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Research Article

CHEMICAL SCREENING AND ANAESTHETIC ACTIVITY OF *PHALLUSIA ARABICA* SAVIGNY, 1816

Kohila Subathra Christy H[1], Jothibai Margret R[2*] and Meenakshi VK[3*]
[1] Department of Chemistry, A.P.C. Mahalaxmi College for Women, Tuthukoodi, Tamil Nadu, India.
[2] Department of Chemistry, Pope's College, Sawyerpuram, Tuthukoodi, Tamil Nadu, India.
[3] Department of Zoology, A.P.C. Mahalaxmi College for Women, Tuthukoodi, Tamil Nadu, India.

*Corresponding Author: Kohila Subathra Christy H ; Email: apcm.research@gmail.com

Abstract: *Phallusia arabica* is a simple ascidian belonging to the family Ascidiidae. It was extracted successively using different solvents such as petroleum ether, benzene, methylene chloride, chloroform, ethanol, and water. Screening indicates the presence of alkaloids, terpenoids, steroids, coumarins, tannins, saponins, flavonoids, quinones, anthroquinone, phenols, aromatic acids, catechins, proteins, carbohydrates and lipids. Ethanolic extract of *Phallusia arabica* was subjected to anaesthetic activity by intracutaneous wheal method. The extract, when administered at a dose of 20 and 40% caused highly significant anaesthetic activity when compared to the standard drug xylocaine (0.5 & 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 arabica, Chemical screening, Anaesthetic, Xylocaine, Aminobarbitone

INTRODUCTION:

Pain is a physiologic response that has been defined in animals as an aversive sensory experience caused by actual or potential injury that elicits progressive motor and vegetative reactions, resulting in learned avoidance, modifying species specific behaviour[1]. Marine organisms are a rich source of structurally novel and biologically active metabolites. Ascidians which are marine organisms rank second with promising source of drugs[2]. Most of the ascidians are utilized as food in various countries and they are known to produce secondary metabolites which prevent bio-fouling and this can be considered as a kind of autogenic protection[3]. The number of natural products isolated from marine biota increase rapidly and now exceeds with hundreds of new compounds being discovered every year[4,5]. A large proportion of these natural compounds have been extracted from marine resources, especially sponges, ascidians, bryozoans and molluscs and some of them are currently in clinical trials[6]. This search for new metabolites has resulted in the isolation of more or less 10,000 metabolites[7].

Chemical screening strategy can benefit from the use of standards or target molecules which can be rapidly characterized by diagnostic parameters[8]. Qualitative and Quantitative estimation of the chemical constituents will be very much useful in the standardization of drugs. In India only very few works has been carried out in ascidians. A review of literature reveals that mainly taxonomical, biofouling, antibacterial, antimitotic, antimicrobial studies, infrared and GC–MS studies of a few species of ascidians are available[9-14]. Hence an attempt has been made to perform chemical screening and anaesthetic study of *Phallusia arabica* Savigny in this present work.

EXPERIMENTAL SECTION:

Collection of animal material:

Collection of *Phallusia arabica* was carried out from Tuticorin coast by SCUBA diving. It was identified, authenticated and a voucher specimen AS 2276 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-628 002 (Figure 1).

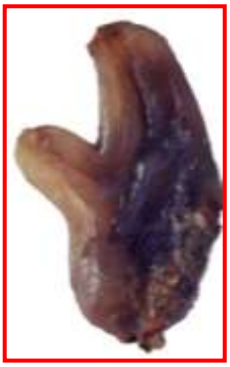

Figure 1: *Phallusia arabica* Savigny, 1816

Preparation of extract:

The whole animal was dried in shade and homogenized to get a coarse powder which was stored in an airtight container and used for further investigations. 100g of powdered animal material was extracted with petroleum ether, benzene, methylene chloride, chloroform, ethanol and water using soxhlet apparatus.

Experimental animal:

Mature adult male wistar albino rats weighing about 120-180 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 '*ad libitum*'.

