DEVELOPMENT OF LICHEN TREATED BANDAGE FOR HEALING CUT WOUND

A PROJECT REPORT

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In partial fulfillment for the award of the degree of

BACHELOR OF TECHNOLOGY

IN

TEXTILE TECHNOLOGY

KUMARAGURU COLLEGE OF TECHNOLOGY

(Autonomous Institution Affiliated to Anna university of Technology Coimbatore)

COIMBATORE

APRIL 2012

BONAFIDE CERTIFICATE

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Submitted for the Project Viva- Voce examination held on 19.04.2012

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ACKNOWLEDGEMENT

We would like to articulate our sincere thanks to **Arutselvar Dr.N. Mahalingam,** Chairman , **Dr.B.K. Krishnaraj Vanavarayar,** Co-Chairman, **Dr.J.Shanmugam,** Director and **Dr.S.Ramachandran,** Principal, KUMARAGURU COLLEGE OF TECHNOLOGY for their kind encouragement.

Our sincere thanks to **Dr.Bhaarathi Dhurai,** Head of the Department, Textile Technology, KUMARAGURU COLLEGE OF TECHNOLOGY, for her remarkable guidance, advice and motivation in completing this project.

We express our gratitude to our project guide **Mr.K. Saravanan**, for his guidance throughout the project. We also thank all the faculty members of Textile Department for their cooperation and guidance in completion of this project.

We are grateful to **Mrs. Radhika** for initiating our project. We express our sincere thanks to **Dr. Saraswathi,** Department of Bio-technology, KUMARAGURU COLLEGE OF TECHNOLOGY for her kind cooperation.

We are also grateful to all teaching and non-teaching staff of Textile Department and our friends who have helped us to complete this project.

We thank our parents who encouraged us physically and mentally for the completion of this project. We dedicate this work to our beloved parents. Finally we thank all those who have helped us directly or indirectly in completion of this project.

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1.ABSTRACT

With the increase in social awareness for the environment there is a greater need to develop nature friendly finishing methods.

Nature is found to be rich with flora and fauna with each of having different characteristics. Discovering the entire species is never been possible as their number are huge. But some known species found to have significant characteristics. In our such case is lichen.

Lichen which is a combination of algae and fungi has found to have distinct characteristics such as anti-microbial and wound healing property.

We choose Xanthoparmelia caperata for our project which was found to have anti-microbial activity. Lichen was collected and crushed into powder. Using 5 types of solutions we extracted the liquid from lichen, in which only two of it found to have more anti-microbial activity.

Then the bandage was treated with the extract. The treated bandage was used over rat for wound healing property at KMCH Pharmacy.

It was found to be highly cost effective and highly efficient. More over the scope of this project not only stops here but also can replace many wound healing chemicals. Therefore, it is a good inclusion in medical textiles.

2.INTRODUCTION

Lichens are unusual creatures. Lichen is not a single organism the way most other living things are, but rather it is a combination of two organisms which live together intimately .Most of the lichen is composed of fungal filaments, but living among the filaments are algal cells, usually from a green alga or a cyanobacterium, that is the Lichens are fungi that live in a symbiotic association with a green alga or a cyanobacterium or both to fulfill its nutrional requirements. In the association the fungal partner is known as mycobiont and the green alga or cyanobacterium is known as the photosynthetic partner or the photobiant. The estimated number of lichen species may range between 15,000 and 20,000 species worldwide. In India, around 2000-2200 species are known to occur and in Tamilnadu a total of lichen species have been reported. Lichens and lichen products have been used in traditional centuries. Lichens are useful as food, medicine, for making dyes, in perfume manufacture and also decorations. Important antibiotics such as Penicillin are of course derived from fungi. Lichens are another type of organism that may hold the potential for medical exploration. Shyam Kumar et.al [2] reported for the first time the presence of antibiotic substances in lichens. After that many lichens are also screened for antibacterial activity. Screening of lichen extracts has revealed the frequent occurrence of these metabolites with antibiotic, antimycobacterial, antiviral, antitumor properties. Lichen produce protective secondary metabolites that serve to deter herbivore and colonization by pathogens. Usnic acid, stictic acid and vulpinic acid are a few of the 700 plus secondary compounds that are produced by lichens. Researchers found that pure extracts of usnic acid, evernic acid and vulpinic acid inhibited the growth of gram positive bacteria like staphylococcus aureus, Bacillus subtillis, Bacillus coli and Pseudomonas aeroginosa.

A wound is a type of injury in which skin is torn, cut or punctured (an *open* wound), or where blunt force trauma causes a contusion (a *closed* wound). In pathology, it specifically refers to a sharp injury which damages the dermis of the skin. In this project we first find out whether the lichen extract is having the antimicrobial property. If it is present then the lichen extract is applied on the different absorbent pads of the bandage. Therefore the present study is to examine the wound healing property of the lichen treated bandage.

2.1 OBJECTIVE:

- Collect the extract from selected lichen (Xanthoparmelia caperata).
- Find out whether the extract is having the antimicrobial property.
- Then the extract is applied on the absorbent pad of the bandage.
- Compare the wound healing property of the lichen treated bandage with the ordinary commercial bandage.

3. LITERATURE REVIEW

3.1 LICHENS

Lichens are unusual creatures. Lichen is not a single organism the way most other living things are, but rather it is a combination of two organisms which live together intimately. Most of the lichen is composed of fungal filaments, but living among the filaments are algal cells, usually from a green alga. Lichens colonize some of the most inhospitable habitats on earth. They can survive in extremely cold areas such as on high mountains and in regions such as the arctic. They may be virtually the only plant form surviving in some of these areas and can be vitally important sources of food for animals. They are also found throughout less extreme climates, inhabiting just about any solid surface. This can range from rocks on sea shores, to walls, trees and concrete. A few are unattached and blow about freely.

3.1.1 CHARACTERISTICS OF LICHEN:

- Lichens are very slow growing organisms – many grow only 1-2 mm/year.
- Lichens are long living organisms - some are thought to be over 1000 years old.
- Lichens lack protective, conductive and assimilatory tissues.
- Lichens are poikilohydric - cannot self-maintain water balance as in higher plants.
- Lichens reproduce by sexual and asexual means. Sexual reproduction of lichens involves the reproduction of the fungal partner.
- Lichenised fungi produce unique and abundant secondary chemical metabolites which are considered to protect the lichen thallus from excess light, drought, insect herb ivory and microbial attack.
- Some lichen compounds are used in pharmaceuticals and cosmetics.
- Lichens are sensitive to air pollutants, primarily sulfur dioxide and heavy metals.

3.1.2 USES OF LICHENS:

Lichens are useful as food, medicine, for making dyes, in perfume manufacture, as decorations and in science. In Japan *Umbilicaria esculenta* is considered a delicacy where it is

eaten as a soup or in salads. Other *Umbilicaria* species are, or have been, eaten in other countries. Some people believe that *Lecanora esculenta* was the original Biblical Manna as it has the habit of coming loose from its substrate (what it was growing on) and being blown around in the wind.

In the northern tundra Reindeer and Caribou eat loads of lichens, mostly species of *Cladonia* and *Cetraria*. Lichens can make up half the food these animals consume during the winter when they are dug up from below the snow by the hungry animals. Eskimos and Lapps both harvest and store these lichens as part of the winter feed for their animals.

Nutritionally, lichens contain practically no fat and only 1-5% protein. They are basically carbohydrate. Deer have a special enzyme called 'lichenase' which helps them to digest the lichens that they eat.

Medicinally Lichens have probably been used by many early civilisations. In Europe records from around the 15th century suggest that by then several lichens were in regular medicinal usage. For example, *Usnea florida* was used for hair problems, *Xanthoria parietina* for jaundice and *Pettigera canina* as a cure for rabies. In some northern places *Cetraria islandica* is still used as a cough remedy. On a more scientific basis, Usnic acid is a known antibiotic and has recently been developed into a salve in Germany. Research is still active into the use of several other lichen products as anti-viral and anti-fungal agents.

3.1.3 LICHENS HAVING ANTIMICROBIAL ACTIVITY:

Roccella belangeriana were extracted from different solvents like Acetone, methanol, diethylether, ethanol, ethyl acetate, petroleum ether, chloroform and aqueous extracts against 12 bacterial strains. The aqueous extract showed no activity against *Pseudomonas aeruginosa, Staphylococcus* sp., *Salmonella* sp. and *Shewanella* sp. The trace activity was recorded against *Escherichia coli, Vibrio flurialis* and *Proteus* sp. The minimum activity was noted against *Streptococcus* sp. (5 mm) followed by *Vibrio splendidus* (6 mm), *Enterococci* sp. (7 mm) and *Vibrio parahaemolyticus* (7 mm). The maximum activity was found against *Klebsiella pneumoniae* (12 mm). The extracts by way of methanol observed no activity *E.coli,*

Streptococcus sp. and *Vibrio parahaemolyticus*. The trace activity was noted against *Klebsiella pneumoniae* (2 mm), *Vibrio flurialis* (2 mm) and *Enterococci* sp. (3 mm), *Shewanella* sp. (3 mm).

