

Study on cephalopod's ink for anti-retroviral activity

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Aqueous extracts of ink from four cephalopods, adult and young *Sepiella inermis* and *Loligo duvaucelli* were tested against Moloney murine leukaemia virus reverse transcriptase (MMLV RT). Ink from young cephalopods, *S. inermis* and *L. duvaucelli* showed strong inhibition of MMLV RT.

Squid, scallop and salmon are commonly used for food in Japan. In India, sepia and squid are delicious seafood. The non-edible parts such as ink gland and other body wastes are discarded. The present study has been undertaken to find out biologically active substances from the non-edible parts of sepia and squid.

In marine red alga, anti-retroviral activity by reverse transcriptase inhibition has been reported by using aqueous extract of *Schizymenia pacifica*¹. Kelletin A, ribityl pentakis (p-hydroxybenzoate, KA), an inhibitor of HTLV-1 replication isolated from *Buccinulum corneum* has shown noncompetitive inhibitory activity with respect to template primer and dTTP in poly (rA) entdot oligo (dT)₁₂₋₁₈ directed reaction of human immunodeficiency virus type-1 (HIV-1), Moloney murine leukemia virus (MMLV) and avian myeloblastosis virus (AMV) reverse transcriptases (RT)². This reverse transcriptase inhibition assay method is an indirect method for HIV inhibition.

Two species of cephalopods *Loligo duvaucelli* (squid) and *Sepiella inermis* (sepia) were taken from Mudasalodai landing centre (11° 29' N; 79° 46' E) on the southeast coast of India for the present study. Fifty fresh animals of each adult and young, were washed with distilled water, dissected carefully to remove ink sac (45g). Ink separated from ink sac and crude extract was prepared³ with some modifications (Fig. 1). The crude extract was screened for anti-retroviral activity. The experiment was conducted in 4 replications.

Electrophoretic RT-inhibition was conducted to screen and identify anti-retroviral potential of ink from squid and sepia. Moloney murine leukaemia

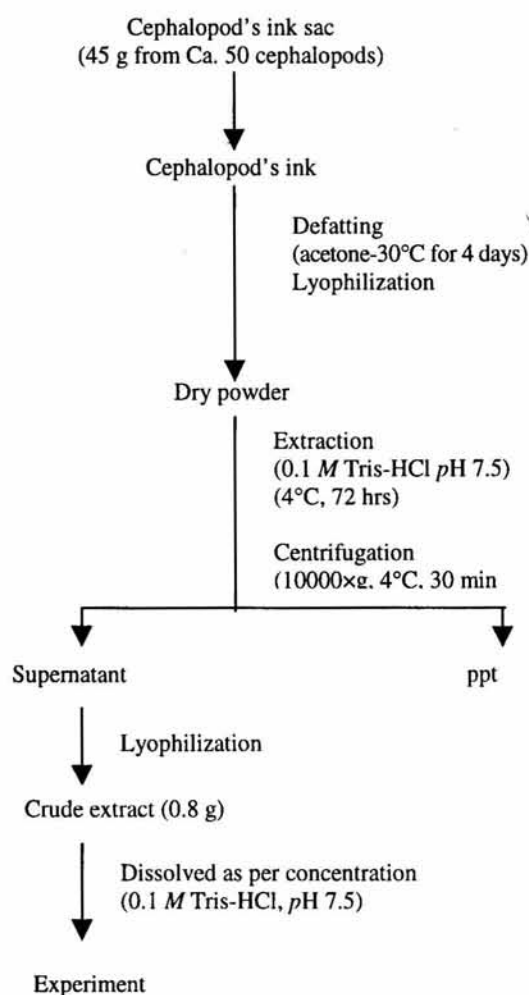


Fig. 1 — Schematic representation of extraction of cephalopod's ink

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virus reverse transcriptase (MMLV RT) was used for DNA synthesis (procured from Amersham International Ltd., UK). Reverse transcriptase (RNA-dependent DNA polymerase) is an enzyme in retroviruses and it plays an important role in multiplication by transcribing viral RNA into cDNA which is required for proviral synthesis. This test is performed to find out the ability of an extract to inhibit RT. Formation of cDNA determines the presence of this property.

Electrophoretic RT-inhibition assay was performed in a reaction mixture containing tris HCl (pH 8.3; 50mM), MgCl₂ (6 mM), KCl (40 mM), poly (rA) entdot oligo (dT)₁₂₋₁₈ (0.5 µg) and MMLV RT (5 units) in a final volume of 25 µL. To this, 2 mL of crude extract of varying concentrations (200, 400, 800, 1600 µg/mL) was added before incubation. Positive control (0.1M tris HCl; pH 7.5) using azidothymidine (AZT; 1 µ/mL) and the solvent control were maintained. All the tubes were incubated at 37°C for 1 hr and the reaction was stopped using EDTA (0.1M). Each assay mixture (6 µL) was loaded on agarose gel (1%) and run for 30 min at 60 v. The gel was stained with ethidium bromide (0.5µg/mL) and viewed in UV transilluminator (Benchtop Transilluminator). The presence or absence of cDNA band indicated non-inhibition or inhibition of RT respectively (Fig. 2). Each sample was tested three times for the reproducibility of its activity with the help of modified cDNA synthesis kit (Amersham International Ltd., Burminghamshire, U K)⁴.

Of the four cephalopod's ink extracts screened, ink from young sepia (*Sepiella inermis*) and squid (*Loligo duvaucelli*) showed maximum inhibition of MMZVRT (Fig. 2; Lanes 5,6) at a concentration of 1600 µg/mL. While ink from adults showed no activity at 1600 µg/mL. Ink secreted by young ones contains high concentration of melanin pigment, which is believed to be alkaloid in nature and restrict the predators, particularly fish, in which it may anesthetize the chemoreceptive senses⁵. Therefore, certain pelagic fishes preferred to eat only small squids⁶ and ejection of ink is an escape response of the young cephalopods. Melanin pigment is formed by oxidation of tyrosine by tyrosinase⁷.

Many of virus inhibitors are polysaccharide (carbohydrate) in nature. So these crude extracts (young cephalopod's ink) may also be containing more polysaccharide components. The use of marine-derived polysaccharides in the treatment of retroviral has been proposed earlier⁸. Popular Japanese squid ink (*Illex argentinus*) which contains peptide (7.8%),



Fig. 2—Electrophoretic RT-inhibition assay [Lane (1)—Molecular weight marker 0.3 to 8.2 kbp; (2)—Reaction mixture; (3)—Positive control (AZT); (4)— Ink of *Sepiella inermis* (adult); (5)— Ink of *Sepiella inermis* (young); (6)— Ink of *Loligo duvaucelli* (young); (7)— Ink of *Loligo duvaucelli* (adult); and (8)— Solvent control]

polysaccharide (57%) and pigment (30%) has exhibited strong antitumor activity against Meth-A fibrosarcoma in BALB/C mice³.

This present study reveals that cephalopod ink has potential antiretroviral activity. Further studies are necessary on their chemical structure to develop effective antiretroviral drug.

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