INTRODUCTION

Oxidation of biological molecules causes a variety of health problems, including carcinogenesis, Parkinson’s disease, atherosclerosis, and aging [1]. Previous studies proved that these damaging pathological events are caused by free radicals [2], commonly associated with cell degeneration, especially in the brain. In addition, oxidative stress results from increased reactive oxygen species (ROS) or a decreased antioxidant capacity from a natural cell in organisms [3].

The antioxidants can inhibit ROS to cause DNA damage, coronary heart disease, and health problems related to advancing age [4]. Moreover, scavenging agents can also be used as inhibitors of free radicals [5]. Many antioxidants were obtained from natural sources such as plants and are used in the food industry due to their health benefits [6]. Therefore, consuming foods rich in antioxidants phytochemicals can decrease degenerative diseases caused by oxidative stress to improve antioxidant capacity in the body [3]. One of the potential sources from natural plants that act as antioxidants is red ginger (Zingiberis officinalis Roscoe var. rubrum).

Ginger is one of the herbs that have been used as a spice, dietary supplement, and traditional medicine in various cultures globally [5, 7]. Several antioxidants compounds such as ascorbic acid, beta-carotene, polyphenols, and terpenoids are contained in ginger [7]. Several studies reported the antioxidant activity of ginger. In an in vitro and animal experiment conducted by Matsuda et al., ginger exhibited antioxidant activity and it has protective properties against free radical damage [8, 9]. The methanolic extract of ginger favorably prevented ROS damage assessed by ABTS assay (91.0±0.96%), nitric oxide assay (86.72±1.51%), and DPPH assay (86.2±0.97%) [10]. Red and white ginger extract can protect the brain by dietary intake through antioxidant activity and prevent oxidative stress, Fe2+ chelating, and OH• scavenging ability [3]. Therefore, it can be a major source of natural or phytochemical antioxidants due to its wide range of antioxidants [11].

Recently, many products have been developed from the gingers, such as skin-lightening cosmetics, tablets, etc. The development of efervescent powder and its antioxidant activity has not been studied. Therefore, this study aimed to formulate the efervescent granules (EG) from red ginger (RG) extract and evaluate its antioxidant activity. EG was chosen as a product because it is soluble, dissolves quickly, and does not have a bad bitter taste. It is one of the most popular oral products due to its stable dosage forms and convenience [12].

MATERIALS AND METHODS

Plant and microbiological material

The RG was collected from Kutawaringin, Bandung, West Java, Indonesia, and authenticated (No. 26/HB/04/2021) by Plant and microbiological material. The RG was collected from Kutawaringin, Bandung, West Java, Indonesia, and authenticated (No. 26/HB/04/2021) by Plant and microbiological material. The RG was collected from Kutawaringin, Bandung, West Java, Indonesia, and authenticated (No. 26/HB/04/2021) by Plant and microbiological material. The RG was collected from Kutawaringin, Bandung, West Java, Indonesia, and authenticated (No. 26/HB/04/2021) by Plant and microbiological material. The RG was collected from Kutawaringin, Bandung, West Java, Indonesia, and authenticated (No. 26/HB/04/2021) by Plant and microbiological material. The RG was collected from Kutawaringin, Bandung, West Java, Indonesia, and authenticated (No. 26/HB/04/2021) by Plant and microbiological material. The RG was collected from Kutawaringin, Bandung, West Java, Indonesia, and authenticated (No. 26/HB/04/2021) by Plant and microbiological material.

Chemicals

Vitamin C and DPPH were purchased from Tokyo Chemical Industry, while other chemicals were purchased from pharmaceutical grade.

Methods

RG extract preparation

The RG powder (300 g) was placed into a round-bottom flask containing 3 L of 96% ethanol as solvent and extracted using the reflux extraction method. Furthermore, the solvent was removed by using the rotary evaporator to obtain the crude extract (37.41 g).

