ARTICLE

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Manufacturing and formulation of natural products based on the Chitosan as active ingredient in the forms of gel and spray

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ABSTRACT

The purpose of this work is to make based on natural polysaccharide compounds such as chitosan for the microorganism testwhich led to the destruction of Escherichia coli bacteria, Candida albicans vegetative cells and Aspergillus niger spores and Herpes simplex virus (HSV-1). Chitosan products in the forms of gel and spray have bactericidal, fungicidal and virucidal properties as antiseptic products. Chitosan polysaccharides with high molecular weight are extracted from aquatic organisms such as shrimp.Physical and chemical analysis, identification test of Chitosan as active ingredient and also non-sensitizing test of these products in the forms of gel and spray have been done on the rabbit's skin that did not show no sensitivity and inflammation. It seems that natural products in the forms of gels and sprays based on chitosan, as alternative products for alcohol and chemical compounds, have a useful and promising role as antiseptic in eliminating bacteria, fungi and viruses left on the skin of the hands.

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Introduction

A wide range of polymers, especially chitosan, is used as a carrier for skin drug delivery. Chitosan is a polysaccharide that consists of acetylglucosamine units and is created from N-glucosamine and alkaline deacetylation of chitin. Chitin is found in large quantities in crustaceans such as crabs and shrimps, insects, fungi and some algae. Chitosan units have a first-type amine group and two free hydroxyl groups that can establish hydrogen and ionic bonds, and for this reason, they have good bioadhesive and mucosal properties (1). The presence of these active groups has made the chitosan molecule undergo chemical changes easily. This phenomenon has created various biological properties for chitosan such as biocompatibility, biodegradability, antibacterial and antifungal, hemostatic, immunoadjuvant, lipid and cholesterol reducing (2-4). For this reason, it is used in many fields such as pharmaceuticals, medicine, chemical engineering, nutrition and agriculture (2). Polymers used for skin formulations should not cause damage and inflammation in the skin. Chitosan is a polymer that does not show any toxic effects and does not cause damage or inflammation (2). On the other hand, it is used as a carrier for skin drug delivery due to its bio adhesion properties, increasing permeability and having suitable physicochemical properties (5). One of the major applications of chitosan is its use in cosmetics and health industries. chitosan has a cationic characteristic and the same its characteristic can be caused to be used as a skin and hair protector. Chitosan is compatible with many compounds used in cosmetics and absorbs many ultraviolet rays. A transparent chitosan solution is placed on the skin and hair as a

flexible coating, increasing softness and softness it becomes. Both chitin and chitosan are used in shampoos, hair dyes, emulsions, sprays, and hair conditioners (6). Both chitin and chitosan have an accelerating effect on wound healing. Recently, a report about a composite of chitin and silver nanoparticles forwound healing has been published, which shows that chitosan has high antibacterial properties as well as great compatibility with has skin (7,8). Chitosan is also considered as a promising candidate for burn treatment. Chitosan can be a durable, water-absorbing and biocompatible coating can be used directly to treat burns. One of the advantages of this type of cover, its high permeability to oxygen accelerates burn healing (9). In this work, the shrimp wastes were brought to the laboratory and the skin and internal organs of the shrimp were separated. The shrimp skin was washed with distilled water and the shrimp skin was dried. Chitosan was extracted by hydrochloric acid and soda. The obtained chitosan material was dried at ambient temperature. antimicrobial activity of chitosan and its derivatives have been reported against various bacteria such as Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa (10). Gram-negative bacteria seem to be more susceptible to chitosan and its derivatives due to a higher negative charge of the cell surface. The mechanism of antibacterial activity of chitosan and its derivatives was intensively studied and discussed; the interaction between chitosan and microorganism appears to be a complex phenomenon that is not fully understood (11). Even if the mechanisms of action are less studied that those involved in the antibacterial activity, Chitosan proved important antifungal activity. Based on the available data, fungi appear to be more sensitive to antimicrobial activity of chitosan than bacteria (12). The antiviral activity of chitosan and its derivatives was less studied and less explored, probably because of difficulties and special requirement in cultivating viruses. In this work, two types of products were made, one in the forms of a gel and the other in the form of a spray. The chitosan-based gel was named as Chitodermic and the chitosan-based spray as Chitoseptic, respectively. Physical and chemical analysis, identification test of Chitosan as active ingredient in the forms of gel and spray have been done in the reference laboratory of Food and Drug Organization, and also non-sensitizing test of these products by the rabbit at department of pharmaceutics, school of pharmacy, Shahid Beheshti University of Medical Sciences in Irancountry. This test to prove the non-sensitizing test of the substances in the spray on the body of the rabbit, sprayed with the control group under the specified protocol and approved within 14 days, checked and done. The results show that there is no irritation on the skin the rabbit was not observed. This experiment has been studied with two replications for 14 days. The used Microorganism species in this work were includedCandida albicans vegetative cells ATCC 10231, Aspergillus niger spores ATCC 6275 and Herpes simplex virus. The antibacterial, antifungal and antiviral activity were done on the products based on natural chitosan compounds in the forms of gel and spray, according to the protocol of the National Standard Organization at the Faculty of Biology of Tehran, University of Medical Sciences. Instead, the water solvent from the sweat of clove and fennel herbals has been used for the complete dissolution of chitosan in topical gel and spray products. The eugenol in clove herbal and anthole in fennel herbal have been identified and analyzed by high performance liquid chromatography instrument at the Jihad University Medicinal Plants Research Institute. The short-term stability has been investigated and studied of 1 to 6 months of eugenol and anthole as auxiliary active ingredients replaced with water for chitosan dissolution. In addition to, physical and chemical analyzes have been doneof short-term stability of 1 to 6 months for Chitoseptic spray (Fig 1).

