Screening of Conotoxins from *Cone Snail* for its potential antimicrobial and anticancer (Vero, C33A,SiHa) activity

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Abstract: The current investigation was accomplished to inspect the antimicrobial, antioxidant .insecticidal and cytotoxic action of isolated venom from the toxin gland of *Cone snail*. The anticancerous activity was studied with, C33A and SiHa cancer cells. Antioxidant activities were determined by Hydrogen peroxide, Superoxide anion radical scavenging and DPPH, assay. Antimicrobial activities were tested with (E.coli, Klebseilla, micrococcus, staphylococcus, Bacillus) and insecticidal activity was tested with C.elegans (worm) injecting isolated conus venom. The conus toxin showed antimicrobial IC50 and IC90 (6 to12ug/ml) S.aureus, (4 to 7 ug/ml) Bacillus, (40.5 to 80 ug/ml) E.coli, (5 to 20ug/ml) Micrococcus, (70 to 115 ug/ml) Klebseilla. Antioxidant assays DPPH (55% at 120ug/ml), SOD (2% at 120ug/ml), HSRA (65% at 150ug/ml). MTT assay for cytotoxic action at 120ug/ml showed C33A (17 %) SiHa (12%) Vero normal cells (40%). *C.elegans* (worm) lethality test showed 100% mortality at 200ug/ml within 3 hours of injecting venom confirmed insecticidal activity

IndexTerms - Conus snail, **Venom protein, Antioxidant, insecticidal, C33A, SiHa.**

I. INTRODUCTION

A massive number of therapeutics have been evolved in pharmaceutical applications from natural sources (Da et al., 2001) Venoms are evolutionary innovations from natural sources, constituting of an array of peptides and biomolecule (proteins), established for paralyzing prey and as protecting against predators (Amador et al., 2003) Since the pharmaceutical value of toxins has been developed from ancient periods, venoms have been highly employed as pharmacological device and as prototype tool for drug development (Sadhasivam et al., 2004). Venoms display a vast and important uninvestigated store of bioactive compounds which may have potential healing disease conditions that do not acknowledge currently accessible therapeutics from bioactive compounds (Kim et al., 2010). Recently, a great curiosity has been observed concerning marine-based bioactive peptides as several pharmaceutical potential, revealed in marine source (Kapono et al., 2003). Although, biological organization of marine creatures has provided numerous bioactive peptides, researchers have developed huge interest for venom based peptides and their potential in human metabolism (Calvete et al., 2009). Marine cone snails contain up to 700-800 species (Livett et al., 2004) evolving an apparatus which synthesize, reserve and eject a distinctive set of venomous mixture containing molecular derived components, which could show 100 constituents in a exclusive crude venom, moreover they are short peptides stretches (10-40 amino acids) acknowledged commonly as conopeptides (Dutertre 2010). Reacting oxygen and nitrogen groups (ROS/RNS) are perpetually generated in the living cells and managed by internal enzymes (superoxide dismutase, peroxidase etc.). But, if there is high generation of these groups, subjecting to external oxidant molecules or damage in the defense system, distruction to essential biomolecules (lipids, nucleic acids, and proteins) may happen (Aruoma 1998).Antioxidant from conus toxin reported might be a valuable approach (Sies 1993). Mostly, conotoxin approaches are considered for analgesic and anti-cancer potentials, however less importance has been given to the efficient insecticidal behaviour for the growth of biological pesticides (Hardy et al.,2013) .The growing concern for antibiotic has lead to investigation of antibacterial activity from several sources (Perumal et al., 2007) and in earlier reports antibacterial activity has been reported

from marine cone snails conotoxin (Anand and Edward 2002) The investigation for anticancerous source from marine product has been searched for long time and the utilization of extracted compounds to cure cancer still in process (Guadalupe et al., 2012). As the life threatening diseases are expanding and developing resistance to current drugs, the marine surroundings approach a novel organization for the expansion of pilot compounds against several diseases (Sheih et al., 2010). The previous work in this area obtained from the curiosity of marine biologists revealed several distinctive toxins present in marine life (Ivan et al., 2007).

In order to identify, the conotoxin protein as potent molecule, the following procedures were carried out (i) antioxidant assays (DPPH, SOD, HRSA), (ii) antimicrobial tests with (gram negative and gram positive microbes), (iii) insecticidal test on *C.elegans* (worm) and (iv) cytotoxic action with (C33A and SiHa (cancer cell lines) compared with Vero (normal cell)) with conotoxins extracted from the venom gland of *C.snail*.

II. Materials and Methods

Preparation of venom extract

Live specimens of *C.snail* were collected from Thoothukudi (Lat.8.9063 N′;Long. 78° 46′ E) District of Tamil Nadu. The natural isolates of venom protein were extracted from the toxin gland by protocol. The crude extract mixture was centrifuged at 17,200 r/m for 10 min at 4^{o}C. The supernatant was collected and preserved at -20^{o}C for later use (Saravanan et al., .2009)

