PHYTOCHEMICAL PROFILES AND POTENTIAL BIOLOGICAL ACTIVITIES OF THE FLOWER EXTRACT OF *Santalum album* L. GROWN IN DAKLAK PROVINCE

Phan Thi Kim Phung[1], Tran Thi Minh Tam[1], Nguyen Thi Hong[1], Le Thi Thu Hong[2], Nguyen Duc Dinh[3], Ho Thi Thanh Thanh[4], Doan Manh Dung[5]

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ABSTRACT

This study explores phytochemical profiles and several biological activities of *Santalum album* L. flowers collected from Dak Lak Province, Vietnam. The qualitative phytochemical analysis of *Santalum album* flower extracts reveals the presence of free triterpenoids, flavonoids, and tannins, particularly in the alcohol and water extracts, suggesting a rich source of bioactive compounds with potential therapeutic benefits, while other compound groups like saponins, alkaloids, coumarins, and essential oils are either absent or present in very low concentrations. Through UHPLC methods, a range of bioactive compounds were identified, including 10 flavonoids and 2 polyphenols compounds. Main compounds identified include salicylic acid, vitexin and catechin. The antioxidant potential of the extracts was evaluated using DPPH and ABTS radical scavenging assays with IC_{50} values of 152.08 ± 0.11 µg/mL and 104.32 ± 0.19 µg/mL, respectively, showing significant free radical inhibition. Additionally, the extracts demonstrated inhibitory activity against *α-glucosidase* and *α-amylase* with IC_{50} value of 233.05 ± 0.32 µg/mL and 124.27 ± 0.17 µg/mL respectively. These findings suggest the potential of the Santalum album L. flower extract in managing diabetes, particularly with its weaker inhibition of α-amylase compared to acarbose, which may help minimize side effects commonly associated with synthetic drugs. This research represents the first comprehensive phytochemical investigation of *Santalum album* flowers, revealing a rich chemical profile that supports further exploration into their pharmacological applications.

Keywords: *Santalum album L., flower extract, phytochemistry, flavonoid, anti-diabetes.*

1. INTRODUCTION

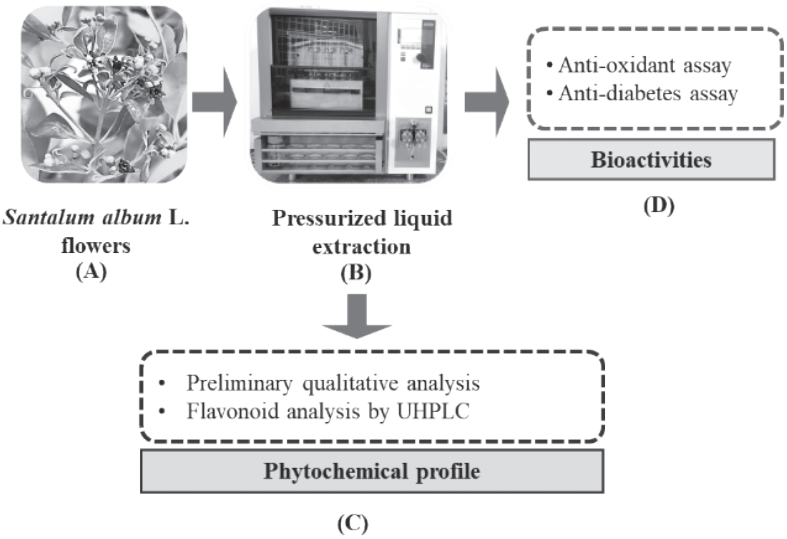
Santalum album L., commonly known as sandalwood, is a species renowned for its fragrant heartwood, traditionally used in perfumery, cosmetics, and religious rituals (Sharma and Kaushal, 2021). Beyond its aromatic properties, *Santalum album* has been the subject of various studies due to its rich phytochemical profile, which includes flavonoids, sesquiterpenes, tannins, fatty acids, esters, aldehydes, vitamin, and minerals (Bisht and Kumar, 2021). These compounds are associated with diverse pharmacological activities, such as antioxidant, anti-inflammatory, anti-cancer, anti-diabetic anti-viral, anti-bacterial, anti-fungal, hepatoprotective and cardio-protective properties (Sharma and Kaushal, 2021). The successful cultivation of *Santalum album* in Dak Lak Province has not only provided a valuable source of herbal medicine but also holds significant economic importance.

