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Chitra Sem R.S.
Abstract

The oceans have proved to be an interminable source of new and effective drugs. Innumerable studies have proved that specific compounds isolated from marine organisms have great nutritional and pharmaceutical value. Polyunsaturated fatty acids (PUFA) in general are known for their dietary benefits in preventing and curing several critical ailments including Coronary heart disease (CHD) and cancers of various kinds. Eicosapentaenoic Acid (EPA) and Docosahexaenoic Acid (DHA) are two PUFA which are entirely marine in origin – and small Clupeoid fishes like sardines are known to be excellent sources of these two compounds. In this study, we selected two widely available Sardine species in the west coast, Sardinella longiceps and Sardinella fimbriata, for a comparative analysis of their bioactive properties. Both these sardines are known to be rich in EPA and DHA, however considerable seasonal variation in its PUFA content was expected and these variations studied. An extraction procedure to isolate PUFA at high purity levels was identified and the extracts obtained thus were studied for anti-bacterial, anti-diabetic and anti-cancerous properties.

Samples of both the sardines were collected from landing centre, measured and their gut content analysed in four different months of the year – viz. June, September, December and March. The fish samples were analyzed for fatty acid using FAME method using gas chromatography to identify the full range of fatty acids and their respective concentration in each of the samples. The fatty acids were expressed in mg/g meat and later converted to percentage values against total fatty acids and total PUFA content. Fatty acids during winter season (Dec-Mar) were found to be generally higher than spawning season (June-Sept). PUFA dominated the
profiles of both species and average PUFA content was also higher during winter. However, it was found that S. longiceps had proportionately higher EPA as compared to S. fimbriata which was DHA rich. Percentage of EPA and DHA also varied across months for both species – the spawning season seemed to show higher EPA content in S. longiceps and higher DHA content in S. fimbriata. Gut content analysis indicate that adult S. fimbriata is partial to zooplanktons which are DHA rich while adult S. longiceps feed mainly on EPA rich phytoplankton. Juveniles of both species, found mainly in winter, had a gut content showing more mixed diet. This difference in the feeding pattern reflect clearly in their PUFA profile – adult S. longiceps, which dominate the catch during the spawn season, feeding mostly on phytoplankton is concentrated with EPA while the juveniles which are found mostly in the winter season has slightly less EPA proportion as compared to adults. The same is true for S. fimbriata adults that are caught mostly in the spawning season; being rich in DHA as they feed mainly on zooplankton while the juveniles caught during winter season has a relatively lower concentration of DHA in their total PUFA.

Various extraction procedures are known to obtain PUFA from fish oil. However, most of them do not give high purity and do not use materials indicated as safe. PUFA extracts have to be edible and should not have harmful substances for applying on mice and human subjects. Some PUFA extraction procedures, though pure and non-toxic, might induce cis-trans conversions during the extraction process. This conversion destroys the benefits of PUFA and at times is harmful to human body. A method free from these limitations has been standardized for this study. Gas Chromatography was performed on the extracts thus made to ensure that it is substantially
pure. EPA: DHA ratios for both samples were derived - for S. longiceps this ratio was 3:2, while it was 3:8 for S. fimbriata.

Eight common strains of gram positive and gram negative bacterial strains were subjected to the PUFA extracts from both species dissolved in acetone solution using Agar Well Diffusion method. The activity was studied against an acetone control. At the end of incubation period, zones of inhibition were measured to estimate the activity. Minimum inhibitory concentration for each of the active combinations was calculated by keeping $p < 0.01$ as significant. Four of the bacteria including multi-resistant Staphylococcus aureus were shown to be inhibited by the fish extracts. It was also found that the extracts from S. fimbriata were better than the one from S. longiceps in annihilating harmful bacteria.

Four groups of mice subjects were studied to evaluate the anti-diabetic properties of the PUFA extracts. Three groups were induced diabetes by administration of alloxan tetra hydrate. One group without diabetes was kept as control and another with diabetes was kept as diabetic control. For two diabetic groups, a prescribed amount of fish extracts were fed from each of the extracts. The biochemical parameters like serum glucose, total cholesterol, LDL & HDL cholesterol, triglycerides, urea and creatinine were sampled from all four groups at regular intervals of 7 days for a period of 28 days. It was found that groups fed with fish extracts had marked improvement in the levels of total LDL & HDL cholesterol, triglycerides and creatinine. Groups fed with extracts from S. fimbriata seem to have fared better as compared to S. longiceps. However, both groups did not show any marked improvement in blood glucose levels or levels of urea.
Cell lines of MCF-7 (Breast Cancer) and DU-145 (Prostate Cancer) were used to analyse the cytotoxicity of the PUFA extracts. Both cell lines were subjected to MTT Assay and later the plates were read using an ELISA reader at a wavelength of 570nm. It was found that both extracts had significant cytotoxic effects against both cell lines and a peak cytotoxicity of 85-90% was apparent. IC\textsubscript{50} values were calculated from the graphs and it was found that S. longiceps extracts had a slightly lower IC\textsubscript{50} value indicating that it is toxic even at a lower concentration as compared to extracts from S. fimbriata.

This study summarizes the bioactivity profile of PUFA extracts and provides recommendation for dietary intake; fish based nutritional industry and indigenous pharmaceutical industry. Possible future directions of this study are also elaborated.