UTTAR PRADESH JOURNAL OF ZOOLOGY

43(9): 62-75, 2022
ISSN: 0256-971X (P)

ANTIOXIDANT PROPERTIES OF TRASH FISH *Odonus niger* LIVER OIL IN COMPARISON WITH COD LIVER OIL

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.56557/UPJOZ/2022/v43i93027

Editor(s):
(1) Dr. Ana Cláudia Correia Coelho, University of Trás-os-Montes and Alto Douro, Portugal.
Reviewers:
(1) Amina Abd Al-Rahman Fayad, University of Baghdad , Iraq.
(2) Wapwera Augustine Jidimma, University of Jos, Nigeria.

Received: 08 March 2022
Accepted: 17 May 2022
Published: 28 May 2022

Original Research Article

ABSTRACT

The present study was undertaken to compare the *in vitro* antioxidant properties of marine trash fish *Odonus niger* liver oil and commercially available cod liver oil. For this *O. niger* fishes were collected and the oil was extracted from the liver mass by following Bligh and Dyer method and it was purified. Further the antioxidant potential of various concentrations (12.5 to 200µg/ml) of the extracted oil was performed through different standard antioxidant assays, simultaneously the same concentrations of commercial cod liver oil were also performed. The antioxidant potential of both oils was compared with appropriate standards like ascorbic acid, gallic acid and quercetin in the respective assays. The different assays performed were hydroxyl radical scavenging activity (23.24%), total antioxidant activity (43.06%), DPPH radical scavenging activity (60.06%), reducing power effect (68.22%), lipid peroxidation inhibitory activity (53.10%) and ferrous ions chelating activity (75%) respectively for *O. niger* at the highest concentration of 200µg/ml. All the above activities showed that *O. niger* liver oil possessed better antioxidant potential, especially at higher concentration (200 µg/ml) than the tested commercial cod liver oil.

Keywords: *Odonus niger*; fish liver oil; antioxidant activity; cod liver oil.

1. INTRODUCTION

"All living organisms including humans have an efficient antioxidant network system protect the body from the free radicals and retard the progress of many chronic diseases" [1,2]. "Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced in the cell by oxidation. ROS and RSN are

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highly reactive and cause damage to biomolecules like lipid, DNA, RNA, proteins and carbohydrates that lead to mutation" [3,4]. If the body does not regulate the production of free radicals properly, "oxidative stress" is resulted, which can stimulate a variety of chronic and degenerative diseases such as antimutagenicity, anticarcinogenicity and antiaging [5,6,7]. Several human diseases are the direct or indirect outcome of oxidative stress [8].

Many antioxidant compounds are naturally occurring in fruits, vegetables, grains, spices, teas, herbs, cereals, sprouts, oils, cocoa shell and seeds [9,10,11]. Also, very little is known about antioxidant compounds present in fish oils and fish meats [12,13,14,15,16]. "Several *in vitro* studies have proved their efficiency in being effective radical scavengers, metal chelators and inhibitors of peroxidation levels in various systems, food stuffs and also oils. Recently, interest has increased considerably in finding naturally occurring antioxidants for use in foods or medical materials to replace synthetic antioxidants, which are restricted due to their side effects such as carcinogenicity" [3,4].

"The use of natural antioxidants is emerging as an effective methodology for controlling rancidity and limiting its deleterious consequences. Many different *in vitro* models have been introduced to evaluate the antioxidant activities so as to assess an antioxidant that would be useful for food and biological system" [17,18]. "Fish oil is different from other oils mainly because of the unique variety of fatty acids, contains high level of polyunsaturated fatty acids, which are essentially required for metabolic activities and possess strong antioxidant abilities" [19,20,21,22,23]. "Marine fish oils contain greater amount of omega-3 fatty acids, whereas seaweeds and shellfish such as crustaceans have potent antioxidants including carotenoids and phenolic compounds" [24]. Vitamin E and carotenoids are antioxidants found in wild salmon oil [25,26]. "Moreover, astaxanthin in salmon oil is associated with reduced risk of diseases such as age-related macular degeneration and ischemic diseases, effects attributed to its potent antioxidant activity" [27].

"The antioxidant activity of fish oil refers to its chemical property of neutralizing free radical damage present in the body" [28]. "Traditionally, fish oil has been produced from by-catch fish and fish specifically caught for fish meal and oil production" [29]. Consumption of these fish oils could reduce the oxidations and other damaging reactions in human beings or other biological systems. In addition the oxidative as well as the hydrolytic stability of fish oils can greatly differed among fish species [30]. In a recent study, *in vitro* antioxidant properties of various fish oils such as Atlantic salmon fish oil by-products from belly part, trimmed muscle and bone oil were studied. Their antioxidant properties were determined by DPPH, Azino-di (3-ethylbenzthiazoline 6-sulfonate) (ABTS), ferric ions (Fe^{3+}) reducing power antioxidant and hydroxyl radical scavenging activities [31]. Cod liver oil can act as a very good antioxidant due to the presence of omega-3 fatty acids. It can be used regularly as a daily health supplement to improve health [32]. The antioxidant properties of blue fin tuna fish *Thunnus thynnus* oil were examined by determining DPPH radical scavenging activity and reducing power effect [33]. Haq and Byung-Soo [34] stated that salmon oil is having higher DPPH and ABTS radical scavenging activity. *Odonus niger* is a marine trash fish of Balistid group, the liver oil of this fish contain high amount of omega-3 and omega-6 polyunsaturated fatty acids as well as being a good source of protein, minerals and vitamins [35]. *O. niger* fish oil has good properties for pharmaceutical and biomedical applications [36]. Considering the above characteristics of fish oil as an antioxidant, the present study was undertaken to determine the antioxidant properties of the trash fish *O. niger* liver oil in *in vitro* condition with a comparison of cod liver oil.

