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The Flavone, Total Phenolic Composition and Antioxidant Activity of Extracts from Bast of *Citrus maxima* and Rind of *Citrus maxima*

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Abstract. In this paper, two extracts were extracted from bast of Citrus maxima (BCM) and the rind of Citrus maxima (RCM) using ethanol and water respectively. Total flavonoids and phenolics of two extracts were detected by UV/visible spectrophotometer and their antioxidant activity were evaluated by scavenging three free radicals (O_2^-•, DPPH • and •OH radical). The flavonoids in ethanol extracts of RCM were analyzed by HPLC. Results showed that all extracts of RCM contained more flavonoids and phenolics than BCM and scavenging free radicals effect of ethanol extract of RCM were stronger than water extract. The ethanol extracts of RCM included four flavonoids of catechins, rutin, naringin and hesperidin. The rind of Citrus maxima as a plant-based antioxidative resource and as raw materials to extract the flavonoid and phenol.

Keywords: Rind of *Citrus maxima* and Bast of *Citrus maxima*, Flavonoids, Total phenol, Antioxidant activity.

1. Introduction

Antioxidants are often added to foods to prevent the radical chain reactions of oxidation, and they act by inhibiting the initiation and propagation step leading to the termination of the reaction and delay the oxidation process [1]. However, the commonly used synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxy toluene (BHT) are restricted by legislative rules because of doubts over their toxic and carcinogenic effects. Natural antioxidant have been found to be safe without side-effects, and natural products from plants offer high potency and selectivity as a result of long evolutionary selection [2]. Therefore, there has been a considerable interest in the food industry to find natural antioxidants to replace synthetic compounds in food applications, and a growing trend in consumer preferences for natural antioxidants, all of which has given more impetus to explore natural sources of antioxidants.

Many herbs and spices have been shown to important antioxidant effects in food. A number of reports have recently been published on the use of natural antioxidants. Various plant extracts have been examined for their antioxidant activity [3], [4]. However, there is a pressing need for new plant-based antioxidants from uninvestigated plants. Using agricultural waste products, such as fruit peels as natural antioxidants or raw material of extraction natural antioxidants is a low-cost and safe from wastewater. Numerous investigators have observed that rich-fiber agricultural waste produces natural antioxidants. Orange peel has been used to extract the essential oil and natural antioxidants [5].

Citrus. maxima (C. maxima) are giant citrus (commonly known as Pummelo or Shaddock) originated from South East Asia, India and cultivated throughout the tropical and temperate regions for the fruits. It is globose, pear-shaped with 11-14 segments. C. maxima fruits are a largest of all citrus variety and are

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consumed in high quantities all over the world. Like other citrus fruits, it has a small edible portion and very large amounts of byproduct wastes, such as peels and they are formed every year.The peel of C. maxima accounted for about 40% of the total C. maxima fruit. These are not only a waste of resource, but also causes environmental pollution. Modern medical research showed that extracts from C. maxima peel could exhibit anti-oxidation, anti-aging, anti-cancer, antimicrobial, antimicrobial enzymes [6]. Therefore, they have potential value and prospect for the development of deep processing and comprehensive utilization of C. maxima peel to investigate the possible chemical constituents and antioxidant activity. At so far, although extraction technology of the flavone from the peels of Citrus maxima have research and reported [7]-[9], but the work of describe a peeled the peels of Citrus maxima into bast of Citrus maxima (BCM) and rind of Citrus maxima (RCM), then extracted and determinated for flavone, total phenol and their antioxidant activity from BCM and RCM is one of very few published reports. In this paper, we report on the antioxidant potential of the extracts from the BCM and theRCM.

2. Materials and Methods

2.1. Laboratory Apparatus

A UV1901 spectrophotometer (Shanghai phenixsi light subject instrument Co., Ltd, China), a LGJ–18S Freeze-Drier Instrument (Henan brother Instrument Inc., China), Rotary vacuum evaporator (RE-52A, Shanghai Yarong Biochemical Equipment Co., Shanghai, China), were used in this study.

