International Journal of
Life science & Pharma Research

PHARMACOLOGICALLY ACTIVE α-LINOLENIC ACID (ALA, 18:3ω3), THE PRIMARY PRECURSOR MOLECULE FOR – ω3 SERIES OF POLYUNSATURATED FATTY ACID(PUFA) FROM MANGROVE ECOSYSTEM

TAPAS KUMAR DAS[1], SAMARESH SAMANTA[1], SUBHASISH CHATTERJEE[1] ANITA GHOSH[2] AND SUSANTA KUMAR CHAKRABORTY[1]*

1Department of Zoology, Vidyasagar University, Midnapore (West), 721102 West Bengal, India.
2Secretary, Drug Research & Development Centre, Kolkata 700028

ABSTRACT

The fatty acid compositions of the three selected mangrove plant leaves and detritus of the leaves have been studied by gas liquid chromatography and other chromatographic techniques. Altogether 25 components were detected and estimated. Major fatty acids recorded were palmitic (16:0), stearic (18:0), oleic (18:1ω9), linoleic (18:2ω6) and α-linolenic (18:3ω3). Of the unsaturated fatty acids, considerably higher levels of pharmacologically active fatty acids, viz. linoleic and α-linolenic acids were recorded. The α-linolenic acid is incorporated into cell membranes, promotes the health of blood vessels and is converted to long-chain omega-3 fatty acids. Such findings have suggested that these plant species studied are also a potential source of pharmacologically active α-linolenic acid, the primary precursor molecule for – ω3 family of fatty acids in animal tissues.

Key words:α-linolenic acid,unsaturated fatty acid,G.L.C mangrove.

1. INTRODUCTION

Biochemical studies on three selected species of mangrove Plants, viz. *Avicennia marina* (Family-Avicenniaceae), *Acanthus ilicifolius* (Family-Acanthaceae) and *Suaeda maritima* (Family-Chenopodiaceae) and mangrove leaves derived detritus is necessary for evaluating their nutritional value as well as their possibility of future usage as natural sources for biologically active components. α-linolenic acid (ALA) is the parent compound of the omega-3 fatty acid family. It must be obtained from our diets because our bodies do not make it(Breanne *et al* 2009). ALA has important roles in human health. It reduces inflammation,. It incorporates into the cell membranes andpromotes the health of blood vessels and is converted to long-chain omega-3 fatty acids(Morris, 2008). ALA has important biologic effects and helps prevent and manage chronic diseases like heart disease, stroke, type 2 diabetes, kidney disease and certain types of cancer(Morris, 2007) ALA helps to promote the proper functioning of blood vessels, which reduces the risk of heart attacks and stroke(Nestel, *et al*.1997). ALA constitutes 75-80% of total omega-3 fatty acids in breast milk, underscoring its importance for infant growth and development(Ratnayake, 1996 and Innis, 2000). ALA is also required for maintaining the nervous system. A deficiency of ALA in humans causes

poor growth, numbness, pain in the legs, difficulty walking and blurred vision(Holman, *et al.*1982). These deficiency symptoms can be alleviated by adding ALA to the diet(Morris, 2007). ALA converts to long-chain omega-3 fatty acids, particularly EPA and docosapentaenoic acid (DPA)(Burdge,2006). Previous studies made from the Sundarban mangrove ecosystem, India, on the sterols and fatty acids from three species of mangrove by a group of researchers (Misra, *et al.* 1984), has shown a rich source of α-linolenic acid . Hydrocarbons and wax esters from seven species of mangrove leaves by (Misra, *et al.*1987) also supported the previous result. Edible wild plants provide α-linolenic acid (ALA) and higher amounts of vitamin E and vitamin C than cultivated plants. In addition to the antioxidant vitamins, edible wild plants are rich in phenols and other compounds that increase their antioxidant capacity(Simopouls, 2004). In the present study, efforts have been made to determine various lipid components of these selected plant species which are very common and abundant in intertidal belt of the coastal mangrove estuarine complex of West Bengal(Chakraborty , 2011) and detritus from the selected study area of Midnapur (East) costal belt, West Bengal, in between the Latitude 21°47′(N) and Longitudes 87°45′(E) with special reference to the fatty acid profile of the lipid.

