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The Phytochemical Analysis and Antioxidant Activity of Oil Extracted from the Skin of the Indian Carp Fish, Labeo Rohita

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Abstract

Checking the phytochemical and anti - oxidant properties of the extracted oil from thrown away fish skins enables its use in the production of a superior food item. This study was conducted to extract the oil from Rohu fish skin and determine what phytochemicals it contains and how effectively it combats free radical damage (Labeo rohita). Powdered dried fish skin was diffused in n-hexane for twenty-four hours, tried to follow by evaporation at the boiling point of the solvent. Phytochemical screening was used to determine the oil's phytochemical constituents. The petroleum product includes alkaloids, glycosides, phenolics, saponins, total sugars, steroids, and coumarin, according to the results. In addition, the results revealed that the oil is devoid of active substance such as flavonoids, tannins, anthrocayanine, anthroquinone, balsams, as well as volatile oil. The antioxidant analysis was performed, and the phenolic element test, the free radical-scavenging DPPH activity test, the ABTS decolorization action test, and even the FRAP assay all indicated that the oil had a high level of antioxidant activity. According to the results, the oil has a total phenolic content of 6.49 0.01 g/mL, DPPH free radical scavenging activity of 3.84 0.02%, ABTS decolorization activity of 3.93 0.02%, and FRAP activity of 995 1.35 g/mL. This research indicates that carp fish skin oil contains phytochemicals and antioxidant activity.

Keywords: Phytochemical, Antioxidant, Labeo rohita, FRAP, DPPH

1. INTRODUCTION

Aquaculture and fishing are two of the world's most rapidly expanding markets. Aquaculture accounted for approximately 46% of the approximately 179 million metric tonnes of fish produced globally in 2018. Since the 1990s, Asia has been the leading source of fish farming, accounting for approximately 90 percent of the worldwide catch. In addition, two-thirds of a world's inland production was located in Asia [1]. India is the number two production of fish farming and the number three producer of wild fish. According to the National Fisheries Data Bank of India (https://nfdb.gov.in/about-indian-fisheries), fisheries and aquaculture account for 1.24 percent of India's total GVA and 7.28 percent of the country's agricultural GVA. There are, however, a number of infectious diseases that often affect these industries, resulting in substantial economic damage. Parasitic illnesses are a significant source of economic loss in Indian aquaculture [2]. Fish in both natural and artificial environments are susceptible to parasite infection. Between $1.05 billion and $9.58 billion annually in parasite-related losses [3].

Fish typically has a fat content of 3-20%, and processing typically results in the waste of around 50% of the fish's initial body weight. The lipid portion of fish & fish by-products is known as fish oil [4]. Fish skins, scales, bone fragments, viscera, gills, dark muscles, and heads account for up to 70% of the waste generated by fish processing industries [5]. Historically, these waste by-products were either sent to landfills or utilised as low-value materials such as animal feed, fertiliser, etc. These leftovers can be used to make high-quality edible products. [6]

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Phytochemicals are naturally occurring, biologically active chemical substances found in plants that have health benefits. Recent research reveals that phytochemicals can help protect humans from disease [7]; plants manufacture phytochemicals to defend themselves. The vast majority of fish oil comes from whole fish. To use fish skin for the synthesising of fish oil reduces the environmental impact of fish processing waste [8]. Because fish ingest aquatic, riverine, and lake vegetation, fish skin oil is likely to contain phytochemicals. L. rohita's crude n-hexane extract was analysed phytochemically for the alkaloid content, flavonoids, tannins, phenols, cardiac glycosides, terpenes, steroids, volatile oils, balsam, coumarin, anthocyanine, and saponins. Oxidative stress constitutes one of the most substantial quality-degrading stages in liposome foods, resulting in substantial economic losses. [9].

Oxidation of lipids reduces the nutritional value and safety of food, in addition to causing rancid odours and flavours. Therefore, it is vital to enhance the scalability of fish oil so that it can be used safely. Antioxidants are chemicals that prevent oxidative damage to biological targets and are a vital element of the body's defence system. Using phenolic content activity, DPPH free radical scavenging activity, ABTS decolorization anti-oxidative action, as well as the FRAP assay, the anti-oxidative property of crude fish skin n-hexane extract was investigated.

2. LITERATURE REVIEW

Nabanita Ghosh et al. (2021) [10] The use of mixtures of fish skin oil and chia seed oil was investigated. Using gluten-free chia flour, nutrient-dense spreads are manufactured. Due to its higher antioxidant capacity and lower toxin content, the 2:1 (CO:FSO) proportion is really the best diet of the 3 blending ratios. Merging raw chia flour, defatted chia flour, raw roasted chia flour, and roasted defatted chia flour with emulsification oil, honey as a natural substance, and egg lecithin as an emulsifier produced four distinct types of spreads. The use of flour that has been marinated imparts a pleasant aroma and a pleasurable colour, which helps to mask the smell of fish in blended oils. The RRC expansion receives the highest rating in a sensory evaluation of all developed spreads. In addition to antimicrobial testing, the RRC spread is compared to the standard rate. All of these analyses increase the spreads' nutritional value. These spreads are high in nutrients, gluten-free, and protein.