Phytochemical screening of the extract

The phytochemical screening was conducted to detect the secondary metabolites contained in ginger such as alkaloids, flavonoids, monoterpenes and sesquiterpenes, steroids/triterpenoids, polyphenols, quinones, saponins, and tannins [1-3].
Formulation of EG from RG extract

The wet granulation method was used to prepare the EG from RG extract [12]; the formula and quantity of each component are shown in table 1. In the acid components, the RG crude extract, citric acid, and tartaric acid were mixed with other ingredients thoroughly to maintain good distribution of the sample. A suitable amount of binding agent was added and the mixture powder was sieved using sieve no. 12 to obtain granules. The obtained granules were dried at 45 °C overnight using the oven to obtain <0.5% of the water content. Similar preparation was also conducted for the base component, which contains sodium bicarbonate and others. Furthermore, both components were mixed, sieved through sieve no. 14, and dried to form granules.

Table 1: The formula of EG from RG extract

| Components            | F1 (%) | F2 (%) | F3 (%) |
|-----------------------|--------|--------|--------|
| RG extract            | 15     | 15     | 15     |
| Mannitol              | 28.3   | 27.8   | 27.3   |
| Citric acid           | 9.4    | 9.4    | 9.4    |
| Tartaric acid         | 18.8   | 18.8   | 18.8   |
| Sodium bicarbonate    | 23.5   | 23.5   | 23.5   |
| Polyvinylpyrrolidone(PVP)| 2      | 2.5    | 3      |
| Sucrose               | 3      | 3      | 3      |

Flowability study

The flow rate and the angle of repose determination

The funnel method was conducted to measure the flow rate and angle of repose. When granules samples are poured onto a horizontal plane, a conical pile will be formed. Furthermore, the angle of repose is the internal angle between the surface of the pile and the horizontal surface. The flow rate and the angle of repose of the sample were calculated as follows:

Flow rate = w/t
Where w is the weight (gram) and t is time (second).

While for the angle of repose:

θ = tan⁻¹(h x r)
Where h is the height of the granules forming the cone and r is the radius of the base [12, 14].

Bulk density (BD) and tapped density (TD)

Two types of BD and TD were determined using the methods outlined in USP. Meanwhile, 25 g of granules were put into a 100 ml measuring cylinder, and the initial volume was measured. The measuring cylinder was tapped and the volume was measured in increments of 10, 50, and 100 taps. From the equation below, BD and TD were calculated [12, 14].

BD = granules weight / ml
Packaging volume

TD = granules weight /
Tapped volume of packing

Compressibility index (CI) and hausner ratio (HR)

CI and HR of granules were measured to provide the flow properties and compressibility of EG from RG [15]. The values were compared with references, as shown in table 2 [12, 14].

CI = [(TD - BD) x 100] / TD

HR = BD / TD

Effervescence time

The effervescent time of granules was measured by adding 1 g of EG from RG to a glass containing 100 ml of water, and the time for obtaining a clear solution was recorded [16].

Physical stability

The physical stability of EG from RG extract was evaluated after 28 d of storage, including organoleptic evaluation, effervescence time, and pH measurement.

Antioxidant activity

The antioxidant activity of EG from RG extract was measured by the DPPH assay [17], and the sample solution was prepared by various concentrations, mixed with DPPH solution in a ratio of 2:3. Furthermore, the absorbance was measured at 517 nm and calculated to obtain the inhibition percentage value using the following equation:

% Inhibition = [1 - (Asample/ADPPH)] x 100

Where % Inhibition is the percentage capacity of free radical inhibition, and A sample is the absorbance of DPPH solution at 515 nm. The linear regression curve between % inhibition and sample concentration (IC₅₀) was calculated and vitamin C was used as a reference compound [18].