2. MATERIALS AND METHODS

The mangrove plant leaves and detritus were collected from the coastal belt of Purusattampur (Dadanpatrabar), Midnapur(East), West Bengal (Latitude 21°47′N and longitudes 87°45′E) . The plant leaves and detritus were immediately frozen and stored at -20°c until analyzed.

2.1 Extraction of Lipids
The total lipids were extracted from the samples following the method of Bligh and Dyer (1959)using methanol chloroform (2:1,v/v), methanol- chloroform – water (2:1:0.8, v/v/v), and then again extracted with the first solvent system. Samples were grounded with the solvent, in a high speed homogenizer, filtered and residues were extracted with, the next solvent system. The process

was repeated. Finally, the three extracts were pooled, diluted with water and layer was allowed to separate in a separating funnel. The chloroform layer at the bottom was withdrawn and dried over anhydrous sodium sulphate in a freezer. The chloroform solution of lipid was evaporated under vacuum, redissolved in distilled n-hexane and kept at -20°C for future use. BHT (Butylated Hydroxy Toluene) was added at a level of 100mg/L to the solvent as antioxidant. After dilution of the pooled extracts, a heavy white precipitate appeared at the junction of the two layers which were kept for further analysis.

2.2 Preparation of Methyl Esters of Fatty Acids
Total lipids were transformed into methyl esters by trans-methylation. The samples were dissolved in anhydrous methanol containing concentrated Sulfuric acid (1.0%, v/v) and the mixture were refluxed (Christie, 1982) for 2 hours. Methanol was evaporated to a small volume and cooled. Distilled water was added to the cooled mixture and the methy1 esters of Fatty acids were extracted 3 times with aliquots of diethy1 ether. The ethereal extracts were pooled and dried over anhydrous sodium sulfate, filtered, vacuum dried, dissolved in n-hexane and kept in a freezer for further use.

2.3 Purification of Fatty Acids Methyl Esters By Thin Layer Chromatography (TLC)
Fatty acid methyl esters were purified (Mangold, 1969) by TLC using a solvent system of n-hexane-diethyl ether (90:10,v/v). A standard methyl ester was also run on the same plate in a separate lane. The location of methyl ester bands corresponding to the standard were marked after placing the TLC plate in an iodine vapour chamber and then scrapped off the methyl ester band from the plate. Methy1 esters were recovered by extracting the recovered bands in a mini glass column with chloroform, the later was evaporated and the Methy1 esters were kept in n-hexane in a freezer, till analyzed by GLC.

2.4 Gas Liquid Chromatography (GLC)
GLC of fatty acid Methy1 esters were done on a Chemito 1000 instrument, equipped with Flame lonization Detector (FID). Quantitation was done by computer using specific clarity lite software.

2.5 Analysis of Fatty Acid Methyl Ester (FAME)

GLC of FAME was done on a BPX-70 megabore capillary column of 30 mt length and 0.53 mm i.d. obtained from SGE, Australia. Oven temperature was programmed from 150°C - 240°C with a rate of 8°C/min. Initial and final times were kept isotheremal for 1 minute and 20 minutes, respectively. Injection port and detector temperatures were 250°C and 300°C, respectively. Nitrogen gas was used as carrier gas, its flow being 6.32 ml/min. Identification of Fatty acids was done by comparing their retention times with those of standards, chromatographed under identical operational conditional of GLC. Confirmation of fatty acids was also done by using the FAME of Cod liver oil fatty acids, as suggested (Ackman, and Burger, 1965).

3.RESULTS AND DISCUSSION

The present investigation has revealed that these plant species contains a considerable amount of pharmacologically active α-linolenic acid, the primary precursor molecule for – ω3 family of fatty acids in animal tissues(Gunstone, 2006). About 25 fatty acids have been found to occur in these leaf extracts and detritus (Table-1& Fig-1,2,3 and 4).

