SUMMARY
In the present study, untapped aquatic and terrestrial sources of animal and plant origin, have been exploited for screening of active constituents of lipids with special reference to fatty and defatty components.

The thesis contains following chapters:

CHAPTER I: An introduction about the work with particular reference to definition, distribution, types, functions and structures of lipids. The current trend of work in the field, the scope and objective of the present study along with a brief account of the work done by others has been given.

Lipids are characterised by molecular heterogeneity and are involved in many vital processes in animals, plants and microorganisms. These are defined as fatty acids and their derivatives and substances related biosynthetically or functionally to these compounds. Lipids are required for insulation, water-proofing, detergency, lubrication and other interfacial phenomenon. In marine mammals these are used to adjust buoyancy and serve as constituents of sonar lens. Mobilization of reserve lipid and protein play an important role in the removal of embryonal dormancy.

Simple lipids on hydrolysis yield alcohol and fatty acids, whereas complex lipids on hydrolysis liberate alcohol, fatty acids, phosphoric acid and carbohydrates.

Fatty acids are monocarboxylic, straight, unbranched chain containing an even number of carbon atoms (between 2 to 36). These may be saturated or unsaturated and sometimes hydroxylated or branched. Triacylglycerols (TAG) being nonpolar in nature are neutral type of lipids. Monoglycerides and diglycerides are important metabolic intermediates. Steroids are a group of lipids derived from cyclopentanoperhydrophenanthrene. Terpenoids act as an intermediates in the synthesis of steroids. The phosphoglycerides include all derivatives of glycerophosphoric acid containing at least one o-acyl or related
group. The galactolipids - monogalactosyl and digalactosyl diglycerides are the plant fats - where diacylglycerols are joined by a glycoside linkage through position 3 to sugar moieties.

Plants are able to synthesize the needed unsaturated fatty acids, which are all incorporated in the phospholipids, which in turn are used for membrane building. The specific physical properties of the membranes is due to the chain length and the degree of unsaturation of the fatty acids. Linoleic and linolenic acids which are not synthesised by several groups of animals are called essential fatty acids.

Comprehensive reviews are available on the occurrence, chemistry, biochemistry and biotechnology of lipids\textsuperscript{8-11} including phospholipids\textsuperscript{12} and glycolipids\textsuperscript{13,14}.

**CHAPTER II :** Methods used in the study of test materials. The methodology include : material identification, extraction, separation and composition determination using chromatographic and spectroscopic methods.

**Extraction :**

For extraction of lipids - hexane or chloroform:methanol is commonly used - depending on the predominance of neutral or polar lipids. There are methods of specialized extraction. Continuous extraction with hot solvents is accomplished by Soxhlet extraction. A short heat treatment is done to inactivate the lipolytic enzymes before homogenization\textsuperscript{15,16}. Precaution to minimize oxidation include the use of peroxide free solvents, shielding from light to prevent photooxidation and photoisomerization and the use of nitrogen to evaporate solvents.
Characterization: Chromatographic methods

By selection of a column of adsorbent - isolation of animal\(^{17, 18}\), plant\(^{19}\) and bacterial\(^{20}\) lipid fractions can be achieved. Complex lipids can be separated on silica gel\(^{21}\).

For thin layer chromatography (TLC) a wide range of functional group specific reagents are available\(^{22-25}\). Quantitation can be achieved by densitometry\(^{26, 27}\). TLC flame ionization detection (TLC-FID) in which TLC is carried out on silica gel fused to quartz rod and this passed through a FID can also be used\(^{28}\). Affinity chromatography\(^{29, 30}\) has been used for the analysis of biochemical molecules.

High Performance Liquid Chromatography (HPLC) has no limitations of thermal stability and is used for separation of molecular species of seed oil triacylglycerols\(^{31}\).

