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RESEARCH ARTICLE

# The Microwave-assisted Extraction of Anthocyanins, Total Phenolic Compounds and the Antioxidant Activity in *Morus nigra L.* (Black mulberry) Grown in the Da Lat City, Lam Dong Province, Vietnam

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## Abstract

*Morus nigra L.* (Black mulberry) is a nutritious fruit that contains many bioactive compounds such as anthocyanins, polyhenol and antioxidant activity. The microwave-assisted extraction method (MAE) is an advanced method with due to its advantages of short extraction time and lower solvent consumption. The objective of this research is to find the appropriate conditions to extract the highest contents of anthocyanin, polyphenol and antioxidant activity in black mulberry by MAE method. The pH-differential method is used to determine the anthocyanin content, the polyphenol content is determined by the Folin-Ciocalteu method and the antioxidant activity is determined by the DPPH method. The highest contents of these coumponds were obtained as follows anthocyanin contents 128.07 mg/L, polyphenol contents 4305.38 mg/L and antioxidant activity 2715.68 µmol/L when extracted under conditions of concentration of ethanol 60o, solid to solvent ratio 1:30, microwave power 600W, microwave-assisted extraction time 2 minutes.

**Keywords:** *Black mulberry, Anthocyanin, Polyphenol, Antioxidant activity, Microwave-assisted extraction.*

## Introduction

Natural food coloring involves the use of colorants that are extracted or processed from natural materials and is relatively safe for consumers [1, 11].Currently there are many natural colorants derived from plants such as carotenoids, betalains, anthocyanins, chlorophylls, turmeric, from insects, such as cochineal and carmine, and from molds such as monascus. Among plant colorants, anthocyanins are a group of natural phenolic compounds and are characterized by red, purple or blue colors. Anthocyanins cause various plants and fruits to appear as red, purple blue or black depending on the pH. Depending on arrangement and methylation of hydroxyl groups constituting anthocyanins molecules, the pigment may take various forms.

In addition, the variation of sugar molecules in terms of binding position, quantity and their connections with aliphatic acid or aromatic acid. On the other hand, the stability of anthocyanin may be altered by the acidity of the solution in which the pigment is dissolved [12, 16].In the food industry as well as human health, anthocyanins have many effects such as anti-cancer, cardiovascular protection, vision improvement, anti-diabetes, anti degenerative and harmless even when being used in high dosage.

Sources for supplementation of anthocyanins are numerous. Of which, black mulberry (Morus nigra L.), a commonly cultivated fruit in Vietnam, especially in Da Lat City, Lam Dong Province is a convenient and potential material. Black mulberry is used for fresh or processed into syrup, jam, ice cream, vinegar, concentrated juice, alcohol [17, 18].Black mulberry is juicy fruit and has a unique color.

It also has sweet and sour, and refreshing taste due to its aroma composition and sugar/acid ratio. The main biochemical compounds found in black mulberry are derivatives of cinnamic and benzoic acid (phenolic acid), quercetin glycoside (flavonoid), cyanidin and pelargonidin glycoside (anthocyanin). Black mulberry has been shown to potentially confer health benefits and exhibit useful bioactivities such as anti-thrombosis, antioxidant, anti-bacterial activity, anti-inflammatory and neuroprotective effects, primarily due to the abundance of phenolic compounds, specifically anthocyanins [19, 23].

Microwave-assisted extraction (MAE) has been an increasingly used extraction routine in various industries recently due to considerable savings the technique may offer in terms of solvent and energy consumption, and extraction times [24, 32].In addition, MAE also has the capability to maintain nutrient composition of the material. Apart from the food field, the applicability of the method also extends to other industries such as medical, rubber, garment, wood and ceramics industries.

