TẠP CHÍ KHOA HỌC VÀ CÔNG NGHỆ ĐẠI HỌC DUY TÂN
DTU Journal of Science and Technology 02(69) (2025) 41-49
ĐẠI HỌC DUY TÂN
DUYTAN UNIVERSITY

A study on the chemical composition and antioxidant effects of *Melodorum fruticosum* Lour. collected in Quang Nam

Nghiên cứu thành phần hóa học và tác dụng chống oxy hóa của cây Dủ dẻ trâu (*Melodorum fruticosum* Lour.) thu hái tại Quảng Nam

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(Date of receiving article: 02/12/2024, date of completion of review: 19/02/2025, date of acceptance for publishing: 01/4/2025)

Abstract

Melodorum fruticosum Lour. (Annonaceae) contains various important bioactive compounds, including flavonoids, heptenes, and essential oils. However, no studies have been conducted to investigate or quantify these compound groups in *Melodorum fruticosum* Lour. from Quang Nam. Several studies have explored its cytotoxic, anti-inflammatory, and antioxidant effects. There is only one research article on the antioxidant effects of flower essential oils. To enrich the existing data on this plant, this study was conducted to determine the chemical components, flavonoid content, and antioxidant effects of leaf and stem extracts of *Melodorum fruticosum* Lour. collected in Quang Nam. Chemical analysis employing chemical reactions and thin-layer chromatography revealed the presence of flavonoids, coumarins, tannins, polysaccharides, lipids, and sterols in both stems and leaves. Additionally, leaves were found to contain carotenes. Total flavonoid quantification using the colorimetric $AlCl_3$ method and quercetin (QE) standard curve yielded 37.80 mg/g for leaves and 15.07 mg/g for stems (calculated based on quercetin equivalence). Antioxidant activity evaluation using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method indicated an IC_{50} value of 5575.60 µg/ml for stems and 2292.62 µg/ml for leaves, based on the dry weight of the medicinal material, demonstrating the superior antioxidant capacity of leaves compared to stems. These findings provide valuable insights into the chemical composition and antioxidant activities of *Melodorum fruticosum* Lour. and suggest further exploration of leaf extracts for in-depth investigations into the plant's chemistry and biological effects.

Keywords: Melodorum fruticosum Lour.; chemical composition; antioxidant effects.

Tóm tắt

Cây Dủ dẻ trâu (*Melodorum fruticosum* Lour.), họ Na (Annonaceae) có chứa nhiều hoạt chất quan trọng như flavonoid, hepten, tinh dầu,... Tuy nhiên, chưa có bài nghiên cứu nào khảo sát sự có mặt hay định lượng các nhóm hợp chất trong cây Dủ dẻ trâu ở Quảng Nam. Một số nghiên cứu đã thử nghiệm tác dụng gây độc tế bào ung thư, chống viêm và chống oxy hóa. Trong đó chỉ có một bài nghiên cứu về tác dụng chống oxy hóa từ tinh dầu hoa. Để góp phần bổ sung thêm các dữ liệu về loài cây này, nghiên cứu này được thực hiện nhằm tiến hành xác định các thành phần hóa học, hàm lượng của flavonoid và tác dụng chống oxy hóa từ dịch chiết lá và thân cây Dủ dẻ trâu thu hái ở Quảng Nam. Các hợp chất chính trong cây được xác định bằng các phản ứng hóa học và sắc ký lớp mỏng cho thấy cả thân và lá đều có flavonoid, coumarin, tanin, polysaccharid, chất béo và sterol; ngoài ra lá cây còn có caroten. Hàm lượng flavonoid toàn phần được xác định

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theo phương pháp tạo màu với $AlCl_3$ và xây dựng đường chuẩn với quercetin (QE), trong lá đạt 37,80 mg/g, trong thân đạt 15,07 mg/g tính theo quercetin. Sử dụng phương pháp 1,1-diphenyl-2-picrylhydrazyl (DPPH) để khảo sát tác dụng chống oxy hóa cho thấy giá trị IC_{50} của thân là 5575,60 µg/ml và cao hơn giá trị IC_{50} của lá là 2292,62 µg/ml. Điều đó cho thấy khả năng chống oxy hóa của thân thấp hơn so với lá. Các kết quả của đề tài góp phần làm tiền đề, gợi ý việc lựa chọn lá để tiếp tục thực hiện thêm các nghiên cứu chuyên sâu hơn về hóa học và tác dụng sinh học của cây Dủ dẻ trâu.

