

## EVALUATION OF ANTIOXIDANT AND XANTHINE OXIDASE INHIBITORY ACTIVITY OF DIFFERENT EXTRACTS OF *GNETUM MONTANUM* MARKGR.

Nguyen Hoang Minh<sup>1,\*</sup>, Ngo Xuan Huy<sup>1</sup>, Nguyen Thi Thu Huong<sup>2</sup>

<sup>1</sup>Research Center of Ginseng and Medicinal Materials,  
National Institute of Medicinal Materials, Ho Chi Minh City, Vietnam;

<sup>2</sup>Hong Bang International University, Vietnam

\*Corresponding author: hoangminhtkd90@gmail.com

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### Summary

#### Evaluation of Antioxidant and Xanthine Oxidase Inhibitory Activities of Different Extracts of *Gnetum montanum* Markgr.

To gout patients, finding the natural drugs, which exhibit high safety and good influences with each step of the disease, is trustly important. The *in vitro* study was performed to investigate DPPH scavenging activity, lipid peroxidation activity and xanthine oxidase inhibitory activity of extracts (aqueous extract, 45% ethanol extract, 80% ethanol extract) from *Gnetum montanum* stem. In 1,1-diphenyl-2-picrylhydrazyl (DPPH) test, 45% ethanol extract from *G. montanum* (GM-EE1) (IC<sub>50</sub>: 6.66 µg/mL) was shown to scavenge DPPH better than 80% ethanol extract from *G. montanum* (GM-EE2) (IC<sub>50</sub>: 13.65 µg/mL), aqueous extract from *G. montanum* (GM-AE) (IC<sub>50</sub>: 81.85 µg/mL) but it was shown to scavenge DPPH weaker than ascorbic acid (IC<sub>50</sub>: 4.18 µg/mL). In lipid peroxidation inhibitory activity test, GM-EE1 had the highest antioxidant effect (IC<sub>50</sub>: 1.18 µg/mL), followed by GM-EE2 (IC<sub>50</sub>: 1.75 µg/mL), and the lowest was GM-AE (IC<sub>50</sub>: 10.16 µg/mL). All extracts were shown to lipid peroxidation inhibitory activity better than Trolox (IC<sub>50</sub>: 32.07 µg/mL). The evaluation of xanthine oxidase inhibition shows that GM-EE1 had the strongest xanthine oxidase inhibitory activity (IC<sub>50</sub>: 1.79 µg/mL), followed by GM-EE2 (IC<sub>50</sub>: 2.21 µg/mL), and the lowest was GM-AE (IC<sub>50</sub>: 17.83 µg/mL). These results indicate that 45% ethanol extract of *G. montanum* was good antioxidant and moderate type xanthine oxidase inhibitor.

**Keywords:** *Gnetum montanum*, Free radical and lipid peroxidation, Xanthine oxidase.

### 1. Introduction

Reactive oxygen species (ROS) include superoxide anion radicals (O<sup>2•-</sup>), hydroxyl radicals (•OH), and peroxy radicals (ROO•), etc. They are continuously generated during aerobic metabolism and exogenous sources such as UV radiation or environmental pollution. ROS are mainly produced by mitochondrial oxidative metabolism and cytochrome P450 systems in hepatocytes. However, when the ROS production is greater than the detoxification capacity of the cell, excessive ROS cause oxidative stress and even induce extensive damage to DNA, proteins, and lipids. It had been confirmed that uric acid, which causes gout, is formed from xanthine in the presence of xanthine oxidase. O<sup>2•-</sup>, generated upon the formation of uric acid, has been found to cause oxidative damage of living tissues. Fortunately, antioxidants may help the body to protect itself from various types of oxidative damage, which are linked to diseases such as cancer, diabetes, cardiovascular disorders, and aging [1]. Recently, searching for antioxidants from plants has been of major interest. However,

scientific studies on antioxidant properties of plants are still rather scarce. The assessment of antioxidant activity of plants remains an interesting and useful task for finding new sources of natural antioxidants.