Despite the well-documented benefits of *Santalum album*, its flowers have received comparatively less attention. Phytochemical investigations into other plant flowers have revealed a richness in bioactive compounds such as flavonoids, tannins, and polyphenols, which are known for their roles in combating oxidative stress, managing diabetes, and preventing neurodegenerative diseases such as Alzheimer's. For example, *Hibiscus rosa-sinensis* flowers are known for their high content of bioactive compounds, which exhibit significant antioxidant and anti-inflammatory properties (Geeganage and Gunathilaka, 2024). Similarly, *Sophora japonica* flowers contain variety of bioactive compounds, including flavonoids (kaempferol, quercetin, rutin, isorhamnetin), isoflavonoids (genistein, sophoricoside), triterpenes, alkaloids, and polysaccharides. These compounds contribute to a range of pharmacological actions, such as cardiovascular benefits, anti-inflammatory, antioxidant, antitumor, anti-diabetic effects (X. He et al., 2016), (Ghatti et al., 2024).

[1]*Faculty of Medicine and Pharmacy, Tay Nguyen University;*
[2]*Faculty of Pharmacy, Lac Hong University;*
[3]*Faculty of Agriculture and Forestry, Tay Nguyen University;*
[4]*Department of Science and Technology of DakLak Province;*
[5]*Institute of Biotechnology and Environment, Tay Nguyen University;*
Corresponding author: Doan Manh Dung; Tel: 0912717202; Email: dmdung@ttn.edu.vn.

To address this gap, we employed Pressurized Liquid Extraction (PLE) and Ultra-High Performance Liquid Chromatography (UHPLC) to extract and profile the bioactive compounds from *Santalum album* flowers. PLE enhances the efficiency of extracting secondary metabolites from plants by reducing both solvent use and extraction time, while UHPLC ensures high resolution and sensitivity for detecting even low concentrations of target molecules (Wianowska and Gil, 2019), (Nováková and Vlčková, 2009). Additionally, antioxidant (DPPH and ABTS assays) and anti-diabetic (α-amylase and α-glucosidase inhibition) assays were employed to evaluate the biological potential of the extracts.

This research represents the first investigation into the bioactive compounds present in *Santalum album L.* flowers. Through UHPLC and bioactivity assays, this study aim to identify the key phytochemicals and explore their pharmacological potential, particularly focusing on antioxidant and anti-diabetic activities (Scheme 1).



Scheme 1. A scheme of the study: (A) Source of extract, (B) Extraction process, (C) Phytochemical profile and (D) Bioactivities

2. MATERIALS AND METHODS

2.1. Materials

Santalum album flowers were collected in 2024 from Dak Lak Province, Vietnam. The plant was authenticated by Nguyen Duc Dinh. The flowers were dried to a constant weight, vacuum-sealed in polyethylene bags, and labeled SAL-DL-2024 (*Santalum album* L.- Dak Lak - year 2024). These specimens were stored at 0-4 °C until extraction.

Pharmaceutical-grade ethanol (Vietnam) was used as the solvent for extraction.

The standards chemicals for UHPLC analysis, including catechin, chlorogenic acid, epicatechin, epicatechin gallate, vitexin, salicylic acid, isovitexin, rutin, apigetrin, quercetin, luteolin, kaempferol were procured from Sigma Chemical Co., USA.

Enzymes *α-glucosidase*, *α-amylase* and reagents 2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were purchased from Sigma Chemical Co., USA.

2.2. Methods

2.2.1. Preparation of the extract from Santalum album flower

The extraction of *Santalum album* flowers was conducted using the pressurized liquid extraction (PLE) technique, following a modified method described by Phan Thi Kim Phung et al (Phan Thi Kim Phung et al., 2023). The process involved soaking 2 grams of dried flower powder in 20 mL of 70% ethanol under pressure 100 bar, temperature extraction 60°C for 50 minutes by PLE system E-916 (SpeedExtractor E-916, Buchi, Switzerland). After that, the extract was filtered, evaporated to remove the solvent, and the concentrated extract was stored at a temperature between 0-4°C for futher analysis.

