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Optimization of the Extraction of Polyphenolic Compounds from Oil palm Bunches and their Potential Application in Fish Feed

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ABSTRACT

Oil palm bunch is one of the biggest agroindustry wastes in Indonesia. Polyphenolic compounds are compounds that could be found in many plants and obtained through extraction, including extraction of oil palm bunch. Polyphenolic compounds have various benefits, including antioxidant properties. This study aims to determine the solvent concentration and the solvent to mass ratio in the extraction of polyphenols from oil palm bunches and then formulate it in fish feed to study the resulting effects. From this research, we found that 50% methanol solvent is the most optimal for oil palm bunches extraction with Total Phenolic Content (TPC) 1.56±0.125 mg Gallic Acid Equivalent (GAE) / g sample, antioxidant activity 3.08±0.08 µmol GAE / g sample (DPPH), and antioxidant activity 3.13±0.96 µmol GAE / g sample (FRAP). We also found that 40:1 is the most optimal extraction mass ratio with TPC 1.62±0.07 mg GAE / g sample, antioxidant activity 5.07±0.09 µmol GAE / g sample (DPPH), and antioxidant activity 3.82±0.35 µmol GAE / g sample (FRAP). When used in fish feed, polyphenols do not affect the length, growth, and mass of molly fish under stress conditions.

Keywords: antioxidant, extraction, molly fish, palm bunches, polyphenols

Abstrak

Tandan kelapa sawit merupakan salah satu limbah agroindustri terbesar di Indonesia. Senyawa polifenol merupakan senyawa yang banyak terdapat pada berbagai bagian tumbuhan dan dapat diperoleh lewat proses ekstraksi, termasuk ekstraksi tandan kelapa sawit. Senyawa polifenol memiliki berbagai manfaat, salah satunya memiliki sifat antioksidan. Penelitian ini bertujuan untuk menentukan konsentrasi pelarut dan rasio pelarut : massa yang paling optimal dalam proses ekstraksi senyawa polifenol dari tandan kelapa sawit kemudian memformulasikannya pada pakan ikan dan mempelajari efek yang dihasilkan. Hasil penelitian menunjukkan bahwa pelarut metanol 50 % merupakan konsentrasi pelarut yang paling optimal dengan kadar total fenolik 1,556 ± 0,125 mg Gallic Acid Equivalent (GAE) / g sampel, aktivitas antioksidan sebesar 3,084 ± 0,084 µmol GAE / g sampel (DPPH), dan aktivitas antioksidan 3,128 ± 0,958 µmol GAE / g sampel (FRAP). Rasio 40 : 1 adalah rasio yang paling optimal dengan kadar total fenolik 1,624 ± 0,073 mg GAE / g sampel, aktivitas antioksidan 5,074 ± 0,090 µmol GAE / g sampel (DPPH), dan aktivitas antioksidan 3,822 ± 0,354 µmol GAE / g sampel (FRAP). Ekstrak polifenol yang diperoleh juga berhasil diformulasikan dalam pakan ikan, namun tidak memberikan pengaruh pada pertumbuhan panjang serta massa ikan molly dalam kondisi stress.

***Kata Kunci**: antioksidan, ekstraksi, ikan molly, kelapa sawit, polifenol*

INTRODUCTION

Oil palm (*Elaeis guineensis*) is one of many plants cultivated in Indonesia as a source of cooking oil. Unfortunately, the bunches of this plant are usually discarded as waste during processing. Oil palm bunches contain high amount of cellulose, lignin, and lignocellulose which makes oil palm bunches degradation difficult (1). Polyphenolic compounds are one of the compounds contained in

oil palm bunches. Study shows that oil palm bunches contain 31.10 g GAE / mL of polyphenols (2). Consuming polyphenolic compounds is considered to have various benefits because of its good antioxidant and anti-Advanced Glycation End-Products (AGE) activity (3). To make better use of this waste, we attempt to extract polyphenols from oil palm bunches for further use cases.

