Preliminary test and antioxidant activity of the *Coptosapelta flavescens* Korth's root extract

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Abstract. The purpose of this study was to examine the phytochemical content and antioxidant activity of *Coptosapelta flavescens* Korth root extract obtained from various places. Phytochemical screening, antioxidant activity testing using DPPH radical reduction, color reaction (ABTS), iron reduction (FRAP), reduction capacity technique (RP), and total antioxidant capacity (TAC). The phytochemical tests revealed that C. *flavescens* water and ethanol extract included alkaloids, coumarins, terpenoids, tannins, and flavonoids. The antioxidant activity results revealed that raw material samples gathered directly in the wild in Ninh Son district (Ninh Thuan province, Vietnam) were more effective than those purchased at the local market with EC50 times value. DPPH, ABTS, FRAP, RP, and TAC techniques yielded 56.02 µg/ml, 54.12 µg/ml, 15.9 µg/ml, 44.85 µg/ml, and 110.94 µg/ml, respectively. The antioxidant impact of raw material samples obtained in Bac Ai district (Ninh Son province, Vietnam) was lower but overall better than that of samples gathered at the local market.

1 Introduction

Coptosapelta flavescens is a species of *Coptosapelta flavescens*. Korth (C. flavescens) or *Coptosapelta tomentosa* Valeton ex K. Heyne is an endemic coffee (Rubiaceae) plant found in Southeast Asia [1, 2]. They are woody vines that can grow to be 5-7m long or more. The

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branches are spherical and dark brown, with hairs on the surface when young. The leaves are opposite, with extremely short stalks (3-12mm), a green upper surface, a pale underside, and veins that are hairy. The cut roots have a strong, disagreeable odor [3].

Previous research on the chemical makeup of C. *flavescens* species has revealed that it contains components such as steroids, phenolics, and flavonoids [4]. C. *flavescens*, in fact, has long been used in folk medicine to alleviate coughs and shortness of breath [5]. The root of this plant is used to cure helminthic infections and colic in Malaysia [6, 7]. Furthermore, Wipapan Kongyen et al. identified four Anthraquinone derivatives and one Naphtoquinone derivative from C. flavescens root acetone extract, 1,4-dimethoxy-2-methylanthraquinone (4), 2-amino-. 3-methoxycarbonyl-1,4-naphthoquinone (5), 1-hydroxy-2-hydroxymethyl-anthraquinone (6), 1-hydroxy-2-methoxycarbonyl-anthraquinone (7), and 2-methoxycarbonyl-anthraquinone (8). Simultaneously, antibacterial activity (with *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus*), dysentery treatment, and cytotoxicity (on kidney cancer cell line - Vero cell) demonstrated that derivative (6) has active Giardiaintestinalis inhibitory activity with a MIC value of 2.5 µg/ml, which was equivalent to that of a positive metronidazole control [9].

The phytochemicals found in plants influence their biological effects. However, the findings to now have lacked a comprehensive data set on phytochemical components and their influence on C. *flavescens* antioxidant activity. As a result, the goal of this study was to analyze the phytochemical composition and antioxidant potential of C. *flavescens* using various methodologies.

2 Material and method

2.1 Material

Coptosapelta flavescens Korth (CF) root was gathered in September 2020 in Ninh Thuan province, Vietnam, for the study. The coding symbols are “CF1” for samples collected in Ninh Son district, “CF2” for samples collected in Bac Ai district, and “CF3” for samples purchased in locally market. The Oriental Medicine Association of Ninh Thuan province, Vietnam, identified and validated plant samples.

After being gathered from local forests, CF’s roots will be preliminarily cleaned, removing excess leaves, cutting into small pieces, and then finely powdered to acquire raw materials for the extraction process. Next, extract the raw components of CF’s root powder three times with a water solvent at 100^{o}C for 48 hours each time. The extraction solution should then be combined, and the solvent should be recovered to a moisture content of 10%. To achieve a sample of uniform size, the product is ground and sieved through a mesh sieve (d = 0.5mm). For analysis, this material was vacuum packed and stored at -20°C.

2.2 Phytochemical tests

Screening of the above six selected medicinal plants for various phytochemical constituents were carried out using standard methods [10, 11] as described in Table 1.

