ABSTRACT

CARBOXYMETHYL KATIRA GEL AND A PROCESS FOR PREPARATION THEREOF

The present invention pertains to a process for preparing carboxymethyl katira gel. Further, the process did not involve conventional heating step that sometimes leads to degradation of katira gum. The optimum proportion of alkali and microwave assisted steps provided in the invention leads to high yield with 0.5 degree of substitution of carboxymethyl katira gel. In addition, the developed carboxymethyl katira gel is advantageous in treating S. aureus induced superficial skin infections in animal model.

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We Claim,

1. A process for the preparation of carboxymethyl katira gel wherein the said process comprises of following steps:-
   a) soaking gum katira in water for a time period of 12 to 18 hrs to obtain gel;
   b) stirring the homogenized gel solution obtained in step a) for 15 to 30 minutes followed by addition of 4 to 20 ml of 45 %w/v ice cold sodium hydroxide solution;
   c) adding 10ml -5ml of 75% w/v 2-Chloro ethanoic acid at a rate of 1-3 ml /min to the alkaline solution obtained in step b),
   d) irradiating the mixture obtained in step c) in a microwave reactor 850 watt for a time period of 1 to 2 minutes followed by a cooling cycle for a time period of 1 to 2 minutes at a temperature of 4 to 10°C in ice bath;
   e) repeating the step d) for seven times followed by precipitation with solvents selected from acetone, methanol, methanol: ethanol,
   f) filtering and drying the precipitates of carboxymethyl katira obtained in step e),
   g) preparing carboxymethyl katira gel by dissolving 1 to 10% of carboxymethyl katira in luke warm water;
   h) sterilizing the carboxymethyl Katira gel at a temperature in the range of 121°C to 118°C for a time period of 20 to 40 minutes.
2. A process as claimed in claim 1 wherein the drying of carboxymethyl katira is carried out using lyophilization at a temperature in the range of -20 to -80^{0}C for a time period of 24 to 72 hours or oven drying at a temperature selected in the range of 45 to 55 ^{0}C for a time period selected in the range of 3 to 4 days or 60 ^{0}C for a time period selected in the range of 1 to 2 days to obtain carboxy methyl katira gel.
3. A process as claimed in claim 1 wherein carboxymethyl katira gel is prepared from a pure katira gum obtained from *Cochlospermum religiosum.*
4. A carboxy methyl katira gel as obtained by the process as claimed in claim 1.
5. A gel as claimed in claim 4 wherein the gel is useful as antibacterial agent against *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Propionibacterium acne.*
6. A gel as claimed in claim 4 wherein the gel has antibacterial activity at a concentration more than 1%w/v.

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7. A formulation for topical application is prepared having 1-10 %w/v carboxy methyl katira gel.

Dated this 27th Day of Feb 2014

(Scientist)

Innovation Protection Unit

Council of Scientific & Industrial Research

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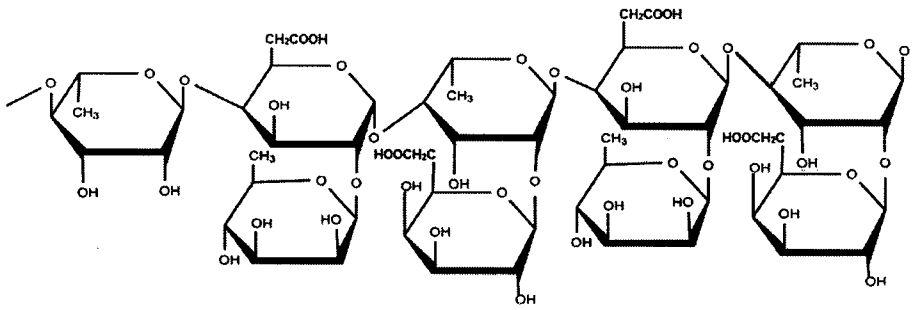

Figure 1

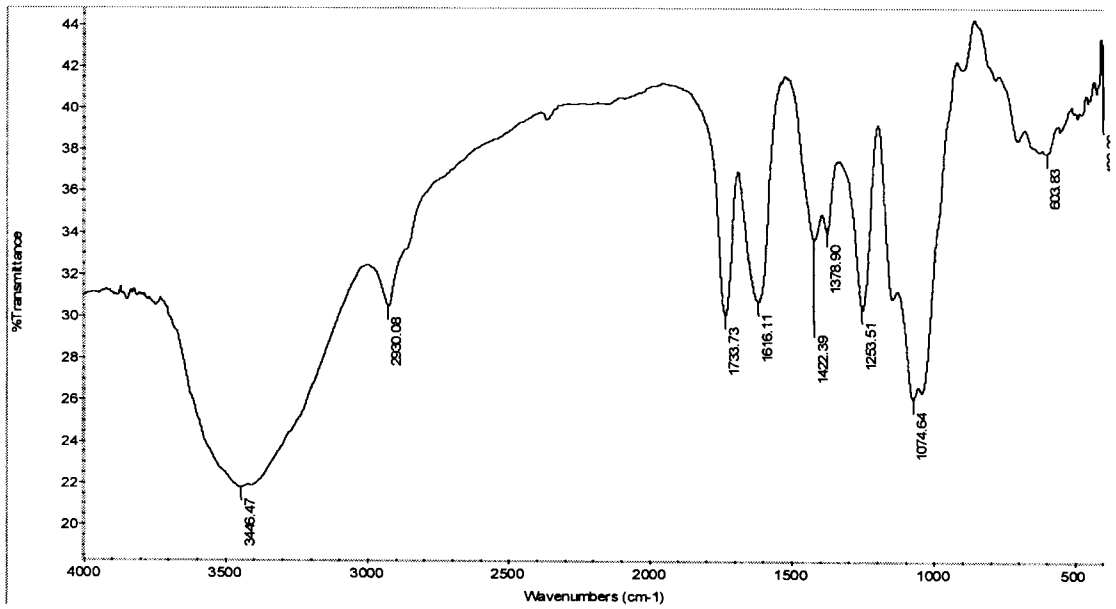

Figure 2

APPLICANT

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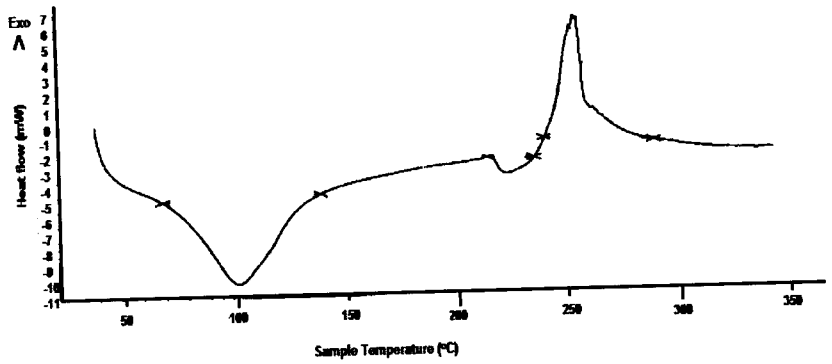

Figure 3

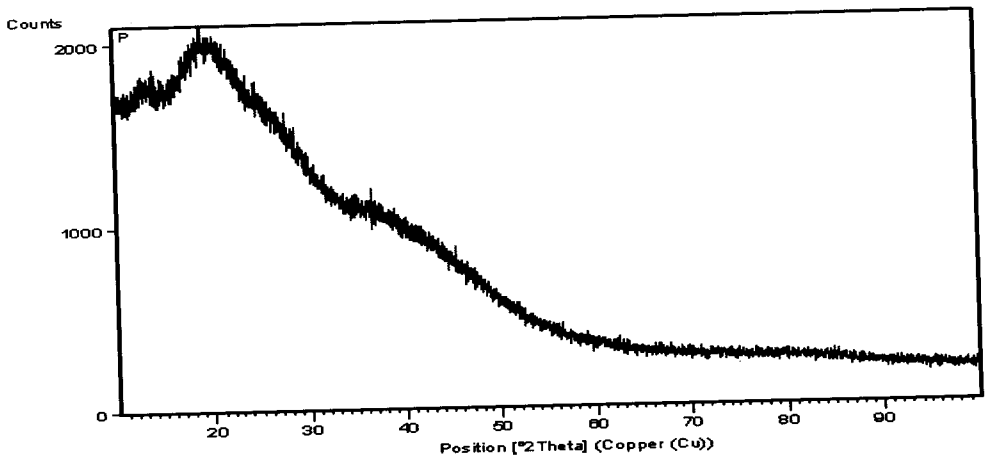

Figure 4

APPLICANT

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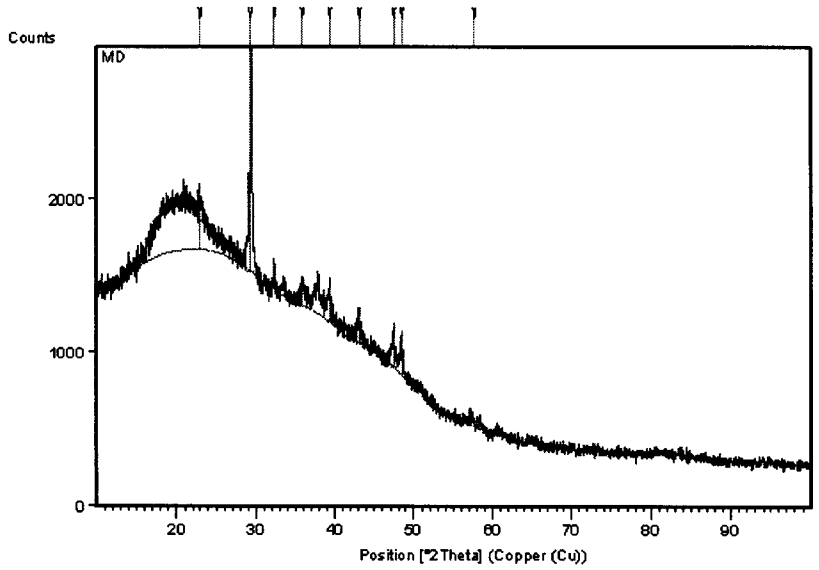