Table-1

Fatty acid compositions of Total Lipid (TL) obtained from L1 (Avicennia marina), L2 (Acanthus ilicifolius), L3 (Suaeda maritima), and Detritus samples as determined by GLC of methyl esters (% w/w of each component in total fatty acids).

Components[a]	L1	L2	L3	Detritus
14:0	0.5	1.1	0.5	6.7
14:1				2.6
15:0	1.4	1.0	1.8	2.6
15:1		0.3	0.5	0.5
16:0	35.2	22.1	29.7	38.3
16:1	1.5	1.8	1.3	10.9
16:2		0.4		1.0
17:0	0.9	0.8	0.4	0.4
17:1	0.4	0.3		0.2
18:0	3.5	4.9	5.1	4.5
18:1ω9	14.4	13.1	2.3	13.1
18:2ω6	10.3	21.9	17.3	9.0
18:3ω6	1.1	0.1	0.2	1.6
18:3ω3	30.1	31.0	39.6	2.7
20:3ω3				0.3
20:4ω6				0.2
22:0	0.1	0.1		0.3
22:1				0.1
20:4ω3	0.1	0.3	0.4	1.2
22:4ω6	0.04	0.1	0.04	
20:5ω3				1.1
22:5ω6				0.03
24:0		0.2	0.2	0.7
22:5ω3				0.02
22:6ω3				0.2

