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# STUDY ON THE ISOLATION AND BIOACTIVITY ASSAY OF SOME COMPOUNDS FROM *Nelumbo nucifera* GAERTN SEEDS COLLECTED AT TINH TAM LAKE, THUA THIEN HUE, VIETNAM

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**ABSTRACT**

**In this study, we isolated and purified compounds from the n-Butanol segment of seed *Concave white Lotus* variety (*N. nucifera*) collected at Tinh Tam Lake, Thua Thien Hue province based on classical column chromatographic methods. Research results have obtained three compounds namely: Nuciferin (NH2 : $C_{19}H_{21}NO_2$), Armepavine (NH8 : $C_{19}H_{23}O_3N$), and Anonaine (NH11 : $C_{17}H_{15}NO_2$). At the concentration of 100 µg/mL, Nuciferin was the highest DPPH free radical scavenging activity substance reaching of 94.620 ± 0.657%, followed by Armepavine (74.019 ± 2.400%) and Anonaine (61.151 ± 2.349 %). The $IC_{50}$ values obtained from these substances were 30.031 ± 1.506 µg/mL (Nuciferin), 43.093 ± 14.215 µg/mL (Armepavine) and 68.217 ± 6.214 µg/mL (Anonaine) respectively. The cytotoxicity results of three active ingredients showed the very low activity of the investigated substances at the studied concentrations. The $IC_{50}$ value cannot be determined in the concentration range from 0.8; 4; 20 and 100 µg/mL. At a concentration of 100 µg/mL, Nuciferin gave the best inhibitory activity against the human gastric cancer cell line MKN7 (23.800%), Armepavine showed the highest inhibitory activity against the oral carcinoma cell line KB (12.320%) and Anonaine showed the best inhibitory activity against the human skin cancer cell line SK-Mel-2 (19.720%). Our results initially show the prospect of using *Concave white Lotus* seeds as functional foods to support and improve human health.**

**Keywords:** *Nelumbo nucifera*; seed; lotus; compound; Thua Thien Hue; Vietnam.

## INTRODUCTION

*Nelumbo nucifera* (*N. nicifera*): *Concave white Lotus* is an aquatic flowering plant. This perennial usually lives in lakes and ponds. Since ancient times, lotus flower has become familiar and close to Vietnamese's life as well as other countries such as India, China and Japan. *Lotus* flowers are not only play an important role in economic and mental areas but also spiritual aspects [1]. All different parts of lotus can be used for preparing various delicious cuisine dishes and valuable traditional herbal medicine formulas [2]. *Lotus* contains vast varieties of potential secondary compounds such as alkaloids, flavonoids, steroids, triterpenoids, glycosides and polyphenols, performing powerful antioxidant activities which are effective in improving human health [3]. There were researches on benefits of consuming *Lotus* seeds on human health, especially during the recent decade while people gained more and more awareness on protecting their health through functional foods [4]. Up to now, *Lotus* seeds have been proved to possess many pharmacological properties such as antioxidant [5,6], anti-inflammatory [7], anti-memory loss [8,9], and anti-tumor activities [10]. Scientists believe that significant biological or medicinal compounds in *Lotus* seeds are naturally alkaloids and flavonoids [11,12].

Recent years, flavonoids and alkaloids have received considerable attentions because of their benefits of human health, especially in preventing a number of chronic diseases, such as cardiovascular diseases, type II diabetes, neurodegenerative diseases and some types of cancer [6].

The scientists in the world have applied assay Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometric (LC/ESI-MS/MS) and classical column chromatography on silica gel or a combination of these methods in solvent systems different media for the extraction, identification characterization and quantitative analysis of alkaloids and flavonoids from different parts of *Lotus* species *N. nucifera* Gaertn, including lotus seeds [11,13,14]. According to Zhu *et al.* (2016), more than 30 flavonoids and 10 alkaloids have been identified in lotus seeds and their content has been quantified in different lotus seed samples [14]. Nuciferine, Armepavine and Anonaine, are alkaloids derived from the *Lotus* plant (*N. nucifera*), which are found in the fruit, seeds and leaves [13,15,16,17]. Studies on the biological activity of these active substances have revealed various interesting pharmacological activities including: anti-tumor, vascular-regulatory, antioxidant, anti-parasitic and antibacterial effects, as well as effects on the central nervous system [18,19,20,21], anti-inflammatory effects [15,22], treatment of liver disease in humans and patients with type 2 diabetes [23] treatment of premature ejaculation [24], and erectile dysfunction [25], brain diseases and various anti-tumor and antiviral pharmacological properties [26,20], immunosuppressive [16] effect on apoptosis in cells leukemia cells [27].