Table 1. Standard methods for various phytochemical constituents

Phytoconstituents	Test	Observation
Alkaloids (Hager's Test)	2ml extract + few drops of Hager's reagent	Yellow precipitate
Saponins (Foam Test)	5ml extract + 5ml H_2O + heat	Froth appears
Anthraquinones (Borntrager's Test)	3ml extract + 3ml Benzene + 5ml NH_3(10%)	Pink, Violet or Red coloration in ammonical layer
Coumarins	2ml extract + 3ml NaOH (10%)	Yellow coloration
Steroids (Salkowski Test)	2ml extract + 2ml CHCl3 + 2ml H_2SO_4 (conc.)	Reddish brown ring at the junction
Terpenoids	2ml extract + 2ml $(CH_3CO)2O$ + 2-3 drops conc. H2SO4	Deep red coloration
Flavonoids	1ml extract + 1ml Pb(OAc)4 (10%)	Yellow coloration
Tannins (Braymer's Test)	2ml extract + 2ml H_2O + 2-3 drops $FeCl_3$ (5%)	Green precipitate
Reducing compound	0.5 ml Fehling A + 0.5ml Fehling B.	Brick red precipitate

2.3 Determination of antioxidant activity Using a radical scavenging process (DPPH)

A modified radical scavenging approach of DPPH was used for creating antioxidants for the CF extract [12]. The mixture of reactants was 40 µL DPPH (1000 µg/mL) and the extract was 960 µL. At 30°C for 30 minutes, the reaction mix has been incubated in the dark. Then DPPH was spectrally absorbed at 517 nm.

2.4 Antioxidant Activity Determination Using the ABTS Free Radical Scavenging Method

ABTS•+ decolorization method published by Nenadis et al. [13] assessed antioxidant activity. ABTS• + is made of potassium persulfate reacted to 7 mM ABTS with 2.45 mM. At room temperature 12-16 hours prior to use, the mixture was incubated in the dark. The blend was diluted to achieve an optical density of 0.70±0.05 to 734 nm. Carry out the examination at room temperature for six minutes by reacting 10 µL of extract to 990 µL ABTS•+. Then, for absorbance spectroscopy at 734 nm the reaction mixture is measured.

2.5 Antioxidant Activity Measurement Using the Ferric Reducing/Antioxidant Power (FRAP) Method

A modified FRAP reduction capacity has been used to test the antioxidant capability of radicular extracts of CF [14]. This approach was based in principle on reducing the complex of ferric-tripyridyltriazine. The FRAP solution (990 µL) for 30 min under dark circumstances interacted with various extracted quantities (10 µL). The optical density of 593 nm was established.

2.6 Determination of Antioxidant Activity Using the Reducing Power (RP) Method

According to the Oyaizu method the reduction power of CF root extracts was done [15]. The mixture of the reaction was 500 µL, 500 µL phosphate buffer (0.2 M, pH = 6.6) and 500 µL K_3FeN_6 1% correspondingly. After incubation of the reaction mixture at 50°C for 20 minutes, CCl3COOH was added at 10 % to 500 µL and centrifuged for 10 minutes at 3000 rpm. The aliquot was drawn 500 µL into 500 µL water after centrifugation and 100 µL of $FeCl_3$ 0.1% well shaken. The spectral absorption was measured at 700 nm of the reaction mixture.

2.7 Determination of total antioxidant capacity (TAC)

Prieto et al. [16] used the phosphomolybdenum technique to assess the total antioxidant activity of CF extracts. Extracts of various concentrations (300 L) were mixed with 900µL of test solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). For 90 minutes, the reaction solution was incubated at 95°C. After cooling to ambient temperature, the reaction mixture was measured at 695 nm.

2.8 Statistical analysis of data

Statgraphics Centurions 18.1.12 and Microsoft Excel 365 were used to do statistical analysis on the findings. To establish the difference between treatments, the analysis of variance (ANOVA) and the LSD test were performed. The findings are shown as mean SEM (standard error of mean).

3 Result

3.1 Preliminary phytochemical

Secondary metabolites are evidence of plants' chemical response to a variety of adverse environmental effects. Furthermore, they act as chemical agents that assist plants in defending, defending, or attacking microbes, insects, or higher herbivores [17]. Furthermore, their presence influences the therapeutic and pharmacological properties of plants [18].

The extract from Khai vine stem was submitted to preliminary phytochemical screening in this study to identify the presence of Alkaloids, saponins, anthraquinones, coumarins, steroids, triterpenoids, reducing chemicals, flavonoids, and tannins in the environment. Table 1 displays the findings of a phytochemical analysis of CF samples. First, alkaloids were identified in both solvents (water and 96 % ethanol) in three CF samples. Alkaloids are naturally occurring nitrogenous chemical molecules with antibacterial potential [19]. This is similar with recent findings in which CF was found to be more efficient than metronidazole against *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus, E. histolytica*, and *G. intestinalis* [20-22].