Hydroxyl radical scavenging activity

The hydroxyl scavenging test was performed according (Smirnoff and Cumbes 1989). The reaction solution contained 1 ml of 1.5 mM FeSO4-EDTA solution, 1 ml of DMSO (dimethyl sulfoxide prepared in phosphate buffer, pH 7.2). 0.7 mL of 6 mM hydrogen peroxide, and 100ul of sample from different venom isolated concentrations (25 to 150 ug/ml). After incubation for 1 hour at 37C, the reaction solution was stopped by adding 1.ml of chilled TCA (18 % w/v). The Nash reagent 3 ml was added in reaction solution contained ammonium acetate 75g, glacial acetic acid 3.5 ml and acetyl acetone 2.5 ml were mixed and made upto 1L with pure water and incubated at 37°C for 10 min. The test solution with no sample was taken as blank. The strength of the color developed was measured at 562 nm against blank. The hydroxyl radical scavenging percentage was estimated by the following equation:

% HRSA = [(Abs of control - Abs of extract or standard)] X100

Antimicrobial activity

Bacterial strains

The antibacterial screening test were done with gram-positive and gram-negative bacteria. Gram-positive were Bacillus subtillis and Staphylococcus aureus, and gram-negative were Klebsiella pneumonia and Escherichia coli.

Growth curves

Pure culturing of microbial strains

The microbial strains were preserved on Nutrient Agar (Merck) by Slant–Streak method for pure cultures. The nutrient Agar composed peptone (5g), Beef extract (2g), Sodium chloride(5g) and Agar-Agar(20g) was melted in1 liter of distilled water and pH was 7.0. The medium were transferred into conical flask and sealed with cotton plug. The conical flask containing medium was autoclaved. Under sterile conditions, 10 ml of medium was poured into sterile test tubes and cooled in Laminar Air Flow by placing in slanting position. The gelatinized medium was streaked with bacterial cultures with inoculation loop.

The slants with cultures were placed in incubator at 37°C for 48 h. The slants with cultures were preserved at 4°C (Mackie and McCartney 1999)

Confirmation of Pure bacterial culture

The bacterial cultures were inoculated to different medium for confirmation. Slant tubes were prepared. (*S.aureus* in mannitol salt agar, *Bacillus subtillis* and *Micrococcus* in nutrient agar, *Klebseilla* in endoagar, E.coli macconkey agar)

Pure bacterial culture in Nutrient Broth

In aseptic environment, pure colonies from slants were inoculated in sterile test tubes constituting 10ml of nutrient broth and placed in incubator at 37°C for 24 h. Presence of turbidity confirmed growth (Ewnetu et al., 2013)

Investigation of antibacterial potency of venom

One ml of microbial broth culture was taken in eppendrof tubes and centrifuged at 4000 g for 3 minutes supernatant was discarded, resuspended in saline water, this was repeated thrice. According to bacterial strain respective antibiotics were used. For, S.aureus (methicillin) Bacillus (chloramphenicol) E.coli (Amoxicillin) Klebseilla (ampicillin) micrococcus (penicillin) (Fennell et al., 1967) Antimicrobial test of the isolated venom was screened using a sterile 96-well microtiter plate. From, different concentrations of crude venom (250, 120, 60, 30, 15, 7, 5 and 2.5 µg/mL) 100 ul was taken in well. Then 100 µl of bacterial suspension was added in first well (Test). Second well contained 100ul of bacterial culture and 100 ul of respective antibiotic (positive control). Third well contained 100ul of test sample and 100 ul of saline water, Fourth well contained 100 ul of bacterial centrifuged suspension and 100 ul of saline water (negative control). The wells were sealed with parafilm. Each test was done in triplicates. Observance was read for 24 hours at 540nm at 37°C employing a microplate multidetection reader (BioTek's PowerWave XS2) for each well (Jose et al., 2008). Change in observance of bacterial growth was based on turbidity. Turbidity was determined by antibiotic and venom concentration and growth curves were obtained the taking control as growth in absence of drug and test. For the measurement of MIC of crude venom at different concentration, percentage of growth was calculated by the following equation (Jafar et al ., 2014)

% Inhibition=100− [OD of venom-OD of control/OD of the growth control-OD of control]×100

Cytotoxicity assay with normal and cancer cell lines (C33A, SiHa)

Cell line Culture

Cell lines were collected from National Centre for Cell Sciences, Pune (NCCS). The cells were preserved in (MEM) minimal essential media with 10% FBS and antibiotics penicillin (100 U/ml) and tetracycline (100µg/ml) in a CO_2 incubator at 37 °C.

Reagents

MEM, Fetal bovine serum (FBS), methylthiazolyl diphenyl- tetrazolium bromide (MTT), Trypsin and Dimethyl sulfoxide (DMSO) were bought from Sigma Aldrich.

MTT Assay

The anticancer potential of venom on, C33A, SiHa & VERO cells were measured by the MTT assay (3-(4, 5-dimethyl thiazol-2yl)-2, 5-diphenyl tetrazolium bromide (Senthilraja and Kathiresan., 2015). The cell lines (1×10^4 cells/wells) were placed in wells with medium (0.3ml) For MTT test the medium was discarded carefully from the wells after incubation. Wells were flushed with MEM without FCS for thrice and 300µl of (5mg/ml) MTT was added. The microtiter plates were kept for 7 hrs in CO_2 (5%) incubator

for cell toxicity assessment. Once, incubation, period was over DMSO which is solublizing agent 1ml was put to wells, mixed and allowed to stand for 1 minute. The suspension was moved to the cuvette and the observance were read at 600nm by taking blank as DMSO. The venom concentration at which 50% of cell mortality was measured by plotting standard graph by taking venom concentration in X axis and correlative cell mortality in Y axis.

