were recorded as 0.602, 0.820, 1.146, 1.300 and 1.440 respectively and for *C. maxima* (Ba), the values for the above mentioned concentrations were 0.447, 0.630, 0.780, 0.850 and 0.950 respectively obtained. The absorbance of different doses of extract was recorded similarly as 1.376, 2.130, 2.350, 2.420 and 2.480 respectively for *C. meyeri* (Chi) variety. The reducing power capacity was enhanced for the above three varieties dose dependently. The higher reducing power capacity for *C. meyeri* (Chi) was observed when compared to ascorbic acid however other varieties of extracts of traits also showed the potent reducing power capacity compared to standard ascorbic acid. All the three varieties of peel showed the higher potency on antioxidative effects and the effects were enhanced in response to the increasing

concentrations although the values were lower than ascorbic acid. Total reducing power capacity was also examined from methanol treatment for another two varieties, *C. sinensis* (Ma) and *C. aurantium* (Ka) where the absorbance were found as 0.850, 1.313, 1.540, 1.640 and 1.743 respectively for *C. sinensis* (Ma) variety for the similar doses of extracts of peels. The values were increased for different extract concentrations respectively when compared to ascorbic acid. The respective absorbances for ascorbic acid were recorded as 2.026, 2.7, 3.015, 3.14 and 3.17 and the values were increased for increasing concentrations. The extract *C. aurantium* (Ka) also caused the higher potency and reducing power capacity (absorbance at 700 nm: 1.53, 2.36, 2.62, 2.76 and 2.84 respectively) during methanol extraction. Therefore, all these different varieties of fruits had the potent reducing power capacity in response to different extract concentrations and the results were appeared to indicate that *C. aurantium* (Ka) and *C. meyeri* (Chi) species of peel played the potential role regarding this phenomenon. Although petroleum ether extract of these species of peel showed the potent effects however methanol extracts cause much higher and pronounced results showing the higher antioxidative effects (Table 4 and 3). The peel extracts after methanol treatment produced higher specificity thereby assumed to be effective extraction strategy for the separation of phytochemicals. The enhanced reducing power capacity of the species of fruit peel might be due to the presence of compounds responsible for the prevention of oxidative stress caused by microorganisms or other environmental stresses.

Table 3. *In vitro* reducing power capacity of petroleum ether extract of *C. assamensis* (Ada), *C. maxima* (Ba), *C. meyeri* (Chi), *C. sinensis* (Ma) and *C. aurantium* (Ka) varieties of citrus fruit peel. Different concentrations of peel extracts or ascorbic acid (20, 40, 60, 80 and 100 µg/mL) were used in the assay and the absorbances were recorded.

	Reducing power capacity (absorbance at 700 nm)					
Concentrations of peel extracts (µg/mL)	Ascorbic acid	Ada	Ba	Chi	Ma	Ka
20	2.032 ± 0.0005	1.108 ± 0.001	1.473 ± 0.005	0.853 ± 0.005	0.460 ± 0.010	0.351 ± 0.0005
40	2.826 ± 0.0050	1.523 ± 0.005	2.113 ± 0.005	1.126 ± 0.015	0.826 ± 0.005	0.580 ± 0.0100
60	3.016 ± 0.0010	1.883 ± 0.011	2.370 ± 0.010	1.310 ± 0.010	1.130 ± 0.010	0.716 ± 0.0050
80	3.144 ± 0.0005	2.133 ± 0.050	2.513 ± 0.005	1.466 ± 0.005	1.243 ± 0.005	0.860 ± 0.0100
100	3.173 ± 0.0010	2.353 ± 0.005	2.676 ± 0.005	1.633 ± 0.050	1.276 ± 0.010	1.213 ± 0.0100

Table 4. *In vitro* reducing power capacity of methanol extract of *C. assamensis* (Ada), *C. maxima* (Ba), *C. meyeri* (Chi), *C. sinensis* (Ma) and *C. aurantium* (Ka) varieties of citrus fruit peel. Different concentrations of peel extracts or ascorbic acid (20, 40, 60, 80 and 100 µg/mL) were used in the assay and the absorbances were recorded.

