Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

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OPEN ACCESS Asian Journal of Plant Sciences

ISSN 1682-3974
DOI: 10.3923/ajps.2020.223.229

Research Article
Evaluation of Antioxidant and Antimicrobial Activities of Mandarin Peel (*Citrus reticulata* Blanco) with Microwave Assisted Extract Using Two Different Solvents

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Abstract

Background and Objective: Mandarin peel is the biggest source of bioactive phenolic compounds, specifically flavonoids, with comparatively higher polyphenol content compared with the edible parts. The flavonoids content in citrus consist of flavones, isoflavones, flavonones, flavonols and anthocyanins. The objective of this study was to determine the antioxidant content as an antibacterial activity of peel mandarin using microwave method and two different solvents (distilled water and ethanol) for extract. **Materials and Methods:** The plant was collected in Kahramanmaras, Turkey. The study performed the extraction methods using water and ethanol with the assist of microwave. The extracts were assessed for yield extraction, total condensed tannins, total phenolic compounds, flavonoid contents, anthocyanin and antimicrobial activity against some antibiotics. **Results:** The yield extraction for water extract was 38.40% which was significantly ($p<0.01$) different than ethanol. The total tannin of ethanol extract content was 0.083% and seems to significantly ($p<0.01$) higher than water. Similarly, the total phenolic and flavonoid contents were 1.862 mg GAE/g and 0.1975 mg CE/g, respectively in ethanol and water extraction. Whereas, anthocyanin content was 26.710 mg kg^{-1} in water extraction method and was higher significantly. Antimicrobial results showed that the peel mandarin extract has not significant effect to all bacteria. The significant results of MIC were obtained for *Bacillus subtilis* at the concentration 125 μg mL^{-1} and *Klebsiella pneumonia* at the concentration of 250 μg mL^{-1}. **Conclusion:** This study showed that mandarin peel can be considered a good natural antioxidant source to human health.

Key words: Peel mandarin, antioxidant, bacteria, free radical scavenging, antibacterial activity

Citation: Holem H. Balaky, Yaseen Galali, Ardalan Abdulhamid Osman, Eyyüp Karaoğul, Ertugrul Altuntas, Metin Tansu Uğuz, Ali Mala Khedir Galalaey and M. Hakki Alma, 2020. Evaluation of antioxidant and antimicrobial activities of Mandarin peel (*Citrus reticulata* blanco) with microwave assisted extract using two different solvents. Asian J. Plant Sci., 19: 223-229.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The world fruit processing manufacture creates large quantities of waste typically composed of stems, peels and pulp, skin, seed and oilseed meals of food[1]. Citrus fruits are considered one of the most grown plants worldwide, with high nutritive and health values. The health advantages of citrus fruits have been attributed mainly to the existence of antioxidants such as ascorbic acid and phenolic[2].

Fruits and vegetables are considered as the most important herbal sources of antioxidants[3]. Mandarin is a group identify for a category of oranges with thin, wide peel, which have been dubbed "kid-glove" oranges. These are dealt with as participants of a distinguished species, *Citrus reticulata* Blanco. The name "tangerine" should be utilized as an alternate identify to the whole group, but in the commerce, is commonly restrained to the kinds with red-orange skin. The Spanish-speaking people in the American tropics call them mandarina, also in the Philippines all mandarin oranges are called naranjita[4]. The *Citrus reticulata* one of the medicinally essential plants belonging to the Rutaceae family[5] for instance, at least 40% of the 1 million t of mandarin produced yearly in South Africa are channeled to juice manufacturing with waste computation for 50-70% of the fresh weight include of pulp (30-35%), peels (60-65%) and seeds (<10%)[6].

The citrus peel sinensis was used as a value-added products in the food industry, because it contains potential antioxidant compounds[7]. Currently, solely a fraction of total peel residue mass is being utilized as beverage bases, candied peel and marmalades. However, citrus peel is the biggest source of bioactive phenolic compounds, specifically flavonoids, with comparatively higher polyphenol content compared with the edible parts. The flavonoids content in citrus consist of flavones, isoflavones, flavonones, flavonols and anthocyanin's[8].

That ethanolic peel extract of *Citrus reticulate* contains bioactive and vindicated, this plant used to treat many diseases in herbal medicine and folk medicine[5], also the plant of water extraction was conducted to extract flavonoids and antioxidant from dried satsuma mandarin peel (Citrus unshiu Markovich) and the ratio of flavonoids recuperated with this extraction[9] was 96.3%.

