Retraction

Retraction: Chlorophyll, polyphenol content and antioxidant activity of Moringa Oleifera Lam. leaf extract by microwave-assisted extraction method (*IOP Conf. Ser.: Mater. Sci. Eng.* 1145 012032)

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IOP Publishing regrets that our usual quality checks did not identify these issues before publication, and have since put additional measures in place to try to prevent these issues from reoccurring. IOP Publishing wishes to credit anonymous whistleblowers and the Problematic Paper Screener [1] for bringing some of the above issues to our attention, prompting us to investigate further.

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Chlorophyll, polyphenol content and antioxidant activity of Moringa Oleifera Lam. leaf extract by microwave-assisted extraction method

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Abstract. Moringa oleifera Lam. is a leafy plant that contains many nutrients and bioactive compounds such as chlorophyll, polyphenol with antioxidant activity. The microwave-assisted extraction (MAE) method is a method that has the advantages of short extraction time, saving organic solvents and safe use. The objective of this study was to investigate the influence conditions of the microwave-assisted extraction on the chlorophyll, polyphenol content and antioxidant activity of moringa leaves. The highest contents of these compounds in Moringa oleifera Lam. leaves were obtained as follows chlorophyll content 12.72 ± 0.17 μg/mL, polyphenol content 5.4 ± 0.09 g/L and antioxidant activity 2895.40 ± 67.38 μmol/L when extracted under conditions of solvent acetone 90°, solid to solvent ratio of 1:30, microwave power of 600W, microwave-assisted extraction time of 2 minutes.

Keywords: Moringa oleifera Lam., microwave-assisted extraction, chlorophyll, polyphenol, DPPH, radical-scavenging activity

1. Introduction
Moringa has 13 species, of which the genus Moringa Adans is one of the most diverse angiosperms in the Moringa family. Moringa has a change from the form of a ‘bottle trees’ to a small shrub, radial symmetrical flowers to two-sided symmetrical flowers. M. Oleifera, also called Moringa Pterygosperma Gaertn, is a species used for research or breeding [1]. Indigenous knowledge and usage of the Moringa beam are referenced in more than 80 countries including Pakistan and are known for over 200 different local languages. Moringa has been used by different countries (Asia and Southeast Asia [2], the Romans, Greece, Egypt, India with a few mentions) for thousands of years with works dating back to 150 BC [3].

Moringa oleifera is a kind of wealth nutrition plant with many nutrients in leaf, bark and seeds. Moringa has 7 times more vitamin C than oranges, 10 times more vitamin A than carrots, 17 times more calcium than milk, 9 time more protein than yogurt and 15 times more potassium than bananas [4]. Traditional medicine can be made from moringa nuts, leaves, oil, resin, bark, roots and flowers[5]. Moringa leaves also contain the good balance of nutrients of vitamins, minerals, fatty acids and amino
acids [6]. In addition, leaves have been reported to comprise antioxidant compounds that are phenolics, carotenoids, ascorbic acid, and flavonoids [7]. Razis et al. concluded that moringa has many nutritional values for human health shown in its balanced nutritional composition as well as antioxidant and antibacterial properties [8]. Calcium that is an important mineral for human growth found in moringa. In addition, Moringa is considered a valuable food that can be used to treat more than 300 diseases [9]. Calcium, potassium, zinc, magnesium, iron and copper are also minerals found in Moringa [10].

Chlorophyll, the most abundant pigment on earth, is a major component of photosynthesis [11]. Chlorophyll is the only green pigment and is present in various kinds of trees, seaweed and cyanophyta [12]. Chlorophyll is composed of two atoms that are carbon and nitrogen, and it has a magnesium ion in the core [13-16].

The two green components are called chlorophyll α and β but nowadays they are called chlorophyll a and chlorophyll b. Several researchers have studied the speed and level of chlorophyll variation. Chlorophyll stability is influenced by temperature and pH scale where the higher pH the more stability [17]. If chlorophyll is lost, it will bring brown tempera that are left behind, unattractive colour [18]. Chlorophyll that may exceed 1000 to 2000 ppm wet weight in some species is the most common of all natural pigments [19]. Traditional medicine uses chlorophyll and its derivatives for therapeutic purposes [20]. Considering the main role of chlorophyll in photosynthesis and its intimate relationships with the famous yellow/orange carotene pigments that are biologically active, these blue pigments are often overlooked. Potential physiological actions and their role in preventing chronic disease [21].

2. Materials and methods

2.1. Moringa leaf powder preparation

Moringa trees were grown in Tien Giang province, Vietnam. The preliminary process was conducted as follows:

Moringa plant → harvest → rinse → dry → grind → sieve → moringa leaf powder.

Moringa leaf powder was stored at 4°C and used throughout the experiment.

