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Research Article

Phytochemical Screening of *Moringa oleifera* Leaf Extracts under Different Solvents

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Abstract

The rich nutrients and essential phytochemical compositions in the Moringa plant make it suitable and useful as a phytogenic feed additive to optimize feed efficiency and boost growth and reproduction in humans and animals. Various metabolites and bio-active ingredients contained in the leaves of *Moringa oleifera* have pharmacological, therapeutic, and physiological values in humans and livestock including fish. This study was intended to investigate the effect of different solvents of extractions on the quantity (yield) and quality (presence) of various phytochemicals in *Moringa oleifera* leaf extracts. Moringa leaves were identified, authenticated, processed, and pulverized following standard procedures. The powdered materials were subjected to a cold extraction method using four solvents: Aqueous, Ethanol, Methanol, and diethyl ether while phytochemical screening was done to determine the yield of the bio-active compounds using UV spectrophotometry and gravimetric methods. Standard qualitative and quantitative tests were conducted to evaluate flavonoids, alkaloids, saponins, tannin, phenol, Coumarin, Terpenoid, Cardiac Glycosides, Quinones, Anthraquinone, and Lignin. Phytochemical investigation of *Moringa oleifera* leaf extracts depicted rich quality and quantity of bioactive compounds including hormonally active phytochemicals such as flavonoids, and saponins which can boost growth and reproduction in animals. The values of Saponin, Flavonoid and phenol ranges respectively across the solvents as follows: Diethyl-ether: 0.94 ± 0.01, 2.16 ± 0.00, 2.78 ± 0.02, Ethanol: 2.24 ± 0.02, 5.36 ± 0.00, 4.76 ± 0.00, Methanol: 2.78 ± 0.02b, 5.02 ± 0.00,4.57 ± 0.02 and aqueous: 4.11 ± 0.01, 6.27 ± 0.01, 4.91 ± 0.00. There was a significant influence ($P < 0.05$) of the extraction solvent on all the phytochemical content of the *Moringa oleifera* leaves analyzed. Aqueous extractions gave the best phytochemical retention in the extracts - Flavonoid (6.27 ± 0.01 mg/g), Phenol (4.91 ± 0.00 mg/g), Saponin (4.11 ± 0.01%), Alkaloid (3.90 ± 0.02%) and Tannin (2.45 ± 0.04 mg/g). Data were analyzed using descriptive statistics and ANOVA at $\alpha_{0.05}$. The study illustrated that the use of a combination of polar and nonpolar solvents increased the extraction efficiency of phytochemicals in terms of yield; different solvents gave varied quantification of phytochemicals in *Moringa oleifera* leave extract. The highly polar aqueous, ethanolic, and methanolic extraction methods performed better than the non-polar diethyl ether in terms of the content yield, activity, and retention of bioactive compounds. Thus the extract can be applied in the diets of fish to boost growth and reproductive performance under varying inclusion levels.

Introduction

Diversification and intensification of aquaculture operations have become the global trend of commercial aquaculture [1]. However, as a result of residual effects, consumer concerns, and strict regulations in many countries, the use of synthetic chemicals, hormones, and antibiotics is becoming unviable, and natural compounds are more acceptable to the public. The medicinal properties of plants are mainly attributed to the presence of Flavonoids, but may also be influenced by their organic and inorganic compounds like coumarin, phenolic acid, antioxidants, and micronutrients like Cu, Mn, and Zn alike. It is known that plants accumulate antioxidant chemicals e.g. Flavonoids as secondary metabolites through evolution as natural means of surviving in a hostile environment [2]. In recent years, there has been a gradual revival of interest in the use of medicinal plants in developed and developing countries because herbal medicines have been reported to be safe and without any adverse side effects especially when compared to synthetic drugs [3].

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The genus Moringa comprises 13 known species among which *M. oleifera* is the most widely used, accepted, and most studied [4]. The high nutritional content of *Moringa* leaves has attracted much interest due to the levels of Minerals such as calcium, iron, potassium, and adequately high protein quality [5]. *Moringa oleifera* leaves' potential in aquaculture has increased recently. According to Momim and Memis [6], *Moringa oleifera* leaves have been used in the feed of different omnivorous, herbivorous, and carnivorous fish to evaluate the effect on several physiological parameters, such as growth performance, reproductive performance, hematological parameters, enzyme activities, and disease resistant performance.

