Chemical composition and Anti-microbial activity of oil extracted from *Channastraita* waste

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Abstract:
Channastraita is a reputed medicinal fresh water fish among the south Asian regions and used to treat wounds, alleviate pain, boosts energy and endowed with remarkable anti-inflammatory, anti-nociceptive, platelet aggregation, as well as mild antimicrobial and antifungal properties. The present work deals with the antimicrobial activity and anti-fungal activity of *Channastraita* fish oil. Their quality was assessed by analysing quality indexes including peroxides, acid, iodine, anisidine and thiobarbituric acid values. Chemical analysis was established by gas chromatography coupled to flame ionization detector. Antibacterial activity was evaluated by disc diffusion method. Fish oil was extracted from *Channastraita* waste and purified, the fatty acids were identified in fish oil. The extracted *Channastraita* oil showed good antimicrobial and anti fungal activity.

Keywords: Antimicrobial properties, Antifungal activity, *Channastraita*, Pharmacological activity
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INTRODUCTION

Channastraita fish is a freshwater fish that live in the slow flowing waters of swamps, rivers, lakes and different small stagnant waters which includes irrigation canals, rice fields, and ditches with dense aquatic plants. This fish in taxonomy belonging to the family *Channidae* extensively disbursed in Central Kalimantan in addition to in different Asia vicinity, has a completely unique capability to live within the peat swamp area

with a low pH and low oxygen content material as it has extra respiratory organ to take oxygen directly from the air [1-4].This fish also can live on in condition of water with excessive ammonia content [5-6] and stay as carnivorous fish that prey on different varieties of fish are smaller. In nature, the snakehead fish spawning occurs once a year in the course of the wet season. This species may be very tough to spawn certainly inside the aquaculture surroundings. Before accomplishing spawning, the fish commonly prepare their nests on floating plant life and grass to region egg producing. Fecundity of the snakehead fish turned into common 6000 eggs per 100 g of broodstock with egg diameter between 1.2 to 1.5 mm [7-9]. Water intensity for the snakehead fish spawning is highly shallow water ranging from 40-80 cm. There are two sorts of the snakehead fish, particularly keeping and without retaining of egg and larvae sorts all through their spawning and early stay stages. Fish spawning is the end result of the interactions among environmental sign and primary nervous system [10].

Bacteria is one of the major health issues in South Asian countries although plant materials have been the major source of natural therapeutic remedies or used to treat various infectious disease including anti-microbial [11] but recently snakehead extract had shown positive results as anti-bacterial and anti-fungal agent. As part of the whole healing processes, anti-microbial activity and anti-fungal in particular, is equally important. Snakehead extracts against 13 filamentous fungus and 3 non-filamentous or yeast species has shown inhibition effects. Although the inhibition is not enough to kill the strain, but the partial inhibition by the snakehead extracts will be of a better use for human consumption to avoid unnecessary repercussion. The antimicrobial properties of the skin and intestinal mucus of different snakehead fish showed a broad spectrum of antibacterial activity against *Aeromonashydrophila, Psedomonasaeruginosa, Vibrio anguillarum, P. Aeruginosa*and *V. fischeri* [12]. Anti-fungal activities of snakehead extract have been demonstrated by an ethanol fillet extract against *Neurosporacrassa, Aleurismakeratinophilum* and *Cordycepsmilitaris* and also inhibited *Botrytis pyramidal* and *Paecilomycesfumosoroseus* on a short-term basis.

MATERIALS AND METHODS

Collection site description

Anantapuramu is a city in Anantapur district of the Indian state of Andhra Pradesh. It is the mandal headquarters of Anantapuramu mandal and also the divisional headquarters of Anantapur revenue division. Anantapuramu is located at 14.68°N 77.6°E. It has an average elevation of 335 m (1,099 ft). It is located at a distance of 356 km from Hyderabad, 484 km from Vijayawada, and 210 km from Bangalore which is the closest international airport.