The antimicrobial for the food industry was hold promise to the extract peel and pulp of mandarin[10]. The addition, that mandarin peel had greater antimicrobial activity than lemon peel[11]. Furthermore, the mandarin segments were retained can after processing, when fresh mandarin fruits are not available, that mandarin cans could avails as a substitute. Due to the presence of the majority of the antioxidant capacity and phenolic compounds[2]. Different traditional methods have been used to extracts biologically active compounds from fruits. However, to the researchers best of knowledge, no research has been done on extracting phenolic compounds from mandarin peel using microwave to assist extraction as a novel technique to increase extraction efficacy. Therefore, the objective of this study was to determine antioxidant and antimicrobial activities of mandarin peel extracts using two different solvents with microwave extracting assistant as a novel technique used in this area.

MATERIALS AND METHODS

Plant collection and powdering: The mandarin peel was collected from local markets in Turkey in February, 2019, after that dried and powdered using new modern grinder YAZICILAR (Model GI, Capacity/hour 15 kg, Capacity 5 letter, Speed 13000 rpm and Cycle 500 g) in Kahramanmaraş Sütçü İmam University laboratory. After that, the powder samples were kept in the plastic bag in the refrigerator at 4°C for further research.

Preparation plant with extraction methods: A quantity of 6.25 g powder of the peel mandarin was soaked in 125 mL of solvents (ethanol and distilled) for 30 min by using the microwave extraction method. After that filtered and evaporate by using Fume Hood.

Yield determination: The yield percentage of the extract was determined by using the following formula for each one of the extraction techniques[12]:

$$\text{Yeild extraction (\%)} = \frac{X}{Y} \times 100$$

Where:

X : Oven-dry weight of extract (g)

Y : Oven-dry weight of the sample (g)

Determination of total condensed tannins: This assay was carried out using Shimadzu UV-vis spectrophotometer. The extraction solution was prepared by mixing 0.05 g of Fe_2SO_4, 95 mL N-butanol and 5 mL HCl (35%). For determining the condensed tannin, 0.01 g of crude peel and mimosa tannin were put separately in the tube and 10 mL of extracted solution was added and heated in water bath for 1 h. The absorbance was read at 580 nm wavelength[13].

Determination of total phenolic compounds: The total phenolic content was measured following Folin-Ciocalteu method as described by Dewanto *et al.*[14]. The diluted extract was added to 20 mL of Folin-Ciocalteu reagent and then to 180 mL of distilled water. The mixture was shaken and allowed to stand for 6 min, before the addition of 1.60 mL of 7% Na_2CO_3. The solution was then regulated with distilled water to a final volume of 3 mL and mixed completely. After storage in the dark, the absorbance was read at 765 nm against a prepared blank. The total phenol content of plant parts was expressed as milligrams of Gallic acid equivalents per gram of dry weight (mg GAE/g DW) from a calibration curve with Gallic acid. All samples were measured in triplicate[14].

Determination of flavonoid contents: The total flavonoid content of individual extracts was measured as per the Dowd method[15]. A quantity of 1 mL of extract solution was placed in a test tube, then 6.4 mL distilled water was added by following 0.3 mL of 10% (w/v) aluminum chloride and 0.3 mL of potassium acetate and finally 2 mL (1 M) of NaOH. The mixture was then incubated for 30 min at room temperature and absorbance was read at 510 nm against the blank. The result data were expressed as mg g^{-1} of quercetin equivalents in mL/g (mg QE/g) of crude extract.

Total anthocyanin measurement using pH differential method: Total anthocyanin was analyzed the following the method described in previous study[16] with a little amendments. The samples of buffer solution of pH 1.0 (0.02 M KCl and 980 mL of distilled water with 1.86 g KCl, with added 27 M HCl) and buffer solution of pH 4.5 (960 mL of distilled water with 54 and 43 g sodium acetate, with added 20 M HCl) added, respectively. The preparation was homogenized and centrifuged twice during 15 min at 4°C at 5000 rpm the absorbance was read at 512 and 700 nm, after the collected supernatant. The anthocyanin were quantified by following the spectrophotometric technique suggested the concentration of anthocyanin was specified stratify the Lambert–Beer law. The recorded of spectra in a Heλios α-spectrophotometer were amount at 25°C, total anthocyanin amount was determined by the following formula[16].

