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# HPTLC ANALYSIS AND ANTIMICROBIAL ACTIVITY OF ETHYL ACETATE EXTRACT OF SEA URCHIN (*ARBACIA LIXULA*) SHELL

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## ABSTRACT

The study aimed to find out the phytochemical expression in shell of sea urchin *Arbacia lixula* by using the solvent ethyl acetate. The ethyl acetate extract was obtained and the presences of phytochemical or micro molecules were found by the HPTLC. Further, the extract was analyzed for its antimicrobial activity against the human pathogens both bacteria and fungi. The growth inhibition of bacteria and fungi was determined using the solvent extract and where the ability was compared with commercial available drugs. The minimum inhibitory concentration of the solvent extract was found against the bacteria and fungi used. As a result, the HPTLC showed 5 spot with different R*f* values which shows the shells of sea urchin also rich with phytochemicals or micro molecules.

**Keywords:** Sea urchin, *Arbacia lixula*, Ethyl Acetate, HPTLC and Antimicrobial Activity

## 1. INTRODUCTION

Phytochemicals are the guardian of our health. It is commonly known that these phytochemicals also are rich and obtained from all plant sources [1, 2]. Many researches says these component possess properties that produce obvious and significant effect in animals [3, 4] and have been created various positive and negative impacts on human health [5-8]. The rate of isolation, purification of various phytochemicals are increased among the researchers [9] and pharmaceutical industries from plant source and these phytochemicals are used as an effective medicine or supplement for human health [10]. But still extracting individual phytochemicals is a challenging promise for researchers and other industries [11].

Phytochemicals are considered to be more important in human benefits since it has innumerable biological properties. It is considered to be a rich antioxidant which traps or scavenge the free radicals with its potential ability of charged particles [12, 13]. It acts effectively against many infectious microbes- both bacteria and fungi [14]. Many studies say it also have antiviral effect [15]. Studies also say it act against various cancer cells and multitudinously *in-vitro* and *in-vivo* studies were carried out in order to prove its strength of ability against cancer [16].

Since it is difficult to isolate a specific phytochemical separately from a plant extract, researchers are more passionate to extract mixture of phytochemicals together by aqueous or solvent as crude. The crude was subjected to characterization to check whether the crude contains phytochemicals by various techniques. The biological activity of the crude phytochemical is more effective than the individual isolation. And to our common understanding, the phytochemicals are present in plant materials and the studies also carried out only targeting the plant material. Hence this present study is focused differently to know whether any phytochemicals or any other compound other than calcium, carbohydrate and protein is present the shell of sea urchin *Arbacia lixula* by extracting the crude using ethyl acetate. The crude was subjected to antimicrobial activity to find the active participation of crude against microbes such as bacteria and fungi.

## 2. MATERIALS AND METHODS

### 2.1. Collection of samples

The shells along with spines of sea urchin *Arbacia lixula* which is considered as waste material was collected along the sides of seashore of Mandapam coastal region, Rameshwaram. The collected shells were tightly packed with sea water in an air tight plastic bag was brought to research laboratory. The spines were separated from the shells and were washed with running tap water to

remove sand, mud and other debris followed by distilled water and finally rinsed with double distilled water. The shells are allowed to air dry in shade at normal room temperature then, finely powdered in blender sieved and stored into deep freezer (-20 °C) for further study.

### 2.2. Preparation of crude extract

Fifty gram of finely powdered shells was subjected to condensation and evaporation in soxhlet apparatus along with the solvent ethyl acetate with boiling temperature of 75-78°C for 72 hours. The crude along with solvent obtained was condensed using rotary evaporator in order to obtain ethyl acetate crude. The crude obtained was weighed and stored.

### 2.3. Preparation of Standard and Sample Solutions HPTLC

A stock solution was prepared by weighing 1mg of solvent extract in 1mL of solvent ethyl acetate and completely dissolved. The TLC was done in order to separate the fractions with solvent ratio of 8:2 (Chloroform: Acetone) and subjected to HPTLC.

HPTLC Finger Printing Chromatography was implemented on 3 × 10 cm HPTLC (Merck, Germany). The plate was prewashed with methanol and activated at 110°C for 5 min. The etethyl acetate extracted sample was applied as 4 mm bandwidth using a Camag (Muttenz, Switzerland) Linomat IV sample applicator equipped with 100 µl Syringe. A constant application rate of 5 µl/sec was used. Mobile phase was methonal:chloroform (1:9) and chromatogram were scanned at 485 nm [17].

