

Original Article

Effect of Microwave Power and Time on Total Phenolic Contents and Antioxidant Characteristics of Microwave Assisted Extracts of Watermelon Rind Powder

Tanzeel Shahid^{*}, Ammar Ahmad Khan¹, Anees Ahmed Khalil¹, Madiha Batool¹, Sundus Khan¹ and Ayesha Aslam¹

¹ University Institute of Diet and Nutritional Sciences, Faculty of Allied Health Sciences, University of Lahore

*tanzeel.shahid11@gmail.com

Abstract:

Watermelon is gaining importance as a functional food due to its therapeutic effect. The therapeutic effect of watermelon has been reported and has been attributed to antioxidant constituents. The major component in watermelon rind is citrulline that has a strong antioxidant effect which protect body from free-radical damage. **Objective:** This study was conducted to investigate the effect of microwave powers (150 W, 300 W & 450 W) and time intervals (1, 3 & 5 minutes) on total phenolic content (TPC) and total flavonoid content (TFC) and antioxidant characteristics i.e. DPPH and ferric reducing antioxidant potential (FRAP) of microwave assisted extracts of watermelon rind powder. **Methods:** The extracts collected after Microwave assisted extraction (MAE) of watermelon rind were analyzed for their antioxidant potential through different tests including total phenolic contents (TPC), total flavonoid content (TFC), DPPH assay and FRAP. **Results:** Microwave assisted extraction by using ethanol as a solvent at different microwave powers and various time intervals showed that total antioxidant potential was significantly higher at low microwave power such as TPC ranges obtained at 150W for 1, 3 & 5 minutes of time intervals show ranges (159.84, 160.04 & 169.71 mg GAE/100 g). While TFC ranges at 150W for time 1, 3 & 5 minutes were (21.31, 24.15 & 42.20 mg CEQ/100g) whereas DPPH ranges at 150W for time 1, 3 & 5 minutes were (53.14, 54.87 & 68.17 % ascorbic acid inhibition) and FRAP values at 150W for time 1, 3 & 5 minutes were (201.71, 221.50 & 326.43 mg FE/100g). While high microwave power 450W can result in disruption of some antioxidants at various time intervals. **Conclusions:** Watermelon rind is a rich source of many antioxidants and microwave assisted extraction technique should be implemented in the food and nutraceutical industries and microwave assisted extracts of watermelon rind should be utilize for the development of new functional food to combat many health related problems.

Keywords: Watermelon rind, microwave assisted extracts, antioxidant characteristics, free-radicals, total phenolic contents, functional food

Introduction:

Our body has an antioxidant defense system which consists of various compounds and enzymes. This antioxidant defense system can activate when the body can get exposed to free-radicals. This defense system can remove free-radicals out of the body to protect tissue

damage. Most of these antioxidants are produced in physiological system and other antioxidants were got from external sources such as food sources and supplements.

Foods contain antioxidant compounds such as carotenoids, polyphenols, gallic acid, carnosol,

catechins, bilirubin, lycopene, hydroxytyrosol, butylated hydroxyanisole and rutin. The strong and most effective antioxidants include vitamin-E and vitamin-C which help to prevent from various diseases including cardiovascular diseases and cancer [1]. Antioxidants can help to remove free radicals from the body hence prevent oxidation process. Free radicals like reactive oxygen species (ROS) are very reactive due to presence of unpaired electrons [2].

In recent years, nutraceutical foods are very common due to their potential health benefits. The term nutraceutical is defined as any substance that is a food or a part of food that can provide medical and health benefits, including the prevention and treatment of diseases. Recently in August 2015, the food and safety standards authority of Bharat defined nutraceuticals as a "naturally present chemical component possess physiological benefit and protect against chronic diseases, that can be isolated from food or non-food sources that can be prepared or sold in markets in form of powder, capsule, tablet, liquid, gel, Sachet, bottle etc." These nutraceuticals have antioxidant, anti-obesity, Anti-diabetic, cardio-protective and anti-inflammatory properties. The use of functional and nutraceutical herbal therapy is increasingly demanding due to its safe management and prophylactic mechanisms. Nutraceutical foods are consists of natural and biologically active components. The demand of dietary supplements is increasing but the arcade of nutraceutical and functional foods is not generally developed in most of the countries [3]. Watermelon also known as "*Citrullus Lanatus*" belongs to the family *cucurbitaceae*. It is the most important crop that grows in warm tropical and sub-tropical regions of U.S, Africa and Russia [4]. Due to its high water content and thirst quenching characteristics, this fruit can be eaten in high quantity in summers.