Figure 5

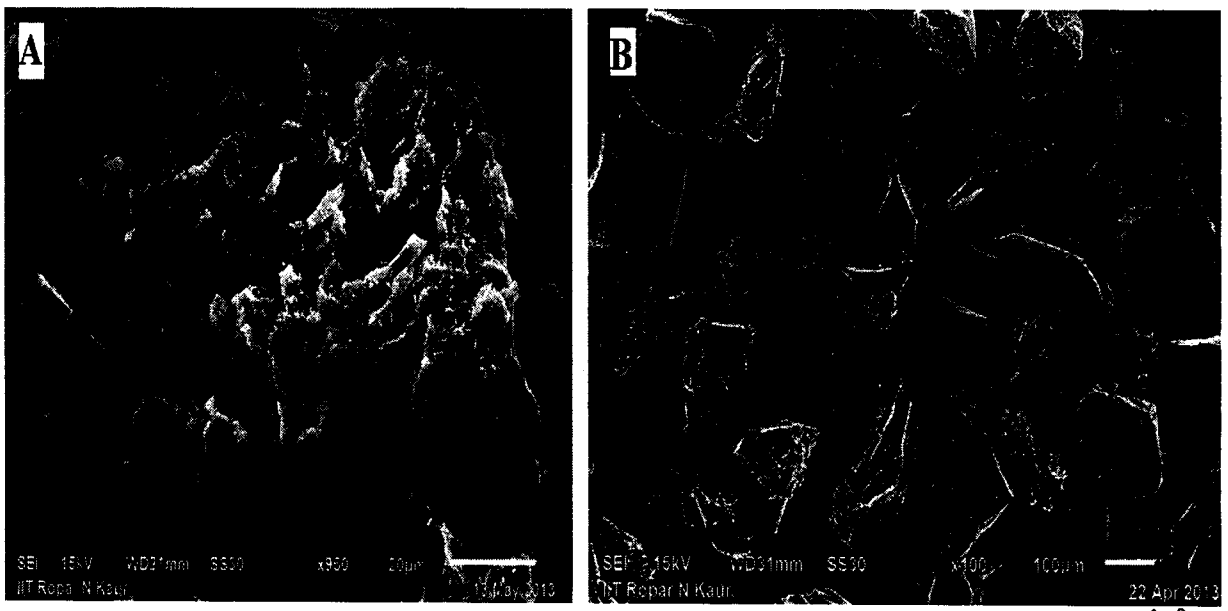

Figure 6

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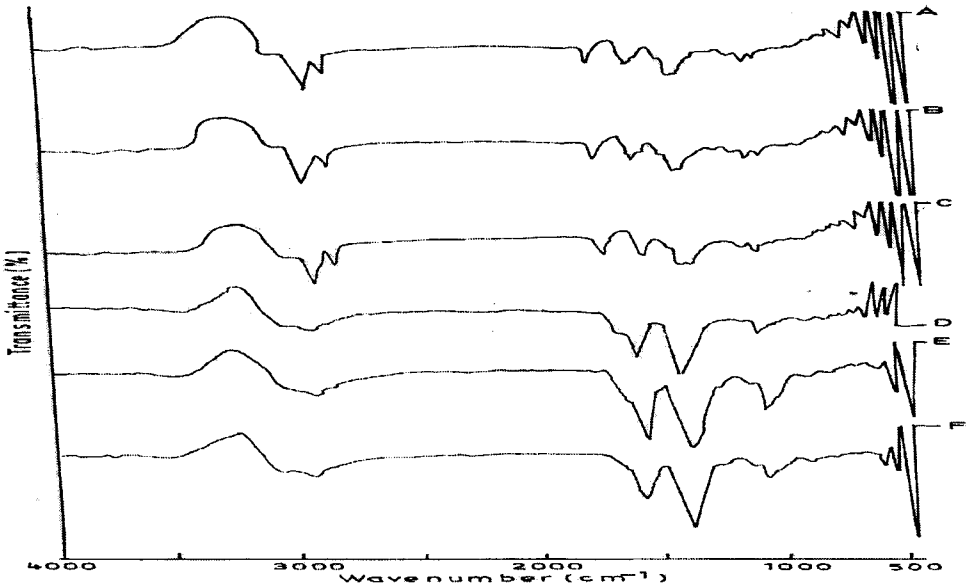

Figure 7

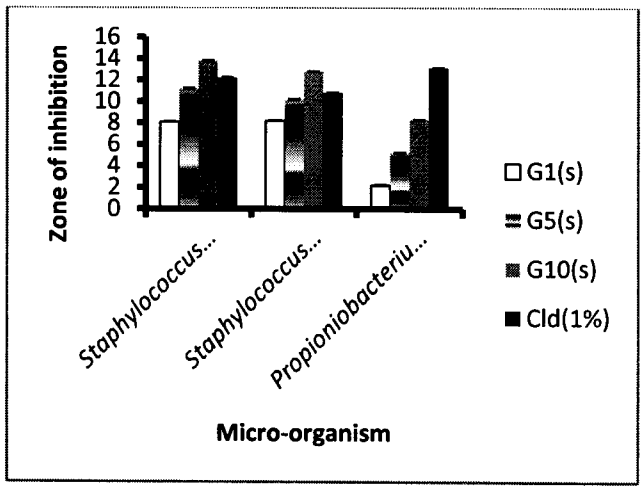

Figure 8

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Code	Volume of sodium hydroxide (45% W/V) added (mL)	Percentage yield of carboxymethyl katira (gm/gm of pure katira gum)
PRL-1	4	Nil
PRL-2	8	0.32±0.11
PRL-3	12	0.38±0.19
PRL-4	16	0.44±0.25
PRL-5	20	0.40±0.20
*Keep all other parameters constant like 7 mL of monochloro acetic acid (75% w/v); microwave treatment: 7 cycles, 850W, 1 min. hot cycle followed by 1 min. cold cycle.		

Table 1

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Table 2

Codes	Independent variables	Level			Codes	Dependent variables	Low	High	Objective
		Low (-1)	Middle (0)	High (+1)					
X_1	Amount of 75% w/v of monochloroacetic acid(ml)	7	10.5	14	Y_1	Degree of substitution	0.41	0.50	Maximum
X_2	No. of cycle with each hot cycle of 1 minute	3	5	7	Y_2	Yield of carboxymethyl katira (gm/gm of gum)	0.44	0.78	Maximum

Details of 3^2 full factorial design

Experimental trials	S1	S2	S3	S4	S5	S6	S7	S8	S9
X_1	-1 (7)	-1 (7)	-1 (7)	0 (10.5)	0 (10.5)	0 (10.5)	+1 (14)	+1 (14)	+1 (14)
X_2	-1 (3)	0 (5)	+1 (7)	-1 (3)	0 (5)	+1 (7)	-1 (3)	0 (5)	+1 (7)
Y_1	0.21±0.30	0.27±1.12	0.28±0.34	0.32±0.40	0.34±0.70	0.38±1.04	0.44±0.31	0.48±1.40	0.50±0.03
Y_2	0.24±0.28	0.34±0.03	0.38±0.12	0.37±0.40	0.44±0.68	0.42±0.15	0.55±0.14	0.65±0.31	0.78±0.34

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Sample	Spindal No	Speed(rpm)	Viscosity (c P)
1% w/v Carboxymethyl Katira gel	61(LV-1)	20	18.06±0.34
	61(LV-1)	50	31.5±0.43
	61(LV-1)	100	41.1±0.37
5% w/v Carboxymethyl Katira gel	63(LV-3)	20	16.5±0.45
	63(LV-3)	50	23.4±0.47
	63(LV-3)	100	24.0±0.52
10% w/v Carboxymethyl Katira gel	63(LV-3)	20	199.5±0.18
	63(LV-3)	50	263.3±0.20
	63(LV-3)	100	252.8±0.26