## MATERIALS AND METHODS

### Plant Materials

The seeds of the *Concave white Lotus* (HueST02) (Accession number: MT903422, MN011709, MN011720, MN011731, MN086254, MT901732, MT901765, MT905226, MT905259 and MZ611977) (5 kg) were collected at Tinh Tam Lake, Thua Thien Hue province, Vietnam (Fig. 1). This study was conducted at the Institute of Biotechnology, Hue University, June, 2018.

**Fig. 1. Flower morphology and seeds of the *Concave white Lotus* variety Thua Thien Hue, Vietnam**

### Methods

### Prepare materials

Seeds of *Concave white Lotus* variety were collected in September 2018 (*Lotus* season from July to September every year). *Lotus* seeds are

peeled, rinse under the tap, then dried in the shade and dried at 50°C to reach a moisture content of less than 10%. *Lotus* seeds are then finely ground and passed through a sieve with size d = 1 mm. A *Lotus* seed powder is stored in PE (Polyethylene) bags placed in sealed plastic containers, stored at room temperature, protected from light and heat moisture to use for further experiments (Fig. 1).

**Isolation of compounds**

The extraction process from seeds of *Concave white Lotus* variety to create the total extraction is shown in Fig. 2. In this study, we used different classical column chromatography methods including thin layer chromatography (TLC, for investigation, small amount preparation), ordinary column chromatography (CC) with a stationary phase of silica gel (Merck) to isolate and purify the compound ingredients from the of the n-Bu segment seed of the *Concave white Lotus* variety collected at Tinh Tam Lake, Thua Thien Hue province on silica gel with a chromatography column of size 60x4 (cm) in the initial elution solvent system was 100% $CHCl_3$, and the next elution solvent was carried out in two systems: - System 1: $CHCl_3$:Acetone:MeOH (100:100:1); Acetone:MeOH with the ratios 100:0; 100:0.75; 100:1.5 and 100:3 and System 2: $CHCl_3$:MeOH with the ratios of 50:50; 30:70 and 0:100.

Thin layer chromatography was performed as described by Bele and Khale (2010) with different solvent systems for each specific isolated compound: (Nuciferin: $CHCl_3$:Acetone:MeOH (50:50:0.5), Armepavine: $CHCl_3$:MeOH:$NH_4OH$ (96:4:0.5) and Anonaine: $CHCl_3$:MeOH:$NH_4OH$ (96:4:0.5), so that the polarity of the solvent systems to obtain $R_f$ values is in the range ≥ 0.5 [28].

