MICROBIALLY SYNTHESIZED BIOACTIVE NANOPARTICLES 
AND THEIR FORMULATION ACTIVE AGAINST HUMAN 
PATHOGENIC FUNGI

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Received: June 26, 2003

Abstract. During artificial culture of some lichens fungi (mycobiont) in synthetic medium for 
bioactive metabolite synthesis, the Usnea longissima lichen (ascomycetes fungi) found to be 
synthesized bioactive nanoparticle (usnic acid) in specified medium under additional conditions. 
The size of the nanoparticle of almost uniform in size, with a diameter of around 50-200 
nanometer across. Such a narrow size distribution is characteristic of microbially synthesized 
nanoparticle. There is an ever-growing need to develop cleaner, non-toxic and environmentally 
friendly procedures for synthesizing the bioactive nanomolecules. So the research turned to 
biological systems for inspiration. This is first ever report of microbially synthesis of bioactive 
nanoparticle from lichen biomass (mycobiont) through in vitro culture. We were used Bio 
nanoemulsion technology for in vitro bioactivity testing of formulated bioactive nanoemulsion 
from lichen metabolites. Detail in vitro analysis of bioactive nanoemulsion was carried out against 
human pathogenic fungi, Epidermophyton floccosum, Microsporum audouini, M. canis, M. 
gypseum, M. nanum, Trichophyton mentagrophytes, T. rubrum, T. tonsurans, T. violaceum, 
causing superficial fungal infection in humans and animals. Nanoemulsion was found to be 
extremely safe and effective for controlling fungal infections (dermatophytoses) during preliminary 
in vivo investigation in humans. Thus, our study will definitely provide an effective natural 
nanomedicine (bio-nanoantifungal) active against microbial infections; in near future after 
successful clinical trial. So, in this way we can exploit the lichen biomass (mycobionts) as 
nanofactories for the production of raw material for nanomedicines (nanoantifungal).

1. INTRODUCTION

The area of nanotechnology, which spans the synthesis of nanoscale matter, understanding/utilizing 
their exotic physicochemical and optoelectronic properties, and organization of nanoscale structures 
into predefined superstructures, promises to play an increasingly important role in many key 
technologies of the new millennium [1,2]. As far as the synthesis of nanoparticle is concerned, there is an 
ever-growing need to develop clean, non-toxic and environmentally friendly (green nanochemistry) 
procedures. Consequently, researches in the field of nanoparticle preparation have been looking at bio-
logical systems for inspiration. The above factors, combined with academic curiosity, have lead to the 
development of biomimetic approaches for the growth of advance materials (bioactive materials). Many 
or ganisms, both unicellular and multicellular, are known to produces inorganic materials either intra-
or extracellularly [3,4]. Even through microbes have been used with considerable success in biotechno-
logical applications, such as remediation of toxic metals, reports on their use in the synthesis of 
nanomaterials are extremely limited. Mukherjee and coworker have demonstrated the formation of gold 
nanoparticle through Verticillium fungus sp [5]. In the present communication we showed that the li-
chen fungi (Usnea longissima) synthesized the bioactive nanoparticle in culture conditions. Lichen 
fungi have diverse range of chemicals and produced some characteristic metabolites during artificial cul-
ture in synthetic medium. Many lichen substances have been detected in cultured lichens: for example, usnic acid [6-8]; depsides and depsidones [9-11]; vulpinic acid [12]; anthraquinones [13] and salazinic acid [14]. Present paper reports, the synthesis of bioactive nanoparticle from Usnea longissima lichen and their formulation as bioactive nanoemulsion active against human pathogenic fungi and preliminary clinical investigations against superficial fungal infections in humans.

2. MATERIAL AND METHODS
Lichen, Usnea longissima Ach. (Fig. 1a) belonging to the family Usneaceae, was collected from Great Himalayan National park, India at the height of 2400-2900 m from the fallen twigs of Quercus semecarpifolia tree. The collected lichen material was deposited in the lichen Laboratory, NBRI, Lucknow, India, voucher specimen no 99 53628.
**Culture methods.** Lichen tissues, mostly mycobionts, were obtained from the fresh lichen thalli by Yamamoto methods [15,16]. Lichen culture was started with in first week after collection. Lichen thallus was thoroughly washed with tap water for 1 hr and then by double distilled water under sterile condition and then dried at room temperature. The washed sterile thallus was cut in small pieces under sterile condition and suspension was formed, which passed through sterilized stainless filter with a 500 µm mesh then passed with filter with a mesh size 150 µm. Small segments from the second filtration were picked up with sterilized bamboo sticks and see under a Trinocular microscope for identification.

