

MOLECULAR DOCKING STUDY, ANTIOXIDANT ACTIVITY, PROXIMATE CONTENT, AND TOTAL PHENOL OF *Lemna perpusilla* Torr. GROWN IN SUMEDANG, WEST JAVA, INDONESIA

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ABSTRACT

Water lentils or duckweeds (Lemnoideae, Araceae family) grow abundantly in the tropical aquatic environments of West Java, Indonesia. There are several types of Lemna plants, however, in this study, we explored *Lemna perpusilla* Torr. grown in Ciparanje Pond, Sumedang, West Java, Indonesia for its potential to be utilized as a fish feed supplement. The antioxidant activity of this small-sized plant is believed to belong to the phenolic compounds or other active secondary metabolites contained in the plant. Methods used were molecular docking study, the proximate analysis, antioxidant activity using DPPH reagent, and determination of the total phenolic compounds of the plant. The molecular docking simulation was performed between zeaxanthin, lutein, carotenoid, and stigmasterol with the NADPH-dependent human carbonyl reductase-1 (hCBR-1), an enzyme that works by protecting cells against cellular damage resulting from oxidative stress. Phytochemical screening showed that our Lemna positively contains alkaloids, flavonoids or carotenoids, polyphenols, tannins, triterpenoids, and steroids. Molecular docking simulation revealed that stigmasterol, lutein, zeaxanthin, and carotenoid in this plant could interact with important residues in the catalytic site of hCBR-1. The proximate analysis indicated a high content of crude protein and fat. The antioxidant activity conveyed a strong activity which is parallel with its total phenolic compounds content. It can be concluded that *Lemna perpusilla* Torr. grown in Ciparanje Pond, Sumedang, West Java, Indonesia is the potential to be utilized as a fish feed supplement.

Keywords: Antioxidant Activity, Duckweeds, *Lemna perpusilla* Torr., Flavonoids, Phenolic Compounds, Fish Feed.

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INTRODUCTION

The free radicals, which play the main role in inducing oxidative stress in the body, are classified according to their corresponding atoms, eg. the reactive oxygen species (ROS) and the reactive nitrogen species (RNS). ROS is produced from the enzymatic reaction within the body, particularly the cellular enzyme NADPH oxidase. Moreover, external factors such as air pollutants and saturated-fatty acids contained environmental stress can also trigger the production of ROS. RNS, in the form of nitric oxide (NO•), is generally created from the oxidation of L-arginine. Antioxidants are molecules that work to neutralize reactive radicals (ROS and RNS) and block or prevent cell damage. Natural antioxidant systems are categorized into enzymatic and non-enzymatic antioxidant groups. The enzymatic antioxidants include catalase and glutathione peroxidase, while the latter are e.g. ascorbic acid, carotenoids, flavonoids, and phenolic compounds.¹

The NADPH-dependent human carbonyl reductase-1 (hCBR-1) (Fig.-1) contributes prominently to the metabolism of endogenous and xenobiotic carbonyl-containing compounds.² This enzyme works by

protecting cells against cellular damage resulting from oxidative stress.³ Its known cofactor is glutathione (GSH).² Interestingly, a recent study reported that flavonoids could interact with hCBR-1 by inhabiting the hydrogen-binding sites adjacent to Ser139 and Cys226, Met234 and Tyr193 or Trp229. These flavonoids also built aromatic–aromatic stacking with Tyr193, Trp229, or NADPH, and van der Waals interaction with Ile140.⁴