Saponins, coumarins, steroids, and flavonoids were found in all three CF samples in the aqueous solvent. For 96% ethanol, the presence of reducing chemicals and flavonoids was detected in two samples, CF1 and CF2. Concerning the biological usefulness of these chemicals, various prior studies have shown that flavonoids constitute the principal group of phenolic compounds that function as antioxidants; additionally, anti-inflammatory, anticancer, and anti-infective actions have been found [23]. Only the presence of alkaloids,

triterpenoids, and polyphenols was detected in sample CF3. Triterpenoids, in particular, a compound used to treat pain, lower fever, protect the liver, calm, and prevent diabetes, were discovered in samples CF1, CF3 (aqueous solvent) and CF2, CF3 (alcohol solvent) [24]. When alcohol solvents were utilized, saponins, anthraquinone, and steroids were not identified in any of the CF samples. Kosala et al. (2019) discovered that CF contains phytochemicals such as polyphenols, saponins, and tannins, as well as demonstrating the relationship of these components with anti-inflammatory potential. Subjects' robustness in comparison to the medication indomethacin [25].

Table 2. Phytochemical test results of C. flavescens's root extract

Compounds	**Aqueous**			**Ethanol 96%**		
	CF1	CF2	CF3	CF1	CF2	CF3
Alkaloids	+	+	+	+	+	+
Saponin	+	+	+	+/–	–	–
Anthraquinons	–	+	+	+/–	+/–	–
Coumarins	+	+	+	+	–	–
Steroid	+	+	+	+/–	–	–
Terpenoids	+	–	+	–	+	+
Reducing compounds	+	+	–	+	+	–
Flavonoids	+	+	+	+	+	–
Tannins	+	+	–	–	+	–
Note: (–): Absent (+/–): In doubt (+) Present						

In general, while completing phytochemical analyses on all three CF samples, ethanol-soluble samples showed no or very little reaction. It is clear from the preceding preliminary screening method that there are variances in the chemicals discovered in two different solvents, water and ethanol. The results in Table 1 showed that utilizing water as the solvent for maximum phytochemical extraction was more efficient than ethanol. This could be because the polarity of the solvent influenced phytochemical extraction during the examination [23]. Indeed, the study of Kokkaiad et al. revealed the presence of most of the substances such as alkaloids, flavonoids, phenolics, saponins, tannins, and terpenoids that only appeared in the methanol extract of D. pentagyna, while acetone extracts only recorded phenolic expression and did not detect any phytochemicals in ethylic extracts [26]. Polar solvents have been demonstrated to be more effective than semi-polar or non-polar solvents at extracting phytochemicals from plant materials [27]. The results in Table 2 show that CF has a wide range of phytochemical components, although there are variances across samples that can be influenced by origin and timing variables gather raw materials.

