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ANTIACNE AND ANTI DANDRUFF POTENTIAL OF GAMMA PEPTIDE ISOLATED FROM CLARIASGARIEPINUSMUCUS

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ABSTRACT

Fish antimicrobial peptides are recognized as a component of the host's defence against infection. The present study was undertaken to isolate the gamma peptides from the epidermal mucus of *Clariasgariepinus*. The isolated gamma peptides were tested against five pathogenic bacteria and it is mainly concentrated on the acne causing microorganism and dandruff causing fungi. The results of the present investigation revealed that the isolated gamma peptide from the mucus of the *Clarias gariepinu*s having remarkable antimicrobial activity. SDS–PAGE of fish mucus showed many protein bands with molecular size ranging from 20 to 100KDa and only one clear band in the mucus was detected in the gel that represented size of 35kDa.

KEYWORDS:*Antimicrobial peptide, Clariasgariepinus, Mucus, Gamma peptide, SDS-PAGE.*

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INTRODUCTION

Fish are a diverse group of animals, highly specialized for their aquatic existence and comprising almost half the number of vertebrate species in existence today. The global trade in animal based medicinal products accounts for billions of dollars per year[1]. Fish by-products are rich in potentially valuable proteins, peptides minerals, enzymes, pigments or flavours[2]. Among the fish by-products fish mucus is considered more valuable as it contains antimicrobial proteins[3]. The fish skin mucus possesses a variety of biologically active substances such as lectins, proteolytic enzymes, flavoenzymes, protein, apolipoprotein A–I, and antimicrobial peptides that are constitutively expressed to provide immediate protection to fish from potential pathogens and also it has antitumor activity[4]. The tremendous development and spread of antibiotic resistance represent a unique challenge to both science and medicine. The increasing resistance of bacteria to the available antimicrobial drugs has resulted in extensive studies of antimicrobial peptides (AMP's) as therapeutic agent.[5] Therefore, several studies were recently carried out in order to identify new antimicrobial agents to overcome such issue. Several natural agents have been shown to exhibit various grade of antimicrobial activity and among them, fish mucus is emerging as one of the most active. Many publications are available to explain the antimicrobial effect of mucus but there is no investigation, regarding the isolation of gamma peptide from the mucus of *Clarias gariepinu*s. In the current investigation, an attempt was made to screen the antimicrobial activity of the isolated gamma peptide from the mucus of *Clarias gariepinu*s.

MATERIALS AND METHODS

Fish collection and maintenance

Mucus sample was collected from Catfish, *Clarias gariepinus* (body weight: 504.5 g) from a pond in Coimbatore. The fish were kept in a tank of 500 L capacity in laboratory for acclimatization for one week. They were fed with commercial feed. Each day half of the water was changed. After a week the healthy fish were used for mucus isolation. Then the fish were stocked into the 500 L capacity circular plastic tanks. The fish acclimatized to laboratory conditions in tap water.Theywere maintained for one week. During this period fish were fed with commercial feed once a day at ad libitum. Every day 50% of the water was changed. After one week of acclimatization the fish were used for mucus collection. Only healthy fish were chosen for mucus collection. Dead fish or fish with skin lesions were removed from the tanks.

Mucus collection

Fish were starved for one day prior to mucus collection. Before collection of mucus fish were kept out of water in specimen tray for 1 hour. After one hour, mucus that had secreted on the epidermal surface of the body of fish was collected as sample. Mucus was carefully scraped from the dorsal body surface using a sterile spatula. Mucus was not collected in the ventral side to avoid intestinal and sperm contamination. The collected fish mucus was stored at 4°C for further use. The pooled mucus sample was then divided into three parts, which were extracted separately with crude, acidic, and aqueous solvent.

Mucus extraction

For crude extract, 5 ml of fish mucus was centrifuged at 5000 rpm for 10 minutes. The supernatant obtained was th en stored in refrigerator at 4°C.The aqueous extract of fish mucus was prepared using a method as described by (Hellio *et al.,* 2002).[6] 5 ml of fish mucus was mixed with 5 ml of distilled water and the mixture was then centrifuged at 30,000g for 30 minutes at 4°C. Supernatant was then collected and filtered with Whatman no.1 filter paper. The filtrate was then collected and stored in refrigerator at 4°C.The acidic extract of fish mucus was prepared using a modified method of (Subramanian *et al.,* 2011).[5] 3 mL of the fish mucus was mixed with 3mL of 0.3% acetic acid and placed in a boiling water bath for 5 minutes. The acid-mucus mixture was then cooled in ice and the mixture was then centrifuged at 18,000 g for 35 minutes at 4°C. Then, the supernatant was collected and stored in refrigerator at 4°C.