Watermelon rind have strong antioxidant effect and have ability to inhibit several oxidative enzymes and stabilize lipid peroxidation and enhance the process of antioxidants which

construct a wide range of phenolic compounds an exceptional target for improving health and associate benefits [5]. Antioxidants from watermelon rind have ability to degrade free radicals and slow down the action of enzymes involved in oxidation reaction [6]. Maximum in-vitro antioxidant essays are predominantly bunce of reducing power. They tend to suppress the activity of di-oxygen and decline the activity of free-radicals and causes complete eradication of free-radicals as well [7]. Antioxidants are compounds that have a strong ability to prevent oxidation processes in food stuffs. To inhibit the process of oxidation, the synthetic antioxidants such as butylated hydroxyanisole (BHA) and terbutylhydroquinone (TBHQ) have substantial role in food additives. Emerging data on conceivable hostile effects on synthetic substances on human health are moving consumers towards the natural constituents. These natural constituents include phenols, flavonoids, carotenoids, and ascorbic acid, but most significant are polyphenols [8].

Another study evaluated the antidiabetic effects of a methanolic extract of watermelon rind in diabetic rats. The results suggested that watermelon rind should be able to normalize some biochemical abnormalities [9]. Another study suggested that watermelon rind serves as an effectual inhibitor on zinc in natural sea water habitat. The results show absorption of inhibitor on zinc surface is exothermic [10]. While a study conducted in 2012, shown that there is a loss of weight in rats fed with watermelon extracts while increase in antioxidant status and decrease in plasma concentrations, while improvement in homeostasis of anti-inflammatory cytokines [11]. Watermelon rind contains citrulline which is a non-essential amino acid that is used by the body to make another amino acid known as arginine. The ammonia from the body is remove by the arginine in urea cycle [12]. Citrulline in watermelon rind has strong antioxidant properties which helps the body to fight against cancer. These extraordinary qualities of watermelon rind explicit us to use it for health

benefits [13]. The ethanolic solvent extract of watermelon rind has more phenolic compounds as compared to water extracts of watermelon rind [14]. So, the use of watermelon rind as a functional food should be needed to get its lavish health benefits rather than disposing it as a waste product.

Methods:

Preparation of watermelon rind powder

Watermelon rind powder was prepared by following six simple steps as shown in Figure 1. Collected watermelon rind was dried in oven drier at $50\pm 5^\circ\text{C}$ to minimize the moisture content. After that they were grinded to reduce the particle size in order to facilitate extraction.

Equipment

Weighing balance, Mixer, Flasks, Microwave Oven, Grinder, Condenser, Rotary evaporator, Orbital shaker, Spectrophotometer.

Chemicals

Ethanol, ascorbic Acid, gallic acid, acetic acid, sodium carbonate (Na_2CO_3), Folin ciocalteu reagent, aluminum chloride (AlCl_3), sodium nitrite (NaNO_2), sodium hydroxide (NaOH), sodium acetate, iron chloride (FeCl_3), 2, 4, 6-tripyridyl-s-triazine (TPTZ), Hydrochloric acid (HCl).

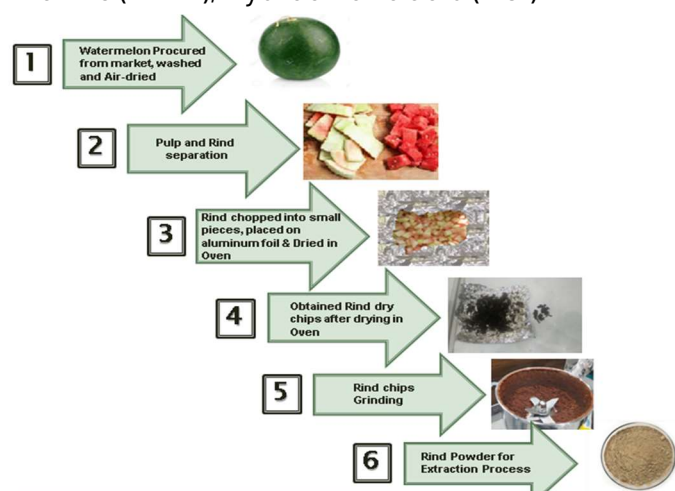


Figure 1: Graphical representation of basic six steps involved in preparation of watermelon rind powder