High performance liquid chromatography (HPLC) analysis was performed with an Agilent 1260 system (Milford, MA, USA) equipped with an HPLC pump separation module, an auto-injector and a 1260 photodiode array detector connected to an Eclipse Plus C18 column (100 mm × 4.6 mm, 3.5 μm).The mobile phase was methanol - 0.4% phosphoric acid in water (45:55, v/v) with a flow rate of 1.0 mL/min, and the column temperature was kept at 20 ℃. Prior to use, all mobile phases were ultrasonically degassed and filtered through a 0.45 μm nylon membrane. The UV spectra were recorded between 190-400 nm for peak characterization, and the detection wavelength was set to 280 nm. The peak area was used to calculate the amount of flavones from the standard curve.

2.2. Chemicals

The following solvents were used for the extractions and analytical procedures: FeSO4·7H2O, 95% ethanol, methanol, acetonitrile, HCl, KOH, H_2O_2, $AlCl_3$·$6H_2O$, NaAc, NaH_2PO_4·$2H_2O$, Na_2HPO_4·$12H_2O$, Tris, Tween, trifluoroacetic acid, trichloroacetic acid, thiobarbituric acid, ethylene diamine tetraacetic acid (EDTA), 2-deoxyribose, Folin–Ciocalteau phenol reagent, pyrogallol and gallic acid (purchased from Shanghai Ling-feng company, Shanghai, China); catechins, rutin, naringin and hesperidin(purchased from Guizhou Dida biological technology co., LTD,Guiyang,China). 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals used were of analytical grade.

2.3. Plant Collection

Citrus maxima fruit were purchased in Huaian city (Jiangsu province, China) in September of 2012. The peel of Citrus maxima fruit were separated into BCM and RCM by hand and the contents of these parts on a dry weight basis were approximately 77.9% and 22.1%, respectively.

2.4. Extraction of the Flavones and Phenolics

The separated BCM and RCM were freeze-dried and then were powdered and sieved through a 0.25 mm (60 mesh) sieve and subsequently packed into plastic bags and stored at -4 ℃ in a refrigerator for later use.

The 4.0g powder was extracted in a round-bottom flask for 3 h with 70% (v/v) ethanol (3×80 mL) at 70 ℃. The extracts were filtered through filter paper and washed three times with 70% ethanol. The liquid portions were concentrated under reduced pressure at 50 ℃ and were subsequently constant volume with ethanol make to 100mL (40mg of dry sample /mL) and analyzed for their phenolics, falvonoids, and their antioxidant capacity. Same methods were used to extract water extracts by using water.

2.5. Phytochemical Analysis of the Each Fraction

Determination of total phenolics: The determination of the total phenolic content in the two extracts was performed as previously described by Negi and Jayaprakasha [10]. The gallic acid (5.00 mg) were dissolved in a 50mL of mixture of methanol:water (6:4 v/v). 0.1mL of the each extract solution and 0.2mL of different concentrations of gallic acid were each mixed with 1.0 mL of ten-fold diluted Folin-Ciocalteu reagent and 0.7 mL of a 7.5% sodium carbonate solution. After standing for 30 min at room temperature, the absorbance was measured at 765 nm using a UV1901 spectrophotometer. Absorbance was compared to a standard curve prepared with gallic acid. Measurements of every sample were taken in triplicate and the results were expressed as milligram gallic acid equivalents (GAE)/g dried weight of plant material (BCM or RCM).

