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Research Article

PHYTOCHEMICALS AND ANTIOXIDANT ACTIVITIES OF METHANOLIC EXTRACT FROM *Callistemon citrinus* LEAVES

Nguyen Thi Nga[1], Ngo Thi Sa Ly[1], Vu Thi Hai Yen[1], Huynh Thi Thu Thao[1], Le Quynh Loan[2], Nguyen Hoang Dung[2*]

[1]Faculty of Medicine Laboratory, Hong Bang International Univerity, Ho Chi Minh City, Vietnam
[2]Institute of Tropical Biology, Vietnam Academy of Science and Technology, Vietnam
[*]Corresponding author: Nguyen Hoang Dung – Email: dung008034@gmail.com
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ABSTRACT

Callistemon citrinus is an evergreen shrub commonly used as an ornamental plant; espescially, it is used as a source of medicinal herbs in folk medicine based on its pharmacological properties. This study was performed to screening of phytochemicals and antioxidant activity of C. citrinus leaf methanolic extract. The results indicated that C. citrinus leaf extracts showed the presence of carbohydrates, tannins, saponins, flavonoids, quinones, terpenoids, phenolics, and steroids; in which total phenolic compounds content is 316.36 mg of GAE/g. In the antioxidant activity evaluation, C. citrinus leaf extracts exhibited strong both DPPH and ABTS scavenging activity, with IC_{50} value of 24.50 µg/mL and 46.64 µg/mL, respectively. It can be concluded that C. citrinus leaves have a remakable bioactivity, could be used as a potential candidate for the development of pharmaceutical and cosmetic purposes.

Keywords: antioxidant; *Callistemon citrinus*; methanolic extract; phenolic compounds

1. Introduction

The disparity between reactive oxygen species (ROS) generation and antioxidant ability could lead to the oxidative stress. It is a significant effect factor in the pathogenesis of numerous chronic diseases. Previously published data have clearly emphasized that free radicals and other reactive oxygen species are recognized as agents involved in many biological complications, with a greatter risk of having certain cancers, neurodegenerative disorders, cardiovascular and metabolic diseases as well as atherosclerosis. Beside that, reactive oxygen species are also said to be responsible for human aging (Chiavaroli, 2011; Kanwar, 2009).

An antioxidant can be defined as any substance that delays or inhibits oxidative damage to a target molecule (Mahdi-Pour, 2012). The main characteristic of an antioxidant

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is its ability to scavenge free radicals. Antioxidant compounds like polyphenols, phenolic acids, and flavonoids trap free radicals (such as peroxide, hydroperoxide, or lipid peroxyl) and inhibit the oxidative mechanisms that could lead to many numerous diseases and aging. (Mahdi-Pour, 2012). Herbal plants may contribuit to the optimization of antioxidant status and therefore offer added preventive compounds for overall health (Alok, 2014).

Callistemon citrinus, is an evergreen tree or shrub belonging to the family Myrtaceae. In addition to being used as an ornamental tree in Asian countries, *C. citrinus* leaves are being used locally in essential oils extraction, farm trees, and land reclamation. Several researchers from across the globe have reported the therapeutic potential of this plant (Cock, 2012; Fayemi, 2017; Petronilho, 2013). *C. citrinus* is known in traditional medicine for its antibronchitis, anticough, and insecticidal effects, and its volatile oils have been used as antimicrobial and antifungal agents (Goyal, 2012). Oyedeji documented the antimicrobial properties of *C. citrinus* against different pathogens of bacteria and fungi strains (Oyedeji, 2009). Recently, Fayemi et al. (2019) investigated the bioactivities of phytochemicals in *C. citrinus* against multi-resistant food borne pathogens, α-glucosidase, and MCF-7 cancer cell line, with promising results. Laganà et al. (2020) have carried out a study to investigate the antioxidant and the biological potential of *C. citrinus* flowers. The results showed a prominent activity, able to actively scavenge DPPH and ABTS radicals. The current study was designed to screen the phythochemical compounds of methanolic extract of *C. citrinus* leaves and then evaluate the antioxidant activity of the extract by using DPPH and ABTS scavenging assay, as well as determination of total phenolics content.

2. Materials and methods

2.1. Sample preparation

Callistemon citrinus leaves were collected from Ho Chi Minh City, Vietnam. The collected leaves were brought to the laboratory. The plant leaves were observed carefully and removed any kind of diseases or infection. The selected parts were washed with distilled water and kept for drying under shade at room temperature (27 ± 2 ^{o}C), prevent direct light for about two weeks till constant weight. Then, the dried leaves were finely grounded using an electric blender.