The minimum activity was recorded against *Pseudomonas aeruginosa* (4 mm), *Salmonella* sp. (4 mm), *Vibrio splendidus* (4 mm). The maximum activity was observed against *Staphylococcus* sp. (23 mm) and *Proteus* sp. (22 mm). Ethyl acetate extract showed no activity against *Streptococcus* sp., *Pseudomonas aeruginosa, Vibrio parahaemolyticus* and *Vibrio splendidus*. The trace activity was noted against *Klebsiella pneumoniae* (2 mm), *Enterococci* sp. (2 mm), *Salmonella* sp. (2 mm), *Shewanella* sp. (2 mm) and *Proteus* sp. (4 mm). The minimum activity was recorded against *Vibrio flurialis* (5mm) and *E.coli* (8 mm). The maximum activity was observed against *Staphylococcus* sp. (16 mm). The chloroform extract showed no activity against *Vibrio splendidus*. The trace activity was observed against *E.coli, Pseudomonas aeruginosa, Vibrio parahaemolyticus* (3mm) and *Staphylococcus* sp., *Streptococcus* sp., *Vibrio Splendidus* (4 mm). The minimum activity was recorded against *Proteus* sp., *Shewanella* sp. (6 mm). The maximum activity was observed against *Enterococci* sp. (29 mm). The ethanol extract showed no activity against *Klebsiella pneumoniae, Streptococcus* sp. And *Pseudomonas aeruginosa, Vibrio parahaemolyticus* and *Salmonella* sp. The trace activity was observed against *Staphylococcus* sp., (2 mm) and *Enterococci* sp., *Shewanella* sp. and *Vibrio flurialis* (3 mm). The minimum activity was recorded against *E.coli* (4 mm). The maximum activity was observed against *Proteus* sp. (18 mm). The diethyl ether extract showed no activity against *Klebsiella pneumoniae, Proteus* sp., *Streptococcus* sp., *Pseudomonas aeruginosa, Vibrio parahaemolyticus* and *Vibrio flurialis*. The trace activity was observed against *Enterococci* sp., *Vibrio Splendidus*, (3mm) and *Staphylococcus* sp., (2mm). The minimum activity was recorded against *Salmonella* sp. (10mm). The maximum activity was observed against *Staphylococcus* sp., (12mm). The petroleum ether extract showed no activity against *Staphylococcus* sp., *Enterococci* sp., *Proteus* sp., *Pseudomonas aeruginosa, Vibrio parahaemolyticus, Vibrio flurialis* and *Vibrio splendidus*. The trace activity was observed against *Klebsiella pneumoniae, Shewanella* sp. (2 mm) and *Streptococcus* sp.,(3 mm). The minimum activity was recorded against *Salmonella* sp. (9mm). The maximum activity was observed against *E.coli* (13mm).[1]

Lichen *P. nilgherrense* belongs to the family Parmeliaceae, was collected from tree bark in the open places and it is a commonly growing species of the locality. Different of five bacteria (gram +ve and -ve) were used in this investigation. Microorganisms (*Bacillus subtilis* MTCC No. 121, *Escherichia coli* MTCC No.40,*Agrobacterium tumefaciens* MTCC No.609). Agar-well diffusion assays of *P. nilgherrense* indicate that crude chloroform, ethanol and methanol extracts have good antibacterial activity against all pathogens tested, while crude aqueous extract exhibited no activity. The chloroform extract showed highest antibacterial activity against all the bacterial strains (*Bacillus subtilis, Erwinia chrysanthemi, Escherichia coli,Agrobacterium tumefaciens* and *Xanthomonas phaseoli*) and it was close to the inhibition zone of commercially available antibiotic drug streptomycin. Ethanol and methanol extract of this lichen showed moderate activity against *Bacillus subtilis, Agrobacterium tumefaciens* and*Xanthomonas phaseoli* while both extracts showed maximum activity against Erwinia chrysanthemi. As aqueous extract were completely inactive against all the pathogenic bacteria tested.[3]

The antimicrobial activity of the acetone, diethyl ether and ethanol extracts of the lichen *Cetraria aculeata* has been investigated. The extracts were tested against twelve bacteria and eight fungi and found active against *Escherichia coli, Staphylococcus aureus, Aeromonas hydrophila, Proteus vulgaris, Streptococcus faecalis, Bacillus cereus, Bacillus subtilis, Pseudomonas aeruginosa, Listeria monocytogenes.* The acetone extract of *C. aculeata* showed the highest inhibitory activity among the other extracts against the bacteria of which their growth is inhibited. The ethanol and diethyl ether extracts were slightly less active compared to that of the acetone extract. For the acetone extract, the antibacterial was detected at a concentration as low as 212 μg/ml (against 107 *A. hydrophila* cells). . In addition they observed bacteriocidal activity against *P. vulgaris, S. feacalis, B. cereus, B. subtilis, L. monocytogenes* and the activity was bacteriostatic against *E. coli, S. aureus, A. hydrophila, P. aeruginosa.*[4]

The antimicrobial interaction between methanol extract of lichen (*Ramalina farinacea* (L) Ach (fam: *Ramalinacea,* a fruticose lichen) and tetracycline was investigated. The combination of methanol extract of lichen *R. farinacea* and tetracycline is hoped to achieve a desirable synergistic effect in order to increase the antibiotic spectrum of tetracycline. Combined drug use is occasionally recommended to prevent resistance emerging during treatment and to achieve higher efficacy in the treatment of infectious diseases.

The results of the interaction studies carried out on the methanol extract and tetracycline against *S. aureus* . This study go a long way to show that combination therapy with these commonly used antibiotic (tetracycline) and lichen methanol extract can possibly improve survival and treatment outcome in some seriously debilitated patients who are afflicted with life threatening *S. aureus* infections.[5].

Lichen samples of. *Cladonia furcata* (Hudson) Schrad., *Lecanora atra* (Hudson) Ach. and *Lecanora muralis* (Schreber) Rabenh., were collected from Kopaonik, Serbia. *Lecanora atra* had largest free radical scavenging activity (94.7% inhibition), which was greater than the standard antioxidants. Moreover, the tested extracts had effective reducing power and superoxide anion radical scavenging. The strong relationships between total phenolic and flavonoid contents and the antioxidant effect of tested extracts were observed. Extract of *Cladonia furcata* was the most active antimicrobial agent with minimum inhibitory concentration values ranging from 0.78 to 25 mg/mL. All extracts were found to be strong anticancer activity toward both cell lines with IC_{50} values ranging from 8.51 to 40.22μg/mL. [6].

The phytochemical analysis of methanol and chloroform extracts of *Umbilicaria cylindrica* was determined by HPLC-UV method. The predominant phenolic compound in both extracts was depsidone, salazinic acid. The methanol and chloroform extracts of the lichen showed significant antioxidant and antimicrobial activity in different assays *in vitro*. Two monocyclic aromatic compounds, two depsidones, one depside, and usnic acid were identified, and salazinic acid was the dominant phenolic compound in the lichen. Although these compounds have already been reported for some other lichen species, this is the first report for *U. cylindrica*. The present study provides data for supporting the use of *U. cylindrica* extracts as natural antimicrobial and antioxidant agents and confirms that these extracts represent a significant source of phenolic compounds. Future investigation will be focused on isolation of phenolic compounds and determination of their biological activities *in vitro* and *in vivo*. Since this lichen has a bigger content of the phenol components which were proven to have numerous biological effects including the antioxidant activity, they can be of a big importance in the food industry, given the fact that they keep the oxidative processes, that way improving the quality and its nutritional value, so they can be used as additives.[7]

The extracts obtained showed the presence of volatile oil, saponins, coumarins and quinines, flavonic glycosides and carotenoids. The ethyl acetate fraction of *E. nepalense* and *U. longifolia* were found to be most effective against all the 8 clinical bacterial pathogens and 5 phytopathogenic fungi tested. The extracts of *Cetraria* spp and *P. milghenensis* were found to be specifically inhibiting the fungal pathogens compared to the bacterial pathogens. Generally the lichen extracts tested demonstrated antimicrobial effect which suggests a possibility of their use in treatment of various diseases caused by these and similar microorganisms.[8].

