



## Antibacterial activity of methanol and water extract of *Loligo duvauceli* ink

<sup>1</sup> Nisha N, <sup>2</sup> Dr. S Suja

<sup>1</sup> Research Scholar, Research and Development Centre, Bharathiar University, Coimbatore, Tamil Nadu, India

<sup>2</sup> Associate Professor, Department of Biochemistry, Bharathiar University, Coimbatore, Tamil Nadu, India

### Abstract

The present study has been under taken to find out the antibacterial activity of methanol and water extract of *Loligo duvauceli* ink. The antibacterial activity was screened against *E. coli* and *S.aureus*. Different concentrations was prepared and tested the bacterial strains for their inhibitory activities using simple disc diffusion method and their percentage of inhibition was calculated. Among the methanol and water forms the water form shows more pronounced antibacterial effect against *E.coli* and less activity in *S.aureus*.

**Keywords:** *loligo duvauceli*, antibacterial activity, percentage of inhibition

### 1. Introduction

Our marine environment is a rich source of biologically and structurally different active natural products, many of them have not been found in terrestrial sources. In medicinal science fields the marine organisms had a very impact and the recent studies mainly focused on their application for the treatment of human diseases [1]. Cephalopod included in the Mollusca group and that group are comprising about 700 species, in which the bacterial association have been known for a long time and can include the reproductive organs (accessory indumenta gland) of myopsids and sepiids and the light organ of sepiolids [2]. Cephalopods are notable for their defense such as jetting escape movements change in coloration, toxic venom and inking [3]. Cephalopod ink is a chemical secretion and which produced and released from ink sac, not a homolog of the ink glands of gastropod but a modified hypobranchial gland [4], organ of defense against predators. In recent years physical activities of cuttle fish and squid ink, like antitumor immunity promotion, antibacterial antioxidant activities has been widely studied [5]. The consumption of cephalopods has recently increased in countries that were traditionally cephalopod consumers, mainly as chilled and frozen meals [6]. General handling processing preservation mainly based on squid which is a major cephalopod species. Inking by cephalopod has been recognized as an adaptive response against predation and physical treat by means of combination of mechanisms which includes sensory disruption and phago mimicry [7]. In Japan the *Loligo duvauceli* ink and cattle fish ink has a traditional application in food products and medicinal application. The present study was carried out the antibacterial activity against two bacterial strains namely *E. coli* and *S. aureus*.

### 2. Materials and methods

The fresh *Loligo duvauceli* was collected from Beypore fort by trawl catch and brought in to the laboratory and cleaned and washed with fresh water to remove impurities. The ink sac

were dissected from the anterior part and the ink was collected by gently squeezing the glands and was air dried using oven. The extraction were done by parallel extraction 25 ml of squid ink with 75 ml of methanol in a sterile glass rod and refrigerated at 4°c for 72 hours<sup>8</sup>. Each preparation was filtered and concentrated under vacuum. The methanol extract was collected and checked the sterility and stored at 4° c in brown bottle. The *Loligo duvauceli* ink was diluted with water and centrifuged and the supernatant was stored for further analysis.

#### 2.1 Bacterial culture

Two bacterial strains *E. coli* and *S. aureus* are used for studying these were clinically isolated from Calicut medical college laboratory.

#### 2.2 Inoculum preparation

Nutrient broth was prepared and sterilized in an autoclave at 15lbs pressure for 15 minutes. The bacterial strains are individually inoculated in the sterilized broth and incubated at 37°c for 24 hours. Himedia (nutrient agar) was prepared and sterilized in an autoclave at 15 lbs for 15 minutes which is then poured into sterile petridishes and incubated 37° c for 24 hours, the bacterial broth cultures was then inoculated in the petridishes using sterile cotton swab.