2.2. Extraction method

Material of 100 g powdered *C. citrinus* leaf was soaked in 1000 mL of methanol and kept for four days with periodic shaking. The crude extract was then filtered using a Whatman filter paper number 1 and then was evaporated in a rotary evaporator at 40^{o}C under reduced pressure to obtain the methanolic extract.

2.3. Phytochemical analysis

Phytochemical analysis of *Callistemon citrinus* leaves was carried out according to the methodology of Harbone JB and Trease GE (Harborne, 1984; Trease, 1989). The color reactions were used to test the presence of common metabolite classes such as carbohydrates,

tannins, saponins, flavonoids, quinones, terpenoids, phenols, and steroids. All measurements were performed in triplicate.

2.4. Total phenolic content

The amount of the total phenolic was determined using the Folin-Ciocalteu assay as described by (López-Mejía, 2021). The crude extract was serially diluted with distilled water to final concentrations of 1.0, 0.5, and 0.25 mg/ml. A 100 µl of the water-diluted extract was mixed thoroughly with 0.5 mL of Folin–Ciocalteu reagent for five minutes, followed by the addition of 400 µl of 7.5% sodium carbonate solution. The mixture was allowed to stand for a further 60 min in the dark at 25 °C and then was centrifuged and aliquoted to a 96-well plate. The absorbance was measured at 760 nm by a microplate reader. Gallic acid (0.01–0.4 mM) was used to calculate the standard curve and the results were expressed as gallic acid equivalents (GAE, mg/g).

2.5. DPPH radical scavenging activity assay

DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay was used to determine the antioxidant activity through its free radical scavenging activity. The total methanolic extract and its fractions were dissolved in ethanol into serial concentration. Reaction mixtures were assayed on a 96-well plate, each well consisting of 100 µl of sample and 100 µl of 600 µM DPPH solution. Each sample was mesured in triplicaite. The absorbance at 517 nm of the solution was measured after 30 minutes. The DPPH radical scavenging activity was calculated using the following equation:

$$\% \text{ Scavenging activity} = \left[1 - \frac{\text{OD (sample)}}{\text{OD (control)}} \right] * 100.$$

The concentration of sample required to scavenge 50% of DPPH radicals (50% of inhibitory concentration value / IC50) were determined by probit-graphic interpolation for at least seven concentration levels. The commercial antioxidant (vitamin C) was used as a positive control.

2.6. ABTS radical scavenging activity assay

The modified technique of Nantitanon et al. (2007) was employed to assess the ABTS (2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) potencies of methanolic extract. The ABTS solution was assayed by mixing an equal volume of a seven mmol/L ABTS stock solution with a 2.45 mM potassium persulfate solution. The mixture was then incubated in the dark at room temperature for 12–16 h. The ABTS solution was diluted with sterilized water to an absorbance of 1.00 ± 0.02 at 734 nm. The reaction mixtures were prepare by adding 150 µL of the sample to 750 µL of ABTS diluted solution. The absorbance at 734 nm of the reaction mixture was measured after five minutes. The ABTS radical scavenging activity was calculated using the equation portrayed for DPPH assay above.

3. Results and discussion

3.1. Sample preparation and extraction

Callistemon citrinus leaf samples were collected and processed into a coarse powder (Figure 1 A-B). The sample was then used for extraction with methanol solvent. Extraction efficiency of the methanolic extract is 10.08% (Figure 1 C).

Figure 1. *Callistemon citrinus samples; (A), leaves; (B), dryed powder; and (C), C. citrinus leaf methanolic extract*

3.2. Phytochemical analysis

Methanolic leaf extract of *Callistemon citrinus* was screened for the phytochemical composition (Table 1). The qualitative phytochemical analysis revealed that the methanolic extract contains carbohydrates, saponins, tannins, flavonoids, quinones, terpenoids, triterpenoids, phenolics, and steroids, and these compounds have been reported to own potential biological activities. The presence of these phytochemical compounds aims to the bioactivity of the extracts from *C. citrinus*. Also, these groups have previously shown to have good antioxidant as well as antibacterial and antifungal activities .