Preparation of watermelon rind extracts

In microwave assisted extraction (MAE) procedure, 25g aliquot of watermelon rind powder was added in round bottom flasks and distilled water about 20ml was added to each

flask to moisturize the sample for 30 minutes. After 30 minutes, the samples were placed in the microwave oven. Heating powers such as 150 W, 300 W and 450 W should be used for each sample for varied extraction time 1, 3 and 5 minutes for each extract as shown in Table 1. The solvent used in this method was ethanol. After the completion of MAE, 200 ml of ethanol was added to each sample. These samples were then subjected to orbital shaker for 24 hours at 3000 rpm then subjected to rotary evaporator. At the end samples were filtered with Whatman filter paper and then centrifuge at 7000 rpm for 15 minutes for the separation of extract [15].

Sample	Time Variation (Min)	Microwave Power (W)	Solvent
1	1 min	150	Ethanol
2	3 min		
3	5 min		
4	1 min	300	Ethanol
5	3 min		
6	5 min		
7	1 min	450	Ethanol
8	3 min		
9	5 min		

Table 1: Time variation, Microwave power and Solvent used in MAE (Microwave Assisted Extraction)

Assessment of Antioxidant Characteristics

The extracts collected after MAE of rind of watermelon were analyzed for their antioxidant potential through various tests such as total phenolic contents (TPC), total flavonoid content (TFC), DPPH (1,1-diphenyl-2-picrylhydrazyl) assay, and (FRAP). All these procedures were formed in three replications.

Determination of Total Phenolic Content (TPC)

For determination of TPC in watermelon rind powder, Folin-Ciocalteu method was used. About 3 ml of sample extract was mixed with Na_2CO_3 (7.5%) about 4 ml and placed in a dark

condition for 5 minutes for incubation. Then, FolinCiocalteu reagent about 4 ml which was pre-diluted, 10 times, was added to sample, vortex and incubated for 30 minutes. After 30 minutes, absorbance of mixture was measured by using UV-VISIBLE spectrophotometer (CECIL CE7200, Japan) at 760 nm. All procedure was formed in three replications. TPC of the sample were expressed as gallic acid equivalent (mg GAE/100 g)[16].

Total Flavonoid Content (TFC) Analysis

About 3 ml of sample extract was mixed with 0.3 ml of (5%) sodium nitrate (NaNO_2). After 5 minutes of time, (10%) AlCl_3 about 0.3 ml was added to the mixture. After 5 minutes, (1 M) NaOH about 2 ml of was added to it. Left this mixture in dark at room temperature for 30 minutes. After 30 min, absorbance was measured by using UV-VISIBLE spectrophotometer at 510 nm. TPC was expressed as mg catechin equivalent per 100 ml of extract (mg CEQ)/100ml)[16].

DPPH (1, 1-diphenyl-2-picrylhydrazyl)

Assay

3 ml of sample extract was mixed with 3.9 ml of DPPH solution (ethanol 0.025 g/L). The mixture in test tube was mixed with vortex mixing and left for 30 minutes at room temperature in the dark room for incubation. After 30 minutes, absorbance was measured by using UV-VIS spectrophotometer at 517 nm. The radical scavenging assay can be illustrated as a calculated reduction of DPPH due to the identified amount of watermelon rind extract [17].

Reduction of absorbance (%) = $[(AB - AA) / AB] \times 100$

AB = Absorbance of blank sample at t = 0 min

AA = Absorbance of tested sample Microwave assisted watermelon rind extract at t = 15 mins

FRAP (Ferric Reducing Antioxidant Power) Assay

Sample extract about 100 μl was added to FRAP reagent about 3ml. Sodium acetate buffer (300 mM) at pH 3.6, 2,4,6-tripyridyl-s-triazine (TPTZ) (10 mM) and iron chloride (20 mM) was dissolved

in HCl (40 mM) at a ratio (10:1:1). The solution was mixed completely and then incubated at room temperature for 5 min. Reagent blank was a mixture of distilled water (100 μl) and FRAP reagent (3 ml) incubated at room temperature for 1 hour. The absorbance of sample extracts, standard and reagent blank was measured against the blank (distilled water) at 593 nm by using UV-VISIBLE spectrophotometer. The FRAP value was expressed as mg of ferrous (Fe^{2+}) equivalent per 100ml (mg FE/100ml)[18, 19].

