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High Flavonoid and Protein Content Obtained from Moringa Oleifera Lam Leaf Extracted via Ultrasound-Assisted Extraction (UAE) Method

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KEYWORDS
Antioxidant; *Moringa oleifera* leaves; UAE; Water extract.

ABSTRACT:

Introduction: Moringa oleifera (MO) leaves are known for their rich nutritional content, including high levels of vitamin C, A, potassium, calcium, iron, and protein, making them a promising plant protein source.

Objectives: The study aimed to extract flavonoids and proteins from MO leaf powder using Ultrasound-Assisted Extraction (UAE) with water as the solvent, and evaluate the extraction method's quality, identify amino acids and antioxidant compounds, and assess its toxicity to HepG2 cells.

Methods: The MO leaves were sourced from East Nusa Tenggara, Indonesia, dried, and extracted using UAE at 40°C and 40 kHz for different durations. The 15-minute extraction yielded the highest protein content, reaching 40%, and essential and non-essential amino acids were identified.

Results: The water extract met safety standards, free from microbial contamination, aflatoxins, abnormal metal levels, and had appropriate water and ethanol content. The major compounds in the extract were kaempferol-3-O-rutinoside, kaempferol-7-O-α-L-rhamnoside, phenylpropionic acid, valine, and tryptophan. Antioxidant testing resulted in an IC50 value of 38.2 ppm, and cytotoxicity testing on HepG2 cells showed a CC50 value of 124 ppm.

Conclusions: This study successfully extracted high-quality water extract (WEMO) from MO leaves using UAE, meeting nutritional standards with potent antioxidant properties and safety for HepG2 cells.

1. Introduction

Moringa, also known as "kelor," has garnered significant attention from researchers due to its abundance of bioactive components and nutritional content. Various parts of the Moringa plant (leaves, roots, stems, seeds, flowers) contain high levels of vitamin C, vitamin A, potassium, calcium, iron, and protein **[1,2]**. Moringa leaves also known to contain phytochemicals such as carotenoids, flavonoids, alkaloids, and amino acids. Due to its rich content, including flavonoids, beta-carotene, and vitamin C, Moringa leaves provide robust defense

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against oxidative damage to lipids, proteins, and nucleic acids caused by free radicals **[3–6]**.

Flavonoid content with high antioxidant activity reported in Moringa leaves includes quercetin, isoquercetin, kaemfericitin, isotiosianat, gallic acid, chlorogenic acid, elegic acid, ferulic acid, kaempferol, proanthocyanidins, and vanillin **[7,8]**. Kaempferol-O-glycosides, quercetin-O-glycosides, and isorhamnetin-O-glycoside are the most commonly found flavonoids in Moringa leaves **[9]**. Isolated kaempferol compounds from Moringa leaves have demonstrated various pharmacological activities, such as inhibiting metalloproteinase activity in in silico studies, potential antivenom effects in in vivo experiments **[10]**, and the ability to enhance lipolysis and suppress adipogenesis in in vitro assays **[5]**.

Apart from its high antioxidant content, Moringa leaves are also reported to contain significant protein content as a source of nutrition. Previous studies have reported protein content in Moringa leaves, with a focus on fresh leaves, Moringa leaf powder, and Moringa seeds. Limited research has explored the protein content in Moringa leaf extracts. Teixeira et al. reported that Moringa leaf powder contains 28.7% crude protein **[11]**, while Dhakad et al. found that Moringa leaves contain 22.9% high-quality protein with a balanced essential amino acid composition **[12]**. Plant-based proteins are known to be trapped within plant cells, necessitating an extraction method to efficiently extract proteins.

The extraction method and type of solvent used significantly influence the quality of flavonoids and the amount of protein that can be extracted from plant cells **[13,14]**. Conventional extraction techniques such as heating, boiling, reflux, and Soxhlet extraction have been used to extract flavonoids and proteins from plant materials. However, these procedures have drawbacks, including high solvent consumption and long extraction times **[15–17]**. In search of more environmentally friendly methods that reduce solvent consumption, shorten extraction times, increase extraction yields, and improve extract quality, several new extraction techniques such as ultrasound, microwave, microwave-assisted extraction (MAE), and high-pressure extraction have been developed **[18]**. Previous studies have shown that UAE can produce much higher concentrations of phenolic compounds in peach and pumpkin **[19]**, flavonoid compounds from *Rosmarinus officinalis* **[20]**, *Moringa stenopetala* Leaves **[21]**, and higher antioxidant activity in *Origanum mayorana* **[22]**, *Moringa stenopetala* Leaves compared to conventional extraction methods (maceration) **[21]**.