*Gnetum montanum* Markgr. (Gnetaceae) is an evergreen vine indigenous to southern China and Southeast Asia, Cambodia, China South-Central, China Southeast, East Himalaya, Laos, Myanmar, Nepal, Thailand, the northern mountainous parts of Vietnam. In traditional clinical practice of Yao medicine, the stems and rhizomes of this plant are used to treat rheumatic arthralgia and bruises. Besides, modern chemistry research shows that the plant contains abundant stilbenes, sterols, flavonoids, alkaloids and other bioactive phytochemicals. Furthermore, modern pharmacological research also proved that the plant exhibited various biological activities such as antitumor, antioxidation, anti-inflammatory, and antibacterial. In the previous pharmacodynamic study, Xianglong Pan *et al.* (2022) found that the stems and rhizomes of *G. montanum* exhibited significant inhibition on the

proliferation of human colon cancer SW480 cells [2]. The leaves, stems and rhizomes of this plant contain abundant stilbenes and stilbene-related alkaloids, and are used as folk medicine for the treatment of lumbago, rheumatic arthralgia and chronic bronchitis [1]. To the best of our knowledge, the antioxidant properties and xanthine oxidase inhibitory effects of *G. montanum* extracts have not been evaluated yet. Therefore, the aim of the present study was to investigate the antioxidant properties of aqueous, 45%, 80% ethanol extracts of *G. montanum*, by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, superoxide radical scavenging and lipid peroxidation inhibition assays. Moreover, xanthine oxidase inhibitory effects were further investigated.

## 2. Materials and methods

### 2.1. Plant materials

*Gnetum montanum* Markgr. (stem) were collected from Binh Chau - Phuoc Buu Nature Reserve-Xuyen Moc, Ba Ria-Vung Tau province, Vietnam in March 2022. It was conserved in Research Center of Ginseng and Medicinal Materials in Ho Chi Minh City (botanical sample and gene conservation, TBSDT-012022DL). Dried powdered material was also extracted two time with distilled water at 100°C for 30 min (ratio 1:20, w:v). The aqueous extracts were combined and concentrated to get crude aqueous extract (GM-AE). At the same time, the material was extracted with 45% ethanol, 80% ethanol at room temperature for 72 hours in percolator apparatus (ratio 1:20, w:v). The ethanol extracts were collected at a rate of 2 mL/min and concentrated using a rotary evaporator at 60°C under reduced pressure to obtain crude ethanol extracts (GM-EE1/GM-EE2). The yields of GM-AE, GM-EE1 and GM-EE2 viscous extracts were respectively 10.70%, 16.37% and 16.02%. The humidity of GM-AE, GM-EE1 and GM-EE2 semi-solid extracts were respectively 14.11%, 17.12% and 19.70%.

### 2.2. Animals

Male *Swiss albino* mice (25 ± 2 gram, aged 5-6 weeks), were supplied by the Institute of Vaccines and Medical Biological Products in Nha Trang City. Before any experience, all animals were kept for 1 week under the same

laboratory conditions of temperature (22 ± 2°C), relative humidity (70 ± 4%), and a 12 hours light/dark cycle, and received a nutritionally standard diet and tap water.

### 2.3. Chemicals- Reagents

Xanthine oxidase (XO), xanthine, allopurinol, 1,1-diphenyl-2-picrylhydrazyl (DPPH), trolox and 2-thiobarbituric acid (TBA) (Sigma-USA); trichloro acetic acid (TCA), ascorbic acid (Merck-Germany); 96% ethanol (OPC-Vietnam). All other chemicals were of analytical grade.

### 2.4. DPPH radical scavenging assay

DPPH, (1,1-diphenyl-2-picrylhydrazyl), is a stable free radical having a maximum absorption 515-517 nm (purple colour). When an antioxidant (as a hydrogen donor) reacts with DPPH, DPPH is reduced to DPPH-H, as a consequence the solution turns from purple to yellow. Therefore, the antioxidant activity of samples can be evaluated by the decrease absorption at 515-517 nm [3].