Chemical screening:
The petroleum ether, benzene, methylene chloride, chloroform, ethanol, and water extracts of *Phallusia arabica* were screened for various chemical constituents such as alkaloids, terpenoids, steroids, coumarins, tannins, saponins, flavonoids, quinones, anthroquinone, phenols, aromatic acids, catechins, proteins, carbohydrates and lipids using standard procedures[15,16].

Acute toxicity studies:
Acute oral toxicity studies were performed to determine minimum sub lethal doses of the animal extract[17]. During the 24 hour observation period no adverse effect or mortality was observed up to 500 mg/kg body weight of ethanolic extract. Hence 100, 200 and 400 mg/kg bw of the ethanolic extract was selected for the study of anaesthetic activity.

Local anaesthetic activity:
Intracutaneous wheal method:
The local anaesthetic activity of the ethanolic extract of *Phallusia arabica* was studied by intracutaneous wheal method in albino rats[18]. One day prior to the study, four cm area of hair on four different areas on the back of albino rats near the midline were clipped and removed. The animals were divided into five groups of six each. Group I, II were treated with 0.5 and 1.0 % standard drug, xylocaine and III, IV, V received 10, 20, 40% of the ethanolic extract. The drugs were injected intracutaneously in equal volumes of 0.2 ml into the shaved areas, 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. Ten pin pricks were then given uniformly every five minutes 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[19,20].

General anaesthetic activity:
Aminobarbitone induced sleeping time and muscle relaxation:
The animals were divided into four groups of six each. Group I was given 10 mg/kg of standard drug, Aminobarbitone and II, III and IV received 100, 200 and 400 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[21].

RESULTS AND DISCUSSION:
Alkaloids, terpenoids, steroids, tannins, flavonoids, quinones, proteins, lipids and carbohydrates have been observed in all the extracts whereas coumarins was not found in any of the extracts Table 1. Saponins were present in petroleum ether, benzene, methylene chloride and chloroform extracts. Except petroleum ether extract all other extracts contain anthroquinone. Phenols and aromatic acids were observed in methylene chloride, ethanol and water. Catechins were detected in methylene chloride and ethanol extracts.

Table 1: Chemical screening of *Phallusia arabica*

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

Key: + Present; - Absent.

The results of the anaesthetic study shows that the extract produced significant anaesthesia in 20% (81.66%) and 40% (90%) dose when compared to standard drug Xylocaine (0.5% (43.33%) and 1% (53.33%) Table 2 and Figure 2. 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 elevated local anaesthetic activity. Dose dependent proportionate increase in sleeping time and muscle relaxation were noted. Maximum sleeping time of 198 ± 5.84 min and 214 ± 7.35 min were observed in group III and group IV respectively compared to that of standard aminobarbitone 179 ± 4.80. Muscle relaxation was also found to be high in group III (75%) and group IV (85%) Table 3 and Figure 3. Some

workers have reported that the local anaesthetic property could be due to the presence of alkylamides[22]. Ascidians are renowned for their overwhelming bias towards the production of nitrogenous secondary metabolites[23]. Rita and coworkers reported that alkaloids and saponins may be responsible for anaesthetic activity[24]. The preliminary chemical screening of the ethanolic extract of *Phallusia arabica* revealed the presence of alkaloids, flavonoids, terpenoids, anthraquinone and one or more of the chemical components may be responsible for the activity. Similar results have been reported using the methanolic extract of *Phallusia nigra*[25].

Table 2: Local anesthetic effect of *Phallusia arabica*

Known drug/Extract/group	Dose (%)	Number of negative responses over time (min)							Total out of 60	Anaesthesia (%)
		0	5	10	15	20	25	30		
Xylocaine - I	0.5	0	8	4	7	4	2	1	26	43.33
Xylocaine - II	1.0	0	7	8	6	3	4	2	32	53.33
Extract - III	10	0	7	6	7	6	5	7	38	63.33*
Extract - IV	20	0	9	8	7	8	6	9	49	81.66**^{aa}
Extract - V	40	0	10	9	9	6	7	10	54	90.00**^{aa}

Data represented as mean of 10 observations, (n=6). Significance between control low dose and extract treated groups * p <0.05; ** p <0.01. Significance between control high dose and extract treated groups ^{a} p <0.05; ^{aa} p <0.01.