Previous studies have also reported the optimization of protein extraction from plants using the UAE method, including *Spirulina platensis* **[23]**, black soybeans (Aquasoya) **[24]**, and green tea leaves **[25]**. The application of UAE in extracting proteins and bioactive compounds from Moringa leaves may be an essential technique for efficiently extracting proteins and bioactive compounds **[26]**. However, no research has been conducted to analyze the effect of UAE extraction on protein and flavonoid content in Moringa leaf powder.

2. Objectives

Our study aims to develop the application of UAE to extract high levels of protein and flavonoids from Moringa leaf powder using water as the solvent (WEMO). We will test the bioactive components, extract quality/safety standards, amino acid content, antioxidant activity, and cytotoxicity of the water extract from Moringa leaves.

3. Methods

Sampel collection

Moringa oleifera leaves were harvested from trees in the Kupang district of East Nusa Tenggara, Indonesia, located at 10°04'22.98" S and 123°51'52.65" E, at an altitude of 25 meters above sea level. The Moringa leaves were collected from local residents' yards and were not specifically cultivated. The harvesting process adhered to the International Union for Conservation of Nature (IUCN) Statement on Research Involving Species at Risk of Extinction, and responsible collection practices were followed. Identification was carried out using references and comparisons with herbarium and plant collections at the Department of Biology, Faculty of Mathematics and Natural Sciences, University of Indonesia (Depokensis Herbarium, Biota Collection Room, University of Indonesia) with certificate number 851/UN2.F3.II/PDP.02.00/2021 and the Scientific Collection Management Directorate of BRIN (Bogoriense Herbarium) with certificate number B-1681/II.6.2/DI.05.07/6/2022.

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Sample preparation

Fresh Moringa leaves (1 kg) were thoroughly cleaned and dried for 24 hours in an oven (UN110 Universal oven, Memmert®) at 40°C. From the dried leaves, 500 g was obtained and finely ground into a powder using a grinding knife, resulting in particles of 100 mesh size. The Moringa leaf powder obtained was carefully stored in a sealed container to maintain its quality.

Ultrasonic assisted extraction (UAE)

Twenty grams of dried Moringa leaf powder were placed in an Erlenmeyer flask and extracted using water as the solvent with an Ultrasonic H-D Ultra Selecta® device operating at a 40 kHz ultrasonic wave frequency and a temperature of 40°C for 15, 45, and 60 minutes. The solid-to-solvent ratio was 1:10 (w/v). The entire filtrate obtained through Whatman No. 1 filter paper filtration was subjected to evaporation using a Buchi® R-215 Rotary Evaporator with B-491 Heating Bath, V700 Vacuum Pump, V850 Vacuum Controller, and F-100 Chiller. Subsequently, freeze-drying (Hypercool® Cooling Trap HC3110) was performed at -110°C and a pressure of 1.3x10-1 Pa for 24 to 48 hours to obtain dry extracts. The dry extract was weighed and stored in light-protected packaging at 4°C.

Protein content determination and WEMO characterization

Proximate analysis, protein content determination, amino acid content, and extract standardization were conducted on WEMO. All tests were performed at the PT. Saraswanti Indo Genetech laboratory in Jakarta (accredited by the National Accreditation Committee KAN with License Number LP-184-IDN). Extract characterization parameters followed the regulations of the Indonesian Food and Drug Authority (BPOM) No. 32 of 2019 concerning the Safety and Quality Requirements for Traditional Medicines.

