

ISOLATION, CHARACTERIZATION AND ANTI-BACTERIAL ACTIVITY OF LECITHIN FROM EGG YOLK

Harith Yaroub Mahmood

Department of Physiology, Pharmacology and Chemistry, College of Veterinary Medicine ,
University of Basrah, Basrah,Iraq.

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ABSTRACT

Generally eggs are considered to be rich source of lecithin. In this current research an attempt has been made to isolate and characterize lecithin from egg yolk. The separation and identification of lecithin with different fatty acyl groups has been the subject of extensive investigation. Lecithin contains choline, which is an important precursor for many biochemical functions. Lecithin was characterized by spectroscopic data such as IR, CHN, and showed good antibacterial activity of the lecithin against: *Bacillus cereus*, *Bacillus subtilus* and *Escherichia coli*.

INTRODUCTION

Eggs contain a large variety of nutrients and are a source of balanced proteins with high nutritional value for humans. They are widely consumed throughout the world and are used in food processing for their technological properties (1). Eggs are also a good source of the antioxidant carotenoids, lutein and zeaxanthin (2). The high nutritional properties of eggs make them ideal for many people with special dietary requirements (3). The egg is protected physically by the eggshell and chemically by antibodies, known as IgYs, mainly concentrated in the egg yolk (4). To minimize infection by microbes, eggs possess physical barriers such as the shell and membranes, and chemical barriers in the form of antimicrobial proteins and peptides known as IgYs (5,6). Although, a little attempt has been made to use it as antimicrobial agent against a variety of microorganisms.

So the aims of this study are to extraction and purification of eggs yolk protein Lecithin showed in (Fig.1)(7) and determine its antimicrobial activity against different bacteria.

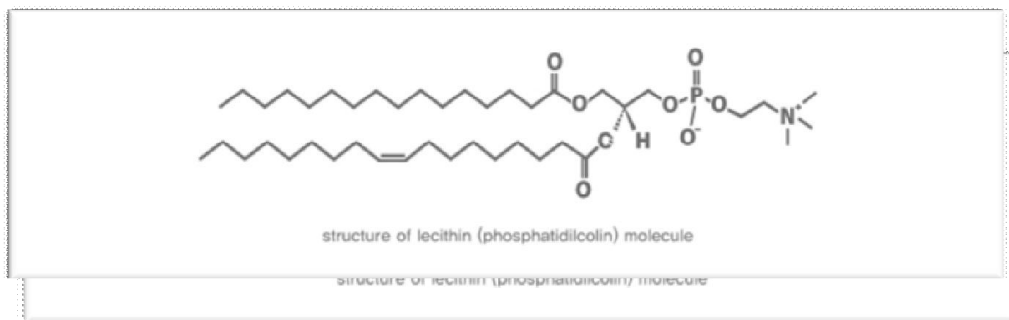


Fig. 1: Chemical structure of Lecithin (phosphatidyl cholin)

MATERIAL AND METHODS

Physical measurement:

Infrared Spectra were recorded as KBr discs in the range of $4000-400\text{cm}^{-1}$ Using a FT-IR Spectrophotometer Shimadzu model at the polymer center research, university of Basrah, Iraq. Microanalysis for Carbon Hydrogen and Nitrogen were carried out by Perkin – Elmer at department of chemistry, college of science, university of Basrah, Iraq.

Isolation of Lecithin (Phospholipid):

Lecithin was isolated using modified and singleton Gray procedure(8). 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 addition of ice cold acetone. The mixture was kept low temperature($-20\text{ }^{\circ}\text{C}$) for required compounds to get precipitated. The precipitated product was separated using vacuum filtration. The Crystals was stored in refrigerator.

Anti-bacterial activity

Different strains of bacteria species (*Bacillus subtilis*, *Bacillus cereus* and *Escherichia coli*) were used for investigating the antibacterial activities of eggs yolk protein lecithin. Strains were obtained from laboratory of Microbiology, Department of Microbiology, College of Veterinary Medicine, University of Basrah, Iraq. In vitro the antibacterial activity was determined by Muller Hinton agar as a culture media for antibacterial activity(9) against two-Gram positive bacteria: *Bacillus subtilis*, *Bacillus cereus* and one Gram negative bacteria: *Escherichia coli*. at a concentration of 100 and 200 μ g/mL using as a solvent reagent. The agar plates were incubated at 37 $^{\circ}$ C for 24 hours. The inhibition zone was measured (mm). Antibacterial anti-biotic disk: Ampicillin concentration (25 μ g/ml) was used as antibacterial standards control(10).