Materials and Methods

Chitosan, distilled water, acetic acid,the sweat of cloves and fennel herbals and polytetrafluoroethylene (PTFE) were purchased from Sadra Pajohesh company. Clove and fennel herbals were prepared by the Research Institute of Medicinal Plants.

Experimental

The Chitin extraction from shrimp shell:

Fresh shrimp shells were used. In the next step, the shells were completely washed with water and soaked in 0.5% caustic soda solution for 4 hours to remove the remains of shrimp meat from the shells. The shells were washed again with water and dried in an oven at 60°C for 2 hours. Then they were turned into powder with a grinder.

The Separation of protein material from the shell:

This procedure was performed using a caustic soda solution at a temperature of 90°C for 2 hours. The weight ratio of shrimp powder to soda solution was 1:20. Then the remains of the shell were filtered and the remaining materials were washed on the filter with distilled water until reaching neutral pH.

The Separation of minerals from the shell:

The residues obtained from the previous step were placed in a 1.4 normal hydrochloric acid solution for one hour. The weight ratio of shell to acid was 1:10. Then the remains of the shell were filtered and the remaining materials were washed on the filter with distilled water until reaching neutral pH. The obtained chitin has a yellow color and must be decolorized.

The Decolorization of chitin:

To produce chitin free from carotenoid pigments, chitin was washed with acetone to make the chitin color clear and white.

The Preparation of chitosan from chitin:

In this step, chitosan was obtained by deacetylation of chitin. deacetylation was performed at 100℃ for 6 hours in a 50% concentrated soda solution. Then the chitosan materials suspended in soda solution were filtered and washed with distilled water until neutral pH was reached. The obtained chitosan was dried in an oven at 60°C for 1 hour. Fig 2 shows all the necessary items for the physical and chemical analyzes of the active ingredient chitosan in the hydrating cream.

Manufacturing and formulation of Chitosan as active ingredient in the form of gel (Chitodermic gel)

The amount of chitosan obtained is weighed by a weighing balance and transferred into the glass container. Acetic acid 1% in water is made and added to weighed chitosan in the glass container. The mixture was stirred for 20 minutes by a stirrer instrument to obtain chitosan in the form of gel.

Manufacturing and formulation of Chitosan as active ingredient in the form of spray (Chitoseptic spray)

The amount of chitosan obtained is weighed by a weighing balance and transferred into the glass container. Acetic acid 1% in water is made and added to weighed chitosan in the glass container. The mixture was stirred for 30 minutes by a stirrer instrument to be dissolve chitosan in acetic acid. The percentage of herbal extracts added to chitosan solution can be including of clove and fennel extracts to the equal proportions. Also, the volume of the sweat of cloves and fennel herbals added to the solution is 3 times the volume of acetic acid dissolved in water. After adding a volume ratio of the extracts to the chitosan solution dissolved in acetic acid, the solution was stirred with a stirrer instrument for 30 minutes. The desired solution and undissolved impurities should be filtered with PTFE filter.

