

EXTRACTION AND CHARACTERIZATION OF OIL FROM OCTOPUS
(*Cistopus indicus*)

Submitted in partial fulfillment of the requirements for the award of
Bachelor of Science degree in Biotechnology

by

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(DEEMED TO BE UNIVERSITY)

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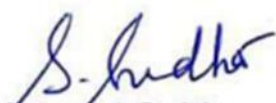
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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of Shakthi Mythili. U.L (40770047) who carried out the project entitled "EXTRACTION AND CHARACTERIZATION OF OIL FROM OCTOPUS (*Cistopus indicus*)" under our supervision from September 2022 to March 2023.



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I **Shakthi Mythili. U.L** (40770047) hereby declare that the Project Report entitled “EXTRACTION AND CHARACTERIZATION OF OIL FROM OCTOPUS (*Cistopus indicus*)” done by me under the guidance of Dr. S. SUDHA (Internal) and Dr. M. MOOVENDHAN (External) at Col. Dr. Jeppiaar Research Park, Centre for Ocean Research, SATHYABAMA INSTITUTE OF SCIENCE AND TECHNOLOGY is submitted in partial fulfillment of the requirements for the award of Bachelor of Science degree in Biotechnology.

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ABSTRACT

In the present study an attempt was made to extract and characterize oil from the whole body tissues of Cephalopod *Cistopus indicus*. Cephalopoda is under the phylum Mollusca with several important marine oceanic habitats. They are mostly well-known for their different uses such as food ingredients and nutraceutical benefits. In this study, the fish oil was extracted using solvent extraction method and the physical properties and chemical properties are characterized and examined. In general, the oil recovery is higher when using solvent extraction method. The physical characteristics such as specific gravity, solidification point, refractive index, saponification value, iodine value and acid value of octopus oil were analyzed and it showed good characters similar to fish oil. The ash and moisture content recorded 9% and 5% respectively, whereas the total protein and carbohydrate recorded 1.6% and 1.9% respectively. The elemental composition such as Carbon, Hydrogen and Nitrogen content recorded 46.3%, 11.2% and 6.9% respectively. The fat soluble Vitamins (A and E) and water soluble Vitamins (B₁, B₂, B₆ and C) were found in good level. Ten fatty acids were found, the oleic acid and α -linolenic acid were recorded maximum levels and the palmitic acid, steric acid, linolenic acid, DHA and EPA were recorded at moderate levels. Whereas margaric acid and morotic acid were recorded at low levels.

Keywords: Cephalopods, *Cistopus indicus*, Octopus oil, Omega-3 fatty acids, Solvent extraction.

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ABBREVIATIONS

ABBREVIATIONS

ALA

AOAC

BSA

CVD

DHA

EFA

EPA

FAME

g

HDL

HPLC

IBD

IDL

IU

IUPAC

KOH

mg

mm

MUFA

PUFA

SFA

µg

MEANINGS

Alpha-linolenic acid

Association of Official Analytical
Communities

Bovine Serum Albumin

Cardiovascular disease

Docosahexaenoic acid

Essential Fatty Acid

Eicosapentaenoic acid

Fatty Acid Methyl Esters

gram

High-density lipoprotein

High Performance Lipid
Chromatography

Inflammatory Bowel Disease

Intermediate-density lipoprotein

International Unit

International Union of Pure and
Applied Chemistry

Potassium hydroxide

milligram

millimeter

Monounsaturated fatty acids

Polyunsaturated fatty acids

Saturated Fatty acids

microgram

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Chapter-1: INTRODUCTION

1. MOLLUSC

Molluscs are made up of over 120000 bilateral species and are the second most varied animal phylum in the environment. Molluscs are among the most well-known species in the world, having dominated nearly all of the planet's terrestrial and aquatic ecosystems (Wanninger and Wollesen, 2019). Molluscs have a clear body design but also exhibit a great deal of variety. Molluscan variety comprises a lengthy, extraordinarily rich and instructive fossil record in addition to affecting all aspects of biology (morphology, physiology, behaviour, genetics and ecology). Molluscs can be found in both freshwater and saltwater habitats and some can also be found on land.

2. SYSTEMATIC POSITION

Phylum: Mollusca

Class: Cephalopoda

Family: Octopodidae

Genes: *Cistopus*

Species: *Cistopus indicus*

Common names: Old lady octopus, Pei Kanava, Pei Kadamba.

3. MAJOR GROUPS (CLASSES AND SUBDIVISIONS) OF MOLLUSCS

❖ Solenogastres (Neomeniomorpha; crawling worm-molluscs) is a tiny (280 species), marine genus of worm-like creatures with bodies ranging in length from 1 mm to 30 cm coated in chitinous cuticles with aragonitic spicules or scales (Hasprunar and Wanninger, 2012).

❖ Caudofoveata (Chaetodermomorpha; burrowing worm-molluscs) is another tiny marine group of worm-like organisms with a body length of 2 mm to 15 cm covered in a chitinous cuticle with spicules or scales (Hasprunar and Wanninger, 2012).

❖ Polyplacophora (Loricata; chitons) include roughly 1000 marine species that range in size from 3 mm to 30 cm and have broad, sucker-like foot soles that are

encircled by a mantle rim that may also include gills (Hasprunar and Wanninger, 2012).

- ❖ Monoplacophora (Tryblidia) has numerous fossil taxa, but there are only a few current species that range in size from 1 to 40 mm and can be found from 200 to 7000 metres of water (Hasprunar and Wanninger, 2012).

- ❖ Bivalvia (Pelecypoda, Lamellibranchia; mussels and clams) comprises of more than 20,000 extant species that range in size from 1 mm to over 150 cm and inhabit a variety of freshwater and marine habitats. Bivalves are extraordinarily diverse and in addition to filter feeders, they also include detritivorous and even predatory species. Many additionally use endosymbiotic bacteria or zooxanthellae for nutrition. This is in addition to their bivalved shell and complete lack of buccal mechanism (Hasprunar and Wanninger, 2012).

- ❖ Scaphopoda (tusk-shells) is a tiny (800 species), purely marine group of animals with typical, elephant tusk-like shells that range in size from 2 mm to up to 20 cm (Hasprunar and Wanninger, 2012).