Then fragments were inoculated in 1000 ml Er- lenmeyer flask containing the Modified MY medium (Malt Yeast Medium) 1% Glucose, 2% malt extract, 0.2% yeast extract, peptone 0.5% at 15 °C under shaking (200 rpm) for 15 days with alternating photoperiod of 14 hrs light (400 lux) and 10 hrs dark with 40-80% relative humidity. After 15 days of fermentation, mycelia were separated from the culture broth by centrifugation (10,000 rpm) at 10 °C for 30 minutes and the settled mycelia were washed three time with sterile distilled water.

After, 15 days of culture (Fig. 1b), we observed the synthesis of light yellow green substances in the medium. Later the particles were aggregate to form a uniform crystalline layer (nanoparticle) below the medium, which was latter identified by chemical analysis.

**Analysis of bioactive metabolites (usnic acid) (Fig. 1c).** Lichen acids were extracted from thallus fragments using by acetone or benzene. For detecting minor chemical substances, the cultured tissues were extracted at first by cold benzene for removing usnic acid, and subsequently extracted by warm acetone. The acetone extract and benzole extract were spotted on Merck’s pre-coated TLC plates. The chromatogram was developed for 20 minutes at room temperature using standard solvent system [17-19] BDA (benzene : dioxane : acetic acid, 180 : 45 : 5; 230 ml) and HEF (hexane : ethyl ether : formic acid, 130 : 80 : 20; 230 ml). Spots were detected by UV light an after spraying with 10% sulphuric acid and heated at 105 °C for 10 minutes. Identification of substances was made by comparison with standard substances. During UV investigation the colour spot confirm the substances as usnic acid (bioactive molecules). Different sized nanoparticle were isolated from the culture at every 2hr (after 15 day of inoculation) and passed through the different sized Millipore filters, ranges from 0.05 µm to 1 µm (50 nm to 1000 nm) filter pore size.

**Preparation of the bioactive nanoemulsion (Fig. 1e).** Isolated lichen substance (usnic acid) is dissolved in the oleic acid with addition of acetone. The acetone was evaporated until complete dryness under reduced pressure using a rotary evaporator. The dry film was hydrated by shaking until lipid was homogenously dispersed in aqueous phase. The dispersion was homogenized for 10 min at 18,000 rpm using a homogenizer. The preparation was then submitted to 15 time of the same shearing. The preparation was then submitted to 15 cycle of high shear homogenization. Egg lecithin as emulsifier and stabilizing agent (Polyacrylic acid) were added in the formulations to provide the stability to the nanoemulsion.

The formulation was filtered through 0.02 µm (20 nm) sterile filter membrane to give uniform sized nanoparticle formulation having bioactive nanoemulsion (Fig. 1e).

**Maintenance of test fungi.** The causal organisms (fungi), *Epidermophyton floccosum*, *Microsporum audouinii*, *M. canis*, *M. gypseum*, *M. nanum*, *Trichophyton mentagrophytes*, *T. rubrum*, *T. tonsurans*, *T. violaceum* were procured from the Department of Microbiology, All India Institute of Medical Sciences (AIIMS), New Delhi, India. Fungal organisms were kept on Sabouraud Dextrose Agar (SDA) medium (dextrose 40 gm, peptone 10 gm and agar 20 gm in 1000 ml distilled water, 5.6 pH, Penicillin G (5 mg) and Streptomycin sulfate (5 mg) were thoroughly mixed in the medium at 40 °C in order to prevent bacterial growth, as suggested by Gupta and Banerjee [20] as test fungi throughout the course of investigation. All these fungi were screened against, using the nanoemulsion prepared from the cultured lichen tissue.

**In vitro bioassay of nanoemulsion.** The antifungal activity of the nanoemulsion were tested by two methods: Modified spore germination inhibition technique of Shahi et al. [21] and Modified Poisoned food technique of Grover and Moore [22].