Briefly, 0.5 mL of various concentrations of each sample or ascorbic acid was added into a tube containing 0.5 mL of 0.6 mM DPPH solution dissolved in methanol and the volume was made uniformly to 4 mL using methanol. The solution was mixed and then allowed to stand in dark at room temperature for 30 min. Absorbance was taken at 515 nm using methanol as blank on UV-visible spectrometer. 0.5 mL of DPPH was added to 3.5 mL of methanol and absorbance was taken for control reading. The potential scavenging abilities of the samples were assessed using ascorbic acid (Merck, Germany) as a positive control. All analyses were run in triplicate [4].

Percentage inhibition (I%) was estimated using the following expression :

$$I\% = \frac{A_c - A_s}{A_c - A_0} \times 100$$

Where  $A_c$ ,  $A_s$  and  $A_0$  are the absorbances of control, sample and blank, respectively.

### 2.5. Lipid peroxidation inhibition assay

Lipid peroxidation is a well-established mechanism of cellular injury in both plants and animals, and is used as an indicator of oxidative stress in cells and tissues. The measurement of malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) has been used as an indicator of lipid

peroxidation. MDA is one of the products of lipid peroxidation on cell membranes. The assay is based on the reaction of MDA with thiobarbituric acid (TBA), forming an MDA-TBA2 adduct that absorbs strongly at 532 nm. The potential lipid peroxidation inhibition power of extract samples was measured using mouse brain homogenate as lipid rich substrate [5].

The mouse brain tissue was immediately placed in ice-cold 5 mM phosphate buffer (pH 7.4) with the brain and phosphate buffer ratio of 1:10 (w/v). The tissue was then homogenized at 13000 rpm in an ice-cold condition. 0.1 mL of various concentrations of each sample or Trolox was added to 0.5 mL of brain homogenate and the volume was made to 2 mL using 50 mM phosphate buffer. The mixture was then incubated at 37°C for 15 min to allow lipid peroxidation and thus MDA production. The reaction was stopped with 1 mL of 10% trichloroacetic acid. The tube was then centrifuged at 10000 rpm in 5°C for 5 min. The supernatant (2 mL) was transferred into a different tube and then allowed to react with 0.8% TBA solution (1 mL) at boiling point of water for 15 min. The control was prepared as above without test sample. Absorbance of the mixture was measured at 532 nm against a blank solution without sample and TBA after the tubes reached room temperature. Trolox, a water-soluble analog of vitamin E, was used as a positive control. All analyses were run in triplicate [4].

Percentage inhibition (I%) was estimated using the following expression :

$$I\% = \frac{A_c - A_s}{A_c - A_0} \times 100$$

Where  $A_c$ ,  $A_s$  and  $A_0$  are the absorbances of control, sample and blank, respectively.

#### 2.6. Xanthine oxidase inhibition assay

Xanthine oxidase (XO) serves as an important biological source of oxygen-derived free radicals that contribute to the oxidative damage of living tissues. XO is involved in the medical condition known as gout, which is characterized by hyperuricemia that leads to uric acid deposition in the joints resulting in painful inflammation. It was known that XO is involved in formation of uric acid from xanthine and hypoxanthine.

Treatment of gout involves the use of therapeutic agents, such as xanthine oxidase inhibitors (XOI) that act by blocking the conversion of purine into uric acid. An increasing number of researchers during the past decade have also suggested that XO plays an important role in various forms of ischemic and other types of tissue and vascular injuries, inflammatory diseases, and chronic heart failure.

The xanthine oxidase activities with xanthine as the substrate were measured spectrophotometrically by the method of Tadataka Noro *et al.* with the following modification [6]. The assay mixture consisted of 0.1 mL of test solution, 0.3 mL of 50 mM phosphate buffer (pH 7.5) and 0.1 mL of enzyme solution. After preincubation of the mixture at 37°C for 15 min, the reaction was initiated by adding 0.2 mL of 0.15 mM xanthine solution. This assay mixture was incubated at 37°C for 30 min. The reaction was stopped by adding 0.2 mL of 0.5 M HCl, and the absorbance of the assay mixture at 295 nm was measured spectrophotometrically. This experiment was assessed using allopurinol (Sigma, USA) as a positive control. All analyses were run in triplicate.

