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Isolated Compounds and Cardiotonic Effect on the Isolated Rabbit Heart of Methanolic Flower Extract of *Nerium oleander* L.

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ABSTRACT

Nerium oleander L. is an evergreen shrub in the dogbane family Apocynaceae and planted throughout the tropical region. It has cardiotonic, antioxidant, anti-inflammatory and anticancer activities. *Nerium oleander* L. has high amount of cardiac glycoside and flavonoids compounds. From methanol extract of flowers of *Nerium oleander* L. grown in vietnam, it has been isolated four compounds. Their structures were identified as (1) D^{16}-Dehydroadynerigenin, (2) D^{16}-Digitoxigenin, (3) Quercetin and (4) Kaempferol on the basis of spectroscopic data and by comparing their physicochemical and spectral data with those published studies. The effect of fraction HF2, which containing compound 1 and 2 on heart function was examined, revealing potent positive inotropic effect on isolated rabbit heart by increasing the contractility and coronary flow heart but does not alter heart rate.

Key words: *Nerium oleander* L., cardiac glycoside, flavonoids, isolated rabbit heart, cardiotonic effect

INTRODUCTION

Vietnam has the tropical monsoon climate with plant species diversity. Vietnam has more than 12000 plant species of which nearly 4000 species can be used in traditional medicine belong to 300 families. *Nerium oleander* L. is a small tree perennial in the dogbane family Apocynaceae, widely distributed in temperate regions throughout the world. It is so widely cultivated as ornamental. The leaf and flower of *Nerium oleander* L. contain some cardiac glycosides that are extremely toxic (Bhuvaneshwari *et al*., 2007; Siddiqui *et al*., 2009; Khan *et al*., 2010; Kumar *et al*., 2013). Flowers of *Nerium oleander* L. has size from 2.5-5 cm, with funnel shape. Traditional medicine has been used in different treatment such as heart failure, asthma, corns, cancer, diabetes and epilepsy (Benson *et al*., 2015). The cardiac glycosides of *Nerium oleander* L. are mainly oleandrin and neriine compounds (Akhtar *et al*., 2014). Some cardenolides in *Nerium oleander* L. are capable of exerting positive inotropic effects on the hearts of animals and humans. The cardiotonic effect of oleanders have been used in therapeutic and also as an instrument of suicide since antiquity (Langford and Boor, 1996). The mechanism of *Nerium oleander* L. cause poisoning by inhibiting plasmalemmal Na^+, K^+-ATPase (Barbosa *et al*., 2008). Some studies have been showed the lethal dose of *Nerium oleander* L. is very small. For mice, the lethal dose of *Nerium oleander* L. leaves ethanolic extract were 520 mg kg^{-1} b.wt. (Saliem, 2010). Vietnam also has many case of poisoning

from misuse, envenom, suicide of some preparations from *Nerium oleander* L. in every year. Identified compound from *Nerium oleander* L. is extremely important to determine the cause of poisoning that help to detoxify patients accurately and quickly. Therefore a study about the chemical composition of *Nerium oleander* L. is very needed. In this study, extracted, isolated and identified four compounds: quercetin, kaempferol, D^{16}-dehydroadynerigenin and D^{16}-digitoxigenin from flower of *Nerium oleander* L. grown in Vietnam.

MATERIALS AND METHODS

Materials: The flowers of *Nerium oleander* L. were collected in August 2013 from Ha Noi, Vietnam. Plant samples were authenticated and stored at the Institute of Medicine, Vietnam.

Chemicals and equipment: Melting points were measured on Mikroskopheiztisch PHMK-50 (VEB Waegetechnik Rapido, Germany). The FT-IR spectra were recorded on an IMPACT-410FT-IR spectrometer (CARL ZEISS JENA). The NMR [^{1}H (500 MHz), ^{13}C (125 MHz) and DEPT-90 and 135 MHz)] spectra were recorded on an AVANCE spectrometer AV 500 (Brucker, Germany) in the Institute of Chemistry, Vietnam Academy of Science and Technology (VAST). Chemical shifts were reported in ppm downfield from TMS with J in Hz. Electrospray Ionization Mass Spectra (ESI-MS) were recorded on a Varian Agilent 1100 LC-MSD mass spectrometer. Analytical TLC was performed on Kieselgel 60 F_{254} (Merck) plates (silica gel, 0.25 mm layer thickness) and RP-18 F_{254} (Merck) plates (0.25 mm layer thickness). Spots were visualized using ultraviolet radiation (at 254 and 365 nm) and by spraying with 10% H_2SO_4 followed by heating with a heat gun. Column chromatography was performed on silica gel (70-230 and 230-400 mesh, Merck). Organic solvents were of analytical grade.

