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BIOREMEDIATION OF INDUSTRIAL MICROBIAL POLLUTANT USING CHITOSAN

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ABSTRACT:

Chitosan is a non-toxic, biodegradable polymer of high molecular weight similar to cellulose (a plant fiber). Chitin is the fiber in shellfish shell such as Crab, Lobster and Shrimp. It is also found in common foods we eat such as grain, yeast, bananas and mushrooms.

Chitin, a naturally abundant polymer consists of 2-acetamide 2-deoxy-ß-D-glucose through a ß-(1-4)linkage.

Chitin is a white, hard, inelastic, nitrogenous polysaccharide and the second most abundant biopolymer and the major source of surface pollution in coastal areas.

The Chitin is deprotenized, demineralised and deacetylated.It is a dietary fiber,meaning that it cannot be digested by the digestive enzymes of a person.(Razdan A & Petterson D.1994)

Chemical chitin extraction has a high efficiency for recovering purified chitin,but the process creates hazardous wastes which are harmful to human health and ecological systems.

Food processing produces large quantities of by-products.Disposal of waste can lead to environment and human health problems,yet often they can be turned into high value,useful products.

In the present study shows antibacterial activity of fish pathogens such as Vibrio cholera,Vibrio parahaemolyticus,Staphylococcus aureus,Escherichia coli,Enterobacter aerogenes,and Salmonella typhi.

To overcome the shortcoming of chemical chitin purification,several biotechnological techniques have been developed that are considered to be efficient alternative approaches for high quality chitin production.As a substitute for chemical and enzymatic processes,lactic acid fermentation combined with microbial deproteinization has been performed.

Key words:Crab shell wastes,Chitin,Chitosan,Portunus pelagicus,Portunus sanguinolentus.

INTRODUCTION:

Chitosan is a natural polysaccharide comprising copolymers of glucosamine and N-acetylglucosamine,and can be obtained by the partial deacetylation of chitin,from crustacean shells, the second most abundant natural polymer after cellulose. Chitin can be converted into chitosan by enzymatic means or alkali deacetylation, this being the most utilized method.During the course of deacetylation, part of polymer N-acetyl links are broken with the formation of D-glucosamine units, which contain a free amine group, increasing the polymers solubility in aqueous means. Typically chitosan is produced from waste generated from crustacean processing (eg. Shrimp and Crab)

Chitin and its derivative chitosan are of commercial interest due to their excellent biocompatibility, biodegradability, non-toxicity, chelating and adsorption power. With these characteristics, especially chitosan has many attractive applications in biotechnology, food and pharmaceutical industry, in cosmetics, environmental engineering, in agriculture and aquaculture.

Chitosan exhibits myrias biological actions such as hypocholesterolemic, antimicrobial and wound healing properties. Since chitosan is a new substance, it is important to carry out precise standardization for its pharmaceutical and biomedical applications like other auxiliary substances.

The Industries reject approximately up to 75% of total weight of raw material, these can create serious pollution and disposal problems. The crustacean shell wastes obtained from sea food industries have only a low economic value and they are used either as a animal feed or organic

manure. The shell fish waste contains 8-10% chitin, 30-65% protein and 10-20% calcium on a dry weight basis.

Chitosan has found wide applicability in conventional pharmaceutical devises as a potential formulation exceipient. The use of chitosan in novel drug delivery as mucoadhesive, peptide and gene delivery, as well as oral enhancer have been reported in the literature.

Scope and approach:

This review summarizes the current state of knowledge of these crustacean shell fish wastes and the various ways to use chitin. This biopolymer and its derivatives, such as Chitosan, have many biological activities eg.anticancer, antioxidant, and immune- enhancing and can be used in various applications (eg.medical,cosmetic,food and textile).

Table:1 Chitin sources

Kingdom/Phylum	Subphylum/class	Groups/species
Arthropods	Insects	Beetles,SilkwormBombyx mori,Aedes aegypti.
	Crustaceans	Crabs,Shrimps,Lobsters,Prowns,Krill.
	Arachnids	Scorpions,Spiders.
Mollusks	Gastropods	Opistho branchia
	Bivalves	
	Cephalopods	Squid pen beaks,Cuttle fish bones.
Fungi	Eurotiomycetes	Aspergillus niger
	Mucormycotina	Mucor rouxii
	Saccharomytes	Candida albicans
Algae	Bacillariophyceae	Diatoms
	Phaeophyceae	Brown algae

	Chlorophyceae	Green algae

References:

Merzendorfer and Zimoch.,2003

Rinaudo.,2006

Grupa and Cody.,2010

Rinaudo.,2006

Martin et al.,2007

Weiss and Schonitzer.,2006

Jothi and Nachiyar.,2012;Sharp.,2013

Walker et al.,2008

Antitumour activity:

The chitosan and its derivatives have antitumor activities using both invitro and invivo models.The antitumour effect of chitosan derivatives is caused by an increase in the secretion of interleukin(IL-1)and 2 which results in the maturation and infiltration of cytotoxic T-lymphocytes.

Antioxidant activity:

Chitosan prevent destruction of membrane lipids,proteins and DNA by the body's reactive oxygen radical molecules.The mechanism of chitosan's antioxidant activity may be through the stabilization of the free radicals by amino and carboxyl groups on chitosan.

Chitosan-based nanoparticles possess large numbers of lone-pair electrons and have high binding power with material with empty orbital.They are used in drugs and gene delivery in biosensor.

Spray drying:

The spray drying method of preparing chitosan nanoparticles involves first dissolving the drug and chitosan together in a solvent.The resulting solution is sprayed through anozzle into a drying champer to form small droplets,which contains hot air to evaporate water to obtain the nanoparticles.The nanoparticles have a uniform and spherical shape.These nanoparticles could play a significant role in the treatment of neurodegenative disorders and pulmonary Tuberculosis.

Chitosan is a biodegradable biopolymer that has the capacity to stimulate an immune response.

Blood coagulation:

Whole blood was mixed with chitin and chitosan suspensions 1.0mg/ml and the platelet rich plasma was mixed chitin and chitosan suspentions,and the platelet aggrecation level induced by chitin was the strongest in all samples including chitosan,cellulose and latex.

Fig:1Portunuspelagicus(Blue swimming crab-Male and Female

Fig:2Portunussanguinolentus(3-spot-Male&Female)

