Research of Service Administration Health and Sains Healthys
Vol. 7 No. 3 September 2026
*p-ISSN:*2830-4748 e-ISSN: 2830-4772
DOI: *10.58258/rehat.v7i3.11490/ http://ejournal.mandalanursa.org/index.php/Rehat*

Formulation and Testing of Antioxidant Activity of Effervesent Granules From Red Pucuk Leaf Extract (Syzygium Myrtifolium Walp.) And Determination of Total Flavonoid Content

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Article Info

Article history:
Accepted: 17 September 2026
Publish: 23 September 2026

Keywords:
Syzygium myrtifolium Walp;
Effervescent Granules;
Antioxidant;
DPPH;
Total Flavonoid.

Abstract

Red-tip leaf (Syzygium myrtifolium Walp*.) is an ornamental plant rich in secondary metabolites, particularly flavonoids, with potential as a natural antioxidant. This study aimed to formulate the ethanolic extract of red-tip leaf into an effervescent granule, evaluate its physical quality, determine its total flavonoid content, and examine the stability of its antioxidant activity using the DPPH method. This laboratory-experimental study used maceration with 70% ethanol, yielding a 24.84% extract recovery with 4.56% moisture content. Phytochemical screening revealed flavonoids, tannins, saponins, steroids, and triterpenoids. Effervescent granules were prepared in four formulas (F0 negative control, F1 1.5%, F2 2%, F3 3%) by separating the acid and base phases. All formulas met the physical quality requirements for organoleptic properties, flow rate (2.5-6 g/s), angle of repose (23.1°-26°), dissolution time (<5 minutes), pH (5.65-6.07), and moisture content (<2%). A hedonic test on 20 panelists showed the highest acceptance for F2 (82.5%). The IC50 values were 4.389 ppm for vitamin C and 18.614 ppm for the pure extract; after formulation, the granules retained very strong antioxidant activity with IC50 values of 22.958-19.459 ppm. The total flavonoid content of the extract was 15.533 mgQE/g. The red-tip leaf extract was successfully formulated into effervescent granules that meet pharmaceutical physical quality standards while retaining very strong antioxidant efficacy.*

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1. INTRODUCTION

Antioxidants are compounds that play a role in protecting cells from damage caused by free radicals, unstable reactive molecules that can trigger oxidative stress in the body. Antioxidants can be obtained endogenously through enzymes such as superoxide dismutase, catalase, and glutathione peroxidase, or exogenously from foods such as vitamin C, vitamin E, and carotenoids [1].

The use of plants as a source of traditional medicine has long been known to the Indonesian people and continues to grow along with the increasing interest in natural materials as alternative therapies [2]. One ornamental plant that has the potential to be developed is the red shoot (*Syzygium myrtifolium*Walp.), a member of the Myrtaceous family, which is widely distributed in Southeast Asia, including Indonesia. Red shoot leaves are rich in flavonoid compounds, one of which is chalcone, which is reported to have cytotoxic activity and other pharmacological bioactivities [3].

The total flavonoid content in an extract can be determined using UV-V is spectrophotometry because flavonoids can absorb visible light in the wavelength range of 400-800 nm through their conjugated aromatic system, and are expressed in units of milligrams of quercetin equivalents per gram of extract (mgQE/g) [4], [5]. The principle of determining flavonoid content using the aluminum chloride (AlCl3) colorimetric method is based on the formation of a complex between

aluminum ions and the keto group at the C-4 atom and the free hydroxyl group at the C-3 or C-5 atom of the flavone and flavonol groups; quercetin is used as a standard compound because it has these functional groups completely [4].

The measured flavonoid levels are directly proportional to the extract's ability to ward off free radicals, so antioxidant activity testing was carried out using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method [6]. This method was chosen because it is simple, fast, sensitive, requires only a small amount of sample and reagent, and can be widely applied to test natural antioxidant compounds. The testing principle is based on the ability of antioxidant compounds to donate hydrogen atoms to DPPH radicals, resulting in a color decay of the solution from purple to pale yellow which can be measured quantitatively using a UV-Vis spectrophotometer at its maximum absorption wavelength.