- ❖ Gastropoda (limpets, snails, whelks, slugs) are by far the most diverse group of molluscs, with about 100,000 species (ranging in size from 0.5 mm to 100 cm long), inhabiting all marine, freshwater and terrestrial habitats. They have a variety of shell types, a freely movable head with eyes, a foot sole and a helical visceral hump, but there are numerous exceptions in the phenotypic expression of all these characters (Hasprunar and Wanninger, 2012).

- ❖ Cephalopoda (nautilids, ammonites, sepias, squids, octopuses) includes more than 30,000 extinct species but only 1,000 are still alive. These marine-only animals have body lengths of 3 cm to 7 m and arms as long as 18 m (Hasprunar and Wanninger, 2012). In Cephalopods, Nautiloidea and Ammonitoidea have external shells, all other (Coleoidea) have internal, reduced or lost shells. These Cephalopods are all predator with large eyes with 8 to 10 (or up to 80 in *Nautilus*) arms for prey capture.

- ❖ Aculifera (scale-bearers) is a major subdivision of Mollusca that has a notum that is at least partially covered by a chitinous cuticle that contains embedded aragonitic scales or spicules (Hasprunar and Wanninger, 2012).

- ❖ Conchifera (shell-bearers) is the second major subdivision of Mollusca, having a dorsal shell that has a number of mineralized layers beneath the exterior

organic layer (periostracum) and is produced by a dorsal shell gland, at least during early ontogeny (Hasprunar and Winninger, 2012).

1.4 STRUCTURE

All molluscs differ from their appearance to their features. There are few features that are so common in mollusc that can be used to differentiate the mollusc from other organisms.

1. Foot - Every mollusc has a unique foot, which varies in shape according to the class of mollusc.

2. Mantle - The body and internal organs of molluscs are covered by a mantle or mass of soft flesh

3. Radula - Many molluscs have radula, scraping organs that resemble rasps and are used for feeding. Radulas are absent in bivalve molluscs.

The molluscan bauplan, which is by definition a combination of the “most significant features of the phylum, frequently resembles a generalized gastropod or pre-gastropod type. It has a shell, head with tentacles, a creeping foot, a visceral hump, a mantle cavity with gills, a complicated stomach and intestinal coils, a gonopericardial system with excretory duct. However, these traits stand in stark contrast to theories about the ‘urmollusc’, the last common ancestor of all molluscs, which were recreated using a variety of inferred phylogenetic trees (Hasprunar and Winninger, 2012). Polyplacophorans and gastropods have single flat foot which can be used for crawling. Some of the bivalves have paddle-shaped foot which are adapted for digging into soft sediments. The foot in octopus and squid is modified into many tentacles which are attached to the head of the animal, which can be used for moving, grasping and holding the prey they capture for food.

1.5 CEPHALOPOD

Any member of the molluscan order Cephalopoda, such as an octopus, a squid, a cuttlefish or a nautilus, can be classified as a cephalopod. They can be found in every ocean on Earth and take up the majority of its depth, from the abyssal plain to the water’s surface. About 660 species of cephalopods, which are marine molluscs that vary in size, shape and behavioral traits, have been identified in the world’s oceans (Moovendhan *et al.*, 2019). Despite retaining some classic

molluscan elements in its body structure, cephalopods evolved a level of sophistication, in particular a sophisticated neurological system and sense organs that, in some aspects reach the standard of vertebrates. Comparable to other varieties of fish and crustaceans, cephalopods include an evenly distributed rich nutritional value. Cephalopods are valuable as food sources, animal models in scientific research and they are the store-house of numerous biologically important substances (Ramasamy *et al.*, 2011). Cephalopods are preferred for their rich flavor, low saturated fat and a substantial amount of minerals including sodium, calcium, potassium, magnesium, iron, zinc, copper and phosphorus (Shalini *et al.*, 2020). Cephalopods can develop high levels of cadmium and lead and have short live span and rapid growth rates (Butamante *et al.*, 2000). These marine animals are distinguished by bilateral body symmetry containing a prominent head with a set of arms or tentacles around the head. Despite some difficult obstacles associated with keeping them, several aquariums have chosen to host them as the main attractions, demonstrating their growing appeal. As a defense mechanism to flee from predators, they frequently possess the ability to squirt ink. Because of their huge brains and sophisticated behavioral traits, the coleoid cephalopods (henceforth cephalopods) have attracted a lot of interest from a variety of scientists (Schnell and Clayton, 2020).

Cephalopods, in contrast to most invertebrates, have a huge, sophisticated brain. Cephalopods have been employed in a variety of scientific studies. Due to the squid's enormous axon's relative accessibility, they played a crucial role in advancing our understanding of the neuron during the first half of the 20th century. A continuously expanding body of studies on several other aspects of cephalopod biology, including genetics, welfare and the effects of climate change, has been done from the end of the 20th century to the present. Additionally, it is significant that cephalopods engage in social interaction with one another. Although these large-brained molluscs do not create social connections, they do engage in comprehensive engagement with one another, especially during reproductive seasons (Schnell and Clayton, 2020). The neural system of cephalopods is unquestionably the most complicated of all invertebrate neurological systems and this complexity is consistent with the creatures (mostly) highly active, fast moving, predatory lifestyles and sophisticated behaviours. The class Cephalopoda consist

of two sub-classes such as Nautiloidea and Coleoidea. Nautiloids consist of Nautilus and Coleoids consist of octopuses, cuttlefishes and squids. Nautiloids and Coleoids have different anatomy, physiology and behavioural activities though they are not often considered in the literature when the term 'Cephalopod' is applied.

1.6 CLASSIFICATION

Class Cephalopoda

- ❖ Subclass Nautiloidea (nautiloids)
- ❖ Subclass Ammonoidea (ammonites)
- ❖ Subclass Coleoidea (octopuses, squids, belemnites, cuttlefishes)

Order Belemnoidea (belemnites)

Order Sepioidea (cuttlefishes and bottle-tailed squids)

Order Teuthoidea (squids)

- ❖ Suborder Myosida
- ❖ Suborder Oegopsida

Order Vampyromorpha

Order Octopoda (octopuses)

- ❖ Suborder Palaeoctopoda (finned octopus)
- ❖ Suborder Cirrata (Cirromorpha)
- ❖ Suborder Incirrata (common octopus)

1.7 OCTOPUS

There are over 300 species of cephalopods, an eight-limbed group of molluscs, in the order Octopoda. The octopuses, like other species, are still poorly understood and new species and genera are identified every year. They have an eight-limbed soft body, two eyes, mouth and are bilateral symmetry. The seafloor is one area of the ocean where octopuses can be found. Every other animal, including little fish, snails, clams and even other octopuses, is killed and eaten by them. Their skin is porous and their bodies are highly protein-rich. Because they have strong hydrostat movement systems, octopuses are rare among animals. Since they lack bones, they move by jet propulsion caused by the muscular mantle's contraction.