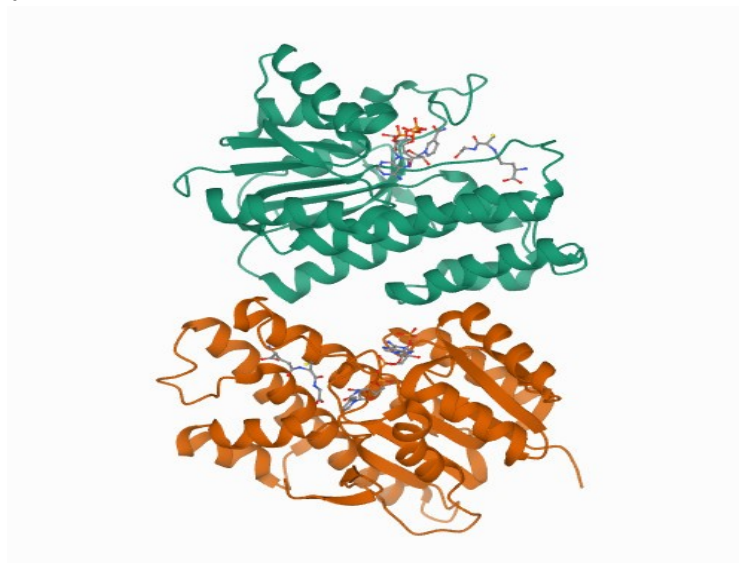


Fig.-1: Human Carbonyl Reductase-1 (PDB ID: 4Z3D) Downloaded from <https://www.rcsb.org/structure/4Z3D>

Many plants have been explored for their secondary metabolites content,⁵ among them are water lentils. Water lentils or duckweeds (Lemnoideae, Araceae family), defined as cosmopolitan plants, grow abundantly in the tropical aquatic environments, particularly in Indonesia.⁶ There are several types of Lemna plants, e.g. *L. aequinoctialis* Welw., *L. perpusilla* Torr., *L. tenera* Kurz, *L. disperma* Hegelm., *L. ecuadoriensis* Landolt, *L. gibba*, *L. japonica* Landolt, *L. minor*, *L. obscura* (Austin) Daubs, *L. trisulca*, *L. turionifera* Landolt, *L. minuta* Kunth, *L. valdiviana* Phil., and *L. yungensis* Landolt. These small-sized plants are the fastest-growing by floating widely on the water and occupying almost all of the lakes or ponds.^{7,8} Due to their protein-richness and other chemical content, water lentils are considered propitious sources for various utilities.⁹ Previous studies of Lemna species confirmed the presence of carotenoids, e.g. zeaxanthin, lutein, and polyphenolic compounds.^{10,11} Moreover, various phenolic compounds, such as caffeic acid, ferulic acid, sinapic acid, ρ -coumaric acid, and phytosterols (campesterol, stigmasterol, β -sitosterol) were also reported contained in *L. paucicostata*.^{12,13}



Fig.-2: (a) *L. perpusilla* Torr. at the Culture Pond; (b) *L. perpusilla* Torr. at 6th Days Culture; (c) Ventral View of *L. perpusilla* Torr.

Duckweeds, particularly *L. perpusilla* Torr., grow rapidly covering the surface of the ponds surrounding Sumedang, West Java, Indonesia. This work aimed to study the potential of *L. perpusilla* Torr. grown in Ciparanje Pond, located in Sumedang, West Java, Indonesia (Fig.-2) to be utilized for fish feed

supplement, by determining its antioxidant activity, the molecular interaction between the phenolic and steroid compounds in the plant with NADPH-dependent human carbonyl reductase-1, the proximate analysis, and total phenolic content.

EXPERIMENTAL

Study Area

This work was carried out at (1) the Faculty of Fisheries and Marine Sciences Universitas Padjadjaran (May 2019-January 2020) and (2) the Center for Computational Research, Faculty of Pharmacy, Universitas Padjadjaran (October-December 2021).

Hardware and Software

The hardware used was MacBook Pro (13-inch, M1, 2020) embedded with macOS Monterey, processor chip Apple M1, memory 8 GB. Software used was MarvinSketch 17.11.0 (Academic License), LigandScout 4.1.4 (Universitas Padjadjaran License), AutoDock 4.2 (Molecular Graphics Laboratory The Scripps Research Institute, downloaded from <http://autodock.scripps.edu>), MacPyMOL: PyMOL 1.7.4.5 Edu.