2. MATERIALS AND METHODS

2.1 Extraction of Liver Oil from the Trash Fish *Odonus niger*

The trash fish *O. niger* was collected from the fish landing centers of Kanyakumari District, South India, in fresh condition. From the fish, the liver samples were aseptically removed and 100 g of liver mass was taken in a 2L beaker. To this 10ml of distilled water, 200ml of chloroform and 400 ml of methanol were added and mixed well, and then the mixture was homogenized for 2min whilst being cooled in ice. Then 200 ml of more chloroform was added and homogenized for one minute, followed by 200 ml of distilled water was added and finally homogenized for 30 seconds. The mixture was centrifuged at 2000 rpm for 20min and then the aqueous layer was removed by suction. The chloroform fraction was evaporated using a rotary evaporator to remove the chloroform completely [37]. Then the extracted oil was stored in refrigerated condition for further use.

2.2 Determination of Antioxidant Properties of *O. niger* Liver Oil

2.2.1 DPPH (2, 2- diphenyl-1- picrylhydrazyl) radical scavenging activity

The free radical scavenging activity of *O. niger* and commercial cod liver oils was measured as a decrease

in the absorbance of the solution of 1-1- diphenyl-2-picryl- hydrazyl (DPPH) [38] Nabavi et al., [39]. Initially a stock solution of DPPH (0.1mM) was prepared in methanol, further different concentrations (12.5, 25, 50, 100 and 200µg/ml) of *O. niger* liver oil and cod liver oil were individually taken. To this 1.48ml of DPPH solution was added and incubated at room temperature for 20 min. After incubation, pale pink colour developed in the oil samples was measured at 517nm in a UV Spectrophotometer (Techcomp-8500; Hong Kong) and compared with the standard (12.5-200 µg/ml ascorbic acid). Free radical scavenging activity was expressed as the percentage inhibition calculated using the following formula

DPPH scavenging activity (%) = (Absorbance of control – Absorbance of test) / Absorbance of control X 100

2.2.2 Lipid peroxidation inhibitory activity (β-Carotene bleaching assay)

The lipid peroxidation inhibitory activity of *O. niger* liver oil and cod liver oils was determined using β-carotene bleaching assay [40,41]. β- Carotene (0.2mg) in 0.2ml chloroform, linoleic acid (20 mg) and Tween 20 (200 mg) were mixed together. After mixing, the chloroform was removed at 40ºC under vaccum condition, and the resulting mixture was diluted with 10 ml of water and mixed well. To this emulsion, 40 ml of oxygenated water was added. 4 ml each of the aliquot of the emulsion was pipetted into different test tubes containing 0.2ml of *O. niger* liver oil and commercial cod liver oil at different concentrations (12.5-200 µg/ml) in ethanol. A control containing 0.2ml of ethanol and 4 ml of the above emulsion was prepared. The tubes were placed at 50ºC in a water bath and the absorbance was measured at 470 nm, after incubation for 180 minutes. Ascorbic acid at 12.5-200 µg/ml was used as standard. All determinations were carried out in triplicate. The antioxidant activity was calculated by the following formula

Lipid peroxidation inhibitory activity (%) = $100\ [1- (A_o – At) / (A^o_o - A_ot)]$

where,

A_o =Absorbance of test sample at zero time incubation

A^o_o = Absorbance of control at zero time incubation

At =Absorbance of test sample after incubation for 180 min

A^ot =Absorbance of control after incubation for 180 min

2.2.3 Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity of *O. niger* liver oil and cod liver oils was measured by the method of Kunchandy and Rao [42]. In different concentrations (12.5 to 200µg/ml) of *O. niger* and cod liver oils, 500µl of the reaction mixture [$FeCl_3$ (100 µM), EDTA (104µM), H_2O_2 (1mM), 2- deoxy- D- ribose (2.8 mM)] was added and the final volume was made up to 1ml with potassium phosphate buffer (20mM, pH 7.4). Further the reaction mixture taken in the test tubes was incubated for 1hr at 37ºC. After incubation 1.0 ml of thiobarbituric acid (1%) and 1.0ml of trichloroacetic acid (2.8%) were added in to the test tubes and incubated again at 100^0 C for 30 min. After cooling, the absorbance was measured at 535nm against a control containing deoxyribose and compared with the standard drug gallic acid (12.5-200 µg/ml). The percentage of scavenging was determined by comparing the result of the test and control using the following formula

Hydroxyl radical scavenging activity (%) = (Absorbance of control – Absorbance of test) / Absorbance of control X 100

2.2.4 Total antioxidant activity

Total antioxidant property of both *O. niger* and cod liver oils was evaluated by the method of Prieto et al. [43]. In brief, 0.3 ml each of both *O. niger* and cod liver oils at different concentrations (12.5 – 200 µg/ml) were taken individually in 15 ml test tubes, to this 3ml each of reagent solution (0.6 M sulphuric acid, 28mM sodium phosphate and 4 mM ammonium molybdate) was added. Then the test tubes were incubated at 95ºC for 90min. After incubation, the absorbance of the reaction mixture was measured at 695 nm using UV-VIS spectrophotometer. The total antioxidant activity was expressed as the number of gram equivalent of the standard ascorbic acid and the calibration curve was prepared using various concentration of ascorbic acid (12.5, 25, 50, 100, 200 µg/ml) as standard.

Total antioxidant activity (%) = (Absorbance of control – Absorbance of test) / Absorbance of control X 100

2.2.5 Reducing power effect

The reducing power effect of the fish liver oils was determined by the modified method of Yamaguchi et al. [44]. Reaction mixture containing different concentrations (12.5- 200 µg/ml) of both *O. niger* and commercial cod liver oils, 2.5 ml of phosphate buffer (0.2M, pH 6.6) and 2.5ml of 1% potassium ferricyanide were taken. The mixture was incubated at

50ºC for 20min. After that, 2.5ml of TCA (10% w/v) was added and centrifuged for 10 min at 2000 rpm. The supernatant was collected, to this 1ml of distilled water and 0.1% (w/v) ferric chloride solution were added and the absorbance was measured at 700 nm. Quercetin at the concentration of 12.5-200 µg/ml was used as standard. The percentage reducing power was calculated by the following formula

> Reducing power activity (%) = (Absorbance of control – Absorbance of test) / Absorbance of control X 100