Bruno et al. (2019) [11] investigated the oxidative continuity and rheological characteristics of oil extracted from the head of Labeo rohita. In contrast to untreated LRH, oil recoveries for microwave and ultrasound pre-treatments ranged from 68.45 to 69.75% and 58.74 to 67.48%, respectively. LRH oil enclosed more monounsaturated and polyunsaturated fats as a result of US pre treatmentAdditionally, MW pre-treatment decreased the oxidative stability of LRH oil, whereas pre-treatment had no effect, as evidenced by the differential in peroxide value, anisidine value, and thiobarbituric acid-reactive compound value. In addition, all oils exhibited Newtonian fluid behaviour. The United States has the lowest crude oil viscosity and temperature sensitivity (Ea = 29.74 kJ/mol). Consequently, MW or US-assisted enzyme processing of LRH oil could be a viable alternative to conventional enzyme extraction.

Ghosh et al. (2019) [12] investigated The oxidative and thermal consistency of the skin oil of Labeo rohita. The skin oil of Labeo rohita was extracted using the Soxhlet method and n-hexane as the solvent. Before as well as one hour after heating the oil to 90 °C, the acid value, the amount of free fatty, and the peroxide value were measured to evaluate the thermostability of the oil. To begin measuring antioxidant activity, the Total Phenolic Content, 2, 2-Diphenyl-1-picryl Hydrazyl-Free Free Radical-Scavenging Activity, and Ferric Reducing Antioxidant Power assays were utilised. The antioxidant potential of the oil was examined by heating it at a constant 90 °C for one, two,

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three, and 4 hours. Two hours were spent heating the oil at temperature changes of 60 °c, 120 °c, and 18 °c. The 2, 2-Diphenyl-1-picryl hydrazyl assay demonstrates that heating increases the radical-scavenging capacity of Labeo rohita skin oil. The viewpoints of the overall phenolic level and Ferric reduction attempted to bolster test lowering as heating rates and times increased (**p0.05, p0.01). Temperature rises the acid value, the concentration of (Free Fatty Acid), and the peroxide value (**p0.01).

3. RESEARCH METHODOLOGY

3.1 Materials

All employed reagents met analytical standards. Ammonia, n-hexane, deionized water, ethanol, sodium hydroxide, potassium iodide, chloroform, glacier acetic acid, sodium thiosulphate, potassium hydroxide, methanol, hydrochloric acid, ferric chloride, and ferric chloride were obtained from regional sources. Additionally, the Maeyer, Dragendroff, and Fehling reagents. Gelatin, Folin-Ciocalteu reagent, and sodium hydroxide are the ingredients. 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid), 2,4,6-tripyridyl-S-triazine, ascorbic acid, gallic acid. 2.2-Diphenyl-1-picrylhydrazyl, 2,2'-a.

3.2 Oil Sample Preparation

3.2.1 Sample Collection

The skin of two kilogrammes of rohu (L.rohita) fish was acquired from the a market in Hazaribag, Jharkhand, India. After cleansing the skin, the metrics were carefully removed. The sterilised skins were kept in a sealed container at -20 ° C until required. Prior to oil extraction, the cooled skin was adjusted to room temperature.

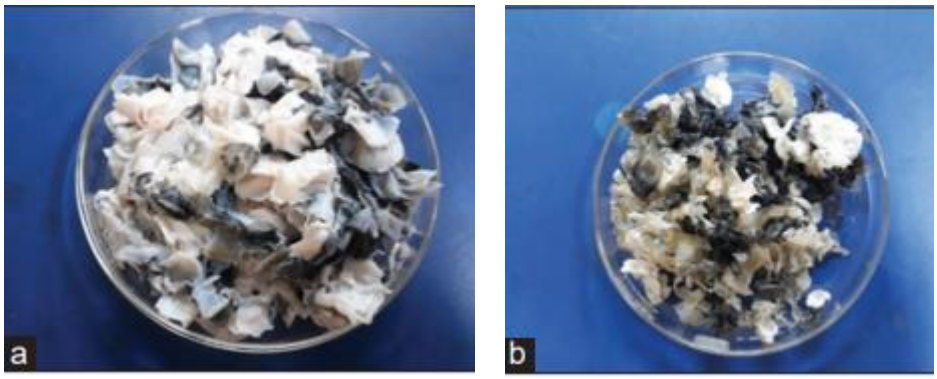

Figure 1(a) fish skin purchased from a local market. (b)Fish skin is separated from scales

3.2.2 Oil Collection

Skin samples were dried using an oven with 60 °C hot air for 24 hours. After samples have been dried, they are ground in a mortarprester, soaked in n-hexane at a ratio of 1:2 for 24 hours, filtered, and then distilled. The oil is kept at four degrees Celsius until further testing can be performed.

