Phytochemical screening and antioxidant potential of crude drug “Cao Khai” in Ninh Thuan Province, Vietnam

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Abstract. For a long time, the ethnic minorities in Ninh Thuan province, Vietnam have mixed Coptosapelta tomentosa Rubiaceae with other types of medicinal herbs to create an esoteric remedy called “Cao Khai”. This study had provided scientific evidence on phytochemical screening and antioxidant potentials of diethyl ether, ethanol and aqueous extracts of “Cao Khai”. Phytochemical screening of crude "Cao Khai” extracts showed the presence of multiple components such as saponins, flavonoids, volatile oils, triterpenoids, organic acids, anthraquinon, coumarin, reducing compounds and polyuronid. The polyphenols and flavonoids content were determined using gallic acid and quercetin as a standard, respectively. 1,1-diphenyl-2-picryl-hydrazyl (DPPH) and 2,2’–azino–bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) methods were used to measure free radical scavenging capacity. Results have shown that the total polyphenol and flavonoids content achieved 49.67 ± 0.35 µgGAE/mg and 5.49 ± 0.84 µgQE/mg, respectively. “Cao Khai” had low IC50 values of 126.06 ± 1.33 µg/mL (DPPH) and 35.4 ± 0.74 µg/mL (ABTS), which indicated that the crude drug “Cao Khai” illustrated significant free radical scavenging activity in a dose-dependent manner, as compared to ascorbic acid.

1. Introduction

Medicinal plants have been used against a number of diseases in traditional medicine as they date back in different regions of the world [1-3]. More than 50% of the medicines and pharmaceuticals used today were first described from conventional medicines and medicinal plants [4,5]. Due to the risk of deforestation and over-exploitation of natural resources around the world, lots of excellent and valuable medicinal plants in many countries are at risk of extinction if not preserved and sustainably exploited in national research investment.

Coptosapelta tomentosa Rubiaceae is a species endemic to Southeast Asia with many uses, typically as a treatment for helminths, abdominal pain, fever, rhinitis, and rheumatoid arthritis. In Kruawan et al study, twenty-one medicinal plants in Thailand were claimed to have anti-diarrhoeal or anti-parasitic anti-protozoa were investigated. In research of Kruawan et al. (2014), twenty one medicinal plants in
Thailand which had been shown to have anti-diarrheal or anti-parasitic activities were investigated the antiprotozoal. These observations provide preliminary evidence that an acetone extract from *Coptosapelta tomentosa* was the most active against both *Entamoeba histolytica* and *Giardia intestinalis* [6]. In 2017, *Coptosapelta tomentosa* was also shown to lower blood pressure. According to this study, the methanol extract vasodilated on a vasoconstrictive model with phenylephedrine in mice. The research suggests that this effect may be caused by the polyphenols or saponins present in *Coptosapelta tomentosa* by activating the release of endogenous NO causing vasodilation [7].

In 2018, the methanol extract from *Coptosapelta tomentosa* roots displayed *in vitro* and *in vivo* anti-inflammatory activities. Its capacity to regulate the hypotonic membrane caused by red blood cells indicated by EC\(_{50}\) value was lower than that of indomethacin. In turn for carrageenan on Wistar rats' left leg, extraction illustrated anti-inflammatory activity at a dose of 1200 mg/kg, equivalent to 20 mg/kg Indomethacin [8]. A researches series of Arnida from 2018 to early 2019 on Hem polymerization inhibiting activity, *Coptosapelta tomentosa* have shown the potential in the treatment of malaria. The leaf extract in hexane and ethanol solvent has the same IC\(_{50}\) value as the antimalarial drug chloroquine diphosphate [9].

The usage of plants as raw products plays a crucial role in discovering different phytotherapeutic compounds. For a long time, the ethnic minorities in Ninh Thuan province, Vietnam have mixed *Coptosapelta tomentosa* with other types of medicinal herbs to create a crude drug called "Cao Khai". The crude drug is dark brown, fragrant and has a slightly bitter taste (Figure 1). It is usually circulated on the market in the form of each dry decoction with a volume of 0.5–1 kg. "Cao Khai" is applied as a traditional medicine for the treatment of osteoarthritis, anti-inflammatory, antibacterial, blood tonic and performance-enhancing drugs [1]. This medicine is common not only in Vietnam but also in Malaysia, Thailand and Lao [4]. The whole organ of *Coptosapelta tomentosa* Rubiaceae is harvested and dried either with a low temperature oven at around 30-40 °C, air-dried for around 3-4 days or until finished. Then, it has been mixed with other types of medicinal herbs. However, people still use "Cao Khai" in treating diseases mainly based on folk experience, lack of scientific basis and have not paid attention to the research on the toxicity of the stems for long-term use [10]. The present research was performed to examine chemical constituents and to determine the total polyphenols and flavonoids contents and free radical scavenging activity of the "Cao Khai".