Many studies have discussed the values of phytochemicals as feed additives. *Moringa oleifera* commonly known as drum stick tree, horse radish tree, or benoil has gained tremendous popularity in recent times due to its unlimited pharmacological, nutraceutical, and functional properties. A wide variety of chemical compounds are found in plants, and many of them have been shown to have beneficial effects on nutrient digestibility, growth, reproduction, and the immune status of fish broodstock and progeny acting through different mechanisms [5]. Phytochemicals are contained in extracts, herbs, spices, and essential oils with biochemical characteristics that may help to improve the general physiological condition of fish. The selection of Phytogenic feed additives for biochemical activities in fish is usually based on the quality and quantity of the extracts and bioactive compound yield considering the solvent of extraction and method. Qualitative and quantitative analysis of the bioactive constituents of a plant is necessary for the determination of potential pharmacological activity [7]. Phytogenic feed additives commonly defined as plant-based feed additives botanicals or phytobiotics represent a group of natural substances used in animal nutrition that are capable of enhancing gonadal development [8]. Phytogenics are a heterogeneous group of feed additives originating from plants and consist of herbs, spices, fruit, and other plant parts [9]. They include many different bio-active ingredients such as alkaloids, bitters, flavonoids, glycosides, mucilage, saponins, tannins, phenolics, polyphenols, terpenoids, polypeptide, thymol, cineole, linalool, anethole, allicin, capsaicin, allylisothiocyanate, and piperine. These feed additives have been tested in the form of extracts, cold-pressed oils, and essential oils in several animals but the results are variable. *Moringa oleifera*, one of the phytogenic feed additives is reported to have a wide range of activities including antimicrobial, anthelminthic, antioxidant, growth enhancer, and immune modulator. Besides these properties, they are also reported to stimulate feed intake and endogenous secretion as well as enhance gonadal development in fish [10].

This research was carried out between January and April 2023. Identification, Authentication, and Processing of *Moringa oleifera* leaves were carried out at the Department of Botany, University of Ibadan, Nigeria, and the Department of Aquaculture and Fisheries Management respectively while the extraction of phytochemicals was done at the Department of Pharmaceutical Chemistry, the University of Ibadan. All the chemicals and reagents used were of optimum analytical grade purchased from Excellence Chemicals LTD, Ibadan, and standardized at the Department of Pharmaceutical Chemistry, University of Ibadan.

Materials and methods

Collection, identification and authentication of plant material

Moringa Oleifera leaves samples of slender trees classification were collected from Adedayo Moringa Farm, Ibadan, identified and further authenticated by a plant taxonomist at the Department of Botany, University of Ibadan, Nigeria where a Voucher Specimen is deposited (Plate 1).

Instrumentation (Chemical reagents)

TECHCOMP Double beam UV-visible spectrophotometer with Hitachi software, and standard Quartz cuvettes with lids were used for measuring the absorbance.

Hypothesis

The extraction methods had a significant effect on the phytochemicals, the content yield, and the compositions of *M. oleifera* Leaf.

Statistical analyses

Data generated from the phytochemical analyses were subjected to Descriptive statistics and One-way Analysis of Variance (ANOVA) to compare the mean values. The Least Significant Difference (LSD) was used to separate the mean values at a 5% threshold of significance ($p < 0.05$).

Processing of plant materials

The processing of the plant was carried out in the Aquaculture and Fisheries Management Laboratory, Faculty of

Plate 1: *Moringa oleifera* leaves (X40).

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Renewable Natural Resources, University of Ibadan following the techniques below:

Sorting

Fresh, green, undamaged, non-insect infested leaves were selected while the bruised, discolored, decayed, and wilted leaves were discarded before washing the leaves.

Washing

The stalks of the leaves were cut from the main branches and the leaves were washed thoroughly with distilled water three times to remove all the adhering dust, dirt, and particles. The sample was then divided into three batches for the drying methods.

