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Research Article

Evaluation of quality parameters and antioxidant activity of commercial melinjo (*Gnetum gnemon*) for functional food

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ABSTRACT

Functional foods are ingredients which provide health benefits. *Gnetum gnemon* Linn (melinjo) seeds contain bioactive compounds with extremely high antioxidant activity and are considered to have the potential to be used as raw materials for functional food. The utilization of melinjo seeds as functional food ingredients must consider the general and specific quality standards to ensure the generated products deliver the intended health benefits. The purpose of this study was to determine the specific and nonspecific quality parameters of commercial melinjo products in the form of extracts and flour as well as evaluate its antioxidant capacity as functional food raw materials. The nonspecific parameters evaluated include drying loss, microbial contamination, moisture, total ash, insoluble acid ash, extract yield, fat, and crude fiber. The specific parameters tested were phenolic and flavonoid content, water- and ethanol-soluble compounds, and marker compounds. The results showed that the nonspecific parameter values extract and flour of melinjo seed met the standard's provisions. Analysis of specific parameters showed that the extract was dark brown in the form of a thick paste, while flour was light brown powdery. Both have a distinctive odor with a slightly bitter taste. The phenolic and flavonoid content of the extract was higher than that of the flour. To the contrary, the water- and ethanol-soluble compounds of the extract were lower than that of the flour. Resveratrol, one of the marker compounds in melinjo seeds, was identified by TLC and HPLC. The melinjo seed extract was found to have strong antioxidant activity with IC50 value of 1.45 mg/ml. On the basis of the nonspecific and specific parameter values together with the antioxidant activity it was concluded that the melinjo seed extract has high potential to be used as a raw material for functional food.

Keywords:
Gnetum gnemon, Functional food, Antioxidant, Phenolic, Resveratrol

1. INTRODUCTION

Gnetum gnemon L. is a plant from the Gnetaceae family spread in various tropical regions. In some countries, this plant is well known as "belinjau" or "bago" in Malaysia, "melinjo" or "belinjo" in Indonesia, "melindjo" in Singapore, "rau danh" in Vietnam, and Thai people call it "pee sae" or "phakmiang"[1-2]. In Indonesia, this plant is cultivated because it has economic value. The leaves, flowers, and seeds of this plant are usually used as food. One of the popular foods is a soup made from the leaves and skin of the melinjo fruit. In addition, melinjo seeds that have been heated are opened. The endosperm is then ground to make crackers or, in Indonesia, called "emping melinjo", a snack often found in traditional markets. These crackers have a slightly bitter taste which is the typical

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taste of melinjo seeds[3]. Meanwhile, in the Philippines, melinjo bean powder is used as an alternative coffee substitute[4]. On the other hand, melinjo seed extract has been commercially used as a dietary supplement in Japan[5]. In addition, behind its popularity, melinjo also has many pharmacological activities. Wazir et al. reported that the leaves, seeds, twigs, and bark of melinjo have antioxidant activity[6]. Fruit peel and leaf extracts from melinjo have anti-microbial[7], uric acid-lowering[8], antimalarial[9], and antidiabetic activities[10-11].

As a plant that produces secondary metabolites, the pharmacological activity of melinjo cannot be separated from its chemical properties. Several bioactive compounds are found in *Gnetum gnemon*, such as saponin, tannin, and flavonoids[12-13]. Moreover, melinjo seeds are rich in stilbenoid dimers such as gnetin C and glucosides (gnemonosides A, C, and D), and a small amount of trans-picked glucosides such as trans-resveratrol (3,5,4'-trihydroxy-trans-stilbene)[3,8,14]. In addition, stilbene derivatives (isorhapontigenin, resveratrol, gnetin D, gnetifolin K, gnetol) and one lignan compound ((+)- lariciresinol B) have been isolated from the fruit-bark of melinjo[1]. Gnemonols K and L (resveratrol trimers), M (isorhapontigenin dimer), and gnemonoside K (glucoside of resveratrol trimer) have been isolated from the root of *Gnetum gnemon* by acetone, methanol and 70% methanol[15]. A novel phenylheptanoid, named gnetumal, was recently identified from the leave of this plant. The compound was shown to have tyrosinase inhibitory activity[16]. MicroRNA analysis of *G. gnemon* indicated that this plant has the potential to be used as a small-RNA based treatment for particular human diseases[17].

Extract of melinjo seed has been reported to have broad biological activity. Ethanol extract has anti-oxidant and radical scavenging capacity equivalent to ascorbic acid and alpha-tocopherol[3,10,18]. Extract of melinjo seed can also inhibit lipase and alpha-amylase activity and shows inhibitory activity against food microorganisms[3]. Furthermore, extract of melinjo seed can inhibit angiogenesis[19], enhance immune response[13], inhibit angiotensin-converting enzyme (ACE) as an antihypertension[20], and prevent endothelial senescence[21].

Gnetin C, the main component of melinjo seed extract, was reported to have activity in suppressing endothelial cell function related to angiogenesis and angiogenesis induced by tumour cells[19]. Gnetin C was also reported to be better than tRV (3,5,4′-trihydroxy-trans-stilbene), a stilbenoid derived from red grape skins in suppressing angiogenesis[22]. Moreover, Gnetin C significantly inhibited the proliferation of pancreatic, prostate, breast, and colon cancer cells in a clinical trial[5]. Other chemical constituents of melinjo seed extract, trans-resveratrol, improve insulin sensitivity and reduces oxidative stress in type 2 diabetic patients[23]. In addition, trans-resveratrol can increase flow-mediated dilatation in obese[24] and immunostimulatory effect[13]. Standardization of herbal medicine from melinjo is necessary given the great potential of melinjo extract as a health supplement. In addition, the efficacy, content of filler, and active compounds in the melinjo extract must meet the applicable food safety standards to ensure the safety and quality of the melinjo extract. Notably, most of the samples on the market in Indonesia come from abroad. Food stability or shelf-life refers to the term of food to resist against the physical, chemical and biological changes under specific period of time. The length of time it undergoes minimal changes or remains acceptable. Physical and chemical changes or microbial in food will cause food spoilage, deleterious effects on the flavor, color, and nutritional value of the food[25]. Moisture content is the most frequently evaluated in food products, especially functional food. Even though it is a small amount, it has a large impact on food stability. Almost all chemical reactions take place in the presence of a solvent. Therefore, chemical changes are very likely to occur when the water content in food is sufficient. In addition, moisture is critical in the fungi and bacteria growth media. High moisture content will activate the bacteria and fungi and also enzyme that lead to spoilage[26]. Therefore, the purpose of this study was to examine the quality parameters and antioxidant activity of commercial melinjo seed extract and flour as a potential source of raw material for functional food. In addition, the commecial melinjo products are widely used for making various types of snacks in Indonesia.

2. MATERIALS AND METHODS

2.1. Materials

Materials used in the present study include: commercial melinjo products (GNR and MR), 70% ethanol, Whatman filter paper, distilled water, Folin Ciocalteu reagent, gallic acid, sodium carbonate, quercetin, 96% ethanol, aluminium chloride solution, sodium acetate, TLC plate with silica gel 60 F245, chloroform, methanol, resveratrol as standard, acetonitrile, trifluoroacetic acid (TFA), methanol, DPPH solution, ascorbic acid, potassium persulfate powder, ethanol p.a, Trolox, ABTS solution, 1% NaOH, H_2SO_4, and petroleum benzene.

