



# Physiological Effects of Rapidus-50 On Chlorophyll, Protein and Lipid Content in *Wolffia sp.*: Implications for Aquatic Plant Health

Safiyya Aliyu Aminu

Department of Biological Sciences, Zamfara State University, Talata Mafara, Zamfara State, Nigeria

Corresponding Authors Email: [Babisofiyya1@gmail.com](mailto:Babisofiyya1@gmail.com)

Received on: April, 2025

Revised and Accepted on: May, 2025

Published on: July, 2025

## ABSTRACT

This study aimed to investigate the physiological effects of varying concentrations of Rapidus-50, a diclofenac-based non-steroidal anti-inflammatory drug (NSAID), on *Wolffia sp.* Over a five-day exposure period, chlorophyll and lipid contents were measured in treatment groups receiving 10  $\mu\text{L}$ , 100  $\mu\text{L}$ , 1000  $\mu\text{L}$ , and 10000  $\mu\text{L}$  of Rapidus-50. The results demonstrated a clear concentration-dependent decrease in both chlorophyll and lipid levels, with the most pronounced reductions observed at higher concentrations. Statistical analysis using ANOVA confirmed the significance of these changes ( $p < 0.05$ ), suggesting that the chemical exposure led to photosynthetic disruption and impaired lipid biosynthesis. These findings highlight the ecological risks posed by pharmaceutical pollutants in aquatic ecosystems.

**Keywords:** *Wolffia sp.*, chlorophyll content, lipid biosynthesis, diclofenac toxicity, aquatic plant stress.

## 1.0 INTRODUCTION

Pharmaceutical contaminants have emerged as a category of persistent and biologically active pollutants in freshwater ecosystems, garnering increasing scientific and legal scrutiny. Their continuous introduction into aquatic systems through domestic waste, medical discharge and agricultural runoff, raises significant ecological issues (Gatidou et al., 2023; Baek et al., 2021). Non-steroidal anti-inflammatory drugs (NSAIDs), particularly diclofenac-based compounds such as Rapidus-50, are extensively documented due to their prevalent use and inadequate elimination in wastewater treatment (Sosa et al., 2024; Farner et al., 2023). Rivers, lakes, and wastewater effluent have detected diclofenac concentrations that pose persistent hazards to aquatic organisms (Ahmed et al., 2021).

Research has shown that diclofenac disrupts the physiological integrity of aquatic organisms, including algae, invertebrates, and fish, resulting in oxidative stress, enzyme inhibition, and histopathological changes (Zhao et al., 2022; Sharma & Kumar, 2022). However, limited research has been conducted on its impact on primary producers such as aquatic plants, which are crucial for oxygenation, nutrient cycling, and serving as bioindicators of aquatic ecosystem health.

Duckweeds, especially those of the genus *Wolffia*, are among the tiniest flowering plants and serve as ideal bioassay organisms due to their rapid growth, clonal reproduction, and elevated surface-area-to-volume ratio (Appenroth et al., 2023; Bernier et al., 2024). Their uncomplicated architecture facilitates the identification of modifications in photosynthesis,

lipid metabolism, and protein synthesis induced by contaminants. They consequently provide early indicators of ecological imbalance and chemical stress.

Numerous studies indicate that drug exposure alters the chlorophyll and lipid profiles in aquatic plants, hence implying potential impairment of photosynthesis and membrane functionality (Arif, 2020; Baek et al., 2021). Diclofenac is particularly recognised for generating reactive oxygen species (ROS) that damage thylakoid membranes, disrupt chloroplast function, and inhibit lipid biosynthesis pathways (Sharma & Kumar, 2022). These physiological abnormalities affect not only plant life but also permeate aquatic food webs. Assessing the ecological effects of diclofenac on aquatic ecosystems is urgently required due to its rising prevalence in Nigerian water bodies and its classification as a contaminant of emerging concern (CEC). This study investigates the concentration-dependent physiological effects of Rapidus-50 on *Wolffia sp.*, focusing on alterations in chlorophyll, protein, and lipid levels. The findings aim to enhance ecotoxicology and biomonitoring by elucidating the impact of pharmaceutical pollutants on the health of aquatic plants.

This study mainly looks at how different amounts of Rapidus-50, a type of pain relief medicine, affect *Wolffia sp.* over five days by measuring chlorophyll, protein, and fat levels. The goal is to find out if Rapidus-50 harms the health of this aquatic plant and to support ongoing research on the impact of pharmaceutical pollutants in freshwater. It specifically looks at how different exposure levels affect chlorophyll, protein, and fat content

throughout five days. It also aimed to ascertain whether Rapidus-50 causes a sub-lethal stress on aquatic plant health and to support continuous ecotoxicological evaluations of pharmaceutical pollutants in freshwater environments.

