RJC

RASĀYAN *J. Chem.*
Vol. 5 | No.3 | 414-419 | July-September | 2012
ISSN: 0974-1496 | CODEN: RJCABP
http://www.rasayanjournal.com

ISOLATION AND CHARACTERIZATION OF LECITHIN FROM EMU EGG AS NOVEL PHARMACEUTICAL EXCIPIENT

T. Sreedevi, Joshny Joseph, D. Ramya Devi, B. N.Vedha Hari *

Department of Pharmaceutical Technology, School of Chemical and Biotechnology, SASTRA University, Thanjavur-613401. Tamilnadu, India

*E-mail: vedhahari@scbt.sastra.edu

ABSTRACT

Lecithin is a compound composed of fatty acids, triglycerides and phospholipids and lecithin plays a very important role in the field of drug delivery. These are mainly used as carriers or as penetration enhancers in different drug delivery systems, major sources are egg yolk and soya bean oil. Emu bird is one of the largest birds available around the globe and their eggs are huge which contain sufficient quantity of yolk which proves to be a rich source of lecithin. The aim of this work is to isolate lecithin from emu egg and characterize these compounds using different methods like Thin Layer Chromatography, GC-MS and NMR. Emu egg yolk contained significant amount of lecithin and it was confirmed with TLC, GC-MS and NMR spectroscopy. Based on the results obtained from the different techniques, we could conclude that the extracted mass contained compounds of lecithin especially, fatty acid, esters of fatty acids, and some amount of phospholipids. Since the amount of yolk is high in emu egg, more amount of lecithin can be extracted. Hence emu egg can be considered as a feasible source of lecithin.

Keywords: Phospholipid, Lecithin, GC-MS, NMR, Thin Layer Chromatography

INTRODUCTION

The Emu (*Dromaius novaehollandiae*) is one of the largest birds, adapted to Northern part of the world, particularly Australia. They are flightless birds which can grow to a height of about 2 meters. They mainly feed on plants and insects and can live without food for many days. These birds have strong claws which they mainly use for defense mechanism. Different subspecies of emu birds are available, while the differentiation between male and female is tedious and is mainly done by means of the particular sounds they produce. Emu products have gained much importance and found to have numerous applications in cosmetic, craft and food industries. The various products used are eggs, meat, feather, oil and so on. Emu eggs are emarald green in colour, with thick shells and average length of 6 inches, weighing around 800 grams. Each egg may contain 400-450 grams of yolk with more quantity of egg white. Moreover the attractive egg shells and weightless feathers may be used for craft work. The main drawback of these eggs is that they are very expensive. But the amount of yolk that can be obtained from a single emu egg is 3 times higher than that of an ordinary egg. Generally eggs are considered to be a rich source of lecithin and in this current research an attempt has been made to isolate and characterize lecithin from emu egg yolk.

EXPERIMENTAL

Emu egg was purchased from Namakkal. GC-MS instrument used was Perkin Elmer Clarus 500 with capillary column (Elite -5MS) of length 30m and internal diameter 250µm. All the chemicals used were of analytical grade.

Isolation of phospholipids

Phospholipid was isolated using Modified and Singleton Gray procedure. Firstly egg yolk was separated from albumin manually. Chloroform: Methanol (2:1) solution was added to the egg yolk and mixed well. The mixture was stirred well using magnetic stirrer for 2 hours, followed by transferring into a separating

funnel. The clear solution which would settle below the mixture would contain the required compounds and this solution was separated and concentrated, followed by the addition of ice cold acetone. The mixture was kept at very low temperature (-20°C) for the required compounds to get precipitated. The precipitated mass was separated using vaccum filtration. The obtained mass was stored in an amber coloured bottle in refrigerator until use.[1]

Table-1: Compounds identified by GC -MS of Emu egg lecithin isolated from Emu egg