Từ khóa: Dủ dẻ trâu; thành phần hoá học; tác dụng chống oxy hóa.

1. Introduction

Melodorum fruticosum Lour. is a species of plant in the Annonaceae [3]. It is native to Southeast Asia, including Vietnam. The fruit is edible, with a sweet or sour taste, and is often used to make beverages or wine [15]. The bark can be used as a betel quid. The leaves, bark, and branches can be used to make a tea that aids digestion. The flowers can be used to make perfume, or eaten raw. They have a sweet taste and are used to treat fever, dizziness, and headaches. They can also be used to treat heart disease and hypertension [11].

Research on the chemistry, as well as the compounds isolated from *M. fruticosum* Lour., is still relatively limited. The parts of the plant that are most often studied are the stems, bark, leaves, or flowers. Studies of the chemical composition of *M. fruticosum* Lour. have shown that heptane derivatives and flavonoids are the most common compounds isolated from the plant. Other groups of compounds that have been isolated or extracted from the plant include steroids, essential oils, and aromatic compounds [5], [9]. Researchers have investigated the activities of these isolated compounds, such as cancer cell inhibition, anti-inflammatory activity, and antioxidant activity [7], [13], [14]. These studies have shown the potential of *M. fruticosum* Lour. as a medicinal plant.

This study aims to analyze and identify the chemical constituents of *M. fruticosum* Lour., with a particular emphasis on quantifying flavonoids and assessing its antioxidant properties. The findings aim to provide valuable insights into the species and reinforce its potential as a medicinal resource and contributing to the advancement of natural medicinal materials in Vietnam.

2. Subjects, time and research methods

2.1. Subjects and research time

Melodorum fruticosum Lour., harvested in Binh Son commune, Hiep Duc district, Quang Nam province, was used as the research subject. The plant includes the following parts: the stems and the leaves. The study was conducted from January 2021 to May 2022.

Based on the morphological characteristics of the research samples, using the genus classification key *Melodorum*, comparing the species description with reference [3], the research samples were accurately identified by their scientific name as *Melodorum fruticosum* Lour. [17], and the specimen is stored at the Department of Medicinal Botany - Pharmacognosy - Traditional Medicine, Faculty of Pharmacy, College of Medicine and Pharmacy, Duy Tan University, under the code KDDTU-01.

Sample preparation: The stems and leaves of the plant were collected, washed, chopped, dried, and ground into powder. The powder was placed in a sealed container and stored in a cool, dry place. The moisture content of the stems was found to be 10.737% and that of the leaves was 12.613%.

2.2. Research methods

2.2.1. Determination by chemical reactions

Specific reagents were used to identify common medicinal herb compound groups in the leaves and stems of *Melodorum fruticosum*

Lour. through chemical reactions using standard methods with modification as reported by Devi et al. [6] and Bhardwaj et al. [4]. Depending on the compound type, the powder was extracted using 80° alcohol (for flavonoids and coumarins), water (for tannins, organic acids, amino acids, reducing sugars), or petroleum ether (for lipids, carotenes, sterols), or other suitable solvents.

2.2.2. Determination by thin-layer chromatography

Determine the conditions for conducting thin-layer chromatography, referring to the procedure in Appendix 5.4 of Vietnam Pharmacopoeia V [8]. Concentrate the petroleum ether extract and the 80° alcohol extract (prepared in the qualitative part by chemical reaction). Dilute the petroleum ether extract with petroleum ether and the alcohol extract with MeOH to obtain test solutions. Subsequently, develop and record the chromatograms.

2.2.3. Survey of flavonoid content

Total flavonoid content (TFC) was determined spectrophotometrically at a wavelength of 510 nm. A standard curve was constructed using quercetin (QE) as the standard [2].

TFC was expressed as milligrams of quercetin equivalent per gram of dried medicine material (mg QE/g medicine material), using the following formula:

$$TFC = \frac{C \times V \times k}{1000 \times m \times (100-H)} \times 100$$

TFC: Total flavonoid content (mg QE/g medicine material);

C: Quercetin concentration determined from standard curve (µg/ml);

V: Volume of test solution (ml);

k: Dilution factor;

m: Mass of medicine material (g);

H: Moisture content of medicine material (%).

2.2.4. Determination of antioxidant activity

The antioxidant activity was determined using the DPPH free radical scavenging method at a wavelength of 517 nm [1]. Antioxidant activity (I%) was calculated using the following formula:

$$I\% = \frac{Ac-At}{Ac} \times 100$$

I%: DPPH antioxidant effect;

Ac: control tube optical density;

At: test tube optical density.

