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Pharmacognostical and Preliminary Phytochemical Evaluation of *Phallusia nigra* Sav.

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Abstract: Pharmacognostical and preliminary phytochemical evaluation of *Phallusia nigra* Sav. has been carried out for the first time. Microscopical examination showed the presence of test, mantle, tentacles, dorsal tubercle, endostyle, dorsal lamina, stigmata, internal longitudinal vessels, branchial papillae, neural gland, accessory openings of neural gland, pyloric glands, ovary and testis. Physico chemical parameters and preliminary phytochemical screening of petroleum ether (40°-60°C), benzene, chloroform, methanol and water extracts were also carried out. Water-soluble ash (4.42%) was found to be greater than that of acid-insoluble ash (0.98%). The extractive value was minimum in petroleum ether (0.64%) and maximum in methanol (12.62%). Alkaloids, steroids, flavonoids, quinones, saponins and anthraquinones were present in almost in all the extracts. In TLC studies, petroleum ether (40°-60°C) and benzene extracts show two spots whereas in chloroform and methanol extracts a maximum of four spots were observed. Amino acids such as serine, norvaline, diiodotyrosine, α-alanine, lysine, aspartic acid and arginine were identified in paper chromatography. These pharmacognostical and phytochemical screening may be used as a diagnostic tool for the correct identification of the marine species.

Key words: *Phallusia nigra* · Pharmacognosy · Physicochemical Parameters · Phytochemical Screening

INTRODUCTION

Marine organisms are a rich source of structurally novel and biologically active metabolites. 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. Since the report of *Phallusia nigra* from Tuticorin coast of India [1], studies on the ecology, distribution, seasonal variation in the occurrence, taxonomy [2], breeding biology, recruitment and succession in the fouling community, role as bioindicators [3], association with coral reef [4], antibacterial activity to human pathogens [5,6], food value [7] and larvicidal potency [8] have been attempted. However, systematic pharmacognosy and phytochemistry of *Phallusia nigra* has not been carried out so far. The present study has been designed to assess the macroscopic and microscopic characters, physicochemical parameters and phytochemical characters of *Phallusia nigra*.

MATERIALS AND METHODS

Animal: Samples of *Phallusia nigra* (Family: Ascidiidae) were collected from Tuticorin coast by SCUBA diving. They were identified and authenticated by Dr.V.K. Meenakshi, Associate Professor, Department of Zoology, A.P.C.Mahalaxmi College for women, Tuticorin-628002. A Voucher specimen (As-2083) has been deposited in the National Collections of Ascidians in the Museum of the Department of Zoology, A.P.C.Mahalaxmi College for women, Tuticorin-628002.

Preparation of Extract: Epibionts adhering to the test were carefully removed, washed with sterile sea water, dried under shade and homogenized to get a coarse powder. The coarse powder was stored in an airtight container and used for further investigations. 100 g of powdered animal material was extracted with methanol using Soxhlet apparatus. The extract was cooled to room temperature, evaporated in a rotary evaporator under reduced pressure and a brown sticky residue was obtained (15 g).

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Table 1: Fluorescence characters of *Phallusia nigra* and their extracts in various solvents

S. No	Treatments	Under ordinary light	Under UV light (365nm)
1	Powder as such	Black	Black
2	Powder + 1N NaOH (aqueous)	Black	Brown
3	Powder + 1N NaOH (ethanolic)	Black	Pale Brown
4	Powder + 1N HCl	Black	Pale Brown
5	Powder + 1:1H_2SO_4	Black	Brownish yellow
6	Powder + 1:1 HNO_3	Black	Brownish yellow
7	Extracts		
	Petroleum ether (40°-60°C)	Yellow	Yellowish brown
	Benzene	Brown	Yellowish brown
	Chloroform	Brown	Yellowish brown
	Methanol	Brown	Dark brown
	Water	Brown	Dark brown

Table 2: Physico-chemical Characters of *Phallusia nigra*

S.No	Particulars	Percentage
1	Loss of weight on drying	83.29
2	Total ash	12.19
3	Acid-insoluble ash	0.98
4	Water-soluble ash	4.42
5	Residue on ignition	11.13
6	Extractive values	
	a) Petroleum ether (40°-60°C)	0.64
	b) Benzene	0.83
	c) Chloroform	2.91
	d) Methanol	12.62
	e) Water	10.14

Macroscopical Characterization: Macroscopical studies were done by naked eye and internal and external appearance have already been reported by Meenakshi [2].