Collection of fish waste

The fish waste was collected from Fish market, Anantapur district, India. The collected fish waste was carefully washed with ambient water and thrice with tap water to remove the adhering soil particles and associate animals. Then it was separated based on the morphological characters. Further, it was dried at 60°C for 24 hrs for further use.

Microorganisms

Microorganisms used included Gram+ and Gram- bacteria. *Staphylococcus aureus* (MTCC 3160), Bacillus cereus (MTCC 1305) *E.Coli* (MTCC 443) and *Pseudomonas aureoginosa* (MTCC 2453) and *Aspergillusfumigatus*. The bacterial strains obtained from Department of Microbiology, Osmania University, were used for evaluating antibacterial activity. The bacterial stock cultures were incubated for 24 hours at 37°C on nutrient agar. The bacteria were grown on Mueller-Hinton agar plates at 37°C. The stock cultures were maintained at 4°C for the growth of fungi potato dextrose agar was used.

Oil extraction

100g of homogenised fish tissues were weighed into beaker (Capacity 1 litre) to this 10ml of

distilled water was added and mixed. Methanol:chloroform was added at the ratio of 1:2V/V and the mixture was thoroughly homogenized. The mixture was centrifuged at 2000rpm for 20 minutes at room temperature. The resultant aqueous layer was removed with the help of separating funnel. The chloroform fraction was evaporated using Rotatory evaporator and finally the yield of obtained oil was recorded.

Determination of quality indexes

The peroxide value was evaluated using standard spectrophotometric method [13]. Determination of thiobarbituric acid value was done following the method recommended by American Oil Chemists' Society [14]. The iodine value was assessed as recommended by AFNOR [15]. The acid value was determined according to standard NFT60–204 of the French Association for Standardization [15]. The anisidine value was evaluated according to AOCS Official Method Cd 18–90 "p-anisidine value".

Fatty acid profile

The fatty acid profile of oil samples were determined by gas chromatography coupled to a flame ionization detector (GC-FID). Briefly, the fatty acid methyl esters were prepared by transesterification, using 2% sulfuric acid in methanol [16]. Analysis were performed on a gas chromatograph (Agilent Technologies, Palo Alto, CA, USA), serial number 7890A, coupled to a flame ionization detector, using a capillary column DB-225 (30 m × 0.25 mm, film thickness 0.25 µm). The initial column temperature was 160 °C for 2 min, then increased to 220 °C (5 °C/min) and maintained for 10 min. Nitrogen was used as mobile phase with flow rate 1.5 ml/min. The temperature of the injector and detector were maintained at 230 °C and 250 °C respectively for 20 min. The identification of fatty acid was based on the comparison of retention time with that of standard reference fatty acid methyl esters performed under same conditions [17].

Antibacterial Activity

Whatman No.1 filter paper discs of 5mm diameter were autoclaved by keeping in a clean and dry Petri plate. The discs were soaked in fish oil for 5 hours were taken as test material. After 5 hours the discs were shade dried. The concentrations of fish oil solutions per disc are accounted for 0.1 grams/1ml. Subsequently they were carefully transferred to spread on cultured Petri plates. Filter paper discs immersed in ethanol, Hexane, benzene and distilled water are prepared and used as control.

To test the anti bacterial activity, LB agar medium was prepared and the medium was sterilized at 121°c for 30 mins. The agar plates were prepared by pouring about 10ml of the medium into 10cm Petri dishes under aseptic condition and left undisturbed for 2hrs to solidify the medium. 1ml of inocculum (containing suspension) of *Staphylococcus aureus* (MTCC 3160), Bacillus cereus (MTCC 1305) *E.Coli* (MTCC 443) and *Pseudomonas aureoginosa* (MTCC 2453) and *Aspergillusfumigatus*was poured on to the plates separately containing solidified agar media. The prepared sterile filter paper discs were impregnated with the compound solutions and shaken thoroughly and these test plates incubated for a period of 48 hrs in BOD at 37ºc for the development of inhibitory zones and the average of 2 independent readings for each organism in different compound solutions were recorded [18]. The inhibition zones were measured after 1 day at 37ºc for bacteria. The diameter of the inhibition zone was measured and recorded with the aid of plastic ruler. Five paper discs placed in one Petri plate.