The chelating of ferrous ions by fish liver oils was estimated by the method of Dinis et al. [45], where in the Fe^{2+} chelating ability of oil was monitored by the absorbance of the ferrous iron- ferrozine complex at 562 nm. For this, 0.4ml of *O. niger* liver oil and commercial cod liver oil at different concentrations (12.5-200 µg/ml) were taken in individual test tubes. To this a solution of 2mM $FeCl_2$ (0.2 ml) was added. The reaction was initiated by the addition of 5mM ferrozine (0.4 ml) and the total volume was adjusted to 4ml of ethanol. Then, the mixture was shaken vigorously and left at room temperature for 10 minutes. Ascorbic acid was used as standard at 12.5-200 µg/ml concentrations. Absorbance of the solution was then measured spectrophotometrically at 562 nm. The percentage of inhibition of ferrozine – Fe^{2+} complex formation was calculated by using the formula

> Ferrous ions chelating activity (%) = [1- (As / Ac)] × 100

Where, As = Absorbance of sample; Ac = Absorbance of control.

2.3 Statistical Analysis

The data obtained in the present study were expressed as Mean±SD and were analyzed using ANOVA at 5% level of significance. Further a multiple comparison test (SNK) was conducted to compare the significant differences among the parameters using a computer software STATISTICA 06 (Statosoft, Bedford, UK).

3. RESULTS

The present study was designed to examine the *in vitro* antioxidant properties of *O. niger* liver oil in comparison with commercial cod liver oil.

3.1 DPPH Radical Scavenging Activity

The result on DPPH radical scavenging activity of both the *O. niger* and cod liver oils possessed better activity (53.26 and 56.02%) at the highest (200 µg/ml) concentration. Here, DPPH radical scavenging activity was increased with increasing concentrations of liver oils (Fig. 1). At the lowest concentration (12.5µg/ml), both *O. niger* (23.24±1.02%) and cod liver oil (24.16±0.84%) showed minimum radical scavenging activity. At the same time, the DPPH radical scavenging activity of the standard drug ascorbic acid displayed 54.02% at the highest concentration of 200µg/ml. The IC_{50} value recorded was 45.285 and 48.885µg/ml respectively for *O. niger* and cod liver oils, however the IC_{50} value observed for standard drug ascorbic acid was 42.993µg/ml. The statistical Two-way ANOVA test revealed that the DPPH radical scavenging activity as a function of variation between different antioxidant sources (F= 6.040) as well as their concentrations (F= 141.080) were statistically more significant (P< 0.05 to 0.001) (Table 1).

3.2 Lipid Peroxidation Inhibitory Activity (β-Carotene Bleaching Assay)

The β-carotene - linoleic acid activity of *O. niger* and cod liver oils are shown in Fig.2 with ascorbic acid as standard. The result exhibited the highest activity of 67.31±2.21% and 62.51±1.86% at the maximum concentration (200 µg/ml) of *O. niger* liver oil and cod liver oil respectively. At the same time, lowest activity of 43.06±1.00% and 31.55±1.02% at the minimum concentration (12.5µg/ml) of both the *O. niger* and cod liver oils, respectively. However, the standard drug ascorbic acid displayed the β-carotene-linoleic acid activity of 56.56±1.82% at the highest concentration of 200µg/ml. The IC_{50} value observed was 55.15, 49.99 and 40.74µg/ml in *O. niger* liver oil, cod liver oil and the standard ascorbic acid, respectively. The Two-way ANOVA test revealed that the β-carotene - linoleic acid activity as a function of variation between different antioxidant sources (F= 21.20), as well as their concentrations (F= 49.135) were statistically more significant (P< 0.001) (Table 1).

3.3 Hydroxyl Radical Scavenging Activity

The *in vitro* hydroxyl radical scavenging effect of the tested oil samples was increased with their increasing concentrations. At the lowest concentration of 12.5µg/ml of *O. niger* liver oil, the hydroxyl radical scavenging activity displayed 6.17±0.48% and it was 5.18±0.62% in cod liver oil. However, when the concentration level increased to 200µg/ml, the hydroxyl radical scavenging activity displayed 60.06±1.42 and 58.33±1.48% in *O. niger*and cod liver oils, respectively (Fig.3). At the same time, in standard drug gallic acid, the hydroxyl radical scavenging activity showed 61.32±1.23% at the highest (200 µg/ml) concentration. The 50%

inhibitory concentration (IC_{50}) of both *O. niger* and cod liver oils determined as 30.863 and 28.173 µg/ml, respectively, whereas it was 30.218µg/ml in standard drug gallic acid. The Two-way ANOVA test revealed that the hydroxyl radical scavenging activity as a function of variation between different sources of antioxidants was statistically non-significant (F= 2.74; P> 0.05), whereas, variation between different concentrations of antioxidants was statistically more significant (F= 155.608; P< 0.0001) (Table 1).

3.4 Total Antioxidant Activity

The result on total antioxidant potential of both *O. niger* and cod liver oils is represented in Fig.4. Concentration dependent antioxidant activity was observed in both *O. niger* and cod liver oils. The minimum antioxidant activity of 15.08±0.84% and 13.07±0.62% was observed at the lowest concentration (12.5µg/ml) of both *O. niger* and cod liver oils respectively. But maximum antioxidant activity of 68.22±2.15 and 65.23±1.96% was observed at the highest (200 µg/ml) concentration of both *O. niger* and cod liver oils respectively. Here, *O. niger* liver oil possessed highest antioxidant capacity when compared with cod liver oil. The result was compared with the standard drug ascorbic acid, which exhibited 88.28±2.40% activity at 200µg/ml concentration. The IC_{50} value of standard drug ascorbic acid as well as both *O. niger* and cod liver oils was observed as 53.544, 43.307 and 41.13µg/ml, respectively. The statistical Two-way ANOVA test revealed that the total antioxidant sources (F= 7.03) as well as different concentrations of antioxidant sources (F= 49.403) were statistically significant (P< 0.05 to 0.001) (Table 1).

