STUDYING CHEMICAL CONSTITUENTS OF *Nelumbo nucifera* PLANTS, CULTIVATED IN HANOI

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ABSTRACT

Nelumbo nucifera *flower oil, collected by stream distillation, contained mainly pentadecane (36.49%), α-terpineol (11.88%)... From the ethanol extracts of* Nelumbo nucifera *leaves dried powders were isolated and by various spectral methods structurally elucidated four compounds: didecylamine, β-sitosterol, hexadecanamide and nuciferine. Nuciferine, a main aporphine-type alkaloid of* Nelumbo nucifera, *showed some activities towards* E.coli, P.aeruginosa, B.substilis, S.aureus *and* C.albicans *(IC_{50}> 128 μg/ml).*

TÓM TẮT

Nghiên cứu thành phần hóa học của cây sen* Nenumbo nucifera *trồng ở Hà Nội

Tinh dầu hoa sen Nenumbo nucifera*, thu hồi theo phương pháp cất cuốn hơi nước, chứa trên 30 cấu tử, chủ yếu là pentadecan (36.49%), α-terpineol (11.88%)... Từ dịch chiết etanol của lá sen, đã chiết tách và xác định cấu trúc của 4 hợp chất: dodexylamine, β-sitosterol, hexadecanamit và nuciferin. Nuciferin, một alcaloit chính trong lá sen, có biểu hiện hoạt tính (yếu) kháng 5 chủng vi sinh vật kiểm định là* E.coli, P.aeruginosa, B.substilis, S.aureus *và* C.albicans *(IC_{50}> 128 μg/ml).*

1. Introduction

Nelumbo nucifera is native to a huge area in Vietnam and considered as the National flower. Flowers, seeds, young leaves, and "roots" (rhizome) are all edible. The rhizome are used as a vegetable in soups and stir-fried dishes. Petals, leaves, and rhizome can also all be eaten raw… In Traditional Medicine, the *Nenumbo nucifera* leaves are boiled with *Mimosa pudica* (Lajjaalu) in goat milk used to treat diarrhea. Leaves and flowers are both useful in many varieties of raktapitta, or bleeding disorders. Flowers are sometimes prescribed to promote conception. The petals alleviate thirst and inflammations. The seed powder mixed with honey is given in cough treatment…[1]. Oral administration of the ethanolic extracted from rhizomes of *Nelumbo nucifera* markedly reduces the blood sugar level of normal, glucose-fed hyperglycemic and streptozotocin-induced diabetic rats, when compared with control group of animals (the extract exhibited activity of

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73 and 67% of that of tolbutamide in normal and diabetic rats, respectively) [7].

Numerous studies on chemical constituents of *Nelumbo nucifera* plants have showed that its main constituents are alkaloids, flavonoids [4,6]. In this report we show our latest investigation on chemical constituents of *Nelumbo nucifera* flower oil and ethanol condensate extracted from leaves.

2. Experiments

2.1. Genaral experimental procedures

IR spectra are recorded on SHIMADZU- FTIR 8101M spectrophotometer using KBr disks. NMR (^{1}H, ^{13}C NMR, DEPT, HSQC) spectra are recorded on Brucker Avance 500MHz. The chemical shift (δ) values are given in ppm with TMS as internal standard, coupling constant *J* : by Hz), EIMS spectra are recorded on HP 5989B mass spectrometer. Silica gel (Merck Co., Germany) is used for flash chromatography. TLC is carried out on pre-coated Si gel GF254 (Merck Co., Germany) and TLC spots are viewed at 254nm and visualized by spraying with valine- 10% H_2SO_4 solution.

2.2. Plant material

Nenumbo nucifera flower and leaves were collected in West Lake, Tayho Distr., Hanoi in April, 2009. A voucher specimen (No.TIN010686) was deposited in the Hebarium of Dept. of Organic Chemistry, HNUE.