Table 3

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Details of the molecular fragments appear during MALDI-TOF/TOF Analysis	
Molecular weight ($gmol^{-1}$)	**Proposed molecular assignments**
22.89	Na^+
38.87	K^+
80.90	$CH_2COO^- Na^+$
226	Na^+ CM Gal*p*
243	K^+CM Gal*p*
919	1→4)- α-D-NaCM Gal*p*-(1→4) α-L-Rha*p*-β -D- NaCM Gal*p*-(1→ 2 2 ↑ ↑ 1 1 β-L-Rha*p* β -D-NaCM Gal*p*
1082	→2)-α-L-Rha*p*-(1→4)- α-D-NaCM Gal*p*-(1→4) α-L-Rha*p*-β -D- NaCM Gal*p*-(1→ 2 2 ↑ ↑ 1 1 β-L-Rha*p* β -D-NaCM Gal*p*
1331	→2)-α-L-Rha*p*-(1→4)- α-D-NaCM Gal*p*-(1→4) α-L-Rha*p*-β -D- NaCM Gal*p*-(1→4)-α-L-Rha*p*-(-1→ 2 2 2 ↑ ↑ ↑ 1 1 1 β-L-Rha*p* β -D-CM Gal*p* β-L-Rha*p*
1541	→2)-α-L-Rha*p*-(1→4)- α-D-NaCM Gal*p*-(1→4) α-L-Rha*p*-β -D- NaCM Gal*p*-(1→4)-α-L-Rha*p*-(-1→ 2 2 2 2 ↑ ↑ ↑ ↑ 1 1 1 1 β-L-Rha*p* β -D- NaCM Gal*p* β-L-Rha*p* β -D-CM Gal*p*
*Na^+=Sodium ion, K^+=Potassium ion, *Rha*p* – Rhamnopyranosyl, *NaCM Gal*p*-sodium carboxymethyl galactopyranosyl, *CM Gal*p*- carboxymethyl galactopyranosyl, *K^+CM Gal*p*- potassium carboxymethyl galactopyranosyl	

Table 4

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Gel formulation code	*Staphylococcus aureus*	*Staphylococcus epidermis*
	%IE	%IE
$G_{1(s)}$	12.8±0.02	22.37±0.02
$G_{5(s)}$	90.83±0.03	89.20±0.03
$G_{10(s)}$	95.61±0.01	94.32±0.01

Table 5

Gel Formulation	Zone of inhibition in mm	
	Staphylococcus aureus	*Staphylococcus epidermis*
Sterilized 1%w/v Carboxymethyl katira Gel ($G_{1(s)}$),	8.1±0.01	8.2±0.03
Sterilized 5%w/v Carboxymethyl katira Gel ($G_{5(s)}$)	11.2±0.03	10.2±0.01
Sterilized 10%w/v Carboxymethyl katira Gel ($G_{10(s)}$)	13.8±0.01	12.8±0.01
Control		
Clindamycine gel (1%) marketed Formulation	12.2±0.02	10.8±0.02
Katira gum (10% w/w)	0.012±0.01	0.02±0.01
Sterilized katira gel (10% w/v)	0.011±0.01	0.01±0.01

Table 6

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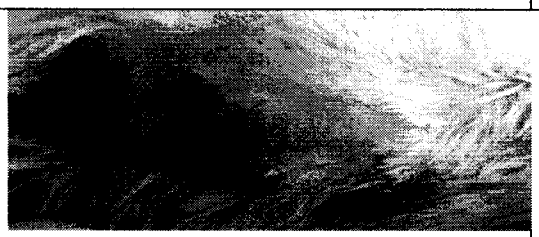
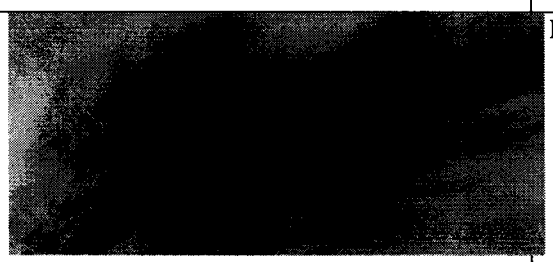
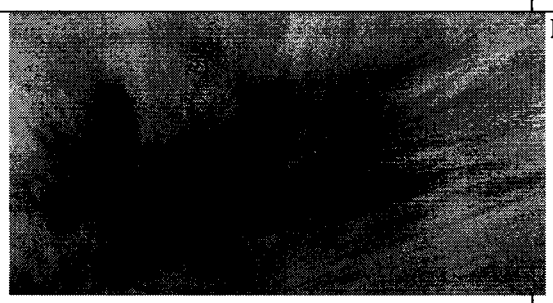
S.No	Observation	Images	Remarks	Inflammation Score
1	0 hrs		No inflammation	0
2	4 hrs		Little inflammation accompanied with redness	1
3	8 hrs		Moderate inflammation with redness and erythema	2
4	16 hrs		Moderate inflammation with redness and erythema	2+
5	24 hrs		Severe inflammation with redness, erythema and other necrotic changes such as visible blood clots.	3+

Table 7

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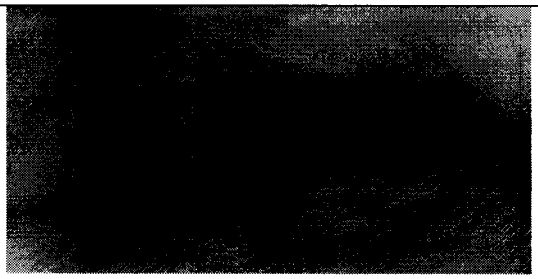
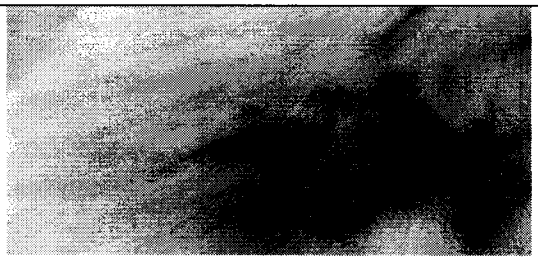
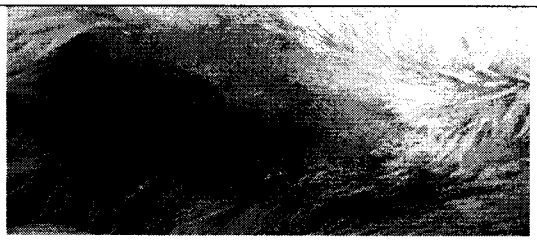
S.No	Observation	Images	Remarks	Inflammation Score
1	0 hrs		No inflammation	0
2	4 hrs		Moderate to severe inflammation with redness and erythema	2+
3	8 hrs		Little inflammation with slight redness	1
4	16 hrs		No inflammation	0
5	24 hrs		No inflammation	0

Table 8

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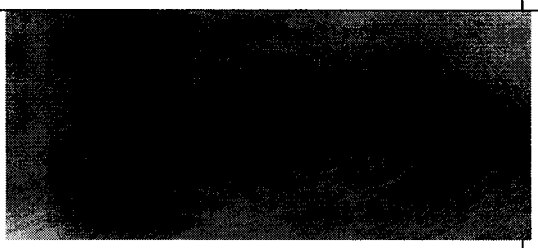
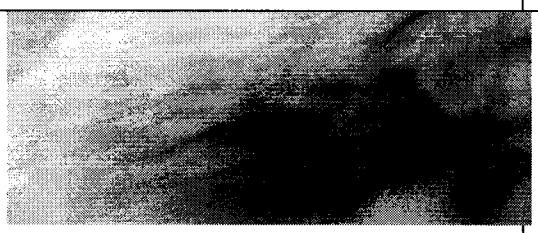
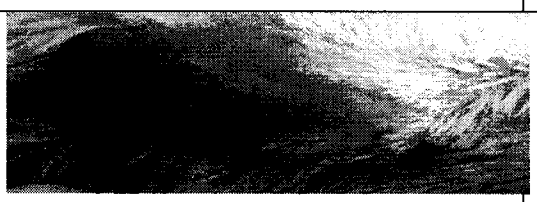
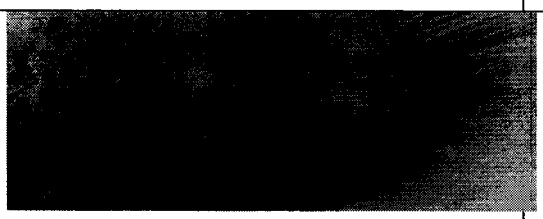
S.No	Observation	Images	Remarks	Inflammation Score
1	0 hrs		No inflammation	0
2	4 hrs		Moderate to severe inflammation with redness and erythema	2+
3	8 hrs		Little inflammation with slight redness	1
4	16 hrs		little inflammation	0
5	24 hrs		No inflammation	0

Table 9

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वरिष्ठ प्रधान वैज्ञानिक / Senior Principal Scientist
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Sheet No.: 13/13
No. of sheets:13

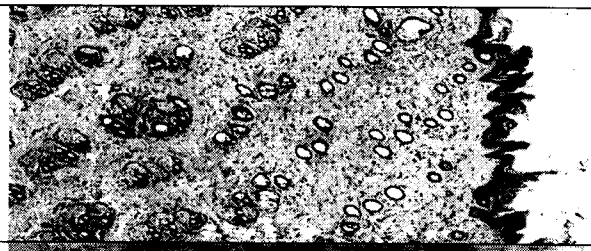
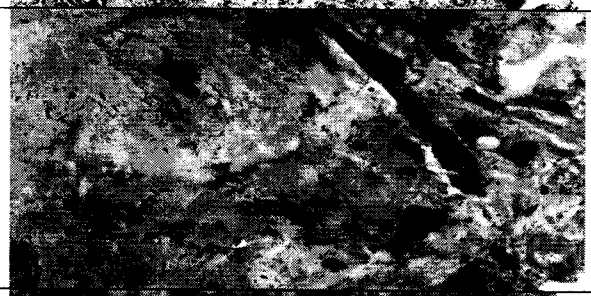
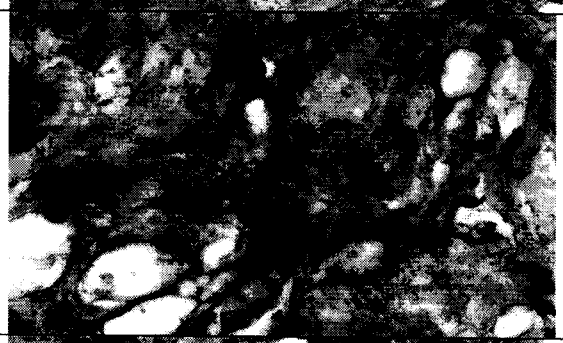
S.No	Tissue sample taken at	Histopathological slide image	Observation	Score*			
				I	N	ML	SA
1	0 hrs		Histological appearance of normal dorsal skin of mice (magnification X100)	0	0	0	0
2	4 hrs		No. of bacterial colonies = two Inflammatory cells=7 No. of neutrophils=7 No. of mononuclear leukocytes= 8	1	1	1	1+
3	8 hrs		No. of bacterial colonies = 4 Inflammatory cells=12 No. of neutrophils=10 No. of mononuclear leukocytes= 11	2+	2+	2+	1+
4	16 hrs		No. of bacterial colonies = 8 Inflammatory cells=12 No. of neutrophils=10 No. of mononuclear leukocytes= 11	2+	1+	1+	2+