Natural ingredient extracts are generally formulated in solid dosage forms such as tablets, capsules, or granules. *Effervescent* was chosen in this study because it is practical to use, has good physical stability, and produces CO2 gas through a reaction between acid and base components when dissolved in water, thus increasing the convenience of consumption compared to ordinary instant granules [7].

Previous studies have reported that red shoot leaves contain alkaloids, triterpenoids, steroids, phenolics, flavonoids, and saponins with various bioactive activities, including antioxidant, antidiabetic, antihypertensive, antibacterial, and antifungal [8]. Another study showed that the ethanol extract of red shoot leaves has very strong antioxidant activity with an IC50 value of 2.195 ppm and a total phenol content of 371.833 mg GAE/g extract [9]. However, this potential has not been widely developed in modern pharmaceutical preparations. Based on this description, this study aims to formulate the ethanol extract of red shoot leaves into an effervescent granule preparation, evaluate its physical quality, prove the stability of antioxidant activity after the formulation process using the DPPH method, and determine the total flavonoid content of the extract.

2. RESEARCH METHOD

This research is an experimental laboratory study conducted in the Natural Materials Laboratory of the Undergraduate Pharmacy Study Program, Faculty of Health Sciences, Duta Bangsa University, Surakarta, for three months (April-July 2026). Fresh red shoot leaf samples (*Syzygium myrtifolium*Walp.) A total of 3,500 grams was obtained from a plantation in the Kedawung area, Sragen, Central Java, and was determined at the Tawangmangu Traditional Health Service Unit to confirm the plant's identity. The study has obtained ethical approval (*ethical clearance*) No. KEPK/UMP/256/V/2026 from Muhammadiyah University of Purwokerto.

The independent variable of this study is the concentration of extract in four effervescent granule formulas, namely F0 (negative control without extract), F1 (1.5%), F2 (2%), and F3 (3%), while the dependent variables are the physical quality of the preparation, antioxidant activity, and total flavonoid content. The operational definition in this study includes red shoot leaves as raw materials taken from the shoots or young red leaves, the extraction process using the maceration method to obtain a thick extract, and the granule formulation, which is evaluated through a series of physical quality tests of the preparation.

The preparation of simplicia is carried out through wet sorting, washing, chopping, drying under the sun covered with black cloth to prevent damage to active compounds due to ultraviolet radiation, pollination, and sieving using mesh 40 [10]. The simplicia obtained were standardized, including organoleptic examination, drying loss, water content, and ash content [11].

Extraction was carried out using the maceration method using 5 L of 70% ethanol for 500 g of the simplex; soaked for the first 6 hours while stirring occasionally, then left for 18 hours and

filtered. The process was repeated once with half the initial solvent volume [10]. The collected macerate was concentrated using a *rotary evaporator* until a thick extract was obtained, then the yield was calculated. The extract is then standardized for water content, alcohol-free testing, and heavy metal contamination (Pb and Cd). Phytochemical screening is carried out qualitatively for alkaloids, saponins, tannins, flavonoids, steroids, and triterpenoids using specific color reactions.

Granule formulation: *effervescent* (Table 1) modified from the reference formula [13] with the addition of sodium benzoate as a preservative to maintain the stability of the preparation [14]. The granules were made by separating the acid phase (extract, citric acid, tartaric acid, PVP, lactose, and aerosil) and the base phase (sodium bicarbonate, sodium cyclamate, sodium benzoate, and lemon essence); each phase was dried at 60°C for 15 minutes, sieved successively with mesh 14 and mesh 16, then mixed until homogeneous in a room at 20-25°C with a relative humidity of 50-53%.

