European Journal of Medicinal Plants
9(2): 1-12, 2015, Article no.EJMP.17761
ISSN: 2231-0894

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Phytochemical Screening of Isolated Compounds from *Nymphaea nouchali* Burm.f. Flowers

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Authors' contributions

This work was carried out in collaboration between all authors. Authors HK and AV deigned and planned the whole research work. Authors AV and SD managed the literature of searches and laboratory reagents. Author HK was provided the analysis data interpretation for all the isolated compounds. Authors HK and AV wrote the protocol, carried out the study and wrote the first manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2015/17761
Editor(s):
(1) Sanjib Ray, Department of Zoology, The University of Burdwan, West-Bengal, India.
(2) Marcello Iriti, Plant Biology and Pathology, Dept. of Agricultural and Environmental Sciences, Milan State University, Italy.
Reviewers:
(1) Mairim Russo Serafini, Pharmacy Department, University Federal of Sergipe, Brazil.
(2) Nyoman Kertia, Department of Internal Medicine, Gadjah Mada University, Indonesia.
(3) Anonymous, Howard University, USA.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=1191&id=13&aid=9714

Original Research Article

Received 25th March 2015
Accepted 29th May 2015
Published 11th June 2015

ABSTRACT

Objective: To learn the phytochemical screening of various compounds from different extracts of *Nymphaea nouchali* Burm.f flowers.
Materials and Methods: *Nymphaea nouchali* flowers were extracted with different solvents (n-hexane, ethyl acetate and ethanol (70%). n - Hexane extract (10 g) was eluted with n-Hexane: $CHCl_3$ in graded mixtures gave fifteen different fractions, which was further rechromatographed to afford two compounds. The ethyl acetate fraction (12 g) was eluted with n-hexane: EtOAc: H_2O (70:30:3). Nineteen fractions were obtained and rechromatographed to give three compounds and ethanolic extract (10 g) was eluted with $CHCl_3$-MeOH-H_2O (90:10:1) to afford three fractions which were rechromatographed to yield a single compound.
Results: 10-eicosenoic acid (1), linoleic acid (2) from n-hexane,7,8 - dihydroxy α-tocopherol-9-O-pyranoside (3), quercetin-3-O-alpha-rhaminoside (4), kaempferol (5) from EtOAc and vasicinone (6) from ethanloic extracts were isolated from *Nymphaea nouchali* flowers for the first time. The structures of the isolated compounds (1-6) were realized on the basis of the spectral data (IR, ^1H

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&^{13}C NMR and Mass).
Conclusion: The obtained compounds of *Nymphaea nouchali* Burm.f. flowers are effective pharmaceutical compounds which will serve as a better alternative to chemical based pharmaceuticals.

Keywords: Nymphaea nouchali; fatty acids; sterols; flavonoids; alkaloids.

1. INTRODUCTION

Nymphaea nouchali Burm.f, (Padma in Sanskrit, Kaluva in Telugu) is a water lily species belonging to the family Nymphaeaceae, genus Nymphaea [1]. *N. nouchali* is a perennial aquatic rooting herb, generally found in tanks and ponds throughout the warmer parts of India, particularly the Eastern Ghats. It grows upto a height of 45 cm in clear, warm, still and slightly acidic waters. Leaves are long-stalked, leathery, green to red-brown, with a deeply heart shaped base and densely hairy beneath. Flowers are fragrant, with 4-5 sepals, cup like calyx, long peduncles, white colored (sometimes with a pink tinge) and 30-250 stamens [2,3,4]. It is used nutritionally as a source of Iron and a fair source of calcium. It is used to treat indigestion (Ayurveda), heart diseases, stomachache and cancer. The rhizome has cooling, sweet, bitter and tonic effects and is useful in diarrhea, dysentery, dipsia and general debility. The flowers are astringent and cardiotonic. The seeds are sweet, cooling, constipating, aphrodisiac, stomachic and restorative [5]. Leaf is used in cutaneous, subcutaneous parasitic infection, eye treatments and pregnancy. It is also used by various tribes in treatment for urinary problems.

Nymphayol, a steroid isolated from the flowers of *Nymphaea stellata* [6] was scientifically proved to responsible for the traditionally claimed antidiabetic activity [7]. 3-O-methyl kaempferol, quercetin, methyl gallate, gallic acid and methyl galloylgallate [8] compounds were isolated from *N. stellata*. To our knowledge, the chemical constituents of the flowers of *Nymphaea nouchali* Burm.f. have not been investigated earlier. The presence of few chemical constituents were [9,10] reported only from the leaves. This paper elucidates the structures of two fatty acids, one sterol**,** two flavonoids and one alkaloid from *Nymphaea nouchali* flowers on the basis of various spectroscopic data.

2. MATERIALS AND METHODS

2.1 General

Column chromatography was carried out by using silica gel (60-120 mesh) (Merck, Bombay) and aluminum sheets and glass-backed TLC plates (20 x 20 cm; Merck, silica gel 60-F254) were used for isolation of compounds. Analytical grade solvents (Sigma Aldrich 32213) were used. IR spectra was recorded using KBr pellets on Thermo Nicolet Nexus 670; ^{1}H NMR spectra were taken on Varian EM-360 (300 MHZ) NMR spectrometer using $CDCl_3$ as solvent; ^{13}C NMR was recorded on Bruker instrument with $CDCl_3$ as solvent at 300 MHz and Mass spectra were recorded on a EI-MS, data on E:ISO/21184-1/QGD.

2.2 Plant Material

The flowers were collected from East Godavari Dist. The plant was authenticated by Dr. B. Prathibha (HOD, Dept. of Botany, Osmania University, Hyderabad).Voucher specimen (MRCP/ N N/12-13/03) was kept at the Malla Reddy College of Pharmacy, Dhullapally, Hyderabad, A.P, India.