[a] First and second figures represent, carbon chain length: number of double bonds. The -ω values represent the methyl end chain from the center of double bond furthest removed from the carboxyl end.
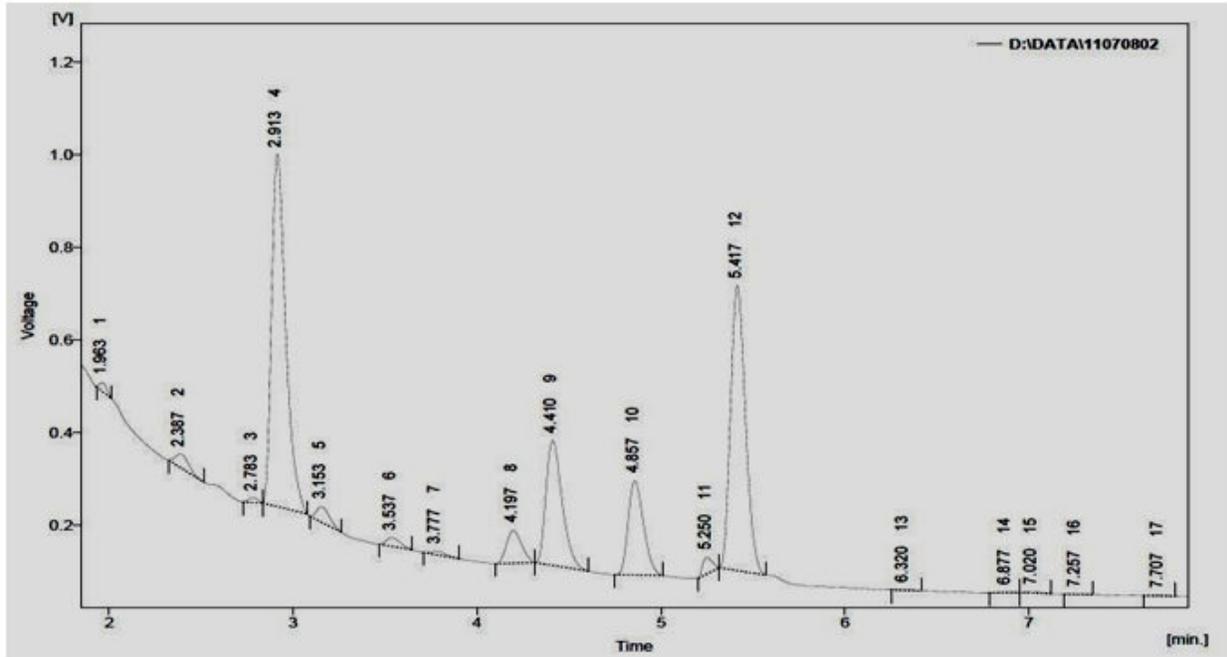
Figure 1
GLC tracing of the fatty acid methyl esters (FAMEs) of the leaf,Avicennia marina(L1). GLC column used was BPX-70 (Polar column)Megabore column (30 meters length x 0.530 mm dia).
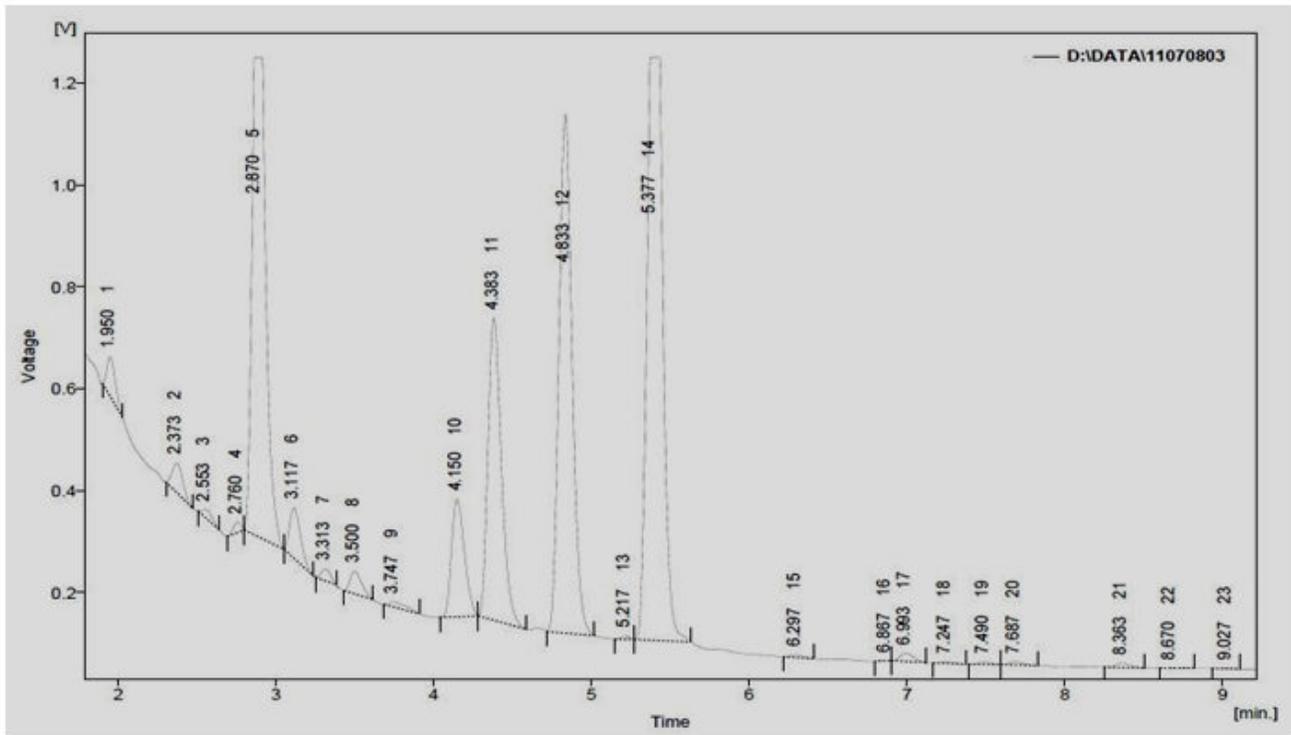