	Reducing power capacity (absorbance at 700 nm)					
Concentrations of peel extracts (µg/mL)	Ascorbic acid	Ada	Ba	Chi	Ma	Ka
20	2.026 ± 0.005	0.602 ± 0.001	0.447 ± 0.001	1.376 ± 0.005	0.850 ± 0.010	1.53 ± 0.01
40	2.700 ± 0.100	0.820 ± 0.010	0.630 ± 0.010	2.130 ± 0.010	1.313 ± 0.015	2.36 ± 0.01
60	3.015 ± 0.001	1.146 ± 0.005	0.780 ± 0.010	2.350 ± 0.010	1.540 ± 0.010	2.62 ± 0.01
80	3.140 ± 0.010	1.300 ± 0.010	0.850 ± 0.010	2.420 ± 0.010	1.640 ± 0.010	2.76 ± 0.01
100	3.170 ± 0.010	1.440 ± 0.010	0.950 ± 0.010	2.480 ± 0.010	1.743 ± 0.015	2.84 ± 0.01

Phytochemical screening and comparative analysis of fruit peels

In Table 5, the two fractions PE (petroleum ether) and Me (methanol) have been shown and their effects on antioxidative activity were compared and evaluated. The effects were demonstrated for maximal concentration of peel extracts (500 µg/mL). Among the petroleum ether fractions, *C. sinensis* (Ma), *C. assamensis* (Ada) and *C. meyeri* (Chi) extract showed higher potency on antioxidative effects when compared to the standard catechin. The other two varieties, *C. maxima* (Ba) and *C. aurantium* (Ka) (PE fraction) although produced potent effects however the values were much lower than the standard catechin. As illustrated in Table 5, the higher antioxidative activity for the varieties, *C. aurantium* (Ka) and *C. maxima* (Ba) after methanol

treatment (Me fraction) were observed when compared to catechin. Although the absorbance values against standard catechin were lower, the extracts showed much higher potency on antioxidative effects because of the increasing absorbance against different concentrations of the extracts of peels during investigation demonstrating that the methanol extract of the fruit peel may have some potential compounds responsible for the prevention of complications caused by the microorganisms. The highest activity was observed in the extract of *C. sinensis* (Ma) (PE fraction) for the maximal concentration of peel extract (500 µg/mL) when compared to the same species in methanol extraction (absorbance at 500 nm: *C. sinensis* (Ma) 0.293 (PE) and 0.223 (Me). The results are appeared to indicate that some species of fruit peel exhibited higher potency on antioxidative effects. Similar trends of antioxidative effects were demonstrated for *C. assamensis* (Ada) and *C. meyeri* (Chi) extract during preparations in petroleum ether extractions when compared to control while methanol fractions for these species of peel show lower potency on antioxidative effects (absorbance at 569: 0.292 and 0.292 (PE fraction); 0.172 and 0.243 (Me fraction). Among the PE fractions, *C. aurantium* (Ka) showed much less absorbance values when compared to the effects of standard catechin. On the contrary, *C. aurantium* (Ka) species (methanol fractions) showed much increased antioxidative effects when compared to catechin and the effects were much lower for petroleum ether fractions (shown in Table 2, Table 3 and Table 5). Among the Me fractions, *C. assamensis* (Ada) showed much less absorbance values when compared to the control while the higher value of the same species during petroleum ether extraction was observed. Therefore, it is obvious that PE extracts of the species may have some essential constituents demonstrating the higher antioxidative effects. The results are good agreement that both these fractions have potential antioxidative effects however the values of absorbance during petroleum ether extraction were assumed to be higher than those of methanol fractions. It is reasonable that the antioxidative activity in some peel extract might be separated by different strategy and extraction procedures.

