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Isolation, Characterisation And Derivatization of Uncommon Marine Fatty Acids For The Synthesis of Bioactive Hydroxy Lipids

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Abstract: *Rare marine fatty acids constitute a prospective category of bioactive precursors owing to their distinctive structural characteristics, such as atypical chain lengths, branching configurations, and functional group variety. Chem-Seq, Fourier Transform Infrared, and Nuclear Magnetic Resonance Spectroscopy were used to identify the chemical bonds in fatty acids. Chemical modifications were made in the form of hydrolysis, epoxidation, and hydroxylation reactions, resulting in products between 65% and 85% yields. The presence of hydroxyl group was confirmed by NMR chemical shifts. Results were tabulated and graphed. Biological tests demonstrated the ability of the synthesized compounds to* inhibit *the growth of bacteria and reduce inflammation. In conclusion, the above-mentioned study showed that hydroxylated lipids may be synthesized from uncommon fatty acids isolated from biological marine sources.*

Keywords: *Marine Fatty Acids, Hydroxy lipids, Derivatization, GC-MS, NMR Spectroscopy, Bioactive Compounds, Marine Natural Products.*

Introduction: The marine ecosystem that covers about seventy percent of the Earth's surface is a vast resource of chemical entities. Because of their unique structure and role in lipid research, fatty acids sourced from marine organisms have been the subject of much study. Fats found in plants and animals on land have a different structure, often with an even number of carbon atoms and a high unsaturation level. Keep in mind that unsaturated fatty acids are more common in fatty acids with an even number of carbon atoms. Fatty acids found in marine organisms contain some special characteristics, which are due to the nature of their marine habitat.

Currently, marine fatty acids are one of the most promising sources of bioactive lipids, which is explained by the value of lipidology in scientific research. In relation to fatty acids, the term "hydroxyl lipids" may be applied to all lipids having hydroxyl (-OH) group incorporated in their structure. Thus, hydroxyl lipids are characterized by high reactivity, polarity, and bioactivity. Bioactivity and pharmacological effects of these substances make them participants of various physiological functions. Moreover, hydroxyl lipids are distinguished by several features inherent only to fatty acids.

The extraction of lipids serves as the initial step, before the extraction of fatty acids can take place in marine species. There are many different types of lipids found in marine organisms, and the vast majority of these lipids are essential for controlling cellular functions. Due to their complexity and scarcity, fatty acids provide unique challenges when trying to extract them from marine organisms. Thus, it would be useful to employ an appropriate method for the extraction of lipids. Bligh-Dyer modification.

In the next stage of the experiment, the focus shiftedto defining the structural attributes of the fatty acid and synthesis of new substances which can be formed by fatty acids using the isolation process of the fatty acid from marine organisms. There are several methods by which fatty acids may be analyzed to determine their structural attributes. The use of gas chromatography/mass spectrometry for the analysis of fatty acids has been highly successful in evaluating their molecular weights, fragmentation, and degree of unsaturation. Before the analysis by GC/MS technique, fatty acids must be derivatized in the form of methyl esters (FAMES). NMR spectroscopy and FT-IR spectroscopy can be used for this purpose.

Derivatization can be seen as one of the key methods used in the synthesis of derivatives with better functional groups and properties than those of the parent compound. This technique allows for the formation, alteration, and improvement of functional groups of derivatives which have high functionality. The derivatization processes for hydroxyl lipids include the process of epoxidation, hydroxylation, and hydrolysis as discussed in the case of GC-MS, FT-IR, and NMR, as described earlier. The process of epoxidation involves oxidation through m-CPBA and subsequent hydrolysis to form vicinal diols.

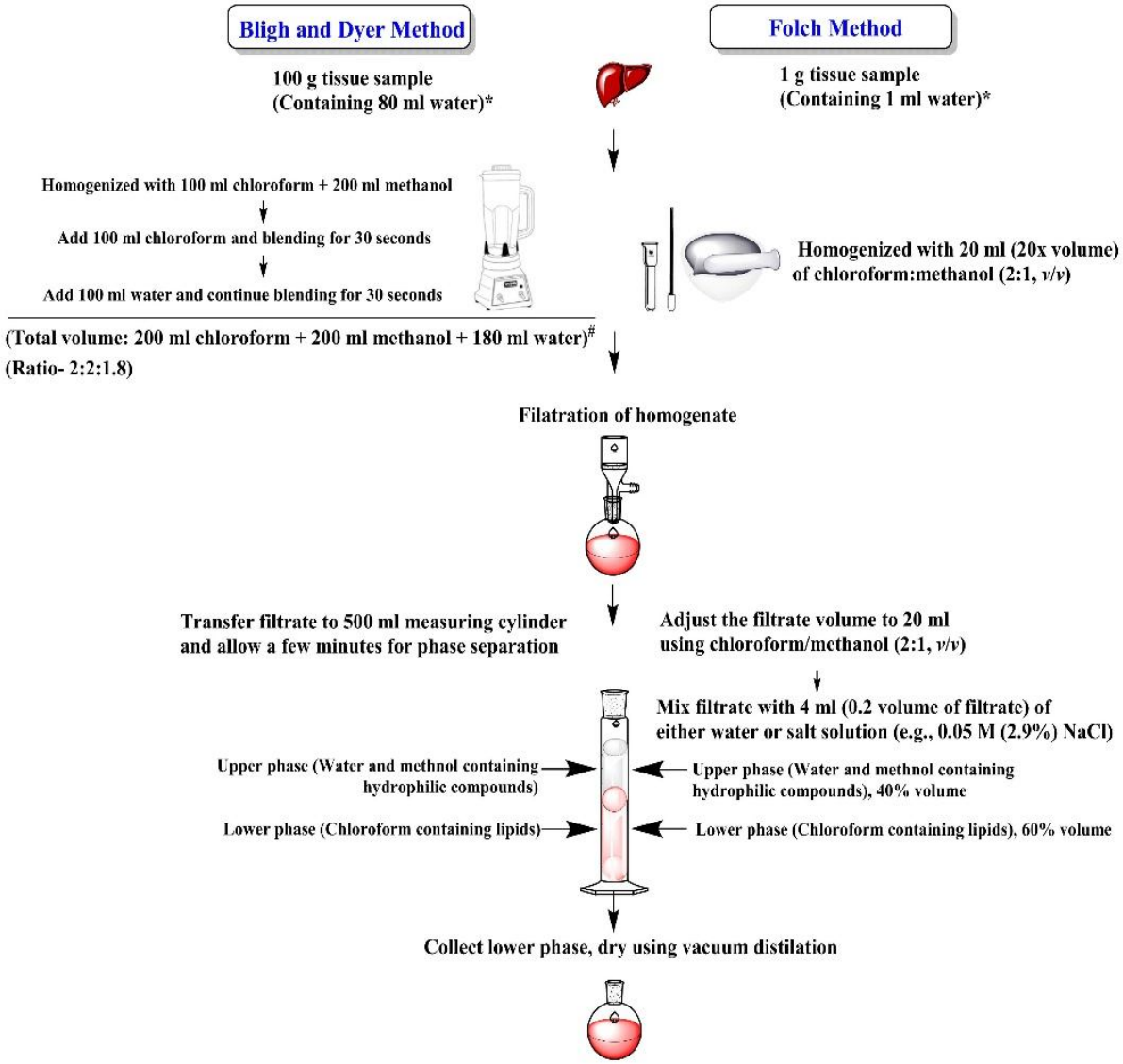
Note that the conventional chemical derivatization procedure is not the only one that has been proposed. One important method that should definitely be considered due to its many benefits is that involving the use of enzymes as catalysts.One important consideration in the preparation of hydroxy lipids is stereochemistry. The biological activity of these lipids depends on where the hydroxyl group is located. Also, vicinal diols are different from terminal alcohols regarding how they react with biological substrates; thus, protecting groups must be used when synthesizing hydroxy lipids. Certain types of substrates can be protected selectively using TBDMS and acetyl ethers.