The emergence of new infectious diseases, the resurgence of several infections that appeared to have been controlled and the increase in bacterial resistance have created the necessity for studies directed towards the development of new antimicrobials. Considering the failure to acquire new molecules with antimicrobial properties from microorganisms, the optimization for screening methods used for the identification of antimicrobials from other natural sources is of great importance.[14] To evaluate technical variants used in screening methods to determine antibacterial activity of natural products. Thus, a varied range of natural products of plant, fungi and lichen origin were tested against two bacterial species, *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922, by two variants of the agar diffusion method (well and disc), two variants of the bioautographic method (direct and indirect) and by microdilution assay. We concluded that the well-variant of the diffusion method was more sensitive than the disc-variant, whilst the direct-variant of the bioautographic method exhibited a greater sensitivity if compared to indirectvariant. Bioautographic and diffusion techniques were found to have similar sensitivity; however the latter technique provided more suitable conditions for the microbial growth. In this study, we also discussed the best conditions for the determination of minimal inhibitory concentration.[15]

3.2 WOUNDS:

Wound is a type of injury in which skin is torn, cut or punctured or where blunt force trauma causes a contusion . Wound includes cuts, scrapes, scratches and punctured skin. They often occur as a result of an accident or injury, but surgical incisions, sutures, and stitches also cause wounds. In pathoiogy, it specifically refers to a sharp injury which damages the dermis of the skin. Minor wounds usually aren't serious, but even cuts and scrapes require care.

3.2.1 TYPES OF WOUND:

- an open wound
- a closed wound

AN OPEN WOUND:

Superficial incision wounds from the claws of a cat. Open wounds can be classified according to the object that caused the wound. The types of open wound are:

- **Incisions** or **incised wounds**, caused by a clean, sharp-edged object such as a knife, a razor or a glass splinter.
- **Lacerations**, irregular tear-like wounds caused by some blunt trauma. Lacerations and incisions may appear linear (regular) or stellate (irregular). The term laceration is commonly misused in reference to incisions.
- **Abrasions** (grazes), superficial wounds in which the topmost layer of the skin (the epidermis) is scraped off. Abrasions are often caused by a sliding fall onto a rough surface.
- **Puncture wounds**, caused by an object puncturing the skin, such as a nail or needle.
- **Penetration wounds**, caused by an object such as a knife entering and coming out from the skin .
- **Gunshot wounds**, caused by a bullet or similar projectile driving into or through the body. There may be two wounds, one at the site of entry and one at the site of exit. generally referred to as a "through-and-through."

CLOSED WOUND:

Closed wounds have fewer categories, but are just as dangerous as open wounds. The types of closed wounds are:

- **Contusions**, more commonly known as bruises, caused by a blunt force trauma that damages tissue under the skin.
- **Hematomas**, also called a blood tumor, caused by damage to a blood vessel that in turn causes blood to collect under the skin.
- **Crush injury**, caused by a great or extreme amount of force applied over a long period of time.
- **Chronic and Acute** Acute or traumatic wounds are the result of injuries that disrupt the tissue. Chronic wounds are those that are caused by a relatively slow process that leads to tissue damage. Chronic wounds include pressure, venous, and diabetic ulcers. Typically, an insufficiency in the circulation or other systemic support of the tissue causes it to fail and disintegrate. Infection then takes hold of the site and becomes a chronic abscess. Once the infection hits a critical point, it can spread locally or become systemic (sepsis).[16].

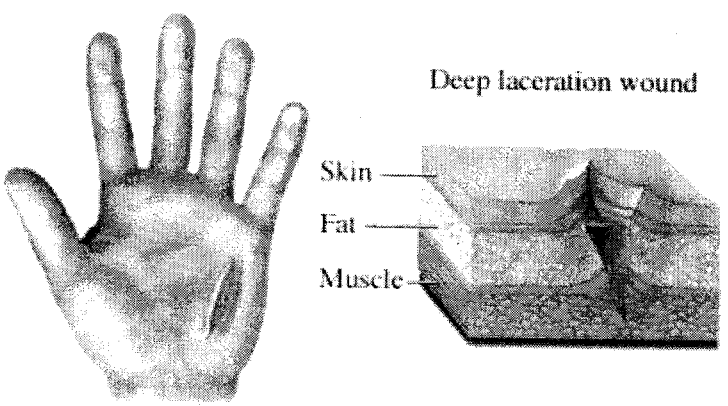


Figure 1: Wound

3.2.2 HEALING OF WOUND:

Wounds heal in three stages.

- **Inflammation** is when blood clots form, bacteria are attacked to prevent infection, and key biochemical cells gather at the site of the wound, causing it to swell. Inflammation begins almost immediately after injury, peaking at three to five days.
- Then there is **proliferation**, when these key cells multiply at the wound site to make new tissue and blood vessels. Open wounds generally heal from the bottom up, as cells multiply to fill in the wound with new tissue.
- Finally, there is the **remodeling phase**, where the wound is healed and the initial scar tissue is gradually restructured.

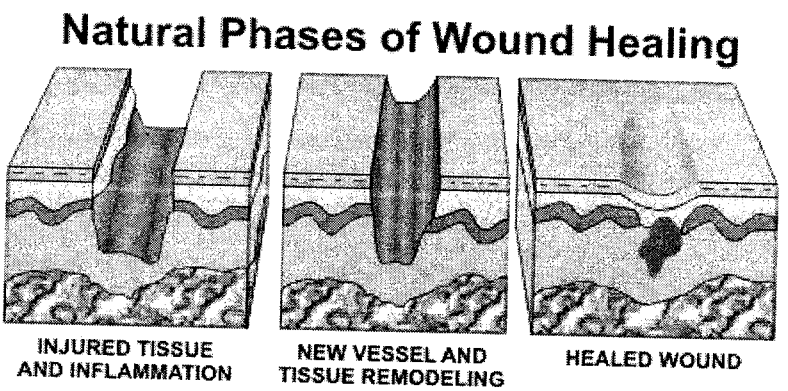


Figure: 2 Phases of wound healing

There are many different types of cells involved in the healing process, including platelets, macrophages and fibroblasts. Platelets are the first to arrive at the wound site and release growth factors: proteins that allow cells to communicate with each other. The growth factors are essential to the healing process. They attract other useful cells and proteins to the wound site, including immune cells to ward off infection, and stimulate and increase the production of connective tissue. They also create a new supply of blood vessels to nourish the wound site, and promote new skin growth across the open area of the wound.[17].

3.3 TYPES OF BACTERIA FOUND IN WOUND CULTURES:

A wound culture is designed to help identify if you're suffering from an infection and the type of bacteria that is causing your infection. Wound cultures may also be ordered to help your physician discern if treatment methods are helping fight your infection, or to help your physician administer additional treatments. There are various types of bacteria that can be responsible for the development of an infection.

STAPHYLOCOCCUS AUREUS:

Staphylococcus aureus is a type of bacteria with nearly 30 different species. Normally, staph lives upon your skin in areas that include your nose, mouth and genital region. But If your skin were to become punctured, staph bacteria could potentially enter your bloodstream through your wound. As result, you can develop a serious staph infection or even methicillin-resistant Staphylococcus aureus (MRSA). According to the Centers for Disease Control and Prevention, MRSA can develop other illnesses such as pneumonia and even result in death.

GROUP A STREPTOCOCCAL:

Streptococcus bacteria is commonly found upon your skin and in your throat. Strep is responsible for causing strep throat. According to the Centers for Disease Control and Prevention, in some instances strep infections can be "life-threatening." Strep bacteria that comes into contact with wounds can result in serious health complications that comprise of toxic shock syndrome (blood pressure loss and organ failure) and necrotizing fasciitis (tissue, skin and muscle death).

ABOUT STAPH INFECTIONS:

Staph infections are caused by the bacteria *Staphylococcus aureus*, which many healthy people carry on their skin and in their noses without getting sick.

But when skin is punctured or broken, staph bacteria can enter the wound and cause infections, which can lead to other health problems.

We can help prevent staph infections in your family by encouraging regular hand washing and daily bathing, and by keeping areas that have been cut clean or covered.

HOW STAPH INFECTIONS SPREAD

Staph bacteria can spread through contaminated surfaces and from person to person. Kids can carry staph bacteria from one area of their body to another or pass it to other people via dirty hands or fingernails. So good hand washing is vital to preventing staph infections.

It's also important to encourage kids to keep their skin clean with a daily bath or shower. If your child has a skin condition such aseczema that makes frequent bathing difficult, ask your doctor for advice.

Keep areas of skin that have been injured such as cuts, scrapes, and rashes caused by allergic reactions or poison ivy clean and covered, and follow any directions given by your doctor.

COMPLICATIONS OF STAPH INFECTIONS

Staph bacteria can cause toxic shock syndrome, cellulitis, staph food poisoning, and these infections:

FOLLICULITIS AND BOILS

Folliculitis is an infection of hair follicles, tiny pockets under the skin where hair shafts (strands) grow. In folliculitis, tiny white-headed pimples appear at the base of hair shafts, sometimes with a small red area around each pimple. This infection often occurs in areas where there's been friction or irritation, such as with shaving.