2.3 Extraction of Plant Material

Around 6 kg of *Nymphaea nouchali* flowers were shade dried, coarsely powdered and subjected for successive extraction process with three different solvents (n-hexane, ethyl acetate and ethanol (70%)) into 15 batches of each 200 g in Soxhlet extractor for 48 hours. After complete extraction, the solvents were distilled off and concentrated under reduced pressure to the dryness in a flash evaporator. The yield was found to be 23 g, 15 g and 13 g respectively.

2.4 Isolation and Purification of Compounds

The concentrated n- hexane extract (10 g) was eluted with n-hexane: $CHCl_3$ in graded mixture i.e. 95:05, 90:10, 85:15, 80:20… upto 50:50. From above elutions, fifteen different fractions were obtained. Fractions 01 to 07 (7.5 g) was rechromatographaed on silica gel column with n-hexane: $CHCl_3$ (85:15) gave four fractions F_{1a}-F_{1d}. Fraction F_{1b}-F_{1c} (719 mg) was rechromatographed over a silica gel eluting with n-hexane: $CHCl_3$ (90:10) to yield compound 1 on

TLC (n-hexane: $CHCl_3$; 8.2:1.8), the compound was further purified with acetone to give pure compound (1) (132 mg). Fractions 08 to 15 (6.2 g) was rechromatographaed on silica gel column with, n-hexane: $CHCl_3$ (gradient) to afford 6 new fractions F_{2a}-F_{2f}. Fractions F_{2a}-F_{2e}(745 mg) was rechromatographed on silica gel with n-hexane: EtOAC (90:10 to 70:30) to yield compound 2on TLC (n-hexane:$CHCl_3$; 8.5:2.5), ascertained as pure compound (2) (59 mg) (Fig. 1).

The ethyl acetate fraction (12 g) was fractionated by column chromatography over a silica gel G (60-120 mesh, Merck) with n-hexane: EtOAc: H_2O (70:30:3) to afford nineteen fractions. Fractions 01 to 06 (5.8 g) was rechromatographed on silica gel column with n-hexane: EtOAc (85:15) gave five fractions F_{3a}-F_{3e}. Fractions F_{3a}-F_{3d} (845 mg) was rechromatographed on silica gel with n-hexane: EtOAc (80:20) to yield compound 3 on TLC (acetone: MeOH; 8:2), ascertained as pure compound (3) (79 mg). Fractions 07 to 13 (7.1 g) was rechromatographed on silica gel column with EtOAc: MeOH (gradient) to afford 5 fractions F_{4a}-F_{4e}. Fractions F_{4a}-F_{4c} (764 mg) was rechromatographed on silica gel with EtOAc: MeOH (85:15) to yield compound 4 on TLC (EtOAc:MeOH ;3.5;1.5), which was recrystallized from $CHCl_3$ to yield pure compound (4) (62 mg). Fractions 14 to 19 (5.8 g) was rechromatographed on silica gel column with EtOAc:MeOH (gradient) to afford 3 fractions F_{5a}-F_{5c}.Fractions F_{5a}-F_{5b} (549 mg) was rechromatographed on silica gel with EtOAc: MeOH (60:40) to yield compound 5 on TLC ($CHCl_3$:MeOH ; 3.5;1.5), which was recrystallized from MeOH to yield pure compound (5) (75 mg) (Fig. 2).

The ethanolic extract (10 g) was fractionated by column chromatography over a silica gel G (60-120 mesh, Merck) with $CHCl_3$-MeOH-H_2O (90:10:1) to afford three fractions. Fraction 1-3 (6.2 g) was rechromatographed on silica gel column with $CHCl_3$: MeOH (95: 05) gave 4 subfractions F_{6a}-F_{6d}. F_{6b}-F_{6c} (2.2g) was rechromatographed on silica gel with $CHCl_3$: MeOH (92:08) to yield compound 6, on TLC (EtOAc: MeOH: NH_3; 8.5:0.5:0.2), which was recrystallized from $CHCl_3$: EtOAc (3:1), ascertained as compound (6) (87 mg) (Fig. 3).

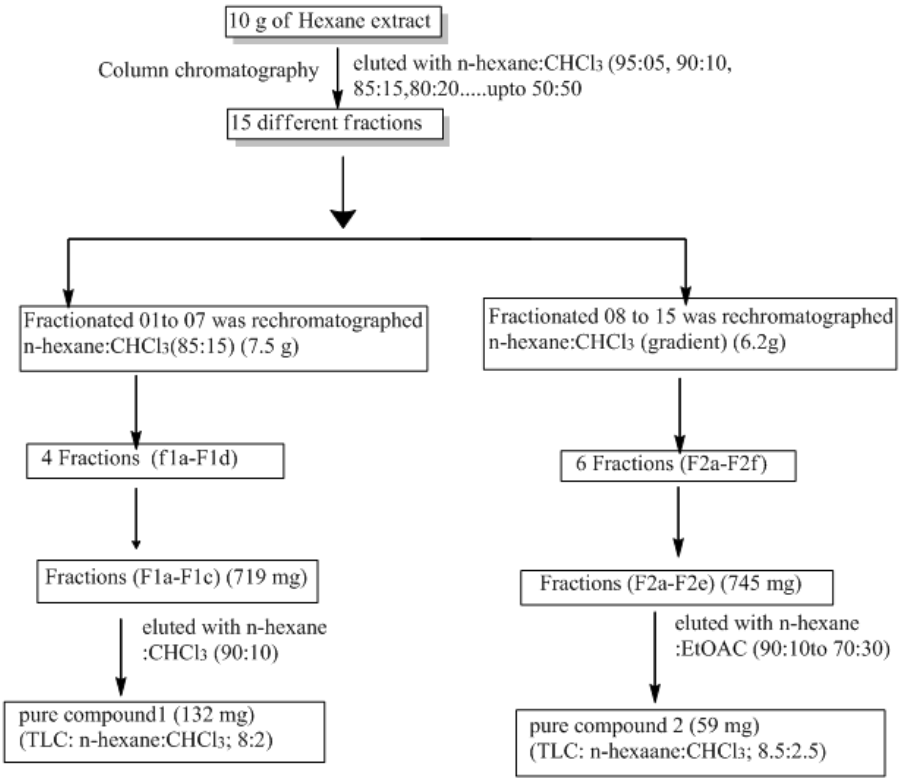

Fig. 1. Schematic diagram of extraction steps of isolated compounds from n-hexane extract of *Nymphaea nouchali* Burm. Flowers