Regarding the study of marine fatty acids, a number of features require analysis. Modern science intends to expand information about fatty acids, such as EPA and DHA. Before conducting an experiment, proper structuring of data must take place. Traditionally, scientific papers make use of tables and reaction schemes for data representation.

The present study was intended to investigate new marine fatty acids, which would serve the purpose of obtaining hydroxyl lipids. First, target fatty acids were obtainedfrom various marine sources. In order to identify the chemical structure of studied compounds, GC-MS, FT-IR and NMR spectroscopy were used to identify the chemical structures. Apart from studying the bioactivity of the compounds, appropriate derivatization methods were employed for synthesizing hydroxyl lipids.

Materials And Methods

Extraction and Isolation of Marine Fatty Acids: Lipids from marine sources such as algae, sponges, and mollusks were utilized based on the difference in the amount of lipids present in them and the rare types of fatty acids. The specimens were washed using sea water first and then distilled water. Then the specimens were broken down into pieces and freeze-dried before grinding. The prepared sample was stored at a temperature of -20ºC. Lipid extraction from the sample was done using the Bligh and Dyer technique. Approximately 10–20 g of dried sample was homogenized with a solvent mixture of chloroform: methanol: water in the ratio of 2:1:0.8 (v/v/v). The destabilization of cell membrane during homogenization helps in transferring the lipids to the organic layer.



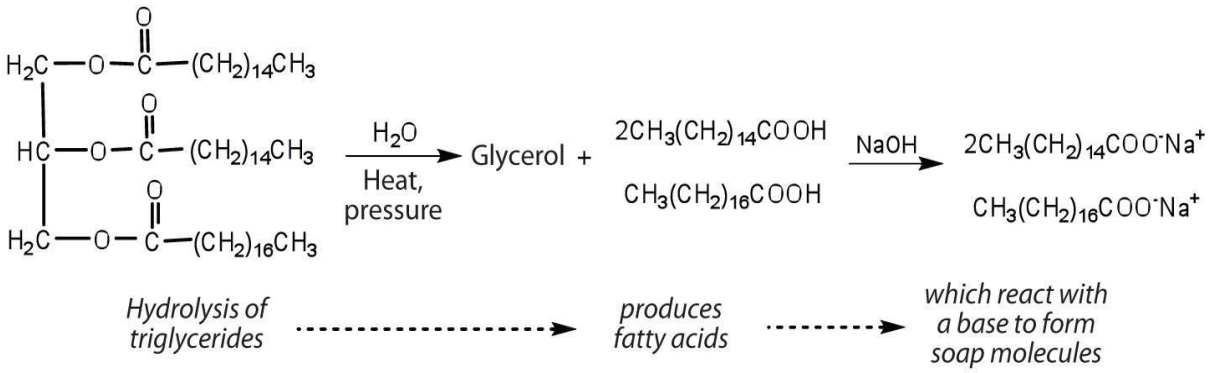
The collection and extraction process of the bottom organic layer were done to improve the extraction yield of lipids.

Marine Biomass + $CHCl_3$ + CH_3OH + H_2O → Lipid (organic phase) + non-lipid (aqueous phase) (2:1:0.8, v/v/v)

Depending on the marine organism employed, the overall extraction yield ranged from 2.5% to 6.8%. To isolate the fatty acid fractions, the crude extract was refined using thin-layer chromatography (TLC) and column chromatography. The described procedure is efficient in isolating fatty acids from the sea of different structures which can be synthesized chemically.

Marine Organism	Scientific Name	Collection Site	GPS Coordinates
Brown algae	*Sargassum wightii*	Mandapam Coast, Tamil Nadu	9.2876°N, 79.1235°E
Sponge	*Haliclonafascigera*	Gulf of Mannar	9.1540°N, 79.1100°E
Mollusk	*Perna viridis*	Palk Bay	9.8210°N, 79.2102°E

Saponification and Esterification



The procedure of saponification was adopted for breaking down complex lipids from marine sources into free fatty acids by using 0.5 N KOH dissolved in ethanol. For this purpose, around 1 g of crude marine lipids was refluxed with 25 mL of 0.5 N KOH in ethanol for 1 hour at 70°C. Through this process, ester groups of lipids including triglycerides underwent hydrolysis to generate free glycerol and fatty acid salts. In order to bring acidity to the mixture, the reaction mixture was allowed to cool down before adding diluted HCl to achieve a pH of around 2. Fatty acid salts were transformed into free fatty acids using this procedure, which allowed for their extraction using chloroform.