RESULTS

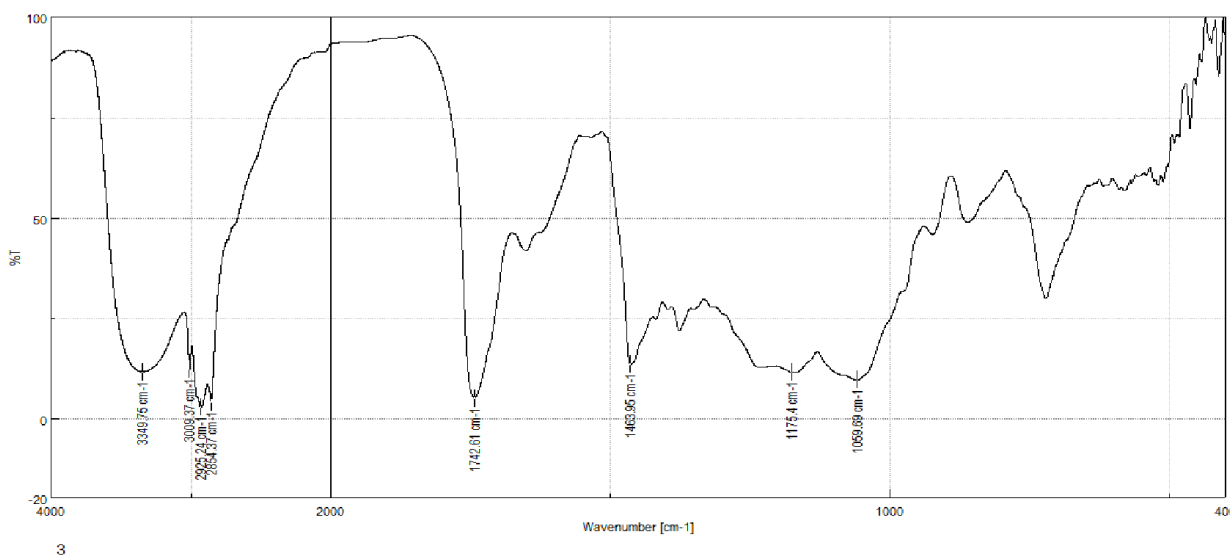


Fig.2 : IR- Spectra of isolated Lecithin

Table (1): Elemental analysis (C.H.N) of lecithin isolated from egg yolk.

Calculated			Found		
C	H	N	C	H	N
65.60	10.79	1.74	66.002	10.92	1.375

Table (2): Antimicrobial activity of Lecithin

Bacterial species	Zone inhibition of antimicrobial sensitivity test of eggs yolk protein Lecithin (mm)		Zone inhibition of Ampicillin disc (mm)(10)
	100µg/mL	200µg/mL	25µg/ml
1- <i>Bacillus cereus</i>	24	30	17
2- <i>Bacillus subtilus</i>	20	25	15
3- <i>Escherichia coli</i>	8	16	20

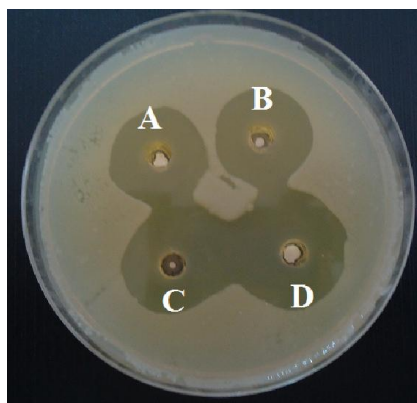


Fig.3: Antimicrobial sensitivity test of eggs yolk protein Lecithin vs. *Bacillus cereus*.

A and B: represent the dilution 100 µg/mL; C and D: represent the dilution 200 µg/mL and the standard antibiotic was (17 mm) in concentration (25µg/ml) of Ampicillin.