Extraction and isolation: *Nerium oleander* L. flowers of 1.2 kg was dried at 60°C and extracted with methanol (5 L×3 times) at room temperature. The methanol extracts were combined and then evaporated to dryness *in vacuo* at 40°C. This crude extract (34 g) was then suspended in H_2O and partitioned successively with n-hexane, chloroform and ethyl acetate. The EtOAc fraction (10,6 g) was chromatographed over a Sephadex LH-20 column using MeOH as the eluting solvent to yield six fractions (HF1 to HF6). Fraction HF2 (4.9 g) was further separated on Sephadex LH-20 column and eluted with MeOH/CH_2Cl_2 (95/5) to yield two fractions HF2.1 (1.2 g) and HF2.2 (200 mg). Fraction HF2.2 was crystallized in solvent system (n-hexane/acetone 4:1) to obtain the compound 1 (30 mg). Fraction Hf2.1 was chromatographed over a silica gel column and eluted with n-hexane/acetone (9:1) to yield compound 2 (10 mg). Fraction HF5 was applied to a silica gel column eluting with CH_2Cl_2/MeOH (95:5) to afford five subfractions (HF5.1-HF5.5). Fraction HF5.1 (420 mg) was further separated on Sephadex LH-20 column and eluted with MeOH/CH_2Cl_2 (4/1) to yield compound 3 (50 mg). Fraction HF5.2 (70 mg) was also separated on Sephadex LH-20 column and eluted with MeOH/CH_2Cl_2 (4/1) to yield compound 4 (50 mg).

Evaluate effect of fraction HF2 (contains compound 1 and 2) on isolated heart rabbit: We used common rabbits (Oryctolagus cuniculus) (1.5-2.5 kg) obtained from a local rabbit breeder. The animals were maintained at ambient temperature (22±1°C) with 12:12 h light-dark cycles and free access to water and food. All procedures were approved by the ethical committee of our institute and the experiments were performed according to international accepted guidelines for the use of

animals. The rabbits were injected intravenously with heparin and anesthetized with ketamine and xylazine. After the heart was quickly removed, the ascending aorta was immediately cannulated and perfused with a Ringer-Locke solution at a constant pressure (60 cm H_2O) at 37°C and continuously bubbled with a mixture of 95% O_2/5% CO_2 according to the Langendorff technique. The Ringer-Locke solution consisted of 154 mM NaCl, 5.63 mM KCl, 2.16 mM $CaCl_2$, 2.10 mM $MgCl_2$, 5.95 mM $NaHCO_3$ and 5.55 mM glucose. Langendorff hearts were allowed to equilibrate for 15 min, hearts presenting any irregularities in function were discarded. The rabbits were randomly divided into three groups (n = 9 for each group):

- HF2 (0.1%): HF2 (0.1%) was added to the Ringer-Locke solution
- HF2 (0.5%): HF2 (0.5%) was added to the Ringer-Locke solution
- HF2 (1%): HF2 (1%) was added to the Ringer-Locke solution

During the experiments each heart served as its own control before injection of each solution. The Heart Rate (HR), contractility and Coronary Flow (CF) were monitored with a physiograph (Ugo-Basile, Italy) by using fluid volume flow through the heart in each 5 min before and after perfused with the sample solution during 30 min.

RESULTS

In order to isolate the compounds from flower of *Nerium oleander* L., the EtOAc-soluble extract of *Nerium oleander* L. was subjected to a succession of chromatographic procedures including Sephadex LH-20, silica gel chromatography, RPC18 and HPLC to afford four compounds (Fig. 1).

Compound 1 (D^{16}-Dehydroadynerigenin): It was obtained as a yellow, solid powder, melting point 209-210°C; IR (KBr) n_{max} 3362 (OH), 2930 (CH, aliphatic), 1742 (g-lactone α,β-unsaturated), 1628, 1443 (C = C) and 1160 (C-O-C) cm^{-1}; ESI-MS: 371 $[M+H]^+$; ^1H NMR (500 MHz, $CDCl_3$) δ_H ppm: 1.04 (3H, s, H-19); 1.21 (3H, s, H-18); 2.57 (1H, dd, J = 20.0; 2.0 Hz, H-15β); 2.61 (1H, dd, J = 20.0; 2.0 Hz, H-15α); 6.07 (1H, t, J = 3,0 Hz, H-16); 5.94 (1H, br s, H-22); 4.97 (1H, dd, J = 16.5; 1.5 Hz, H-21α); 4.91 (1H, dd, J = 16.5; 1.5 Hz; H-21β); 4.12 (1H, br s, H-3); ^{13}C NMR (500 MHz, $CDCl_3$) δ_H ppm: 15.63 (C-11); 19.91 (C-18); 24.51 (C-6); 24.58 (C-19); 26.78 (C-7); 28.08 (C-2); 29.60 (C-1); 33.04 (C-15); 33.16 (C-4); 33.32 (C-12); 35.94 (C-9); 36.00 (C-5); 36.94 (C-10), 44.74 (C-13); 65.12 (C-8); 66.49 (C-3); 70.10 (C-14); 71.40 (C-21); 112.82 (C-22), 132.24 (C-16); 143.01 (C-17); 157.68 (C-20); 174.28 (C-23).