Processing

Air drying: The leaves were spread on cotton sheets and kept in a well-ventilated room at room temperature. The leaves were shade-dried for 7 – 10 days [11] for proper drying to be achieved. Upon drying, the leaves were pulverized under aseptic conditions using an Orange Legend Gold electric grinder into a fine powdery form, weighed, sieved, labeled, and stored in a dry dark airtight sealed glass jar and kept at -20 ^{o}C for phytochemical, proximate, and mineral analyses.

Preparation of plant extracts for phytochemical analysis

The extraction of active bioactive compounds was carried out at the Department of Pharmaceutical Chemistry, University of Ibadan. Under the cold maceration method, four solvents of extraction were used to determine the solvents with the best phytochemicals in terms of quality and quantity.

I. **Solvent extraction processeAqueous (Water) extraction:** The extraction process used was according to the modified method of Makanjuola *et. al.*, [12] as follows: Five hundred grams of the powdered leave sample was soaked in 1.5 liter of de-ionized warm water at the temperature of 60^{o}C. Each solution was then allowed to stand for 24 hours, after which was sieved with a muslin cloth and filtered using a Buchner funnel, centrifuged at 6000 rpm/min, then applied in a freeze dryer to concentrate it. The filtrate was collected in a conical flask and allowed to cool. The obtained semi-solid extracts (residue) were preserved in an airtight bottle and kept in a freezer at -20 ^{o}C until further use.

II. **Ethanolic extraction:** Fifty grams of the powdered sample was soaked in 500 ml of absolute ethanol and held for 24 hours. The mixture was stirred occasionally. After 24 hours, the sample was double-filtered using cheesecloth. Extracts (filtrate) were concentrated at 40 ^{o}C under reduced pressure using a rotary vacuum evaporator and then kept in a glass flask. The filtrate was then dried in a hot-air oven at a temperature of 45 ^{o}C [13,14]. The obtained semi-solid extracts (residue) were preserved in an airtight bottle and kept in a freezer at -20 ^{o}C until further use.

III. **Methanolic extraction:** Following Isitua, et al. [11] method, the powdered plant material sample (100g) was macerated in 98 % methanol (500 ml), modified by heating the water bath for 45 minutes, and vortexed. The methanol extract then was concentrated using a water bath to evaporate the extracting solvent (methanol) and later transferred to a hot air oven set at 40 to evaporate any trace of the solvent to obtain a greenish-brown residue. The extracts obtained were stored in the refrigerator at - 4^{o}C until when required for phytochemical screening.

IV. **Diethyl ether extraction:** The process followed the already established extraction procedure of plant samples [15], using ether as solvent. 200g of plant powder was added into 500 ml diethyl ether (98%) in a glass jar. The mixture was shaken at two-hour intervals during the daytime for 3 days. The mixture was decanted and filtered using Whatman's No.1 filter paper in a Buchner funnel using a suction pump. The residual solvent was removed by oven drying at 105^{o}C for 3hrs. The extracts obtained were stored in the refrigerator at - 4^{o}C until when required for phytochemical screening.

Phytochemical screening

The extracts of the powdered leaves of *Moringa oleifera* were analyzed for the presence of various Phyto constituents like saponins, alkaloids, flavonoids, tannin, and phenolic compounds, etc. by using standard phytochemical procedures. Both qualitative and quantitative phytochemical analyses of the *Moringa oleifera* leaf extracts were exploited. Tests for the presence of the bio-active compounds including the following plant secondary metabolites alkaloids, flavonoids, saponins, phenols, glycosides, lignin, and tannins were carried out on the powdered samples using standard procedures as described by Mayuri [16].

Qualitative phytochemical analysis

This analysis revealed the presence or absence of bio-active compounds usually expressed with "+" or "-" indicating presence and not detection respectively.

Qualitative tests

i. **Detection of alkaloids:** Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

Mayer's test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). The formation of a yellow-colored precipitate indicates the presence of alkaloids.

Wagner's test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). The formation of a brown/reddish precipitate indicates the presence of alkaloids.

ii. **Detection of saponins**

Froth test: Extracts were diluted with distilled water to 20ml and shaken in a graduated cylinder for 15 minutes.

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The formation of a 1 cm layer of foam indicates the presence of saponins.

