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Research Article

Effect of microwave assisted extraction conditions on mineral and antioxidant properties of *moringa oleifera* lam

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ABSTRACT

Moringa oleifera Lam provides a source of essential minerals and antioxidative substance for human health. The study was performed to determine the effect of solvent, extraction time, microwave power, and liquid-solid ratio of minerals and antioxidants. The mineral (iron and calcium) contents were determined using atomic absorption spectrophotometric, and antioxidative substance were using a spectrophotometer. The highest extraction yiled of calcium (2631.53 mg/kg) was achieved using water with 10 ml/g liquid-solid ratio and 450 watts microwave power for 5 minutes. The optimum conditions for iron extraction used water with 12.5 ml/g liquid-solid ratio and 600 watts microwave power for 3 minutes, yielding total iron of 30.87 mg/Kg. The optimum conditions for antioxidant extractions were using 96% ethanol with a liquid-solid ratio of 12.5 ml/g, microwave power of 900 watts for 2 minutes. It achieved the antioxidant activity (IC_{50}) 5.29 µg/ml, total phenolic content 201 mgGAE/g, and total flavonoid content 76.29 mgQE/g.
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Key words: *Moringa oleifera Lam; Microwave assisted extraction; Antioxidant properties; calcium and iron content; Total phenolic and flavonoid content*

INTRODUCTION

Moringa oleifera Lam is a species from the Moringaceae family. It is edible and used in medicine. Some tribes in the eastern region of Indonesia consume the plant as a vegetable. It can be found easily in countries with subtropical and tropical such as India, Philippines, Pakistan, Ethiopia, Sudan, and Indonesia [1]. In the Philippine, the leaves of this plant contain relatively high iron and calcium, and it is known as "the best friend of the mother" because it stimulates lactation and is sometimes prescribed for anemia [2]. Moreover, some studies have reported the plant possesses pharmacological properties, such as antihypertensives, diuretics, anti-cholesterol, antispasmodic, hepatoprotection [1], antibacterial [3], anti-anemia [4,5], antioxidant [6], and immunomodulatory [7]. Moringa leaves have been shown to be a rich source of calcium, iron, potassium, β-carotene, and protein. Moreover, it also provides such antioxidants composed of phenolics, flavonoids, carotenoids, and ascorbic acid [6,8].

Calcium is a critical element playing diverse roles in the human body, including many enzymatic reactions (where calcium is required as a cofactor), nerve function, and strengthening teeth and bones. Moreover, calcium is also necessary for the regulation of sodium ion permeability in cell membranes (including nerve cells), and muscle contraction [9].

Iron is generally in the ionic form (Fe^{2+} and Fe^{3+}) an essential mineral that regulates the body's metabolism and the formation of red blood cells. However, when consumed excessive amounts, iron becomes harmful to health, in particular, causing liver damage, diabetes, and heart blood vessel blockage [10]. An appropriate method is required for optimally extracting these elements from the plant.

An antioxidant is defined as compounds capable of delaying or inhibiting oxidative reactions [11]. It works in the body to bind free radicals, which are known to induce cancer, arteriosclerosis, and aging through oxidative damage to tissues. Moreover, free radicals naturally occur in the body as a byproduct of metabolism and can absorb from outside the body through the respiratory pathway or skin [12].

Bioactive compounds from natural products can be extracted by a different method, either modern or conventional [13,14]. Modern extraction methods include countercurrent extraction (CE), ultrasound-assisted extraction (UAE), supercritical fluid extraction (SFE), pulsed-electric field extraction (PEF), microwave-assisted extraction (MAE), and pressurized liquid extraction (PLE) [13]. The MAE methods for extracting active compounds from the plant, after improvement over the years, have great potential [15]. Its extraction process is rapid, effective, and efficient using a small amount of solvent. Additional advantages include its protection of thermolabile [14]. The MAE uses the microwave with a frequency 2.45 MHz in the form of non-ionizing electromagnetic radiation [16]. This energy causes molecules to move along with ion migration and spin around the two poles altering molecular structure. The energy generated by the microwave radiation the cell walls so that the analyte to be extracted diffuse into the solvent [17,18].

