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Research Article

HPTLC and IR Spectral studies of the ethanolic extract of *Phallusia nigra*

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Abstract

Ascidians, commonly called as 'Sea Squirts' are sedentary tunicates. *Phallusia nigra* is a simple ascidian belonging to the family Ascidiidae found in plenty throughout the year. The ethanolic extract of *Phallusia nigra* was subjected to HPTLC and IR spectral analysis to determine the possible bioactive components. In HPTLC studies, gallic acid, ferulic acid, caffeic acid and flavonoids such as rutin, isoquercitrin and quercetin were found to be present. The interpretation of the spectrum showed the presence of aliphatic bromo compounds, phenol or tertiary alcohols, carbonyl compound, carboxylic acids, lipids, proteins, alkanes and aromatic compound.

Keywords: *Phallusia nigra*, ascidian, HPTLC, IR

1. Introduction

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. Previous studies show that the animal possesses antipyretic, analgesic, anaesthetic, anti-inflammatory, woundhealing, antimicrobial, antibacterial, chemical investigation, HPLC and IR studies, pharmacognostical, antioxidant, antidiabetic, hepatoprotective, antiproliferative, antitumour, immunomodulatory, larvicidal, antifertility, CNS depressant, cardioprotective and hyperlipidemic activities [1-55]. No reports are available on the HPTLC Analysis of the simple ascidian *Phallusia nigra*. Hence the present study aims to investigate the HPTLC and IR spectral studies of ethanol extract *Phallusia nigra.*

2. Materials and Methods

2.1 Collection of animal material

Phallusia nigra (Family: Ascidiidae) was collected from Tuticorin coast in the month of October 2010 by SCUBA diving. Molluscan shell, calcrete rock fragments attached to the foot of the animal was carefully removed. They were identified using key to identification of Indian ascidians [56]. A voucher specimen AS-2083 has been submitted in the ascidian collection of museum of the Department of Zoology, A. P. C. Mahalaxmi College for women, Tuticorin – 628002, Tamilnadu, India.

2.2 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 (40^0-60^0 C), benzene, chloroform, ethanol and water. The extracts were concentrated in a rotary evaporator under reduced pressure and used for further chemical investigations.

2.3 HPTLC studies

Shimadzu CLASS-VP V6. 13 SP2 instrument was used to carry out HPTLC analysis employing the following conditions: Column 4.6 x 75 mm Zorbax Eclipse XDB-C18,3.5 im; Mobile phase A=water, B=methanol, Gradient at 0 min 90% B, at 20 min 100% B, Column wash at 21 min 90% B; Flow rate-1.0 ml/min; UV detector-

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variable wave length detector 210 nm, standard cell, Column compartment temperature 200C; Stop time 21 min, Post time 5 min; Injection volume – 5 micro liter.

2.4 IR spectral studies

Extracts were analyzed in a liquid cell. This is a small container made from KBr (or other IR-transparent material) which can be filled with liquid, such as the extract for EPA 418.1 analysis. This creates a longer path length for the sample, which leads to increased sensitivity. Sampling methods include making a mull of a powder with a hydrocarbon oil (Nujol) or pyrolyzing insoluble polymers and using the distilled pyrolyzate to cast a film. Materials are placed in an Attenuated Total Reflectance (ATR) cell and gases in gas cells. The following conditions were employed; Perkin Elmer Model spectrum RXI; Range 4000nm-400nm; Resolution 4; Transmittance test mode.

3. Results and Discussion

3.1 HPTLC Studies

HPTLC studies have been performed for the methanol extract of *Phallusia nigra*. Fig. 1 shows the peak area of various phytochemical constituents identified and their concentrations. R_f values of the different spots were measured and are presented in Table 1.

In HPTLC studies, six peaks were noticed in the chromatogram. Gallic acid, ferulic acid, caffeic acid and flavonoids such as rutin, isoquercitrin and quercetin were found to be present. A maximum peak area (27773.44) corresponding to quercetin was observed with 221.89 µg/g concentration.