Statistical Analysis

Statistical analysis was performed by using SPSS (9.0). All data was reported as mean \pm standard deviation of three replicates. Difference between means was determined with ANOVA using Tukey-HSD test. Statistical significance was evaluated at level of $p \leq 0.05$.

Results:

Total Phenolic contents

The values obtained by the assessment of antioxidant potential of MAE of watermelon rind extract were shown in Table 2 and are expressed as mean \pm standard deviation. From table 2 it is concluded that the maximum TPC 169.71 ± 0.29 mg GAE/100g was recorded at microwave power of 150 W for 5 minutes of time intervals, whereas minimum was 92.03 ± 0.34 mg GAE/100g recorded at power of 450 W for 5 minutes of time intervals. The results indicate that the grand mean values of 150 W has highest TPC while 300 W (99.12 ± 0.47) has low compared to 150 W which is (163.19 ± 0.37) but higher than 450 W (117.70 ± 0.44).

Total Flavonoid contents

The highest mean value observed for TFC of watermelon rind extract is (42.20 ± 0.04 mg CEQ/100g) at power of 150 W and time interval of 5 minutes. whereas lowest (0.2 ± 0.36 mg CEQ/100g) obtained with time interval of 5 minutes at power of 450 W. By observing the grand values of all powers, it is cleared that power 300 W has low TFC mean values 10.67 ± 0.43 compare to 150 W which is (92.22 ± 0.44) but high mean values than 450 W (6.83 ± 0.39) shown in Table 3.

DPPH antioxidant scavenging assay

From table 4, the grand mean values of 150 W power 58.72 ± 0.32 shows highest DPPH capacity as compared to 300 W (31.46 ± 0.28) and lowest (25.04 ± 0.36) at power 450 W. The mean value observed for DPPH assay 68.17 ± 0.45 should be the most highest antioxidant capacity obtained at power 150 W for 5 minutes of time. The least capacity obtained at power 450 W for 5 minutes of time interval is 9.51 ± 0.27 .

FRAP (Ferric Reducing Antioxidant Power) assay

In FRAP assay from the Table 5, the highest antioxidant potential 326.43 ± 0.21 mg FE/100g should be obtained at the time interval of 5 minutes and power of 150 W. The lowest antioxidant potential as 105.71 ± 0.72 was obtained at power of 450 W in 5 minutes of time interval. By observing the grand values of all powers, it is cleared that power 150 W has high mean values 249.88 ± 0.33 compare to 300 W and 450 W which is 164.94 ± 0.78 and 145.57 ± 0.45 .

Microwave Powers (W)	Time Intervals			Means
	1 min	3 min	5 min	
150 W	159.84 ± 0.97	160.04 ± 0.17	169.71 ± 0.29	163.19 ± 0.37^a
300 W	159.05 ± 0.34	135.96 ± 0.90	102.37 ± 0.19	99.12 ± 0.47^c
450 W	156.27 ± 0.73	104.81 ± 0.25	92.03 ± 0.34	117.70 ± 0.44^b
Means	158.38 ± 0.68^a	133.60 ± 0.44^b	121.37 ± 0.27^c	

Table 2: Analysis of Total Phenolic Contents (expressed as mg GAE/100g) in MAE of Watermelon rind extracts, Values are expressed as mean \pm standard deviation of three replications

Microwave Powers (W)	Time Intervals			Means
	1 min	3 min	5 min	
150 W	21.31 ± 0.57	24.15 ± 0.71	42.20 ± 0.04	92.22 ± 0.44^a
300 W	20.65 ± 0.60	6.54 ± 0.17	4.84 ± 0.52	10.67 ± 0.43^b
450 W	19.6 ± 0.24	0.7 ± 0.59	0.2 ± 0.36	6.83 ± 0.39^c
Means	20.52 ± 0.47^a	10.46 ± 0.49^c	15.74 ± 0.30^b	

Table 3: Analysis of Total Flavonoid Contents (expressed as mg CEQ/100g) in MAE of Watermelon rind extracts, Values are expressed as mean \pm standard deviation of three replications