Phytic acid content

Phytic acid examination was carried out on the Moringa leaf powder and WEMO. The Davies and Reid method was used to determine the phytic acid content 27. One gram of the sample was suspended in 50 mL of 0.5 M HNO3 solution. Phytic acid content determination was carried out as follows: 0.5 mL of the filtrate was added to 0.9 mL of 0.5 M HNO3 and 1 mL of 0.3 mM FeCl3, then immersed in boiling water for 20 minutes. After cooling, 5 mL of amyl alcohol and 1 mL of 0.1 mM ammonium thiocyanate solution were added, and the sample was centrifuged at 1500 rpm for 10 minutes. The absorbance of the amyl alcohol layer was measured using a spectrophotometer at a wavelength of 465 nm with amyl alcohol as the blank, 15 minutes after the addition of ammonium thiocyanate.

Phytochemical analysis

The identification of phytochemical components in WEMO was performed using LC-MS/MS in positive mode. The extract was dissolved in water, with an injection volume of 1 µL. The ESI mode was set to full scan m/z from 100-1200. The mobile phase was a mixture of H2O + 0.1% formic acid and acetonitrile + 0.1% formic acid. The analysis was conducted using a Waters TM Acquity UPLC-1 Class and XEVO G2-XS QTof mass spectrometer (Shimadzu LCMS-8060) with an ACQUITY UPLCTM BEH C18 column (1.7 µm, 2.1 x 50 mm), and mass spectrometry TOF XEVO G2-XS (Shimadzu LCMS-8060).

Antioxidant activity determined by DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) radical assay (DPPH Test)

The antioxidant activity of WEMO was assessed using the DPPH assay following Desmarchelier and Rahman's methods. Extracts at various concentrations (12.5–150 g/mL) were mixed with 2.4 mL of 0.1 mM DPPH. The mixture was vortexed and kept in a dark room at room temperature for 30 minutes. Absorbance was measured by spectrophotometry at 517 nm, with butylhydroxytoluene (BHT) as the reference compound.

Citoxicity activity determined by MTT assay

The MTT (3-(4,5-dimethylthiazolyl-2) -2,5 diphenyltetrazolium bromide) assay followed the procedures of Barhoi D **[28]** and Abd Rabou AA **[29]** with modifications. HepG2 cells were cultured in complete media consisting of RPMI medium, 10% FBS, 1% streptomycin-penicillin as antibiotics, and amphotericin B as an antifungal agent. HepG2 cells were incubated in a 5% CO2 incubator and observed every 2-3 days. When the HepG2 cell confluence line was reached, the cells were harvested chemically and physically by adding 0.25% trypsin EDTA and slight agitation. The cells were then incubated in a 5% CO2

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incubator for 3-5 minutes. A total of 10,000 cells were placed in a 96-well plate per well. After 24 hours, if the cells had grown to the bottom of the well plate, the sample (N-alkylgalamide) could be added with different concentrations of Moringa leaf water extract (7.81 - 1000 µg/mL). The samples were incubated for 24 hours, followed by the addition of MTT reagent, and the absorbance was read using an ELISA reader (λ= 590 nm). A concentration-inhibition percentage curve was created, and the concentration value to inhibit 50% of free radicals (CC50) was determined.

4. Results and discussion

The production of WEMO revealed that the protein content obtained from ultrasonic-assisted extraction (UAE) for 15 minutes, 45 minutes, and 60 minutes were 40%, 39%, and 38.5%, respectively (Figure 1). We developed a UAE extraction method that we believe effectively extracts proteins bound within cells. Thus far, UAE methods have been widely developed for the extraction of chemical components from various plants. Fatima et al., 2023 reported that the protein extraction yield from MO seeds using the UAE method was 39.12% with an optimal time of 20 minutes **[30]**. Kingwascharapong et al., 2022 also reported that ultrasonic extraction can enhance protein yields by improving extraction efficiency **[31]**.

Moringa oleifera (MO) extraction

The UAE method has been developed as a novel technique with low cost, low temperature, minimal solvent requirements, environmentally friendly characteristics, short extraction time, and maximum extraction yields for bioactive compound extraction from various plant materials **[32,33]**. UAE has been widely applied for the extraction of active compounds such as hawthorn seed oil **[34]**, Chinese medicine ChanSu **[35]**, flavonoids from MO leaves, apple, onion, ascorbic acid (orange) **[36,37]**, *Artemisia sphaerocephala* **[32]**, *Nothapodytes nimmoniana* plant **[38]**, polysaccharides from Kangxian flowers **[39]**, *Acanthus ilicifolius* **[40]**, and alkaloids from *Moringa stenopetala* Leaves **[21]**.

