Chemical Composition and Antioxidant Activities of Extracts of *Combretum quadrangulare* Kurz Leaves Grown in An Giang Province, Vietnam

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Abstract. *Combretum quadrangulare* leaves are an important herbal in traditional Vietnamese medicine due to their ability to cure various diseases and improve health. This study provided the investigation results of different organic solvents that affected the chemical composition, the extraction of polyphenol and flavonoid content, the antioxidant activity of *Combretum quadrangulare* extract obtained through the maceration method. Several classes of constituents in the extract of *Combretum quadrangulare* leaves were detected including flavonoids, volatile oils, tannins, etc. The ethanol extract had the highest polyphenol content found at 18.45 ± 0.12 mg GAE/g extract. The diethyl ether leaves extract observed the highest falonoid with a value of 21.19 ± 0.05 mg QE/g in. The antioxidant activities were shown through the IC₅₀ values of 1514.50 ± 25.65 µg/ml (DPPH) and 685.15 ± 8.58 µg/ml (ABTS), individually.
1. Introduction
For the treatment of various diseases, natural products such as herbs or their extracts play a very important role [1, 2]. Herbs are broadly grown in many parts of the world, especially in tropical and subtropical countries [3]. *Combretum quadrangulare*, commonly known as "tram Bau" (Vietnamese) is a medicinal herb commonly used in traditional medicine [4]. The secondary metabolites in the plant have been proven to be very diverse and abundant by many studies. In Thailand and other countries, various parts of *Combretum quadrangulare* have been purposed for traditional use such as anti-inflammatory, anticancer, and improve health. Previous researches demonstrated that the roots and seeds of *Combretum quadrangulare* had a good alpha-glucosidase inhibition, against methicillin-resistant *Staphylococcus aureus* (MRSA) and extended-spectrum beta-lactamase-producing *Escherichia coli* (ESBL-producing *E. coli*). [5]. Herbal and medicine may cure Reactive Oxygen Species (ROS) compounds, which are responsible for many diseases. The human body regularly produces unstable molecular fragments called free radicals. These radicals destroy cells, tissues, organs of the body, and cause blockage of arteries, develop diseases such as cancer, inflammation, and hundreds of other diseases that cause rapid aging and death of the body [6]. Traditional herbal plants often contain lots of antioxidant compounds which are capable of neutralizing free radicals such as polyphenols, flavonoids, carotenoids, vitamins, etc. They can protect the human body against free radical disorders by promoting scavenging free radicals, inhibiting invasion, and killing tumor cells [7, 8]. Recently, *Combretum quadrangulare* has gained public attention in cultivation due to the leaves’s essential oil [9]. Not only do essential oils have applications, but leaves extract also has many pharmacological effects. For example, in the study of Chittasupho et al., the leaves extract encapsulated in nanoparticles were cytotoxic to the human A549 lung cancer cell line [10]. In the research of Nguyen Huu Hung et al (2021), combretanones G and H from *Combretum quadrangulare* exhibited moderate cytotoxicity against leukemia, carcinoma, and cancer cell lines [11]. To provide more scientific evidence on the chemical composition and antioxidant activities of *Combretum quadrangulare*, this study was conducted.

2. Materials and Methods
2.1 Plant extraction
In February 2019, leaves of *Combretum quadrangulare* were harvested in An Giang province, Vietnam. After being washed, the raw materials were dried at 45°C - 50°C until the moisture content was below 10%. Next, the material was finely ground to a size < 0.5 mm. The dried leaves powder of *Combretum quadrangulare* was extracted according to the procedure shown in Figure 1. First, the powder was extracted by soaking at room temperature with diethyl ether for about 24 hours. Next, the mixture was filtered to obtain the extract. It was evaporated to recover the solvent and the dried diethyl ether extract. The upper residue was further soaked in
ethanol 96% following the same procedure to obtain the dried alcohol extract. After that, the residue was also hot-extracted by reflux with distilled water for 30 min at 80 ± 5°C. Finally, the mixture was filtered and evaporated to obtain the dried aqueous extract.

