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Phytochemical Screening, Antioxidant and Xanthine Oxidase Inhibitory Activities of *Vitis heyneana* Schult. Stem Extracts From Vietnam

Linh K.H. Nguyen[1], Chen V. Tran[2]*, Nguyen D. Pham[1], Tan V. Tran[3]

[1]*Faculty of Pharmacy, Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam.*
[2]*Faculty of Traditional Medicine, University of Medicine and Pharmacy at Ho Chi Minh City, Ho Chi Minh City, Vietnam.*
[3]*Faculty of Pharmacy, University of Medicine and Pharmacy at Ho Chi Minh City, Ho Chi Minh City, Vietnam.*

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ABSTRACT

Vitis heyneana Schult. (family Vitaceae) is locally known as 'Wild Grapes'. It has long been used traditionally as a remedy for irregular menstruation, furuncle, bronchitis, and arthritis-related diseases. Antioxidant and xanthine oxidase (XO) inhibitory activities have not been reported in this species. Therefore, the present study aim to investigate the antioxidant and XO inhibitory activities of the plant. Phytochemical screening of the ethanol stem extract was carried out using standard method. The antioxidant activity was evaluated using the 2,2-diphenyl-1-picryl hydrazyl (DPPH) radical scavenging method. The XO inhibitory activity was evaluated using standard procedure. Phytochemical screening showed the presence of carbohydrates, essential oils, amino acids, triterpenoids, saponins, coumarins, flavonoids, and tannins. The ethyl acetate fraction showed the highest antioxidant activity with IC_{50} value of 21.90 ± 0.17 µg/mL, followed by chloroform fraction (IC_{50} = 35.92 ± 0.28 µg/mL), petroleum ether fraction (IC_{50} = 52.22 ± 0.20 µg/mL), and aqueous fraction (IC_{50} = 143.37 ± 1.12 µg/mL). The plant extract exhibited significant XO inhibitory activity with the ethyl acetate fraction showing the highest activity (IC_{50} = 11.28 ± 0.41 µg/mL) followed by the aqueous fraction (IC_{50} = 17.51 ± 0.38 µg/mL), chloroform fraction (IC_{50} = 35.75 ± 0.19 µg/mL), and the petroleum ether fraction (IC_{50} = 130.20 ± 0.24 µg/mL). These results suggest the potential use of *V. heyneana* stems in the management of gout as well as an antioxidant to eliminate free radicals and reduce the oxidative stress associated with gout.

Keywords: *Vitis heyneana*, Antioxidant, Anti-gout, Phytoconstituents, Xanthine oxidase.

Introduction

Gout is a chronic disease caused by a disorder of uric acid metabolism that is characterized by hyperuricemia. Gout patients have joint pain as a result of the long-term increase in serum uric acid level, which causes monosodium urate crystals to form in the joints and subcutaneous tissues.[1,2]

Oxidation is a process that occurs normally in the body. However, oxidative stress is caused by increased production of reactive oxygen species (ROS), e.g., hydrogen peroxide, superoxide, hydroxyl, hydroperoxyl, and peroxyl radicals, and reactive nitrogen species (RNS), e.g., nitroxyl anion, nitrosonium cation, higher oxides of nitrogen, S-nitrosothiols, and dinitrosyl iron complexes, which leads to the unbalance between the free radical activity and the antioxidant systems within the body cells and tissues.[3,4] Particularly, xanthine oxidase (XO) is a crucial enzyme of oxygen-derived free radicals that contribute to oxidative damage.[5] XO uses oxygen and purine to produce ROS ($O2^-$, H_2O_2) and uric acid through oxidative hydroxylation.[6] In other words, XO catalyzes the oxidation of hypoxanthine to xanthine and subsequently uric acid.[7]

*Corresponding author. E mail: tvchenpharma@ump.edu.vn, tvchenpharma@gmail.com
Tel: +84866486674

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Generally, these agents have the ability to oxidize proteins, lipids, and nucleic acids and produce hazardous byproducts. These byproducts can cause cellular damage, such as changes in membrane structure, damage to organelles, damage to DNA, and dysfunction of cells, in addition to a number of serious illnesses (e.g., diabetes, hypertension, gouty inflammation, dyslipidemia, atherosclerosis, etc.).[4,8-10] Previous reports have demonstrated that gout is associated with comorbidities such as hypertension, renal disease, diabetes mellitus, metabolic syndrome, hyperlipidemia and atherosclerosis, cancer, and aging.[2,11] Therefore, antioxidant therapy using bioactive compounds that inhibit XO activity is frequently suggested as an effective method to prevent gout complications and related ailments.