Microorganism species activity on Chitoseptic spray:

The Antibacterial activity of Chitoseptic spray:

The purpose of this test is to evaluate the ability of antiseptic to eliminate bacterial contamination from hands based on the quantitative suspension test. Bacterial suspension culture medium: tryptic soy broth (TSB) according to standard 8512 for maintaining bacterial strains and counting bacterial strains. In the test conditions, the number of Escherichia coli NCTC10538K12 bacteria in the test suspension was 1.5×10^8. Verification of neutralizer and verification of non-toxicity of the neutralizer was done according to the standard. Evaluating the effectiveness of the antiseptic dilution is done by calculating the logarithmic reduction. Reduction means the difference between the infectious titer obtained before exposure to the antiseptic Chitoseptic spray and the infectious titer obtained after exposure to the antiseptic Chitoseptic spray at the specified contact time, as well as the antiseptic introduced in the standard at the same time. When the product is evaluated according to the test method, it should show a reduction of at least 5 $\log_{10}$ in the Escherichia coli bacteria titer. After the test, the plates were heated at 37°C for 24 hours and then the colonies were counted. The number of bacteria counted was multiplied by the dilution factor (Tables 1&2).

The Antifungal activity of Chitoseptic spray:

The purpose of this test is to evaluate the ability of antiseptic to eliminate fungal and yeast contamination from contaminated hands. To examine the test material, Candida albicans vegetative cells ATCC 10231 and Aspergillus niger spores ATCC 6275 were used to examine the test material. The evaluation of the effectiveness of the Chitoseptic antiseptic was done using the logarithmic reduction calculation. In the test conditions, the reduction of $4\log_{10}$ should be checked. The organisms were exposed to the tested hand antiseptic for 30 seconds and then neutralized by dilution method. Dilutions prepared from microorganisms were heated for 48 hr at 30℃, and then the colonies were counted and the log reduction rate was calculated based on standard 16119. All tests were repeated three times to confirm the reproducibility of the results (Table 3).

The Antiviral activity of Chitoseptic spray:

The purpose of the test is to evaluate the ability of the hand antiseptic to inactivate HSV virus particles under a specific contact time with the titration method. The cell name is VERO, the number of cell passages is 4 to 6 times, the culture medium for cell cultures is DMEM, the characteristics of the virus strain and the number of passages are HSV-1 virus with a titer of 10^9. The evaluation of antiseptic dilution efficiency was done using logarithmic reduction calculation. The reduction means the difference between the infectious titer obtained without exposure to the Chitoseptic solution (Virus Recovery Control) and the infectious titer obtained after exposure to the Chitoseptic solution at the specified contact time and whenever the product is evaluated according to the test method should show a decrease of at least $4\log_{10}$ in the viral titer. According to the evaluation carried out on vero cell culture in proximity to HSV-1 virus as an enveloped virus model, Chitoseptic solution during proximity of 30 and 120 seconds in clean and dirty conditions, the ability to neutralize the virus and decrease 4 $\log_{10}$ in the virus titer after from the duration of the exposure. According to the results, Chitoseptic spray is evaluated as effective against HSV-1 test strain in terms of activity (Table 4).

MethodologyAcute Dermal Irritation/Corrosion for Chitoseptic product:

Good Laboratory Practice compliance statement: This study was conducted in compliance with current OECD Good Laboratory Practices Standards. **Study Title:** Acute Dermal Irritation Study in Rabbits. **Irritation Test:** In Vivo. **Test Animal:** Male Albino Rabbit. **Test Guideline:** Based on OECD Guideline for the Testing of Chemicals. **Performing Laboratory:** Quality Control Laboratory, Department of Pharmaceutics, school of pharmacy, Shahid Beheshti University of Medical Sciences, 2660 Vali-e-Asr Ave., Tehran, Iran.

Results: The sample showed no irritation.

The aim of this study was to evaluate the skin irritation/ corrosive potential and the reversibility of dermal effects of the Chitoseptic spray following a 4-hour dermal exposure in albino rabbit. The results of the study were used to determine the approximate toxicity classification. Dermal erythema and edema are evaluated and scored at approximately 60 minutes, and 24, 48, and 72 hours following the removal of the test substance at the end of the 4-hour exposure in rabbit. The reversibility of any dermal effects is assessed for up to 14 days. If necessary. Dermal effects are quantified according to the Draize scale (Table 5) (13). In this study, one male adult New Zeland albino rabbit was obtained from the Animal House of School of Pharmacy, Shahid

