



Isolation and Characterization of Zygadenine-N-ribofuranoside from the Leaves of *Nymphaea alba* Lily

Peter Chidi Njoku^{1*}, Johnbull O. Echeme², Rosemary I. Uchegbu¹
and Emeka C. Ogoko³

¹Department of Chemistry, Alvan Ikoku Federal University of Education, P.M.B. 1033, Owerri, Imo State, Nigeria.

²Department of Chemistry, Michael Okpara University of Agriculture, Umudike P.M.B. 7267, Umuahia, Abia State, Nigeria.

³Department of Chemistry, National Open University of Nigeria, P.M.B.80067, Lagos, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author JOE conceptualized and designed the study. Author PCN wrote the protocol and the first draft of the manuscript. Authors RIU and ECO managed the literature search. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ACSJ/2016/19929

Editor(s):

(1) Mazeyar Parvinzadeh Gashti, Department of Chemistry, Université Laval, 1045 Avenue of Medicine, Canada.

(2) Say Leong Ong, Civil and Environmental Engineering Department and NUS Environmental Research Institute, National University of Singapore (NUS), Singapore.

Reviewers:

(1) Bhaskar Sharma, Suresh Gyan Vihar University, Rajasthan, India.

(2) Anonymous, Howard University, USA.

(3) Nyoman Kertia, Gadjah Mada University, Indonesia.

Complete Peer review History: <http://sciencedomain.org/review-history/13011>

Original Research Article

Received 3rd July 2015
Accepted 14th December 2015
Published 19th January 2016

ABSTRACT

Aims: To isolate and characterize a new secondary metabolite, zygadenine-N-ribofuranoside derivative from the leaves of water lily (*Nymphaeae alba* L.).

Methodology: The ethanol extract of the dried leaves was partitioned between chloroform and water (1:1), then the chloroform fraction was further partitioned between petroleum ether (60-80°C) and aqueous methanol (1:1). Column chromatography of the chloroform fraction over silica gel (200 mesh) by a gradient elution technique from petroleum ether to chloroform, followed by methanol was carried out.

Results: A yellow solid 0.22 g was isolated which was characterized by employing IR, 1D and 2D

*Corresponding author: E-mail: chidinjoku71@gmail.com;

NMR techniques spanning ^1H , ^{13}C , ^1H - ^1H COSY, ^1H - ^{13}C COSY, HMBC and TOF ESI-mass spectrometry.

Conclusion: Zygadenine-N-ribofuranoside derivative isolated, gave m/z 622.4631, corresponding to molecular formula $\text{C}_{33}\text{H}_{54}\text{O}_9\text{N}_2$.

Keywords: *Nymphaea alba*; ribofuranoside; secondary metabolite; zygadenine.

1. INTRODUCTION

Nymphaea alba Lily belongs to family Nymphaeaceae; commonly known as white water lily. Its Yoruba name is *Osibada* or *Bado* [1].

White water lily is a vivacious water plant, has a blackish, large fleshy perennial rhizome, growing in mud, where the water is from 91.44 cm to 304.8 cm in depth. The leaves are floating, orbicular; heart shaped up to 39 cm in diameter. The plant grows in ponds, lagoons and slow streams in forest regions.

Nymphaea contain tannic and gallic acids, with starch, mucilage, resin, sugar, tartaric acid and several non toxic alkaloids. Three novel flavonoids, myricetin -3'-O-6''-p-coumaroyl glycoside and two epimeric mhacrocyclic d-rhamnoside and pentagalloyl glucose, have been isolated from the wild water lily, *Nymphaea lotus* [2].