## 2.0 MATERIALS AND METHODS

### 2.1 Materials

Rapidus-50 the test compound containing diclofenac potassium was obtained from a commercial pharmacy. All analytical-grade reagents necessary for biochemical analyses, including acetone, chloroform, methanol and various buffer solutions, were sourced from established chemical suppliers.

The *Wolffia* specimens were cultured in sterile, transparent one-liter plastic containers that functioned as experimental chambers. Each container was labeled according to one of the applied Rapidus-50 concentrations: 10  $\mu\text{L}$ , 100  $\mu\text{L}$ , 1000  $\mu\text{L}$ , and 10000  $\mu\text{L}$ . A nutrient-rich aquatic solution, prepared using Hoagland's formula, was used to simulate semi-natural conditions and support healthy plant growth (Sosa et al., 2024).

### 2.2 Methods

Under carefully regulated laboratory circumstances, the experimental process took five days. To guarantee homogeneity, all treatment groups experienced same light (12-hour light/dark photoperiod), temperature (kept at  $25 \pm 1^\circ\text{C}$ ), and humidity conditions. White fluorescent tubes (around 4000 lux) were used to give light, positioned at 30 cm above the cultures to prevent photodegradation of the test chemical and yet offer enough illumination for photosynthesis (Appenroth et al., 2023). To guarantee suitable sample size for biochemical investigation, each treatment group included 50–60 fronds of *Wolffia* sp.

Daily determination of chlorophyll content applied Arnon's (1949) technique. To get a clean supernatant, homogenised approx 0.5 grammes of *Wolffia* tissue in 80% acetone was centrifuged. A UV-visible spectrophotometer measured the extract's absorbance at 645 nm and 663 nm. Arnon's calculations allowed one to determine total chlorophyll; the findings were given in micrograms per millilitre ( $\mu\text{g}/\text{mL}$ ).

Using a generally established method for lipid extraction from plant materials, the Bligh and Dyer (1959) method, gravimetrically examined lipid content. In a 2:1:0.8 ratio, homogenised fronds were mixed with water, methanol and chloroform. The layer of chloroform separated and was gathered and evaporated under low pressure. Dried to constant weight in a desiccator, the lipid residue was calculated as a percentage of dry weight.

Mean values of chlorophyll and lipid contents among treatment groups were compared by means of one-way analysis of variance (ANOVA). Considered statistically significant was a p-value below 0.05. To guarantee the dependability and repeatability of the data, all experiments were run triplicates. Post hoc comparisons and computations were done using statistical software SPSS v23.0.

## 3.0 RESULTS

### 3.1 Effect of Rapidus-50 on *Wolffia* sp.

Table (3.1) provides a detailed account of how *Wolffia* sp. responded to varying concentrations of Rapidus-50 (10  $\mu\text{L}$ , 100  $\mu\text{L}$ , 1000  $\mu\text{L}$ , and 10000  $\mu\text{L}$ ) at days 1 to 5. Key parameters such as protein content, chlorophyll content, lipid content, absorbance at 750 nm and the number of fronds were tracked. Significant trends emerged, especially in the reduction of chlorophyll and lipid content at higher concentrations of Rapidus-50.