Table 1. Granule Formulation *Effervescent* Red Shoot Leaf Extract

Material	F0 (%)	F1 (%)	F2 (%)	F3 (%)	Information
Ethanol extract of red shoot leaves	0	1,5	2	3	Active ingredients
Citric acid	15	15	15	15	Source of acid
Tartaric acid	15	15	15	15	Source of acid
Sodium bicarbonate	34,8	34,8	34,8	34,8	Language source
Sodium cyclamate	0,1	0,1	0,1	0,1	Sweetener
PVP	4	4	4	4	Fastener
Aerosil	0,5	0,5	0,5	0,5	Sliding
Lemon essence	1	1	1	1	Seasoning
Natrium benzoat	0,2	0,2	0,2	0,2	Preservative
Dye	qs	qs	qs	qs	Dye
Lactose	Ad 100	Ad 100	Ad 100	Ad 100	Filler

The total flavonoid content was determined using the AlCl3 colorimetric method with quercetin as a standard, including determining the maximum wavelength, *operating time*, calibration curve, and extract absorbance measurements, then calculated using the standard curve linear regression equation (y = ax + b) and expressed in mgQE/g [4].

Antioxidant activity was tested using the DPPH method, comparing pure extract, vitamin C as a positive control, and the four granule formulas. A 500-ppm DPPH solution was reacted with a series of sample concentrations, incubated for the *specified time*, and then the absorbance was measured at the maximum wavelength using a UV-Vis spectrophotometer. The percentage of inhibition was calculated using the equation %Inhibition = [(Control Abs - Sample Abs)/Control Abs] x 100%, and the IC50 value was obtained from the linear regression equation of the curve of the relationship between concentration and percentage of inhibition. The smaller the IC50 value, the higher the antioxidant activity of a compound, with categories: very strong (<50 ppm), strong (50-100 ppm), moderate (101-250 ppm), weak (250-500 ppm), and very weak (>500 ppm) [12].

Evaluation of the physical quality of granules includes organoleptic tests, flow rate and angle of repose using a *flow tester* [18], dissolving time with a standard <5 minutes [19], pH using a pH meter with standard 5-7 [12], water content using a *moisture analyzer*, as well as a hedonic test on 20 panelists with a preference scale of 1 (very dislike) to 5 (very like) for color, aroma, taste, and texture. All quantitative data were analyzed descriptively using Microsoft Excel software.

3. RESEARCH RESULTS AND DISCUSSION

3.1 Research Results

The results of plant determination confirmed that the sample used was indeed a red shoot plant from the Myrtaceae family. Research ethics approval was declared to have met the ethical principles of health research based on certificate No. KEPK / UMP / 256 / V / 2026. Fresh leaf samples collected from the Kedawung area, Sragen, amounting to 3,500 grams were processed into 792 grams of dry simplicia and 516 grams of simplicia powder, with a simplicia yield of 22.62%.

The results of the standardization of the simplex and ethanol extract of red shoot leaves are presented in Table 2. The simplex showed drying loss, water content, and ash content that all met the required quality standards. The maceration process of 500 g of simplex with 5 L of 70% ethanol produced 124.2 g of thick extract with a yield of 24.84%. The extract was declared free of alcohol and free of Pb and Cd metal contamination.

Table 2. Results of Standardization of Simplex and Ethanol Extract of Pucuk Merah Leaves

Parameter	Simple ingredients	Extract	Quality Requirements
Yield (%)	22,62	24,84	>10%
Drying shrinkage (%)	7,23 ± 0,23	-	<10%
Water content (%)	7,95 ± 0,05	4,56 ± 0,67	<10%
Ash content (%)	13,56 ± 0,50	-	<16%
Alcohol-free	-	Negative (free)	Ethanol free
Metal contamination (Pb, Cd)	-	Negative (free)	Metal-free

Phytochemical screening (Table 3) showed that the ethanol extract of red shoot leaves contained flavonoids, tannins, saponins, as well as steroids and triterpenoids, but did not show a positive reaction to alkaloids.

Table 3. Phytochemical Screening Results of Ethanol Extract of Pucuk Merah Leaves

Compound	Reagent	Results	Information
Alkaloid	Mayer, Dragendorff, Bouchardat	(-)	No sediment formed
Saponin	Aquadest + HCl 2N	(+)	Stable foam formed
Land	Metanol + FeCl3 10%	(+)	Changes color to black
Flavonoid	Mg powder + concentrated HCl	(+)	Turns orange
Steroid & Triterpenoid	CH3COOH + concentrated H2SO4	(+)	Greenish brown color

Evaluation of the physical quality of granules (Table 4) shows that all four formulas have a light green to dark green color (F0 is white), with a distinctive aroma and taste of the extract whose intensity increases with increasing extract concentration. The flow rate values range from 2.5-6 g/s, angle of repose 23.1°-26°, dissolution time 0.22-1.27 minutes, pH 5.65-6.07, and water content 1.35-2.04%, all of which meet the physical quality requirements of the granules *effervescent* good. The hedonic test on 20 panelists showed the highest level of acceptance at F2 (82.5 ± 1.11%).