Foam test: 0.5 gm of extract was shaken with 2 ml of water. The persistence of foam produced for up to ten minutes indicated the presence of saponins.

iii. Detection of phenols

Ferric chloride test: Extracts were treated with 3-4 drops of ferric chloride solution. The formation of a bluish-black color indicates the presence of phenols.

iv. Detection of tannins

Gelatin test: To the extract, 1% gelatin solution containing sodium chloride was added. The formation of a white precipitate indicated the presence of tannins.

v. Detection of flavonoids

Alkaline reagent test: Extracts were treated with a few drops of sodium hydroxide solution. The formation of an intense yellow color, which became colorless with the addition of dilute acid, indicated the presence of flavonoids.

Lead acetate test: Extracts were treated with a few drops of lead acetate solution. The formation of a yellow color precipitate indicated the presence of flavonoids.

The solvents whose extract has the best qualitative and quantitative phytochemical constituents were selected and used for further studies following standard procedures.

Quantitative estimation of phytoconstituents

This analysis revealed significant variations in the contents of the extracts i.e. the amount of the phytochemicals present in terms of the quantity measured in grams (g) or milligrams (mg) (Kokate *et. al.*, 2004).

A. Flavonoids: One gram of the plant extract sample was weighed into 10ml of 80% Methanol; left to stand for 2 hours, filtered into a weighed Petri dish, and left to dry in the oven at 40°C until it attained a constant weight. Thereafter, the weight of the Petri dish was recorded. A volume of 0.5ml of 2% $AlCl_3$ Methanol solution was added to 0.05ml sample solution. After 1hr at room temperature, the absorbance was measured at A420 nm with a UV/VIS spectrophotometer. Rutin was used as a standard flavonoid and results were expressed in terms of Rutin equivalent.

B. Alkaloids: One gram of the sample (W) was added to 20ml of 10% Acetic Acid in Ethanol, shaken, allowed to stand for 4 hours, and filtered. The filtrate was allowed to evaporate to about a quarter of its original volume and one drop of concentrated Ammonia was added. The precipitate formed was filtered through a weighed (W1) filter paper. The filter paper dried in the oven at 60°C and weighed when it had attained a constant weight (W2).

$$\%\text{Alkaloids} = \frac{W2 - W1 \times 100}{W}$$

C. Tannins: One gram of the sample was extracted with 25ml of the solvent mixture of 80:20 Acetone: 10% Glacial Acetic Acid for 5 hours. The supernatant was filtered and the Absorbance of the filtrate as well as the reagent blank measured at 500nm Absorbance. A standard graph was produced with 10, 20, 30, 40, 50 mg/100g of Tannic Acid. The concentration of Tannin was read off taking into addition of dilute acid.

D. For phenols/phenolics: 2g of extract was mixed with 20 ml of 80:20 Acetone: 0.5% formic Acid and allowed for 2 min and filter. 2ml of the extract was mixed with 0.5ml of Folin-Ciocalteau Reagent and 1.5ml Sodium Carbonate (20%) mix for 15 sec and allowed to stand at 40°C for 30min to develop color. Measured at A765. Expressed as GAE/g (Gallic Acid Equivalent).

E. Saponins: One gram of sample was added to 5ml of 20% Ethanol in a conical flask and placed in a water bath at 55°C for 4 hours. This was followed by filtering and washing the residue with 20% Ethanol twice and reducing the extract to about 5ml in the oven. The extract was further treated successively Petroleum Ether, butanol, and 5% Sodium Chloride.

Authors hereby emphasize that there were no significant hazards or risks associated with this work only that the cost of reagents and chemicals used were impacted due to the high inflation rate.

Results

Qualitative and quantitative indices of Phytochemicals of *Moringa oliefera* leave extracts

Tables 1, 2A,B as well as Figure 1 showed the phytochemical screen effect of different extraction solvents on the phytochemical presence of *Moringa Oleifera* leaves and the Phytochemicals content yield of various secondary metabolites. The values of Saponin, Flavonoid, Alkaloid, Tannin, and phenol ranges respectively across the solvents as follows: Ethanol: 2.24 ± 0.02, 5.36 ± 0.00, 3.43 ± 0.02, 2.04 ± 0.01, 4.76 ± 0.00, Methanol: 2.78 ± 0.02b, 5.02 ± 0.00, 2.92 ± 0.02, 2.13 ± 0.01, 4.57 ± 0.02 and aqueous: 4.11 ± 0.01, 6.27 ± 0.01, 3.90 ± 0.02, 2.45 ± 0.04, 4.91 ± 0.00. There was a significant influence (P <

Table 1: Percentage yield of the crude extracts of *Moringa oleifera* leaves by different solvents.