2.2.2. Phytochemical analysis

Preliminary qualitative analysis: The phytochemical composition of *Santalum album* flowers was investigated using ether, alcohol, and water extracts. These extracts were tested for the presence of bioactive compounds such as alkaloids, saponins, coumarins, anthranoids,

terpenoids, steroids, tannins, flavonoids, carotenoids, and cardiac glycosides etc, using standard phytochemical screening methods (Shaikh and Patil, 2020), (Cyril et al., 2019).

Ultra High-Performance Liquid Chromatography method: The components of flavonoids and phenolics in the *Santalum album* flower extract were analyzed using a UHPLC system (Thermo Ultimate 3000, USA) based on the method described by Doan Manh Dung et al (Doan et al., 2023). The extract was prepared at a concentration of 10.0 mg/mL in methanol (MeOH) and filtered through a 0.45 µm Polyvinylidene difluoride membrane filter before injection. Separation was performed using a Hypersil GOLD aQ column (3 µm, 150 × 2.1 mm) at 30°C. The mobile phase consisted of MeOH and water with 0.10% H_3PO_4, with a flow rate of 0.2 mL/min. The gradient elution program ranged from 5% to 95% MeOH over 26 minutes, with peaks detected at 265 nm.

2.2.3. Bioactivity assays

For the evaluation of biological activities, several assays were employed:

Antioxidant Activity: The DPPH and ABTS radical scavenging assays were used to assess antioxidant potential, following the protocols detailed by Nguyen Quang Vinh et al (Nguyen Van Bon et al., 2018). In brief, samples at various concentrations were mixed with DPPH or ABTS solutions, incubated, and the absorbance was measured at 517 nm and 734 nm, respectively. Ascorbic acid served as the positive control.

Anti-glucosidase and Anti-amylase Assays: These assays were performed based on the method described by Nguyen et al (Nguyen Van Bon and Wang, 2018), using acarbose as a control.

All tests were conducted in triplicate. The inhibitory activity was measured and presented as percentage inhibition, with IC_{50} values calculated to determine the concentration required to inhibit 50% of the enzyme activity. Lower IC_{50} values indicate stronger inhibitory potential.

2.2.4. Statistical Analysis

Data were analyzed using Microsoft Excel and SPSS software. The analysis involved the calculation of means and standard deviations (SD) to summarize the data. A p-value of <0.05 was considered statistically significant.

3. RESULTS AND DISCUSSIONS

3.1. Chemical profiles of Santalum album flower extract

3.1.1. Preliminary qualitative analysis

The qualitative phytochemical analysis of *Santalum album* flower extracts is summarized in Table 1. The results indicate the presence or absence of various phytochemical groups across different solvents used for extraction.

Table 1. Qualitative phytochemical analysis of *Santalum album* flower extracts

Test	Reagent/Method	Qualitative Results		
		Ether Extract	Alcohol Extract	Water Extract
Lipids	Spot test	-		
Carotenoids	H_2SO_4	-		
Essential Oils	Evaporation to residue	-		
Free Triterpenoids	Libermann-Burchard reagent	+		
Alkaloids	General alkaloid reagent	-	-	-
Courmarins	Fluorescence in alkali	-	-	-
Anthranoids	10% KOH	-		
Flavonoids	Mg/HCl	-	++	-
Cardiac Glycosides	Lactone ring reagent		-	-
	2-deoxy sugar reagent		-	-
Anthocyanosid	HCl			-
	KOH			-
Proanthocyanidin	HCl/temperature			-
Tanins	Ferric Chloride		+++	+++
	Gelatin		-	+

Test	Reagent/Method	Qualitative Results		
		Ether Extract	Alcohol Extract	Water Extract
Saponins	Saponin		-	-
Reducing Substances	Fehling's reagent		-	-

Note: (-) Not present, (±) Not clear, (+) Slightly present, (++) Present, (+++) Abundantly present, (++++) Highly abundant

There may be a reaction, but it was not performed

The compound group is not present in the extract

The detection of free triterpenoids in the ether extract of *Santalum album* flowers suggests potential therapeutic applications related to these compounds.