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Fig 2: Steps for Oil collection from Fish Skin

4. PHYTOCHEMICAL SCREEINING

The bioactive components found in the FSO were evaluated qualitatively using standard procedures. The presence or absence of Alkaloid, flavonoids, tannins, phenols, glycosides, terpenoids, steroids, volatile oils, balsam, coumarine, anthocyanine, and saponins was determined using qualitative methods [13-17].

4.1 Test for alkaloids:

The mix of 0.5 ml of the sample and 8 ml of 1% HCl is heated and filtered. The existence of alkaloids in the turbidity or precipitation was determined by trying to treat 2 ml of the filter paper seperate with Maeyer's and Dragendorff's reagents.

4.2 Examine for glycosides (Keller-kilani test):

Two millilitres of glacier acetic, two falls of a 5% solution of ferric chloride, and two ml of concentrated H2SO4 were used to treat the oil. A brown ring formed between the two layers, implying the coronary glycosides were present.

4.3 Test for phenolic compound:

Combine 2 millilitres of a 5% solution of FeCl3 with oil. The colour green demonstrated the phenolic molecules were present.

4.4 Test for saponins (Foam test): The collection tube has been filled with one millilitre of oil. The formation of foam after adding 1 millilitre of filtered water and zealously rolling the combination for 2 minutes suggested the presence of saponins.

4.5 Test for reducing sugar (Fehling test):

With the oil, an equal mixture of Fehling solutions A and B was heated. The appearance of reducing sugars was indicated by the presence of a red precipitate.

4.6 Test for steroid:

With 2 millilitres of chloroform and H2SO4 solution, the oil was combined. The appearance of steroids was indicated by red pigment in the lower chloroform layer.

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4.7 Test for coumarine:

Oil has been treated with 10% sodium hydroxide and chloroform. Yellow was showing the existence of coumarine.

4.8 Test for Flavonoids:

There has been 2ml of sodium hydroxide in 2ml of oil. Once it was believed that yellow indicated the flavonoids were present.

4.9 Test for tannin(Gelatin test):

In five millilitres of distilled water, 50 mg of oil is dissolved, then 2 ml of a 1% gelatin solution that contains 10% sodium chloride is added. A white precipitate demonstrates tannins are present.

4.10 Test for Anthocyanin:

Two millilitres of oil, two ml of HCl (2N), and two millilitres of ammonia were mixed. The existence of a colour ranging from pinkish-red to violetish identifies anthocyanine.

4.11 Test for Anthroquinones (Borntrager's Test):

A few minutes were spent keeping approximately 5 mg of an oil warm in a 10% HCl-containing water bath. Afterward, it was cooled and filtered. A adequate number of CHCl3 is added to the filtrate. Prior to heating, some few falls of 10% NH3 were included in the mixture. The existence of anthroquinones was demonstrated by the existence of a pink color.

4.12 Test for Balsams:

Two ml of oil and two ml of ethanol were combined. Two drops of a ferric chloride solution in alcohol were added. The existence of balsams is demonstrated by their presence of a dark green hue.

4.13 Test for Volatile oils:

0.1 ml of dissolved hydroxide solution was added to 2 ml of oil, followed by a minute amount of diluted hydrochloric acid solution. Precipitation that is white suggests the presence of oils.

5. ANTIOXIDANT ACTIVITY

5.1 Determination of Total Phenolics

Using the Folin-Ciocalteu assay [19], the total phenolic content was determined. In test tubes, one millilitre of an oil and 0.5 millilitres of Ciocalteu's Folin-reagent have been placed (diluted 10 times with water). After five minutes in the dark, one millilitre of sodium carbonate (7.5 percent by weight) was included in the solution. One hour was spent in the dark with the tubes. Absorption at 765 nm was determined using a Jasco V-630 spectrophotometrically and compared specifically to a Gallic acid calibration plot. Sample gallic acid concentration levels were reported in milligrams per gramme.