Cell mortality (%) = [Mean OD/Control OD] x 100%

Insecticidal Bioassay

Nematode *C.elegans* (worms) were collected in between growth stage of 3rd and 4th instar. Chicken feed, was provided as worm's food. The desired final concentration (20 ug, 50 ug and 200 ug) of the isolated conotoxins were diffused in 0.8% freshly prepared saline immediately before use. Conotoxins and negative control (0.8% saline) were injected using a micro-injector in a volume of 10 ul, followed by 20uL of 0.8% saline to clean any impurities in the injector. The assay was conducted in triplicates of 10 mealworms, used for different conotoxin concentrations and control. After 48 hours of injection, the mortality of mealworms were determined (Bruce et al., 2011)

III. Results and Discussion

Radical scavenging activity using DPPH assay of extract from *conus snail* venom

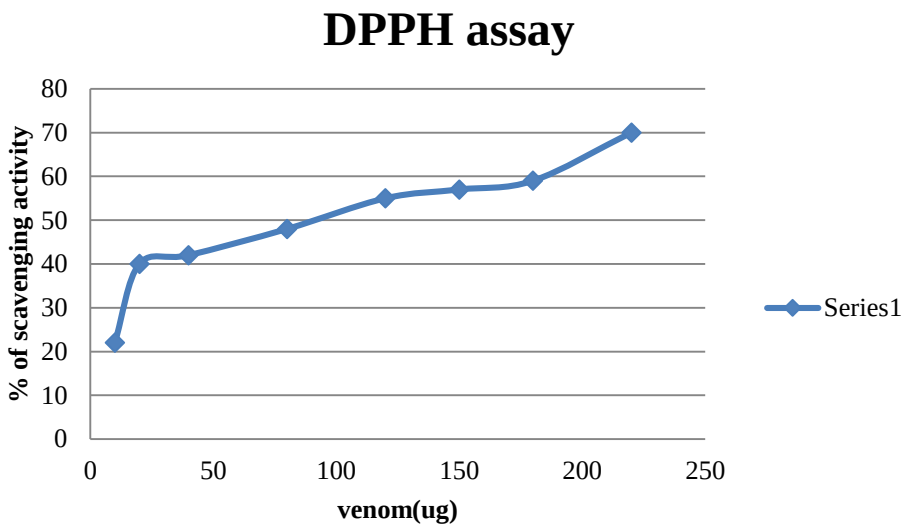

Figure 1: DPPH radical scavenging assay at different concentration venom of *C.snail*

The venom protein hydrolysate from *Conus snail* displays the potential to extinguish DPPH radicals at several concentration (10–220µg/ml) as depicted in Fig. 1. The results were observed where isolates of conus toxin from *Conus snail* showed 55% scavenging potential at120µg/ml concentration whereas standard antioxidant α-tocopherol showed 55% activity at less than 80 ug/ml concentration (Aziz et al., 2007). *Conus amadis* has reported 46% scavenging activity at 120 ug/ml (Ramesh et al., 2014) The potential of *C.snail* venom isolates were observed having DPPH radical scavenging significant activity which evidence the isolate proton donating efficiency and could probably act as free radical scavenger activity as initial antioxidant.

Superoxide anion radical scavenging assay

Superoxides are generated by oxidative enzymes from molecular oxygen and also by non-enzymatic reactions like self-oxidation by catecholamines. Superoxide anions acts important part in the production of several reactive oxygen such as hydroxyl radical, hydrogen peroxide and single oxygen that influence oxidative destruction in protein lipids and DNA (Hemmami and Parihar., 2014) The superoxide scavenging

potential of *Conus snail* venom was observed, having efficient scavenging potential of superoxide anion radicals. Fig. 2.

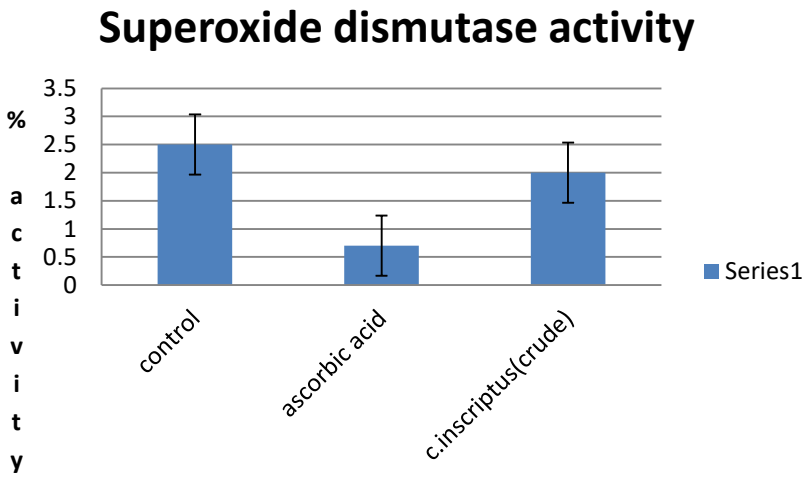

Figure 1: SOD activity of C.snail venom

Superoxide radical minimized NBT and blue color developed observed at 560nm. At 120 ug/ml concentration of venom from *C.snail* showed 2% of scavenging activity, whereas earlier reported in *C.amadis* was 0.9% at 100ug/ml concentration. In ascorbic acid at 100ug more or less 0.7 % was reported (Ramesh et al., 2014). The results of SOD activity reveals concentration based radical scavenging potential, as inhibition percentage increased when sample concentration was higher.