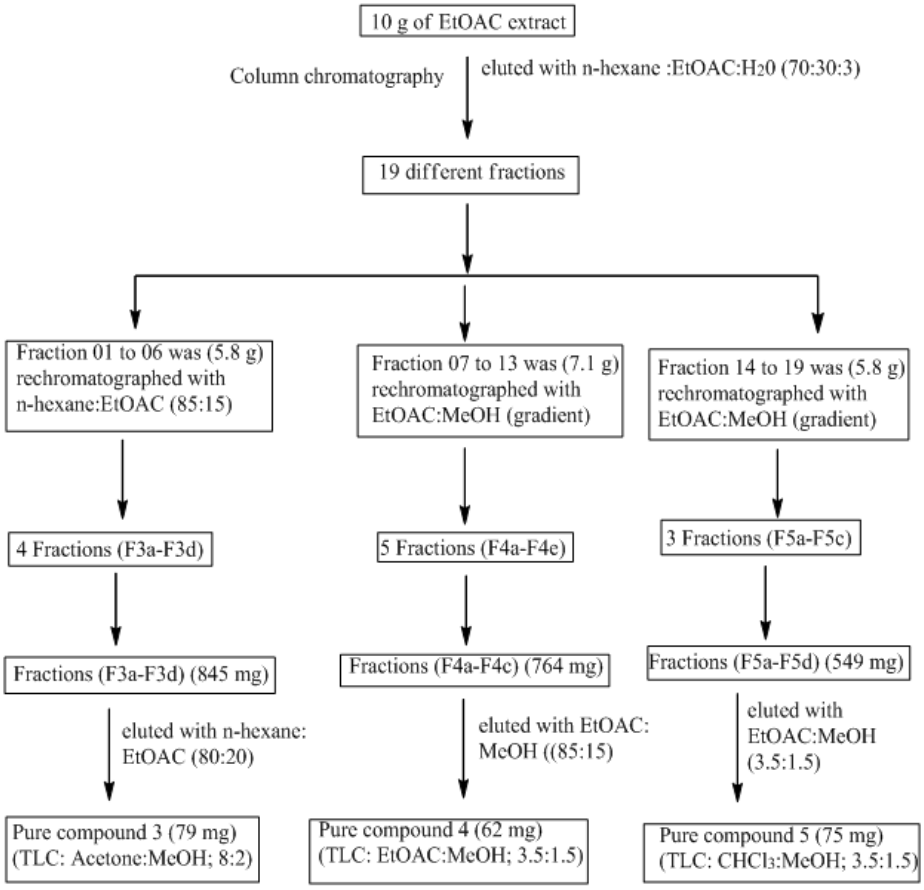

Fig. 2. Schematic diagram of extraction steps of isolated compounds from ethyl acetate extract of *Nymphaea nouchali* Burm. Flowers

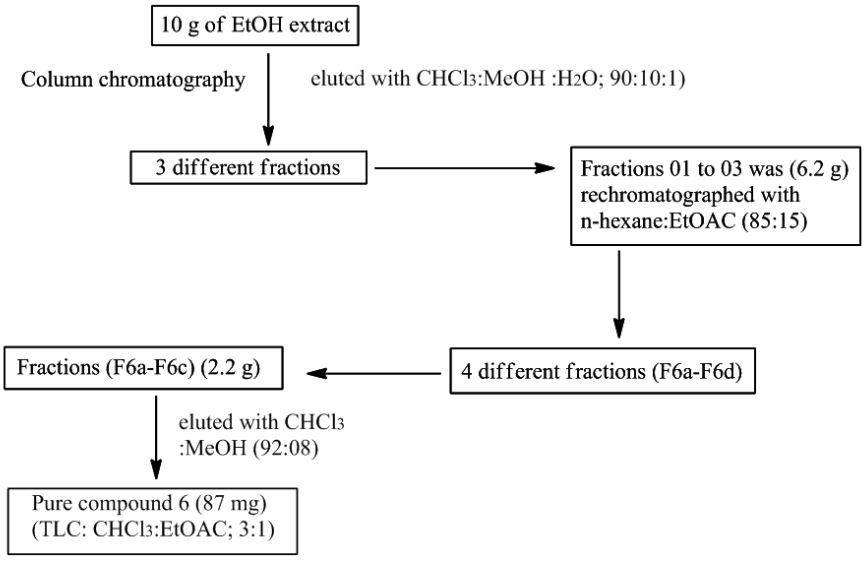

Fig. 3. Schematic diagram of extraction steps of isolated compounds from ethanolic extract of *Nymphaea nouchali* Burm. Flowers

3. RESULTS

10-eicosenoic acid (1): Green color powder, mp:28ºC; IR (KBr, cm^{-1}): 3170, 2919, 2850, 1737, 1643,1166, 722 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): *δ* 10.27(1H, s, COOH group), *δ* 5.37 (2H,d,olefinic proton at H-10), *δ* 2.31 (2H, m, H-2), *δ* 1.68 to 1.25 (26H, m, CH_2 group), *δ* 2.07 (2H, m, H-13), *δ* 0.89 (3H, s, CH_3 group H-20); ^{13}C NMR ($CDCl_3$, 75 MHz): Table 1; $C_{20}H_{38}O_2$; EIMS *m/z*: 310[M^+], 333 [M+Na]$^+$ (Fig. 4).