Determination of total flavonoids: The content of total flavonoids was assessed using the colorimetric aluminum chloride assay described by Jia *et al*. [11] with minor modifications. To start, 0.1 mL of the two extract solution was added to a 25 mL volumetric flask and 60% ethanol was added to make a volume of 10 mL. At time zero, 1 mL of 5% $NaNO_2$ was added to the flask, after 5 min, 1 mL of 10% $AlCl_3$ was added. Then after 6 min, 10 mL of 1 M NaOH was added and the solution was mixed. The solution was then brought up to a volume of 25 mL with 60% ethanol and mixed thoroughly. The absorbance of the mixture was determined at 510 nm using a UV1901 spectrophotometer versus a 60% ethanol blank. Following the same method, a rutin solution was used to make the calibration curve. Rutin was used to make the calibration curve and the flavonoids content was expressed as milligram rutin equivalents (RE)/g dried plant powder. Samples were analyzed in triplicate.

2.6. Radical-Scavenging Activity

DPPH assay: The free radical scavenging activity of the extracts was determined as described by Singh et al [12] and Brand-Williams et al [13]. Different concentrations of the extracts were added to test tubes. The sample volume was adjusted to 0.1mL with MeOH. Five mL aliquots of 0.1 mM methanolic solution of DPPH were added to these tubes and shaken vigorously. The tubes were allowed to stand at 27 ℃ for 20 min. The control tube was prepared as above without the addition of sample. Methanol was used for the baseline correction. The changes in the absorption of the samples were measured at 517 nm. The test was performed in triplicate, and the radical scavenging activity was expressed as the inhibition percentage, calculated using the following formula:

$$\% \text{ Inhibition} = (1 - A_1/A_0) \times 100 \quad (1)$$

where A_0 is the absorbance of the control, and A_1 is the absorbance of each fraction. The IC_{50} (the concentration of sample required to inhibit 50% of the DPPH in the assay) were used to determine the antioxidant activity of the crude extracts and the positive control.

Hydroxyl radical scavenging assay (OH assay): Hydroxyl radical scavenging activity was analyzed using the 2-deoxyribose oxidation assay as described by Chung and Osawa [15]. A 0.2ml solution of $FeSO_4.7H_2O$ (10 mM) and EDTA (10 mM) was prepared in a screw-capped test tube. Then 0.2 ml of a 2-deoxyribose solution (10 mM), the sample solutions, and a sodium phosphate buffer (pH 7.4, 0.1 M) were added to give a total volume of 1.8 ml. Finally, 200 µl of H_2O_2 solution (10 mM) were added to this reaction mixture and incubated at 37 ℃ for 4 h. After incubation, 1 ml each of a trichloroacetic acid solution (2.8%) and thiobarbituric acid solution (1.0%) were added to the reaction mixture. The sample was boiled at 100 ℃ for 10 min, cooled on ice, and its absorbance was measured with a UV–VIS 751-GD spectrophotometer at 515 nm. The capability to scavenge hydroxyl radical was calculated by the following equation:

$$\text{Scavenging effect (\%)} = [1–(\text{absorbance of sample} / \text{absorbance of control})] \times 100\%. \quad (2)$$

Superoxide anion radical scavenging assay: Superoxide anion radicals ($O_2^{-}\bullet$) were measured by a spectrophotometric method based on the inhibitory action of the auto-oxidation of pyrogallol. The scavenging ability on the auto-oxidation of pyrogallol of all extracts were investigated using the method as described by S.Marklund & G. Marklund [14] with minor modifications. Different concentrations of the

sample solutions were mixed with 2.8 mL of 0.05M Tris–HCl buffer (pH 8.0) containing 1mM EDTA and gallic acid (0.2mL, 6mM) and shaken rapidly at room temperature. The absorbance of the mixture was measured at 325nm per 30s for 4min on an UV–VIS 751-GD spectrophotometer against a blank, and the slope was calculated as absorbance/min. Determinations were replicated three times. The scavenging ability on the auto-oxidation of pyrogallol of all fractions was calculated using the equation

$$\text{Scavenging effect (\%)} = (1\text{-slope of sample/slope of control}) \times 100\% \quad (3)$$

2.7. Statistical Analysis

The radical-scavenging capacity of all extracts was expressed as the mean ± standard deviation (SD) of triplicate measurements. The results were processed using Origin 75 software, and the data was fitted by nonlinear or linear regression analysis. IC50 values (the effective concentration at which 50% of radicals are scavenged) were calculated by nonlinear or linear regression equations. The level of statistically significant difference was set at $p < 0.05$.

