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In vitro Anti-inflammatory Activity of *Nephrolepis biserrata* (Sw.) Schott Rhizome and *Angiopteris palmiformis* (Cav.) C. Chr. Leaf Extracts

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Abstract

Increasing inflammation-mediated health issues have driven the search for more natural anti-inflammatory drug sources. In this study, the anti-inflammatory activity of *Nephrolepis biserrata* rhizome and *Angiopteris palmiformis* frond extracts were determined via inhibition of pro-inflammatory enzymes, 15-lipoxygenase (15-LOX) and cyclooxygenase-2 (COX-2). Extraction with absolute ethanol was done followed by subsequent partitioning with hexane, ethyl acetate, and water. Results revealed that the ethyl acetate-soluble partition (Nb-EtOAc) and aqueous partition (Nb-Aq) of *N. biserrata* and the ethanolic extract (Ap-EtOH) of *A. palmiformis* exhibit active inhibition against the 15-LOX enzyme. All of the *N. biserrata* extracts (Nb-EtOH, Nb-Hex, Nb-EtOAc, and Nb-Aq) and the hexane-soluble partition (Ap-Hex) of *A. palmiformis* were found to be active and selective towards inhibition of the COX-2 enzyme. The observed anti-inflammatory activity of *N. biserrata* rhizome and *A. palmiformis* frond extracts suggests that *N. biserrata* rhizomes and *A. palmiformis* fronds are potential sources of natural anti-inflammatory components.

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Introduction

Inflammation is a natural defense mechanism of a body against noxious conditions such as microbial infection and tissue injury. Inflammation enables the removal of harmful stimuli as well as the healing of damaged tissue (Ahmed, 2011). However, prolonged and chronic inflammation is linked to the development of various harmful and fatal diseases such as cancer, diabetes, cardiovascular, pulmonary, and neurological diseases (Aggarwal *et al.*, 2006). Studies have associated the occurrence of a variety of diseases such as atherosclerosis, diabetes mellitus, obesity, cancer, asthma, and psoriasis with inflammation. For the past thirty years, the number of inflammation-mediated diseases has increased rapidly (Bosma-den Boer *et al.*, 2012).

While several non-steroidal anti-inflammatory drugs (NSAIDs) have become available for the treatment of inflammation-related conditions, they also come with unwanted harmful side effects (Rainsford, 1999). Even with the advancements in technology, nature remains a primary source of various potent drugs responsible for the treatment of a wide range of diseases, inflammation-related or not. Hence, continued screening of natural sources for biologically active compounds is encouraged (Schumacher *et al.*, 2011).

Nephrolepis biserrata (Sw.) Schott, under the family Lomariopsidaceae – fringedferns, is a perennial fern commonly found in shaded and wet places such as river banks and swamps. It has been used as a treatment for microbial infections, wounds, stomach pain, bleeding, boils, blisters, abscess, and sores (Shah *et al.*, 2014; Kormin *et al.*, 2018). Moreover, the plant has been found to be composed of a number of several phytochemicals that play various important roles in the body, such as beta carotene, steroids, phenols, terpenoids, tannins, alkaloids, anthraquinones, phytosterol, saponins, and flavonoids (Shah *et al.*, 2014; Shorinwa and Ogeleka, 2016). *Angiopteris palmiformis*, under the Family Marattiaceae, is a tropical fern with large, pinnately compound fronds measuring up to three meters in length and is commonly found in Taiwan, Philippines, and Tahiti. In the Philippines, it is known as Elephant fern or *"pakong kalabaw"*. Some biologically important compounds have also been isolated from *A. palmiformis* such as triterpenoids which were found to be cytotoxic against cancer cells (Chudzik *et al.*, 2015).

To date, there are still very limited studies on the anti-inflammatory activity of *N. biserrata* and *A. palmiformis*. Hence, the potential of *N. biserrata* rhizomes and *A. palmiformis* fronds to inhibit 15-LOX, cyclooxygenase-1 (COX-1) and COX-2 pro-inflammation enzymes was evaluated in this study.

Materials and methods

Sample collection, preparation and extraction

Fresh rhizomes from healthy and mature *N. biserrata* samples (Fig. 1) were collected from the Rubber Plantation Project, Central Mindanao University (CMU), University Town, Musuan, Maramag, and Bukidnon, Philippines (N $07^0$51.688 'E $125^0$02.590'.