Figure 1: HPTLC chromatogram of ethanolic extract of *Phallusia nigra*

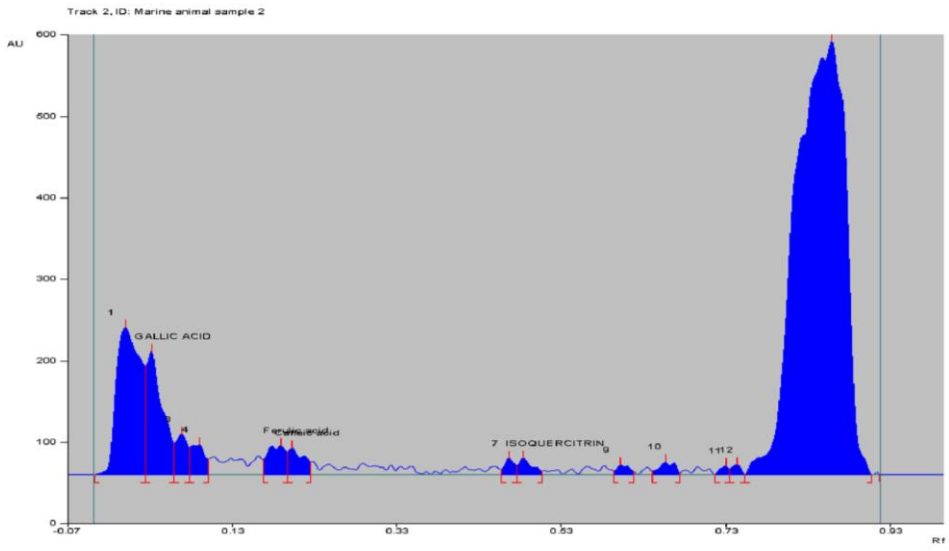

Table 1: Phytochemical constituents identified in the ethanol extract of *Phallusia nigra* by HPTLC

Phytochemical constituents	Rf	Peak area	Concentration (µg/g)
Gallic acid	0.03	2623.65	9.50
Rutin	0.12	Not Detected	Not Detected
Ferulic acid	0.19	693.36	0.97
Caffeic acid	0.21	565.60	0.31
Isoquercitrin	0.49	287.95	0.118
Quercetin	0.86	27773.44	221.89

IR spectral studies

Figure 2 Shows the IR spectrum of ethanolic extract of *Phallusia nigra*. The spectrum is interpreted and the results are presented in Table 2.

Figure 2. IR spectra of ethanolic extract of *Phallusia nigra*

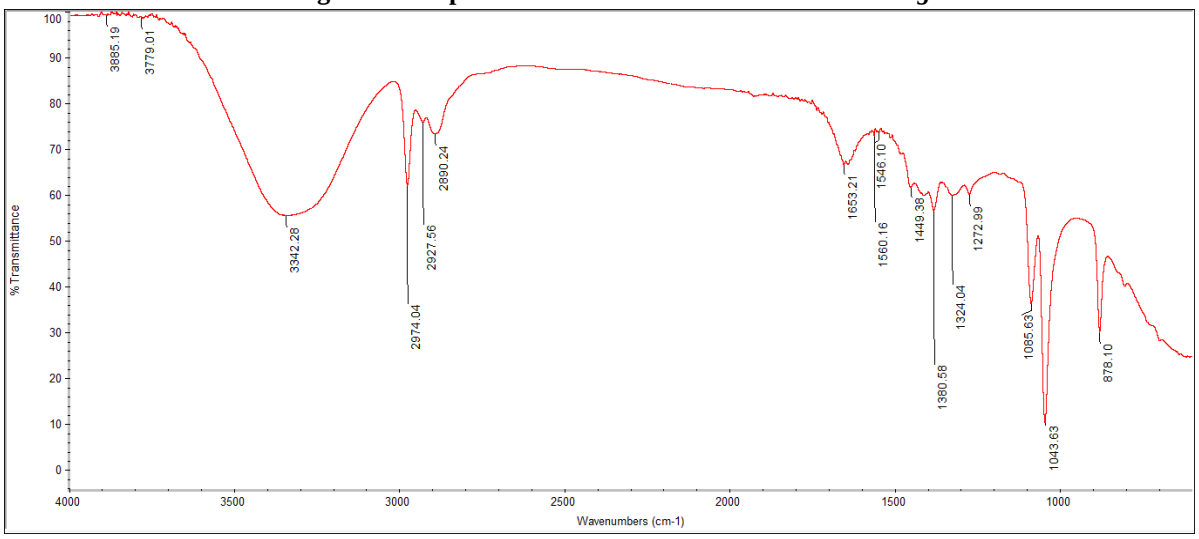

Table 2: IR spectral data

No	Group Frequency cm^{-1} of the sample compounds	Functional group assignment and compound	Group frequency cm^{-1}
1	878.10	Aliphatic compounds, C-H out of plane bend	800–900
2	1043.63	Phosphate ion	1100-1000
3	1380.88	O-H bend, Phenol or tertiary alcohol	1410-1310
4	1653.21	C=O stretch, carbonyl compound	1650-1600
5	2890.24	-CH-CH_2 asymmetric stretch- lipids, protein	2865-2845
6	2974.04	C-H stretch, alkanes	3000-2850
7	3342.28	O-H stretch, H bonded-Alcohols, Phenols	3570-3200
8	3885.19	C-H stretch, C=C stretch, Aromatic compound	>3000

Interpretation of Infrared Spectra has been done by the methods suggested by John Coates [57]. The interpretation of the spectrum showed the presence of aliphatic bromo compounds, phenol or tertiary alcohols, carbonyl compound, carboxylic acids, lipids, proteins, alkanes and aromatic compound.

4. Conclusion

The study clearly indicates that the ethanolic extract of *Phallusia nigra* was rich in many bioactive chemical components. However further studies such isolation, purification and structure determination is required for the development of new drug.

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