ASSESSMENT OF ANTIOXIDANT CAPACITY IN VIETNAMESE MEDICINAL PLANTS, VEGETABLES, AND FRUITS USING 2,2-DIPHENYL-1-PICRYLHYDRAZYL (DPPH)

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Abstract

Medicinal plants have been utilized since ancient times, serving as foundational sources for modern medicine. Plant-derived compounds remain a vital source for medicinal formulation. Scientists have discovered that medicinal plants, vegetables, and fruits that humans consume daily contain compounds that fight free radicals and antioxidants. These free radical and antioxidant compounds play a crucial role in enhancing health and preventing or treating diseases in humans.

Over the decades, scientists worldwide have used various methods to determine the content of antioxidants in medicinal plants. The 2,2-Diphenyl-1-picrylhydrazyl (DPPH•) free radical scavenging method is the most commonly used measure of antioxidant capacity.

Recently, Vietnamese scientists have conducted numerous studies to determine the antioxidant and anti-free radical activity of Vietnamese food crops, medicinal plants, and herbs. Commonly used methods include the DPPH (1,1-diphenyl-2-picrylhydrazyl) assay and other measurements such as ABTS• and PFRAP. Research results have been published in many Vietnamese journals.

This article presents the results of several studies published in scientific journals in Vietnam.
Keywords*: antioxidants; 1,1-diphenyl-2-picrylhydrazil; DPPH; antioxidant assay.*

Nghiên cứu xác định khả năng chống oxy hóa tổng thể của một số cây thuốc, rau, quả Việt Nam bằng phản ứng với 2,2-Diphenyl-1-picrylhydrazyl (DPPH)

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Tóm tắt

Từ thời cổ đại, con người đã biết sử dụng cây thuốc và thậm chí có thể được coi là nguồn gốc của y học hiện đại. Các hợp chất có nguồn gốc thực vật đã và vẫn là nguồn hợp chất quan trọng để làm thuốc. Các nhà khoa học đã phát hiện trong cây thuốc, rau, củ, quả con người dùng hằng ngày có chứa các hợp chất chống gốc tự do, chống oxy hóa. Chất chống gốc tự do và chống oxy hóa có vai trò đặc biệt trong tăng cường sức khỏe, ngăn ngừa và điều trị bệnh tật cho con người.

Những thập kỷ qua, các nhà khoa học trên thế giới đã sử dụng nhiều phương pháp khác nhau để xác định hàm lượng chất chống gốc tự do, chống oxy hóa trong cây thuốc. Phương pháp loại bỏ gốc tự do 2,2-Diphenyl-1-picrylhydrazyl (DPPH•) là phép đo khả năng chống oxy hóa được sử dụng phổ biến nhất.

Gần đây, các nhà khoa học Việt Nam đã có nhiều công trình nghiên cứu xác định hoạt tính chống oxy hóa, chống gốc tự do trong cây lương thực, thực phẩm và dược liệu Việt Nam. Phương pháp hay dùng là phương pháp DPPH (1,1-diphenyl-2-picrylhydrazyl) và một số phép đo như ABTS• và PFRAP. Kết quả nghiên cứu được đăng trên nhiều tạp chí Việt Nam.

Trong bài báo này, chúng tôi giới thiệu kết quả một số nghiên cứu được đăng trên các tạp chí khoa học ở Việt Nam.
Từ khóa*: Chất chống oxy hóa, 1,1-diphenyl-2-picrylhydrazil, DPPH, xét nghiệm chống oxy hóa.*

1. Introduction

Over the past two decades, there has been a growing trend towards using herbal products for disease prevention and treatment. Pharmacopoeias in Asian countries such as Vietnam, South Korea,

China, and Japan all contain monographs on medicinal plants. Some of these monographs have also been included in the US and European Pharmacopoeias. It is estimated that 70% of the global population still uses herbal medicines in primary healthcare within the community. Therefore, the World Health Organization has stressed that ensuring the quality of these drugs must be based on modern analytical techniques, with the use of appropriate reference standards. But significant challenges remain in scientific validation, consistent quality control, protection of indigenous knowledge, standardization and regulatory oversight (Vũ & Thành, 2016).