Figure 2
GLC tracing of the fatty acid methyl esters (FAMEs) of the leaf, Acanthus ilicifolius(L2). GLC column used was BPX-70 (Polar column)Megabore column (30 meters length x 0.530 mm dia).
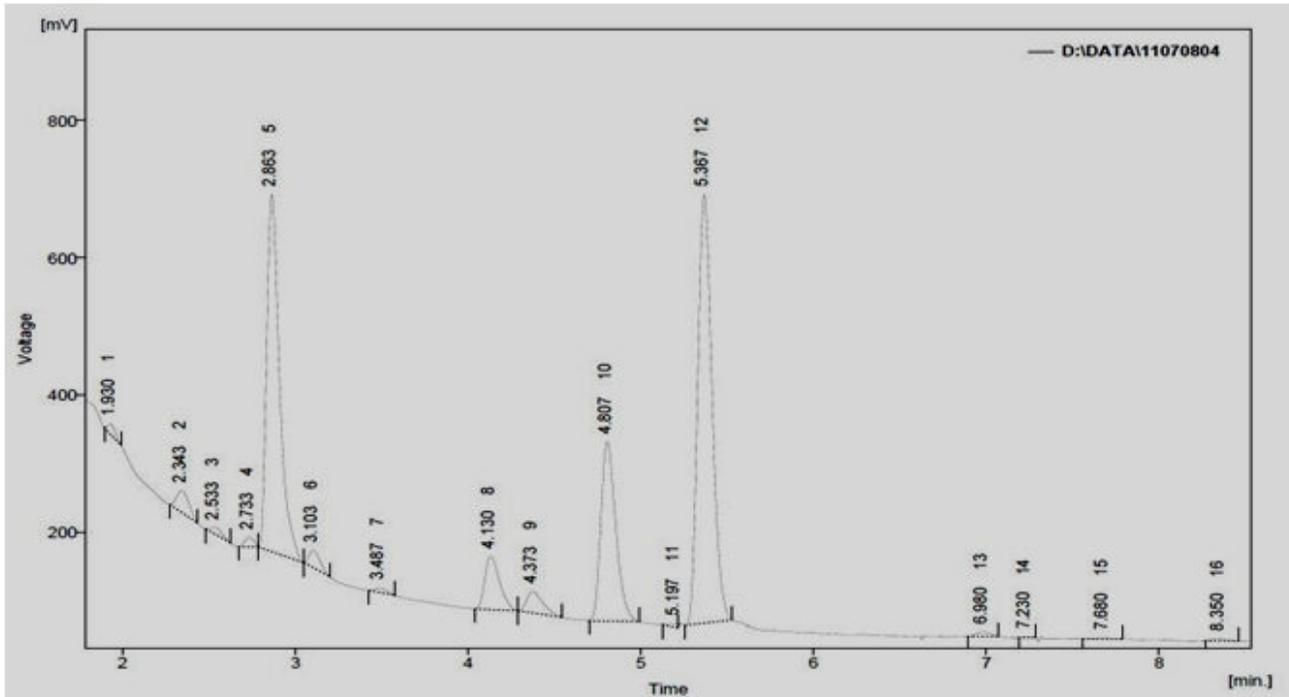
L - 37

Figure 3
GLC tracing of the fatty acid methyl esters (FAMEs) of the leaf Suaeda maritima(L3).GLC column used was BPX-70 (Polar column)Megabore column (30 meters length x 0.530 mm dia)
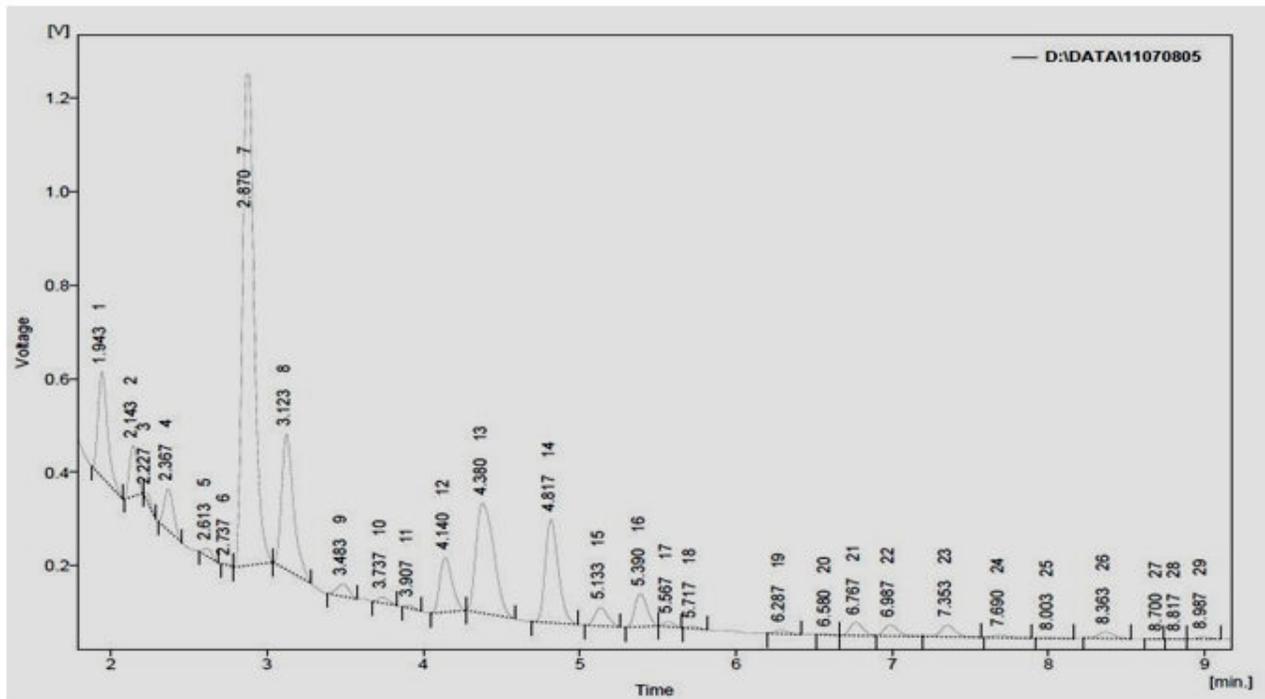