In the field of environment, the MAE method is also used to treat sludge, medical waste, contaminated soil, wastewater, recycles activated carbon and be a source of heat/energy for integrated treatment methods. In this study, we explored the possibility of using MAE to extract anthocyanins from black mulberry and determined optimal extraction conditions that give the optimal extraction efficiency

## Materials and Method

### Materials

Black mulberry (*Morus nigra* L.) was harvested from Da Lat city, Lam Dong Province, Vietnam. Obtained black mulberry was washed and blanched at 70°C in 60 seconds to inactivate enzyme.

Following that the material was dried at 60°C to reach the moisture content of <5%, followed by grinding and filtration through a 60-mesh sieve. The obtained powder was stored at 4°C in plastic jars prior to analysis. Common chemicals such as distilled water, ethanol, methanol, HCL, KCl, $CH_3COONa.3H_2O$, Folin-Ciocalteu, $Na_2CO_3$, gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and trolox were of analytical grade.

### Procedure

Microwave-aided extraction commenced under parameters that were allowed to vary through experiment attempts. To be specific, one gram of black mulberry powder was extracted using ethanol solvent with different concentrations ($0^o$ - $100^o$) and solid to solvent ratio (1:20, 1:30, 1:40). The microwave power varied from 100 to 800W and duration of the process lasted 30 seconds to 4 minutes. The extract was then diluted to 100mL, followed by filtration using cloth and subsequently using Whatman No.1 filter paper to afford the filtrate, which was used for analysis.

### Analytical Method

#### Determination of Total Monomeric Anthocyanin Content (TAC)

The pH-differential method described by Giusti & Wrolstad was adopted to determine the total monomeric anthocyanin content [33]. First, the filtrate was first adjusted either to pH 1.0 using 0.025M KCl or to pH 4.5 using 0.04M $CH_3CO_2Na.3H_2O$. The two samples were allowed to equilibrate in the dark for 15 minutes before being measured for absorbance at 520 nm and 700 nm. Calculated TAC was recorded as mg cyanidin-3-glucoside equivalent per volume of the sample (mg/L).

#### Determination of Total Polyphenol

The total polyphenol was measured using a modified Folin-Ciocalteau method described by Torres [34]. The diluted extract (1ml) was added with 1mL of Folin reagent and was allowed to stand in the dark for 5 minutes when 1mL of $Na_2CO_3$ 20% was added. Before being measured for absorbance at 765 nm, samples were incubated in the dark for 30 minutes. Calculated total polyphenol was described as mg acid Gallic equivalent per volume of the sample (mg/L).

### Determination of the Antioxidant Activity (DPPH)

To determine antioxidant activity of the samples, the scavenging free radical (DPPH) method described by Brand-Williams was carried out [35]. The general principle of the method bases on the ability of antioxidants to scavenge free radical, in turn causing discoloration to the purple color of DPPH solution. An aliquot of 0.2ml of diluted extract first introduced into 3ml of DPPH solution, followed by 30 minutes of incubation in the dark and then measurement for absorbance at 515 nm. The results were expressed as µmol trolox equivalent per volume of the sample (µmol/L).

## Results and Discussion

### Effects of Ethanol Concentration on the Extraction Process

Solvents of varying concentrations have a different polarity, which exerts a significant effect on the content of the compounds obtained during the extraction process. Figure 1 and Figure 2 indicated that the contents of anthocyanin, polyphenol and DPPH achieved the maximum values, at 111.05 mg/L, 4330.24 mg/L and 2483.77 µmol/L respectively, with the sample extracted at ethanol 60° . Obtained anthocyanins and polyphenols tended to increase when rising the concentration of ethanol from 0° to 60°. Afterwards, as the ethanol concentration continued to increase from 60° to 100°, the content showed a decline and reached the lowest content at ethanol concentration of 100°.

This trend is attributable to the discrepancy between of water and ethanol in terms of dielectric constant and hydrogen bond value, resulting in the mixture having different levels of polarity. A previous study also suggested the use of the solvent system, rather than individual solvent, to achieve favorable extraction conditions and to yield higher extraction efficiency [36].