The novel anthocyanin, cyanidin 3-O-(6''-acetyl beta galactopyranoside) (25%), cyaniding 3-O-(2''-galloyl-6''-acetylgalactoside) 7%, delphinidin 3-O-(2''-galloyl galactoside) 3% and cyanadin 3-O-galactoside (2%) have been identified in extract of reddish leaves of white flowered water lily, *Nymphaea alba*. [3]. Two 5-glycosyl isoflavones, 7,3',4'-trihydroxy-5-O-beta-D-(2''-acetyl)xylopyranosylisoflavone and rhamnopyranosylisoflavone together with triterpene saponins were isolated from four Mexican *Nymphaea* species, *N. ampla*, *N. pulchella*, *N. gracillis* and *N. elegans* [4]. Assay- guided fractionation of the ethanol extract of *Nymphaea odorata* resulted in the identification of keamferol 3-O- α -1-rhamnopyranoside (afzelin), quercetin-3-O- α -rhamnopyranoside, myricetin 3-O- α -1-rhamnopyranoside(myricitrin), quercetin 3-O-(6''-O-acetyl)- β -d-galactopyranoside [5]. Studies reveal that *N. alba* is potent chemopreventive agent and suppresses Fe-NTA (ferric nitrilotriacetate)-induced oxidative stress, hyperproliferative response and renal carcinogenesis in Wistar rats [6].

Medically, water lily have been indicated for the following conditions: acne, adenopathy, bleeding,

boil, burn, cancer, cold, cough, cytositis, dermatosis, diarrhea, dysentery, enterosis, flu, freckle, furuncle, gonorrhoea, inflammation, Leukorrhoea, nephrosis, pain, pharyngitis, pulmosis, scrufole, sore throat, sore, spermatorrhoea, stomatitis, swelling, toothache, tuberculosis, tumor, uterosis, vaginosis, whitlow, [7]. However, the leaves of *Nymphaea alba* have been used in herbal preparations to treat inflammation, wound, tumor and boil. The flowers of *N. alba* showed anti *S. aureus* activity [8].

The medicinal value of the aquatic plant, *Nymphaea alba* lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents include alkaloids, tannins, flavonoids, saponins and phenols. These compounds are termed secondary metabolites. This paper describes the isolation and characterization of a new secondary metabolite zygadenine-N-ribofuranoside derivative from the leaves of *Nymphaea alba*.

2. MATERIALS AND METHODS

2.1 Collection of Sample

Leaves of *Nymphaea alba* L. (white water lily) were harvested from Efur stream in Umuchi, Isiala Mbano, Imo State. The plant material was authenticated at the department of Forestry, Michael Okpara University of Agriculture, Umudike, Abia state.

2.2 Preparation of Sample

The leaves of *Nymphaea alba* were air dried on the laboratory bench for four weeks. The dried sample was then milled into powder.

2.3 Extraction and Isolation

The milled sample (1 kg) was percolated in 98% ethanol for 48 hours after which it was filtered. A greenish liquid which was collected as the filtrate was concentrated with Rotary evaporator (Heildoph model) at 40°C to obtain a dark green crude extract (38.6 g). The ethanolic crude

extract (26.32 g) was partitioned between chloroform and water. The fraction soluble in chloroform (25.4 g) was obtained. The chloroform fraction (15.2 g) was further partitioned between petroleum ether (60-80°C) and aqueous methanol.

Thin-layer chromatography of the chloroform soluble fraction in elution solvent mixture of chloroform (90): Pet. Ether (5): methanol (5). Gave one band R_f 0.89.

The chloroform fraction (6.80 g) was subjected to column chromatography over silica gel (200 mesh) by a gradient elution technique with Pet. Ether : chloroform (100:0; 90:10; 80:20; 70:30; 60:40; 50:50; 40:60; 30:70; 40:60; 30:70; 20:80; 10:90; 0:100) then chloroform: methanol (90:10; 80:20; 70:30; 60:40; 50:50; 40:60; 30:70; 20:80; 10:90; 0:100).

A yellow solid 0.22 g was isolated which gave one defined spot at R_f 0.81. Spectroscopic techniques (IR, ^1H NMR, ^{13}C NMR and MS) were employed to characterize the isolated compound.

3. RESULTS AND DISCUSSION

The compound isolated labeled NJI is a yellow oily substance (0.22 g). It gave one defined spot at R_f 0.81 with Thin Layer chromatography using silica gel in CHCl_3 : MeOH: Pet. Ether (50:30:20).

IR analysis showed that NJI contained OH (3380 cm^{-1}), broadening of the band at that wavelength is due to hydrogen bonding between the oxygen of OH at 13' and neighbouring H_{12} . C-H stretching vibrations was observed at 2919 cm^{-1} and C-N bending vibrations at 1155 cm^{-1} .

See Table 1 for IR analysis and Table 2, for ^1H NMR and ^{13}C NMR analysis.