2.1.1. Sample Preparation and Pre-processing

Extract (GNR) and flour (MR) of melinjo seeds (*Gnetum gnemon*), produced by PT Kalbe Farma, Jakarta, Indonesia, were obtained commercially. Before testing, the sample underwent a pre-processing stage to minimize the sample's filler material (excipient). In the early stages, melinjo seed samples (GNR and MR) were extracted using ethanol through maceration. As much as 30 g of melinjo sample was added to 300 mL of 70% ethanol. The mixture was soaked for 4 hours with stirring at 30°C. After that, the mixture was allowed to settle for one hour

and filtered. The sample precipitate was separated. The ethanol extract was then evaporated at room temperature to obtain melinjo seed concentrate without losing the essential chemicals. The concentrated extract was stored in a closed dark container and protected from direct sunlight.

2.1.2. Determination of Extract Nonspecific Parameters

Extract standardization parameters were determined through specific and nonspecific approaches. Determination of nonspecific parameters of the extract refers to the guidelines set by The Ministry of Health of the Republic of Indonesia. Nonspecific parameters include drying loss, microbial contamination, moisture content, total ash content, acid-insoluble ash content, extraction yield, fat content, and crude fiber. The drying loss was measured by weighing 1-2 g of sample followed by a drying process at 105°C for 30 min. Every hour the weight of the sample was measured until constant weight was achieved[27]. Microbial contamination was tested using the standard agar plate method[28]. Moisture content was measured by drying 10 g of sample at 105°C for 5 hours. The weight of the sample was then measured every hour until constant weight was achieved. The percentage of water content was then calculated based on the weight loss during drying[27]. The total ash content was measured by weighing 2-3 g of sample. The sample was dried and then burn at 800°C until constant weight was achieved and total ash content was calculated[27]. Acid insoluble ash content was measured by dissolving the ash obtained from ash content test in 25 mL of diluted sulfuric acid. The undissolved part was collected, filtered, and burn at 800°C until constant weight was achieved. Acid insoluble ash content was then calculated[27]. The extraction yield was calculated as a percentage of extract weight against sample weight[27]. Fat content was determined by using the Micro Soxhlet method. As much as 1-2 g of sample was placed in the Micro Soxhlet. Following solvent extraction, the weight of the fat recovered was measured[27]. Crude fiber content was measured by using the gravimetric method. Briefly, as much as 2-4 g of sample was acid-digested using 200 mL of 25% of sulfuric acid. After acid removal, alkali digestion was carried out by adding 200 mL of 1.25% sodium hydroxide. The crude fiber content was expressed as the percentage of the weight of undigestible components of the sample[27].

2.1.3. Determination of Extract Specific Parameters

The specific parameter measurement consisted of extract identity, organoleptic evaluation (shape, odor, taste, and color), total of water-soluble extractive value, total ethanol-soluble extractive value, and phytochemical compounds. Water-soluble extractive value was determined by macerating with shaking of 5 g sample with 100 mL of chloroform watery in a closed flask for six hours and then allowing standing for eighteen hours. The suspension was filtered. As much as 20 mL filtrate was evaporated until dry at 105°C until constant weight was achieved. The percentage of water-soluble extractive was then calculated[27]. Similarly, the ethanol-soluble extractive value was determined by macerating with shaking of 5 g sample with 100 mL of 96% ethanol in a closed flask for six hours and then allowing standing for eighteen hours. The suspension was filtered. As much as 20 mL filtrate was evaporated until dry at 105°C until constant weight was achieved. The percentage of ethanol-soluble extractive was then calculated[27]. Determination of phytochemical compounds was carried out by quantitative tests of total phenolic content (TPC) and total flavonoid content (TFC), then identification of marker compounds by thin-layer chromatography (TLC) and quantification of compounds by High-performance liquid chromatography (HPLC).

2.1.4. Analysis of Total Phenolic Content

The total phenolic content (TPC) of the extract was tested based on the method of Singleton et al. (1999)[29]. The extract was dissolved in distilled water and made at a concentration of 10,000 mg/ml As much as 50 µl of the extract was added with 50 µl of distilled water. The solution was then added with 50 µl of the 10% Foline-Ciocalteu reagent and 50 µl of the bicarbonate solution (60 g/L). The mixtures (in microplates) were then incubated at room temperature for 60 minutes. The absorbance was read with a spectrophotometer at a wavelength of 750 nm. The gallic acid standard was made by dissolving gallic acid in distilled water and made at a concentration of 3.125; 6.25; 12.5; 25; 50; 100 (g/mL). Distilled water was also used as a blank. Standards and blank are tested in the same way as samples. Total phenolics are calibrated against the gallic acid standard and expressed as mg gallic acid equivalent (GAE) per gram of plant extract.

2.1.5. Analysis of Total Flavonoid Content

Total flavonoid content (TFC) was determined by aluminium chloride colorimetric assay adapted from Chang et al. (2002)[30] with slight modification. Standard solution of quercetin at concentrations of 30, 40, 50, 60, 70, 80, 90, and 100 µg/ml were prepared in 96% ethanol. As much as 50 µl of extract (1 mg/ml) or standard solution was added to 10 µl of 10% aluminium chloride solution, followed by 150 µl of 96% ethanol. As much as 10 µl of 1 M sodium acetate was added to the mixture in a 96-well plate. Ethanol (96%) was used as a reagent blank. All reagents were mixed and incubated for 40 min at room temperature, protected from light. The absorbance was measured at 415 nm with a microplate reader. Total

flavonoid contents were expressed as mg quercetin equivalents (QE) per gram of plant extract.

2.1.6. Analysis of Marker Compound

Thin-layer chromatography (TLC) was employed to analyze chemical composition and determine marker compound of melinjo seed products. The TLC used silica gel 60 F245 as the stationary phase and a mixture of chloroform:methanol (4:1 v/v) as the mobile phase. During the preparation process, resveratrol as the marker compound was diluted in acetonitrile with 0.1% trifluoroacetic acid (TFA). The melinjo seed extract (GNR) and flour (MR) were diluted in methanol total of 20 µL (1,000 ppm) of test samples and 2 µL of standard resveratrol solution were spotted on a 60 F245 silica gel plate and let dry. The TLC plate was eluted into the TLC chamber (Chamag), which the mobile phase saturated. The spots were eluted for 6 cm and then air-dried. The spots were read under UV light at 365 nm, and the retention factor (Rf) value was calculated.

Determination of the resveratrol and the phytochemical profile of the sample in melinjo extract was carried out using a reverse-phase HPLC system, with the following chromatographic setup and conditions: ODS column C-18 (4.6 mm × 250 mm × 5 µm), using methanol-water as mobile phase (30:70, v/v), injection volume 20 µL, column flow rate 0.8 mL/min, run time 45 minutes, and chromatogram were recorded using a UV detector at 300 nm. The identification of the resveratrol peak was based on the retention time of the standard/marker, and then the spectral position of the sample was confirmed concerning the marker compound[21]. The calculation method was based on a standard curve of resveratrol.