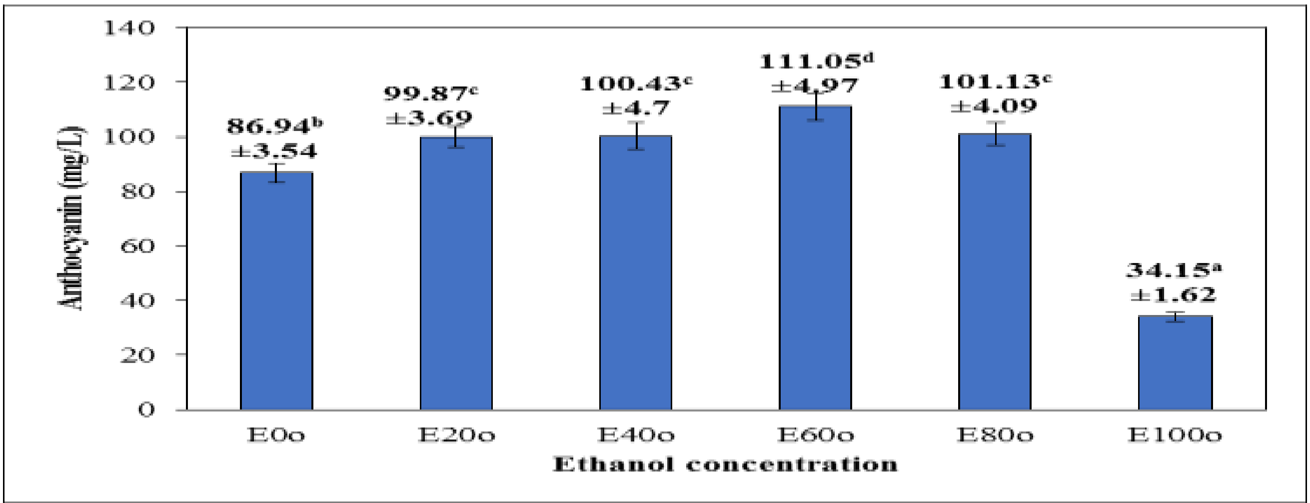


**Figure 1: Effect of ethanol concentration on anthocyanin contents in black mulberry extract (a, b, c show significant differences at significance levels P <0.05)**