Protein Content

The protein concentration present in the three different extracts of mucus was determined by Lowry's method using bovine serum albumin as standard.

Thin-layer Chromatography

Thin-Layer chromatography profiling was done for the crude, aqueous and acidic mucus extract in solvent system of Butanol, Acetic acid and Water (B: A: W) in proportions of 5:1:4 as developing solvent. After development, compounds were visualized as purplish pink spots on spraying within ninhydrin.

Purification of gamma peptide and conformation of the purified sample

To subject the compounds for various spectral studies, a simple andinexpensive purification procedure was followed. Two TLC plates of these compounds were run simultaneously using TLC sheets. One was developed with the ninhydrin reagent. The corresponding region of the TLC sheet on the other containing the ninhydrin positive compounds was cut into pieces and eluted in 80% ethanol. The compounds thus obtained were subjected to spectral studies such as UV absorption and also for antimicrobial studies. The eluted compounds were again run on TLC sheet for the conformation of purity.

SDS-PAGE

The *C. gariepinus* skin mucus sample was subjected to SDS-PAGE to estimate the molecular weight of proteins present in it. Different standard were used to determine the molecular weight of mucus proteins.

ANTIMICROBIAL ASSAY

The potential of antimicrobial activity of mucus of *C. gariepinus* was screened against seven microorganisms including *Staphylococcus aureus, Bacillus subtili, Proteus species, Klebsiellapneumoniae, Pseudomonas aeruginosa*, *Escherichia coli, Staphylococcus epidermidis* (acne causing bacteria), *Candida albicans* (dandruff causing fungi). The aqueous extract of *C.*

gariepinus mucus was mixed with equal quantity of sterilized physiological saline (0.85% NaCl) and the mixture was centrifuged at 5000 rpm for 15 minutes. The supernatant was collected and stored at 4°C for studying the antimicrobial activity.

RESULT AND DISCUSSION

Mucus of Clarias gariepinus

The amount of mucus collected was 30ml from fish of 504.5g body weight. The amount of mucus secretion varies with different fish species which had been observed to play a role in the susceptibility of the fish to infection.The biochemical substances of mucus have been shown to differ depending on the ecological and physiological conditions such as salinity, pH, handling stress and stages of growth and maturity[4]

Protein

The pooled mucus sample was then divided into three parts, which were extracted separately with crude, acidic, and aqueous solvent and the obtained protein content of the three mucus extracts was shown in **(**Figure 1**).** From figure 1, it was observed that the highest amount of protein content (2.38mg/ml) was present in crude extract, followed by aqueous (1.52mg/ml) and acidic extract (0.48 mg/ml) respectively.Protein quantification results revealed that *C. gariepinus* contain a high amount of protein that may be a potential antimicrobial source[7] Our findings are in accordance with the observation of Wei [4] where the highest amount of protein content was observed in crude extract followed by aqueous and acidic extract.

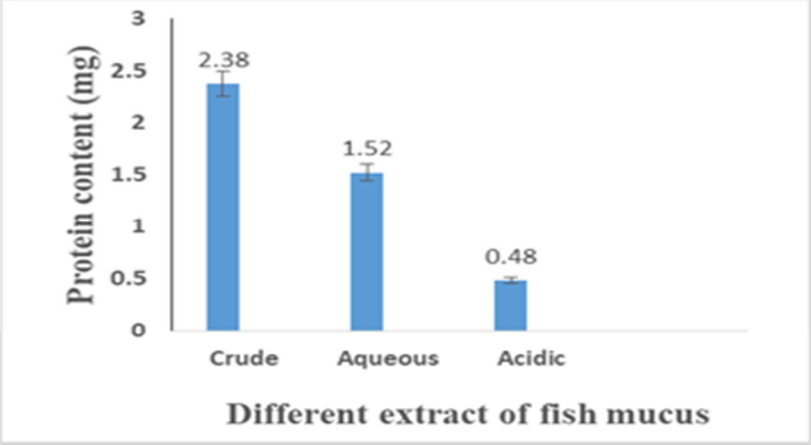


Figure 1
Protein Content in Clariusgariepinusmucus

Conformation of the Peptides by TLC profiling

Thin-Layer chromatography profiling was done for the crude, aqueous and acidic mucus extract in solvent system of Butanol, Acetic acid and Water (B: A: W) in proportions of 5:1:4. The plates when developed in the solvent systems showed light pink spots, when the TLC plate is sprayed with ninhydrin showing pink spots indicated the presence of peptides (plate 2).