Compound 2 (16-dehydrogitoxigenin or D^{16}-Digitoxigenin): It was obtained as a white, solid powder, melting point 240-241°C; IR (KBr) n_{max} 3484 (OH), 2930 (CH, aliphatic), 1727 (γ-lactone α, β-unsaturated), 1619, 1456 (C = C) and 1170 (C-O-C) cm^{-1}; ESI-MS: 373 $[M+H]^+$, 355 $[M-H_2O+H]^+$; ^1H NMR (500 MHz, $CDCl_3$) δ_H ppm: 0.99 (3H, s, H-19); 1.27 (3H, s, H-18); 2.34 (1H, dd, J = 18.5; 3.5 Hz, H-15β); 2.72 (1H, br d, J = 18.5 Hz, H-15α); 4.07 (1H, br s, H-3), 5.00 (1H, dd, J = 16.5; 1.5 Hz; H-21β); 5.08 (1H, dd, J = 16.5; 1.5 Hz, H-21α); 5.94 (1H, br s, H-22); 6.20 (1H, br s, H-16). ^{13}C NMR (500 MHz, $CDCl_3$) δ_H ppm: 16.85 (C-18); 20.46 (C- 7); 21.71 (C-11); 24.29 (C-19); 27.10 (C-6); 28.20 (C-2); 30.32 (C-1); 33.72 (C-4); 35.88 (C-10); 36.71 (C-9); 36.99 (C-8); 39.13 (C-15); 40.78 (C-12); 41.54 (C-5); 52.93 (C-13); 67.05 (C-3); 73.01 (C-21); 86.13 (C-14); 111.74 (C-22), 134.20 (C-16); 144.49 (C-17); 161.13 (C-20); 176.76 (C-23).

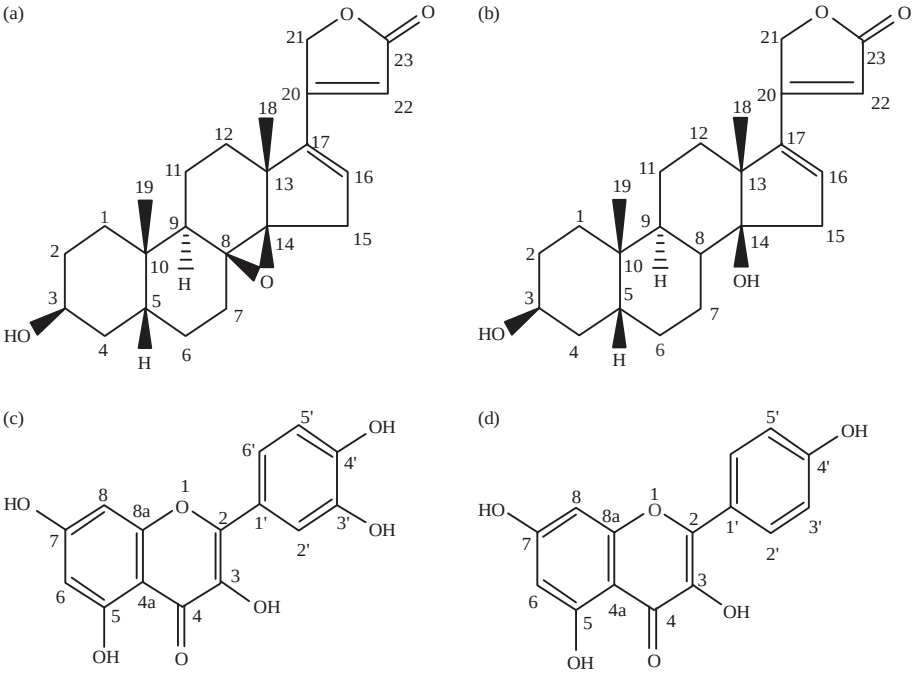

Fig. 1(a-d): Chemical structure of compounds 1-4 isolated from the flower of *Nerium oleander* L.

