International Research Journal of Engineering and Technology (IRJET) e-ISSN: 2395-0056
Volume: 06 Issue: 02 | Feb 2019 www.irjet.net p-ISSN: 2395-0072

PHYTOCHEMICAL SCREENING AND ANTIOXIDATIVE ACTIVITY OF OIL EXTRACTED FROM INDIAN CARP FISH (*Labeo rohita*) SKIN

Nabanita Ghosh[1], Saheli Ghosal[2], D.K Bhattacharyya[3]

[1,2]Research Scholar, School of Community Science and Technology, Indian Institute of Engineering Science & Technology, Shibpur Howrah, West Bengal-711103
[3]Adjunct Professor, School of Community Science and Technology, Indian Institute of Engineering Science & Technology, Shibpur Howrah, West Bengal-711103

Abstract:- *Fish skin which is discarded as a waste can be utilized for better food application after ascertaining the presence of Phytochemicalsand Antioxidant activity of the oil extracted from the discarded fish skin. The present study was conducted to extract and evaluate the kinds of Phytochemicals present and Antioxidative activity of the oil, extracted from Rohu Fish skin (Labeorohita).Extraction and Physicochemical characterization of fish skin oil (FSO) was carried out by dissolving the dried fish skin powder into n-hexane for 24 hours, followed by distillation at the boiling point of the solvent. Phytochemical screening was conducted to detect the Phytochemicals present into the oil. Results revealedthat the crude oil contains Alkaloids, Glycoside, Phenolic compounds, Saponins, Reducing sugar, Steroid, Coumarine as active compounds qualitatively. Results also showed that the oil does not contain Flavonoid, Tannin, Anthrocayanine, Anthroquinone, Balsams, Volatile oil as active compounds. The antioxidant assay was conducted and the oil showed a good antioxidant activity via Total Phenoilc component test, DPPH free radical scavenging activity test, ABTS decolorization activity test and FRAP assay. Results showed the oil has 6.50 ± 0.02 µg GAE/mL,TotalPhonolic content, DPPH free radical scavenging activity (3.84 ± 0.02) % , ABTS decolorization activity (3.92 ± 0.02) % and (990 ± 1.32) µg of AAE /mL FRAP activity. From this study, it can be implied that the carp fish skin oil has appreciable Phytochemiclas and Antioxidant activity which makes it beneficial for health.*

Keywords: ***Labeo rohita*, Fish skin, Phytochemicals, Antioxidant, DPPH, TPC, FRAP, ABTS**

1. INTRODUCTION

Generally, fish contains 2-30% fat, and about 50% of the body weight is generated as waste during the fish processing operation. Fish oil is the lipid fraction extracted from fish and fish by-products[1]. Fish processing industries generate large amounts of wastes or by-products, as much as 70% of the original materials, in the form of skins, scales, bones, viscera, gills, dark muscles and heads[2]. These waste by-products have traditionally been discarded as waste or used as low-value by-products like animal feed, fertilizers, etc. These waste material can be properly utilized for making value added food products[3]

Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits. Plants produce these chemicals to protect themselves but recent research suggests that phytochemiclas can also protect humans against disease[4].Fish oil is mainly produced from whole fish. Utilizing fish skin as a raw material for fish oil production reduces environmental pollution caused by fish processing waste[5].As fish live on sea, river and pond weed, it can be hypothesized that fish skin oil can also have some phytochemicals The phytochemical analysis was done for the detection of the alkaloid, flavonoids, tannins, phenols, cardiac glycosides, terpenes and steroids, volatile oils, balsam, coumarine, anthocyanine, saponins, present in crude n-hexane of *L.rohita*

Lipid oxidation is one of the most important quality deteriorating processes in lipid bearing foods and leads to great economic losses in the food industry[6].Oxidation of lipids not only produces rancid odours and flavours but can also decrease nutritional quality and safety. Therefore, it is important to improve the stability of fish oil for safe consumption. Antioxidants are molecules that protect biological targets against oxidative damage and are a major part of the individual defence mechanism. Antioxidative property of the crude n-hexane extract of fish skin was determined via total phenolic acitivity, DPPH free radical scavenging activity, ABTS decolorization antioxidativeacitivity, FRAP assay.

This work describes the study performed on the extraction, physico-chemical characterization phytochemical screening and antioxidative activity of fish oil from the discardedfish skin during fish selling to use it as an alternative to regular edible oil.