S. No.	Peak Name	% Peak Area	Retention Time
1	Heptyl phthalate	0.9029	23.36
2	Methyl tridecanoate	4.0977	24.87
3	Palmitic acid	17.8708	26.01
4	Oxalic acid, allyl octyl ester	0.9968	28.27
5	Methyl oleate	3.5481	28.76
6	Methyl stearate	1.4579	29.24
7	Sulfurous acid, isohexyl 2-propyl ester	1.0407	29.61
8	Myristoleic acid	8.5082	29.69
9	Stearic acid	2.8541	30.11
10	4-fluoro-1-methyl-5-carboxyl acid,ethyl ester	1.0743	31.02
11	Palmitoyl chloride	11.7923	31.44
12	Tetradecanoyl chloride	8.2137	32.18
13	Palmitoylethanolamide	3.9707	32.57
14	22-Tricosenoic acid	0.0837	33.89
15	Glyceryl oleate (lysophosphatidylcholine)	12.6589	34.16
16	1-Glyceryl stearate	5.2426	34.56
17	9-Octadecanal	7.7643	34.87
18	Palmitoyl chloride	7.6325	35.22
19	Cholesta-4,6-diene-3-ol	0.2896	41.27

Characterization Techniques

Thin Layer Chromatography (TLC)

Laboratory experimental glass slide was cleaned thoroughly with alcohol followed by distilled water and air dried. Slurry of silica was prepared by taking appropriate quantity of silica, dissolved in distilled water. The prepared slurry was uniformly and evenly spread onto the glass slide and dried to get the required TLC plate. The required solvent system, Chloroform: Methanol: Water in the appropriate ratio of 65:25:4 for TLC was prepared for the detection of lecithin[3]. A chamber was made which was saturated with the solvent system for carrying out TLC. The isolated phospholipids was dispersed in the appropriate solvent (chloroform) and loaded as a small spot on the prepared TLC plate, which is about 1.5mm from the bottom with the help of a thin capillary tube. For comparison, pure soya lecithin was used as the standard[2].

After loading of sample the TLC plate was kept in saturated chamber of solvent system. The solvent system was allowed to ascend through the plate till it had travelled three- fourths of the plate. Then the plate was taken out, air dried and kept in saturated iodine chamber for 15 minutes for identification of the sample.

Calculation of Retardation factor (R_f value)

Retardation factor was calculated using the formula:

Retardation factor = Distance travelled by compound in the substance / Distance travelled by the solvent.

The R_f value obtained for the standard was compared with that of the obtained sample.

Fig.-1: Comparative Photographic image of Thin Layer Chromatography. A-Emu egg lecithin, B- Pure soy lecithin

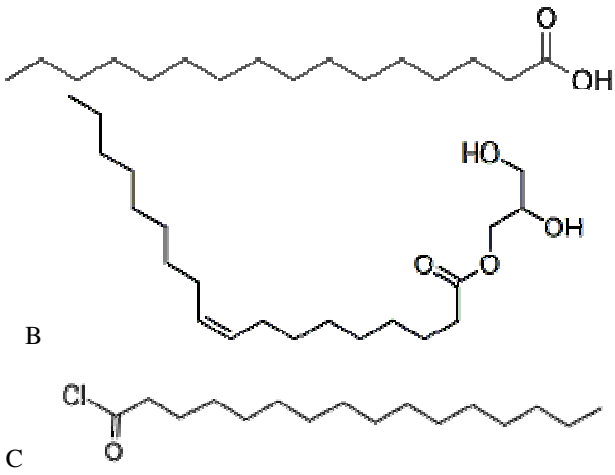

Fig.-2: A-Structure of palmitic acid, B-Structure of glyceryl oleate, C-Structure of palmitoyl chloride

Gas Chromatography Mass Spectroscopy (GC-MS)

GC-MS was performed in order to the separate the components present in the extracted lecithin and also to produce a spectral output. Gas chromatography was done by injecting 1µl of the sample dissolved in appropriate solvent at an injection temperature of 290°C, using Helium as carrier gas at a constant flow rate of 1ml per min. The GC oven was programmed from 50°C at 7°C per minute to 200°C (5 min) at 7°C per minute and again to 290°C (for 10 min). Mass spectroscopic analysis was done at the range of 40-600 amu, with the help of electron ionisation along with a voltage of 70eV[3, 4].