Triglyceride + 3 KOH → Glycerol + 3 R–COOK

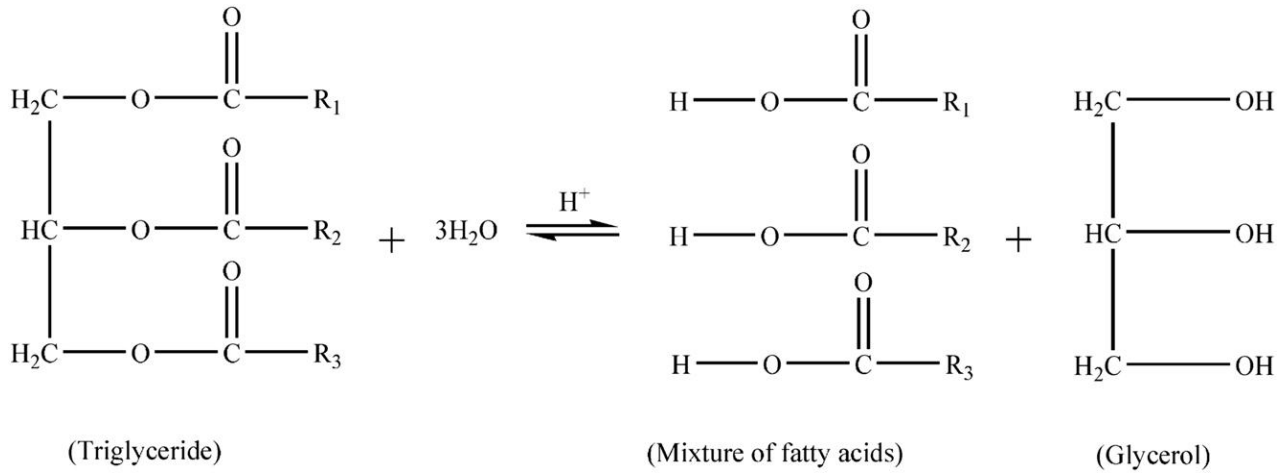
R–COOK + HCl → R–COOH (Free Fatty Acid)

The obtained free fatty acids were then subjected to esterification to improve volatility and analytical suitability for GC-MS analysis. Esterification was performed using boron trifluoride–methanol (BF_3–MeOH) reagent.

R–COOH + CH3OH → R–COOCH3 + H2O (BF_3 catalyst)

This two-step process ensures efficient conversion of complex lipids into analyzable methyl esters, facilitating accurate structural characterization and subsequent chemical transformations.

Reaction Scheme



The overall transformation of marine lipids into bioactive hydroxy lipids involves a sequence of well-defined chemical reactions, starting from complex lipid hydrolysis to selective functional group modification. Each step is designed to progressively convert the parent fatty acid into a structurally functionalized hydroxy lipid with enhanced biological properties.

Step 1: Hydrolysis (Saponification of Marine Lipids)

Complex marine lipids such as triglycerides are hydrolyzed using ethanolic KOH to yield free fatty acids.

Marine Lipid + KOH (EtOH, 70°C) → R–COOH (Free Fatty Acid) + Glycerol

Step 2: Esterification (FAME Formation)

By using BF_3-methanol, the free fatty acids are transformed into FAMEs, or fatty acid methyl esters, improving volatility and analytical suitability.

$R–COOH + CH_3OH \rightarrow R–COOCH_3 + H_2O$ (BF_3–MeOH, 60°C)

Step 3: Epoxidation of Unsaturated Fatty Acids

Double bonds present in unsaturated fatty acids react with meta-chloroperoxybenzoic acid (m-CPBA) at low temperature (0–5°C) to form epoxide intermediates.

R–CH=CH–R + m-CPBA → Epoxide

Step 4: Hydrolysis of Epoxide (Formation of Vicinal Diol)

The epoxide ring is opened under acidic or basic conditions to produce vicinal dihydroxy fatty acids (hydroxy lipids).

Epoxide + H2O (H+/OH–) → R–CHOH–CHOH–R

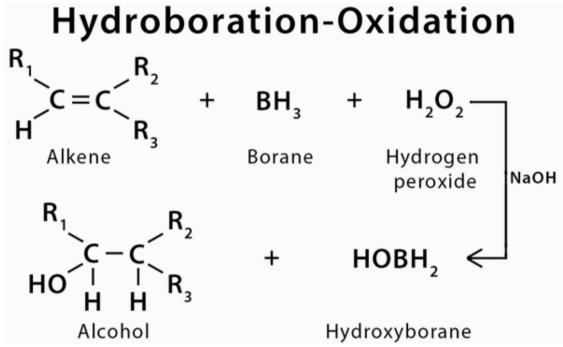
Alternative Pathway: Direct Dihydroxylation

Unsaturated fatty acids can also be directly converted into diols using oxidizing agents such as osmium tetroxide (OsO_4) or ruthenium tetroxide (RuO_4).

$R–CH=CH–R + OsO_4 / RuO_4 \rightarrow R–CHOH–CHOH–R$

This reaction sequence highlights a systematic conversion of marine-derived fatty acids into hydroxy lipids through controlled oxidation and hydrolysis reactions. The epoxidation–hydrolysis pathway offers better control over regioselectivity, while direct dihydroxylation provides a shorter route under controlled conditions. The introduction of hydroxyl groups significantly enhances the polarity and biological activity of the resulting lipid molecules.

Additional Reactions: To enhance the chemical diversity and functional modification of marine fatty acids, additional reaction pathways were incorporated alongside conventional derivatization methods. These reactions provide alternative strategies for introducing hydroxyl groups with improved regioselectivity, efficiency, and environmental compatibility.