The use of conventional methods (maceration) with 70% ethanol obtained a yield of 1.323 mg/g total phenolic content, 0.62 mg/g total flavonoid content, and 62.94 µg/mL antioxidant activity with IC_{50} [19]. Non-conventional extraction has also been conducted to determine total phenolic content using MAE and PLE [20]. However, the application and optimization of MAE for extracting of iron and calcium content from *M. oleifera* has not been reported.

The study aims to obtain the optimum condition of MAE considering the combination of several factors in the experimental design: microwave power, solvent types, liquid-solid ratio, and extraction time. Their effect on the extraction efficiency of mineral (iron and calcium) and antioxidant constituent of Moringa leaves were determined.

MATERIAL AND METHODS

Plant Material

The Moringa leaves were collected from Sukabumi, West Java, Indonesia and were identified at the Herbarium Bogoriense, Botanic Garden, Bogor, West Java, Indonesia. The voucher specimen was deposited at Pharmacognosy and Phytochemistry Laboratory, Universitas Indonesia, Depok, West Java, Indonesia.

Chemical Materials and General Equipment

The chemical and apparatuses used in this study, including ethanol, aqua demineralisation (aqua DM), and methanol were purchased from PT. Smart Lab Indonesia. Sodium carbonate, gallic acid standard, Aluminium chloride, calcium carbonate, iron standard, and sulfuric acid were purchased from PT. Merck, Germany. 2,2-diphenyl-1-pikrylhydrazyl), quercetin standard, Folin-Ciocalteu P, Nitric acid, perchlorate acid, potassium hexacyanoferrate (II) (K4Fe(CN)6) LP, potassium hexacyanoferrate (III) (K3Fe(CN)6) LP, ammonium carbonate, percholic acid, and sodium hydroxide were purchased from Sigma-Aldrich, Germany. Microwave Assisted Extraction (Modena MV series, USA), Rotary vacuum evaporator (Buchi, Japan), Furnace (Watberthrem, USA), Atomic Absorption Spectrophotometric (Shimadzu AA-6300 PC, Japan), Spectrophotometer UV-VIS (Shimadzu, Japan), and other.

Extraction Process

Moringa leaves powder was extracted using MAE following the literature [14,20] with slight modification. Briefly, the sample (100 g) was extracted using microwave oven (domestic microwave with slight modified) at several combinations of multiple factors, including liquid-solid ratio, the type of solvent, extraction time, and microwave power (shown in **Table 1**). The resulting extract was evaporated by vacuum evaporator to obtain a dry extract, and then the yield value was calculated.

Iron and Calcium Content Analysis

Iron and calcium were analyzed using AAS based on methods reported in the literature [21,22] with modification. Briefly, the extract (1 g each) was mixed with diluted HNO_3 (incubated overnight) and was dry-ashed in a muffle furnace at 550 – 600 ^oC for 10h. The ash sample was dissolved with aqua DM after filtration. The filtrate was analyzed at their respective wavelengths of 248.3 nm and 422.7 nm. Total iron and calcium content were examined using the equation of regression linear results from standard

solution at their equation of Y = 0.0404X + 0.0026 (R^2 = 0.9994) and Y = 0.0291X + 0.0031 (R^2 = 0.9992), successively.

Antioxidant Activity

The extract (25 mg each) was first dissolved in 50 mL methanol and applied for the actual experiment at six-fold dilution. The antioxidant activity assay using 2,2-diphenyl-1-picrylhydrazyl (DPPH) was conducted as previously described by literature [23,24,30].

Total Phenolic Content

The total phenolic content of the extract under optimal condition was performed using Folin-Ciocalteu method [19,24] and gallic acid as a standard. Briefly, a sample of the extract (1.0 ml, three replicates) was mixed 9.5 ml of deionized water and 5.0 ml 0.2 N Folin-Ciocalteu reagent. The mixture incubated for 5 minutes, and then 4 mL of saturated sodium carbonate (7.5% w/v) was added. The absorbance was measured at the wavelength of 765 nm using spectrophotometer after incubation at 30^{o}C for 1.5 hour. Determination of total phenolic content was performed using the linear regression of gallic acid standard (10, 20, 30, 50, and 60 ppm) with the following equation: Y = 0.010X + 0.005 (r = 0.999).