Xanthine oxidase inhibitors (XOIs) e.g., allopurinol, topiroxostat, febuxostat, and pyranostat are a class of medications used for lowering uric acid level in the management of gout.[12,13] Allopurinol, introduced in 1966, is the most commonly used drug to lower urate levels in serum and urine.[14] It has been shown to be effective in reducing flares and tophi as urate levels decrease, reducing cardiovascular mortality, the risk of developing chronic kidney disease, the risk of prostate cancer,[15-17] as well as anti-inflammatory, and analgesic effects.[12] However, due to the long-term treatment, allopurinol may lead to many adverse effects including gastrointestinal bleeding, renal toxicity,[5,18] hypersensitivity syndrome and rashes,[13] toxic epidermal necrolysis syndrome, and liver function abnormalities.[5] Therefore, the search for new XO inhibitors, especially natural compounds, is still necessary. Studies have reported that bioactive compounds such as polyphenols (e.g., flavonoids, tannins, and coumarins), saponins, terpenoids, and alkaloids showed uric acid-lowering effects and were effective in inhibiting XO.[19,20] Furthermore, these compounds may also contribute to the improvement of gout by impeding oxidative stress.[20]

The genus *Vitis* L. (belonging to the family Vitaceae) contains 81 accepted species,[21] and they are well known for their many uses in

traditional medicine as well as in foods, beverages, and wines. In Vietnam, *V. heyneana* Schult. commonly called 'Wild Grapes' was discovered in the northern part of the country (Lang Son, Cao Bang, and Lao Cai provinces).[22] In Vietnamese folk medicine, *V. heyneana* is used as a remedy for arthritis, bronchitis, carbuncles and other inflammatory symptoms, and menstrual irregularities.[22] Characteristics of 'Wild Grapes' are the climbing vines, stems, and young branches that have yellow-white hairs. Leaves are simple, alternate, heart-shaped base, leaf margin consists of 18-22 pairs of sharp serrated teeth. Peduncle (2-6 cm long), the underside of the leaves, and fruit clusters are yellow-brown and hairy. Tassels (twining) and leaves are opposite, split at the apex, hairy, then smooth. Berries are globose, irregular size in the same cluster, 0.9-1.4 cm in diameter, dark purple when ripe, smooth, sour taste.[23] Many studies have reported phytochemical components isolated from various parts of the plant such as stilbenoids, megastigmane glucosides, cycloartane triterpenoid, benzyl diglycoside, lignan glycoside, and phenolic acid, etc.[22,24] Additionally, Ha *et al.*[22] reported that compounds isolated from the aerial part of V. heyneana have anti-inflammatory effects via suppression of the NF-*κ*B activation in RAW 264.7 cells.[22] However, except for the anti-inflammatory activity, the *in vitro* XO inhibitory activity of the plant has not been investigated and there is still not much evidence to prove the pharmacological effects of this herb.
Therefore, the present study is aimed at substantiating the antioxidant and anti-gout effects of the crude and fractionated extracts obtained from *V. heyneana* stems. This is the first experimental attempt to investigate the XO inhibitory activity of *V. heyneana* stems extracts.

Materials and Methods

Chemicals and reagents
Ethanol, petroleum ether (PE), chloroform ($CHCl_3$), ethyl acetate (EtOAc) (VN-Chemsol, Vietnam); methanol, dimethyl sulfoxide (DMSO) (Merck, Germany); 2,2-diphenyl-1-picrylhydrazyl (DPPH), trolox, allopurinol, xanthine, xanthine oxidase (Sigma, USA). Other analytical-grade chemical reagents were used in this experiment.