Table 10

APPLICANT

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FIELD OF THE INVENTION

The present invention relates to a carboxymethyl katira gel and a process for preparation. The present invention also relates to a microwave assisted process for the preparation of carboxymethyl katira gel. The present invention particularly relates to the carboxymethyl katira gel prepared by the said process. More particularly, the present invention relates to carboxymethyl katira gel having antibacterial activity useful for gel based formulations, treatment of dermal infections, etc.

BACKGROUND OF THE INVENTION

The prospects of natural gums are brighter as synthetic polymers have certain disadvantages such as high cost, toxicity, environmental pollution during synthesis, non-renewable sources, side effects, less patient compliance etc. Hence, natural gums are widely used for conventional and novel dosage forms since they are chemically inert, nontoxic, less expensive, biodegradable and widely available and these natural gums are being modified in different ways to obtain better materials for drug delivery system and thus can compete with available synthetic excipients. Various kinds of gums are used in food industry and are regarded as safe for human consumption. Gums have a variety of applications in pharmacy. They are used in medicine for their demulcent properties of cough suppression. They are ingredients of dental and other adhesives and can be used as bulk laxatives. These hydrophilic polymers are used as tablet binders, disintegrants, emulsifiers, suspending agents, gelling agents, stabilizing agents, thickening agents etc. (T. Mahendran, P. A. Williams, G. O. Phillips, S. Al-Assaf, T.C. Baldwin. New insights into the structural characteristics of the arabinogalactan-protein (AGP) fraction of gum arabic. Journal of Agriculture and Food Chemistry. 56:9269–9276 (2008)). Exudate gums are polysaccharides produced by plants as a result of stress, including physical injury and fungal attack. Gum Arabic (*Acacia Senegal*), gum Tragacanth (*Astragalus gummifer*), gum Karaya (*Sterculia urens*), gum Ghatti (*Anogeissus latifolia*) and gum Katira (*C. religiosum*) have been used by humans for thousands of years in various food and pharmaceutical applications. Natural gums have been modified to overcome certain drawbacks like uncontrolled rate of hydration, thickening, drop in viscosity on storage, solubility and microbial contamination. Various methods are available to modify the state of molecular interaction between polymers (V. Rana,

P. Rai, A. K. Tiwary, R. S. Singh, J. F. Kennedy, C. J. Knill Modified gums: Approaches and applications in drug delivery. Carbohydrate Polymers, 83, 1031-1047 (2011)).

Katira gum is an exudate gum, polysaccharide produced by plants as a result of stress, including physical injury and fungal attack. The gum is secreted by *Cochlospermum religiosum* (A small or medium sized, deciduous, soft wooded tree)

Katira gum is pale and semi-transparent, insoluble in water but swells into a pasty transparent mass with water. The gum is sweet, thermogenic, anodyne, sedative and effective in cough, dysentery, diarrohoea, gonorrhea, syphilis, trachoma and antilice.

The heteropolysaccharide isolated from the gum (Katira) was found to consist of D-galactose, D-galactrouronic acid and L-rhamnose in a molar ratio 2:1:3 (A. K. Ojha, D. Maiti, K. Chandra, S. Mondal, D S. Roy, K. Ghosh and S. S. Islam. Structural assignment of a heteropolysaccharide isolated from the gum of *Cochlospermum religiosum* (Katira gum). Carbohydrate Research. 343: 1222–1231(2008)).

A polysaccharide grafted katira gum was synthesized by graft acrylamide on katira gum in presence of varying concentration of ceric ammonium nitrate (CAN) as initiator. This modified gum was found to be useful as an excipient for colon targeting of drugs (B. Bharaniraja, K. Jayaram Kumar, C. M. Prasad, A. K. Sen. Modified Katira Gum for Colon Targeted Drug Delivery. Journal of Applied Polymer Science.119:2644–2651 (2011); B. Bharaniraja, K. J. Kumar, C. M. Prasad, A.K. Sen. Different approaches of katira gum formulations for colon targeting. International Journal of Biological Macromolecules 49 305– 310 (2011)). The synthesis of acrylamide grafted katira gum requires a heating step at 60 °C for maximum of 5 hr on a water bath.

A green synthesis of gold nanoparticles using aqueous solution of a hetero-polysaccharide, extracted from the katira gum and found to be useful as a efficient heterogeneous catalyst in the reduction of 4-nitrophenol to 4-aminophenol (S. Maity, I. K. Sen, S. S. Islam. Green synthesis of gold nanoparticles using gum polysaccharide of *Cochlospermum religiosum* (katira gum) and study of catalytic activity. Physica E45130–134 (2012). Therefore, the method involves catalytic reduction of gum katira employing direct heating at 70 °C for 6 hr to prepare gold nanopartciles.

The katira gum has been successfully used as a gelling agent in tissue culture media for *in vitro* shoot formation and rooting in *Syzygium cuminii* and somaic embryogenesis in *Albizzia lebbeck*

(N. Jain & S. B. Babbar. Gum katira – a cheap gelling agent for plant tissue culture media. Plant Cell, Tissue and Organ Culture. 71: 223–229 (2002)).

In a different investigation methanolic extracts of leaves and flowers of *Cochlospermum ereligiosum* linn. was found to show antibacterial activity against eight strains of bacterial species, viz., *Staphylococcus aureus*, *Salmonella typhi, Enterobacter aerogenes, Pseudomonas aeruginosa, Xanthomonas oryzae* pv. *oryzae*, *Xanthomonas axonopodis* pv. *malvacearum, Bacillus cereus* and *Micrococcus* sp. (J.A. Bai, R.V. Rai and P.V. Samaga. Evaluation of the antibacterial activity of three medicinal plants of South India. Malaysian Journal of Microbiology.7:14-18(2008)). The extract obtained from leaves and flowers of *Cochlospermum religiosum* linn was found to contain alkaloids, steroids, glycosides, saponins, flavonoids, tannins and phenols. However, no reports have been available for any anti bacterial activity of gum katira.

OBJECTIVES OF THE INVENTION

The main object of the present invention is to provide a process for the preparation of carboxymethyl katira gel.

Another object of the present invention is to provide a microwave assisted process for the preparation of carboxymethyl katira gel.

Still another object of the present invention is to provide carboxymethyl katira gel by using the said process.

Yet another object is to provide a carboxymethyl katira gel formulation effective against *S. aureus, S. epidermis* and *Propionibacterium acne* dermal infection.

Yet another object is to provide a carboxymethyl katira gel formulation effective against *S. aureus* dermal infection as compared to clindamycin gel formulation available in the market.

SUMMARY OF THE INVENTION

Accordingly, the present invention relates to a process for the preparation of carboxymethyl katira gel, a novel gel in pharmaceutical and nutraceutical in general and for the treatment of skin infection.

In an embodiment of the present invention a process for the preparation of carboxymethyl katira gels wherein the said process comprises of following steps:-

a) soaking gum katira in water for a time period of 12 to 18 hrs to obtain gel;
b) stirring the homogenized gel solution obtained in step a) for 15 to 30 minutes followed by addition of 4 to 20 ml of 45 %w/v ice cold sodium hydroxide solution;
c) adding 10ml -5ml of 75% w/v 2-Chloro ethanoic acid at a rate of 1-3 ml /min to the alkaline solution obtained in step b),
d) irradiating the mixture obtained in step c) in a microwave reactor 850 watt for a time period of 1 to 2 minutes followed by a cooling cycle for a time period of 1 to 2 minutes at a temperature of 4 to 10°C in ice bath;
e) repeating the step d) for seven times followed by precipitation with solvents selected from acetone, methanol, methanol: ethanol,
f) filtering and drying the precipitates of carboxymethyl katira obtained in step e),
g) preparing carboxymethyl katira gel by dissolving 1 to 10% of carboxymethyl katira in luke warm water;
h) sterilizing the carboxymethyl Katira gel at a temperature in the range of 121°C to 118°C for a time period of 20 to 40 minutes.

In another embodiment of the present invention a process where the drying of carboxymethyl katira is carried out using lyophilization at a temperature in the range of -20 to -80^{0}C for a time period of 24 to 72 hours or oven drying at a temperature selected in the range of 45 to 55 ^{0}C for a time period selected in the range of 3 to 4 days or 60 ^{0}C for a time period selected in the range of 1 to 2 days to obtain carboxy methyl katira gel.