Healthy fronds of *A. palmiformis* (Fig. 2) were collected from the Center for Ecological Development and Recreation (CEDAR), Impalutao, Bukidnon, Philippines (N $08^0$14.590' E $125^0$01.520'). The collected samples were washed, air-dried, and homogenized. The ground samples were soaked exhaustively in absolute ethanol for 72 hours. The collected extracts (EtOH) were then concentrated under *vacuo* at 40°C.

Sequential partitioning

Sequential partitioning of the crude ethanolic extract (EtOH) of *N. biserrata* rhizome and *A. palmiformis* frond samples was carried out using hexane, ethyl acetate, and water (Otsuka, 2016). Hexane and water (1:1 v/v) were added to the reconstituted ethanolic extract in a separatory funnel.

The hexane-soluble partition (Hex) was then collected after an hour. The addition of hexane and water was repeatedly done until the hexane-soluble partition was clear in color. The aqueous layer was then added

with equal parts of water and ethyl acetate (1:1 v/v). The ethyl acetate-soluble partition (EtOAc) was collected and dried *in vacuo* at 40°C, while the aqueous partition (Aq) was concentrated *in vacuo* at 40°C followed by freeze-drying.

15-LOX inhibition assay

The determination of the anti-inflammatory activity of the crude ethanolic extract and solvent partitions of *N. biserrata* rhizomes and *A. palmiformis* fronds were carried out via inhibition of pro-inflammatory enzymes 15-LOX and COX. The 15-LOX inhibition assay was done at the Bioorganic and Natural Products Laboratory (BNPL) of the Institute of Chemistry, University of the Philippines, Diliman, Quezon City, Philippines.

The lipoxygenase inhibitory assay was conducted via spectrophotometric method using lipoxidase from Glycine max as the enzyme source (Auerbach *et al.*, 1992; Axelrold *et al.*, 1981). A 260 uL of 0.1 M phosphate buffer (pH 7.4) was added to the wells of a 96-well quartz microplate. Then, 10 uL of 1000 ug/mL *N. biserrata* and *A. palmiformis* extracts, positive control Nordihydroguaiaretic acid (NDGA), and 10% negative control Dimethyl sulfoxide (DMSO,) were then added accordingly. A 15 uL of the 15-LOX enzyme (4,740 U/mL) was added. The mixture was then shaken and incubated at 25°C for five (5) minutes. Lastly, linoleic acid (15 uL) was added to start the reaction, and absorbance measurements at 234 nm were observed to obtain 22 readings with 30-second intervals. Lipoxygenase activity was calculated using equation (1):

$$\%\,Inhibition = \frac{Slope_{uninhibited} - Slope_{inhibited}}{Slope_{uninhibited}} \times 100 \quad (1)$$

where $Slope_{uninhibited}$ is the slope of the line from the absorbance vs. time plot of the negative control group and the $Slope_{inhibited}$ is the slope of the line from the absorbance vs. time plot of the samples/positive control. Samples with a percent inhibition of ≥50% were considered active. Each sample was read using two trials with two replicates.

COX inhibition assay

The cyclooxygenase inhibitory assay employed the spectrophotometric method of Fry and Bonner (2012). COX-1 from sheep and COX-2 from humans were used as enzyme sources. The assay was conducted at the Terrestrial Natural Products Laboratory (TNPL) of the Institute of Chemistry, University of the Philippines, Diliman, Quezon City, Philippines. An enzyme-cofactor solution was first prepared by mixing 5184 uL of 100 mM Tris buffer (pH 8.00), 480 uL of 20 uM Hematin, and 96 uL of 250 U/mL COX-2 or COX-1 enzyme in a scintillation vial. To each test well that has 50 uL of the 100 mM Tris buffer, 120 uL of the enzyme-cofactor mixture was added followed by 10 uL of 200 ug/mL of *N. biserrata* and *A. palmiformis* extracts in DMSO. The positive control used was 8 mM indomethacin (IND) in 100% DMSO and the negative control was 5% DMSO (final well concentrations). The mixture was incubated at 25 ^{0}C for fifteen (15) minutes. After incubation, 10 uL of 200 uM 10-acetyl-3,7-dihydroxyphenoxazine (Amplex Red) was added to each well, followed by 10 uL of 2000 uM arachidonic acid. The reaction mixture was mixed and purged with N_2 gas. The reaction was monitored for two (2) minutes using CLARIOstar® (BMG LABTECH) multifunctional microplate reader at an excitation wavelength of 535 nm and emission wavelength of 590 nm. Fluorescence intensity was measured at 12-s intervals. The percent COX-1 and COX-2 inhibition of the samples and the positive control were determined based on the averaged slope of each replicate by using equation (2):