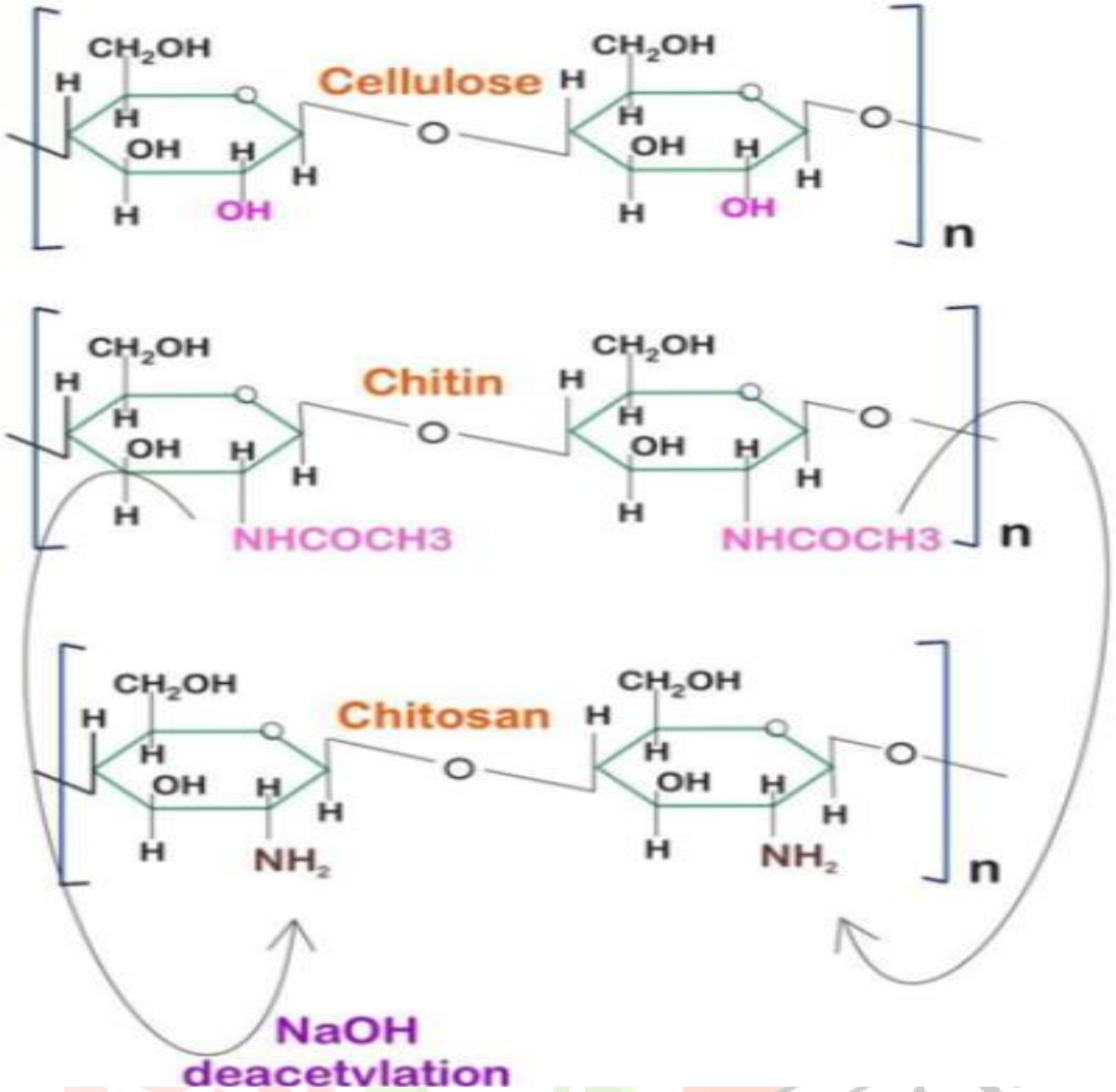


Fig:3 Shows Chitin and its derivatives

Fig:4CHEMICAL CONVERSION OF CRAB HARD SHELL TO SOFT SHELL BY BLENDING WITH HYDROCHLORIC ACID IN BLENDER

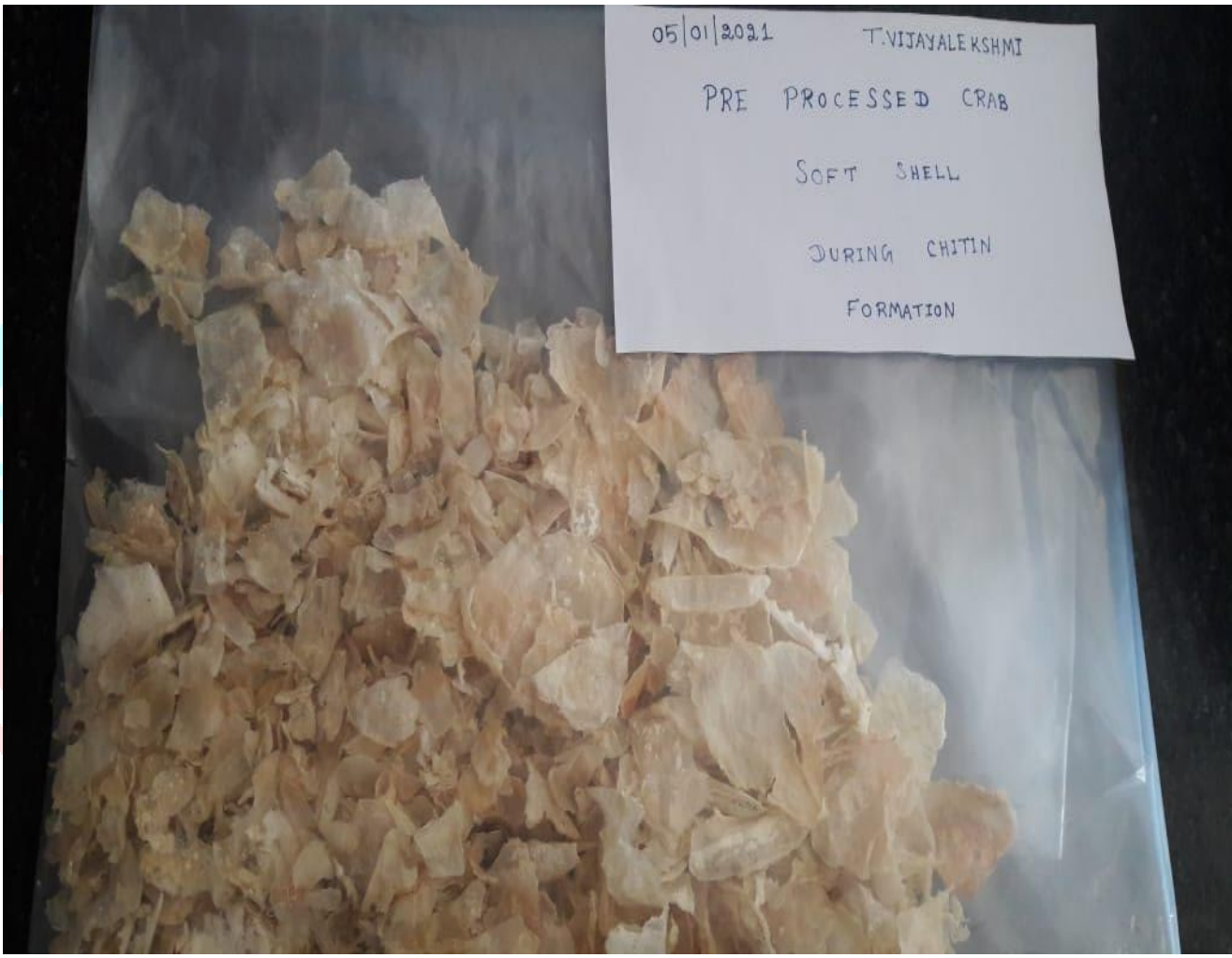


Fig:5PRE-PROCESSED CRAB SOFT SHELL FROM WHICH CHITIN AND CHITOSAN IS EXTRACTED

OBJECTIVE:

The objective of this research is to find the anti-bacterial effects of chitin and chitosan against some harmful pathogenic bacteria.

Scope of study:

The scope of this study covers the protection of our environment from pollutants and waste to useful commercial valuable products.

Materials and Methods

Crab shells from Portunus pelagicus and Portunus sanguinolentus were provided by local processing factory**.** The crab shells were collected in big plastic bags and kept cool in the dark to avoid effects of direct sunlight. After arrival, the Crab shell wastes were directly used to produce chitosan.

Chitin extraction process is basically composed of demineralization and deproteinization stages.Chemical and biological methods may be employed for the two major stages of this extraction process.In the chemical process ,acid and alkaline reagents are employed for the removal of inorganic material and hydrolysis of protein respectively. On the other hand , the biological method makes use of enzymatic extracts or isolated enzymes and biological fermentation.

Fig:6 BIOLOGICAL EXTRACTION(Fermentation) OF CHITIN AND CHITOSAN FROM Portunus pelagicus AND Portunus sanguinolentus

Preparation of Chitin and Chitosan

The preparation of chitin was followed by (2) treatment steps namely demineralization, deproteinization and the production of chitosan by the additional treatment step called deacetylation.

Demineralization

Demineralization of shell wastes were treated with 3% Hcl with a solvent to solid ratio 5:1(v/w) at room temperature (28±2°c) for 16 hrs. The residual Hcl was removed by repeated washing by portable water to reach the neutral pH.

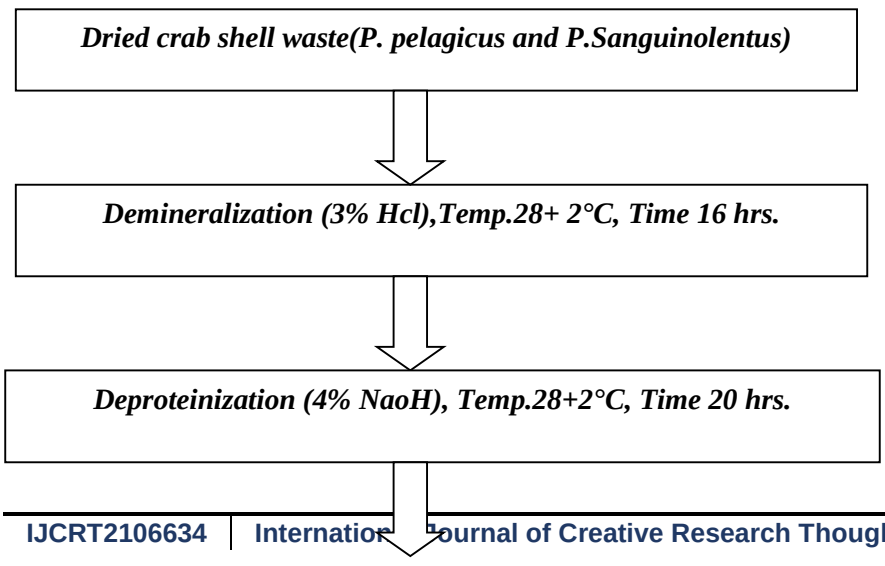
Deproteinization

After demineralization,4%NaOHwith a solvent to solid ratio 5:1 (v/w) for 20 hours at ambient temperature (28±2°c) carried out for the deproteinization of shells. The residual NaOH was removed by repeated washing by portable water to reach the neutral pH.The filtered chitin was dehydrated and made in to powder to enable deacetylation process.

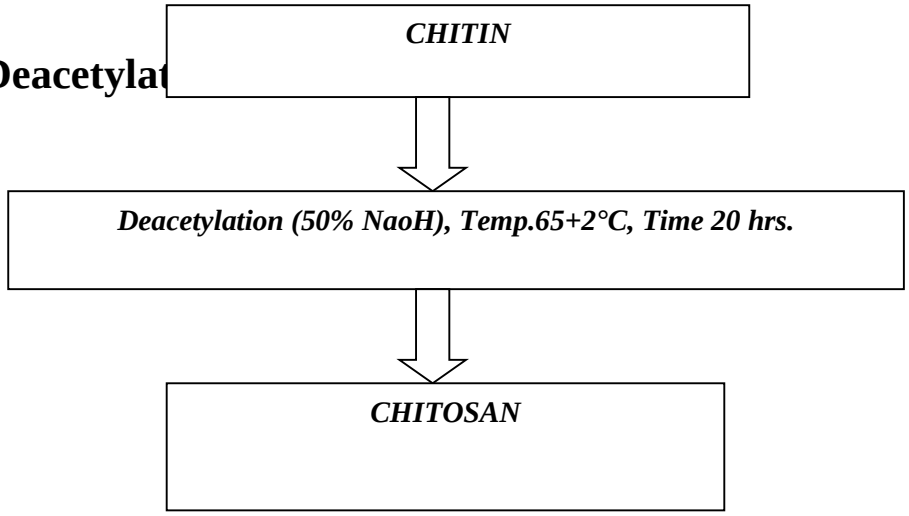
Deacetylation

Removal of acetyl groups from chitin obtained from the shell wastes were treated with 50% NaOH with a solvent to solid ratio 10:1(v/w) for 20 hours at 65°C temperature . The residual NaOH was removed by repeated washing by portable water to reach the neutral PH.The filtered chitosan was dehydrated at hot air oven for 4 hours at 65±50°C to enable the characterization.

Fig:7Schematic flow diagram for production of chitin and chitosan



Deacetylat



Solubility:

Unlike chitosan, chitin is insoluble in organic solvents but chitosan is soluble in acidic condition. Organic acids such as acetic, formic and lactic acid can solubilise the chitosan.

Microorganisms:

The Lactic acid bacterium **Lactobacillus acidophilus isolated from curd.**

Biological chitin purification:

A 24hr culture of **Lacobacillus acidophilus** fermentation was performed by addition of 10% glucose with 500gms of powdered crab shell collected from Portunus pelagicus and Portunus sanguinolentus in a 1000ml conical flask at a pH of 6.1 has been maintained and kept in room temperature for 30 days.

The chitin obtained from biologically purified chitin had a high viscosity compared with chitosan prepared from chemically processed chitin.

During our biological chitin purification we noticed a pH of 8.9 & TDS of 980ppm for chitin solution derived from Portunus pelagicus and a pH of 7.9 & TDS of 1572ppm for Portunus sanguinolentus.

Anti-Microbial activity:

Method:

The method used for this particular experiment was the agar cup diffusion method. The microbial pathogens which have been selected for experiment were as follows:

1) Vibrio parahaemolyticus:
   Colony morphology:
   Blue green colonies with darken centers on Thiosulphate citrate bile salt sucrose agar.

2) Escherichia coli:

Colony morphology:

Circular, non mucoid flat colonies with well defined circle occupying major portions as yellow with pinkish tinge color on Tergitol-7-agar.

3)Vibrio cholera:

Colony morphology:

Colonies will be large, smooth yellow and slight flattened with opaque center and translucent peripheries on Thiosulphate citrate bile salt sucrose agar.

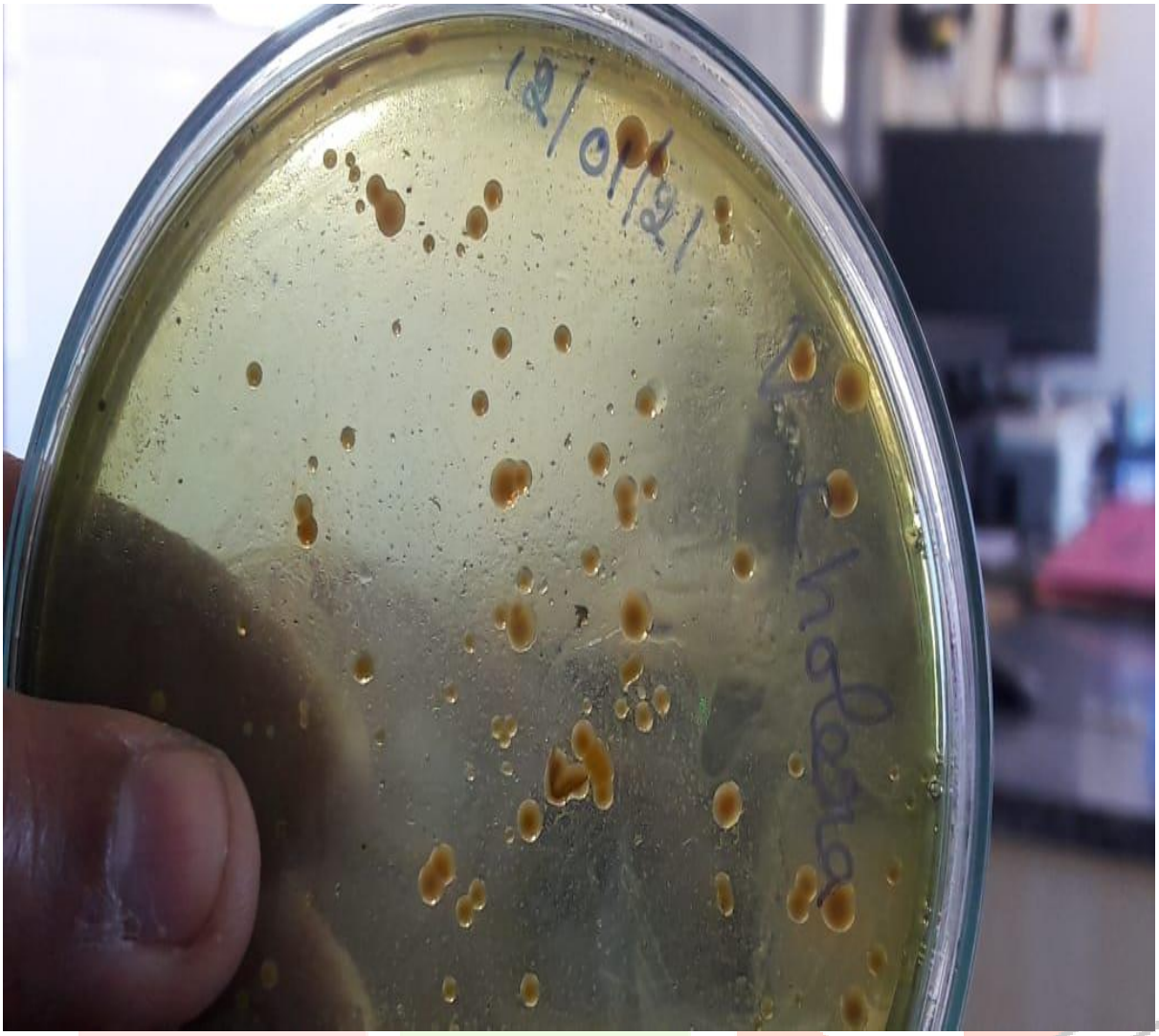


Fig:8 COLONY ISOLATION OF Vibreo cholera IN THIOSULPHATE CITRATE BILE SALTS SUCROSE AGAR

4)Staphylococcus aureus:

Colony morphology:

Black, convex, narrow white entire margin and surrounded by clearing zone on Baird parker agar.