2. MATERIALS AND METHODS

2.1. Materials

All reagents that used were of analytical grade (AR). n-hexane, distilled water, ethanol, sodium hydroxide(NaOH), potassium iodide, chloroform, glacial acetic acid, sodium thiosulphate, potassium hydroxide, methanol, hydrochloric acid(HCl), ferric chloride($FeCl_3$), ammonia were purchased from local suppliers. Maeyer's reagent, Dragendroff's reagent, Fehling reagent, Gelatin,Folin-Ciocalteu reagent, 2.2-Diphenyl-1-Picrylhydrazyl(DPPH), 2,2'-azinobis (3-ethylbenzenethiazoline-6-sulfonic acid) [ABTS],2,4,6-tripyridyl-S-triazine, ascorbic acid, Gallic acid were brought from Sigma Chemical Co.,USA.

2.2. Oil Sample Preparation

2.2.1. Sample Collection

Fish skin from rohu fish (*L.rohita*) of 2kg weight was collected from the local market of Chatra, Serampore town, Hooghly, West Bengal, India. The skin was then washed and the scales were separated from the skin properly. The washed skins were kept in anair tightcontainer and stored at -20^{o}C until further use. Before oil extraction, the skin was thawed to room temperature.

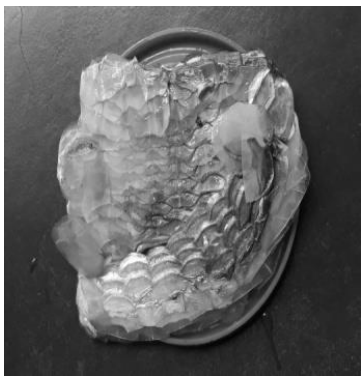

Fig 1. Collected skin from local market

Fig 2. Scales are separated from the skin

2.2.2. Oil Collection

Stored skinsamples were dried at 60ºC for 24 hours in a hot air oven. The dried samples are then grinded in a morterprester and soaked in n-hexane in 1:2 ratios for 24 hours followed by filtration and distillation. The oil collected is stored at 4 ºC for further analysis.

Fig3. Steps for Oil collection from Fish Skin

3. PHYTOCHEMICAL SCREEINING

The qualitative screening of bioactive molecules presentsin the FSO was carried out via standard methods. Qualitative methods were applied to detect the presence or absence of alkaloid, flavonoids, tannins, phenols, cardiac glycosides, terpenes, steroids, volatile oils, balsam, coumarine, anthocyanine, saponins, Anthroquinone[8-12]

3.1. Test for alkaloids:

0.5 sample was mixed in 8 ml of 1% HCl, warmed and filtered. 2 ml of the filtrate were treated separately with both reagents (Maeyer's and Dragendorff's), after which it was observed whether the alkaloids were present or absent in the turbidity or precipitate formation.

3.2. Test for glycosides (Keller-kilani test):

A mixture of Acetic acid glacial (2 ml) with 2 drops of 5% $FeCl_3$ solution and concentrated$H_2SO_4$was added to the oil. A brown ring produced between the layers indicated the entity of cardiac glycosides.

3.3. Test for the phenolic compound:

2 ml of 5% solution of$FeCl_3$ mixed with oil. Green color indicated the presence of phenolic compounds.

3.4. Test for saponins (Foam test):

About 1ml of oil was introduced into a tube containing 1 ml of distilled water, the mixture was vigorously shaken for 2 min, and formation of foam indicated the presence of saponins.

3.5. Test for reducing sugar (Fehling test):

A mixture of equal volumes of Fehling solutions A and B were boiled with the oil . A red color precipitate indicated the presence of reducing sugars.

3.6. Test for steroid:

2 ml of chloroform and concentrated H_2SO_4 were mixed with the oil. In the lower chloroform layerwas produced redcolor that indicated the presence of steroids.

3.7. Test for coumarine:

10% NaOH was added to the oil and chloroform was added. Fomation of yellow colour indicated the presence of coumarine.

3.8. Test for Flavonoids:

2ml of sodium hydroxide was added in 2ml of oil. Appearence of yellow color was regarded as the presence of flavonoids.

3.9. Test for tannin (Gelatin test):

50 mg of oil wasdissolved in 5 ml of distilled water and 2 ml of 1% solution of Gelatin containing 10% NaCl is added to it. A white precipitate indicates the presence of tannin.

3.10. Test for Anthocyanin:

2 ml of oil were mixed with 2ml HCl(2N) and ammonia. Appearence of pinkish red to blush violet colour indicated the presence of anthocyanine.

3.11. Test for Anthroquinones(Borntrager s Test):

About 5 mg of the oil was boiled with 10% HCl for a few minutes in a water bath. It was filtered and allowed to cool. An equal volume of $CHCl_3$ was added to the filtrate. Few drops of 10% NH_3 were added to the mixture and heated. Formation of pinkcolour indicated that the presence ofanthroquinones.

3.12. Test for Balsams:

2 ml of oil was mixed with an equal volume of 90% ethanol. 2 drops of alcoholic ferric chloride solution added to it. Appearence of dark green colour shows the presence of balsams.