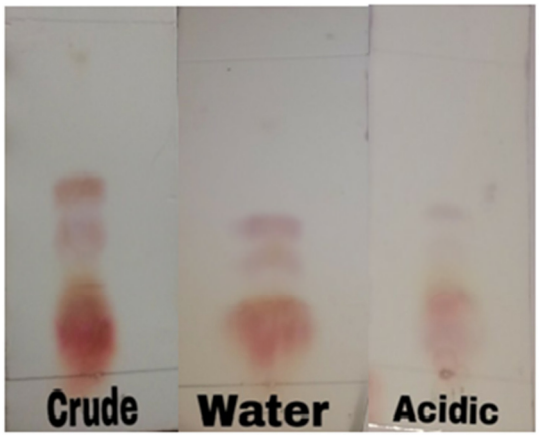


Plate 1
Thin layer chromatography

Since the three compounds were separated perfectly in the aqueous extract of the mucus, it was taken for the further study. The aqueous extract of mucus was again run on TLC sheets several times for the confirmation of the 3 compounds (Plate 4).

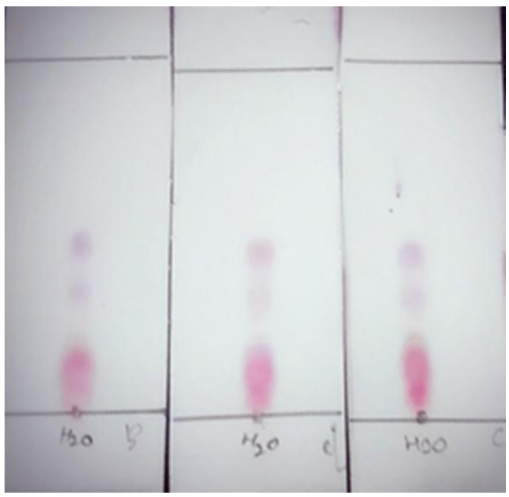


Plate 2
Thin layer chromatography on aqueous extract of mucus

Cu (II)-ninhydrin reaction

The aqueous extract of mucus was run on TLC and sprayed with Cu (II)-ninhydrin reagent. Absence of yellow colour indicates the absence of alpha peptide (Plate 5). Only alpha peptides produce a yellow colour with the Cu (II)-ninhydrin reagent. All gamma peptides produce red colour. All the three compounds separated by TLC appear to be gamma peptides.

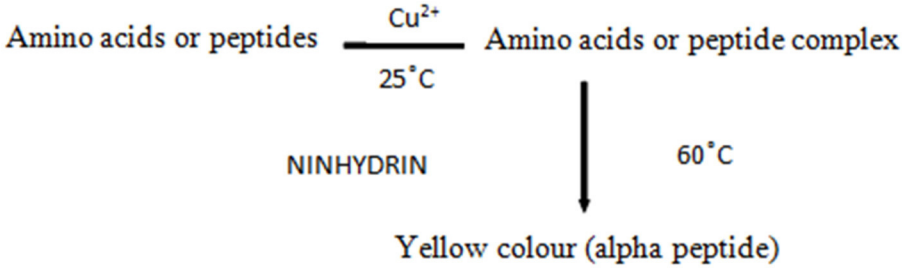
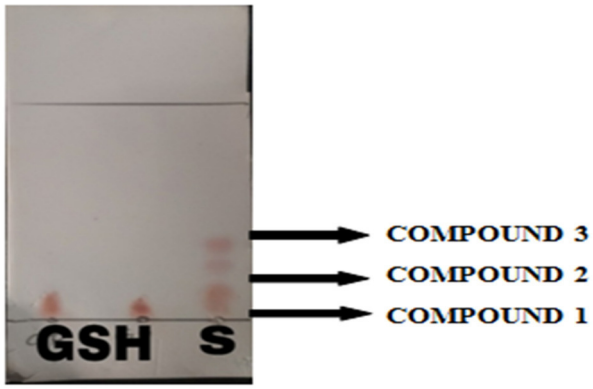


Plate 3
Cu (II)-Ninhydrin reaction

Conformation of the gamma peptide

The aqueous extract of mucus was run with the standard gamma peptide, Glutathione. The first compound of the aqueous extract of mucus was similar to that of glutathione (plate 6).The other two compounds were eluted with ethanol and they were also confirmed as peptides by UV visible spectrophotometry. They may be a different type of gamma peptides but all of them exhibited an adsorption peak at about 210nm in the UV region.[8]



GSH –Glutathione (standard), S–Aqueous extract of mucus

Plate 4
Conformation of the glutathione peptide

Purification of gamma peptide and conformation of the purified sample

The other two compounds were purified by thin layer chromatography moved as a single band on the TLC sheet. The movement of the compound as single band also indicates its purity (Plate 5).