3. Research and discussion

3.1. Qualitative results of compound groups using chemical methods

Table 1. Qualitative results of organic compound group detection in the *Melodorum fruticosum* Lour. stems and leaves using chemical reactions.

Serial	Compound	Chemical reactions	Result		Conclude	
			Stems	Leaves	Stems	Leaves
1	Alkaloids	Mayer's reagent	(-)	(-)	(-)	(-)
		Dragendorff's reagent	(-)	(-)		
2	Cardiac glycosides	Liebermann-Burchard reaction	(-)	(-)	(-)	(-)
		Legal's reaction	(-)	(-)		
		Baljet reaction	(-)	(-)		
3	Anthranoids	Borntraeger reaction	(-)	(-)	(-)	(-)
4	Flavonoids	Cyanidin reaction	(+)	(+)	(+)	(+)
5	Coumarins	Lactone ring opening reaction	(+)	(+)	(+)	(+)

6	Saponins	Foaming reaction	(+)	(+)	(+)	(+)
7	Tannins	Reaction with 5% $FeCl_3$	(+)	(+)	(+)	(+)
		Reaction with 1% gelatin	(+)	(+)		
8	Reducing sugar	Fehling's test	(-)	(-)	(-)	(-)
9	Polysaccharides	Bouchardat's test	(+)	(+)	(+)	(+)
10	Lipids	Evaporation of the solvent by heating	(+)	(+)	(+)	(+)
11	Sterols	Liebermann-Burchard reaction	(+)	(+)	(+)	(+)
12	Carotenes	Reaction with H_2SO_4	(-)	(+)	(-)	(+)

Note: (-) negative; (+) positive

Preliminary chemical reactions revealed the presence of the following compound groups in the stems of the *M. fruticosum*: flavonoids, coumarins, saponins, tannins, polysaccharides, lipids, and sterols. Additionally, the leaves were found to contain flavonoids, coumarins, saponins, tannins, polysaccharides, lipids, sterols, and carotenes.

Chemical studies on *M. fruticosum* primarily focus on isolation and extraction of compound groups such as flavonoids, steroids, essential oils, and aromatic compounds. The results of the investigation confirm the presence of corresponding compound groups as indicated in the literature. Qualitative analysis reveals the presence of flavonoid groups in both the stems and leaves, consistent with previous studies [7], [9], [10], [18]. Additionally, qualitative assessment confirms the presence of sterol groups in both the stems and leaves, whereas earlier studies had only isolated sterol compound groups from the stem bark [10], [16]. The research has provided supplementary insights into the presence of other compounds such as coumarins, saponins, tannins, polysaccharides, and lipids in both the stems and leaves, as well as the presence of carotenoid groups in the leaves.

3.2. Results of qualitative analysis of compound groups by thin-layer chromatography

After extraction, the following extracts were obtained: petroleum ether extract of stems (PT), petroleum ether extract of leaves (PL), alcohol extract of stems (CT), and alcohol extract of leaves (CL). After several trials on different solvent systems, it was found that the petroleum ether extracts (PT, PL) separated well on the petroleum ether: chloroform: ethyl acetate (12:3:1) system, and the alcohol extracts (CT, CL) separated well on the toluene: ethyl acetate: formic acid (5:3:0.2) system (Figure 1, Figure 2).

System I: Petroleum ether: Chloroform: Ethyl acetate (12:3:1): Both leaf and stem ether extracts showed spots I9 and I15 after development on the system I. The remaining spots appeared independently on each extract type. Spot I9 turned purple after spraying with 10% H_2SO_4 reagent, suggesting the presence of steroid compounds.