5) Enterobacter aerogenes:

Colony morphology:

Red colored colonies on Tergitol-7-agar differentiation media**.**

These organisms were taken as test strains and inoculated in Tryptone broth and Incubated for 24hrs at 35°C.

Fig:9 COLONY ISOLATION OF Enterobacter aerogenes IN TERGITOL-7-AGAR

The bacterial strains were spread over the Mueller hinton agar medium. one plate for each set has been taken. Two wells of 6mm diameter were dug into each plate and 12µl of chitosan were poured into each well.The plates were incubated at 35°C for 24 hrs and kept in normal position(not being kept in a inverted position)

BIOCHEMICAL TEST:

Gram,s staining:

Prepare a smear ,put a drop of sterile saline ,aseptically add a little of the colony for staining (18 -24 hours culture) mix well in the saline ,air dry,fix by passing the slide 2-3 times through a Bunsen flame.Flood the smear with crystal violet for 1 minute.Wash with tape water.Flood the smear with crystal violet for 1 minute .Wash with tape water.Flood the smear with gram,s iodine for 1 minute.Wash with ethyl alcohol(75%).Wash with tape water.

Flood with Safranin for 30 seconds.Wash with tape water.Airdry.Observe under Microscope.Gram-positive organisms stain violetcolour.

Gram-Negative organisms stain red colour.

Motility test:

Tube method:

Inoculate motility medium by stabbing into the top of a tube of the semi solid medium to a depth of about 5mm.Incubate at 35-37°c for 48 hrs and observe for spreading of the growth through the medium.

Slide motility method:

Hanging drop method:

Place vasaline in four corners of a coverslip.Using a sterile loop place a drop of an 18 hrs nutrient broth culture centre of the cover slip.Place a clean microscope slide(cavity slide) over the top of the coverslip.Invert the slide so that the drop is upside down.Observe under microscope using x 40 objectives.The bacterium can be said to be motile when it is seen to move from one side of the field of view to the other.

Indole test:

Inoculate tubes of Tryptone broth incubate tubes at 37°c for 24 hours.Add 0.2-0.3 ml of indole (Kovac,s)reagent to the tube and shake.Allow 10 minutes and observe the results .A dark red colour in the amyl alcohol surface layer constitutes a positive test.

Methyl test:Inoculate tubes of MRVP medium inoculate tubes at 37°c for 5 days .Add five drops of methyl red solution and shake .Record a distinct red as methyl red positive,a distinct yellow as methyl red negative.

Voges -Proskauer test:

Inoculate tubes of MRVP medium and incubate at 37°c 48 hrs.Pipette 1 ml of each culture to ₄a separate empty culture tube and add 0.6 ml of α-naphthol solution and 0.2ml of potassium hydroxide solution.Shake

the tubes and let them stand 2-4 hours .Record the development of pink colour in the mixture as a positive test.

Simmon's citrate Agar:

Inoculate in Simmon's Citrate agar slant and slab the butt and incubate for 96 hrs at 37°c.Usually accompanied by colour change from green to blue.

Catalase test:

Place 30%Hydrogen Peroxide (H_2O_2) in a clean slide .Add a 18 hrs young culture use platinum loop and mix well,Bubbles formation in slide means Positive reaction.If bubbles are absent Negative reaction.

Oxidase test:

Place a piece of filter paper into an empty petridish and add 3 drops of tetramethyl paraphenylene diamine dihydrochloride solution to its centre with a sterile glass rod smear cells thoroughly into the reagent.The oxidase test is positive if transferred cells turn dark purple in5-10 seconds.

Urease test:

Urea:20g

Yeast extract :0.1g

Na_2HPo_4:9.5g

K_2HPo_4:9.1g

Phenol red:0.01g

Distilled water:1 liter

Inoculate growth from each presumed positive TSI slant culture into tubes of Urea broth inoculated tubes of Urea broth.Dissolve the ingredients in distilled water .Do not heat.Sterilize by filtration through 0.45µm membrane .Aseptically dispence 1.5 -3.0ml portions in sterile test tubes.Incubate 24 hrs at 35°c turn at purple red positive.

Coagulase positive Staphylococci:

Add 0.5ml of Rabbit plasma to a small test tube .Transfer 2 drops of the suspended 24 hrs culture .Incubate at 37°c and observe for coagulation at 1hour.

Slide coagulation method:

Pick off a minimum of five colonies of Staphylococcus and do coagulase test in a slide ,add one drop of plasma,coagulation occur within 1 minute positive reaction.

Compared with Bergey's manual and identified the microorganisms.

MINIMAL INHIBITORY CONCENTRATION:

Determination of mechanisms of antibiosis (bacteriostatic or bactericidal).

The minimal inhibitory concentrations(MIC) was determined by the broth dilution method.Appropriate chitosan filtered extract was diluted in tryptone broth or peptone water and filled up to the brim of each well and negative control were used to validate the inferences. The plates were incubated at 37°c for 24 hrs,after incubation the bioactivity was determined by measuring the diameter of inhibition zone.

Fig 10: 2.4mm ZONE FORMATION IN E.coli DURING INHIBITION OF CHITIN AND ITS ANTI-BACTERIAL ACTIVITY CONFIRMATION

Fig:11COLONY MORPHOLOGY OF Staphylococcus aureus FROM BAIRD PARKER AGAR

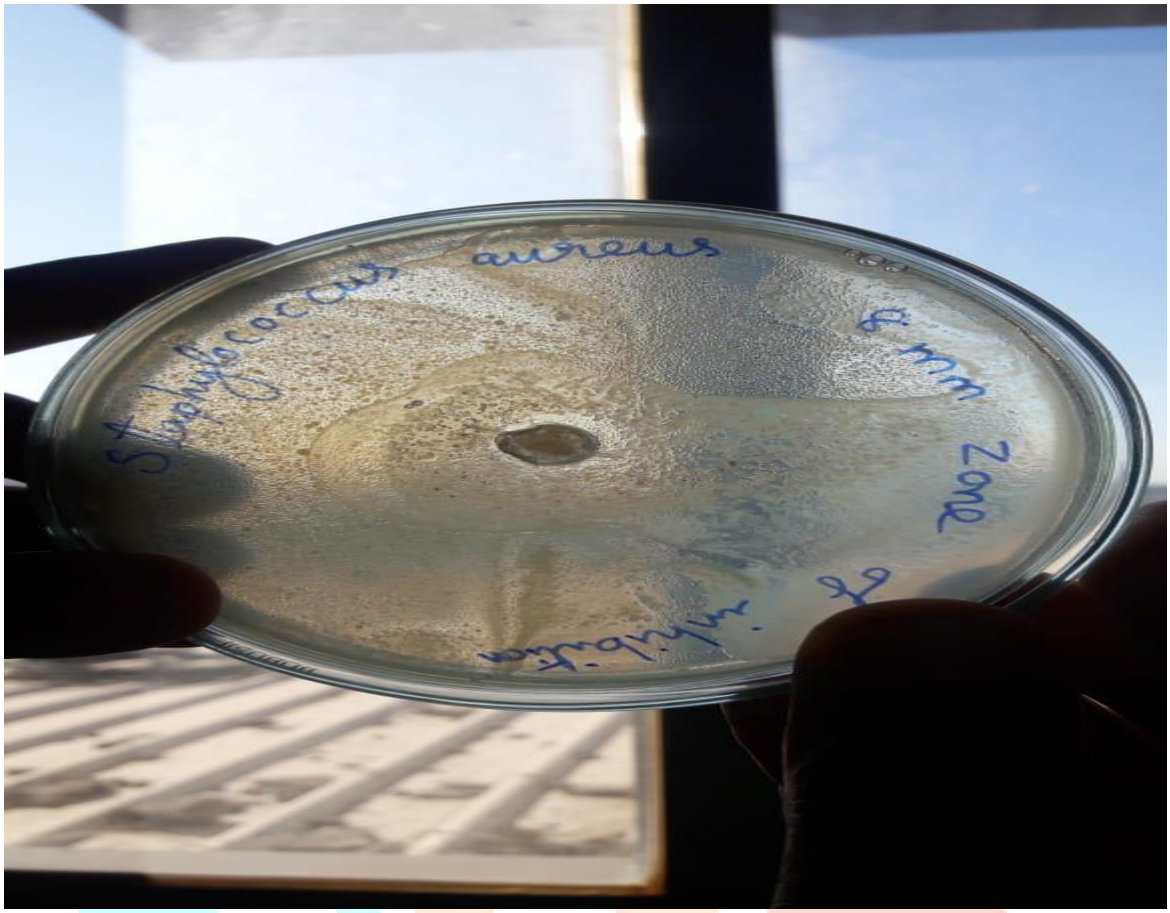


Fig:12 0.8mm ZONE FORMATION IN Staphylococcus aureus DURING INHIBITION OF CHITIN AND ITS ANTI-BACTERIAL ACTIVITY CONFIRMATION