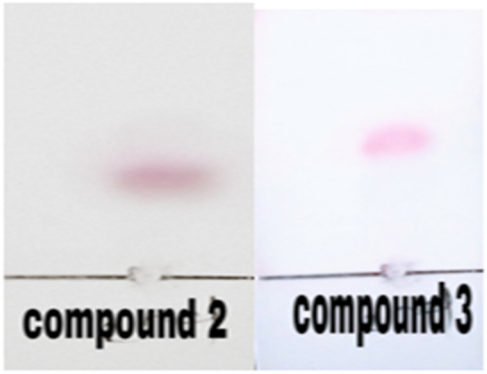


Plate 5
Conformation of the purified sample

Scanning in UV-VIS spectrophotometer

The compounds purified from the aqueous extract of mucus were scanned for their absorption properties, from 200 nm to 400 nm in a UV-VIS spectrophotometer. The second compound purified fromthe aqueous extract of mucusshowed a peak at 206 nm (Figure 5) and the third compound purified from the aqueous extract of mucus showed a peak of 204.5 nm (Figure 6). The results clearly indicated the peptide nature of these compounds [8,9].

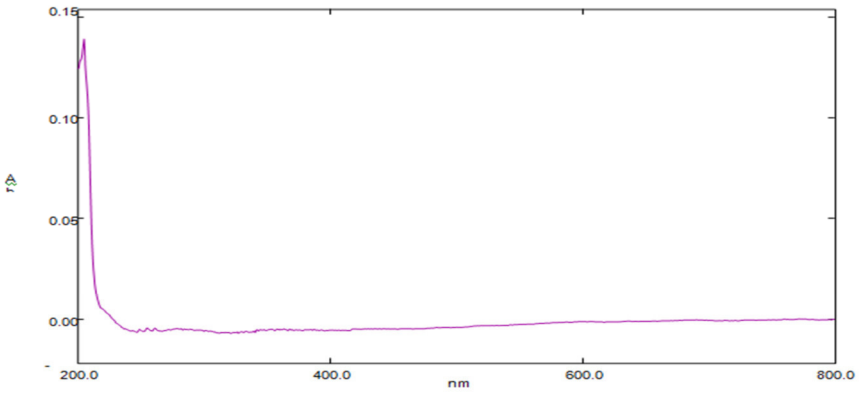


Figure 2
UV-VIS spectrum of the second compound purified from the aqueous extract of mucus

Figure 2 shows the absorption spectrum of the purified second compound from the aqueous extract of mucus. A peak around 206 nm in the UV region is an indicative of the presence of a peptide bond in the compound.

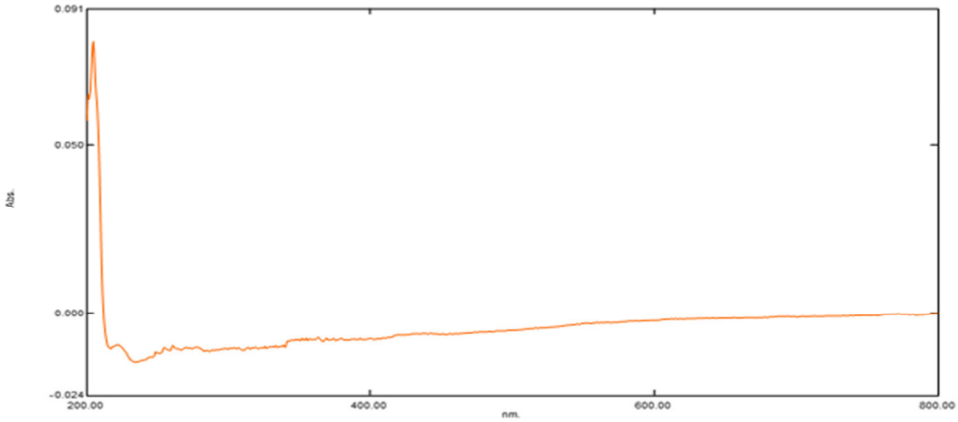


Figure 3
UV-VIS spectrum of the third compound purified from the aqueous extract of mucus

Figure 3 shows the absorption spectrum of the purified third compound from the aqueous extract of mucus. A peak around 204.5nm in the UV region is indicative of the presence of a peptide bond in the compound.

