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METHODS OF EXTRACTION OF MUCIN FROM GIANT AFRICAN SNAIL *ACHATINA FULICA* BOWDICH

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ABSTRACT

***Achatina fulica* Bowdich is one of the most notorious pests in crop fields. However, the mucin secreted by this terrestrial mollusc has magnificent pharmaceutical attributes. In the present study, solvent and mechanical methods were explored to find out the best method of mucin extraction from this gastropod pest under laboratory conditions. In case of both medium and large sized snails, dichloromethane registered the maximum (2.79 ml and 2.94 ml, respectively) mucin collection out of the six solvents tested. Volume wise comparison showed that the maximum amount of mucin recovery was possible in the highest volume (3 ml) of solvents applied for both the age groups. Of all the mechanical methods tested, smoking method yielded maximum (2.05 ml and 3.02 ml) amount of mucin from both medium and large sized snails, respectively. However, the minimum mucin recovery was registered when the snails were allowed to move over rough tiles.**

Key words: *Achatina fulica*, mucin, terrestrial mollusc, pharmaceutical, solvent, mechanical methods, gastropod pest, dichloromethane, smoking method, rough tiles, acharan sulfate

Giant African snail *Achatina fulica* Bowdich (Stylommatophora: Achatinidae) has emerged as one of the most important pestiferous land snails causing heavy damage to various economically important crops of India. This pest has already been registered as one of the 100 worst invasive species in the world (Lowe et al., 2000). This snail has been spreading across the globe at an alarming rate primarily through human activities (Raut and Barker, 2002). Barring its prodigious damage potential, the mucin secreted by *A. fulica* has drawn global attention due to presence of several pharmaceutical attributes like some other gastropods. Since time immemorial, gastropods mucin had been used in ethno-medicinal system in different parts of the world. Basically, snail mucin is a naturally produced glycoprotein rich slimy substance that acts as a lubricant to protect delicate epithelial body surfaces against any kinds of mechanical and physical injuries and also makes slippery track for locomotion. The major glycoprotein component of snail mucin contains polysaccharide-hexosamines conjugation (Adikwu and Okafor, 2006).

Advanced biochemical and medical studies revealed that, acharan sulfate; a novel glycosaminoglycan compound is an important compound of snail mucin which represents 3-5 % of dry weight of the soft body tissue of *A. fulica* (Kim et al., 1996; Jeong et al., 2001; Vieira et al., 2004). Mucin of *A. fulica* has already been reported to heal wound twice faster than the normal saline solution and acharan sulfate has been considered as the key factor of wound healing (Harti et al., 2016). Acharan sulfate is also known to contain some antiangiogenic properties which can suppress the tumour growth as reported by Lee et al. (2003). Furthermore snail mucin exhibits positive antibacterial activity against both gram positive and negative bacteria like *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* (Iguchi et al., 1982; Ehara et al., 2002). The standard methods of mucin extraction from the live snails for both analytical and industrial exploration are however very scanty. Perusal of available literature also revealed that most of the methods attempted so far for mucin extraction leads to death of the snails. Pertinent to above, the present investigation was carried out to know the best method of mucin extraction from *A. fulica* by both solvent and mechanical means.

MATERIALS AND METHODS

Hand collection of snails of different age groups was made during their active foraging period (7-9 am and 7-9 pm) from different ecological habitats

of Jorhat (26°74´N, 94°20´E, 91.0 masl) districts of Assam. Collected snails were washed properly with tap water to remove the dirt, sand and soil particles in the laboratory and kept them on the plastic tray (39x 27x 7 cm) to eliminate the excess water present on the shells. Collected snails were reared in the Soil Arthropod Pests Laboratory, Department of Entomology, Assam Agricultural University, Jorhat during 2017-19. Three sets of both large (59x 44.5 cm) and medium (30x 44 cm) sized rectangular shaped single chamber iron netted cages with one lid were selected for rearing of the collected snails. Soils with high organic matter content was collected, ground finely and mixed with lime powder as a source of calcium (30 g/ kg of soil) and placed at the bottom of the cage up to the height of 5 cm. Food materials like carrot, cabbage and kitchen wastes were placed above the soil. Snails were then released gently inside the cage. Moisture content of the soil was regularly checked. Removal of snail excreta was carried out in regular interval as a sanitary measure. Two different age groups of snails i.e. medium and large sized (based on weight) were selected for the laboratory test. Snails weighing 25-30 g and 55-60 g were considered as medium and large sized, respectively.

