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Anti-advanced glycation end-products and antibacterial inhibitory activities of *Neonauclea formicaria* (Rubiaceae)

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ABSTRACT

Neonauclea species have been studied for their phytochemical and potential pharmacological uses. However, many of its species still remained unexplored. The Philippine endemic *Neonauclea formicaria* has been documented for its ethnobotanical use, but little has been reported on its phytochemical and pharmacological profile. Hence, this study evaluates the total phenolic (TPC) and flavonoid (TFC) contents and assesses its ethanolic leaf extracts' antiglycation, antioxidant, and antibacterial activities. *N. formicaria* leaf extracts at 1000 ppm gave 63.02±5.82 mg GAE/g DW TPC and 31.25±4.24 mg QE/g DW TFC. The extract showed a concentration-dependent activity in inhibiting AGE formation for the antiglycation assay, with an IC_{50} value of 2823.5 ppm. In antioxidant assay, the extract exhibited the highest TEAC value of 419.5±14.3 mg TE/kg DW at 250 ppm. *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa* were shown to be the most susceptible to the leaf extract (1000 ppm), with zones of inhibition at 16.56±5.65 mm and 14.06±2.65 mm, respectively. The results highlight that *N. formicaria* bears promising properties with pharmacological and nutraceutical applications.

KEYWORDS: AGE, Antioxidants, Antibacterial, ESKAPE, Philippine medicinal plant, Phytochemistry

INTRODUCTION

There is a growing burden of chronic diseases associated with advanced glycation end-products (AGEs) and oxidative stress, contributing to the progression of various diseases and medical disorders that amplify in complexity each day. AGE formation, a spontaneous or enzymatic process, stiffens tissues and disrupts cellular function, while oxidative stress damages biomolecules caused by an imbalance between free radicals and antioxidants. The consequence of AGE over-accumulation and oxidative stress potentially leads to the development of diabetes, carcinogenesis, neurodegenerative disorders, cardiovascular diseases, and a compromised immune system, increasing susceptibility to bacterial infections (Baig *et al.*, 2017; Pizzino *et al.*, 2017). AGEs can activate inflammatory pathways, and oxidative stress can trigger the release of pro-inflammatory mediators, leading to a chronic inflammatory state that can suppress the immune system's ability to mount an effective response against pathogens while also disrupting the balance of the gut microbiota, creating a window of opportunity for infection (Prata *et al.*, 2024). Thus, there is a continuous search for plants with promising pharmacological properties to combat these diseases.

Plants from the family Rubiaceae are well-known for their medicinal application in treating complications such as malaria, diarrhea, digestive issues, skin diseases, fever, bleeding, urinary and respiratory infections, headache, and inflammation of the eyes and gums (Wong *et al.*, 2015). The genus *Neonauclea*, has attracted considerable interest for their diverse biologically active compounds which may be responsible for their relevant pharmacological activities (Karaket *et al.*, 2012; Chang *et al.*, 2019; Vergara *et al.*, 2021). However, only a handful of its known species are studied, thus, demonstrating a need for further investigations in the direction of natural resources and their potential contributions to healthcare.

Neonauclea formicaria, a Philippine endemic plant, is distributed primarily in the central and southern regions of the archipelago (Ordas *et al.*, 2021). This species was reported to be an ethnomedicinal plant utilized by locals for Surigao del Norte to treat swelling, relapse, and fever (Demetillo *et al.*,

2019). Vergara *et al.* (2021) also reported its angio-preventive potential. Herein, we report the phytochemical analysis (total phenolic and flavonoid contents), antioxidant activity, anti-AGE activity using the BSA-dextrose model, and antibacterial activities utilizing the ESKAPE bacteria

MATERIALS AND METHODS

Plant Collection

Fresh *Neonauclea formicaria* leaves were collected in Cagdianao, Dinagat Islands, Philippines (10°10'53" N, 125°38'8"). Voucher specimens (USTH 018549) were prepared and deposited in the University of Santo Tomas Herbarium (USTH). Confirmation of species identity was performed using the taxonomic key of myrmecophytic *Neonauclea* species in the Philippines (Ordas *et al.*, 2017).