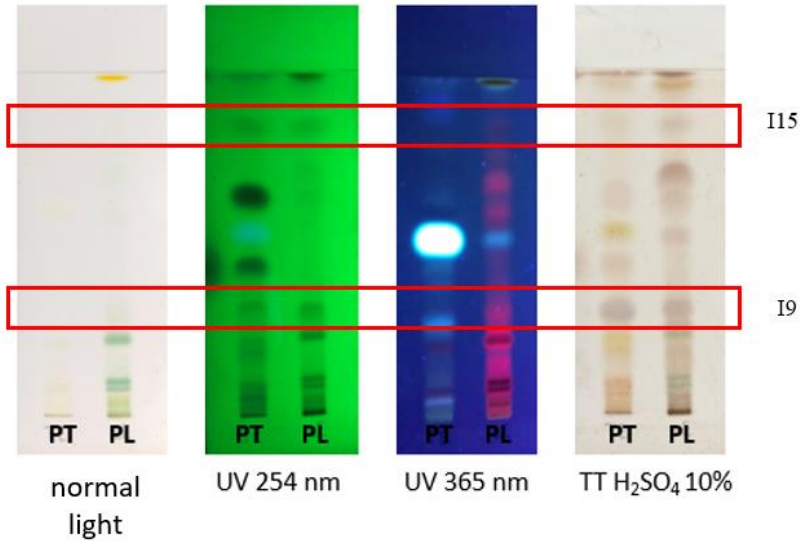

Figure 1. Chromatogram of stem and leaf petroleum ether on the solvent system Petroleum ether: Chloroform: Ethyl acetate (12:3:1)

System II: Toluene: Ethyl acetate: Acid formic (5:3:0.2): Leaf and stem alcohol extracts showed spots II1, II2, II11, and II14 after developing on system II. The remaining spots appeared independently on each extract type. Spots II3, II4, II5, II8, and II11 were visible under both UV 254 nm and UV 365 nm light and after spraying with reagents. Spot II5 turned blackish-blue after spraying with $FeCl_3$ reagent, suggesting the presence of polyphenol compounds.

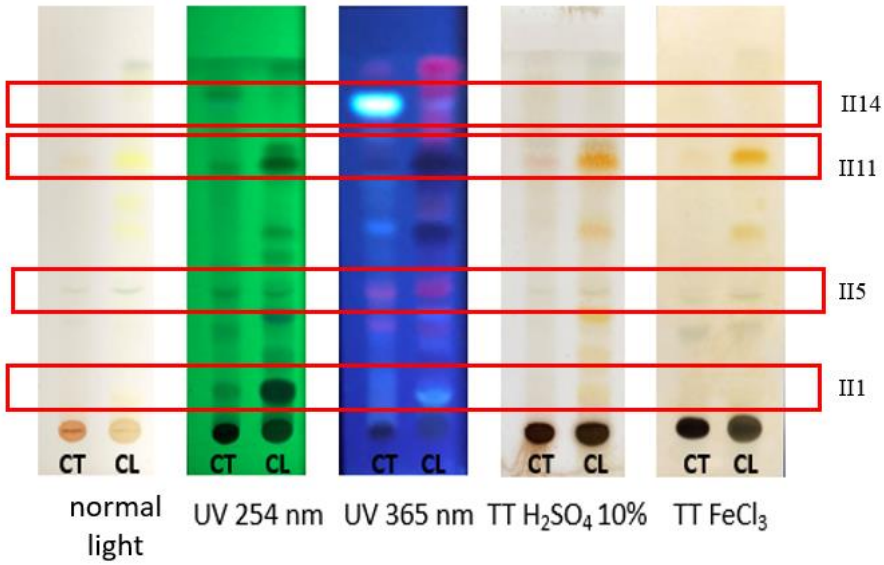

Figure 2. Chromatogram of stem and leaf alcohol on Toluene: Ethyl acetate: Formic acid solvent system (5:3:0,.2)

$FeCl_3$ reagent was not utilized in the System I (to detect traces of polyphenol compound groups) as the primarily non-polar compounds are present in this spot. Chromatographic results indicated the potential presence of steroid compound groups in the petroleum ether extract of the plant and polyphenol groups in the alcohol extract, consistent with the findings of chemical methods. Furthermore, the results revealed differences between the stems and leaves.

Specifically, out of the 15 separated spots in system I of petroleum ether, only 2 spots were present in both stems and leaves (I9, I15), while out of the 15 spots separated in system II of alcohol, only 4 spots appeared in both parts (II1, II5, II11, II14).

The results of chromatography show that in the ether extract of the plant there may be a group of steroid compounds, and in the alcohol extract of the plant there may be a group of polyphenols, corresponding to the results of the chemical method. Furthermore, the results also showed differences between stems and leaves.

3.3. Results of total flavonoid content survey

A series of standard solutions for quercetin was used to make the standard curve, and the correlation between the quercetin concentrations and their absorbance value was deduced by Excel software (Figure 3).