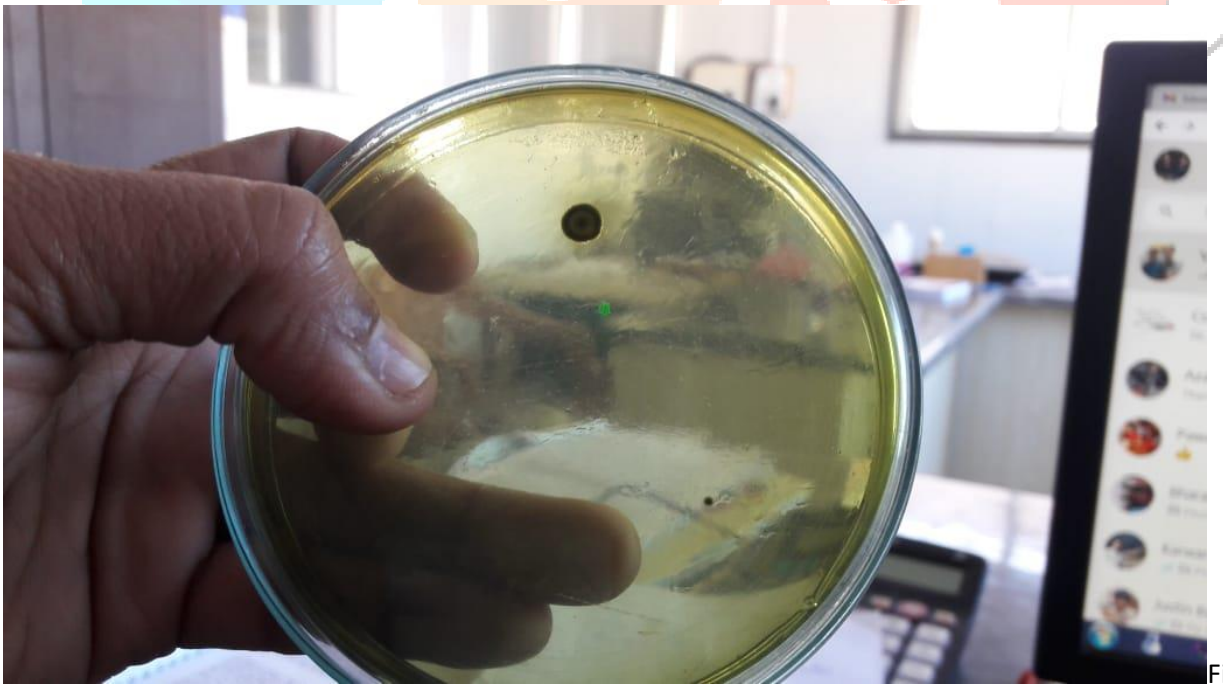


Fig:13 V.parahaemolyticus growth on Thiosulphate citrate bile salt sucrose agar

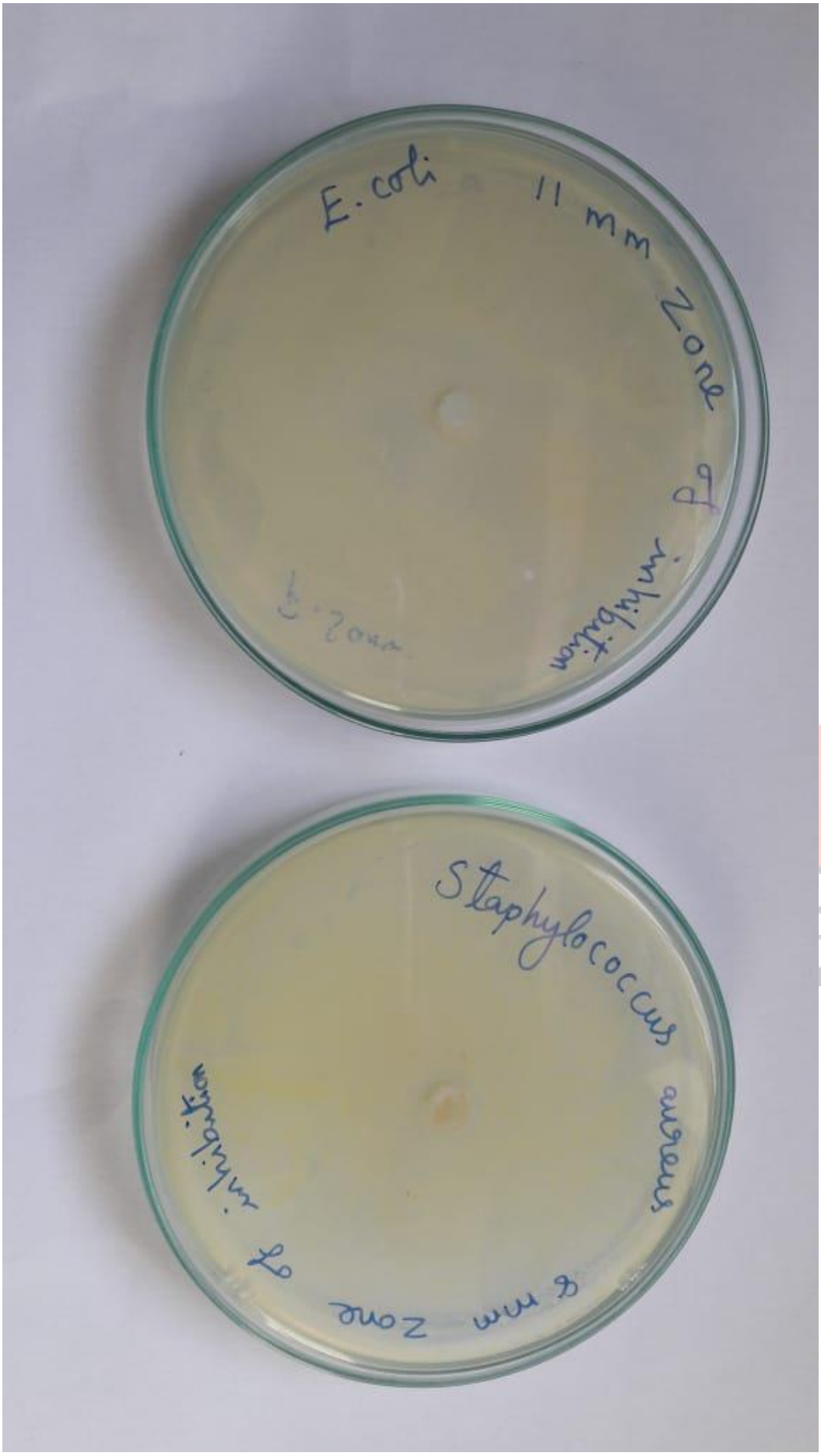


Fig:14 Antibacterial activity of chitosan against pathogenic microorganisms

INVITRO ASSAYS :

DIFUSSION AGAR TEST:

The method widely used to evaluate antibacterial activity is the well diffusion method. Soluble extracts diffuse into the culture medium generally Muller hinton agar used for bacteria. The plates were incubated under the optimal conditions37°C for each microorganisms ,and the presence or not of an inhibition zone was determined.

Fig:15 Indole test conformative test for E.coli

BIOCHEMICAL TEST	ENTEROBACTER AEROGENES	ESCHERICHIA COLI	VIBRIO CHOLERA	VIBRIO PARAHEMOLYTICUS	STAPHYLOCOCUS AUREUS	
INDOLE TEST	-VE	+VE	+VE	+VE	+VE	
METHYL REDTEST	-VE	+VE	_VE	+VE	+VE	
VOGES PROSKAUER	+VE	-VE	+VE	_VE	+VE	
MOTILITY TEST	MOTILE	MOTILE	MOTILE	MOTILE	NON-MOTILE	
SIMMON'S CITRATE TEST	-VE	-VE	+VE	+VE	+VE	
CATALASE TEST	+VE	+VE	+VE	+VE	+VE	
UREASE TEST	-VE	-VE	_VE	+VE	+VE	
TRIPLE SUGAR IRON TEST	+VE	+VE	_VE	_VE	_VE	
GRAM'S STAINING	-VE,ROD	-VE,ROD	_VE,C0MMA	_VE,COMMA	+VE,COCCI,GRAPE LIKE CLUSTERS	
H_2S PRODUCTION	-VE	-VE	_VE	_VE	_VE	
COAGULASE TEST	-VE	-VE	_VE	_VE	+VE	
OXIDASE	_VE	_VE	+VE	+VE	_VE	

Table:2 Biochemical test results(Compared with Bergey's manual of systemic Bacteriology.

Results and Discussion:

If there is any anti-microbial activity in the extracts then a zone of clearance will be formed around the corresponding well, which occurs due to diffusion of the extract through the agar.

Table:3 Comparative Zone formation between P.pelagicus and P.sanguinolentus :

Organisms	**Portunus pelagicus(zone of inhibition)**	**P.sanguinolentus(zone of inhibition)**
V.cholerae	0.8mm	2.1mm
V.parahaemolyticus	0.9mm	2.0mm
Escherichia coli	1.3mm	2.4mm
Staphylococcus aureus	0.8mm	1.8mm
Enterobacter aerogenes	1.2mm	1.9mm

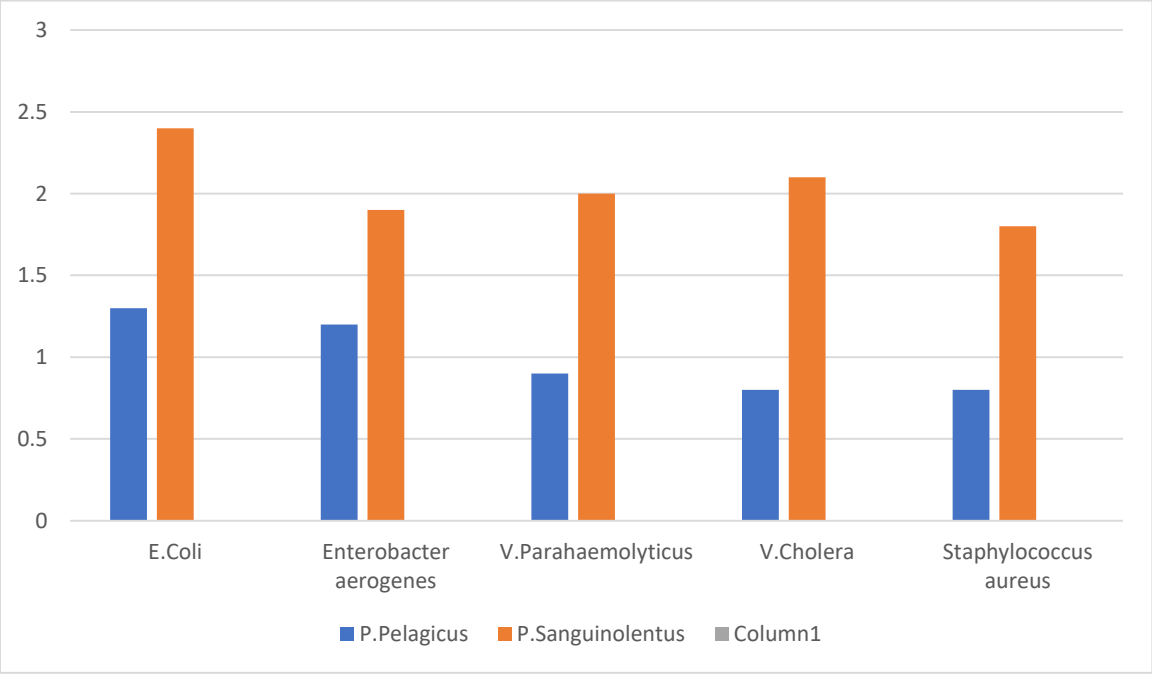


Fig:16 Trend analysis showing comparison between P.pelagicus and P.sanguinolentus

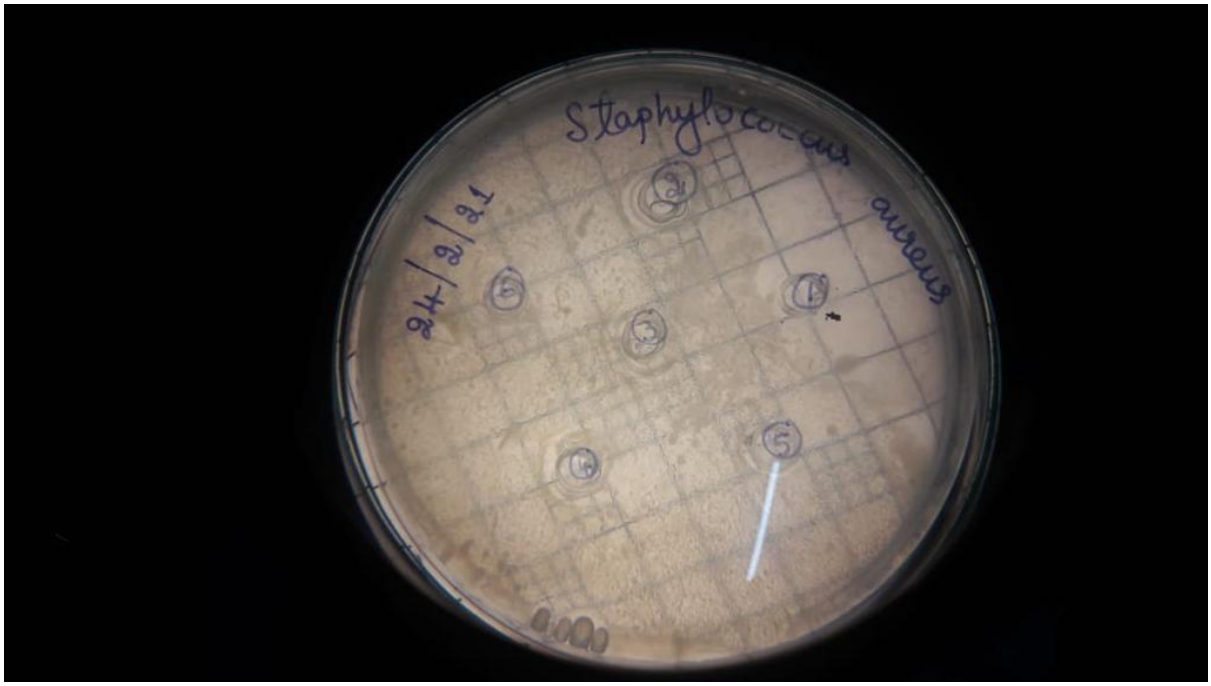


Fig:17 MIC 10^{-1} TO 10^{-6} against Staphylococcus aureus

Fig:18 MIC REPORT FOR TEST PATHOGENS

Table:4MIC results of Chitosan using Portunus pelagicus and Portunus sanguinolentus