Extraction of the Plant Material

The *N. formicaria* leaves were washed, air-dried, and ground into a fine powder with a dry weight of 354 g. The ground leaves were placed in a percolator and extracted with technical grade EtOH. After 24 h, the EtOH extract was collected. The extraction process was repeated thrice using a total volume of 5.7 L EtOH. The EtOH extracts were combined and dried under reduced pressure to yield the crude *N. formicaria* extract (18.59 g). The crude extract was kept in an amber bottle at 4 °C until further use.

Phytochemical Analysis - Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

The TPC and TFC of *N. formicaria* were determined using the modified Folin-Ciocalteu (FC) Assay (Seawan *et al.*, 2014) and Aluminum Chloride Colorimetric Method (Aryal *et al.*, 2019), respectively. For TPC, the ethanolic crude extract (1000 ppm) was dissolved in water in a 96-well plate. The FC reagent, sodium carbonate, and samples were incubated in the dark for 1 h, and the absorbance at 750 nm was measured using a microtiter-plate multimode detector (Promega-Glomax Multi Detection System). TPC was quantified against a gallic acid standard curve and expressed as mg gallic acid equivalents (GAE)/g dry weight (DW). For TFC, the crude extract (1000 ppm) was dissolved in MeOH. The blank solution and samples (extract and positive control) were incubated for 30 min with aluminum chloride, potassium acetate, and water, and absorbance at 415 nm was measured using the spectrophotometer mentioned. TFC was quantified against a quercetin standard curve and expressed as mg quercetin equivalents (QE)/g DW.

Antioxidant Assay - ABTS Radical Decolorization

The antioxidant activity and capacity of *N. formicaria* were investigated using the ABTS (2,2'-Azino-bis-3-ethylbenzthiazoline-6-sulphonic acid) Radical Decolorization Assay (Moreira *et al.*, 2019) with modifications. The crude extract (4 mg) was dissolved in MeOH to obtain a 1000 ppm stock solution. The mixture was shaken thoroughly via sonication to completely dissolve the extract. Extract solutions of 1000, 500, and 250 ppm were added on a 96-well microtiter plate as the working concentrations. Wells containing 10 µL of phosphate buffered saline (PBS) and 190 µL H_2O were used as blank solutions (control). Excluding the references, 190 µL of the ABTS Radical Solution was added to the sample mixtures and incubated in the dark at room temperature for 5 min. The absorbance at 734 nm was measured using a Promega GloMax microplate reader. The percentage decolorization of the extract was calculated using the following equation:

$$\%Decolorization = \left(\frac{Abs_{Control} - Abs_{Sample}}{Abs_{Control}} \right) x100$$

The calculated percentage values were subsequently used to determine the antioxidant activity expressed as Trolox Equivalent Antioxidant Capacity (TEAC) using a calibration curve generated with various concentrations of the standard Trolox.

Anti-Advanced Glycation End-Products (AGEs) (Antiglycation) Assay

The capacity of *N. formicaria* against the formation of AGEs was evaluated based on Muñiz *et al.* (2018) with modifications. A 100 mM Phosphate Buffer (pH 7.4) containing 50 mg/mL of Bovine Serum Albumin (BSA), 0.5 M Dextrose, and 5 mM Sodium Azide (NaN_3) was prepared. From this solution, 1.8 mL was mixed with varying concentrations of the crude extract (0.2 mL) (100 ppm, 500 ppm, 1000 ppm, 3000 ppm, and 5000 ppm) and quercetin as the positive control. The BSA-crude extract solutions were incubated at room temperature in the dark for 2 weeks. After incubation, the fluorescence was measured at excitation and emission wavelengths of 365 nm and 440 nm, respectively, using a Promega GloMax microplate reader. The percentage AGE inhibition was calculated using the formula:

$$\%AGE\,Inhibition = \left(\frac{Abs_{Control} - Abs_{Sample}}{Abs_{Control}} \right) x100$$

Antibacterial Analyses

Disk diffusion assay following the protocol of Balouiri *et al.* (2016) was performed, testing the leaf extract (1000 ppm) against the ESKAPE organisms *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Klebsiella pneumoniae* ATCC 13583, *Acinetobacter baumannii* NCIMB 12457, *Pseudomonas aeruginosa* ATCC 27853, and *Enterobacter cloacae* ATCC13047. Gentamicin was used as the positive control, while EtOH was used as the negative control. The diameters of the zones of inhibition (ZOI) were measured using a vernier caliper. ZOIs were qualitatively categorized as inactive (<10 mm), partially active (10-13 mm), active (14-19 mm), and very active (>19 mm) (Quinto & Santos, 2005).