**Spore germination inhibition.** The minimum inhibitory concentrations (MICs) of the nanoemulsion were determined following the modified spore germination inhibition technique (MSGIT) of Shahi et al [21]. The nanoemulsion (stock nanoemulsion) in 0.05, 0.1, 0.2, 0.3, 0.4, and 0.5 ml was mixed in 1000 ml standardized inoculum suspension separately, to get the desired concentration, 0.05, 0.1, 0.2, 0.3, 0.4 and 0.5 µl ml⁻¹ respectively. In con-
trols, sterile water was used in place of the nanoemulsion. Experiments (treatment as well as control sets) were incubated at 27 ± 1 °C and observations were recorded at the interval of 24 hrs up to 96 hrs, by counting the number of germinated spores. Percentage of spore germination inhibition (SGI) was calculated as per equation of Shahi et al. [21].

\[ SGI (\%) = \frac{(Gc-Gt)}{Gc} \times 100 \]

where, Gt = number of spore germination in control sets, Gc = number of spore germination in treatment sets.

**Nature of antifungal activity.** The nature of activity (fungicidal/fungistatic) of the nanoemulsion at MICs was determined by the method of Shahi et al. [21]. This was done by reinoculating 1ml inhibited inoculum suspension at MICs in 5 ml medium (broth). Spore germination after 24 hrs of incubation at 27 ± 1 °C, indicated fungistatic nature and absence of spore germination indicated fungicidal action of the nanoemulsion at MICs.

**Mycelial growth inhibition.** The minimum inhibitory concentrations (MICs) of the nanoemulsion against test pathogens were determined following the poisoned food technique [22] with slight modification. The nanoemulsion (stock suspension) in 0.05, 0.1, 0.2, 0.3, 0.4 and 0.5 ml was mixed in 1000 ml medium separately, to get the desired concentration, 0.05, 0.1, 0.2, 0.3, 0.4 and 0.5 ml l−1 respectively. In control sets, sterilized water was used in place of the nanoemulsion. Mycelial discs of 5-mm diameter, cut out from the periphery of 7-day old cultures, were aseptically inoculated upside down on the agar surface of the medium. Inoculated petri plates were incubated at 27 ± 1 °C and the observations were recorded on seventh day. Percentage of mycelial growth inhibition (MGI) was calculated as per formula.

\[ MGI (\%) = \frac{(dc-dt)}{dc} \times 100 \]

where, dc = fungal colony diameter in control sets, dt = fungal colony diameter in treatment sets.

**Nature of antifungal activity.** To find out the minimum fungistatic/fungicidal concentrations of the nanoemulsion at minimum inhibitory concentrations (MICs) were ascertained by the method of Garber and Houston [23]. This was done by reinoculated the inhibited fungal discs at MICs on SDA medium. Observations were recorded after 7-day incubation. Fungal growth on seventh day indicated fungistatic nature while absence of fungal growth denoted fungicidal action of the nanoemulsion.

**Effect of nanoemulsion against inoculum density.** The effect of inoculum density on antifungal activity of the nanoemulsion (at MICs) was determined following the procedure outlined by Shahi et al. [24]. Fungal discs of 5 mm diameter cut out from 7 day old cultures of the test fungi, were inoculated in culture tubes containing the liquid medium with nanoemulsion at MICs. In controls, using sterile water in place of the nanoemulsion. The number of fungal discs in the test and control sets were increased progressively upto 40 in multiples of five. Absence of fungal growth after 7th day of incubation in treatment sets indicated the lichen nanoemulsion potential to heavy doses of inocula of the test fungi.

**Effect of nanoemulsion on human skin.** To determined the maximum tolerable concentrations (MTCs) and long-term toxicity for irritant activity, if any, of the prepared nanoemulsion by their topical application on human skin, following the patch test method as described by Shahi et al. [24].

People of different sex, from 8 to 70 years were selected randomly and a group of 20 individuals of each sex was constituted. Circular areas of 5 cm² on upper hairy and lower glabrous surface of palms, nail and 3 cm² of neck region of each individuals were first washed with distilled water followed by 70% ethyl alcohol and then allowed to dry for five minute. Testing solution (nanoemulsion), 0.2 ml of the graded concentrations, was applied to each individual separately twice in a day for three week. The volunteers were not allowed to wash the applied area. The qualitative observations were recorded after the interval of 24 hrs up to three week.

**Clinical response of the nanoemulsion (nanoantifungal) in the form of lotion.** The preliminary study was designed to see the activity of the nanoemulsion in the form of lotion (1% concentration v/v) on human patients for the control of fungal infections [24]. The patients were treated with 1% lotion. Medication was administered twice a day for 3 week. The patients were not allowed to take any other systemic or topical therapy during the course of study.