As shown in Table 6, the reducing power capacity of different species of fruit peel, *C. assamensis* (Ada), *C. maxima* (Ba), *C. meyeri* (Chi), *C. sinensis* (Ma) and *C. aurantium* (Ka) for petroleum ether (PE) and methanol (Me) fractions have been shown and the effects were demonstrated for maximal concentrations of peel extracts (100 µg/mL). Among the petroleum ether fractions, *C. maxima* (Ba), *C. assamensis* (Ada) and *C. meyeri* (Chi) extract showed higher potency on reducing power capacity when compared to the standard ascorbic acid. The other two varieties, *C. sinensis* (Ma) and *C. aurantium* (Ka) also produced potent effects because of their increasing absorbance however the values were much lower than the standard ascorbic acid. On the contrary, the reducing power capacity for Ka (*C. aurantium*), Chi (*C. meyeri*) and Ma (*C. sinensis*) in methanol extraction had much higher potency when compared to ascorbic acid. While the other two varieties, Ada and Ba caused much lower effects when compared to control although the extracts were effective because of their increasing concentrations. The maximal activity was observed in the extract of *C. aurantium* (Ka) (Me fraction) for the maximal concentration of peel extract (100 µg/mL) when compared to the same species in petroleum ether extraction (absorbance at 700 nm: *C. aurantium* 1.213, PE fraction and 2.84, Me fraction). The results are appeared to indicate that some species of fruit peel exhibited higher potency on reducing power effects depending on the solvent extraction. Similar trends of reducing power capacity were demonstrated for *C. meyeri* (Chi) and *C. sinensis* (Ma) during preparations in petroleum ether extractions when compared to control while methanol fractions for these species of peel showed higher potency on antioxidative effects respectively (absorbance at 700 nm: 1.633 and 1.276 for PE fraction); 2.48 and 1.743 for Me fraction) (Table 6). Therefore, it is substantial to make strategy for the extraction of the compounds present in fruit peel and both PE and Me fractions may produce much better results on the purification of the compounds having potent reducing power capacity.

It was observed that the higher antioxidant capacity in petroleum ether extract of citrus peel was given by *C. sinensis* (Ma), *C. assamensis* (Ada) and *C. meyeri* (Chi) peel against the standard catechin (Table 1, Table 5). On the other hand, the peels of *C. maxima* (Ba) *and C. aurantium* (Ka) showed lower antioxidant capacity (Table 5). The trend of overall antioxidative effects of petroleum ether extract of citrus peels according to their absorbance is given bellow:

Ma > Ada > Chi > Ba > Ka

Among the methanol extract of citrus peel, the higher antioxidant capacity was observed by *C. aurantium* (Ka) and *C. maxima* (Ba) peel when compared *to* the standard catechin (Table 2, Table 5) and the lower effects were given by the peel of *C. meyeri* (Chi), *C. sinensis* (Ma) and *C. assamensis* (Ada) against the maximal response of peel extract (500 µg/mL) (Table 5). The trend of overall antioxidative effects of methanol extract of citrus peel according to their absorbance is given bellow:

Ka > Ba > Chi > Ma > Ada

It was observed from the investigation that the higher reducing power capacity in petroleum ether extract of citrus peel was given by *C. maxima* (Ba), *C. assamensis* (Ada) and *C. meyeri* (Chi) peel against the standard ascorbic acid (Table 3, Table 6). On the other hand, the peels of *C. sinensis* (Ma) *and C. aurantium* (Ka)

showed lower reducing power capacity (Table 3, Table 6). The trend of overall reducing power capacity of petroleum ether extract of citrus peel according to their absorbance is given bellow:

Ba > Ada > Chi > Ma > Ka

Among the methanol extract of citrus peel, the higher reducing power capacity were recorded by *C. aurantium* (Ka), *C. meyeri* (Chi) and *C. sinensis* (Ma) peel when compared to the standard ascorbic acid (Table 4, Table 6) and the lower absorbance were given by the peel of *C. assamensis* (Ada) and *C. maxima* (Ba) (Table 4 and Table 6). The trend of overall reducing power capacity of methanol extract of citrus peel according to their absorbance is given bellow:

Ka > Chi > Ma > Ada > Ba

Table 5. Comparative efficacy on total antioxidant capacity of petroleum ether and methanol extract of different varieties of citrus fruit peel. The effects of 500 µg/mL peel extracts of different varieties of fruit were shown. The effects of catechin (control) on total antioxidant capacity were similarly done except giving peel extract. The results are means of ± standard deviation for three values in each group of sample.

Name of sample	Total antioxidant capacity (absorbance at 695 nm)	
	Petroleum ether (PE) fraction	Methanol (Me) fraction
Catechin	0.370 ± 0.0100	0.370 ± 0.010
C. assamensis (Ada)	0.292 ± 0.0005	0.172 ± 0.001
C. maxima (Ba)	0.262 ± 0.0010	0.262 ± 0.001
C. meyeri (Chi)	0.292 ± 0.0005	0.243 ± 0.005
C. sinensis (Ma)	0.293 ± 0.0005	0.223 ± 0.005
C. aurantium (Ka)	0.192 ± 0.0008	0.330 ± 0.001