Preparation of Ligands and Molecular Docking Simulation

The X-ray crystallographic 3D structure of Human Carbonyl Reductase-1 (PDB ID: 4Z3D) was downloaded from the online Protein Data Bank <https://www.rcsb.org/structure/4Z3D>.² The 2D structure of the ligands (Fig.-3) was generated using MarvinSketch of ChemAxon, then converted to the 3D structure by applied energy minimization using MMFF94 forcefield partial charges in LigandScout. The physicochemical properties (Lipinski Ro5) were predicted using LigandScout.¹⁴ Molecular docking validation was carried out by re-docking the nature ligand (GSH) into the active pocket of hCBR-1. The grid box position and size of the active site were determined automatically by the LigandScout software based on the position of GSH. Molecular docking simulation was carried out for the bioactive compounds towards the catalytic site of hCBR-1. Molecular docking simulation was performed by employing AutoDock 4.2 which is embedded in the LigandScout program. At the end of docking, the structures were ranked by energy, as were the clusters, and the binding affinity of the receptor/ligand complexes was expressed in terms of docking scores.¹⁴

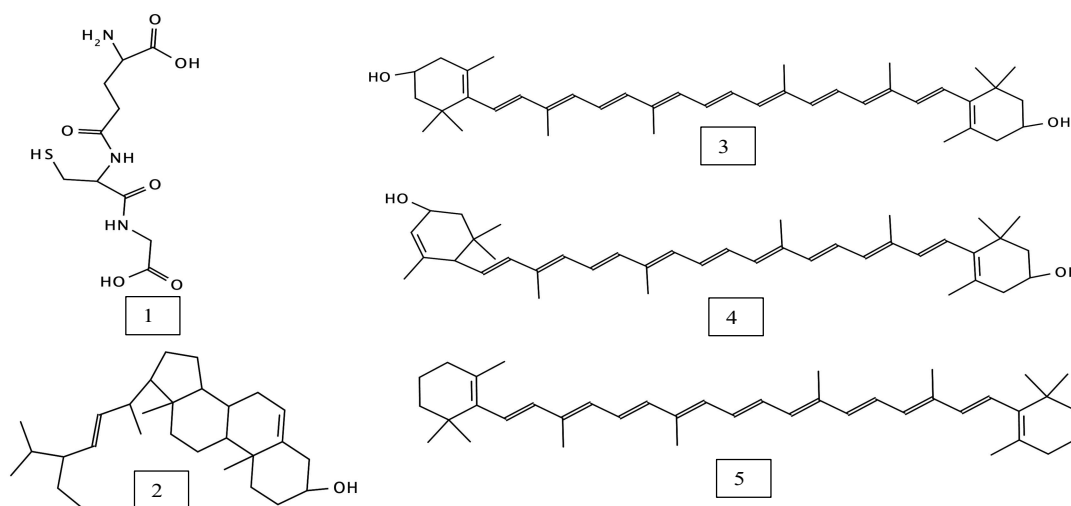


Fig.-3: 2D Structure of (1) Glutathione; (2) Stigmasterol; (3) Zeaxanthin; (4) Lutein; (5) Carotenoid

Instruments

Instruments utilized in this study were digital analytical balance (Sartorius® BP 221 S), microplate reader (BioTek Epoch™), microplate 96-well (NEST®), micropipette (Socorex Acura® 20-200 µL, and Eppendorf® 10-100 µL), micro tips (Gilson), and analytical glassware.

Materials

The plants were obtained from Ciparanje Pond, Faculty of Fisheries and Marine Sciences, Universitas Padjadjaran, Indonesia. The plant samples were identified at the Herbarium Bandungense (FIPIA) SITH Bandung Institute of Technology (ITB), Indonesia (5245/IT1.C11.2/TA.00/2021).

Extraction

Extraction of the plants, using 722 kg of wet *L. perpusilla* Torr. (Fig.-2), was carried out by following previous procedures with a few modifications.¹⁵⁻¹⁷

Proximate Analysis

Proximate content was analyzed according to the protocol of the Indonesian National Standard SNI 01-2891-1992.

Phytochemical Screening

The plant extracts were screened for the presence of secondary metabolites using standard procedures of analysis.¹⁸

DPPH Radical Scavenging Assay

The antioxidant activity of the plant extracts was investigated by using DPPH radical scavenging assay. This assay is based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants. The experiment was carried out in triplicates. Quercetin was used as the standard. The IC₅₀ was calculated by using GraphPad Prism 8.4.0.¹⁸

RESULTS AND DISCUSSION

The prediction of physicochemical properties of the bioactive compounds indicated that all compounds are hydrophobic, and the most hydrophilic compound is stigmasterol (cLogP=7.801), followed by lutein (cLogP=10.403), zeaxanthin (cLogP=10.547), and carotenoid (cLogP=12.606), respectively. Validation of the molecular docking simulation revealed that both GSH molecules overlapped with RMSD 1.273 Å (Fig.-4), which confirms the validity.