Fluorescence Analysis: The animal powder and their extracts in various solvents were examined under ordinary light and UV light (365 nm). The powder was also treated with 1N NaOH (aqueous), 1N NaOH (ethanolic), 1N HCl, 1:1 H_2SO_4 and 1:1 HNO_3 and changes in colour were recorded and are presented in Table 1. The fluorescence characters were determined according to the method of Chase and Pratt [9].

Physicochemical Parameters: The percentage of loss of weight on drying, total ash, acid-insoluble ash, water-soluble ash and residue on ignition were obtained by employing standard method of analysis described in Pharmacopoeia of India [10] and the results are presented in Table 2.

Preliminary Phytochemical Screening: Standard procedures as suggested by Brindha *et al*. [11] Trease and Evans [12] and Harborne [13] were followed for preliminary phytochemical screening and the results are presented in Table 3.

Chromatographic Studies

Thin Layer Chromatography: Thin layer chromatographic studies have been performed for the petroleum ether (40°-60°C), benzene, chloroform and methanol extracts using pre-coated plates of Silica gel for TLC (E-Merck, Germany). The Silica gel-G for TLC was poured as thin layers on glass plates by preparing semi-solid slurry with distilled water. The plates were dried until they are free from moisture and activated in an air-oven at about 110^0C for about 3 hours. Different solvent systems were employed for various extracts of the samples. The plates were viewed under UV light (365nm) using a UV visible viewing cabinet. The fluorescence spots were located and R_f values were measured. The plates were then developed in an iodine chamber and the R_f values of the spots were calculated. The solvent systems for each extracts were standardized after trial and error. TLC analysis of the various solvents tried for the thin layer chromatographic techniques, no common solvent could be identified for all the extracts. The solvent system employed and the R_f values obtained are presented in Table 4.

Table 3: Preliminary phytochemical screening of the various extracts of *Phallusia nigra*

S. No	Test	Petroleum Ether (40°-60°C)	Benzene	Chloroform	Methanol	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 4: R_f values of the various extracts of *Phallusia nigra*

			Rf values of the spot	
S.No	Extracts	Solvent system used	Under UV light (365 nm)	In Iodine chamber
1.	Petroleum ether (40°-60°C)	100% Chloroform	0.29 (Yellowish-brown)	0.21?,0.29?
2.	Benzene	Chloroform: Ethanol (9.5:0.5)	0.29 (Yellowish-brown)	0.10?,0.29?
3.	Chloroform	Chloroform: Ethanol (8.5:1.5)	0.65 (Yellowish-brown)	0.31?, 0.44?0.65?,0.72?
4.	Methanol	Chloroform: Ethanol (8.5:1.5)	0.73 (Yellowish-brown)	0.26?,0.4?,0.61?,0.73?
5.	*Water	Butanol: Acetic acid: Water (8.5:0.5:1.0)	0.73 (Yellowish-brown)	0.19?, 0.31?,0.44?,0.64?, 0.65?,0.73?

*For water extract paper chromatography was carried out.

▲-thick spot; ?-moderate spot; ?-mild spot

Paper Chromatography: Paper chromatographic studies have been performed for the water extract of *Phallusia nigra*. The solvent system used was a mixture of n-butanol: acetic acid: water (8.5:0.5:1.0). The solvent mixture was shaken well in a separating funnel and allowed the two phases to separate. The upper organic layer was used for developing chromatogram. Whatmann No.1filter paper was used for this purpose. Fluorescence spots were located first and the R_f values were calculated by observing the paper chromatogram in a UV-viewing cabinet (365 nm). The paper chromatogram was then developed in an iodine chamber and R_f values of the spots were calculated and the results are presented in Table 4.

RESULTS

Microscopical Study: The test covers the animal on the outside which is thick and cartilaginous. Mantle forms a covering to the inner organs and lies inside the test. Slender, filamentous tentacles are present at the base of the oral siphon. Dorsal tubercle is a small, flat cushion, protruding into the pharynx. Endostyle is a groove extending from along the pharyngeal sac. Dorsal lamina is situated on the dorsal margin of the branchial sac. Stigmata are oval or slit like openings helping in respiration. Accessory openings of neural gland are additional openings of the neural duct into the atrial cavity. Pyloric glands are the digestive glands. Ovary is numerous and tubular. Testis are small, branched and numerous. Microscopic characters are presented in Fig. 1a to Fig. 1g.