Patient's selection – Patients within the age group of 8 to 40 years suffering from tinea corporis, tinea mannum, tinea cruris or tinea pedis were selected randomly after voluntary consultation of patients and a group of ten individuals was formed. The diagnosis was confirmed by KOH microscopic examination of the scraping (from the infected area), showing inoculum will be designated as KOH positive and their absence will be recorded as KOH nega-
Table 1. Inhibition of human pathogenic fungi by the prepared nanoemulsion.

<table>
<thead>
<tr>
<th>Fungi</th>
<th>Percent inhibition at different concentrations µl ml⁻¹</th>
<th>MGI (%)</th>
<th>SGI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.05</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td><em>Ef</em></td>
<td>67.9</td>
<td>100⁺</td>
<td>100⁺</td>
</tr>
<tr>
<td><em>Ma</em></td>
<td>76.9</td>
<td>100⁺</td>
<td>100⁺</td>
</tr>
<tr>
<td><em>Mc</em></td>
<td>87.8</td>
<td>100⁺</td>
<td>100⁺</td>
</tr>
<tr>
<td><em>Mn</em></td>
<td>76.9</td>
<td>100⁺</td>
<td>100⁺</td>
</tr>
<tr>
<td><em>Mg</em></td>
<td>98.0</td>
<td>100⁺</td>
<td>100⁺</td>
</tr>
<tr>
<td><em>Tm</em></td>
<td>73.3</td>
<td>100⁺</td>
<td>100⁺</td>
</tr>
<tr>
<td><em>Tr</em></td>
<td>67.9</td>
<td>100⁺</td>
<td>100⁺</td>
</tr>
<tr>
<td><em>Tt</em></td>
<td>87.9</td>
<td>100⁺</td>
<td>100⁺</td>
</tr>
<tr>
<td><em>Tv</em></td>
<td>56.9</td>
<td>100⁺</td>
<td>100⁺</td>
</tr>
</tbody>
</table>

*Ef*, *Epidermophyton floccosum*, *Ma*, *Microsporum audouini*, *Mc*, *M. canis*, *Mg*, *M. gypseum*, *Mn*, *M. nanum*, *Tm*, *Trichophyton mentagrophytes*, *Tr*, *T. rubrum*, *Tt*, *T. tonsurans*, *Tv*, *T. violaceum* s = fungistatic, c = fungicidal, MGI= Mycelial growth inhibition, SGI= spore germination inhibition

tive. Only KOH positive cases were enrolled in the study. To minimize the inconvenience off the patients another 10 subjects (patients) were selected as control.

Methods – Patients were examined just before therapy was initiated and weekly for 3 week of treatment. Where the coetaneous fungal disease occurred in more than one body area, all affected areas could be treated, but only one was selected and designated as the reference lesion. At each visit, the same reference lesion was scraped for fungal culture to identify the organism and for demonstration of hyphae by microscopic examination of the scrapings covered with 10% KOH preparations. Signs and symptoms of disease i.e. erythema, oedema, scaling, maceration, vesiculation, pustulation, crusting and itching were recorded as absent (-), mild (+), moderate (++), or severe (+++) and noted at every visit and scores were added. Less than 25% improvement was considered no improvement, 25-50% as mild, 50-75% as moderate, >75% as significant and complete cure when there was total clearance of signs and symptoms. Any adverse systemic or local reaction was noted at each visit. Satisfactory response with KOH negative cases after three week were reexamined after two months later to find out the relapse rates if any.

3. RESULTS

During artificial culture of lichen, *Usnea longissima* (mycobiont) in a specific medium, rod shaped yellowish green crystalline substance (Usnic acid) (Fig. 1c) was synthesized, which was confirmed as the usnic acid by TLC analysis. The unique method of production of this substance in the form of different sized particle that can be isolated in the nanoparticle form or nanoparticulate size stage. As previously discussed in method it was clearly stated that the production of different sized particle was depended on the time of harvesting and at a particular culminate stage the synthesized substance was no more in the particulate form, but rather become in the crystal form as showed in Fig. 1c, that the rod shaped crystal of the synthesized usnic acid in the artificial condition (Figs. 1c and 1d).

Nanoemulsion prepared as antimicrobial formulation using the synthesized nanoparticulated usnic acid showed good results both during in vitro and in vivo investigations. During in vitro investigation, the minimum inhibitory concentrations of the nanoemulsion were found to be 0.1 µl ml⁻¹ for *Epidermophyton floccosum*, *Microsporum audouini*, *M. canis*, *M. gypseum*, *M. nanum*, *Trichophyton mentagrophytes*, *T. rubrum*, *T. tonsurans*, *T. violaceum* with fungicidal in nature by both the method tested (Table 1). The nanoemulsion was found effective against heavy doses (40 mycelial discs, each of 5 mm in diameter) of fungal inoculum at 0.1 µl ml⁻¹ concentration.