Hydroxyl radical scavenging activity

The potential of Conus venom from *Conus snail* to prevent hydroxyl radical was determined at different concentration 25 to 150 ug/ml. The conus venom displayed least activity of 12% at 25ug/ml and highest activity of 65% at 150 ug/ml, revealed dose dependent activity of hydroxyl radical scavenging activity (Fig. 3). The result shows the scavenging efficiency of *C.snail.*against hydroxyl radicals. Standard ascorbic acid was used to compare the graphical presentation of values obtained. In earlier studies, with conotoxin of *C.amadis* at 50µg/ml concentration 9% the scavenging activity was reported (Ramesh et al., 2014). Hydroxyl radical scavenging potential was analysed by producing hydroxyl radicals utilizing ascorbic acid,iron EDTA (.Hemmami and Parihar., 1998 ,Wickens 2001) Hydroxyl radicals produced on oxidation generates formaldehyde on reacting with DMSO, using Nash reagent for analyzing the activity was found easy method for detecting the activity (Gulcin 2006) Hydroxyl radicals are extremely efficient oxidants, which on reacting with biological molecules in viably cells can cause high damage (Bhavani et al., 1998)

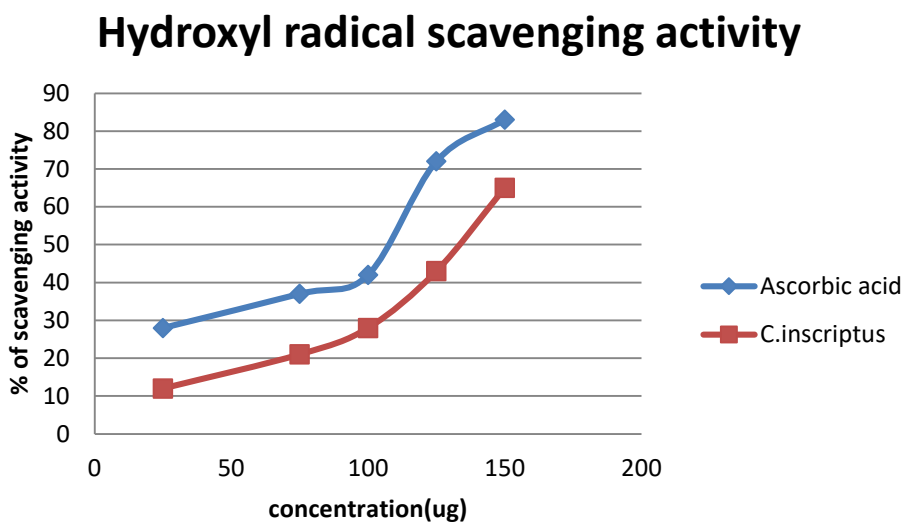

Figure 2: HSRA of C.snail venom compared with standard

Antimicrobial

C.snail crude venom isolate showed a valuable result against selected gram-positive and negative strains of bacteria used in this study. The bacterial growth inhibitory concentration of venom compared with respective antibiotic are shown in Figure 4a,b,c. The minimum inhibitory concentration of the crude venom and respective antibiotic(5ug/ml) was turbidity based observance for growth curves recorded for microtitre wells were found to be 80ug/ml(E.coli), 8ug/ml(s.aureus), 7ug/ml(bacillus) 20ug/ml (micrococcus) and 80ug/ml(Klebseilla) using the microdilution method.. The minimum inhibitory concentration 50% and 90% of the venom isolate against bacteria determined were 6 and 12 µg/mL for S. aureus; 4 and 7 µg/mL for B. subtilis; 40.5 and 80 µg/mL for E. coli; and 5 ug/ml and 20ug/ml for micrococcus 70.5 ug/ml and 115.5 µg/mL for K. pneumonia Figure 5. Antimicrobial has been reported earlier with poultry pathogens and antimicrobial activity observed (Periyasamy et al., 2012). In the present study conus snail showed more activity for gram positive bacterial strains. In earlier study with venom of honey bee similar results were seen (Dani et al., 2003). Among assay with bacterial strains M.luteus and B. subtilis were observed most sensitive to the venom. Therefore, the study shows that the isolated venom is more sensitive for gram-positive bacteria.

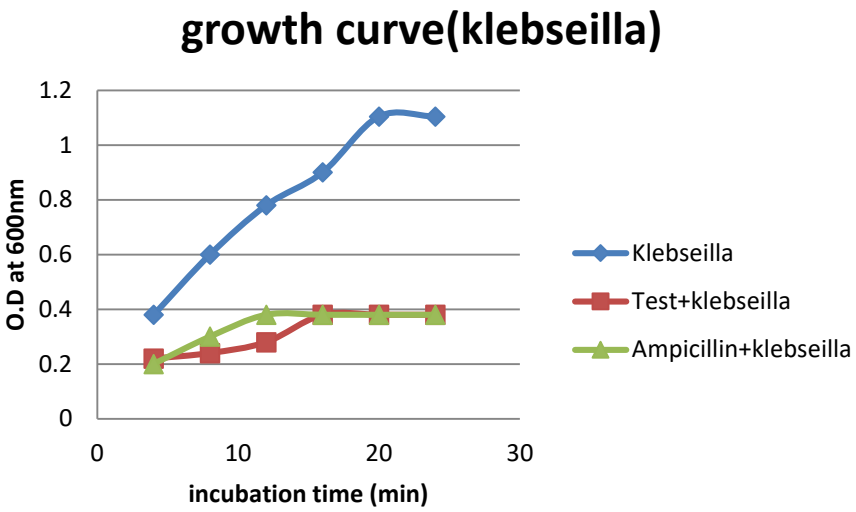

Figure 3(A): growth curve of klebseilla with venom compared with Antibiotic (Ampicillin)