Total Flavonoid Content

Total flavonoid content of the extract under of optimal condition was recorded using aluminum chloride method [19,25]. Briefly, a sample of optimal extract (2.0 ml in methanol, three replicates) was mixed 0.1 ml of aluminium chloride solution (2%w/v) and 0.1 ml potassium acetate solution (0.1 mM). The absorbance was read using a spectrophotometer at a wavelength of 415 nm after incubation at room temperature for 30 minutes. Determination of Flavonoid content was performed using the linear regression of quercetin standard (6, 8, 10, 12, 14, and 16 ppm) with the following equation: Y = 0.033X – 0.026 (r = 0.997).

RESULTS AND DISCUSSION

The extraction process was performed using a Modena MV Series microwave, which was modified using a condenser to prevent evaporation of solvents and reduce pressure in the microwave. The microwave was able to set extraction time and microwave power. Moringa leaf powder was extracted with a combination factors designed for MAE. Total yields of the extracts were calculated using the following equation: (%) = EW/SW x 100%. Were EW is extract weight; and SW is sample weight.

Effect of Microwave Power

Five different power levels were attempted, including 90, 270, 450, 630, dan 900 watts. Moringa leaves were extracted using 70% ethanol, the liquid-solid ratio of 5 ml/g, and 3 minutes of extraction time. The solvent was selected because it efficiently attracted phenolic and flavonoid compound from this plant [19]. The yield, iron, calcium, and DPPH inhibition in Moringa extract using MAE with five different levels of microwave power are shown in Figure 1. Microwave power affected the extraction yields, Total yield and DPPH inhibition varied from 17.6 to 20.1% and 63,63 to 74.82%, respectively, while both iron and calcium content changed in the range 20.07 to 30.87 mg/Kg and 1833.87 to 2631.53 mg/Kg, respectively.

The highest total iron content (30.87 mg/Kg) were obtained with a microwave power of 630 watts (shown in Figure 1B). Both the highest total calcium content (2631.53 mg/Kg) and the highest total yield (20.1 %) were achieved with a microwave power of 450 watts (shown in Figure 1A and 1C). These results are consistent with the previous study [26], the level of a microwave power has been commonly used in the range of 400-700 watts of MAE. Meanwhile, The most active DPPH inhibition (74.83%) was observed at the microwave power of 90 watts (shown in Figure 1D). Changing microwave power was sufficient to affect the total content of iron and calcium but failed to alter the yield and DPPH inhibition.

Effect of Type of Solvent

The solvent used in this study included aqua DM, ethanol (30%, 50%, 70%, and 96%). The extraction of Moringa leaves was performed with the microwave power of 630 watts, the liquid-solid ratio of 5 ml/g, and extraction time of 3 minutes.

The polarity and type of solvent significantly affected yields, the resulting antioxidant activity and mineral content (notably iron and calcium) as shown in Figure 2. The use of aqua DM as a solvent generated the highest iron, calcium, and DPPH inhibition such as 59,54 mg/Kg, 20063,76 mg/Kg, and 80,8 %, respectively. Meanwhile, the use of 30% ethanol gave the highest yield, iron, calcium, and DPPH inhibition. The solvent that is most widely recommended for the extraction of bioactive compounds with antioxidant activity is ethyl acetate, acetone, methanol, water, ethanol, and a mixture of organic solvents in water [27]. Additionally, iron and calcium have polar properties. Moreover, extraction using MAE should use a solvent that can convert the energy into heat by its dielectric properties [28]. In this study, the use of aqua DM is optimal for the

extraction of iron, calcium, and the DPPH inhibition with this plant.