Although conventional extraction methods (maceration, percolation, soxhlet, and stirring methods) can still extract flavonoids and proteins, as previously reported in some studies, they are known to have drawbacks, including the need for more solvents/reagents, daily solvent replacement, increased waste generation and environmental impact, longer extraction times (3-5 days), and lower final extract yields **[38]**. Our research results showed a high yield from the UAE method as the final extract product reached 20% (Figure 1).

The primary mechanism of UAE is based on the phenomena of cavitation and mechanical mixing effects. Ultrasonic energy can lead to the disruption of plant cell walls due to the implosion of cavitation bubbles on the solid matrix's surface. Subsequently, mass transfer is enhanced, allowing for greater solvent penetration into the solid matrix and increasing the contact surface area between the solid and liquid phases, thereby resulting in rapid diffusion of solutes from the solid phase to the solvent phase **[38,41,42]**.

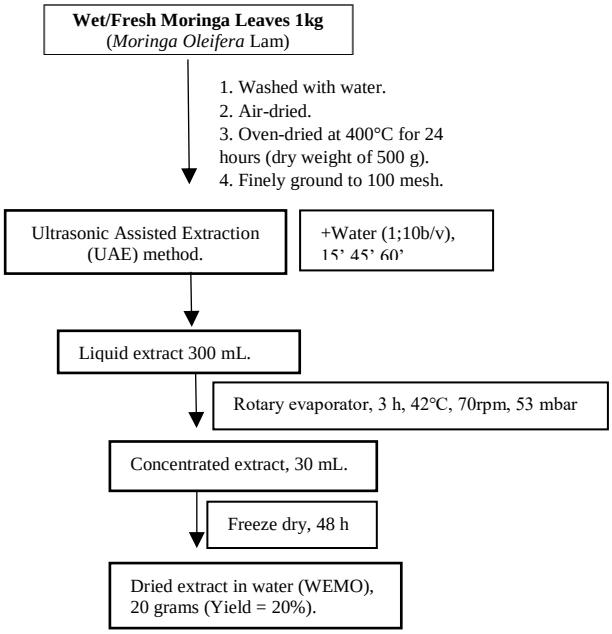

Figure 1. UAE extraction process from fresh MO leaves to dry extract.

Protein analysis, proximate, and characterization of WEMO

We selected the best WEMO (15 minutes) with the highest protein content for further analysis in this study. Both *Moringa oleifera* leaf powder and WEMO (15 minutes) underwent proximate testing to determine the comparison of protein, water, and carbohydrates content (Table 1).

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Table 1. Proximate analys is results of leaf powder and WEMO (15 minutes)

Sample	· Leaf Powder	· WEMO (15 minutes)
Moisture content	· **5,35 %**	· **4,73 %**
Ash content	· **13,82 %**	· **10,3 %,**
Carbohydrates	· **34,15 %**	· **40,56 %**
Protein	· **28,76 %**	· **39 %**
Crude fat	· **7,49 %**	· **2,8 %**
Crude fiber	· **10,94 %**	· **3,56 %**

The proximate test results above also indicate that the moisture content in both leaf powder and WEMO meets the requirements, which should not exceed 10% (Ministry of Health, Indonesia, 2017). Furthermore, the protein content in the extracted result is significantly higher, approximately 1.35 times more than in the Moringa oleifera leaf powder without extraction. This finding supports the UAE method's capability to extract proteins from *Moringa oleifera* leaf cells. Moringa leaf powder has been widely used as a raw material for making cakes, meatballs, wet noodles, cookies, milk, biscuits, etc., but with relatively low protein content. We found that the appropriate extraction process can yield higher protein content, making it a recommendation as a source of plant-based protein.

Testing is required to ensure that the WEMO (15 minutes) we produced meets quality and safety standards for herbal plant extract. It is known that herbal drug standardization includes two aspects: specific and non-specific. The specific aspect focuses on the compounds or groups of compounds responsible for pharmacological activity. Meanwhile, the non-specific aspect focuses on chemical, microbiological, water content, ethanol content, heavy metal contamination, and physical aspects that affect consumer safety and product stability **[43]**.