Percentage inhibition (I%) was estimated using the following expression:

$$I\% = \frac{A_c - A_s}{A_c - A_0} \times 100$$

Where  $A_c$ ,  $A_s$  and  $A_0$  are the absorbances of control, sample and blank, respectively.

#### 2.7. Data analysis

The results were expressed in terms of mean  $\pm$  SEM (Standard error of the mean) using MS Excel 2016 software. Data were analyzed by Systat statistical software (version 3.5, Inc. SigmaStat) using t-test and One-way ANOVA following by Student-Newman-Keuls test.

### 3. Results

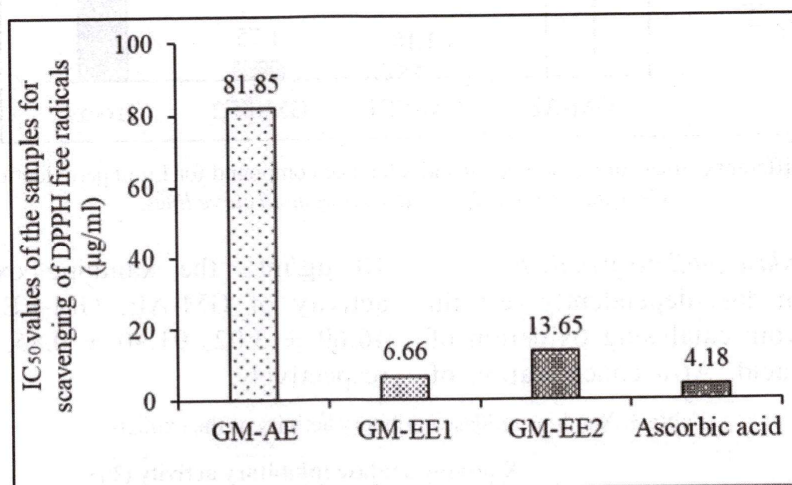
#### 3.1. DPPH radical scavenging activity of crude extracts

Free radical scavenging activity of different extracts of *Gnetum montanum* was determined according to the DPPH radical scavenging method and is shown in Table 1. Different concentrations of three different extracts i.e. GM-AE, GM-EE1 and GM-EE2 extracts were analyzed.

**Table 1.** Free radical scavenging activity of the extracts

| Extracts                           | DPPH radical scavenging activity (%) |                    |                    |                    |                    |
|------------------------------------|--------------------------------------|--------------------|--------------------|--------------------|--------------------|
|                                    | 6.25                                 | 12.50              | 31.25              | 62.50              | 125.00             |
| Concentration ( $\mu\text{g/mL}$ ) |                                      |                    |                    |                    |                    |
| <b>GM-AE</b>                       | $8.11 \pm 0.88$                      | $16.49 \pm 5.54$   | $23.52 \pm 1.39^a$ | $37.24 \pm 1.84$   | $70.16 \pm 2.37$   |
| Concentration ( $\mu\text{g/mL}$ ) | 1.56                                 | 3.13               | 6.25               | 12.50              | 31.25              |
| <b>GM-EE1</b>                      | $37.21 \pm 0.66^a$                   | $41.33 \pm 1.31^a$ | $50.11 \pm 0.44^a$ | $65.09 \pm 0.44^a$ | $93.63 \pm 0.20^b$ |
| <b>GM-EE2</b>                      | $14.51 \pm 0.76^b$                   | $22.57 \pm 0.58^b$ | $31.66 \pm 0.93^b$ | $49.71 \pm 1.51^b$ | $87.34 \pm 0.12^c$ |

(a-c)  $p < 0.05$  significantly different as compared to others at same concentration. Results are mean  $\pm$  SEM ( $n = 3$ ).