#### 2.3 Antibacterial activity by disc diffusion method

The antibacterial activity was carried out by agar disc diffusion method. The Himedia was transferred aseptically into sterilized petriplates. The plates were left at room temperature for solidification. Each plate a single well was made by using a sterile borer. The extract were reconstituted with solvents and tested at various concentrations. The samples were introduced into the well by using a micropipette. Then the plates were incubated overnight at 37°c. The bacterial growth was determined by measuring the diameter zone of inhibition, silver nitrate is used as a positive control

and water is used as negative control.

### 2.4 Determination of percentage of inhibition

The methanol and water extract of *Loligo duvauceli* showed significant antibacterial activity. The percentage of inhibition concentration values were determined using the method of two fold serial dilution. The nutrient broth, the tested samples and controls were inoculated and the bacterial strains were incubated for 24 hours at 30°C, and the growth was monitored visually and spectrophotometrically. The minimum inhibitory concentration was defined as the low concentration required to arrest the growth of bacteria at the end of 24 hours of incubation.

### 3. Result and discussion

The antibacterial activity of methanol and water extract of *Loligo duvauceli* ink was evaluated against two bacterial strains *E. coli* and *S. aureus*. The highest inhibition zone was recorded in *E. coli* in water extract of *Loligo duvauceli* ink at concentration 200mg and which was 22mm and for *S. aureus* 15mm. the diameter zone of inhibition for water extract having the concentration 100mg for *E. coli* and *S. aureus* recorded as 20mm and 15mm respectively and the diameter zone inhibition having 50mg of water extract of *Loligo duvauceli* ink was recorded as 11 for *E. coli* and 10 for *S. aureus*, which was represented in table 1. The methanol extract of *Loligo duvauceli* ink shows no activity for *E. coli* and *S. aureus*

The percentage of inhibition zone was recorded and which represented in table 2, the percentage of inhibition for water extract at concentration 200mg for *E. coli* recorded as 62.85 and for *S. aureus* recorded as 60. Percentage of inhibition for water extracts *Loligo duvauceli* ink at concentration 100mg 62.5 for *E. coli* and 42.85 for *S. aureus*, percentage of inhibition at concentration 50 recorded as 39.28 for *E. coli* and 40 for *S. aureus*. Whereas for methanol extract was not seen to inhibit the growth for *E. coli* and *S. aureus* was represented in table 2. Compared to methanol extract of *Loligo duvauceli* ink, the water extract has more antibacterial activity and percentage of inhibition for *E. coli* and *S. aureus*

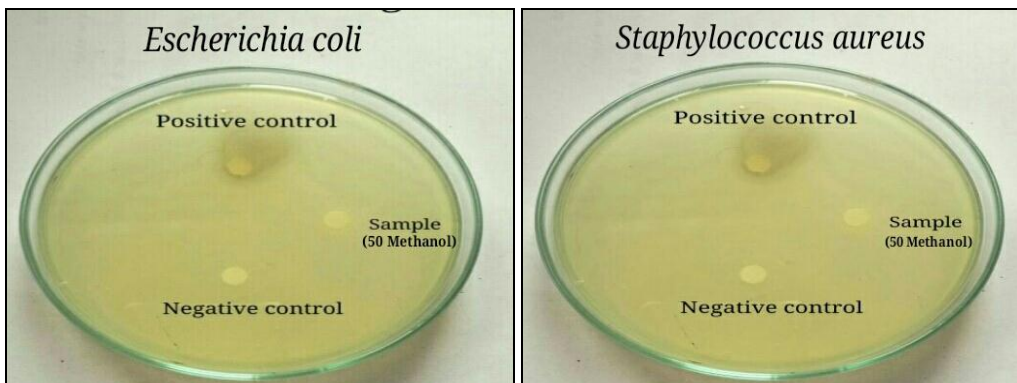
**Table 1:** diameter zone of inhibition for test organisms

Test organisms	<i>Loligo duvauceli</i> ink water extract			<i>Loligo duvauceli</i> ink methanol extract		
	Concentration(mg/ml)			Concentration(mg/ml)		
	50mg	100mg	200mg	50mg	100mg	200mg
<i>E. coli</i>	11mm	20mm	22mm	0	0	0
<i>S. aureus</i>	10mm	15mm	15mm	0	0	0