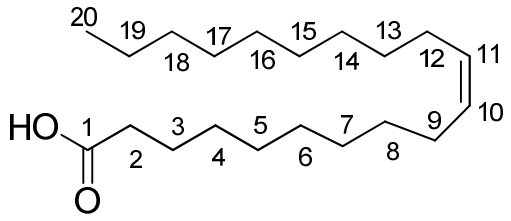

Fig. 4. 10-eicosenoic acid or 9,12-doenoic acid

Linoleic acid (2): Light green amorphous compound, mp: 25-26ºC; IR (KBr, cm^{-1}): 3260, 2919, 2851,1738, 1462, 722 cm^{-1}; ^{1}HNMR (300 MHz, $CDCl_3$) *δ*10.34 (1H, s, COOH group), *δ* 5.36 & 5.42 (2H,d, olefinic proton at H-9&10), *δ* 5.45 & 5.40 (2H, d, δ 2.31,olefenic proton at H-12&13), *δ* 1.95 to 1.05 (22H, m, CH_2 protons), *δ* 0.98 (3H, s, CH_3 group); ^{13}C NMR ($CDCl_3$, 75 MHz): Table 1; $C_{18}H_{34}O_2$; EIMS *m/z*: 281[M+1], 304 [M+Na]$^+$ (Fig. 6).

7,8 - dihydroxy α-tocopherol-9-O-pyranoside (3): yellow amorphous compound, mp: 44-45ºC; IR (KBr, cm^{-1}): 3309, 2913, 2871, 1708, 1436, 721 cm^{-1}; ^{1}HNMR (300 MHz, $CDCl_3$) *δ* 6.53 (1H, d, ar-proton, H-10), *δ* 5.39 (2H, s, OH groups, H-7 & 8), *δ* 5.81 (1H, m, H-1^I), *δ* 4.15 & 4.10 (2H, m, H-6^I), *δ* 3.72 to 3.39 (6H, m. Pyranose ring), *δ* 3.58 to 3.51 (3H, m, 3-OH groups of pyranose ring), *δ* 2.82 to 2.75 (4H, m, 2XCH_2 group, H-3 &4),*δ* 1.64 to 1.03 (11H, m, CH_2 and CH groups), *δ* 1.53 (3H s, CH_3 group, H-22), *δ* 1.21 (3H, s, CH_3 group, H-26), *δ* 0.97 to 0.88 (9H, s, 3XCH_3 group, H-23, 24, 25). ^{13}C NMR ($CDCl_3$, 75 MHz): Table 1; $C_{31}H_{58}O_9$; EIMS *m/z*: 568[M^+], 591[M+Na]$^+$ (Fig. 8).

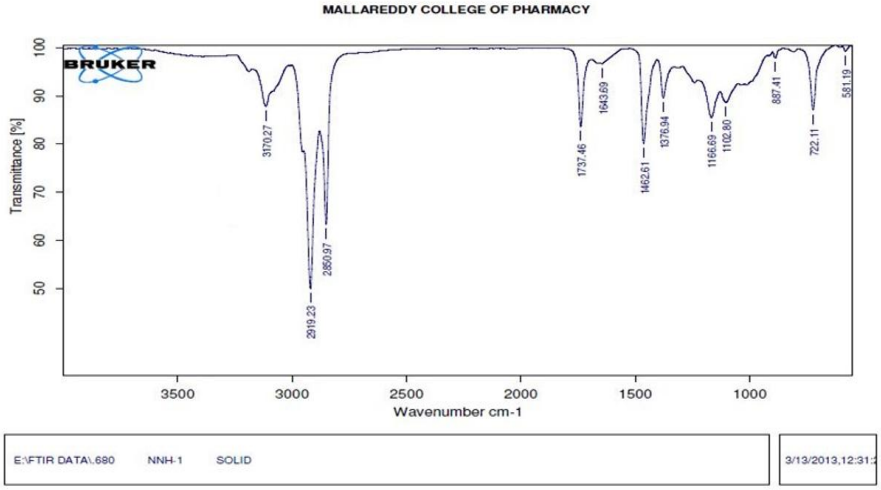

Fig. 5. IR spectra of 10-eicosonoic acid (NNH-1)

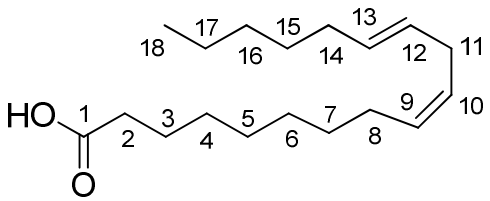

Fig. 6. Linoleic acid

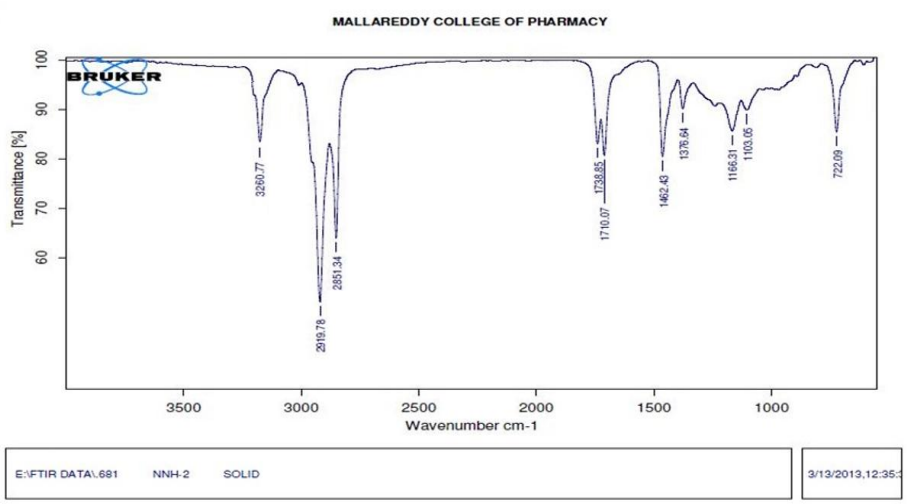

Fig. 7. IR spectra of linoleic acid (NNH-2)