The concentration (mg L^{-1}) of every anthocyanin was measured according to the following:

$$A = \frac{A \times MV \times DF \times 1000}{\epsilon \times 1}$$

Where:

A : Absorbance = (Aλvis-max) pH 1.0-(Aλvis-max) pH 4.5
MW : Molecular weight (g mol^{-1}) = 449.2 g mol^{-1} for Cy-3-glc
DF : Dilution factor (0.2 mL sample is diluted to 2 mL DF = 100)
ϵ : Extinction coefficient (L × cm^{-1} × mol^{-1}) = 26,900 for Cy-3-glc, where L (path length in cm) = 1

For comparison, the same extinction coefficient was used for other standards to calculate the concentration of each anthocyanin and thus results reported is expressed as Cy-3 glc equivalents.

Measurement of antioxidant activities of plant extracts compounds using DPPH assay: Antioxidant activity was measured by 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay with slight adjustment. Briefly, 0.1 mM of DPPH was prepared with ethanol. Then 0.1, 0.2 and 0.3 mL of extracts were placed in test tubes and ethanol were added to complete 3 mL. Then 1 mL of DPPH was added to the mixture, shaken vigorously and placed for 30 min in dark room. The prepared reagent was measured by Shimadzu UV-VIS 1240 spectrophotometer at 517 nm. Furthermore, the butylated hydroxyl toluene (BHT) was used as a standard. Free radical scavenging activity was expressed as the inhibition and calculated with the following equation[17,18]:

$$\text{Inhibition of DPPH radical scavenging activity (\%)} = \frac{A - B}{A} \times 100$$

Where:

A : Absorbance of DPPH
B : Absorbance in the existence of sample and BHT

Antibacterial susceptibility testing disc diffusion method: To activate stock bacteria, media was prepared by mixing 21 g (Mueller-Hinton broth) with 100 mL of distilled water. Whereas, for preparing bacteria culture 38 g of (Mueller-Hinton agar) was mixed with 100 mL of water. The prepared media was sterilized in autoclaving for 15 min at 121°C. After that, the disk diffusion method, known as the Kirby Bauer method[19]. Inoculum suspension was ready utilized colony suspension homogenized to match the turbidity of a McFarland 0.5[20,21] and inoculum of 24 h culture was swabbed (rubbed) on the plate by using cotton swab. Wells were punched on each plate using sterile borer. Different plant extract concentrations (50, 70 and 100) were added to the wells. The plates were incubated in a straight position for

24 h at 37°C. Antibacterial activity was calculated by measuring the diameter of inhibited zone (mm).

Identification of Minimum Inhibitory Concentration (MIC): The MIC is the less concentration of an antimicrobial that prevents the visual growth of a micro-organism after put in incubation during[22] 24 h. MICs were known as the less concentration of boric acid and borax preventing the visual growth of the micro-organism. A quantity of 1 mL of extract concentration of 100 mg mL^{-1} for five extract solvents were mixed with agar in serial decimal dilutions diluting 1 mL in 9 mol of agar to get concentration range from 0.5-512 mcg mL^{-1}, this is usually hours. All the bacteria suspensions were prepared by suspending 24 h bacteria culture in sterile no of the bacteria suspension was adjusted to 1.0 McFarland standard (equivalent to 15 io CFU mL^{-1}). May also be standardized based on Mueller-Hinton broth. The bacterial suspend was adjusted to the logarithmic-phase increase to in shape the turbidity of a 0.5 McFarland standard[20,21],the yielding about 108 CFU mL^{-1}. The identical quantities of micro-organism were added to all tubes and the tubes were then incubated at 37°C for 24 h. Every tube was examined for growth and compared to the control. The bacterial suspension brought to a tube filled with the nutrient broth was once used as positive growth control. A tube now not containing nutrient broth was once used as negative growth control. The absence of growth used to be described as antibacterial activity.

Statistical analysis: The peel mandarin was analyzed and expressed as values of Means±SE (standard errors) of triplicate calculated all parameter. The results of the two groups were compared using the analysis of independent-samples t-test by IBM SPSS for Windows (version 20.). An eventuality value of $p<0.01$ was depend on as the standards for significant differences.