Solvent	Weight of powdered leaves	Weight of Crude Extracts	Yield (%)
Aqueous	910	166.5	18.3
Ethanol	910	152.8	16.8
Methanol	910	121.9	13.4
Di-ethyl ether	910	52.8	5.8

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Table 2A: Qualitative phytochemical screening of *Moringa oleifera* leave Extract

Phytochemical	Test	Colour	Methanol	Ethanolic	Aqueous	Diethyl Ether
1. Saponin	(Froth's Test)	White Ppt	+	+	+	+
2. Alkaloid	(Hager's Test)	Yellow Ppt	+	+	+	+
3. Flavonoid	(Lead acetate Test)	Yellow	+	+	+	+
4. Tannin	(Braymer's Test)	White Ppt	+	+	+	+
5. Coumarin	(Reaction with 10% NaOH)	Yellow	+	+	+	-
6. Steroid	(Salkowski's Test)	Red	+	+	+	-
7. Terpenoid	(Salkowski's Test)	Reddish Brown	+	+	+	-
8. Cardiac Glycosides	(Legal's Test)	Blue	+	+	+	-
9. Glycosides	(Antimony Trichloride Test)	Blue	+	+	+	-
10. Quinones	(Polarography)	Yellow Crystalline	+	+	+	-
11. Anthraquinone	(Borntrager's Test)	Red to Blue	+	-	-	-
12. Phenol	(Ferric Chloride Test)	Bluish Black	+	+	+	+
13. Lignin	(Wiesner Test)	Red	-	-	-	+

Table 2B: Quantitative phytochemical screening: Effect of Different Extraction Methods on Phytochemical Content of *Moringa Oleifera* leaves.

	Extraction Method			
	Ethanol	Methanol	Aqueous	Diethyl Ether
Saponin (%)	2.24 ± 0.02^{c}	2.78 ± 0.02^{b}	4.11 ± 0.01^{a}	0.94 ± 0.01^{d}
Flavonoid(mg/g)	5.36 ± 0.00^{b}	5.02 ± 0.00^{c}	6.27 ± 0.01^{a}	2.16 ± 0.00^{d}
Alkaloid (%)	3.43 ± 0.02^{b}	2.92 ± 0.02^{c}	3.90 ± 0.02^{a}	1.69 ± 0.01^{d}
Tannin (mg/g)	2.04 ± 0.01^{c}	2.13 ± 0.01^{b}	2.45 ± 0.04^{a}	0.59 ± 0.01^{d}
Phenol (mg/g)	4.76 ± 0.00^{b}	4.57 ± 0.02^{c}	4.91 ± 0.00^{a}	2.78 ± 0.02^{d}

Mean ± SD values in the same row with different superscripts are significantly different at $p < 0.05$

Legends: Superscripts a, b, c, and d show the significant level in terms of ranking, a (highest), d (lowest)

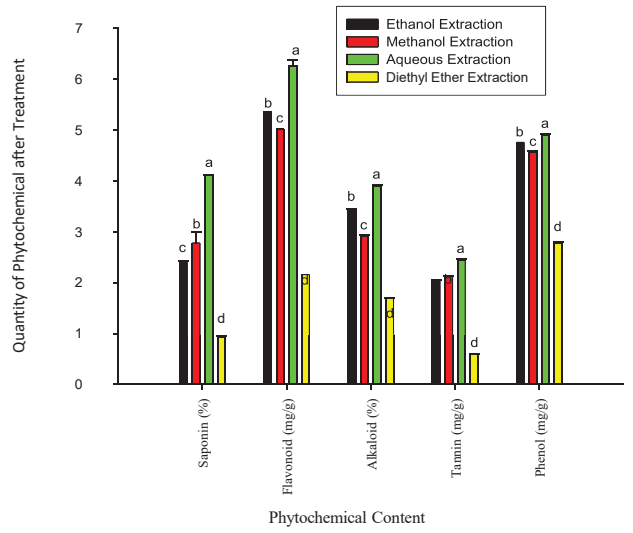

Figure 1: Phytochemicals yield with respect to solvent of extraction.