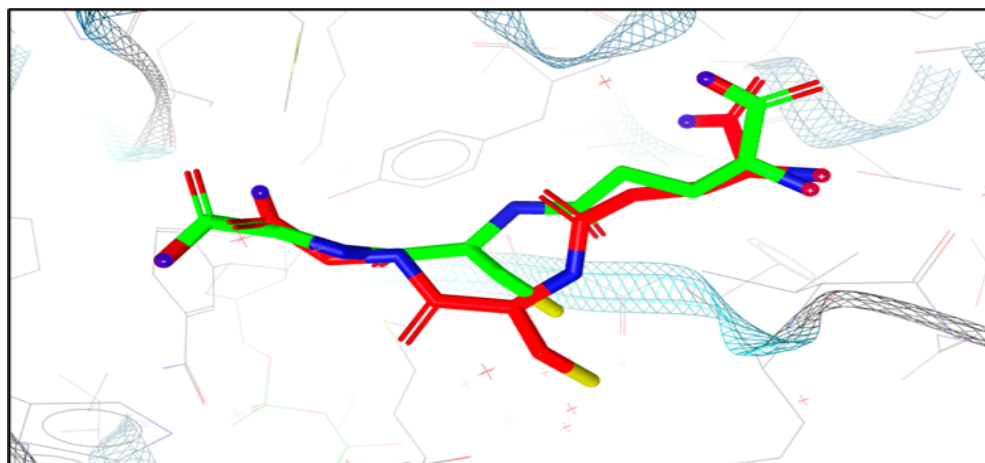


Fig.-4: Overlapped GSH Molecules (Coloured in Green and Red; RMSD value = 1.273 Å) which Confirms the Validity of the Molecular Docking Method

The molecular docking simulation results indicate that all compounds contained in the Lemna plant could occupy the catalytic site of hCBR-1, moreover, important residues, e.g. Tyr193, Trp 229, and NADPH, are also observed in the molecular docking result of all docked compounds, zeaxanthin (Fig.-5) reveals the best affinity (docking score -9.13 kcal/mol and Ki: 0.20 µM). Molecular docking simulation of stigmasterol, lutein, zeaxanthin, and carotenoid into the catalytic site of hCBR-1 in which GSH was cocrystallized, is presented in Table-1.

Interestingly, the binding modes of stigmasterol, lutein, zeaxanthin, and carotenoid are similar to those reported previously by Carloquist and co-workers.⁴ Carloquist and co-workers confirmed that flavonoids

interact with hCBR-1 by settling in the hydrogen-binding sites adjacent to certain residues, such as Tyr193 or Trp229, and also built aromatic–aromatic stacking with Tyr193, Trp229, or NADPH.

Table-1: The Binding Mode and Affinity of the Ligands in Catalytic Site of hCBR-1

No.	Ligand	Binding Affinity in terms of Docking Score (kcal/mol), inhibition constant (Ki), and Amino Acid Residues Involved in the Interaction
1.	Glutathione (GSH)	Docking score: -7.57 kcal/mol Ki: 2.84 μ M Hydrogen bond: Phe94, Val96, Gln105, Thr109, Tyr193 Amino acid residues involved in the hydrophobic interaction: Val96, Met141, Tyr193, Trp229
2.	Stigmasterol	Docking score: -7.18 kcal/mol Ki: 5.48 μ M Hydrogen bond: Not detected Amino acid residues involved in the hydrophobic interaction: Val96, Met141, Tyr193, Trp229
3.	Lutein	Docking score: -8.16 kcal/mol Ki: 1.05 μ M Hydrogen bond: Not detected Amino acid residues involved in the hydrophobic interaction: Ile92, Ala93, Phe94, Lys95, Val96, Thr109, Ile140, Met141, Trp229, Ala235
4.	Zeaxanthin	Docking score: -9.13 kcal/mol Ki: 0.20 μ M Hydrogen bond: Cys226 Amino acid residues involved in the hydrophobic interaction: Ile92, Ala93, Phe94, Lys95, Val96, Thr109, Ile140, Met141, Tyr193, Trp229, Ala235, NADPH
5.	Carotenoid	Docking score: -8.33 kcal/mol Ki: 0.79 μ M Hydrogen bond: Not detected Amino acid residues involved in the hydrophobic interaction: Ile92, Ala93, Phe94, Lys95, Val96, Met141, Trp229, Ala235