3.5 Reducing Power Effect

The reducing power effect of both liver oils was increased with increasing concentrations (Fig.5). At the lowest concentration of 12.5µg/ml, the reducing power effect was recorded as 35.27±1.20% and 32.08±1.14% in *O. niger* and cod liver oils respectively. But at the highest concentration (200µg/ml), the reducing power effect was correspondingly increased to 53.10±1.41% in cod liver oil and 55.10±1.76% in *O. niger* liver oil. The IC_{50} value of *O. niger* and cod liver oils was recorded as 44.905 and 41.862µg/ml, respectively. The reducing power effect of both liver oils was compared with the standard drug quercetin, the result displayed the highest activity of 60.13±2.14% at 200µg/ml concentration and this standard drug has the IC_{50} value of 50.41µg/ml. The Two-way ANOVA test revealed that the reducing power activity as a function of variation between different antioxidant sources (F=125.445), as well as their concentrations (F= 564.841) were statistically more significant (P< 0.0001) (Table 1).

3.6 Ferrous Ions Chelating Activity

The ferrous ion chelating effect displayed with concentration dependent variation (Fig.6). At the lowest concentration of 12.5 µg/ml fish liver oils, the chelating activity recorded was 34.68±1.13% and 32.31±1.06, respectively in *O. niger* and cod liver oils. Whereas it was 75±2.52% and 74.61±2.26% in the highest concentration (200 µg/ml) of *O. niger* and cod liver oils respectively. In variably, there was a highest level of chelating activity of 79.17±2.36% 100% recorded in the standard drug ascorbic acid at 200µg/ml concentration. The IC_{50} value was recorded respectively as 56.729, 55.180 and 60.119µg/ml for *O. niger* liver oil, cod liver oil and the standard ascorbic acid. The Two-way ANOVA test revealed that the ferrous ions chelating activity as a function of variation between different antioxidant sources (F= 14.104), as well as their concentrations (F= 435.707) were statistically more significant (P< 0.05 to 0.0001) (Table 1).

4. DISCUSSION

Natural defense mechanism of an organism is overwhelmed by an excessive generation of reactive oxygen species (ROS), thereby oxidative stress occurs. Consequently cellular and extracellular macromolecules suffer through oxidative damage [46]. Antioxidant supplementation is necessary to support the defense system against variety of diseases and environmental stresses. Antioxidant compounds in food play an important role as a health-protecting factor and hence interest on antioxidants in diet has been increased. Synthetic antioxidants such as butylatedhydroxyanisole (BHA), butylatedhydroxytoluene (BHT), t-butylhydroquinone (TBHQ) and propyl gallate could be used to retard lipid peroxidation in foods [47]. However, the use of synthetic antioxidants is under strict regulation due to their potential health hazards and toxicity [48]. Moreover, consumers are increasingly avoiding foods supplemented with preservatives of chemical origin; therefore, the importance of exploiting natural antioxidants from various sources and replacing synthetic antioxidants with natural ingredients has attracted increasing attention. Carotenoids, tocopherols, ascorbates and polyphenols are strong natural antioxidants present in variety of plants and animals based food sources, similarly essential oils are also present in these food sources and they were

shown to protect against oxidative stress by playing the total antioxidant defense system. The antioxidant properties of certain essential oils have been demonstrated, which may be important in suppressing lipid oxidation [49,50,51]. Fish oils are having good antioxidant properties [52] and fish oils have been demonstrated to play an important role as free radical scavengers and antioxidants for the prevention of oxidative damage in living organisms. In the present study *in vitro* assays were carried out to evaluate the antioxidant properties of *O. niger* liver oil in comparison with cod liver oil. *In vitro* antioxidant activity of these oils were evaluated by DPPH radical scavenging activity, lipid peroxidation inhibitory activity, hydroxyl radical scavenging activity, total antioxidant activity, reducing power assay and ferrous ions chelating activity.

DPPH assay is widely used as a model system in the assessment of scavenging activities of several natural compounds [53]. In the present study DPPH free radical scavenging activity of both *O. niger* and cod liver oil was recorded as 53.26% and 56.02% respectively at the highest concentration of 200 µg/ml, at the same time DPPH scavenging activity of the standard drug ascorbic acid was recorded with 54.02% at the same concentration. Similarly, Haq et al. [31] reported that Atlantic salmon fish pressed belly part oil and hexane extracted belly part oil exhibited 54.790% and 55.5% DPPH radical scavenging activity at 100 µl concentration. Akmal and Roy [32] suggested that the cod liver oil exhibited 42.45% DPPH scavenging activity at 0.6mg/ml concentration. Likewise, Unlu et al. [54] observed that essential oil from the fisch *Thymus pectinatus* exhibited 14.7% DPPH scavenging activity at 9.5µg/ml concentration. In the present study, the DPPH assay of *O. niger* and cod liver oil get confirmed that these oils have better free radical scavenging potential.

Lipid peroxidation inhibitory activity is determined by measuring the inhibition of the volatile organic compounds and the conjugated dienehydroperoxides arising from linoleic acid oxidation [55]. β- Carotene reacts with a peroxyl radical to form a resonance-stabilized carbon-centered radical within its conjugated alkyl structure, thereby inhibiting the chain propagation effect of ROS [56]. In the present study maximum (67.31%) lipid peroxidation inhibitory activity was shown by *O. niger* liver oil at 200 µg/ml concentration, whereas commercial cod liver oil exhibited 62.51% inhibition at the same concentration. Comparatively it was higher than that of the standard drug ascorbic acid (56.56%). Baroty et al. [57] portrayed that essential oils from cinnamon and ginger showed the lipid peroxidation activity of 66.5% and 82.3% respectively at 120µg/ml concentration. At the same time Luterotti et al. [58] reported that salmon oil exhibited 172.0ng/ml lipid peroxidation activity at 100mg/ml concentration. Akmal and Roy [32] stated that at the concentration of 0.5mg/ml cod liver oil, the lipid peroxidation activity was observed to be more (50.70%). Sellami et al. (2018) suggested that the ray fish *Dasyatis pastina* liver oil exhibited good effect on lipid peroxidation activity at 22.72mg/g concentration. Nasri et al. [59] stated that *Zosterissessor ophicephalus* fish protein exhibited 64.8% lipid peroxidation activity at 5mg/ml concentration. Similarly, Kumari et al. [60] pointed out that fish oil with ginger oil inhibit β-carotene activity of 52.94% at 5% concentration. In accordance with that of the above studies, the present finding proved that the antioxidant activity of *O. niger* liver oil possessed better effect on β-carotene- linoleic acid activity than cod liver oil.