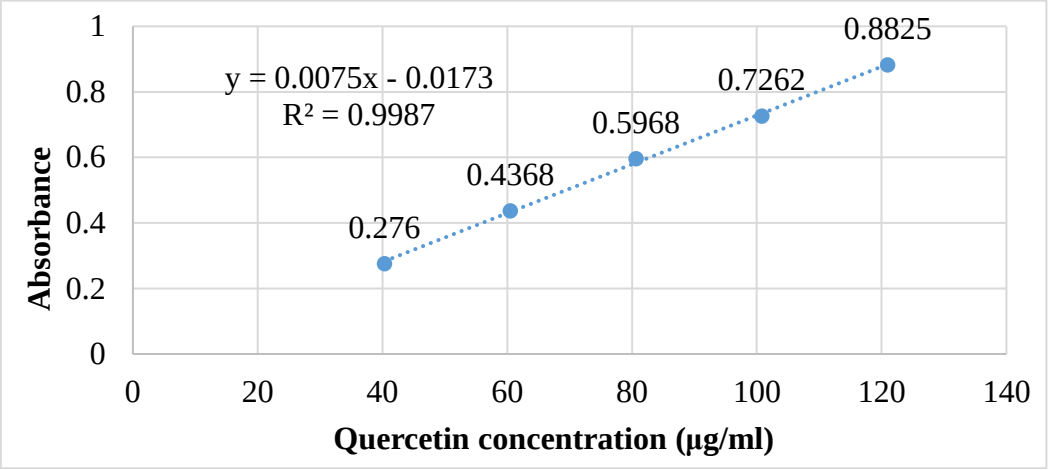

Figure 3. Calibration curve of the quercetin standard solution

Based on the standard curve, the analysis of total flavonoid content revealed no significant difference between the three stem samples and the three leaf samples. On average, the total flavonoid content in the stems was 15.07 mg/g, which was lower than that in the leaves, measured at 37.80 mg/g based on the dry weight of the medicinal material.

Table 2. Results of total flavonoid content in leaves and stems of *M. fruticosum* Lour.

	Sample	**A_T**	**C (µg/ml)**	**V (ml)**	**k**	**m (g)**	**H (%)**	**TFC (mg/g)**
Stems	1	0.6234	85.43	25	62.5	10.088	10.737	14.82
	2	0.6204	85.03	25	62.5	10.066	10.737	14.79
	3	0.6636	90.79	25	62.5	10.187	10.737	15.60
	Average content in the stems							15.07
Leaves	1	0.4628	64.01	25	625/3	10.097	12.603	37.78
	2	0.4539	62.83	25	625/3	10.092	12.603	37.10
	3	0.4756	65.72	25	625/3	10.174	12.603	38.50
	Average content in the leaves							37.80

(AT: Absorbance of the samples, C: Quercetin concentration determined from the standard curve, V: Test solution volume, k: Dilution factor, m: Sample weight, H: Sample moisture, TFC: Total flavonoid content)

3.4. Antioxidant activity test results

To establish a scientific basis for explaining the medicinal properties of this herbal remedy in traditional medicine, the study assessed its antioxidant effects using the DPPH free radical scavenging method. This method is a well-established approach for evaluating the antioxidant activity of test samples.

The antioxidant activity of the stem and leaf extracts of *M. fruticosum* Lour. was measured using the DPPH radical scavenging assay, with ascorbic acid serving as the positive control.

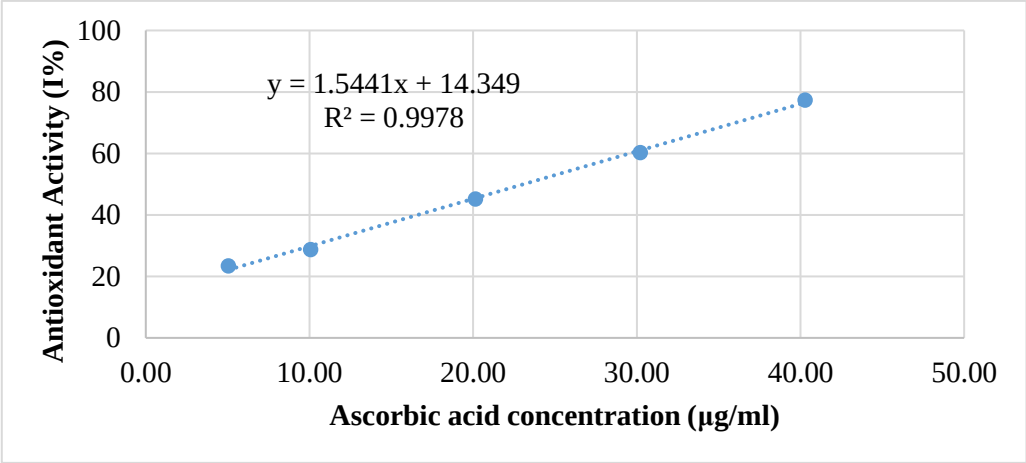

Figure 4. DPPH radical scavenging activity of ascorbic acid

From the linear equation y = 1.5549x + 14.349, the IC_{50} value of ascorbic acid was calculated to be 22.93 µg/ml.

