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QUALITATIVE AND QUANTITATIVE ESTIMATIONS OF CHEMICAL CONSTITUENTS IN A SIMPLE ASCIDIAN *PHALLUSIA NIGRA*

***D. Shanmuga Priya, H. Kohila Subathra Christy and S. Sankaravadivu**

Department of Chemistry, A.P.C. Mahalaxmi College for Women, Tuticorin 628 002, Tamilnadu, India.

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***Correspondence for Author**
Dr. D. Shanmuga Priya
Department of Chemistry, A.P.C. Mahalaxmi College for Women, Tuticorin 628 002, Tamilnadu, India.

ABSTRACT

Ascidians are marine sedentary organisms. *Phallusia nigra* is a simple ascidian belonging to the family Ascidiidae found in plenty throughout the year along the Thoothukudi coast of India. Preliminary chemical screening of petroleum ether (40^0- 60^0C), benzene, chloroform, ethanol and water extracts revealed the presence of chemical constituents such as alkaloids, steroids, flavonoids, phenolic compounds, quinones, saponins and anthraquinones. Quantitative estimation of alkaloids, phenolic compounds, saponins and flavonoids were carried out using uv-vis spectrophotometer and found that the ethanolic extract contains the higher amount of steroids and flavonoids.

KEYWORDS: *Phallusia nigra*, phytochemical screening, uv-vis spectrophotometer.

INTRODUCTION

Phytochemicals are natural bioactive compounds found in plants and animal foods that work with nutrients and dietary fibre to protect against diseases. Ascidians are marine sedentary organisms and they belong to biofouling community. They are found in piers, pilings, harbour installations, materials used in aquaculture operations etc. *Phallusia nigra* is a simple ascidian belonging to the family Ascidiidae. 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] Because of the number of phytochemicals and the complexity of the chemical processes that are involved in, researchers face a challenging task in trying to determine the type of phytochemicals in food that may fight cancer and other diseases, which have no effect and those that may even be harmful. Researchers have shown much interest in phytochemical supplements in animal source. Studies in cell cultures and animals have shown that certain phytochemicals have pharmacological activity. Previous studies show that the animal possesses antipyretic[5],analgesic, anaesthetic[6], anti-inflammatory[7],wound healing[8] and antimicrobial activities.[9,10] The present study focuses on the chemical investigation of the chemical constituents of the different extracts of *Phallusia nigra* responsible for these pharmacological activities.

MATERIALS AND METHODS

Collection and identification: *Phallusia nigra* (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.[11] A voucher specimen (AS 2083) was deposited in the Museum of the Department of Zoology, A.P.C. Mahalaxmi College for Women, Tuticorin 628002, Tamilnadu, India.

Fig. 1: *Phallusia nigra* Sav.

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 (40^0-60^0C), benzene, chloroform, ethanol, methanol and water.

Preliminary phytochemical screening

Standard procedures as suggested by Brindha *et al*., Trease and Evans and Harborne[12,13,14] were followed for preliminary phytochemical screening.

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

Ethanolic 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^{o}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±2^{0}C 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 Preliminary chemical screening of petroleum ether (40^0- 60^0C), benzene, chloroform, ethanol and water extracts were presented in the table 1. Quantitative estimation of major chemical constituents such as alkaloids, steroids, phenolic compounds, saponins and flavonoids of ethanolic extract and aqueous extract were presented in the table 2.

Table 1. Preliminary chemical screening of the various extracts of *Phallusia nigra*

S. No	Test	Petroleum Ether (40^0-60^0C)	Benzene	Chloroform	Ethanol	Water
1.	Alkaloids	+	-	+	+	+
2.	Terpenoids	+	+	+	+	+
3.	Steroids	+	+	+	+	+
4.	Coumarins	-	-	-	-	-
5.	Tannins	-	-	-	-	-
6.	Saponins	+	+	-	+	+
7.	Flavonoids	+	+	+	+	+
8.	Quinones	+	+	+	-	-
9.	Anthraquinones	+	+	+	-	+
10.	Phenols	-	-	-	+	+
11.	Catechins	-	-	+	-	-
12.	Aromatic acids	-	-	-	-	-
13.	Proteins	-	+	-	+	-
14.	Lipids	-	+	+	+	-
15.	Carbohydrate	-	+	+	+	+

Table 2: Quantitative estimation of chemical constituents

S.No	Chemical constituent	Standard	Ethanolic extract	Aqueous extract
1	Alkaloids	Atropine	89.09 µg	111.65 µg
2	Saponin	Diosgenin	22.01 µg	-------
3	Phenolic compound	Catechol	108.12 µg	-------
4	Steroids	Cycloartenol	221.52 µg	78.06 µg
5	Flavonoids	Catechin	182.89 µg	65.43 µg

Preliminary chemical screening of petroleum ether (40^0- 60^0C), benzene, chloroform, ethanol and water extracts revealed the presence of chemical constituents such as alkaloids, steroids, flavonoids, phenolic compounds, quinones, saponins and anthraquinones. Among different solvents used for extraction in a series, ethanolic extract and aqueous extract showed +ve results for many numbers of chemical compounds. It contains Steroids, Saponins, Alkaloids, Carbohydrates, flavonoids and phenolic compounds. From the results of quantitative estimation of alkaloids, steroids, phenolic compounds, saponins and flavonoids it was found that the ethanolic extract contains the higher amount of steroids and flavonoids.

CONCLUSION

Qualitative and Quantitative estimations of chemical constituents in a simple ascidian *Phallusia nigra* revealed the presence of chemical constituents such as alkaloids, steroids, phenolic compounds, saponins and flavonoids. Further detailed study is required to target the isolation of the pure compound.

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