

# Estimation of Heavy Metals and Chemical Constituents in a Colonial Ascidian DIDEMUM PSAMMATHODES

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**Abstract:--** The study aims at estimating the heavy metals and chemical constituents in the marine colonial ascidian *Didemnum psammathodes*. Accumulation of heavy metals like copper, cadmium, lead, arsenic, zinc and mercury were determined. Analysis of the heavy metal in the selected animal sample was performed by atomic absorption spectrophotometer (AAS). Measurements were made using a hollow electron discharge lamp (EDL) for copper, cadmium, lead, arsenic, zinc and mercury at wavelengths of 220.62 nm, 228.80 nm, 283.31 nm, 193.70 nm, 240 nm and 253.7 nm respectively. Chemical constituents were analysed by spectrophotometric methods. This study confirms that the risk of heavy metals contamination in the ascidian appears low when compare to high amount of phenols and flavonoids.

**keywords:--** *Didemnum psammathodes*, ascidian, heavy metal

## INTRODUCTION

Ascidians are marine sedentary organisms and they belong to biofouling community. *Didemnum psammathodes* is a colonial ascidian belonging to the family Didemnidae. Ascidians are consumed as food in many parts of the world and there are coastal aqua farms in Japan as well as Thailand for the culture of ascidians. *Microcosmus sulcatus*, *Styela plicata* and *Polycarpa pomaria* are taken as food in the Mediterranean.[1] *Halocynthia roretzi* in Japan, is even cultured in the North of Honsyu[2] for human consumption and *Pyura chilensis* is popular in South America[3] as a food source. Margalino and Destefano found that the flesh of *Microcosmus sulcatus* is almost as digestible as whole egg and the protein content higher[4]. Antioxidants play a significant role in the prevention of diseases and do have a capacity to reduce oxidative stress by chelating trace elements or scavenging free radicals and protecting antioxidant defenses [5]. Various species of ascidians from Indian water has been proved to exhibit potent pharmacological activities [6-40]. No reports are available for the determination of heavy metal in a colonial ascidian *Didemnum psammathodes*. The aim of this work is to investigate the magnitude of heavy metals contamination and chemical constituents in the animal under study by Atomic Absorption Spectroscopy and spectrophotometric studies.

## MATERIALS AND METHODS

### *Collection and identification*

*Didemnum psammathodes* (Fig.1) was collected from Green Gate area (8°48'N and 78°11'E) of Thoothukudi Port, Tamil Nadu by SCUBA diving and identified using Key to identification of Indian ascidians.[41] A voucher specimen was deposited in the Museum of the Department of Zoology, A.P.C. Mahalaxmi College for Women, Tuticorin 628002, Tamilnadu, India.



**Figure 1: colony of *Didemnum psammathodes***

### *Preparation of extract*

The whole animal was dried in shade and homogenized to get a coarse powder. The powder was successively extracted

with various solvents such as petroleum ether (400-600 C), benzene, chloroform, ethanol, methanol and water.

#### **Standard preparation**

The selected metals were copper, cadmium, lead, arsenic, zinc and mercury. For each of the selected metals a standard linear calibration curve of various concentrations ranging from 0.5000 ppm, 1.0000 ppm and 1.5000 ppm (three points) were analysed by AAS and used as the stock solutions in a quartz flask.

#### **Instrumentation**

Analysis of the heavy metal in the selected animal sample was performed by Varian model AA 240 FS atomic absorption spectrophotometer (AAS). Measurements were made using a hollow electron discharge lamp (EDL) for copper, cadmium, lead, arsenic, zinc and mercury at wavelengths of 220.62 nm, 228.80 nm, 283.31 nm, 193.70 nm, 240 nm and 253.7 nm respectively. Analysis was performed by testing samples at three different concentrations 0.5000 ppm, 1.0000 ppm and 1.5000 ppm to ensure that the method has wide adaptability and good accuracy.

#### **Chemical constituents**

##### **Quantitative Estimation of Alkaloids**

To 1ml of methanolic extract 5 ml pH 4.7 phosphate Buffer was added and 5 ml BCG solution and shake a mixture with 4 ml of chloroform. The extracts were collected in a 10-ml volumetric flask and then diluted to adjust volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm against blank prepared as above but without extract. Atropine is used as a standard material and compared the assay with Atropine equivalents.

##### **Quantitative estimation of Saponins**

Ethanollic and aqueous extract was dissolved in 80% methanol, 2ml of vanilin in ethanol was added, mixed well and the 2ml of 72% sulphuric acid solution was added, mixed well and heated on a water bath at 600°C for 10min, absorbance was measured at 544nm against reagent blank. Diosgenin is used as a standard material.