Jet propulsion is utilized to move objects on the sea floor, capture visually detected prey and escape from scavengers (Mather and Alupay, 2016). They can walk on the ocean floor with the help of their powerful tentacles, which they also utilize to jet about the sea. Octopuses and many other cephalopods have a system that allows them to instantly change many different aspects of their appearance (Messenger, 2001; Mather and Kuba, 2013). They have chromatophores, which are elastic sacs in the skin's top layer that hold pigments that can aid in millisecond changes in appearance. Below the chromatophores is a layer of leucophores that reflect light of all wavelengths. This layer will be used to match the wavelength of light surrounding the animals. As a cephalopod molluscs, marine octopuses are highly sought-after commercial fisheries products on the global market due to their high nutritional and bioactive contents. It was thought that cephalopod molluscs were plentiful source of bioactive substance with potential therapeutic effects (Nair *et al.*, 2011). Among cephalopods, marine octopuses (family Octopodidae) have been extensively studied for their biological effects, including their anti-oxidant, anti-inflammatory, anti-hypertensive and anti-hyperglycemic properties. Despite being employed in several research, marine octopus species have just lately been identified as possible sources for bioactive chemicals.

1.8 *Cistopus indicus*

Cistopus indicus (Rapp [in A. Férussac & d'Orbigny], 1835) is a benthic species known as “old women octopus” can be found in depths of up to 50 meters. The species is found throughout the Indo-Pacific, primarily in the Indo-Malayan region and in countries like China, Bangladesh, India and Pakistan as well as the Philippines (Roper *et al.*, 1984). Due to the low demand for this resource in the past, octopus now has a strong market due to its strong market due to its increased export value. Because of increased fishing pressure brought on by rising demand, overexploitation may occur, which could result in the depletion of stock. For the wise exploitation of this crucial resource, actions should be done. This species greatest mantle length is 180mm and its total length is approximately 600 mm (Sundaram, 2011).

9. HEALTH BENEFITS

Octopus is a delicacy in many cultures and a significant protein source for coastal people all over the world. It is said to be a folk medicine for impotence and to increase libido. Scientists remark that several nutrients in octopus can be beneficial to reproductive health, despite the lack of evidence to support these assertions. Due to the octopus distinctive biology, there is a large body of current research (Mather and Alupay, 2016). The octopus, often known as the chameleon of the sea, has several hearts and brains, can regenerate its limbs and many change its shape and color. Many point to the health-improving properties of the nutrients in octopus, including several that are frequently lacking in our diets, while studies on the possible health advantages of eating octopus continue. Octopus is a good source of iron, omega-3 fatty acids, copper, selenium and Vitamin B₁₂. Additionally, it's a great provider of calcium, magnesium and potassium. According to studies, these minerals work together to lower the risk of stroke and support heart health. Octopus is rich in vitamins and minerals. It is a fantastic source of complete protein for persons seeking to manage their weight because it is also low in fat (Sundaram, 2011). However, how it's prepared may affect this. Octopus can increase the amount of fat and calories in your dinner if you fry it or cook it in butter or oil.

10. FISH OIL

Fish oil is extracted from various types of fishes. Fish oil is a good source of high quality animal protein, essential fatty acids and micronutrients, all of which are present in fish at significantly higher levels than in terrestrial animal dietary sources (El-Rahman *et al.*, 2018). Fish oil may be derived from the whole body as well as the liver. Due to their complex composition of saturated, unsaturated and polysaturated fatty acids, fish oils are more complex than those found in terrestrial animals and plants (Immanuel *et al.*, 2002; Adeniyi and Bawa, 2006). Fish oil also includes cholesterol, carotenoids, free fatty acids and the lipid-soluble Vitamins A and D (Aidos, 2002). Large amounts of fish waste are produced by the fishing and fish processing sectors and when this material is incorrectly disposed off, it is thought to constitute a harm to the environment. The consumption of fish viscera varies depending on the species, seasonality, age, sex, food intake and environmental factors. Fish viscera have significant protein and fat levels as well as

some minerals (Kris-Etherton *et al.*, 2003). Enzymatic hydrolysis, wet rendering, dry rendering, autolysis, solvent extraction and supercritical fluid extraction are a few of the methods that can be used to extract fish oil. Fish oil is now more widely produced and available than ever before, nevertheless (Kris-Etherton *et al.*, 2003). Additionally, farm animals like pigs, chickens, cattle and sheep are fed fish oil. Along with other industries, fish oil is also used in pet foods. Due to its high concentration of omega-3 polyunsaturated fatty acids (PUFA), including docosahexanoic acid (DHA) and eicosapentaenoic acid (EPA), which are used to treat and prevent various autoimmune and cardiovascular disorders, as well as improve eye sight, fish oil is a vital substance (Weitz *et al.*, 2010). Many of the omega-3 fatty acids that our body needs are not produced by it. Previously a by-product of fish meal used for animal feed, fish oil is now understood to be the main source of these fatty acids (Mardones *et al.*, 2012). Incorporating fish oil into foods is a crucial strategy for increasing the consumption of the fatty acids included in these oils as well as a very efficient technique to raise the value of fish oils. Therefore, the level of EPA and DHA in fish oil is very important. Several different species of fish can be used to obtain fish oil. There are numerous techniques for obtaining fish oil, including biological, chemical and mechanical extraction (Gamez-Meza *et al.*, 2003).

1.11 BENEFICIAL EFFECTS OF FISH OIL TO HUMAN HEALTH

Aliphatic monocarboxylic acids known as fatty acids are those that are generated from or that are present in esterified form in an animal or vegetable fat, oil or wax. One class of fatty acids are Essential Fatty Acids (EFA). They cannot be created in our bodies from scratch. Because certain fatty acids cannot be produced by our body due to a lack of enzymes, they must be consumed in the diet from either plant or animal sources. But because they are involved in crucial biological processes, they are incredibly vital to us. The omega-6 EFAs and the omega-3 EFAs are two groups of polyunsaturated essential fatty acids. The body can change one omega-3 to another, but it cannot produce an omega-3 from an omega-6 or a saturated fat. Essential fatty acids are so-called because we cannot survive without them (Das *et al.*, 2009).