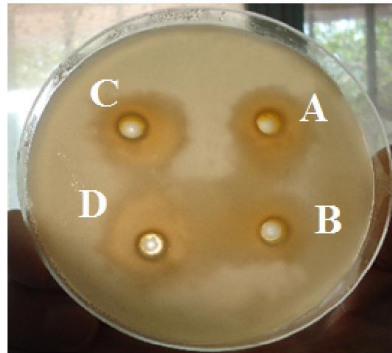


Fig.4: Antimicrobial sensitivity test of eggs yolk protein Lecithin vs. *Bacillus subtilis*.

A and B: represent the dilution 100 µg/mL; C and D : represent the dilution 200 µg/mL and the standard antibiotic was (15mm) in concentration (25µg/ml) of Ampicillin.

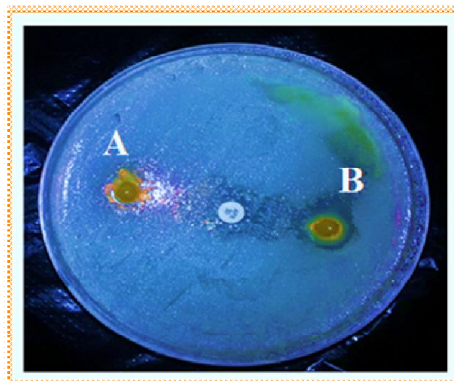


Figure 4: Antimicrobial sensitivity test of eggs yolk protein Lecithin vs. *Escherichia coli* . A and

B: represent the dilution 100 µg/mL and the standard antibiotic was (20mm) in concentration (25µg/ml) of Ampicillin.

DISCUSSION

Spectroscopic result:

The I.R spectra of extracted lecithin (Fig.2) displayed common features in certain regions and characteristic bands in the finger print and other regions. The spectra showed broad strong bands in 3349.75 cm^{-1} due to OH stretching. IR spectra showed bands in the range $2925\text{-}2854.37\text{ cm}^{-1}$ due to (CH-aliphatic) stretching. IR spectra of compound showed sharp strong band in 1742.61 cm^{-1} attributed to carbonyl group C=O (ester) stretching.

The Carbon, Hydrogen and Nitrogen (C.H.N) analytical data for lecithin isolated from egg yolk is given in table(1), the elemental analysis (C.H.N) data is agreement with structure of Lecithin as showed (Fig.1) and the result showed that there was compatible between the calculated theoretically and that found practically and I haven't find any study to determine this elemental analysis just UV-Vis. and powder X-ray diffractions (7) and TLC (12).

Anti-Bacterial activity:

The eggs yolk protein lecithin have been tested for their antibacterial activities using the Muller Hinton agar as a culture media for antibacterial activity(10) by measuring the inhibition zone in mm. Antibiotic drug ampicillin was used as control. The antibacterial activity of the eggs yolk protein Lecithin was tested The results of the antimicrobial activity are shown in tables (2). It is observed that the eggs yolk protein lecithin show activity against all tested bacterial species. However, the eggs yolk protein lecithin had the highest effect against the Gram positive *Bacillus cereus*, *Bacillus subtilus* and showed moderate effect against *Escherichia coli*. It is observing that the eggs yolk protein lecithin exhibits antimicrobial activity against both Gram-positive and Gram-negative bacteria, and it's activity was increases with an increase in the concentration of the solutions. However, the eggs yolk protein lecithin shows highest effect against *Bacillus cereus*, and moderate activity against *Escherichia coli*.

عزل وتشخيص وقياس الفعالية ضد البكتيريا لليسيثين من صفار البيض

حارث يعرب محمود

الخلاصة

يعتبر البيض بصورة عامة مصدرا غنياً بالليسيثين. تم في البحث الحالي فصل وتشخيص الليسيثين من صفار البيض. يحتوي الليسيثين على الكولين والذي يعتبر مهماً في عدد من الوظائف الحيوية وتم تشخيص الليسيثين بواسطة القياسات الطيفية مثل ولوحظ لليسيثين فعالية جيدة ضد بكتيريا *Bacillus subtilus* و *Bacillus cereus* و *Escherichia coli* و IR و CHN.

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