##### **Quantitative estimation of Phenolic Compounds**

The total phenolics content in different solvent extracts was determined with the Folin- Ciocalteu's reagent (FCR).

Different concentrations of the extracts were mixed with 0.4 ml FCR (diluted 1:10 v/v). After 5 min 4 ml of sodium carbonate solution was added. The final volume of the tubes were made upto 10 ml with distilled water and allowed to stand for 90 min at room temperature. Absorbance of sample was measured against the blank at 750 nm using a spectrophotometer. A calibration curve was constructed using catechol solutions as standard and total phenolic content of the extract was expressed in terms of milligrams of catechol per gram of dry weight.

##### **Quantitative Estimation of Steroids**

1ml of ethanolic extract of steroid solution was transferred into 10 ml volumetric flasks. Sulphuric acid (4N, 2ml) and iron (III) chloride (0.5% w/v, 2 ml), were added, followed by potassium hexacyanoferrate (III) solution (0.5% w/v, 0.5 ml). The mixture was heated in a water-bath maintained at 70±20C for 30 minutes with occasional shaking and diluted to the mark with distilled water. The absorbance was measured at 780 nm against the reagent blank.

##### **Quantitative Estimation of flavonoids**

Total flavonoid content was determined by Aluminium chloride method using catechin as a standard. 1ml of test sample and 4 ml of water were added to a volumetric flask (10 ml volume). After 5 min 0.3 ml of 5 % Sodium nitrite, 0.3 ml of 10% Aluminium chloride was added. After 6 min incubation at room temperature, 2 ml of 1 M Sodium hydroxide was added to the reaction mixture. Immediately the final volume was made up to 10 ml with distilled water. The absorbance of the reaction mixture was measured at 510 nm against a blank spectrophotometrically. Results were expressed as catechin equivalents (mg catechin/g dried extract).

## **RESULTS AND DISCUSSION**

The results of heavy metals determination in a selected colonial ascidian *Didemnum psammathodes* was presented in Table 1.

**Table 1. Contamination levels of heavy metals in *Didemnum psammathodes***

| <b>Copper</b> | <b>Cadmi<br/>um</b> | <b>Lead</b> | <b>Arsen<br/>ic</b> | <b>zinc</b> | <b>Merc<br/>ury</b> | <b>MDLa</b>   |
|---------------|---------------------|-------------|---------------------|-------------|---------------------|---------------|
| ND            | ND                  | ND          | ND                  | ND          | ND                  | 0.03mg/<br>kg |

## REFERENCES

ND: Not detected; MDL: Minimum detection limit; an=3.

Atomic absorption spectrometry detection was carried out on positive ionisation mode because this mode gave sharp and sensitive signals. It was optimised by using a standard linear calibration curve for various concentrations ranging from 0.5000 ppm, 1.0000 ppm and 1.5000 ppm (three points). The calibration curves were constructed by plotting the response against the concentration. A linear relationship was obtained for each compound. The heavy metals (cadmium, lead, arsenic, and mercury) were analysed at their particular wavelength and the ion with the uppermost intensity was selected as the basic ion. The study revealed that no resultant spectral peaks of Cd, Pb, As and Hg in *Didemnum psammathodes* was observed (Table 1).

**Table 2: Quantitative estimation of chemical constituents**

| S. No | Chemical constituent | Standard     | Ethanol extract | Aqueous extract |
|-------|----------------------|--------------|-----------------|-----------------|
| 1     | Alkaloids            | Atropine     | 90.09 µg        | 10.65 µg        |
| 2     | Saponin              | Diosgenin    | 12.01 µg        | -----           |
| 3     | Phenolic compound    | Catechol     | 90.32 µg        | 43.10 µg        |
| 4     | Steroids             | Cycloartenol | 21.12 µg        | 58.03 µg        |
| 5     | Flavonoids           | Catechin     | 152.89 µg       | 65.43 µg        |

Quantitative estimation of chemical constituents was carried out by spectrophotometric methods. Flavonoids were present in large amount followed by phenol, alkaloids, steroids and saponins in the ethanolic extract. Aqueous extract show the high amount of flavonoids, steroids, phenols and alkaloids respectively.

## CONCLUSION

The above reported that no heavy metals were in colonial ascidian *Didemnum psammathodes*. High amount of flavonoids and phenols were observed in ethanol and water extracts. It exhibited antioxidant property.

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