5.2 DPPH (2, 2-Diphenyl-1-picrylhydrazyl) free Radical Scavenging activity:

The test tubes are then filled with two ml of an oil methanolic extracts and two ml of a 1 mM DPPH. One hour has already been spent in the dark with the tubes. Using a Jasco V-630 spectrophotometer, the absorption spectrum at 517 nm was measured in contrast to an ascorbic

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calibration curve. This data was also provided in milligrams of ascorbic per gramme of sample.. Calculated proportion of the free radicals in DPPH.

$$I\% = \frac{(A}{\qquad}$$

Where I = DPPH inhibition (percent), A0 = absorption coefficient of control sample (0 h), and A = absorbance of test sample at the end of the reaction (1 h).

5.3 ABTS (2, 2'-azinobis 3-ethylbenzenethiazoline-6-sulfonic acid) decolorization Method:

Using ABTS method, the antioxidant activities was determined by combining, at room temperature for 10 minutes, 20 L (1:10) of disintegrated oil with 2 mL of ABTS solution. The 734 nm absorption spectrum was determined using a spectrophotometer (Jasco V-630). The results were compared with the normal curve of distribution (butyl hydroxy toluene)

$$\frac{\qquad}{\qquad}$$

In which Ac and As depict the standard and sample absorbances, respectively.

5.4 Determination of FRAP (Ferric Reducing/Antioxidant Power) activity:

Using a customized form of the FRAP assay methodology developed by Benzie and Strain in 1996, the antioxidant activity was measured. 40 minutes were spent storing 20 L of specimen and 180 L of FRAP reagent in a dark room at 37 degrees Celsius. Using an ultraviolet spectrophotometer, the absorbances of the eventual results solution was measured at 593 nm (Jasco V-630). The FRAP value was determined using distinction in absorption between both the sample's final count (4-min reading) and totally empty reading (0-min reading) relative to standard ascorbic acid.

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6. Result

6.1 Yield of FSO from fish skin

25.50% was the yield of FSO from fish skin

6.2 Phytochemical Screening

The L.rohita skin oil phytochemical analysis shows the existence of a number of interesting secondary metabolites. The first table demonstrates the qualitative determination of the existence and absence of countless phytochemicals using established procedures.

Tests	Confirmatory Indication	Findings
Keller-Killiani Test	Reddish brown ring	+
Mayers Test	White precipitation	+
Foam Test	Bubble formation	+
Ferric chloride Test	Green colouration	+
Sulphuric acid Test	Reddish-brown colouration at lower layer	+
Fehling Test	Yellow Colouration	+
NaOH Test	Yellow colour	+

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Gelatin Test	No white precipitation	-
Alkali Test	No yellow colour	-
HCl & Ammonia Test	No Pinkish red-violet colouation	+
Borntranger's Test	Red colouration	+
FeCl3 Test	No dark green colouration	-
HCl	No white precipitation	-

Table 1: Evaluation of Present Phytochemicals Qualitatively

6.3 Anti-oxidant Activity analysis

Fish skin Oil is examined to anti-oxidant assays employing the Total Phenolic Content assay with Gallic acid as that of the standard, the DPPH radical removal action assay, the ABTS decolorization action assay, and the FRAP test with Ascorbic acid as the standard. The unique anti-oxidative properties of Fish Skin oil are listed in Table 2.

Table 2: FSO anti-oxidative assays

Anti-oxidant Test	Amount
TPC assay	6.49 ±0.01 µg GAE/mL
DPPH assay	(3.85 ±0.02) %
ABTS assay	(3.93 ± 0.02) %
FRAP assay	(995 ±1.35)%

Conclusion

The physicochemical analyses of the FSO were satisfactory. The qualitative phytoconstituent related to task that FSO consists of a variety of secondary bioactive metabolites. Plants produce secondary metabolites as an adaptive reaction to stresses from the environment. Thus, phytochemicals could be utilised in numerous fields, including food, medicine, and even cosmetics, all of which improve the well-being of individuals. Some of the phytochemicals found in FSO include: alkaloids, glycosides, phenolic compounds, saponins, reducing sugar, steroids, and coumarine; all of these compounds are either directly or indirectly involved in promoting health. Nonetheless, analysis of the oil reveals that it is devoid of the active components flavonoid, tanin, anthropocayanine, anthropoquinone, balsams, and volatile oil. It is believed that alkaloids can kill bacteria, and they are used to treat a variety of skin conditions, including eczema, seborrheic dermatitis, & neurodermatitis. Creams, soaps, and ointments are all made with steroids in the cosmetic, soap, and pharmaceutical industries. The phenyl-propanoid biosynthesis pathway is responsible for the production of phenolic chemicals, which are then utilised to construct plant pigments that are incorporated into the cellular walls of 67 organisms There is evidence that glycosides, sugars, and steroids have antimicrobial, antifungal, and anti - carcinogenic effects against a broad spectrum of microorganisms, fungi, and cancer-causing chemicals. These beneficial phytochemicals found in FSO have multiple applications, including in nutritional supplements and pharmaceuticals.

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