**Table 3.1: Effect of Rapidus-50 at Different Concentrations on *Wolffia* sp. from day 1 to 5.**

Day	Concentration ( $\mu\text{L}$ )	Protein Content (mg/g)	Chlorophyll Content ( $\mu\text{g}/\text{mL}$ )	Lipid Content (%)	Absorbance @ 750 nm
Day 1	10	$1.2 \pm 0.05$	$2.4 \pm 0.08$	$8.6 \pm 0.12$	$0.150 \pm 0.005$
Day 1	100	$1.3 \pm 0.06$	$2.3 \pm 0.07$	$8.3 \pm 0.10$	$0.140 \pm 0.006$
Day 1	1000	$1.1 \pm 0.04$	$2.0 \pm 0.06$	$8.0 \pm 0.15$	$0.130 \pm 0.008$
Day 1	10000	$0.9 \pm 0.03$	$1.8 \pm 0.05$	$7.5 \pm 0.11$	$0.120 \pm 0.007$
Day 2	10	$1.3 \pm 0.06$	$2.5 \pm 0.09$	$8.7 \pm 0.13$	$0.155 \pm 0.004$
Day 2	100	$1.4 \pm 0.07$	$2.4 \pm 0.10$	$8.5 \pm 0.12$	$0.145 \pm 0.005$
Day 2	1000	$1.2 \pm 0.05$	$2.1 \pm 0.07$	$8.1 \pm 0.10$	$0.135 \pm 0.006$
Day 2	10000	$1.0 \pm 0.03$	$1.9 \pm 0.06$	$7.6 \pm 0.14$	$0.125 \pm 0.005$
Day 3	10	$1.4 \pm 0.07$	$2.6 \pm 0.08$	$8.8 \pm 0.15$	$0.160 \pm 0.005$
Day 3	100	$1.5 \pm 0.08$	$2.5 \pm 0.09$	$8.6 \pm 0.11$	$0.150 \pm 0.006$
Day 3	1000	$1.3 \pm 0.05$	$2.2 \pm 0.07$	$8.2 \pm 0.12$	$0.140 \pm 0.007$

Day 3	10000	1.1 ± 0.04	2.0 ± 0.05	7.7 ± 0.12	0.130 ± 0.005
Day 4	10	1.5 ± 0.06	2.7 ± 0.09	8.9 ± 0.16	0.165 ± 0.004
Day 4	100	1.6 ± 0.09	2.6 ± 0.07	8.7 ± 0.14	0.155 ± 0.007
Day 4	1000	1.4 ± 0.05	2.3 ± 0.08	8.3 ± 0.15	0.145 ± 0.005
Day 4	10000	1.2 ± 0.03	2.1 ± 0.06	7.8 ± 0.11	0.135 ± 0.006
Day 5	10	1.6 ± 0.07	2.8 ± 0.10	9.0 ± 0.17	0.170 ± 0.005
Day 5	100	1.7 ± 0.08	2.7 ± 0.09	8.8 ± 0.12	0.160 ± 0.007
Day 5	1000	1.5 ± 0.06	2.4 ± 0.07	8.4 ± 0.14	0.150 ± 0.006
Day 5	10000	1.3 ± 0.04	2.2 ± 0.05	7.9 ± 0.13	0.140 ± 0.005

### 3.2.1 Protein Content

The protein content of *Wolffia* sp. steadily increased in the lower concentrations of Rapidus-50 across the five-day period. On day 1, the protein content at 10  $\mu\text{L}$  was 1.2 mg/g, increasing to 1.6 mg/g by day 5. This increase was observed across all concentrations, although the rate of increase was slightly higher at lower concentrations. The highest protein content was recorded at 100  $\mu\text{L}$  on day 5, at 1.7 mg/g. At 10000  $\mu\text{L}$ , the protein content remained lower throughout the experiment, reaching only 1.3 mg/g by day 5. Figure (3.1), illustrates the pattern of protein content changes, showing that higher concentrations of Rapidus-50 resulted in a slower increase in protein synthesis.

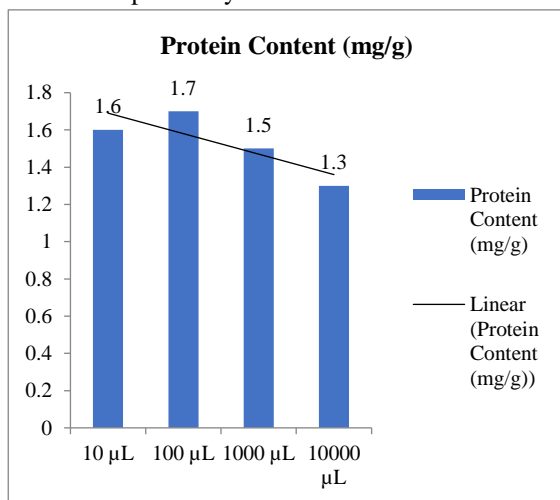


Fig. 3.1: Protein content of *Wolffia* sp. at Day 5

### 3.2.2 Chlorophyll Content

Chlorophyll content showed a clear trend of reduction as Rapidus-50 concentration increased. At day 1, chlorophyll content at 10  $\mu\text{L}$  was 2.4  $\mu\text{g/mL}$ , and it increased to 2.8  $\mu\text{g/mL}$  by day 5. However, at higher concentrations, such as 10000  $\mu\text{L}$ , chlorophyll content declined from 1.8  $\mu\text{g/mL}$  on day 1 to 2.2  $\mu\text{g/mL}$  by day 5. This reduction suggests that higher concentrations of Rapidus-50 have a negative impact on photosynthesis in *Wolffia* sp., possibly by disrupting chloroplast function. (Figure 3.2)

highlights the overall decrease in chlorophyll content at higher concentrations.