Table 1. *Qualitative phytochemical analysis of C. citrinus methanolic leaf extract*

Phytochemical compounds	**Results***
Carbohydrate	++
Saponin	+
Tannin	+
Flavonoid	++
Quinone	+
Terpenoid	+
Triterpenoid	+
Phenolic	++
Steroid	+

*Legend: +, Rare; ++, Abundant

This result is consistent with previous studies that many species in the genus Callistemon contain abundant phenolics, triterpenoids, flavonoids, steroids, and saponins (Goyal, 2012). It is noteworthy that in this study, the qualitative results showed methanolic leaf extract of *C. citrinus* is rich in carbohydrate, flavonoid and phenolic compounds. Phenolic compounds have received much attention for their effective antioxidant properties, and their beneficial effects are attributed to their donating electrons, scavenging free radicals, and reducing power (López-Mejía, 2021). Further experiments will focus on the determination of the total phenolic content and antioxidant activity of the *C. citrinus* leaf extract.

3.3. The total phenolic content and antioxidant activity

The amount of the total phenolic was determined using the Folin-Ciocalteu assay. The phenolic content was determined to reach 316.36 mg of GAE/g in the crude methanol extract of *C. citrinus* leaf. Pham Ngoc Khanh et al. (2016) isolated the phenolic compound from the leaves and stems of *C. citrinus*, this plant was analyzed phytochemical and isolated eight phenolic compounds, including two flavonoids (eucalyptine and 8-demethyleucalyptine), two alcohols (blumenol A, tetratriacontanol), three benzoic acid derivatives (gallic acid, methyl gallate, and protocatechuic acid), and one sterol (β-sitosterol). Phenolic compounds are one of the major contributors to the antioxidant activity of plants due to their redox properties, which can play an remarkable role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Zheng, 2001).

The ABTS and DPPH assays are two widely used methods for investigating the *in vitro* antioxidant capacities of medical plants and herbs. They both have the same reaction mechanism, the quenching of stable colored radicals (ABTS or DPPH) and show the radical scavenging ability of antioxidants by spectrophotometric techniques. Thus, these methods could dectective activity of antoxidants even when present in complex biological mixtures of natural products. As can be seen in Figure 2, the methanolic extract showed a well-defined dose-dependent antioxidant and free-radical scavenging activity towards DPPH• and ABTS•+, the IC_{50} value was 24.50 µg/mL and 46.64 µg/mL, respectively (Figure 2).