Microwave Powers (W)	Time Intervals			Means
	1 min	3 min	5 min	
150 W	53.14 ± 0.31	54.87 ± 0.20	68.17 ± 0.45	58.72 ± 0.32^a
300 W	51.02 ± 0.11	25.68 ± 0.53	17.69 ± 0.21	31.46 ± 0.28^b
450 W	50.12 ± 0.20	15.49 ± 0.63	9.51 ± 0.27	25.04 ± 0.36^c
Means	51.42 ± 0.20^a	32.01 ± 0.45^b	31.79 ± 0.31^b	

Table 4: Analysis of DPPH Scavenging Assay (expressed as % ascorbic acid inhibition) of MAE of watermelon rind extracts, Values are expressed as mean \pm standard deviation of three replications

Microwave Powers (W)	Time Intervals			Means
	1 min	3 min	5 min	
150 W	201.71±0.65	221.50±0.15	326.43±0.21	249.88±0.33 ^a
300 W	201.29±0.78	169.38±0.61	124.15±0.96	164.94±0.78 ^b
450 W	200.19±0.44	130.81±0.20	105.71±0.72	145.57±0.45 ^c
Means	201.06±0.62 ^a	173.89±0.32 ^c	185.43±0.63 ^b	

Table 5: Analysis of Ferric Reducing Antioxidant Potential (FRAP) assay (expressed as mg FE/100g) of MAE of watermelon rind extracts, Values are expressed as mean ± standard deviation of three replications

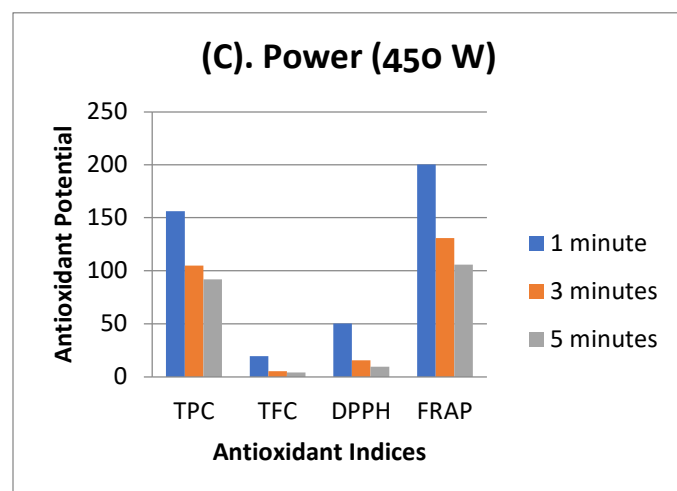
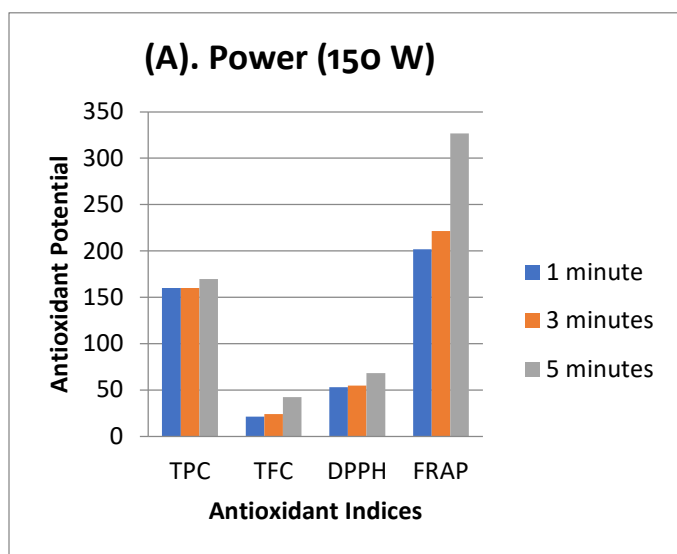
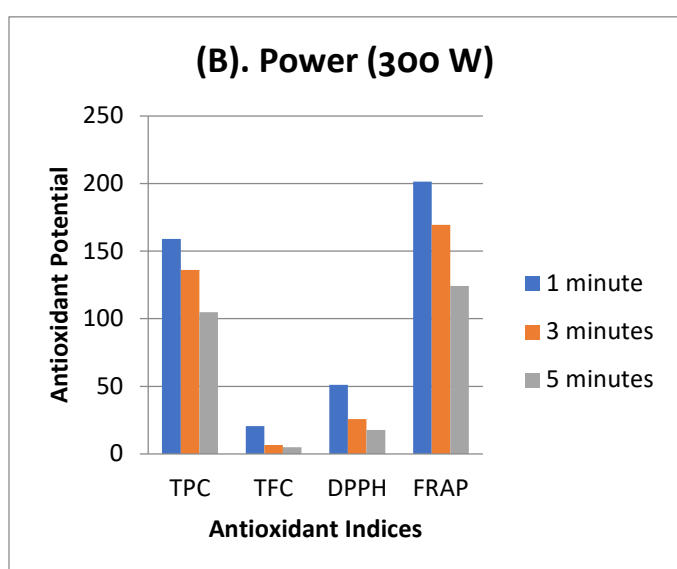