2.3. Flower oil collection

1.0 kg of fresh *Nenumbo nucifera* flowers were subjected to stream distillation. The collected essential oils (n_D^{25} = 1.456, d^{25} = 0.789) were dried by waterless Na_2SO_4 powder and were subjected to GC-MS to identify chemical constituents.

2.4. Extraction and isolation

The air-dried leaves of *Nenumbo nucifera* (2.0 kg) were ground into powder and extracted with 80% methanol (5L x 7 days x 3 time). After evaporation of collected percolate, the crude one was extracted in *n*-hexan: H_2O (1:1), ethyl acetate: H_2O (1:1) biphasic solvent system. Evaporating the organic phase was giving 120g (F1), 80g (F2) of condensates, respectively. The crude condensate F1 was subjected to column chromatography over silica gel and eluted with *n*-hexan: ethyl acetate (100: 0, 50: 1, 10: 1, 1:1, 1:2) solvent systems, giving fractions F1.1, F1.2, F1.3, F1.4, F1.5. From crude condensate F2, by CC fractionation, eluting by ethyl acetate: MeOH (4: 1, 1:1, 1: 2, 1: 4) systems, giving fractions F2.1, F2.1, F2.3, F2.4. From F1.2 precipitated compound **1** (18mg), F1.3 – compound **2**, F2.3 – compound **3**.

The other part of air-dried *Nenumbo nucifera* leaves (1.5kg) were extracted with 95% ethanol. After evaporation of the collected percolate, the crude extract was acidified with dilute HCl (4%) to pH 1-2 and partitioned between CH_2Cl_2 and water. The aqueous part was then basified with aqueou NH_3 to pH 9-10 and extracted with CH_2Cl_2 to afford 12,75g of crude alkaloids. Crude alkaloids (5g) were subjected to column

chromatography over silica gel and eluted gradiently with petroleum ether – acetone, and then methanol. Twelve fractions were obtained. From fraction 3 was precipitated compound **4** (21mg).

*Didecylamine (**1**):* white crystals, m.p.: 81.8°C; dissolved in MeOH, EtOH, $CHCl_3$, R_f (*n*-hexan): 0.4. IR (KBr, cm^{-1}): 3600-3100 (wide), 2924, 2852, 1724 (weak), 1625, 1467, 1131, 1041, 860, 724, 606, 434; ^1H NMR (δ, ppm): 3.58 (1H, m), 1,25-1,57 (36H, m), 0.88 (6H, t, J=7.0Hz). EIMS (m/z, %): 297 ($[M]^+$,12), 278 (3) , 157 (25), 139 (6), 125 (10), 97 (63), 83 (100), 69 (67), 57 (80), $C_{20}H_{43}N$.

β-Sitosterol (**2**): white needles, m.p.: 139-140°C; dissolved in $CHCl_3$, ethanol, R_f (in *n*-hexan: EtOAc = 5:1): 0,45

IR (KBr, cm^{-1}): 3426 (OH), 2938, 2873 (C-H, Csp^3), 1641, 1461, 1378 (C=C), 1060, 958 (*trans*-C=C), 592, 451. EI-MS (m/z): 414 $[M]^+$, $C_{29}H_{50}O$.

^1H NMR ($CDCl_3$, σ ppm): 3.521 (1H, t, J=5, H-3), 5.356 (1H, brd m, H-6), 0.682 (3H, s, H-18), 1.000 (3H, s, H-19), 0.917 (3H, d, J=5.5Hz, H-21), 0.833 (1H, d, J=7.1Hz, H-25), 0.823 (3H, d, J=6.5 Hz, H-26), 1.109 (3H, m, H-17), 0.848 (3H, d, J=7.5, H-29).