2.1.7. Determination of Antioxidant Activity

2.1.7.1. Determination with DPPH method

Samples were diluted in ethanol and prepared in various concentrations as a sample solution. 100 µL of the sample solution and 100 µL of 125 µM DPPH solution were put into a 96-well plate. Samples were incubated at room temperature for 30 min. After that, the absorbance was measured at a wavelength of 517 nm using a microplate reader. Ascorbic acid was used as a positive control. Each sample concentration and positive control was tested in triplicate[31].

2.1.7.2. Determination with ABTS method

Approximately 7.100 mg ABTS powder and 3.500 mg potassium persulfate powder were dissolved in 5 ml ethanol. The solution was incubated for 12 hours in a dark room. The two solutions were mixed, and then added ethanol to the volume of up to 25 ml. The test procedure is based on Arnao (2000)[32]. Several samples of extracts were made with concentrations of 10, 20, 30, 40, and 50 ppm. Trolox was prepared at concentrations 5, 10, 15, 20, and 25 ppm. The ABTS solution and the sample were pipette 1:1 into a 96-well transparent polystyrene microplate and then homogenized. Then the absorption of the mixture was measured with a microplate reader at a wavelength of 734 nm. Trolox as a positive control was treated the same as the sample.

2.1.8. Data Analysis

Statistical analyses were conducted using triplicate sub-samples. All results were expressed as mean±standard deviation (SD).

3. RESULTS AND DISCUSSION

Functional foods are intended to provide health benefits such as protecting against disease, preventing nutrient deficiencies and promoting proper growth and developments. The melinjo seeds have the potential to be developed as a standardized antioxidant herb with great potential as ingredients of functional food. The standardization of melinjo seed products is intended to ensure that they have both nonspecific and specific parameter values of standardized quality levels. Standard parameter refers to the general standard value of medicinal plant extract provided by the Ministry of Health of the Republic of Indonesia.

3.1. Nonspecific Parameters of Melinjo Seed Endosperm

Data of the nonspecific parameters of extract (GNR) and flour (MR) of the commercial melinjo seeds is presented in Table 1.

The values of non-specific parameters of extract and flour of melinjo seeds are presented in Table 1. It was shown that the melinjo seed extract has a lower dry loss (2.80%) than the melinjo seed flour (3.10%). Differ from water content which refers to the amount of water present in the commecial melinjo products, the dry loss or loss on drying (LOD) value include water content and other volatile substances present in the the commecial melinjo products. Dry loss value is defined as the loss of sample weight expressed as percentage w/w resulting from water and volatile matter of any kind that driven off after drying at a high temperature (105°C) until a constant weight is obtained. The dry loss value and moisture content of melinjo seed extract and flour met the referenced parameter standards, according to which, the dry loss value of the sample should not exceed 11%, and the water content should not exceed 10%. Notably, the optimal drying process must be achieved due to the sensitive materials are prone to contamination by bacteria and/or fungi. The maximum limit for microbial contamination must meet

Table 1. Standardization of nonspecific parameters of extract (GNR) and flour (MR) of industrial melinjo (*G. gnemon*) seed.

No	Non-specific Parameter	Samples		Standard of food ingredient*
		Melinjo Seed Extract (GNR)	Melinjo Seed Flour (MR)	
1	Drying loss	2.80%	3.10%	≤11%
2	Bacterial total plate number	<3000 CFU/g	<3000 CFU/g	10^4 CFU/g
3	Moisture content	3.34%	4.45%	≤10%
4	Total ash	0.93%	5.25%	16.6%
5	Total acid insoluble ash	0.24%	0.51%	≤0.7%
6	Extraction yield	29.27%	>>87%	-
7	Total fat	0.38%	0.03%	-
8	Crude fiber	0.29	0.15	-

*The second edition of the Indonesian herbal pharmacopoeia 2017

Table 2. Standardizing specific parameters of extract (GNR) and flour (MR) of industrial *G. gnemon* seeds.

No	Analysis	Results	
		Melinjo Seed Extract (GNR)	Melinjo Seed Flour (MR)
1	Organoleptic	Thick, characteristic odor, slightly bitter taste, and dark brown color	Powder, characteristic odor, slightly bitter taste, and light brown color
2	water-soluble extractive value	5.04%	78.72%
3	ethanol-soluble extractive value	38.27%	72.88%

the established standards, which should not exceed 10^4 CFU/g. The samples of both melinjo seed extract and flour were classified as safe because their bacterial total plate numbers were below the standard-setting limit of <3,000 CFU/g.

The physical properties of samples are strongly influenced by the mineral content (inorganic material). It is therefore necessary to measure the mineral content of the sample which is expressed as ash content. The melinjo seed extract and flour contained 0.93% and 5.25% total ash, respectively, indicating that they met the established standards (<16.6%). Analysis of acid-insoluble ash content was intended to determine the amount of the sandy matter and plant body parts such as calyx, leaves, etc., which contain higher content of non-combustible acid insoluble matter. According to the reference standard, the acid-insoluble ash value should not exceed 0.7%. As shown in Table 1, the values of both melinjo seed extract (0.24%) and flour (0.51 %) met the standard (Table 1).

Extract yield refers to the maximum ability of a solvent to extract the test material. The extract yield of melinjo seed extract was lower (29.27%) than that of melinjo seed flour (>> 87%). Total fat and crude fiber melinjo seed extract and flour are presented in Table 1. Overall, the test results showed that the nonspecific parameters of extract and flour of the melinjo met the required standard provisions of The Ministry of Health of the Republic of Indonesia.

3.2. Specific Parameter

Results of the specific parameter examination of extract (GNR) and flour (MR) of commercial melinjo seed are presented in Table 2.

The endosperm of *G. gnemon* seed was subjected to specific parameter tests because it was reported to have high level of resveratrol and was specified as a raw material with antioxidant activity[33]. Specific parameters examined included: sample identity (organoleptic), ethanol and water-soluble compounds, phytochemical content, resveratrol as a marker compound and other active compounds. The extract of melinjo seed was found to be thick and the flour was powdery. The extract and flour of melinjo seed have characteristic odor, slightly bitter taste, and brown color. As shown in Table 2, the water-soluble extracts of melinjo seed extract (GNR) and flour (MR) were 5.04% and 78.72%, respectively. The values of their ethanol-soluble extracts were 38.27% and 72.88%, respectively. The water-soluble extractive value is the proportion of the biomass which is lost as a result of extraction with water. Similarly, the ethanol- soluble extractive value is the proportion of the biomass which is lost following extraction with ethanol.

As shown in Figure 1, the total phenolic and flavonoid contents of melinjo seed extract (GNR) were higher than those of the flour (MR). These are due to the fact that in the extract the metabolites are more concentrated because they are already separated and released from plant matrix during the extraction process. In the flour, on the contrary, those phytochemicals are still trapped in the matrix and hence, less concentrated[34-35].

