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## Anaesthetic activity of *Phallusia nigra* Savigny

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### ABSTRACT

Methanolic extract of the simple ascidian, *Phallusia nigra* Sav. was subjected to anaesthetic activity by intracutaneous wheal method. The extract, when administered at a dose of 10% and 15% caused highly significant anaesthetic activity when compared to the standard drug xylocaine (1%). The mean sleeping time and percentage relaxation of muscle was also highly significant in the extract treated groups compared to the standard drug, Aminobarbitone.

**Key words:** *Phallusia nigra*, Anaesthetic, Xylocaine, Aminobarbitone, Sleeping time.

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### INTRODUCTION

Marine organisms are a rich source of structurally novel and biologically active metabolites. Primary and secondary metabolites produced by these organisms may be potential bioactive compounds of interest in the pharmaceutical industry. 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 studied. However, systematic pharmacological screening of the crude extract of *Phallusia nigra* has not been carried out so far. The present study has been designed to assess the anaesthetic activity of the methanolic extract 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 a Soxhlet apparatus. The extract was cooled to room temperature, evaporated in a rotary evaporator under reduced pressure when a brown sticky residue was obtained (15 g).

**Experimental animal:** Mature adult male Wistar Albino rats weighing about 180-200 g were selected for the study. They were maintained in a well ventilated animal house with constant 12 hours of darkness and 12 hours of light schedule. Clean water and standard pellet diet (Hindustan Lever Ltd., India) were given to them 'ad libitum'.

**Phytochemical screening:** The methanolic extract of *Phallusia nigra* was screened for various chemical constituents such as tannins, saponins, alkaloids, steroids, terpenoids, reducing sugars, proteins, lipids, etc using established methods [9, 10].

**Acute toxicity studies:** Acute oral toxicity studies were performed to determine minimum sub lethal doses of the animal extract. During the 24 hour observation period no adverse effect or mortality was observed up to 500 mg/kg bw of methanolic extract. Hence 50, 100, and 150 mg/kg bw of the methanolic extract was selected for the anaesthetic activity study [11].

#### Local Anaesthetic activity

**Intracutaneous wheal method:** The local anesthetic activity of the methanolic extract of *Phallusia nigra* was studied by intracutaneous wheal method [12] in albino rats as described by Burn *et al*. The animals were divided into four groups of six rats each. Group I was given 1% of standard drug, xylocaine. Group II, III and IV received 5%, 10% and 15% of the extract. The results are presented in Table 1. On the day prior to the study, the hair on the back of albino rats near the midline (four different areas of 4 cm each) were clipped and removed. The drugs were injected intracutaneously in equal volumes of 0.2 ml into the shaved areas and wheals were marked with ink and the time of injection was noted. The normal responses of the animals were observed first by applying pin pricks in the midline. Six pin pricks were then given uniformly every five min at an interval of four seconds on the wheal areas. The responses were recorded up to 30 min. A localized skin twitch, usually accompanied by squeak, was considered as the normal response to pin prick. When the animal failed to respond either by twitching of the muscle or squeaking following a pin prick, a negative response was recorded [13, 14].

#### General Anaesthetic activity

**Aminobarbitone-induced sleeping time and muscle relaxation:** The animals were divided into four groups of six rats each. Group I was given 10 mg/kg of standard drug, Aminobarbitone. Group II, III and IV received 50 mg/kg bw, 100 mg/kg bw and 150 mg/kg bw of the extract. The mean sleeping time and muscle relaxation (% of rats unable to grasp the board with fore paws) were noted [15].

### RESULTS AND DISCUSSION

In intracutaneous wheal model in albino rats, the 5%, 10% and 15% of the extracts produced 58.33%, 66.66% and 75.0% anesthesia respectively compared to 48.33% anaesthetic effect produced by the standard drug, xylocaine (1%). The negative responses of the extract treated groups showed a highly significant increase when compared to that of the standard. An increased concentration of the test drug produced an increase in local anaesthetic activity. A dose dependent sleeping time and muscle relaxation was observed. A maximum sleeping time of  $172 \pm 5.90$  min and  $184 \pm 6.55$  min was observed in group III and group IV respectively when compared to that of the standard, aminobarbitone ( $169 \pm 5.40$ ). Muscle relaxation was also found to be high in group III (65%) and group IV (80%) when compared to that of the standard, aminobarbitone (55%) (Table 2).

**Table 1. The local anaesthetic effect of methanolic extract *Phallusia nigra***

Known drug/ Extract	Dose (%)	Number of negative responses over time (min)							Total out of 60	Anaesthesia (%)
		0	5	10	15	20	25	30		
Xylocaine	1	0	6	7	5	2	6	3	29	48.33
Group I	5	0	5	6	7	6	5	6	35	58.33
Group II	10	0	7	9	6	8	7	6	40	66.66
Group III	15	0	8	9	8	5	7	8	45	75.00

**Table 2. Effect of the methanolic extract on aminobarbitone induced sleeping time and muscle relaxation in rats**

Group	Drug/Dose (mg/kg bw)	Sleeping Time (Min +SD) Mean time	Muscle Relaxation (% of rats unable to grasp board with fore paws) (%)
Group I	Aminobarbitone 10	$169 \pm 5.40$	55
Group II	50	$164 \pm 5.25$	35
Group III	100	$172 \pm 5.90$	65
Group IV	150	$184 \pm 6.55$	80

## CONCLUSION

The present investigation reveals that the methanolic extract of *Phallusia nigra* is found to be a good natural anaesthetic agent. Attempts will be made in future to isolate and identify the phytochemical constituents of the methanolic extract responsible for the anaesthetic activity.

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