The Ferric Chloride test shows a strong positive reaction (+++) in both alcohol and water extracts, indicating a significant presence of phenolic compounds. The presence of flavonoids predominantly in the alcohol extract, along with tannins in water extracts, highlights the effectiveness of polar solvents in extracting these bioactive compounds. Flavonoids, known for their powerful antioxidant properties, have potential roles in cancer prevention, diabetes management, and anti-Alzheimer's activities, warranting further study (Nguyen Van Bon et al., 2023), (Phan Thi Kim Phung et al., 2023)In this research, the specific components of flavonoids in *Santalum album* flowers will be analyzed using the UHPLC method.

The absence of sapoin, alkaloids, coumarins, and essential oils etc suggests they are either not present or are in very low concentrations in *Santalum album* flowers, which may help narrow the focus of future studies.

This study adds valuable information to the chemical profile of *Santalum album*, especially since the phytochemical profiles of its flowers have not been previously reported. The presence of bioactive compounds highlights the potential for further research to isolate and characterize these compounds, as well as investigate their specific bioactivities.

3.1.2. UHPLC Analysis

The qualitative phytochemical analysis indicated that flavonoids were predominantly present in the alcohol extract ofn*Santalum album* flowers. To further investigate these findings, UHPLC was conducted to determine the flavonoid and phenolic components present in the extract. The UHPLC analysis of *Santalum album* flower extract revealed a profile of phenolic compounds, with a significant presence of flavonoids and polyphenols (shown in Table 2 and Figure 1).

Table 2. Content of major compounds identified from *Santalum album* flower extract

No	Compound	Group/Subgroup	Content (mg/g) (n=3)
1	Catechin	Flavonoid/Flavanol	15.178 ± 0.013^{b}
2	Chlorogenic acid	Polyphenol	9.117 ± 0.01^{d}
3	Epicatechin	Flavonoid/Flavanol	7.014 ± 0.011^{e}
4	Epicatechin gallate	Flavonoid/Flavanol	5.832 ± 0.005^{f}
5	Vitexin	Flavonoid/Flavone	15.425 ± 0.004^{b}
6	Salicylic acid	Polyphenol	50.792 ± 0.023^{a}
7	Isovitexin	Flavonoid/Flavone	11.512 ± 0.004^{c}
8	Rutin	Flavonoid/Flavonol glycoside	4.371 ± 0.003^{g}
9	Apigetrin	Flavonoid/Flavonol glycoside	0.311 ± 0.001^{h}
10	Quercetin	Flavonoid/Flavonol	0.063 ± 0.002^{i}
11	Luteolin	Flavonoid/Flavone	0.06 ± 0.001^{i}
12	Kaempferol	Flavonoid/Flavonol	0.038 ± 0.001^{k}

Values in the same column with the different letters are significantly different

A total of 12 **(1-12)** distinct compounds were identified in the analysis. Among the polyphenols, salicylic acid was the most concentrated, with a content of 50.792 mg/g. Salicylic acid is widely recognized for its anti-inflammatory and skin-soothing effects, making it a valuable component in both therapeutic and cosmetic applications (Phan Thi Kim Phung et al., 2023). Chlorogenic acid (9.118 µg/g), another polyphenol, is known for its antioxidant, anti-inflammatory, and anti-diabetic, anticardiovascular, antimutagenic and anticancer effects. (Miao and Xiang, 2020). Its significant presence reinforces the potential health benefits of *Santalum album* extract, especially in managing oxidative stress-related conditions.