Figure 2: Presentation of analysis of Antioxidant potential of MAE of Watermelon rind extract at various Powers (150, 300 and 450 W) and Time intervals (1, 3 and 5 minutes). (A), (B) and (C) clearly represents the maximum antioxidant capacity of MAE of watermelon rind extract at power 150 W for 5 minutes of time interval **(A)**, 300 W for 5 minutes **(B)** and minimum at 450 W **(C)**



Discussion:

The analysis of the antioxidant potential of watermelon rind was performed in this study to highlight the lavish benefits of rind. From our results findings, the extraction technique and solvent has a great impact on antioxidant potential. According to another researchers, long exposure to heat can increase redox activity that result in the degradation of most phenolic compounds lead to decrease in antioxidant potential [20].

The highest total phenolic compounds (TPC) in this present study were found (169.71±0.29 mg

GAE/100g) microwave assisted extraction power 150 W for 5 minutes of time interval which is much higher than that of TPC contents findings of Anwar *et al.*, [21]. The results of TPC analysis by Ho *et al.*, are much relevant to our findings and shows that increasing the temperature of hot air drying from 40°C to 60°C results in the elevation of antioxidant potential of watermelon rind extracts. The ethanolic extract obtained at low temperature which is 1677.45±0.29 mg GAE/100g of TPC contents shows high significance compared to high temperature extract 195.8±0.47 mg GAE/100g of TPC contents. The ethanolic extracts has high TPC compared to methanolic and acetone [14]. Moreover, another study suggested that the extraction yield of TPC with methanol as a solvent is much higher than that of ethanol [22].

The DPPH scavenging capacity is more in our study findings compared to other previous studies carried out by Shotorbani *et al.*, showed that drying method can breakdown the free radical scavenging capacity due to high temperature [23]. In our study DPPH scavenging capacity shows significant increase in value (68.17±0.45%) at low microwave power 150 W and for 5 minutes of time interval. In our findings both power and time had great impact on extraction yield. According to another study, the heating and drying time has great impact on extraction yield. Longer the time, minimum the extraction yield due to degradation of some antioxidant compounds whereas, lower the time, maximum the antioxidant activity [24]. This finding is closely related to our study.

The results of the total flavonoid contents (TFC) in this present study are much relevant to the findings of the study conducted by Ho *et al.*, that reported high yield of TFC as (48.63±1.04 mg CEQ/100g) dry matter obtained at low power with ethanol as a solvent [14]. On the other hand, a study reported that flavonoids are water-soluble antioxidants so give high yield by using water as a solvent [25]. The variation in yield % is due to different methods used for extraction and different solvents as well as different

temperature and time variations. According to Chan *et al.*, freeze-drying techniques for TFC extraction shows high yield compare two hot air drying or microwave extraction methods which shows reduced TFC results [26].

The antioxidant potential of watermelon rind determined by FRAP assay for samples show 326.43±0.21 mg FE/100g at 150 W power and 5 minutes of time interval. These results of our finding were similar with another study in which extracts using a solvent methanol have less efficiency of antioxidant potential compared to extraction with ethanol [27]. In present study the total antioxidant potential was significantly higher at low microwave power while high microwave power can result in disruption of some antioxidants at various time intervals.

Conclusions:

Based on the results of this study, it is concluded that watermelon rind is enriched with many phytochemicals and antioxidant compounds that exhibit various health benefits. Microwave assisted extraction by using ethanol as a solvent at different microwave powers and various time intervals showed minimum to maximum extraction yield of many phytochemical contents of watermelon rind powder. Microwave power, time and solvent had great impact on extraction yield. The best extraction yield of antioxidants was obtained at low microwave power. Thus, microwave assisted extraction technique should be implemented in the food and nutraceutical industries and microwave assisted extracts of watermelon rind should be utilize for the development of new functional food to combat many health related issues.

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