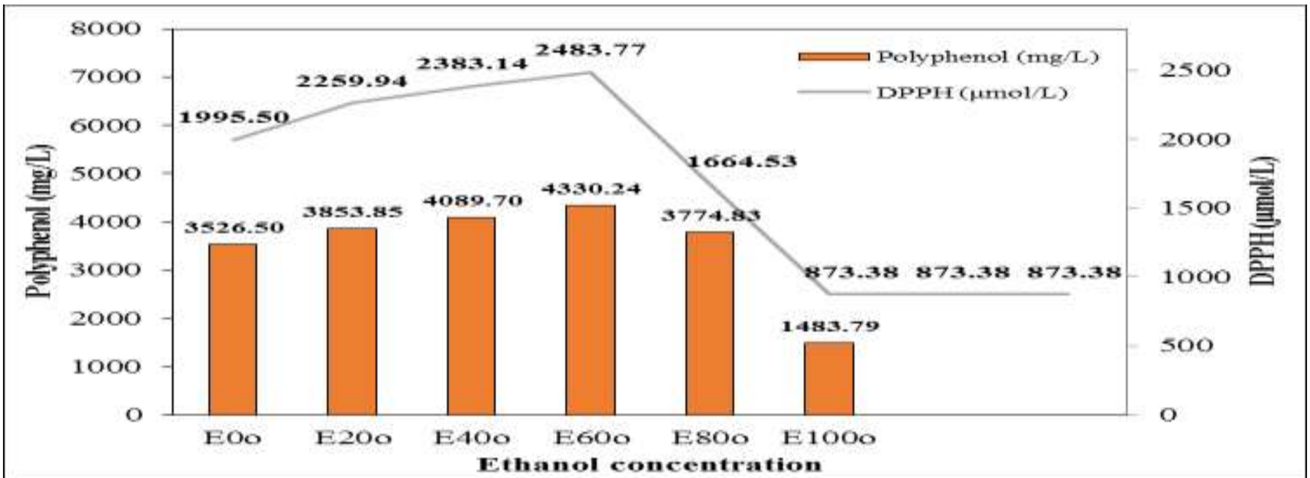


**Figure 2: Effect of ethanol concentration on polyphenol and DPPH contents in black mulberry extract (a, b, c show significant differences at significance levels P <0.05)**

### Effects of Solid-to-solvent Ratio on the Extraction Process

The solid to solvent ratio significantly affects the efficiency of the extraction process.

Three ratios, 1:20, 1:30 and 1:40 were investigated for their influence on polyphenol and anthocyanin content in this experiment. Figure 3 and Figure 4 showed that the contents of anthocyanin, polyphenol and DPPH obtained with samples extracted at ratio 1:30 achieved the highest contents, respectively at 119.21 mg/L, 4303.46 mg/L and 2273.62 µmol/L for anthocyanin, polyphenol and DPPH. This relationship is elaborated by the study Rostagno et al. [37] which suggested that insufficient amount of solvent required for material submersion may hinder extraction efficiency. However, the same study also articulated that, given a fixed microwave power, higher solvent quantity may lower extraction efficiency because of dissipated microwave energy, which lowers temperature of the mixture. Therefore, in addition to increased amount of solvents, it is necessary to apply larger microwave power and prolong extraction time to achieve the temperature required for the extraction.

The large amount of solvents can also cause the dissolution of unwanted compounds, which reduces the selectivity for the desired compounds. The results of this experiment are similar to the result of microwave-assisted extraction of anthocyanin from lavender 38], which concluded the solid to solvent ratio of 1:30 for maximal extraction efficiency.

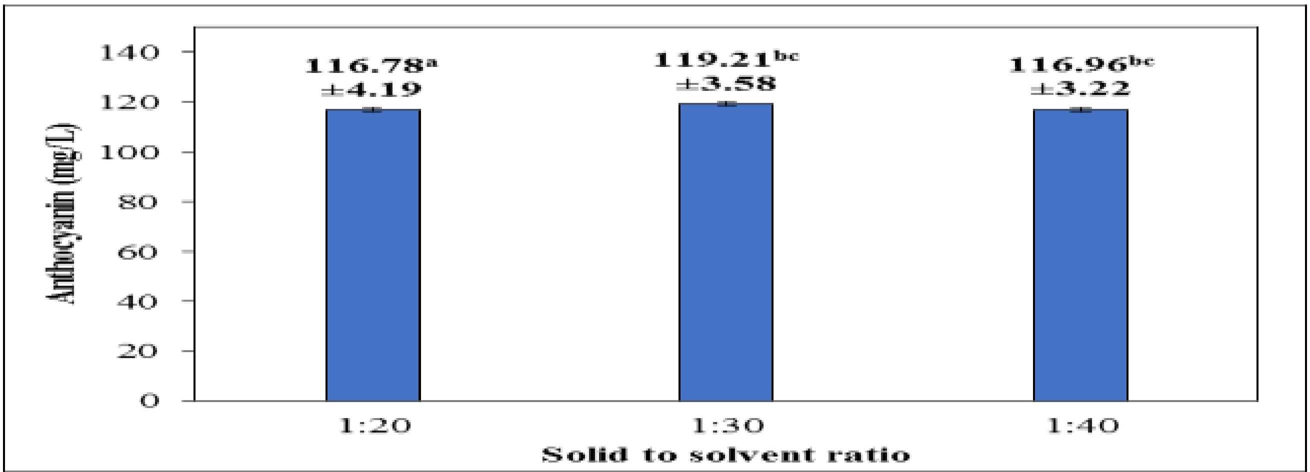


**Figure 3: Effect of solid to solvent on anthocyanin contents in black mulberry extract (a, b, c show significant differences at significance levels P <0.05)**