Table 1: Effect of fraction HF2 on rate of isolated rabbits heart

HR (beats/min)	Without fraction HF2	With fraction HF2	Percentage changed (%)	p-value (with-without)
HF2 0.1%	57.4±10.1	56.9±11.1	Decreased 0.9	>0.5
HF2 0.5%	41.9±8.2	39.9±6.8	Decreased 4.8	>0.1
HF2 1%	62.0±9.2	57.1±9.1	Decreased 7.9	>0.05

We observed that three concentration of fraction HF2 did not influenced significantly (p>0.05) to heart rate isolated rabbit, HR: Heart rate

Compound 3 (Quercetin): It was obtained as a yellow, solid powder, melting point 304-305°C; IR (KBr) n_{max} cm^{-1}: broad absorbance band 3426 (-OH), 1664 (C = O), 1614 (C = C), 1523, 1386 (C-H), 1020 (C-O-C) cm^{-1}; ESI-MS: 301 $[M-H]^+$; ^1H NMR (500 MHz, $CDCl_{3+}$ MeOH-d_4) δ_H ppm: 6.13 (1H, d, J = 2.5 Hz, H-2'); 6.28 (1H, d, J = 2.0 Hz, H-8); 6.80 (1H, d, J = 8.5 Hz, H-5'); 7.52 (1H, dd, J = 8.5; 2.5 Hz, H- 6'); 7.61 (1H, J = 2.0 Hz, H-6).^{13}C NMR (500 MHz, $CDCl_{3+}$ MeOH-d_4) δ_H ppm: 93.40 (C-8); 98.09 (C-6); 102.98 (C-4a); 114.42 (C-2'); 114.83 (C-5'); 120.32 (C-6'); 122.44 (C-1'); 135.29 (C-3); 144.25 (C-3'); 146.07 (C-2); 146.78 (C-4'); 156.46 (C-8a); 160.32 (C-5); 163.55 (C-7); 175.22 (C-4).

Compound 4 (Kaempferol): It was obtained as a yellow powder, melting point 275-276°C; IR (KBr) n_{max} cm^{-1}: 3421 (OH phenol), 2992 (-CH-), 1661 (C = O), 1612, 1507, 1385 (C = C), 1178, 1007 (C-O-C), 821 (C-H); ESI-MS: 285 $[M-H]^+$; ^1H NMR (500 MHz, methanol-d_4) δ_H ppm: 6.19 (1H, d, J = 2,0 Hz, H-6); 6.41 (1H, d, J = 2.0 Hz, H-8); 6.91 (2H, dd, J = 7.0; 2.0 Hz, H-5' va H-3'); 8.01 (2H, dd, J = 7.0; 1.5 Hz, H- 6' va H-2').^{13}C NMR (500 MHz, metanol-d_4) δ_H ppm: 148.05 (C-2); 137.13 (C-3); 177.37 (C-4); 162.52 (C-5); 99.2 (C-6); 165.58 (C-7); 94.46 (C-8); 158.26 (C-8a); 104.54 (C-4a); 123.73 (C-1'); 130.68 (C-2'); 116.30 (C-3'); 160.55 (C-4'); 116.30 (C-5'); 130.68 (C-6').

Effect of fraction HF2 on isolated rabbit heart: The results of fraction HF2 to heart rate, contractility heart (mm) and coronary flow on isolated rabbit heart were showed in Table 1-3.

Table 2: Effect of fraction HF2 on contractility of isolated rabbits heart

Contractility heart (mm)	Without fraction HF2	With fraction HF2	Percentage changed (%)	p-value (with-without)
HF2 0.1%	18.0±3.6	22.9±2.9	Increased 21.2	<0.001
HF2 0.5%	16.0±3.6	21.7±5.2	Increased 35.7	<0.005
HF2 1%	14.5±1.8	20.4±3.3	Increased 41.9	<0.001

Table 3: Effect of fraction HF2 on coronary flow of isolated rabbits heart

Coronary flow (mL/5 min)	Without fraction HF2	With fraction HF2	Percentage changed (%)	p-value (with-without)
HF2 0.1%	18.7±2.6	19.5±3.1	Increased 4.5	<0.05
HF2 0.5%	22.0±4.4	23.4±4.6	Increased 6.5	<0.001
HF2 1%	24.7±2.4	27.2±2.5	Increased 13.0	<0.005

We observed that three concentration of fraction HF2 did not influenced significantly ($p>0.05$) to heart rate isolated rabbit.

In Table 2 we observed that the three concentrations of HF2 significantly increased the contractile of isolated rabbits heart ($p<0.005$-$p<0.001$). When the concentration increased from 0.1-1%, the contractility of isolated rabbit's heart was increased from 21.2-41.9%. As the concentration of HF2 increases, the contractility also increases.