Hydroxyl radical is particularly a reactive free radical formed in biological systems and has been involved as highly damaging free radical pathology [61]. This has a capacity to join nucleotides in DNA and cause damage that contributes to carcinogenesis, mutagenesis and cytotoxicity [62]. In the present study, the hydroxyl radical scavenging activity of *O. niger* and cod liver oils was determined with the their concentrations. Here, the maximum (60.06%) hydroxyl radical scavenging activity was shown by the liver oil of *O. niger* at 200µg/ml concentration; however the cod liver oil displayed 58.33% scavenging activity at the same concentration. Similarly Haq et al. [31] stated that the hydroxyl radical scavenging activity of Atlantic salmon bone oil had 57.56% at 100µl concentration. Jeevitha et al. [63] reported that the body oil of *Sardinella longiceps* exhibited better hydroxyl radical scavenging activity and its IC_{50} value was found to be 52.98% at 5mg/ml concentration. Similarly Je et al. [64] stated that the tuna fish *Katsuwonus pelamis* liver exhibited good hydroxyl radical scavenging activity of 60% at 2 mg/ml concentration. The present study indicated that *O. niger* liver oil comparatively possessed good hydroxyl radical scavenging activity.

The total antioxidant assay measures the amount of peroxide produced during the initial stages of oxidation, which is the primary product of lipid oxidation [65]. In the present study *O. niger* and cod liver oils possessed a better total antioxidant capacity (68.22 and 65.23%) at the maximum concentration of 200µg/ml, however the standard antioxidant ascorbic acid showed comparatively high (88.28%) antioxidant capacity at the same concentration. Wu and Bechtel [66] noticed that the pink salmon oil inhibit total antioxidant activity of 25% at 0.89µMole/g

concentration. Karthikeyan et al. [67] reported that marine diatoms powder inhibited total antioxidant activity of 45.03% at 0.1mg/ml concentration. Gulcin et al. [68] stated that clove oil exhibited total antioxidant activity of 99.7% at 45µg/ml concentration.

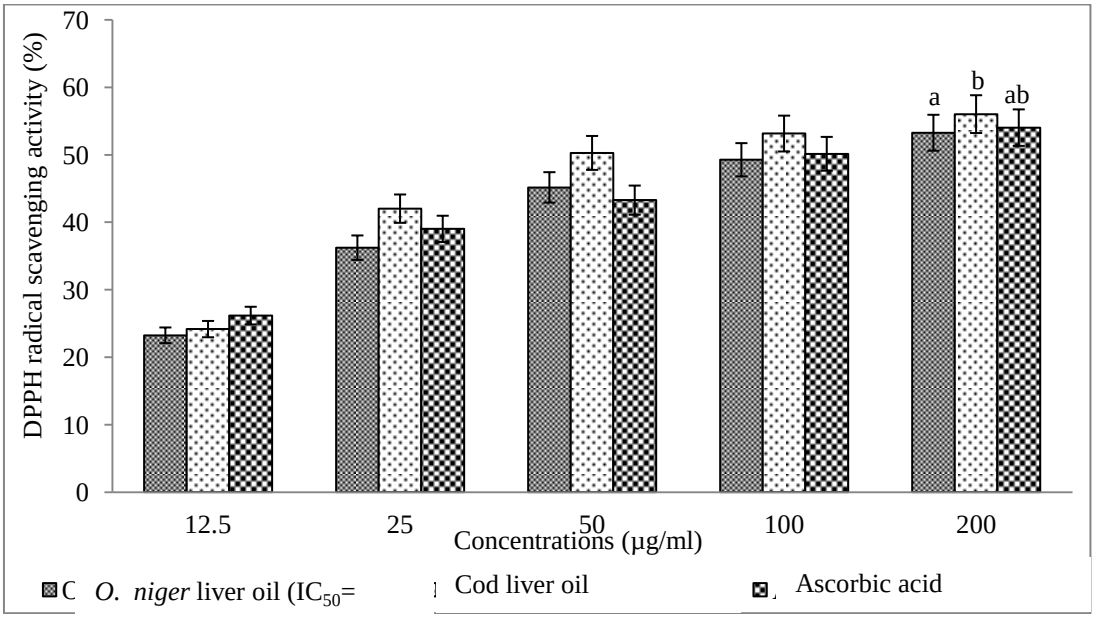

Fig. 1. *In vitro* DPPH radical scavenging activity of *O. niger* and commercial cod liver oils at different concentrations in comparison with the standard ascorbic acid

Each value is the Mean ± SD of triplicate analysis; Bars with different superscript alphabets are statistically significant (One-Way ANOVA test; P< 0.05 and subsequently post hoc multiple comparison with SNK test)