RESULTS AND DISCUSSION

The results of the yield extraction, total tannin, total phenol, total flavonoid and total anthocyanin of mandarin peel extracts of both solvent is shown in Table 1. The result of yield extraction of water was 39.04 of was appeared to be highly significant comparing to ethanol which was 24.66%. On the other hand, extraction with ethanol of total tannin, total phenol and total flavonoid were 0.082, 1.871 and 0.197, respectively and were higher than water extraction which were 0.077, 0.527 and 0.101, respectively. Regarding anthocyanin, using distilled water extracted 26.71 and using ethanol could not extract any. These findings are in agreement with those reported by Pfukwa *et al.*[10] and Djilas *et al.*[1] and Liew *et al.*[7] disagreement with Kelebek and Selli[23] since the method was different but the same plant. The recovery of phenolic compounds were purely dependent on the solvent used and its polarity for the different plant materials. The recovery of polyphenolic compounds from plant materials are affected by their solubility in that specific solvent. Also, the solubility of solvent performs a pivotal function in increasing the phenolic compounds solubility in it[24].

Antioxidant assay: The antioxidant activities with 2,2-diphenyl-1-picrylhydrazyl was assessed though measuring free radical scavenging activities of peel extracts of both solvents is presented in Table 2. It can be seen that free radical scavenging of water extracts of all three concentrations 0.1, 0.2 and 0.3 were 42.79, 47.90 and 52.32, respectively. On the other hand, the free radical scavenging activity of ethanol extraction for aforementioned concentrations were 75.53, 77.41 and 77, 82, respectively (Table 2).

The results suggested that antioxidant activity of ethanol extraction is higher than water extraction and more close to standard BHT. As, it was mentioned earlier in this study that ethanol extraction was higher and again ethanol extraction

Table 1: Extraction yield, total tannin and total phenolic content of mandarin peel solvent extracts

	Microwave extraction (Mean±SE)		Statistic	
Analysis	DW-solvent	E-solvent	t-test	p-value
Yield extraction (%)	39.02±0.0060	24.65±0.0080	1342.677	0.000
Total tannin	0.077±0.0004	0.082±0.0006	-5.911	0.001
Total phenol	0.527±0.0006	1.871±0.0000	-532.148	0.000
Total flavonoid	0.101±0.0002	0.197±0.0004	-171.283	0.000
Total anthocyanin	26.710±0.000	ND	-171.283	ND

Values are Mean±SE of triplicate samples, Independent-samples t-test significantly different ($p<0.01$), ND: Not detected, DW: Distilled water, E: Ethanol

Table 2: DPPH free radical scavenging ability of mandarin peel extracts compared with the standard BHT

Concentration (%)	Water	W-BHT	Ethanol	E-BHT
0.1	42.79	78.37	75.53	88.05
0.2	47.90	79.53	77.41	91.73
0.3	52.32	81.86	77.82	92.30

W: Distilled water, E: Ethanol, BHT: Butylated hydroxy Toluene CN_{10}

Table 3: Inhibition zones (IZ mm) and Minimum Inhibitory Concentration (MIC) of the mandarin

MWM	DW (IZ mm)	E (IZ mm)	Antibiotic (antimicrobial agent)/susceptible			
Bacteria	100 µL/disk	100 µL/disk	P_{10} (mm)	AMP_{10} (mm)	Eyr_{10} (mm)	(Ge) CN_{10} (mm)
B1	ND	14.23±1.06	12.94±0.006	12.83±0.0058	11.60±0.0577	16.90±0.0577
B2	ND	ND	11.09±0.006	10.59±0.0058	16.45±0.0058	10.19±0.0058
B3	17.25±0.460	ND	14.30±0.058	ND	11.37±0.0060	11.10±0.0580
B4	ND	ND	8.27±0.006	7.80±0.0577	13.14±0.0058	10.71±0.0058
B5	ND	ND	10.02±0.005	11.42±0.0058	8.80±0.0577	17.66±0.0058
B6	15.76±0.890	ND	10.61±0.005	12.64±0.0058	12.98±0.0058	14.21±0.0058

Solvents extract and synthetic antibiotic activities against the bacteria dilution assay, Values are Mean±SE of triplicate samples, ND: Not detected, MWM: Microwave method, DW: Distilled water, E: Ethanol, ZI: Zone of inhibition, MIC: Minimum inhibition concentration, B1: *Staphylococcus aureus* (ATCC 29213), B2: *Pseudomonas aeruginosa* (ATCC 27853) B3: *Bacillus subtilis* (ATCC 6633), B4: *Enterococcus faecalis* (ATCC 29212), B5: *Enterobacter aerogenes*, B6: *Klebsiella pneumonia*, Antibiotic (Antimicrobial agent): P_{10}: Penicillin, AMP_{10}: *Ampicillin* (other), Eyr_{10}: Erythromycin, (Ge) CN_{10}: Gentamicin