Antibacterial activity of C. gariepinus(mucus and isolated gamma peptide)
Antibacterial activity of purified gamma peptides (glutathione, second and third compound) and aqueous extract of mucus by agar well diffusion method. The obtained results were shown in table 1.

Table 1
Antibacterial activity of C. Gariepinus

Micro organisms	The diameter of inhibition zone (mm) *clariasgariepinus*				
	Purified gamma peptide compounds			Aqueous extract of mucus	Gentamycin
	1(40µl)	2(40µl)	3(40µl)	C(40µl)	G(20µl)
Staphylococcus aureus	12	2	4	6	14
Bacillus subtilis	10	7	5	6	8
Proteus species	7	5	4	6	8
Klebsiellapneumoniae	7	6	8	9	13
Pseudomonas aeruginosa	5	6	3	8	12
Escherichia coli	16	17	7	11	19
Staphylococcus epidermidis	9	8	3	10	15

1 -First compound (Glutathione)
2 -Second compound (gamma peptide)
3 - Third compound (gamma peptide)
C -Aqueous extract of mucus
G -Gentamycin (standard)

Antiacne activity of C. gariepinus (mucus and isolated gamma peptide)
Antiacne activity of purified gamma peptides (glutathione, second and third compound) and aqueous extract of mucus by disc diffusion method were shown in table 2.

Table 2
Antiacne activity of C. gariepinus

Micro organisms	The diameter of inhibition zone (mm) *clariasgariepinus*				
	Purified gamma peptide compounds			Aqueous extract of mucus	Gentamycin
	1(40µl)	2(40µl)	3(40µl)	C(40µl)	G(20µl)
Candida albicans	9	8	3	10	15

Antifungal activity of C. gariepinus mucus (Disc diffusion method)
The antidandruff activity was assayed *in vitro* on SDA agar plate against *Candida albicans.* The parts used for the study were aqueous extract of mucus of *C.gariepinus* and the isolated gamma peptides. Zone of inhibition formed was presented in the table 1

Table 3
Antidandruff activity of C. gariepinus

Micro organisms	The diameter of inhibition zone (mm) *clariasgariepinus*				
	Purified gamma peptide compounds			Aqueous extract of mucus	Ketoconazole
	1(40µl)	2(40µl)	3(40µl)	C(40µl)	K(20µl)
Candida albicans	2	5	-	4	10

Thus from the results obtained above, it clearly shows that the aqueous extract of mucus and the isolated peptides from *C. gariepinus* have certain degree of antifungal activity.The antimicrobial property of epidermal mucus against infectious pathogen has been demonstrated previously by[1] who stated that observed variation in antimicrobial activity among examined fish species may be due to the diverse composition of the secreted mucus. Although the epidermal mucus from several fish has been explored for antimicrobial components, to date little information is available on the antimicrobial activity of the catfish species

PROTEIN PROFILING BY SDS PAGE

The *C. gariepinus* skin mucus sample was subjected to Sodium Dodecyl Sulphate-Poly Acrylamide Gel Electrophoresis to estimate the molecular weight of proteins present in it. Different standard were used to determine the molecular weight of mucus proteins.The standard gel revealed that the mucus contained a simple population of proteins from 20-100kDa. Only one clear band in the mucus was detected in the gel that represented peptide of 35kDa plate.

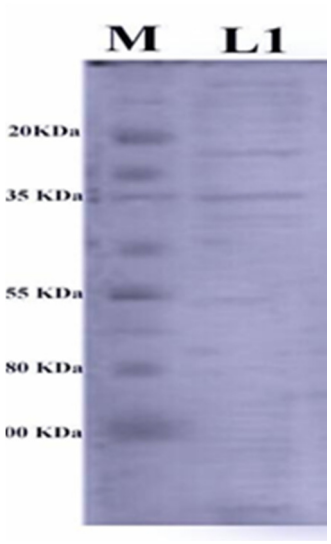


Plate 6
Protein profiling by SDS-PAGE

The study by Shepherd showed that molecular weight of protein from the isolated mucus of other fish species such as winter flounder (2,711.0Da) Catfish (2,000.4Da). The observed variation in antimicrobial activity among fish species examined is thought to be due to the diverse composition of the secreted mucus [10]