![Image of (a) Coptosapelta tomentosa Rubiaceae and (b) crude drug “Cao Khai”](image)

**Figure 1.** Image of (a) *Coptosapelta tomentosa* Rubiaceae and (b) crude drug “Cao Khai”.

2. Materials and methods

2.1. Materials and chemicals

In this study, “Cao Khai” was collected from traditional medicine shops in Ninh Thuan province, Vietnam. Reagents and chemicals such as diethyl ether, ethanol, gallic acid, quercetin, L-ascorbic acid,
DPPH and ABTS were of analytical grade. Sigma Chemical Company (St. Louis, MO, USA) supplied all the chemicals used.

2.2 Methods and procedures
To analyse the chemical constituents, “Cao Khai” was extracted with three different solvents, including diethyl ether, ethanol 96% and water following the procedure shown in Figure 2. About 25 g of dried “Cao Khai” powder was used during the extraction process.

Figure 2. Extraction process of “Cao Khai”.

2.2.1. Analysis of chemical components. The compound groups were defined in each extract using different chemical reactions, such as hydrochloride acid, ferric chloride, chloroform, NaOH, anhydride acetic, KOH, aluminium chloride and Fehling’ solution reagent.[11-13].

2.2.2. Total polyphenol content determination (TPC). The procedure of Zivic et al. with some modification was used to determine the TPC in “Cao Khai” [14-16]. First, the sample of 0.5 mL of ethanol was pipetted into a test tube comprising 10% (v / v) of the Folin-Ciocalteu reagent. Then, the sample was applied to Na2CO3 7.5 per cent (w / v) after 5 minutes and then vigorously shaken, followed by 1-hour incubation in the dark. The absorbance was eventually calculated at 765 nm, and the findings were seen in µg of gallic acid equivalents per sample dry weight (µgGAE/mg).

2.2.3. Total flavonoid content (TFC). The content of TFC was estimated by Ebrahimzadeh et al. [16, 17]. After 5 minutes, 0.5 mL of the extract and 0.1 mL of AlCl3 10% were mixed, vigorously shaken with 0.1 mL of CH3COOK 1M and distilled water. The mixture was then incubated in the dark for 30 minutes. The absorbance was calculated at 415 nm with quercetin was a standard. The TFC results were determined in µg of quercetin equivalents per dry weight of the sample (µg QE/mg).

2.2.4. Evaluation of antioxidant potential. The antioxidant activity of ethanol extract was evaluated by IC50 values of DPPH and ABTS scavenging activity assay by Islam et al. with some modification [18, 19]. Stock solutions of DPPH and ABTS were diluted in order to have working solutions, its absorbance 1.1 ± 0.02 at 517 nm and 714 nm, respectively. Then, the 1 mL ethanol extraction was pipetted into 3 mL working solution and the mixture was stable at RT in the dark within 30 minutes. The optical measurement of mixture was using UV/VIS–1800 Shimadzu Spectrometer. In blank sample, 0.5 mL sample was replaced ethanol 96%. Vitamin C (0.1 g ± 0.01) absorbed ethanol 96% into the 100mL
volume flask was used as the standard sample. The percent DPPH and ABTS scavenging effect were calculated by using following Equation (1):

\[
\text{Scavenging effect (\%) or percent inhibition (\%) } = \frac{A_b - A_s}{A_b} \times 100
\]  

whereby \( A_b \) - Blank sample absorbance, \( A_s \)-sample absorbance, \( \%I \)-Percent inhibition. The IC\(_{50}\) meaning was the sample concentration that inhibited the level to 50%. Thus, IC\(_{50}\) values are negatively proportional to the antioxidant activity, the lower IC\(_{50}\) value indicates the maximum antioxidant activity of the sample being evaluated.

2.2.5. **Statistical analysis.** All experiments were carried out in triplicate. The findings were presented as standard deviation and mean values. ANOVA was conducted using SPSS (version 23, IBM, USA) and the variations were measured using Tukey's Test (P <0.05).