Figure 4
GLC tracing of the fatty acid methyl esters (FAMEs) ofthe detritus of the leaves. GLC column used was BPX-70 (Polar column)Megabore column (30 meters length x 0.530 mm dia).

L - 38

Of the saturated fatty acids, the highest amount was represented by palmitic acid (38.3%) in the detritus ,35.2% in L1(*Avicennia marina*) and 29.7% in L3(*Suaeda maritima*) but such fatty acid was 22.1 % in the L2(*Acanthus ilicifolius*) as obtained. Among the unsaturated fatty acids, the major component was α-linolenic acid (39.6%) in the L3(*Suaeda maritima*) , 31.0% in L2(*Acanthus ilicifolius*) , 30.1% in L1(*Avicennia marina*) and it was 2.7% in the detritus . The role of ALA in human nutrition becomes important in terms of long-term dietary intake. One advantage of the consumption of ALA over omega-3 fatty acids from fish is that the problem of insufficient vitamin E intake does not exist with high intake of ALA from plant sources(Simopouls, 2004). In clinical studies, ALA contributed to lowering of blood pressure (Berry, 1986). In a prospective epidemiological study, (Ascherio, *et al.*1996) showed that ALA is inversely related to the risk of coronary heart disease in men. Preliminary research has found evidence that α-linolenic acid is related to a lower risk of cardiovascular disease(Penny, *et al.*2002 and Connor, 2000). A study found that daily administration of α-linolenic acid significantly reduced both self-reported anxiety, stress levels, and objective measured cortisol levels in college age students(Yehuda, *et al*2005). In contrast, α-linoleic acid was recently shown to negatively regulate the growth of cancer cells, but not healthy cells, in vitro(Deshpande, *et al.*2013). Basic research has also suggested a major neuroprotective effect of α-linolenic acid in in vivo models of both global ischemia and kainate-induced epilepsy(Lauritzen, *et al.*2000). A longitudinal study of over 50,000 women, conducted at Harvard University, over a period of ten years, found that a higher intake of α-linolenic acid (combined with a lower intake of linoleic acid) was positively associated with a significant reduction in depression in the same group (the same study also found that by contrast an intake of EPA and DHA found in fish oils did not reduce depression)(Lucas, *et al.*2011). More specifically, ALA also reduces the levels of inflammation-associated adhesion molecules (Thies, *et al.*2001) and can down regulate the expression of pro-inflammatory genes through the suppression of nuclear factor κB and activation of peroxisome