Figure 5 presents the results of antioxidant activity tests performed on both the stems and leaves of *M. fruticosum*. The findings demonstrate a proportional increase in DPPH free radical inhibition with increasing sample concentrations, indicating a direct correlation between sample concentration and antioxidant effectiveness within the linear range. Analysis of the standard curves revealed an IC_{50} value of 5575.60 µg/ml for the stems, which exceeds the IC_{50} value of the leaves at 2292.62 µg/ml, based on the dry weight of the medicinal material. This discrepancy highlights a superior antioxidant capacity in the leaves compared to the stems.

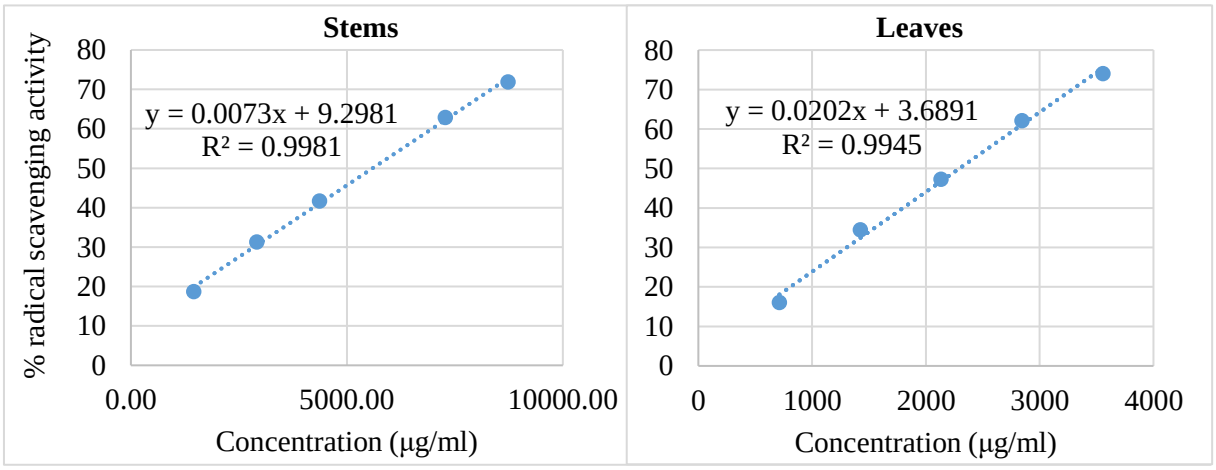

Figure 5. DPPH radical scavenging activity of the stem and leaf extracts of *M. fruticosum*

To date, only one research article has explored the antioxidant effects of *M. fruticosum*, specifically focusing on its flowers [12]. Among the tested extracts, dichloromethane, hexane, methanol, and flower essential oil exhibited varying levels of antioxidant activity, with IC_{50} values of 87.60, 118.00, 194.50, and 100.13 µg/ml, respectively. In contrast, the synthetic antioxidants BHT and α-tocopherol demonstrated higher efficacy, with IC_{50} values of 30.53 and 50.23 µg/ml, respectively. The dichloromethane extract showed the highest radical-scavenging activity among the natural extracts [12]. However, flowers are often less utilized in herbal medicine due to their limited availability and seasonal nature. Therefore, our preliminary study on the antioxidant effects of both the stems and leaves of *M. fruticosum* provides valuable additional information on the properties of these two plant parts.

4. Conclusion

This study identified the presence of several compounds, including flavonoids, coumarins, tannins, polysaccharides, lipids, sterols, and carotenes, in the leaves and stems of *Melodorum fruticosum* Lour. collected in Quang Nam, Vietnam. Quantitative analysis revealed that the leaves contained a significantly higher total flavonoid content (37.80 mg/g) compared to the stems (15.07 mg/g). Antioxidant activity, assessed using the DPPH method, demonstrated the superior antioxidant capacity of the leaf extracts (IC_{50} = 2292.62 µg/ml) compared to the stem extracts (IC_{50} = 5575.60 µg/ml), based on the dry weight of the medicinal material.

These findings suggest further investigation into isolating specific compounds from the leaves and exploring their potential applications in antioxidant therapy.

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