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Research Article**Herbal formulation of *Cocos nucifera* L. for treatment of eczematic infections: An *in vitro* study**Sharad Bhandarkar^a, Ajay Shukla^a, Alok Pal Jain^{a*}^aDepartment of Pharmacy, Guru Ramdas Khalsa Institute of Science and Technology, Jabalpur, M.P.

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Abstract

Objective: In the present study we describe in-vitro efficacy of *Cocos nucifera* L. (family Areaceae) husk fiber extract ointment for growth inhibition of four microbes (*Candida albicans*, *Aspergillus fumigatus*, *Staphylococcus aureus* and *Trichosporon asahii*) that may cause severe infection in eczema patient. **Materials and methods:** The stability parameters of extract ointment such as physical stability, spreadability, centrifugation etc. would be performed and found applicable results. The therapeutic effect was compared with marketed product Tacrolimus 0.1%w/w ointment. **Results:** The shells of *Cocos nucifera* L. has rich in polyphenolic compounds and has been reported for arthritis, diarrhoea, antibacterial, antiviral and also has inhibitory lymphocyte production. Catechins are flavonoids that were present in *Cocos nucifera* L shells, already have antioxidant, powerful cellular growth inhibitor and anti-inflammatory activity which might be correlated with this study. **Conclusions:** The aqueous extract ointment of *Cocos nucifera* L was found effective and require further *in-vivo* study for eczema as well as isolation of responsible active constituents for this activity.

Keywords: *Cocos nucifera*, eczema, Tacrolimus, polyphenols, ointment

Introduction

Herbal medicines are being used worldwide, particularly in the developing countries for primary health care, because they have stood their safety, efficacy, cultural acceptability and lesser side effects. The chemical constituents present in them are a part of the physiological function of living flora and hence they are believed to have better compatibility with human body (Marcus and Grollman, 2002; Kamboj, 2000).

Atopic eczema is a common skin disorder affecting between 5% and 15% of school age children and also with other atopic diseases, the prevalence appears to be increasing. The disease causes sleep disturbance and misery to sufferers with reduced quality of life, psychological problems, and disruption to family life. There are mainly four triggers of eczema- irritants, allergens, infection and environmental factors. Infection may be bacterial, viral (fungal, cold) and stomach flu (Ahuja et Al., 2003). In this study we have selected four microorganisms (*Candida albicans*, *Aspergillus fumigatus*, *Staphylococcus*

aureus and *Trichosporon asahii*) that may cause allergic eczema as well as severe infection in eczema patient.

The British Association of Dermatologists' guidelines on the management of eczema, stress on the importance of the patient education, explanation and discussion about aspects of treatment. Topical corticosteroids, with or without topical calcineurin inhibitors, are the mainstay of treatment for atopic eczema (Chinn and Poyner, 2002; Krejci-Manwaring et al., 2007). In eczematous condition, to take good skin care and maintain moist skin condition because dry skin facilitate microorganisms growth. Therefore in present study we formulated ointment (which has little water content) instead of lotion which has more water than oil.

The species *Cocos nucifera* L. (Palmae) is a member of Areaceae family, commonly known as Coconut palm and widely distributed over the Brazilian northeastern coast. The popular medicinal use of *Cocos nucifera* aqueous extract as a tea has been reported in large extent (Duke, 1992) but the knowledge of its potential beneficial or adverse biological effect in human is still very preliminary.

Recent results show that aqueous extract from husk fiber of *Cocos nucifera* exhibits antibacterial, antiviral (Esquenazi et al., 2004) and antileishmanial property (Mendonca-Filho et al., 2004). In addition *Cocos nucifera* extract has

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antiproliferative effect on animal lymphocytes (Kirszberg et al., 2003). Chromatographic technique coupled with mass spectrometry technique revealed that aqueous extract of *Cocos nucifera* mostly contains catechin and epicatechin together with condensed tannins (β - type procyanidins) (Esquenazi et al., 2004). These classes of compound have been associated with analgesia and antioxidant activity in several experimental models. In addition *Cocos nucifera* water has antioxidant properties, which was correlated with the ascorbic acid (Alviano et al., 2004).