### 2.4. Preparation of Test Organism

The bacterial strains included Gram positive *Staphylococcus aureus*; Gram negative *Escherichia coli, Klepsiella pneumoniae* and *Pseudomonas aeruginosa* and clinical fungi isolates of *Candida albicans, Cryptococcus neoformans, Aspergillus fumigatus, Histoplasma capsulatum*. All microorganisms were obtained from the Microbiology lab, Voluntary Health Service, Taramani, Chennai, Tamil Nadu, and India. The pathogens were very carefully identified using standard microbiological method. All bacterial strains were maintained in MHA slants, stocks were stored at -20 °C and the fungi were maintained on Sabouraud dextrose agar (SDA) slants at 10 °C until use and all strains were sub-cultured monthly throughout this study.

### 2.5. Antimicrobial activity

Antimicrobial activity of the ethyl acetate extract from the shell of sea urchin were assessed using well diffusion and the minimum inhibitory concentration (MIC) was determined by examining the inhibition zones of bacterial and fungal growth [18].

### 2.6. Determination of antimicrobial activity by well diffusion method

An agar-well diffusion method was employed for determination of antimicrobial activities [19]. The ethyl acetate extract of shells of sea urchin samples were dissolved in ethyl acetate to the final concentration of 100 mg/mL and sterilized by filtration through 0.22 µm sterilizing Millipore filter. All bacterial species were suspended in peptone water and diluted to ~$10^6$ CFU/mL. The bacterial suspension (100 µL) was spread onto the surface of Mueller Hinton agar medium whereas the fungal suspension was spread onto surface of Sabouraud dextrose agar medium. Wells were made in each plate using 5 mm diameter of sterile gel puncher and different concentration of 100 µL extract solutions were drop-shipped into each well. Negative controls were prepared using PBS solution and Gentamicin (100 µg/well) as positive control for bacteria and Clotrimazole (100 µg/well) as positive control for fungi. The inoculated plates of bacteria were incubated at 35 °C for 24 h and fungi at 35°C for 48 h. Antibacterial and antifungal activity was evaluated by measuring the diameter inhibition zone (DIZ) using zone of inhibition scale in mm. Experiment was performed in triplicate and in sterile condition.

### 2.7. Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) was performed according to the standard reference method NCCLS. The extracts were dissolved in ethyl acetate (stock solution). A stock solution of the extract was serially diluted in 96-well microtiter plate with Mueller Hinton broth for bacteria and Sabouraud dextrose broth for fungi to obtain a concentration ranging from 500 mg/mL to 10 mg/mL. A standardized inoculum for each bacterial strain and fungal strain was prepared so as to give an inoculum size of $10^5$ CFU/mL in each well. Gentamycin and Clotrimazole were used as a standard antibiotic for comparative analysis with the effectiveness of ethyl acetate extracts against tested clinical isolates. 96 well-plates was kept at 37°C and incubated for 24 h.

## 3. RESULT & DISCUSSION

### 3.1. HPTLC

In the HPTLC fingerprinting of ethyl acetate extract gave five spots at different Rf values Figure 1. Purity of the

sample extract was confirmed by comparing the absorption spectra at start, middle and end position of the band (Table 1). HPTLC is an irreplaceable quality assessment tool for the assessment of compounds in crude extract. It allows for the analysis of a broad number of compounds both efficient and cost effective. The corresponding HPTLC chromatograms are presented in (Fig. 2).

**Table 1: Absorption spectra at start, middle and end position of the band obtained from ethyl acetate extract of *Arabacia lixula* shell**

| Peak | Start | | Maximum | | | End | | Area | Area % | Assigned substance |
|---|---|---|---|---|---|---|---|---|---|---|
| | Rf | Height | Rf | Height | % | Rf | Height | | | |
| 1 | -0.07 | 3.7 | 0.11 | 156.6 | 14.87 | 0.21 | 1.7 | 17618.6 | 17.79 | Unknown* |
| 2 | 0.25 | 3.0 | 0.28 | 12.6 | 1.19 | 0.30 | 6.6 | 308.6 | 0.31 | Unknown* |
| 3 | 0.32 | 0.6 | 0.45 | 283.1 | 26.88 | 0.55 | 237.6 | 26086.9 | 26.33 | Unknown* |
| 4 | 0.60 | 229.0 | 0.74 | 327.1 | 31.06 | 0.82 | 269.7 | 40689.7 | 41.08 | Unknown* |
| 5 | 0.88 | 273.1 | 0.88 | 273.8 | 26.00 | 0.99 | 59.5 | 14356.1 | 14.49 | Unknown* |