**Fig. 2.** IC<sub>50</sub> values of different extracts of *G. montanum* and reference compound for scavenging of DPPH free radicals. IC<sub>50</sub> values were calculated from sigmoid curve lines

Table 1 shows the free radical scavenging activity of GM-AE, GM-EE1, GM-EE2 extracts. Among the extractives, GM-EE1 possessed the highest activity. At a concentration of 31.25  $\mu\text{g/mL}$ , the scavenging activity of GM-AE, GM-EE1, GM-EE2 was  $23.52 \pm 1.39$ ,  $93.63 \pm 0.20$ ,  $87.34 \pm 0.12\%$ , respectively. The IC<sub>50</sub> values of GM-AE, GM-EE1, GM-EE2 were 81.85  $\mu\text{g/mL}$ , 6.66  $\mu\text{g/mL}$ , 13.65  $\mu\text{g/mL}$ , respectively. GM-EE1 extract showed higher free radical scavenging activity than GM-AE and GM-EE2 extracts. The results were compared with ascorbic acid. The IC<sub>50</sub> of ascorbic acid (positive control) was 4.18  $\mu\text{g/mL}$ . Ascorbic acid exhibited highest free radical scavenging activity and the overall

ranking of the tested extracts and reference compounds were in the order of: ascorbic acid > GM-EE1 > GM-EE2 > GM-AE.

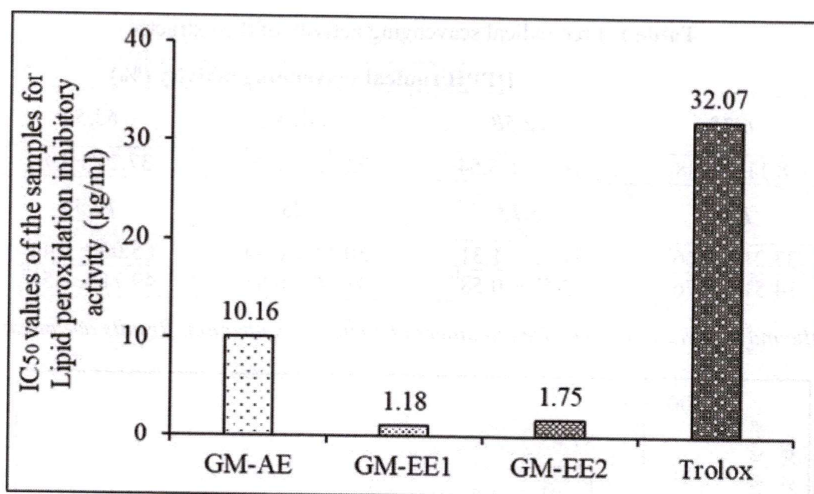
### 3.2. Lipid peroxidation inhibitory activity

The lipid peroxidation inhibition activity of GM-AE, GM-EE1, GM-EE2 was compared with Trolox. At a concentration of 12.5  $\mu\text{g/mL}$ , the inhibitory activity of GM-AE, GM-EE1, GM-EE2 was  $56.78 \pm 2.34$ ,  $92.78 \pm 0.43$  and  $90.67 \pm 1.32\%$ , respectively (Table 2). IC<sub>50</sub> values of GM-AE, GM-EE1, GM-EE2 were 10.16  $\mu\text{g/mL}$ , 1.18  $\mu\text{g/mL}$ , 1.75  $\mu\text{g/mL}$ , respectively (Fig. 2). GM-EE1 had higher inhibitory activity than other extracts and Trolox. The IC<sub>50</sub> of Trolox (positive control) was 32.07  $\mu\text{g/mL}$ .