![Figure 1. The extraction process of three types of extracts from Combretum quadrangulare leaves](image)

2.2 Procedures
2.2.1 Phytochemical scavenging
The active ingredient groups in each extract were determined by specific reactions or reagents, presented in studies of Takaidza et al and Purushotham et al [12, 13]. When only one of the three extracts was positive, the leaves material was considered to contain these compounds.

2.2.2 Determination of total polyphenol content (TPC)
All extracts were diluted with alcohol 96%, then filtered to obtain a solution for evaluation. A sequential evaluation of each extract was conducted. Accurately pipetted 1 mL of extract. Next, added 5 mL of Folin-Ciocalteu 10% reagent, shaken well, and waited for a reaction in 5 minutes. After the above time, added in 4 mL of Na₂CO₃ 20% (w/v), mixed well, and incubated for 30 minutes. All the above operations were done in a protected from the light condition. Finally, measured the absorbance with a spectrophotometer at 765 nm [1, 14]. Calculation results were presented as milligram gallic acid equivalents per gram dry sample (mgGAE/g).
2.2.3 Determination of total Flavonoid Content (TFC)

Similar to the method for determination of total polyphenol content, the extract was evaluated as a dried extract diluted with alcohol 96%. First, accurately aspirated 0.2 mL of 10% AlCl$_3$ and 0.2 mL of 1M CH$_3$COOK. Mixed 1.0 mL of the extract into the above mixture and incubated in the dark for 5 min. Then, added 8.6 mL of distilled water, well shaken, continue incubation, and protected from light for a half of hour. Absorbance was measured spectrophotometrically at 415 nm and calculated based on the equivalent quercetin standard [15].

2.2.4 DPPH Scavenging Activity

Pipetted exactly 1500 μL of DPPH working solution (OD 517 nm = 1.1 ± 0.02) into a test tube containing 500 μL extract. Allowed the mixture to stabilize protected from light for half of hour. The spectrophotometer of the mixture was measured 517 nm. The blank sample was a solution in which the extract was replaced with 96% alcohol [14]. The percentage of DPPH scavenging activity or percentage inhibition concentration (% IC) was calculated:

\[
% \text{ IC} = \frac{(\text{Ab-As})}{\text{Ab}} \times 100 \quad (1)
\]

Note: Ab - Absorbance of the blank sample, As - Absorbance of the sample, % IC – Percentage of DPPH scavenging activity.

2.2.5 ABTS scavenging activity

First, by adding 20 mL of K$_2$S$_2$O$_8$ 2.6 mM to 20 mL of ABTS 7.4 mM solution, the ABTS stock solution was prepared. After 15 hours, the working ABTS solution was ready by adding 2 ml of stock solution to 120 ml of distilled water and adjusting accordingly to obtain an ABTS working solution (OD$_{734\text{nm}}$ = 1.1 ± 0.02). Prepared the spectrometric sample by adding 2 mL of sample to 6.0 mL of ABTS working solution. For an half hour, the above mixture was incubated protected from light. Finally, the mixture was measured using a UV-VIS spectrophotometer at 734 nm [16]. The percentage of ABTS scavenging activity was determined by the formula:

\[
% \text{ IC} = \frac{(\text{Ab-As})}{\text{Ab}} \times 100 \quad (2)
\]

Note: Ab - Absorbance of the blank sample, As - Absorbance of the sample, % IC - Percentage of ABTS scavenging activity.