The ^1H - ^1H COSY showed coupling between methylene protons of H_6 at $\delta 1.27\text{ ppm}$ and methane protons of H_5 at $\delta 1.41\text{ ppm}$. It also revealed coupling between the 3 methylene protons of H_{15} at $\delta 2.70\text{ ppm}$ and methine proton of H_{16} at $\delta 1.67\text{ ppm}$ and protons of tetramethylsilane (TMS) are shown not coupled to any other protons.

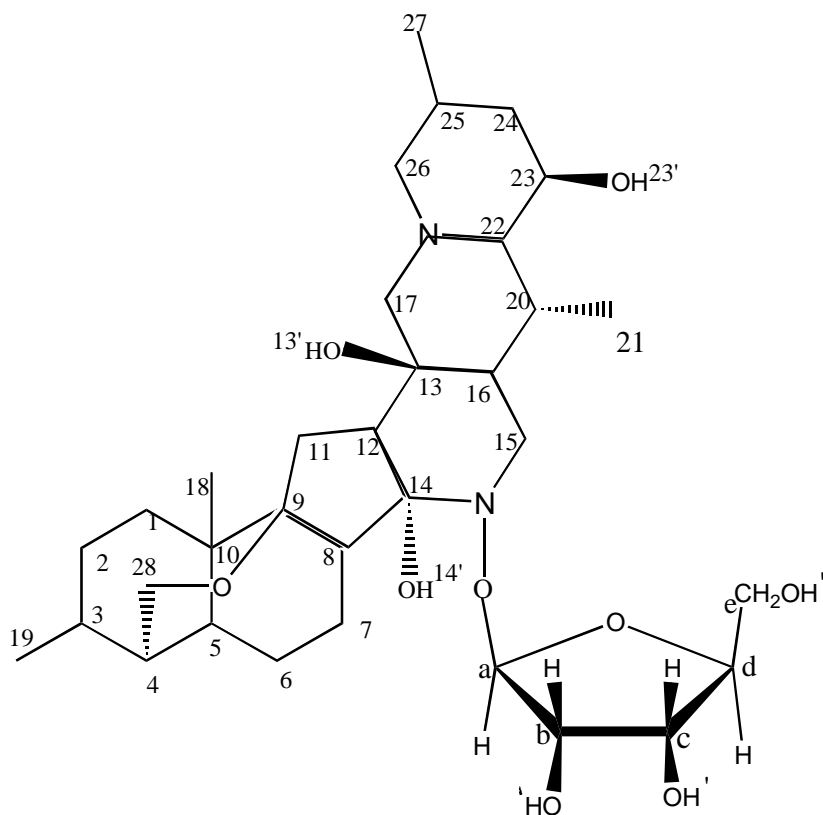


Fig. 1. Zygadenine-N-ribofuranoside derivative isolated from *Nymphaea alba*

$^1\text{H} - ^{13}\text{C}$ COSY reveals correlation between C_{16} at $\delta 30.4$ ppm and H_{16} at $\delta 1.67$ ppm. HMBC data shows from contour peaks, connections between C_5 at $\delta 28.6$ ppm and H_8 at $\delta 1.43$ ppm; C_{12} at $\delta 42.1$ ppm and H_{16} at $\delta 1.67$ ppm are also connected.

Mass spectrometry of NJI employing TOF ESI MS shows m/z 622.4631. The molecular formula is $\text{C}_{33}\text{H}_{54}\text{O}_9\text{N}_2$.

Table 1. Infra-red analysis of *Nymphaea alba*

IR-absorption (cm^{-1})	Functional group
3380.8 broad	O-H stretching, alcohol
2919.2 sharp	C-H stretching, aliphatic
2850.3 sharp	C-H stretching, aliphatic
1451.2	C-H bending, aliphatic
1192.0 weak	C-N 3° amine
1155.1	C-N bending
1029.2	C-O