In polyphenol extraction, study shown that different solvent concentrations and solvent volume to mass ratio can affect the polyphenol extraction result (4). A study has also shown that the Ultrasound-Assisted Extraction (UAE) method is good for polyphenol extraction (5). Optimization was carried out on solvent concentration and sample mass ratio to ensure that polyphenolic compounds can be obtained with high efficiency and cost-effectiveness.

Polyphenolic compounds are known to contain activity as natural antioxidant (6). Antioxidant like astaxanthin shows ability to help maintaining fish's cell condition from oxidative stress (7). In this study, polyphenols extracted from oil palm bunches were also experimented as an additive ingredient to fish feed, which is expected to provide better nutrition to molly fish for life quality improvement, especially in terms of resistance against stress conditions. This study aims to optimize the processing of oil palm bunches waste by extracting polyphenolic compounds, implementing polyphenol to fish feed and studying its resulting effects against stress conditions in fish life.

METHODS

This study was performed in Purification and Molecular Laboratory and Food Biotechnology Laboratory, Faculty of Technobiology, University of Surabaya.

Preparation and solvent optimization

Oil palm bunches waste bought from oil palm garden in Sampit, Central Kalimantan were cleaned with distilled water and cut into small pieces. Pieces of oil palm bunches were heated at 50°C for 3 days in cabinet dryer. Dry pieces were blended and filtered with 40-mesh filter. Optimization of solvent concentration was performed using the UAE method with 50% ethanol, 60% ethanol, 70% ethanol, 50% methanol, 60% methanol, and 70% methanol as solvents variation. The extraction was done with 5 g of powder in 100 mL of solvent. The UAE process was performed at room temperature for 30 minutes. Stirring was done during UAE using magnetic bar (8). Extracts were analyzed for yield percentage, Total Phenolic Content (TPC), flavonoids, and antioxidant activity. After the optimization of solvent and its concentration, we compare different solvent to mass ratio (30:1, 40:1, and 50:1) to analyze yield percentage, TPC, flavonoids, and antioxidant activity.

Yield percentage analysis

Yield percentage was obtained by calculating the mass of the extract obtained after the extraction process is complete and the existing solvent is evaporated using a rotary evaporator machine until no solvent drips anymore (5). Calculation was done using formula

$$\text{Yield } \% = \frac{\text{Mass obtained}}{\text{Mass of initial extract}} \text{ x } 100\% \text{ (9).}$$

Total phenolic content analysis

TPC was analyzed using the Folin-Ciocalteu method with gallic acid as standard (8). A total of 0.1 mL extract was mixed with 0.2 mL reagent Folin-Ciocalteu, 2 mL of distilled water, and 1 mL sodium anhydrate 15% (w/v). The solution was incubated for 1 hour in dark room and analyzed with spectrofotometer at 765 nm. The calculation was done using the formula $\text{Total Fenolic} = \frac{\text{GAE (mg)}}{\text{sample dry mass (500 g)}}$ with GAE as Gallic Acid Equivalent (8)

Flavonoid content analysis

Flavonoid content was analyzed using catechin as standard (10). A total of 0.1 mL extract (1000 mg / L) was mixed with 0.15 mL $NaNO_2$ 15% and 0.15 mL $AlCl_3$ 10%. The solution was incubated for 6 minutes in room temperature. Solution was then mixed with 2 mL NaOH 1 M and diluted with distilled water until 5 mL. The solution was reincubated for 15 minutes in room temperature. After incubation, solution was analyzed with spectrophotometer at 510 nm. Calculation was done using $\text{Total Flavonoid} = \frac{\text{CE (mg)}}{\text{sample dry mass (500 g)}}$ with CE as Catechin Equivalent (10)

Antioxidant activity analysis

Antioxidant activity was analyzed by 2,2-diphenyl-1-picrylhydrazil (DPPH), Ferric Reducing Ability of

