Journal of Applied Pharmaceutical Science

Available online at: https://japsonline.com

The UHPLC-HRMS profiling, *in vitro*-antioxidant and pancreatic lipase inhibitory activities of *Peronema canescens* leaves extract and fractions from Indonesia

Ade Erma Suryani[1,2], Khoirun Nisa[1], Sri Handayani[1], Cici Darsih[1], Hastari Wuryastuty[3], Yanuartono Yanuartono[3]

[1]Research Center for Food Technology and Processing, National Research and Innovation Agency (BRIN), Yogyakarta, Indonesia.

[2]Doctor of Veterinary Science Program, Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia.

[3]Department of Veterinary Internal Medicine, Faculty of Veterinary Medicine, Gadjah Mada University, Yogyakarta, Indonesia.

Doi: http://doi.org/10.7324/JAPS.2025.214965

SUPPLEMENTARY MATERIAL

DPPH radical scavenging assay

The free radical scavenging capacity of the extract samples and all fractions of *P. canescens* was determined *in vitro* by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, with the procedure described by Mahmood et al [20] with slight modifications. Twenty microliters of each extract and fraction, as well as the standard solution, were placed into a 96-well microplate and then added with 180 microliters of DPPH reagent solution. The mixture solution was incubated for 40 minutes at ambient temperature while being shielded from any exposure to light. Following the incubation period, the absorbance at a wavelength of 517 nm was calculated using the microplate reader (Multiskan Go, Thermo ScientificTM). A standard curve was established using ascorbic acid as a positive control, covering a concentration range of 1–30 µg/mL. The antioxidant activity was calculated using the following formula to obtain the percentage of DPPH radical capture:

$$\%DPPH\ radical\ scavenging\ effect = [\frac{(Abs.control - Abs.sample)}{Abs.control}] \times 100$$

Next, the scavenging capacity was assessed by calculating the amount of DPPH that was scavenged compared to the concentration of the antioxidant standard reference. The calculation of linear regression was used to determine the half-maximal inhibitory concentration value (IC_{50}) of the extract.

ABTS radical scavenging assay

The formation of the $ABTS^{\cdot+}$ compound involved the chemical combination of the ABTS reagent with potassium sulphate in a 1:1 proportion, and the mixture was then stirred overnight. In a 96-well microplate, 180 microliters of $ABTS^{\cdot+}$ working solution were combined with 20 microliters of extract and fractions. After mixing, the solution was incubated at room temperature without the presence of light for 15 minutes. After the incubation period, the absorbance at a wavelength of 734 nm was measured using the microplate reader. Trolox standard by concentration range of 5-120 µg/mL was employed to generate a calibration curve. The scavenging activity percentage was determined by this equation:

$$\%ABTS\ radical\ scavenging\ effect = [\frac{(Abs.control - Abs.sample)}{Abs.control}] \times 100$$

Next, the scavenging activity was established by calculating the percentage of $ABTS^{\cdot+}$ particles scavenged towards the standard concentration of antioxidants. The value of IC_{50} from the sample extract and fraction was determined by the method of linear regression.

Ferric-reducing antioxidant power (FRAP) assay

Twenty microliters of either the sample of extract/fraction or a solution of the standard were put into a 96-well microplate. Trolox was used as standard. This microplate was then added 180 µL of a FRAP solution. This solution is a combination of different

components. It includes an acetate buffer with a pH of 3.6, along with 2,4,6-tripyridyl-s-triazine in hydrochloric acid and ferric chloride hexahydrate. The components are mixed in ratios 10:1:1 to create the FRAP solution. The reaction mixture in the microplate was incubated at 37°C for 30 minutes under light-free conditions. Following the incubation period, the absorbance of the solution was quantified using a microplate reader at a wavelength of 593 nm. A standard curve was generated using Trolox solution at 40 to 200 µg/mL concentrations. The data were presented as milligrams of Trolox equivalent (TE) per gram plant of extract or fractions.