During in vivo investigation, the nanoemulsion when tested for the irritant activity and long-term toxicity on human skin, the nanoemulsion didn’t
show any irritation or adverse effect at 5% concentration up to 3 weeks. After preliminary topical application (clinical investigation) of the nanoemulsion, improvements were observed right from the first week; 50.0% showed moderate improvement and 30.0% mild improvement, while 20.0% showed no improvement. After second week, 30% and 25% patients exhibit significant and moderate improvement respectively. However, at the end of medication (i.e. after third week), 60.0% of the patients were classed as complete clear and 30.0% as significant improvement (Table 2). Majority of the controls showed no improvement and none of them showed more than a mild improvement.

During cost-benefit analysis [24] of the nanoemulsion based lotion compared with synthetic antifungal drugs, the lotion was found to be most effective, less expensive (INR. 1.0/ml), long shelf life (18 months as tested) and absence of any adverse effects.

4. DISCUSSION

Gentles was to revolutionize the therapy of ringworm infections. In 1958 he reported that the oral administration of griseofulvin cured experimental dermatophytosis (fungal infections) in a guinea pig [25]. Clinical trials were carried out in many parts of the world and included the whole gamut of dermatophytic disease. These trials, conducted by various workers [26,27] and others, have been reviewed by Blank [28] and are the standards for therapy. Recently several new topical drugs have been introduced. Of these, tolinafate (Tinactin) has gained wide popularity but is of limited value in some types of infection. More recently a few of the imidazoles clotrimazole, ketoconazole, miconazole, and econazole have been used as topical agents in the treatment of certain dermatophyte infections, however, these agents are associated with skin sensitization and drug induced eruptions. Treatment failures and relapses occur with all presently available drugs. The need for better therapeutic agent is apparent.

The field of medicine has been revolutionized by the application of nanotechnology science. Advanced extra effective medicine become today’s demand, which was hopefully provided by the most advance technique of 21st century the ‘nanotechnology’. Scientists from all over the world are worked to develop nanoproduct to boon the field of medicine. Nanotechnological studies provide some good results in the cosmetics, dermatological, pharmacological and ophthalmological fields. In recent years, much progress has been made to improve the performance of skin care products. New excipients, refined processing techniques and a better knowledge of the physiochemical properties have led to the development of new concepts. Some of them such as liposome are well established in the market and lipid nanoparticles are currently introduced and particle-stabilized emulsion is on the way to be implemented in new products. Drug delivery system (DDS) using liposome-encapsulated antibiotics could improve the antimicrobial activities. Various formulations for amphotericin B (antifungal substances) have been used clinically for fungal infection in Europe and the United states. Lopez-Berstein

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patients showing clinical response for 3 weeks</th>
<th>T</th>
<th>C</th>
<th>T</th>
<th>C</th>
<th>T</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>No improvement</td>
<td></td>
<td>20</td>
<td>100</td>
<td>10</td>
<td>100</td>
<td>00</td>
<td>65</td>
</tr>
<tr>
<td>Mild improvement</td>
<td></td>
<td>30</td>
<td>00</td>
<td>30</td>
<td>00</td>
<td>10</td>
<td>35</td>
</tr>
<tr>
<td>Moderate improvement</td>
<td></td>
<td>50</td>
<td>00</td>
<td>35</td>
<td>00</td>
<td>00</td>
<td>00</td>
</tr>
<tr>
<td>Significant improvement</td>
<td></td>
<td>00</td>
<td>00</td>
<td>25</td>
<td>00</td>
<td>40</td>
<td>00</td>
</tr>
<tr>
<td>Complete clear</td>
<td></td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>60</td>
<td>00</td>
<td>00</td>
</tr>
<tr>
<td>T test paired difference</td>
<td></td>
<td>48.9</td>
<td>41.3</td>
<td>47.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SE of Mean</td>
<td></td>
<td>21.9</td>
<td>18.5</td>
<td>21.2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

T – treatment; C – control

Table 2. Ten patients showing preliminary clinical response to prepared nanoemulsion (1% lotion).
et al. [29] studied treatment and prophylaxis of disseminated infection due to *Candida albicans* in mice with liposome-encapsulated Amphotericin B. Hospenthal et al. [30] studied the liposomal system for the treatment of murine infection and against mycobacterial infection by Koga et al. [31]. But according to the recent study liposomal nanodispersed system (lipid bilayer enclosing an aqueous core) was not found more acceptable in comparison to the recently developed nanodispersed system, i.e., nanoemulsions (lipid monolayer enclosing lipid core). Which found to be more suitable for the transport of lipophilic compounds than liposomes. Furthermore nanoemulsions gain increasing interest due to their own bioactive effect. Kohno et al. [32] develop a new antifungal drug delivery system, lipid nanosphere and studied it against aspergillosis in rat.