IV. DISCUSSION

The comparative efficacy and evaluation on total antioxidant capacity and reducing power capacity of five citrus fruit peel varieties; *C. assamensis* (Ada), *C. maxima* (Ba), *C. meyeri* (Chi), *C. sinensis* (Ma) and *C. aurantium* (Ka) have been demonstrated in the current study. Fruit peels are the major sources of phytochemicals and have been recognized to be involved in prevention of diverse complications caused by microorganisms. The current investigation regarding this phenomenon therefore has been undertaken and different fruit peels have been shown to exert their potential antioxidative effects. To identify the compounds regarding this phenomenon, different approaches and strategies have been employed. In the current study, however the extractions were carried out by petroleum ether and methanol as potent organic solvents. In response to different concentrations of extract (100, 200, 300, 400 and 500 µg/mL) of the varieties of fruit peel, *C. assamensis* (Ada), *C. maxima* (Ba), *C. meyeri* (Chi), *C. sinensis* (Ma) and *C. aurantium* (Ka), the total antioxidant capacity were increased dose dependently (Table 1) showing that the petroleum ether extract of peels were very active fractions during separation of the compounds. Among the different varieties of peel, the total antioxidant capacity was much higher for *C. sinensis* (Ma), *C. assamensis* (Ada) and *C. meyeri* (Chi) however for other varieties of peel (Ba and Ka), the absorbance values were comparatively lower although the peels were very active ingredient because of their increasing absorbance. Therefore, it is assumed that petroleum ether causes extractions of some essential compounds from the peels and thereby is recognized to be as a potent organic solvent for purification of the compounds having antioxidative effects. It has been demonstrated from the previous investigations that phytochemicals were extracted through petroleum ether and were found to play the role on antioxidative effects [12][13]. Singh *et al.* [14] examined the antioxidant activity and found that the free radical scavenging activity of the different fractions of petroleum ether extract of *P. nigrum* (PEPN) had increased in a concentration dependent manner. The current findings are compatible to their investigation and the results are strongly supported by them. The previous studies revealed that the antioxidant activity of plant extracts is strongly dependent on the solvent due to the different antioxidant potentials of compounds with different polarity [15][16]. Similarly, methanol extraction also causes the findings of antioxidant capacity of

different fruit peels and the extract of different concentrations (100, 200, 300, 400 and 500 µg/mL) of different peels, *C. aurantium* (Ka), *C. maxima* (Ba), *C. meyeri* (Chi), *C. sinensis* (Ma) and *C. assamensis* (Ada) shows the higher absorbance and the absorbance were found to increase dose dependently (Table 2). Therefore, methanol extract is also very active fraction and may have some potent compounds showing antioxidant capacity. Among the different varieties of peels, *C. aurantium* (Ka) and *C. maxima* (Ba) were shown to have higher absorbance demonstrating the higher antioxidant capacity. Although the absorbance were lower when compared to catechin, the peel extract of *C. meyeri* (Chi), *C. sinensis* (Ma) and *C. assamensis* (Ada) were very active and potent during preparation with methanol and show antioxidative effects since their absorbance were increased similarly dose dependently. The higher antioxidant capacity and antioxidative effects in fruits were observed after methanol extractions as demonstrated by the previous investigation [17]. The results are very compatible to their findings and strongly supported by their research investigation. Therefore, both petroleum ether and methanol are considered to be very potent organic solvents and cause the extractions of compounds from the peels. Although not identified, it is assumed that some phytochemicals are present in the fruit peels and show antioxidative effects. Several lines of evidences are pointed to suggest that fruit peels are very active ingredients and cause antioxidative effects [18][19].

Citrus fruit is popular due to its characteristic flavor, taste, aroma and numerous health benefits. Processing of citrus fruits into different products or their consumption as such produce by-products such as peel, seed and pulp which are usually wasted. This waste is an important source of bioactive compounds such as ascorbic acids. Citrus peels contain significant amounts of phenolic compounds especially phenolic acids and flavonoids. Citrus fruit residues, which are usually discarded as waste can be used as nutraceutical resources. The previous studies reported that the peel of pomelo fruit had contained a higher amount of antioxidant content and antioxidant capacity as compared to its pulp [20]. More importantly, the prevention of many chronic diseases, such as cancer, diabetes and cardiovascular disease, has been suggested to be associated with the antioxidant activity [6]. Therefore, identification and characterization of natural antioxidants, from fruits and vegetables, are of great importance to human health and clinical purposes. Plants are the major source of natural antioxidants due to the presence of various biophenolic compounds like phenolic acids, saponins, flavonoids and tocopherols [21]. Plant materials which are rich in phenol contents, are widely used as medicinal remedies due to their various pharmacological properties [21]. Flavonoids are naturally-occurring compounds of plants and account for different phenolic compounds. They have been shown to effectively scavenge most oxidizing molecules, which include singlet oxygen and other free radicals [22]. The antioxidative effects observed in different varieties of fruit peels in the current study are therefore, very important findings in the field of elucidation of phytochemicals as demonstrated by the above several lines of investigations.