The tissues of oily fish are used to make fish oil. It contains the omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are the

building blocks of eicosanoids, which reduce inflammation throughout the body (Sargent, 1997). Small amounts of fish oils must be supplied for maximum health benefits, cellular metabolism and regular physiological processes (Sau *et al.*, 2004). When Danish physicians noticed that Greenland Eskimos had an extraordinarily low incidence of heart disease and arthritis while eating a high-fat diet, scientists learned about various benefits of fish oils. This was in the early 1970s. Deep investigation quickly revealed that EPA and DHA, two of the fats they consumed in considerable amounts, were actually quite healthy. There are several health benefits of fish oil for humans. It guards against peripheral vascular disease, atherosclerosis, angina, heart attacks, congestive heart failure and arrhythmias. Clinical studies have demonstrated its efficacy in treating a variety of disorders; such as rheumatoid arthritis, diabetes, cancer etc., (Connor, 2000). Numerous significant disorders have been reported to emerge as a result of an imbalance in the consumption of omega-3 and omega-6 fatty acids. Therefore, these issues can be avoided by altering eating practices and using fish oil.

12. ESSENTIAL FATTY ACIDS (EFAs)

In general, the lipids of marine fish species are characterized by high quantities of long chain n-3 polyunsaturated fatty acids and low levels of linoleic acid (18:2, n-6) and linolenic acid (18:3, n-3). Omega-3 and omega-6 are the two families of essential fatty acids (EFAs). Alpha-linolenic acid, the most prominent member of the omega-3 family, can be found in flaxseed and canola oils as well as green, leafy vegetable, nuts and legumes. The omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are also present in fish and fish oil (Steffens, 2006).

13. OMEGA-3 FATTY ACIDS

When counting from the methyl end of the fatty acid molecule, the first of many double bonds in omega-3 fatty acids, which have lengthy chains (18-22 carbon atoms in length), begins at the third carbon atom. The three primary types of omega-3 fatty acids are eicosapentaenoic acids (EPA), docosahexaenoic acids (DHA) and alpha-linolenic acids, with EPA and DHA having the biggest health benefits. While the omega-3 is concentrated across the food chain, cold-water fish oils, including those of mackerel, salmon, sardines, herring and cod, have the

highest concentration (Adeniyi and Bawa, 2006). EPA is transformed into biochemical intermediates that are anti-inflammatory. DHA is the most abundant omega-3 fatty acid in most tissues and is produced from EPA which is particularly plentiful in the brain and retina (Das *et al.*, 2009). DHA is necessary for healthy brain growth and function, the development of the central nervous system and optimal visual acuity. Through a series of enzymatic processes, alpha-linolenic acid (ALA) is transformed into EPA and DHA (Wirth *et al.*, 1997).

14. OMEGA-6 FATTY ACIDS

Omega-6 fatty acids are occasionally thought of as pro-inflammatory mediators, however the physiological effect depends on the kind and amount of omega-6 fatty acids taken. An essential fatty acid with cardioprotective properties is linoleic acid. It has been discovered that the risk of cardiovascular disease is inversely correlated with the amount of linoleic acid in adipose tissue; the lower the risk, the higher the amount. Through enzymatic processes, linoleic acid transforms into arachidonic acid, which is the main omega-6 fatty acid (Das *et al.*, 2009).

15. BIO-CHEMISTRY OF PUFA

Fish oil includes omega-3 and omega-6 fatty acids, its mechanism of action mimics that of these EFA. Both cannot be produced by vertebrates from scratch, thus they must be received through nutrition. Important physiological and developmental processes can be supported by either type. They can produce eicosanoids like lipoxins, leukotrienes and prostaglandins. These omega-3 and omega-6 fatty acids are esterified from tissue glycerolipids and hydrolyzed and they can be biologically extended and desaturated to a variety of highly unsaturated fatty acids. Linoleic acids and alpha-linolenic acids are in conflict with one another. High levels of linoleic acids prevent alpha-linolenic from being converted to its long-chain derivatives. These fatty acids with a longer chain are crucial because they are precursors of eicosanoids (Das *et al.*, 2009).

16. ADVANTAGES OF CONSUMPTION OF FISH OIL OVER WHOLE FISH

Over eating fish directly, using fish oil supplements has many advantages. Fish oil can be consumed by eating the fish directly or by drinking it; it is frequently sold

as a supplement in the form of tablets, capsules, pills, soft gels, etc. Here is a list of a few advantages of fish oil:

- ❖ Regular fish oil consumption may cause the body to produce less Vitamin E and other antioxidants. Several fish oil supplements that have incorporated Vitamin E help us combat this issue (Das *et al.*, 2009).
- ❖ Occasionally, in severe circumstances, when we need big dosages of fish oil for therapy and try to take the fish oil by eating fish, we will have to consume a lot of fish. This convenience is made possible by the rich fatty acid found in fish oil supplements (Das *et al.*, 2009).
- ❖ Supplements with fish oil are quite useful. To get our daily dose of fish oil, all we need to do is open the jar, choose a tablet and swallow it. On the other hand, cooking fish every day might be a real hassle. Furthermore, we need to be aware of the exact dosage of fish oil (Das *et al.*, 2009).

1.17 POSITIVE EFFECTS

❖ **Cardiovascular disease:**

It is widely known that populations with a high intake of oily fish have a lower incidence of heart disease and numerous studies have verified that the beneficial components of fish oils, EPA and DHA, are present. Cardiovascular disease (CVD) incidence can be decreased by ω -3 (Das *et al.*, 2009). Fish oil, which is abundant in ω -3 fatty acids, reduces the risk of heart diseases and heart arrhythmias. It helps to maintain the elasticity of artery walls, prevent blood clotting, reduce blood pressure, stabilize heart rhythm and help combat inflammation. It lowers the levels of LDL cholesterol, which is bad cholesterol and increases the HDL levels, which is good cholesterol. Fish oil prevents accumulation of triglycerides and further reduces the levels of excess triglycerides. Blood pressure, serum lipid levels and platelet function are significantly affected by arachidonic acid, which is obtained from silver carp (Wirth *et al.*, 1997). Fish oil is therefore useful in treating heart attacks, avoiding atherosclerosis in patients who have coronary disease and averting numerous sudden cardiac deaths through daily use (Mori *et al.*, 1997). To keep your heart healthy, you must consume enough EPA and DHA each day (approximately 1 gram). It is claimed that fish oil can lower triglyceride and serum cholesterol levels and enhance blood circulation. The Ecologic, cross-sectional and case-control studies

have typically revealed an adverse relationship between stroke risk and fish and fish oil consumption. Fish oils offer anti-arrhythmic properties and reduce the risk of blood clots. Supplementing with fish oil will increase plaque stability and assist shield against heart attacks and stroke. Therefore, consuming fish oil helps prevent stroke (Das *et al.*, 2009).