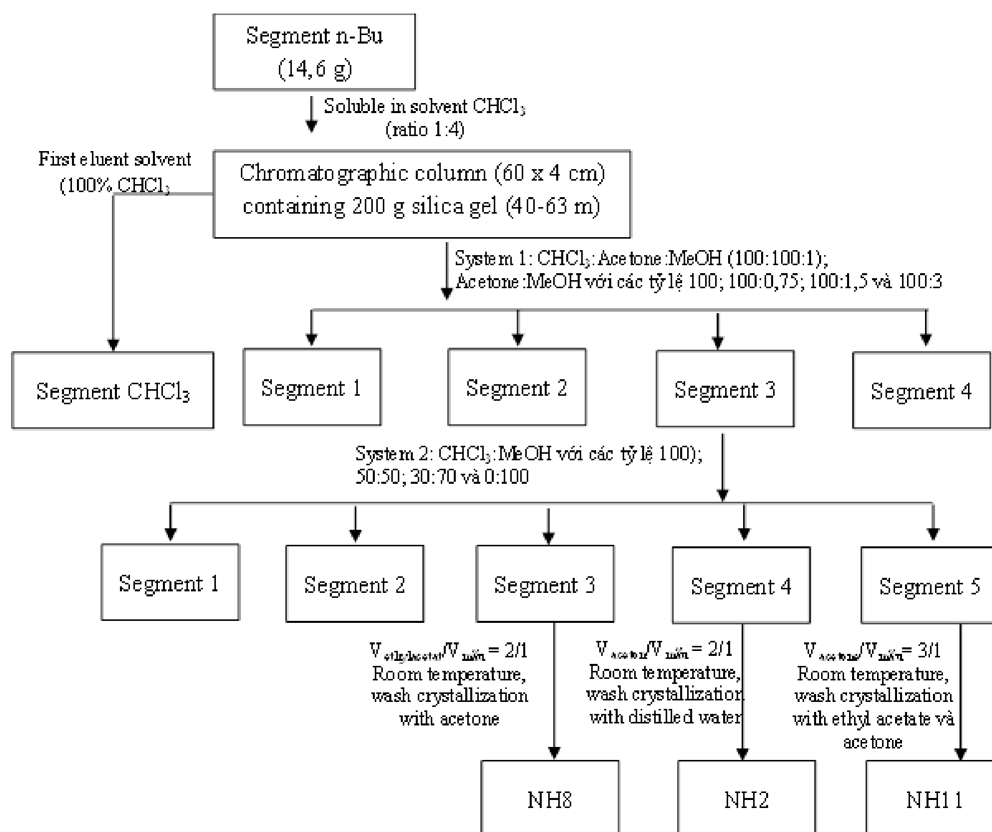


**Fig. 2. Diagrammatic of compound extract from n-Bu segment the seed of the *Concave white Lotus* variety**

## Chromatographic and Mass Spectrometry Conditions

*Sample Preparation*: Using 1 µg of compound solution in 500 µL methanol (50%) and 500 µL of formic acid and then vortex-mixed. Afer centrifugation at 14000 rpm for 10 min. Finally, 10 µL supernatant was injected into the LC-MS/MS system for analysis.

The HPLC-MS/MS assays of compounds perform base on the machine Q Exactive™ Plus Hybrid Quadrupole-Orbitrap™ Mass Spectrometer with an electrospray ionization (ESI) source. The chromatographic separation was achieved on a TCC3000 column, and the column temperature was kept at 30°C. Mobile phases which consisted of 0.1% formic acid in water (A) and acetonitrile (B) were used in the following gradient elution method: 0 - 0.5 min, 0% - 2% B; 0.5 - 2 min, 2% - 40% B; 2 - 20 min, 40% - 95% B; 20 - 23 min, 95% B, 23-25 min, 95% - 2% B. The flow rate was set at 0.3 mL/min, and the injection volume was 10 µL. All data were analyzed by Mass Hunter workstation software (Agilent Technologies, USA).

The mass spectrometer was carried out in both positive and negative ionization multiple-reaction monitoring (MRM) mode. The source parameters were as follows: the capillary voltage set at 300 V for positive ionization mode and -300 V for negative ionization mode, the drying gas temperature was 320°C, the flow was 11 L/min, and nebulizing gas pressure was 30 psi. The mass spectrometer data of compounds were searched for comparison on the NIST2017 spectrum library of the American Academy of Science and Technology (https://chemdata.nist.gov/) and refer to previous publications.

## Determination of antioxidant activity

The antioxidant activities of the compounds isolation from seeds of the *Concave white Lotus* variety were measured by 1,1-diphenyl-2-picrylhydrazyl (DPPH) method as described in Vuong et al. [2]. An amount of 1 mL sample was added to test tube, followed by 1 mL of DPPH of 0.2 mM and 30 minutes incubation in the darkness. Absorbance was measured at 517 nm. The ascorbic acid was used as control. The compounds were prepared serial dilutions of 0.8, 4, 20 and 100 µg/mL. DPPH solution 0.2 mM was prepared in 70% ethanol and ascorbic acid was prepared as of 0.08; 4; 2.0 and 10 µg/mL.