**Table 2.** Lipid peroxidation inhibitory activity of the extracts

| Extracts                           | Lipid peroxidation inhibitory activity (%) |                    |                    |                    |                    |
|------------------------------------|--------------------------------------------|--------------------|--------------------|--------------------|--------------------|
|                                    | 1.25                                       | 2.50               | 5.00               | 12.50              | 25.00              |
| Concentration ( $\mu\text{g/mL}$ ) |                                            |                    |                    |                    |                    |
| <b>GM-AE</b>                       | $3.44 \pm 0.75^a$                          | $8.44 \pm 1.72^a$  | $27.44 \pm 2.68^a$ | $56.78 \pm 2.34^a$ | $84.67 \pm 0.29$   |
| Concentration ( $\mu\text{g/mL}$ ) | 0.63                                       | 1.25               | 2.50               | 5.00               | 12.50              |
| <b>GM-EE1</b>                      | $28.11 \pm 0.66^a$                         | $43.67 \pm 0.78^b$ | $61.78 \pm 2.74^b$ | $75.44 \pm 1.27^b$ | $92.78 \pm 0.43^b$ |
| <b>GM-EE2</b>                      | $22.33 \pm 2.97^b$                         | $38.67 \pm 2.31^c$ | $61.22 \pm 1.73^b$ | $70.22 \pm 2.09^c$ | $90.67 \pm 1.32^b$ |

(a-c)  $p < 0.05$  significantly different as compared to others at same concentration. Results are mean  $\pm$  SEM ( $n = 3$ ).



**Fig. 2.** IC<sub>50</sub> values of different extracts of *G. montanum* and reference compound for Lipid peroxidation inhibitory activity. IC<sub>50</sub> values were calculated from sigmoid curve lines.

### 3.3. Xanthine oxidase inhibitory activity

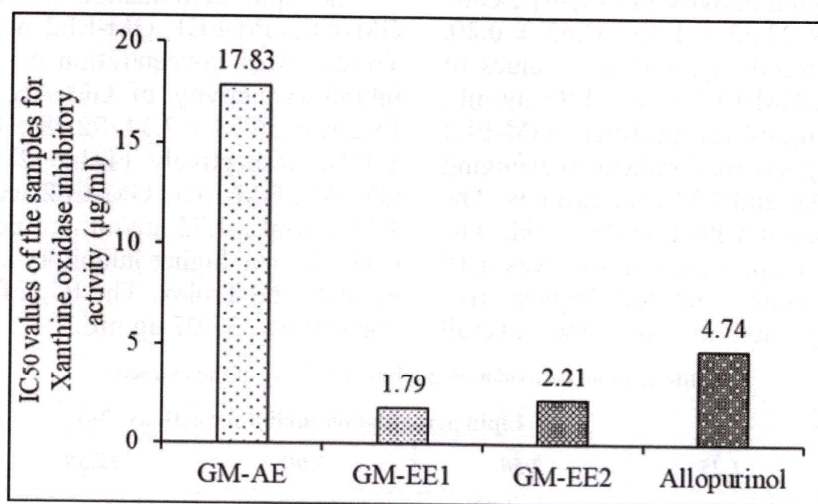
The extracts can dose-dependently restrain xanthine oxidase from catalysing oxidation of xanthine into uric acid. At a concentration of

10 µg/mL, the xanthine oxidase inhibitory activity of GM-AE, GM-EE1, GM-EE2 was  $46.69 \pm 3.02$ ,  $91.90 \pm 0.85$ ,  $63.66 \pm 2.04\%$ , respectively.

**Table 3.** Xanthine oxidase inhibitory activity of the extracts

| Extracts              | Xanthine oxidase inhibitory activity (%) |                    |                    |                    |                    |
|-----------------------|------------------------------------------|--------------------|--------------------|--------------------|--------------------|
|                       | 1.250                                    | 2.50               | 5.00               | 10.00              | 25.0               |
| Concentration (µg/mL) |                                          |                    |                    |                    |                    |
| GM-AE                 | $12.23 \pm 2.65^a$                       | $18.95 \pm 0.72^a$ | $33.62 \pm 4.12^a$ | $46.69 \pm 3.02^a$ | $59.35 \pm 0.59$   |
| Concentration (µg/mL) | 0.625                                    | 1.25               | 2.50               | 5.00               | 10.00              |
| GM-EE1                | $23.29 \pm 2.53$                         | $34.26 \pm 2.55^b$ | $54.35 \pm 1.97^b$ | $77.44 \pm 2.10^b$ | $91.90 \pm 0.85^b$ |
| GM-EE2                | $21.51 \pm 4.32$                         | $30.25 \pm 1.61^b$ | $45.18 \pm 2.09^c$ | $56.28 \pm 1.94^c$ | $63.66 \pm 2.04^c$ |