Statistical Analysis

Data are reported as mean±standard deviation in triplicate experiments. For the antiglycation assay, the IC_{50} values for both the extract and the positive control were determined using GraphPad Prism 10 and ATT Bioquest.

RESULTS AND DISCUSSION

Determination of the TPC and TFC

Phenolic compounds found in plants offer numerous health benefits, including lowering blood sugar, reducing cell damage, preventing proteins from sticking to sugar molecules, and blocking enzymes involved in carbohydrate breakdown (de la Rosa *et al.*, 2019; de Paulo Farias *et al.*, 2021). Polyphenols such as phenolic acids and flavonoids are known phytochemicals that correlate strongly with a plant's antioxidant activity (Rodríguez-Yoldi, 2021) by initiating the scavenging action that neutralizes free radicals through electron donation, ultimately preventing oxidative stress.

The TPC of the crude leaf extract of *N. formicaria* was determined using the regression equation of the standard calibration curve of Gallic Acid ($y=0.0036x+0.1008$; $R^2=0.9948$). *N. formicaria* gave a TPC of 63.02±5.82 mg GAE/g DW at 1000 ppm. The TFC of the leaf extract was determined using the regression equation of the standard calibration curve of Quercetin ($y=0.0072x-0.0124$; $R^2=0.998$). The extract exhibited a TFC of 31.25±4.24 mg QE/g DW at 1000 ppm.

Antioxidant Activity of *N. formicaria* Crude Leaf Extract

The antioxidant activity of *N. formicaria* was assessed by measuring the decrease in intensity of the initial bluish-green color of the ABTS radical solution utilizing 250, 500, and 1000 ppm concentrations (Figure 1). The extract exhibited the highest decolorization percentage of 85.9±2.63% at 1000 ppm. At 500 ppm, the extract revealed a decolorization percentage of 68.74±4.33%, while the lowest decolorization percentage of 52.01±3.97% was observed at 250 ppm.

Figure 2 gave the corresponding TEAC (Trolox Equivalent Antioxidant Capacity) values of the crude extract. The extract exhibited the highest TEAC value of 419.5±14.3 mg TE/kg DW at 250 ppm. At 500 ppm, the extract revealed a TEAC of 282.1±8.7 mg TE/kg DW, while the extract at 1000 ppm displayed a TEAC value of 178.2±5.7 mg TE/kg DW, which was the lowest TEAC value observed. The decrease in the TEAC values as the concentration increases may be attributed to the amount of the antioxidants present which at 250 ppm is not enough to neutralize the ABTS radicals available, reflecting significant antioxidant activity in the process. The low TEAC value at 1000 ppm can be attributed to the greater amount of antioxidants present relative to the available ABTS radicals, reflecting the decrease in the antioxidant activity (Mira *et al.*, 1999).

Quantifying the phenolic and flavonoid content of the crude extract in *N. formicaria* leaves reveals substantial amounts of these phytochemicals, as exhibited by the concentration-dependent increase of polyphenols. Their presence in its leaves indicates its ability to combat oxidative stress and ultimately provide preventive benefits against degenerative diseases. This is substantiated by the ABTS radical decolorization assay results, exhibiting increasing antioxidant activity of the leaf extract with increasing concentration. Many Rubiaceae plants found in the Philippines have been documented to exhibit potent antioxidant capacity as well (Castro *et al.*, 2018; Aureada *et al.*, 2023; Esguerra *et al.*, 2024).