Table 4. Results of Physical Quality Evaluation of Granules *Effervescent*

Parameter	F0	F1	F2	F3
Color	White	Bright green	Green	Dark green
Smell	Odorless	Weak extract	Extract	Strong extract
Flavor	Sour	Weak acid	Sour	Slightly sour and bitter
Flow rate (g/s)	4,2 ± 2,06	6 ± 1,56	2,5 ± 0,08	3,9 ± 0,41

Parameter	F0	F1	F2	F3
Angle of repose (°)	23,1 ± 2,92	24,5 ± 2,44	26 ± 0,88	25,5 ± 1,21
Dissolving time (minutes)	0,22 ± 0,04	1 ± 0,26	1,27 ± 0,15	0,96 ± 0,25
pH	6,07 ± 0,19	5,77 ± 0,19	5,79 ± 0,11	5,65 ± 0,18
Water content (%)	1,92 ± 0,91	1,35 ± 0,32	1,43 ± 0,48	2,04 ± 1,03
Hedonic test (%)	77,75 ± 2,68	72 ± 11,66	82,5 ± 1,11	78,40 ± 2,68

Testing *operating time DPPH* showed stable absorbance starting from the 4th minute, with a maximum wavelength of 516 nm. The IC50 values from the antioxidant activity test results are presented in Table 5. Vitamin C, as a comparison, has an IC50 of 4.389 ppm, pure extract 18.614 ppm, and formula F0 (without extract) 136.63 ppm. After being formulated into *effervescent granules*, the IC50 values of F1, F2, and F3 were 22.958, 19.718, and 19.459 ppm, respectively, all of which were classified as very strong antioxidants (IC50 <50 ppm). Determination of total flavonoid levels using a quercetin standard curve at a maximum wavelength of 422 nm obtained a total flavonoid content of 15.533 mgQE/g.

Table 5. IC50 Value of Antioxidant Activity Using DPPH Method

Sample	IC50 value (ppm)	Category
Vitamin C (positive control)	4,389	Very strong
Ethanol extract of red shoot leaves	18,614	Very strong
F0 (negative control, without extract)	136,63	Currently
F1 (1.5% extract)	22,958	Very strong
F2 (extrak 2%)	19,718	Very strong
F3 (extra 3%)	19,459	Very strong

3.2 Discussion

The process of making simplicia begins with washing to remove dirt and attached residue, followed by drying under sunlight covered with black cloth. This treatment aims to absorb some of the direct ultraviolet radiation so that thermolabile active compounds, such as flavonoids and phenolic compounds, do not experience excessive degradation during drying [10]. The yield of simplicia of 22.62% indicates good efficiency of the drying and pollination process because this value far exceeds the minimum quality threshold of 10% [11]. The drying loss value (7.23%) and water content (7.95%), which are below 10%, indicate that the simplicia has a low bound water content, so the risk of microbial growth and enzymatic degradation during storage can be minimized. The ash content of 13.56%, which describes the mineral content and inorganic residues in the simplicia, is also still within the required range (<16%) [11], indicating that the washing process of the raw material has been carried out adequately to remove contaminants attached to the leaf surface.

The selection of the maceration method in the extraction stage is based on its nature as a cold extraction technique without heating, so it is able to maintain the stability of thermolabile active compounds such as flavonoids, phenolics, and other antioxidant compounds that are susceptible to decomposition at high temperatures [10]. The yield of the thick extract of 24.84% indicates that the 70% ethanol solvent is quite effective in extracting polar to semipolar secondary metabolite compounds contained in the red shoot leaf simplicia, and this value has met the quality standards for good extract yield (>10%). The results of the extract standardization, with a water content of 4.56%, alcohol-free, and free from Pb and Cd heavy metal contamination, confirm that the resulting extract meets the safety and pharmaceutical quality requirements for use in the next formulation stage.