0.05) of the extraction solvent on all the phytochemical content of the *Moringa oleifera* leaves analyzed. The result depicted that the extracts performed better quantitatively in descending order as follows- aqueous extraction, ethanolic extraction, methanolic extraction, and diethyl ether. The Aqueous methods gave the best phytochemical retention in hormonally active phytochemicals such as Flavonoid (6.27 ± 0.01 mg/g), Phenol (4.91 ± 00 mg/g), Saponin (4.11 ± 0.01 %), Alkaloid (3.90 ± 0.02%) and Tannin (2.45 ± 04).

Discussion

The following notable phytochemicals were extracted from the processed *Moringa oliefera* leaves: saponin, flavonoid, alkaloid, tannin, and phenol. Four solvents were selected and used for extraction: ethanol, aqueous, methanol, and diethyl ether. Two hormonally active phytochemicals: saponin and flavonoid were identified to be useful bioactive compounds due to their reported influence on the growth and reproductive performance of fish [17]. The best solvents of extraction were considered so because they have the highest phytochemicals in terms of the quality and quantity of yield. There was a significant influence $p < 0.05$ of the extraction methods on all the phytochemical content of the *Moringa oleifera* leaves analyzed.

The result depicted that aqueous extraction methods gave the best phytochemical retention in hormonally active phytochemicals of interest- Flavonoid (6.27 ± 0.01 mg/g) and Saponin (4.11 ± 0.01%) followed by ethanolic extraction with Flavonoid and Saponin was (5.36 ± 0.00 mg/g) and Saponin (2.24 ± 0.02%) while methanolic extraction method was: Flavonoid, 5.02 ± 0.00 and Saponin (2.78 ± 0.02,). The aqueous extraction method had the best retention of phytochemicals quantitatively and the lowest significant reduction ($p < 0.05$). Relative to the ethanol extraction method, methanol extraction significantly reduced ($p < 0.05$) the level of flavonoid, alkaloid, and phenol while saponin and tannin content were significantly reduced by the ethanol extraction method It was noticed that aqueous and ethanolic extracts were richer in total phenol and flavonoid content than other extracts, which suggested the presence of water-loving (polar) phenol. Al-Owaisi, et al. [18] reported that *M. oleifera* leaves exhibited the richest phenolic compounds and flavonoid contents among different species of Moringa. Ethanolic extract of *M. oleifera* leaves showed higher phenolic compounds and flavonoid content than that reported by Nagarani, et al. [19]. In the previous study by Adebayo, et al. [208], *M. oleifera* leaves showed higher phenolic content than what was reported by Leone [21] who used 80% ethanolic extract and obtained 8.21 µg/100 g, while in contrast, the results of this study were less than theirs in the flavonoid content with the same solvent (5.36 mg/g). That study on *M. oleifera* leaves was supported by Maqsood [22] regarding the ethanolic extract in phenolic and flavonoid content determination.

Conclusion

Aqueous solvent with the highest yield of crude extract (18.3%) gave the highest flavonoid concentration of 6.27 ± 0.01 mg/g, followed by Ethanol solvent (16.8% yield) with a

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flavonoid concentration of 5.36 ± 0.00 mg/g while Diethyl-Ether solvent with 5.8 % crude extracts yield had the least flavonoid concentration of 2.16 ± 0.01 mg/g. This implies that highly polar aqueous and ethanolic extraction methods performed better than diethyl ether in terms of the content yield, activity, and retention of bioactive compounds. Due to its rich source of phytochemicals, this plant is a good candidate for phytogenic feed additives for boosting animal health, and reproductive and growth performance and is also capable of being considered a biotechnological and bio-fortification tool useful for sustainable aquaculture productivity.

Authors' contributions

***ONUOHA Stanley Obialo:** The Postgraduate Researcher who contributed ideas in designing and executing the work, Analysed and put together the data, and developed part of the article.

AJANI Emmanuel Kolawole: Contributed majorly to designing the experiment, Supervised and participated in the writing of the article

JENYO-ONI Adetola: Key Supervisor who contributed to editing the write-up of the article.

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