Antiglycation Activity of the *N. formicaria* Leaf Extract

Figure 3 illustrates the percentage inhibition of the *N. formicaria* crude extract against the formation of AGEs using the BSA-dextrose model. A dose-dependent trend was observed in the antiglycation assay. At 5000 ppm, crude extract showed 36.3±0.59% inhibition. At 100 ppm, 6.28±2.77% inhibitory activity was observed. The IC_{50} of the crude *N. formicaria* extract was determined to be and the positive control Quercetin is elucidated in Figure 2. The IC_{50} of the ethanolic crude extract was 2823.5 ppm. This was significantly different with the positive control quercetin with an IC_{50} of 11.30 ppm.

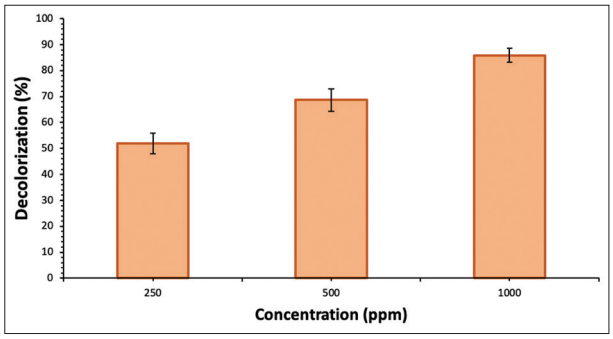

Figure 1: Decolorization percentage (%) of *N. formicaria* ethanolic extracts in varying concentrations. Values are expressed as mean±standard deviation (n=3)

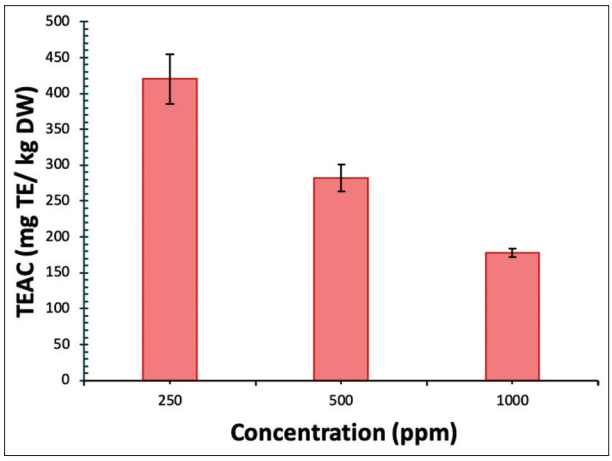

Figure 2: TEAC (mg TE/kg DW) of *N. formicaria* ethanolic extracts in varying concentrations. Values are expressed as mean±standard deviation (n=3)

Table 1: Antibacterial activity of *N. formicaria* crude ethanolic extracts (1000 ppm) against the ESKAPE microorganisms. Values are expressed as mean±standard deviation (n=3). Gentamicin and Ethanol were used as the positive and negative controls, respectively

ESKAPE Microorganisms	Zone of inhibition (mm)		
	Crude extract (1000 ppm)	Gentamicin (Positive control)	Ethanol (Negative control)
Escherichia coli	11.30±1.07	37.9	0
Staphylococcus aureus	16.56±5.65	50.9	0
Klebsiella pneumoniae	10.10±4.36	38.1	0
Acinetobacter baumannii	9.50±0.52	43.5	0
Pseudomonas aeruginosa	14.06±2.65	37.2	0
Enterobacter cloacae	11.40±4.85	37.8	0

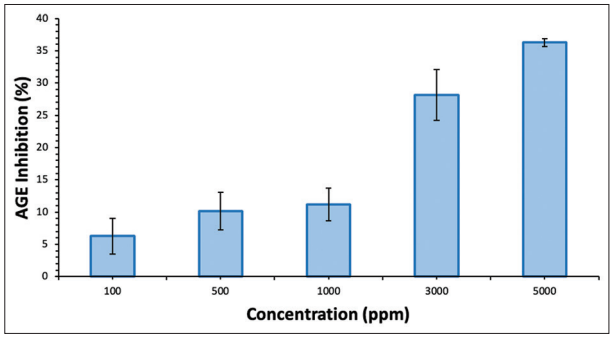

Figure 3: AGE Inhibition (%) of *N. formicaria* ethanolic extracts in varying concentrations. Values are expressed as mean±standard deviation (n=3)