Plant material
The stems of *V. heyneana* used in this study were collected from Tam Dao mountain (21°34'23.81"N 105°32'39.58"E), Vinh Phuc Province (north of Vietnam) in December 2019. The specimen was botanically identified by Dr. Vo Van Leo. A voucher specimen (VH-1219) has been deposited at the Faculty of Pharmacy, University of Medicine and Pharmacy at Ho Chi Minh City.

Preparation of crude and fractionated extracts
The stems were washed with clean water, shade dried and then pulverized into a midding powder. Stem powder (500 g) was macerated with 5.0 L of 96% ethanol for three days. The fractions were obtained from the crude extract by liquid-liquid partitioning using solvents in order of increasing polarity, including petroleum ether (PE), chloroform ($CHCl_3$), ethyl acetate (EtOAc), and water. The extracts and fractions were concentrated using a rotary evaporator at 42 ± 5°C, resulting in the crude and fractionated extracts of *V. heyneana* stems. These extracts were stored at 4-8°C until when needed.

Preliminary phytochemical screening
The crude ethanol extract of *V. heyneana* stems was tested for the presence of carbohydrates, essential oils, amino acids, fats, steroids, cardiac glycosides, triterpenoids, saponins, tannins, flavonoids, alkaloids, coumarins, and polyuronides. The qualitative analysis of these phytoconstituents was performed using standard protocols as reported by Amalia *et al.*[25] and Van Chen *et al.*[26] with slight modifications.

Antioxidant assay by DPPH inhibition
The DPPH free radical scavenging method was used to investigate the antioxidant activity of the fractionated extracts of *V. heyneana* stems according to the procedure described by Van Chen *et al.*[10] and Tran *et al.*[27] with some modifications. First, the the fractionated extracts were dissolved in methanol and then diluted to obtain the following concentrations; 6.25, 12.5, 25.0, 50.0, 100, and 200 µg/mL. Each sample dilution (0.5 mL), was added to 3.0 mL of 0.6 mM DPPH solution and 0.5 mL of methanol, and incubated for 30 minutes in the dark at room temperature. As the positive control, Trolox was used at concentrations of 1.25, 2.5, 5.0, 10.0, and 20.0 µg/mL. A mixture of 3.5 mL methanol and 0.5 mL 0.6 mM DPPH solution was used as the negative control. While, the blank was 4.0 mL methanol without extract or trolox. The absorbance of the sample was measured at 517 nm using a ultraviolet-visible spectrophotometer.
The percentage DPPH free radical scavenging activity was calculated with the follwing formula:

DPPH free radical scavenging activity (%)
= [(Control absorbance - Sample absorbance)/(Control absorbance)] x 100

The 50% DPPH free radical scavenging capacity (IC_{50}) of the fractionated extracts was calculated by a linear regression.[10,27]
The antioxidant activity of the fractionated extracts was represented by IC_{50} values (µg/mL).

Xanthine oxidase (XO) inhibitory activity assay
The *in vitro* xanthine oxidase (XO) inhibitory assay of the stem extract of *V. heyneana* was performed according to the procedure of Abdulhafiz et al.28 and Falodun *et al.*[29] with slight modifications. Briefly, 100 µL of extract at different concentrations (6.25, 12.5, 25, 50, 100, and 200 µg/mL prepared in 1% DMSO) was added to 290 µL of phosphate buffer, and 10 µL of enzyme solution (0.1 U/mL XO in phosphate buffer, pH 7.5). After pre-incubation at 25°C for 15 min, the reaction was initiated by the addition of 200 µL of 0.15 mM xanthine solution. This reaction mixture was further incubated at 25°C for 30 min and then the reaction was stopped by the addition of 100 µL of 0.5 N hydrochloric acid. Finally, the absorbance of this reaction mixture was measured at 290 nm by using a UV spectrophotometer. Allopurinol (0.3125, 0.625, 1.25, 2.5, and 5.0 µg/mL) was used as positive control. The XO inhibitory activity of the test samples was shown as the percentage inhibition of XO. The IC_{50} value was determined based on the percentage of inhibition at different concentrations.
The formula below was used for calculating the percentage XO inhibition (% I):