Nuclear Magnetic Resonance (NMR) Spectroscopy

NMR spectroscopy was done to find out the physical and chemical properties of compounds contained in the crude sample. This technique is based on the phenomenon of nuclear magnetic resonance and can give detailed information about the structure and chemical properties of different compounds present in the sample. Both 1-dimensional proton and C-13 NMR spectroscopy was done inorder to find out the number of protons and carbon atoms and the pattern in which they are aligned, thereby giving an idea about the compounds present in the sample. NMR spectroscopy was done by dissolving the crude extract in chloroform (containing deuterium as isotope of hydrogen) with reference as trimethylsilane(TMS).

RESULTS AND DISCUSSION

With the Thin layer chromatography the brownish yellow spots appeared after the plate was kept in iodine chamber. R_f factor was calculated for both standard and the sample. It was found to be the same for the

soya lecithin standard and the test sample. From this we could conclude that the test sample used also contained compounds of lecithin. The spot formed is shown in Figure.1 (A= Emu lecithin, B= Soy lecithin). R_f value was found to be 0.8 for both standard and the sample.

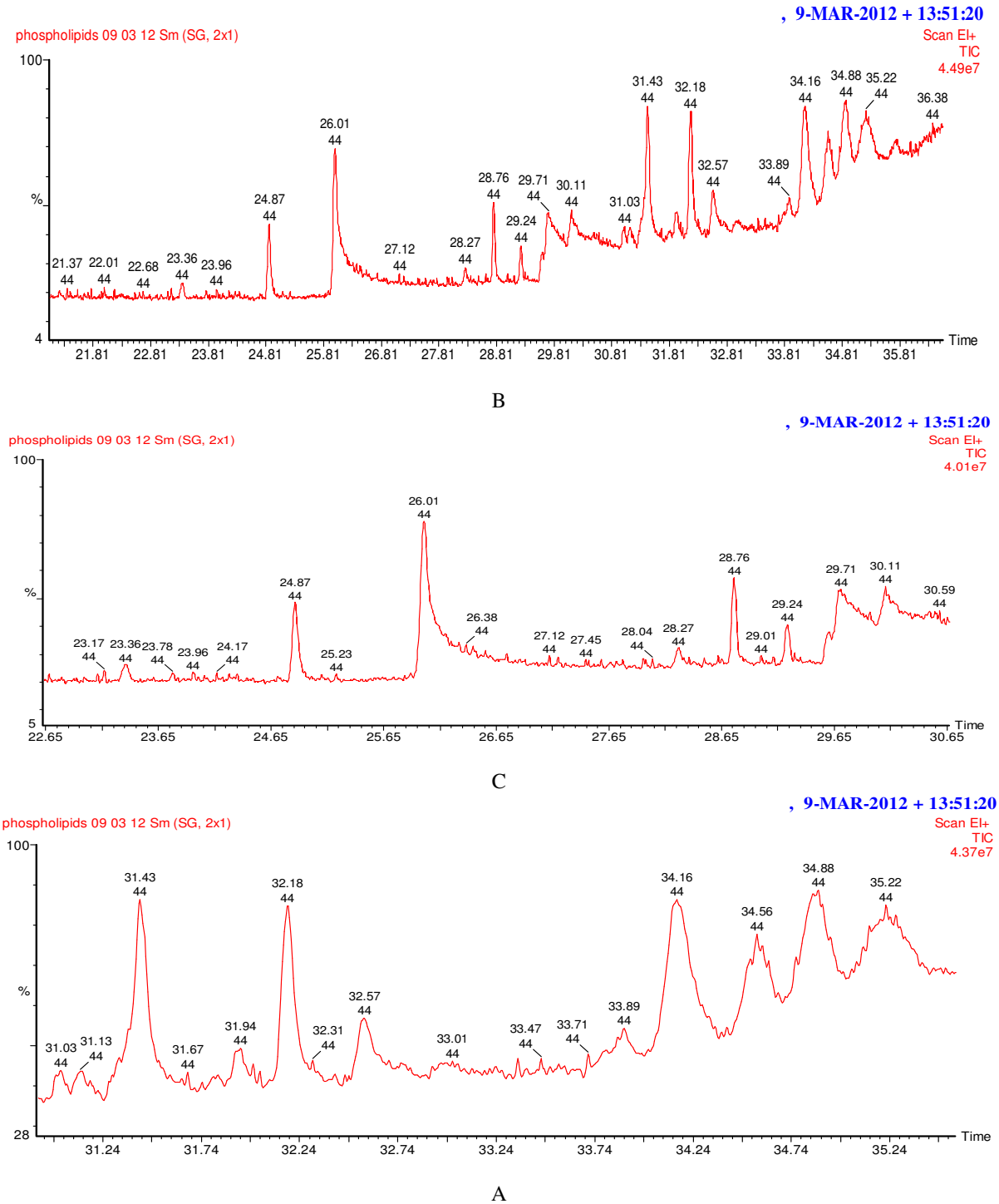

Fig.-3: GC MS spectra of Emu egg Lecithin isolated from Emu egg (A)-Full Chromatogram of isolated sample, (B) - Chromatogram with extended peaks, (C)-Chromatogram with further extended peaks.