For solvent method, six different organic solvents (both polar and non-polar) were used for extraction of mucin viz., hexane, petroleum ether, acetone, ethanol, methanol and dichloromethane, respectively, where distilled water was used as the control treatment. Five different volumes of each solvent were selected i.e., 0.5, 1, 1.5, 2 and 3 ml. Two hours before the experimentation, snails were washed properly with distilled water to avoid probable mixing of mucin with any kind of impurities. Food particles and soil that were noticed on the shells were removed gently by using camel hair brush. The snails were then kept on plastic tray (without food) and covered with black cloth so that they could resume their normal activity. Then, the individual snail was firmly held by one hand and the desired volume of the solvent was applied by pouring it gently on the foot portion of the snail. Immediately after the pouring, treated snail was placed at the middle of a glass petri plate (9 cm dia) and waited for 5 min for the release of the mucin by the snail. The mucin released by the snail was collected carefully by using spatula and was then measured in measuring cylinder (5 ml capacity). Five replications were kept for each of the solvents, including the control. The mucin so collected was kept treatment wise in self-standing centrifuge tube (50 ml capacity) and stored in deep freeze for further use. After the end of the experimentation, the treated snails were washed with tap water and kept in plastic tray for one day and allowed to feed. Next day, the snails were again released in iron netted rearing cages.

Four mechanical means with six replications for the extraction of mucin from *A. fulica* were attempted. In case of rough tiles method, both large and medium sized snails were allowed to move over the rough surface of the floor tiles for maximum of 30 min during the peak activity period of the snails (7-9 pm). The mucin was collected with camel hair brush and measured. For smoking method, snail was firmly held by one hand and then smoke emanating from an incense stick was directed towards the fleshy portion for 15 min so as to excite and compel the snail to secret mucin. Secreted mucin was collected directly in self-standing centrifuge tube (50 ml). To induce an electrical shock, a simple voltage shocker of 10 v capacity was made by using two sealed lead acid battery (6v- 5, 4v-0.5 Amp Hour), a multimeter and one positive and one negative each of electric probe with banana plug. Batteries were connected with electric wire in series connection and to check the voltage the wires were connected to the multimeter. At positive and negative plug points positioned in multimeter, two electric probes with banana plug were connected to complete the arrangement. Before giving the electrical shock, the snail was kept in inverted position on a petri plate (9 cm in dia) facing the flashy portion upward. Then the 10v shock was applied to the snail by projecting the pointed electric probes on in the fleshy part of the snail for 30 sec. The treated snail was then kept in normal position for 3 min and the released mucin was collected and measured. Stroking of snails using Pasteur pipette was the last mechanical way of mucin collection. For that the snail was firmly held and the foot region of was stroked (4-5 times) by the terminal end of a Pasteur pipette (3 ml capacity) as a means of mechanical excitement. The mucin secreted by the snail was then collected and measured. Data obtained from solvent method were analyzed by two factorial completely randomized design. One way completely randomized design was employed to evaluate the best mechanical methods of mucin extraction. The significance of difference between mean values was ascertained by Duncan's Multiple Range Test (DMRT). All statistical analysis were performed using IBM SPSS statistical 21 (IBM Corp. 2012).