In Vietnam, the combination of modern medicine and traditional medicine is being given importance. Therefore, the State and the Ministry of Health have strict regulations on quality control of raw materials and medicines in traditional medicine (Luật dược, 2025; Bộ Y tế, 2020; Bộ Y tế, 2021). Currently, science has proven that antioxidants and free radical scavengers in medicinal plants and food have the effect of preventing and treating diseases. Therefore, research on antioxidants and free radical scavengers in medicinal plants and food is developing worldwide, contributing to the modernization of traditional medicine. In recent years, Vietnamese scientists have shown great interest in this research direction.

Compounds in medicinal plants and foods fight oxidation and free radicals through different mechanisms (Irina & Constantin, 2021): Anti-free radical action via HAT (HAT-Hydrogen-Atom-Transfer) mechanism, SET (SET-Single-Electron-Transfer) mechanism and combined HAT/SET mechanism. Therefore, to determine the total antioxidant capacity (TAC) of a medicinal plant, vegetable, or fruit, it is often necessary to use multiple methods simultaneously, each method having multiple measurements. Specifically, the mechanism-based antioxidant capacity (HAT) is determined by four types of measurements: ORAC (Oxygen Radical Absorption Capacity), HORAC (Hydroxyl Radical Antioxidant Capacity), TRAP (Total Peroxyl Radical Trapping Antioxidant Parameter), and TOSC (Total Oxyradical Scavenging Capacity).

The SET electron donation mechanism has three types of measurements: CUPRAC (Cupric Reducing Antioxidant Power) measurement, FRAP (Ferric Reducing Antioxidant Power) measurement, and Folin-Ciocalteu measurement.

The HAT/SET combination mechanism has two types of measurements: ABTS measurement (-2,20-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid); and DPPH measurement (2,2-diphenyl-1-picrylhydrazyl).

In 2017, in Vietnam, based on the DPPH measurement - Determination of antioxidant activity by reaction with 2,2-diphenyl-1-picrylhydrazyl (DPPH), the National Standard Technical Committee TCVN/TC/F6 Nutrition and Dietary Foods compiled, the General Department of Standards, Metrology and Quality assessed, and the Ministry of Science and Technology published it as National Standard TCVN 11939:2017 (Tiêu chuẩn quốc gia TCVN 11939, 2017). Based on this method, researchers in Vietnam have determined the total antioxidant capacity (TAC) of many medicinal plants, vegetables, and fruits. In this paper, we present some recent research studies determining the antioxidant activity in medicinal plants and herbs in Vietnam using the DPPH method - National Standard TCVN 11939:2017.

2. Some studies on the antioxidant capacity of certain medicinal plants and fruits and vegetables in Vietnam

Antioxidants in medicinal plants and food play an important role in preventing and treating diseases, contributing to improved health. In addition, antioxidants and anti-free radicals also have the effect of preserving food. The antioxidant capacity of medicinal plants in the research works presented below was determined by reaction with 2,2-diphenyl -1-picrylhydrazyl (DPPH) (Irina & Constantin, 2021).

Results of research on the antioxidant activity of tea (Hương & Chính, 2024) showed that the total polyphenol content of Thai Nguyen tea sample was the highest (139.06 mg GAE/g), followed by Japanese green tea sample (89.07 mg GAE/g), and the lowest was black tea sample (77.57 mg GAE/g). However, the antioxidant activity of Thai Nguyen tea was the lowest (IC50 = 2.97 μg/mL), the antioxidant capacity of Japanese green tea was better (IC50 = 2.11 μg/mL) and the highest was black tea (IC50 = 1.22 μg/mL). The study's authors suggest that while tea has a high

total polyphenol content, the antioxidant capacity of tea extracts may be reduced because, in addition to polyphenols which determine tea's antioxidant capacity, tea also contains other antioxidants such as vitamin C, vitamin E, and L-ergothioneine.

It is essential to determine the antioxidant capacity of the studied sample through reaction with 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Tiêu chuẩn quốc gia TCVN 11939, 2017) is determining the total antioxidant capacity (TAC) of the study sample, and it is not possible to know the antioxidant capacity of the individual antioxidants in the study sample.