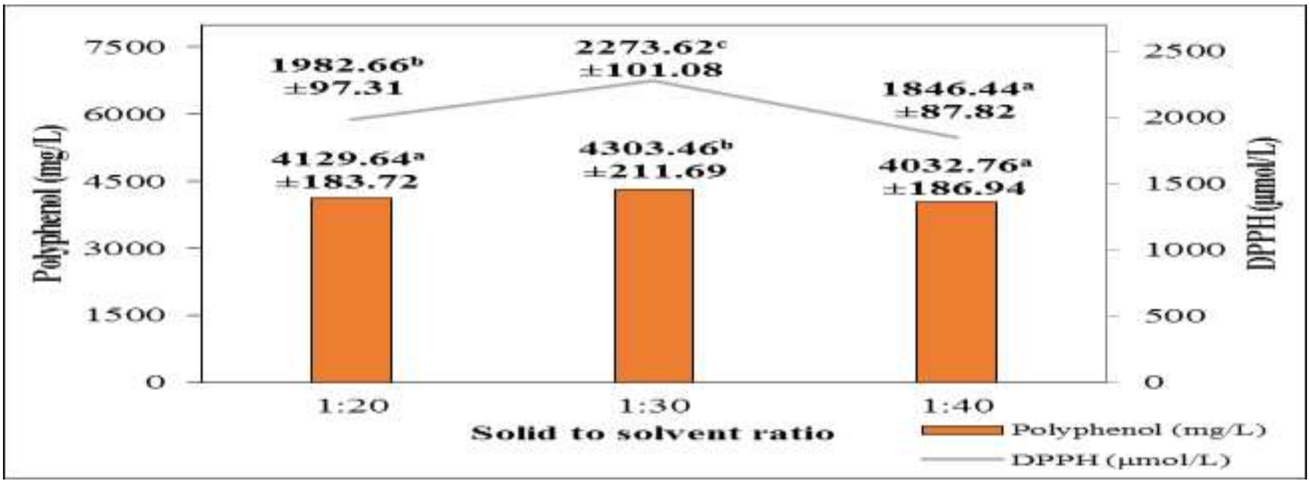


**Figure 4: Effect of solid to solvent on polyphenol and DPPH contents in black mulberry extract (a, b, c show significant differences at significance levels P <0.05)**

### Effects of Microwave Power on the Extraction Process

The microwave power affects the temperature of the extraction mixture, thus affecting the contents of the compounds obtained. The differences in anthocyanin, polyphenol and DPPH contents were shown in Figure 5 and Figure 6. The microwave power of 600W seemed to give the highest extraction contents with the anthocyanin value of 130.89 mg/L, polyphenol of 4554.76 mg/L and DPPH of 2687.9 µmol/L. According to experimental results, rising the microwave power from 100 to 600W caused extracted contents to improve. However, rising the power from 600 to 800W induced decline in obtained contents. The importance of microwave power is highlighted in a previous study [37] elaborating that inappropriate temperatures may lead to overpressure inside the closed vessel and the loss of thermally sensitive compounds.

As a result, the microwave power of about 250W will be suitable for open extraction systems and the range from 600W to 1000W is advised for closed extraction systems. Current experimental result is similar to the result of microwave-assisted extraction of polyphenol from pomegranate peel [39] in which the microwave power of 600W gave the highest polyphenol contents.

This finding supports the current optimal microwave power of 600W. When the microwave power reaches 800W, the temperature of the mixture was elevated to a very high level, resulting in decomposition of heat-unstable compounds, which is also consistent with the study of Tsai et al. [21] which mentioned that anthocyanin is unstable when being treated at high temperatures.