In gas liquid chromatography (GLC) due to non specificity of flame ionization detector it is widely used for lipid analysis. With the commercial availability of fused silica capillary columns there has been a rise in the use of capillary gas chromatography\(^{32, 33}\). In recent studies capillary gas liquid chromatography has been used for the separation of phospholipids\(^{34}\) and phytosterols\(^{35}\).

Spectroscopic Methods

Spectroscopy of fatty acids has been reviewed by Gunstone\(^{36, 37}\).

The work on Mass Spectroscopy of acyl lipids is dominated by fatty acid analysis\(^{38, 39}\). Dommes\(^{40}\) suggests trimethylsilyl ether derivatives as the best derivatives for the analysis of polyunsaturated fatty acids. Direct determination of phospholipid structures in microorganism have been reported by fast atom bombardment triple quadruple mass spectrometry\(^{41}\). Combination of LCMS
and LCNMR has been used as a tool for the structural determination\textsuperscript{42} of natural products.

For lipids the most commonly studied nuclei in Nuclear Magnetic Resonance (NMR) are \(^1\)H and \(^{13}\)C. The structure and stereochemistry of the glycosides of fatty alcohols and sterols have been determined by \(^1\)H NMR\textsuperscript{43}.

The application of Ultraviolet spectroscopy are limited though it has extensive use in detection of lipid classes separated by HPLC\textsuperscript{44}.

Infrared detection has been used in commercial shortenings and in product of partial hydrolysis of seed oils\textsuperscript{45}.

Degradative Methods

These constitute analysis of constituent moieties of complex lipid moieties degradation of acyl chain\textsuperscript{46} and stereospecific analysis\textsuperscript{47,49}.

Studies on Active Principles of Lipids From Aquatic Sources

CHAPTER III A : Studies on \textit{Labeo rohita} Lipids

The potential of various fresh water fish species as sources of low fat, high protein food is being realised\textsuperscript{50}. The polyunsaturated fatty acids particularly of n-3 family are known to be more in the lipids of aquatic animals than terrestrial ones. These shows a beneficial effect against heart diseases\textsuperscript{51}. Of the various components that affect edible quality attributes, the lipids are most important.

In the literature there are reports on fatty acids of \textit{Anaplopoma fimbria}\textsuperscript{52,53} and \textit{Scomber scombrus}\textsuperscript{54,55}. Wolfe\textsuperscript{56} studied the fatty composition of Cray fish lipids. Kinsella\textsuperscript{57} that of fin fish and Saha\textsuperscript{58} of \textit{Hilsa ilisa}. Low temperature deodorizations of fish oils with volatile acidic and basic steam sources have been reported\textsuperscript{59}. Recently the National Marine Fisheries Service
of America has started producing encapsulated fish oil derivatives for use in biomedical research.\(^{69}\)

Fatty acid analysis, total protein content and subsequent amino acid profile from *Labeo rohita* fillets and effect of smoking (used for preservation) on the proximate composition and lipid stability has been studied in this laboratory.\(^{60}\) A branched chain fatty acid has also been characterised from the non urea complex fraction in our laboratory.\(^{61}\)

The present work involves:

**CHAPTER III A(i) : Rapid determination of polyunsaturated fatty acid by \(^1\)H NMR**

In the analysis of n-3 fatty acids by GLC artifacts are formed during transmethylation step. In this method, which is quick, the difference of chemical shift observed for methyl resonance of n-3 polyunsaturated fatty acid with respect to methyl resonance of all other fatty acids is the basis of determination.

**CHAPTER III A (ii) : Increase in polyunsaturated fatty acid content**

Keeping in view the vital role of polyunsaturated fatty acids, enrichment of this content from the oil was attempted by treating it with lipase, to which the esters involving polyunsaturated fatty acyl groups are stable whereas the once with nonpolyunsaturated fatty acyl groups are not. Thus the esters of nonpolyunsaturated fatty acids get hydrolysed (lipase destroys these) resulting in increase of polyunsaturated fatty acid content.