The biological activity of melinjo seed products depends on the phenolics and flavonoids content. phenolics and flavonoids contribute to the antioxidant capacity. According to Yang and team, phenolic content and antioxidant activity are important aspect as a source of functional food[36]. It was recently reported that phenolic compounds have the potential to be used to develop functional foods for obesity[37]. Better food processing techniques are essential to retain bioactive phenolic compounds[38]. Flavonoids are natural substances produced by plants with high antioxidant activity and ability

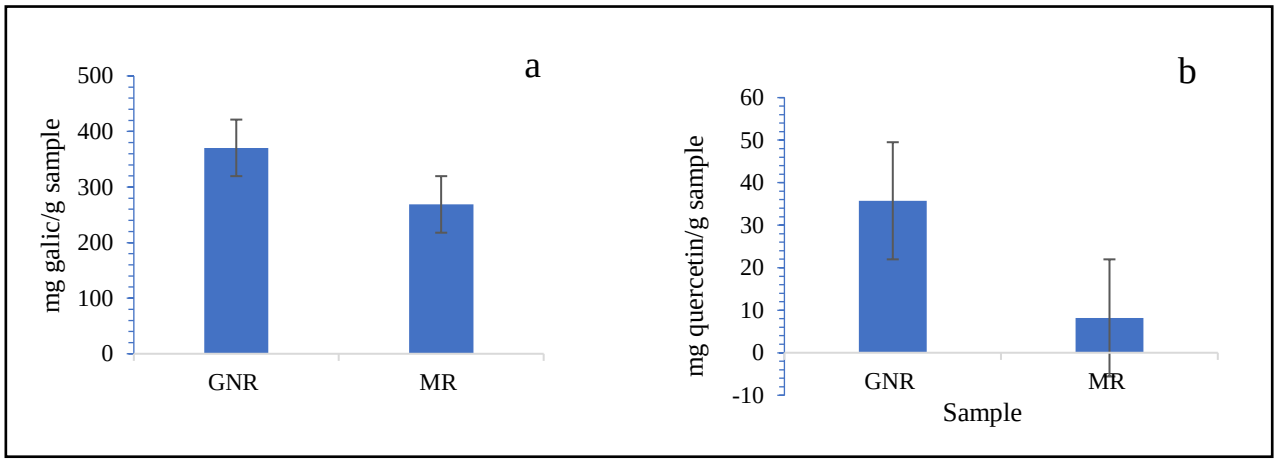


Figure 1. (a) Total phenolic of extract (GNR) and flour (MR) of commercial melinjo seed. (b) Total flavonoid of extract (GNR) and flour (MR) of commercial melinjo seed. Data are representative of values from at least three independent experiments.

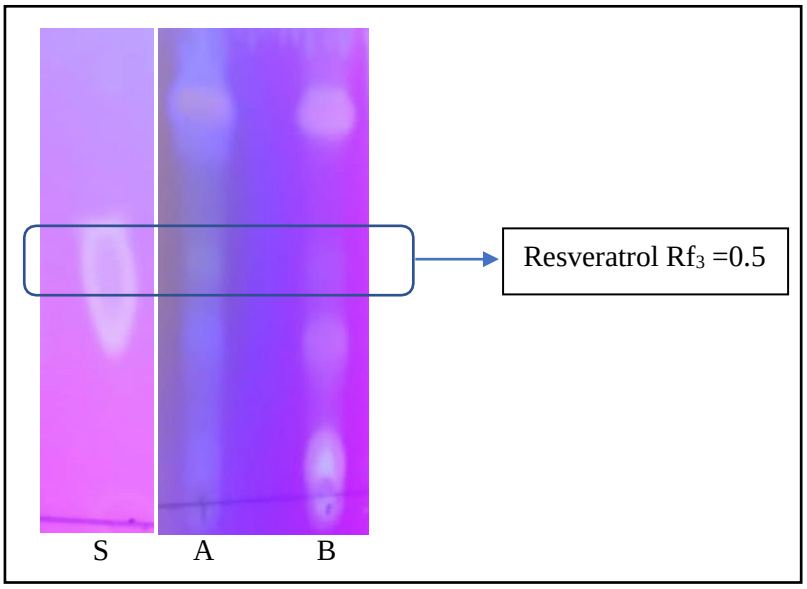


Figure 2. TLC results of resveratrol standard (left) and sample (right) under 365 nm UV light, S: resveratrol standard, A: melinjo seed extract (GNR), and B: melinjo seed flour (MR).

to reduce ROS accumulation[39]. One of the plant flavonoids, the quercetin, has recently been shown to have antiviral and immunomodulatory activities[40]. Flavonoids are a family of polyphenolic compounds. In general, polyphenol can reduce the impact of oxidation in the human body, and hence, prevent organ deterioration and maintain their proper functionality[41]. Techniques employed for extraction was found to determine the phenolic and flavonoid contents of plant extracts and their antioxidant activity[42].

3.3. Marker compound

The results of chemical content analysis using TLC are shown in Figure 2.

TLC can be used to detect nonvolatile compounds present in the sample. In this study, TLC was employed to detect the present of resveratrol as a marker compound candidate for melinjo seed products. In this context, marker compound is chemical constituent within melinjo seed products that can be used to verify its potency or identity. Melinjo seed extracts have been known to be rich is resveratrol which may function as melinjo seed marker compound. A marker compound is useful for detection of adulteration and can also be used as an indicator of product quality during manufacturing, handling, and storage. It can, therefore, be used as a quality control measure for plant mixtures, bioactivity and as a shelf-life indicator[15].

In the present study, determination of chemical content of the melinjo seed products was aimed to identify the compounds extracted from melinjo seeds. The melinjo main compound, resveratrol, was used as a marker compound in the sample assay using TLC. Results showed that resveratrol was successfully detected. However, other bioactive compounds were also detected. The sample spots on the TLC plate indicated that resveratrol had been successfully extracted into the melinjo seed extract (GNR)