3. **Results and discussions**

3.1. **Analysis of chemical components**

It is essential to analyse the content of main active components in raw materials and phytomedicines, as it enables evaluation of the product quality as well as their efficacy and safety for therapeutic use [20, 21]. Phytochemical screening of “Cao Khai” showed the presence of multiple components like saponins, flavonoids, volatile oils, triterpenoids, organic acids, anthraquinon, coumarin, reducing compounds and polyuronid (Table 1). Polyphenols and flavonoids were presented most in ethanol extraction.

| Compounds          | Diethyl ether | Ethanol 96% | Aqueous |
|--------------------|---------------|-------------|---------|
| Alkaloids          | –             | –           | –       |
| Antraquinons       | +             | +           |         |
| Cardiac glycosides | +             | +           | +       |
| Carotenoids        | –             | //          | //      |
| Coumarins          | +             | +           | //      |
| Flavonoids         | –             | +           | –       |
| Lipid              | –             | //          | //      |
| Organic acid       | //            | +           | +       |
| Polyphenols        | //            | –           | –       |
| Polyuronid         | //            | //          | +       |
| Reducing compounds | //            | +           | +       |
| Saponin            | //            | +           | +       |
| Tannins            | //            | –           | –       |
| Triterpenoids      | +             | +           | +       |
| Volatile oils      | +             | //          | //      |

“+” indicating presence, “–” indicating absence and “//” indicating not examined

3.2. **TPC and TLC of crude drug “Cao Khai”**

Phenolic is one of the most important components and makes up a large proportion of plants. These are strong antioxidants. Therefore, this indicator is quite important in studying the antioxidant activity of plants. Moreover, according to Marja et al., drugs with a total phenolic content greater than 20 \( \mu \)GAE/mg are considered to have strong antioxidant activity [22, 23]. Antioxidant activity of phenolics causes by their redox properties. Phenolics act as hydrogen donors, reducing agents, and singlet oxygen quenchers. Therefore, this compound effect on the interaction of dietary health in the human body [24, 25].
Quantitative results show that the presence of relatively high levels of polyphenol and flavonoid compounds, "Cao Khai" has the potential to be a rich source of natural antioxidants. Total polyphenol and flavonoids content were 49.67 ± 0.35 µgGAE/mg and 5.49 ± 0.84 μgQE/mg, respectively.

3.3. Evaluation of antioxidant potential

Crude "Cao Khai" antioxidant function was calculated by calculating the free radical scavenging capability. The DPPH and ABTS free radical scavenging tests were performed by measuring the absorbance at 517 nm and 734 nm on UV–Vis spectrophotometer, respectively. Figure 3 illustrates the antioxidant potential of crude drug “Cao Khai” by DPPH and ABTS radical scavenging activity. In comparison to aqueous extract, in scavenging ABTS and DPPH free radicals, the ethanolic extract showed better response. The IC50 value was the sample concentration that inhibited the rate to 50%. Thus, IC50 values are negatively associated with the antioxidant activity. "Cao Khai" had low IC50 values 126.06 ± 1.33 µg/ml (DPPH) and 35.4 ± 0.74 µg/ml (ABTS). As contrasted with vitamin C (i.e. the standard sample), the rudimentary drug "Cao Khai" displayed substantial dose-dependent free radical scavenging activities. This study had provided scientific evidence on phytochemical screening and antioxidant potential of the crude drug “Cao Khai”.

![Figure 3](image_url)

**Figure 3.** The antioxidant potential of crude drug “Cao Khai” by DPPH and ABTS radical scavenging activity.

4. Conclusions

The crude drug “Cao Khai” has been used to treat osteoarthritis, anti–inflammatory, antibacterial, blood tonic and performance–enhancing drugs in many countries for a long time. In this study, we assessed the chemical elements, complete polyphenol material, flavonoids and the antioxidant function of this crude product. Phytochemical screening of crude drug "Cao Khai” showed the presence of multiple components like saponins, flavonoids, volatile oils, triterpenoids, organic acids, anthraquinon, coumarin, reducing compounds and polyuronid. Total polyphenol and flavonoids content were 49.67 ± 0.35 µgGAE/mg and 5.49 ± 0.84 µgQE/mg, respectively. "Cao Khai” had low IC50 values 126.06 ± 1.33 µg/ml (DPPH) and 35.4 ± 0.74 µg/ml (ABTS).
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