Vitexin (15.425 mg/g) and catechin (15.181 mg/g) were identified as the most abundant flavonoids. Vitexin, a flavone, is known for its antioxidant, anti-diabetic, anti-inflammatory, and neuroprotective effects (M. He et al., 2016), while catechin, a flavanol, is recognized for its strong antioxidant properties and its role in cancer and cardiovascular diseases (Ohishi et al., 2022). The high concentration of these compounds suggests that *Santalum album* flowers could have potent health-promoting properties. The extract also contains notable amounts of epicatechin (7.013 mg/g), epicatechin gallate (5.831 mg/g), and isovitexin (11,512 mg/g), all of which are flavonoids with documented antioxidant, anti-inflammatory, and potential cardioprotective effects (M. He et al., 2016), (Phan Thi Kim Phung et al., 2023). Minor constituents such as rutin, apigetrin, quercetin, luteolin, and kaempferol were also identified.

Overall, the UHPLC analysis reveals that *Santalum album* flowers are a rich source of bioactive phenolic compounds, particularly flavonoids and polyphenols. The high concentrations of vitexin, catechin, and salicylic acid suggest that the extract could be utilized for its antioxidant, anti-diabetic and potential therapeutic properties. Further studies could explore the specific biological activities of these compounds to better understand their synergistic effects and potential health benefits.

3.2. Novel medical effects of Santalum album flower extract

The bioactivity analysis of *Santalum album* flower extract was conducted to assess its inhibitory effects on various biological targets, including free radical scavenging activities (DPPH and ABTS), and carbohydrate-metabolizing enzymes (*α-amylase* and *α-glucosidase*). The IC_{50} values for these activities are presented in Table 3.

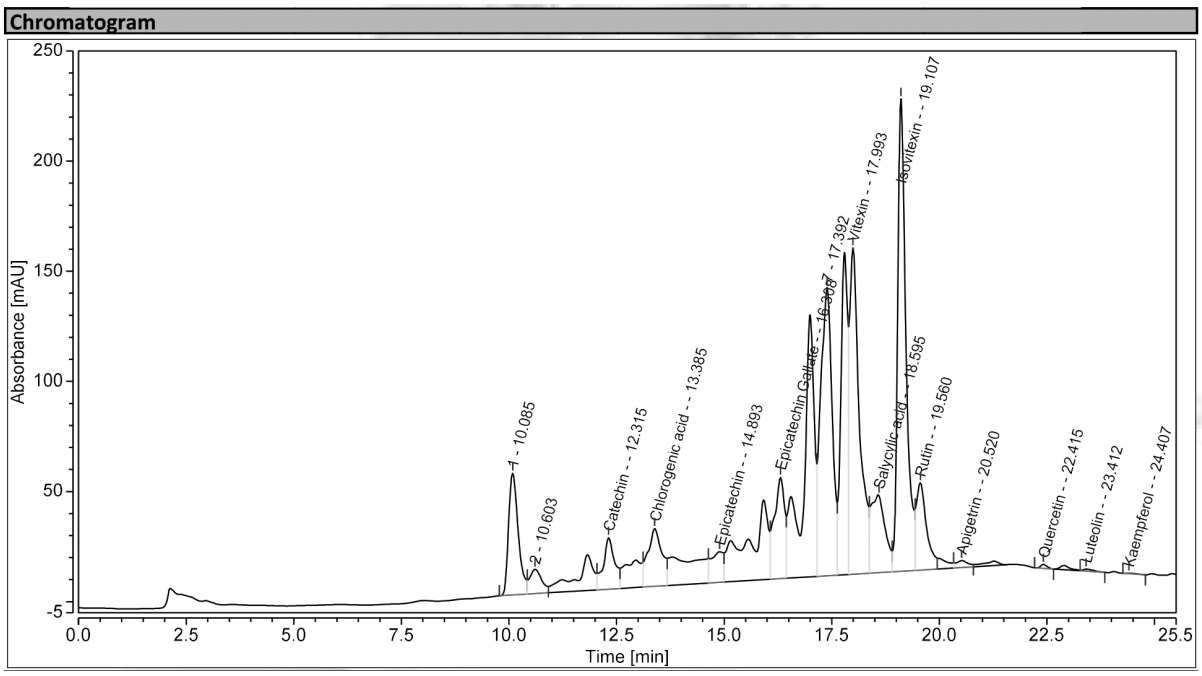


Figure 1. UHPLC finger printing of *Santalum album*'s flower extract.