Table 3 showed that the three concentrations of HF2 also significantly increased the coronary flow of isolated rabbits heart ($p<0.05$-$p<0.001$). When the concentration of HF2 increased from 0.1-1%, the coronary flow of isolated rabbit's heart was increased from 4.5-13.0%. The effect of HF2 on coronary flow is concentration-dependent.

DISCUSSION

Compound 1 was isolated as a yellow solid, melting point at 209-210°C. The molecular formula was established as $C_{23}H_{30}O_4$ based on a molecular ion peak at m/z 371 $[M+H]^+$. The IR spectrum showed typical absorption bands arising from hydroxyl (n_{max} 3372 cm^{-1}), γ-lactone α, β-unsaturated group (n_{max} 1789, 1742 cm^{-1}) and double bond C = C (n_{max} 1628 cm^{-1}). The ^{13}C-NMR spectrum indicated the presence of 23 carbon atoms in the molecule. They are distinguished by DEPT 90 and DEPT 135 spectrum. There are a carbonyl carbon (δ_C 174.28), 4 carbons of two double bond (δ_C 143.01 and 132.24, 157.68 and 112.82), two methyl carbons (δ_C 19.91 and 24.58), 9 methylene carbons in which one oxymethylene carbon (δ_C 71.40), three sp^3 methine carbon including a hydroxymethine carbon (δ_C 66.49) and four quaternary carbon sp^3 including two carbon bond to oxygen. The ^{1}H-NMR spectrum showed the presence of two methyl singlet group relatively in up-field at δ_H 1.04 (3H-19) and δ_H 1.21 (3H-18), two signals double doublet appear at d_H 2.57 (J = 20; 2.0 Hz, H-15β) and d_H 2.61 (J = 20.0; 2.0 Hz, H-15α) belong to two gemminal proton of methylene group. Additionally, the spectrum indicated at down-field a singlet broad signal of a proton hydroxymethine at d_H 4.12, this signal has a configuration. The signal of two protons in oxymethylene group appears as two double doublet at δ_H 4.97 (J = 16.5, 1.5 Hz, H-21 α) and 4.91 (J = 16.5; 1. 5 Hz; H-21β). Signal of proton of unsaturated hydrocarbon appears as a triplet at δ_H 6.07 (J = 3.0 Hz, H-16) and a broad singlet at δ_H 5.94 (H-22). In addition, the ^{1}H- NMR spectrum had signals as multiplets at up-field of methine and methylene group at d_H 1.10-2.20 ppm region. Furthermore, from molecular formula $C_{23}H_{30}O_4$ of compound 1, it can inferred the total rings and double bonds in the molecule is 9, which identify the molecule containing 3 double bonds and 6 rings and then this compound containing steroid ring with γ-lactone α, β-unsaturated ring. The steroid ring with γ-lactone α, β-unsaturated ring and a double bond contain 5 rings and 3 double bonds. Thus there is only one oxygen atom corresponding to a loop in the molecule, which indicates that the molecule must contain an epoxy ring. This is totally consistent with the presence of two

carbons sp^3 bond with oxygen at δ_C 65,12 (C-8) and δ_C 70,10 (C-14) on ^{13}C NMR spectrum. Based on the above evidence and the literature data (Yamauchi *et al*., 1973; Siddiqui *et al*., 1986, 1997), compound 1 was identified as 3β-hydroxy-8, 14β-epoxy-5β-carda-16, 20(22)-dienolide or D^{16}-Dehydroadynerigenin.