The results of phytochemical screening showed that the ethanol extract of pucuk merah leaves contained flavonoids, tannins, saponins, steroids, and triterpenoids, in line with previous reports on the phytochemical content of this plant [8]. The non-detection of alkaloids in this study was likely due to the levels of compounds being below the detection limit of the test tube method used, considering that the levels of secondary metabolites in plants are greatly influenced by environmental factors where they grow, such as geographic conditions, altitude, soil type and nutrients, sunlight intensity, and harvest time. Differences in sampling locations and harvest seasons between studies may explain the variations in phytochemical screening results reported in various studies related to pucuk merah plants.

Evaluation of physical quality of granules aims to ensure that the preparation meets aesthetic standards, homogeneity, and consumer acceptability. Organoleptic tests show that the intensity of the green color, aroma, and distinctive taste of the extract increases with increasing extract concentration in formulas F1 to F3, while formula F0, as a negative control, appears white without the distinctive aroma of the extract, confirming that the organoleptic characteristics of the granules are directly influenced by the content of the incorporated extract. The flow rate values of the four formulas (2.5-6 g/s) and the angle of repose (23.1°-26°) indicate good granule flow properties, because granules with an angle of repose below 40° and a short flow time are categorized as having adequate flow properties for the packaging process [18]. The dissolution time of all formulas is below 5 minutes, meeting the solubility requirements of the preparation [19], while the pH value ranges from 5.65-6.07, which is considered a normal pH according to the range required for the preparation *effervescent* (pH 5-7) [12], so it is safe from the risk of gastrointestinal irritation when consumed. The water content of the granules, which ranges from 1.35-2.04%, also supports the physical stability of the preparation, considering that high water content can trigger reactions *and e*arly deterioration due to contact with air humidity during storage. The hedonic test results showed the highest level of preference for formula F2 (82.5%), indicating that the 2% extract concentration provided an optimal balance between the sweet and sour taste of the *effervescent powder,* with a distinctive extract taste that is not too dominant or bitter, compared to the F3 formula, which has a slightly bitter taste due to the higher extract concentration.

The working mechanism of the DPPH method is based on the ability of antioxidant compounds to donate hydrogen atoms to DPPH radicals, indicated by a change in the color of the solution from purple to yellow and a decrease in absorbance at the maximum wavelength [15]. Vitamin C is used as a positive control because it has electron donor groups on the C-2 and C-3 atoms that enable it to effectively capture free radicals; the IC50 value of vitamin C of 4.389 ppm confirms its very strong antioxidant activity, although this compound is susceptible to oxidation by light, temperature, and air, so that storage of the test solution needs to be done carefully and measured immediately after preparation [16]. The ethanol extract of pucuk merah leaves shows an IC50 value of 18.614 ppm, which is classified as very strong (<50 ppm), confirming the great potential of this plant as a source of natural antioxidants, in line with the findings in previous studies on pucuk merah leaf extracts using total phenol parameters and other antioxidant testing methods [9].

The IC50 value of formula F0 (136.63 ppm; medium category) indicates that the granule excipient matrix, *effervescent* without the addition of extract, still has relatively low free radical scavenging activity, possibly originating from the mild reducing properties of the organic acid components (citric acid and tartaric acid) in the mixture. After being incorporated with the extract, the antioxidant activity of the granules increased drastically and was consistently in the very strong category, with IC50 values of F1, F2, and F3 of 22.958; 19.718; and 19.459 ppm, respectively. This pattern shows an inverse relationship between the concentration of the incorporated extract and the IC50 value of the preparation; the higher the extract concentration, the lower the IC50

value obtained, which means the stronger the antioxidant activity. This finding proves that the formulation process into a granule preparation, *effervescent*, including the heating stage at 60°C during the drying of the acid and base phases, did not cause significant degradation of the active compounds of the extract, so that its antioxidant efficacy was maintained even after going through the pharmaceutical formulation process.