In another embodiment of the present invention a process where carboxymethyl katira gel is prepared from a pure katira gum obtained from *Cochlospermum religiosum.*

In still another embodiment of the present invention a carboxy methyl katira gel as obtained by the process as mentioned.

In still another embodiment of the present invention a gel where the gel is useful as antibacterial agent against *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Propionibacterium acne.*

In yet another embodiment of the present invention a gel where the gel has antibacterial activity at a concentration more than 1%w/v.

In yet another embodiment of the present invention a formulation for topical application is prepared having 1-10 %w/v carboxy methyl katira gel.

BRIEF DESCRIPTION OF THE DRAWING & FIGURES

Fig.1 Structure of Carboxy methyl katira.

Fig. 2 Illustrates FTIR-ATR of carboxymethyl katira.

Fig. 3 DSC thermogram of carboxymethyl katira.

Fig. 4 XRD spectra of katira gum.

Fig. 5 XRD spectra of carboxymethyl katira.

Fig. 6 Illustrates scanning electron microscopy of pure (a) katira gum and (b) CM katira

Fig. 7 Illustrates FTIR-ATR spectra of unsterilized carboxymethyl Katira gel formulations prepared from A) 1% B) 5% C) 10% and sterilized carboxymethyl Katira gel formulations prepared from D) 1% E) 5% F) 10%.

Fig. 8 Impact of different carboxymethyl katira gel formulations on Staphylococcus aureus, Staphylococcus epidermis and Propionibacterium acne employing agar well diffusion method

Table 1 Effect of concentration of sodium hydroxide on carboxymethyl katira

Table 2 3^2 Full factorial design for the synthesis of carboxymethyl katira gel

Table 3: Viscosities of different concentrations of Carboxymethyl Katira gel

Table 4 Details of the molecular fragments appear during MALDI-TOF/TOF Analysis

Table 5: Results obtained when antibacterial activity studied employing broth dilution method where %IE= percentage inhibition

Table 6: Results obtained when antibacterial activity studied employing agar well diffusion method

Table 7: Results of visible inflammatory response observed during evaluation of *Staphylococcus aureus* induced superficial skin infection in mice belongs to group 2 (infected with *Staphylococcus aureus* bacteria)

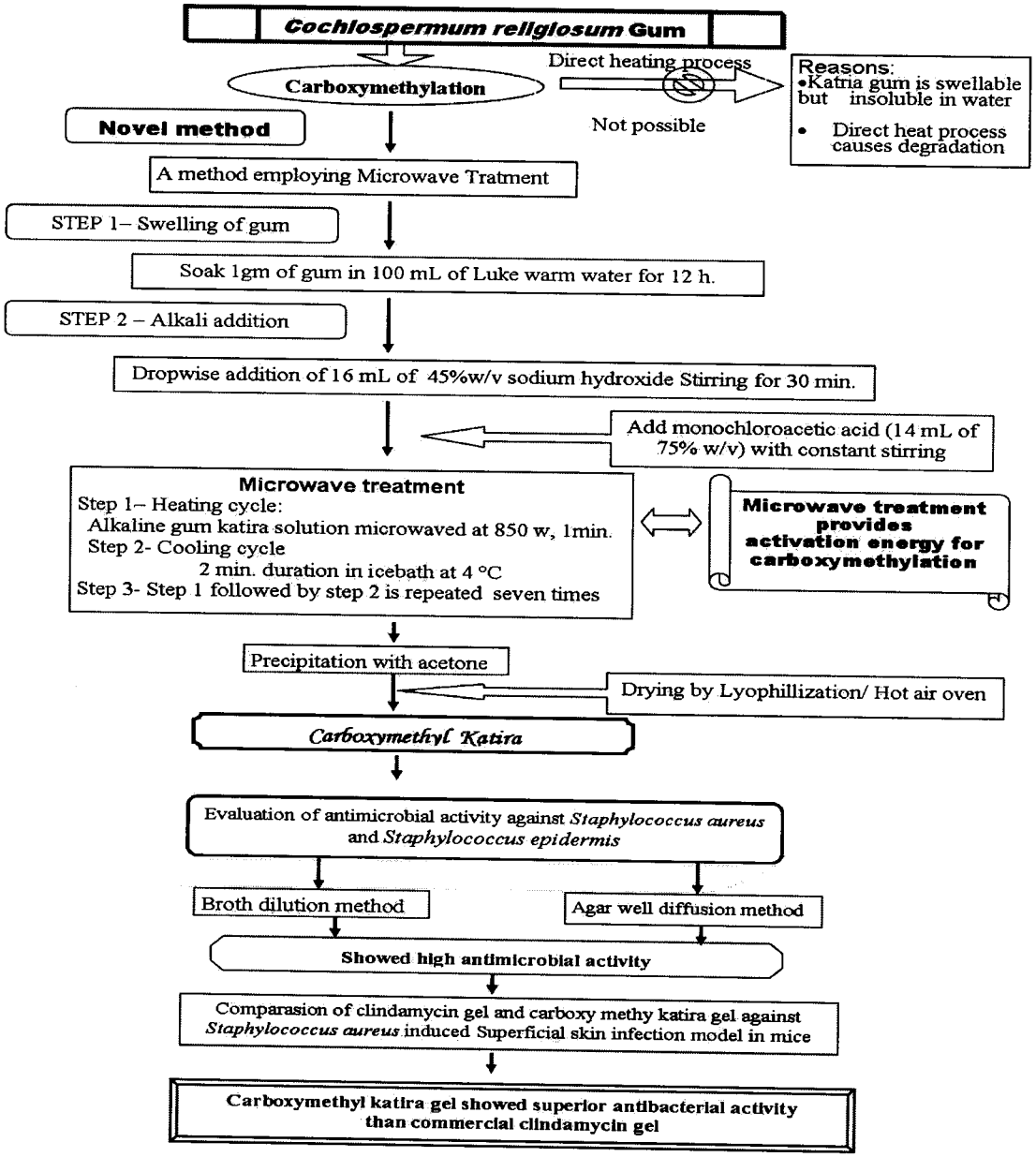
Table 8 Results of visible inflammatory response observed during evaluation of *Staphylococcus aureus* induced superficial skin infection in mice belongs to group 3 (treated with carboxymethyl katira gel).

Table 9 Results of visible inflammatory response observed during evaluation of *Staphylococcus aureus* induced superficial skin infection in mice belongs to group 4 (treated with Clindamycin 1% gel)

Table 10 Histopathological evaluation of mice skin collected after different time intervals belongs to group 2 (infected with *Staphylococcus aureus*) *Scores for inflammatory response (I), infiltrating neutrophils (N), mononuclear leukocytes(ML) and presence of bacteria

DETAILED DESCRIPTION OF THE INVENTION

The katira gum used in the present invention was purchased from "Monu Di Hatti" Kiryana store, Badala Rode, Kharar, Bill No. 521, Dated: 7th January, 2013



Flow diagram: Synthesis and advantage of carboxymethyl katira gel

Cochlospermum religiosum is commonly called as Butter cup tree, Yellow silk cotton tree; Golden silk cotton tree and is a native of India, Burma and Thailand. The flowers of this tree are used for temple offerings. The synonyms of this plant are *Bombax gossypium, Cochlospermum gossypium, Maximilianea gossypium. Cochlospermum religiosum* is a small or medium sized, deciduous, soft wooded tree. The tree

yields a gum that exudes from the fibrous, deeply furrowed bark, which is known as Katira gum (N. D. Prajapati, S. S. Purohit, A. K. Sharma. A hand book of Medicinal plants. A complete source book 1 st ed. Daya Publishing House. Delhi. India, p. 149-544 (2003)). *Cochlospermum religiosum* (L.) is extensively used in Ayurvedic medicines and other uses. Every part of this plant is used medicinally. It is also used for its anti-inflammatory activity in Siddha Drug 'Kalnar Parpam'. Gum katira is used as a cheap gelling agent for plant tissue culture and has wide application in pharmaceutical and food industries. Katira gum is pale and semi-transparent, insoluble in water, but swells into a pasty transparent mass with water. This gum has assumed great importance in recent years and exported annually from India for use in the cigar paste and ice-cream industry (A. K. Ojha, D. Maiti, K. Chandra, S. Mondal, D S. Roy, K. Ghosh and S. S. Islam. Structural assignment of a heteropolysaccharide isolated from the gum of *Cochlospermum religiosum* (Katira gum). Carbohydrate Research. 343: 1222–1231(2008)).

Thus, the present invention relates to the method of converting water insoluble, low spreadability and high swellable katira gum in to water soluble, high swellable, high spreadable and with gelling property carboxymethyl katira gel.

Microwaves comprise electromagnetic radiation in the frequency range of 300 MHz to 300 GHz. On exposure to microwaves, the charged or polar particles tend to align themselves with electric field component of the microwaves which rapidly reverses its direction e.g. at the rate of 2.4 × 109/s at 2.45 GHz microwave frequency. As the polar or charged particles in a reaction medium fail to align themselves as fast as the direction of the electric field of microwaves changes, friction is created to heat the medium (S.A. Galema. Microwave chemistry. Chemical Society Reviews. 26:233–8 (1997)). The heat generated could be utilized to provide activation energy for the reaction. In addition, in the microwave heating process, the high temperatures attained and the ability to work under high pressure conditions for relatively short times make reactions faster than under conventional thermal conditions, and limit the ocurrence of slower side reactions. Thus, greater yields are usually obtained. (C. O. Kappe. Controlled microwave heating in modern organic synthesis. Angewandte Chemie International. 6250–84. (2004)). Hence, the present invention utilized alternative microwave assisted method to prepare carboxymethyl katira gel from katira gum.