Characterization testing was conducted on the WEMO (15 minutes) with the highest protein content to ensure that it meets The Indonesian Food and Drug Authority (BPOM) standards including chemical, microbiological, heavy metals, water content, and ethanol content (Table 2). Our findings indicate that the WEMO we produced meets the standards set by the Regulation of the Indonesian National Agency of Drug and Food Control Number 32 of 2019. There was no detection of heavy metal contamination, microbial contamination, or aflatoxin contamination, thus making it suitable for preclinical and clinical testing as a herbal medicine product.

Table 2. Standardization examination results of WEMO

No	Parameter	Content	Quality Standard*
1	Aflatoxin G2	ND	≤ 20 µg/kg
2	Aflatoxin B2	ND	≤ 20 µg/kg
3	Aflatoxin G1	ND	≤ 20 µg/kg
4	AflatoxinB1	ND	≤ 20 µg/kg
5	Total Aflatoxin	ND	≤ 20 µg/kg
6	Moisture Content	≤10%	≤10%
7	Ethanol Content	ND	<1%
8	Total Plate Count	≤ 10^5 colony/g	≤ 10^5 colony/g
9	Yeast and Mold Count	≤ 10^3 colony/g	≤ 10^3 colony/g
10	*Escherichia coli*	≤ 10 colony/g	≤ 10 colony/g
11	*Enterobacteriaceae*	≤ 10^3 colony/g	≤ 10^3 colony/g
12	*Salmonella*sp	Negative	negative/g
13	*Shigella* sp	Negative	negative/g
14	*Clostridium* sp	Negative	negative/g
15	Arsenic (As)	ND	≤ 5 mg/kg
16	Mercury (Hg)	ND	≤ 0,5 mg/kg
17	Cadmium (Cd)	ND	≤ 0,3 mg/kg
18	Lead (Pb)	ND	≤ 10 mg/kg

ND : Not detection

*Regulation of the Indonesian National Agency of Drug and Food Control Number 32 of 2019

Amino acid and flavonoid content of WEMO

The examination of the essential and non-essential amino acid composition of WEMO was conducted to reinforce the information regarding its quality as a source of nutrition, as shown in Table 3. Eight essential amino

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acids were found, with the highest content in consecutive order (Phenylalanine, valine, leucine, threonine, histidine, isoleucine, lysine, and tryptophan). Eight non-essential amino acids were also found with the highest content in consecutive order (Glutamic acid, aspartic acid, alanine, glycine, proline, serine, arginine, and tyrosine). A complete composition of amino acids in food is crucial to meet nutritional requirements, especially the presence of essential amino acids that greatly determine nutritional quality. Previous research has reported complete amino acid contents in Moringa oleifera plants. Although they may vary in amino acid levels among different parts of the plant, our findings indicate that WEMO can be a source of plant-based amino acids.

Table 3. Amino acid content in WEMO

Amino Acid Type	WEMO content (g/kg)
*** Essential Amino Acids**	
1. Histidine	6,62
2. Isoleucine	5,68
3. Leucine	7,93
4. Lysine	3,61
5. Phenylalanine	21,03
6. Threonine	7,89
7. Tryptophan	2,1
8. Valine	12,47
**** Non-Essential Amino Acids**	
1. Glutamic acid	51,14
2. Aspartic acid	28,08
3. Arginine	4,34
4. Alanine	12,55
5. Serine	5,43
6. Proline	7,25
7. Glycine	7,93
8. Tyrosine	2,81

LC-MS/MS analysis of WEMO was conducted to identify its chemical composition. LC-MS/MS analysis of WEMO showed the presence of several bioactive chemical compounds in the chromatogram, as depicted in Figure 2 and listed in Table 4. Compounds such as kaempferol-3-O-rutinoside, kaempferol-7-O-α-L-rhamnoside, phenyl propionic acid, valine, and tryptophan were found.