**CHAPTER IIIA (iii) : Positional distribution of n-3 fatty acids by \(^{13}\)C NMR**

\(^{13}\)C NMR studies the lipid mixture noninvasibly. The positional distribution of polyunsaturated fatty acids have been attempted from the spectrum of carbonyl carbon, methylene carbon and glycerol carbon regions.
CHAPTER IIIB : Polyunsaturated fatty acids from *Achlya ambisexualis*

Microbial production of polyunsaturated fatty acids particularly eicosapentaenoic acid and docosahexaenoic acid - due to their effectiveness in blood cholesterol reduction and in functioning of eye, is gaining importance\(^\text{62,63}\). Eicosapentaenoic acid has earlier been detected from a fungus *Rhizopus oryzae* in our laboratory\(^\text{64}\). This prompted us to screen the aquatic fungus *Achlya ambisexualis*. Docosapentaenoic and Docosahexaenoic acids have been isolated from the fungus. Looking to the specific function of these acids - the finding of a new source gains significance.

**Studies on Active Principles of Lipids from Terrestrial Sources**

CHAPTER IV : Studies involving seed fats

CHAPTER IV A : Preparation and identification of derivatized unusual fatty acids from seed oil of *Artemisia vestita*

Genus *Artemisia* (family Compositae) has attracted the attention of various workers for a longtime\(^\text{65-69}\). The genus comprises of 280 species of which 34 are found in Jammu and Kashmir. *Artemisia vestita* grows abundantly in Srinagar at 5000 ft and is a shrub with fern like structure.

Literature survey reveals work on acetylenic hydrocarbon\(^\text{70,71}\), phenolic constituents\(^\text{72}\), ketone synthesis\(^\text{66,73}\), ether\(^\text{74,75}\), monoterpenes\(^\text{76,77}\), flavonoids\(^\text{78}\) and essential oils\(^\text{69,79-81}\).

The seed fat of *Artemisia vestita* has been earlier analysed in our laboratory\(^\text{82}\). Epoxyoctadecenoic acid has been identified in the present study from the seed oil. The epoxyoctadecenoic acid rich oil has been used to prepare following derivatives:
CHAPTER IVA (i) : Preparation of Epoxy amide (Butyl epoxyoctadecenamide)

Fatty amides are important chemical intermediates for commence with applications in paper coatings, print ink and additives for polyethylene films. In the present work the epoxy rich fraction of the seed oil has been converted into an oleochemical-epoxy amide by direct amidation, with retention of the epoxy group.

The formation of the derivative prepared by reacting epoxy rich fraction of the seed oil with butylamine has been confirmed by elemental analysis followed by $^{1}$H NMR and mass spectrometry.

CHAPTER IVA (ii) : Preparation of Aminoundecanoic acid

Epoxydised triglyceride fatty acids find use for synthesis of chemical intermediates e.g. resins and plasticizers. The intermediates are dimensional stable and vibration resistant and find utility in fabrics and electrical sports equipments.

The aminoundecanoic acid was prepared from epoxy rich fraction of the seed oil. The reaction was carried under several steps and the end formation of the product was confirmed by $^{13}$C NMR and mass spectrometry.

CHAPTER IV B : Studies on derivatized unusual fatty acid from Wrightia tinctoria

Wrightia tinctoria is a small deciduous shrubs distributed in Madhya Pradesh and Rajasthan. The material was collected locally and authenticated at the Botany Department of this University. The seed fat has been studied in our laboratory.
In the present study hydroxy-octadecenoic acid has been identified from the seed oil. The hydroxy-octadecenoic acid rich oil has been used to prepare following derivatives:

CHAPTER IVB (i) : Preparation of amide (Butyl hydroxy-octadecenamide)

In the present work, the unusual fatty acid (hydroxy octadecenoic acid) containing oil has been directly subjected to amidation because of importance of fatty amide derivative as intermediates for coating and print ink additives. The amide has been separated from other formed products by silica gel partitioning.