Reducing power capacity was determined from petroleum ether and methanol fractions of the different fruit peels, *C. assamensis* (Ada), *C. maxima* (Ba), *C. meyeri* (Chi), *C. sinensis* (Ma) and *C. aurantium* (Ka). The absorbance of different concentrations of peel extract (20, 40, 60, 80 and 100 µg/mL) was increased dose dependently showing the higher antioxidative effects of the fruit peels. For the different peel species, *C. maxima* (Ba), *C. assamensis* (Ada) and *C. meyeri* (Chi) showed higher effectivity on reducing power capacity and thereby higher antioxidative effects were demonstrated when purified through petroleum ether extraction. Although the effects of other two varieties of peels (Ma and Ka) were lower when compared to ascorbic acid; however they were also considered to be potent varieties of fruit peel and showed antioxidative effects because of their increasing absorbance against ascorbic acid. Therefore, all the varieties of fruit peel are biologically active and may have some essential bioactive compounds. Several lines of evidences are strongly suggested in this connection that foods containing phytochemicals, such as fruits and vegetables containing antioxidants, have protective effects against disease. It has been shown from the previous study that the reducing power capacity were determined from the biological sample extracted with petroleum ether and were found to be enhanced [12][23]. Moreover, the absorbance of all extracts and standard is a function of their concentrations, and generally, increases linearly with the increase in concentration as shown by some researchers [24]. The results recorded after petroleum ether treatment in the present study, are supported by their findings as the reducing power capacity in the extract were enhanced in their experiment. Similarly, methanol extractions of different fruit peels were examined to find the reducing power capacity and the absorbance of peel extracts of different concentrations (20, 40, 60, 80 and 100 µg/mL) were enhanced dose dependently showing the peels were very active and exhibited antioxidative effects. Among the different varieties of peels, *C. aurantium* (Ka), *C. meyeri* (Chi) and *C. sinensis* (Ma) were shown to have higher reducing power capacity and played the potential role however other varieties of peel (Ada and Ba) also showed potent antioxidative effects although the values were comparatively lower against the standard ascorbic acid. Therefore, petroleum ether and methanol extracts of peels were found to have diverse antioxidative effects and may have some essential bioactive compounds. The antioxidative effects were enhanced in response to the extracts of peel through methanol solvent extraction as demonstrated by the previous observations [25][13]. Dar *et al*. [26] demonstrated that the methanolic root extract of *Mentha arvensis* L. had showed good reducing power when compared to the standard

ascorbic acid. The results are compatible and are strongly supported by their findings. The separation of the compounds from the fruit peels with petroleum ether and methanol are therefore, very essential extraction strategy causing the purification of phytochemicals and thereby the higher antioxidative effects were observed.

The increased antioxidant capacity and reducing power capacity of peel varieties are of great importance in biological system. In adverse environmental circumstances, reactive oxygen species (ROS) are produced thereby metabolic alterations and cellular impairment are observed however phytochemicals present in the plants play the pivotal role because of their antioxidative effects. Excess production of ROS in response to biotic and abiotic stresses has been shown to cause oxidative stress leading to cellular damage and ultimately cell death. To prevent or alleviate the ROS induced damage allowing the beneficial functions of ROS to continue, plants have evolved an intriguing antioxidant defence system which functions to keep levels of reactive or active oxygen species under control. Antioxidant defence systems comprise both enzymatic as well as non-enzymatic components. The production of ROS in response to environmental stress is reduced through these defense systems and thereby the stress tolerance is observed. Phytochemicals are major ingredients available in plant kingdom and are considered to be involved in the prevention of oxidative stress caused by both biotic and abiotic stress in the environment. These environmental stimuli affect the living organisms and cause metabolic alterations however, because of the presence of antioxidative effects, phytochemicals in fruits or fruit peels were found to neutralize the effects. The ROS is very powerful chemicals causing diverse biochemical and biological adverse effect in the organisms. Phytochemicals present in the plant kingdom plays the critical role through the antioxidative functions. The enhanced antioxidative effects in the fruit peels in the present study might be due to the presence of some essential phytochemicals. It is assumed that fruit peel having active ingredients and causes antioxidative effects in the biological system.