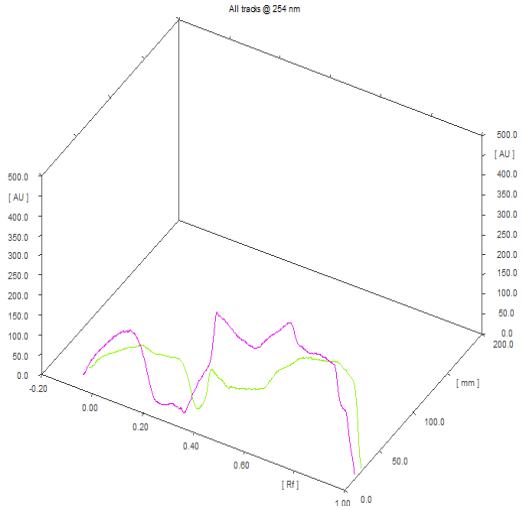


**Fig. 1: Densitograms of Sea Urchin *Arbacia lixula* shell ethyl acetate extract scanned at 485 nm**

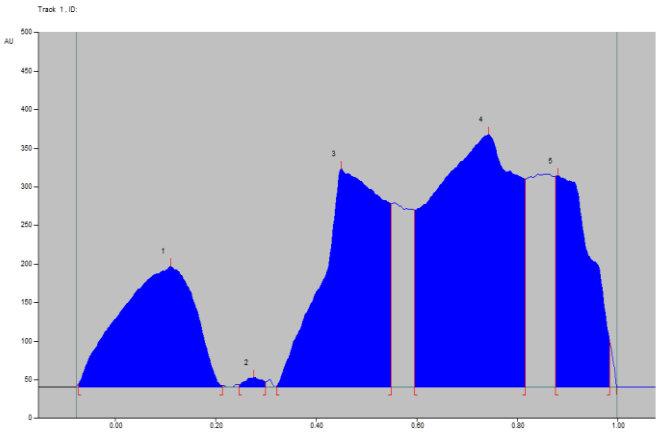


**Fig. 2: HPTLC fingerprinting profile of ethyl acetate extract of *Arabacia lixula* shell**

### 3.2. Antimicrobial activity

The ethyl acetate extract isolated from the sea urchin (*Arbacialixula*) shell was screened for antimicrobial activity in bacteria as well as fungi and the minimum inhibitory concentration of the extract for all the microbes were screened and compared with the commercial available drugs.

The antibacterial activity of the ethyl acetate shell extract against 4 human pathogenic bacteria was tabulated in Table 2. The maximum zone of inhibition was taken to consideration at a concentration of 500mg/mL and measured using zone scale was compared with Gentamycin. The values of the extract was not upto the bench mark as like Gentamycin but the extract showed minimal activity against *S. aureus, E. coli, K. pneumonia, P. aeruginosa* whose zone of inhibition was between 10-15 mm at the concentration of 500 mg/mL.

The antifungal activity of the shell ethyl acetate extract against selected fungi was tabulated (Table 3). The zone of inhibition was very less when compared to the commercial available drug Clotrimazole. The zone of inhibition of ethyl acetate extract was between 5 – 13mm in diameter whereas for *A. fumigatus,* the extract was absent to show zone of inhibition which proves there is no action of antifungal activity of the extract against the fungi *A. fumigatus*.

The zone of inhibition in fungi observed as in descending order such as *H. capsulatum* (13mm)>*C. albicans* (8mm) >*C. neoformans* (5mm). Hence with the present study it is clear that antifungal property of the extract is very less which states the inhibition rate of extract against bacteria and fungi was taken place with milligram amount. When compared the inhibition rate against bacteria was pointable with few mg of the extract used, when compared to the inhibition rate of extract amount against fungi. The results were also compared with the Gentamycin and Clotrimazole in µg/mL.

**Table 2: Antibacterial activity of ethyl acetate extract of *Arbacia lixula* shell**

| Compound | Maximum Zone of inhibition (mm) | | | |
|---|---|---|---|---|
| | *S. aureus* | *E. coli* | *K. pneumoniae* | *P. aeruginosa* |
| Ethyl acetate Extract (mg/ml) | 13 | 12 | 13 | 10 |
| Gentamycin | 28 | 32 | 24 | 29 |

**Table 3: Antifungal activity of ethyl acetate extract from *Arbacia lixula* shell**

| Compound | Maximum Zone of inhibition (mm) | | | |
|---|---|---|---|---|
| | *C. albicans* | *C. neoformans* | *A. fumigatus* | *H. capsulatum* |
| Ethyl acetate Extract (mg/ml) | 08 | 05 | NZ | 13 |
| Clotrimazole | 18 | 25 | 22 | 28 |

**Table 4: MIC result ethyl acetate extract of *Arbacia lixula* shell against bacteria**

| Compound | *Minimum inhibitory concentration | | | |
|---|---|---|---|---|
| | *S. aureus* | *E. coli* | *K. pneumoniae* | *P. aeruginosa* |
| Ethyl acetate Extract (mg/ml) | 43 | 79 | 47 | 83 |
| Gentamycin | 54 | 16 | 42 | 81 |