The results obtained in the study evaluating the antioxidant capacity of onion, garlic, and shallot extracts with 99% ethanol (Hương & Chính, 2024) showed that all three materials had the highest extraction efficiency at a ratio of 1:4 (W/V) and the reactivity with 2,2-diphenyl-1-picrylhydrazyl (DPPH) of garlic extract was higher than shallot and onion at all concentrations investigated, 300, 600 and 900 µg/L The higher the concentration of the extract, the greater the rate of free radical scavenging. The authors of this study used onion, garlic, and shallot extracts in their research on peanut oil preservation. Experimental results showed that adding garlic extract at a concentration of 900 µg/L to traditional peanut oil extended its shelf life by 2 months.

A group of researchers also studied the interaction with the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) and the anti-inflammatory activity of the extract by carrageenan-induced paw edema test (Nhân & Hương, 2015). The results showed that all three 45%, 60% and 96% alcoholic extracts from Moringa seeds showed antioxidant activity at a maximum concentration of 250 µg/ml. The 70% alcoholic extract from Moringa seeds showed acute anti-inflammatory effects at a dose of 0.814 g/kg in a carrageenan-induced paw edema experiment in mice. All three alcoholic extracts (45%, 60%, and 96%) at doses of 1.080 g/kg, 0.814 g/kg, and 0.587 g/kg, respectively, also exhibited anti-inflammatory activity. The anti-inflammatory properties of the three alcoholic extracts (45%, 60%, and 96%) derived from Moringa seeds indicate that this herbal medicine may be beneficial in both treating and supporting treatment of inflammatory diseases.

To determine the antioxidant capacity of green and ripe pineapple, the author of the work (Hậu et al, 2020) chopped, dried the flesh and peel of green pineapple, ground the flesh and peel of ripe pineapple and soaked it in 99.5% ethanol at a material-solvent ratio of 1/4 (w/v). The antioxidant capacity of the extracts was determined by reaction with DPPH. The research results showed that at the same concentration, the extract of the ripe peel sample had the highest antioxidant capacity compared to the other 3 extracts.

In the study on the antioxidant and antibacterial activity of ethanol extract from white toad leaves (Yến et al, 2021), the author collected white toad leaves, washed them, cut them into small pieces and dried them. The dried white toad leaves were soaked in 99% ethanol. The extract was concentrated and white toad leaf extract was obtained in 99% ethanol. The antioxidant capacity of white toad leaf extract was determined by DPPH, ABTS, RP, and TAC measurements, with IC50 or OD0.5 values of 81.734 ± 1.009 µg/mL, 20.461 ± 0.5211 µg/mL, and 113.108 ± 0.094 µg/mL, respectively. In addition, the extract from white toad leaves inhibited four common pathogenic bacterial strains in aquatic animals.

In the research study investigating the antioxidant and α-glucosidase inhibitory and cytotoxic effects on two breast cancer and cervical cancer lines and the anti-free radical effects of marigold petal extract (Dung & Tường, 2020), researchers at Can Tho University soaked 100 g of each petal powder sample at room temperature in two types of solvents: 70% ethanol and 96% ethanol. The extracts were concentrated under reduced pressure at 400°C until the extracts reached the concentration requirements specified in the Vietnamese Pharmacopoeia V. Four samples of marigold petal extracts were obtained: two samples of yellow marigold petals with 70% and 96% ethanol solvents, and two samples of orange marigold petals with 70% and 96% ethanol solvents. The authors of this study determined the α-glucosidase inhibitory activity and the antioxidant capacity using DPPH reaction for all four samples. The results showed that all four extracts exhibited significant antioxidant, α-glucosidase inhibitory, and cytotoxic activity. The extract of yellow marigold flowers with 70% ethanol showed the best antioxidant activity

(IC50 = 82.03 ± 1.37 µg/mL) and the highest α-glucosidase inhibitory activity and cytotoxicity to breast cancer cells. These results demonstrate the potential of marigold petals for many beneficial biological effects in supporting the treatment of free radical-related diseases, inhibiting α-glucosidase, and inducing cytotoxicity in breast cancer cells.

Methanol extract from short-stemmed tea plant (Camellia kissi, theacceae) collected from Lam Dong (Hương et al, 2023) was studied to determine its antioxidant capacity using 3 free radical scavenging methods including DPPH, ABTS free radical scavenging, and PFRAP reduction capacity evaluation. The research results showed that the extract had antioxidant effects and the antioxidant effect was concentration-dependent.