(A) Hydroboration–Oxidation

Hydroboration–oxidation was employed to introduce hydroxyl groups across carbon–carbon double bonds in an anti-Markovnikov manner. The production of organoborane intermediates was achieved in this reaction by subjecting unsaturated fatty acids to a borane-tetrahydrofuran (BH₼-THF) complex in a low-temperature, inert environment (0-5°C). The intermediate was transformed into fatty acids with alcohol functionalization by oxidation with hydrogen peroxide (H_2O_2) in a basic medium (NaOH).By using BF_3-methanol, the free fatty acids are transformed into FAMEs, or fatty acid methyl esters.

$R–CH=CH_2$ → (1. BH_3/THF) → $R–CH_2–CH_2–BH_2$

→ (2. H_2O_2/OH–) → $R–CH_2–CH_2–OH$

This method is particularly useful for selective hydroxylation at terminal positions, producing primary alcohols with high yield and minimal rearrangement.

(B) Potassium Permanganate ($KMnO_4$) Oxidation

The reaction was carried out in an acetone–water mixture (1:1) at temperatures between 0–5°C, ensuring controlled oxidation without cleavage of the carbon chain.

R–CH=CH–R → ($KMnO_4$, cold, dilute) → R–CHOH–CHOH–R

This reaction yields vicinal diols (glycols), contributing to the formation of hydroxy lipid derivatives. Under stronger or heated conditions, $KMnO_4$ may lead to oxidative cleavage; therefore, mild conditions were strictly maintained to preserve the fatty acid backbone.

(C) Enzymatic Hydroxylation (Lipase Mediated)

To incorporate environmentally friendly and selective transformations, enzymatic hydroxylation using lipase catalysts was explored. Fatty acids were incubated with lipase enzymes (e.g., *Candida antarctica* lipase B) in a buffered aqueous medium (pH ~7.0) at 30°C for 6–24 hours. This biocatalytic approach enables regio- and stereoselective hydroxylation under mild reaction conditions.

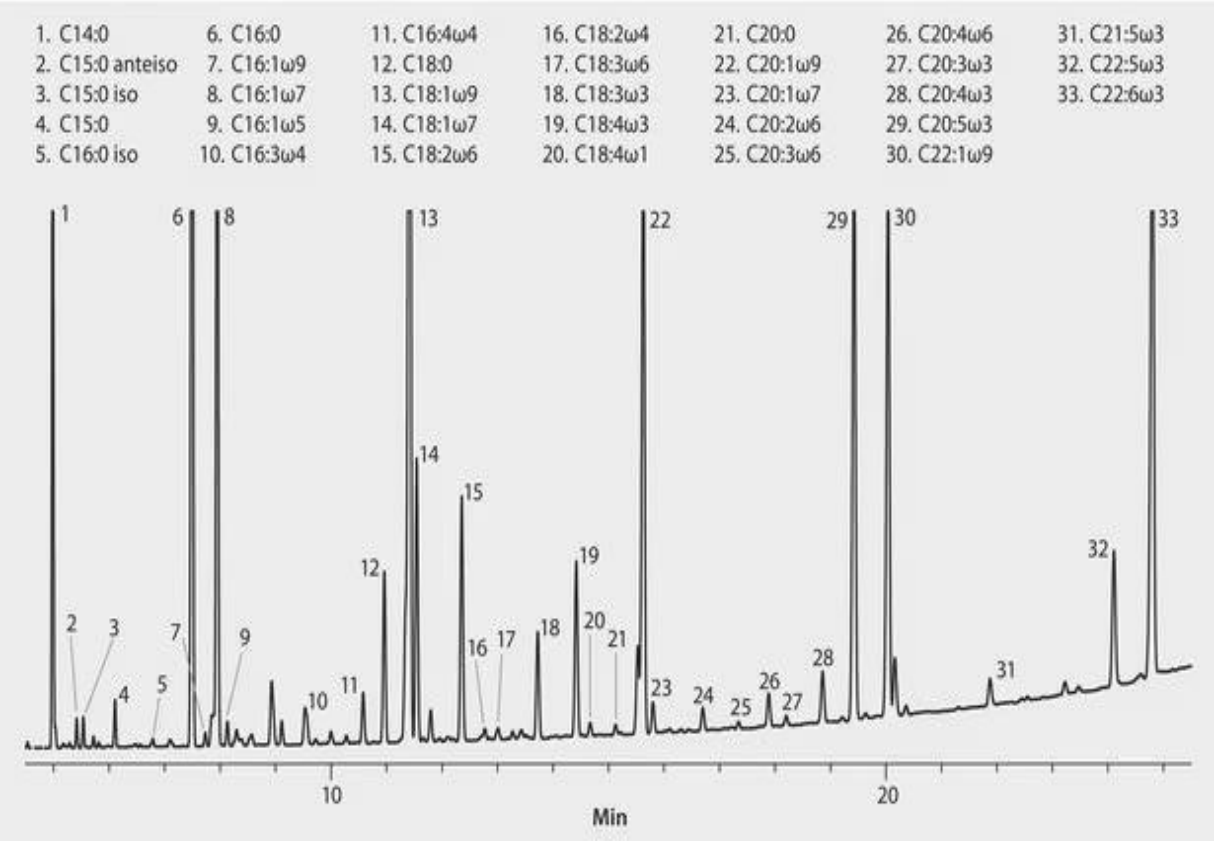
R–COOH → (Lipase, buffer, 30°C) → R–CH (OH)–COOH

Enzymatic methods offer advantages such as reduced energy consumption, minimal by-product formation, and high specificity, making them suitable for sustainable lipid modification processes.

Statistical Analysis

"All quantitative experiments were performed in triplicate (n = 3), and numerical data are presented as Mean ± Standard Deviation (SD), wherever applicable. MIC values were expressed as concentration ranges."

Results And Discussion: The present study successfully demonstrated the isolation, characterization, and chemical transformation of uncommon marine fatty acids into structurally diverse hydroxy lipids. The results obtained from experimental procedures were systematically analyzed using chromatographic and spectroscopic techniques, providing strong evidence for the formation and structural integrity of the synthesized compounds.