Folliculitis often clears up on its own with good skin hygiene. Sometimes, it can progress to become a **furuncle**, or a boil. With a boil, the staph infection spreads deeper and wider, often affecting the skin's **subcutaneous** tissue (deeper tissue under the skin) and the oil-producing glands, which are called **sebaceous** glands.

In the first stage, which parents and kids often miss, the area of skin either begins to itch or becomes mildly painful. Next, the skin turns red and begins to swell over the infected area.

Finally, the skin above the infection becomes very tender and a whitish "head" may appear. The head may break, and the boil may begin to drain pus, blood, or an amber-colored liquid. Boils can occur anywhere on the skin, especially under the arms or on the groin or buttocks in kids.

To help relieve pain from a boil, try warm-water soaks, a heating pad, or a hot-water bottle applied to the skin for about 20 minutes, three or four times a day. Make sure that the washcloths used for the soaks are washed after each use. Boils are occasionally treated with oral antibiotics and in some cases need to be surgically drained.

IMPETIGO

Impetigo can affect skin anywhere on the body but commonly occurs around the nose and mouth. It usually affects preschoolers and school-age kids, especially in the summer months.

Impetigo caused by staph bacteria is characterized by large blisters containing fluid that is first clearing, then cloudy. The blisters may burst, ooze fluid, and develop a honey-colored crust. Impetigo may itch and can be spread by scratching.

Doctors usually prescribe a topical ointment to treat it and may, depending on the severity, add oral antibiotics.

MRSA

You may have heard about methicillin-resistant *Staphylococcus aureus* (MRSA), a type of staph bacteria with a resistance to the antibiotics usually used to treat staph infections. Although MRSA infections can be harder to treat, in most cases they heal with proper care.

Most MRSA infections involve the skin, but sometimes MRSA can cause more serious problems, such as bone infections or pneumonia. MRSA pneumonia is rare, but is more of a risk for kids already sick with the flu.

SCALDED SKIN SYNDROME

Scalded skin syndrome (SSS) most often affects newborns and kids under age 5. The illness usually starts with a localized staph skin infection, but the staph bacteria manufacture a toxin that affects skin all over the body. The child has a fever, rash, and sometimes blisters. As blisters burst and the rash passes, the top layer of skin is dislodged and the skin surface becomes red and raw, like a burn.

SSS is a serious illness that needs to be treated and monitored in a hospital. It affects the body in the same way as serious burns. After treatment, most kids make a full recovery.

TREATING STAPH INFECTIONS

Most localized staph skin infections can be treated by washing the skin with an antibacterial cleanser, warm soaks, applying an antibiotic ointment prescribed by a doctor, and covering the skin with a clean dressing. To keep the infection from spreading, use a towel only once when you soak or clean an area of infected skin, and then wash it.

Our doctor may prescribe an oral antibiotic for your child's staph skin infection. If so, give the antibiotic on schedule for as many days as the doctor directs. More serious staph infections may require hospitalization.[18]

4. MATERIALS AND METHODS

4.1 COLLECTION OF LICHEN MATERIALS:

The lichen thalli of *Xanthoparmelia caperata* [18]were collected from the tree barks at Ooty forest (TamilNadu, India).

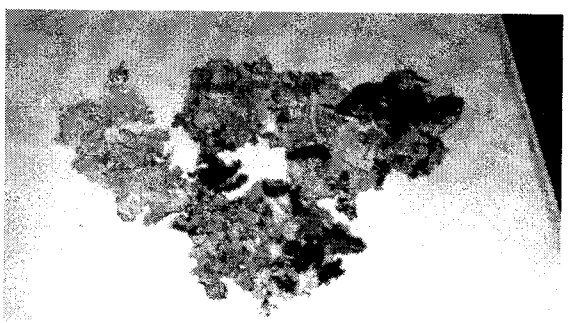


Figure: 3 Xanthoparmelia caperata

4.2 EXTRACTION PROCEDURE:

The lichen thalli were thoroughly washed, spread on paper sheet and dried at room temperature (20±2°C) in the lab. The dried material was powdered in an electric grinder.

Figure: 4 Xanthoparmelia caperata in powder form

To prepare stock solution 10 g of this powder were taken and added to 100 ml of solvents of petroleum ether, ethanol, chloroform, diethyl ether and acetone (w/v, 10g/100ml) separately in a conical flask and leave it for two days. After that each extract was passed through filter paper and the final filtrate is allowed to dry in a room temperature ant thus the crude extract 10 % thus obtained was utilized for the antimicrobial test.

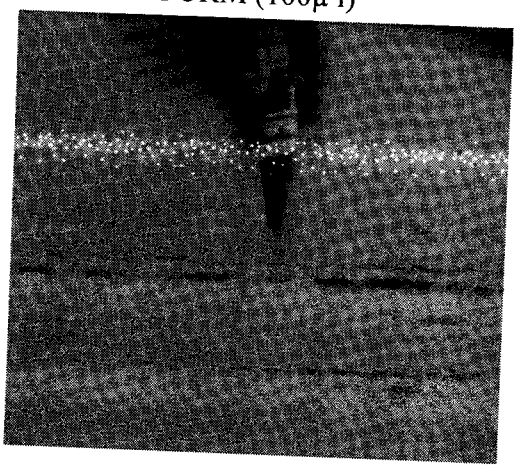
4.3 TEST ORGANISMS:

Two types of Bacteria both gram positive and gram negative bacteria are used for screening of antimicrobial property. We took E.coli and Staphylococcus aureus for accessing antibacterial activity for our lichen extract.

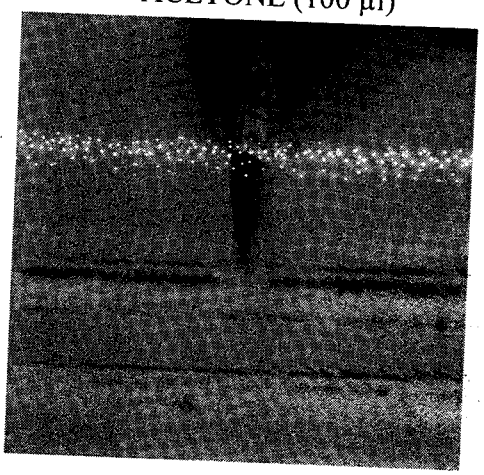
4.4 SCREENING OF ANTIMICROBIAL ACTIVITY:

Antibacterial test of selected microorganism were carried out using Agar well diffusion method (Perez et al. 1990). Nutrient agar media was poured into the Petri plates. A small sterile cotton swab was dipped into the 24 hour old culture of bacteria. Then the dried surfaces of plates were inoculated by streaking the swab over the entire sterile agar surface. This process is repeated by streaking the swab 2 or more times rotating the plates approximately 60° each time to ensure even distribution of inoculums. After inoculation the plates were allowed to dry at room temperature (20±2°C) for 15minutes in laminar chamber for absorption to take place. Wells were made in agar plates (1cm diameter) and 200 µl of the extract was added into each well. For the control we use the particular chemicals used in the extract. The plates were incubated at 37°C in dark and observed for inhibition zone (a circular zone formed due to killing effects of extracts,) after 24 hours. The experiments were carried out in triplicate. The diameter of the inhibition zones were measured in millimeter (including well size 1cm).

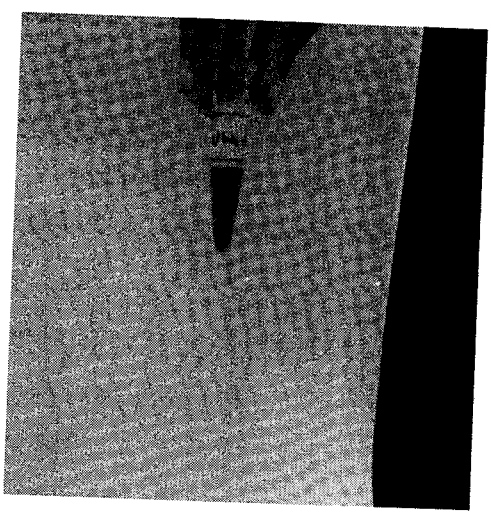
CHLOROFORM (100µ l)



ACETONE (100 µl)



ETHANOL(100µ l)



DIETHYL ETHER(100µ l)

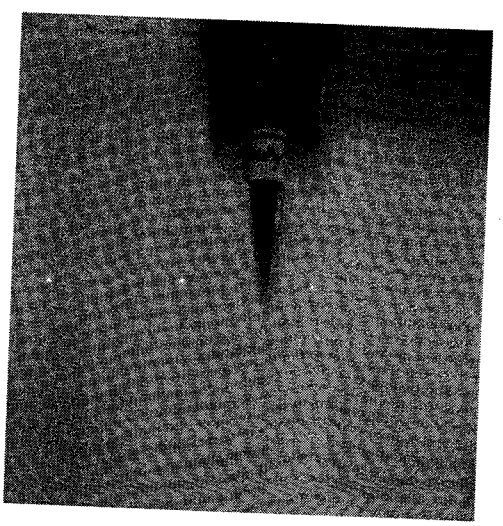


Figure 5 : Lichen extracts

4.5 APPLICATION OF EXTRACT ON BANDAGE CLOTH

The purpose of the bandage is to heal the wound. We are preparing a bandage to heal the cut wound. We have found that s.choccus bacteria develops the wound. In order to find the best healing method we have used 5 chemicals and the results prove that, the antimicrobial property of lichen is good in chloroform and acetone extraction than diethyl ether , petroleum ether, ethanol. So we choose acetone and chloroform for bandage preparation. The extracted liquid is applied on the normal cotton bandage.