2.2. Chemicals and reagents

Folin-Ciocalteu reagent and DPPH (2,2-diphenyl-1-picrylhydrazyl) were derived from Merck (Germany), acid galic and Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid) reagent were bought from Sigma-Aldrich (USA). Acetone, methanol, ethanol, Na2CO3, distilled water was originated from China, and all chemicals met analytical standards.

2.3. Extraction process

2 g of dried moringa leaf powder were extracted under surveyed conditions for each parameter of the experiment. Solvents (ethanol, methanol, acetone), material-solvent ratios (1:15, 1:30, 1:45), microwave powers (100W - 800W), microwave-assisted extraction times (30 seconds - 4 minutes) were researched factors. Extracts were dissolved with the solvent to volume of 100 mL and clarified by vacuum filtration. The filtered solution was then analyzed for chlorophyll, polyphenol content and DPPH radical-scavenging activity.

2.3.1. Determination of total polyphenol content (TPC). The total polyphenol content (TPC) was calculated by using a modification of the Folin-Ciocalteau method described by Torres [22]. Put 1 mL of diluted extract into a testtube, then add 1 mL of Folin-Ciocalteau reagent and place in the dark for 5 minutes, then 1 mL of Na2CO3 20% was added. At 765 nm, the absorbance was measured after putting in 30 minutes in dark place. The outcome was interpreted to mg acid Gallic equivalent per volume (mgGAE/L), the measurement unit of polyphenol.
2.3.2. Determination of chlorophyll content. 662 - 665 nm is the maximum wavelength and 645 - 652 nm is the minimum wavelength that were used to measure and determine chlorophyll content. The chlorophyll content when extracted with acetone 90° and methanol 100° was calculated according to Raymond J. Ritchie, 2006 [23] and when extracted with ethanol 96°, chlorophyll content was calculated according to Joanna Fabrowska et al., 2017 [24-28].

2.3.3. Determination of the antioxidant activity (DPPH). The moringa extract has antioxidant compounds that has the ability to scavenge free radicals so moringa extract discolors purple in DPPH solution. The method of determination of resistance to oxidation is based on Brand-Williams method [29]. After making the extract weaker by adding suitable solvent, 0.2 mL of mixture was dissolved with 3 ml DPPH solution, then putting in dark place for 30 minutes. The solution was poured into cuvet and quantified at 515 nm, the absorbance of DPPH. The outcome was interpreted as μmol Trolox equivalent per volume of the sample (μmolTE/L).

2.3.4. Data analysis. Each experiment was carried out 3 replicates, results are shown as mean ± standard deviation (SD). Data was analyzed by IBM SPSS Statistics 20 software. ANOVA variance analysis and Duncan test were applied to conclude about the difference between the mean of all treatments.

3. Results and discussion

3.1. Affection of solvent type on chlorophyll and polyphenol extraction of moringa leaf powder

Each type of solvent has a different polarity from those of other solvents, so the solvent directly affects the diffusion of dissolved substances in the material.

Figure 1. Chlorophyll, polyphenol content and DPPH antioxidant activity of moringa leaves when extracted with different solvents.

Chlorophyll a (9.33 ± 0.15 μg/mL), chlorophyll b (3.28 ± 0.09 μg/mL), total chlorophyll (12.57 ± 0.28 μg/mL), polyphenol content (5.24 ± 0.5 gGAE/L) and DPPH radical scavenging activity (2707.12 ± 67.5 (μmolTE/L) are these highest values shown in figure 1 when moringa leaf powder was extracted with acetone 90°. According to the basic theory, according to the dielectric constant of a solvent, solvents can be divided into polar and non-polar solvent [30]. Water, acetone, ethanol and methanol are all highly polar solvents, so the fact that they can work together. On the other hand, the polarization and dielectric constant of solvent increase rapidly when a quantity of water is added to the solvent. Adding water to different types of polar solvents causes different extracted efficiency. In addition, if individual solvents are used, the extraction efficiency is less effective than combined solvent systems [31,32]. Therefore, a mixture of acetone and water at the concentration of 90 is the best extraction solvent to obtain polyphenol, chlorophyll, DPPH. This result is also consistent with
research of R. J. Ritchie in natural assemblages of aquatic plants, acetone (90°) produces the highest chlorophyll content in comparison to ones of ethanol and methanol [33].

### 3.2. Affection of microwave power on chlorophyll and polyphenol extraction of moringa leaf powder

The power of microwave is one of important factors that attain the extraction effectiveness, because it involves the movement of molecules in the solvent.

![Figure 2](image)

**Figure 2.** Chlorophyll, polyphenol content and DPPH antioxidant activity of moringa leaves when extracted with different powers of a microwave oven.