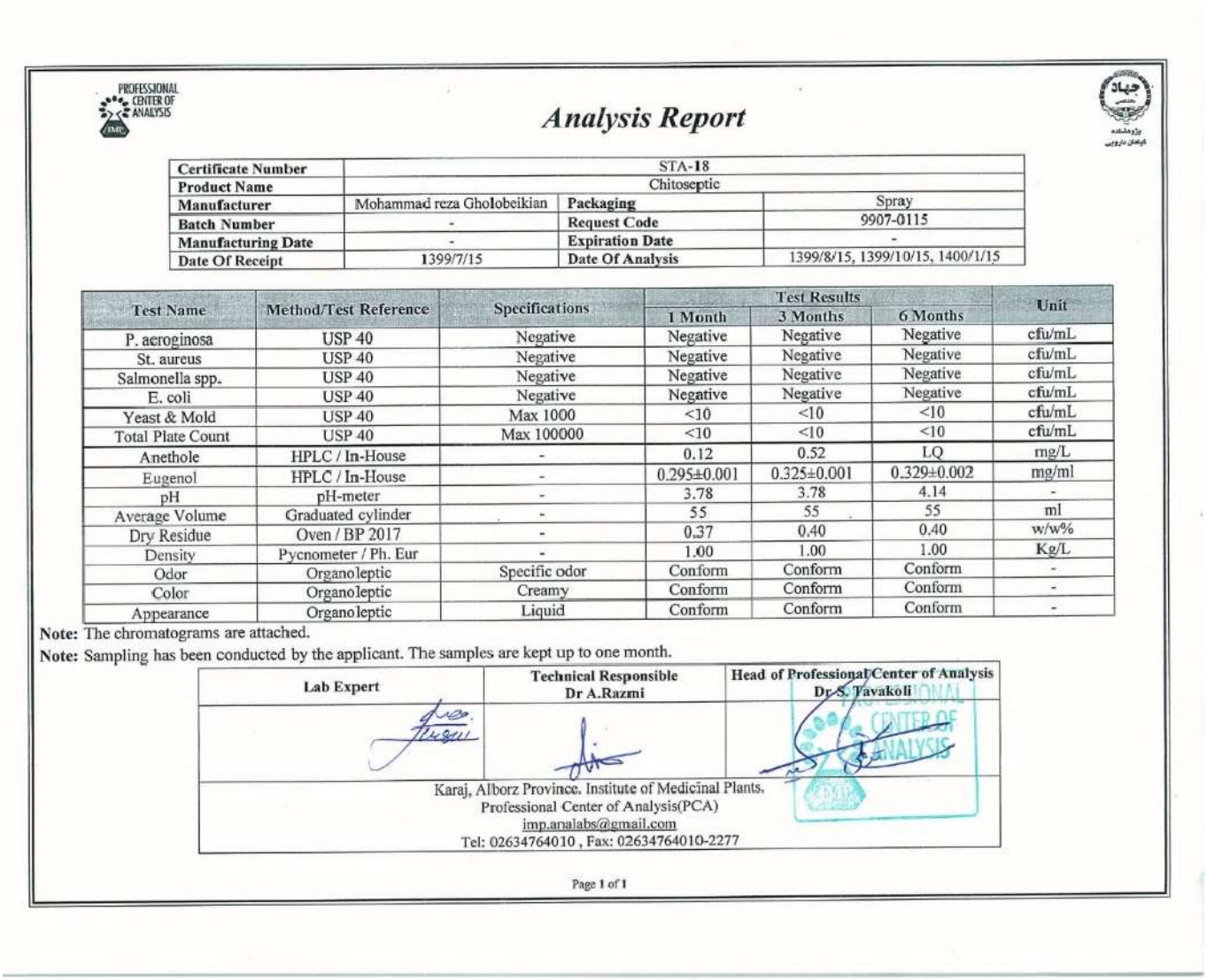
Beheshti University of Medical Sciences, Tehran, Iran. The rabbit was housed singly in stainless steel, wire-mesh cages and kept under standard animal laboratory conditions, 12 h of light and dark cycles, at controlled temperature 22±2℃, with a relative humidity of 50±5%, and free access to food and water. Around 24 hours prior to treatment, the four of male New Zealand White rabbit was closely shaved to exposure the skin (approximately 2.5×2.5) from scapular to the lumbar region of the back.