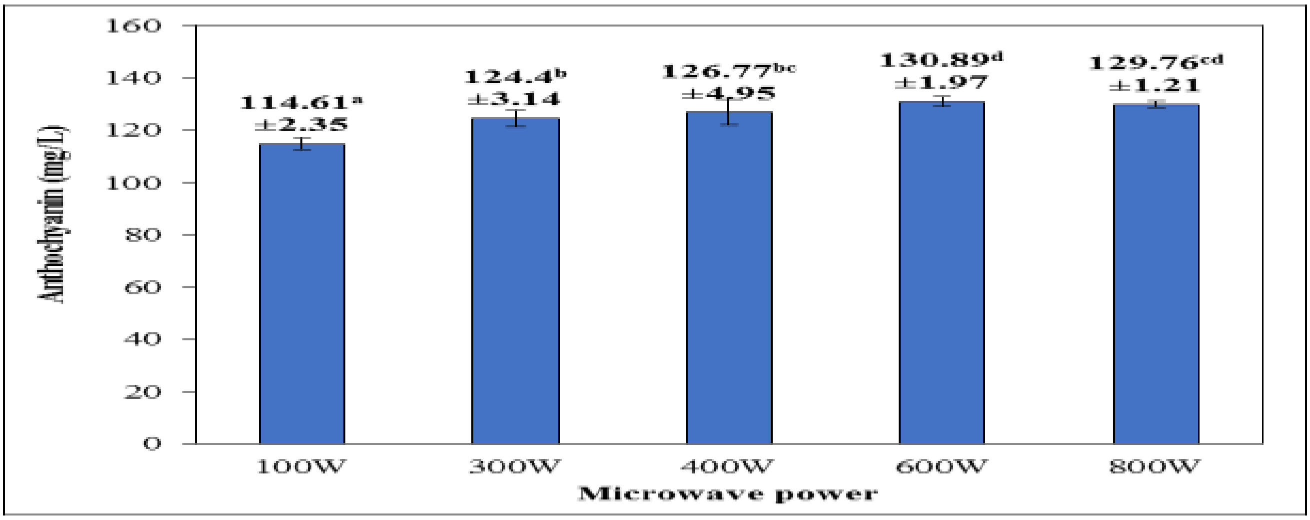


**Figure 5: Effect of microwave power on anthocyanin contents in black mulberry extract (a, b, c show significant differences at significance levels P <0.05)**