<sup>(a-c)</sup>  $p < 0.05$  significantly different as compared to others at same concentration. Results are mean  $\pm$  SEM ( $n = 3$ ).



**Fig. 3.** IC<sub>50</sub> values of different extracts of *G. montanum* and reference compound for xanthine oxidase inhibitory activities. IC<sub>50</sub> values were calculated from sigmoid curve lines.

The IC<sub>50</sub> of allopurinol was 4.74 µg/mL. The GM-EE1 exhibited the highest potency (IC<sub>50</sub> = 1.79 mg/mL), followed by GM-EE2 (IC<sub>50</sub> = 2.21 mg/mL) and GM-AE (IC<sub>50</sub> = 17.83 mg/mL) (Fig.

3). GM-EE1, GM-EE2 and GM-AE showed strong xanthine oxidase inhibitory activities. Xanthine oxidase is an important enzyme in nucleic acid metabolism; it catalyses oxidation of xanthine and hypoxanthine into uric acid and generates oxidative free radicals. Therefore, inhibiting xanthine oxidase activity reduces uric acid formation, effectively halts or delays gout and its complications and weakens tissue damage caused by free radicals, all of which are critical to human health.

#### 4. Discussion

There is considerable evidence that free radicals induce oxidative damage to biomolecules and play an important role in cardiovascular disease, aging, cancer, inflammatory diseases and a variety of other disorders. Antioxidants that scavenge free radicals are now known to possess preventive as well as therapeutic potential in free radical-mediated disease conditions. The free radical-scavenging activity of the extracts is ascribed to their hydrogen donating ability. Antioxidants are believed to intercept the free radical chain during oxidation and to donate hydrogen from the phenolic (flavonoid, stilbenes) hydroxyl groups, thereby forming a stable end product, which does not initiate or propagate further oxidation of the lipid [7].

The data of this study reveal that the extracts of *G. montanum* (stem) are free radical inhibitors and acted as primary antioxidants that react with free radical: DPPH radical scavenging, lipid peroxidation inhibition activity and xanthine oxidase inhibitory activity. *G. montanum* contains abundant stilbenes, sterols, flavonoids, alkaloids. Many studies have suggested that flavonoids exhibit biological activities, including antiallergenic, antiviral, anti-inflammatory, and vasodilating actions. These pharmacological effects are linked to the antioxidant properties of flavonoids. Protective effects of flavonoids are

ascribed to their capacity to suppress ROS formation by inhibiting some enzymes or chelating trace elements involved in free radical production, scavenge radical species and more specially the ROS, and improve regulation antioxidant defense [8]. Vu Thi Lan Phuong (2019) reported the isolation and structural determination of 3 compounds including transresveratrol, resveratrololide and isorhapontigenin-13-glucoside from *G. montanum* collected in Tuyen Quang province, Vietnam [9]. Acquaviva R. *et al.* (2002) investigated the free radical scavenging capacity of resveratrol, its effects on xanthine oxidase activity, spontaneous membrane lipid oxidation, and DNA cleavage. Resveratrol showed a dose-dependent free radical scavenging activity, significant inhibition of xanthine oxidase activity, an anti-lipoperoxidative capacity, and a protective effect on DNA cleavage [10]. The antioxidant capacity of resveratrol is ascribed to the concomitant activities of scavenging free radicals, metal chelating, and inhibition of some enzymes involved in free radical generation. These results showed that 45% ethanol extract had the highest antioxidant effect and xanthine oxidase inhibitory activity as compared to other extracts. This suggests the research direction of the topic that needs to be tested to find the key marker (quantitative, qualitative) in the 45% ethanol extract from *G. montanum* that has antioxidant effects.