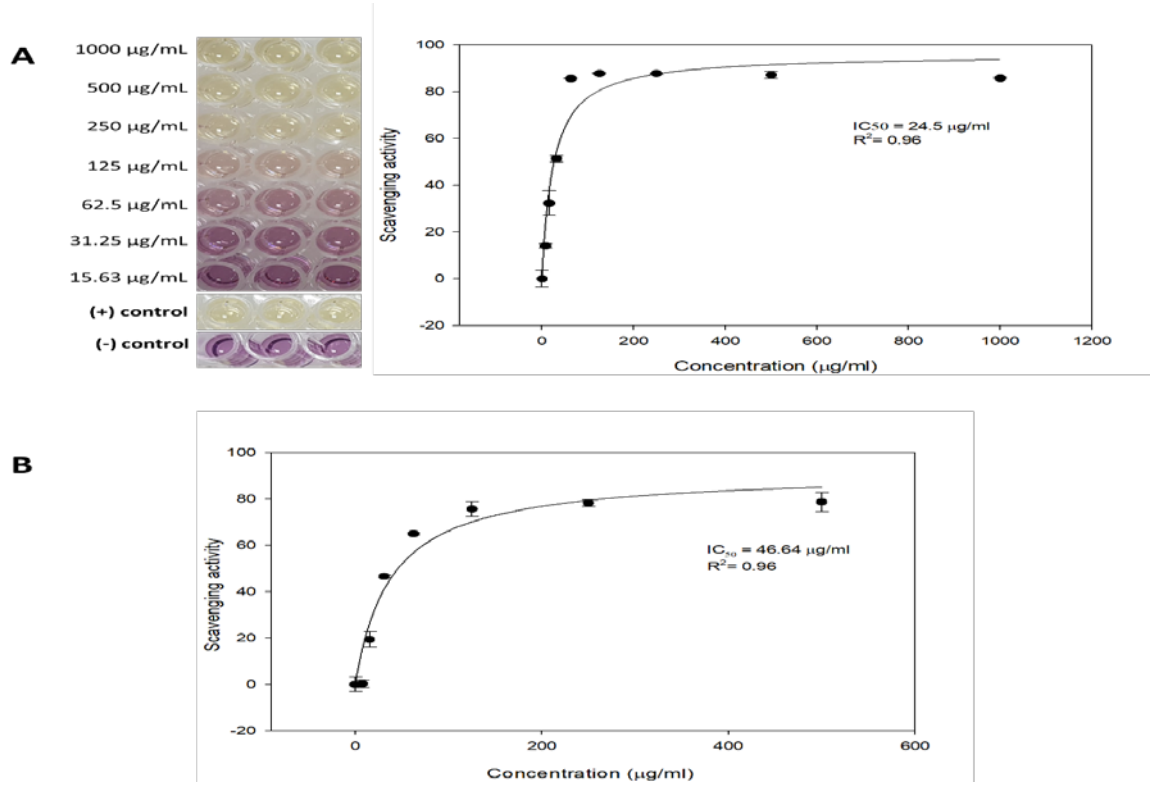

Figure 2. *Antioxidant activity of C. Citrinus methanolic extract; (A), DPPH free radical scavenging activity; and (B), ABTS•+ free radical scavenging activity*

Difference parts of *C. citrinus* tree were reported about the antioxidant activity. Stem bark extract showed a strong DPPH free radical scavenging activity. The IC_{50} reached 10.50 µg/mL. *C. citrinus* flowers contain 250.15 mg GAE/g of phenolic compounds and maximum 92.50% inhibition of DPPH radical at 250 µg/ml (López-Mejía, 2021). Comparison with the published data, the DPPH free radical inhibition of leaf extract (in this study reached a maximum >90% at 88 µg/ml) is higher than the one in flower extract and lower than the one in stem bark. Besides, harvesting leaves will give a high yield and is more convenient than harvesting flowers or stem bark. From this investigation, it can be concluded that, the *C. citrinus* leaves have a remarkable potential role in scavenging free radicals due toantioxidant properties.

4. Conclutions

The above results showed the antioxidant potential of methanolic extract from *C. citrinus* leaves was based on the abundant presence of phenolic compounds (316.36 mg of GAE/g) and strong DPPH and ABTS free radical scavenging activity (IC_{50} = 24.50 µg/mL and 46.64 µg/mL, respectively). It can be concluded that the methanolic extract of *C. citrinus* leaves could be a promising material in the production of pharmaceutical and cosmetic purposes. More studies are in the process performing to investigate chemical and biological propeties as well as to isolate and characterize the bioactive compounds of this species.

❖ ***Conflict of Interest:*** *Authors have no conflict of interest to declare.*

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REFERENCES

Alok, S., Jain, S. K., Verma, A., Kumar, M., Mahor, A., & Sabharwal, M. (2014). Herbal antioxidant in clinical practice: A review. *Asian Pac J Trop Biomed, 4*(1), 78-84.

Chiavaroli, V., Giannini, C., De Marco, S., Chiarelli, F., & Mohn, A. (2011). Unbalanced oxidant-antioxidant status and its effects in pediatric diseases. *Redox Rep, 16*(3), 101-107.

Cock, I. (2012). Antimicrobial Activity of Acacia aulacocarpa and Acacia complanta Methanolic Extracts. *Pharmacognosy Communications, 2*(1), 66-71.

Fayemi, P. O., Ozturk, I., Kaan, D., Özcan, S., Yerer, M. B., Dokumaci, A. H., . . . Yetim, H. (2019). Bioactivities of phytochemicals in Callistemon citrinus against multi-resistant foodborne pathogens, alpha glucosidase inhibition and MCF-7 cancer cell line. *Biotechnology & Biotechnological Equipment, 33*(1), 764-778.

Fayemi, P. O., Öztürk, I., Özcan, C., Muguruma, M., Yetim, H., Sakata, R., & Ahhmed, A. (2017). Antimicrobial activity of extracts of Callistemon citrinus flowers and leaves against Listeria

monocytogenes in beef burger. *Journal of Food Measurement and Characterization, 11*(2), 924-929.

Goyal, P. K., Jain, R., Jain, S., & Sharma, A. (2012). A Review on biological and phytochemical investigation of plant genus Callistimon. *Asian Pac J Trop Biomed, 2*(3, Supplement), S1906-S1909.

Harborne, J. B. (1984). A Guide to Modern Techniques of Plant Analysis. In *Phytochemical Methods* (1 ed., Vol. 1, pp. XII, 288). Springer Book Archive: Springer Dordrecht.

Kanwar, J. R., Kanwar, R. K., Burrow, H., & Baratchi, S. (2009). Recent advances on the roles of NO in cancer and chronic inflammatory disorders. *Curr Med Chem, 16*(19), 2373-2394.