Plasma (FRAP), and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS). DPPH analysis was done by mixing 90 µL DPPH reagent 0.15 mM with 30 µL of extract. Solution was incubated for 30 minutes in dark condition and room temperature. After incubation, solution was analyzed with spectrophotometer at 575 nm. Control was created by mixing 4.9 mL DPPH and 0.1 mL methanol. Control was analyzed without incubation. The calculation was done using the formula $\text{Inhibition\%} = \frac{\text{CA}-\text{SA}}{\text{CA}}\ x\ 100\%$ with CA as control absorbance and SA as sample absorbance to calculate inhibition percentage and the formula $\text{Ant. Act.} = \frac{\text{GAE (mg)}}{\text{sample dry mass (500 g)}}$ with GAE as Gallic Acid Equivalent to calculate antioxidant activity (11)

FRAP analysis was analyzed with gallic acid as standard. First of all, a total of 187 mg $C_2H_3NaO_2.3H_2O$ was dissolved with 16 mL acetate buffed (pH 3.6) and diluted with distilled water until 250 mL. FRAP reagent was made by mixing 25 mL of natrium acetate trihydrate, 2.5 mL TPTZ (2,4,6-tripyridyl-s-triazine in 40 mmol / L HCl), and 2.5 mL $FeCl_3.6H_2O$ 20 mM (in distilled water) (12). After mixing, the reagent was diluted until 100 mL using distilled water. FRAP analysis was done by mixing 60 µL FRAP reagent and 60 µL of extract. Solution was analyzed with spectrophotometer at 593 nm. The calculation was done using the formula $\text{Ant. Act.} = \frac{\text{GAE (mg)}}{\text{sample dry mass (500 g)}}$ with GAE as Gallic Acid Equivalent to calculate antioxidant activity (10).

ABTS analysis was analyzed with gallic acid as standard. First, ABTS stock 7.4 mM and potassium persulfate 2.6 mM was mixed in 1:1 ratio and incubated for 12 hours in dark condition. A total of 150 µL extract and 2850 µL reagent was mixed and incubated for 2 hours in dark condition. Solution was analyzed with spectrophotometer at 734 nm (13). The calculation was done using $\text{Inhibition\%} = \frac{\text{CA}-\text{SA}}{\text{CA}}\ x\ 100\%$ with CA as control absorbance and SA as sample absorbance to calculate inhibition percentage (10).

Fish growth analysis

Polyphenols were incorporated by spraying 5 mL of the extract into 5 g of commercial fish feed, which was then dried for 15 minutes in closed container. Fish growth was monitored in molly fish (*Poecilia spehnops*) aged about two months. All fish were purchased at the Gunungsari Surabaya fish market center. Each test batch consisted of 20 fishes.

Analysis was done in three different salt concentrations, which is 15%, 30%, and 0% as control (14). Salt concentration was created using commercial salt. Reverse osmosis water was used in every tank for the fish to live, which was drained and replaced every 1 week. The temperature used for fish growth is room temperature (27–30^{o}C) Body weight and length were analyzed every three weeks (15). (16)

Data analysis

Data analysis was performed using Shapiro-Wilk normality test, one-way ANOVA, and Tukey's significance test at α=0.05 with Minitab 18 Software (16). Each of the test in solvent optimization and solvent to mass ratio was done with 3 times repetition.

RESULT

Every data in the table are mean values ± SD and different letters indicate significant differences based on the results of the One-Way ANOVA test with a confidence level of 5%.

While there was no statistically significant variation in the percentage yield of the extract produced after extraction with different solvent types and concentrations, there was statistically significant difference between total phenolic compound analysis between solvent. The highest result came from 50% and 60% MeOH, which is significantly different from 60% EtOH, 70% EtOH, and 70% MeOH. Based on the results, MeOH 50% is considered the best solvent for UAE extraction of polyphenolic compounds from oil palm bunches due to needing less MeOH than 60% concentration (Table 1).