3.2 The antioxidant capacity of CF's root extract

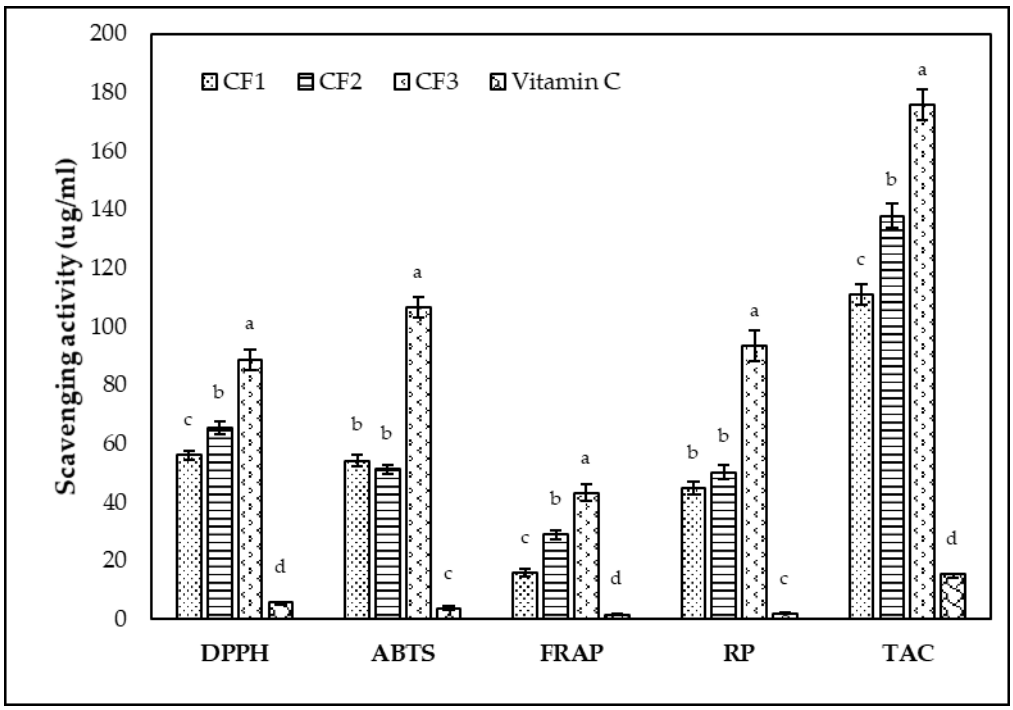

Fig. 1. Antioxidant activity of CF samples at different test methods

Antioxidant activity (DPPH, ABTS•+, RP, FRAP, and TAC) of CF is assessed by measuring its ability to neutralize or decrease free radicals formed in the reagents. Distinct measurement methods can be employed to quantify the antioxidant activity of plant extracts; in practice, at least two different methods should be utilized to be objective and successful in comparing results [28, 29]. Figure 1 depicts the antioxidant capacity of CF extracts as evaluated by the DPPH, ABTS•+, RP, FRAP, and TAC test techniques.

The chemical DPPH contains a proton moiety and exhibits a distinctive violet color with maximum absorbance at 517 nm that gradually declines as the proton moiety is neutralized. The greater the bleaching action, the greater the antioxidant activity, resulting in a lower EC_{50} [30]. Many studies have linked the flavonoid content and their free radical scavenging action to this feature of DPPH [31]. CF1 extract had a stronger antioxidant capacity than the other samples, with an EC_{50} value of 56,029 µg/ml, although it was still 9.8 times worse than the normal vitamin C substance (5,671 µg/ml). All three CF samples had stronger antioxidant activity than *Coptosapelelta tomentosa* (Blume) of Bohari et al report, which had previously been measured (EC_{50} = 93,166 µg/ml) [32]. Concerning the antioxidant activity as measured by ABTS, the molecule is oxidized by oxidizing agents to form the dark blue ABTS•+ cation. The ability to decolorize the antioxidant contained in CF from the reaction of ABTS•+ radicals and the extract is used to determine antioxidant capacity in this approach [33]. The difference in EC_{50} values between CF1 and CF2 was not statistically significant ($p>0.05$), with EC_{50} values of 54.12 µg/ml and 51.27 µg/ml, respectively. When compared to vitamin C, the antioxidant effect of the CF3 sample was superior (EC50 = 3.70 µg/ml). When comparing *Mitragyna speciosa* species (of the same Rubiaceae family) to CF, the recorded value of this species is greater, indicating that its antioxidant capacity is lower than CF [34]. This could be explained by the fact that C. *Flavescens* root extract has a high concentration of hydrogen donors, resulting in lower free radical generation and decolorization in both the DPPH and ABTS assays. In a recent

investigation, we found that the product "Cao Khai" made from CF roots had good DPPH and ABTS free radical scavenging activity [35].

For the quantitative approach of oxidation resistance using reduction energy (RP), sample CF3 had the highest EC_{50} value of 93.34 µg/ml among the three tested samples (oxidative resistance was poor). The antioxidant capacity of all three CF samples was shown to be similar using the FRAP and TAC techniques. CF1 had the best iron reduction ability (EC_{50} = 15.9 µg/ml) while CF3 had the worst (EC_{50} = 43.11 µg/ml). The FRAP test determines the potential of phenolic antioxidant compounds to diminish the blue hue of the iron 2,4,6-tripyridyl-s-triazine complex [Fe3+-(TPTZ)2]3+. In an acidic media, a dark blue iron complex [Fe2+ - (TPTZ)2]2+ [36]. The iron-reducing capacity of CF in this investigation was greater than that of *Catunaregam tomentosa*, *Haldina cordifolia, Mitragyna diversifolia*, and *Mitragyna rotundifolia* (all in the Rubiaceae family) reported by Suksungworn et al (2021) [37]. Overall, sample CF1 had the highest antioxidant value across all five test techniques. This discrepancy may be due to the existence of phenolic components in each type of raw material; also, the influence of the collection, processing, and extraction procedure is a factor affecting the potential of CF to have innate antioxidant capability.

4 Conclusion

C. *flavescens*'s root material was gathered from three distinct locations and tested for phytochemical content and antioxidant activity using various methods in this study. Based on the findings, we infer that C. *flavescens*'s root extract is high in phytochemicals and has strong antioxidant activity. This is a useful and potentially medicinal herb that could become a component of the food chain to aid in human health care.

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