Chitoseptic spray was applied as a single dermal dose to the shaved intact skin of the rabbit. The application area was covered with a 2-ply gauze square which was held in place with non-irritating tape. The rabbit was returened to cage after treatment. No other substances were tested on the rabbit. The rabbit was exposed to the test substance for 4 hours after which the test substance was removed. Test sites were evaluated and scored by the method of Draize (Table 5) for erythema, edema, and other evidence of dermal effects approximately 60 minutes, and 24, 48, and 72 hours after test substance removal. Additionally, the rabbit was examined for clinical signs of toxicity at each observation period. The test substance appeared to be stable under the conditions of the study. No evidence of instability, such as a change in color or physical state, was observed. For data analysis and interpretation of the results, values for each lesion (erythema and edeme) were calculated from numerical scores obtained at the 60 minutes, 24, 48, and 72 hour observations (Table 6). The final results was calculated as a primary irritation index (PII). The mean scores were summed and averaged to obtain the primary irritation index (Table 7). No dermal irritation, erythema and edema was observed at 60 minutes, and 72 hours after removal of the substance in the rabbit. No clinical signs were observed. The dermal scores from rabbit with respect to observation time are presented in Table 8.

Result:

According to the comparison of the results of the left and right hands and the exposure of the Chitoseptic spray product to isopropanol as a reference solution in Tables 1&2, Chitoseptic spray is more efficient and effective than isopropanol as a antiseptic solution and can replace the chemical composition of isopropanol as a antiseptic solution. Also, there was not no dermal irritation in Irritation Test in Male Albino Rabbit. No clinical signs were observed. Under the conditions of this study, Chitodermic gel and Chitoseptic spray, produced no erythema and no edema when applied to the skin of rabbit. Chitodermic gel and Chitoseptic spray showed a negligible irritation response.

According to the Antibacterial activity of Chitoseptic spray, the $\log_{10}$ reduction for the test strain was calculated based on standard 8512. According to the analysis of the Chitoseptic test solution with laboratory code 99/H/119, it has the ability to kill bacteria in a period of 30 seconds for Escherichia coli reference strains. Based on the results of the Tables 1&2, Chitoseptic spray is evaluated as effective against the tested strains. According to the Antifungal activity of Chitoseptic spray, the test combination has the ability to reduce ≥4 log10 with the tested strains of Candida albicans and Aspergillus niger in 30 seconds. According to the results, the Chitoseptic compound with the laboratory code 99/A/119 is evaluated as effective against Candida albicans and Aspergillus niger (Table 3). According to the Viral evaluation carried out on vero cell culture in proximity to HSV-1 virus as an enveloped virus model, Chitoseptic solution during proximity of 30 and 120 seconds in clean and dirty conditions, the ability to neutralize the virus and decrease 4 log10 in the virus titer after from the duration of the exposure. According to the results, Chitoseptic spray is evaluated as effective against HSV-1 test strain in terms of activity (Table 4).



PROFESSIONAL CENTER OF ANALYSIS

Analysis Report

Certificate Number	STA-18		
Product Name	Chitoseptic		
Manufacturer	Mohammad reza Gholobeikian	**Packaging**	Spray
Batch Number	-	**Request Code**	9907-0115
Manufacturing Date	-	**Expiration Date**	-
Date Of Receipt	1399/7/15	**Date Of Analysis**	1399/8/15, 1399/10/15, 1400/1/15

Test Name	Method/Test Reference	Specifications	Test Results			Unit
			1 Month	3 Months	6 Months	
P. aeroginosa	USP 40	Negative	Negative	Negative	Negative	cfu/mL
St. aureus	USP 40	Negative	Negative	Negative	Negative	cfu/mL
Salmonella spp.	USP 40	Negative	Negative	Negative	Negative	cfu/mL
E. coli	USP 40	Negative	Negative	Negative	Negative	cfu/mL
Yeast & Mold	USP 40	Max 1000	<10	<10	<10	cfu/mL
Total Plate Count	USP 40	Max 100000	<10	<10	<10	cfu/mL
Anethole	HPLC / In-House	-	0.12	0.52	LQ	mg/L
Eugenol	HPLC / In-House	-	0.295±0.001	0.325±0.001	0.329±0.002	mg/ml
pH	pH-meter	-	3.78	3.78	4.14	-
Average Volume	Graduated cylinder	-	55	55	55	ml
Dry Residue	Oven / BP 2017	-	0.37	0.40	0.40	w/w%
Density	Pycnometer / Ph. Eur	-	1.00	1.00	1.00	Kg/L
Odor	Organoleptic	Specific odor	Conform	Conform	Conform	-
Color	Organoleptic	Creamy	Conform	Conform	Conform	-
Appearance	Organoleptic	Liquid	Conform	Conform	Conform	-

Note: The chromatograms are attached.