RESULTS AND DISCUSSION

Solvent method: The experimental findings revealed that in case of both medium and large sized

snails, dichloromethane registered the maximum (2.94 ml from large sized and 2.79 ml from medium sized snails) amount of mucin and it was found to be significantly superior to rest of the solvents tested. The mean amount of mucin recorded in acetone and ethanol were 1.97, 1.92 ml and 2.52, 2.57 ml for medium and large sized snails, respectively, which showed statistical parity with each other. It is worthy to mention that out of all solvents tested, hexane and petroleum ether were the non-polar solvents which gave minimum amount of mucin. Considering the different volumes of solvents applied (3, 2, 1.5, 1 and 0.5 ml), mean maximum (2.72 ml and 1.93 ml in large and medium sized snails, respectively) amount of mucin was at the highest dose of volume (3ml). The performance order of the solvents was dichloromethane>ethanol = acetone>methanol >hexane = petroleum ether (Table 1). Perusal of the data revealed that irrespective of solvents and their volumes tested, maximum amount of mucin was registered in large sized snails. It might be due to the large foot area along with the pedal muscles of the snail where the different types of secretory glands were located. The present findings corroborate the observations of Greistorfer et al. (2017), who in a histochemical study reported the presence of five different mucin secretory glands on *Helix pomatia* foot. Campion (1961) also noticed four mucus secretory glands in case of *Helix aspersa* foot. However, the study on the type of secretory glands of *A. fulica* is yet to be reported.

Considering the polarity of the solvents, a clear-cut difference in the mucin extraction from the snails was observed. All the four polar solvents (acetone, ethanol, methanol and dichloromethane) registered more amounts of mucin extraction than the two non-polar solvents tested. Among the polar solvents, dichloromethane registered the maximum amount of mucin indicating that the shock due to the application of dichloromethane might be higher. However, dichloromethane extracted mucin immediately changed its colour and a whitish foamy mucin was noticed. But, no changes in mucin colour were noticed in case of acetone, ethanol and methanol extracted mucin and all these three solvents yielded very light yellowish watery mucin. Moreover, the possibility of precipitation of major macromolecules present in the raw mucin extracted by polar solvents also cannot be denied. Mucin glycoproteins are the major macromolecules present in epithelial mucus of the gastropods (Swapna and Reddy, 2015). Various workers (Iguchi et al., 1982; Adikwu, 2005; Gabriel et al., 2011;

Table 1. Mean amount of mucin (ml) extracted by using different solvents from medium and large sized *A. fulica*

Treatments (T)	Mucin extracted (ml) (mean* ± SE)					Mean (T- wise)
	0.5 ml	1 ml	1.5 ml	2 ml	3 ml	
A. From medium sized snails						
T_1 (Hexane)	0.36± 0.05	0.30± 0.03	0.58± 0.06	0.70± 0.40	1.16± 0.14	0.62
T_2 (Petroleum ether)	0.18± 0.04	0.40± 0.05	0.58± 0.09	0.88± 0.07	0.90± 0.17	0.59
T_3 (Acetone)	2.00± 0.16	1.78± 0.07	1.56± 0.11	2.30± 0.18	2.20± 0.37	1.97
T_4 (Ethanol)	1.52± 0.19	1.74± 0.07	1.82± 0.06	2.74± 0.19	1.78± 0.27	1.92
T_5 (Methanol)	0.96± 0.14	1.16± 0.16	1.14± 0.22	1.62± 0.19	1.86± 0.26	1.35
T_6 (Dichloromethane)	2.92± 0.37	2.06± 0.29	2.62± 0.15	2.94± 0.16	3.40± 1.51	2.79
T_7 (Distilled water) (Control)	0.36± 0.02	0.58± 0.04	0.82± 0.06	1.26± 0.11	2.24± 0.11	1.05
Mean (Volume wise)	1.19	1.15	1.30	1.78	1.93	
CD (p≤0.05)	Solvent (Treatments): 0.23		Volume: 0.20		Solvent× Volume: 0.52	
B. From large sized snails						
T_1 (Hexane)	0.62± 0.14	0.66± 0.11	1.16± 0.10	1.62± 0.14	2.10± 0.17	1.23
T_2 (Petroleum ether)	0.60 ± 0.15	0.72 ± 0.17	0.92 ± 0.15	1.56 ± 0.17	1.94 ± 0.20	1.15
T_3 (Acetone)	2.94 ± 0.16	2.38 ± 0.21	1.96 ± 0.19	2.06 ± 0.40	3.26 ±0.28	2.52
T_4 (Ethanol)	1.94 ± 0.15	2.54 ± 0.33	2.64 ± 0.44	3.10 ± 0.34	2.62 ± 0.31	2.57
T_5 (Methanol)	1.06 ± 0.14	1.58 ± 0.24	1.84 ± 0.34	2.44 ± 0.22	3.60 ± 0.37	2.10
T_6 (Dichloromethane)	2.42 ± 0.24	2.30 ± 0.22	2.96 ± 0.25	3.06 ± 0.39	3.96 ± 0.42	2.94
T_7 (Distilled water) (Control)	0.20 ± 0.05	0.42 ± 0.04	0.84 ± 0.10	1.02 ± 0.04	1.56 ± 0.15	0.81
Mean (Volume wise)	1.40	1.51	1.76	2.12	2.72	
CD (p≤0.05)	Solvent (Treatments): 0.30		Volume: 0.25		Solvent× Volume: 0.67	