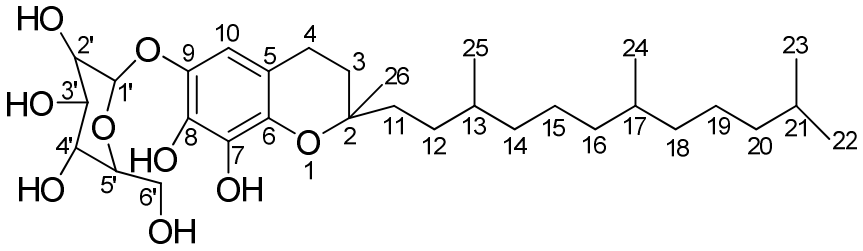

Fig. 8. 7,8 - dihydroxy α-tocopherol-9-O-pyranoside

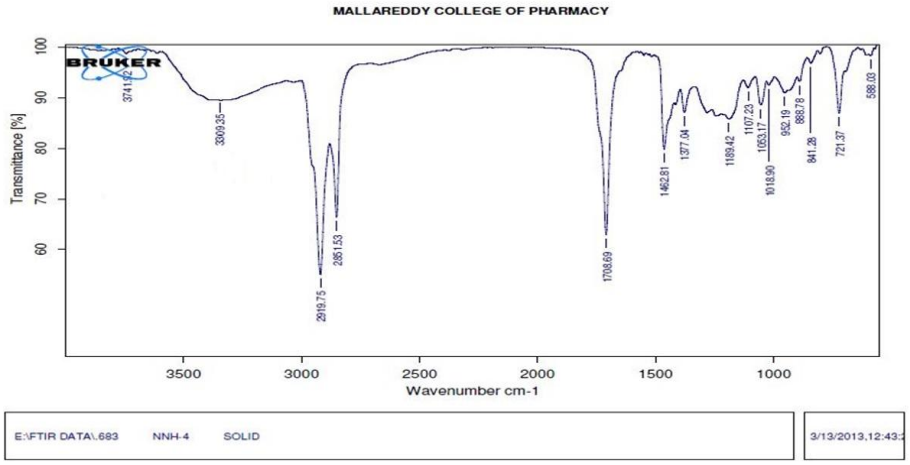

Fig. 9. IR spectra of 7,8-dihydroxy-α-tocopherol-9-O-pyranoside (NNH-4)

Quercetin-3-O-alpha-rhaminoside (4): Yellowish needles, mp: 319-322ºC; IR (KBr, cm^{-1}):3396, 2922, 2852, 1738, 1715, 1610, 1513, 1094 cm^{-1}; ^{1}HNMR (300 MHz, $CDCl_3$): *δ* 12.77 (1H, br, s, OH group, H-3), *δ* 7.41 to 6.05 (5H, d, Ar-protons, H-8, 6, 2', 5', 6'), δ 5.71 (1H, m, H-1"), *δ* 5.35 (4H, br, s, OH-group, H-5,7, 3'&4'), *δ* 4.05 to 3.37 (6H, m, CH &CH_2 group of pyranose

ring), *δ* 3.49 (3H, s, OH-group of pyranose moiety), *δ*1.12 (-CH_3 group in rhaminose moiety). ^{13}C NMR ($CDCl_3$, 75 MHz): Table 1; $C_{21}H_{20}O_{11}$; EIMS *m/z*: 448[M^+], 471[M+Na]$^+$ (Fig. 10).

Kaempferol (5): pale white color compound, mp: 227-229ºC; IR (KBr, cm^{-1}): 3339, 2923, 2852, 1737, 1411, 1371 cm^{-1}; ^{1}HNMR (300 MHz, $CDCl_3$): *δ* 12.48 (1H, s, OH group, H-5), δ 10.70 (1H, s, OH group, H-3), *δ* 10.11 (1H, s, OH group, H-7), *δ* 9.41 (1H, s, OH group, H-4'), *δ* 8.06 to 6.49 (6H, d, Ar-protons, H-6,8,2',3',5',6'). ^{13}C NMR ($CDCl_3$, 75 MHz): Table 1; $C_{15}H_{10}O_6$; EIMS *m/z*: 286[M^+], 309 [M+Na]$^+$ (Fig. 12).

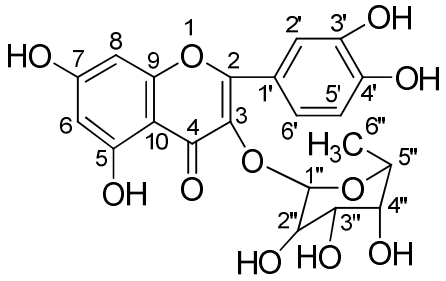

Fig. 10. Quercetin-3-O-alpha-rhaminoside

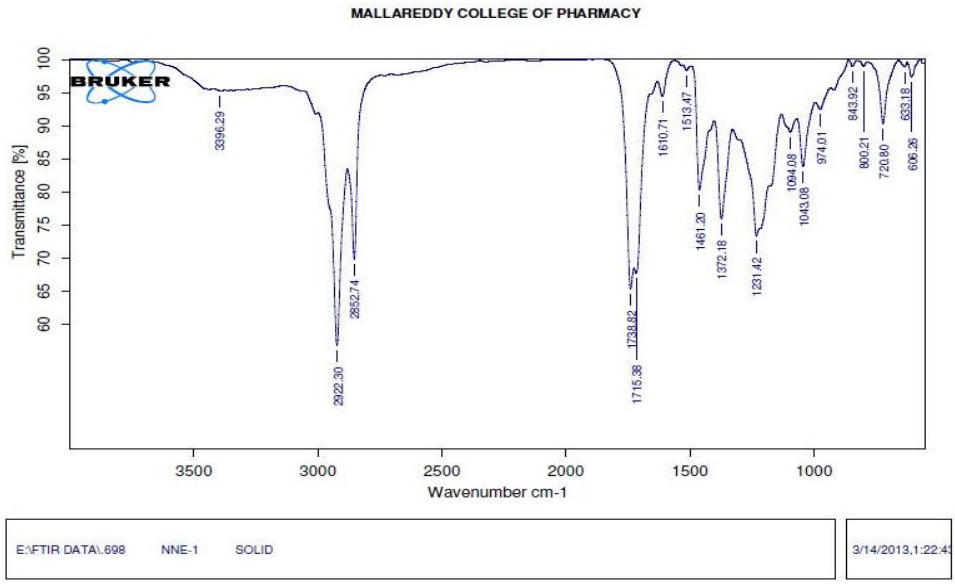

Fig. 11. IR spectra of quercetin-3-O-alpha-rhamnoside (NNE-1)