Recipe :

- M:L ratio - 1:20
- Liquor - Acetone and chloroform
- Method - Dip method
- Time - 1 hour
- pH Range - 6
- Temperature - Room temperature

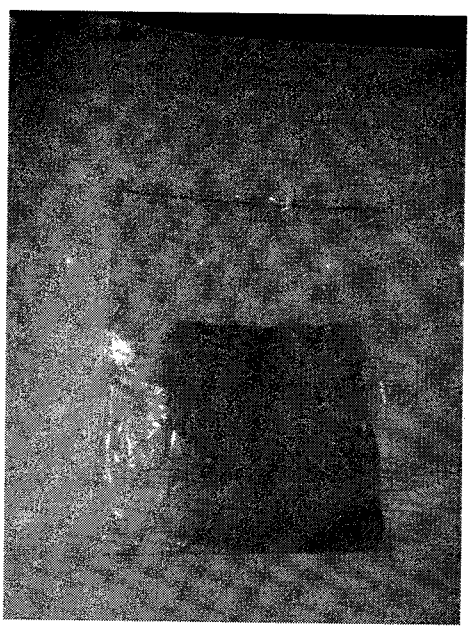


Figure 6: Acetone extract

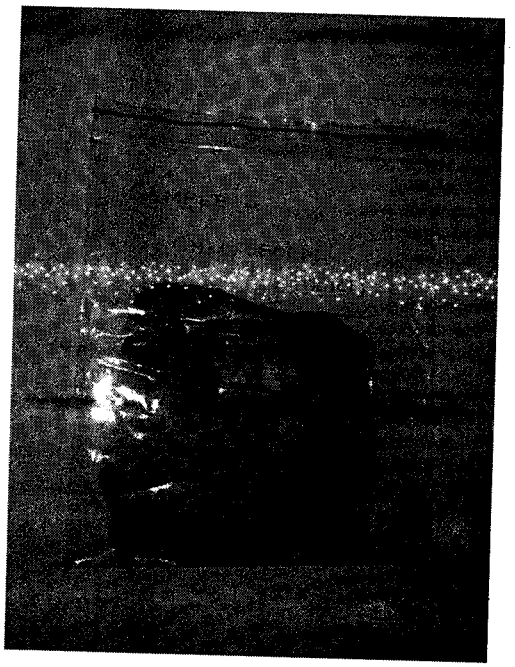


Figure 7 : Chloroform extract

4.6 ANIMAL TESTING

Animal research has provided valuable information about many physiological processes that are relevant to humans and has been fundamental in the development of many drugs, including vaccines, anesthetics, and antibiotics. Animals and humans are similar in many ways. Animal behavior can be as complex as human behavior, and the cellular structures, proteins, and genes of humans and animals are so similar that the prospect of using animal tissues to replace diseased human tissues is under intense investigation for patients who would otherwise never receive a potentially life-saving transplant.

However, the way in which animals and humans react to their environments, both physiologically and behaviorally, can be drastically different, and the conditions under which laboratory animals are kept can influence and alter experimental results. The husbandry and treatment of laboratory animals has been and continues to be a major topic of ethical debate. While animal testing is not always the most efficient way to test the toxicity of a chemical or the efficacy of a pharmaceutical compound, it is sometimes the only way to obtain information about

how a substance behaves in a whole organism, especially in the case of pharmaceutical compounds. The testing of pharmaceuticals is aimed at determining whether a compound is able to produce a desired effect, such as killing cancer cells. Studies of pharmacokinetic effects (effects of the body on a drug) and pharmacodynamic effects (effects of a drug on the body) often require testing in animals to determine the most effective way to administer a drug. How a drug behaves in the body is largely determined by its chemical properties, such as size, chemical constituents, and solubility. While the results of in vitro experiments on human cells are sometimes applicable to determining the expected outcomes of animal studies, there are often unexpected effects in animals, and whether these effects will be relevant to humans remains uncertain until clinical trials in human subjects have been performed. . Even though public concern for the welfare of laboratory animals is greater than it used to be, most people still think that it would be better for an experimental drug to kill a few animals than for it to kill a few humans. While it is difficult to measure the intrinsic value of alternatives to animal testing, the message that pursuing and investing in these technologies sends is positive and should encourage and inspire innovative thought and research.

4.7 EXCISION WOUNDS

Excision of wounds was made as described by Morton and Malone. Animals were anaesthetized with anaesthetic ether and placed in operation table in its natural position . A square wound of about 1.5cm (width) x 0.2cm (depth) was made on depilated ethanol-sterilized dorsal thoracic region of rats. Made infection on wound by staphylococci aureus and separate the animals in to groups . Male albino Wistar rats weighing 110 to 150g were divided in to four groups of 6 rats each, Group I animals were considered as the control; Grouped II animals were served as the standard and were treated with cipladin ointment; Group III animals were treated with 500mg/kg body weight of chloroform extract ; and Group IV animals were treated with 500mg/kg body weight of ethanolic extract . Cipladin and extracts were topically applied once a day, till the epithelialization was complete. The wound contraction was studied by tracing the raw wound area subsequently on day 4, 7, 14, 21 on graph paper. Scar residue, area and time of complete epitheliasation were also measured. The percentage of wound closure and period of epithelialisation were recorded.

5. RESULT AND DISCUSSION

5.1 RESULT OF ANTIMICROBIAL ACTIVITY TEST:

- The inhibition zone in the *Staphylococcus aureus* is high in the chloroform and acetone extract.
- Antimicrobial testing in the *E. coli* is not up to the expected level.
- As *staphylococcus aureus* is one of the main cause for the cut wound infection, we planned to proceed our bandage preparation with chloroform and acetone extracts since lichen extracts has more antimicrobial activity on *s. aureus*.

Figure 8: Antimicrobial Test - Staphylococcus aureus

Figure 9: Antimicrobial Test – E.coli

Table 1: comparision of s.coccus and E.coli

Bacteria	Inhibition zone in cm (diameter)			
	Acetone	Chloroform	Di ethyl ether	Ethanol
S.coccus	1.2	1.6	0.4	0.7
E.coli	1	0.9	0.1	0.5

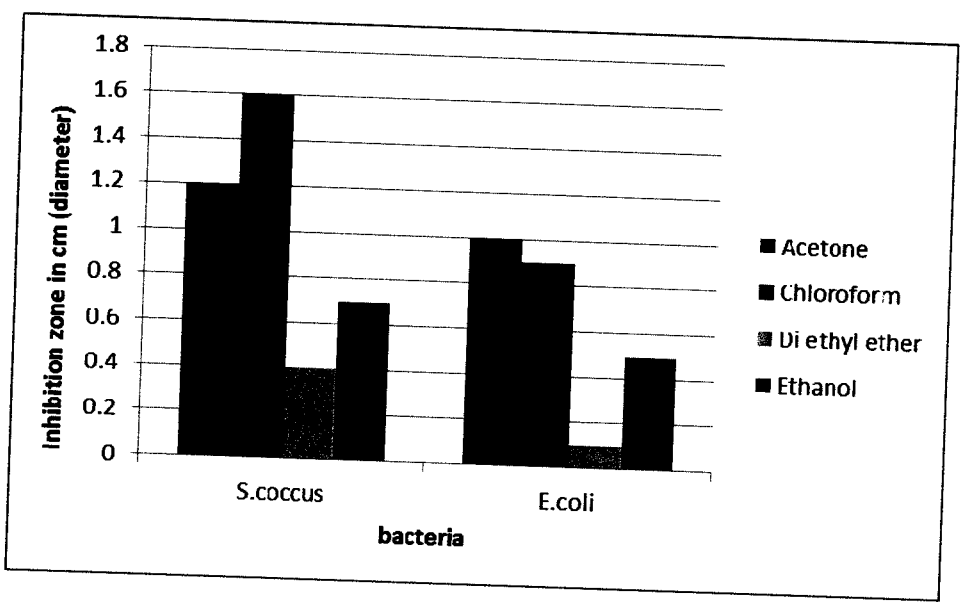


Figure 10: comparision of s.coccus and E.coli

The results obtained in the present study can be concluded that *Xanthoparmelia caperata* have broad spectrum of antibacterial potentiality. All the organic solvent extract of this lichen *viz.*, chloroform, ethanol and acetone possess significant inhibitory activity against the gram positive and gram negative bacteria. Hence, this lichen *Xanthoparmelia caperata* can be a potential source for evolving newer antibacterial compounds.