Analysis shows the best ratio for extraction process was 40:1, as the yield showed consistently optimal results (Table 2). Polyphenols extract with MeOH 50% as solvent with 40:1 solvent/mass ratio was used for mixing process of fish feed. However, results shows that the addition of polyphenolic compound extracts did not help fish grow in water conditions with high salinity (Figure 1).

Table 1. Several parameters used to select optimum solvent

Parameter	Solvent Concentration					
	EtOH			MeOH		
	50%	60%	70%	50%	60%	70%
% Extract yield	11.16± 0.72[a]	9.18± 0.86[a]	11.55± 1.88[a]	10.69± 0.83[a]	10.69± 0.83[a]	9.91± 0.68[a]
Total phenolic[1]	1.47± 0.08[a]	1.19± 0.14[b]	0.96± 0.07[b]	1.57± 0.07[a]	1.57± 0.07[a]	1.00± 0.08[b]
Total flavonoid concentration[2]	0.46± 0.10[a]	0.36± 0.02[ab]	0.26± 0.05[b]	0.27± 0.06[ab]	0.27± 0.06[b]	0.39± 0.05[ab]
% DPPH Inhibition	20.35± 5.07[abc]	10.58± 3.59[c]	27.4± 3.35[a]	21.68± 3.45[ab]	21.86± 3.45[ab]	17.37± 4.16[bc]
DPPH antioxidant activity[1]	2.06± 0.38[bc]	1.33± 0.27[c]	2.58± 0.25[ab]	3.01± 0.33[a]	3.01± 0.33[a]	2.60± 0.39[ab]
FRAP antioxidant activity[1]	2.87± 0.62[a]	3.00± 0.97[a]	3.01± 0.48[a]	2.06± 0.38[a]	2.06± 0.38[a]	2.18± 0.28[a]
% ABTS inhibition	5.24± 2.97[c]	29.52± 6.44[a]	15.7± 1.43[bc]	23.17± 4.40[a]	23.17± 4.40[ab]	21.14± 3.73[ab]

*1 (µmol GAE/500 g sample); 2 (mg CE/500 g sample)

Table 2. Several parameters used to select the optimum solvent/mass ratio

Parameter	Solvent: Mass ratio		
	30:1	40:1	50:1
% Extract yield	10.82±0.31[b]	17.06±1.56[a]	14.57±1.04[a]
Total phenolic[1]	1.39±0.14[b]	1.62±0.07[a]	1.58±0.04[ab]
Total flavonoid concentration[2]	0.35±0.04[b]	0.51±0.03[a]	0.25±0.03[c]
% DPPH inhibition	30.95±2.29[a]	34.66±1.43[a]	20.14±3.31[b]
DPPH antioxidant activity[1]	4.48±0.14[a]	5.07±0.09[a]	4.16±0.21[b]
FRAP antioxidant activity[1]	1.56±0.23[b]	3.82±0.35[a]	1.86±0.20[b]
% ABTS inhibition	31.45±21.3[ab]	42.47±6.05[a]	20.70±5.25[b]

*1 (µmol GAE/500 g sample); 2 (mg CE/500 g sample)