3. Results and Discussion

3.1. The total Phenolic, and Total Flavonoid Content of the Ethanol Extract

BCM and RCM were extracted with 70% (v/v) ethanol and water repectively. The total phenolic, and flavonoid content of BCM and RCM were shown in Table 1. In the ethanol extracts, the phenolic content was analyzed using the Folin–Ciocalteu method and the flavonoid content was evaluated using the colorimetric aluminum chloride assay. From table1, the contents of total flavonoids and total phenolics in RCM were mroe than BCM. This has indicated that RCM is good raw materials to extract flavonoids and total phenolics.

Table. 1. The various solvent extracts, the contents of the total flavonoids and total phenolics

	Kinds of the extracts	Total flavonoids (mg/g, dry weight) [a]	Total phenolics (mg/g, dry weight) [b]
BCM	Water	14.38±2.13	56.44±3.28
	Ethanol	16.39 ± 1.62	70.78±2.32
RCM	Water	18.10 ± 3.03	110.98±14.4
	Ethanol	20.35 ± 2.01	139.72±15.47

a As rutin equivalents present in corresponding extracts
b As gallic acid equivalents present in corresponding extracts

3.2. Antioxidant Activity

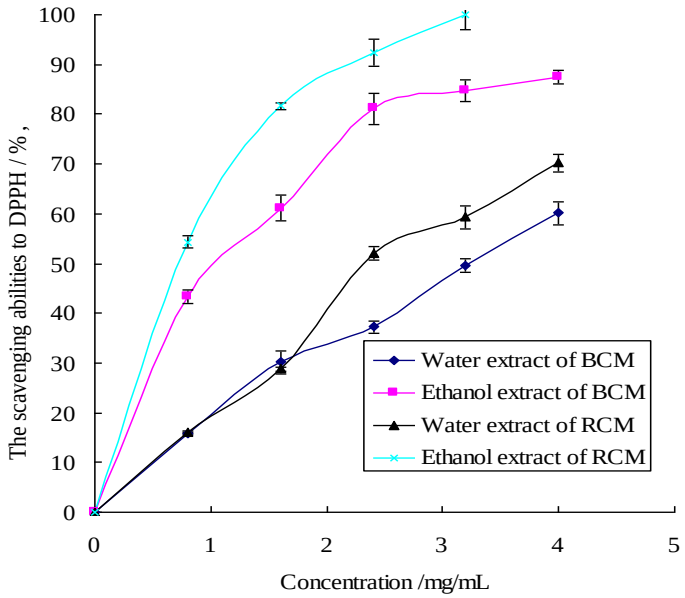
Scavenging ability on DPPH radicals: In order to evaluate the scavenging capacity of the two extracts, the DPPH radical-scavenging ability of each samples was performed. The results of DPPH reduction by these extracts are shown in Fig 1(a).

Each extract exhibited different scavenging activity corresponding to the different ranges of concentrations (Fig. 1a). The Ethanol extract of RCM showed a higher capacity of scavenging the DPPH radical in the range of 0.8-4.0mg/mL concentration. At 1.6-4.0 mg/mL, Ethanol extract of BCM showed scavenging abilities of 43.35–87.7%, and at 2.4-4.0 mg/ml, Ethanol extract of RCM showed scavenging abilities of 52.13–70.19%.However, Water extract of RCM at <3.2 mg/mL did not exhibit good scavenging abilities. The relative effectiveness of all fractions in scavenging DPPH radicals was as follows: ethanol extract of RCM> Ethanol extract of BCM > water extract of RCM > Ethanol extract of BCM. These results indicated that the water extract of RCM and BCM may be used as DPPH radical scavengers.70% Ethanol was a good solvent of extracting antioxidant from RCM and BCM. In order to further assay the scavenging abilities of DPPH, their IC50 values were calculated by fit equations and presented in Table 2.