Phenolics and flavonoids, with their antioxidant properties, have been found to inhibit AGE formation by preventing glycoxidation (Khan *et al.*, 2020). The results of the antiglycation assay of *N. formicaria* leaves reveal a concentration-dependent trend in the inhibition of AGE formation, with higher concentrations exhibiting greater inhibition capability (Harris *et al.*, 2014). This prompts further investigation into its potential as a therapeutic agent for combating AGE-related diseases. Replacing synthetic drugs with phytochemicals promotes therapeutic strategies for the prevention and treatment of diseases such as diabetes, ovarian cancer, and Alzheimer's disease (Fernandes *et al.*, 2022).

Antibacterial Activity of the *N. formicaria* Crude Extracts

The *N. formicaria* leaf crude extract exhibited varying antibacterial activities against the ESKAPE organisms (Table 1). The extract was observed to be partially active in *E. cloacae* (ZOI 11.40±4.85 mm), *E. coli* (ZOI 11.30±1.07 mm), and *K. pneumoniae* (ZOI 10.10±4.36 mm), and active against *S. aureus* (ZOI 16.56±5.65 mm) and *P. aeruginosa* (ZOI 14.06±2.65 mm).

The observed antibacterial activities of the crude extract against the ESKAPE pathogens may attribute to the potential bioactive compounds that can more readily interact with Gram-positive bacteria's simpler cell wall structure than with Gram-negative bacteria's complex lipopolysaccharide outer membrane (Breijyeh *et al.*, 2020). Several native Rubiaceae in the Philippines have demonstrated antibacterial activities against Gram-positive bacteria, such as *Psychotria luzoniensis*, *Psydrax puberula*, and *Uncaria cordata* var. *circa* (Castro *et al.*, 2016). Thus, while the *N. formicaria* extract exhibits promising antibacterial properties, further exploration are warranted to elucidate their full therapeutic potential and optimize their application in combating infectious diseases.

CONCLUSIONS

The present study demonstrates the antiglycation, antioxidant, and antibacterial potentials of the *N. formicaria* leaf extracts in association with its phenolic and flavonoid contents. Hence, its leaves may be used for other ethnomedicinal treatments and hold promising pharmacological importance for developing functional foods and novel drugs against various AGE-related and bacterial diseases. Future studies should purify and characterize the metabolites and compounds found in the crude extracts for further investigation using guided bioassays and quantitative tests. Additional research regarding other native *Neonauclea* or Rubiaceae species in the Philippines that may aid in developing novel pharmaceuticals is also recommended.

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AUTHORS' CONTRIBUTION

All authors contributed to the conceptualization of the research design. MZPL, JNFD, ECS, CDC, RALB, and JADO performed the field collection. MZPL, JNFD, ECS, CDC, RALB, and MAT performed the experimentation. MZPL, JNFD, and ECS performed data curation and analysis. MZPL, JNFD, ECS, CDC, and RALB wrote the first draft of the manuscript. MZPL, JNFD, ECS, JADO, and MAT revised and finalized the manuscript. JADO and MAT supervised the project. All authors approved the final version of the manuscript.

REFERENCES

Aryal, S., Baniya, M. K., Danekhu, K., Kunwar, P., Gurung, R., & Koirala, N. (2019). Total phenolic content, flavonoid content and antioxidant

potential of wild vegetables from western Nepal. *Plants, 8*(4), 96. https://doi.org/10.3390/plants8040096

Aureada, M. Y. M., Duran, J. D. R., Falcatan, S. R. A., Pornillos, K. M. T., Villanueva, M. A. G., Ordas, J. A. D., & Tan, M. A. (2023). Correlation of total phenolic and flavonoid contents on the antioxidant activity of *Psychotria gitingensis* and *Psychotria pilosella*. *Journal of Phytology, 15*, 110-115. https://doi.org/10.25081/jp.2023.v15.8545