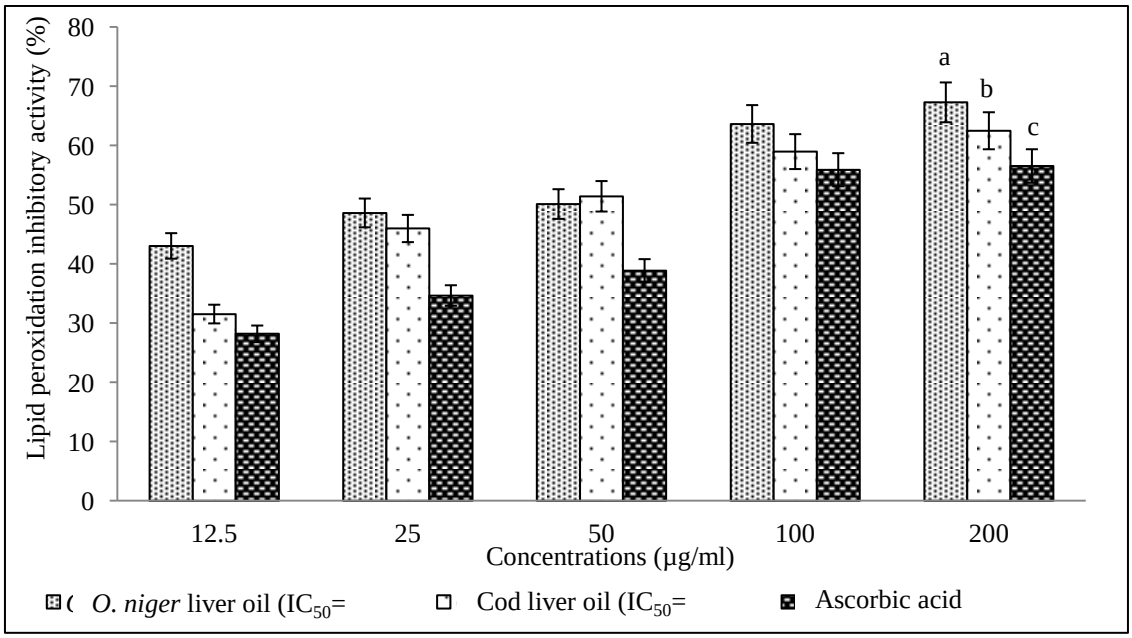

Fig. 2. *In vitro*lipid peroxidation inhibitory activity of *O. niger* and commercial cod liver oils at different concentrations in comparison with the standard ascorbic acid

Each value is the Mean ± SD of triplicate analysis; Bars with different superscript alphabets are statistically significant (One-Way ANOVA test; P< 0.05 and subsequently post hoc multiple comparison with SNK test)

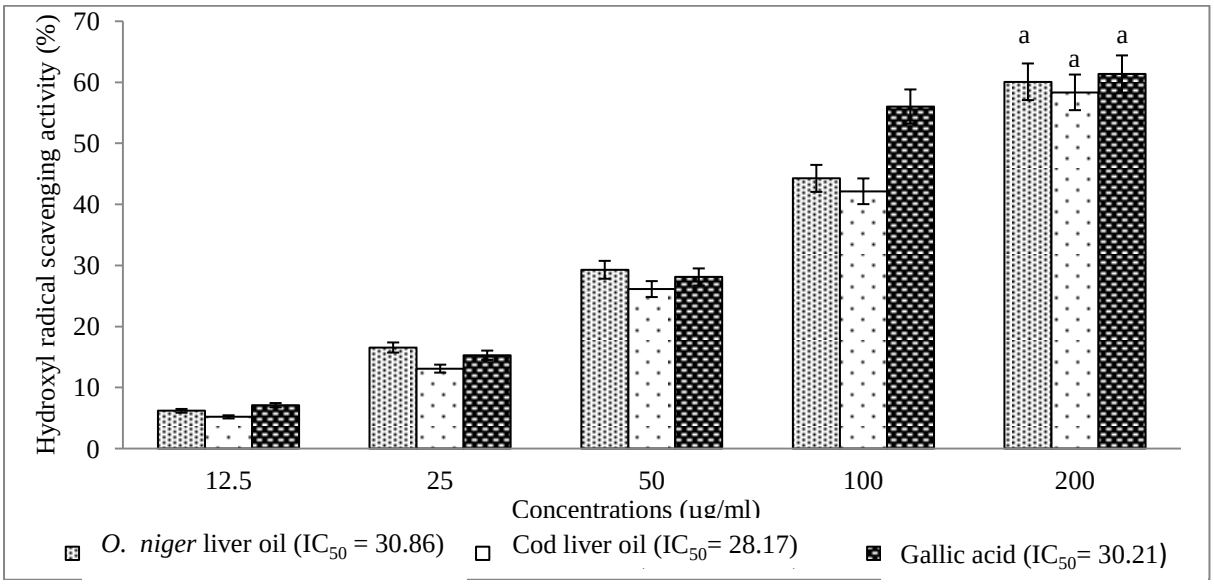

Fig. 3. *In vitro* hydroxyl radical scavenging activity of *O. niger* and commercial cod liver oils at different concentrations in comparison with the standard gallic acid

Each value is the Mean ± SD of triplicate analysis; Bars with same superscript alphabets are statistically non-significant (One-Way ANOVA test; P> 0.05 and subsequently post hoc multiple comparison with SNK test)

