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EVALUATION OF ANTIOXIDANT AND ANTIFUNGAL PROPERTIES OF PALU SHALLOT (*Allium ascalonicum* L VAR. *Aggregatum*)

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KEYWORDS

Total phenolics

Total flavonoids

Quercetin

DPPH

Candida albicans

Allium ascalonicum

ABSTRACT

Shallot is one of the typical plants at Palu, Central Sulawesi, Indonesia, famous by local people as fried Shallot. It is used as a cooking spice and traditional medicine for treating various diseases. This study was carried out to assess the phytochemical constituent including total phenolics, total flavonoids, and quercetin content of the Palu shallot (*Allium ascalonicum* L var. *aggregatum*), and to determine the antifungal and antioxidant properties of this plant ethanolic extract. Total phenolics/ flavonoids and quercetin concentration were determined by spectrophotometry UV-Vis and Reverse Phase - High-Performance Liquid Chromatography (RP-HPLC) methods. Antifungal activity and antioxidant capacity of the ethanolic extract was assayed by using diffusion agar and 2,2-diphenyl-1-picrylhydrazyl (DPPH) methods. Results of the study revealed that the total flavonoids content of the ethanolic extract was 0.3634 ± 0.018 mg QE/100 mg while total phenolics content was 0.4834 ± 0.003 mg GAE/100 mg. Meanwhile, the quercetin content was 65.46±0.0002 mg/kg. Further, ethanolic extract of Palu shallot also showed the radical scavenging activity with IC_{50} of 0.1398 mg/mL and growth inhibition on *Candida albicans* with inhibitory zone diameter range from 7.57 to 16.51 mm. This study confirms the high quality of Palu shallot as it has high total flavonoids, represented by the high quercetin concentration, and it is proposed to be a source for an antioxidant and antifungal medicinal herb.

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1 Introduction

Shallot (*Allium ascalonicum* Linn.), belonging to the family Liliaceae, is the most widely consumed vegetable in the world and widely used as a food, spice and folk medicine. The bioactive compounds and pharmacological activities such as antimicrobial (Kyung, 2012), antiviruses (Mohamed, 2010), antioxidant (Raeisi et al., 2016), antifungal (Moghim et al., 2014), haematological effects (Owoyele et al., 2004), anticancer and anti-inflammatory (Mohammadi-Motlagh et al., 2011) have been well studied so far. Meanwhile, ascalonicoside A1/A2 and ascalonicoside B (Fattorusso et al., 2002), four sulfur-containing compounds (Ogra et al., 2005), and ascalin (Wang & Ng, 2002) had also been reported from bulbs of Shallot. Besides, the flavonoid fractions of Shallot contained high amounts of free quercetin, glycoside form such as quercetin 4'-glucoside and quercetin 7-glucoside, and isorhamnetin (Fattorusso et al., 2002).

In Central Sulawesi with a dry climate, there is a type of shallot that can grow and produce well. This type of local shallot is known as Palu shallot and has been processed into a ready-to-eat product commonly called Palu fried shallot (Figure 1). Palu shallot has advantages like a dense texture, a savoury taste and an unchanging aroma even though it is stored for a long time. Besides, Palu shallot has many nutrients, including protein, fat, carbohydrates, vitamin A, vitamin E, and a small number of vitamins B (thiamine, riboflavin, niacin, pantothenic acid, and pyridoxine) (Sulfina, 2020).

Quercetin was found as one of the primary compounds in some varieties of Shallot (Sittisart et al., 2017). The antioxidant capacity is always associated with the properties of quercetin because of its ability to capture free radicals and reactive oxygen such as superoxide anions and hydroxyl radicals (Zhang et al., 2011). In addition, flavonoids compounds can also disrupt the cell proteins and shrink the cell walls causing apoptosis induction in several *candida* species (Seleem et al., 2017).

Considering the more advantage of consuming Palu shallot as daily food and the wide cultivation of this plant in Palu and its surrounding area, there is a need for extract material standardization based on phytochemical characteristics and pharmacological activity information. Therefore, this study aims to determine the total phenolics/flavonoids of extract and continuing by determination of its quercetin concentration. The antioxidant and antifungal activities of ethanol extract of bulbs of Palu shallot were also discussed.