Effect of Extraction Time

The extraction time used in this study were 2, 3, 5, 7, and 10 minutes. The sample was extracted using a microwave power of 630 watts, 5 ml/g (liquid-solid ratio), and aqua DM as a solvent.

The results of yields, iron, calcium, and the DPPH inhibition can be seen in Figure 3. The highest of iron and calcium were obtained with an extraction time of 3 minutes (59.47 mg/Kg) and 5 minutes (21951.75 mg/Kg), respectively (Shown in Figure 3B and 3C). Whereas, the highest yield value and the most potent DPPH inhibition were found for the shortest time (2 minutes, Figure 3A and 3D). The extraction time is inversely proportional to the microwave power, the higher of the power level was, the faster of the time MAE required. By contrast, the lower the microwave power was, the longest the time MAE needed [15]. These results are study reported by Khajeh (2006) [29], the levels of zinc and copper in food increased in the 9th minute and then decreases with increasing the time of MAE.

Effect of Liquid-Solid Ratio

The ratio between the solvent and the powder sample used was 5, 7.5, 10, 12.5, and 15 ml/g, successively. The powder sample was extracted for 3 minutes using a microwave power 630 watts and using aqua DM as a solvent.

Figure 4 shows that the highest of yields, iron, calcium and the strongest of DPPH inhibition (32%, 164.17 mg/kg, 22727.93 mg/kg, and 68.66%, respectively) were achieved using a liquid-solid ratio of 12.5 ml/g. The extraction utilizing the rate was adequate to make a substantial contact between powder and solvent so that it could attract the components contained in the sample. The proportion of 10 to 20 ml/g was an optimum ratio used in the MAE [15]. Based on the combination of four factors, the optimum conditions for iron and calcium used water solvent in both, the liquid-solid ratio of 12.5 and 10 ml/g, microwave power of 630 and 450 watts, and the extraction time of 3 and 5 minutes, respectively. The levels of iron contained in dried Moringa leaves of 191.57 mg/Kg and calcium levels of 25254.27 mg/kg, Whereas the MAE method extracted 164.17 mg/kg (85.70% of that in dried leaves) and 22727.93 mg/kg calcium (89.99% of that in dried leaves). Furthermore, the optimum condition to extract the antioxidative content used 96% ethanol, 12.5 m/g liquid-solid ratio, 900 watts microwave power, and 2 minutes of extraction time.

Antioxidative, flavonoid, and phenolic constituent of the optimum extract

Antioxidant activity of the optimum extract was measured using DPPH, total flavonoid content was analyzed by an aluminum chloride colorimetric method with quercetin as standard, and total phenolic content was calculated using Folin-Ciolcalteu reagent with gallic acid as standard. Each absorbance was measured using spectrophotometer at the appropriate wavelength. In **Table 2**, the test results of the antioxidative properties, flavonoid, and phenolic constituent showed that the optimum extracts from MAE method outperform that using the conventional extraction methods (maceration) as reported by Vongsak et al. (2013) [19]. These results are consistent with research has been published by Rodriguez-Perez et al. (2016) [20]. In this work, the increase in the phenolic and flavonoid contents in the optimum extract is directly proportional to the increased antioxidant activity reported previously [8,30.]

CONCLUSION

Based on the results presented here, the application of MAE method for extract mineral and antioxidant constituents in Moringa leaves is improved. The optimal condition of MAE was identified based on the attempts in different combinations of experimental factors including extraction time, microwave power, solvent types, and liquid-solid ratio. The MAE method provides many edge, such as less energy, less solvent, shorter time, and better target constituents with the lowest cost compared to conventional methods. Hence, it is feasible for the MAE method to replace concentional extraction methods. However, in this study, the optimization was performed only by comparing the levels of target compounds and did not the extraction optimize using response surface of each factor/analysis multifactor, which can provide a systematic view on how the exerimental conditions affect the outcome. It can be addreeesed in future study.