DPPH free radical scavenging abilities were calculated as:

$$\%SC = \frac{OD_c - OD_m}{OD_c} \times 100$$

Where: $OD_m$: optical density of sample after deducting blank (without DPPH); $OD_c$: optical density of blank sample after deducting blank (without DPPH).

The standard curve was developed with percentages of DPPH inhibitions, obtaining at different concentrations. From there, calculating the value of $IC_{50}$ based on the standard curve equation ((y) = ax + b) with y = 50% to find x (x is the value of $IC_{50}$ which need to find) [3].

## *In vitro* cancer cell line culture

Cancer cell lines were cultured as a monolayer in DMEM culture medium (Dulbecco's Modified Eagle Medium) with 2 mM L-glutamine, 1.5 g/L sodium bicarbonate, 4.5 g/L glucose, 10 mM HEPES, and 1.0 mM sodium pyruvate, adding 10% fetal bovine serum-FBS (Gibco, Invitrogen). Cells were transplanted after 3-5 days at a ratio (1:3) and kept in $CO_2$ incubators at 37°C, 5% $CO_2$.

Cytotoxic assay was confirmed by the National Cancer Institute (NCI) as a standard test to select and detect substances that can inhibit growth of cancer cells or kill them *in vitro*. The assay was carried out based on method of Monks (1991) [29], and was conducted to determine total cellular protein content based on the optical density (OD) measured when the protein components of the cell were dyed with Sulforhodamine B (SRB, Sigma-Aldrich, USA). Measured OD values were proportional to the amount of SRB attached to the protein molecule, thus the more cells give (the more the protein) the larger the OD value. SRB assay was performed on KB (human carcinoma in

the mouth), SK-Mel-2 (human melanoma), and MKN7 (human gastric adenocarcinoma) cell lines.

Reagent (10 µL) diluted in 10% DMSO (in sterile distilled water, Merck, Germany) was fed into the wells of the 96-well disk (Corning, USA) for a selective concentration of 100 µg/mL. The active reagents were determined $IC_{50}$ by using serial dilution of 100; 20; 4; and 0.8 µg/mL. Then, trypsinized cancer cells at appropriate concentrations were added into the wells (180 µL medium), incubating in an incubator for 48 hours. Another 96-well tray without reagents but with cancer cells (180 µL medium) was prepared in 3 columns for control on day 0. After 1 hour, the control plate on day 0 would be fixed with Trichloroacetic acid (TCA, Sigma-Aldrich, USA).

After the development stage in the $CO_2$ incubator, the cells were fixed to the bottom of the wells with TCA during 60 minutes, dyed with SRB for 30 minutes at 37°C. Discard the SRB and the tested wells were washed 3 times with 5% acetic acid and allowed to dry in air at room temperature.

Finally, using 10 mM Tris (hydroxymethyl) aminomethane solution to dissolve the amount of SRB that has been attached and dyed the protein molecules, put it on a gently shaker plate for 10 minutes and using the ELISA Plate Reader (Bio-Rad) to read the result of the color content of the SRB dye through the absorption spectrum at 515 nm. The percentage inhibiting cell growth in the presence of the test substance would be determined through the following formula:

$$\% \text{ Alive cells} = \frac{[OD_{reagent} - OD_{day0}]}{[OD_{negativecontrol} - OD_{day0}]} \times 100$$

$$\% \text{ inhibited cells} = 100 - \% \text{ alive cells}$$

The assay was carried out in triplicated. Ellipticine (Sigma-Aldrich, USA) was used as positive control at concentrations of 10; 2; 0.4 and 0.08 µg/mL. DMSO 10% was used as a negative control. The value of $IC_{50}$ would be determined by using TableCurve 2Dv4 software (System software Inc., San Jose, California, USA). According to the standard National Cancer Institute (NCI), the residue was considered to have good activity with $IC_{50} \leq 20$ µg/mL, while the pure substance was considered to have good activity when $IC_{50} \leq 5$ µM [30].

## Statistical Analysis

The data were analyzed with Excel 2010, IBM SPSS Statistics 20. Means were separated by Duncan's multiple range test, $p \leq 0.05$.