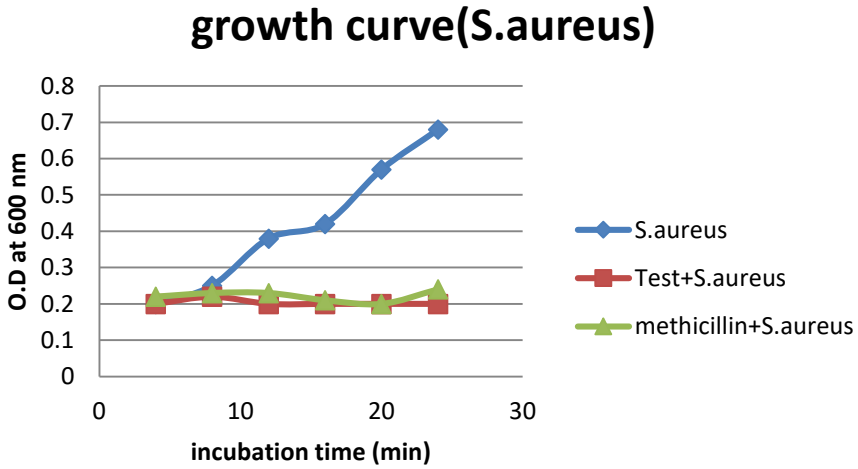

Figure 4(B): *S.aureus* growth curve with venom compared with antibiotic (Methicillin

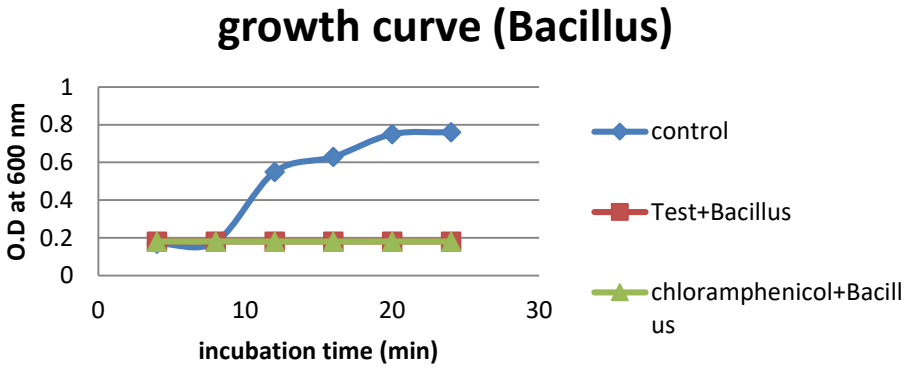

Figure 4(C): Growth curve of Bacillus with venom compared with antibiotic (Chloramphenicol)

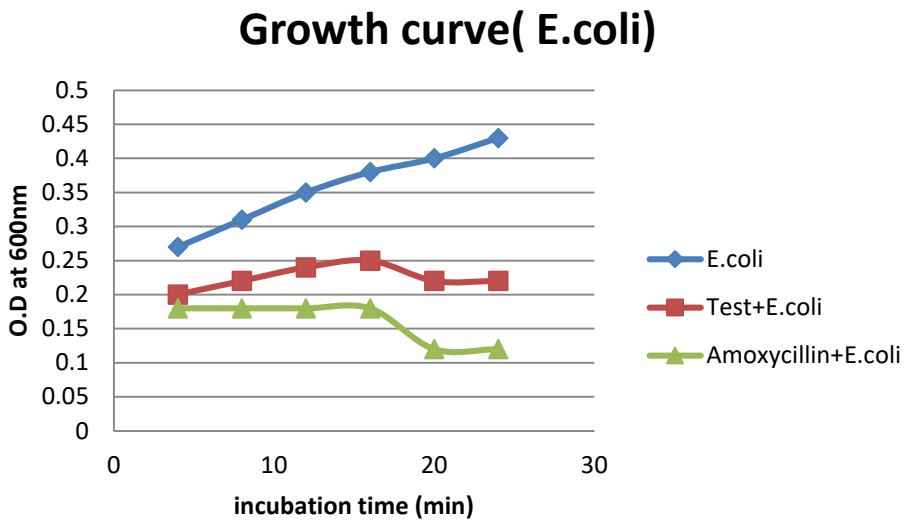

Figure 4(D): Growth curve of *E.Coli* with venom compared with antibiotic (Amoxcyllin