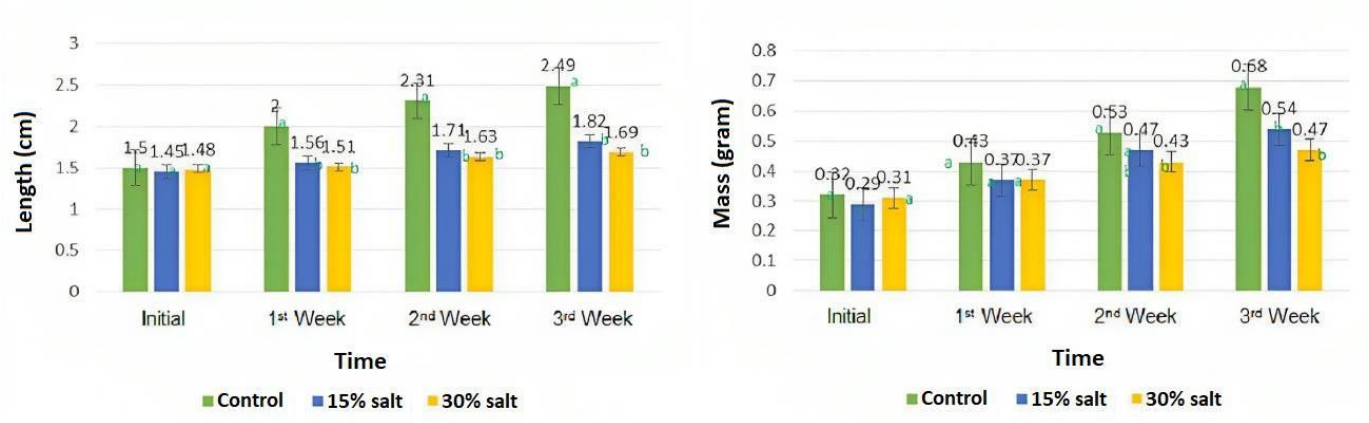

Figure 1. Effect of supplemented fish feed on mass and length growth of molly fish. Polyphenols used in fish feed supplement were obtained using 50% methanol as solvent with 40:1 solvent to mass ratio extraction.

DISCUSSION

The aim of this study was to find the optimal solvent concentration and solvent/mass ratio for the extraction of polyphenols from oil palm bunches, which were later also experimented in this study to be added to fish feed. UAE method was used for extraction because of its time efficiency, convenience of operation, and ability to break down the cell walls of oil palm bunches to improve solvent penetration (5,17).

Several factors were considered to select the most optimal solvent concentration, including extract mass, total phenolics, total flavonoids, and antioxidant activity that were measured using three methods, namely DPPH, FRAP, and ABTS. According to the data, there was no statistically significant variation in the percentage yield of the extract produced after extraction with different solvent types and concentrations. This can be caused by the chemical structure of the two types of solvents used being relatively similar to each other and the solvent concentration ranges also being similar (4).

Total phenolics analysis shows that the most optimal solvent is methanol 50% or 60% and ethanol 60%. These results contradict other study that said that the greater the concentration of organic solvent, the greater the total levels of phenolics and flavonoids obtained (18). These different results can be caused by the presence of water in organic solvent used. Water cause plant cell walls to swell, which will eventually burst and make it easier for the organic solvent to carry out mass transfer according to the like dissolves like principle (19).

Antioxidant analysis with DPPH, FRAP, and ABTS method also shows different results from literature. Literature shows that the greater the concentration of ethanol and methanol solvents used, the greater the antioxidant activity obtained (18,20). However, data shows that the use of 50% methanol solvent gave the most optimal results consistently and was equivalent to the total phenolics obtained, while the results with ethanol solvent using the ABTS method gave less than optimal results. This can be caused by the characteristics of the ABTS test which has a slow reaction time so that the reaction endpoint for each type of solvent can be different due to different antioxidant composition and activity (21–23). Data also shows that there are some examples of higher antioxidant activity result when measured using the DPPH method, while other data suggest higher antioxidant activity from FRAP method. If the antioxidant compounds are higher when measured using the DPPH method, it means that the antioxidant compounds contained in the extract are hydrophobic, while higher antioxidant from FRAP analysis shows more antioxidant compounds that are hydrophilic (24). ABTS method was carried out as the final confirmation method for the test results using the DPPH and FRAP methods. This was done because ABTS method can measure antioxidant compounds that are both hydrophilic and lipophilic, but sometimes gives less consistent results due to the unstable nature of the reagent (21). Based on all of these parameters results and economic consideration, methanol 50% was chosen as the optimum solvent and was used for mass ratio analysis.