# RESULTS AND DISCUSSION

## Isolation and Identification Compounds

Alkaloids in *Lotus* seeds (*N. nucifera*) are usually extracted using methanol/water, ethanol/water, or acidifying solvents because of their alkalinity [11,31]. However, new methods for the extraction of metabolites are rarely used in the analysis of lotus seed metabolites [32]. In this study, we used different classical column chromatography methods base on silica gel with a chromatography column of size 60 x 4 (cm) in the different elution solvent systems to isolate and purify the compound ingredients. The results of the thin layer chromatography (TLC) (Fig. 3A, 3B and 3C) shows that alkaloids in seeds of *Concave white Lotus* have only one color stain when viewed under $UV_{254\ nm}$ and after spraying Bouchardat reagent and drying at 105°C, this stain gives a characteristic red-brown color for alkaloids with $R_f$ of 0.651; 0.724 and 0.651, respectively. Thus, these solvent systems can be used for the development of TLC chromatography to identify alkaloids in the construction and isolation of alkaloid active substances present in the seeds of the *Concave white Lotus* (Fig. 3).

The results on High-performance liquid chromatography (HPLC) at 350 nm show that each compound has one peak chromatography structure and has good resolution, the corresponding retention time of the compounds are 12.065 minutes (NH2), 5.686 minutes (NH8), 9.620 minutes (NH11), respectively (Table 1 and Fig. 4). To identify these alkaloids, LC-MS/MS experiments were carried out by using mass spectrometers analysis (HPLC-MS/MS) on the Q Exactive™ Plus Hybrid Quadrupole-Orbitrap™ Mass Spectrometer with TCC-3000 column, data including retention time, molecular ions and some important fragmentation ions are listed of

results MS/MS shows that Table 1 and Fig. 5 as follows:

High-resolution mass spectrometry recorded positive ion $[M+H]^+$ of compounds NH2, NH8 and NH11 with m/z = 296.9706, m/z = 314.1750 and m/z = 266.1595, respectively $[C_{19}H_{21}NO_2 + H]^+$, $[C_{19}H_{23}NO_3 + H]^+$ and $[C_{17}H_{15}NO_2 + H]^+$ consistent with a molecular formula NH2 = $C_{19}H_{21}NO_2$, NH8 = $C_{19}H_{23}NO_3$ and NH11 = $C_{17}H_{15}NO_2$. The results of MS/MS analysis of ion fragmentation in positive ion measurement mode and comparing, contrasting on the NIST2017 (https://www.sisweb.com/software/ms/nist.htm) data spectrum on natural active ingredients and reference by Deng et al. [33], Xu et al. [34], Tan et al. [35], Lima et al. [36], Gontijo et al. [37] analysis compounds this, We can determine the compounds- NH2 is Nuciferine, NH8 is Armepavine and NH11 is Anonaine corresponding to the chemical formula in Fig. 2 (Table 1 and Fig. 5). This is compounds have been carried out by many researchers and found to be present in many different parts of the lotus plant *N. nucifera* such as leaves, seeds, and fruits [38,39,40,41,42,17,43].

The scientists in the world have applied assay Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometric (LC/ESI-MS/MS) and classical column chromatography on silica gel or a combination of these methods in solvent systems different media for the extraction, identification characterization and quantitative analysis of alkaloids and flavonoids from different parts of *Lotus* species *N. nucifera* Gaertn, including lotus seeds [14,11,13]. According to Zhu et al. (2016), more than 30 flavonoids and 10 alkaloids have been identified in lotus seeds and their content has been quantified in different *Lotus* seed samples [14].

## Biological Activities

### Antioxidant activity

The antioxidant activity of three active ingredients isolated from seeds of the *Concave white Lotus* variety collected at Tinh Tam Lake, Thua Thien Hue province in the n-Bu segment showed that Nuciferin (NH2 : $C_{19}H_{21}NO_2$) was the highest DPPH free radical scavenging activity substance reaching of 94.620 ± 0.657% at the concentration of 100 µg/mL, followed by Armepavine (NH8 : $C_{19}H_{23}O_3N$) (74.019 ± 2.400%). Meanwhile, Anonaine (NH11 : $C_{17}H_{15}NO_2$) was the lowest free radical scavenger activity substance with only 61.151 ± 2.349%. The $IC_{50}$ values obtained from these substances were 30.031 ± 1.506 µg/mL (Nuciferin), 43.093 ± 4.215 µg/mL (Armepavine) and 68.217 ± 6.214 µg/mL (Anonaine) respectively (Table 2).