I (%) = [((Ac - Aoc) - (At - Aot))/ (Ac - Aoc)] x 100

Where, Ac is the absorbance of the control (with enzyme, without assayed extract); Aoc is the absorbance of the control blank (without enzyme and assayed extract); At is the absorbance of the assayed sample (with enzyme and assayed extract); Aot is the absorbance of the test blank (without enzyme, with assayed extract).

Data analysis
The results were calculated using Microsoft Excel 2023 and presented as mean values ± S.D (Standard Deviation) of three replicates.

Results and Discussion

Phytochemical constituents
Phytochemical screening of *V. heyneana* stem extract showed the presence of various groups of phytoconstituents including carbohydrates, essential oils, amino acids, triterpenoids, saponins, coumarins, flavonoids, and tannins. However, lipids, carotenoids, alkaloids, cardiac glycosides, and polyuronides were not detected in the stem extract. Phytochemical screening results of *V. heyneana* stems extract is presented in Table 1. Phytochemical screening is known as an analytical method that uses several reagents to perform a chemical reaction to identify secondary metabolites present in plants.[10,26,27]
In recent years, phytochemical compounds found in herbs have received much attention from the public due to their significant benefits to human health.[31] However, many herbs have not been

studied for their phytochemical composition as well as biological effects. Therefore, research on the chemical composition and biological effects of different herbs is still being done to identify biologically active natural compounds.[30,32] For example, *V. heyneana* is used in Traditional Vietnamese Medicine to treat arthritis, bronchitis, boils, inflammation, and menstrual irregularities. These biological effects in the stems of *V. heyneana* may result from the bioactive phytochemical compounds.[22] Generally, the major compounds such as triterpenoids, saponins, tannins, coumarins, and flavonoids present in *V. heyneana* stems are also found in other *Vitis* species such as *Vitis labrusca* L., *Vitis vinifera* L., *Vitis riparia* Michx.,[33,34] *Vitis labruscana* L.H. Bailey, *Vitis rotundifolia* (Lam.) Deflers, etc.[21,34]

Structurally, flavonoids contain a benzo-γ-pyrone derivative bearing a phenolic or poly-phenolic group at different positions.[35] The anthoxanthins (flavanone and flavanol), flavanonols, flavans, chalcones, anthocyanidins, and isoflavonoids are flavonoid subclasses classified on the basis of their chemical structure, degree of unsaturation, and oxidation of the carbon ring.[27,36] Flavonoids are used as anticancer, antibacterial, antiviral, anti-angiogenic, antiparasitic, antioxidant, anti-inflammatory, antispasmodic, neuroprotective, antitumor, and antiproliferative agents.[36,37] Furthermore, Mazidi *et al*.[38] reported that flavonoids have a preventive effect on nonalcoholic fatty liver disease and cardiovascular metabolic disorder-related diseases.[38]

Besides other polyphenol compounds, tannins are also found in the stems of *V. heyneana*. Previous studies have reported tannins as compounds with various therapeutic properties as well as pharmacological effects such as antioxidant, anti-inflammatory, antibacterial, antiviral, antitoxic, anticancer, antiallergic, antiparasitic, wound healing, and antidiarrhoeal activities.[39-41]

Coumarin compounds are commonly found in many medicinal plants. In terms of structure, coumarins belong to the benzopyrone family and consist of two six-membered rings with lactone carbonyl groups. Natural coumarins are classified into various types depending on the differences in substituent locations and characteristics of their chemical structures. Simple coumarins, isocoumarins, furanocoumarins, pyranocoumarins, bis-coumarins, and other coumarins (e.g., phenylcoumarins) are the different subgroups of coumarins.[42,43] In the present study, phytochemical screening revealed the presence of coumarins in the stems of *V. heyneana*, which contributes to the stems of *V. heyneana* as a promising source of medicinal herbs. Coumarins were demonstrated to have multiple pharmacological effects including anti-inflammatory, anticoagulant, antitumor, anti-HIV, antibacterial, antimalarial, antifungal, antiviral, neuroprotective, casein kinase-2 inhibitory, anti-Alzheimer, anticonvulsant, anti-gout, and antihypertensive effects.[42-45]