5.2 RAT TEST PHOTOGRAPH

Wound was created on the rat and it was covered with the treated bandage and kept for further observation.

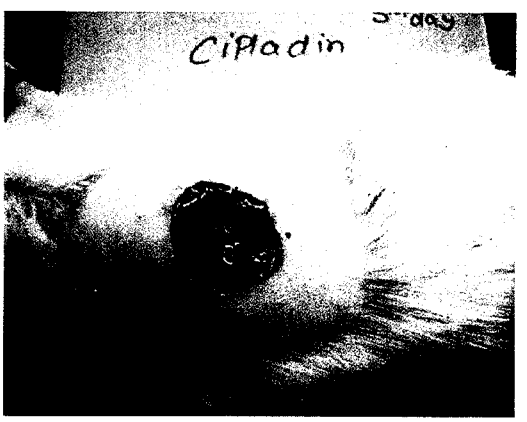
A-Group I – Normal control

B- GroupII – Cipladin

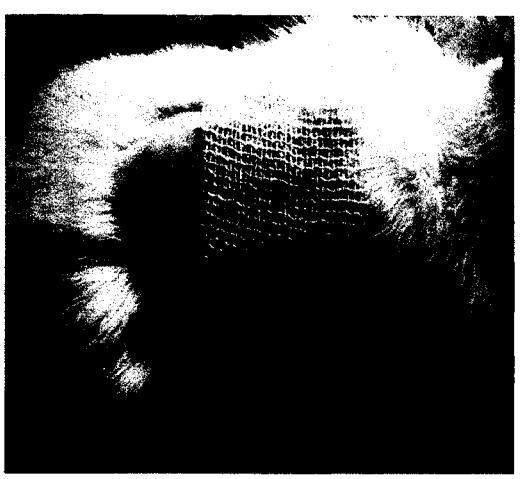
C-GroupIII – Chloroform extract

D-Group IV – Acetone extract

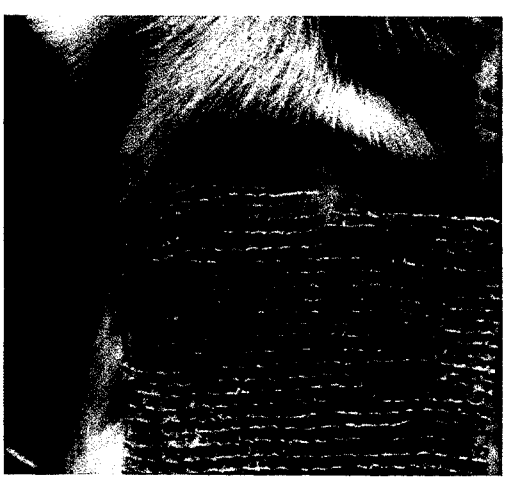
GROUP -A



GROUP-B



GROUP-C



GROUP-D

Figure 11 : Macroscopic observation of excision wounds on day- 0

On the 7th day under observation it was found that the recovery rate of the wound wrapped by the treated bandage was faster than the wound wrapped by the ordinary bandage.

A-Group I – Normal control

B- GroupII – Cipladin

C-GroupIII – Chloroform extract

D-Group IV – Acetone extract

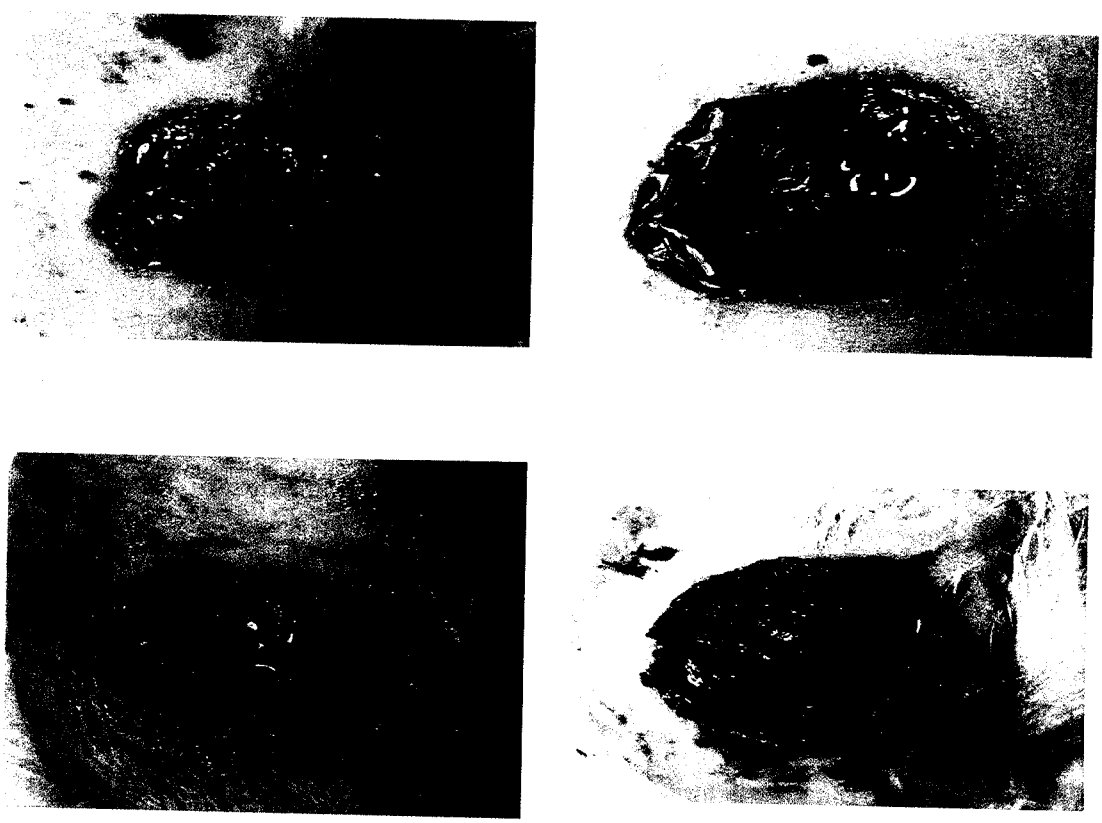


Figure 12 : Macroscopic observation of excision wounds on day- 7

On day 14 it was found that more than half of the wound which was covered by the treated bandage has been healed , whereas the wound covered by ordinary bandage healed in a lower rate.