The results of the optimum solvent : mass ratio for extraction obtained were in accordance with literature carrying out the optimization process for the extraction of oil palm seeds (5). The solvent : mass ratio of 40 : 1 provides consistent results for the yield mass of the extract, total phenolics, total flavonoids, to the antioxidant activity obtained. Result shows a decrease in yield at a ratio of 50:1 for several parameters tested, such as total flavonoids and antioxidant activity. This can occur because the ratio is too high, so the time required for the reaction occurring in the solvent to reach equilibrium (when reaction reaches the end point) will be longer (25). Research also states that the effect of the solvent : mass ratio in an extraction process can reach a saturation point and then the efficiency of the extraction process can be reduced (19). The most optimal polyphenol extraction results were then added to the fish feed for molly fish.

The purpose of using molly fish in this research is to improve the quality of this fish. Because this type of fish is widely used as ornamental fish, if the quality of this fish can be improved, its selling value can also be increased (26). In general, molly fish live in ideal conditions in fresh water with a salt concentration of 0 ppm (26). However, not all places have ideal water conditions for the growth of molly fish. Molly fish will have stunted growth and die in increasingly high salt water conditions (26). This happens because water conditions with high salt levels force fish to carry out the osmoregulation process more regularly in their cells, thus requiring a lot of energy and making the

nutrients they obtain not utilized optimally to support fish growth (15).

The antioxidant activity is known to helps fish cells to reduce the oxidative stress conditions (7). However, the results in Figure 1 showed that the addition of polyphenolic compound extracts did not help fish grow in water conditions with high salinity. This result can be caused by several factors, for example poor absorption rate of polyphenol compounds into the fish's digestive system, which is related to the chemical structure of the polyphenol compounds contained being too complex for fish to digest (27). Another influencing factor is level of compounds in the extract. Flavonoid content that is too high in polyphenolic compounds will not have an effect on fish growth, but will help increase fish immunity (28). These factors contributed to the results showing that fish growth in the control condition was more optimal, considering that the appropriate salt content in the control conditions did not cause stunted fish growth like in other salt treated tank (26).

There are many variables that were not carried out in the optimization process in the research, either in the extraction process, during feed formulation, or during fish rearing, which could also be a factor in the inability of feed formulated with polyphenolic compounds to increase the growth of molly fish. In the extraction process, factors such as temperature, time, and the age of oil palm bunches can also influence the extract results obtained (20,29). Finally, the age and gender of the fish can also influence fish growth (26).

CONCLUSION

Methanol 50% as a solvent with a solvent volume to mass ratio of 40:1 is optimal for polyphenol extraction from oil palm bunches based on total phenolic content, flavonoid, and antioxidant analyses. Fish feed with a polyphenol formula did not stimulate the length growth and mass of molly fish.

REFERENCE

[1] Puspaningsih NNT. Pengolahan Limbah Tandan Kosong Kelapa Sawit (TKKS) dengan Excelzyme. 2023 [Cited 2024 Jul 18]. Available from: https://unair.ac.id/pengolahan-limbah-tandan-kosong-kelapa-sawit-tkks-dengan-excelzyme/

[2] Yahaya A, Naim A, Sohrab Hossain M, Edyvean R. Phenolics in EFBs. Vol. 12, *BioResources*. 2017. doi: 10.15376/biores.12.3.4594-4605

[3] Nimse SB, Pal D. Free Radicals, Natural Antioxidants, and Their Reaction Mechanisms. *RSC Adv*. 2015;5(35):27986–8006. doi: 10.1039/c4ra13315c

[4] Mokrani A, Madani K. Effect of Solvent, Time and Temperature on the Extraction of Phenolic Compounds and Antioxidant Capacity of Peach (Prunus persica L.) Fruit. *Sep Purif Technol*. 2016;162:68–76. doi: 10.1016/j.seppur.2016.01.043

[5] Tsouko E, Alexandri M, Vieira Fernandes K, Maria Guimarães Freire D, Mallouchos A, Koutinas AA. Extraction of Phenolic Compounds from Palm Oil Processing Residues and Their Application as Antioxidants. *Food Technol. Biotechnol.* 2019;57(1):29-38. doi: 10.17113/ftb.57.01.19.5784