#### 5. Conclusion

Several methods were employed for the evaluation of antioxidant potential of *G. montanum* extracts. It can be seen that various extracts of *G. montanum* prepared by different solvents exhibited various degrees of antioxidant activity. In view of overall discussed results, it was concluded that 45% ethanol extract of *G. montanum* were good antioxidant and moderate type xanthine oxidase inhibitor.

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### DIVERSITY OF MEDICINAL PLANTS FROM DONG NAI PROVINCE

Pham Thanh Huyen<sup>1,\*</sup>, Lai Viet Hung<sup>1</sup>, Phan Van Truong<sup>1</sup>, Nguyen Van Hieu<sup>1</sup>, Dang Minh Tu<sup>1</sup>,  
 Nham Minh Phuc<sup>1</sup>, Nguyen Quynh Nga<sup>1</sup>, Nguyen Hoang<sup>1</sup>, Nguyen Minh Khoi<sup>1</sup>, Le Van Minh<sup>1</sup>,  
 Nguyen Thu Hang<sup>1</sup>, Nguyen Duy Van<sup>2</sup>, Nguyen Hoang Hao<sup>3</sup>, Vo Quang Trung<sup>3</sup>

<sup>1</sup>National Institute of Medicinal Material, Hanoi, Vietnam; <sup>2</sup>Dong Nai Department of Health, Vietnam;

<sup>3</sup>Dong Nai Culture and Nature Reserve, Vietnam

\*Corresponding author: huyenptnimm@gmail.com

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#### Summary

#### Diversity of Medicinal Plants from Dong Nai Province

A survey of the medicinal plant resources in Dong Nai province has been carried out from April 2019 to December 2021. A total of 1,086 species belonging to 591 genera, 163 families of seven phyla of vascular plants (Podypodiophyta; Pinophyta; Cycadophyta; Gnetophyta; Pteridophyta; Magnoliophyta) and Fungi were recorded. Among them, 18 species conservation were listed in the Vietnam Red Data Book, Decision No 84/2021/NĐ-CP and Red List of Medicinal Plants of Vietnam, 2019. Life forms are identified as: herbaceous plant (38.30%); shrub and subshrub (18.97%); vine (Lianas and nonwoody vines) (13.08%); Tree (26.33%); palm-like plant: (0.65%), Epiphyte - parasitic (2.67%). The study highlighted the richness and importance of medicinal plant species in Dong Nai province.

**Keywords:** *Dong Nai, Medicinal plants, Diversity.*

#### 1. Background

Dong Nai is a province with diverse and abundant plant resources and medicinal plant resources from the Southeast region as well as Vietnam. The research and conservation of natural medicinal plant resources have become an urgent need to serve the development goals of the socio-economic sectors and the local pharmaceutical industry. Investigating and researching diverse medicinal plant resources has important scientific and practical significance to provide new data to contribute to the protection and sustainable development of this resource.

This study documents valuable ethnobotanical information on the medicinal plants used by Dong Nai province.

#### 2. Main methods

1,086 specimens of the medicinal plant were collected in the fieldwork from April 2019 to

December 2021). Transect lines research is applied in the field to identify and collect medicinal plant species in the study area: at each survey site, establishing the routes for specific topography types and ecological forms to collect data on species. The Collected specimens were kept at the National Institute of Medicinal Material Herbarium (NIMM)

The number of transect lines: Nature Reserve (6 route-based); Chua Chan mountain (2 route-based), Cat Tien National Park (3 route-based); Tan Phu guard forest (01 route - based).

Synthesize, analyze and use published data related to the research area on medicinal plants resources

Interview survey: Investigate the experience of using medicinal plants and remedies from people who are staff of National Park, Nature Reserve, healers, physicians