

Investigating the Effect of *Lymnaea auricularia* (snail) Powder on Different Species of Pathogenic Bacteria

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Abstract

The aim of this study was to investigate the antibacterial activity of *Lymnaea auricularia* (snail) tissue powder and shell powder against different human pathogenic bacteria (*Escherichia Coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia*). Four concentration was tested (75,100,125,150) mg/ml of the powder with well diffusion assay. The highest activity of shell powder was in *E. Coli* at 75mg\ml concentration(19mm) inhibition zone, while the highest activity of tissue powder was in *P. aeruginosa* at 150mg/ml concentration with inhibition zone (28 mm). The powder extracts ' antimicrobial activity reveals that they may have biologically active metabolites.

Keywords: *Lymnaea auricularia*. Antibacterial Activity. Human Pathogens.

Introduction

Mollusca phylum is considered for example the following generality widespread brute phylum including 100,000-200,000 types through over 52,000 types be situated specified and recognize ^(1,2). Mollusca integrate a diverse extent of faunae and it is divided into Scaphopoda, Snail, Cephalopoda, Bivalvia, Polyplacophora, Caudofoveata, Solenogastres, and Monoplacophora ^(1,3). Gastropod (snails) is the most diverse group of Mollusca, evaluated approximately 80,000 to 100,000 evaluated sorts ^(4,5). Snails have colonized very different ecosystems from an ecological point of view, representing unified marine, aquatic and terrestrial taxonomic orders ^(6,7). The snail includes Algae eater, deposit feeders, detritophages, herbivores, predators, filter- eater, ecto- and endoparasites ⁽⁸⁾. those are smooth body beasts that are shielded via a solitary helical also limy shell that differs in volume, constitute and color. Snail be situated of economic importance for example a protein origin, ornament, coloring also

remedy, Moreover, as a part of the natural diet for fish and birds, these species are important in the marine food chain ^(9,10).

Material and Methods

Snail Collection

Snails were purchased from Al-ghazel market in the middle of Baghdad governorate. The samples were taken to the laboratory, water cleaned to remove sand and other dust particles ⁽¹¹⁾.

Identification of snails

Specimens were confirmed and identified by Dr.Muhannad Ramzi , the snail was identified according to the whorls number and the opening position⁽¹²⁾.

Preparation of snail powder

With the aid of a small hammer, the shells of the snail are broken and the meat was collected in clean containers or sterile beakers, then the meat was kept for 72 hours in the incubator at 55 ° C. The dried samples of snails were powdered into fine powder using mortar ⁽¹¹⁾. The shell was washed, cleaned, dried in an incubator at 55°C and crushed before it was blended into fine powder

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using commercial milling machine⁽¹³⁾.

Culture media preparation

The media was prepared according to manufacturing companies' instructions, then disinfected via pressure chamber at 121 °C for 15 minutes under pressure 15bar/Inch², then poured in to sterilized petridishes after cooling to 45°C, incubated at 37°C for 24 periods for sterility then stocked by 4°C till utilize.

The tested microorganisms

The tested bacteria used in this study were pathogenic bacteria which were obtained from culture collection of higher studies Labrotory / department of biology /college of science / mustansiyrih university, which included three species of bacteria: (*Kleibssla pneumonia*, *Pseudomonas auergunosa*, *Escherichia coli*).

preparation of the concentrations of snail powders against tested bacteria

The snail powder was prepared as mentioned and four concentration prepared by adding distil water to powder. These concentrations prepared as following: 75mg/ml was prepared by adding 375mg of the powder to 5ml distil water. 100mg/ml was prepared by adding 500mg of the powder to 5ml distil water.

125mg/ml was prepared by adding 625mg of the powder to 5ml distil water. 150 mg/ml was prepared by adding 750mg of the powder to 5ml distil water⁽¹⁴⁾.

Antimicrobial activity of the prepared snail powder against tested bacteria

Muller-Hinton a gelatinous substance dishes be situated utilized of vulnerability experiment through a gelatinous substance fully distribution procedure. Antibacterial activity was carried out by using standard well diffusion method^(15,16). This method is based on the observation the effect on the growth of microorganisms in plate. Bacteria used in this test are: (*K. pneumonia. auergunosa*, *E. coli*). Bacterial suspension was prepared by picking 4-5 colonies of each original bacterial isolate and suspended in a test tube containing 5 ml of normal saline, then adjusting turbidity to approximately 1.5×10^8 CFU/ml (MacFarland tube). A portion of the bacterial suspension was carefully and evenly transformed on

the Mueller-Hinton a gelatinous substance medium by a sterile cotton swab. Wells was made in a gelatinous substance medium with the use of a cork borer. and then it was left for 10 min. After 10 min, about 100 µl of varying concentrations of each powder (75mg/ml, 100 mg/ml, 125 mg/ml, and 150 mg/ml) be additional in the hole and the dishes be situated nurtured aimed at 24 periods by 37 ° C. A scale was used to measure the diameters of the inhibition region⁽¹⁴⁾.