MIC	Enterobacter aerogenes		Eschrichia coli		S.aureus		V.parahaemolyticus		V.cholerae		
	Portunus pelagicus	Portunus sanguinolentus	Porunus pelagicus	P.sanguinolentus	P.pelagicus	P.sanguinolentus	P.pelagicus	P.sanguinolentus	P.pelagicus	P.sanguinolentus	
10^{-1}	0.8mm	1.4mm	0.9mm	2.0mm	0.8mm	1.8mm	0.8mm	1.3mm	0.6mm	1.9mm	
10^{-2}	0.7mm	1.3mm	0.8mm	1.8mm	0.7mm	1.2mm	0.7mm	1.2mm	0.5mm	1.1mm	
10^{-3}	0.6mm	1.3mm	0.7mm	1.2mm	0.7mm	0.8mm	0.7mm	1.0mm	0.5mm	0.7mm	
10^{-4}	0.5mm	1.2mm	0.6mm	1.0mm	0.6mm	0.7mm	0.6mm	0.8mm	0.5mm	0.6mm	
10^{-5}	0.5mm	0.9mm	0.5mm	0.7mm	0.4mm	0.6mm	0.5mm	0.7mm	0.5mm	0.6mm	
10^{-6}	-	0.6mm	-	0.6mm	-	0.6mm	-	0.7mm	-	0.6mm	

conversion of chitin into chitosan by employing enzymatic or chemical method.Biological conversion process have some eco-friendly procedure and application also more than the chemical process.

Portunus pelagicus common name blue swimming crab,these crab have hard shell wall.

Portunus sanguinolentus common name 3-spot crab,these crab have soft shell .After lactic acid fermentation process TDS 1572 and Portunus sanguinolentus shell powder completely dissolved in lactic acid.

The present study crab shell waste contain large quantities of chitin,this biopolymer and its derivative such as chitosan, have biological activities anticancer,antioxidant and immune -enhancing and can be used in various applications medical,cosmetic,food and textile.

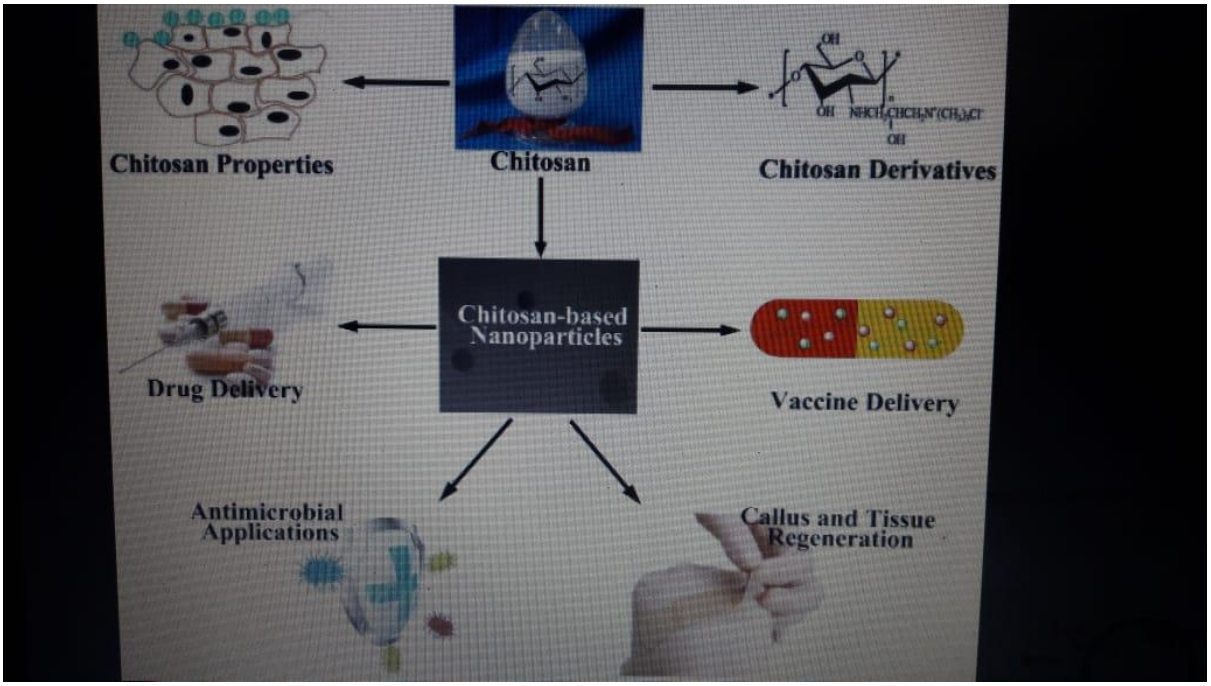


Fig:19Chitosan and its applications

Elisa test Procedure

1) **Homogenize a representative sample amount completely–**
2) **Add 3 ml of distilled water and 6 ml ethyl acetate to 3 g of homogenized sample– and mix shake for 10 min upside down–**
3) **Centrifuge: 10 min / 3,000 g / room temperature (20 - 25 °C)**
4) **Transfer 4 ml of supernatant (corresponding to 2 g of sample) into a new vial– and evaporate at 60 °C to complete dryness by nitrogen or air reconstitute the dried residue in 1 ml n-hexane– add 500 µl wash buffer and vortex for 1 min– centrifuge: 10 min / 3,000 g / room temperature (20 - 25 °C)**
5) **use 50 µl of the lower aqueous phase per well in the assay**

Test procedure:

Carefully follow the recommended washing procedure. Do not allow microwells to dry between working steps. 1. Insert a sufficient number of wells into the microwell holder for all standards and samples to be run in duplicate. Record standard and sample positions.

2. Add 50 µl of each standard or prepared sample to separate duplicate wells.

3. Add 50 µl of the conjugate to the bottom of each well, mix gently by shaking the plate manually and incubate for 30 min at room temperature (20 - 25 C).

4. Pour the liquid out of the wells and tap the microwell holder upside down vigorously (three times in a row) against absorbent paper to ensure complete removal of liquid from the wells. Fill all the wells with 250 µl washing buffer (see 10.1.) and pour out the liquid again. Repeat two more times.

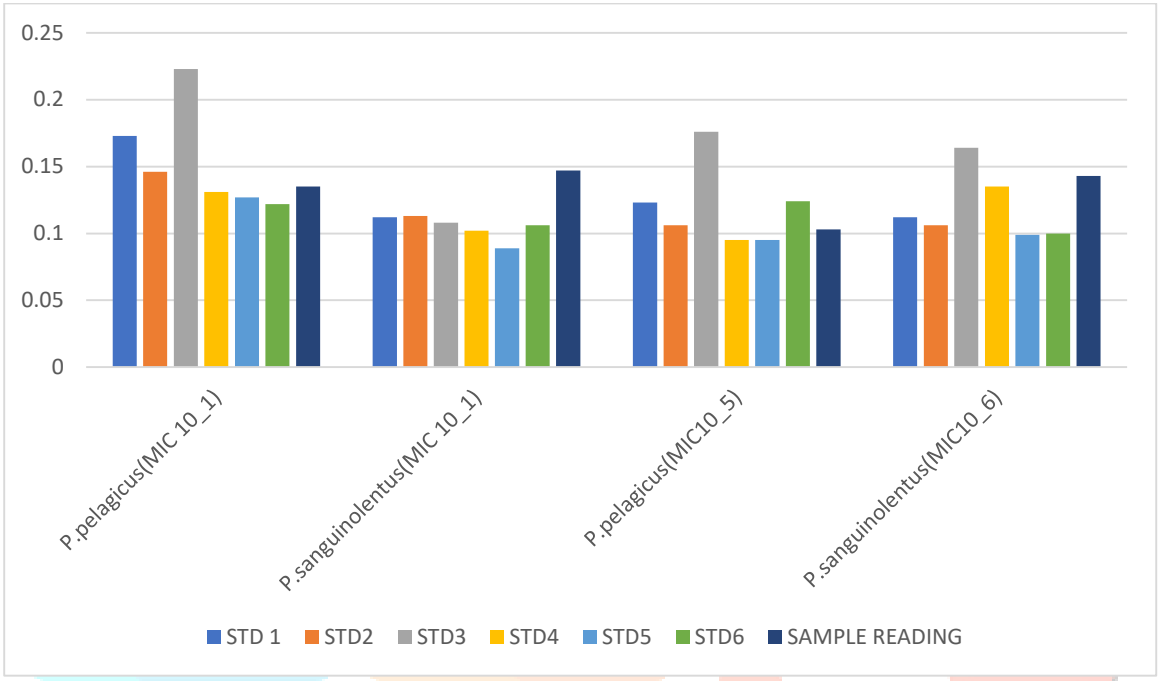
5. Add 100 µl of substrate/chromogen solution to each well. Mix gently by shaking the plate manually and incubate for 15 min at room temperature (20 - 25 °C) in the dark.

6. Add 100 µl of the stop solution to each well. Mix gently by shaking the plate manually and measure the absorbance at 450 nm. Read within 30 minutes after addition of stop solution. 11. Results

A special software, the RIDA®SOFT Win.NET (Art. No. Z9996), is available to evaluate the RIDASCREEN® enzyme immunoassays. The course of the standard curve is shown in the Quality Assurance Certificate enclosed in the test kit. Remark for the calculation without software: absorbance standard (or sample) absorbance zero standard x 100 = % absorbance The zero standard is thus made equal to 100 % and the absorbance values are quoted in percentages. The values calculated for the standards are entered in a system of coordinates on semilogarithmic graph paper against the chloramphenicol concentration [ng/L]. In order to obtain the chloramphenicol concentration in ng/L / ng/kg (ppt) or µg/L /

µg/kg (ppb) actually contained in a sample, the concentration read from the calibration curve must be further multiplied by the corresponding dilution factor.