❖ **Brain development:**

One of the main "consumers" of DHA is the human brain. DHA makes up 30% to 60% of the retina and 15% to 20% of the cerebral cortex respectively. For the pregnancy and baby to develop normally, it is therefore necessary. More than 20 grams of DHA can be found in a healthy adult human brain. Low brain serotonin levels, which are also tied to an increased propensity for depression, suicide and violence, have been linked to low DHA levels. A high fish diet has been associated with a considerable reduction in age-related memory loss and cognitive function impairment as well as a lower chance of Alzheimer's disease development. An omega-3-rich supplement was provided to Alzheimer's sufferers, according to a recent study and this significantly improved their quality of life (Kalmijn *et al.*, 1997).

❖ **Immunity:**

The immune system of humans may be strengthened by fish oil. Our immunity is boosted when we regularly consume fish oil. Therefore, it aids in preventing the spread of common infections including the flu, cough and cold. Fish oil contains omega-3 fatty acids, which influence our body's cytokines and eicosanoids to enhance the immune system. Patients with lupus, a disease marked by attacks of the body's immune system on numerous organs and tissues, can also benefit from fish oil (Das *et al.*, 2009).

❖ **Prevents Cancer:**

Today, cancer is the most serious illness. Many people lose their lives to cancer each year, including cancers of the kidney, colon and pancreas. The primary components of fish oil, long-chain omega-3 polyunsaturated fatty acids (EPA and DHA), have been demonstrated in several *in vitro* and animal studies to significantly slow the development and spread of cancer. Their curative impact is most noticeable

in cancer that depend on hormones, such as breast and prostate cancer (Das *et al.*, 2009).

According to additional research, consuming more than 2.3 grams of EPA + DHA daily helps to reduce the development of superoxide, a powerful cancer-promoting compound. In addition, fish oil reduces the level of the COX-2 enzyme, whose overexpression is linked to colon cancer. Greek medical researchers now claim that fish oil supplements significantly lengthen cancer patients' lives with widespread malignancy. Patients with end-stage metastatic cancer may benefit significantly from supplementation with dietary omega-3 polyunsaturated fatty acids, particularly fish oils combined with an antioxidant like Vitamin E (Das *et al.*, 2009).

❖ **Inflammation:**

Fish oil is good at reducing tissue and blood inflammation because it possesses anti-inflammatory effects. Fish oil supplements, tablets, pills and capsules are beneficial for people with chronic inflammatory illnesses when taken regularly. Sprue, short bowel syndrome, inflammatory bowel disease (IBD), including Crohn's disease and ulcerative colitis and other common gastrointestinal illnesses can all be effectively treated with fish oil (Das *et al.*, 2009). It should be mentioned that fish oil only has anti-inflammatory qualities that reduce inflammation. Inflammation prevention benefits from fish oil are modest. Additionally, studies are being done on how to improve the anti-inflammatory effects of fish oil by combining it with other dietary supplements and medications.

❖ **Arthritis:**

Fish oil can also be used in treating arthritis, rheumatism, Raynaud's symptoms and other comparable illnesses (Sales *et al.*, 2008). Fish oil can be beneficial in lowering the effects of the enzymes that damage cartilage in cases of osteoarthritis. Inflammatory eicosanoids are not produced as much when omega-3 fatty acids are present. As a result, they can reduce the pain.

❖ **Mental disorder:**

Cholesterol, gangliosides, phospholipids and sphingolipids are all found in the brain. These are important for the composition and operation of brain cell membranes. A significant component of the brain's glycerophospholipids are

polyunsaturated fatty acids (PUFA). Alpha-linolenic acid and linoleic acid are the sources of this. Docosahexaenoic acid (DHA), alpha-linolenic acid, arachidonic acid and docosatetraenoic acid are the primary PUFA in the brain. DHA is made of omega-3 fatty acids. Animal experiments have demonstrated that diets lacking in ω -3 PUFA cause significant disruptions in brain function (Capper *et al.*, 2007). The addition of ω -3 PUFA to the diet can reverse this neuronal impairment. Fish oil is beneficial for reducing depression, sadness, anxiety, restlessness, mental exhaustion, stress, diminished sexual desire, suicidal tendencies and other nerve illnesses since it contains ω -3 fatty acids. Alzheimer's disease can be effectively treated with fatty acids. Fish oil aids in the treatment of Alzheimer's disease since it is one of the best sources of critical fatty acids like EPA and DHA (Das *et al.*, 2009).

❖ **Asthma:**

Asthma causes the pharynx, larynx and lungs to become inflamed. Populations with a high diet of fish oils had a decreased prevalence of inflammatory disorders like asthma, according to epidemiological studies. In the Western world, asthma is a disease that affects a rising number of people. Between 20% and 25% of all children are thought to experience one or more asthma symptoms at some stage. In many people, dietary intake of polyunsaturated fatty acids (PUFAs) may be helpful in lowering asthma symptoms. Asthma treatment options may include dietary supplementation with fish oils or other enhanced sources of ω -3 PUFAs (Das *et al.*, 2009).

❖ **Eye disorder:**

It is generally known that fish oil is beneficial for enhancing vision. Macular degeneration is one of the most typical degenerative eye diseases. It also aids in preventing macular degeneration brought on by aging. The DHA in fish oil may be the component that is most crucial for maintaining eye health as one matures. The retina contains a significant amount of omega-3 fatty acids. DHA is essential since it makes up 60% of the fatty acids in the retina (Das *et al.*, 2009).