The objective of the present study was to formulate an ointment containing aqueous extract of *Cocos nucifera* shells and evaluate their efficacy for growth inhibition of microorganisms that cause infection in atopic eczema.

Material and methods

Plant material

Cocos nucifera L. (Palmae) shells were collected from local market of Jabalpur and were authenticated in the, JNKVV, Jabalpur, (M.P.) India. A specimen herbarium was submitted in Botany Department.

Extraction and fraction preparation

Husk fiber of *Cocos nucifera* L were dried, powdered and extracted with water by simple extraction (maceration) method using boiling distilled water. Yield was found to be 10%w/v. The extract was filtered, lyophilized and stored at 5 °C. The polyphenolic rich extract was separated by thin layer chromatography methods (Mendonca-Filho et al., 2004). The polyphenolic rich extract was used to formulate ointment dosage form for topical application.

Microorganisms and culture

The study was carried out on four causative microorganism stains that are *Candida albicans*, *Aspergillus fumigatus*, *Staphylococcus aureus* and *Trichosporon asahii* were kindly provided by Department of Microbiology, Dr. H. S. Gour University, Sagar, India. They were grown on Sabouraud agar media and stored at 4°C for 18 hrs.

Formulation preparation

Simple ointment B.P. was prepared by fusion method (Anonymous, 1953). The lyophilized aqueous extract (5%w/w) was mixed uniformly with simple ointment base at room temperature. Simple ointment base was used as control group. Marketed formulation Tacrolimus 0.1%w/w ointment (Aurochem Lab Pvt. Ltd.) was used for comparisons.

Stability study of formulation

Stability of prepared ointment formulations was evaluated (Prasad and Dorle, 2006) in terms of the changes in physical and chemical parameters, which were likely to affect the stability and acceptability of the formulations.

Physical stability

Ointment formulations were evaluated in terms of physical changes like changes in color, odour and consistency, thereby affecting their stability and other desired formulation properties. Samples of the ointment formulations were kept at different temperature like 40°C, 37 °C and room temperature for 45 days. They were periodically observed for physical changes development of objectionable color and odour.

Centrifugation

Centrifugation is believed to be an excellent tool for the evaluation of accelerated deterioration of ointments. Stability of formulated ointments on centrifugation was determined in 10 ml-graduated cylinder at 10,000 rpm for 10 min using a sigma centrifuge. The formulations, which were resistant towards centrifugation, were selected for further evaluation.

Spreadibility

Spreadibility was determined by modified wooden block and glass slide apparatus. The apparatus consisted of a wooden block, with fixed glass slide and a pulley. A pan was attached to another glass slide (movable) with the help of a string. For the determination of spreadibility, measured amount of ointment was placed in the fixed glass slide and the movable glass slide was placed over the fixed glass slide, such that the ointments were sandwiched between the two slides for 5 min. Now about 50 g of weight was added to the pan and time taken for the slides to separate was noted.

Spreadibility was determined using the following formula:

$$S = M/T$$

Where S is the spreadibility in g/s, M is the mass in grams and T is the time in seconds.

Irritant effect

Six healthy male albino rabbit, weighed 2-2.5 kg were selected for the study. Rabbits were caged individually. Food and water given *ad libitum* during the test period. 24 hr prior to the test, the hair from the back of rabbit were shaved on both sides of the spine to expose sufficiently large skin areas, which could accommodate three test sites of 2.5 cm² each on each side of the spine. The test sites were cleaned with surgical spirit and the developed formulations, simple ointment base (control) and extract ointment were applied on both side of spine of both group (control and test) rabbits respectively. The test group animals were observed for erythema and edema for 48 hr after application.

In-vitro study

The bacterial pellets were incubated at 23°C for 1 h to facilitate diffusion, and then incubated at 35°C for 24 hrs.