Chlorophyll a (9.11 ± 0.16 μg/mL), chlorophyll b (3.85 ± 0.17 μg/mL), total chlorophyll (12.83 ± 0.26 μg/mL), polyphenol content (5.06 ± 0.12 gGAE/L) and DPPH radical scavenging activity (2688.28 ± 45.2 (μmolTE/L) are these highest values shown in figure 2 when moringa leaf powder was extracted with microwave power of 600W. In microwave extraction, according to the authors Vivekananda Mandal and Rostagno, when the microwave power is too high (800W-1000W), the temperature rises, the heating unstable compounds will be degraded, the cell wall is broken down quickly. This will quickly generate undesirable impurities that can dissolve into the extract, resulting in low extraction efficiency. In addition, as microwave capacity increases, longer extraction times can lead to solvent loss due to evaporation, which reduces extraction efficiency [34]. If the extraction process is conducted at too low power (100W), the interaction between the particles and the molar diffusion rate of the solvent and the material is insufficient, resulting in reduced extraction efficiency. When the capacity is increased moderately (300-600W), the extraction efficiency is higher, at this time both temperature and pressure increase, making the interaction between molecules in the solvent increasing moderately, the cell membranes are broken. Diffusion rate increases, in turn it helps to dissolve desirable substances well. Therefore, choosing the right microwave capacity takes a leading role in the extraction process. This result is close to the study of Farid Dahmoune et al., the authors choose the range of microwave power of (400W-600W) to optimization the extraction of phenolic compounds from *Pistacia lentiscus* leaves.

### 3.3. Affection of solid-to-solvent ratio on chlorophyll and polyphenol extraction of moringa leaf powder

The concentration difference between solvent and material is important in extraction steps; therefore, solid-to-solvent ratio significantly affects extraction efficiency.
Chlorophyll a (9.37 ± 0.17 μg/mL), chlorophyll b (3.45 ± 0.19 μg/mL), total chlorophyll (12.9 ± 0.2 μg/mL), polyphenol content (5.26 ± 0.07 gGAE/L) and DPPH radical scavenging activity (2884.28 ± 49.65 (μmolTE/L) are these highest values shown in figure 3 when moringa leaf powder was extracted with the material-to-solvent ratio of 1:30. In microwave-assisted extraction, when the quantity of material remains constant, the solvent volume increases, the pressure and temperature acting on the sample will decrease. Since a decrease in temperature leads to a decrease in the mass transfer factor at a certain liquid / solid ratio, this decrease cannot be recompensed for by an increase in concentration [35]. The volume of solvent that is enough larger than the number of material will always increases the extraction efficiency [36]. When the material-to-solvent ratio increases to 1:45, the volume of solvent is too large, the temperature impact on the mixture decreases, so the concentration of the extracts tends to decrease. Conversely, if using too little volume of solvent, the ratio of raw material-to-solvent of 1:15, the little volume of solvent leads to poor material expansion, the concentration difference of soluble substances between raw material and solvent is poor, soluble substances in the material difficulty dissolved in the solvent, so the extraction efficiency decreases. The research of Deng Kong et al. on the extraction of flavonoids from *Cyclocaryapaliurus* also showed that the ratio of raw materials-to-solvents of 1:30 also yielded the highest levels of substances [37].

**3.4. Affection of extraction time on chlorophyll and polyphenol extraction of moringa leaf powder**

Chlorophyll a (9.14 ± 0.1 μg/mL), chlorophyll b (3.61 ± 0.17 μg/mL), total chlorophyll (12.72 ± 0.17 μg/mL), polyphenol content (5.4 ± 0.09 gGAE/L) and DPPH radical scavenging activity (2895.4...
± 67.38 (μmol/TE/L) are these highest values shown in figure 4 when moringa leaf powder was extracted with the microwave-assisted extraction of 2 minutes. Microwave-assisted extraction (MAE) has many advantages in comparison to traditional extraction methods, they are shortening extraction time, using a small volume of solvent with high extraction efficiency [38]. The microwave-assisted extraction time increases, the rupture level of the cell walls also increases, the solubility in the solvent of chlorophyll increases, so the extraction efficiency also increase. However, prolonged microwave action time will decrease the extraction yield because long microwave-assisted time leads to chlorophyll degradation or conversion. On the other hand, the chlorophyll content will easily decompose if it is stored at high temperature and long extraction time [39]. This result is similar to that of a study of F. Dahmoune when extracting phenolic compounds from pistachio leaves (Pistacia lentiscus), microwave-assisted extraction time of 2 minutes brings the highest extraction yield [40].

4. Conclusion
Moringa is a popular ingredient and has many nutritional benefits for human health. Polyphenol and chlorophyll were two obtained components supported by microwave in extraction. The experimental data indicated that the contents of polyphenol and chlorophyll reached the highest values of 5.4 gGAE/L and 12.72 µg/mL, respectively. To achieve the highest values and antioxidant ability of 2895.4 µmol/TE/L, the selected extraction parameters were acetone 90°, microwave capacity of 600W, the moringa powder-to-acetone ratio of 1:30 and the extraction time with microwave of 2 minutes. This research was supported in terms of facilities by Nguyen Tat Thanh University, Vietnam.

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