2 Materials and methods

2.1 Materials

Fresh bulbs of Palu Shallot (*A. Ascalonicum* var. *aggregatum)* were collected from Soulove village, Sigi Regency (± 25 km from Palu City, Central Sulawesi, Indonesia). The plant identification was done in the Plant Biosystematical Laboratory, Department of Biology, Science Faculty, Tadulako University, Palu, Indonesia where a voucher specimen was deposited. Other commonly used materials are gallic acid, quercetin (≥95% purity), *1,1-difenil-2-pikrilhidrazil* (DPPH), potato dextrose agar (PDA) medium, ethanol 96%, nystatin 0.1%, NaCl 0.9% physiologic solution, sodium nitrite, sodium hydroxide, sodium carbonate, ascorbic acid sodium carbonate, aluminium chloride, Folin-Ciocalteau 50% reagent, *Dimetil Sulphoxide* (DMSO), methanol, methanol pro-HPLC, water pro-HPLC, and aqua dest. All chemicals were purchased from Sigma Aldrich.

Figure 1 Palu shallot (left), ready to eat product of Palu fried shallot (right)

2.2 Extraction

Bulbs of Palu shallot were cleaned of skin, epidermis and leaves and then chop them into smaller pieces. It was dried at room temperature protected from direct sunlight. Around 1 kg of dried bulbs was weighed and then extracted by maceration using 2.5 L of 96% ethanol. The macerator was then tightly closed and stored in a place protected from sunlight for 3 x 24 hours and concentrated using a rotary evaporator until obtained the viscous extract (64.67 g).

2.3 Total Flavonoids Determination

The measurement of total flavonoids were conducted by the aluminium chloride colorimetric method as suggested by Karagiorgou et al. (2016) and Sulastri et al. (2018,)**.** About 10 mg of sample was dissolved in 10 mL ethanol (p.a) and then diluted to 100 µg/mL. Meanwhile, quercetin as standard was also prepared by dissolving 10 mg in 10 mL ethanol p.a (1000 µg/mL) and then diluted to 5, 10, 20 and 40 µg/mL. Sample and each concentration of standard solutions (1:1) were mixed with 0.2 mL 1 M potassium acetate, 0.2 mL 10% aluminium chloride, 3 mL 96% ethanol, and 5.6 mL distilled water. Then, the mixture was incubated at room temperature for 10 minutes. The absorbance was measured by using spectrophotometer UV-Vis Cecil CE7410 at 447 nm, along with a blank solution. Total flavonoid was calculated and resulted in milligrams of quercetin equivalent (QE) per 100 mg extract. The experiment was performed in triplicates.

2.4 Total Phenolics Determination

The measurement of total phenolics was conducted by using Folin–Ciocalteu method as described by Hossain & Rahman (2011) and Sulastri et al. (2018). For this, 10 mg gallic acid as standard was dissolved in 10 mL ethanol p.a (1000 µg/mL). This solution was diluted to obtain standard solutions with the concentration series of 5, 10, 20 and 40 µg/mL. Similarly, 10 mg plant sample was also dissolved in 10 mL ethanol p.a. About 0.5 mL standards and sample solutions were mixed with 50% Folin–Ciocalteu and distilled water (1:1) and added 2 mL of sodium carbonate (7.5%, w/v) after incubation for 5 min. The mixture was then shaken and incubated for 15 min at room temperature. The absorbance of standard and sample solutions were measured by using spectrophotometer UV-Vis Cecil CE7410 at 755 nm. Total phenolic content was calculated and resulted in milligrams of gallic acid equivalents (GAE) per 100 mg extract. The experiment was performed triplicates.