Gels are popular dosage forms as a potential topical drug delivery system over other pharmaceutical dosage forms in terms of ease of application and drug release characteristics in the development of topical/dermal formulations containing analgesic or anti-inflammatory drugs due to the fact that the possibility of delivering these drugs through the skin for local pain and inflammations at low doses is attractive. The contact time of a gel formulation on skin or mucosa is typically much longer than that of an aqueous solution owing to the more favourable adhesive and/or rheological properties. An extended contact time at the site of administration might increase the absorption of the drug substance,

bioavailability, might render high patient compliance (P. Batheja, L. Sheihet, J. Kohn, A. J. Singer, B. Michniak-Kohn. Topical drug delivery by a polymeric nanosphere gel: Formulation optimization and in vitro an in vivo skin distribution studies. Journal of Controlled Release. 149:159–167 (2011)). However, the gels prepared with carboxymethyl katira gel for topical drug delivery possess prolonged physicochemical stability, with less systemic side effects and skin or gastric irritation compared to the oral administration. Thus, the present study pertains to novel gel from a carboxymethyl katira and its advantage as a potent antibacterial agent in dermal infections caused by *Staphylococcus aureus/ Staphylococcus epidermis/ Propionibacterium acne.*

The initial step of the present invention relates to carboxymethylation of katira gum, comprise katira gum, monochloroacetic acid (2-Chloro ethanoic acid, $ClCH_2COOH$; as reactant), heating system as microwave operated at different conditions, precipitation stage is carried out with different solvents (Methanol, ethanol, methanol:ethanol or acetone), drying phase in oven at different conditions (45 °C for 3 days, 60 °C for two days, 65 °C for 36 hrs or 70 °C for 24 hrs). The present invention pertains inter alia to dermal gel compositions that treat S. *aureus/S. epidermis* associated skin infection.

The drying phase may be one lyophilization at different conditions (Inlet temperature of -80 °C), spray drying at different conditions (aspiration flow rate of 40, sample flow rate of 1-10 mL/min).

Carboxymethylation can be performed in water as a solvent or in water miscible organic solvents containing small amount of water. The reaction parameters, which influence the carboxymethylation parameters, are the solvent system, solvent composition, concentration of sodium hydroxide and monochloroacetic acid, reaction time and reaction temperature.

The carboxymethylation was performed employing microwave technique, else conventional techniques which not provide carboxymethylation of water insoluble katira gum.

The present invention includes reaction steps as:

$$\text{Katira-OH} + \text{NaOH} \longrightarrow \text{Katira-ONa}$$

$$\text{Katira-ONa} + ClCH_2COOH \xrightarrow{\text{Microwave treatment}} \text{Katira-OCH}_2\text{COONa} + \text{ClCH2COOH}$$

The present invention detailed the process of carboxymethylation by varying the process parameters such as concentration of sodium hydroxide, concentration of monochloroacetic acid, microwave radiation, microwave radiation time, precipitation solvent system and drying stage. The effect of variation of sodium hydroxide concentration from microwave radiation (850W for 5 cycles, each cycle 1 min/cycle followed by 2 min cooling cycle) on degree of substitution was examined. The degree of substitution (DS) of a polymer is the (average) no. of substitution groups ($-OCH_2COONa$) attached per base unit

(Example 5) Further, the increase in sodium hydroxide concentration up to optimum concentration, the yield (gm/gm of pure gum) also increases and then level decreases (Table 2). Further, the present investigation detailed at optimum concentration of sodium hydroxide, the carboxymethylation of katira prevails over its competitive reaction (Table 2). The present investigation detailed, further, above optimum concentration of sodium hydroxide, the DS formation increases (Table 2). Accordingly, the present investigation prevailed maximum degree of substitution, when optimum concentration of monochloroacetic acid as reactant at different microwave radiation conditions. The present investigation detailed deviation from optimized concentration of monochloroacetic acid leads to lower DS (Table 2). The microwave radiation and microwave time drastically influence integrity, stability of katira. The point when gel live consistence of a reaction mixture, turned down to clean, thick, solution after microwave radiation and time is the end point. The 1 min/cycle microwave radiation duration followed by cooling cycle for 2 min enhanced DS and yield of product. The total 3-8 cycles at 100% (850w), 90% (850w), 80% (850w), 70% (850w), 60% (850w) microwave radiation is required to attain end point.

A second part of the present investigation pertains to *in vitro* antibacterial/antimicrobial performance evaluation employing agar diffusion method (Example 10). The dermal gel may inhibit microbial growth, defined using inter alia the corrected zone of inhibition (Example 10).

The zone of inhibition method involves placing a dermal gel on sterile mannitol salt agar surface in 25 ml/ 9 cm plates that produces an agar depth of 4mm, which has been seeded with the test microorganism (*Staphylococcus aureas*). Diffusion of antibacterial agent into the agar results in inhibition of growth, which appears as a clear zone on the agar. The corrected zone of inhibition is determined as the diameter of the whole inhibition zone minus the size of the whole.

The dermal gel inhibits the growth of microbes on the skin of mouse model (Example 12).

EXAMPLES

The following examples are given by way of illustration of the working of the invention in actual practice and should not be construed to limit the scope of the present invention in any way.

EXAMPLE-1

Screening of the concentration of alkali for obtaining maximum yield of carboxy methyl katira.

The gum katira (1gm) was soaked in 100 mL of water until the gum completely swells. The homogenized gum solution was stirred for 30 min with the slow addition of different volume (4ml, 8ml, 12ml, 16ml or 20ml) of 45%w/v ice cold sodium hydroxide solution (Table 1). Separately, 7 ml of monochloro acetic acid (75% w/v) i.e. 2-Chloro ethanoic acid, $ClCH_2COOH$ was prepared and mixed with alkaline katira gum solution with constant stirring at a rate of 1ml/min. The prepared mixture was microwaved (7 cycles,

850 W) for 1 min of hot cycle followed by 1min cooling cycle. This solution was precipitated with acetone. The 16ml of 45% w/v concentration of sodium hydroxide was selected.

EXAMPLE-2

The gum katira (1gm) was soaked in 100 mL water until the gum completely swells. The homogenized gum solution was stirred for 30 min with the slow addition of 16 ml of 45%w/v ice cold sodium hydroxide solution (Table 1). Separately, 7 ml of monochloro acetic acid i.e 2-Chloro ethanoic acid, $ClCH_2COOH$ (75% w/v) was prepared and mixed with alkaline katira gum solution with constant stirring at a rate of 1ml/min. The prepared mixture was microwaved (7 cycles, 5cycles or 3cycles (any one as per table2), 850 W) for each cycle of 1 min followed by 1min, cooling cycle (Table 2). This solution was precipitated with acetone.

EXAMPLE- 3

The precipitates of carboxymethyl katira were filtered and dried using lyophilization or oven dry at 45°C for 3 days or 60°C for 2 days.

Procedure was as follow:

1 gm of precipitates was dissolved with 100 mL water and deep freezed (-20 to -80°C) for 24 hrs. The sample was lyophilized using lyophilization (Allied frost, New Delhi) at the vacuum pressure of 0.05 mm Hg other batch was another way of drying the precipitates.

EXAMPLE- 4

The process for the synthesis of carboxymethyl katira gel was conducted as per 3^2 full factorial designs. The X_1 was amount of monochloroacetic acid added and X_2 was number of cycles. During preliminary studies, it was observed that the optimum concentration of sodium hydroxide i.e. 16 ml of 45 % w/v is required to complete the reaction. After this concentration of sodium hydroxide, no significant change in degree of substitution was obtained. Therefore, the composition of sodium hydroxide was fixed to 16 ml of 45% w/v. The amount of monchloroacetic acid and number of heating cycles during microwave treatment was found to show significant effect on the degree of substitution and % yield of carboxymethyl katira. Thus, both the factors were taken to optimize the synthesis of carboxymethyl katira. The results were shown in the Table 2.The equation generated after multiple linear regression are:

$$Y_1 = 0.36+0.95X_1+0.27X_2 \quad (R^2 = 0.99)$$

$$Y_2 = 0.46+0.87X_1+0.36X_2 \quad (R^2 = 0.90)$$

The equations showed direct correlation of Y_1 (degree of substitution) and Y_2 (yield) on X_1 and X_2. Further, the increase in amount of monomer (X_1) and no. of cycles during microwave treatment (X_2) increased the yield and degree of substitution. The maximum yield and degree of substitution was found to obtain at 14 ml of 75% w/v amount of monomer and 7 cycles of microwave treatment (850W, 1

min/cycle). i.e synthesis S_9. Therefore, further carboxymethyl katira was synthesized with method of S_9 to get 0.78 gram of yield per gram of pure gum.

EXAMPLE-5

Determination of Degree of substitution (D.S.)

The carboxymethyl gum (1.5gm) was dispersed in 50 mL of 2 M HCl (used 70 % v/v methanol as solvent). This suspension was kept under magnetic stirring for 24 hrs for the conversion of sodium carboxymethyl katira into H-carboxymethyl katira. H-carboxymethyl katira was washed with 95% v/v ethanol and dried at 60 °C for 2 hr after filtration.