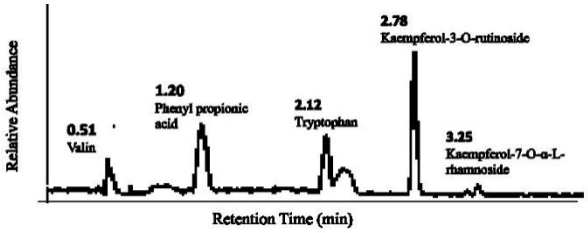
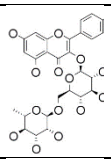
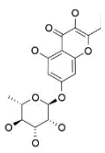
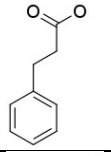
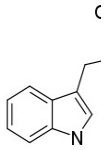
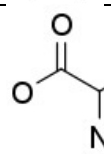


Figure 2. LC-MS/MS chromatogram showing the presence of bioactive compounds in WEMO

Two dominant flavonoid derivatives were found in our study, namely kaempferol-3-O-rutinoside (KOR) and kaempferol-7-O-α-L-rhamnoside. Both compounds have been reported to have high antioxidant, anti-inflammatory, antimicrobial, anticancer, heart disease prevention, neurological disease prevention, antidiabetic, antiosteoporotic, antiestrogenic, analgesic, and hypoallergenic activities **[44,45]**. KOR is also a potent compound that can inhibit hyaluronidase enzyme **[44]**.

Table 4. Phytochemical compounds in WEMO

No	Compound	Chemical Formula	Chemical Structure	Observed m/z	Retention Time (min)
1	Kaempferol-3-O-rutinoside	$C_{27}H_{30}O_{15}$		595.1684	2.78
2	Kaempferol-7-O-α-L-rhamnoside	$C_{21}H_{20}O_{10}$		433.1143	3.25
3	Phenyl propionic acid	$C_9H_{11}NO_2$		166.0862	1.19
4	Tryptophan	$C_{11}H_{12}N_2O_2$		205.0970	2.12
5	Valin	$C_5H_{11}NO_2$		118,0859	0,51

Furthermore, our research showed that WEMO contains two dominant water-soluble protein derivatives, valine and tryptophan, both of which are essential amino acids.

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This finding further supports the UAE extraction method's ability to extract proteins from within Moringa oleifera leaf cells. It also demonstrates that Moringa leaves have the potential to be a source of plant-based amino acids.

The presence of anti-nutritional factors in Moringa leaves is a topic that requires further study to ensure it does not hinder the absorption, digestion, and metabolism of nutrients in the body. It has been previously reported that Moringa leaves contain low levels of anti-nutritional factors such as saponins, phytates, and tannins. The phytate content previously reported was 22.3 mg/g of dry leaves **[46]**. Our research indicates that the phytic acid content in Moringa leaf powder is 1.73 g/100 g, and in the water extract, it is 0.13 g/100 g.

High antioxidant activity and low toxicity of WEMO

The antioxidant activity against DPPH free radicals from various concentrations of WEMO was compared with the standard antioxidant, ascorbic acid. The results depicted in Figure 3 show that the IC50 value of WEMO was 38.03 ppm, while ascorbic acid had an IC50 value of 1.58 ppm.

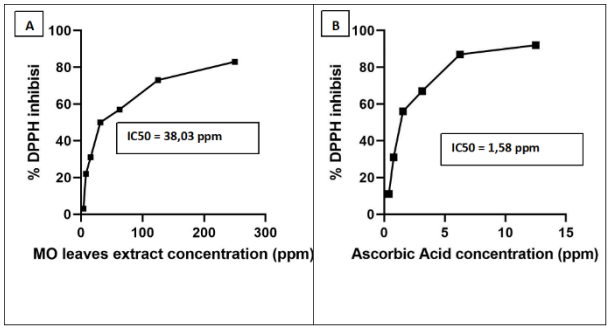

Figure 3. Antioxidant activity of A. WEMO; B. Ascorbic acid

The antioxidant activity of various parts of the Moringa oleifera plant has been reported previously, including leaves **[47–52]**, seeds **[3,49,53–55]**, and roots **[55–57]**. The evaluated compounds with antioxidant activity are polyphenols, flavonoids, phenolics, and carotenoids **[1,58]**. The UAE method has also been reported to extract antioxidant compounds. Several previous studies have reported that the UAE extraction method can yield higher antioxidant activity compared to conventional methods (maceration), including in extracts of *Moringa stenopetala* Leaves **[21]**,

One of the successfully cultivated hepatocarcinoma cells, widely used in drug metabolism research, is HepG2. HepG2 cells have the advantage of high morphological and functional differentiation and are more stable and uniform compared to primary liver cells. The CYP3A4 enzyme, which can metabolize more than 50% of drugs, can be produced through HepG2 cells in vitro **[59]**.