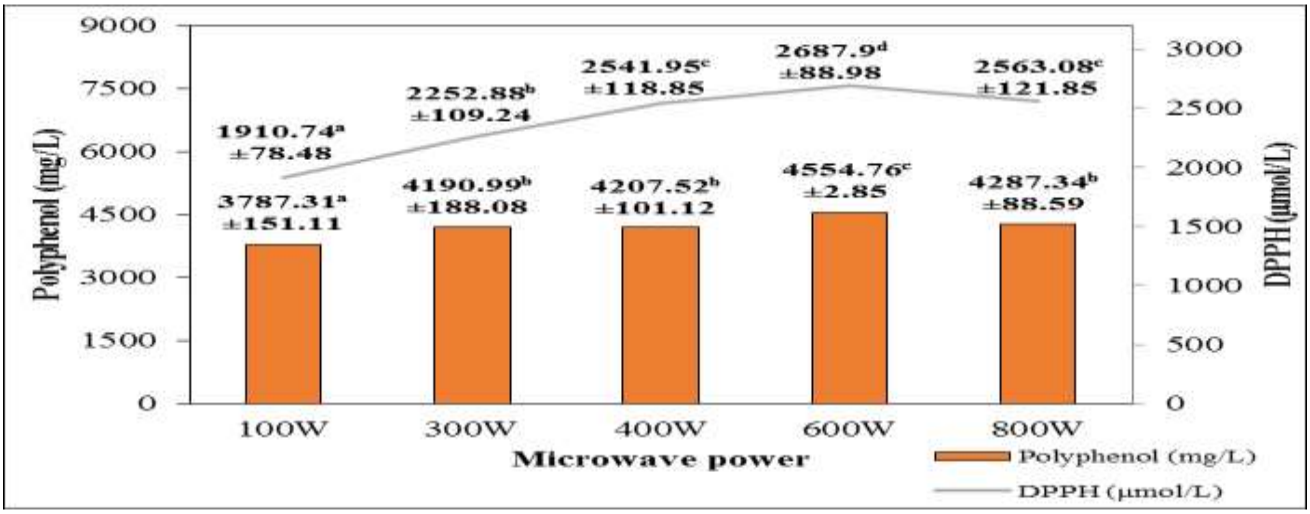


**Figure 6: Effect of microwave power on polyphenol and DPPH contents in red cabbage extract (a, b, c show significant differences at significance levels P <0.05)**

## Effects of Microwave Extraction Time on the Extraction Process

The microwave irradiation duration influences the temperature of the extraction mixture, thus significantly affecting the extraction efficiency. To maintain temperature stability, microwave irradiation was carried out in 30-second intervals, alternated by idle periods of 1 min. The contents of the extracts are shown in Figure 7 and Figure 8. It was shown that the extracted anthocyanin, polyphenol and DPPH contents are highest, at 128.07 mg/L, 4305.38 mg/L and 2715.68 µmol/L respectively when irradiating the sample for 2 minutes. The increasing relationship between microwave irradiation time and the outcomes was also observed when rising the time from 30 seconds to 2 minutes, at which the reverse trend began to appear afterwards.

Rostagno et al. [37] maintained that even though microwave-assisted extraction has a very short extraction time, required irradiation period may depend on the desired compounds. When the extraction time is too short, the temperature impacts on the solvent and the material is may be insufficient and excessive long irradiation may cause heat-unstable compounds in the materials to degra. Tsai et al. [21] also mentioned that anthocyanin are unstable during heat treatment. The pattern of alternating cooling and heating period adopted in this study was also suggested by Quan et al. [40] when extracting polyphenol with microwave-assisted extraction from fresh tea buds with a period of 2 minutes of irradiation and 1 minutes of cooling.

It can be explained that at the time of insufficient microwave processing, the temperature is not high enough, so the extraction efficiency is low. If the duration of microwave extraction is too long the temperature could be excessively elevated and recovery of compounds could be undermined. Therefore, within 2 minutes, the highest content of compounds is obtained.