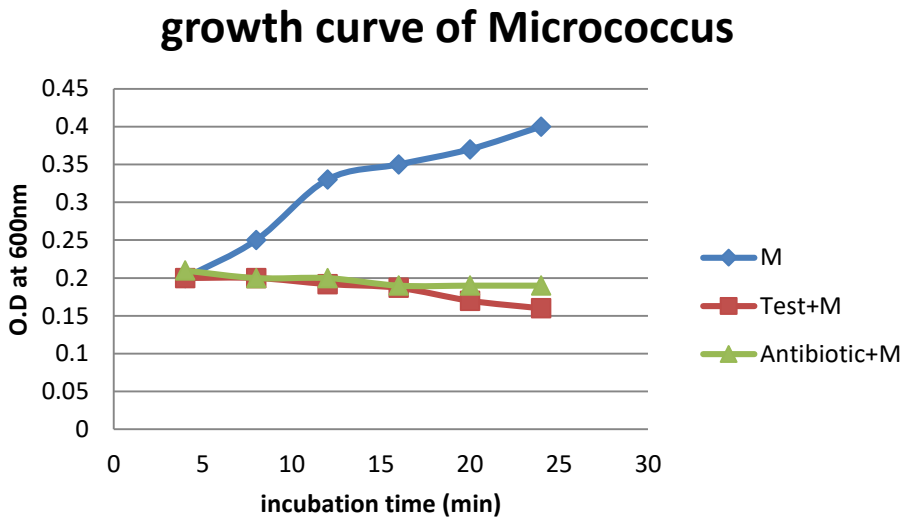

Figure 4(E): Growth curve of Micrococcus with venom compared with Penicillin

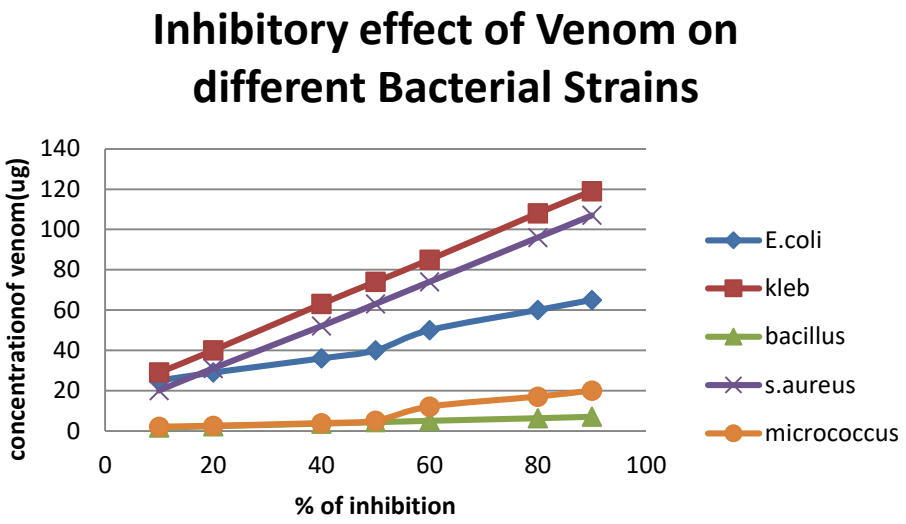

Figure 4: Inhibitory effect of venom from C.inscriptus on different bacterial strains

Anticancer

The measurement of the anticancer potential of conus venom from *C.snail* was determined by cytotoxic assay (MTT assay) against cancer cell line and Vero cell line (Horiuchi et al., 1988) were represented in Figure 6. The conus venom observed cell toxicity on C33A, SiHa cancer cell compared with Vero normal cell at concentrations 120µg/mL, 80µg/mL, 32µg/mL, 12µg/mL, 6µg/mL, and 3µg/mL. At highest concentration 120ug/ml of venom 17% for C33A, 12 % for SiHa and vero normal cell 40% was observed. The present investigation shows that the conus venom isolates of *C.snail* could suppress the proliferation of cervical cancer cells SiHa (HPV positive) and C33A cell (HPV negative) and *in vitro*. C33A and SiHa with bee venom cervical tumor cells growth suppressing activity has been reported (Lee et al., 2015) Earlier with Sf9 cells toxicity assay from conus venom has been reported (Yu et al., 2012)

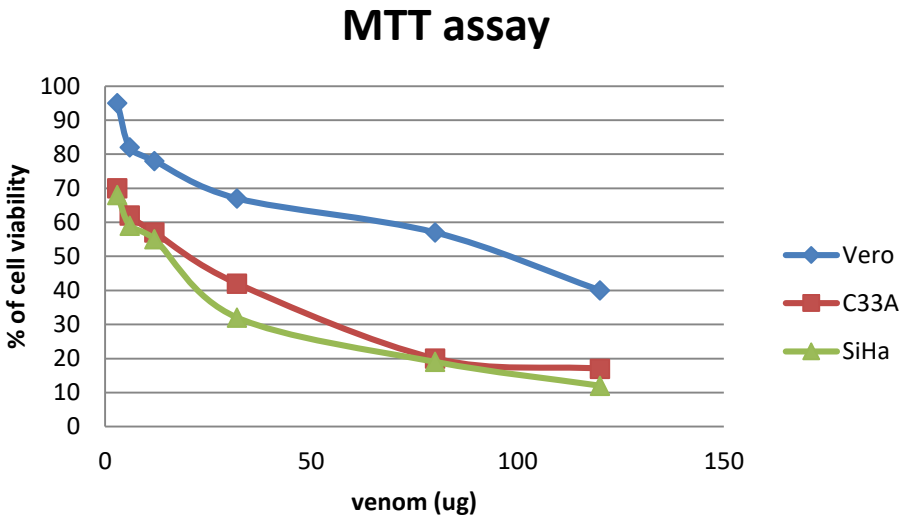

Figure 6: Showing cell viability using the MTT assays on (Vero, C33A, SiHa, cells)