Note: Sampling has been conducted by the applicant. The samples are kept up to one month.

Lab Expert	Technical Responsible Dr A.Razmi	Head of Professional Center of Analysis Dr S. Tavakoli

Karaj, Alborz Province. Institute of Medicinal Plants.
Professional Center of Analysis(PCA)
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Figure 1: Short-term stability of 1 to 6 months of Chitoseptic spray

شرکت شیمی توان آریا

شماره نامه:۰۰۰۳

تاریخ: ۱۳۹۹/۰۶/۳۰

Analysis Report

Name: Chitosan **Chemical name : (1,4)-2-Amino-2-desoxy- beta-D-glucan**

Test	Standard Limit	Result	
Appearance A	White or almost white	Conform	✓
Appearance B	fine powder	Conform	✓
Appearance of solution	Complies with reference	Conform	✓
Identification A (IR)	Complies with reference	Conform	✓
Identification B (Chloride)	Complies with reference	Conform	✓
Hg	Complies with reference	<0.5	✓
Pb	Complies with reference	<0.1	✓
Cd	Complies with reference	<.05	✓
As	Complies with reference	<1.0	✓
Solubility	Sparingly soluble in acid acetic 0.1%	Conform	✓
Mater Insoluble in Water	NMT 0.5%	0.05%	✓
Loss on Drying (LOD)	Not More Than 10 % (105°C, 3h)	2.17 %	✓
Sulfated ash	Not More Than 1% (600°C, 4h)	0.3 %	✓
Heavy Metals	Not More Than 40 ppm	Conform	✓
Viscosity	Brookfield viscosity 200-800 cP	Conform	✓
pH	4.0 to 6.0 for solution S.	4.82	✓
Chloride	10.0 to 20.0%	17.3%	✓
Degree of Deacetylation	70.0-95.0%	95.3%	✓

Formula : $(C_6H_{11}NO_4)_n$ **CAS Number:9012-76-4**

شرکت شیمی توان آریا با مسئولیت محدود

آدرس : استان تهران – منطقه ۱۸ –شهرستان ری – بخش کهریزک – دهستان کهریزک – محله محمد آباد چمبورک – کوچه [illegible] کیمیایی ادیب – جاده [illegible] پلاک ۰

Figure 2: items for the physical and chemical analyzes of chitosan

Table 1: Comparison of Antibacterial activity of isopropanol with Chitoseptic spray on the right hand

Chitoseptic spray						Isopropanol							
Log limit Reduction of bacterial titers	Logarithmic reduction rate	logarithm	The number of live bacteria after use for 30 seconds	logarithm	Live bacteria count before use	Logarithmic reduction rate	logarithm	The number of live bacteria after use for 30 seconds	logarithm	Live bacteria count before use	Test items	Test bacteria	Entry
$4 \geq \log_{10}$	5.15	1.97	9.5×10	7.12	1.33×10^{7}	4.88	2.2	1.6×10^{2}	7.08	1.23×10^{7}	Right hand	Escherichia coli NCTC10538K12	1
	5.13	1.98	9.6×10	7.11	1.31×10^{7}	4.92	2.19	1.55×10^{2}	7.11	1.23×10^{7}			
	5.18	1.97	9.5×10	7.15	1.43×10^{7}	4.94	2.21	1.65×10^{2}	7.15	1.42×10^{7}			
	5.08	2.09	1.24×10^{2}	7.17	1.48×10^{7}	4.93	2.24	1.75×10^{2}	7.17	1.48×10^{7}			
	5.2	1.97	9.5×10	7.17	1.50×10^{7}	5.09	2.08	1.23×10^{2}	7.17	1.50×10^{7}			
	5.15	1.97	9.5×10	7.12	1.32×10^{7}	4.9	2.22202	1.67×10^{2}	7.12	1.32×10^{7}			
	4.87	2.18	1.54×10^{2}	7.05	1.13×10^{7}	4.84	1	1.65×10^{2}	7.05	1.13×10^{7}			
	4.97	2.19	1.56×10^{2}	7.16	1.45×10^{7}	4.94	2.22	1.66×10^{2}	7.16	1.45×10^{7}			
	4.89	2.16	1.47×10^{2}	7.05	1.14×10^{7}	4.92	2.13	1.35×10^{2}	7.05	1.14×10^{7}			
	4.97	2.2	1.59×10^{2}	7.17	1.50×10^{7}	4.92	2.23	1.75×10^{2}	7.17	1.50×10^{7}			
	5.18	1.97	9.5×10	7.15	1.43×10^{7}	4.94	2.21201	1.65×10^{2}	7.15	1.43×10^{7}			
	4.96	2.19	1.55×10^{2}	7.15	1.43×10^{7}	5.02	3	1.35×10^{2}	7.15	1.43×10^{7}			
	5.07	2.09	1.25×10^{2}	7.16	1.45×10^{7}	5.03	2.13	1.35×10^{2}	7.16	1.45×10^{7}			
	5.01	2.16	1.45×10^{2}	7.17	1.50×10^{7}	5.08	2.09	1.25×10^{2}	7.17	1.50×10^{7}			
	4.94	2.21	1.65×10^{2}	7.15	1.44×10^{7}	5.09	2.09	1.25×10^{2}	7.15	1.44×10^{7}			
	4.93	2.22	1.69×10^{2}	7.15	1.43×10^{7}	4.95	2.2	1.60×10^{2}	7.15	1.43×10^{7}			
	5.19	1.98	9.6×10	7.17	1.50×10^{7}	5.03	2.14	1.40×10^{2}	7.17	1.50×10^{7}			
	5.20	1.97	9.6×10	7.17	1.50×10^{7}	4.97	2.2	1.60×10^{2}	7.17	1.50×10^{7}			
	5.06 ± 0.11					4.96 ± 0.07					Average Reducelogarithmic ±standard deviation		