❖ **Pregnancy:**

Fish oil is highly beneficial for pregnant women since the DHA it contains aids in the development of the baby's eyes and brain. Premature births, low birth weights

and miscarriages can all be prevented with its aid. Additionally, it is thought that women who do not consume enough EPA and DHA in their diet have postpartum depression because, during the last trimester of pregnancy, some brain matter is transferred from the mother to the fetus. By ingesting the recommended dosage of fish oil while pregnant, low birth weight can be prevented. There is some proof that consuming insufficient amounts of omega-3 fatty acids may raise the risk of premature birth and low birth weight (Das *et al.*, 2009).

Chapter-2: REVIEW OF LITERATURE

1. Moovendhan *et al.* (2021), work aims to analyze the physical characteristics such as specific gravity, solidification point, saponification value, iodine and acid value of liver oil. He also found the heavy metal, ash, moisture, protein and carbohydrate content, bad and good cholesterol levels. Ten fatty acids were recorded in which the oleic acid, ω -3, EPA, DHA, and α – linolenic acid were recorded maximum level. The essential and non- essential amino acids were estimated and the fat soluble Vitamins (A and E) were noticed. The minerals Zn and Fe were also found. Further, he also analyzed the antibacterial effect of liver oil against 7 bacterial strains and 6 antioxidant activities of liver oil which showed concentration depended.

2. Moovendhan *et al.* (2019), work aims is to evaluate the nutritional fact and anticancer effect of liver oil extracted from the squid *Loligo duvacei*. He noted the ash and moisture content as 3.21% and 2.13% whereas the total protein, lipid and carbohydrate recorded as 23.44, % 21.23% and 2.13% respectively. The water and fat soluble Vitamins and minerals were found in good level. The lipid profile was noted similarly to cod liver oil at the same time the good cholesterol was found in high level. He noted ten fatty acids especially DHA, EPA, omega-3 in high level. The essential amino acids were recorded at high level whereas Non-essential acids were recorded at moderate level. The anticancer ability of oil against lung cancer cell (A549) showed better activity and there cytotoxicity effect was seen in Vero (normal) cell lines. He concludes his present study by the statement, that *L. duvauceli* liver oil is a good source of nutrition and provides good health benefits to human.

3. Moovendhan *et al.* (2019), estimated the ash, moisture, protein and carbohydrate content of the extracted oil as 4%, 7%, 4% and 1% respectively. The Vitamins (A and E) was found and nine fatty acids especially the linoleic acid, DHA and EPA were recorded higher. The specific gravity, solidification point, refractive index, saponification value, iodine value and acid value were also calculated and found. He then studied the antioxidant activity that includes total antioxidant activity, DPPH & Hydroxyl radical scavenging assay, reducing power and ferrous ion chelating activity of liver oil by following standard methods and results of all the assays was found increasing with increase in concentration. Further purification of liver oil and

studying its antioxidant property in animal models shall form the basis for its use as a potent alternative natural antioxidant and nutrition supplements.

4. Immanuel *et al.* (2009), work aims to characterize the physico-chemical properties of liver oil from lost cost Balistid fish *Sufflamen capistratus*. He extracted the oil by Soxhlet, Bligh and Dyer, direct steaming, solar extraction, Mc Gill and Moffat methods. The physical properties such as solidification point, melting point, refractive index and moisture content of the extracted oil samples were differed between methods. The chemical properties like the acid value, free fatty acid (FFA) level and carotenoid content of the individual oil samples were showed extraction methods dependant variation. Fatty acid content was also showed variation between methods. In all oil samples, fat soluble Vitamin K was more when compared with the other tested Vitamins. Irrespective of methods of extraction, permissible level of heavy metal and minerals were recorded in the oil samples.

5. Das *et al.* (2009), work aims to talk about the beneficial effects of fish oil to human health. The Omega (α)-3 and α -6 poly unsaturated fatty acids are dietary essential constituents of human diet. As our body cannot de novo synthesize it, so, it must be consumed through diet. Fish oil is a major source of ω -3 and ω -6 essential fatty acids. It prevents major heart diseases, cancer, diabetes, mental disorder, arthritis and many other health problems. Health, fish oil contributes a large number of beneficial effects to us. It must be consumed as per the recommended dosages. Consumption of 1.0g fish oil daily will help the human being to combat the coronary heart disease. Fish oil supplements have numerous benefits over consumption of fish directly.

6. Schnell and Clayton (2021), work aims to talk about the large brained molluscs Cephalopod, their ability of rethinking cognition. Traditional approaches in comparative cognition have a long history of focusing on a narrow range of vertebrate species. However, in recent years the range of model species has expanded. Despite this development, invertebrate taxa are still largely neglected in comparative cognition, which limits our ability to locate the origins of cognitive traits. The time has come to rethink cognition and develop a more comprehensive understanding of cognitive evolution by expanding comparative analyses to include a diverse range of invertebrate taxa. In this review, he contend that cephalopod are

suitable ambassadors for rethinking cognition. Cephalopods have large complex brains, exhibit sophisticated behavioral traits and increasing evidence suggests that they possess complex cognitive abilities once thought to be unique to large-brained vertebrates. Comparing cephalopods with vertebrates, whose cognition has evolved independently, provides prominent opportunities to circumvent current limitations in comparative cognition that have arisen from traditional vertebrate comparisons. Increased efforts in investigating the cognitive abilities of cephalopods have also led to important welfare-related improvements. These large-brained molluscs are paving the way for a more inclusive approach to investigating cognitive evolution that we hope will extend to other invertebrate taxa.

Chapter-3: AIM AND OBJECTIVE

1. AIM

Extraction and characterization of octopus oil

2. OBJECTIVE

1. Extraction of oil by solvent extraction method
2. To analyze the fatty acid profile
3. Determination of physical properties of oil
4. Determination of chemical properties of oil

Chapter-4: MATERIALS AND METHODS

4.1 COLLECTION OF OCTOPUS TISSUE

Octopus tissue was collected from fresh specimens of *Cistopus indicus* in Kasimedu, Chennai, Tamil Nadu, India. The tissue was washed thoroughly with distilled water to remove the ink and the adherents.



Fig 4.1.1 Octopus sample dorsal and ventral view

4.2 EXTRACTION OF OCTOPUS OIL

The Octopus tissue was cut into small pieces and dried in sunlight. The dry tissue obtained was used for oil extraction by Bligh and Dyer method using chloroform and methanol (2:1). After 7 days the tissues are grinded and again solvents are added in 2:1 ratio. After 7 days the solvents were removed in hot air oven at 40°C and the solvent free oil was stored in brown coloured amber bottles.