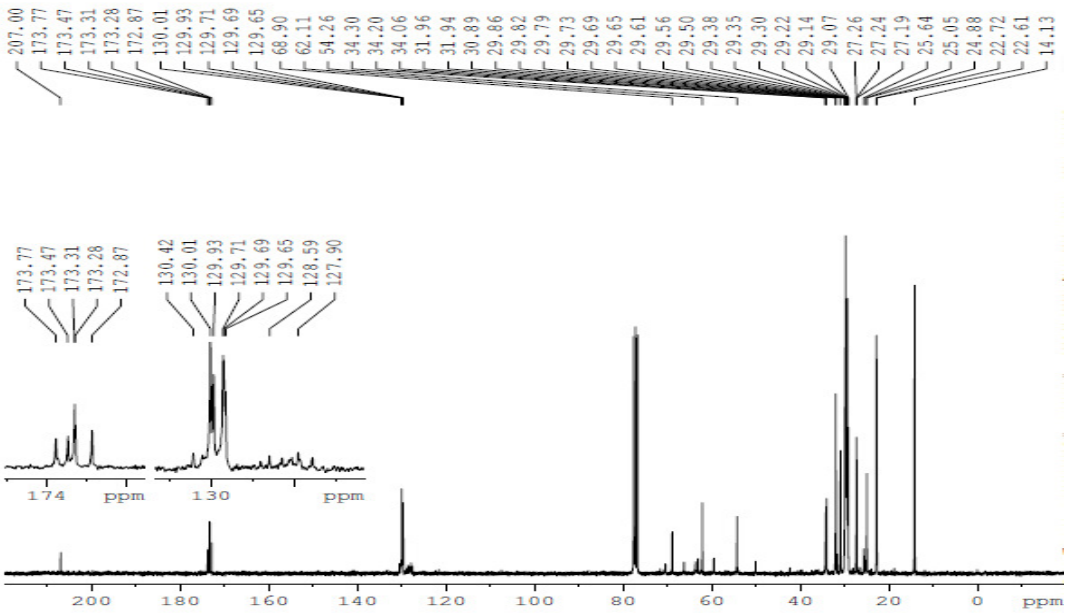

Fig.-4: Carbon-13 NMR Spectrum of Emu egg lecithin isolated from emu egg

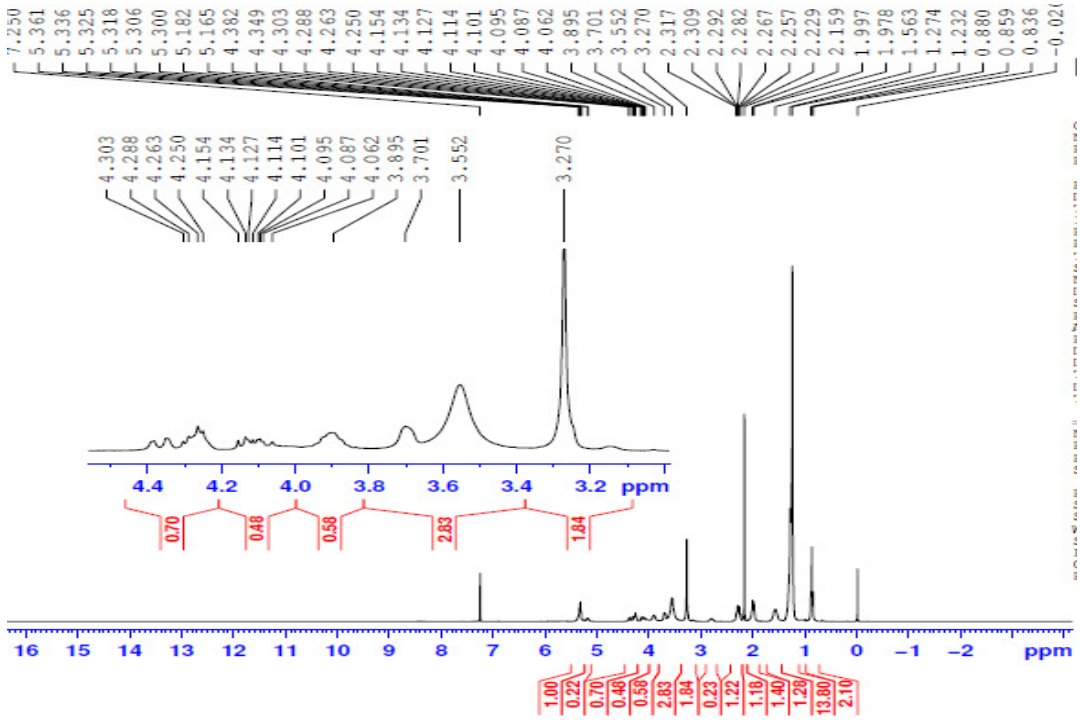

Fig.-5: Proton NMR spectrum of Emu egg lecithin isolated from emu egg

The output produced by GC-MS was interpreted and the identified compounds are given in table 1. The identification of compounds present in the extracted mass was based on direct comparison of the retention times and spectral data with that of standard compounds and by matching with that of NIST 2005 library. The quantitative estimation of each peak obtained in the spectrum was done with the help of area obtained by means of the computer which is attached to the GC-MS instrument.[3] The amount of palmitic acid was found to be the highest (17%), followed by glyceryl oleate (12%) and palmitoyl chloride (11%), which

could be classified as fatty acid and fatty acid esters respectively. Thus we could conclude that the extracted mass contained compounds of lecithin (group of fatty acids, esters, and phospholipids). The structure of these compounds is shown in figure 2 [5-7]. The GC-MS spectra obtained is given in figure 3. NMR spectra obtained for the crude sample is given in figure 4 and 5.The peaks obtained in both proton and carbon NMR spectrum revealed that both hydrogen and carbon containing compounds are present in the crude sample.

CONCLUSION

With the above obtained data we could conclude that the lecithin was isolated successfully by well established technique from Emu egg and identified qualitatively with the help of modern analytical instruments. It can be assured that the Emu egg lecithin can be used as a carrier for drug delivery systems and as an excipient in Pharmaceutical industry.

ACKNOWLEDGEMENTS

The authors wish to express their sincere gratitude and thanks towards the authorities of SASTRA University for availing the necessary facilities to make this project a success.

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[RJC-950/2012]