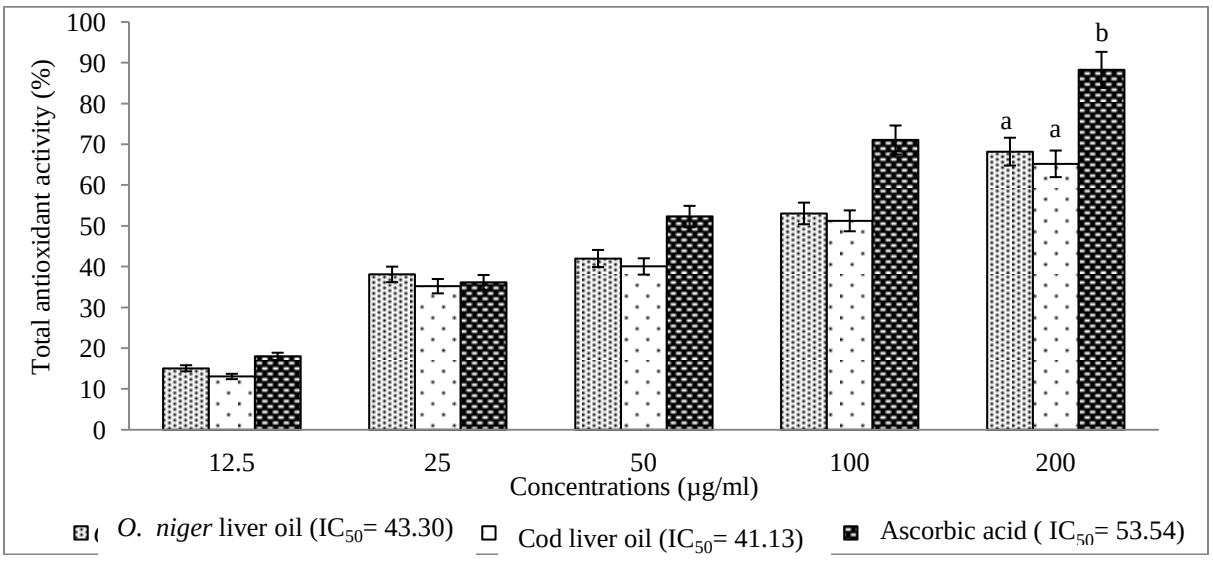

Fig. 4. *In vitro* total antioxidant activity of *O. niger* and commercial cod liver oils at different concentrations in comparison with the standard ascorbic acid

Each value is the Mean ± SD of triplicate analysis; Bars with different superscript alphabets are statistically significant (One-Way ANOVA test; P< 0.05 and subsequently post hoc multiple comparison with SNK test)

"Reducing power activity is often used to evaluate the ability of natural antioxidant to donate electron" [69,70]. "The existence of reductones are the key of the reducing power, which exhibit their antioxidant activities through the action of breaking the free radical chain by donating a hydrogen atom" [71]. Natural antioxidants are believed to break free radical chain reaction by donating an electron or hydrogen atom to free radicals. Therefore the reducing power of a compound is a significant indicator of its potential antioxidant activity. In the present study, the reducing power effect of *O. niger* and cod liver oils was recorded with 55.10 and 53.10% at the highest (200 µg/ml) concentration, when compared to the standard drug quercetin (60.13%). There was a stable increase in reductive potential of the oil was observed with increase in the concentration. Haq et al. [72] reported that Atlantic salmon fish bone oil exhibited an

antioxidant activity against reducing power with 39.42% at 100µl concentration. Similarly, Bordbar et al. [73] stated that the reducing power effect of oil from *Actinopyga lecanora* fish tissue was recorded as 23.95 mmol/100 ml. Likewise, Politeo et al. [74] pointed out that the essential oil from *Ocimum basilicum* L. expressed the reducing power effect of 44% at 20µg/ml concentration. The present finding concludes that both *O. niger* and cod liver oils possessed good reducing power effect, which may be due to the characteristic quality of fatty acids.

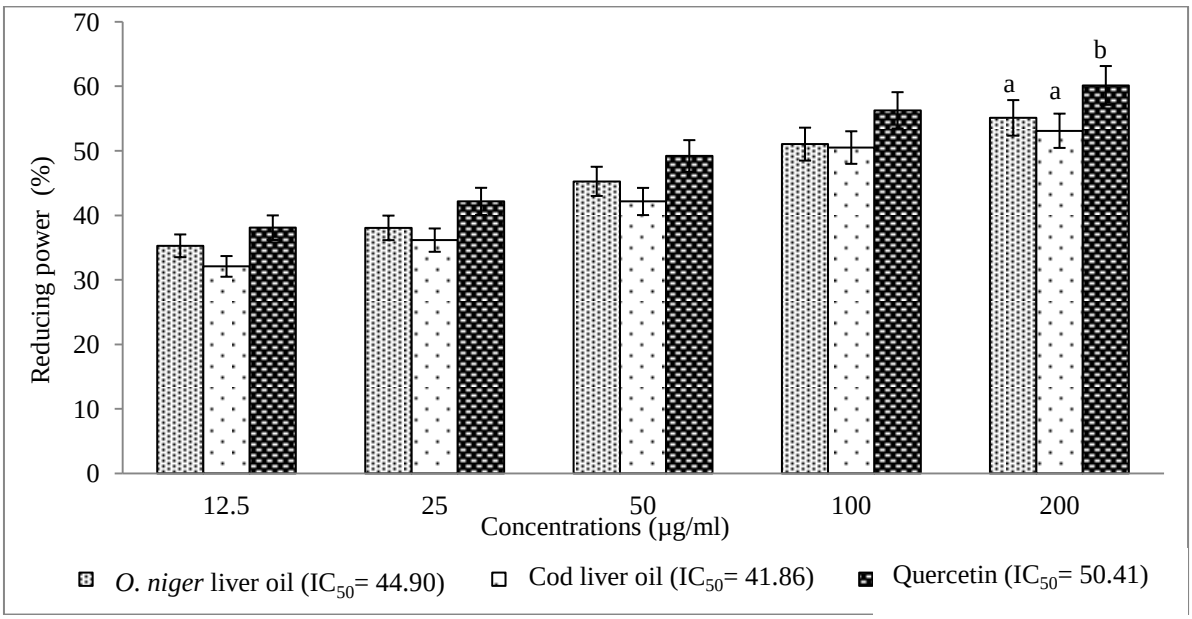

Fig. 5. *In vitro* reducing power activity of *O. niger* and commercial cod liver oils at different concentrations in comparison with the standard quercetin

Each value is the Mean ± SD of triplicate analysis; Bars with different superscript alphabets are statistically significant (One-Way ANOVA test; P< 0.05 and subsequently post hoc multiple comparison with SNK test)