Baig, M. H., Jan, A. T., Rabbani, G., Ahmad, K., Ashraf, J. M., Kim, T., Min, H. S., Lee, Y. H., Cho, W.-K., Ma, J. Y., Lee, E. J., & Choi, I. (2017). Methylglyoxal and advanced glycation end products: insight of the regulatory machinery affecting the myogenic program and of its modulation by natural compounds. *Scientific Reports, 7*, 5916. https://doi.org/10.1038/s41598-017-06067-5

Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis, 6*(2), 71-79. https://doi.org/10.1016/j.jpha.2015.11.005

Breijyeh, Z., Jubeh, B., & Karaman, R. (2020). Resistance of Gram-negative bacteria to current antibacterial agents and approaches to resolve it. *Molecules, 25*(6), 1340. https://doi.org/10.3390/molecules25061340

Castro, G. C., Alejandro, G. J. D., Ramos, M. C. R., & Ysrael, M. C. (2018). Phytochemical analysis, antioxidant, and antimutagenic potentials of *Greeniopsis sibuyanensis* Elmer. *Acta Manilana, 66*, 25-32.

Castro, S. G., Cid, J. E. V., Ibañez, W. A. S., Alejandro, G. J. D., & Tan, M. A. (2016). GC-MS metabolite profiling of the hexane extract and antimicrobial characterization of the Philippine endemic Rubiaceae species *Uncaria cordata* var. *circa*, *Psychotria luzoniensis*, and *Psydrax puberula*. *Acta Manilana, 64*, 9-16.

Chang, F.-P., Huang, S.-S., Lee, T.-H., Chang, C.-I., Kuo, T.-F., Huang, G.-J., & Kuo, Y.-H. (2019). Four new iridoid metabolites have been isolated from the stems of *Neonauclea reticulata* (Havil.) Merr. with anti-inflammatory activities on LPS-induced RAW264.7 cells. *Molecules, 24*(23), 4271. https://doi.org/10.3390/molecules24234271

de la Rosa, L. A., Moreno-Escamilla, J. O., Rodrigo-García, J., & Alvarez-Parrilla, E. (2019). Phenolic compounds. In E. M. Yahia (Eds.), *Postharvest Physiology and Biochemistry of Fruits and Vegetables* (pp. 253-271) Sawston, UK: Woodhead Publishing. https://doi.org/10.1016/b978-0-12-813278-4.00012-9

de Paulo Farias, D., de Araújo, F. F., Neri-Numa, I. A., & Pastore, G. M. (2021). Antidiabetic potential of dietary polyphenols: A mechanistic review. *Food Research International, 145*, 110383. https://doi.org/10.1016/j.foodres.2021.110383

Demetillo, M. T., Betco, G. L., & Goloran, A. B. (2019). Assessment of native medicinal plants in selected mining area of claver Surigao Del Norte, Philippines. *Journal of Medicinal Plants Studies, 7*(2), 171-174.

Esguerra, J. D. J., Bernardo, J. M. M., Gimao, K. M. S., Peralta, M. E. T., Tiu, C. J. A., Hernandez, G. G. A., Alejandro, G. J. D., & Tan, M. A. (2024). Mechanism-based antioxidant activity of Rubiaceae species collected from Ilocos Norte, Philippines. *Notulae Scientia Biologicae, 16*(2), 11888. https://doi.org/10.55779/nsb16211888

Fernandes, A. C. F., Melo, J. B., Gênova, V. M., Santana, Á. L., & Macedo, G. (2022). Phytochemicals as potential inhibitors of advanced glycation end products: Health aspects and patent survey. *Recent Advances in Food, Nutrition and Agriculture, 13*(1), 3-16. https://doi.org/10.2174/2212798412666210528130001

Harris, C. S., Cuerrier, A., Lamont, E., Haddad, P. S., Arnason, J. T., Bennett, S. A. L., & Johns, T. (2014). Investigating wild berries as a dietary approach to reducing the formation of advanced glycation endproducts: Chemical correlates of *in vitro* antiglycation activity. *Plant Foods for Human Nutrition*, *69*, 71-77. https://doi.org/10.1007/s11130-014-0403-3