Antioxidants are those substances that prevent most of the oxidation reactions which are initiated with the production of free radicals. Antioxidants captured free radicals thereby delaying or preventing damage to the cells and tissues of the living organisms. Various rich sources of antioxidant compounds such as carotenoids, flavonoids, lutein, and polyphenols are vegetables and fruits; plant extraction or isolation produces some potent compounds or substances that are accountable for biological activity [27]. Flavonoids are one of the most diverse and widespread group of natural compounds and are probably the most important natural phenolics. These compounds possess a broad spectrum of chemical and biological activities including radical scavenging properties [28]. Previously, Jayaprakasha *et al.* [29] indicated that the total antioxidant activity of citrus was due to the presence of phenolics and flavonoids. Our data was supported with the findings of Li *et al*. [30] and Guo *et al*. [31] who observed that fruit peel of mango, kiwifruit, guava, and orange among others contains high concentration of phenolics, flavonols, and antioxidant activities than pulp and seed extracts.

Most of the bioactivities of these crude extracts in our investigation might be due to the high amounts of flavonoids and phenolic content. Flavonoids are good and highly effective scavengers for most of the oxidizing compounds such as singlet oxygen, and various types of other free radicals are implicated or involved in several diseases. Flavonoids suppress reactive oxygen formation; in free radical production, chelate elements are specially used, and scavenge reactive species up regulate and protect antioxidant defenses [32]. Similarly, phenolic compounds confer oxidative stress tolerance in plants. Such types of crude extracts of fruits, herbs, vegetables, cereals, and other plant materials are fulfilled by phenolic contents which are highly used in the food industry and medicine industry for their antioxidative properties and for better health. Collectively, our findings are useful and good agreement for the evaluation of antioxidative effects of peel extract in petroleum ether and methanol which may play the potential role on the elucidation of phytochemicals having the beneficial and clinical importance.

Table 6. Comparative efficacy on reducing power capacity of petroleum ether and methanol extract of different varieties of citrus fruit peel. The effects of 100 µg/mL peel extracts of different varieties of fruit were shown. The effects of ascorbic acid (control) on reducing power capacity were similarly done except giving peel extract. The results are means of ± standard deviation for three values in each group of sample.

Name of sample	Reducing power capacity (absorbance at700 nm)	
	Petroleum ether (PE) fraction	Methanol (Me) fraction
Ascorbic acid	3.173 ± 0.001	3.170 ± 0.010
C. assamensis (Ada)	2.353 ± 0.005	1.440 ± 0.010

C. maxima (Ba)	2.676 ± 0.005	0.950 ± 0.010
C. meyeri (Chi)	1.633 ± 0.050	2.480 ± 0.010
C. sinensis (Ma)	1.276 ± 0.010	1.743 ± 0.015
C. aurantium (Ka)	1.213 ± 0.010	2.840 ± 0.010

V. CONCLUSIONS

Five different varieties of fruits peels, *C. assamensis* (Ada), *C. maxima* (Ba), *C. meyeri* (Chi), *C. sinensis* (Ma) and *C. aurantium* (Ka) were examined and the antioxidative effects particularly total antioxidant capacity and reducing power capacity were assayed and evaluated through organic solvent extraction. Among the five different varieties of species of peel, both petroleum ether and methanol fraction show the potent antioxidative effects. The separation of the compounds through these solvents shows higher specificity on antioxidative effects showing the presence of some phytochemicals in the fruit peel extracts. In petroleum ether extraction, the following species of fruits were potential: Ma, Ada, Chi, Ba and Ka; while in methanol extraction, the varieties of peels are assumed to be predominant: Ka, Chi, Ma, Ada and Ba. It is assumed that the compounds present in the variety of peels may play the potential role on the prevention of clinical and biochemical perspectives caused by microorganisms or other environmental stresses.

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