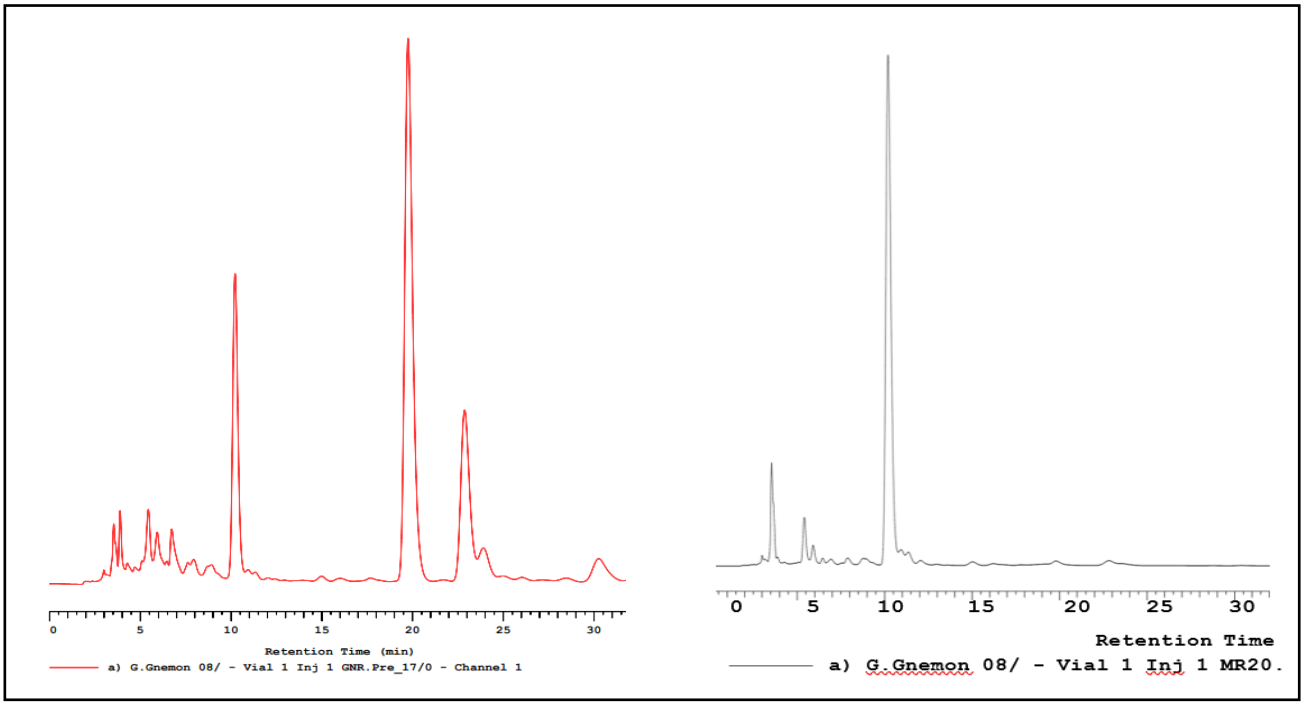


Figure 3. GNR (a) and MR (b) chromatogram profile of melinjo herb (*Gnetum gnemon* L.)

and flour (MR) (slightly faint). The resveratrol spots of the samples were detected under UV light at 365 nm with an Rf value of 0.50 cm.

Similarly, in the standard solution (resveratrol standard), the vital spots were detected on Rf=0.50 cm. The overall identification results showed that more than two active compounds had been extracted into the melinjo seed extract with Rf values (except resveratrol) were Rf1=0.15 cm, Rf2=0.35 cm, and Rf4=0.90 cm (Figure 2).

Melinjo is a plant known for its main phytochemical content, resveratrol. Resveratrol is a phenolic group that has been known to have high antioxidant activity[14-15]. In the present experiments, analysis of resveratrol content of melinjo seed products was conducted using HPLC equipped with a UV detector, and the resulting chromatogram profile of melinjo seed extract (GNR, (a)) and flour (MR, (b)) is shown in Figure 3 that indicates a different composition of each compound.

Phytochemicals contained in the melinjo seed products *Gnetum gnemon* extract separated through the chromatographic conditions are shown in Figure 4. However, some peaks could not be identified due to lack of standard compounds. The HPLC profile showed that the standard resveratrol was eluted at 19.67 minutes. At the same time, the resveratrol in the extract (GNR) and flour (MR) was eluted at 19.76 and 19.78 minutes, respectively. From the chromatograms of the standard and samples it can be seen that the resveratrol peak intensity of the GNR was higher than that of the MR which indicated that GNR is rich in resveratrol. The concentration of resveratrol in the GNR and MR calculate based on standard curve of resveratrol and it was showed at 45.10 ppm and -41.42 ppm, respectively. The total resveratrol levels from both samples are presented in Figure 5. The result seen that MR has low concentration is negative value compared than GNR. These results indicate that the hplc parameters used are less sensitive and need to be validated. The intercept obtained in the linear line equation shows a very high value. This indicates that there are still matrix disturbances in the analysis process. the analyte concentration obtained in the analysis process will be negative if the sample being analyzed has a low analyte content[43].

Resveratrol in the melinjo seed may have antidiabetic effects[44]. In addition, resveratrol has been indicated to have antiinflammatory[45], anticancer[46] and myocardial ischemia protective[47]. Microwave-assisted extraction has been shown to be an efficient technique for recovering resveratrol from food products[48]. Resveratrol food supplement products should be accurately labeled to ensure food safety for consumers[49].

3.4. Antioxidant activity

The antioxidant capacity of *Gnetum gnemon* extract was evaluated using DPPH and ABTS methods. Antioxidant measurement using ABTS and DPPH is based on hydrogen-donating antioxidants against nitrogen radicals[50-51]. DPPH and ABTS radical scavenging activity indicates the ability of a compound to donate electrons or hydrogen to ABTS or DPPH radicals, then turn them into more stable compounds and lower the absorbance value. The decrease in absorbance value is interpreted as the ability of a compound as an anti-oxidant or free radical scavenger[52-54]. The effect of different concentrations of *Gnetum gnemon* extract on the antioxidant activity is shown in Figure 6. The scavenging ability of the melinjo