This dried carboxymethyl katira (1g) was dissolved in distilled water (100 ml). An excess of 1 N NaOH (10-15 ml) solution was added with stirring and stirring was continued for 15 more minutes before solution was heated to boil for 30 min. While the solution was hot excess NaOH was back titrated with 0.5 N HCl to a phenolphthalein end point. The amount of acid consumed was recorded and the D.S. was calculated according to equation reported by Whistler *et al.* (Whistler, R. L. (Ed.) Methods in Carbohydrate Chemistry, vol. 3, Academic Press Inc., New York, USA. (1963)).

$$D.S. = 0.72\ A/(1-0.058A) \quad \text{---------- (a)}$$

Where

$$A = (BC-DE)/F$$

A= acid consumed per gram of sample; **B**= NaOH solution Added, ml; **C**= Normality of NaOH; **D**= HCl required for titration of the excess NaOH, ml; **E**= Normality of HCl, **F**= CMG used, g; **72**= grams molecular mass of pure gum and **58**= net increase in molecular mass of pure gum for each The maximum degree of substitution was found to be 0.5 (Table 2)

EXAMPLE 6

Procedure: Carboxymethyl katira gum powder is dispersed in luke warm Purified water while stirring to allow full swelling to obtain homogeneous solution.

Preparation of carboxymethyl katira gel: Carboxymethyl katira gel is prepared using the following formula

Unsterilized Carboxymethyl katira gel Formulations			
Composition Code	G_1	G_5	G_{10}
Carboxymethyl katira gum Powder	1gm	5 gm	10 gm
Purified water (Quantity Sufficient to)	100 ml	100 ml	100 ml

Sterilized Carboxymethyl katira gel Formulations			
Composition Code	$G_{1(s)}$	$G_{5(s)}$	$G_{10(s)}$
Carboxymethyl katira gum Powder	1gm	5 gm	10 gm
Purified water (Quantity Sufficient to)	100 ml	100 ml	100 ml

Procedure: Carboxymethyl katira gum powder is dispersed in luke warm purified water while stirring to allow full swelling to obtain homogeneous solution. This homogeneous solution was transferred to 10 ml bottles and sealed. These Bottles were sterilized at 121°C, 15 pound pressure for 30 min in an autoclave

EXAMPLE 7

Characterization of Carboxymethyl Katira

A Fourier transform infrared spectroscopy

The FTIR spectra of Carboxymethyl Katira was recorded in solid state using KBr pellets with a Thermo Nicolet 310i FTIR spectrophotometer. FTIR spectra were scanned in the wavelength range of 400-4000cm^{-1}. The FTIR spectra of carboxymethyl katira showed characteristic peaks at 1733.71cm^{-1}, 1616.11cm^{-1}, 1422.38 cm^{-1}, 1378 cm^{-1}, 1253 cm^{-1}, 1074.64 cm^{-1} indicated chemical linkage of carboxymethyl moiety with pure katira gum. (Figure 2)

B. Differential scanning calorimetry analysis

DSC measurement wasperformed on powdered samples of Carboxymethyl Katira employing differential scanning calorimeter (812E, Mettler Toledo, Switzerland) in the range of 40 to 350 ° C at a heating rate of 10°C/min. The DSC thermogram of carboxymethyl katira showed two exothermic transitions at 90.738°C (Δ H=238.22kg) and 287.24 °C (ΔH= -120.78 J/g (Figure 3).

C. X-Ray Diffraction analysis

The X-ray powder diffraction data was acquired by Analytical X'Pert PRO diffractometer with step-scan technique using Johansson monochromator to produce pure Cu k[α]1 radiation (1.5406 A°;45V ; 40 mA). The sample was crushed to a fine powder and pressed into a sample holder. The XRD scans were recorded from 2θ = 10° to 80° using a step size of 0.0170° The XRD analysis of pure katira gum showed amorphous nature (Figure 4). However, the appearance of characteristic peaks at 2θ of 22.9°, 29°, 32.2°, 35.8°, 39.2, 43°, 47.4°, 48.42°, 57.6° indicated the crystalline nature of carboxymethyl katira (Figure 5).

D. Scanning electron microscopy

Morphological features of the pure Katira gum (Figure 6(a) or Carboxymethyl Katira (Figure 6(b)) were studied with a Joel JSM-6610 LV scanning electron microscope. The dried sample was mounted on a metal stub and sputtered with gold in order to make the sample conductive and the images were taken at an accelerating voltage of 10KV and at varied magnification. The SEM images of pure katira gum is shown in Figure 6A.The results suggested irregular shaped, rough surfaced pure katira gum particles. However, crystals of carboxymethyl katira was observed in SEM image suggesting crystalline nature of carboxymethyl katira (Figure 6B).

E Viscosity Determination

The different concentrations of carboxymethyl katira were analyzed for viscosity using Brookfield viscometer (Brookfield LV DV-II Plus Pro, Bruker, Berlin, Germany) spindle No. S-61(LV-1) or 63(LV-3). The temperature of the sample was maintained at 25 °C using refrigerated circulating water bath. The shear stress and shear rate correlation was determined to observe the flow behavior. (Table 3)

F. Molecular weight determination of carboxymethyl Katira and its fragments obtained during MALDI-TOF/TOF Analysis

The molecular weight of biggest fragment present in the carboxymethyl Katira sample was evaluated employing MALDI TOF/TOF. For this purpose 1%w/v solution of carboxymethyl Katira was prepared. Each of 2µl of 1%w/v carboxymethyl Katira and 2µl of matrix solution (2,5-dihydroxybenzoic acid 10mg/ml in TFA:CH_3CN:: 1.75:0.75 v/v). The total of 2µl of above solution was applied to a stainless steel sample slide and dried under vacuum. MALDI-TOF mass spectra of the generated

Oligomer/polysaccharide were recorded on a Bruker Daltonics ultraflex TOF/TOF Analysis inmthe molecular weight range of 0-3000 gm/mol. Spectra were recorded on Reflactor mode. Results are depicted in table 4. The largest fragment appeared during analysis was of molecular weight 1541g/mol indicated for the molecular weight of the carboxymethyl Katira.

EXAMPLE-8

The carboxymethyl katira was analyzed for different powder characteristics like bulk and tap densities, Hausner's ratio, Compressibility index, Angle of repose, Swelling capacity and Effective pore radius ($R_{eff\cdot P}$). The Bulk, density, tap density, Hausner's ratio, Carrs index, Angle of repose, Swelling capacity and Effective pore radius ($R_{eff\cdot P}$) was found to be 0.50±0.12g/ml, 0.74±0.03g/ml, 1.55±0.03, 32.43±0.10, 52, 200 and 1.44±0.0013 mm, respectively.

EXAMPLE-9

Determination of Stability of gel formulation after autoclaving employing FTIR-ATR

The stability of gel for its resistance to autoclaving temperature was examined using FTIR-ATR spectral analysis. The sterilized carboxymethyl Katira gel formulation (1-10%w/v) was subjected to FTIR-ATR and the spectra obtained was compared with unsterilized carboxymethyl Katira gel formulation (1-10%w/v) (Figure 7).

The gel formulation obtained were sterilized employing autoclaving at 15 psi for 30 minutes. The stability of autoclaved gel formulation was examined by using FTIR-ATR analysis. The FTIR-ATR spectra of unsterilized 1% w/v gel formulation showed peaks at 1039.84 cm^{-1}, 1230.30 cm^{-1}, 1423.72 cm^{-1}, 1515.44 cm^{-1}, 1649.22 cm^{-1}, 1744.45 cm^{-1} and 3742 cm^{-1}(Figure 7A). A similar peak with enhanced magnitude was observed in the FTIR-ATR spectra of gel formulation prepared with 5% or 10% w/v (Figure 7B and 7C). The FTIR-ATR spectra of sterilized 1%, 5%, and 10% w/v showed the similar prominent characteristic peaks at 1074 cm^{-1}, 1403 cm^{-1} and 1580 cm^{-1} indicating the gel formulation was stable even after autoclaving (Figure 7D, E and F).

EXAMPLE-10

Estimation of antibacterial activity employing Broth dilution method

The pure bacterial strains (*Staphylococcus aureus or Staphylococcus epidermis*) were obtained from Rajendra Medical College, Patiala. Sterile nutrient broth was prepared by mixing definite volumes of peptone (0.6%), yeast extract (0.15%) and di-potassium dihydrogen phosphate (0.36%) in distilled water, pH adjusted to 7.2 as per Indian Pharmacopoeia (2010) and autoclaved at 15 psi for 30 minutes. The sterile broth was inoculated with the *Staphylococcus aureus* or *Staphylococcus epidermis* under aseptic conditions and incubated for 24 hrs at 37° C to obtain 24 hr old culture. The test was performed as per the method reported by Jorgensen *et al.* (J. H. Jorgensen, S. A. Crawford, M. L. McElmeel and K. R.