Table 3. The bioactivities of *Santalum album* flowers

Sample	DPPH (IC_{50}, µg/mL)	ABTS (IC_{50}, µg/mL)	*α-amylase* Inhibition (IC_{50}, µg/mL)	*α-glucosidase* Inhibition (IC_{50}, µg/mL)
***Santalum album* flowers extract**	152.08 ± 0.11	104.32 ± 0.19	233.05 ± 0.32	124.27 ± 0.17
Trolox	60.42 ± 0.15	38.17 ± 0.28	Nd	Nd
Acarbose	Nd	Nd	24.80 ± 0.13	192.35 ± 0.28

3.2.1. Antioxidant Activity (DPPH and ABTS Assays)

Antioxidant activity is crucial in combating oxidative stress, which is linked to various chronic diseases such as amyotrophic lateral sclerosis, Parkinson's and Alzheimer's diseases, cardiovascular diseases and diabetes (Jamshidi-kia et al., 2020). The DPPH and ABTS assays measure the extract's ability to neutralize free radicals.

The *Santalum album* flower extract exhibited notable DPPH and ABTS radical scavenging activities with IC_{50} values of 152.08 ± 0.11 µg/mL and 104.32 ± 0.19 µg/mL, respectively. When compared to Trolox, a well-known antioxidant used as a positive control, the extract shows around 2.5 times higher IC_{50} values, as Trolox had 60.42 ± 0.15 µg/mL for DPPH and 38.17 ± 0.28 µg/mL for ABTS. Despite this difference, the antioxidant potential of the extract remains notable, suggesting the presence of bioactive compounds capable of neutralizing free radicals. Further studies are warranted to isolate and characterize the specific bioactive compounds responsible for these activities, which could lead to the development of extract rich in key bioactive compounds that can be applied as natural therapeutic agents.

3.2.2. Inhibition of Carbohydrate Hydrolyzing Enzymes

To assess the anti-diabetic potential of *Santalum album* flower extracts, their inhibitory effects on key carbohydrate-hydrolyzing enzymes, namely *α-amylase* and *α-glucosidase*, were examined. These enzymes play a crucial role in the digestion of dietary carbohydrates, and their inhibition is a widely recognized strategy for managing postprandial blood glucose levels in type 2 diabetes (T2D) (Kaur et al., 2021).

The extract demonstrated moderate inhibition against *α-amylase*, with an IC_{50} value of 233.05 ± 0.32 µg/mL, which is significantly higher than the IC_{50} value of the standard drug acarbose, recorded at 24.80 ± 0.13 µg/mL. This suggests that while the extract inhibits *α-amylase*, its activity is weaker compared to acarbose (9.4 times). Interestingly, the extract exhibits an IC_{50} of 124.27 ± 0.17 µg/mL, lower than acarbose at 192.35 ± 0.28 µg/mL, meaning the extract is about 1.5 times more effective in inhibiting α-*glucosidase*. This is an important finding, as it indicates superior selectivity for α-*glucosidase* inhibition. This selective inhibition is advantageous for managing postprandial blood glucose levels in type 2 diabetes (T2D). Synthetic drugs like acarbose, a common *α-glucosidase* inhibitor, strongly inhibit both enzymes, often leading to gastrointestinal side effects such as abdominal distention, flatulence, and diarrhea. These side effects are believed to result from excessive pancreatic *α-amylase* inhibition, which can cause abnormal bacterial fermentation of undigested carbohydrates in the colon (Oboh, Akinyemi, and Ademiluyi, 2012).

All in all, the milder inhibition of *α-amylase* by *Santalum album* flower extract suggests a lower probability of such adverse effects, while its strong inhibition of *α-glucosidase* remains effective in controlling postprandial hyperglycemia. This combination positions *Santalum album* flower extract as a potential natural therapeutic agent that can manage blood glucose levels with reduced risk of gastrointestinal discomfort, making it a promising alternative to conventional drugs for T2D management. The findings align with other studies, such as those found in *T. occidentalis*, which also exhibit mild *α-amylase* inhibition alongside strong *α-glucosidase* inhibition (Oboh, Akinyemi, and Ademiluyi, 2012). Moreover, other research has indicated that extracts rich in flavonoid compounds like vitexin, catechin, isovitexin and kaempferol possess notable anti-diabetic properties (Phan Thi Kim Phung et al., 2023) (Xiao et al., 2013).