### Anticancer activity

The cytotoxicity results of three active ingredients isolated from the *Concave white Lotus* seeds showed the low activity of the investigated substances at the studied concentrations. The $IC_{50}$ value cannot be determined in the concentration range from 0.8; 4; 20 and 100 µg/mL. At a concentration of 100 µg/mL, Nuciferin gave the best inhibitory activity against the human gastric cancer cell line MKN7, with an inhibitory capacity of 23.800%, and the lowest inhibitory ability on the human skin cancer cell line SK-Mel-2 (11.960%). Armepavine showed the highest inhibitory activity against the oral carcinoma cell line KB (12.320%) and the lowest on the human skin cancer cell line SK-Mel-2 (8.980%). In addition, Anonaine showed the best inhibitory activity against the human skin cancer cell line SK-Mel-2 (19.720%) and the lowest against the oral carcinoma cell line KB (9.010%) (Table 3).

**Table 1. Results of mass spectrometry analysis by HPLC-MS/MS of the compounds**

| Compound symbol | Retention time ($T_R$, min) | Molecular Formula | Predicted Compound Name | $[M+H]^+$ Molecular Weigh | $[M+H]^+$ Error (ppm) | MS/MS Ion fragmentation in positive ion measurement mode |
|---|---|---|---|---|---|---|
| NH2 | 12.065 | $C_{19}H_{21}NO_2$ | Nuciferine | 296.9706 | >5 | 233.9; 250.1; 265.2; 296.2 |
| NH8 | 5.686 | $C_{19}H_{23}NO_3$ | Armepavine | 314.1750 | 1.9 | 107; 145.2; 173.9; 189.3; 239.1; 251.1; 268.2; 314.1 |
| NH11 | 9.620 | $C_{17}H_{15}NO_2$ | Anonaine | 266.1595 | >5 | 178.3; 189; 190; 191; 219 |

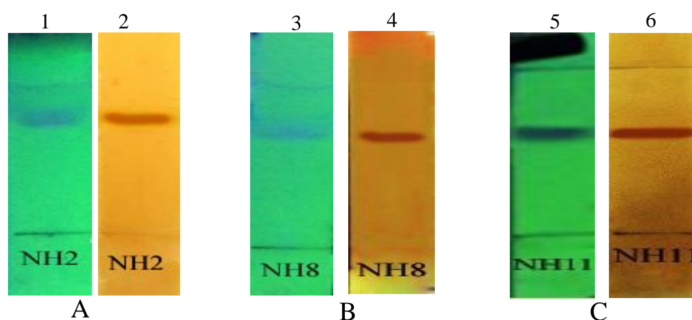


**Fig. 3. Thin layer chromatography (TLC) of compounds: Figs. 1, 3 and 5 are compounds NH2, NH8 and NH11 when viewed under $UV_{254\ nm}$, Figs. 2, 4 and 6 compounds are spraying Bouchardat reagent and drying at 105°C**

A
12.065
0.089
Intensity [counts] (10^6)
RT [min]

B
5.686
Intensity [counts] (10^6)
RT [min]

C
9.620
Intensity [counts] (10^6)
RT [min]