$$\%\,Inhibition = \frac{Slope_{uninhibited} - Slope_{inhibited}}{Slope_{uninhibited}} \times 100 \quad (2)$$

where $Slope_{uninhibited}$ is the slope of the line from the fluorescence vs. time plot of the negative control group and the $Slope_{inhibited}$ is the slope of the line from the fluorescence intensity vs. time plot of the samples/positive control. A sample is considered "active" at the screening concentration used if (1) the COX-2 inhibition is greater than or equal to 50% and (2) if the COX-2/COX-1 inhibition ratio is greater

than 1.00. Each sample was read using two trials with two replicates.

Statistical analysis

The statistical analyses employed include Analysis of Variance with Completely Randomized Design and Scheffe's Test.

Results and discussion

The anti-inflammatory activity of the crude ethanolic extract (EtOH), hexane- (Hex), ethyl acetate- (EtOAc), and aqueous (Aq) partitions of *N. biserrata* rhizomes and *A. palmiformis* fronds were determined through inhibition of the pro-inflammatory enzymes 15-LOX and COX-2. Percent inhibition of COX-1 was also obtained to determine the selectivity of the extracts towards COX-2.

Anti-inflammatory activity of N. biserrata rhizome extracts

Results of the 15-LOX and COX inhibition assays for *N. biserrata* rhizome extracts are presented in Fig. 3.

Fig. 1. *N. biserrata* (Sw.) Schott found in Bukidnon, Philippines. (A) Upper part. (B) Lower part.

The aqueous (Nb-Aq) and ethyl acetate (Nb-EtOAc) partitions both exhibited greater than fifty percent (>50%) inhibition of the 15-LOX enzyme with 88.90 ± 4.62% and 80.00 ± 1.48% inhibition, respectively.

Moreover, the ethanolic extract and all of the solvent partitions of *N. biserrata* rhizomes were found to be active and selective in inhibiting the COX-2 enzyme. The highest % COX-2 inhibition was shown by the ethyl acetate (EtOAc) partition at 88.76±1.21% followed by the ethanolic (EtOH) extract, aqueous- (Aq), and hexane (Hex) partitions with 67.93±1.90%, 66.21±2.74%, and 51.69±5.85% COX-2 inhibition, respectively. Consequently, the ethanolic (EtOH) extract, hexane- (Hex), ethyl acetate- (EtOAc), and aqueous (Aq) partitions of *N. biserrata* rhizomes gave 1.04, 1.35, 1.02, and 1.09 COX selectivity index, respectively. The observed results of the 15-LOX and COX inhibition assays suggest that *N. biserrata* rhizomes possess anti-inflammatory activity through active and selective inhibition of the 15-LOX and COX-2 enzymes which were reported to be involved in the inflammation process. These results primarily support the use of *N. biserrata* as treatment for various common ailments such as boils, abscess, sore, blisters, jaundice, and other skin disorders.

Fig. 2. *A. palmiformis* (Cav.) C. Chr. plant found in Bukidnon, Philippines.