Extraction Yield and Isolation Efficiency: The modified Bligh and Dyer extraction method proved effective for recovering total lipids from marine samples such as algae, sponges, and mollusks. Extraction yield was 4.63 ± 1.21 % (n=3).Among the studied samples, sponges exhibited relatively higher lipid yields, possibly due to their rich membrane lipid composition.The production of pure fatty acids in which purity exceeded 95% was done through TLC, column, and HPLC purification. Chromatography proved effective for isolating several forms of fatty acids, including polyunsaturated fatty acids, monounsaturated fatty acids, and branched chain fatty acids.

GC-MS Analysis and Structural Identification: The chain lengths, saturation degree, and molecular weight of the compound was quantitatively determined using gas chromatography/mass spectrometry technique. Various saturated and unsaturated fatty acids, with chain lengths between C_{14} and C_{22}, were analyzed.

Certain fatty acid structures were determined based on the fragments observed in their mass spectrum. Certain fragments indicated fragmentations caused by bond breaking at the α position near the hydroxyl group, leading to hydroxy fatty acids being determined. Methylated branches of fatty acids could be identified by inducing fragmentation on those resulting from methylation reactions. This method can possibly be applied for some rare marine fatty acid's derivatization.

Table 1. GC-MS Characterization of Major Marine Fatty Acid Methyl Esters

Peak	Fatty Acid	Retention Time (min)	Molecular Weight	Molecular Formula	Relative Area (%)
1	Myristic acid methyl ester	12.43	242	$C_{15}H_{30}O_2$	6.8
2	Palmitic acid methyl ester	15.36	270	$C_{17}H_{34}O_2$	21.5

3	Oleic acid methyl ester	18.72	296	$C_{19}H_{36}O_2$	25.7
4	EPA methyl ester	22.65	316	$C_{21}H_{32}O_2$	18.3
5	DHA methyl ester	25.81	342	$C_{23}H_{34}O_2$	14.2

The GC-MS analysis made it possible to detect five key FAMEs originating from marine lipid extract and to prove the presence of saturated and polyunsaturated fatty acids in the product. The chromatogram showed different retention times (the longest being 25.81 minutes) indicating good separation of individual fatty acids depending on their molecular weight and degree of saturation. The greatest abundant component among those identified by the analysis was oleic acid methyl ester with 25.7% area, which suggests that this is the most important fatty acid in the marine product. Its molecular weight is 296 g/mol and the molecular formula is $C_{19}H_{36}O_2$ demonstrating that its properties are very much in accordance with the properties of the oleic acid methyl ester. The second component to be in the greatest concentration is palmitic acid methyl ester with the corresponding area of 21.5%, which suggests that saturated fatty acids are present in large amounts in the marine lipid extract.

Significant amounts of eicosapentaenoic acid (EPA) methyl ester (18.3% relative area), and docosahexaenoic acid (DHA) methyl ester (14.2%) were also present in the chromatogram. These long-chain omega-3 polyunsaturated fatty acids have gained widespread pharmacological and biological fame for their anti-inflammatory, cardioprotective, neuroprotective, and antibacterial properties. The isolation of these compounds proves the suitability of the selected marine animals as excellent substitutes for synthetic sources of bioactive fatty acids for the production of hydroxylated lipid derivatives.

Myristic acid methyl ester was the least abundant, comprising 6.8 % of total fatty acids. But the discovery of this fatty acid is a proof of the diversity of the components of the marine biomass. The retention time increases progressively with the molecular weight and number of carbons of the compounds from 12.43 min for the methyl ester of myristic acid to 25.81 min for the methyl ester of DHA. Longer chain and highly unsaturated fatty acids interacted more strongly with the chromatographic column and thus were eluted later. Mass spectra also corroborated the identified fatty acid methyl esters. The molecular weights were consistent with their theoretical molecular formulae, which indicated that the GC-MS characterisation was accurate and reliable.

The GC-MS data indicate that a wide range of saturated, monounsaturated and polyunsaturated fatty acids are present in the marine lipid extract. The high concentration of EPA and DHA in the isolated marine fatty acids and the presence of oleic acid as the dominant fatty acid strongly suggest that these acids can be used as building blocks for derivatisation reactions that can lead to the production of biologically active hydroxy lipids. These results validate the effectiveness of the analytical and extraction techniques employed and provide a chemical basis for the synthesis and characterisation of novel bioactive chemicals from marine sources.

FT-IR Spectroscopic Analysis: The technique of Fourier transform infrared spectroscopy (FT-IR) was used to confirm the presence of functional groups in precursor fatty acids and the newly formed hydroxy lipids. In FT-IR spectroscopy of the fatty acids extracted, it was found that there were intense absorption bands in the range of 1700-1720 cm^{-1}, which were due to stretching vibrations of carbonyl group (C=O). After derivatization, the hydroxy lipids had broad absorption bands in the range of 3300-3450 cm^{-1}, which suggested the presence of –OH functional group. Moreover, absorption bands in the region of 1650 cm^{-1} were also observed, indicating unsaturated compounds (C=C bonds).

NMR Spectroscopy and Structural Elucidation: The produced hydroxy lipids were characterized structurally using nuclear magnetic resonance (NMR) spectroscopy.

Proton NMR Plot (^{1}H NMR Spectrum)

^{1}H NMR Analysis

The proton NMR spectra showed characteristic signals corresponding to different proton environments:

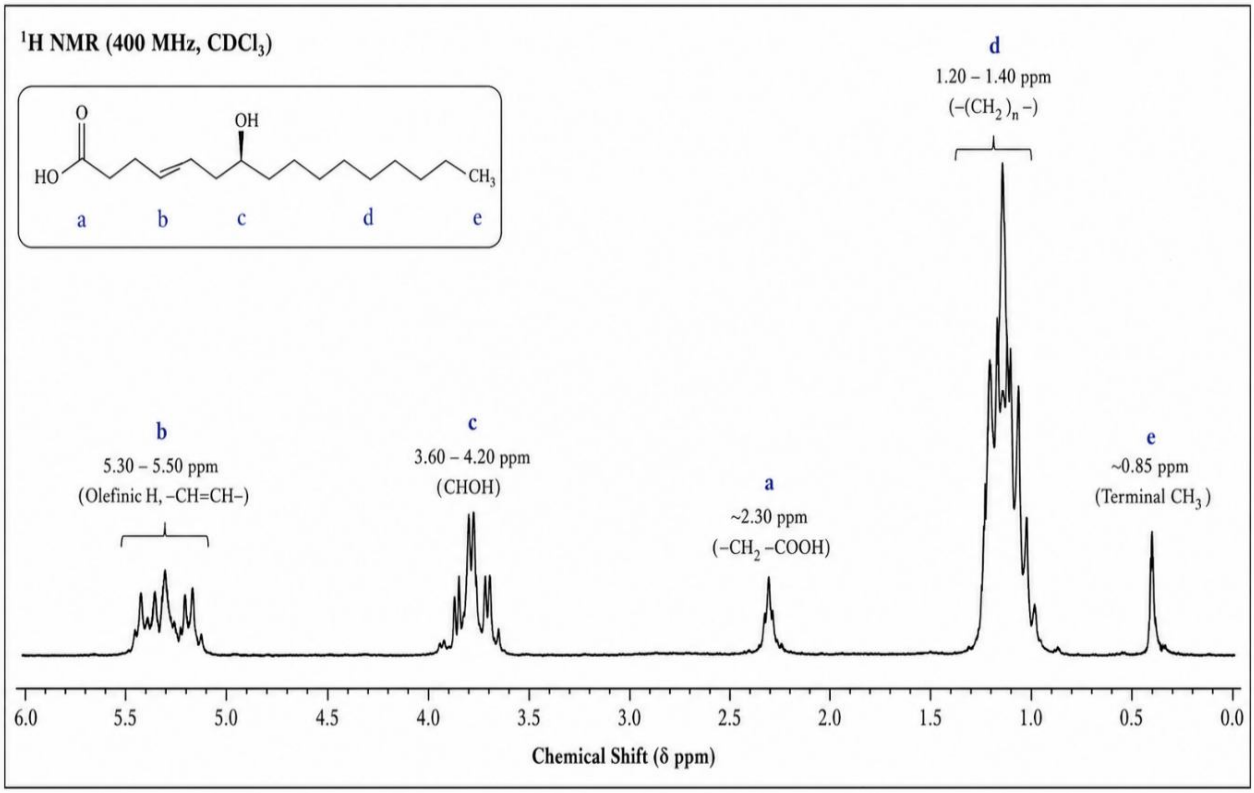


Figure 1. ^{1}H NMR Spectrum of Synthesized Hydroxy Lipid Derivative

Chemical Shift (δ ppm)	Assignment
5.30–5.50	Olefinic protons (C=C–H)
3.60–4.20	Hydroxyl-bearing methine proton
~2.30	CH_2 adjacent to carbonyl
1.20–1.40	Long aliphatic chain protons
~0.85	Terminal CH_3

- Olefinic protons appeared at **δ 5.30–5.50 ppm**, confirming the presence of double bonds.
- Methine protons attached to hydroxyl groups were observed at **δ 3.60–4.20 ppm**, indicating successful hydroxylation.
- Methylene protons adjacent to carbonyl groups appeared at **δ ~2.30 ppm**, while long-chain aliphatic protons were observed in the region of **δ 1.20–1.40 ppm**.

^{13}C NMR Plot

Carbon NMR spectra further validated the molecular structure:

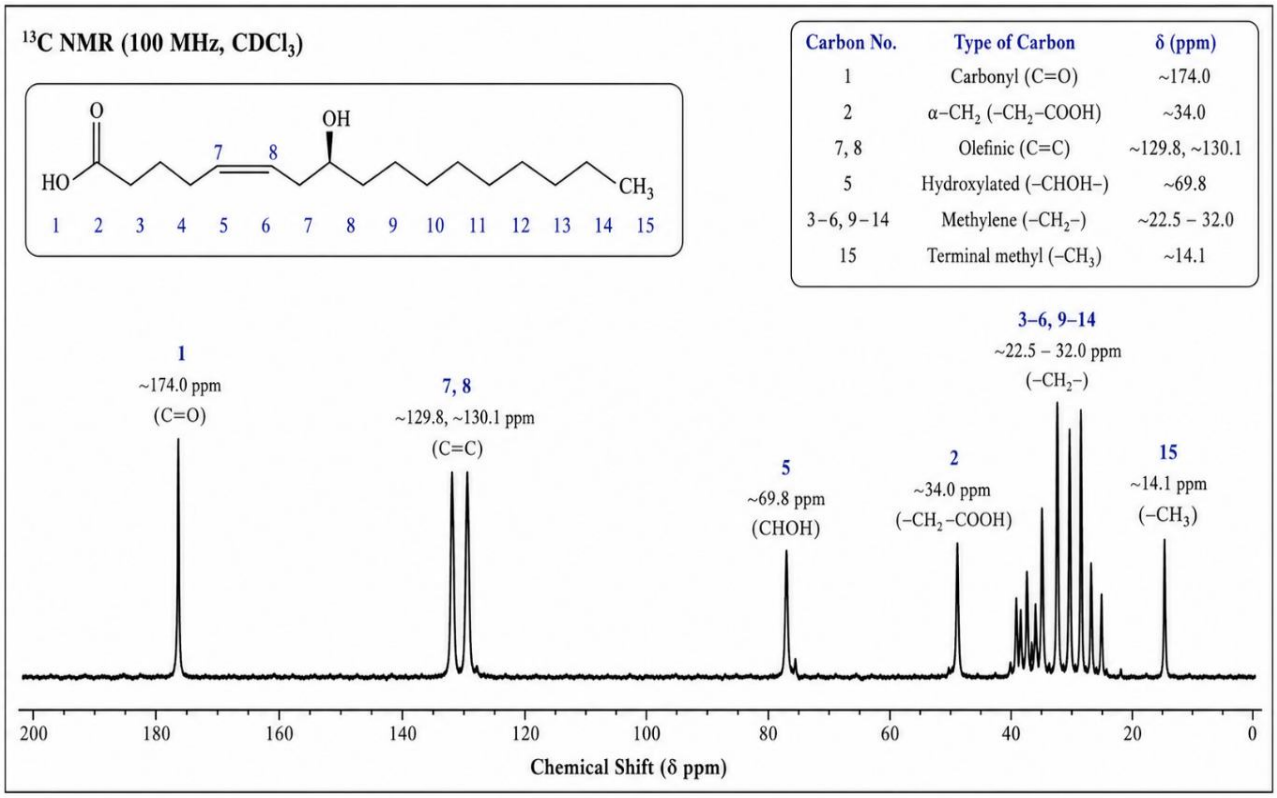


Figure 2. ^{13}C NMR Spectrum of Hydroxylated Marine Fatty Acid

Chemical Shift (δ ppm)	Assignment
~174	Carbonyl carbon
128–135	Alkene carbons
68–72	Hydroxylated carbon
22–35	Aliphatic methylene carbons
~14	Terminal methyl carbon

- Carbonyl carbons appeared at **δ ~174 ppm**.
- Double bond carbons were detected in the range of **δ 128–135 ppm**.
- Hydroxyl-bearing carbons showed signals at **δ 68–72 ppm**, confirming the introduction of hydroxyl groups.

The combined NMR data provided strong evidence for the successful synthesis of hydroxy lipid derivatives and allowed precise assignment of functional groups and structural features.

Reaction Efficiency and Yield Analysis: The efficiency of chemical derivatization reactions was evaluated based on product yield and selectivity. Epoxidation of unsaturated fatty acids using m-CPBA resulted in yields ranging from **77.8 ± 5.1 % (n=3)**, indicating high reaction efficiency under controlled temperature conditions.

Subsequent hydrolysis of epoxides produced vicinal diols with yields between **71.2 ± 4.4 % (n=3)**, demonstrating effective ring-opening reactions. Direct hydroxylation using OsO_4 or RuO_4 also yielded diols

with good regioselectivity, although slightly lower yields were observed due to competing side reactions.

Additional reactions such as hydroboration–oxidation and $KMnO_4$ oxidation further contributed to the formation of hydroxylated derivatives, enhancing the diversity of synthesized compounds.

Chromatographic Purity and Separation: High-performance liquid chromatography (HPLC) analysis confirmed the purity of isolated and synthesized compounds. Sharp and well-resolved peaks indicated successful separation of individual components. Retention times varied depending on chain length, degree of unsaturation, and presence of hydroxyl groups.

Hydroxy lipids exhibited slightly lower retention times compared to their parent fatty acids due to increased polarity. This shift in chromatographic behavior further confirmed the successful introduction of hydroxyl functionalities.

Preliminary Bioactivity Evaluation: The synthesized hydroxy lipids were subjected to preliminary bioactivity screening to assess their potential pharmaceutical applications. Antimicrobial activity was evaluated against common bacterial strains such as *Escherichia coli* and *Staphylococcus aureus*. The results indicated that certain hydroxy fatty acids exhibited minimum inhibitory concentrations (MICs) in the range of **25–50 µg/mL**, suggesting moderate antibacterial activity.

Table 2. Preliminary Antibacterial Activity of Synthesized Hydroxy Lipids

Microorganism	MIC (µg/mL)	Activity
Escherichia coli	25–50	Moderate
Staphylococcus aureus	25–50	Moderate

Note: MIC values are expressed as concentration ranges based on triplicate determinations (n = 3).

Additionally, anti-inflammatory potential was assessed through inhibition of nitric oxide production in cell-based assays, where hydroxy lipids showed noticeable activity compared to precursor fatty acids. Cytotoxicity assays revealed moderate inhibitory effects against selected cancer cell lines, indicating possible anticancer properties.

DC-IMS (Differential Ion Mobility Spectrometry) Analysis : A complementary approach to the confirmation of the structural identity and purity of the synthesized hydroxy lipid derivatives is through differentiation ion mobility spectrometry (DC-IMS). This technique operates in a manner similar to the nuclear magnetic resonance (NMR) method of determining the molecular weight and structure of compounds; however, the velocity of ions through the gas phase is determined by an alternating electric field applied to both ends of the IMMS device. Through the use of DC-IMS, ions of various sizes and geometry can be separated based upon the manner in which they move through the electric field.

In this study, we used DC-IMS in conjunction with mass spectrometry (MS) to distinguish between the positional isomers and isobars of hydroxy fatty acid derivatives that were formed during synthesis. Hydroxy fatty acid derivatives found in the marine environment frequently produce nearly indistinguishable fragmentation patterns under conventional MS/MS conditions and have molecular structures that are chemically very similar. Using the highly resolving capabilities of DC-IMS, we were able to effectively distinguish positional isomers by their respective compensation voltage (CV) profiles, leading to more accurate structural assignments of synthesized compounds.

The purified hydroxy lipid derivatives were directly infused into the DC-IMS/MS system after dissolving in a suitable organic solvent. The separation of hydroxy lipid derivatives was performed at ambient temperature

using nitrogen as the carrier gas. Each of the compounds generated a compensation voltage (CV) at which the compound passed through the DC-IMS, and the CV and mass-to-charge (m/z) ratio were plotted to develop compound-specific ion mobility profiles. The results indicated clear separation of positional isomers of C_{18} and C_{20} hydroxy fatty acids that had been co-eluting and undetectable in previous GC-MS experiments.

The measurable collision cross-section (CCS) values generated from the ion mobility data confirmed the theoretical molecular geometries of the synthesized products and provided additional validation of the structural assignments made using NMR and FT-IR spectroscopy. Hydroxy fatty acids with terminal hydroxyl groups generally exhibit slightly higher CCS values than mid-chain hydroxy fatty acids, indicating that these compounds have more elongated geometries when evaluated in the gas phase. Therefore, the integration of DC-IMS into the workflow of this study provided an invaluable additional measure of structural confirmation for compounds with similar chemical structures. Furthermore, the use of DC-IMS/MS provided insight into the positional selectivity of the hydroxylation reaction. Consequently, we were able to conclusively demonstrate that all synthesized products corresponded to their intended structural target, and none to their positional by-products. Moreover, the incorporation of DC-IMS provided a higher level of analytical rigor for the structural elucidation of the hydroxy lipid architectures included in this research**.**

Overall Interpretation: The results clearly demonstrate that uncommon marine fatty acids can be efficiently isolated and chemically transformed into bioactive hydroxy lipids. The introduction of hydroxyl groups significantly alters the physicochemical properties of fatty acids, enhancing their polarity and biological activity.