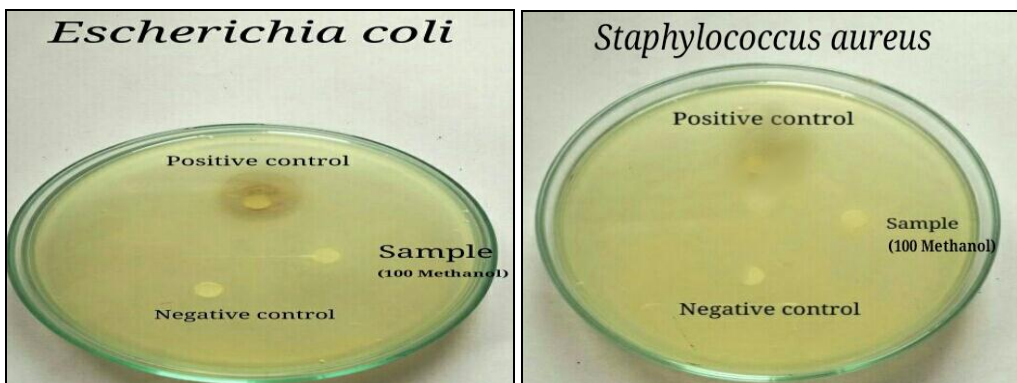
**Table 2:** percentage of inhibition

Test organisms	<i>Loligo duvauceli</i> ink water extract			<i>Loligo duvauceli</i> ink methanol extract		
	Concentration(mg/ml)			Concentration(mg/ml)		
	50mg	100mg	200mg	50mg	100mg	200mg
<i>E. coli</i>	39.28	62.5	62.85	0	0	0
<i>S. aureus</i>	40	42.85	60	0	0	0

Pictures for diameter zone of inhibition for methanol extract of *Loligo duvauceli* ink



**Fig 1**



**Fig 2**

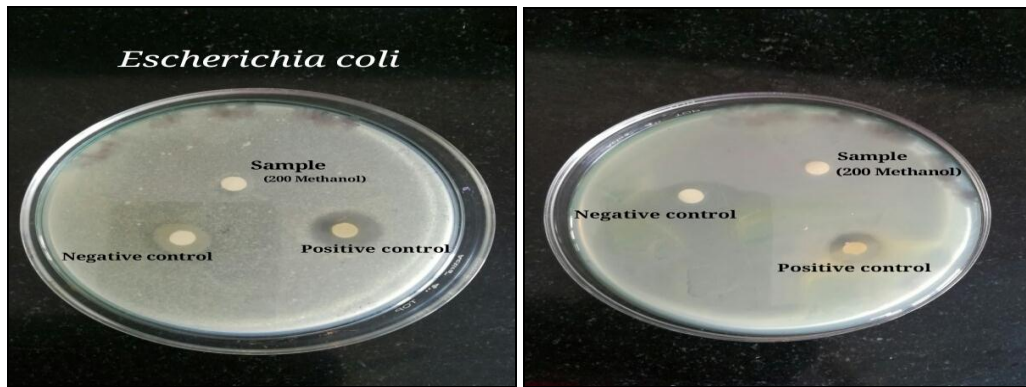


Fig 3

Pictures for diameter zone of inhibition for water extract of *Loligo duvauceli* ink:

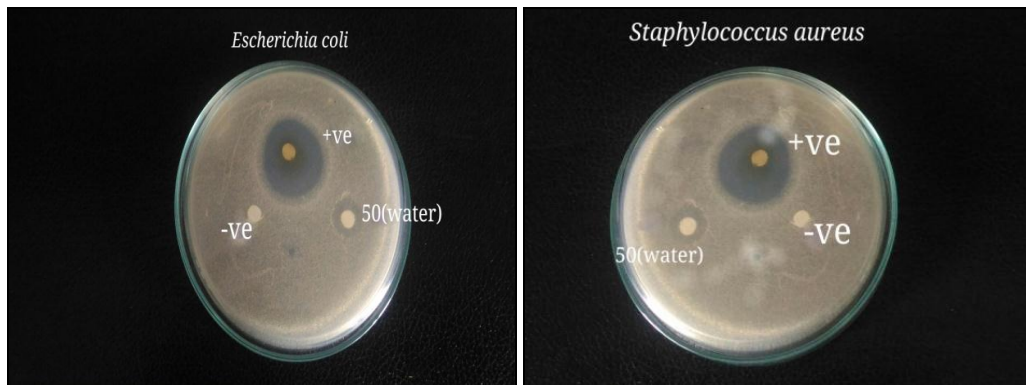


Fig 4

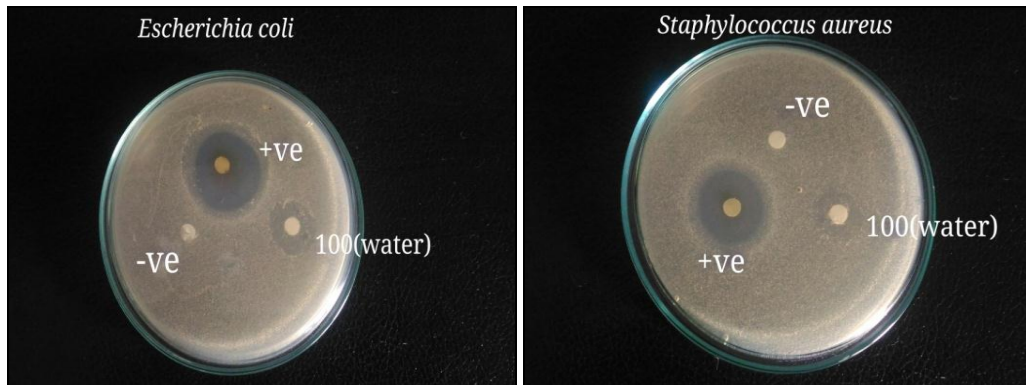


Fig 5

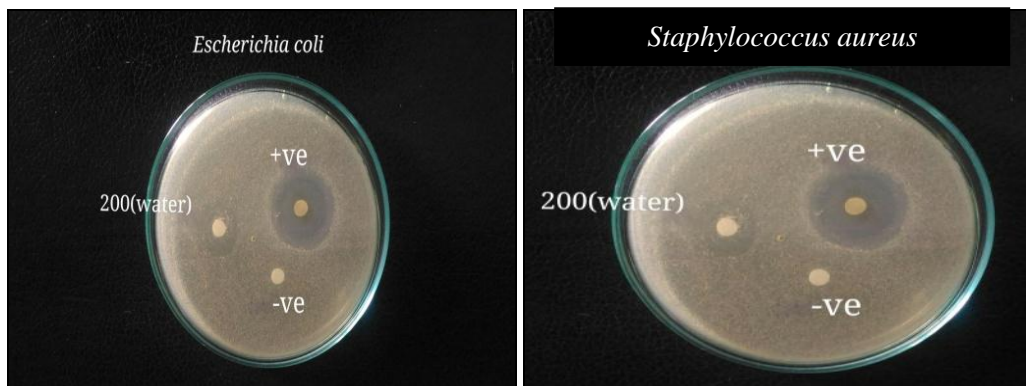


Fig 6

#### 4. Conclusion

In this studies the antibacterial activity of *Loligo duvauceli* ink in methanol and water extract for *E. coli* and *S. aureus* were tested, compared to methanol extract water have more antibacterial activity. The studies related to antioxidant and anticancer activity of *Loligo duvauceli* ink are going on. Thus this study recommends the use of squid ink has available biopharmaceutical product with antibacterial activity.

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