Insecticidal Assay

The results of insecticidal assay has been depicted in Table 1. The conotoxins and controls were injected into the abdomen of nematode *C.elegans* (worm) to estimate insecticidal activity (Bingmiao et al.,

2017). The *C.elegans* (worm) mortality in the blank control (without injection) and the negative control was 0.7% saline injected was 0%, whereas mortality in the isolated conotoxin group was high in contrast to the controls, specifying injection method as feasible process to analyze the insecticidal test of conotoxins. At 200 ug/ml concentration 100 % mortality was observed after 3 hours. As conotoxins are known to be neurotoxic peptides (Richard and McIntosh., 2006). After injecting venom the specimen (worm) mobility was gradually decreased leading to paralysis followed by death

Table 1: **Mealworm lethality test for crude venom of *C.inscriptus***

Aliquots of sample injected (ul)	Control (10 (worms)	Dead cells triplicates			Mortality
		(i)	(ii)	(iii)	(%)
25	10	4	3	4	36
50	10	7	6	7	66
200	10	10	10	10	100

Conclusion

In present study, antimicrobial, antioxidant, insecticidal and cytotoxic tests of the crude and fractions of conus venom from *C.snail* were investigated. In this investigation, it was observed that the conopeptides from *C.insriptus* has antimicrobial, antioxidant, insecticidal and cytotoxic activity. Therefore, these conotoxin exhibited better activity in C33A, SiHa (cancer cell lines) cell lines at lower concentration and does not damage the (normal) Vero cell lines. Thus, concluded that the conotoxin from *Conus insritpus* can be employed as an accessible origin of therapeutic potential.

Conflict of interest

No conflict of interest

References

Amador, M.L., J. Jimeno, L. Paz-Ares, H. Cortes-Funes and M. Hidalgo, 2003. Progress in the development and acquisition of anticancer agents from marine sources. Ann. Oncol., 14: 1607-1611.

Aziz Turkoglu , Mehmet Emin Duru, Nazime Mercan ,Ibrahim Kivrak , Kudret Gezer 2007 Antioxidant and antimicrobial activities of Laetiporus sulphureus (Bull.) Murril Food Chemistry 101:267–273

Anand PT, and Edward JKP. 2002 Antimicrobial activity in the tissue extracts of five species of cowries Cyprea sp. (Mollusca: Gastropoda) and an ascidian, Didemnum psammathodes (Tunicata: Didemnidae). Indian J. Mar. Sci.; 25: 239-242.

Aruoma IO, J. 1998 Free radicals, oxidative stress and antioxidants in human health and disease. J Oil Chem Soc;75:199-212.

Akrima Abu Bakar et al. Turbidity Method to Measure the Growth of Anaerobic Bacteria Related to Microbiologically Influenced Corrosion solid state phenomena 227(2015):298-301

Bruce, C; Fitches, E.C; Chougule, N.; Bell, H.A.; Gatehouse, J.A.2011 Recombinant conotoxin, TxVIA, produced in yeast has insecticidal activity. Toxicon, 58:93–100.

Bhavani B, Pogozelski WK, Tullius WK., 1998 DNA strand breaking by hydroxyl radical is governed by the accessible surface area of the hydrogen atoms of the DNA backbones. J Proc National Acad Sci;95:9738-43.

Bingmiao G, Chao Peng, Bo Lin, Qin Chen, Junqing Zhang, and Qiong Shi, 2017 Screening and Validation of Highly-Efficient Insecticidal Conotoxins from a Transcriptome-Based Dataset of Chinese Tubular Cone Snail Toxins 2017, 9:214;

Calvete JJ, Sanz L, Angulo Y, Lomonte B, Gutiérrez JM.2009 Venoms, venomics, antivenomics. FEBS Lett ; 583: 1736-1743.

Dutertre S, Biass D, Stöcklin R, Favreau P. 2010 Dramatic intraspecimen variations within the injected venom of Conus consors: an unsuspected contribution to venom diversity. Toxicon; 55(8): 1453-1462.

Da Rocha B.A., Lopes R.M. and Schwartsman G., *Curr. Opin. Pharmacol.*, 2001; **1**: 364-369 Kim SK, Wijesekara I. 2010 Development and biological activities of marine-derived bioactive peptides: a review. J Funct Foods; 2(1): 1-9.

Dani MP, Richards EH, Isaac RE, Edwards JP, 2003 Antibacterial and proteolytic activity in venom from the endoparasitic wasp Pimpla hypochondriaca (Hymenoptera: Ichneumonidae). J Insect Physiol, 49(10):945–954.

Ewnetu Y, Lemma W, Birhane N., 2013 Antibacterial effects of *Apis mellifera and stingless* bees honeys on susceptible and resistant strains of *Escherichia coli, Staphylococcus aureus and Klebsiella pneumonia* in Gondar, Northwest Ethiopia BMC Complement Altern Med 19;13:269

Fennell J F, Shipman WH, Cole LJ: 1967 Antibacterial action of a bee venom fraction (melittin) against a penicillin-resistant Staphylococcus and other microorganisms. USNRDL-TR-67-101. Res Dev Tech Rep,5:1–13

Guadalupe-Miroslava Suarez-Jimenez, Armando Burgos-Hernandez and Josafat-Marina Ezquerra-Brauer 2012 Bioactive Peptides and Depsipeptides with Anticancer Potential: Sources from Marine Animals *Mar. Drugs* , *10*, 963-986;

Gulcin I. 2006 Antioxidant and antiradical activities of L-carnitine. Life Sci.;78:803-811

Hardy, M.C.; Daly, N.L.; Mobli, M.; Morales, R.A.; King, G.F. 2013 Isolation of an orally active insecticidal toxin from the venom of an Australian tarantula. PLoS ONE , 8, 73136.