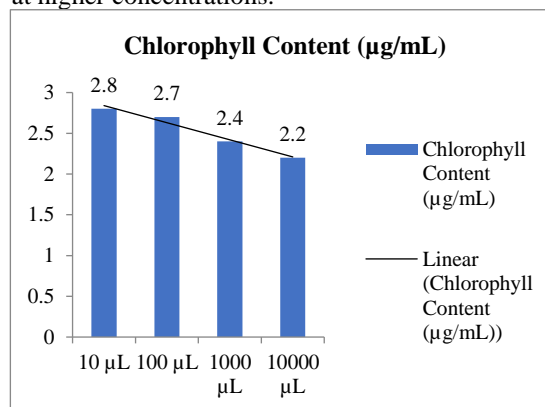


Fig. 3.2: Chlorophyll content of *Wolffia* sp. at Day 5

### 3.2.3 Lipid Content

The lipid content also displayed significant changes across the five-day period, with lower lipid accumulation at higher concentrations. On day 1, the lipid content at 10  $\mu\text{L}$  was 8.6%, increasing slightly to 9.0% by day 5. At 10000  $\mu\text{L}$ , lipid content decreased from 7.5% on day 1 to 7.9% on day 5. These results suggest that lower concentrations of Rapidus-50 enhance lipid synthesis, whereas higher concentrations inhibit it. (Figure 3.3) visualizes these findings, showing the decline in lipid content as the concentration of Rapidus-50 increases.

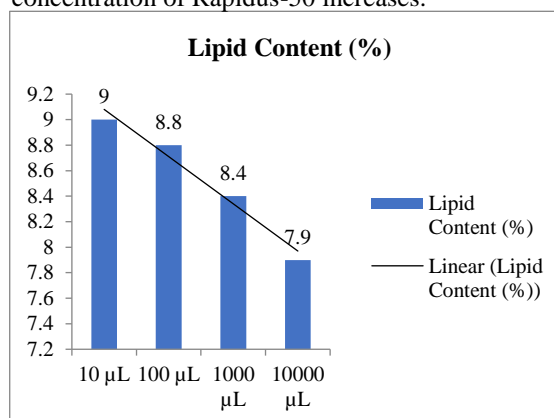


Fig 3.3: lipid content of *Wolffia* sp. at day 5

### 3.2.4 Absorbance at 750 nm

Absorbance at 750 nm, which measures the turbidity and potential pigment accumulation, decreased with higher concentrations of Rapidus-50. Absorbance values decreased from 0.150 at 10  $\mu$ L on day 1 to 0.170 by day 5, while at 10000  $\mu$ L, absorbance decreased from 0.120 on day 1 to 0.140 by day 5. These results indicate that lower concentrations of Rapidus-50 promote better pigment formation, potentially due to reduced cellular stress.

### 4.0 DISCUSSION

The noticed reduction in chlorophyll points to limitation of photosynthesis, most likely resulting from oxidative stress or chloroplast damage, a phenomena recorded in related research involving aquatic plants subjected to pharmaceuticals (Zhao et al., 2022; Sosa et al., 2024). Reduced chlorophyll content is usually associated with the breakdown of thylakoid membranes or disturbance of chlorophyll production enzymes, therefore compromising light absorption and energy conversion (Bernier et al., 2024). Similarly, the reduced lipid production seen in higher concentration treatments with Rapidus-50 indicates potential metabolic disruption, possibly involving enzymatic pathways regulating fatty acid synthesis or NADPH availability (Arif, 2020; Baek et al., 2021). In plant cells, these alterations may influence energy storage and membrane integrity. Further corroborating the current findings, Baek et al. (2021) emphasised that lipid alterations in duckweeds reflect physiological stress. The results line up with other studies on duckweed species such *Lemna* and *Wolffia*, which demonstrate sensitivity to environmental toxins and micropollutants. Low amounts of pharmaceutical residues can clearly affect physiology and growth, according to Appenroth et al. (2023) and Gatidou et al. (2023). Therefore, the physiological defects found in *Wolffia* sp. subjected to Rapidus-50 fit known toxicological reactions in related aquatic angiosperms. For protein at higher concentrations (especially 10000  $\mu$ L), protein accumulation was consistently lower, indicating a possible toxic or inhibitory effect of Rapidus-50 at elevated doses. The reduced protein content at this concentration suggests stress or impaired metabolic function, possibly due to chemical overload or physiological disruption.