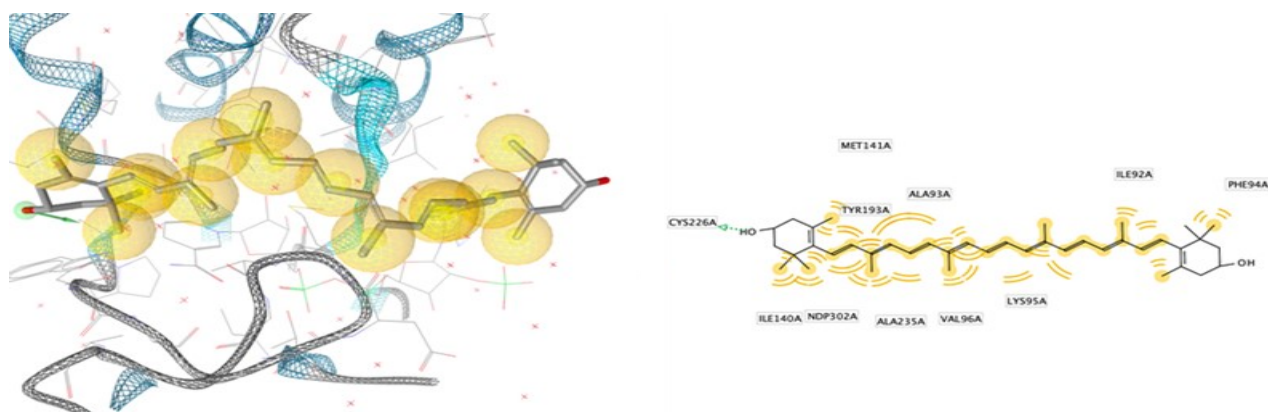


Fig.-5: Molecular Interaction of Zeaxanthin with Important Amino Acid Residues in the Catalytic Site of hCBR-1

Furthermore, the extraction of 722 kg wet *L. perpusilla* Torr. resulted in 28.21 g (3.65% w/w) of a concentrated extract. This extract was further analyzed for its proximate content, antioxidant activity, and total phenolic compounds. The proximate composition of *L. perpusilla* Torr. is presented in Table-2.

The proximate analysis indicated that our *L. perpusilla* Torr. contains a higher percentage of crude protein and fat compared to those of reported *L. gibba* obtained from the middle basin of Papaloapan River, Southeast Mexico.¹⁹ Another advantage of our Lemna is that the ash content is lower than the other Lemna sp. The high content of crude protein and fat in our Lemna is beneficial for its prospect to be utilized as a fish feed supplement. The phytochemical screening of *L. perpusilla* Torr. resulted in the

presence of alkaloids, flavonoids or carotenoids, polyphenols, tannins, steroids, and triterpenoids. Additionally, the total phenolic compounds in *L. perpusilla* Torr. is 18.5 mg/L and the strong antioxidant activity of *L. perpusilla* is confirmed by its IC_{50} at 54.51 ppm (Fig.-6).

Table-2: Proximate Content in *L. perpusilla* Torr.

Proximate Content of <i>L. perpusilla</i> Torr.	
Energy (kcal/kg)	3412
Ash (%)	14.56
Crude protein (%)	24.93
Fat (%)	5.11
Total dietary fiber (TDF) (%)	13.40