Result

Identification of the snail

The snail has been described as *Lymnaea auricularia* based on its characteristics of morphology and physiology, which was confirmed by Assistant Prof. Dr Muhannad Ramzi, the length of the *L. auricularia* ranged from 1-4 cm and the weight of the whole body ranged from 2-6 g.



Figure(1): *Lymnaea auricularia* snail

The effect of *Psudodontopsis euphraticus* snail shell powder and tissue powder on pathogenic bacteria

L. auricularia powder was tested for antibacterial activities against three pathogenic bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *klebsiella pneumonia*). Four concentration of the snail shell powder and snail tissue powder (75,100,125,150) mg/ml with well diffusion assay was used. The results were summarized in Table (1) and table (2)

Table (1): Effect of different concentration of snail shell powder

Pathogenic Bacteria	Concentration & inhibition zone			
	75	100	125	150
Klebsiella pneumonia	12	14	15	12
Pseudomonas Aeruginoa	11.4	14	16	16.5
Escherichia coli	19	14.8	16.7	18

At 75mg/ml concentration *E. Coli* also showed the largest inhibition zone (19 mm), *K. pneumonia* (12 mm) and *P. aeruginosa* (11.4 mm). In concentration of 100mg/ml *E. Coli* showed the largest inhibition zone (14.8 mm) while both of *K. pneumonia* and *P. aeruginosa* were (14 mm). At 125 mg/ml concentration appeared high inhibition effect in *E. Coli* which was (16.7 mm) then *P. aeruginosa* (16 mm) and *K. pneumonia* (15 mm). The highest activity of powder at 150 mg/ml concentration was appeared in *E. coli* with inhibition zone (18 mm) followed by *P. aeruginosa* with inhibition zone (16.5 mm) *K. pneumonia* (12 mm).as shown in table (1) and figure (2).

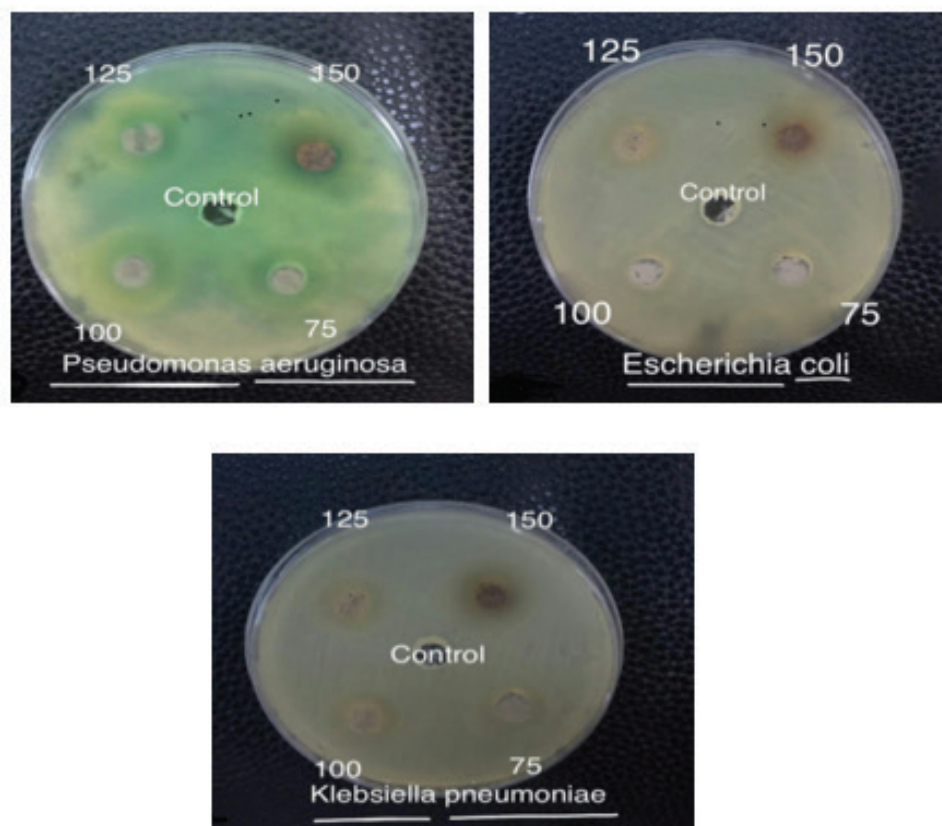
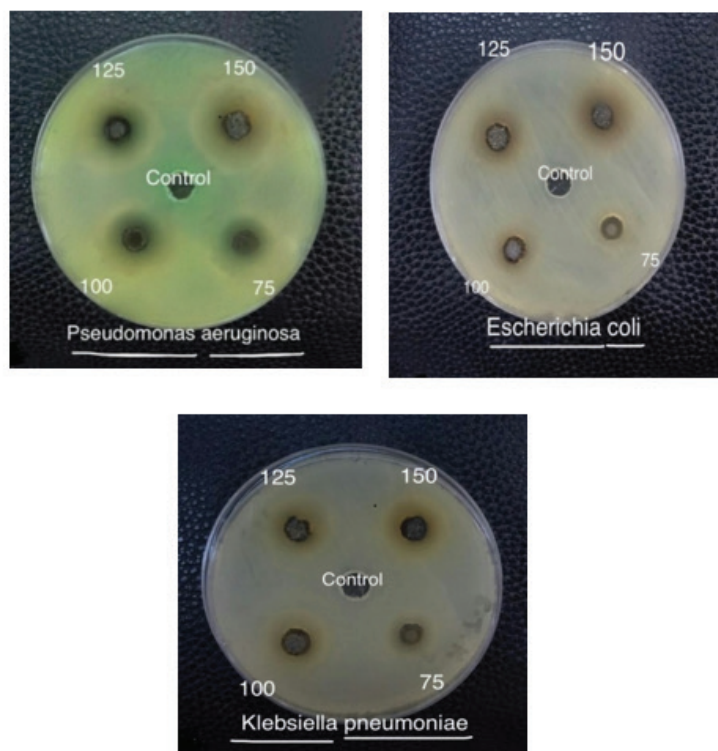
**Figure (2): Antibacterial activity of *Lymnaea auricularia* snail shell powder against bacterial isolates**