2.5 Quercetin Concentration Determination

The concentration of quercetin on ethanol extract was determined by RP-HPLC. 20 mg dried ethanol extract was dissolved in 10 mL methanol and sonicated for 15 minutes. Similarly, 10 mg quercetin as standard was dissolved in 10 ml methanol and then diluted to obtain the concentration series of 1.2, 2.4, 4.8, 9.6 and 19.2 µg/mL. Samples and standard solutions were filtered through a 0.45 µm millipore filter and then injected into the column (C18 size 250 mm × 4.6 mm) on HPLC Cecil CE4201 with UV visible detector. Methanol: water (90:10, v/v) with parameters: injection volume 20 µL, the flow rate of 1 mL/min, and detection wavelength at 370 nm was used to obtain the optimum efficiency of separation. Quercetin concentration was calculated by linear regression analysis using SPSS 17.0 (SPSS. Inc, Chicago IL, USA).

2.6 Antioxidant Activity Determination

The antioxidant activity of Palu shallot ethanol extracts was determined by using DPPH radical method (Karimi & Moradi, 2015). About 3 mL of both 0.1 mM DPPH solution and ethanol solutions of extracts (concentration series of 50 – 150 µg/mL) were mixed and incubated for 30 min at the darkroom. The decreasing absorbance of the mixture was monitored at 515 nm. Blank sample and vitamin C (concentration series of 1, 2, 4, 6, and 8 µg/mL) as positive control were also prepared and measured at the same wavelength. The experiment was carried out in triplicate. The calculation of the percentage of inhibition was done by the following formula:

$$\%\text{inhibition} = \left(\frac{\text{Absorbance of blank solution} - \text{Absorbance of sample solution}}{\text{Absorbance of blank solution}}\right) x\ 100\%$$

Meanwhile, the 50% inhibitory concentration (IC_{50}) was calculated by probit analysis correlating the extract concentrations against their inhibition percentage.

2.7 Determination of antifungal activity

2.7.1 Test microorganisms

Candida albicans, isolated from candidiasis patient at Palu Health Laboratory Office, were cultured on potato dextrose agar. Incubation was performed at 37°C for 18-24 h.

2.7.2 Disc diffusion assay

The antifungal activity of ethanol extracts of Palu shallot was performed by the well-diffusion method (NCCLS, 2012). *C. albicans* fungi were cultured overnight at 37°C on Potato Dextrose broth. Inoculums consisting of 0.5 McFarland was prepared in physiologic saline. Fungi inoculums in potato dextrose agar medium (2000 µL) was poured in Petri dishes with a Potato Dextrose Agar solid medium (1000 uL) as basis layer. The sample was prepared by dissolving 1 g extract on 1 mL DMSO (1000 mg/mL) and then dilute to 250, 500 and 750 mg/mL. Sterile wells (6 mm diameter) were deposited on medium and impregnated with 50 µL of extract solutions. The plates were inverted and incubated

for 3 x 24 h at 37°C. The negative control was performed with discs containing 50 µL of DMSO and the positive control was nystatin 1 mg/mL. Each experiment was performed in triplicate. The diameters of the clear zone of growth inhibition around each disc were measured and recorded. The scale of measurement (disc diameter included) is as follow: < 12 mm is no inhibitory activity, < 20-12 mm is moderate inhibitory activity; and ≥ 20 mm is strong inhibitory activity (Espina et al., 2011).

3 Results and Discussion

In the Province of Central Sulawesi, particularly in Soulove village, Sigi Regency, there is a local commodity of superior shallots, which is already well known as a source of typical fried onion ingredients with more distinctive taste compared to other shallots in the country. It is known as local Palu shallot or better known as Palu fried shallot. Palu shallot varieties in Soulove village are cultivated traditionally by farmers. Shallot farming has been started for decades, especially around the Soulove village where shallot can adapt well to lowland areas with dry climates (Yusuf et al., 2016).

In this study, phytochemical analysis was performed to measure the amount of total phenolics/flavonoids and quercetin concentration on an ethanol extract of Palu shallot bulbs. The results can be seen in Table 1. Total phenolics was calculated according to equation from gallic acid graph with y = 0.016x + 0.048 (R^2 = 0.998). The total phenolic, expressed as gallic acid equivalents (GA), was found of 0.4834 ± 0.003 mg GA/100 mg of dry extract. Meanwhile, the total flavonoid, expressed as quercetin equivalents (QE), was 0.3634 ± 0.018 mg/100 mg of dry extract obtained from the equation of quercetin graph, y = 0.011x + 0.024 (R^2 = 0.996). *A. ascalonicum* was reported to contain more total flavonoids than other types of onion varieties (Fattorusso et al., 2002). The total flavonoid of ethanol extract of onion (*Allium cepa*) and garlic (*A. sativum*) was reported in the range of 0.015 – 0.02 mg CE/100 mg and the total phenolic of ethanol extract of garlic (*A. sativum*) was 0.6 mg GAE/ 100 mg (Priecina & Karlina, 2013).