4. CONCLUSIONS

This work first reports the phytochemical profiles and several medical effects of *Santalum album* flower extract. The qualitative phytochemical analysis uncovered a rich presence of flavonoids, particularly in the alcohol extract, and substantial levels of tannins in water extracts. Based on UHPLC, 10 flavonoids and 2 polyphenols compounds were detected and identified. Of these, salicylic acid, vitexin and catechin were found as major compounds. In bioactivities tests, *Santalum album* flower extract demonstrated a high antioxidant effect. This extract also was found to show moderate inhibition of *α-amylase* and potent inhibition of *α-glucosidase*. The result obtained in

this work suggested that *Santalum album* flower extract is a rich source of bioactive substances with potential antioxidant and anti-diabetes effects.

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THÀNH PHẦN HÓA THỰC VẬT VÀ MỘT SỐ HOẠT TÍNH SINH HỌC TIỀM NĂNG CỦA CAO CHIẾT HOA ĐÀN HƯƠNG TRẮNG (*Santalum album* L.) TRỒNG TẠI TỈNH ĐẮK LẮK

Phan Thị Kim Phụng[1], Trần Thị Minh Tâm[1], Nguyễn Thị Hồng[1], Lê Thị Thu Hồng[2], Nguyễn Đức Định[3], Hồ Thị Thanh Thanh[4], Đoàn Mạnh Dũng[5]

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TÓM TẮT

Nghiên cứu được thực hiện nhằm phân tích thành phần hóa thực vật và hoạt tính sinh học của hoa *Santalum album* L. thu hái ở tỉnh Đắk Lắk, Việt Nam. Kết quả phân tích sơ bộ cho thấy trong cao chiết từ hoa có triterpenoid tự do, flavonoid và tannin, đặc biệt là trong các cao chiết cồn và nước, gợi ý về một nguồn giàu hợp chất sinh học có tiềm năng trị liệu. Các nhóm hợp chất khác như saponin, alkaloid, coumarin, và tinh dầu hiện diện ở nồng độ rất thấp hoặc không có. Thông qua phương pháp UHPLC, nhiều hợp chất sinh học đã được xác định, bao gồm 10 flavonoid và 2 hợp chất polyphenol, trong đó, các hợp chất chính là acid salicylic, vitexin và catechin. Tiềm năng chống oxy hóa của cao chiết được đánh giá bằng thử nghiệm DPPH và ABTS với giá trị IC_{50} lần lượt là lượt là 152,08 ± 0,11 µg/mL và 104,32 ± 0,19 µg/mL, cho thấy khả năng ức chế gốc tự do đáng kể. Ngoài ra, cao chiết cũng thể hiện hoạt tính ức chế enzyme *α-glucosidase* và *α-amylase* với IC_{50} lần lượt là 233,05 ± 0,32 µg/mL và 124,27 ± 0,17 µg/mL, cho thấy tiềm năng kháng đái tháo đường với ít tác dụng không mong muốn. Đây là nghiên cứu đầu tiên về thành phần hóa thực vật của hoa *Santalum album* L., góp phần cung cấp thông tin cho các nghiên cứu sâu hơn về tiềm năng ứng dụng của loài hoa này.

Từ khóa: *Santalum album L, chiết xuất hoa, thành phần hóa thực vật, flavonoid, kháng đái tháo đường.*

[1]Khoa Y Dược, Trường Đại học Tây Nguyên;
[2]Khoa Dược, Trường Đại học Lạc Hồng;
[3]Khoa Nông Lâm nghiệp, Trường Đại học Tây Nguyên;
[4]Sở Khoa học và Công nghệ tỉnh Đắk Lắk;
[5]Viện Công nghệ Sinh học và Môi trường, Trường Đại học Tây Nguyên;
Tác giả liên hệ: Đoàn Mạnh Dũng; ĐT: 0912717202; Email: dmdung@ttn.edu.vn.

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