Additionally, among secondary metabolites, saponins are known to be bioactive compounds with interesting beneficial health effects such as anti-inflammatory, anticancer, cholesterol-lowering, and other biological properties.[46,47]

In the present study, important phytochemical compounds such as essential oils, triterpenoids, saponins, coumarins, tannins, and flavonoids were found in the stem extract of *V. heyneana*. The beneficial effects of these compounds including anti-gout, anti-arthritis, antioxidant, anti-inflammatory, anticancer, antibacterial, antiviral, and other biological effects have been reported.[28,48-50] Moreover, flavonoids, coumarins, tannins, terpenoids, and saponins were reported to inhibit the activity of xanthin oxidase enzyme and also act as antioxidant agents.[51-53]

In addition, phenolic compounds (e.g., coumarins, tannins, and stilbene, etc.) and flavonoids are known to be essential secondary metabolites of plants. Previous studies have reported that these compounds have many pharmacological benefits such as antioxidant, anti-cancer, anti-bacterial, cardioprotective, immune system promoting and anti-inflammatory, and skin protective effect from UV radiation, etc.[28,54] The finding of this study suggested that the stems of *V. heyneana* are rich in phenolic and flavonoids contents, which could be the main agents for their *in vitro* XO inhibitory activity.

Antioxidant activity of crude and fractionated extracts of V. heyneana

The DPPH free radical scavenging activity of the crude and fractionated extracts of *V. heyneana* stems showed a concentration-dependent activity. The antioxidant activity of extract are classified as strong, moderate, and weak according to their IC_{50} (µg/mL) values; 50-100, 100-150, and 151-200 µg/mL, respectively.[55] Among the stem extracts of *V. heyneana*, the crude extract had the highest DPPH radical scavenging capacity (with the lowest IC_{50} value of 17.82 ± 0.15 µg/mL, R^2 = 0.9840) and all the fractions studied exhibited strong DPPH radical scavenging activity in the following order; EtOAC fraction (IC_{50} = 21.90 ± 0.17 µg/mL, R^2 = 0.9920) > $CHCl_3$ fraction (IC_{50} = 35.92 ± 0.28 µg/mL, R^2 = 0.9907) > PE fraction (IC_{50} = 52.22 ± 0.20 µg/mL, R^2 = 0.9819) > aqueous fraction (IC_{50} = 143.37 ± 1.12 µg/mL, R^2 = 0.9817). However, the IC_{50} value of the plant extracts was higher than that of Trolox (IC_{50} = 9.08 ± 0.32 µg/mL, R^2 = 0.9818). The results of the antioxidant activity of *V. heyneana* stems extracts based on DPPH assay are summarized in Table 2.

Previous reports demonstrated that the antioxidant effect of plant extracts is related to total phenolic and total flavonoids contents. Additionally, extraction solvents polarities are also related to phenolic, flavonoids contents and antioxidant activity.[28,56,57] Antioxidants play a major role in preventing the oxidation of biomolecules. They protect healthy cells from the attack of free radicals, unstable molecules, metabolic byproducts, and other stresses occurring in the human body.[28,58]

Table 1: Phytochemical constituents of *V. heyneana* stems extract

Constituents	Test	Inference
Lipids	Stain test	-
Carbohydrate	Fehling's test, Molisch's test	+
Carotenoids	H_2SO_4 test	-
Essential oil	Scent test	+
Triterpenoids	Salkowski test	+
Alkaloids	Dragendoff's test, Wagner's test	-
Amino acids	Na_2CO_3 test	+
Steroid/Cardiac glycosides	Liebermann Burchard test/ Raymond's test, Xanthydrol's test	-
Saponins	Foam test	+
Coumarins	Lactone ring test	+
Flavonoids	Cyanidin's test	+
Tannins	Gelatin's test, $FeCl_3$ test	+
Polyuronides		-

Note: "+" indicates the presence and "-" indicates the absence of the phytoconstituent.