*Hexadecanamide (**3**)* white crystals, m.p.: 103-104°C; dissolved in MeOH, EtOH, $CHCl_3$, R_f (*n*-hexan: EtOAc = 1:4): 0.48. IR (KBr, cm^{-1}): 3426 (wide, strong), 2938, 2873, 1641 (weak), 1625, 1461, 1378, 1060, 958, 803, 592, 451; ^1H NMR (δ, ppm): 5.3 (2H, m), 2.2 (2H, t, J =7.5Hz), 1,65 (2H, t, J =7.0Hz), 1.25-1.3 (24H, m), 0.88 (3H, t, J=6.5 Hz); ^{13}C NMR (δ, ppm): 154 (C=O), 35.94, 35.2, 31.94, 29.69, 29.61, 29.48, 29.42, 29.37, 29.34, 29.26, 26.80, 25.55, 22.70 (CH_3). EIMS (m/z, %): 256 ($[M]^+$,15), 212 (1), 128 (3), 86 (8), 72 (48), 59 (100), $C_{16}H_{33}ON$.

Nuciferine (**4**): yellowish crystals, m.p.: 163-164°C; dissolved in MeOH, EtOH, $CHCl_3$, R_f (*n*-hexan: EtOAc = 1:3): 0.42. IR (KBr, cm^{-1}): 3440 (OH), 3002, 2952, 2880, 2809, 2802, 1597, 1569, 1449, 1418, 1324, 1254, 1104, 764 ; ^1H NMR and ^{13}C NMR (δ, ppm): see the Table 3.1. EIMS (m/z, %): 295 ($[M]^+$,100), 280 (80), 264 (65), 252 (25), 237 (33), 221 (45), 208 (18), 194 (20), 178 (30), 165 (55), 152 (13), 139 (12), 118 (8), 69 (7), 55 (4), 51 (3), $C_{19}H_{22}O_2N$.

2.5. Test on biological activity

Compounds **1**, **3**, **4** were subjected to testing on inhibiting bacterial and fungal growths, such as *E.coli, P.aeruginosa, B.substilis, S.aureus, C.albicans* following the methods, described in [5].

3. Results and Discussion

3.1. Chemical constituents of Nenumbo nucifera *flower oils*

Nenumbo nucifera flower oils, collected by stream distillation (with yield: 0.0158% of fresh flower weight), contained more than 30 compounds, mainly pentadecane (36.49%), α-terpineol (11.88%), *trans*-carryophyllen (9.28%)…

3.2. Identifying structures of isolated compounds

Compound 1:

From the EI-MS data of **1** afforded

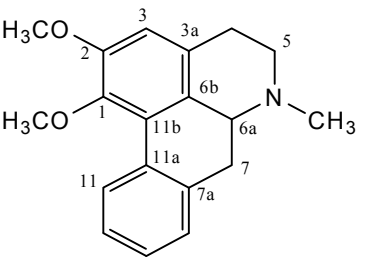
m/z $[M]^+$ m/z= 297. According to "odd mass number" of *N*-containing compounds, **1** must be an amine or an alkaloid. The strong IR absorption at 3311 (with small curved at 3203 cm^{-1}), 1467 cm^{-1} (assigned for –NH- band absorption), together with ^{1}H-NMR signal at 3.58ppm (weak) suggests the presence of **1** amino group -NH-. The ^{1}H NMR of **1** showed 42 protons: two methyl signal at 0.88 ppm (6H, t, J=7.0); 36 methylen protons, belonging to 2 symmetrical alkyl groups, at 1.25-1.57 ppm . From above analysis of IR, NMR, MS spectra and melting point of **1** we made a suggestion that **1** is didecylamine, $(CH_3[CH_2]_9)_2NH$. This amine is the first time isolated from leaves of *Nenumbo nucifera*.