3.13. Test for Volatile oils:

2 ml of oil were added with 0.1 ml of diluted sodium hydroxide and a smallamount of diluted hydrochloric acid. The formation of a white precipitate indicates volatile oils.

Fig4. Different Phytochemical Screening

4. ANTIOXIDANT ACTIVITY

4.1. Determination of Total Phenolics

The total phenol content (TPC) was measured using the Folin-Ciocalteuassay[7]. 0.2 mL of the oil was taken into test tubes followed by 0.5 mL Folin-Ciocalteu's reagent (diluted 10 times with water). The solution was then kept at dark for 5 min and then 1 mL sodium carbonate (7.5% w/v) was added. The tubes were kept in the dark for 1 h. Absorption at 765 nm was measured with a spectrophotometer (Jasco V-630) and compared to a Gallic acid calibration curve. The results were expressed as mg Gallic acid/g of sample.

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

A volume of 2 mL of a methanolic solution of the oil was put into test tubes and 2 mL of 1 mM DPPH solution was added. The tubes were kept in the dark for 1 h. Absorbance at 517 nm was measured with a spectrophotometer (Jasco V-630) and compared to an ascorbic acid calibration curve. The results were expressed as mg ascorbic acid/g of sample. The percentage inhibition of the DPPH radical was calculated.

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

The antioxidant activity by the ABTS method was performed by using a volume of 20 µL (diluted 1:10) of oil extract added to 2 mL of ABTS solution, and the mixture was kept at a room temperature for 10 mintues. The absorbance was measured at 734 nm with a spectrophotometer (Jasco V-630). The values were compared with those of the BHT (butyl hydroxy toluene) standard curve.

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

The antioxidant activity by FRAP assay was carried out by the method of the ferric reducing ability of plasma (FRAP) assay of Benzie and Strain 1996 with slight modifications. 20 µL of sample and 180 µL of FRAP reagent were and incubated at 37 ◦C for 40 min in the dark. The absorbance of the resulting solution was measured at 593 nm in a UV spectrophotometer (Jasco V-630). The change in absorbance between the final reading (4-min reading) and blank reading (0 min reading) of the sample and the same of standard Ascorbic acid was selected for the calculation of FRAP value.

5. RESULT

5.1. Yield of FSO from fish skin

The yield of FSO from the fish skin was 25.40%.

5.2. Phytochemical Screening

The result of the Phytochemical Screening of*L.rohita*skin oil shows that the oil contains several important secondary metabolites. Table-1 indicates the presence and absence of different Phytochemicals which are confirmed qualitatively by standard methods.

Table-1: Qualitative Assessment of Phytochemicals present

Phytochemical	Test	Confirmatory Indication	Results
Alkaloids	Mayers Test	White precipitation	+
Glycoside	Keller-Killiani Test	Reddish brown ring	+
Phenolic Compounds	Ferric chloride Test	Green colouration	+
Saponins	Foam Test	Bubble formation	+
Reducing Sugar	Fehling Test	Yellow Colouration	+
Steroid	Ejikene*et al*.	Redish –brown colouration at lower layer	+
Coumarine	NaOH Test	Yellow colour	+
Flavonoid	Alkali Test	No yellow colour	-
Tanins	Gelatin Test	No white precipitation	-
Anthrocyanine	HCl& Ammonia Test	No Pinkish red-violet colouration	-
Anthroquinone	Borntranger's Test	Red colouration	+
Balsams	Ferric chloride Test	No dark green colouration	-
Volatile Oil	HCl Test	No white Precipitation	-

5.3. Anti oxidant Activity analysis

Fish skin Oil (FSO) is subjected to anitioxidant assay via Total Phenolic Cntent (TPC) assay using Gallic acid as standard, DPPH free radical scavenging activity assay, ABTS decolorization activity asaay and FRAP assay using Ascorbic acid as standard. Table-2 reveals the different antioxidative capacity of the Fish Skin oil.

Table-2: Antioxidativeassays of FSO

Antioxidant Test	Amount
TPC assay	6.50 ± 0.02 µg GAE/mL,
DPPH assay	(3.84 ± 0.02) %
ABTS assay	(3.92 ± 0.02) %
FRAP assay	(990 1.32) µg of AAE /mL

6. STATISTICAL ANALYSIS

All statistical analysis was done using standard deviation. All analyticaltests were performed in triplets.