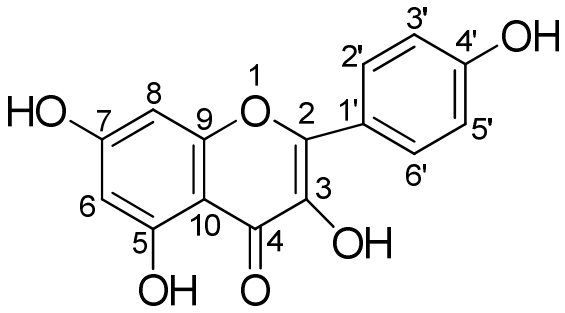

Fig. 12. Kaempferol or 3,5,7-trohydroxy-2-(4'-hydroxyphenyl)-4 flavone

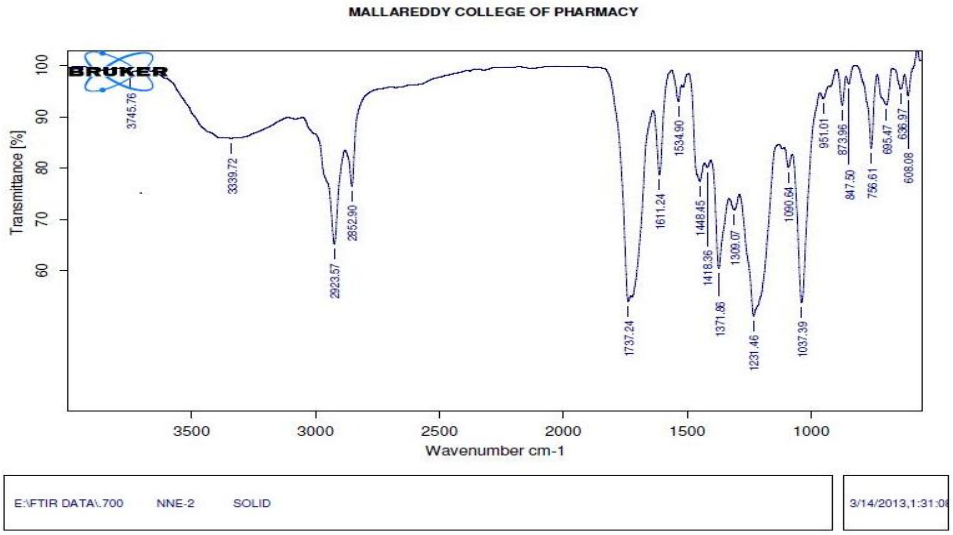

Fig. 13. IR spectra of kaempferol (NNE-2)

Vasicinone (6): white amorphous powder; IR (KBr): 3375, 2923, 2854, 1606, 1470, 1024 cm^{-1}; ^{1}HNMR (300 MHz, $CDCl_3$): δ 8.62 to 7.18 (4H, d, Ar-proton, H-6,7,8,9), *δ* 5.35 (1H, s, OH group, H-11), *δ* 4.22 (1H, m, CH group, H-11), *δ* 3.25 & 2.83 (2H, m, CH_2, H-13), *δ* 1.75 & 1.23 (2H, m, -CH_2- group, H-12);^{13}C NMR ($CDCl_3$, 75 MHz): Table 1; $C_{11}H_{10}N_2O_2$; EIMS *m/z*: 202 [M^+], 235 [M+Na]$^+$ (Fig. 14).

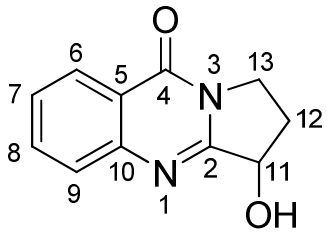

Fig. 14. Vasicinone

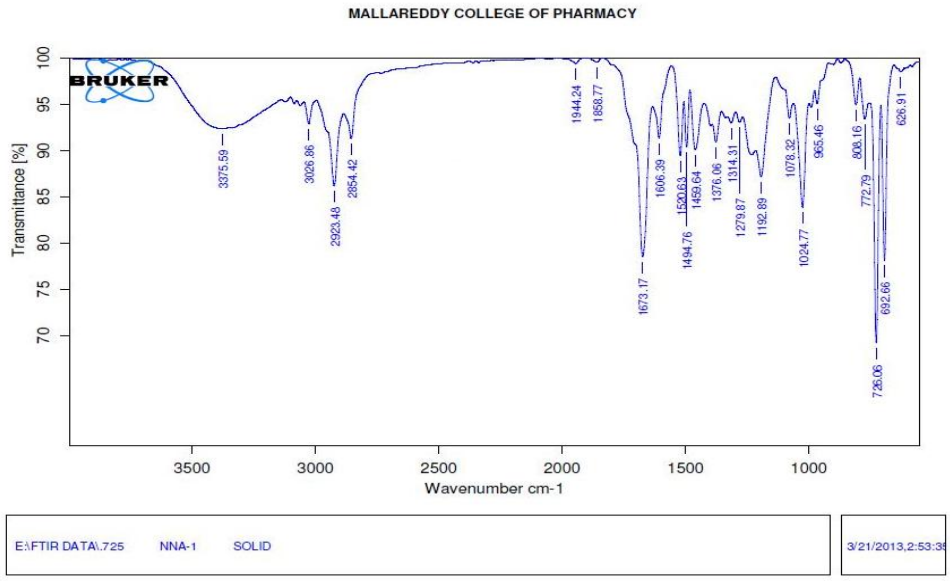

Fig. 15. IR spectra of vasicinone (NNA-1)

4. DISCUSSION

Total six compounds were isolated from n-hexane, EtOAc and ethanolic extracts of *Nymphaea nouchali* flowers. The above obtained isolated compounds were characterized on the basis of spectral evidences (IR, ^{1}H &$^{13}CNMR$ and Mass).