Table 2: Flow properties of the angle of repose [12, 14]

| Angle of repose | Flow properties |
|-----------------|-----------------|
| <20             | Excellent       |
| 20-30           | Good            |
| 30-40           | Slightly poor   |
| >40             | Very poor       |

Table 3: Table of compressibility index [12, 14]

| Flow characters | Carr’s index |
|-----------------|--------------|
| Excellent       | 1-10         |
| Good            | 11-15        |
| Fair            | 16-20        |
| Slightly poor   | 21-25        |
| Poor            | 26-31        |
| Very poor       | 32-37        |
| Extremely poor  | >38          |

Table 4: Table of hausner’s ratio of granules [12, 14]

| Hausner’s ratio | Flowing properties |
|-----------------|--------------------|
| <1.25           | Good               |
| 1.25-1.6        | Moderate           |
| >1.6            | more cohesive powders |

RESULTS AND DISCUSSION

The result of phytochemical screening showed that RG extract contained flavonoids, monoterpenes and sesquiterpenes, steroids/triterpenoids, polyphenols, quinones, saponins, and tannins. Meanwhile, phenolic compounds protect the human body from free radicals due to their antioxidants capacity. The antiradical
activity of phenols and flavonoids is based on the structural relationship between different parts of their chemical structure [19, 20]. Natural polyphenols can remove free radicals, activate antioxidant enzymes, reduce α-tocopherol radicals, and inhibit oxidases [21, 22]. The dose of EG from RG extract used in this study was 15%, and the concentration of the antibacterial activity against Staphylococcus aureus and Escherichia coli was reported [23]. In addition, 15% red ginger extract was used, and it had an antibacterial activity of 12.9 mm and 13.5 mm against Staphylococcus aureus and Escherichia coli, respectively.

In the formula of EG from RG extract, mannitol was added as filler, binder, and lubricant to improve flowability and reduce friction [24]. PVP is a nontoxic and hydrophilic excipient used in various pharmaceutical formulations, especially in the solid dosage form. In this formula, PVP was added as a binder to form granules materials and improve the properties of RG extract [25]. In this formula, sucrose was used as a sweetening agent and a diluent [26]. The combination of sodium bicarbonate-citric and tartaric acid was commonly used as effervescent material in the formulations of effervescent [27].

Table 5 showed the results of the flowability study of EG from RG extract, and the values of the angle of repose from EG were found in the range 25.72-26.32 with a flow rate of 4.87-5.27. The value of bulk and tapped density were in the range of 39.17-45.27, and 36.97-42.67, respectively. The result of the Carr's index of EG from RG extract was in the range of 5.78-5.95, and the Hausner's ratio values were found in the range of 1.04-1.06. These results demonstrated that all the formulas have good flow properties, and the granules showed excellent flow properties based on Carr's index results. The good or excellent flow properties of granules were attributed to the successful method of preparation using wet granulation [12].

The physical stability was monitored from organoleptic evaluation, effervescent time, and pH measurement, and the sample appearances (fig. 1), color, and odor of granules did not change after 28 d of storage. Moreover, the effervescence time was still in the acceptable range according to USP [28].

The ginger and EG from RG extract showed antioxidant activity at IC50 values of 144.42 g/ml and 283.28 g/ml, respectively, while vitamin C was 16.36 g/ml. Furthermore, the IC50 of ginger effervescent granules was lower compared to extracts as well as vitamin C as standard. However, these granules can remove free radicals since their IC50 showed moderate antioxidant activity. This indicated that flavonoids and phenols remain in ginger effervescent granules. Therefore, EG from RG extract can be used as a food supplement to protect the human body from free radicals and inhibit oxidases.

**CONCLUSION**

This study explains the formulation of EG from RG extract and its antioxidant activity. The flowability study and effervescent measurement showed that EG was successfully prepared by wet granulation. Furthermore, the DPPH assay demonstrated its moderate antioxidant activity. Therefore, this study provided fundamental insight that EG from RG extract can be used as food supplements to protect the human body from free radicals and inhibit oxidases.
ACKNOWLEDGMENT
This research was supported by Hazanah Foundation, Indonesian School of Pharmacy through the Fundamental Research Grant number 009/SPK/YHZ/II/2019.

FUNDING
Nil

AUTHORS CONTRIBUTIONS
All the authors have contributed equally.

CONFLICT OF INTERESTS
Declared none

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