But in contrast to liposomes and nanoemulsions, it is not necessary to develop completely new products if one intended to use lipid nanoparticle. Due to their good physical stability and compatibility with other ingredients they can often be added to exist formulations without any problems. Bender et al. [33] studied the efficiency of nanoparticle as a carrier system for antiviral agents in human immunodeficiency virus infected human monocytes / macrophages in vitro. Hossain et al. [34] develop a novel lipid nanosphere against *Cryptococcus neoformans*. Recently some firm patents Nanoperal and Lipopearl two lipid nanoparticles. Amselem, et al. [35] patented a solid fat nanoemulsions preparation for the parental, oral, intranasal, rectal, or topical delivery of both fat-soluble and water soluble drugs. Different firm applied various other patents based on nanoemulsion preparation. Recently Simonnet et al. [36], patented the nanoemulsion based on alky ether citrates and its uses in the cosmetics, dermatological, pharmacological and ophthalmological fields. Recently, Robert Ebisch, of the University of Michigan, filed a patent a nasal spray for flu to prevent transmission of the virus, which was also based on the nanoemulsion. Thus, from the above discussion, the application of antimicrobial nanoemulsion technology (developed by Dr. James R. Baker at the University of Michigan) in the various field of the medical can be understand and we can stated that the nanoemulsion preparation were mainly based on the accepted active ingredient but in the present study it was first time that the active ingredient was also synthesized in the laboratory under artificial condition in artificial medium. More interestingly that the bioactive ingredient was synthesized by the mycobiont of the Lichen, *Usnea longissima* under artificial condition which can be isolated at the nanosized stage. In best of our knowledge it was first claim of this type. However some scientists were artificially synthesized nanoparticles (and not the bioactive nanoparticle) by the studied living entity. *Bacillus subtilis* 168, was utilized for the synthesis of the gold nanoparticle by Beveridge and Murray [37] and
Pseudomonas stutzeri AG259 was used for the synthesis of the silver nanoparticle by Klaus et al. [38,39]. In the present study the living bio entity was a fungal or mycobiont of the Lichen Usnea longissima. The nanoparticle synthesized artificially by the mycobiont of the Usnea longissima found to be very effective against tested pathogens. The nanoemulsion prepared by using the synthesized bioactive agent found effective during both in vitro and in vivo study against the tested pathogens causing various dermatophytic diseases.

The size of the nanoparticle of almost uniforms in size, with a diameter of around 50 to 200 nanometer across. Such a narrow size distribution is characteristic of microbially synthesized nanoparticle. Detail in vitro analysis of bioactive nanoemulsion was carried out against human pathogenic fungi, the minimum inhibitory concentrations of the nanoemulsion were found to be 0.1 μl ml⁻¹ against Epidermophyton floccosum, Microsporum audouinii, M. canis, M. gypseum, M. nanum, Trichophyton mentagrophytes, T. rubrum, T. tonsurans, T. violaceum, causing fungal infection in humans and animals. Nanoemulsion was found to be extremely safe and effective for controlling fungal infections (dermatophytoes) during preliminary in vivo investigation in humans. Thus, our study will definitely provide an effective natural nanomedicine (bio-nanoantifungal) active against microbial infections; in near future after successful clinical trial. So, in this way we can exploit the lichen biomass (mycobionts) as nanofactories for the production of raw material for nanomedicines.

ACKNOWLEDGEMENT

We thanks to Head, Department of Botany, University of Allahabad for providing the facilities; to Dr. Uma Benerjee, Division of Microbiology, All India Institute of Medical Sciences, New Delhi, India, Dr. G. Midgely, St. Thomas hospital, London, UK for providing the culture of fungi and Dr. D.K Upreti, Lichen Laboratory, national Botanical Research Institute, Lucknow for identification of lichen species. We also thankful to Dr. K.G Singh, Department of Dermatology, M.L.N Medical College, Allahabad for providing clinical trials and to DST, New Delhi for financial assistance

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