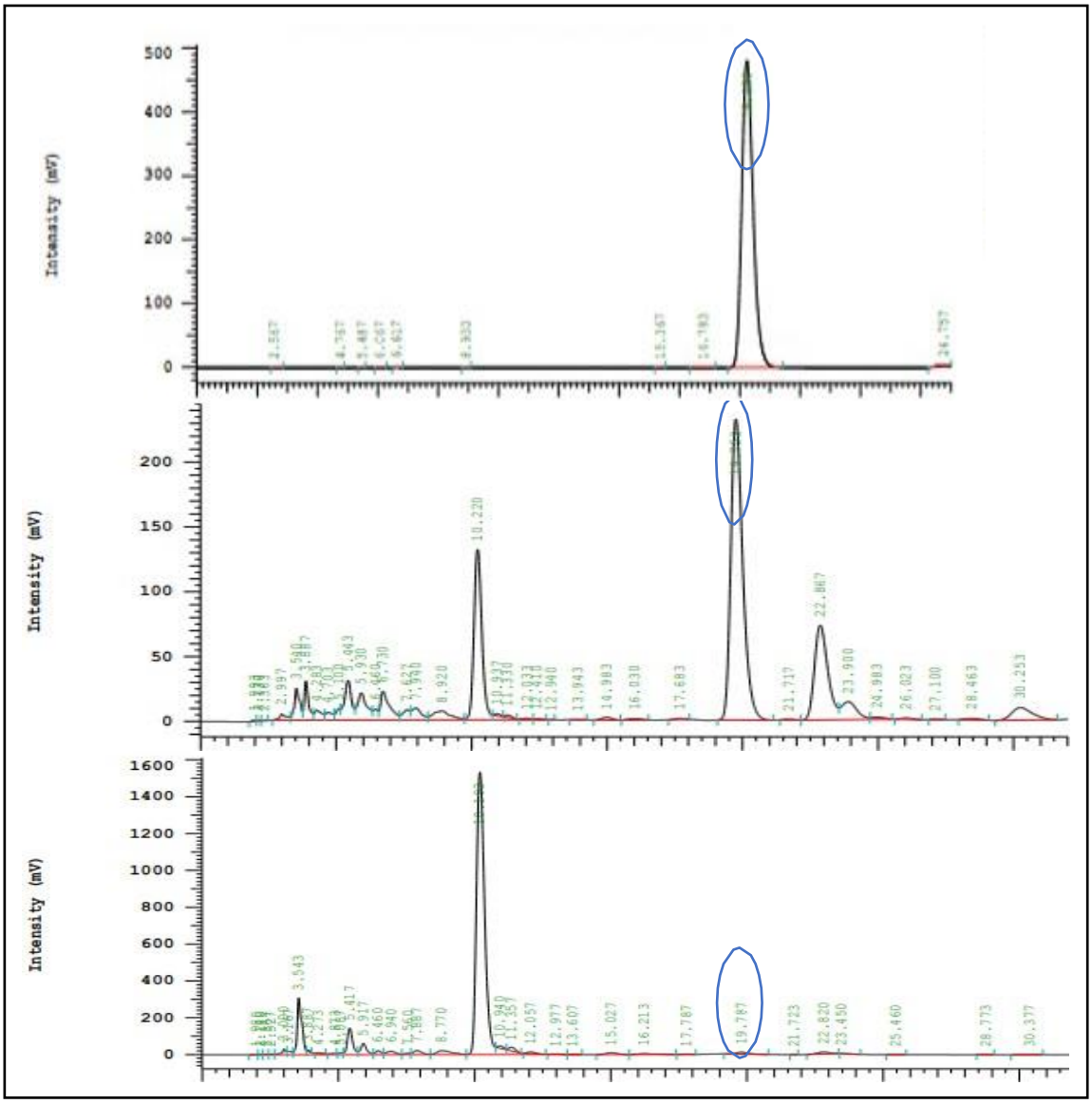
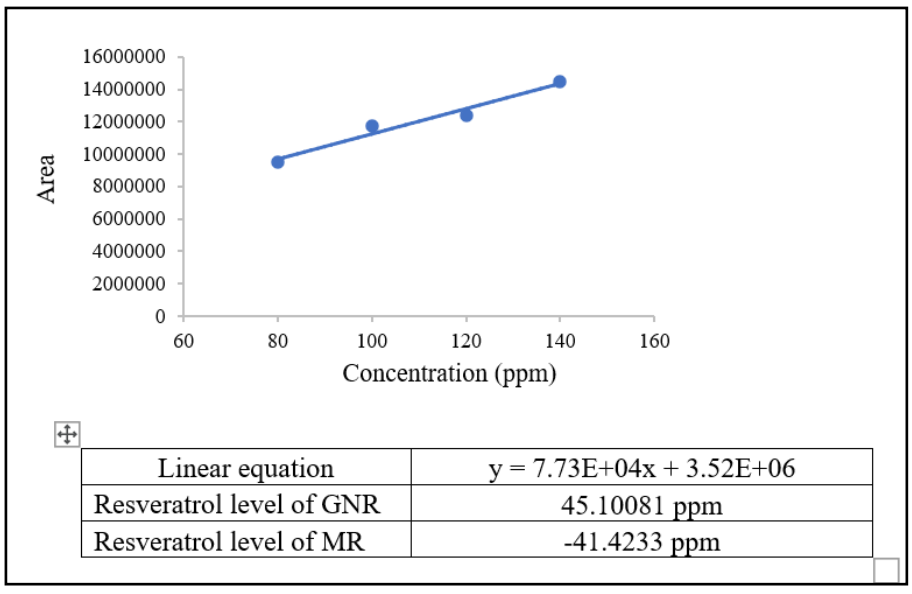


Figure 4. Chromatogram of standard resveratrol (top), GNR of melinjo herbs (mid), MR of melinjo herbs (bottom) at a retention time of ±19.78 min.



Linear equation	y = 7.73E+04x + 3.52E+06
Resveratrol level of GNR	45.10081 ppm
Resveratrol level of MR	-41.4233 ppm

Figure 5. Standar curve of resveratrol and the concentration in the samples (GNR and MR).

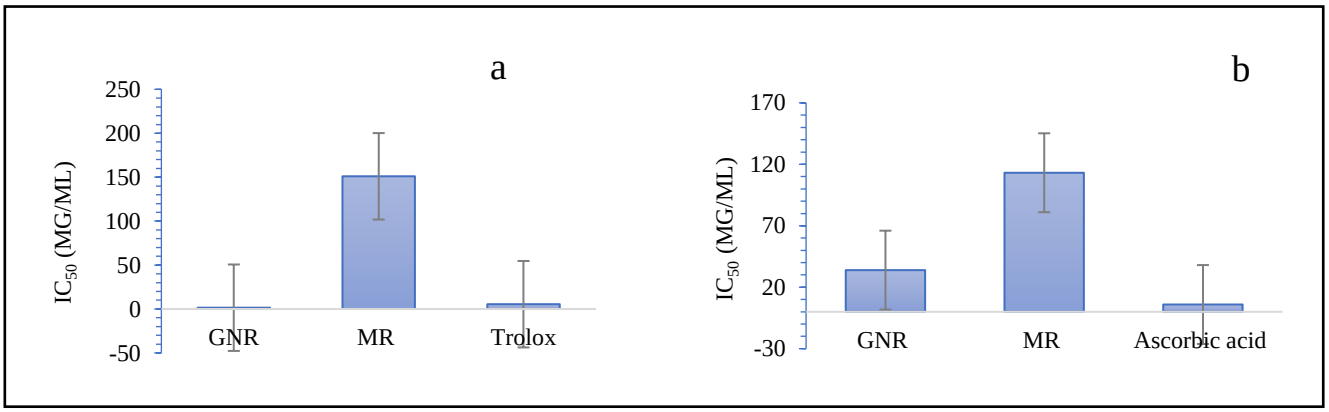


Figure 6. Antioxidant activity of GNR (melinjo seed extract), MR (melinjo seed flour), and positive control based on ABTS (a) and DPPH (b) methods. Data are representative of values from at least three independent experiments.

seed extract (GNR) based on DPPH method showed a lower IC50 value than that of melinjo seed flour (MR), with values of 33.89 ppm and 113.12 ppm, respectively. The IC50 value of the ascorbic acid as a positive control was 5.93 ppm indicating its higher radical scavenging capacity. Notably, based on ABTS method, the radical scavenging ability of *Gnetum gnemon* extract was higher than that of the Trolox as a positive control. The IC50 value of the melinjo seed extract (GNR) was 1.45 ppm. This value is up to 3.7 times lower than of Trolox which was 5.43 ppm. Interestingly, the ability of melinjo seed flour (MR) to reduce ABTS radicals was much lower than the others, with an IC50 value of 151.02 ppm. This indicated that melinjo seed extract has better potential than melinjo seed flour as a source of antioxidant agents.