Horiuchi N, Nakagava K, Sasaki Y, Minato K, Fujiwara Y, Nezu K, et al. 1988 In vitro antitumor activity of mitomycin C derivative (RM-49) and a new anticancer antibiotic (FK973) against lung cancer cell lines determined by tetrazolium dye (MTT) assay. Cancer Chemother Pharmacol; 22:246-50.

Hemmami T, Parihar MS. 1998 Reactive oxygen damage., Ind J Physiol Pharmacol ;42:440-452.

Hye Lim Lee et al , Bee venom inhibits growth of human cervical tumors in mice www.impactjournals.com/oncotarget/ Oncotarget, Vol. 6, No.9

Ivan lopez-exposito, ana quiros, lourdes amigo, isidra recio.2007 casein hydrolysates as a source of antimicrobial, antioxidant and antihypertensive peptides. Le lait, inra editions, 87 (4-5),.241-249.

Jose a. Rufian-henares, francisco j.morales 2008 microtiter plate-based assay for screening antimicrobial activity of melanoidins against e. Coli and s. Aureus food chemistry, .11(4): 1069-1074

Jafar jalaei, mehdi fozeli, hamid rajaian, seyed shahram, shekarforoush 2014 in vitro antibacterial effect of wasp (vespa orientalis) venom journal of venomous animals and toxins including tropical diseases, 20:22

Kapono ca, thapa p, cabalteja cc, guendisch d, collier ac, bingham jp.2013 conotoxin truncation as a post-translational modification to increase the pharmacological diversity within the milked venom of conus magus. Toxicon; 70: 170-178.

Livett.B, k. Gayler, & Z. Khalil. 2004. Drugs from the sea: conopeptides as potential therapeutics. Current medicinal chemistry, 11 (13), 1715-1723.

Mackie and McCartney 1999. Practical Medical Microbiology. In:R.S. Miller, S.G.B Amyes, fourth Edition. Lab. Control of Antimicrobial Therapy. 151-178

Nenadis N, Wang LF, Tsimidou M, Zhang HY. 2002 Observations on the estimation of scavenging activity of phenolic compounds using rapid 1-diphenyl2-picrylhydrazyl (DPPH) tests. J Oil Chem Soc 79; 1:1191-5.

Periyasamy.N, S Arularasan, S Gayathri Antibacterial activity of the tissue extracts of Conus betulinus and Conus snail Asian Pacific Journal of Tropical Disease 2012 S914-S919 Linnaeus, 1758 (Mollusca: Gastropoda) from Nagapattinam, Southeast coast of India

Perumal SR, Gopalakrishnakone P, Thwin MM, Chow TK, Bow H, Yap EH, Thong TW:2007 Antibacterial activity of snake, scorpion and bee venoms:a comparison with purified venom phospholipase A2 enzymes. J Appl Microbiol, 102(3):650–659.

Richard.T., J.Michael Mclntosh, 2006 Conotoxins: Therapeutic Potential and Application, Mar.Drugs, 4(3) 119-142

Ramesh. s, E. dilipan, P. mayavu" 2014 Effects of drugs against antioxidant and cytotoxic (hep 2 cell line) activity compounds from marine animals *conus amadis* venom (gmelin, j.f, 1791) International Journal of Pharmacy and Pharmaceutical Sciences, 6(7)

Sadhasivam.G, Arumugam Muthuvel, Ramya Rajasekaran, Abirami Pachaiyappan, Balasubramanian Thangavel 2014 Studies on biochemical and biomedical properties of Conus betulinus venom Asian Pac J Trop Dis; 4(Suppl 1): S102-S110

Sies H, J. 1993 Strategies of antioxidant defense. J Eur ;215 (2):213-9.

Sheih, I.; Fang, T.; Wu, T.; Lin, P. 2010 Anticancer and antioxidant activities of the peptide fraction from algae protein waste. *J. Agric. Food Chem*, *58*, 1202–1207.

Saravanan R., Sambasivam S., Shanmugam A., Sathishkumar D., Tamilvannan T. and Nazeer RA., 2009 Isolation, purification and biochemical characterization of conotoxin from *Conus fugulinus* (1758). Indian J Biotechnol 1758;8:266-71.

Smirnoff N, Cumbes QJ. 1989 Hydroxyl radical scavenging activity of compatible solutes. Phytochemistry.;28:1057-1060.

Senthilraja.P, K. Kathiresan 2015 *In vitro* cytotoxicity MTT assay in Vero, HepG2 and MCF -7 cell lines study of Marine Yeast Journal of Applied Pharmaceutical Science 5 (03) 080-084

Venkatachalam, Suriyavathana, Muthukrishnan 2012 Free radical scavenging activity of ethanolic extract of Desmodium gangeticum Journal of Acute Medicine 2:36-42

Wickens AP.2001 Aging and the free radical theory. Respir Physiol;128:379-391.

Yu, R.; Kaas, Q.; Craik, D.J. 2012 Delineation of the unbinding pathway of alpha-conotoxin ImI from the alpha7nicotinic acetylcholine receptor. J. Phys. Chem. B, 116, 6097–6105