proliferator-activated receptors(Zhao, *et al.*2005). Like EPA and DHA, ALA consumption is associated with a decreased risk of cardiovascular disease, potentially by affecting aspects of cardiac function as well as lowering cholesterol levels(De Caterina, 2011). ALA also reportedly reduces the risk of insulin resistance through an anti-oxidant function as well as by promoting membrane fluidity(Anderson, 2000). The effect of α-linolenic acid deficiency on neurological function supports the role of α-linolenic acid as a precursor to longer chain n-3 PUFA which are critical in the function of the central nervous system(Alessandri, 2004). Fifty percent of children and 30% of adults receiving long-term total parenteral nutrition lacking α-linolenic acid exhibited visual dysfunction, which suggests decreased availability of DHA for incorporation into neural membranes(Vinton, 1990) . The offspring of monkeys fed an n-3 PUFA deficient diet during pregnancy show visual impairments(Neuringer, *et al.*1986). Supplementation of the infant monkeys with α-linolenic acid resulted in an increase in the concentration of DHA in neural tissues and an improvement in visual function (Connor, 1988). This suggests that a deficit in the availability of α-linolenic acid for conversion to, in particular DHA was the principal mechanism underlying the deficiency symptoms

4.CONCLUSION

In the present study, it has been established that, considerably higher levels of α-linolenic acid (ALA) have been found in these selected mangrove species , particularly in the *Suaeda maritima*(39.6%), the primary precursor molecule for – ω3 family of fatty acids and also have various therapeutic effects.

5.ACKNOWLEDGEMENT

6.REFERENCES

1. Ackman, R.G.and Burger, R.D. Cod liver oil fatty acids as secondary reference standards in the GLC of polyunsaturated fatty acids of animal origin: Analysis of a dermal oil of the Atlantic leather-back turtle. J. Am. Oil Chem Soc.,1965,42, 38-42.

2. Alessandri, J.M.;Guesnet, P.;Vancassel, S.;Astorg, P.; Denis, I.;Langelier, B.; Aid, S.;Poumes-Ballihaut, C.;Champeil-Potokar, G. and Lavialle, M. Polyunsaturated fatty acids in the central nervous system: evolution of concepts and nutritional implications throughout life. Reprod Nutr Dev 2004, 44: 509–538.

3. Anderson, B.M. and Ma, D.W.L. Are all n-3 polyunsaturated fatty acids created equal?.*Lipids In Health and Disease* 8, 33 (2009).

4. Ascherio, A.; Rimm, E.B.; Giovannucci, E.L.; Spiegelman, D.; Stampfer, M. and Willett, W.C. Dietary fat and risk of coronary heart disease in men: cohort follow up study in the United States. BMJ.1996, 313: 84-90

5. Berry, E.M. and Hirsch, J. Does dietary linoleic acid influence blood pressure? Am J Clin Nutr. 1986,44: 336-340

6. Bligh, E.G. and Dyer, W.J. A rapid method of total lipid extraction and purification. Can J Biochem Physiol.1959, 37:911-917

7. Breanne M. Anderson and David WL, Ma (2009). "Are all n-3 polynsaturated fatty acids created equal?".Lipids in Health and Disease 8 (33):33.

8. Burdge, G.C. Prostaglandins Leukot Essent Fatty Acids 2006;75: 161-168.

9. Chakraborty, S.K. .In Mangroves: Ecology, Biology and Taxonomy. Ed. James N. Metras (publ.NOVA publishers), 2011, pp.83-112.

10. Christie, W.W. Lipid analysis. 2nd edn. Pergamon Press, 1982, Oxford, England.

11. Connor, W.E. and Neuringer, M. The effects of n-3 fatty acid deficiency and repletion upon the fatty acid composition and function of the brain and retina. Prog Clin Biol Res 1988, 282: 275–294 .

12. De Caterina, R. n-3 fatty acids in cardiovascular disease. *N. Engl. J. Med.* 364, 2439-2450 (2011).

13. Deshpande, R.; Mansara, P.; Suryavanshi, S. and Kaul-Ghanekar, R. "Alpha-linolenic acid regulates the growth of breast and cervical cancer cell lines through regulation of NO release and induction of lipid peroxidation". Journal of Molecular Biochemistry. 2013, 2 (1): 6–17.