Fig 4.2.1 Sun dried sample



Fig 4.2.2 Solvent extraction

3. YIELD OF OIL

The weight of the total yield was taken into account to calculate the percentage yield against the amount of tissue used for the extraction through simple arithmetic calculation.

4. PHYSICAL PROPERTIES

❖ Specific Gravity and Solidification Point

The specific gravity and solidification point of the *Cistopus indicus* oil were measured following IUPAC protocol (Bruschweiler and Dieffenbacher, 1991).

❖ Refractive Index

The refractive index of *Cistopus indicus* oil was estimated using Spectrometer-Hollow prism method described by Gaithersburg (1984).

❖ Saponification Value

The Saponification value of *Cistopus indicus* oil was calculated using the titration method described by Adeyemo (2004).

❖ **Iodine Value**

The iodine value of *Cistopus indicus* oil was determined by using titration method described by Abdulkadir *et al.* (2010) in which starch was used as an indicator.

❖ **Acid Value**

The acid value of *Cistopus indicus* oil was determined by following the titration method described by Abdulkadir *et al.* (2010). in which Phenolphthalein was used as an indicator.

4.5 CHEMICAL PROPERTIES

❖ **C, H and N analysis**

The determination of Carbon, Hydrogen and Nitrogen in octopus oil was done using Elemental Analyzer (Perkin Elmer-2400, series II).

❖ **Ash and moisture**

The ash and moisture content of octopus oil was analyzed as described by Gaithersburg (1984).

❖ **Estimation of biochemicals**

The biochemical content such as protein (Lowry *et al.*, 1951) and carbohydrate (DuBios *et al.*, 1956) of octopus oil was estimated and the BSA and D-Glucose was used as a standard for the biochemical assays.

❖ **Estimation of Vitamins content**

The fat-soluble Vitamins (A and E) and water-soluble Vitamins (B₁, B₂, B₆ and C) of octopus oil were analyzed in HPLC (Merck, Hitachi L-7400) following the method described by Sadasivan and Manickam (1996).

❖ **Fatty acid analysis**

The FAME (Fatty Acid Methyl Esters) was prepared by following the protocol of Metcalfe *et al.* (1966). Fatty acid analysis was achieved by Gas chromatography (ASHMACO, Japan).

Chapter-5: RESULTS AND DISCUSSION

5.1 YIELD OF OCTOPUS OIL

Oil yield is an important parameter for the large-scale production of octopus oil and high oil yield reduces the cost of oil extraction. In the present study, the yield of oil from *Cistopus indicus* was found moderate, i.e., 13%.



Fig 5.1.1 Oil yield



Fig 5.1.2 Oil in amber bottle

5.2 PHYSICAL PROPERTIES OF OCTOPUS OIL

To evaluate the market value, human consumption and potential applications of fats and oils, a physical property relates to the quantification of quality (Impurities) features. In the present finding, the specific gravity of *Cistopus indicus* oil was found to be 1.124. Similarly, Young (1986), investigated the specific gravity of herring fish oil in three different temperature conditions such as 15°C, 30°C and 45°C and reported it as 0.92, 0.91 and 0.90 respectively. When compared to the above reports, the *Cistopus indicus* showed higher specific gravity than fish oils described above. The saponification value determined the fatty acid chain length and the *Cistopus indicus* oil has showed 47.8 mg, this is very lesser than the finding of Abdulkadir *et al.* (2010), who estimated the saponification value of *Mormyrops deliciousus*, *Bagrus docmac niger*, *Tilapia dagati* and *Clarias anguilloris* liver oil as 549.78, 367.46, 347.82 and 318.48 mg respectively. In the present evaluation for the solidification point of *Cistopus indicus* oil, it was found to be 1.1°C. Immuanuel

et al. (2002), reported the solidification point of liver oil from *O. niger* extracted by four different protocols and it was found to be $29\pm0.5^{\circ}\text{C}$ in all extraction methods respectively. The refractive index of *Cistopus indicus* oil was recorded as 1.176. The results of the present study are agreed with the four fish species such as *M. deliciousus*, *B. docmac*, *T. dagati* and *C. anguilloris* and the refractive index was noticed 1.6642, 1.6769, 1.5990 and 1.6240 respectively along with standard cod liver oil (1.472-1400) (Abdulkadir *et al.*, 2010). The acid and iodine value of *Cistopus indicus* oil was displayed accepted level of 0.064 mg KOH/g and 23.4 respectively. Paul *et al.* (2013), calculated the higher acid and iodine value of fish oils (*C. striatus* and *C. marulius*) as (1.76 ± 0.03 and 1.87 ± 0.05) (87.34 ± 0.64 and 1.87 ± 0.05) respectively.

5.2.1 Table; Physical properties of *Cistopus indicus* oil

S.NO	PROPERTIES	SAMPLE (IU)
1.	Specific gravity	1.124
2.	Solidification point	1.1°C
3.	Refractive index	1.176μ
4.	Saponification value	47.8mg
5.	Iodine value	23.4
6.	Acid value	0.064 mg KOH/g oil

5.3 CHEMICAL PROPERTIES OF OCTOPUS OIL

The elemental composition such as Carbon, Hydrogen and Nitrogen content in *Cistopus indicus* oil were found to be 46.3%, 11.2% and 6.9% respectively, it determined the structure and function of fatty acids. In addition, the ash and moisture was noticed 9% and 5% respectively in *Cistopus indicus* oil. Likewise, Abdulkadir *et al.* (2010), pointed the moisture content in crude fish oil from four species of fishes such as *M. deliciousus*, *B. domac niger*, *T. dagati* and *C. anguilloris*, it was found to be 30.22%, 6.72%, 14.52% and 17.93% respectively. The refining operation has the aim to remove undesirable components from the oil regarding commercialization and human consumption (Biohaz, 2010). *Cistopus indicus* oil has showed trace amounts of protein and carbohydrate of 1.6% and 1.9% respectively.