The in vitro toxicity test we used is an enzymatic assay (colorimetry) with the MTT assay reagent. This test is used to measure the ability of live cells based on mitochondrial activity. The MTT/toxicity test of Moringa leaf extract has been reported in various cells, including gingival fibroblast cells **[60]**, HepG2 **[61,62]**, breast cancer cells (MCF-7 **[63,64]**, T47D **[65,66]**, MDA-MB-231 **[67]**), colorectal cancer cells (HCT-116 **[63,68]**, Colo-205 **[65]**, Caco2 **[63]**), and leukemia cells (THP-1, HL **[60]**, K562 **[65]**, EAC **[61]**).

Cytotoxicity testing was conducted with eight series of WEMO concentrations (0–1000 ppm) using a 1% DMSO solvent. DMSO is a (semi-polar) compound that is not toxic and does not exert any activity on the dissolved substance **[69]**. Cell death is characterized by cell shrinkage, chromatin condensation, abnormal plasma membrane, and cells becoming circular. Cell death was predominantly observed at concentrations of 1000 ppm, 500 ppm, and 250 ppm. Concentrations of 15.62 ppm and 7.81 ppm showed minimal cell death. The cytotoxicity test of WEMO shows dose-dependent behavior, meaning cell viability decreases with increasing extract concentration.

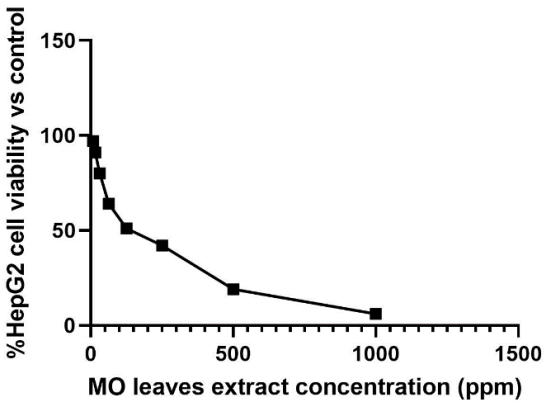

Figure 4. Depicts the cytotoxic activity of WEMO on HepG2 cells.

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The tested WEMO had a CC50 value of 124.59 ppm (Figure 4). Our study results indicate that the selectivity index value (CC_{50}/IC_{50}) of WEMO is 3.27. This value means that the CC_{50} value is three times higher than the antioxidant dose on HepG2 cells. Meanwhile, the IC_{50} value falls into the low inhibitory category (IC_{50} >100 - 1000 µg/mL). According to the US National Cancer Institute (NCI), the toxicity level of a compound is classified into four categories: high cytotoxic activity (IC_{50} <20 µg/mL), moderate activity (IC_{50} 21-200 µg/mL), low activity (IC_{50} >200 - 1000 µg/mL), and no cytotoxic activity (IC_{50} > 1000 µg/mL) **[70]**. However, some studies use different IC_{50} values for categorizing the toxicity level of an extract: highly active ($IC_{50} \leq 20$ µg/mL), moderately active (IC_{50} > 20 - 100 µg/mL), weakly active (IC_{50} >100 - 1000 µg/mL) and inactive (IC_{50} > 1000 µg/mL) **[71,72]**. This indicates that the WEMO we produced has low toxicity towards HepG2 cells

Conclusion

The UAE method on WEMO successfully obtained an extract containing high levels of protein (valine, tryptophan) and flavonoids (Kaempferol-3-O-rutinoside (KOR) and kaempferol-7-O-α-L-rhamnoside) and met the standard characterization requirements for medicinal plant materials. WEMO also demonstrated antioxidant effects with a high selectivity index.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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