*Mean of five replications

Harti et al., 2016) also attempted to precipitate the macromolecules of raw mucin using polar solvents like acetone and ethanol for further biochemical analysis of the mucin. Therefore, the present study suggests that for the extraction of mucin from large sized *A. fulica* snails as well as for their further macromolecule precipitation, polar solvents like acetone and ethanol can be applied. Non-polar solvents like hexane and petroleum ether not only extracted less amount of mucin as compared to polar solvents but also exhibited oil like yellowish sticky appearance. It might be due to the extraction of fat portion from the exposed fleshy part of the snail. Presence of fat parts on the fleshy meat portion of *A. fulica* was studied by Babalola and Akinsoyinu (2009) and according to them, the fresh meat of *A. fulica* possessed on an average 1.61 g of fat (extracted in ether)/100 g of fresh meat.

Mechanical method: Of the four mechanical means of mucin extraction used, the maximum amount of mucin could be extracted in case of smoking method (2.05 ml and 3.02 ml from medium and large sized snails, respectively). It was found to be significantly superior to rest of the methods. Extraction of mucin from large sized snails with the application of electric shock and stroking of Pasteur pipette amounts to 1.30 ml and 0.80 ml, respectively, which showed significant difference. The mucin obtained using electric shock and Pasteur pipette was watery and clear. Works in similar line were earlier conducted by Simkiss and Wilbur (1977), where they applied electric shock of 20v (50-100 shocks/ min) on the dorsal epithelium of the snail *H. pomatia*. It was reported that the mucin produced under above condition, was clear and watery, with a viscosity only 25% greater than that of water. While assaying the mucin extraction by exploring four mechanical means the order of performance was: Smoking > electric shock >Pasteur pipette > rough tiles (Table 2).

Results of the present study indicated that polar solvents have the ability to extract more amount of mucin from both large and medium sized snails in which dichloromethane led to maximum mucin extraction. However, change in colour of the mucin extracted by using dichloromethane has to be further analyzed. Barring dichloromethane, either acetone or ethanol can be considered as the best polar solvent for the purpose of mucin extraction from *A. fulica*. In case of mechanical means of mucin extraction, application of smokes of incense stick for 15 min showed the best results in both the age groups of the snail. Nevertheless, further biochemical analysis of the mucin extracted from *A. fulica* by exploring different methods needs to be investigated to ascertain the quality of the mucin.

Table 2. Amount of mucin (ml) extracted by mechanical means from *A. fulica*

Treatments	Mucin (ml) extracted (mean± SE)	
	medium sized	large sized
T_1 (Use of rough tiles)	0.11± 0.01	0.23± 0.06
T_2 (Application of smoke)	2.05± 0.20	3.02± 0.24
T_3 (Application of electric shock)	0.50± 0.11	1.30± 0.19
T_4 (Stroke using Pasteurpipette)	0.47± 0.02	0.80± 0.07
CD ($p \leq 0.05$)	0.34	0.48

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