5.3.1 Table; Chemical properties of *Cistopus indicus* oil

S.NO	Name of the Chemical characters	Composition (%)
1.	Carbon (C)	46.3
2.	Hydrogen (H)	11.2
3.	Nitrogen (N)	6.9
4.	Ash	9
5.	Moisture	5
6.	Total Protein	1.6
7.	Total Carbohydrates	1.9

❖ Vitamin content of octopus oil

Organic chemical substances known as Vitamins are necessary for promoting development, reproduction and healthy physiological functions (Dean, 1990). The Vitamins content of *Cistopus indicus* oil, the fat soluble Vitamins A and E were found to be 3951.7 IU and 3.784 mg respectively. The water soluble Vitamins such as B₁, B₂, B₆ and C were found in moderate level (0.102 mg, 0.219 mg, 0.247 mg, 0.248 mg/100 g respectively). Cod liver oil contained high amount of fat soluble Vitamins A and E (Jobling and Leknes, 2010). Mathew and Mathew (2010), reported the Vitamin A and E content of shark liver oil (*Neoharriotta raleighana* and *Centrosymnus crepidaiier*) as A-0.96%, E-2.55% in *Neoharriotta raleighana* liver oil and A-0.27%, E-0.35% in *Centrosymnus crepidaiier* liver oil.

5.3.2 Table; Vitamins content of *Cistopus indicus* oil

S.NO	Name of the Vitamins	Quantity (units/100 g)
1.	Vitamin A	3951.7 IU
2.	Vitamin E	3.784mg
3.	Vitamin B ₁	0.102mg
4.	Vitamin B ₂	0.219mg
5.	Vitamin B ₆	0.247mg
6.	Vitamin C	0.248mg

❖ Fatty acids composition of octopus oil

The primary constituents of lipids are fatty acids and the location of double bonds, chain length, degree of unsaturation and geometry vary depending on the type of organism (Gutnikov, 1995). In the present investigation, the *Cistopus indicus* oil is reported to contain the valuable fatty acids such as oleic acid and α - Linolenic acid at high levels (21.47 and 9.855 gm/100gms respectively) and the palmitic acid, steric acid, linolenic acid at moderate levels (5.478, 5.766 and 6.404 gm/100gms respectively). Whereas margaric acid and morotic acid were recorded at low levels (0.884 and 1.862 gm/100gms respectively) and the commercially important fatty acids DHA and EPA were recorded in moderate level (1.147 and 0.925 gm/100gms respectively). The results of the present study are supported very well by the following finding: Joseph (2007), accounted the SFA, MUFA, PUFA, EPA and DHA as 38.3, 15.6, 17.4, 4.9 and 2.5% respectively in *S. pharonis* liver oil.

5.3.3 Table; Fatty acid profile of *Cistopus indicus* oil

S.NO	Name of the fatty acid	Structure	gm/100gms
1.	Palmitic acid	C16: 0	5.478
2.	Margaric acid	C17: 0	0.884
3.	Stearic acid	C18: 0	5.766
4.	Oleic acid	C18: 1	21.47
5.	Linolenic acid		6.404
6.	α - Linolenic acid	C18: 3	9.855
7.	Morotic acid	C18: 4	1.862
8.	DHA	C22: 6	1.147
9.	EPA	C20: 5	0.925
10.	Other fatty acids		1.784

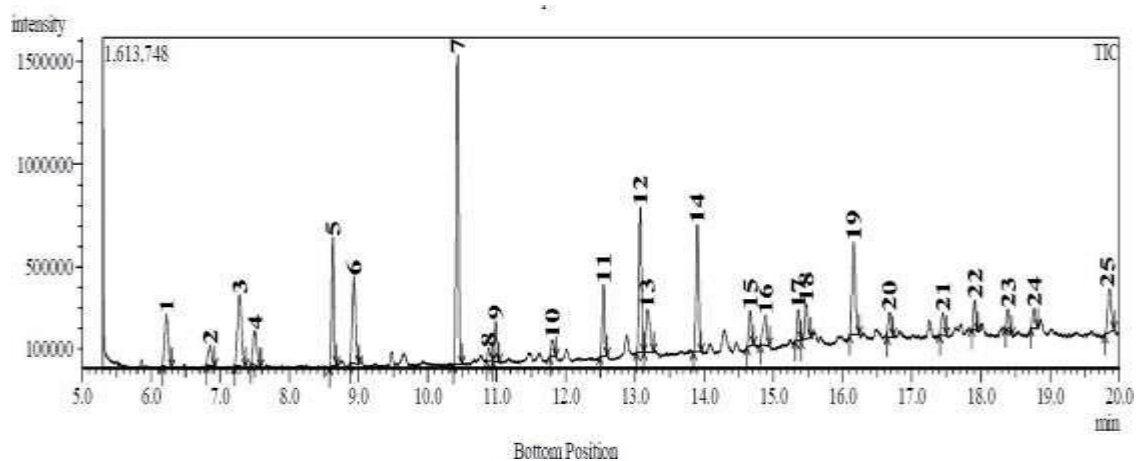


Fig 5.3.4 GC-MS Chromatogram

Chapter-6: SUMMARY AND CONCLUSION

The present study was based on the extraction and characterization of octopus oil. This project aims in finding out the physical and chemical properties of the freshly extracted octopus oil. First, the Indian species *Cistopus indicus* was collected from Kasimedu, Chennai, Tamil Nadu, India. The tissue was washed thoroughly in distilled water to remove the ink and the adherents. Then the sample was dried in sunlight for a week. The sample was taken to the laboratory and weight is noted. Then the sample was transferred to the 1000ml beaker and the solvents are added (chloroform and methanol in 2:1 ratio). After 7 days, the solvents were added again (chloroform and methanol in 2:1 ratio). After 7 days the sample with the solvents was kept in hot air oven at 40°C to remove the solvents. Once the solvents were evaporated, the crude oil is precipitated at the bottom of the beaker. The crude oil is then scraped and then transferred to the amber bottle and sent for characterization.

Traditionally fish oil was extracted from Sardine, Shark, Tuna, Salmon fishes etc., due to its demand and health benefits, target fisheries was highly developed and most of the species was yet to be endangered.

This study was focused to extract the oil from the alternate and sustainable waste source of octopus because it's not edible in India and million tonnes of octopus and its waste was discarded as waste to environment. It's a cheap and potential source of oil for human supplement.

CONCLUSION

The present study explicitly reveals that the octopus oil from *Cistopus indicus* has good nutritional factors. The physical and chemical properties revealed the low impurities of oil and also it having long time storage characters. Based on the findings, the *Cistopus indicus* oil is an alternative source of functional food from a cheap source in nutrition supplementation and biomedical applications instead of fish oils.

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