**Fig. 4. HPLC Chromatograms of compounds: A (NH2), B (NH8) and C (NH11)**

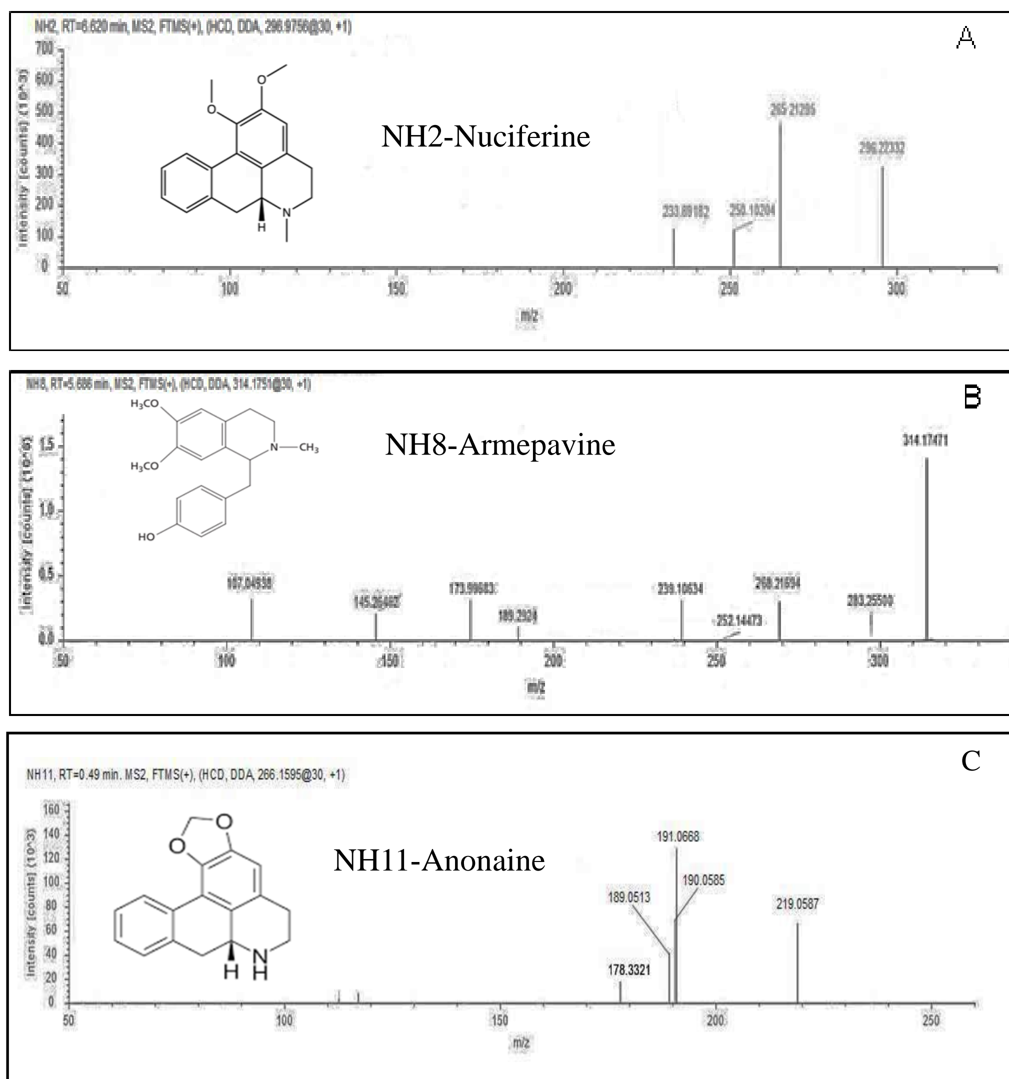


**Fig. 5. Q-TOF mass spectrometer MS/MS of compounds NH2, NH8 and NH11 : A, B and C, respectively**

**Table 2. The antioxidant activity of compounds extraction from seeds of the *Concave white Lotus* variety**

| Concentration (µg/mL) | Compounds | | | |
|---|---|---|---|---|
| | Nuciferin | Armepavine | Anonaine | Acid ascorbic |
| | $\overline{SC\%} \pm SD$ | | | |
| 100 | $94.620^{d} \pm 0.657$ | $74.019^{c} \pm 2.400$ | $61.151^{d} \pm 2.349$ | $99.483^{d} \pm 0.896$ |
| 20 | $46.894^{c} \pm 3.329$ | $39.827^{b} \pm 3.324$ | $37.368^{c} \pm 2.028$ | $68.172^{c} \pm 1.561$ |
| 4 | $35.587^{b} \pm 2.725$ | $33.750^{b} \pm 6.236$ | $27.928^{b} \pm 3.975$ | $51.822^{b} \pm 2.607$ |
| 0.8 | $25.916^{a} \pm 1.340$ | $16.722^{a} \pm 3.133$ | $15.003^{a} \pm 2.256$ | $16.059^{a} \pm 3.414$ |
| **$IC_{50}$ (µg/mL)** | **30.031 ± 1.506** | **43.093 ± 4.215** | **68.217 ± 6.214** | **1.734 ± 0.151** |