Scavenging ability on hydroxyl radicals: Scavenging activity on hydroxyl radicals was assessed using the 2-deoxyribose oxidation assay. The scavenging activities of all extracts on hydroxyl radicals are shown in Fig. 1(b).

The scavenging activity of the all extracts were directly correlated with increasing concentrations within a large range of values. The scavenging effect of the ethanol extract of RCM and BCM were respectively 62.69-87.29%, 52.77-72.27% for concentrations of 8.0-12.0 mg/mL, water extract of RCM and BCM were respectively 54.42-60.23%, 50.85-75.88% at concentrations of 10.0-12.0 mg/mL. Thus, the scavenging activities of the ethanol extract of RCM and BCM were stronger than the water extract of RCM and BCM. These results indicate that all extracts have free radical scavenging potential,but scavenging ability of all extracts on hydroxyl free radicals were weaker than on DPPH.

Scavenging activity on the self-oxidation of 1,2,3-phentriol: all extracts from RCM and BCM scavenging ability on the self-oxidation of 1,2,3-phentriol shown Fig. 1(c). The all extracts were found that the ability to scavenge activity increased with increasing concentration and the scavenging activities were stonger.The scavenging effect of the all extracts of RCM and BCM were between 49.03-96.73% for sample concentrations of 0.20-0.80 mg/mL. These results indicated that the ethanol extract of RCM and BCM may be used as radical scavengers and RCM and BCM may be used the raw material of extract antioxidant.



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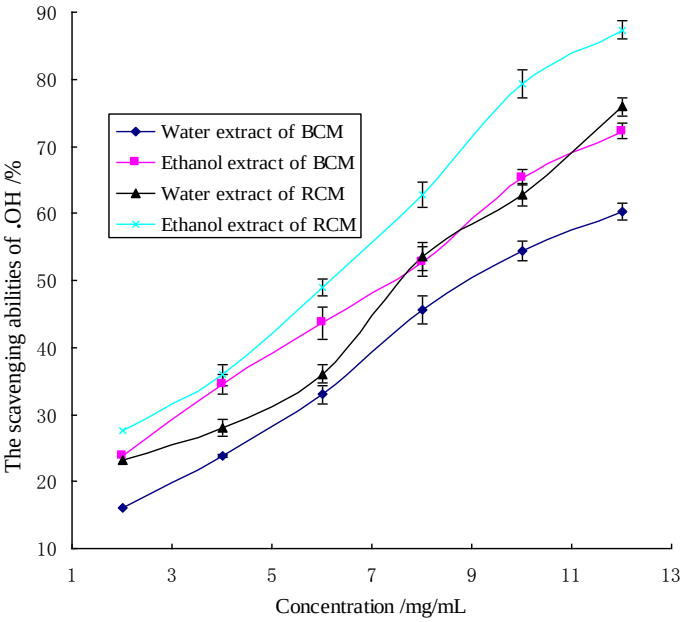

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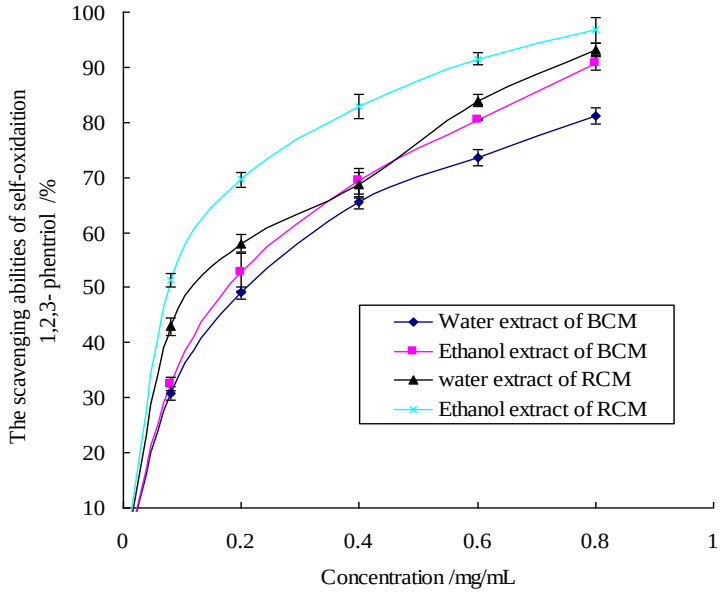