The author of the research work "Investigation of the antioxidant activity of flavonoid extract from the leaves of Sonneratia caseolaris" (Hậu et al, 2024) extracted the active substance in Sonneratia caseolaris with 70% ethanol, then concentrated it under low pressure into a dry extract and determined the antioxidant capacity of the dry extract by measuring DPPH and ABTS. Experimental results showed that the antioxidant capacity of the dry extract from Sonneratia caseolaris leaves was very low.

In the research work "*antioxidant active ingredients of extracts from Siegesbeckia orientalis L.0*" (Nhi, 2022), the author extracted the active ingredients from Siegesbeckia orientalis L.0 using methanol and determined the antioxidant capacity of the extract by measuring DPPH, ABTS and PFRAP. Based on experimental results, the authors of the study concluded that antioxidant activity depends on the concentration of the extract, and that the plant Hyacinthus orientalis has great potential in the research and production of antioxidant preparations.

The antioxidant capacity of watercress was also studied (Duy et al, 2024). Watercress plants harvested in Hung Hoi commune, Vinh Loi district, Bac Lieu province were dried and coarsely ground. The powdered medicinal material was thoroughly soaked in 40% alcohol at a ratio of 1:10. The extract was concentrated by water bath. The antioxidant capacity of watercress extract was determined by DPPH and MDA measurements. The IC50 values were 1706.52 µg/ml (DPPH measurement) and 1081.17 µg/ml (MDA measurement), respectively. The results showed that watercress has antioxidant activity, although which is lower than the two reference substances, ascorbic acid and trolox. However, since it is an edible vegetable, watercress still has the ability to contribute to supplementing antioxidants for the body.

3. Discussion

Vietnam is one of the countries with a long-standing tradition of traditional medicine in Asia. Over thousands of years, the wealth of folk medical knowledge passed down from our ancestors has been compiled into a system of theories, diagnostic and treatment methods, prescriptions, and a rich array of medicinal herbs. Vietnam currently possesses hundreds of inherited remedies and thousands of valuable folk medicines preserved in traditional villages, clans, and ethnic minority communities. Researching, verifying, and standardizing this knowledge is an urgent task, aiming to integrate traditional values into the formal healthcare system while simultaneously protecting the rights and intellectual property of the people.

To preserve, promote, and develop traditional medicine and integrate it effectively with modern medicine, it is necessary to go hand in hand with innovation, creativity, and the application of science and technology to standardize medicinal herbs and prescriptions. This is especially significant in the current context, when people are increasingly interested in natural, safe, and body-friendly treatment methods.

In recent decades, scientists have discovered that free radicals, oxidants, and antioxidants are closely related to the pathogenesis, prevention, and treatment of diseases. Scientists worldwide have conducted numerous studies on this issue. In Vietnam, however, this topic has not received much attention, especially in traditional medicine. We believe that if the antioxidants in medicinal plants, herbs, and prescriptions are identified through research, it will not only improve treatment outcomes but also provide a basis for closer integration of traditional medicine with modern medicine. Therefore, the research works on antioxidants in medicinal herbs, vegetables, and fruits introduced above, although still in their early stages, are a necessary starting point. In particular, the research results on antioxidants from

some works have practical applications. Specifically, garlic extract (Tiên et al., 2022) was found to have a high antioxidant content, so when this extract is added to traditional peanut oil, the shelf life of peanut oil increases.

In our opinion, a medicinal plant or food contains many different antioxidants. These antioxidants can fight oxidation by donating a hydrogen atom (HAT) or donating an electron (SET). Therefore, to determine the overall antioxidant capacity, multiple measurements should be taken simultaneously on a single sample.

On the other hand, although the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical removal method is the most commonly used method in studying the antioxidant capacity of the sample, its main drawback is its dependence on a spectrophotometer. To overcome this drawback, a colorimetric method for determining antioxidant activity using a scanner and the free Image J software has been developed (Akar et al, 2017). In this new method, a mixture of DPPH solution and standard antioxidants or extracts from common herbs is dropped onto a thin-layer chromatography (TLC) plate after an incubation period. The spots are then evaluated using Image J software to determine the CSC50 value, the concentration of the sample that produces a 50% color reduction, which is very similar to the SC50 value obtained by spectrophotometry. The advantages of this new method include the use of less reagent and solvent, the elimination of the need for an expensive spectrophotometer, thus significantly reducing costs and making it easy to implement in any environment and situation.

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