Compound 2 was isolated as a white solid with melting point at 240-241°C. Spectrum of compound 2 is very similar to compound 1. The ^{13}C-NMR spectrum also indicated the presence of 23 carbon atoms in the molecule and distinguished by DEPT 90 and DEPT 135 spectrum. There are two methyls carbons (d_C 16.85 va 24.29), nine methylene carbons including one oxymethylene carbons (d_C 73.01), six methine carbons including two methine carbons unsaturated (d_C 111.74 va 134.20), one hydroxymethine carbon (d_C 67.05) and two sp^3 methine and six quaternary carbon including a carbonyl carbon (d_C 176.76), two quaternary carbon unsaturated (d_C 144.49 va 161.13), one sp^3 carbon bonds to oxygen (d_C 86.13). The ^1H-NMR spectrum of compound 2 has many similarities with the ^1H-NMR spectrum of compound 1 as showed the signals of two singlets of two methyl groups at d_H 0.99 and d_H 1.27, two gemminal proton of methylene group as one signal of double doublet at d_H 2.34 (J = 18.5; 3.5 Hz, H-15β) and one broad doublet at d_H 2.72 (br d, J = 18.5 Hz, H-15α). In addition, at the down-field of spectrum there was a signal of hydroxymethine as a broad singlet at d_H 4.07, two protons of a oxymethylene group as two double doublet at d_H 5.00 (J = 16.5; 1.5 Hz; H-21β) and d_H 5.08 (J = 16.5; 1.5 Hz, H-21α). Signals of two protons of double bonds appear as two broads singlet at d_H 5.94 (br s, H-22) and d_H 6.20 (br s, H-16). Additionally, there were also signals as multiplets of the methylene group and methine group at up-field d_H 0.88-2.03. The molecular formula was established as $C_{23}H_{32}O_4$ based on a molecular ion peak at m/z 373 $[M+H]^+$. From molecular formula $C_{23}H_{30}O_4$ of compound 2, we can inferred the total rings and double bonds in the molecule is 8. Based on spectrum data on, the compound has three double bonds, then it has five rings in the molecule. Compound 2 has spectrum data is very similar of compound 1, differing only in that compound 2 has more two hydrogen but less a ring and a carbon quaternary bonds to oxygen as compared to compound 1. Combining all the data, it can be concluded that the compound 2 has structure of compound 1 with epoxy ring opening. Based on the above evidence and the previous studies (Yamauchi *et al*., 1975; Siddiqui *et al*., 1986), compound 2 was identified as 16-anhydrogitoxigenin or D^{16}-Digitoxigenin.

Compound 3 was isolated as a yellow powder melting point at 304-305°C. The ^1H NMR spectrum showed only signal of five protons of 2 spin-spin interactions at down-field of proton in aromatic ring. The first interaction is an AX system of two protons in *meta* position in a benzene ring with four substituted [d_H 6.28 (1H, d, J = 2.0 Hz, H-6) va 7.61 (1H, d, J = 2.0 Hz, H-8)]. The second interaction is an ABX system of three protons in a benzene ring with three substituted [d_H 6.13 (1H, d, J = 2.5 Hz, H-2'); 6.80 (1H, J = 8.5 Hz, H-5') va 7.52 (1H, dd, J = 8.5, 2.0 Hz)]. The ^{13}C-NMR spectrum showed the presence of 15 carbon atoms at down-field (d_C 93.40-175.24) in the molecule, in which 5 carbons belong methine group of double bond (= CH) [d_C 93.40 (C-8), 98.09 (C-6), 114.42 (C-2'), 114.83 (C-5') and 120.32 (C-6')] seven quaternary carbons bond to oxygen (d_C 135.29-175.22) and 2 other quaternary carbons sp^2. The signal at d_C 175.22 indicated that the molecule containing a carbonyl group.

The presence of carbonyl group in the molecule was confirmed by the presence of the absorption band at n_{max} 1664 cm^{-1} in IR spectrum. Also the IR spectrum showed typical absorption bands arising from some hydroxyl groups (n_{max} 3200-3500 cm^{-1}) and benzen ring through absorption band for C = C bonds in aromatic rings (n_{max} 1614, 1523 cm^{-1}). In addition, there is also a typical absorption band for C-O-C bond at n_{max} 1020 cm^{-1}. The molecular formula was established as

$C_{15}H_{10}O_7$ based on a molecular ion peak at m/z 301 $[M+H]^+$ and combining all the data, it can be concluded this compound is a flavonoid. Based on the above evidence and the study which reported by Ahmedova *et al*. (2012), compound 3 was identified as quercetin. Quercetin is a flavonol with has various biological activities and it is very common in some plants. This compound has strong antioxidant, anti-inflammatory and anti-cancer activities (Lee *et al*., 2007).