Compound (1) was obtained as green colour amorphous powder. Its molecular formula was determined to be $C_{20}H_{38}O_2$on the basis of positive-ion peak EIMS (*m/z*) 310 [M^+]. IR spectral data showed absorption bands for hydroxyl group (3170 cm^{-1}), C-H stretching (2919.23 & 2850.97 cm^{-1}), for C=O stretching (1737 cm^{-1}), for C=C stretching (1643 cm^{-1}), for C-O stretching at (1166 cm^{-1}) functionalities. The FTIR spectrum of methyl ester presented no absorption in 980-960 cm^{-1} region, indicating cis rather than trans unsaturation. This data indicates the new acid is 10, 15-eicosadienoic acid which has not been previously recognized to exist in nature [11]. ^{1}H NMR data of 1 indicated the presence of peak at *δ* 10.27 as singlet for COOH group, one double bond absorption peak at *δ* 5.37, intense peak at *δ* 2.3 due to methylene group α- to the carbonyl group and other methylene in fatty acyl chain found as multiplet in between *δ* 1.68 to 1.24 and three proton peak for -CH_3 group as singlet at*δ* 0.89. ^{13}C NMR spectrum of 1 exhibited the presence of 20 carbon signals are there in respective δ ppm (see Table 1). The ^{13}C NMR spectrum of the compound 1 showed olefinic carbon signals at *δ* 131.9 and 131.6 at 10^{th} and 11^{th} positions respectively. Further characterization of mass spectrum for corresponding fatty acid methyl ester displayed molecular peak ion at *m/z* 310 [12].

Compound (2) was obtained as light green amorphous compound. Its molecular formula was determined to be $C_{18}H_{32}O_2$ on the basis of positive-ion peak EIMS (*m/z*) 282 [M+2]. IR spectral data showed absorption bands for hydroxyl group (3260 cm^{-1}), for C-H stretching (2851 cm^{-1}), C=O stretching (1738 cm^{-1}) and C=C stretching (722 cm^{-1}) functionalities. ^{1}H NMR data of 2 indicated the presence of peak at *δ* 10.34 as singlet for COOH group, the unsaturated olefinic proton peak at *δ* 5.36& 5.42 for 9^{th} and 10^{th} position and another olefinic proton peak at *δ*5.45 & 5.40 for 12^{th} and 13 position respectively, hydrogen attached methylene groups are appeared at *δ* 4.32 to 40 4.10, bis-allylic protons resonance at δ 2.31 and allylic protons resonance at *δ* 1.95 to 1.05 and intensities of methyl resonance peak at δ 0.98 represents linoleic acid. The signals of terminal methyl group can be used to determine the amount of linoleic acid with proximity of the C_{12}-C_{13} double bond, the signals of terminal CH_3 is shifted downfield to approximately *δ* 0.95 (anisotropic effect) [13].^{13}C NMR spectrum of 2 exhibited the presence of 18 carbon signals (see Table 1). The ^{13}C NMR spectrum of the compound 2 showed olefenic carbon signals at δ 131.9 and 129.6 at 9^{th} and 10^{th} positions and another olefinic carbon signals at δ 129.6 and 131.6 for 12^{th} and 13^{th} position respectively. Carbons in lineloic acid C-9, 10, 12&13 which are attached accordingly to C-1/3 or C-2 position of glycerol. For instance, C-10 &12 signals are about 2ppm more upfiled than the corresponding C-13&9 signals in α–linoleic acid [14].

Compound (3) was obtained as yellow amorphous compound. Its molecular formula was determined to be $C_{31}H_{58}O_9$ on the basis of positive-ion peak EIMS (*m/z*) 568 [M^+].Mass fragment ions occurred at *m/z* 205 & 165 resulting from the cleavage of side chain accompanied by the breakdown of the chroman structure with hydrogen rearrangement and loss of methyl acetylene fragment [15].

IR spectral data showed absorption bands for broad OH stretching (3309 cm^{-1}), for C-H stretching for methyl group (2913 cm^{-1}), C=O stretching (1708 cm^{-1}) and CH bending of aromatic ring (721 cm^{-1}) functionalities. ^{1}H NMR data of compound 3 indicated the aromatic proton peak at *δ* 6.53 as doublet. One proton peak found as broad singlet at δ 5.39 for two OH groups at 7^{th} and 8^{th} position. Broad peak in between *δ* 3.72 to 3.39 indicates the presence of CH group and δ 3.58 to 3.51indicates OH group protons for pyranose ring, a peak at *δ* 1.53, 1.21 and 0.97 to 0.88 integrating 15 protons due to presence of methyl group at 22^{nd}, 26^{th} and 23^{rd}, 24^{th} & 25^{th} positions respectively. ^{13}C NMR spectrum of 3 exhibited the presence of 31 carbon signals are there in respective δ ppm (see Table 1). Compound 3,α-tocopherol-O-D-mannopyranoside is represented by a signal at *δ* 110.1 and the other values being *δ* 73.5, 72.9, 68.9, 81.6, 62.6 respectively [16].