When viewed from the total phenol-flavonoid content of GNR and MR (Figure 1), the high antioxidant capacity of the GNR samples was directly proportional to the total phenol-flavonoid content. GNR, which has higher total phenol-flavonoid content, shows a high antioxidant capacity, and vice versa, MR with a lower total phenol-flavonoid content has a lower antioxidant capacity. In addition, the resveratrol content may have affected the two samples' antioxidant capacity. Analysis of the sample resveratrol content showed that the resveratrol content of GNR was higher than that of MR. This is directly proportional to the antioxidant capacity of the two samples. Kato et al.[3] reported that the activity of gnetin L, gnetin C, gnemonoside A, gnemonoside C, gnemonoside D, and resveratrol isolated from the seed of *Gnetun gnemon* could maintain the DPPH radical scavenging activity in concentration-dependent manner. In addition, several researchers have also revealed that high resveratrol content increases antioxidant capacity and several other biological activities[55-56]. Thus, the antioxidant capacity of *Gnetum gnemon* is strongly influenced by the total phenol-flavonoid and resveratrol contents.

4. CONCLUSION

In general, both the commercial melinjo seed extract and flour meet the quality parameter standards as herbal ingredients for functional food preparation. The concentration of antioxidant compounds in the extract was higher than that in the dry powder. The polyphenols and flavonoids of the melinjo seeds significantly contribute to the antioxidant activity in a dose-dependent fashion.

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Conflicts of Interest

The authors declare no conflict of interest.

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Ethics approval

None to declare. This study did not involve human or animal specimens.

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LIMITATIONS

The limitations of this study include lack of analyses such as sample stability and water activity.

REFERENCES

1. Cahyana AH, Ardiansah B. Antioxidative and cytotoxic effects of prenylated stilbene derivative-rich Melinjo (*Gnetum gnemon* L.) fruit rind. AIP Conf Proc. 2016;1729:020057.
2. Bhat R, binti Yahya N. Evaluating belinjau (*Gnetum gnemon* L.) seed flour quality as a base for development of novel food pro-

ducts and food formulations. Food Chem. 2014;156:42-9.
3. Kato E, Tokunaga Y, Sakan F. Stilbenoids isolated from the seeds of melinjo (*Gnetum gnemon* L.) and their biological activity. J Agric Food Chem. 2009;57(6):2544-9.
4. Lim TK. Edible medicinal and non medicinal plants. Netherlands: Springer; 2012.
5. Narayanan NK, Kunimasa K, Yamori Y, Mori M, Mori H, Nakamura K, et al. Antitumor activity of melinjo (*Gnetum gnemon* L.) seed extract in human and murine tumor models *in vitro* and in a colon-26 tumor-bearing mouse model *in vivo*. Cancer Med. 2015;4(11):1767-80.
6. Wazir D, Ahmad S, Muse R, Mahmood M, Shukor MY. Antioxidant activities of different parts of *Gnetum gnemon* L. J Plant Biochem Biotechnol. 2011;20:234-40.
7. Parhusip AJN, Sitanggang AB. Antimicrobial activity of melinjo seed and peel extract (*Gnetum gnemon*) against selected pathogenic bacteria. Microbiol Indones. 2011;5(3):103-12.
8. Konno H, Kanai Y, Katagiri M, Watanabe T, Mori A, Ikuta T, et al. Melinjo (*Gnetum gnemon* L.) seed extract decreases serum uric acid levels in nonobese japanese males: A randomized controlled study. Evid Based Complement Alternat Med. 2013;2013:1-9.
9. Dutta PP, Bordoloi M, Roy S, Narzary B, Gogoi K, Bhattacharyya DR, et al. Antiplasmodial activity of *Gnetum gnemon* leaves and compounds isolated from them. Nat Prod Commun. 2018;13(10): 1263-5.
10. Supriyadi A, Arum LS, Nugraha AS, Istri Ratnadewi AA, Siswoyo TA. Revealing antioxidant and antidiabetic potency of melinjo (*Gnetum Gnemon*) seed protein hydrolysate at different stages of seed maturation. Curr Res Nutr Food Sci J. 2019;7(2): 479-87.
11. Latif R, Wang CY. Andrographolide as a potent and promising antiviral agent. Chin J Nat Med. 2020;18(10):760-69.
12. Santoso M, Naka Y, Angkawidjaja C, Yamaguchi T, Matoba T, Takamura H. Antioxidant and DNA damage prevention activities of the edible parts of *Gnetum gnemon* and their changes upon heat treatment. Food Sci Technol Res. 2010;16(6):549-56.
13. Kato H, Samizo M, Kawabata R, Takano F, Ohta T. Stilbenoids from the melinjo (*Gnetum gnemon* L.) fruit modulate cytokine production in murine Peyer's patch cells *ex vivo*. Planta Med. 2011;77(10):1027-34.
14. Tatefuji T, Yanagihara M, Fukushima S, Hashimoto K. Safety assessment of melinjo (*Gnetum gnemon* L.) seed extract: Acute and subchronic toxicity studies. Food Chem Toxicol. 2014;67: 230-5.
15. Iliya I, Ali Z, Tanaka T, Iinuma M, Furusawa M, Nakaya K, et al. Stilbene derivatives from *Gnetum gnemon* Linn. Phytochemistry. 2003;62(4):601-6.
16. Le TH, Van Do TN, Nguyen HX, Dang PH, Nguyen NT, Nguyen MTT. A new phenylheptanoid from the leaves of *Gnetum gnemon* L. Nat Prod Res. 2021;35(21):3999-4004.
17. Krishnatreya DB, Ray D, Baruah PM, Dowarah B, Bordoloi KS, Agarwal H, et al. Identification of putative miRNAs from expressed sequence tags of *Gnetum gnemon* L. and their cross-kingdom targets in human. BioTechnologia. 2021;102(2):179-95.
18. Tani H, Hikami S, Iizuna S, Yoshimatsu M, Asama T, Ota H, et al. Pharmacokinetics and safety of resveratrol derivatives in humans after oral administration of melinjo (*Gnetum gnemon* L.) seed extract powder. J Agric Food Chem. 2014;62(8):1999-2007.
19. Kunimasa K, Ohta T, Tani H, Kato E, Eguchi R, Kaji K, et al. Resveratrol derivative-rich melinjo (*Gnetum gnemon* L.) seed extract suppresses multiple angiogenesis-related endothelial cell functions and tumor angiogenesis. Mol Nutr Food Res. 2011;55 (11):1730-4.
20. Mun'im A, Munadhil MA, Puspitasari NA, Yanuar A. Angiotensin converting enzyme inhibitory activity of melinjo (*Gnetum gnemon* L.) seed extracts and molecular docking of its stilbene constituents. Asian J Pharm Clin Res. 2017;10(3):243-8.
21. Ota H, Akishita M, Tani H, Tatefuji T, Ogawa S, Iijima K, et al. trans-Resveratrol in *Gnetum gnemo*n protects against oxidative-stress-induced endothelial senescence. J Nat Prod. 2013;76(7): 1242-7.
22. Aggarwal BB, Takada Y, Oommen OV. From chemoprevention to chemotherapy: Common targets and common goals. Expert Opin Investig Drugs. 2004;13(10):1327-38.
23. Brasnyó P, Molnár GA, Mohás M, Markó L, Laczy B, Cseh J, et al. Resveratrol improves insulin sensitivity, reduces oxidative stress and activates the Akt pathway in type 2 diabetic patients. Br J Nutr. 2011;106(3):383-9.
24. Wong RH, Howe PR, Buckley JD, Coates AM, Kunz I, Berry NM. Acute resveratrol supplementation improves flow-mediated dilatation in overweight/obese individuals with mildly elevated blood pressure. Nutr Metab Cardiovasc Dis. 2011;21(11):851-6.
25. Bell LN. Moisture effects on food's chemical stability. In: Gustavo V. Barbosa-Cánovas, editors. Water activity in foods: Fundamentals and applications. New York: John Wiley & Sons, Inc.; 2020. p. 227-53.
26. Hageman MJ. The role of moisture in protein stability. Drug Dev Ind Pharm. 1988;14(14):2047-70.
27. Ministry of Health of the Republic of Indonesia. Farmakope herbal Indonesia. Jakarta : Kementerian Kesehatan RI.; 2017.
28. Sundari S, Fadhliani. Uji Angka Lempeng Total (ALT) pada Sediaan Kosmetik Lotion X di BBPOM Medan. J Biol Samudra. 2019;1:25-8.
29. Singleton VL, Orthofer R, Lamuela-Raventós RM. [14] Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Meth Enzymol. 1999;299: 152-78.
30. Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colometric methods. J Food Drug Anal. 2002;10(3):178-82.
31. Molyneux P. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating anti-oxidant activity. Songklanakarin J Sci Technol. 2004;26:211-9.
32. Arnao MB. Some methodological problems in the determination of antioxidant activity using chromogen radicals: A practical case. Trends Food Sci Technol. 2000;11:419-21.
33. Siswoyo TA, Ardyati T, Hosokawa K. Fermentation-induced changes in antioxidant activities and oxidative DNA damage protection of melinjo (*Gnetum gnemon*) flour. J Food Biochem. 2017;41(4):e12382.
34. Saraswaty V, Ketut Adnyana I, Pudjiraharti S, Mozef T, Insanu M, Kurniati NF, et al. Fractionation using adsorptive macroporous resin HPD-600 enhances antioxidant activity of *Gnetum gnemon* L. seed hard shell extract. J Food Sci Technol. 2017;54 (10):3349-57.
35. Siswoyo TA, Mardiana E, Lee KO, Hoshokawa K. Isolation and characterization of antioxidant protein fractions from melinjo (*Gnetum gnemon*) seeds. J Agric Food Chem. 2011;59(10):5648-56.
36. Yang L, Zhang Z, Hu X, You L, Khan RAA, Yu Y. Phenolic contents, organic acids, and the antioxidant and bio activity of wild medicinal berberis plants-as sustainable sources of functional food. Molecules. 2022;27(8):2497.
37. Duque-Soto C, Expósito-Almellón X, García P, Pando ME, Borrás-Linares I, Lozano-Sánchez J. Extraction, characterization, and bioactivity of phenolic compounds—a case on *Hibiscus* genera. Foods. 2023;12(5):963.
38. Kasote D, Tiozon RN Jr, Sartagoda KJD, Itagi H, Roy P, Kohli A, et al. Food processing technologies to develop functional foods with enriched bioactive phenolic compounds in cereals. Front Plant Sci. 2021;12:771276.
39. Dias MC, Pinto DCGA, Silva AMS. Plant flavonoids: Chemical characteristics and biological activity. Molecules. 2021;26(17): 5377.
40. Shorobi FM, Nisa FY, Saha S, Chowdhury MAH, Srisuphanunt M, Hossain KH, et al. Quercetin: A functional food-flavonoid incredibly attenuates emerging and re-emerging viral infections through immunomodulatory actions. Molecules. 2023;28(3):938.
41. Rathod NB, Elabed N, Punia S, Ozogul F, Kim SK, Rocha JM.