Compound 3:

From the EI-MS data of **3** afforded *m/z* $[M]^+$ m/z= 256. The strong, double IR absorption at 3361 and 3203 cm^{-1} (assigned for $–NH_2$ band absorption), together with ^{1}H-NMR signal at 5.3 ppm (weak), ^{13}C NMR signal at 154 ppm (signed for amide C=O group) suggests the presence of **3** amide group $–CONH_2$. The ^{1}H NMR of **3** showed 31 protons: one methyl signal at 0.88 ppm (3H, t, J=6,5); 28 methylen protons (1.25-136, 2.06 and 2.22 ppm). The ^{13}C NMR of **3** showed 13 carbon signals: one methyl signal at 14.11 ppm, thirteen methylen signals at 22.67-35.20 ppm, one carbonyl C=O (mentioned above) signal at 154 ppm. From above analysis of IR, NMR, MS spectra and melting point of **3** we made a suggestion that **3** is hexadecanamide, $CH_3[CH_2]_{14}CONH_2$. This amide is the first time isolated from leaves of *Nenumbo nucifera*.

Compound 4:

From the EI-MS data of **4** afforded *m/z* $[M]^+$ m/z= 295. According to "odd mass number" of *N*-containing compounds, **4** may be an amine or an alkaloid. **4** affords yellow precipitate; it means that **4** must be an alkaloid. The IR absorption at 2802cm^{-1} (signed for CH_3-NH^+ band absorption), together with ^{1}H-NMR signal at 2.54 ppm (3H) suggests the presence of N-methyl alkaloid. The ^{1}H NMR of **4** showed totally 21 protons: three methyl signals at 2.54 ppm (3H, s, CH_3-N), 3.66 and 3.88 ppm (6H, s, $2CH_3O$-), three cyclic metylen signals at 2.58-3.19, five methin signal at 6.66 - 8.37ppm. The ^{13}C-NMR of **4** shows 19 carbon signals: three methyl signals at 44.5 55.5 and 59.6 (signed for N-CH_3, 2 OCH_3, respectively), four unsaturated cyclic carbon signals at 28.9 34.8 and 52.8ppm, twelve aromatic carbon signal at 126.9-152.0ppm.From above analysis of IR, NMR, MS spectra, melting point of **4**, in comparison with those of nuciferine [6,7] we made a conclusion that **4** is nuciferine, a main alkaloid from *Nenumbo nucifera* leaves.



Nuciferine

Table 3.1. ^{1}H and ^{13}C NMR data of 4 in comparison with those of nuciferine [2,3]

STT	^{1}H NMR, δ ppm		^{13}C NMR, δ ppm	
	4	**Nuciferine [3]**	**4**	**Nuciferine [2]**
1	-	-	145.19	144.6
2	-	-	152.00	151.4
3	7.24 1H s	7.25 1H	111.32	110.9
3a	-	-	128.74	128.1
4	2.54 2H	2.51 2H	29.22	28.9
5	3.12 2H	3.19 2H	53.32	52.8
6a	3.02 2H	3.00 1H m	60.24	61.9
6b	-	-	128.06	127.0
7	2.61 2H	2.62 2H	35.14	34.8
7a	-	-	136.52	136.9
8	6.66 1H	6.62 1H, d, 8.0 Hz	128.35	127.7
9	7.32 1H, m	7.24 1H, m	127.31	126.7
10	7.21 1H, m	7.21 1H, m	126.99	126.4
11	8.37 1H, d 8Hz	8.34 1H, m	126.99	127.3
11a	-	-	132.18	131.6
11b	-	-	127.86	126.3
N-CH_3	2.54 3H, s	2.53 3H, s	44.01	43.5
1-OCH_3	3.66 3H, s	3.65 3H, s	62.37	59.6
2-OCH_3	3.89 3H, s	3.86 3H, s	55.37	55.5

3.3. Biological activity

Results of testing on biological activity of **1, 3, 4** towards five experimental bacteria and fungi: *E.coli, P.aeruginosa, B.substilis, S.aureus* and *C.albicans* showed that only nuciferine showed activities towards mentioned bacteria and fungi (IC_{50}> 128 μg/ml).

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