Compound (4) was yellow needles. Its molecular formula was determined to be $C_{31}H_{58}O_9$ on the basis of positive-ion peak EIMS (*m/z*) 465 [M+1]. IR spectral data showed absorption bands for hydroxyl groups and C-H stretching (3396 cm^{-1})

& (2922 & 2825 cm^{-1}) respectively. For C=C and C=O stretching, absorption peak was found to be at (1738 cm^{-1}) and (1610 cm^{-1}) respectively. ^{1}H NMR data of 4 reveals, the presence of hydroxylic group as singlet at *δ* 12.77 for 3rd position integrating for one proton and other hydroxyl groups peak found as broad singlet at *δ* 5.35 for 5,7,3'&4' positions respectively, the A ring protons at C-6 &C-8 appear at *δ* 7.21 and *δ* 7.39 ppm respectively. The OH proton resonates at *δ* 12.77 for 3rd position. The protons at C-2′ and C-6′ appear as doublet at *δ* 6.51 and δ 6.59 respectively and proton at 5' appear at *δ*6.89 in ring B. The H-1″ of the rhamnoside resonates at *δ* 5.08. The remaining sugar protons appear in the range *δ* 4.05 to 3.37. Hydroxylic group of pyranose moiety found as singlet at *δ*3.49 [17]. Flavonols possessing free-OH group at the C-3, C-3′ and C-4′ –positions are known to be unstable in NaOMe [18]. A glycone signals typical for quercetin nucleus as well as the presence of anomeric protons of glucose & galactose with coupling constants characteristic for β–configuration [19]. ^{13}C NMR spectrum of 4 exhibited the presence of 21 carbon signals are there in respective *δ* ppm (see Table 1).

Table 1. ^{13}C-NMR Spectral data of fatty acids (1& 2), sterol (3), flavonoids (4&5) and alkaloid (6)

C	1	2	3	4	5	6
1	174.5	180.1	-O-	-O-	-O-	-N-
2	35.2	35.5	76.5	155.6	146.7	165.9
3	25.5	26.7	30.5	135.1	135.5	-N-
4	39.7	31.9	23.5	179.1	175.7	161.9
5	32.7	31.5	118.5	160.7	160.6	120.2
6	32.5	31.3	142.1	100.1	98.1	137.3
7	31.8	30.5	132.2	160.5	163.7	108.6
8	29.6	29.9	130.7	95.2	93.3	144.7
9	29.2	131.9	139.5	155.4	156.1	128.3
10	130.9	129.3	105.1	104.9	103.3	165.7
11	130.6	29.2	37.4			76.9
12	27.1	129.3	29.0			28.9
13	34.3	131.6	33.8			40.3
14	37.3	27.6	37.7			
15	36.5	22.6	24.6			
16	36.0	33.3	37.5			
17	22.6	22.7	33.2			
18	22.5	14.0	37.3			
19	22.1		24.5			
20	14.0		40.0			
21			28.9			
CH_3			23.8			
CH_3			23.3			
CH_3			21.9			
CH_3			21.5			
CH_3			27.8			
1'			110.1	124.8	121.5	
2'			73.5	123.1	129.5	
3'			72.9	142.2	115.3	
4'			68.9	141.9	159.1	
5'			81.6	112.2	115.7	
6'			62.6	121.5	129.9	
1″				105.1		
2″				73.5		
3″				72.9		
4″				68.9		
5″				81.6		
6″				18.6		

Compound (5) was obtained as pale white crystals. Its molecular formula was determined to be $C_{15}H_{10}O_6$ on the basis of positive-ion peak EIMS (*m/z*) 286 [M^+]. IR spectral data showed absorption bands for hydroxyl groups (3339 cm^{-1}), ester carbonyl group (1737 cm^{-1}) and aromatic ring (1411 & 1371 cm^{-1}). ^{1}H NMR data of 2 reveals, the presence of hydroxylic group as singlet at *δ* 12.41 for 5th position integrating for one proton and remaining three hydroxyl group peaks found as broad singlet at δ 10.70, δ 10.11 and *δ* 9.41 for 3rd, 7th and 4' positions of flavones skeleton. The characteristic signals of kaemferol nucleus, two doublets at *δ* 6.25 & *δ* 6.95 assigned to 2' & 6' position respectively and a pair of aromatic protons in ring C, *δ* 6.55 and *δ* 8.03 are assigned to 3', 5' and 6', 8' positions respectively [20].

^{13}C NMR spectrum of 5 exhibited the presence of 21 carbon signals are there in respective *δ* ppm (see Table 1). This structure was further confirmed by ^{13}C NMR spectral studies. The ^{13}C NMR spectrum of the compound showed a total of 15 signals for 15 carbons. A signal was observed at *δ* 175.7to C-4. An additional 2 signals were observed resonating at *δ* 129.3 and *δ* 129.9 attributed to C-2' & C-6' respectively [21].

Compound (6) was obtained as white amorphous powder. Its molecular formula was determined to be $C_{11}H_{10}N_2O_2$. IR spectral data showed absorption bands for broad OH stretching for free OH group (3375 cm^{-1}) and C-H stretching at (2923 & 2854 cm^{-1}) respectively. The absorption band at (1673 cm^{-1}) might be the presence of saturated cyclic C-O or keto-enol conformation. Also bands at wave numbers (1024 cm^{-1}) characteristic absorption might be due to indicative of C-N bond at (1024 cm^{-1}) might be of aromatic hydrogen present in the suspected molecule. ^{1}H NMR data of 3 indicated the aromatic proton peak exhibited four singlet between *δ* 8.62-7.18 resonances at 7.95 (1H, m, Ar H-8), 7.12 (1H, m, Ar H-7), 8.15 (1H, m, Ar H-6), 8.25 (1H, m, Ar H-9) which were attributed to the four aromatic protons. One singlet proton peak for *δ* 5.35 at 11th position [16]. ^{13}C NMR spectrum of 6 exhibited the presence of 11 carbon signals are there in respective *δ* ppm (see Table 1).

5. CONCLUSION

In the investigation of chemical compounds from Natural products is fundamentally important for the development of new drugs, especially in view of the vast worldwide flora. Based on the results, the obtained compounds of *Nymphaea nouchali* Burm. flowers are reported the first time. The structure of isolated compounds were characterized by IR, ^{1}H-NMR, ^{13}C-NMR and Mass spectral studies.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
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