Table 2: Percentage inhibition (%) and IC_{50} (µg/mL) values for antioxidant activity by DPPH free radical scavenging of the crude and fractionated extracts of *V. heyneana* stems and Trolox.

***V. heyneana* stems extract**	**Concentration (µg/mL)**						**IC_{50} (µg/mL)**	**Linear regression equation (y; R^2)**
	6.25	**12.5**	**25**	**50**	**100**	**200**		
	% Inhibition							
Crude extract	19.32±0.53	37.06±0.66	62.50±0.53	84.82±0.56	94.87±0.24	-	17.82±0.15	y = 28.689ln(x) - 32.634, R^2 = 0.9840
PE fraction	-	15.88±0.72	29.41±0.74	45.45±0.47	63.45±0.51	90.10±0.26	52.22±0.20	y = 26.326ln(x) -54.131, R^2 = 0.9819
$CHCl_3$ fraction	-	24.48±0.88	38.43±0.58	55.92±0.20	80.45±0.23	93.83±0.61	35.92±0.28	y = 26.072ln(x) - 43.374, R^2 = 0.9907
EtOAc fraction	20.09±0.32	34.56±0.43	49.63±0.19	72.34±0.63	91.25±0.24	-	21.90±0.17	y = 25.983ln(x) - 30.062, R^2 = 0.9920
Aqueous fraction	-	4.59±1.08	13.12±1.18	25.92±1.27	41.37±0.98	59.73±1.07	143.37±1.12	y = 19.986ln(x) - 49.238, R^2 = 0.9817
Trolox (positive control)								
Concentration (µg/mL)	1.25	2.5	5.0	10	20		IC_{50} (µg/mL)	Linear regression equation (y; R^2)
% Inhibition	9.85±0.15	18.21±0.88	32.37±0.35	62.02±0.64	96.85±0.15		9.08±0.32	y = 4.6336x + 7.9496, R^2 = 0.9818

Note: (-) Not tested; Mean ± S.D (n = 3).

Table 3: Percentage inhibition (%) and IC_{50} (µg/mL) values for XOI activity of the fractionated extracts of *V. heyneana* stems and allopurinol.

***V. heyneana* stems extract**	**Concentration (µg/mL)**						**IC_{50} (µg/mL)**	**Linear regression equation (y; R^2)**
	6.25	**12.5**	**25**	**50**	**100**	**200**		
	% Inhibition							
PE fraction	-	19.84±1.58	27.93±1.41	38.25±1.20	46.48±1.48	55.48±0.99	130.20±0.24	y = 12.96ln(x) -13.103, R^2 = 0.9988
$CHCl_3$ fraction	23.50±0.57	33.29±0.91	44.52±0.79	52.48±1.02	65.4±0.79	79.77±1.12	35.75±0.19	y = 15.894ln(x) - 6.8441, R^2 = 0.9924
EtOAc fraction	35.90±1.70	53.66±1.31	65.54±0.60	86.68±1.04	94.39±1.31	-	11.28±0.41	y = 21.64ln(x) - 2.4238, R^2 = 0.9849
Aqueous fraction	27.81±0.94	44.26±1.02	55.74±1.04	71.80±1.85	88.77±1.38	-	17.51±0.38	y = 21.568ln(x) - 11.731, R^2 = 0.9963
Allopurinol (positive control)								
Concentration (µg/mL)	0.3125	0.625	1.25	2.5	5.0		IC_{50} (µg/mL)	Linear regression equation (y; R^2)
% Inhibition	36.16±1.58	54.17±1.04	60.31±1.07	74.54±1.63	87.97±1.55		0.62±0.32	y = 17.888ln(x) + 58.638, R^2 = 0.9845

Mean ± S.D (n = 3).