Moreso, the results can be attributed to the presence of Vitamin C and E, proteins, minerals, and phytochemicals such as beta-carotene, cardiac glycosides, steroids, phenols, saponins, phytosterol, alkaloids, terpenoids, tannins, and flavonoids in *N. biserrata* ethanolic extract which are known to possess high nutritional value and antioxidant activities (Shah *et al.*, 2014; Ofoego, 2015). It is also notable that the ethyl acetate (Nb-EtOAc) and aqueous (Nb-Aq) partitions of *N. biserrata* fronds were both active against the 15-LOX and COX-2 enzymes which could indicate the possible presence of metabolites that can actively inhibit both of the pro-inflammatory enzymes. Some of the compounds having various important biological activities that were reported to be found in *N. biserrata* include benzeneacetaldehyde (antioxidant and anti-inflammatory), 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (antimicrobial, anti-inflammatory, antiproliferative), 4,6-dimethyl-2H-pyran-2-one (anti-inflammatory, antiviral), catechol (anti-inflammatory), 2,3-dihydrobenzofuran (antihelminthic, anti-inflammatory, antidiarrheal), phytol (anticancer, anti-inflammatory, hepatoprotective), gamolenic acid (anti-inflammatory), and octadecanoic acid (antioxidant, antimicrobial) (Shah *et al.*, 2014). Other reports also supported the anti-inflammatory property of *N. biserrata*. For instance, the antinociceptive and anti-inflammatory activities of *N. biserrata* have been reported in which its leaf ethanolic extract significantly inhibited acute inflammation caused by egg albumin-induced rat paw edema (Shorinwa and Ogeleka, 2016). Various studies also evaluated the anti-inflammatory activity of other Nephrolepis species including *N. cordifolia* (Amoroso *et al.*, 2014) and *N. falcata* (Komala *et al.*, 2020).

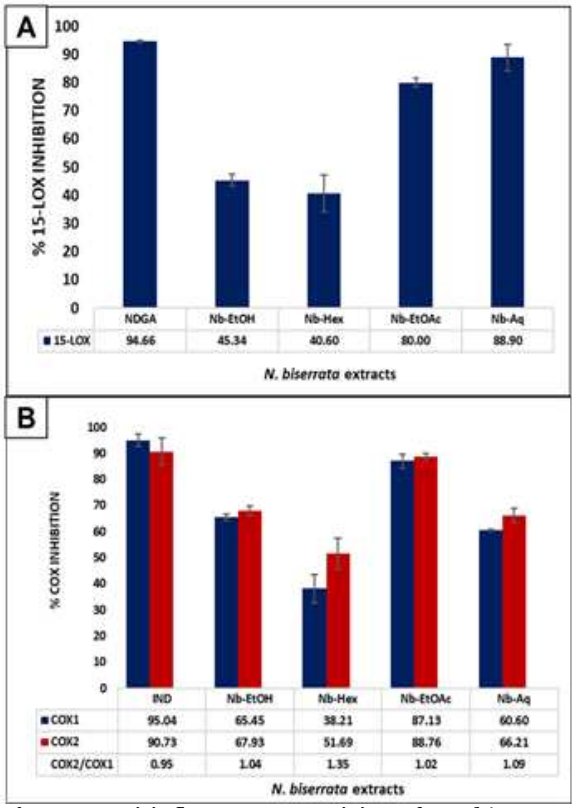

Fig. 3. Anti-inflammatory activity of *N. biserrata* rhizome extracts. (A) Percent 15-LOX inhibition at 33 ug/mL. (B) Percent COX-1 and COX-2 inhibition at 10 ug/mL. Error bars are standard deviation (n=4).

Anti-inflammatory activity of A. palmifomis frond extracts

Percent 15-LOX and COX inhibition of *A. palmiformis* frond extracts are summarized in Fig. 4. Based on the results, the crude ethanolic extract of *A. palmiformis* (Ap-EtOH) is found to be active in inhibiting the 15-LOX enzyme with 59.23±6.10% inhibition. Upon partitioning, however, the 15-LOX and COX inhibition activity were no longer observed as all hexane- (Ap-Hex), ethyl acetate- (Ap-EtOAc), and aqueous- (Ap-Aq) partitions of *A. palmiformis* frond extracts exhibited less than fifty percent (<50%) inhibition against the 15-LOX enzyme. However, all of

the *A. palmiformis* extracts showed active inhibition of the COX-2 enzyme with 52.55±3.53% (Ap-EtOH), 77.56±9.39% (Ap-Hex), 75.96±2.81% (Ap-EtOAc), and 54.68±10.38% (Ap-Aq) inhibition, respectively. However, only the hexane-soluble partition of *A. palmiformis* (Ap-Hex) is considered both active and selective (1.24) towards COX-2 inhibition.