In general, these physiological disturbances could lower oxygen generation, impede nutrient cycling and affect aquatic food webs, thus highlighting the importance of ecological surveillance and pharmaceutical waste control (Zhang et al., 2024; Gatidou et al., 2023).

### 5.0 CONCLUSION

The results of this study show that Rapidus-50 influences the physiological properties of *Wolffia* sp. in line with its concentration. Low amounts may somewhat accelerate the synthesis of lipids and chlorophyll but increases in dosage significantly impede these processes. The results show that the plant is definitely sensitive to pharmaceutical pollutants thereby indicating that it is fit for use as an experimental organism for aquatic toxicity research.

### 6.0 ACKNOWLEDGEMENT

The authors are very grateful to the Tertiary Education Trust Fund (TETFund, Nigeria) for funding support through the Institutional Based Research (IBR) intervention (TETF/DR&D/CE/UNI/ZAMFARA/IBR/2025/VO L.I).

### REFERENCES

- Ahmed, M., Yusuf, A. A., & Okonkwo, J. O. (2021). Occurrence, fate, and ecotoxicological risk of diclofenac in African aquatic systems: A review. *Environmental Science and Pollution Research*, 28(16), 20334–20352. <https://doi.org/10.1007/s11356-020-11858-6>
- Appenroth, K.-J., Sree, K. S., Bog, M., Ecker, J., Seeliger, C., Böhm, V. & Tolzin-Banasch, K. (2023). Nutritional value of duckweeds (Lemnaceae) as human food. *Frontiers in Chemistry*, 11, Article 1134924. <https://doi.org/10.3389/fchem.2023.1134924>
- Appenroth, K.-J., Sree, K. S., Bog, M., Ecker, J., Seeliger, C., Böhm, V., ... & Tolzin-Banasch, K. (2023). Nutritional value of duckweeds (Lemnaceae) as human food. *Frontiers in Chemistry*, 11, Article 1134924. <https://doi.org/10.3389/fchem.2023.1134924>
- Arif, A. (2020). Water pollution and industries. *Pure and Applied Biology*, 9(4), 2072–2081. <https://doi.org/10.19045/bspab.2020.90219>
- Baek, G. Y., Saeed, M., & Choi, H. K. (2021). Duckweeds: Their utilization, metabolites and cultivation. *Applied Biological Chemistry*, 64(1), 1–17. <https://doi.org/10.1186/s13765-021-00644-z>
- Bernier, M.-È., Thibodeau, J., & Bazinet, L. (2024). Enzymatic hydrolysis of water lentil (duckweed): An emerging source of proteins for the production of antihypertensive fractions. *Foods*, 13(2), 323. <https://doi.org/10.3390/foods13020323>
- Farner, J. M., Margolis, A., & Wilkinson, J. L. (2023). Mapping global pharmaceutical pollution in rivers. *Nature*, 602, 483–488. <https://doi.org/10.1038/s41586-022-04584-2>



- Gatidou, G., Oursouzidou, M., Stefanatou, A., & Stasinakis, A. S. (2023). Fate of antimicrobials and benzotriazoles in duckweed (*Lemna minor*) wastewater treatment systems. *Science of the Total Environment*, 896, 165284. <https://doi.org/10.1016/j.scitotenv.2023.165284>
- Sharma, R., & Kumar, R. (2022). Toxicological effects of NSAIDs on aquatic plants: A review. *Aquatic Toxicology*, 248, 106232. <https://doi.org/10.1016/j.aquatox.2022.106232>
- Sosa, L., Liu, Y., & Smith, D. (2024). Duckweed protein: Extraction, modification, and potential application. *Journal of Agricultural and Food Chemistry*. Advance online publication.
- Zhang, X., & Rai, P. K. (2024). Heavy metals/-metalloids (As) phytoremediation with *Landoltia punctata* and *Lemna* spp.: Coupling with biorefinery prospects for sustainable phytotechnologies. *Environmental Science and Pollution Research International*, 31(11), 16216–16240. <https://doi.org/10.1007/s11356-024-32177-5>
- Zhao, Z., Thingujam, D., Mukhtar, M. S., Pajerowska-Mukhtar, K. M., & Farid, M. (2022). Physiological response, microbial diversity characterization, and endophytic bacteria isolation of duckweed under cadmium stress. *Chemosphere*, 304, 135262. <https://doi.org/10.1016/j.chemosphere.2022.135262>