Table 2: Comparison of Antibacterial activity of isopropanol with Chitoseptic spray on the left hand

Chitoseptic spray						Isopropanol							
Log limit Reduction of bacterial titers	Logarithmic reduction rate	logarithm	The number of live bacteria after use for 30 seconds	logarithm	Live bacteria count before use	Logarithmic reduction rate	logarithm	The number of live bacteria after use for 30 seconds	logarithm	Live bacteria count before use	Test items	Test bacteria	Entry
$4 \geq \log_{10}$	4.94	2.18	1.54×10^{2}	7.12	1.32×10^{7}	4.84	2.21	1.64×10^{2}	7.05	1.13×10^{7}	Left hand	Escherichia coli NCTC10538K12	1
	4.92	2.2	1.59×10^{2}	7.12	1.33×10^{7}	4.98	2.19	1.75×10^{2}	7.17	1.50×10^{7}			
	5.18	1.97	9.5×10	7.15	1.44×10^{7}	4.81	2.24	1.65×10^{2}	7.05	1.12×10^{7}			
	5.08	2.09	1.24×10^{2}	7.17	1.47×10^{7}	4.93	2.24	1.75×10^{2}	7.17	1.48×10^{7}			
	5.2	1.97	9.5×10	7.17	1.50×10^{7}	5.09	2.08	1.23×10^{2}	7.17	1.50×10^{7}			
	5.15	1.97	9.5×10	7.12	1.32×10^{7}	4.09	2.22	1.67×10^{2}	7.12	1.32×10^{7}			

	4.87	2.18	1.54×10^{2}	7.05	1.13×10^{7}	4.87	2.21	1.65×10^{2}	7.08	1.23×10^{7}		
	4.97	2.19	1.56×10^{2}	7.16	1.45×10^{7}	4.94	2.22	1.66×10^{2}	7.16	1.45×10^{7}		
	4.89	2.16	1.47×10^{2}	7.05	1.14×10^{7}	4.92	2.13	1.35×10^{2}	7.05	1.13×10^{7}		
	4.97	2.2	1.59×10^{2}	7.17	1.50×10^{7}	4.93	2.24	1.75×10^{2}	7.17	1.50×10^{7}		
	5.18	1.97	9.5×10	7.15	1.43×10^{7}	4.84	2.21	1.65×10^{2}	7.05	1.23×10^{7}		
	4.96	2.19	1.55×10^{2}	7.15	1.43×10^{7}	5.02	2.13	1.35×10^{2}	7.15	1.43×10^{7}		
	5.07	2.09	1.25×10^{2}	7.16	1.45×10^{7}	4.97	2.09	1.25×10^{2}	7.06	1.15×10^{7}		
	5.01	2.16	1.45×10^{2}	7.17	1.50×10^{7}	5.08	2.09	1.25×10^{2}	7.17	1.50×10^{7}		
	4.94	2.21	1.65×10^{2}	7.15	1.44×10^{7}	5.06	2.09	1.25×10^{2}	7.15	1.44×10^{7}		
	4.93	2.22	1.69×10^{2}	7.15	1.43×10^{7}	4.85	2.2	1.60×10^{2}	7.15	1.23×10^{7}		
	4.95	2.22	1.69×10^{2}	7.17	1.48×10^{7}	5.00	2.14	1.40×10^{2}	7.14	1.40×10^{7}		
	4.95	2.22	1.69×10^{2}	7.17	1.47×10^{7}	4.94	2.21	1.70×10^{2}	7.15	1.42×10^{7}		
	5.00 ± 0.1					4.89 ± 0.21					Average Reducelogarithmic ±standard deviation	