Table 4: Determination of Minimum Inhibitory Concentration (MIC) different extracts against bacteria strains

Bacteria	DW (MIC) (µg mL^{-1})	E (MIC) (µg mL^{-1})
Staphylococcus aureus (ATCC 29213)	ND	500
Pseudomonas aeruginosa (ATCC 27853)	ND	ND
Bacillus subtilis (ATCC 6633)	125	ND
Enterococcus faecalis (ATCC 29212)	ND	ND
Enterobacter aerogenes	ND	ND
Klebsiella pneumonia (CCM 2318)	250	ND

MIC: Minimum inhibition concentration, DW: Distilled water, E: Ethanol, Antibiotic of DW and E extracts (Antimicrobial agent)/Susceptible, 500, 250, 125, 62.5, 31.25, 15.62, 7.81, 3.90 and 1.95

could be more effective against free radicals. Moreover, the antioxidant activity of phenolic content is significantly influenced by properties of the solvent extracts [25]. In contrast, antioxidant value of the ethanol extract correlated well to compare with the extract of distilled water, 80%. That observed the antioxidant activity was optimum to 77 and 50% in each of ethanol and distilled water extracts. The antioxidant activity of phenolic content is significantly influenced by properties of the solvent extracts[25]. The phenolic compounds are essentially known to dissolve preferable in solvents with higher polarity[26]. In agreement with this study the yield extraction enhanced the solvent of water proportion inside every solvent extraction. This result is similar to Liew *et al.*[7] and disagreement with Al-Sayyed *et al.*[27].

Antibacterial assay: Results of the disc diffusion assay expressed as zone inhibition and the MIC against all bacteria testes were between different concentration as 500, 250, 125, 62.5, 31.25, 15.62, 7.81, 3.90 and 1.95 the solvent extracts are presented in Table 3 and 4. It can be seen that the highest antibacterial activity was obtained with the water extract of mandarin peel against *Bacillus subtilis* and *Klebsiella pneumonia* with inhibition zone diameters are 17.25±0.460 mm and 15.76±0.890 mm, respectively. Whereas, inhibition zone of ethanol extraction showed to be 14.23±1.06 mm against *Staphylococcus aureus.* No inhibition zone was observed neither by water nor ethanol against other bacteria species (Table 3). It should be taken into account that the area of inhibition of bacterial strain depends on the ability of the extract to diffuse uniformly through the agar[28].

Furthermore, the antibacterial activities of the plant extract was assessed against some bacteria and compared with four synthetic antibacterial disc including P_{10}, AMP_{10}, Eyr_{10} and CN_{10} as shown in Table 4. The data showed positive, that is mean the peel of mandarin is a significant natural plant might be used instead of these antibiotics. This inhibitory effect has been attributed to phenolic compounds present in the plant extracts[29]. The mandarin peel had greater antimicrobial activity than lemon peel. It was reported that an ethyl acetate extract of sea buckthorn seed had lower antibacterial activities than an acetone extract, although it had the lower phenolic contents than the acetone one. That is for reason findings of this study are similar to Turkmen *et al.*[11], because this researcher was used acetone it is one of the polar solvent same ethanol and distilled water, also antimicrobial finding is not agreement with Yashaswini *et al.*[30] and in agreement with Wu *et al.*[31].

CONCLUSION

This study observed that the yield extraction and total anthocyanin from water extract were higher significantly ($p<0.01$), but the total tannin, total flavonoid and total phenolic were higher significantly ($p<0.01$) ethanol. Similar to that, the free radical scavenging activity of ethanol was higher comparing to water 80%. Moreover, the mandarin peel extract

not significant effect to all bacteria, but the maximum inhibition zone and the significant results of minimum inhibition concentration were obtained significant value against with 3 bacteria according solvent extracts. Finally, the mandarin peel will be a good provenance of antioxidant to human health.

SIGNIFICANCE STATEMENT

The study discovered that the extract of mandarin peel is a good antioxidant to human health that will be used as alternative a medicine antibiotic to treat some diseases and killing some bacteria by either using water or ethanol. So, this information can be beneficial researchers, herbalists and community get idea about health benefits of mandarin peel extracts.

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