Compound 4 was isolated as a yellow crystal, melting point at 275-276°C. The ^{1}H NMR spectrum showed signal of six protons at down-field of proton in aromatic ring. There is an interaction AX system of two protons in a benzene ring with four substituted [d_H 6.19 (1H, d, J = 2.0 Hz, H-6); 6.41 (1H, d, J = 2.0 Hz, H-8)]. Other signal belonging to four protons in *para* position in a benzene ring with two substituted [d_H 6.91 (2H, dd, J = 7.0; 2.0 Hz, H-5' and H-3'); 8.01 (2H, dd, J = 7.0; 1.5 Hz, H- 6' and H-2')]. The ^{13}C-NMR spectrum showed the presence of 15 carbon atoms at down-field (d_C 94.46-177.39) in the molecule, in which 6 carbons belong methine group of double bond, nine quaternary carbons including one carbonyl carbon (d_C 177.39), six quaternary carbons bond to oxygen and 2 other quaternary carbons sp^2. The IR spectrum showed typical absorption bands arising from some hydroxyl groups (n_{max} 3300-3500 cm^{-1}). The presence of methine double bond (= CH) group in the molecule was confirmed by the presence of the typical absorption band of C-H bond at n_{max} 2929 cm^{-1} in IR spectrum. The carbonyl group in the molecule gives an absorption band at n_{max} 1661 cm^{-1}. Also the IR spectrum showed typical absorption bands arising from benzen ring through absorption band for C = C bonds in aromatic rings (n_{max} 1612, 1507 and 1385 cm^{-1}). Additionally, there is also a typical absorption band for C-O-C bond at n_{max} 1178 cm^{-1}. Combining all the data, we can conclude this compound is a flavonoid. The molecular formula was established as $C_{15}H_{10}O_6$ based on a molecular ion peak at m/z 285 $[M+H]^+$. Based on the above evidence and the study which reported by Furusawa *et al*. (2005), compound 4 was identified as kaempferol. Kaempferol is a flavonol and presents in some plants. This compound has strong anti-inflammatory, diuretic, antioxidant activity and inhibition of topoisomerase-II, adenosine deaminase, tyrosine kinase, xanthine oxidase enzyme (Lee *et al*., 2007).

In this study, methanolic extract of Nerium oleander elicited to increase the force of contraction, coronary flow heart but does not influence to heart rate. Present data can be used to explain the possible benefit of this herb in cardiovascular diseases. The increased force of contraction of the heart will cause the heart to expel more blood into the arterial system with each beat. The patient with failing heart will be beneficial by this properties of the herb (Adome *et al*., 2003). Mechanism of action of cardiac glycosides can explain by capacity of inhibit Na^+-K^+ pumps in the cardiac myocytes, then increased intracellular sodium concentration. The raises intracellular sodium levels lead to inhibit Na^+/Ca^{2+} exchanger, then calcium ions are not extruded and begins to increase inside the cell. Increased cytoplasmic calcium concentrations allow for greater calcium release on stimulation so that myocytes could achieve faster and more powerful contraction by cross-bridge cycling (Radzyukevich *et al*., 2009). Our data was agreed with previous study of Adome *et al*. (2003). These authors have shown that the crude ethanolic extracts of the dried leaves of *Nerium oleander* increased the force of contraction and cardiac flow on the isolated guinea pig hearts in manner dose-dependent. When the force of cardiac contraction is increased, the failure is partially relieved, also reducing the size heart rate. In our present work the heart rate was not influenced, it is contradiction with Adome *et al*. (2003), which they showed the heart rate was increased. In other study, Gayathri *et al*. (2010) have shown the cardioprotective effect of hydroalcoholic extract of Nerium oleander flowers against isoproterenol-induced myocardial toxicity by improving the antioxidant defense system in rats model. The positive inotropic effect of fraction HF2 may due to

present of two major compounds 1 and 2, which we have isolated from this fraction. In fact, dehydrogitoxigenin and digitoxigenin glucoside have been showed the inotropic activity in previous study (Altman *et al*., 1988; Packer *et al*., 1991). The pretreatment with extract of *Nerium oleander* prevented the elevation of marker enzymes such as lactate dehydrogenase, γ-glutamyl transferase, creatine kinase, aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase in plasma and also attenuated the lipid peroxidation and increased the levels of enzymatic such as superoxide dismutase, glutathione peroxidase and nonenzymatic antioxidants including reduced glutathione and nitrite (Gayathri *et al*., 2010).

CONCLUSION

From the flowers of *Nerium oleander* L. grown in Hanoi, Vietnam, we have isolated and identified successfully four compounds: (1) D^{16}-Dehydroadynerigenin or 16-dehydrogitoxigenin, (2) D^{16}-Digitoxigenin, (3) Quercetin and (4) Kaempferol based on IR, MS, NMR spectrum. This is the first time that four compounds were isolated from flower of *Nerium oleander* L. Fraction HF2 of flowers of *Nerium oleander* L. showed potent inotropic effect on isolated rabbit heart by increasing the contractility and coronary flow heart but does not alter heart rate.

REFERENCES

Adome, R.O., J.W. Gachihi, B. Onegi, J. Tamale and S.O. Apio, 2003. The cardiotonic effect of the crude ethanolic extract of *Nerium oleander* in the isolated guinea pig hearts. Afr. Health Sci., 3: 77-82.