A-Group I – Normal control

B- GroupII – Cipladin

C-GroupIII – Chloroform extract

D-Group IV – Acetone extract

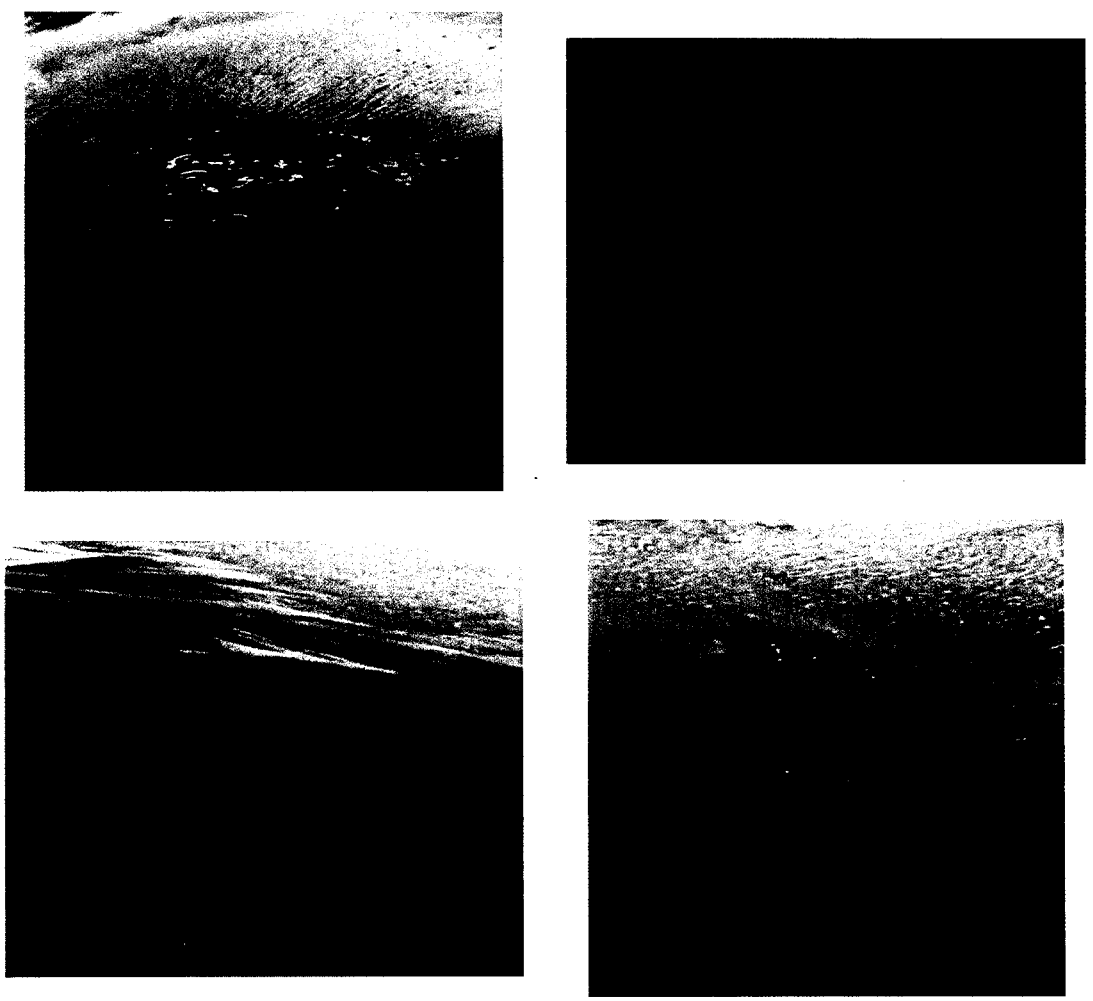


Figure 13 : Macroscopic observation of excision wounds on day- 14

On 21st day the wound which was wrapped by the treated fabric has healed completely , whereas only ¾ th of the wound was healed by the normal bandage. This behavior explains the quick recovery of wound which was due to the antimicrobial activity of Xanthoparmelia caperata.

A-Group I – Normal control

B- GroupII – Cipladin

C-GroupIII – Chloroform extract

D-Group IV – Acetone extract

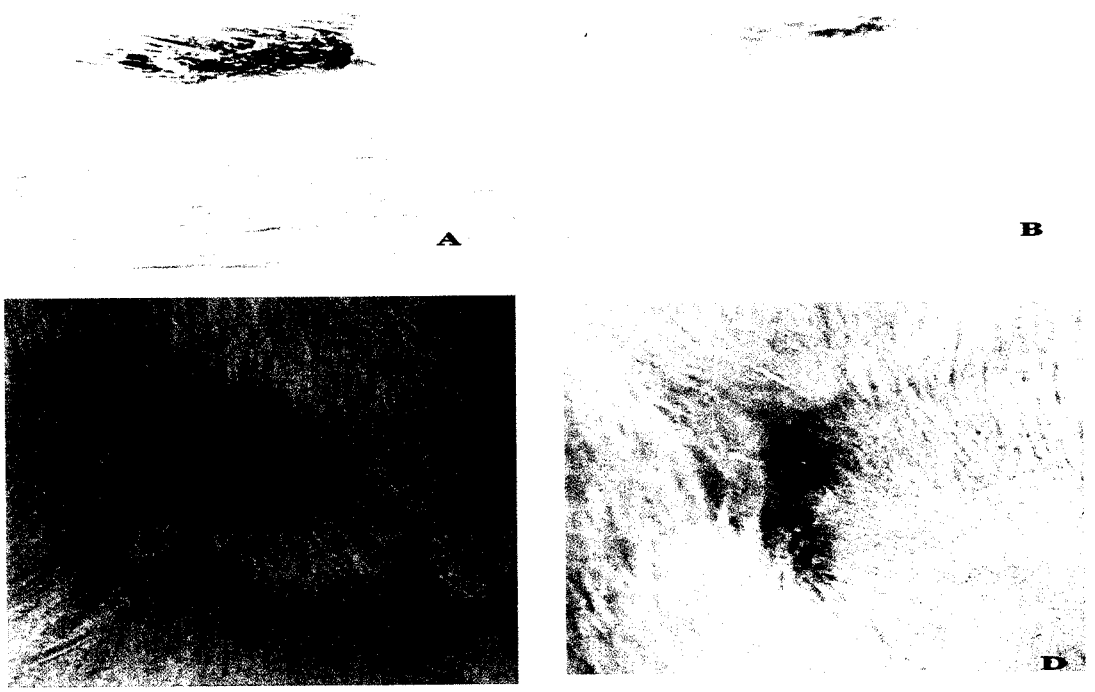


Figure 14 : Macroscopic observation of excision wounds on day- 21

5.2.1 ONLY WOUND:

Table 2: Cut wound size percentage (only wound)

No of days	Cut wound size percentage					
	Rat 1	Rat2	Rat3	Rat4	Rat5	Rat6
0 day	100	100	100	100	100	100
7th day	98.95	147.4	139.47	119.17	136.12	135.05
14th day	113.54	103.6	128.42	105.6	99.47	77.31
21st day	42.18	74.22	48.42	66.83	68.06	52.61

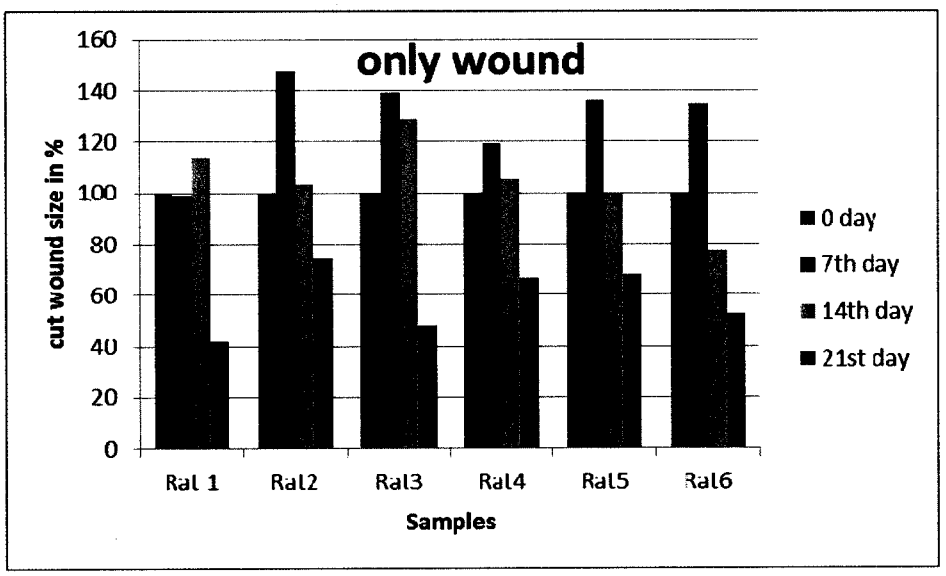


Figure 15: Graph for Cut wound size percentage (only wound)

Initially the wound was created on the rat .In general the wound get expanded after 7th day, which was same even in this case. But on 14th day, it was found that the wound has started to heal. Normally on the 21st day, the wound size got reduced with the range of 40-60%.

5.2.2WOUND + STANDARD:

Table 3: Cut wound size percentage (wound + standard)

No. of days	Cut wound size percentage					
	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Rat 6
0day	100	100	100	100	100	100
7 th day	96.31	89.94	90.9	128.2	59.27	56.99
14th day	32.63	15.8	40.4	28.86	21.6	19.6
21st day	7.6	4.2	10.1	5.6	0	7.2

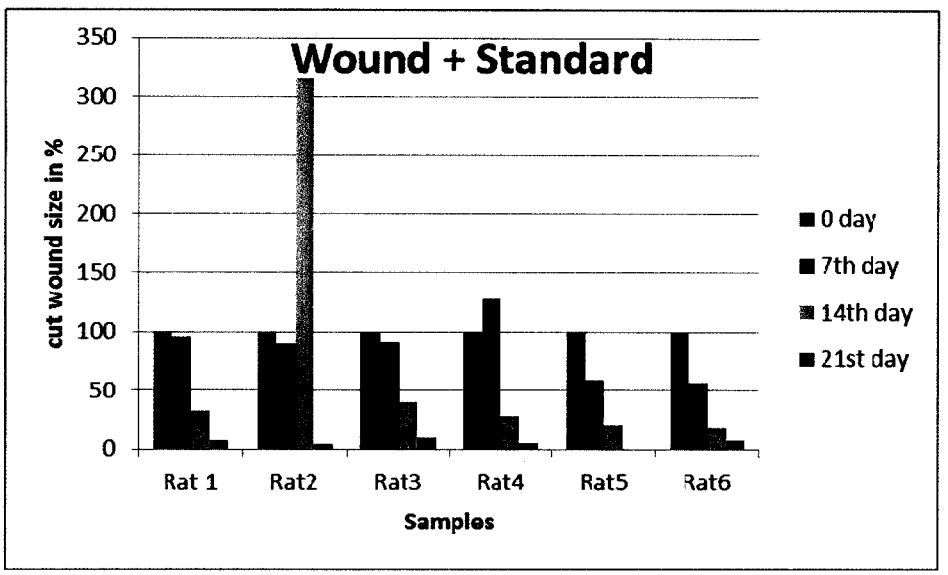


Figure 16: Graph for Cut wound size percentage (wound + standard)

The above table and graph indicates the wound size % after the application of standard antiseptics (Cipladin).Normally this is used as standard for all types of rat. Initially the wound is created. On the 7th day the Rat2 shows the unexpected growth of wound size. But rest of them shows similar size. So, the unexpected growth of Rat2 wound size will not affect the result. On 21st day, the wound size got reduced in the range of 4-10%.