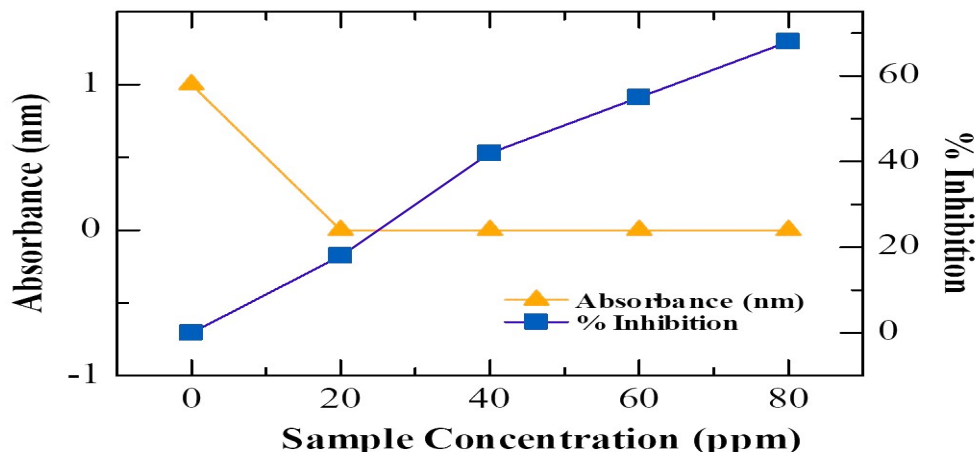


Fig.-6: Percentage of Inhibition Extract of *L. perpusilla* Torr. Using DPPH Reagent Method

Our result is compared to those of previous studies. The presence of carotenoids, e.g. zeaxanthin and lutein, and polyphenolic compounds were reported in Lemna plants.^{10,11} Moreover, various phenolic compounds, such as caffeic acid, isoferulic acid, sinapic acid, p -coumaric acid, and phytosterols (campesterol, stigmasterol, β -sitosterol) were also reported contained in *L. paucicostata*.^{12,13} Flavonoids and phenolic compounds are well recognized as plant secondary metabolites that possess an aromatic ring with a minimum of one hydroxyl group attached. These compounds have been announced for their capability in scavenging radicals, and thus can work as antioxidants.²⁰ Flavonoids can function as antioxidants, immunomodulators, and anti-inflammatory agents, while some other substances are thought to increase immunity, i.e. vaccines, immunostimulants, probiotics, and antioxidants.²¹ Active compounds, especially flavonoids, carotenoids, and amino acids, of plant extracts including the extract from Lemna plants are able to play roles in stimulating leukocytes as a non-specific defense, hence functioning as immunostimulants.²² Our study is compared to a previous study, that confirmed the antioxidant activity of both water extract and ethanol extract of *L. minor* using various methods (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid radical scavenging, 1,1-diphenyl-2-picryl-hydrazyl free radical scavenging, total antioxidant activity by ferric thiocyanate, total reducing power by potassium ferricyanide reduction method, superoxide anion radical scavenging, hydrogen peroxide scavenging, and ferrous ions chelating activities).²³ Increasing sample concentration and decreasing absorbance values will increase the % inhibition value which can scavenge DPPH free radicals. The smaller IC_{50} value indicates higher antioxidant activity. The antioxidant levels of a compound based on IC_{50} values are: very strong ($IC_{50} < 50$ ppm), strong (IC_{50} 50-100 ppm), medium (IC_{50} 100-150 ppm), and weak ($IC_{50} > 150$ ppm).²⁴ However, fish farming is prone to diseases, e.g. stress and immunity in fish, which are caused by the interaction between fish and an unbalanced environment. Fish is very susceptible to ROS which can damage the fish tissue. To prevent that, fish must be fed with effective antioxidants. *Lemna* sp contains more flavonoids, vitamin C, and vitamin E. Vitamin E as an antioxidant works by protecting fat or fatty acids contained in cell membranes to remain unoxidized.²⁵

CONCLUSION

Tropical aquatic plant *Lemna perpusilla* Torr. grown in Ciparanje Pond, Sumedang, West Java, Indonesia confirmed its strong antioxidant activity by scavenging the DPPH radical. The phytochemical screening of *L. perpusilla* Torr. resulted in the presence of alkaloids, flavonoids or carotenoids, polyphenols, tannins, steroids, and triterpenoids. The total phenolic content is 18.5 mg/L. Molecular docking simulation revealed that stigmasterol, lutein, zeaxanthin, and carotenoid in this plant could interact with important residues in the catalytic site of human carbonyl reductase-1 (hCBR-1). Zeaxanthin shows the best affinity by building hydrophobic interaction with Tyr193, Trp229, and NADPH. Moreover, the proximate analysis indicated a high content of crude protein and fat. Based on this finding, *Lemna perpusilla* Torr. can be used as a potential source of antioxidants as well as a good supplement for fish feed.

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