RESULTS AND DISCUSSION

The *Channastriata* and fish waste was collected from fish market, Anantapur district, India. The striped snakehead has a long body characterized with dark black-brown on the upper section of its body, and bands of a white on its belly. The striped snakehead length was 90cm and 3 kg - growth studies reported that they reached an average body mass was reported as 60g by 12 weeks past the fingerling

stage. The female striped snakehead is larger than the male. As juveniles, the striped snakehead has a tan coloring with dark brown stripes. Striped snakeheads have a long dorsal fin, a pectoral fin, a pelvic fin that is almost directly under the pectoral fin, and a long anal fin. They also have large mouths with sharp visible teeth with 4-7 canines, located on the bottom row of their mouths (Fig 1).

Fig 1: Collection of *Channastriata*

Fish oil extraction yields

The average yield of oil extracted using extraction technique was 96ml and showed significant variation between the species and various methods. According to Linder et al. [19-23], fishes with oil content less than 5% are lean fishes, those containing 5 to 10% are semi fatty fishes and beyond 10% are considered fat. Therefore, *C. nigrodigitatus* with a yield of 5.8% - 6.52% is a semi fatty fish unlike *H. odoe* which is a lean fish (yield of 4.31%).

Quality Assessment of Fish Oil

Since higher yield of fish oil was obtained in *Channastriata*was alone recorded here so as to avoid redundancy (Table 1). All the analytical values are well within the acceptable standard values for all the methods. The moisture content values and free fatty acid value in modified Bligh and Dyer method were found to be superior to that of other methods. It is important to note that the results of various analytical parameters did not exhibit profound variation between the methods. Theanisidine value reflects the secondary oxidation products, specifically 2-alkenals and 2.4-dienals unlike thiobarbituric acid value which allows highlighting the malondialdehydes which characterise the last steps of fatty acid oxidation [24].

Table 1: Quality analysis of fish oil produced from *Channastriata* employing different extraction procedures

S. No.	Parameters analysed	B & D	MB & D	M & M	DS
1.	Moisture content (%)	0.90± 0.12	0.96± 0.14	0.92± 0.15	0.94± 0.22
2.	Free fatty acid (mg KOH/g)	3.4 ± 0.32	2.87 ± 0.16	2.65 ± 0.27	2.4 ± 0.13
3.	Iodine Value I2/100g)	164 ± 4.0	175 ± 3.0	185 ± 2.0	189 ± 3.0
4.	Peroxide Value (mEq/Kg)	1.98 ± 0.13	2.45 ± 0.27	2.26 ± 0.11	2.87 ± 0.32
5.	Saponification Value (mg KOH/g)	220 ± 1.2	210 ± 1.3	212 ± 1.6	208 ± 1.7
6.	Specific Gravity at RT	0.73 ± 0.05	0.84 ± 0.03	0.79 ± 0.02	0.85 ± 0.04
7.	Refractive index	1.43 ± 0.04	1.54 ± 0.04	1.48 ± 0.03	1.76 ± 0.02
8.	Colour	Yellow	Yellow	Yellow	Yellow