Karaket, N., Supaibulwatana, K., Ounsuk, S., Bultel-Poncé, V., Pham, V. C., & Bodo, B. (2012). Chemical and bioactivity evaluation of the bark of *Neonauclea purpurea*. *Natural Product Communications, 7*(2), 169-170. https://doi.org/10.1177/1934578X1200700208

Khan, M., Liu, H., Wang, J., & Sun, B. (2020). Inhibitory effect of phenolic compounds and plant extracts on the formation of advance glycation end products: A comprehensive review. *Food Research International, 130*, 108933. https://doi.org/10.1016/j.foodres.2019.108933

Mira, L., Silva, M., Rocha, R., & Manso, C. F. (1999). Measurement of relative antioxidant activity of compounds: a methodological note. *Redox Report: Communications in Free Radical Research, 4*(1-2), 69-74. https://doi.org/10.1179/135100099101534666

Moreira, D. C. (2019). ABTS decolorization assay - in vitro antioxidant capacity. https://doi.org/10.17504/protocols.io.42xgyfn

Muñiz, A., Garcia, A. H., Pérez, R. M., García, E. V., & González, D. E. (2018). *In vitro* inhibitory activity of *Acca sellowiana* fruit extract on end products of advanced glycation. *Diabetes Therapy, 9*, 67-74. https://doi.org/10.1007/s13300-017-0335-7

Ordas, J. A. D., Banag, C. I., & Alejandro, G. J. D. (2017) *Neonauclea viridiflora* (Rubiaceae), a new species of Naucleeae from Eastern Samar, with notes on myrmecophytic species in the Philippines. *Systematic Botany, 42*(2), 364-370. https://doi.org/10.1600/036364417X695592

Ordas, J. A. D., Razafimandimbison, S. G., Moran, C. B., & Alejandro, G. J. D. (2021). Phylogeny and the evolutionary origins of myrmecophytism in the *Neonauclea* clade (Rubiaceae) revisited, with particular emphasis on the Philippine lineages. *Plant Systematics and Evolution, 307*, 31. https://doi.org/10.1007/s00606-021-01752-5

Pizzino, G., Irrera, N., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V., Squadrito, F., Altavilla, D., & Bitto, A. (2017). Oxidative stress: harms and benefits for human health. *Oxidative Medicine and Cellular Longevity, 2017*, 8416763. https://doi.org/10.1155/2017/8416763

Prata, C., Angeloni, C., & Maraldi, T. (2024). Strategies to counteract oxidative stress and inflammation in chronic-degenerative diseases 2.0. *International Journal of Molecular Sciences, 25*(9), 5026. https://doi.org/10.3390/ijms25095026

Quinto, E., & Santos, M. A. (2005). Microbiology. In B. Q. Guevara (Eds.), A Guidebook to Plant Screening: Phytochemical and Biological (pp. 65-95). Manila, Philippines: UST Publishing House.

Rodríguez-Yoldi, M. J. (2021). Anti-Inflammatory and antioxidant properties of plant extracts. *Antioxidants, 10*(6), 921. https://doi.org/10.3390/antiox10060921

Seawan, N., Vichit, W., Thakam, A., Thitipramote, N., Chaiwut, P., Pintathong, P., & Thitilertdech, N. (2014). Antioxidant capacities, phenolic, anthocyanin and proanthocyanidin contents of pigmented rice extracts obtained by microwave-assisted method. *Suranaree Journal of Science and Technology, 21*(4), 301-306. https://doi.org/10.14456/sjst.2014.32

Vergara, J., Demetillo, M., Ombat, L., & Rosal, J. (2021). *Neonauclea formicaria* (Rubiaceae) leaf extract inhibits vascularization in the chorioallantoic membrane of duck embryos. *International Letters of Natural Sciences, 83*, 22-31. https://doi.org/10.56431/p-gvak39

Wong, K.-Y., Vikram, P., Chiruvella, K. K., & Mohammed, A. (2015). Phytochemical screening and antimicrobial potentials of *Borreria* sps (Rubiaceae). *Journal of King Saud University -Science, 27(4)*, 302-311. https://doi.org/10.1016/j.jksus.2014.12.001