Ahmedova, A., K. Paradowska and I. Wawer, 2012. ^{1}H, ^{1}3C MAS NMR and DFT GIAO study of quercetin and its complex with Al(III) in solid state. J. Inorg. Biochem., 110: 27-35.

Akhtar, T., N. Sheikh and M.H. Abbasi, 2014. Clinical and pathological features of Nerium oleander extract toxicosis in wistar rats. BMC Res. Notes, Vol. 7. 10.1186/1756-0500-7-947

Altman, P.M., R. Einstein, A.H. Goodman and R.E. Thomas, 1988. Inotropic activity of digitoxigenin glucoside and related glycosides. Arzneimittel-Forschung, 38: 1115-1119.

Barbosa, R.R., J.D. Fontenele-Neto and B. Soto-Blanco, 2008. Toxicity in goats caused by oleander (*Nerium oleander*). Res. Vet. Sci., 85: 279-281.

Benson, K.F., R.A. Newman and G.S. Jensen, 2015. Antioxidant, anti-inflammatory, anti-apoptotic and skin regenerative properties of an *Aloe vera*-based extract of *Nerium oleander* leaves (NAE-8®). Clin. Cosmetic Invest. Dermatol., 8: 239-248.

Bhuvaneshwari, L., E. Arthy, C. Anitha, K. Dhanabalan and M. Meena, 2007. Phytochemical analysis and antibacterial activity of *Nerium oleander*. Ancient Sci. Life, 26: 24-28.

Furusawa, M., T. Tanaka, T. Ito, K.I. Nakaya and I. Iliya *et al*., 2005. Flavonol glycosides in leaves of two *Diospyros* species. Chem. Pharmaceut. Bull., 53: 591-593.

Gayathri, V., S. Ananthi, C. Chandronitha, G. Ramakrishnan, R.L. Sundaram and H.R. Vasanthi, 2010. Cardioprotective effect of *Nerium oleander* flower against isoproterenol-induced myocardial oxidative stress in experimental rats. J. Cardiovasc. Pharmacol. Therapeut., 16: 96-104.

Khan, I., C. Kant, A. Sanwaria and L. Meena, 2010. Acute cardiac toxicity of *Nerium oleander*/indicum poisoning (Kaner) poisoning. Heart Views, 11: 115-116.

Kumar, A., T. De, A. Mishra and A.K. Mishra, 2013. Oleandrin: A cardiac glycosides with potent cytotoxicity. Pharm. Rev., 7: 131-139.

Langford, S.D. and P.J. Boor, 1996. Oleander toxicity: An examination of human and animal toxic exposures. Toxicology, 109: 1-13.

Lee, E.J., E.J. Choi, C.I. Choi, J.S. Park and M.K. Sung, 2007. Effects of anti-inflammatory quercetin and kaempferol on cell growth and the production of angiogenic factors in HT-29 human colon cancer cells. FASEB J., Vol. 21.

Packer, M., J.R. Carver, R.J. Rodeheffer, R.J. Ivanhoe and R. DiBianco *et al*., 1991. Effect of oral milrinone on mortality in severe chronic heart failure. N. Engl. J. Med., 325: 1468-1475.

Radzyukevich, T.L., J.B. Lingrel and J.A. Heiny, 2009. The cardiac glycoside binding site on the Na,K-ATPase α 2 isoform plays a role in the dynamic regulation of active transport in skeletal muscle. Proc. Natl. Acad. Sci. USA., 106: 2565-2570.

Saliem, A.H., 2010. Effect of the median lethal dose of the ethanolic extract of (*Nerium oleander*) leaves on the histopathology features of the vital organs in mice. Iraqi J. Vet. Med., 34: 103-109.

Siddiqui, B.S., R. Sultana, S. Begum, A. Zia and A. Suria, 1997. Cardenolides from the methanolic extract of *Nerium oleander* leaves possessing central nervous system depressant activity in mice. J. Nat. Prod., 60: 540-544.

Siddiqui, B.S., N. Khatoon, S. Begum and S.A. Durrani, 2009. Two new triterpenoid isomers from *Nerium oleander* leaves. Nat. Prod. Res., 23: 1603-1608.

Siddiqui, S., F. Hafeez, S. Begum and B.S. Siddiqui, 1986. Isolation and structure of two cardiac glycosides from the leaves of *Nerium oleander*. Phytochemistry, 26: 237-241.

Yamauchi, T., Y. Mori and Y. Ogata, 1973. Δ16-Dehydroadynerigenin glycosides of *Nerium odorum*. Phytochemistry, 12: 2737-2739.

Yamauchi, T., N. Takata and T. Mimura, 1975. Cardiac glycosides of the leaves of *Nerium odorum*. Phytochemistry, 14: 1379-1382.