Table 1 Phytochemical analysis of ethanol extract of Palu Shallot

Analysis	Ethanol extract
Total phenolics (mg/100 mg) in GAE	0.4834±0.003
Total flavonoids (mg/100 mg) in QE	0.3634±0.018
Quercetin concentration (mg/kg)	65.46±0,0002

Shallot was characterized by the presence of flavonoid compounds of quercetin. Therefore, quercetin concentration in ethanol extract of Palu shallot bulbs was conducted by using RP-HPLC. The quercetin concentration on the extract was quantified based on the obtained quercetin standard calibration curve, y = 9.368x + 11.82 (R^2 = 0.993). HPLC chromatogram showed that a peak for quercetin on the ethanol extract can be comparable with the peak of quercetin standard with the retention time (RT) of 2.53 (Figure 2). Using calibration curve plotting between concentration and peak area, Palu shallot was found to contain quercetin of 65.46±0.0002 mg/kg.

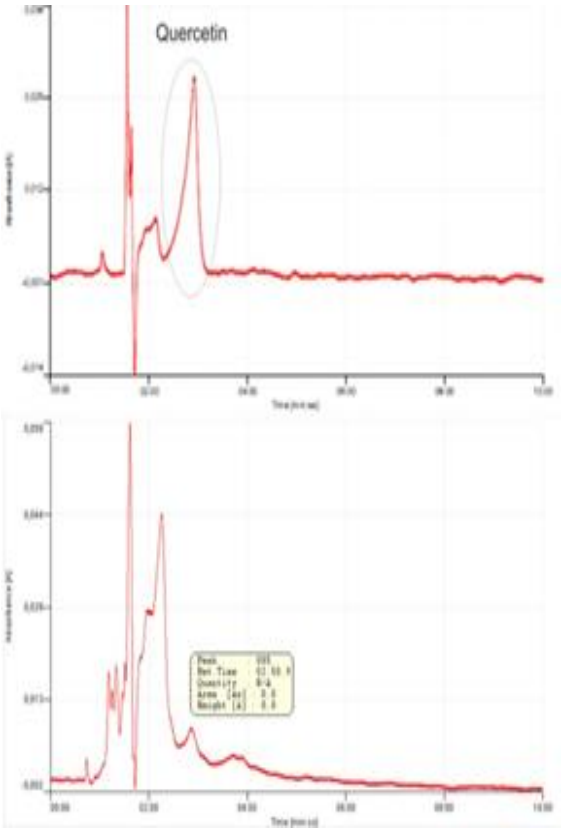

Figure 2 HPLC chromatogram of ethanol extract of Palu shallot bulbs.

This is the first report of the concentration of quercetin on Palu shallot. Pobłocka-olech et al. (2016) had studied the quercetin level on Onion (*A. cepa*) and Shallot (*A. ascalonicum*) from Polland and found that the edible part of onion only contains 11, 17, and 24 mg/kg for gold onion type red baron, amstrong, and exhibition, respectively and for shallot with types of ambition and matador did not contain quercetin (Pobłocka-olech et al., 2016). The quercetin level of red onion was reported as 30.0±0.00 mg/kg (Kwak et al., 2017). This study supports the high quality of Palu Shallot according to quercetin content on ethanol extract.