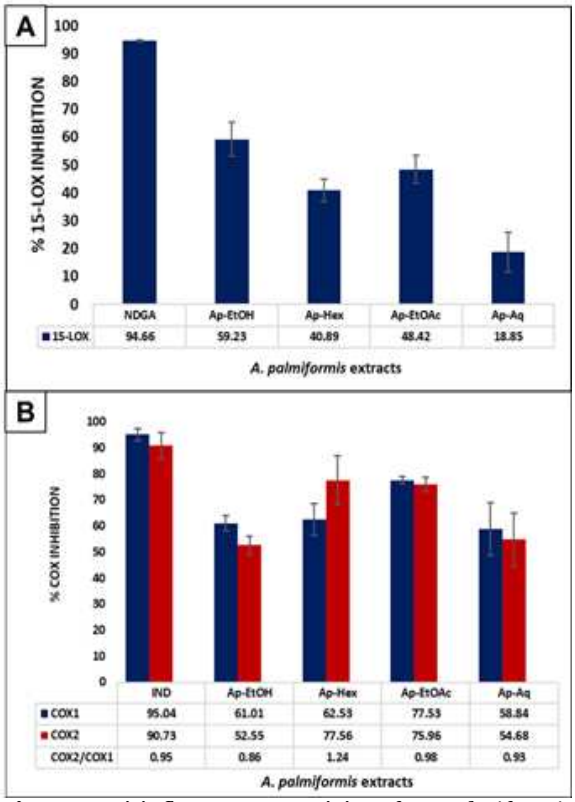

Fig. 4. Anti-inflammatory activity of *A. palmiformis* frond extracts. (A) Percent 15-LOX inhibition at 33 ug/mL. (B) Percent COX-1 and COX-2 inhibition at 10 ug/mL. Error bars are standard deviation (n=4).

A study by Amoroso *et al.* (2017) has also reported the anti-inflammatory and antioxidant activity as well as the high protein content of *A. palmiformis*. Moreover, natural compounds that can be found in *Angiopteris* like triterpenoids have been reviewed with numerous biological activities such as anti-inflammatory, antioxidative, anti-viral, antibacterial, anticancer and chemopreventive (Chudzik *et al.*, 2015). A number of species belonging to the same genus have also been reported to possess anti-inflammatory activity. For instance, *A. evecta* has been used as traditional medicine for beriberi, backache, abdominal pain, stomachache and swellings (De Winter and Amoroso, 2003). Also, compounds isolated from the ethyl acetate fraction of *A. helferiana* methanolic extract have been found to have potent adipogenic and anti-inflammatory activity (Lamichhane *et al.*, 2020).

While Ap-EtOH exhibited active inhibition of the 15-LOX enzyme, the observed activity was no longer observed upon partitioning. This may suggest that the observed anti-inflammatory activity could be synergistic in nature. Zhang *et al.* (2019) have reported the synergistic anti-inflammatory effects of combined phytochemicals in plants. Partitioning causes the components to segregate. Hence, the probability for the synergistic anti-inflammatory effect to be disrupted may exist.

Conclusion

The results of this study have shown that *N. biserrata* rhizome and *A. palmiformis* frond ethanolic extracts and solvent partitions are potential sources of active and selective 15-LOX and COX-2 inhibitors.

The observed significant anti-inflammatory activity of the extracts has also provided scientific evidence for their use as traditional medicinal plants. Further investigation to identify the secondary metabolites responsible for their exhibited bioactivity is hereby recommended.

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References

Aggarwal B, Shishodia S, Sandur S, Pandey M, Sethi G. 2006. Inflammation and cancer: How hot is the link? Biochemical Pharmacology **72(11),** 1605-1621.
http://dx.doi.org/10.1016/j.bcp.2006.06.029

Ahmed A. 2011. An overview of inflammation: mechanism and consequences. Frontiers in Biology **6(4),** 274-281.
http://dx.doi.org/10.1007/s11515-011-1123-9

Auerbach B, Kiely J, Cornicelli J. 1992. A Spectrophotometric Microtiter-Based Assay for the Detection of Hydroperoxy Derivatives of Linoleic Acid. Analytical Biochemistry **201(2),** 375-380.
https://doi.org/10.1016/0003-2697(92)90354-a