7. DISCUSSION

All physic-chemical analysis of the FSO was satisfactory. The results of the qualitative screening of Phytochemicals show that FSO contains different secondary bioactive metabolites. Secondary metabolites are produced by the plants as a part of the defence system of the plant. So phytochemicals have intense health benefits and can be used in food products, pharmaceutical products as well as in cosmetic products. Among various phytochemicals, FSO contains Alkaloids, Glycoside, Phenolic compounds, Saponins, Reducing sugar, Steroid, Coumarine as active compounds qualitatively which have health benefits. On the other hand, it is revealed that the oil does not contain Flavonoid, Tanin, Anthrocayanine, Anthroquinone, Balsams, Volatile oil as active compounds. Alkaloids are said to active against bacteria and they are also used in the treatment of skin disorders such as eczema, seborrheic dermatitis, and neurodermatitis. Steroids are used in the cosmetic, soap and pharmaceutical industries to produce creams, soaps, and ointments. Phenolic compounds are one of the most common and widespread secondary metabolites, earlier research reveals that these compounds are produced through the phenyl-propanoid biosynthetic pathway and are the building blocks for plant pigments which are used for 67 cell wall development. Glycosides, Sugars, Steroids are proven to exhibit anti-bacterial, anti-fungal and anti-carcinogenic properties against various bacteria, fungus, and carcinogenic materials. FSO containing these important phytochemiclas can be utilized in value added food products and also for medicinal purposes.

Antioxidative activity analysis also states that FSO can withstand oxidative damage as all the results of TPC, DPPH test, ABTS assay and FRAP assay are under the range of satisfaction.

From the above discussion, it can be concluded that FSO can be used as a health beneficiaryfood product ingredient.

8. ACKNOWLEDGEMENT

The authors are thankful to the Indian Institute of Engineering Science & Technology (IIEST), Shibpur, Howrah, West Bengal, India for providing all the necessary facilities and cooperation during the work. The authors are also thankful to all the research fellow of the School of Community Science & Technology, IIEST, Shibpur lab for rendering help when needed.

9. REFERENCES

1) F. Guerard, L. Guimas, A. Binet. Production of tuna waste hydrolysates by a commercial neutral protease preparation, Journal of molecular catalysis B: Enzymatic, (2002). 19-20,489-498.

2) GhalyAE ,Ramakrishnan VV, Brooks MS, Budge SM and Dave DFish Processing Wastes as a Potential Source of Proteins, Amino Acids and Oils: A Critical Review, Microbial & Biochemical Technology, (2013)-5 (4), 107-129.

3) Lawrence Abbey, Mary Glover-Amengor, Margaret O. Atikpo, Amy Atter&JogeirToppe. Nutrient content of fish powder from low value fish and fish byproducts, Food Science & Nutrition. (2017). 5(3):374–379**,** doi: 10.1002/fsn3.402.

4) Pravinkumar Moorthy, Eugien Xavier Lawrence, Viswanathan Chinnathambi and Raffi Mohammad Sirajudeen Extraction of fish body oil from Sardinellalongicepsby employing direct steaming method and its quantitative and qualitative assessment, Journal of coastal Life Medicine, (2015). doi:10.12980/jclm.3.201514j71.

5) Vincenza Ferraro, Ana P. Carvalho, Clara Piccirillo, Manuela M. Santos, Paula M. L. Castro, Manuela E. PintadoExtraction of high added value biological compounds from sardine, sardine-type fish and mackerel canning residues — A review, Materials Science and Engineering C . (2013). 33, 3111–3120.

6) Kim JS, Mendis E, Bioactive compounds from marine processing by the products-a review, Food Research International, (2006). 39(4), 383-393.

7) Akinsanmi A. Odujeet. al. Assesment of the nutritional, anti nutritional, and antioxidant capacity of Uripe, ripe and over ripe Plantain (Musaparadisiaca) peels, International Journal of Advanced Research, (2015). 3(2), 63-72.

8) Harborne, A.J. Phytochemical methods a guide to modern techniques of plant analysis, (1998). ISBN 978-0-412-57260-9.

9) Kalpana Devi Rajesh, N.V Rajesh. Qualitative & quantitative phytochemical analysis in four pteridophytes, International Journal of Pharmaceutical Sciences Review and Research, (2014) article no. 72, pages 408-412.

10) MeryemSeladji ,ChahrazedBekhechi, FawziaBeddou, Hanane Dib, NassimaBendimerad. Antioxidant activity and phytochemical screening of Nepetanepetellaaqueous and methanolic extracts from Algeria, Journal of Applied Pharmaceutical Science. 4 (02), 012-016, February, 2014, DOI: 10.7324/JAPS.2014.40203.

11) UmerQadir, V.I. Paul, P. Ganesh. Preliminary phytochemical screening and in vitro antibacterial activity of Anamirtacocculus (Linn.) seeds, Journal of King Saud University- Science, (2015). 27, 97–104.

12) Abiona O. Oladapo, Shola H. Awojide. Quality evaluation of oil extracted from catfish and mackerel as compared with commertial cod liver oil, J. Food Chem. Nutr., (2015). 03 (01),13-18.