Fig:20COMPARITIVE IMMUNOASSAY GRAPH OF ELISA TEST BETWEEN P.pelagicus AND P.sanguinolentus (WITH STANDARD VALUES)



Portunus pelagicus	**Portunus sanguinolentus**
10^{-1}=0.135ppt	**10^{-1}=0.147ppt**
10^{-5}=0.103ppt	**10^{-6}=0.143ppt**

Table:5 ELISA reading(sample)

Fig:21 comparative study of ELISA SAMPLE READING

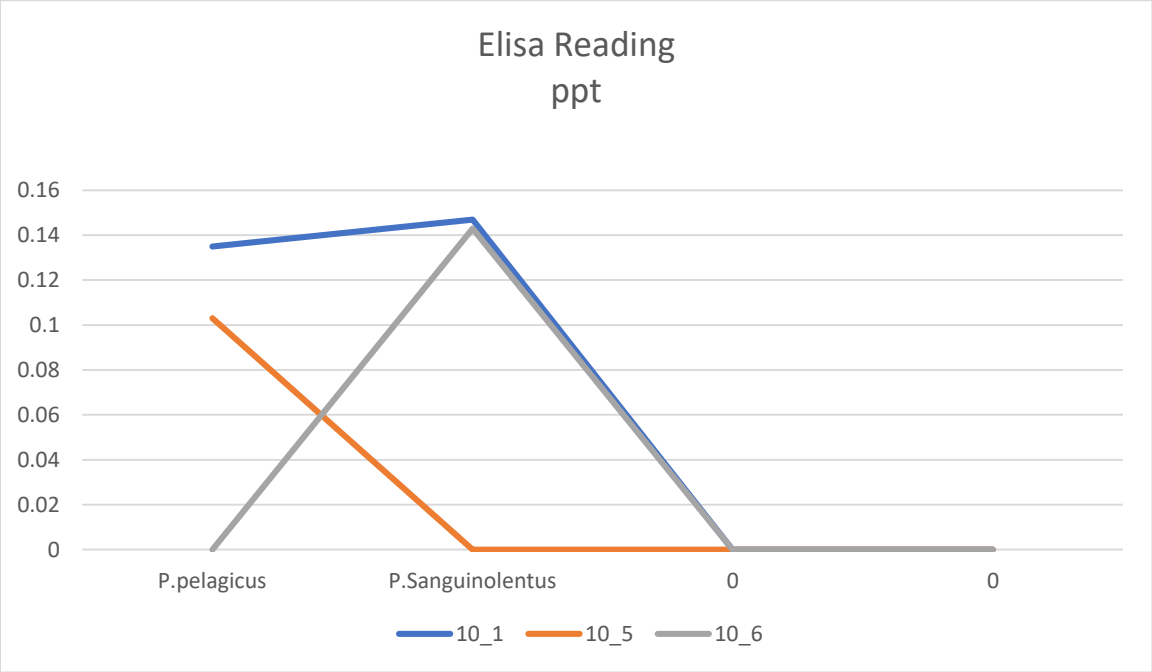
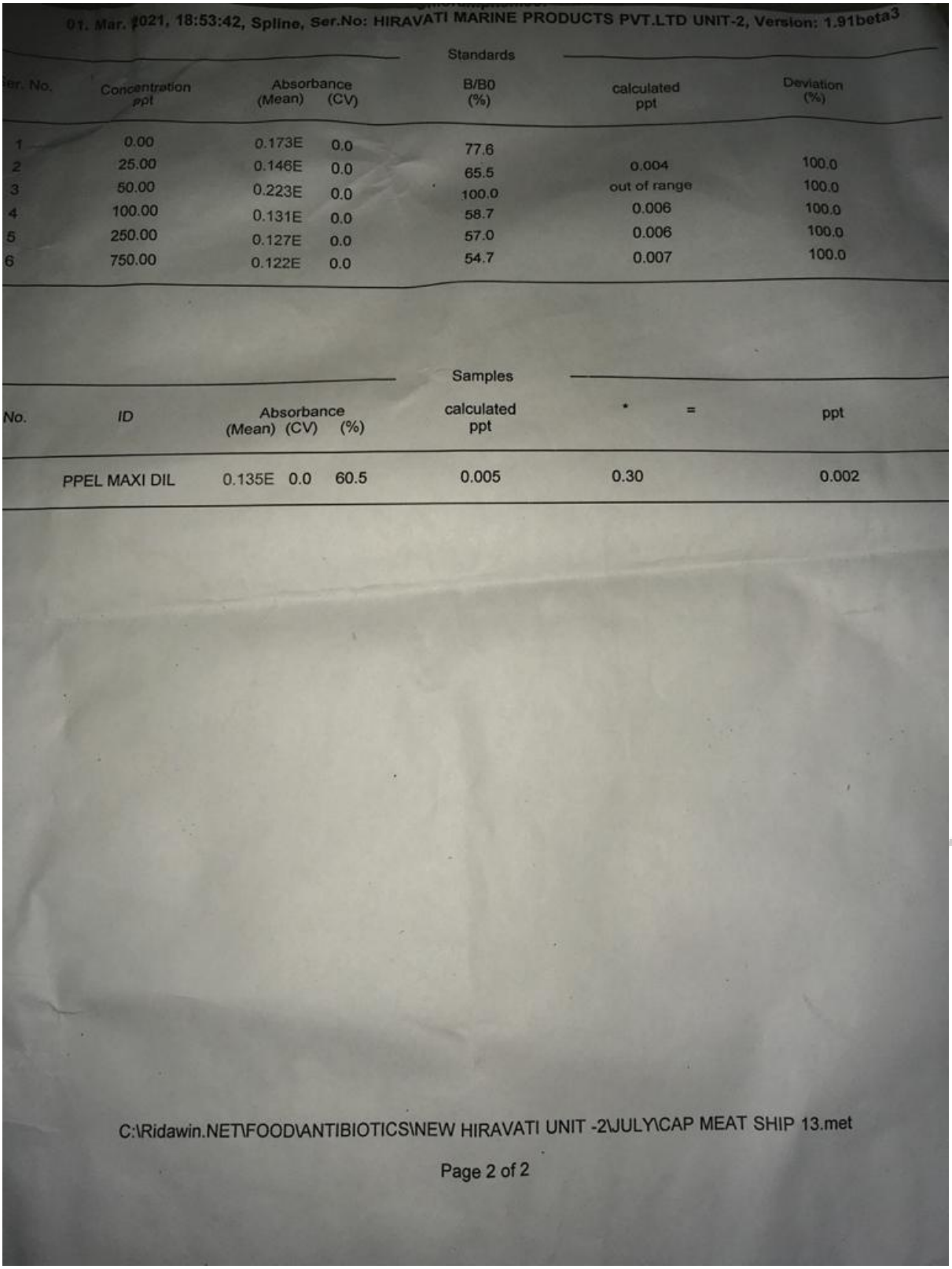


Fig:22 MICROPLATE ELISA READER



01. Mar. 2021, 18:53:42, Spline, Ser.No: HIRAVATI MARINE PRODUCTS PVT.LTD UNIT-2, Version: 1.91beta3

Standards

Ser. No.	Concentration ppt	Absorbance (Mean)	(CV)	B/B0 (%)	calculated ppt	Deviation (%)
1	0.00	0.173E	0.0	77.6		
2	25.00	0.146E	0.0	65.5	0.004	100.0
3	50.00	0.223E	0.0	100.0	out of range	100.0
4	100.00	0.131E	0.0	58.7	0.006	100.0
5	250.00	0.127E	0.0	57.0	0.006	100.0
6	750.00	0.122E	0.0	54.7	0.007	100.0

Samples

No.	ID	Absorbance (Mean)	(CV)	(%)	calculated ppt	*	=	ppt
	PPEL MAXI DIL	0.135E	0.0	60.5	0.005	0.30		0.002

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Page 2 of 2

Fig:23 ELISA MICROPLATE READER REPORT(P.pelagicus 10^{-1}MIC)

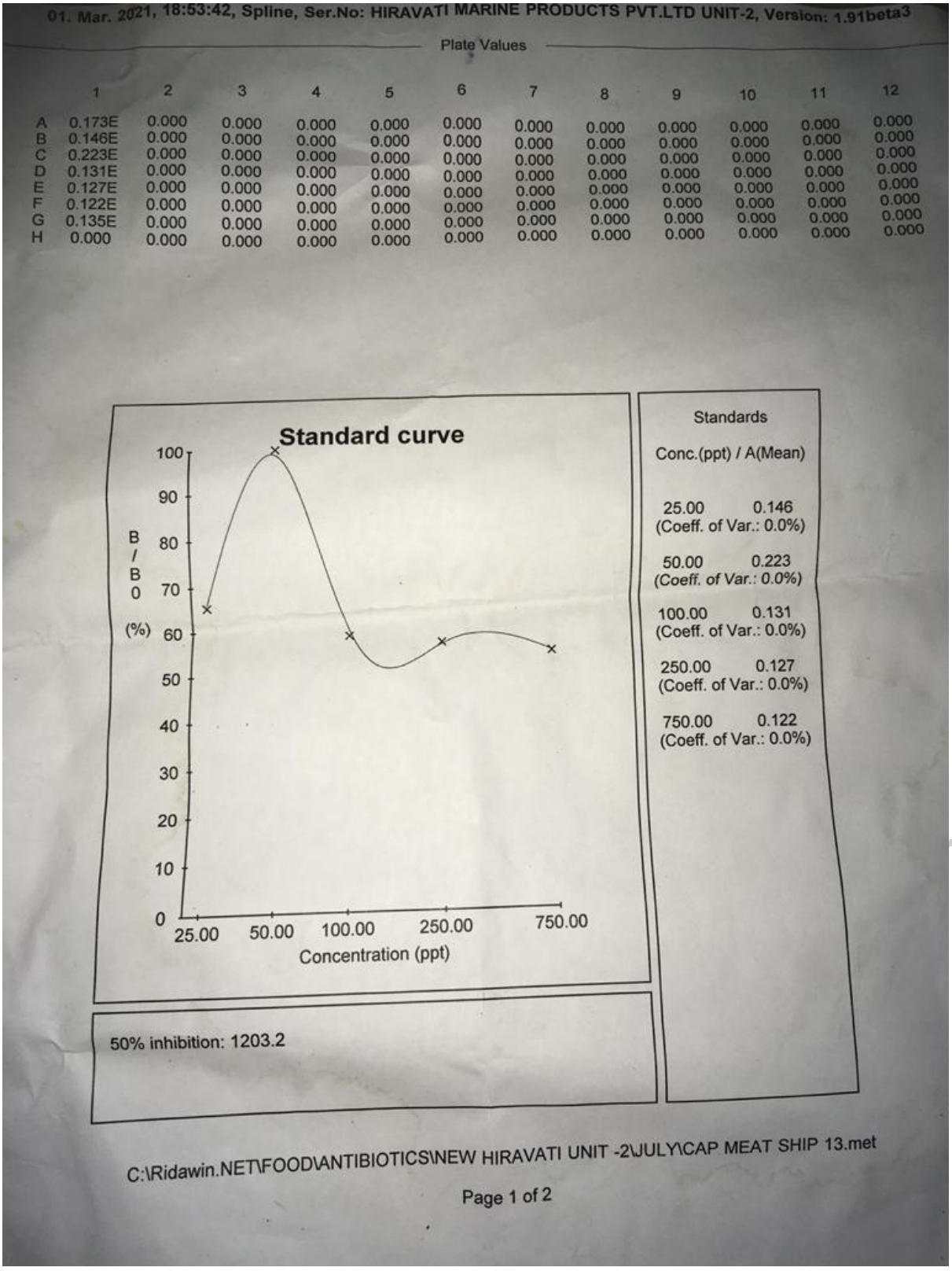
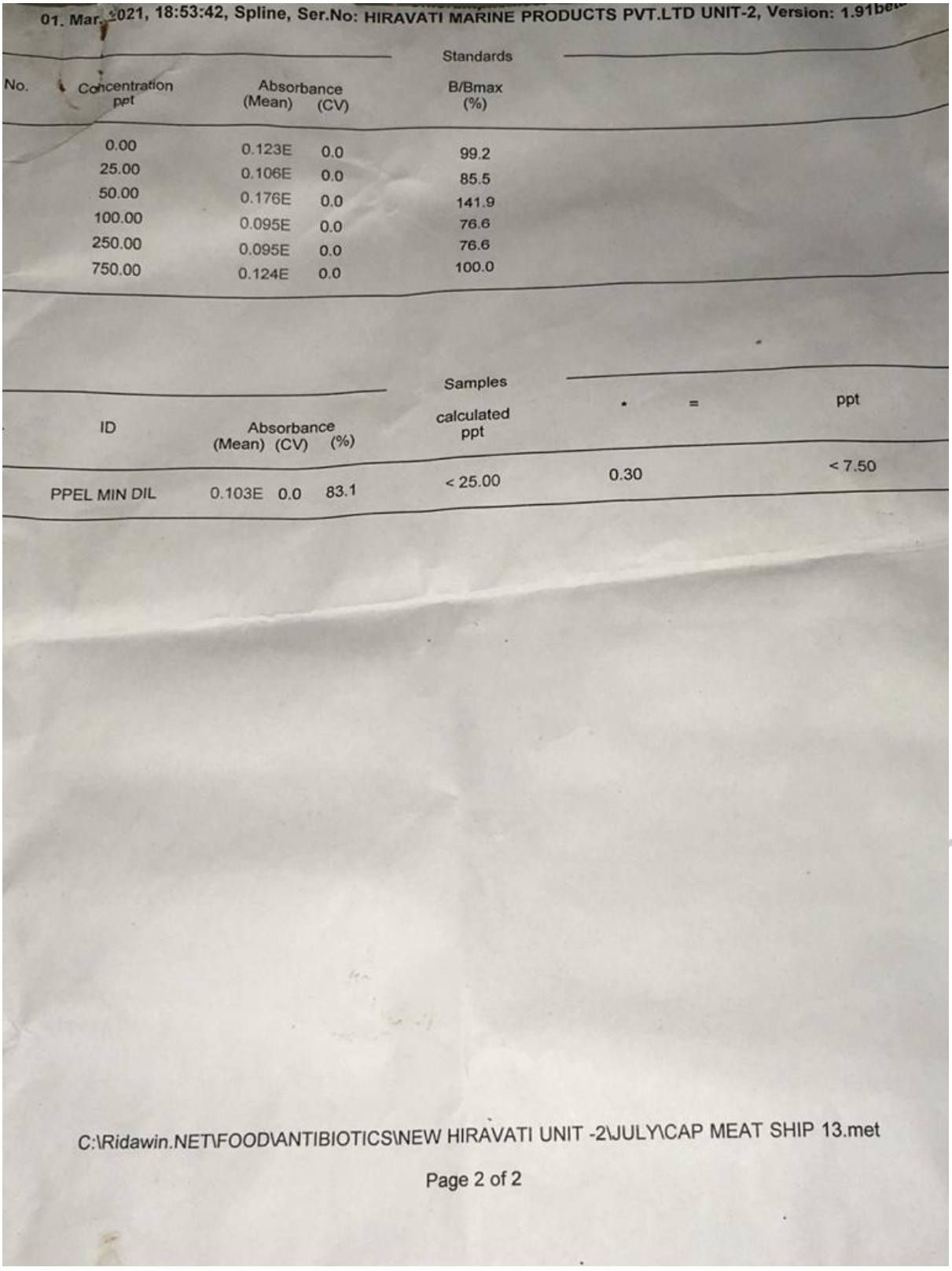