All bacterial strain were suspended in sterile water and diluted to 10^6 CFU/ml. Then suspension (100 μ l) was spread on to the surface of Sabouraud agar medium. Wells (6 mm in diameter) were cut from the agar with a sterile borer and 60 mg ointment formulations were incorporated into wells. Tacrolimus 0.1%w/w ointment (60 mg/well) was used as positive reference standard for comparison and simple ointment base B.P. was used as control. The inoculated plates were incubated at 37 °C for 24 h for bacterial strain, 48 h for yeast and 72 h for fungi isolates. Activity was evaluated by measuring inhibition zone against the test organisms. Each assay was repeated twice (Sahin et al., 2003).

Measurement of inhibition zone diameter

After incubation all plates, inhibition zone were measured to an accuracy of 0.1 mm and the effect was calculated as a means of triplicate tests.

MIC determination

Each well of a sterile 96 well micro plate was filled with 100 μ l aliquot of Na-HI broth. The first column of wells in each micro titer plate received a 100 μ l of broth containing herbal ointment tested. After mixing of pipetting the mixture (100 μ l) were transferred to the next column of wells in a process of 1:1 serial dilution, then the plates were incubated at 30°C for 30 min. before bacterial incubation. Inoculum was prepared by diluting the overnight bacterial culture to level of 10^6 CFU/ml. Each well in the micro titer plate was inoculated with a 100 μ l of the inoculums and incubated at 30°C for 24 h. The growth or survival of the bacterium after incubation was examined. A 100 μ l of the mixture from each well was spread on the Sabouraud agar and incubated at 37 °C for 24 h. The concentration in the lowest serial dilution of the species and herbal ointment at which growth did not occur on Sabouraud, was recorded as the minimum inhibition concentration(MIC)(Sahin et al., 2003).

Results

Stability of formulation

The ointment formulation was found stable at room temperature, 37 °C and 40 °C for 45 days. No change in color and odor was found. Spreadability was determined in repeated experiment and data are tabulated in Table 1. After centrifugation we don't found any separation and deterioration in ointment.

In-vitro study

The results were given in table 2-3; show that MIC for all microorganisms between 6 and 7 mg/ml and inhibition zone diameter of *Candida albicans* and *S. aureus* were more efficient than of other bacterial strain.

Irritant effect

Erythema and edema was observed visually on rabbit skin. We don't found any change in skin color (redness) in both control as well as extract ointment groups.

Table 1 Spreadability of extract ointment formulation (g/s)

| S.N | Temperature (°C) | Extract ointment | Standard drug ointment |
|-----|------------------|------------------|------------------------|
| 1 | Room temp. | 6.320 | 6.645 |
| 2 | 37 | 6.155 | 5.982 |
| 3 | 40 | 5.914 | 6.452 |

Table 2 The MIC value of *Cocos nucifera* L. extract ointment against the pathogens tested in micro dilution assay (MIC in mg/ml)

| S.N | Microorganisms | Extract ointment | Standard drug ointment |
|-----|------------------------------|------------------|------------------------|
| 1 | <i>Aspergillus fumigatus</i> | 7.0 | 5.76 |
| 2 | <i>Trichosporon aschii</i> | 6.5 | 3.40 |
| 3 | <i>Candida albicans</i> | 6.0 | 1.25 |
| 4 | <i>Staphylococcus aureus</i> | 6.0 | 3.55 |

Table 3 The antimicrobial activity of *Cocos nucifera* L. extract ointment against different pathogens tested based on diffusion method

| S.N. Microorganisms | Inhibition zone diameter (mm) | | |
|--------------------------------|-------------------------------|----------------------|------------------------|
| | Extract ointment | Simple ointment base | Standard drug ointment |
| 1 <i>Aspergillus fumigatus</i> | 20 | - | 21 |
| 2 <i>Trichosporon asahii</i> | 19 | - | 22 |
| 3 <i>Candida albicans</i> | 24 | - | 24 |
| 4 <i>Staphylococcus aureus</i> | 27 | - | 28 |