Identification of compounds by GC-MS

The compounds were identified in the fish oil using GC-MS method. The fatty acid composition of fish oil (*Channastriata*and Fish waste) undergone different steps of purification are tabulated separately in Table 12. The major SFAs found in sardine oil during refining are C16:0, C18:0, C14:0, C15:0, C17:0, C24:0, C23:0 and C22:0. The MUFAs and PUFAs are C18:1ω-9, C14: 1ω-4, C24:1ω-3, C16:1ω-5 and C18:3ω-3, C18:2ω-6,C18:4ω-3, C20:5ω-3, C20:4ω-6, C22:5ω-3 and C22:6ω-3. Among them Palmitic acid (C16:0, 14.86) was found significantly higher among all other SFAs at the end of purification, followed by stearic acid (C18:0, 6.25), myristic acid (C14:0, 2.67), pentadecyclic acid (C15:0, 2.39), Margaric acid (C17:0, 2.0), lignoceric acid (C24:0, 1.98), Tricosanic acid (C23:0, 1.75) and pehenicacid (C22:0,1.13)followed by others in trace quantities. AmongMUFA'sOleic acid (C18:1ω-9, 14.16) are found to be dominant followed by myristoleic acid (C14:1ω-4, 4.55) and cis-3-Tetracosenoic acid (C24:1ω-3, 1.61) and others in the order of descend. Among PUFA's docosohexaeneic acid (C22:6ω-3, 5.61) predominates followed by alfalinoleic acid (C18:3ω-3, 2.788), docosopentaenoic acid (C22:5ω-3, 2.166), linoleic acid (C18:2ω-6, 1.43), stearidonic acid (C18:4ω-3, 1.28) and eicosapentaenoic acid (C20:5ω-3, 1.16) followed by others in its order of descend. The total composition of SFAs, MUFAs and PUFAs were determined as 45.776%, 28.59% and 22.918% in crude oil and 34.16%, 22.61% and 17.759% in purified oil respectively. The major loss in yield took place during the degumming process. The total fatty acid composition of the crude fish oil decreased from 97.284 to 74.529% (w/w). There were losses incurred during different steps of purification for MUFAs (28.59 to 22.61% w/w) and PUFA's (22.918 to 17.759% w/w), whereas a significant reduction was occurred for Saturated Fatty Acids (45.776 to 34.16% w/w).Polyunsaturated (PUFA) and monounsaturated (MUFA) fatty acids have been known to provide varied health benefits such as minimizing inflammation and/or acting as antioxidants. Recent studies highlighted n-3 PUFA and their ester derivatives antibacterial activity against various oral pathogens, including *S. mutans, C. albicans, A. actinomycetemcomitans, F. nucleatum*, and *P. gingivalis*. The major n-6 PUFAs, (linoleic acid, γ-linolenic acid, arachidonic acid), the n-7 MUFA, palmitoleic acid, the n-9 MUFA and oleic acid (OA) were found to be responsible for this activity [25-28].

Table 2: Fatty Acid profile of fish oil (*Channastriata*) during various steps of Refining (w/w%)

Carbon chain	Fatty acids	Crude	Degummed	Neutralised	Bleached	Deodourised
C10:0	Capric acid	0.59	0.47	1.43	1.30	0.21
C11:0	Undecyclic acid	0.47	0.45	0.30	0.21	0.11
C12:0	Lauric acid	0.246	0.167	0.030	-	-
C13:0	Tri decyclic acid	0.30	0.24	0.16	0.01	-
C14:0	Myristic acid	6.01	3.65	4.06	3.0	2.67
C15:0	Pentadecyclic acid	3.21	2.54	2.49	2.43	2.39
C16:0	Palmitic acid	16.0	14.12	13.0	14.98	12.86
C17:0	Margaric acid	2.16	2.09	2.06	2.01	2.0
C18:0	Stearic acid	6.57	5.51	4.49	4.36	6.25
C19:0	Nonadecyclic acid	0.51	0.45	0.38	0.35	0.30

C20:0	Arachidic acid	0.72	0.68	0.64	0.55	0.51
C22:0	Pehenic acid	0.69	1.62	0.58	1.49	0.13
C23:0	Tricosanic acid	2.0	0.99	1. 87	1.80	1.75
C24:0	Lignoceric acid	2.30	2.26	2.23	2.0	0.98
Sum of SFAs		45.776	41.237	37.72	34.940	34.16

Anti microbial activity

In disc diffusion method the nutrient agar was poured into Petri plate and allowed to solidify. After 1ml of microbial suspension was poured into plates, fish oil was applied on to the discs and placed in Petri plate and incubated for 48 hours. The results from the agar plates revealed all compounds showed inhibitory zones. The fish oil showed best inhibitory zones against gram positive. The inhibitory zones represent the sensitiveness of microorganisms in culture to the fish oil against gram negative. In this study, *Staphylococcus aureus* was found to the most sensitive among the tested microorganisms. Bacteria including *S. parathyphi* A, *K. pneumoniae* and *S. aureus* are responsible for food intoxications. In addition to suffering and death, these bacteria cause considerable economic losses [29]. *S. aureus* and *S. parathyphi* A are two microorganisms responsible for food intoxication and typhoid fever respectively [30].