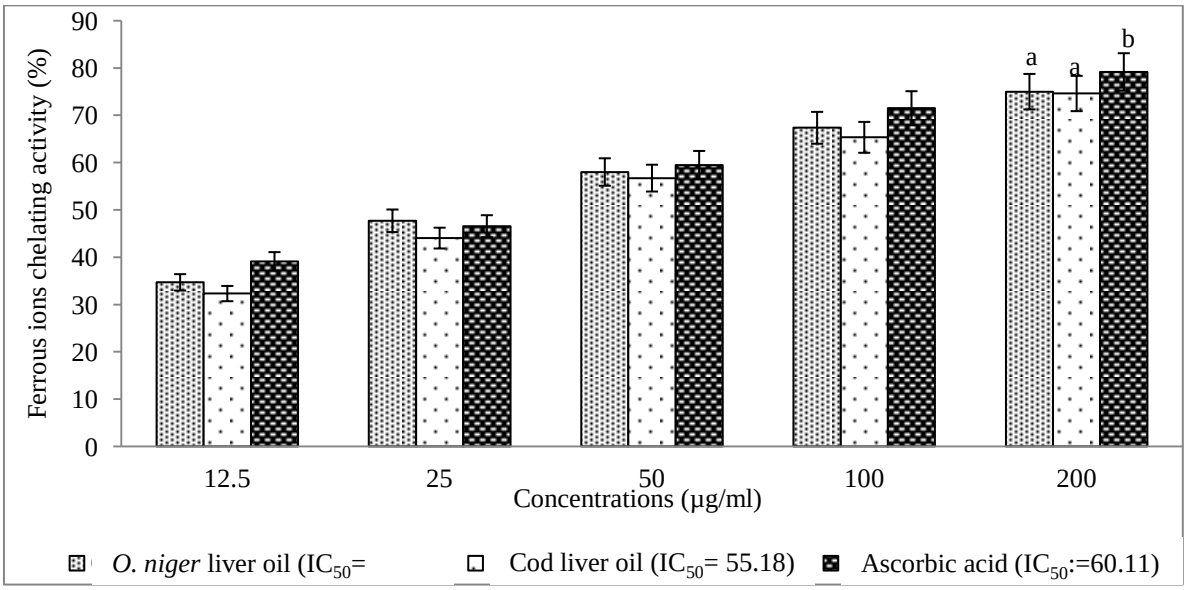

Fig. 6. *In vitro*ferrous ions chelating activity of *O. niger* and commercial cod liver oils at different concentrations in comparison with the standard ascorbic acid

Each value is the Mean ± SD of triplicate analysis; Bars with different superscript alphabets are statistically significant (One-Way ANOVA test; P< 0.05 and subsequently post hoc multiple comparison with SNK test)

Table 1. Two-way ANOVA for the data on various antioxidant activities as a function of variation between different antioxidant sources and their individual concentrations

Antioxidant activity	Source	Sum of square	Degree of freedom	Mean square	F-Value	P-Value
DPPH radical scavenging activity	Total variance	1738.520	14	-	-	-
	Variance due to antioxidant sources	35.940	2	17.970	6.040	P<0.05*
	Variance due to different concentrations of antioxidant sources	1678.781	4	419.695	141.080	P<0.0001***
	Error variance	23.798	8	2.9748	-	-
Lipid peroxidation inhibitory activity	Total variance	2033.514	14	-	-	-
	Variance due to antioxidant sources	349.170	2	174.585	21.200	P<0.001**
	Variance due to different concentrations of antioxidant sources	1618.465	4	404.616	49.135	P<0.001**
	Error variance	65.878	8	8.234	-	-
Hydroxyl radical scavenging activity	Total variance	6102.294	14	-	-	-
	Variance due to antioxidant sources	52.670	2	26.335	2.744	P>0.05
	Variance due to different concentrations of antioxidant sources	5972.856	4	1493.214	155.608	P<0.0001***
	Error variance	76.767	8	9.595	-	-
Total antioxidant activity	Total variance	6547.7241	14	-	-	-
	Variance due to antioxidant sources	419.476	2	209.738	7.037	P<0.05*
	Variance due to different concentrations of antioxidant sources	5889.812	4	1472.453	49.403	P<0.001**
	Error variance	238.435	8	29.804	-	-
Reducing power activity	Total variance	1054.181	14	-	-	-
	Variance due to antioxidant sources	105.026	2	52.513	125.445	P<0.0001***
	Variance due to different concentrations of antioxidant sources	945.804	4	236.451	564.841	P<0.0001***
	Error variance	3.348	8	0.418	-	-
Ferrous ions chelating activity	Total variance	3311.00	14	-	-	-
	Variance due to antioxidant sources	52.499	2	26.249	14.104	P<0.05**
	Variance due to different concentrations of antioxidant sources	3243.61	4	810.903	435.707	P<0.0001***
	Error variance	14.888	8	1.861	-	-

P<0.05 : Significant*
*P<0.001** : highly significant*
*P<0.0001***: highly significant*
P>0.05 : Non significant

Elemental sources such as ferrous iron (Fe2+) can facilitate the production of ROS within animal and human systems. Hence, the ability of substances to chelate iron can be a valuable antioxidant capability. The effective ferrous ion (Fe2+) chelators may also afford protection against oxidative damage by removing iron that may otherwise participate in HO generating Fenton type reaction. Ferrous ions are the most powerful prooxidant, among the various species of metal ions [75]. Minimizing ferrous ions may give the protection against oxidative damage by inhibition of ROS and lipid peroxidation. Metal chelation is an important antioxidant property [76]. In the present study ferrous ions (Fe2+) chelating activity of *O. niger* liver oil and cod liver oil (75.0% and 74.61%) exhibited much inhibition at 200µg/ml concentration, when compared to the standard ascorbic acid (79.17%). Gulcin et al. [68] reported that at the concentration of 15µg/ml, the clove oil was active (58.2%) in ferrous ions chelating activity. The present findings revealed that *O. niger* and cod liver oils demonstrated a marked capacity for iron binding, suggested that their main action as peroxidation protector, which may be related to their iron binding capacity.

5. CONCLUSION

The present study indicated that *O. niger* and cod liver oils are the highly potent antioxidants to improve the antioxidant capacity, which are considered as the rich sources of ω-3 PUFAs. Future *in vivo* studies are also needed to find out the mechanism of action as antioxidants. Further, *O. niger* and cod liver oils could be used as a natural antioxidant in dietary supplement.

ACKNOWLEDGEMENT

The first author of this manuscript gratefully acknowledge that this research was supported by Basic Science Research Fellowship in Science for Students, funded by the University Grants Commission (UGC), New Delhi.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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