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CONFLICT INTEREST

All author declared that no have conflict of interest

Table 1 Design experimental of microwave assisted extraction (MAE)

Factors	Range and Level				
Extraction time	1 minute	3 minutes	5 minutes	7 minutes	10 minutes
Microwave power	90 watts	270 watts	450 watts	630 watts	900 watts
Solvent types	Aqua DM	Ethanol 30%	Ethanol 50%	Ethanol 70%	Ethanol 96%
Liquid-solid Ratio	3 ml/g	5 ml/g	7 ml/g	10 ml/g	12.5 ml/g

Table 2 Antioxidant activity and total phenolic and flavanoid content of the optimum extract

Test	Results
Antioxidant activity (IC_{50})	5.29 µg/ml
Total Phenolic Content	201 mg GAE/g sample
Total Flavonoid Content	76.29 mg QE/g sample

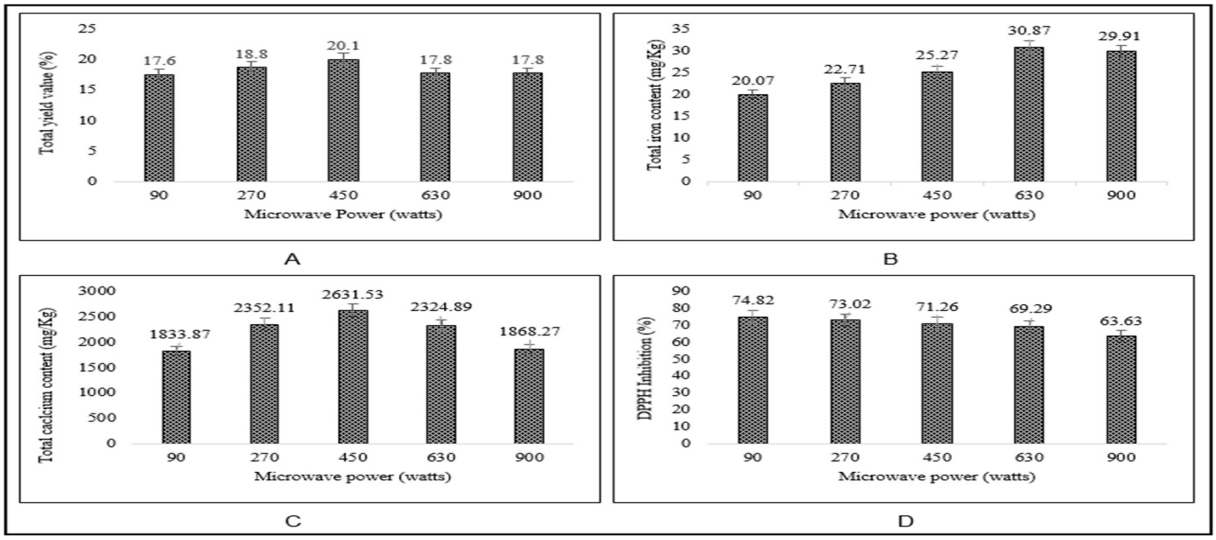

Figure 1 : Total yield, total iron and calcium, and DPPH inhibition Based on the levels of Microwave Power used