Discussion and conclusion

Atopic eczema is a chronic inflammatory skin condition characterized by an itchy red rash that favors the skin creases such as folds of elbows or behind the knees. The eczema lesions themselves vary in appearance from collections of fluid in the skin to grass thickening of skin (lichenification). Atopic eczema also associated with other atopic diseases such as hay fever and asthma (Hoare et al., 2000). *Staphylococcus aureus* play a pathogenic role in atopic eczema in which a non specific reaction of protein component of bacterium with immune cells occurs and produces specific endotoxins called super antigens which are capable of large population of T-lymphocytes distant from the site of colonization, giving rise to widespread activation of eczematous lesions (McFadden et al., 1993). The density of *S. aureus* tends to increase with the clinical severity of atopic eczema lesions. It has been suggested that the dry skin of atopic eczema is deficient in certain inhibitory fatty acids, which may encourage growth of the organism and increase adherence properties to skin cells obtain from atopic eczema suffers compared with normal control (Noble, 1995).

The pathogenesis of atopic eczema is multifactorial with many allergic and non-allergic triggers involved (Jones,

2002). Allergic triggers include food, pollens, molds, house dust, mites, cockroach allergens and microbial superantigens. Circumstantial evidence exists that the mold *Aspergillus fumigatus* may play a role in pathogenesis of atopic eczema, concomitant with increased tendency of patient with atopic eczema to react to common environmental antigens (Schmid-Grendelmeier et al., 2005; Crameri et al., 2006). Silk or silver coated textiles show antimicrobial properties that can significantly reduce the burden of *S. aureus*, leading to positive effect on eczema. In-vitro studies of these silver coated textiles demonstrated a significant decrease in *S. aureus* and *Candida albicans*. Silk has been increasingly implemented in medicinal treatment of atopic eczema and produce smoothness that reduce irritation. The combination of smoothness of silk and antimicrobial finish appears to make an ideal textile for patient suffering from atopic eczema (Hang et al., 2006). Recently *Trichosporon asahii* yeast like fungus to be mimicking hand eczema during chemotherapy for patient with chronic myelocytic leukemia (Nakagawa et al., 2000).

Recently some Chinese herbs have become more popular for atopic eczema. It was also reported that Chinese herbs exert their anti eczematic activity by acting as antioxidants (Kirby and Schmidt, 1997). A number of Indian herbs have also been used topically due to their antioxidant or free radical scavenging property. The *Cocos nucifera* L. also have number of therapeutic properties like antibacterial, antiviral, antinociceptive and in leishmaniasis (Mendonca-Filho et al., 2004; Esquenazi et al., 2004; Alviano et al., 2004). It is also reported that alcoholic extract of coconut shell was effective against ring worm infection (Kirtikar and Basu, 1956). In other studies it has been reported that *Cocos nucifera* husk fiber rich in polyphenolic compounds contains high amount of catechins, epicatechins and epicatechin(4-2)-phloroglucinol units (Mendonca-Filho et al., 2004). These active constituents of *Cocos nucifera* are responsible for free radical scavenging action (Alviano et al., 2004). Some authors reported that catechins are powerful cellular growth inhibitor (Yang et al., 1998; Paschka et al., 1998).

In the present study we describe efficacy of *Cocos nucifera* husk fiber extract ointment for growth inhibition of allergens and infection causing microbes in eczematic patients. Our results reveal that *Cocos nucifera* husk fiber containing polyphenols may be more efficient for secondary infection of eczema if they formulated in suitable topical formulation.

In conclusion, this is the first in vitro study to report positive relationship between eczema and phenolic content of plant extract. We proposed that phenolic compounds may be helpful in eczema treatment by inhibiting allergens and microbial infection

during eczema through free radical scavenging mechanism. They could be a potential source for inhibitory substance for secondary infection in eczema. This study may be helpful in eczema treatment for combination therapy with anti-inflammatory steroids and antimicrobials.

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Conflict of interest

Authors are only responsible for article content. Authors did not have any conflict of interest.

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