2.2.6 Statistical analysis

Three replicate results of each experiment were entered using SPSS software IBM version 26 and Microsoft Excel (Office 365). Compare mean values using Anova and post-test as LSD to find out the difference of results in the study.
3. Results and Discussions

3.1 Evaluation of chemical components

Table 1. Phytochemical results of leaves of Combretum quadrangulare in three different solvents

| Solvents Tests | Diethyl ether extract | Ethanol extract | Aqueous extract | Conclusion |
|----------------|----------------------|-----------------|-----------------|------------|
| Alkaloids      | +                    | +               | +               | +          |
| Anthocyanosid  | +                    | +               | +               | +          |
| Anthraglycosides| +                    | -               | +               | +          |
| Cardiac glycosides| +                    | -               | +               | +          |
| Coumarins      | +                    | +               | +               | +          |
| Flavonoids     | +                    | +               | +               | +          |
| Lipid          | +                    | +               | +               | +          |
| Organic acid   | +                    | +               | +               | +          |
| Polyphenols    | +                    | +               | +               | +          |
| Polysaccharide | +                    | +               | +               | +          |
| Prolactoside   | +                    | +               | +               | +          |
| Reducing sugar | +                    | +               | +               | +          |
| Saponins       | +                    | +               | +               | +          |
| Tannins        | +                    | +               | +               | +          |
| Triterpenoids  | +                    | +               | +               | +          |
| Volatile oils  | +                    | +               | +               | +          |

Legend: 
(-) Absent 
(+) Present 
(+-) In doubt 
May have a reaction but do not perform
There is no presence of this compound

Phytochemical composition in each extract of Combretum quadrangulare's leaves was conducted analysis and recorded the results in Table 1. The raw powder was respectively extracted with three solvents of increasing polarity, diethyl ether, ethanol, and water to obtain groups of compounds with different polarities. As long as one of the three solvents contained a group of active ingredients, the leaves of plant A were concluded to have that. Similar to the study of Ly Hai Trieu et al, the analysis results showed that the plant contained many diverse compounds such as alkaloids, flavonoids, saponins, tannins, etc [4]. Among the extracts, the ethanol extract was the extract containing the most groups of compounds (9 groups).

3.2 Total polyphenol and flavonoid content
Polyphenols compounds in herbs have the ability to neutralize free radicals [17]. Medicinal plants with high polyphenol content are often appreciated. Because
those are potential sources not only for the production of dietary supplements but also widely applied in cosmetics, food, and pharmaceuticals. Figure 2 showed the total polyphenol and flavonoid content in the three extraction solvents with an increasing polarity like diethyl ether, ethanol 96%, and water. The ethanolic dried extract contained the highest polyphenol content with a value of 18.45 ± 0.12 μg GAE/mg. The highest flavonoid value was 21.19 ± 0.05 μg QE/mg of diethyl ether dried extract in *Combretum quadrangulare* leaves.

![Figure 2. The contents of polyphenol and flavonoid in *Combretum quadrangulare* leaves](image)

3.3 Antioxidant activity

Herbal plants contain antioxidant compounds, which have the ability to partially neutralize free radicals, thereby reducing the risk of chronic diseases and slowing down the aging process. With the diversity of active ingredient groups in the plant, only assessing the antioxidant capacity based on the content of a specific group will not give a comprehensive view. To estimate the antioxidant activity of compounds, various methods are available such as free radical neutralization assays, hydrogen atom transfer, metal chelation, and so on [18]. Among that, DPPH and ABTS assays are quick and low-cost methods [19]. They are widely used to evaluate the antioxidant activity potential of different natural herbs. The IC$_{50}$ values of extracts by DPPH and ABTS scavenging activities were illustrated in Figure 3. The results showed that the ethanolic extract had the highest antioxidant activity among the three extracts with a value of 1514.50 ± 25.65 μg/ml (DPPH) and 685.15 ± 8.58 μg/ml (ABTS).
4. Conclusions
The dried leaves powder of *Combretum quadrangulare* was alternately extracted through three solvents with increasing polarity. The results showed that *Combretum quadrangulare* leaves contained a variety of compounds, such as flavonoids, volatile oils, tannins, etc. The leaves extracts of *Combretum quadrangulare* content 18.45 ± 0.12 mg GAE/g (polyphenol) and 21.19 ± 0.05 mg QE/g (flavonoid). One of the therapeutic effects of *Combretum quadrangulare* leaves was evaluated as an antioxidant activity through DPPH and ABTS methods. This shows that this is a potential source of medicinal herbs with many therapeutic effects.

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