Fig:24 ELISA MICROPLATE READER REPORT (standard curve report)



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Standards

No.	Concentration ppt	Absorbance (Mean)	(CV)	B/Bmax (%)
	0.00	0.123E	0.0	99.2
	25.00	0.106E	0.0	85.5
	50.00	0.176E	0.0	141.9
	100.00	0.095E	0.0	76.6
	250.00	0.095E	0.0	76.6
	750.00	0.124E	0.0	100.0

Samples

ID	Absorbance (Mean)	(CV)	(%)	calculated ppt	•	=	ppt
PPEL MIN DIL	0.103E	0.0	83.1	< 25.00	0.30		< 7.50

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Page 2 of 2

Fig:25 ELISA MICROPLATE REPORT (P.pelagicus 10^{-5}MIC)

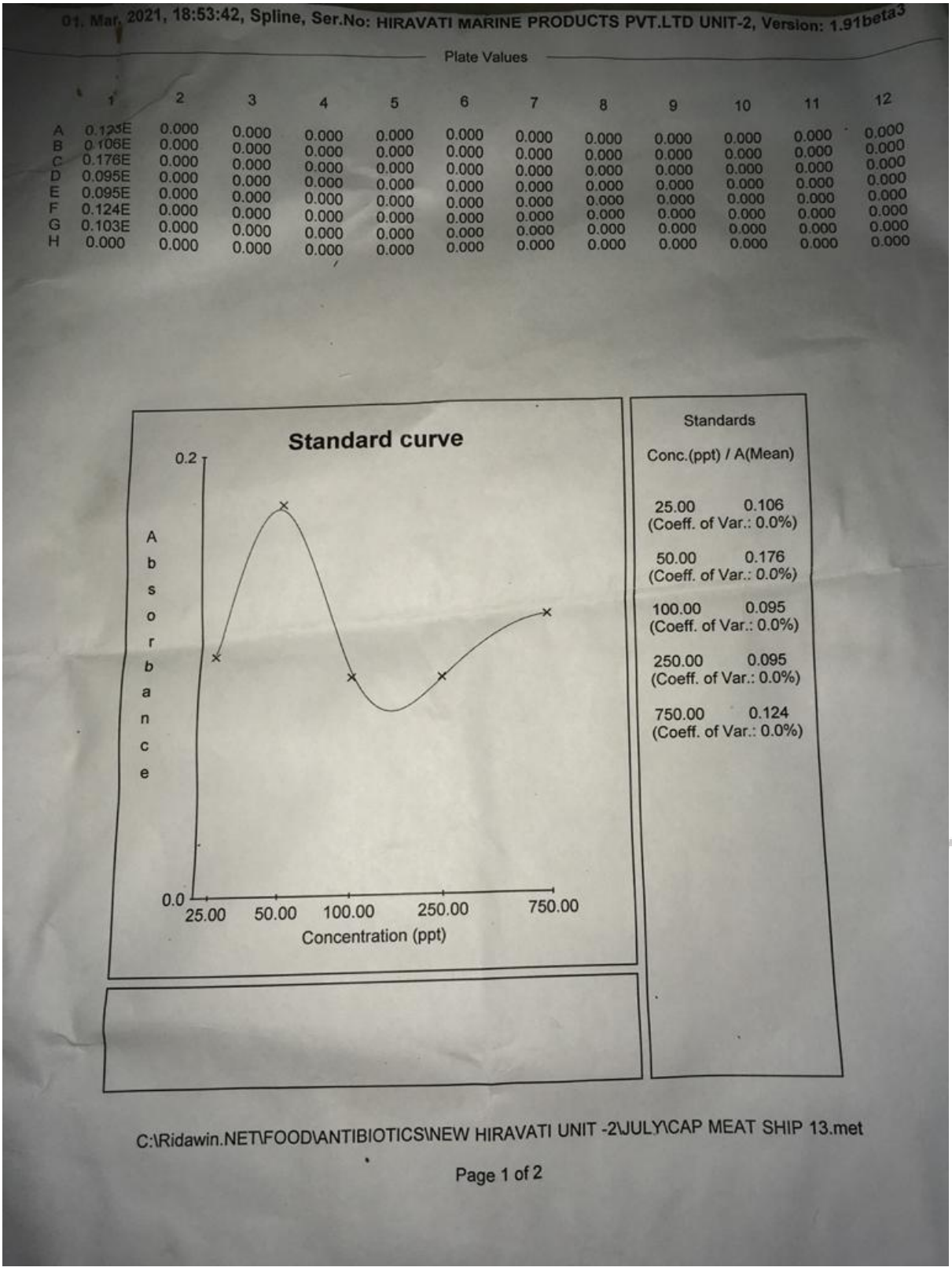
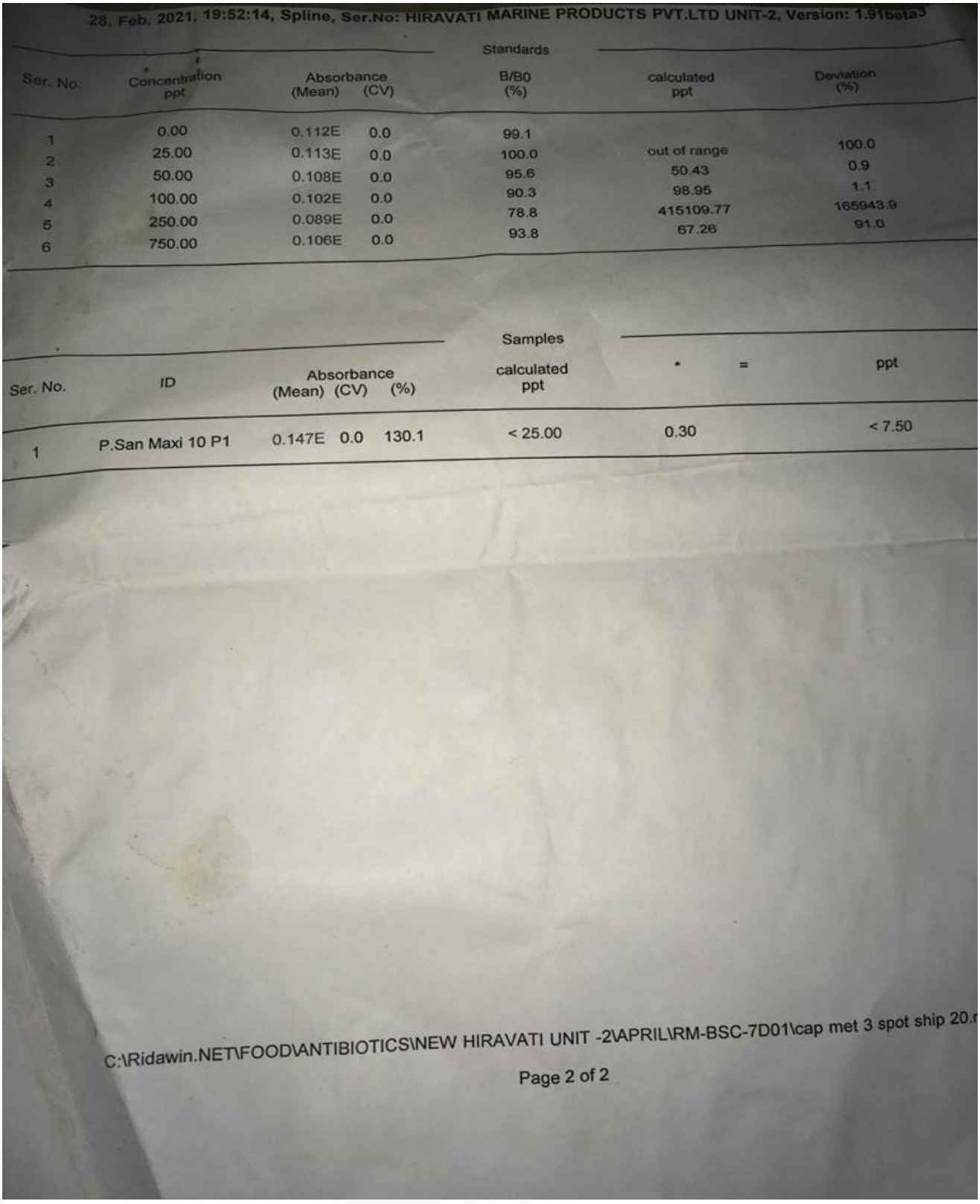


Fig:26 ELISA MICROPLATE READER REPORT (STANDARD CURVE)



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Standards

Ser. No.	Concentration ppt	Absorbance (Mean)	(CV)	B/B0 (%)	calculated ppt	Deviation (%)
1	0.00	0.112E	0.0	99.1		
2	25.00	0.113E	0.0	100.0	out of range	100.0
3	50.00	0.108E	0.0	95.6	50.43	0.9
4	100.00	0.102E	0.0	90.3	98.95	1.1
5	250.00	0.089E	0.0	78.8	415109.77	165943.9
6	750.00	0.106E	0.0	93.8	67.26	91.0

Samples

Ser. No.	ID	Absorbance (Mean)	(CV)	(%)	calculated ppt	*	=	ppt
1	P.San Maxi 10 P1	0.147E	0.0	130.1	< 25.00	0.30		< 7.50

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Page 2 of 2

Fig:27 ELISA MICROPLATE READER REPORT P.sanguinolentus (10^{-1}MIC)