Fluorescence Characters: Many drugs give fluorescence when the cut surface or the powder is exposed to UV radiation. The powder was also treated with various chemical reagents and the changes in colour was recorded. The fluorescence characters were determined according to the methods of Chase and Pratt [9] and the results are presented in Table 1. A characteristic yellowish-brown fluorescence was noticed in petroleum ether (40°-60°C), benzene and chloroform extracts and a dark brown fluorescence was observed in methanol and aqueous extracts under UV light (365 nm). This characteristic brown fluorescence can be used as a diagnostic tool for the correct identification of the species and also to test adulteration (if any).

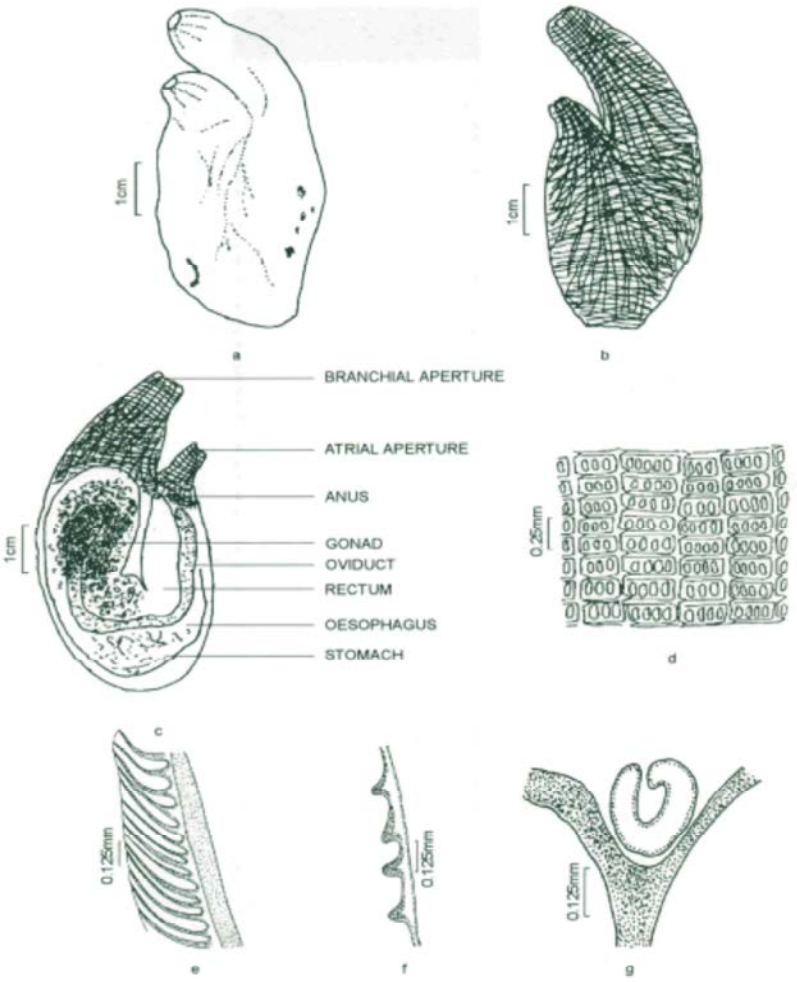

Fig. 1: *Phallusia nigra*. a. external appearance b. right side of the body showing musculature c. left side of the body showing gut and gonad d. a portion of branchial sac e. dorsal lamina f. branchial papilla g. dorsal tubercle (AS-2083)

Physico-Chemical Characters: The determination of ash values is useful for detecting low grade products, exhausted drugs and excess of sandy or earthy matter. The percentage of total ash, acid-insoluble ash, water-soluble ash, residue on ignition and extractive values are presented in Table 2. The total ash value is three times higher than that of water soluble ash value. The acid insoluble ash value is less than one percent. The extractive value was found to be minimum in petroleum ether (0.64%) and maximum in methanol (12.62%).