5.2.3 WOUND + LICHEN EXTRACTED FROM CHLOROFORM:

Table 4: Cut wound size percentage (wound + Lichen extracted from chloroform)

No. of days	Cut wound size percentage					
	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Rat 6
0day	100	100	100	100	100	100
7th day	65.1	83.1	96.2	87.8	67	68.4
14th day	32.2	40.8	16.4	27.8	42.9	27.3
21st day	6.7	8.6	4.2	13.6	1	2.1

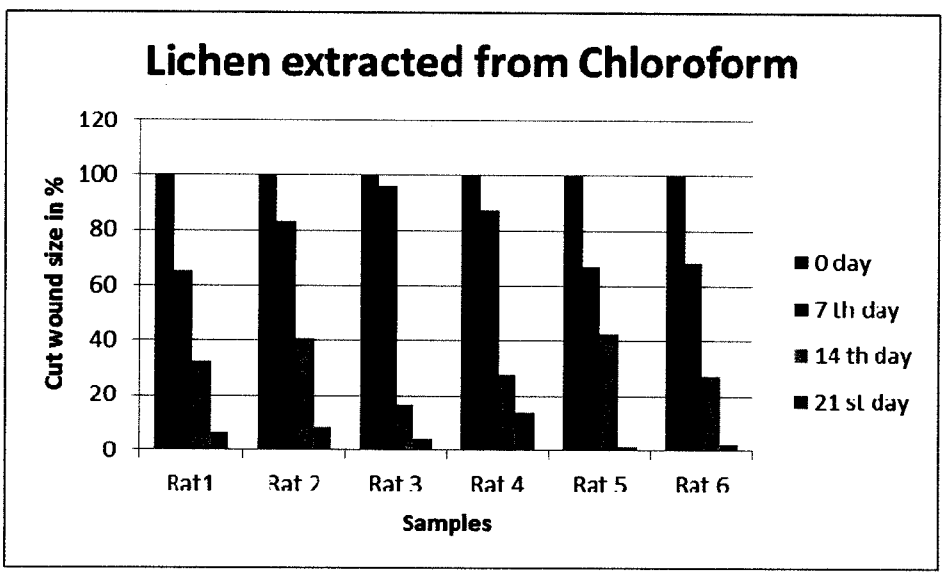


Figure 17: Graph for Cut wound size percentage (wound +Lichen extracted from chloroform)

The above table and graph indicates the wound size % after the application of lichen treated bandage (chloroform extract).Initially the wound was created. On 7th day instead of expansion, the wound healing activity has begun. On 14th day it was much recovered. On 21t day, the wound size got reduced in the range of 2-8%.

5.2.4WOUND + LICHEN EXTRACTED FROM ACETONE:

Table 5: Cut wound size percentage (wound + Lichen extracted from Acetone)

No. of days	Cut wound size percentage					
	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Rat 6
0day	100	100	100	100	100	100
7 th day	135.6	144.2	100.5	115.18	102.1	90.06
14th day	86.7	57.89	26.56	79.7	48.42	45.59
21st day	7.44	11.57	11.45	10.47	10.5	9.3

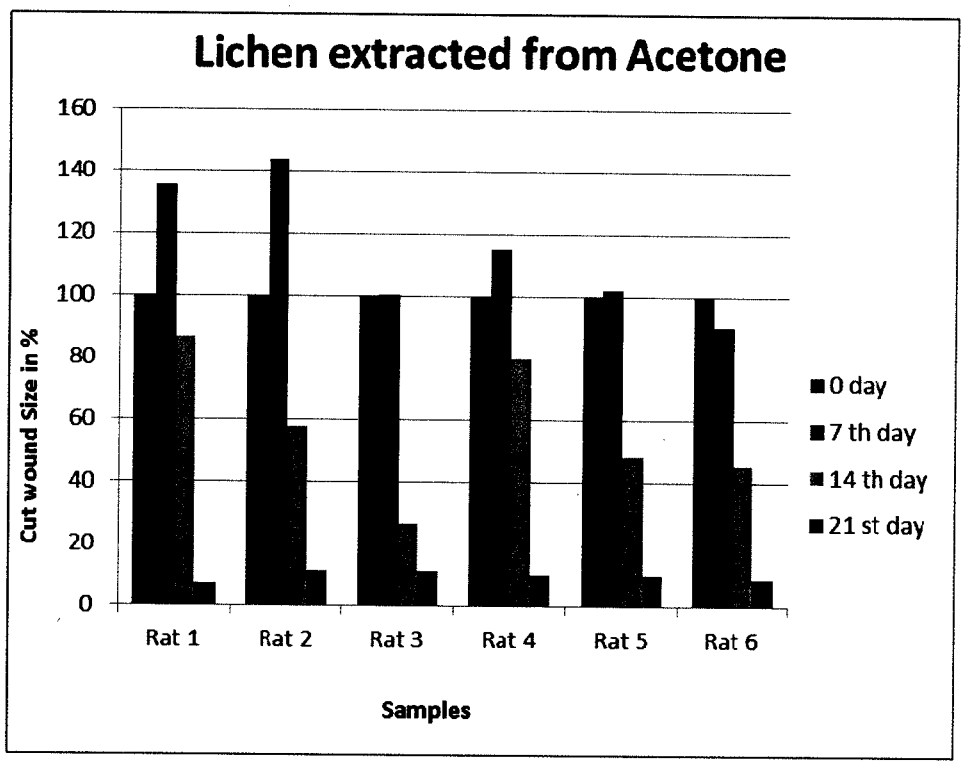


Figure 18:Graph for Cut wound size percentage (wound + Lichen extracted from Acetone)

The above table and graph indicates the wound size % after the application of lichen treated bandage (Acetone extract). Initially the wound was created. On 7th day instead of expansion, the wound healing activity has begun. On 14th day it was much recovered. On 21st day, the wound size got reduced in the range of 7-11%.

6. CONCLUSION

During the antimicrobial test it was found that chloroform and acetone extract shows good result.

The chloroform extract and acetone extract solution was prepared and it is applied on bandage cloth.

On the 21 st day the average size of wound got reduced to 40-60% without any application of antiseptics.

On the 21 st the average wound size got reduced to 4-10% after the application of standard antiseptic solution (cipladin)

On the 21 st the average wound size got reduced to 2-8% after the application of lichen treated from Chloroform extract

On the 21 st the average wound size got reduced to 7-11% after the application of lichen treated from Acetone extract

When compare all the above, the lichen extracted from chloroform is having higher wound healing capacity.

So, it is concluded that lichen extracted from chloroform can be effectively utilized in the field of medical as a cut wound healing bandage.

7. FUTURE SCOPE OF THE PROJECT

- Different species of lichen can be explored for the possibility of utilizing in the medical field.
- The lichen can also be extracted with some other solvents like methanol, benzo proponal, hexane etc.
- Other than cut wound it can be experimented for its healing nature of different kinds of wound.
- Apart from S.coccus and E.coli, bacterias like *Klebsiella pneumonia, Aeromonas hydrophila, Bacillus cereus, Bacillus subtilis, Listeria monocytogenes, Proteus vulgaris, Yersinia enterocolitica, Staphylococcus aureus, Streptococcus faecalis, Candida albicans, Candida glabrata, Aspergillus niger, Aspergillus fumigatus, and Penicillium notatum* can also be experimented for the inhibition zone of antimicrobial activity can be verified.
- It can also be explored to make tablets for its internal healing nature.

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April 18, 2012.

TO WHOMSOEVER IT MAY CONCERN

This is to certify that **Mr. AYYANAR R, Mr. ARUN R, Ms. ANANTHI R and Ms. JESSIMA H,** Department of Textile Technology, Kumaraguru College of Technology has undertaken the animal experimental study titled "**Excision wound of Lichen extract + Chloroform extract and Lichen extract + Acetone extract in experimental rats**" at our organization during the month of March, 2012 under my guidance and supervision.

18/4/12

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