**Table 5: MIC result ethyl acetate extract of *Arbacia lixula* shell against fungi**

| Compound | Maximum Zone of inhibition (mm) | | | |
|---|---|---|---|---|
| | *S. aureus* | *E. coli* | *K. pneumoniae* | *P. aeruginosa* |
| Ethyl acetate Extract (mg/ml) | 121 | 203 | ND | 117 |
| Clotrimazole | 33 | 49 | 27 | 58 |

The minimum inhibitory activity of the ethyl acetate shell extract of sea urchin was determined and the values were found to follow in mg/mL whereas the commercial available drugs were proved to be active in µg/mL. The present study results are more or less similar with previous study in which the methanol extract of shells of purple sea urchin (*Salmacis virgulata*) was used as an antimicrobial agent against different strains of bacteria and fungi but this study doesn't show any evidence of presence of any compounds with any analytical method [20]. No other study was done with the solvent extract isolated from the shells of any marine organisms.

## 4. CONCLUSION

The results of this study proved that not only the plant materials contain phytochemicals but also animal waste too possesses that specificity.

The ethyl acetate extract from the shells of sea urchin *Arbacia lixula* showed antibacterial and antifungal activity against clinical pathogens. The activity was comparatively less when compared to the phytochemicals isolated from plant source. A deep study is needed to find the phytochemical individually present in the extract so that in future the waste shells along the seashore can be effectively used for human welfare.

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## 6. REFERENCES

1. Azwanida NN. *Med Aromat Plants*, 2015; **4(196)**:2167-0412.
2. Kaisar MA, Rahman MS, Rahman MZ, Hasan CM, Rashid MA. *J PharNutri. Sci*, 2011; **1**:87-95.
3. Kwon KH, Barve A, Yu S, Huang MT, Kong AN T. *Acta Pharmacologica Sinica*, 2007; **28(9)**:1409-1421.
4. Surh YJ. *Nature Reviews Cancer*, 2003; **3(10)**:768.
5. Awika, Joseph M, Lloyd W Rooney. *Phytochemistry,* 2004; **65(9)**:1199-1221.

6. Brandt K, Christensen LP, Hansen-Møller J, Hansen SL, Haraldsdottir J, Jespersen L, Kobæk-Larsen M. *Trends in Food Science & Technology*, 2004; **15(7-8)**:384-393.
7. Seeram NP. *Journal of Agricultural and Food Chemistry*, 2008; **56**:627-629.
8. Rochfort S, Panozzo J. *Journal of Agricultural and Food Chemistry*, 2007; **55(20)**:7981-7994.
9. Sasidharan S, Chen Y, Saravanan D, Sundram K M, Latha L Y. *African Journal of Traditional, Complementary and Alternative Medicines*, 2011; **8(1)**:1-10.
10. Raskin I, Ribnicky DM, Komarnytsky S, Ilic N, Poulev A, Borisjuk N., O'Neal JM. *TRENDS in Biotechnology*, 2002; **20(12)**:522-531.
11. Corrêa RC, Peralta RM, Haminiuk CW, Maciel G M, Bracht A, Ferreira IC. *Critical reviews in food science and nutrition*, 2018; **58(6)**:942-957.
12. Prior RL, Cao G. *HortScience*, 2000; **35(4)**:588-592.
13. Ayoola GA, Coker HA, Adesegun SA, Adepoju-Bello AA, Obaweya K, Ezennia EC, Atangbayila TO. *Tropical Journal of Pharmaceutical Research*, 2008; **7(3)**:1019-1024.
14. Igbinosa OO, Igbinosa EO, Aiyegoro OA. *African Journal Of Pharmacy and Pharmacology*, 2009; **3(2)**: 058-062.
15. Naithani R, Huma LC, Holland LE, Shukla D, McCormick DL, Mehta RG, Moriarty R M. *Mini Reviews In Medicinal Chemistry*, 2008; **8(11)**:1106-1133.
16. Liu RH. *The Journal of Nutrition*, 2004; **134(12)**: 3479S-3485S.
17. Kumar D, Sharma U. *Separation Science Plus*, 2018; **1(2)**:100-134.
18. National Committee for Clinical Laboratory Standards. Approved standard M38-A. Wayne, PA: 2002.
19. National Committee for Clinical Laboratory Standards (1999) Nineth Information Supplement, M100–S9. Wayne, PA: NCCLS.
20. Shankarlal S, Prabu K, Natarajan E. *Am-Euras J Sci Res*, 2011; **6**:178-81.