The formation of the derivative prepared by reacting epoxy rich fraction of the seed oil with butyl amine has been confirmed by Elemental analysis, TLC, IR and NMR spectrometry.

CHAPTER IVB (ii) : Preparation of methyl octadecadienoate

Octadecadienoic acid has been reported to have anticarcinogenic and anticholesterolemic properties. Attempt has been made to synthesise it from methyl hydroxy-octadecenoate. The formation of the product (methyl octadecadienoate) was confirmed by Mass Spectrometry.

These syntheses have potential application in that the starting material is a raw material of a plant origin and the reaction sequence is simple.

CHAPTER V : Miscellaneous Studies Involving Lipids

CHAPTER VA : Composition of acid water from seed oil soapstock of Buchanania lanza

Soapstock is formed during refining - a process in edible oil industry. Due to high fat and protein content, presence of essential fatty acid, amino acids and possible edible attributes based on nutritional evaluation, the seed
oil of *Buchanania lanzan* was chosen for the study. Acidification of soapstock separates acid water. The present study aims at its composition which may give idea about the left behind components after removal of high concentration materials during refining. The components (phosphate compounds, phosphoric acid, inositol, lactic acid, glycerol) have been identified by HPLC.

CHAPTER V B (i) : Studies on nontraditional seeds of *Boswellia serrata*

The shortage of oil production for human consumption as well as industrial purposes, has prompted to find alternatives to conventional sources of edible oils as well as bringing in of non edible oils for industrial purposes, which could save an appreciable amount of edible oils that are used for non edible purposes. The forest of Madhya Pradesh are rich sources of plant raw materials. The fatty acid compositions of the seed oils of commercial importance have been reviewed\(^9\). Utilisation of the full potential of minor oil seeds of tree origin, and byproducts including marine sources, has been included in discussion of India's edible oil deficits and possible remedies\(^99-102\). The minor oil seeds belong to less cultivated category of species and serve as nontraditional sources of vegetable oils. The main objectives of the screening programme is to determine what amounts and general classes of fatty acids are contained in different seed oils.

Genus *Boswellia serrata* (Family Burseraceae) is a large branching tree mainly found in dry hilly areas. The plant was collected in Nowrozabad (Shahdol) and authenticated at the Botany Department of this University (Herbarium No. B\(_{15}\))\(^{103}\).

In the present study the fatty acid composition of seed of *Boswellia serrata* has been identified by GC and Mass Spectrometry. The unusual acid has been identified to be saturated branched (14 methyl 16:0) fatty acid. Another acid with retention time of 10.4 minutes and molecular ion m/z 371 has been found to be present along with traces of long chain saturated fatty acids.
CHAPTER V B (ii) : Analysis of unsaponifiable matter

The unsaponifiable matter of the oil, idented by HPLC and GC, contains wax esters and stigmastadiene.

CHAPTER V C (i) : Analysis of lipase treated C_{18:0} solid glyceride by HPLC

Enzymatic modification of a triglyceride requires the use of a method of analysis - capable of detecting and quantitating the enzyme mediated products. The progress of lipolysis of triolein has earlier been studied in this laboratory\textsuperscript{104}. The earlier study was monitored by TLC. In the present study HPLC has been used and monostearin followed by stearic acid distearin and tristearin have been separated. Tristearin was found to have high and stearic acid low response.

CHAPTER V C (ii) : Analysis of C_{18:1} isomer acids by GLC

Isomeric fatty acids affect human lipoprotein composition\textsuperscript{105} and subsequently lead to coronary problems\textsuperscript{106,107}. Usually the fatty acid (as their methyl esters) are separated by GLC with polar phases wherein the separation between cis and trans is not complete\textsuperscript{108}. In the present study DMOX derivatization of fatty acids give better results than methyl esters in separating 18-carbon trans and cis isomers by GLC.
REFERENCES