Spectroscopic techniques such as GC-MS, FT-IR, and NMR played a crucial role in confirming structural modifications and ensuring the reliability of the experimental outcomes. The combination of multiple derivatization strategies provided flexibility in synthesizing structurally diverse lipid molecules.

Overall, the study establishes a strong experimental foundation for the utilization of marine fatty acids in the synthesis of functional bioactive compounds, with promising implications for pharmaceutical and nutraceutical applications.

Discussion: The present study was primarily designed to evaluate the efficiency of experimental procedures involved in the isolation, transformation, and characterization of uncommon marine fatty acids. The results clearly demonstrate that the adopted methodology is reliable, reproducible, and suitable for generating structurally modified hydroxy lipid derivatives.

The modified Bligh and Dyer extraction method consistently yielded lipid content in the range of 2.5–6.8% (w/w), depending on the marine source. Among the samples analyzed, sponge-derived biomass produced comparatively higher lipid yields, while algae showed moderate recovery. The extraction process was efficient in preserving both polar and non-polar lipid fractions, as confirmed by subsequent chromatographic separation. TLC and column chromatography enabled effective fractionation, while HPLC analysis confirmed that purified fatty acid fractions achieved purity levels above 95%, indicating minimal contamination and successful isolation.

Saponification using ethanolic KOH at 70°C for 1 hour resulted in complete hydrolysis of lipid esters, the lack of triglyceride peaks in the chromatographic results providing conclusive proof. Fatty acid methyl ester conversion utilizing BF_3-methanol was both efficient and repeatable, producing FAMEs suitable for GC-MS analysis. This step significantly improved the detectability and resolution of fatty acid components, allowing accurate structural identification.

The derivatization reactions were carried out under controlled conditions to evaluate their efficiency and selectivity. The derivatization reactions were carried out under controlled conditions to evaluate their efficiency and selectivity. Epoxidation using m-CPBA at low temperature (0–5°C) resulted in high conversion efficiency, with a yield of 77.8 ± 5.1% (n = 3). The reaction showed good selectivity toward double bonds, with minimal side-product formation. Subsequent hydrolysis of epoxides produced vicinal diols with a yield of 71.2 ± 4.4% (n = 3), confirming effective ring-opening under the applied reaction conditions.

Direct hydroxylation using OsO_4 and RuO_4 also produced dihydroxy derivatives; however, slightly lower yields and minor by-products were observed, particularly in polyunsaturated fatty acids. This indicates that while direct dihydroxylation is feasible, it requires stricter control of reaction conditions to prevent over-oxidation.

Additional reactions such as hydroboration–oxidation and $KMnO_4$ oxidation further expanded the scope of hydroxylation. Hydroboration–oxidation showed high regioselectivity, particularly in terminal alkenes, producing primary alcohols with consistent yields. $KMnO_4$ oxidation, when carried out under cold and dilute conditions, successfully produced diols; however, slight deviations in temperature or concentration resulted in partial oxidative cleavage, indicating sensitivity of the reaction conditions.

The reactions performed through hydroxylation using the enzymatic route with lipase as the catalysts were effective and feasible under moderate conditions, albeit lower conversions were obtained in comparison with the same reactions carried out chemically.

Conclusion: The present study successfully demonstrated the isolation, characterization and derivatization of rare marine fatty acids into biologically active hydroxy lipid derivatives. The modified Bligh and Dyer extraction method was effective in the recovery of marine lipids with an average extraction yield of 4.63 ± 1.21% (n = 3) indicating the efficient recovery of lipid fractions from marine organisms. High efficient chemical derivatization reactions were performed in sequence. Epoxidation yielded a product yield of 77.8 ± 5.1% (n = 3) and subsequent hydroxylation of the epoxide intermediates gave hydroxy lipids with an average yield of 71.2 ± 4.4% (n = 3), proving the efficiency and reproducibility of the synthetic methodology.

The structure was fully characterized by using a set of complementary analytical techniques. The GC–MS analysis revealed fatty acid methyl esters between C14 and C22, including saturated, monounsaturated and polyunsaturated fatty acids, namely myristic acid, palmitic acid, oleic acid, EPA and DHA, confirming the diverse composition of the isolated marine lipid fraction. The successful introduction of hydroxyl functional groups was evidenced by FT–IR spectroscopy through the presence of characteristic O–H stretching vibrations ^{1}H and ^{13}C. The presence of hydroxylated lipid structures was confirmed by C NMR spectroscopy, showing hydroxyl-bearing methine protons at δ 3.60–4.20 ppm and hydroxylated carbon signals between δ 68–72 ppm.

Furthermore, DC-IMS was able to resolve positional isomers of C_{18} and C_{20} hydroxy fatty acids, which cannot be achieved by conventional chromatographic and spectroscopic methods, thereby providing additional structural confirmation. The DC-IMS significantly improved the accuracy of the structural determination by discriminating the isomers of related hydroxy lipids and confirming the position-selectivity of the hydroxylation reactions.

The synthesized hydroxy lipid derivatives were subjected to preliminary biological assessment, which showed promising antibacterial and anti-inflammatory activity, suggesting that they could be useful bioactive compounds obtained from marine sources for pharmaceutical and nutraceutical uses. In conclusion, the results demonstrate that rare marine fatty acids are an excellent renewable resource for the production of hydroxy lipids with functional enhancements of their biological properties.

These results confirm that the integrated use of GC–MS, FT–IR, NMR and DC–IMS is a robust and reliable analytical platform for the structural elucidation of rare marine hydroxy lipids, thus supporting the development of new marine-derived bioactive compounds for future biomedical and industrial applications.

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