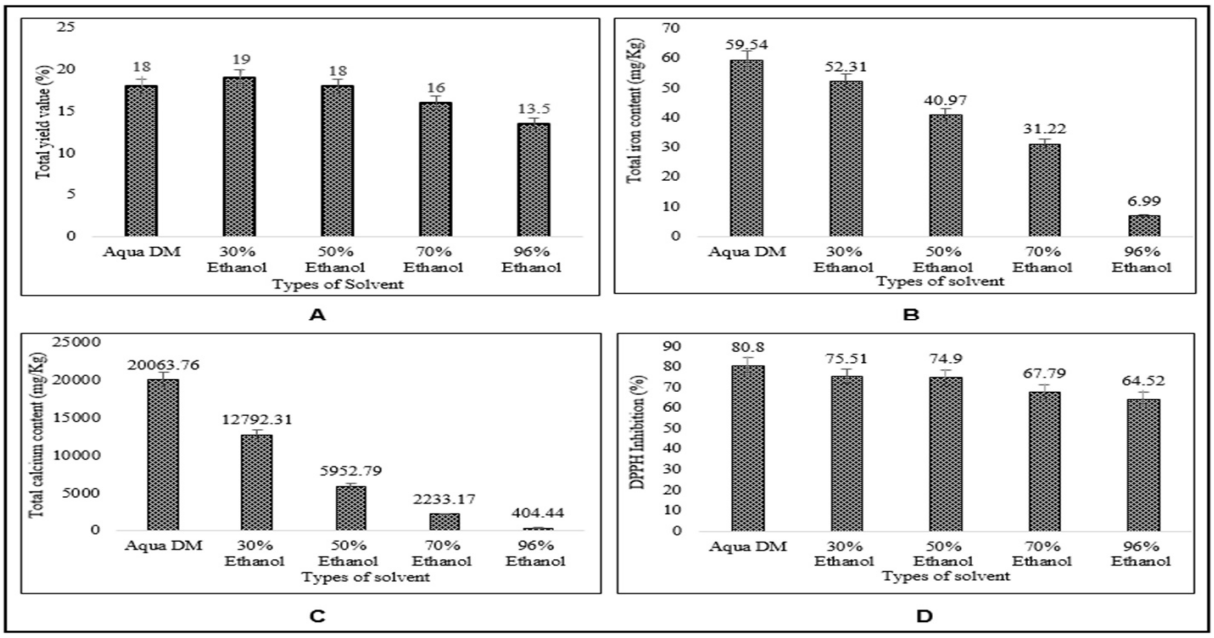

Figure 2: Total yield value, Total iron and calcium content, and DPPH Inhibition based on the types of solvent used