Laganà, G., Barreca, D., Smeriglio, A., Germanò, M. P., #039, Angelo, V., . . . Trombetta, D. (2020). Evaluation of Anthocyanin Profile, Antioxidant, Cytoprotective, and Anti-Angiogenic Properties of Callistemon citrinus Flowers, *9*(8), 1045.

López-Mejía, A., Ortega-Pérez, L. G., Magaña-Rodríguez, O. R., Ayala-Ruiz, L. A., Piñón-Simental, J. S., Hernández, D. G., & Rios-Chavez, P. (2021). Protective effect of Callistemon citrinus on oxidative stress in rats with 1,2-dimethylhydrazine-induced colon cancer. *Biomed Pharmacother, 142*, 112070.

Mahdi-Pour, B., Jothy, S. L., Latha, L. Y., Chen, Y., & Sasidharan, S. (2012). Antioxidant activity of methanol extracts of different parts of Lantana camara. *Asian Pac J Trop Biomed, 2*(12), 960-965.

Nantitanon, W., Chowwanapoonpohn, S., & Okonogi, S. (2007). Antioxidant and Antimicrobial Activities of Hyptis suaveolens Essential Oil, *75*(1), 35-54.

Oyedeji, O. O., Lawal, O. A., Shode, F. O., & Oyedeji, A. O. (2009). Chemical composition and antibacterial activity of the essential oils of Callistemon citrinus and Callistemon viminalis from South Africa. *Molecules, 14*(6), 1990-1998.

Petronilho, S., Rocha, S. M., Ramírez-Chávez, E., Molina-Torres, J., & Rios-Chavez, P. (2013). Assessment of the terpenic profile of Callistemon citrinus (Curtis) Skeels from Mexico. *Industrial Crops and Products, 46*, 369-379.

Pham, N. K, Ho, V. D., Tran. T. H., Vu, T. H., Doan, T. V., Son, N. T., . . . Nguyen, M. C. (2016). Phenolic compounds from Callistemon citrinus leaves and stems. *Vietnam Journal of Science and Technology, 54*(2), 190-197.

Trease, G. E., & Evan, W. C. (1989). *Pharmacognosy* (11 Ed.). London: Brailliar Tridel and Macmillan Publishers.

Zheng, W., & Wang, S. Y. (2001). Antioxidant Activity and Phenolic Compounds in Selected Herbs. *Journal of Agricultural and Food Chemistry, 49*(11), 5165-5170.

NGHIÊN CỨU THÀNH PHẦN HÓA THỰC VẬT VÀ HOẠT TÍNH KHÁNG OXY HÓA TỪ CAO METHANOL CỦA LÁ CÂY TRÀM BÔNG ĐỎ *Callistemon citrinus*

Nguyễn Thị Nga[1*], Ngô Thị Sa Ly[1], Vũ Thị Hải Yến[1],
Huỳnh Thị Thu Thảo[1], Lê Quỳnh Loan[2], Nguyễn Hoàng Dũng[2*]
[1]Trường Đại học Quốc tế Hồng Bàng, Thành phố Hồ Chí Minh, Việt Nam
[2]Viện Sinh học Nhiệt đới, Viện Hàn lâm Khoa học Công nghệ, Việt Nam
[*]Tác giả liên hệ: Nguyễn Hoàng Dũng – Email: dung008034@gmail.com
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TÓM TẮT

Cây Tràm bông đỏ (Callistemon citrinus) là loại cây bụi thường xanh thường được sử dụng như cây cảnh; đặc biệt, được sử dụng như một nguồn dược liệu trong y học dân gian nhờ vào đặc tính dược lí. Trong nghiên cứu này, việc định tính các thành phần hóa thực vật và hoạt tính kháng oxy hóa của cao chiết methanol từ lá cây C. citrinus được thực hiện. Kết quả cho thấy lá C. citrinus chứa carbohydrates, tannins, saponins, flavonoids, quinones, terpenoids, phenolics và steroids. Hàm lượng phenolic tổng số là 316,36 mg GAE/g. Cao chiết lá C. citrinus có hoạt tính ức chế mạnh đối với gốc tự do của cả DPPH và ABTS với giá trị IC_{50} đạt lần lượt là 24,50 µg/mL và 46,64 µg/mL. Từ kết quả nghiên cứu có thể kết luận rằng lá cây C. citrinus có hoạt tính sinh học đáng chú ý, có thể được sử dụng làm nguyên liệu để phát triển các loại dược phẩm và mĩ phẩm.

Từ khóa: kháng oxy hóa; *Callistemon citrinus;* cao methanol; các chất phenol