Preliminary Phytochemical Screening: Phytochemical constituents like alkaloids, terpenoids, steroids, coumarins, tannins, saponins, flavonoids, quinones, anthraquinones, phenols, aromatic acids, catechins, proteins, xanthoprotein, amino acids, carbohydrate, starch and lipids were tested qualitatively using the petroleum ether (40^0-60°C), benzene, chloroform, methanol and water extracts. The results are presented in Table 3. The petroleum ether (40^0-60°C) and benzene extracts showed the presence of alkaloids, terpenoids, steroids, saponins, flavonoids, quinones, anthraquinones, proteins, carbohydrate and lipids. The chloroform extract showed the presence of alkaloids, terpenoids, steroids, saponins, flavonoids, quinones, anthraquinones and carbohydrate. The methanol extract showed the presence of alkaloids, terpenoids, steroids, flavonoids, quinones, phenols, protein, aminoacids, carbohydrate, sugar and lipids. Water extract showed the presence of alkaloids, terpenoids, steroids, saponins, flavonoids, quinones, anthraquinones, phenols, protein, aminoacids, carbohydrate, sugar and lipids.

Chromatographic Studies

Thin Layer Chromatography: Crude extracts of petroleum ether (40°-60°C), benzene, chloroform and methanol of *Phallusia nigra* has been subjected to thin layer chromatographic study using pre-coated plates of silica gel G (E-Merck, Germany). The solvent systems were chosen after trial and error of the various solvents tried for the thin layer chromatography. No common solvent could be identified for all the extracts. Different solvent systems are found to be effective to get the maximum number of spots for the various extracts.

The developed TLC plates have been first viewed through ultraviolet fluorescence viewing cabinet (365 nm) before keeping in an iodine chamber and the R_f values of the fluorescing spots were measured. The results are presented in Table 4.

Paper Chromatography: Paper chromatographic study has been performed for the aqueous extract of *Phallusia nigra.* The solvent system used is a mixture of n-butanol: acetic acid: water (8.5:0.5:1.0). The R_f values of the spots are calculated and presented in the Table 4.

DISCUSSION

Many phytocompounds exhibit fluorescence when suitably illuminated. This is specific for each compound. A characteristic yellowish-brown fluorescence was noticed in petroleum ether (40°-60°C), benzene and chloroform extracts of *Phallusia nigra* and a dark brown fluorescence was observed in methanol and aqueous extract under UV light (365 nm).

The physical constant evaluation of the drug is one of the important parameters in detecting adulteration or improper handling of drugs [14]. Equally important in the evaluation of crude drugs is the ash value and acid insoluble ash value determination. The total ash is particularly important in the evaluation of purity of drugs i.e the presence or absence of foreign organic matter such as metallic salts and or silica [15]. The total ash value is three times higher than that of water soluble ash value. The acid insoluble ash value is less than one percent. The extractive value was found to be minimum in petroleum ether (0.64%) and maximum in methanol (12.62%).

A knowledge of the phytochemical screening is desirable, not only for the discovery of therapeutic agents, but also because such information may be of value disclosing new sources of such economic materials as tannins, oils, gums, precursors for the synthesis of complex chemical substances etc. Major phytochemical constituents such as alkaloids, steroids, flavonoids, quinones, saponins and anthraquinones are present in almost all the extracts. Phenolic compounds, saponins and flavonoids may be linked or suggested to be involved with antibacterial, antiviral and antidiarrhoeal activity as suggested by Majaw and Moirangthem [16], in plants. Investigations on the mode of action by Enzo [17] in plants indicate that flavonoids increase colonic water, electrolyte reabsorption and other chemicals act by inhibiting intestinal mobility while some components have been shown to inhibit particular entero pathogens. Alkaloids are reported to have cardio vascular effects by Juge *et al.* [18]. As ascidians are sedentary animals the same role may be suggested in these animals as defense mechanism. In order to avoid confusion of misidentification and adulteration, it is essential to give details of morphology, anatomy of the different parts used in the official preparations, their chemical composition and also fixing the identity of the animal source.

In TLC studies, petroleum ether (40°-60°C) and benzene extracts show two spots whereas in chloroform and methanol extracts a maximum of four spots were observed. Separation of amino acid was carried out for the water extract of *Phallusia nigra* using paper chromatography. On comparison with the standard amino acids with the water extract the amino acid present in the aqueous extract was determined. Serine, norvaline, diiodotyrosine, α-alanine, lysine, aspartic acid and arginine were found to be present in *Phallusia nigra* in the water extract. These pharmacognostical and phytochemical characters of *Phallusia nigra* can be used as a diagnostic tool for the correct identification of the animal species.

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