Axelrold B, Cheesebrough T, Laakso S. 1981. Lipoxygenase from Soybeans. Methods in Enzymology Lipids Part C. **71**, 441-451.
http://dx.doi.org/10.1016/0076-6879(81)71055-3

Bosma-den Boer M, van Wetten ML, Pruimboom L. 2012. Chronic Inflammatory diseases are stimulated by current lifestyle: how diet, stress levels and medication prevent our body from recovering. Nutrition and Metabolism **9(1),** 1-14.
http://dx.doi.org/10.1186/1743-7075-9-32

Chudzik M, Korzonek-Szlacheta I, Krol W. 2015. Triterpenes as Potentially Cytotoxic Compounds. Molecules **20(1),** 1610-1625.
http://dx.doi.org/10.3390/molecules20011610

de Winter WP, Amoroso VB. 2003. Cryptogams: ferns and fern allies. Plant Resources of South-East Asia - Backhuys Publishers **15(2).**
https://edepot.wur.nl/411315

Fry M, Bonner A. 2012. Development of a fluorescent-based assay to detect cyclooxygenase inhibitory activity of *δ*-lactone derivatives. 22nd Annual Argonne Symposium for Undergraduates. Central States Incorporated, Argonne National Laboratory, Argonne, Illinois, USA.

Komala I, Yardi A, Betha OS, Muliati F, Ni'mah M. 2015. Antioxidant and anti-inflammatory activity of the Indonesian ferns, *Nephrolepis falcata* and *Pyrrosia lanceolata*. International Journal of Pharmacy and Pharmaceutical Sciences **7(12),** 162-165.
https://innovareacademics.in/journals/index.php/ijpps/article/view/8751

Kormin F, Khan M, Shafie NSM, Nour AH, Yunus RM. 2018. Statistical mixture design: Study of solvent performance in temperature-controlled microwave assisted extraction system on antioxidant properties of *N. biserrata* (Schott.) Sw. frond extract. International Journal of Engineering and Technology **7(3.7)**, 166-172.
http://dx.doi.org/10.14419/ijet.v7i3.7.19061

Lamichhane R, Pandeya PR, Lee KH, Kim SG, Devkota HP, Jung HJ. 2020. Anti-Adipogenic and Anti-Inflammatory Activities of (−)-epi-Osmundalactone and Angiopteroside from *Angiopteris helferiana* C. Presl. Molecules **25(6),** 1337.
https://doi.org/10.3390/molecules25061337

Ofoego EU. 2015. The phytochemical analysis and evaluation of the antioxidant and antidiabetic potentials of ethanolic leaf extract of *Nephrolepis biserrata*. Thesis submitted to the Department of Biotechnology Technology, FUTO.
https://librarian67.wixsite.com/futo-oer/project-reports

Otsuka H. 2006. Purification by Solvent Extraction Using Partition Coefficient. Methods in Biotechnology **20,** 269-273.
http://dx.doi.org/10.1385/1-59259-955-9:269

Rainsford KD. 1999. Profile and mechanisms of gastrointestinal and other side effects of non-steroidal anti-inflammatory drugs (NSAIDs). The American Journal of Medicine **107(6A),** 27S-35S.
http://dx.doi.org/10.1016/s0002-9343(99)00365-4

Schumacher M, Juncker T, Schnekenburger M, Gaascht F, Diederich M. 2011. Natural compounds as inflammation inhibitors. Genes and Nutrition **6(2)**, 89-92.
http://dx.doi.org/10.1007/s12263-011-0231-0

Shah MD, Yong YS, Iqbal M. 2014. Phytochemical investigation and free radical scavenging activities of essential oil, methanol extract and methanol fractions of *N. biserrata*. International Journal of Pharmacy and Pharmaceutical Sciences **6(9),** 269-277.

Shorinwa OA, Ogeleka NO. 2016. Antinociceptive and Anti-inflammatory Activities of Aerial Part of *N. biserrata* (Sw) Schott **5**, 246–254

Zhang L, Virgous C, Si H. 2019. Synergistic anti-inflammatory effects and mechanisms of combined phytochemicals. The Journal of nutritional biochemistry **69,** 19-30. http://dx.doi.org/10.1016/j.jnutbio.2019.03.009