Table 2. ^1H NMR and ^{13}C NMR chemical shifts of *Nymphaea alba*

Position	^{13}C chemical shift (δ) in ppm	Type of carbon	^1H chemical shift (δ) in ppm	Multiplicity	Number of H
a	109.2	CH	5.10	t	1H
b	71.1	CH	2.58	t	1H
b'	—	—	4.20	s	1H
c	72.2	CH	2.57	t	1H
c'	—	—	4.10	s	1H
d	75.2	CH	2.10	d	1H
e	85.4	CH_2OH	3.40	d	2H
e'	—	—	4.62	s	1H
1	25.7	CH_2	1.24	t	2H
2	26.8	CH_2	1.25	q	2H
3	27.3	CH	1.30	m	1H
4	28.4	CH	1.40	m	1H
5	28.6	CH	1.41	q	1H
6	27.4	CH_2	1.27	q	2H
7	27.5	CH_2	1.28	q	2H
8	28.7	CH	1.43	t	1H
9	102.1	C	—	—	—
10	101.0	C	—	—	—
11	40.8	CH_2	1.80	d	2H
12	42.1	CH	2.80	t	1H
13	105.1	C	—	—	—
13	—	—	3.50	s	1H
14	106.0	C	—	—	—
14	—	—	3.60	s	1H
15	41.2	CH_2	2.70	d	2H
16	30.4	CH	1.67	q	1H
17	31.2	CH_2	1.90	s	2H
18	21.2	CH_3	0.89	s	3H
19	21.0	CH_3	0.87	d	3H
20	28.8	CH	1.42	t	1H
21	22.3	CH_3	0.90	d	3H
22	40.0	CH	1.99	t	1H
23	42.5	CH	2.90	q	1H
23	—	—	3.30	s	1H
24	27.6	CH_2	1.29	t	2H
25	29.3	CH	1.45	m	1H
26	42.2	CH_2	2.74	d	2H
27	20.9	CH_3	0.88	d	3H
28	61.3	CH_2	3.84	d	2H

s = Singlet, d = Doublet, t = Triplet, q = Quartet, m = Multiplet

Based on information from combined spectra data and ACD elucidator, compound NJI was proposed to be Zygadenine-N-ribofuranoside derivative.

The use of *Nymphaea alba* to treat inflammation, ulcer and tumor suggests that the plant contains anti-inflammatory and cytotoxic chemical agents which can serve as useful medicinal compounds to be exploited by pharmaceutical industries. The medicinal compounds can be used as leads to the synthesis of potent pharmaceutical drugs.

4. CONCLUSION

Zygadenine –N-ribofuranoside derivative isolated, gave m/z 622.4631, corresponding to molecular formula $C_{33}H_{54}O_9N_2$.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. Bhavani Joshi of Brucker Centre, Lucknow, India for his assistance in running the spectra of this work.

COMPETING INTERESTS

Authors declare that no competing interests exist.

REFERENCES

1. Umberto Q. CRC World dictionary of plant names. (M-Q) CRC. 2000;III:1843.
2. Awatif AE, Bates C, Alexander IG, Simon PM, Graham GS, Roger DW. Two very unusual macrocyclic flavonoids from the water lily *Nymphaea lotus*. Photochemistry. 2003;63(6):727-731.
3. Torgils F, Øyvind MN. Cyaniding 3-(6''-acetylgalactoside) and other anthocyanins from reddish leaves of the water lily, *Nymphaea alba*. J. Horticultural Sci. and Biotech. 2001;76(2):213-215.
4. Silvia Marquina, Jaime Bonilla-Borbosa, Laura Alvarez. Comparative phytochemical analysis of four Mexican *Nymphaea* species. Phytochemistry. 2005;66(8): 847-950.
5. Zhizhen Zhang, Hala N Elsohly. Phenolic compounds from *Nymphaea odorata*. J. Nat. Prod. 2003;66(4):548-540.
6. Khan N, Sultana S. Anticarcinogenic effect of *Nymphaea alba* against oxidative damage, hyperproliferative response and renal carcinogenesis in Wistar rats. Mol. Cell. Biochem. 2005;271(1-2):1-11.
7. Duke JA. Handbook of medicinal herbs 2nd ed. Boca Raton: CRC; 2002.
8. Shadidi Bonjar GH. Inhibition of three isolates of *Staphylococcus aureus* mediated by plants used by Iranian native people. J. Med. Sci. 2004;4(2):136-141.

© 2016 Njoku et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
<http://sciencedomain.org/review-history/13011>