Further study was continued to assay the antioxidant and antifungal activities. The DPPH antioxidant activity test of ethanol extract showed the percentage of inhibition of 33.50%, 44.27%, and 54.45% at concentration series of 100, 125, and 150 µg/mL, respectively (Figure 3). Moreover, it also inhibited the growth of *C. albicans* at concentrations of 250, 500, 750, and 1000 mg/mL with the inhibitory zone diameter of 7.57 mm, 9.88 mm, 14.39 mm, and 16.51 mm, respectively (Figure 4). Acheampong and colleagues (2016) have reported that total phenolics have a correlation with the antioxidant activity of several vegetables, including *A. ascalonicum*. The higher phenolic content will affect the higher antioxidant capacity. The methanol extract of *A. ascalonicum* was reported to contain a total phenolic of 0.124 mg TAE/DW, and DPPH scavenging activity was 2.2708 mg/mL (Acheampong et al., 2016). Comparing this study, the ethanol extract of Palu shallot also showed high antioxidant activity where the IC_{50} was 0.1398 mg/mL (Table 2).

Table 2 IC_{50} of ethanol extract of Palu Shallot

Sample	IC_{50} (mg/mL)
Ethanol extract	0.1398
Vitamin C	0.0037

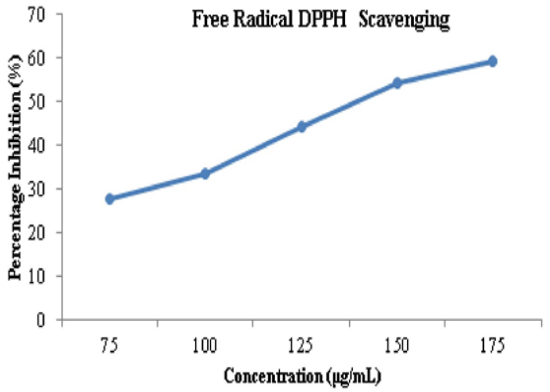

Figure 3 Antioxidant activity of ethanol extract of Palu shallot

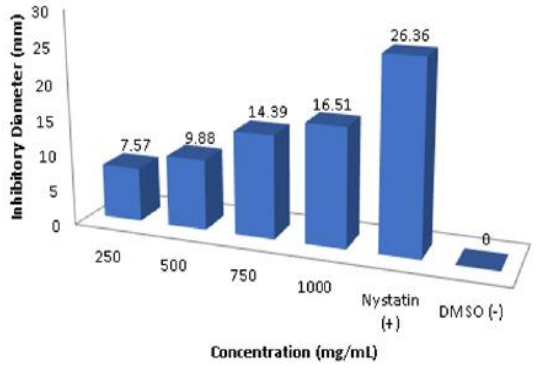

Figure 4 Inhibitory zone diameter (mm) of ethanol extract of Palu shallot against *C. albicans*

Shallot was reported to have the highest antifungal activity against the *C. albicans*. The minimum fungicidal concentration (MFC) of its ethanol extract on *C. albicans* was 20 mg/ml (Moghim et al., 2014). It is also found that shallot has more effect on saphrophyte than *C. albicans* (Mahmoudabadi & Naser, 2009). In this research, Palu shallot showed antifungal inhibition on all concentrations used with the range of inhibitory zone diameter range from 7.57 to 16.51 mm. The result is following the previous reports regarding the antifungal activity of Shallot. Wang & Ng (2002) reported ascalin as the main compound that responsible for the inhibition of mycelial growth in several fungi.

This study supports the application of Palu shallot as a potential natural antioxidant and antifungal agent based on total phenolics, total flavonoids, and quercetin content. Although it has been used as a food flavor in daily seasoning, it has also been widely applied as a raw material in the food industry (snacks production and cooking seasoning) (Sun et al., 2019). It is suggested to develop Palu shallot as raw material for drug formulation or as functional food products.

Conclusion

Palu shallot was analyzed for total phenolics, total flavonoids, and quercetin content with the amount of 0.4834±0.003 mg/100 mg GAE, 0.3634±0.018 mg/100 mg QE, and 65.46±0.0002mg/kg, respectively. Examination of DPPH scavenging activity and agar diffusion methods showed antioxidant activity with the IC_{50} of 0.1398 mg/mL and growth inhibition of *C. albicans* at all concentrations. This study supports the potency of local Palu shallot to be an antioxidant and antifungal medicinal herb.

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Conflict of Interest

The authors declare no conflict of interest

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