Table (2): Effect of different concentration of snail tissue powder on bacteria

Pathogenic Bacteria	Concentration & inhibition zone			
	75	100	125	150
Klebsiella pneumonia	12	15	16	16.2
Pseudomonas aeruginosa	15	22	25	28
Escherichia coli	12	12.5	16	20

At 75mg/ml *P. aeruginosa* (15 mm), both of *E. Coli* and *K. pneumonia* were (12 mm). At 100mg/ml concentration *P. aeruginosa* (22 mm), *K. pneumonia* (15 mm) and *E. Coli* (12.5 mm). The concentration of 125mg/ml showed activity in *P. aeruginosa* (25 mm), both of *K. pneumonia* and *E. Coli* were (16 mm). The highest activity appeared in *P. aeruginosa* (28 mm) at 150mg/ml concentration followed by *E. Coli* (20 mm) then *K.pneumonia* (16.2 mm) as shown in table (2) and figure (3).

**Figure(3): Antibacterial activity *Lymnaea auricularia* snail tissue powder against bacterial isolates**

Discussion

Molluscs are commonly used for various studies in international research institutions, but those possess be situated identified as per possibility origin about antiseptic and antimycotic metabolites only recently. It has identified and characterized some of the molecules

involve in the antimicrobial activities⁽¹⁷⁾.

Every year, extra than 100 novel antimicrobial composites own stayed isolated as of aquatic, animal lacking a backbone like snail and bivalves, which exhibit a wide range of antimicrobial properties^(18,19). extra than 1,000 novel composites own be situated identified as of

aquatic animal lacking a backbone for instance peptides, terpenes, polypropionates, nitrogen component, polypeptides, macrolides, prostaglandins also greasy acidulous output, sterols and other components⁽¹⁹⁾.

In this study the highest activity of snail shell powder appeared in *E. coli* (19mm), and the highest activity of snail tissue powder exhibited in *P. aeruginosa* (28mm). This powder may have the potential to destroy the bacterial cell by inhibiting gene expression or have the ability to destroy cell membrane by pore formation, altering the level of intracellular ions or changing the trans membrane potential.

Agreed with Lekshmi *et al.*⁽¹⁴⁾ who investigated the antibacterial activity of *Pomacea insularium* snail tissue powder against (*Klebsiella sp*, *E. coli*, *Pseudomonas sp*, *Proteus sp*, *Salmonella sp*, *Aeromonas sp*, *Streptococcus sp*, *Staphylococcus sp*, *Bacillus sp* and *Enterobacter sp*). the maximum activity was found against *Proteus sp*. (30.16±0.76 mm), agreed by Elezabeth *et al.*⁽²⁰⁾ Record peak antibacterial activity against *klebsiella sp* and *Enterobacter sp* (20 mm).

The aim of the research by Darwin *et al.*⁽²¹⁾ be situated to explore the germicide vigor of *Purpura bufo* aquatic gastropod similar with this study. Estimated antimicrobial activity in *Purpura bufo* body tissue extracts against *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae*, the extreme suppression region stayed detected in *K. pneumonia* (26 mm). The *Purpura bufo* species displayed possible antimicrobial activity against pathogenic microorganisms.

Gayathri *et al.*⁽²²⁾ concluded that *Pila viren* freshwater snails contain different bioactive compounds (proteins, peptides and sterols) and may be recommended as pharmaceutical-relevant freshwater snail.

Disagreed with Anand & Edward⁽²³⁾ examined five *Cypraea* species (*Cypraea errones*, *C. arabica*, *C. onyx*, *C.tigris* and *C.vitellus*) which stayed tested for those antiseptic and antimycotic effectiveness, the water solvent showed no activity against *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Escherichia coli*.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of

both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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