CONCLUSION

Fish mucus is attractive in the world of scientific community due to its antimicrobial effect. The antibacterial activity was assayed *in vitro* on nutrient agar plates against different bacterial strains and it mainly focused on acne causing bacteria (*Staphylococcus epidermidis*).Our results showed that, compound1 (Glutathione) exhibited higher Antibacterial activity in all microbial species when compared to other compounds.Thus, the mucus isolated from *C. gariepinus*shows an inhibiting effect on the selected microorganisms. The antibacterial activity of fish mucus may be due to the presence of antibacterial glycoproteins and the ability to kill bacteria by forming large pores in the target membrane. The antifungal activity was assayed *in vitro* on SDA agar plate against dandruff causing *Candida albicans.* It clearly shows that the aqueous extract of mucus and the isolated peptides from *C. gariepinus* have certain degree of antifungal activity. These facts thus open new arenas which provide the scope for research of antimicrobial peptides that may contribute to the development of novel therapeutic agents. However, further investigations are needed to confirm the mode of action and efficacy of *Clariasgariepinus* mucus in order to explore in therapeutic efficacy.

AUTHOR CONTRIBUTION STATEMENT

BrindhaDurairaj and Santhi R conceived of the presented idea. They developed the theory and work was performed by Reni Sheron. All the authors verified the analytical methods. BrindhaDurairaj and Santhi R encouraged Reni Sheron to do the research work and the work was supervised by BrindhaDurairaj and Santhi R. All authors discussed the results and contributed to the final manuscript.

CONFLICT OF INTEREST

Conflict of interest declared none.

REFERENCES

1. Haniffa MA, Viswanathan S, Jancy D, Poomari K MS. Antibacterial studies of fish mucus from two marketed air-breathing fishes – Channastriatus and Heteropneustesfossilis. Int Res J Microbiol. 2014;5(2):22–7.
2. Rustad T. Utilization of marine By-products. Electron. J. Environ. Agric. Food Chem. 2003;2(4):458-463.
3. Effiong BN FJ. Proximate and mineral content of traditional smoked species from Lake Kainji, Nigeria. Bulletin of Environment. Pharmacol Life Sci [Internet]. 2012;1:43–5. Available from: http://bepls.com/march2012/9.pdf
4. Wei OY, Xavier R, Marimuthu K. Screening of antibacterial activity of mucus extracts of snakehead fish, Channastriatus (Bloch). Eur Rev Med PharmacolSci. 2010;14:675–81.
5. Bragadeeswaran S, Priyadharshini S, Prabhu K, Rani SRS. Antimicrobial and hemolytic activity of fish epidermal mucus Cynoglossus arel and Arius caelatus. Asian Pac J Trop Med [Internet]. 2011;4(4):305–9. Available from: http://dx.doi.org/10.1016/s1995-7645(11)60091-6
6. Hellio C, Pons AM, Eaupoil C, Bourgougnon N, Legal Y. Antibacterial, antifungal and cytotoxic activities of extract from fish epidermis and epidermal mucus. IntJAntimicrob Agents 2002;20:214-19.Availablefrom:https://doi.org/10.1016/S0924-8579(02)00172-3
7. Fernandes J. Isolation and characterisation of oncorhyncin II, a histone H1-derived antimicrobial peptide from skin secretions of rainbow trout, Oncorhynchus mykiss. Dev Comp Immunol [Internet]. 2004;28(2):127–38. Available from:http://dx.doi.org/10.1016/s0145-305x(03)00120-4
8. Goldberg DJ, Hussain M, Fazeli A, Berlin AL. Treatment of skin laxity of the lower face and neck in older individuals with a broad-spectrum infrared light device. J Cosmet Laser Ther [Internet]. 2007;9(1):35–40. Availablefrom:http://dx.doi.org/10.1080/14764170601186107
9. Ramachandramurty B SV. Isolation and partial characterization of a small alpha peptide from CuminumcyminumL. Seeds as detected by Cu(II)- ninhydrin reagent. Int J Chem Sci [Internet]. 2009;7(4):2872–82. Available from: https://www.cabdirect.org/cabdirect/abstract/20103109049
10. Shephard KL. Functions for fish mucus. Rev Fish Biol Fish [Internet]. 1994;4(4):401–29. Availablefrom:http://dx.doi.org/10.1007/bf00042888