Fiebelkorn. Detection of inducible clindamycin resistance of staphylococci in conjunction with performance of automated broth susceptibility testing. Journal of Clinical Microbiology. 42: 1800–1802 (2004)). Various test tubes were prepared each containing gel formulation (1-10% w/v), 24hr old culture broth (0.5ml), pure culture media (5ml) as shown in Table 3. In addition to test samples positive control, negative control and respective blanks were also prepared. All the test tubes were incubated in oven at 37°C for 18 hr. The absorbance of the culture broth was measured at 540 nm or 276 nm, respectively for *Staphylococcus aureus* and *Staphylococcus epidermis* against respective blanks. The microbial inhibition effect was calculated as follows;

Inhibition effect % = (Ac- As)/Ac x 100

Where, Ac = Absorbance of control & As = Absorbance of sample

Results

The results suggested more than 90% of percentage inhibition when *Staphylococcus aureus* or *Staphylococcus epidermis* grown in the presence of sterilized 5%w/v Carboxymethyl gel or sterilized 10%w/v Carboxymethyl gel. Thus, findings pointed towards anti-microbial effect of carboxymethyl katira formulations. Results are depicted in Table 5.

EXAMPLE-11

Estimation of antibacterial activity employing Agar well diffusion method

Antibacterial activity against Gram positive aerobic bacteria (*Staphylococcus aureus or Staphylococcus epidermis)* were examined as per method reported in I.P. (2010).

Preparation of 24hr old culture

For the preparation of 24hr old culture, sterile nutrient broth was prepared by mixing definite volumes of peptone (0.6%), yeast extract (0.15%) and di-potassium dihydrogen phosphate (0.36%) in distilled water, pH adjusted to 7.2 as per I.P. (2010) and autoclaved at 15 psi for 30 minutes. The sterile broth was inoculated with the *Staphylococcus aureus* and *Staphylococcus epidermis* under aseptic conditions and incubated for 24 hrs at 37 °C to obtain 24 hr old culture.

Preparation of sterile Mannitol Salt agar media

38 grams of solid media was dissolved in one litre of lukewarm distilled water in a conical flask. The prepared culture media and petriplates were sterilized separately at 15 psi for 30 minutes. 1ml of 24hr old microbial suspension was added to sterilized mannitol salt agar media at 40°C and poured in to the petriplates (90 mm×15 mm) till solidify under aseptic conditions. The wells were prepared with the help of sterile borer. For each petriplate four wells were created. Each of the well was filled with 1ml of gel formulation ($G_{1(s)}$, $G_{5(s)}$, $G_{10(s)}$), while the fourth well was filled with 1ml clindamycin 1 % gel marketed

formulation. After incubation at 37 °C for 24 hrs, the zones of inhibition were recorded. Results are depicted in Table 6.

EXAMPLE-12

Determination of activity against *Staphylococcus aureus* induced superficial skin infection model in mice

Animal infection experiments were performed at the animal house PGIMER, Chandigarh (160012) in accordance with institutional animal ethics committee and institutional biosafety level 2 guidelines (Ref No. 67/IAEC/405). Six- to 8-week-old swiss albino mice of either sex were used for the experiment. The tape stripping infection model was used for the study as per the method reported by Kugulberg *et al.* (Kugelberg, E., T. Norström. Establishment of a superficial skin infection model in mice by using *Staphylococcus aureus* and *Streptococcus pyogenes*. Antimicrobial agents and chemotherapy. 49, 3435-3441. (2005). In brief, abdominal hairs were removed employing the epilatory. An area of $4cm.^2$ was tape stripped. After stripping of the skin, an infection of *Staphylococcus aureus* ATCC No.29213 was initiated by placing on the skin a 5µl droplet containing 10^7 cells concentrated from an overnight bacterial culture in stationary phase. In each experiment, a group of mice were killed 4 h after infection to control the infectious dose. The mice were treated with carboxymethyl katira gel (10%w/v). The first application of carboxymethyl katira gel (10%w/v) to the stripped skin of the mice was at 4 h post infection. Thereafter, beginning at 8th after the first treatment, additional carboxymethyl katira gel (10%w/v) applications were made twice (16th hour and 24th hour, with an 8 h interval) for a period of 1 day and was compared with marketed clindamycin (1%) gel formulation for the treatment of infection. For each treatment 10 ml of gel was applied. Two infection control groups were included for each experiment: one consisted of untreated mice and the other consisted of mice treated with gel. For all experiments in which untreated or topically treated groups were included, the experiments were terminated at 24 h after the last topical treatment. The study was conducted in the four groups with minimum five animals per group.

(a) Group 1 was a control group (novel gel was applied without infection for skin irritation studies).

(b) Group 2 was mice infected with *Staphylococcus aureus* bacteria only (no treatment)

(c) Group 3 received a carboxymethyl Katira gel (10%w/v) formulation application post infection.

(d) Group 4 received a clindamycin gel treatment post infection.

In all the groups the skin surface was observed for erythema, redness, pimples and other necrotic changes. Tissue specimen from the infected site was taken after 4hr, 8hr, 16hr and 24 hr for histopathological evaluation (Table 7, 8, 9 and 10).

EXAMPLE- 13

Antimicrobial activity against anaerobic *Propionibacterium acne*

Ampoule containing freeze dried culture of *Propionibacterium acne* was aseptically transferred to sterilized brain heart infusion media after breaking the seal at the marked position. The inoculated broth was incubated at 37°C in anaerobic chamber for three days. Subculturing was done on fourth day and again incubated in anaerobic chamber for next three days. Growth was observed on 7th day and used as inoculum for further studies.

The revived 7 day old *Propionibacterium acne* culture was further incubated in brain heart infusion broth (BHI) containing additional 1% glucose and incubated for 48 h under anaerobic conditions and adjusted to yield approximately 1.0×10^8 CFU/ml.

Aliquots of molten BHI with glucose agar were used as the agar base for preparing petriplates. A prepared inoculum was added to the molten agar, mixed, poured over the surface of the agar base and left to solidify. A sterile 8 mm borer was used to cut wells of equidistance in each of plates. 1 ml of clindamycin 1% marketed formulation was introduced in to the well at randomly and the gum solution in rest of three wells. The plates were then incubated at 37°C for 48 h under anaerobic conditions in an anaerobic jar (Hi-Media) with gas pack and indicator strip and the jar was kept in an incubator for 48 h at 37 ± 1°C. Gas packs containing citric acid, sodium carbonate and sodium borohydride were used to maintain and check the anaerobiosis, where citric acid releases carbon dioxide and sodium borohydride releases hydrogen when they come in contact with oxygen. An indicator strip of methylene blue, when introduced into the jar, changes in colour from white to blue in the absence of anaerobiosis. The antibacterial activity was evaluated by measuring the diameter of zones of inhibition (in mm).

The growth of *Propionibacterium acne* into brain heart infusion agar media change the brown coloured petriplate into yellow coloured. The results suggested that carboxymethyl Katira gel formulated in the form of gel formulation $G_{5(s)}$ and $G_{10(s)}$ is effective against *Staphylococcus aureus*, *Staphylococcus epidermis* and *Propionibacterium acne*. The effect of carboxymethyl katira gel follow the order *Staphylococcus aureus*> Clindamycin > *Staphylococcus epidermis* > *Propionibacterium acne*. Thus, the effect of carboxymethyl katira gel was found to be equivalent to the effect produced by Clindamycin on *Staphylococcus aureus*, *Staphylococcus epidermis* and *Propionibacterium acne*. Therfore, the finding pointed towards the utilization of carboxymethyl katira gel for the treatment of diseases caused by *Staphylococcus aureus*, *Staphylococcus epidermis* and *Propionibacterium acne*.

ADVANTAGES OF THE INVENTION

The main advantages of the present invention are:

1. The carboxymethyl katira gel is prepared from katira gel employing microwave assisted method. Till today, no reports available for the synthesis of carboxymethy katira. This is probably due to degradation of katira gum during a conventional thermal heating step that provides non-uniform distribution of heat.
2. The main reaction parameters which influence the carboxymethylation of katira gum are the concentration of sodium hydroxide, monochloroacetic acid, microwave power and microwave cycle. The present invention provides optimum concentration of sodium hydroxide and monochloroacetic acid necessary for the synthesis carboxymethy katira gel.
3. The microwave power used and microwave hot cycle followed by cold cycle leads to enhanced degree of substitution along with high yield.
4. In addition, the microwave used in the present invention uniformly distributes heat into the reaction mixture that further decreases reaction time and, increases yield and degree of substitution.
5. The stability of autoclaved gel formulation were examined employing FTIR-ATR analysis suggested gel is stable and can withstand autoclave conditions.
6. The rheological profile of carboxymethy katira gel with variable concentration suggested newtonian behaviour. This behaviour is not affected/influenced by autoclave conditions.
7. The results of zone of inhibition suggested carboxymethyl katira gel formulation was effective against *Staphylococcus aureus*, *S. epidermis*.
8. Further, the effect of carboxymethy katira gel was equivalent to clindamycin marketed gel formulation against *S. aureus and S. epidermis*.
9. The carboxy methyl katira gel is effective in treating inflammation, redness erythema and edema effectively in comparison to standard marketed clindamycin gel (1%).
10. The score for inflammation response, infiltrating neutrophils, mononuclear leukocytes and number of bacteria with histopathological images obtained at different time intervals of 24 hr study period confirmed that enhanced activity of 5% w/v carboxymethy katira gel for the treatment of *S. aureus* induced superficial skin infections in animal model (Table 9). Interestingly, no signs of inflammation, redness, erythema and edema observed during evaluation of carboxymethy katira gel in mice for three days skin irritation studies (Table 7-10).

11. Further, histopathological evaluation of mice skin confirmed carboxymethyl katira gel (5% w/v) is safe and effective and can be used for the treatment of *S. aureus* skin infection in human as an alternative to topical clindamycin 1 % gel (Table 9).