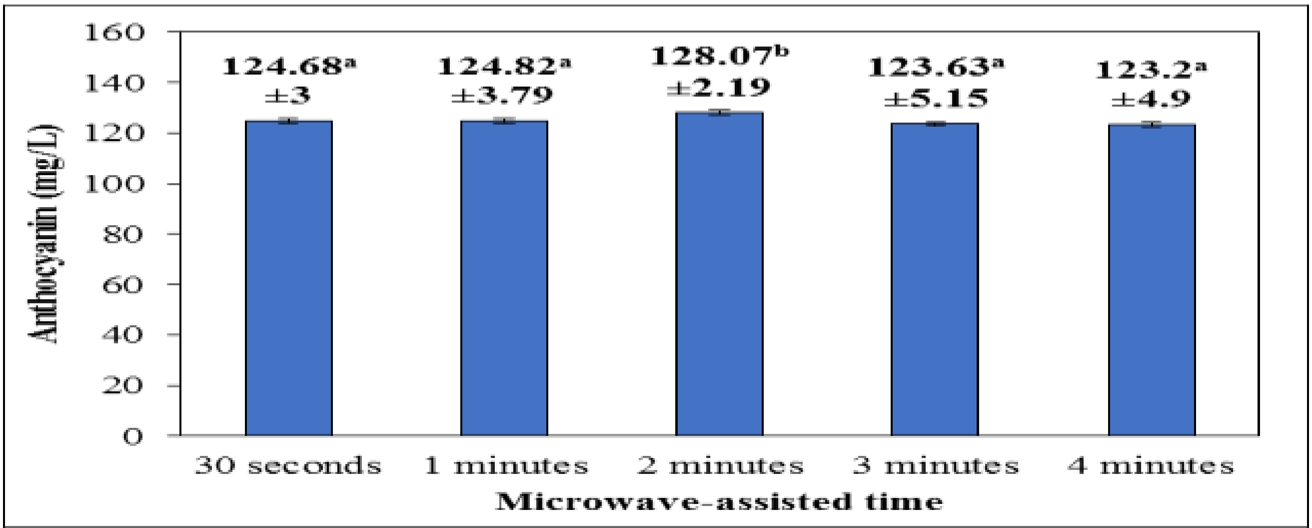


**Figure 7: Effect of microwave-assisted extraction time on anthocyanin contents in black mulberry extract (a, b, c show significant differences at significance levels P <0.05)**

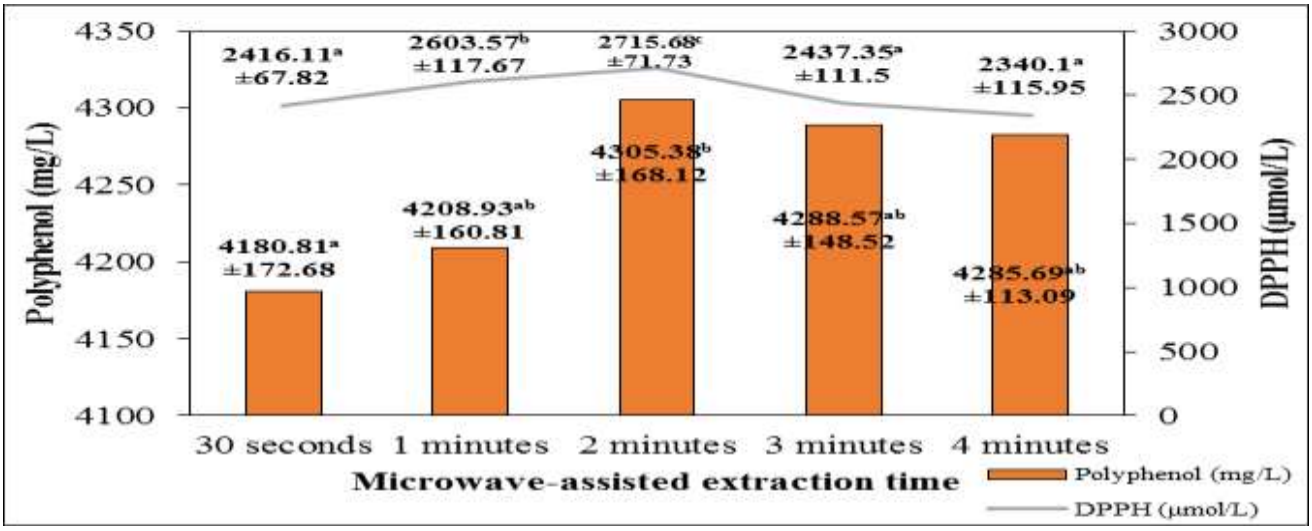


**Figure 8: Effect of microwave-assisted extraction time on polyphenol and DPPH contents in black mulberry extract (a, b, c show significant differences at significance levels P <0.05)**

## Conclusion

In this study, we have found the optimal conditions for maximal recovery of polyphenols and anthocyanins from the black mulberry material. Parameters consisted of ethanol concentration of 60°, solid to solvent ratio of 1:30, microwave power of 600W and microwave-assisted extraction time of 2 minutes. Thereby, it is also proved that the microwave-assisted extraction method is a method that requires a short time, less effort, is suitable for the extraction of heat-unstable compounds and also provides better extraction efficiency than the conventional extraction methods. This result may act as a precursor for natural colorant production studies that utilize black mulberry.

## Acknowledgements

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