The key mechanism of antioxidant compounds in disease prevention is their ability to resist oxidative damage or cell death.[28,59] Therefore, the present study has documented that *V. heyneana* stem extracts contain active components (e.g., flavonoids, coumarins, and tannins, etc.) capable of scavenging DPPH free radicals and therefore protect cells from oxidative damage.

Xanthine oxidase inhibitory activity of the plant extracts

In this study, the crude and fractionated extracts of *V. heyneana* stems were investigated as potential XO inhibitors. The results of the XO inhibition assay of *V. heyneana* stem extracts are shown in Table 3. The degree of XO inhibition was evaluated for the extracts of *V. heyneana* stems at concentrations of 6.25, 12.5, 25.0, 50.0, 100, and 200 µg/mL. All the fractionated extracts (PE, $CHCl_3$, EtOAc, and aqueous fractions) exhibited XOI activity with IC_{50} values of 130.20 ± 0.24 µg/mL (R^2 = 0.9988), 35.75 ± 0.19 µg/mL (R^2 = 0.9924), 11.28 ± 0.41 µg/mL (R^2 = 0.9849), and 17.51 ± 0.38 µg/mL (R^2 = 0.9963), respectively. Meanwhile, allopurinol, the positive control had an IC_{50} value of 0.62 ± 0.32 µg/mL (R^2 = 0.9845).

Interestingly, the extracts of *V. heyneana* stems all showed XOI activity, and this represents the first report of XOI activity in this species. Moreover, the genus *Vitis* L. is well known as an important source of phenolic compounds and several common species, for example, *V. vinifera*, were previously reported to have XOI activity.[60,61] Additionally, flavonoids, saponins, and terpenoids have shown uric acid-lowering effects. These compounds inhibit the enzyme XO and regulate uric acid transporters, which could lead to a decrease in blood uric acid.[20]

Vitis sp. is widely distributed in the Northern Hemisphere[21] and has been used as traditional raw materials for food (e.g., juice, wine, etc.), as well as for pharmaceutical applications. Previous studies have reported that the roots, and stems of *Vitis* sp. (i.e, *V. labrusca*, *V. vinifera*, *V. heyneana*, etc.) have anti-inflammatory, antioxidant, anti-tumor, antimicrobial, cardioprotective, hepatoprotective, anticonvulsant, and neuroprotective activities.[22,34,62] These pharmacological effects are due to the presence of several stilbenoid and resveratrol oligomers,[22,24,34] as well as the flavonoid compounds isolated from the stem extracts of *Vitis* sp. (e.g., cyanidin-3-*O*-glucoside, peonidin-3-*O*-glucoside, rutin, quercetin-3-*O*-galactoside, quercetin-3-*O*-glucoside, and quercetin-3-*O*-glucuronide, etc.).[33,34]

Nowadays, scientists are increasingly interested in isolating natural compounds from different plants due to their many positive effects on human health. Therefore, this study has suggested that further research on *V. heyneana* should be focused on the isolation of active compounds.

To the best of our knowledge, this is the first report on the phytochemical constituents, antioxidant activity, and *in vitro* XOI activity of *V. heyneana* stems from Vietnam. The crude and fractionated extracts of *V. heyneana* stems contained various phytochemical groups with high phenolic content, as well as potential antioxidant properties and other beneficial pharmacological effects. Moreover, these extracts are able to inhibit xanthine oxidase in a concentration-dependent manner and this effect was closely related to the phenolic content in the stems of *V. heyneana*.

Conclusion

From the results of the present study, it could be concluded that *V. heyneana* possesses many bioactive compounds, as well as a strong antioxidant and xanthine oxidase inhibitory properties. The ethyl acetate fraction exhibited the strongest antioxidant and xanthine oxidase inhibitory effects. Therefore, the stems of *V. heyneana* could be a source of natural compounds for the treatment of gout and other illnesses associated with xanthine oxidase. However, further studies are required to confirm the antioxidant capacity and xanthine oxidase inhibitory activity *in vivo*, and to isolate bioactive compounds from the stems of *V. heyneana*.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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