[6] Zhu W, Han M, Bu Y, Li X, Yi S, Xu Y, et al. Plant Polyphenols Regulating Myoglobin Oxidation and Color Stability in Red Meat and Certain Fish: A Review. *Crit Rev Food Sci Nutr*. 2022;64(8):2276–88. doi: 10.1080/10408398.2022.2122922

[7] Shastak Y, Pelletier W. Captivating Colors, Crucial Roles: Astaxanthin's Antioxidant Impact on Fish Oxidative Stress and Reproductive Performance. *Animals.* 2023;13(21):3357. 10.3390/ani13213357

[8] Vianney YM, Amanda N, Pieknell K, Johan CW, Hardjo PH. Evaluation of the Antioxidant and Antibacterial Activity of Breadnut (*Artocarpus camansi* Blanco) Leaf Extracts. Vol. 9, *Indian Journal of Natural Products and Resources.* 2018;9(2):151-159.

[9] Badriyah L, Farihah, DA. Analisis Ekstraksi Kulit Bawang Merah (*Allium cepa* L.) Menggunakan Metode Maserasi. *J. Sintesis*. 2022;3(1):30-37. doi: https://doi.org/10.56399/jst.v3i1.32

[10] Putra LS, Sukweenadhi J, Nathania C, Wibowo ES, Buschle-Diller G, Marianti Purwanto MG. Comparative Study of Polyphenolic Compound Extraction from Empty Palm Fruit Bunches and Sugarcane

Pulp. *Heliyon.* 2022;8(2):e08951. doi: 10.1016/j.heliyon.2022.e08951

[11] Abozed SS, El-kalyoubi M, Abdelrashid A, Salama MF. Total Phenolic Contents and Antioxidant Activities of Various Solvent Extracts from Whole Wheat and Bran. *Annals of Agricultural Sciences.* 2014;59(1):63–7. doi: 10.1016/j.aoas.2014.06.009

[12] Al-Rimawi F, Rishmawi S, Ariqat SH, Khalid MF, Warad I, Salah Z. Anticancer Activity, Antioxidant Activity, and Phenolic and Flavonoids Content of Wild *Tragopogon porrifolius* Plant Extracts. Evidence-Based Complementary and Alternative Medicine. 2016;2016:9612490

[13] Fashakin OO, Tangjaidee P, Unban K, Klangpetch W, Khumsap T, Sringarm K, et al. Isolation and Identification of Antioxidant Peptides Derived from Cricket (*Gryllus bimaculatus*) Protein Fractions. *Insects.* 2023;14(8):674. doi: https://doi.org/10.3390/insects14080674

[14] Taugbøl A, Solbakken MH, Jakobsen KS, Vøllestad LA. Salinity-induced Transcriptome Profiles in Marine and Freshwater Threespine Stickleback After an Abrupt 6-Hour Exposure. *Ecol Evol.* 2022;12(10). doi: 10.1002/ece3.9395

[15] Pamulu TWP, Koniyo Y, Mulis. Pemberian Cacing Sutera untuk Pertumbuhan dan Kelangsungan Hidup Benih Ikan Black Molly. *Nikè: Jurnal Ilmiah Perikanan dan Kelautan*. 2017; 5(4):98-106.