Table 3: The Antifungal activity of Chitoseptic spray

Reference method	The limit of number reduction in terms of log	Number of organisms after contact with the test sample	Number of inoculated control organisms	Test organism	Entry
Standard 16119	≥4 $\log_{10}$	0	2.8×10^{7}	Candida albicans	1
Standard 16119	≥4 $\log_{10}$	1.7×10^{2}	2.9×10^{7}	Aspergillus niger	2

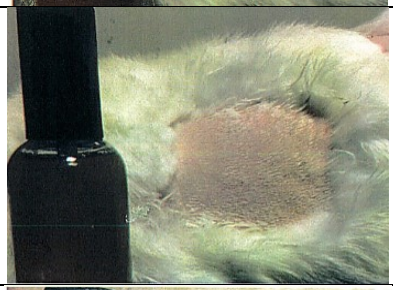
Table 4: The Antiviral activity of Chitoseptic spray:

Reference method	Reduction limit Virus titer according to log	Virus titer with the product (2 minutes after contact)	Virus titer with the product (30 seconds after contact)	Control virus titer, dose inoculated into cells	The type of virus tested	Entry
Standard 16676, E1052, 17981	≥4 $\log_{10}$	0	6.2×10	16.3×10^{9}	HSV-1 (clean condition)	1
Standard 16676, E1052, 17981	≥4 $\log_{10}$	0	6.2×10	16.3×10^{9}	HSV-1 (dirty condition)	2

Table 5. Dermal effects quantified according to the Draize scale

scores	Arrhythmia rate
0	no erythema
1	Very mild erythema
2	Specific arrhythmia
3	Moderate to severe erythema
4	Severe erythema to scar formation

Table 6: Comparison of the test group and the control group on the rabbit body with Chitoseptic spray

Time	Test group	Control group
After 24 hr		
After 60min		
After 48 hr		
After 72 hr		

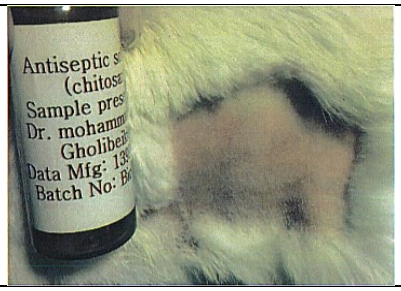
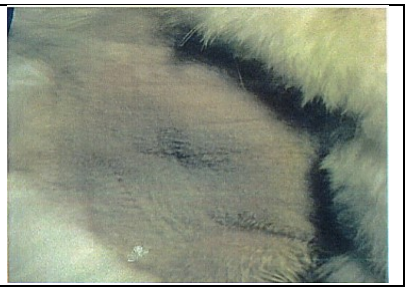
After 14 day		

Table 7: Descriptive rating for mean primary irritation index (PII).

Primary irritation index (PII)	Classification
0	Negligible
0<PII≤2	Slight
0<PII≤5	Moderate
5<PII	Severe

Table 8: Dermal response observed in Rabbit

	Evaluation after removal of test substance				
	0 minutes	60 minutes	24 hours	48 hours	72 hours
Erythema	0	0	0	0	0
Edema	0	0	0	0	0

Conclusion:

Chitodermic gel and Chitoseptic spray containing the active ingredient chitosan can be good products for moisturizing and softening the skin. According to the results, the effective ingredient chitosan in gel and spray topical products can have bactericidal, fungicidal and virucidal properties.These products can be well formulated with a mixture of herbal extracts added to chitosan solution can be including of clove and fennel extracts to the equal proportions.

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