14. Gunstone, F.D.Modifying lipids for use in food.CRC Press .2006.Boca Raton Boston. New York Washington, DC

15. Holman RT, et al. *Am J ClinNutr*. 1982;35: 617-623.

16. Lauritzen, I.;Blondeau, N.;Heurteaux,C. Widmann,C.;Romey, G. and Lazdunski,M. "Polyunsaturated fatty acids are potent neuroprotectors". *The EMBO Journal* . 2000,19 (8): 1784–1793.

17. Innis, S.M. Am J Clin Nutr. 2000;71(suppl): 238S-244S.

18. Lucas, M.; Mirzaei, F.; O'Reilly, E. J.; Pan, A.; Willett, W. C.; Kawachi, I.; Koenen, K. and Ascherio, A. "Dietary intake of n-3 and n-6 fatty acids and the risk of clinical depression in women: a 10-y prospective follow-up study". Am J Clin Nutr. 2011,93 (6): 1337–43.

19. Mangold, H.K. Aliphatic lipids. In: Sthal E (ed) Thin layer Chromatography. Springer, New York, 1969, pp 363-415.

20. Misra, S. Choudhury, A. Dutta, A.K. Ghosh, A. Sterols and fatty acids from three species of mangrove. Phytochemistry. Volume 23, Issue 12, 1984, Pages 2823–2827.

21. Misra, S. Dutta, A.K. Chattopadhyay, S. Choudhury, A. Ghosh, A. Hydrocarbons and wax esters from seven species of mangrove leaves. Phytochemistry. Volume 26, Issue 12, 1987, Pages 3265–3268.

22. Morris, D.H. *Flax* – A Health and Nutrition Primer. Winnipeg, MB: Flax Council of Canada, 2007.

23. Morris, D.H. Flax – A Health and Nutrition Primer. Winnipeg, MB: Flax Council of Canada, 2008.

24. Nestel, P.J. et al. Arterioscler Thromb Vasc Biol. 1997;17: 1163-1170.

25. Neuringer, M.; Connor, W.E.; Lin, D.S. Barstad, L. and Luck, S. Biochemical and functional effects of prenatal and postnatal omega 3 fatty acid deficiency on retina and brain in rhesus monkeys. Proc Natl Acad Sci. USA, 1986, 83: 4021– 4025.

26. Penny, M.;Etherton, K.; William, S. Harris.and Lawrence, J. Appel, for the Nutrition Committee "Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease".Circulation . 2002,106 (21): 2747–2757

27. Ratnayake, W.M.N. Chen Z-Y. Lipids. 1996;31: S279-S282.

28. Simopouls, A.P.,Omega-3 Fatty Acids and Antioxidants in Edible Wild Plants. Biol Res2004,37: 263-277,

29. Thies, F. et al. Influence of dietary supplementation with long-chain n-3 or n-6 polyunsaturated fatty acids on blood inflammatory cell populations and functions and on plasma soluble adhesion molecules in healthy adults. Lipids . 2001,36, 1183-1193

30. Vinton, N.E.;Heckenlively, J.R.; Laidlaw, S.A.; Martin, D.A.;Foxman, S.R.; Ament, M.E. and Kopple, J.D. Visual function in patients undergoing parenteral nutrition. Am J Clin Nutr 1990, 52: 895–902.

31. Connor , W.E. "Importance of $n-3$ fatty acids in health and disease". American Journal of Clinical Nutrition. 2000,71 (1 Suppl.): 171S–175S.

32. Yehuda, S.;Rabinovitz, S. andMostofsky, D.I. "Mixture of essential fatty acids lowers test anxiety". Nutritional Neuroscience . 2005,8 (4): 265–267.

33. Zhao, G., Etherton, T.D., Martin, K.R., Vanden, H.J.P., Gillies, P.J., West, S.G. & Kris-Etherton, P.M. Anti-inflammatory effects of polyunsaturated fatty acids in THP-1 cells. Biochem. Biophys. Res. Commun. 2005,336, 909-917