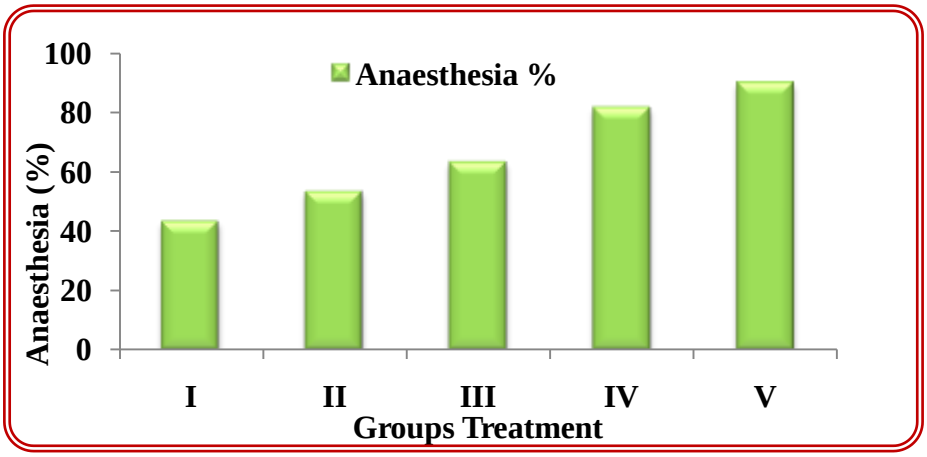

Figure 2: Local anaesthetic effect of *Phallusia arabica*

Table 3: The general anaesthetic effect of *Phallusia arabica*

Groups	Drug/Dose (mg/Kg bw)	SleepingTime (Min±SD) Mean Time	Muscle Relaxation (% of rats unable to grasp board with fore paws)
I	Aminobarbitone 10	179±4.80	60%
II	100	182±4.55ns	40%
III	200	198±5.84**	75%
IV	400	214±7.35***	85%

Data represented as mean± S.E.M, (n=6). Significance between control and extract treated groups. * p <0.05; ** p <0.01; *** p <0.001.

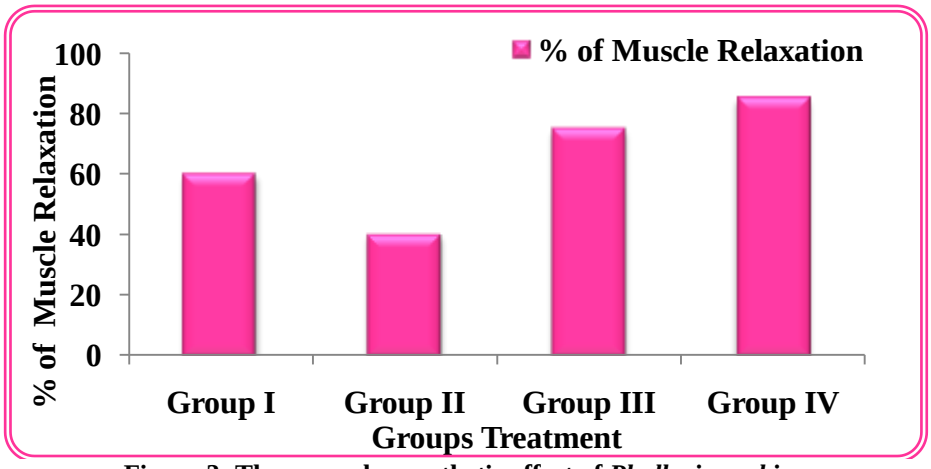

Figure 3: The general anaesthetic effect of *Phallusia arabica*

CONCLUSION

It can be concluded, that the ethanolic extract of *Phallusia arabica* has significant anaesthetic properties. However further detailed study is needed in order to understand the active principle and the mode of action responsible for this activity.

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