Recent developments in polyphenol applications on human health: A review with current knowledge. Plants. 2023;12(6):1217.
42. Nurcholis W, Alfadzrin R, Izzati N, Arianti R, Vinnai BÁ, Sabri F, et al. Effects of methods and durations of extraction on total flavonoid and phenolic contents and antioxidant activity of Java cardamom (*Amomum compactum* Soland Ex Maton) Fruit. Plants. 2022;11(17):2221.
43. Pum JA. Practical guide to validation and verification of analytical methods in the clinical laboratory. Adv Clin Chem. 2019; 90:215-81.
44. Ariyanto EF, Danil AS, Rohmawaty E, Sujatmiko B, Berbudi A. Effect of resveratrol in melinjo seed (*Gnetum gnemon* L.) extract on type 2 diabetes mellitus patients and its possible mechanism: A review. Curr Diabetes Rev. 2023;19(2):e280222201512.
45. Meng T, Xiao D, Muhammed A, Deng J, Chen L, He J. Anti-inflammatory action and mechanisms of resveratrol. Molecules. 2021;26(1):229.
46. Ren B, Kwah MX, Liu C, Ma Z, Shanmugam MK, Ding L, et al. Resveratrol for cancer therapy: Challenges and future perspectives. Cancer Lett. 2021;515:63-72.
47. Li T, Tan Y, Ouyang S, He J, Liu L. Resveratrol protects against myocardial ischemia-reperfusion injury via attenuating ferroptosis. Gene. 2022;808:145968.
48. Setyaningsih W, Guamán-Balcázar MDC, Oktaviani NMD, Palma M. Response surface methodology optimization for analytical microwave-assisted extraction of resveratrol from functional marmalade and cookies. Foods. 2023;12(2):233.
49. Bensa M, Vovk I, Glavnik V. Resveratrol food supplement products and the challenges of accurate label information to ensure food safety for consumers. Nutrients. 2023;15(2):474.
50. Xiang J, Li W, Ndolo VU, Beta T. A comparative study of the phenolic compounds and i*n vitro* antioxidant capacity of finger millets from different growing regions in Malawi. J Cereal Sci. 2019;87:143-9.
51. Munteanu IG, Apetrei C. Analytical methods used in determining antioxidant activity: A review. Int J Mol Sci. 2021;22(7):3380.
52. Shimada K, Fujikawa K, Yahara K, Nakamura T. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. J Agric Food Chem. 1992;40(6):945-8.
53. Huang W, Deng Q, Xie B, Shi J, Huang F, Tian B, et al. Purification and characterization of an antioxidant protein from Ginkgo biloba seeds. Food Res Int. 2010;43:86-94.
54. Liu YW, Han CH, Lee MH, Hsu FL, Hou WC. Patatin, the tuber storage protein of potato (*Solanum tuberosum* L.), exhibits antioxidant activity *in vitro*. J Agric Food Chem. 2003;51:4389-93.
55. Delmas D, Jannin B, Latruffe N. Resveratrol: Preventing properties against vascular alterations and ageing. Mol Nutr Food Res. 2005;49:377-95.
56. Kovacic P, Somanathan R. Multifaceted approach to resveratrol bioactivity: Focus on antioxidant action, cell signaling and safety. Oxid Med Cell Longev. 2010;3:86-100.