Table 3: Antimicrobial and anti fungal studies of fish oil

Sample	**Gram (+) ve Bacillus cereus**	**Gram (+)*Staphylococcus aureus***	**Gram (–) ve*E,coli***	**Gram (–) ve P.aureoginosa**	***Aspergillus fumigatus***
Fish oil	5.6mm	6 mm	5 mm	3.3 mm	4mm
Streptomycin	8mm	8.5mm	7mm	4mm	6mm

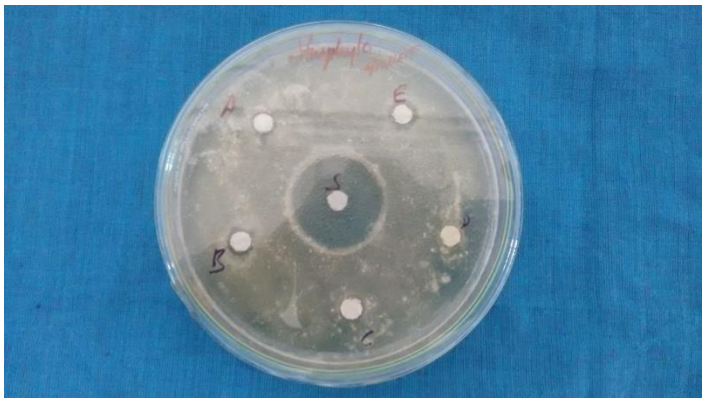

Fig 2: Antimicrobial activity of fish oil

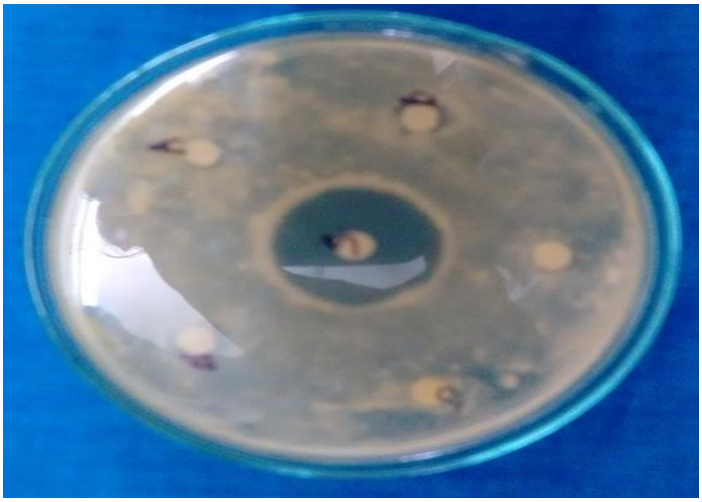

Fig 3: Anti fungal studies of fish oil

CONCLUSION

*Channastraita*fish oil is largely used for food, traditional medicines and pharmacological therapeutics including anti-microbial, anti-inflammatory, cell proliferation, induction of platelet accretion and anti-nociceptive activities. It'snutraceutical value is outstanding and essentially contributes, at least in part, to the bioactive compounds, engaging in clinical trials, therapeutics and nutritional supplements. *Channastraita* extracted oil may also have a role in other non-traditional uses such as in treating neurological diseases and in inducing regenerative potential of organs and cells. Therefore, *Channastraita* fish oil has a high potential to be used as a promising acceptable source of medicines and nutrients for the treatment of serious diseases as well as for the improvement of general body tones of human beings to a greater extent.

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CONFLICT OF INTEREST

Authors declare that there is no conflict of interest

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