Determination of total flavonoid levels using the AlCl3 colorimetric method is based on the principle of the formation of colored complexes between aluminum ions with keto groups at the C-4 atom and adjacent free hydroxyl groups at the C-3 or C-5 atoms of the flavone and flavonol groups, which are measured for absorbance at a maximum wavelength of 422 nm with stable solution conditions in the 20th to 21st minute range after incubation. The total flavonoid content of the ethanol extract of red shoot leaves obtained at 15.533 mgQE/g confirms that flavonoids are one of the major compound groups that contribute to the antioxidant activity of this extract. Mechanistically, flavonoids act as primary antioxidants (*chain-breaking antioxidants*) that stabilize free radicals by donating hydrogen atoms from their active hydroxyl groups, thereby stopping the chain reaction of new radical formation. The relationship between total flavonoid levels and antioxidant activity is directly proportional (positive correlation): the higher the flavonoid levels in an extract, the greater its ability to reduce free radicals, which is reflected in the smaller IC50 value [17]. This finding is consistent with the IC50 values of the extract and all granule formulati*ons.* In this study, which is generally classified as a very strong antioxidant, it can be concluded that the flavonoid content of red shoot leaves is the main contributor to the antioxidant activity observed, both in pure extracts and after being formulated into effervescent granular preparations.

In general, the results of this study strengthen the scientific evidence reported in previous literature reviews that pucuk merah leaves are a source of bioactive compounds with consistently strong antioxidant activity, even though they were measured using different parameters and methods, such as total phenol content and IC50 of 2.195 ppm in previous studies [9], as well as comprehensive phytochemical and bioactivity studies that reported flavonoid content as one of the dominant secondary metabolite groups in this plant [8]. The IC50 value of the extract in this study (18.614 ppm) is indeed slightly higher than several previous reports, which can reasonably be caused by differences in growing location, age of the leaves harvested, extraction method and time, and solvent used, considering that the content of secondary metabolites in plants is strongly influenced by agroclimatic and post-harvest factors. However, all IC50 values obtained in this study, both from pure extracts and granular formulati*ons (*F1 to F3, remain far below the threshold of the very strong category (<50 ppm), so that the success of this formulation can be used as a scientific basis for the development of functional food products or antioxidant supplements based on red shoot leaves that are more practical, stable, and preferred by consumers.

4. CONCLUSION

Based on the results of the research that has been conducted, several things can be concluded as follows. First, the ethanol extract of red shoot leaves (*Syzygium myrtifolium* Walp.) was successfully pharmaceutically formulated into a granular *effervescent preparat*ion that meets all the required physical quality standards, including organoleptic, flow rate and angle of repose, dissolution time, pH, and water content. Second, variations in the concentration of acid components (citric acid and tartaric acid) and base (sodium bicarbonate) as foaming agents (*effervescent agent*) had a significant effect on the physical characteristics of the resulting granules, including flow rate, angle of repose, and dissolution time, although all formulas remained within the good quality range. Third, the antioxidant activity test using the DPPH method proved that the red shoot leaf extract had very strong antioxidant activity (IC50 18.614 ppm) and this activity was

consistently maintained after being formulated into granules *effervescent*, with IC50 values that vary proportionally to the concentration of the incorporated extract, namely 22.958 ppm in F1 (1.5%), 19.718 ppm in F2 (2%), and 19.459 ppm in F3 (3%), all of which are classified as very strong. Fourth, qualitative phytochemical analysis shows that the red shoot leaf extract contains flavonoids, saponins, tannins, as well as steroids and triterpenoids, while quantitative analysis using the UV-Vis spectrophotometry method with quercetin as a standard shows the total flavonoid content of the extract is 15.533 mgQE/g.

Overall, this study proves that red shoot leaves, which are better known as ornamental plants, have significant pharmaceutical potential as a source of natural antioxidants and can be developed into modern preparations in the form of granules without losing their bioactive efficacy. For further research, it is recommended to identify specific flavonoid compounds using more sophisticated analytical methods, such as LC-MS or HPLC, as well as testing their pharmacological activity *in vivo* in test animals to support further validation of the therapeutic potential of red shoot leaf extract.

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