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Fig. 1: Scavenging ability of all extracts from BCM and RCM on DPPH radicals(a), hydroxyl free radicals(b) and O_2^{-}•.

IC_{50} values and antioxidant capablities: IC_{50} was obtained by extrapolation from fit equation of nonlinear regression or linear regression analysis.The fit equation and IC_{50} values from the present assays are summarized in Table 2. With regard to the scavenging ability on DPPH, ·OH radicals and self-oxidation of 1,2,3-phentriol, the IC_{50} value of all extracts of RCM and BCM, were as follows: ethanol extracts of RCM < ethanol extracts of BCM ≤ water extract of RCM < water extract of BCM. The ethanol extracts of RCM showed that the scabenging ability on all radicalsa were stronger than other extracts and the its IC_{50} values were significantly less than other extract ($p < 0.05$).

Table. 2: IC_{50} values of all extracts from BCM and RCM in antioxidant properties

Samples and scavenging radicals		Equation	R^2	IC_{50} (mg/mL)
Water extract of BCM	to DPPH ·	Y=14.61X+2.935,	0.9888	3.23
	to O_2^{-} ·	Y=-155.6X ³+212.19X+7.717,	0.9607	0.24
	to OH	Y=4.649X+6.304,	0.9909	9.39
Water extract of RCM	to DPPH	Y=1.791+17. 99X,	0.9809	2.68
	to O_2^{-} ·	Y=152.6X^2+217.4X+13.09,	0.912	0.15
	to OH	Y=5.5109X+8.004,	0.9762	7.63
Ethanol extract of RCM	to DPPH	Y=-12.28X ³+69.04X+2.347,	0.9913	0.81
	to O_2^{-} ·	Y=15.31+273.4X-222.72X^2,	0.8961	0.14
	to OH	Y=6.319X+12.75,	0.9905	5.89
Ethanol extract of BCM	to DPPH	Y=-7.258X ³+49.79X+2.629,	0.9903	1.79
	to O_2^{-} ·	Y=-154.0X ³+222.0X+8.311,	0.9623	0.22
	to OH	Y=4.920 X+14.27,	0.9966	7.26

a Means with different letters within a row are significantly different ($P < 0.05$).

The ethanol extract of RCM in particular showed an especially low IC_{50} value for its scavenging ability of self-oxidation of 1,2,3-phentriol. Superoxide anion free radical may be formed self-oxidation of 1,2,3-phentriol and the former was also the precursor of hydroxyl free radicals. This result that RCM were good material to extract antioxdant than BCM.

Phenolic compounds and flavonoids offer important sensory and nutritional qualities that responsible for the colours, flavours and tastes of many plants. Phenolic compounds are dominant antioxidants distributed widely in the plant kingdom that exhibit scavenging efficiency on free radicals and reactive oxygen species, and flavonoids in food and beverages in particular have obvious antioxidant stress effects [16]. The contents of phenolic compounds and flavonoids of ethanol extract from RCM were the highest.Therefore it showed the effective antioxidant activity.