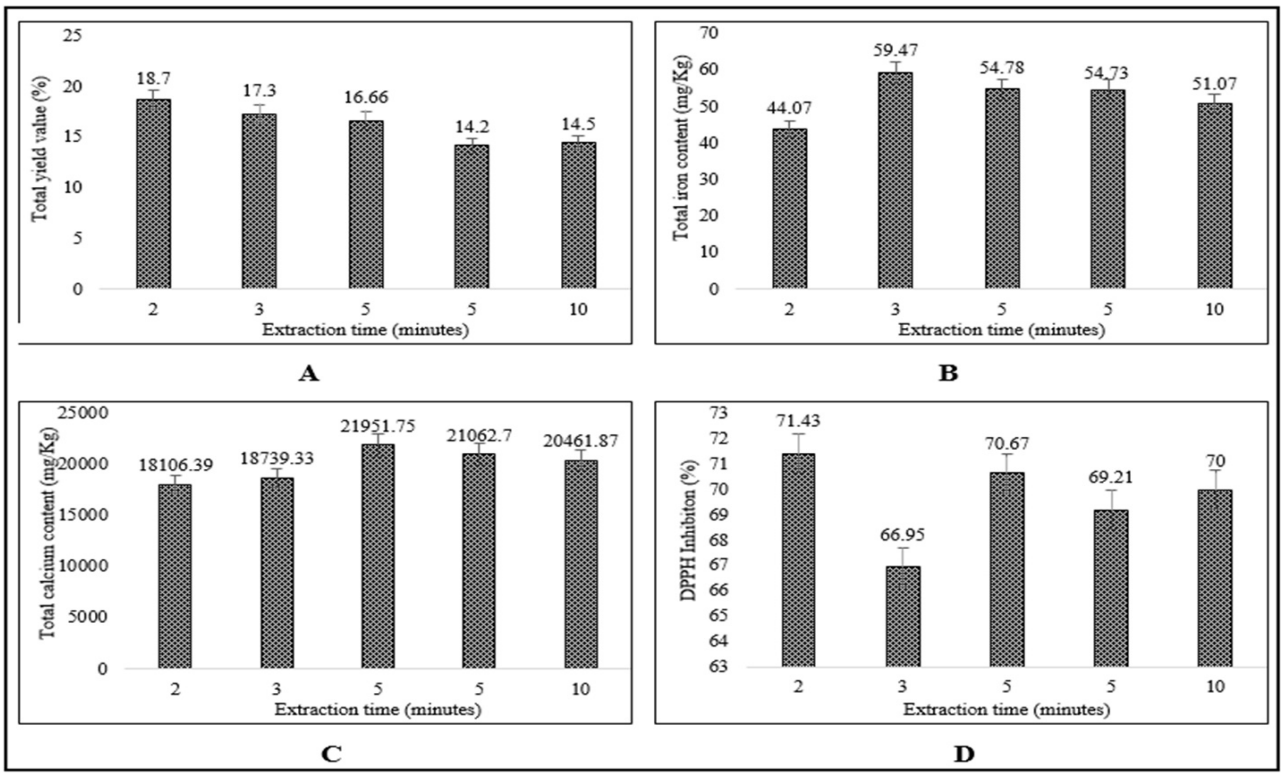

Figure 3: Total yield value, total iron and calcium content, and DPPH Inhibition based on the levels of extraction time used

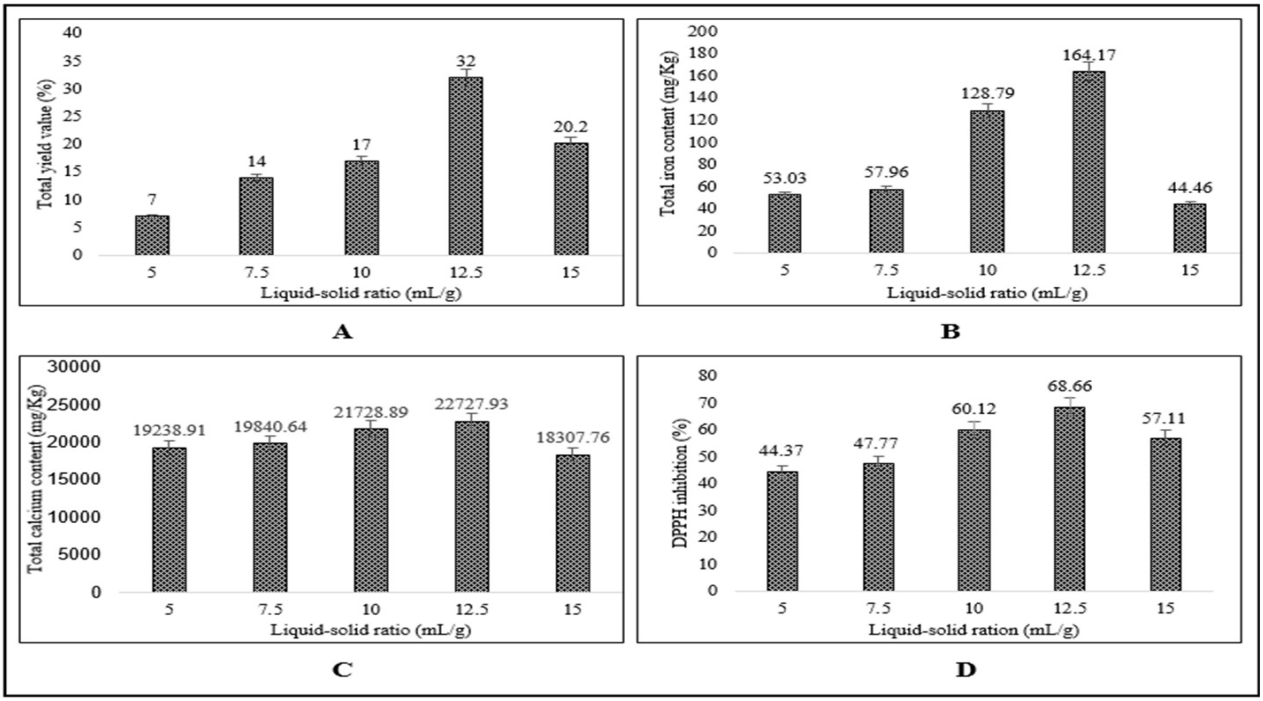

Figure 4: Total yield value, total iron and calcium content, and DPPH Inhibition Based on the levels of liquid-solid ratio

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