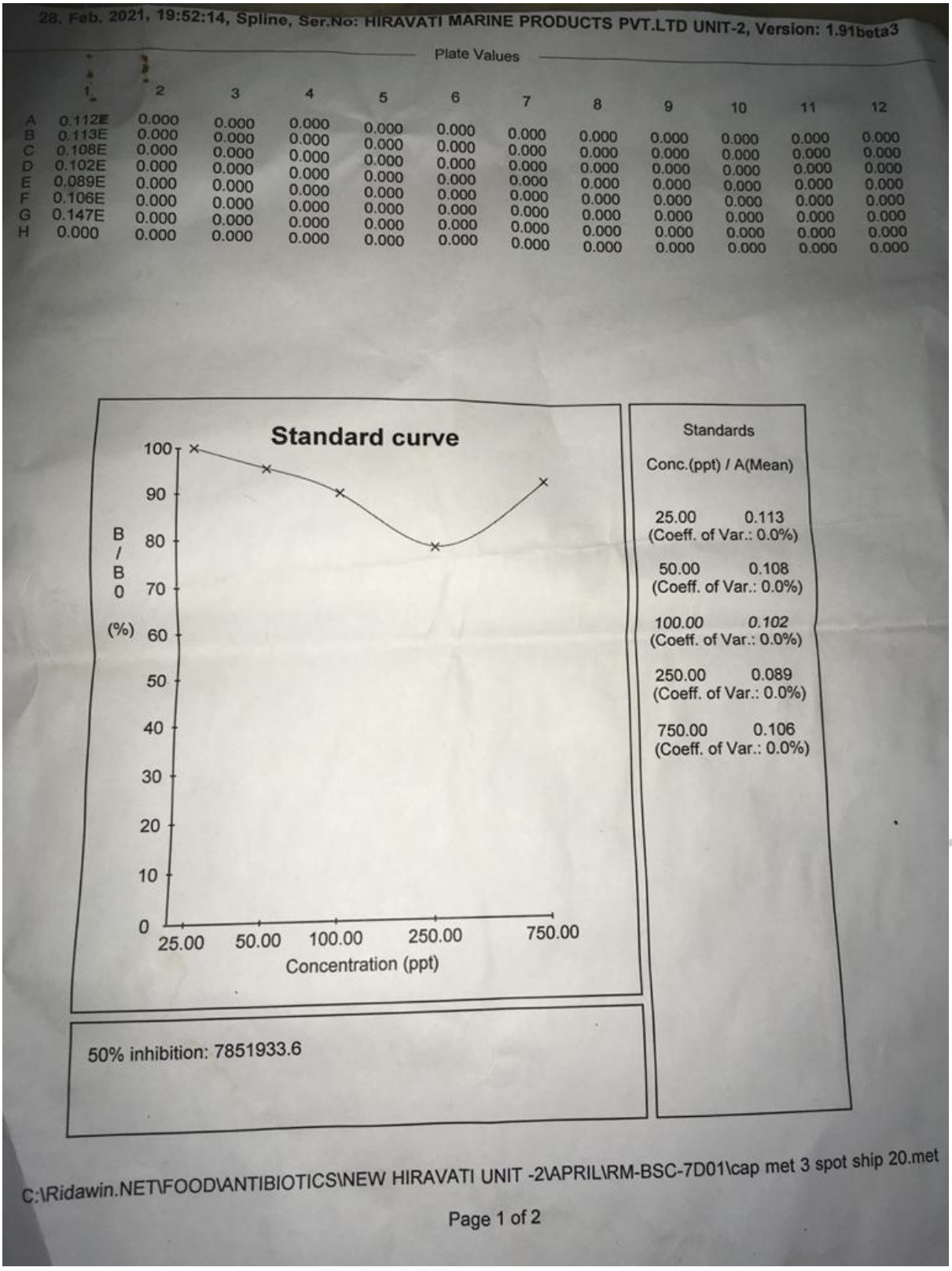
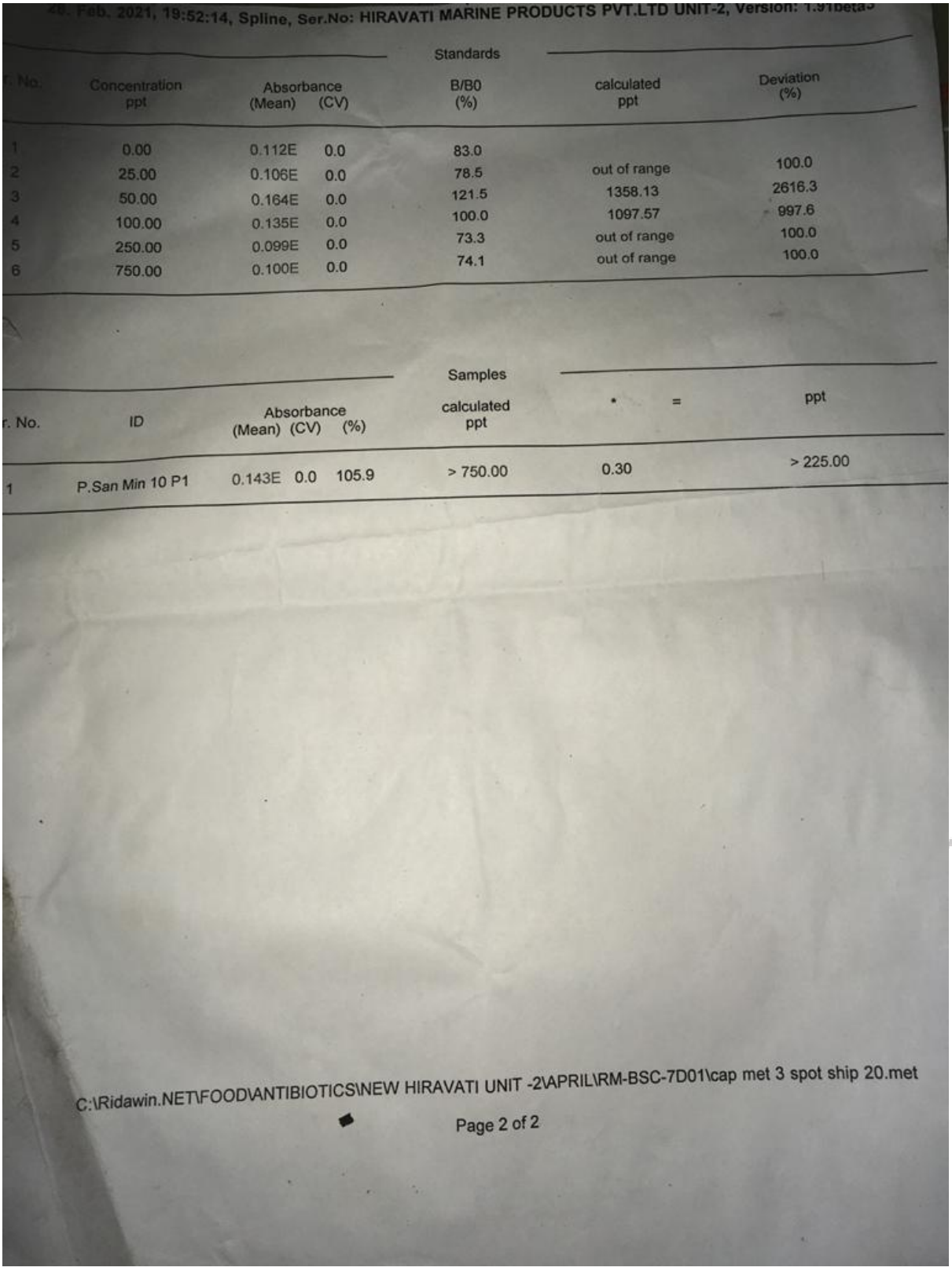


Fig:28ELISA MICROPLATE READER REPORT P.pelagicus(standard curve)



26. Feb. 2021, 19:52:14, Spline, Ser.No: HIRAVATI MARINE PRODUCTS PVT.LTD UNIT-2, Version: 1.91beta3

Standards

r. No.	Concentration ppt	Absorbance (Mean)	(CV)	B/B0 (%)	calculated ppt	Deviation (%)
1	0.00	0.112E	0.0	83.0		
2	25.00	0.106E	0.0	78.5	out of range	100.0
3	50.00	0.164E	0.0	121.5	1358.13	2616.3
4	100.00	0.135E	0.0	100.0	1097.57	997.6
5	250.00	0.099E	0.0	73.3	out of range	100.0
6	750.00	0.100E	0.0	74.1	out of range	100.0

Samples

r. No.	ID	Absorbance (Mean)	(CV)	(%)	calculated ppt	*	=	ppt
1	P.San Min 10 P1	0.143E	0.0	105.9	> 750.00	0.30		> 225.00

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Page 2 of 2

Fig:29 ELISA MICROPLATE READER REPORT P.sanguinolentus(10^{-6}MIC)

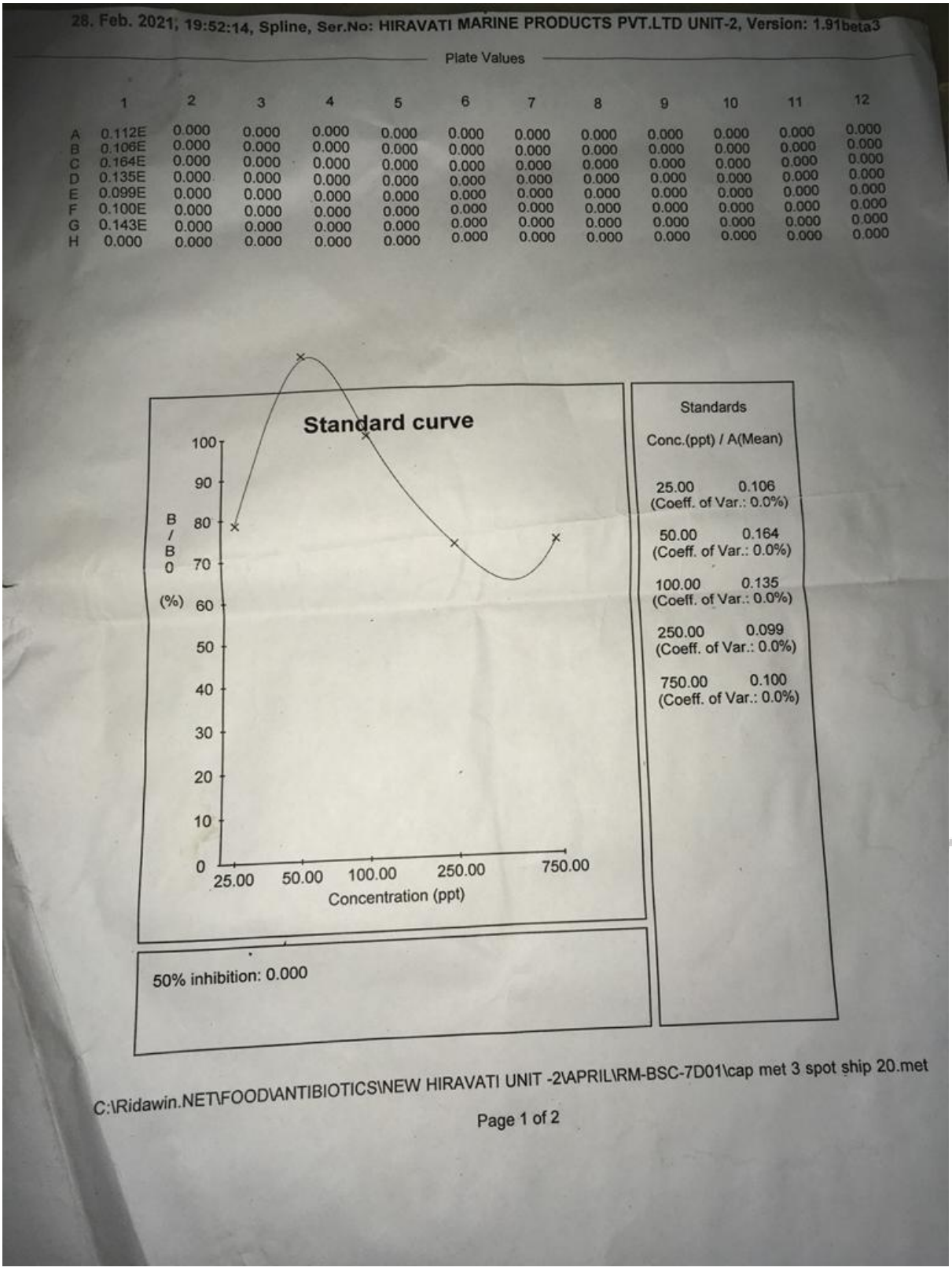

Fig:30 ELISA MICROPLATE READER REPORT P.sanguinolentus(standard curve)

Conclusion:

Utilisation of shell waste for the production of chitin and chitosan will give more economical and biological value along with reduction of environmental pollution.

The potential value and the application of the crustacean shell waste still under utilized and need to be further research .At the same time the various industries need to be encouraged to incorporate many of these applications commercially.

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