4. Conclusions

The present study showed that all extracts of bast of Citrus maxima (BCM) and the rind of Citrus maxima (RCM) contained predominantly flavonoids and phenolics.The antioxidant activity in vitro showed that the ethanol extract of RCM in particular displayed low IC_{50} values and possessed good antioxidant properties. These antioxidant activities can be attributed to the actions of flavonoids and phenolics, but in particular were the actions of flavonoids,which were the catechins, rutin, naringin and hesperidin within the ethanol extract of RCM. The results of this study have point to the importance of the rind of Citrus maxima as a plant-based antioxidative resource and as raw materials to extract the flavonoid and phenol.

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6. References

[1] F.Shahidi, P.K.Janitha, P.D.Wanasundara. Phenolic antioxidants. *Crit Rev Food Sci*. 1992,32:67–103.

[2] N. Hutadilok-Towatana, P. Chaiyamutti, K. Panthong, et al. Antioxidativeand free radical scavenging activities of some plants used in Thai FolkMedicine.*PharmlBiol*.2006,44:221–2286.

[3] H.Tanabe, M.Yoshiad, N.Tomita. Comparison of the antioxidant activities of 22 commonly used herbs and spices on the lipid oxidation of pork meat. *Animal Science Journal*.2002,73:389–393.

[4] J.-Y.Shi, J.-Y.Gong, Ji'er.Liu, X.-Q.Wu, Y.Zhang. Antioxidant capacity of extract from edible flowers of Prunus mume in China and its active components. LWT- *Food Sci Technol*.2009,42:477–482.

[5] S.M.S.Sawalha, D.Arra′ez-Roma′n, A.Segura- Carretero. Quantification of main phenolic compounds in sweet and bitter orange peel using CE–MS/MS. *Food Chem*.2009,116:567–574.

[6] B.A. Arias, L.L. Ramon. Pharmacological properties of citrus and their ancient and medivial uses in the Mediterrean region. *J Ethnopharmacol*.2005,97:89–95.

[7] T. Wu, Y.-Q Guan, J.-N. Ye. Determination of flavonoids and ascorbic acid in grapefruit peel and juice by capillary electrophoresis with electrochemical detection. *Food Chem*.2007,100:1573–1579.

[8] J. Londo ño-Londo ño, V.R. Lima, O. Lara, A. Gil,T. B. C. Pasa, G. J. Arango, J. R. Ramirez Pineda. Clean recovery of antioxidant flavonoids from citrus peel: Optimizing an aqueous ultrasound-assisted extraction method. *Food Chem*.2010,119: 81–87.

[9] M. Igual, E.Gar c á-Mart ínez, M.M. Camacho, N.Mart ínez-Navarrete. Jam processing and storage effects on β-carotene and flavonoids content in grapefruit. *J Funct Foods*.2013,5:736 –744.

[10] P.S.Negi, G.K.Jayaprakasha. Antioxidant and antibacterial activities of Punica granatum peel extracts. *J Food Sci*.2003,68:1473–1477.

[11] Z.S. Jia, M.C. Tang, & J.M.Wang. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem*.1999,64(4):555–559.

[12] R.P.Singh, K.N.Chidama bara Murthy, G.K.Jayaprakasha. Antioxidant activity of Pomegranate (Punica garanatum) peel and seeds extracts using in vitro models. *J Agr Food Chem*.2002,50:81–86.

[13] W.Brand-Williams, M. E.Cuvelier, & C. Berset. Use of a free radical method to evaluate antioxidant activity. *Lebensmittel-Wissenschaft & Technologie*.1995,28(1):25–30.

[14] S.Marklund, & G. Marklund. Involvement of superoxide anion radicals in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur J Biochem*.1974, 47:469–471.

[15] S. K.Chung, & T.Osawa. Hydroxyl radical scavengers from white mustard (Sinapis alba). *Food Sci Biotechnol*. 1998,7: 209–213.

[16] C.W.Song, S.M. Wang, L.L. Zhou, F.F. Hou, K.J. Wang, Q.B. Han, N.Li, and Y.X. Cheng. Isolation and Identification of Compounds Responsible for Antioxidant Capacity of Euryale ferox Seeds. *J Agr Food Chem*.2011, 59:1199–1204.