Note: Different letters on the same row indicate a statistically significant difference of the sample mean with $p < 0.05$ (Duncan's test). The concentration of Acid ascorbic used in the test was 10-2-0.4-0.08 µg/mL

**Table 3. Carcinogenic effects of compounds extraction from seeds of the *Concave white Lotus***

| Concentration (µg/mL) | | 100 | 20 | 4 | 0.8 | $IC_{50}$ |
|---|---|---|---|---|---|---|
| **Cell lines** | **Compuonds** | **Inhibitions of compounds on cell lines (%)** | | | | **Concentration (µg/mL)** |
| MKN7 | Nuciferin | 23.800 | 10.020 | 5.460 | 2.760 | >100 |
| KB | | 13.710 | 7.370 | 3.750 | 1.730 | >100 |
| SK-Mel-2 | | 11.960 | 9.010 | 5.790 | 2.460 | >100 |
| MKN7 | Armepavine | 12.100 | 9.710 | 6.390 | 3.380 | >100 |
| KB | | 12.320 | 8.560 | 5.580 | 3.700 | >100 |
| SK-Mel-2 | | 8.980 | 5.140 | 3.780 | 1.630 | >100 |
| MKN7 | Anonaine | 12.100 | 9.710 | 6.390 | 3.380 | >100 |
| KB | | 9.010 | 5.220 | 4.400 | 2.820 | >100 |
| SK-Mel-2 | | 19.720 | 14.010 | 9.230 | 2.040 | >100 |
| MKN7 | Ellipticine | 96.060 | 82.780 | 49.030 | 20.710 | 0.360 ± 0.030 |
| KB | | 100.200 | 85.910 | 51.220 | 23.170 | 0.360 ± 0.030 |
| SK-Mel-2 | | 103.290 | 88.500 | 52.610 | 27.720 | 0.300 ± 0.030 |

Note: The concentration of Ellipticine used in the test was 10 -2-0.4-0.08 µg/mL

An important approach to cancer therapy is angiogenesis inhibition. The study of extracting compounds from different parts of lotus and testing the cytotoxic activity of cancer has been studied by many scientists [44,45,46]. Add in, Nuciferine, Armepavine and Anonaine, are alkaloids derived from the lotus plant (*N. nucifera*), which are found in the fruit, seeds and leaves [13,15,17,16]. Studies on the biological activity of these active substances have revealed various interesting pharmacological activities including: anti-tumor, vascular-regulatory, antioxidant, anti-parasitic and antibacterial effects, as well as effects on the central nervous system [18,20,19,21], anti-inflammatory effects [22,15], treatment of liver disease in humans and patients with type 2 diabetes [23], treatment of premature ejaculation [24], and erectile dysfunction [25], brain diseases and various anti-tumor and antiviral pharmacological properties [20,26], immunosuppressive [16], effect on apoptosis in cells leukemia cells [27].

## CONCLUSION

In this study, our results also indicate in the *Nelumbo nucifera* Gaertn seeds contains three compound ingredients appearance, namely Nuciferin (NH2 : $C_{19}H_{21}NO_2$), Armepavine (NH8 : $C_{19}H_{23}O_3N$), and Anonaine (NH11 : $C_{17}H_{15}NO_2$). Some biological activities of the 3 compounds showed that, DPPH free radical scavenging activity substance at 50% ($IC_{50}$ values) were 30.031 ± 1.506 µg/mL (Nuciferin), 43.093 ± 14.215 µg/mL (Armepavine) and 68.217 ± 6.214 µg/mL (Anonaine) respectively. The cytotoxicity results of three active ingredients showed the low activity of the investigated substances at the studied concentrations. The $IC_{50}$ value can not be determined in the concentration range from 0.8; 4; 20 and 100 µg/mL.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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