[16] Minitab, LLC. Minitab [Internet]. 2021 [Cited 2024 Jul 19]. Available from: https://www.minitab.com

[17] Nile SH, Nile AS, Keum YS. Total Phenolics, Antioxidant, Antitumor, and Enzyme Inhibitory Activity of Indian Medicinal and Aromatic Plants Extracted with Different Extraction Methods. *3 Biotech*. 2017;7(1):76. doi: 10.1007/s13205-017-0706-9

[18] Nandasiri R, Eskin NAM, Thiyam-Höllander U. Antioxidative Polyphenols of Canola Meal Extracted by High Pressure: Impact of Temperature and Solvents. *J Food Sci.* 201;84(11):3117–28. doi: 10.1111/1750-3841.14799

[19] Tay P, Tan C, Abas F, Yim H, Ho C. Assessment of Extraction Parameters on Antioxidant Capacity, Polyphenol Content, Epigallocatechin Gallate (EGCG), Epicatechin Gallate (ECG) and Iriflophenone 3-C-β-Glucoside of Agarwood (Aquilaria crassna) Young Leaves. *Molecules*. 2014;19(8):12304–19. doi: 10.3390/molecules190812304

[20] Wong WH, Lee WX, Ramanan RN, Tee LH, Kong KW, Galanakis CM, et al. Two Level Half Factorial Design for the Extraction of Phenolics, Flavonoids and Antioxidants Recovery from Palm Kernel By-product. *Ind Crops Prod.* 2015;63:238–48. doi: 10.1016/j.indcrop.2014.09.049

[21] Munteanu IG, Apetrei C. Analytical Methods Used in Determining Antioxidant Activity: A Review. *Int J Mol Sci.* 2021;22(7):3380. doi: 10.3390/ijms22073380

[22] Dong JW, Cai L, Xing Y, Yu J, Ding ZT. Re-evaluation of ABTS*+ Assay for Total Antioxidant Capacity of Natural Products. *Nat Prod Commun.* 2015;10(12):2169–72.

[23] El Mannoubi I. Impact of Different Solvents on Extraction Yield, Phenolic Composition, In Vitro Antioxidant and Antibacterial Activities of Deseeded *Opuntia stricta* Fruit. *Journal of Umm Al-Qura University for Applied Sciences*. 2023;9(2):176–84. doi: 0.1007/s43994-023-00031-y

[24] Ulewicz-Magulska B, Wesolowski M. Total Phenolic Contents and Antioxidant Potential of Herbs Used for Medical and Culinary Purposes. *Plant Foods for Human Nutrition.* 2019;74(1):61–7. doi: 10.1007/s11130-018-0699-5

[25] Durdun C, Papuc C, Gajaila I, Valentin Goran G. The Influence of Solid-to-Solvent Ratio and Extraction Method on Total Phenolic Content, Flavonoid Content and Antioxidant Properties of Some Ethanolic Plant Extracts. *REV. CHIM.* (*Bucharest*). 2016;67(10):1922-27.

[26] Sirimanna SR, Dissanayake C. Effects of Culture Conditions on Growth and Survival of *Poecilia sphenops* and *Poecilia reticulata*. *International Journal of Aquatic Biology*. 2019;7(4):202–10. doi: 10.22034/ijab.v7i4.570

[27] Ahmadifar E, Yousefi M, Karimi M, Fadaei Raieni R, Dadar M, Yilmaz S, et al. Benefits of Dietary Polyphenols and Polyphenol-Rich Additives to Aquatic Animal Health: An

Overview. *Reviews in Fisheries Science & Aquaculture*. 2021;29(4):478–511. doi: 10.1080/23308249.2020.1818689

[28] Jahazi MA, Hoseinifar SH, Jafari V, Hajimoradloo A, Van Doan H, Paolucci M. Dietary Supplementation of Polyphenols Positively Affects the Innate Immune Response, Oxidative Status, and Growth Performance of Common Carp, *Cyprinus carpio* L. *Aquaculture*. 2020;517:734709. doi: 10.1016/j.aquaculture.2019.

[29] Bayona-Rodriguez CJ, Romero HM. Physiological and Agronomic Behavior of Commercial Cultivars of Oil Palm (*Elaeis guineensis*) and OxG Hybrids (*Elaeis oleifera* x *Elaeis guineensis*) at Rainy and Dry Seasons. *Aust J Crop Sci.* 2019;13(3):424–32. doi: 10.21475/ajcs.19.13.03.p1354