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# FORMULATION OF ANTI-FUNGAL CREAM FOR JOCK ITCH (TINEA CRURIS) DISEASE FROM PLANTS AND EVALUATION OF ITS SAFETY AND EFFICACY THROUGH ANIMAL AND HUMAN CLINICAL TRIALS, AND OTHER BIOLOGICAL AND PHYSICAL PARAMETERS

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## ABSTRACT

Jock itch (tinea cruris) is a disease caused by dermatophytic fungus *Trichophyton rubrum* which covers lower abdominal external area and under buttocks. Disease is very common in society. Fluconazole an effective medication, have some issues i.e., it is cost effective, have side effects and arose resistant in fungus, so to find solution of this problem ethnobotanical survey was done to find plants with anti-fungal activity. Fungal strains were isolated from patients and identified through various test to confirm the disease's causative agent which was *Trichophyton rubrum*. *Curcuma longa*, *Azadirachta indica*, *Allium sativum*, apple cider vinegar, *Aloe vera* gel and *Lavandula Lavandula* were selected as suggested in ethnobotanical survey, Disc diffusion method was used to test their anti-fungal activities and compared with fluconazole as positive control. Ethanolic extracts have shown higher anti-fungal actives than water (individually and mixed plants extracts). cream was formulated then from ethanolic herbal extracts of all plants and apple cider vinegar mixed in equal ratios. Cream was evaluated through various physical and biological parameters. Pre-Clinical trials were run on four rabbits 1 cm square shaved skin to test cream safety and judged as safe. In Phase 1 Clinical Trial two healthy skin volunteers participated to test the medication safety and result proved it safe. In Phase 2 Clinical Trial three patients participated voluntarily and used the medication. Results proved

this antifungal cream as effective as it cured all the three patients in very short time. This type of study is performed for the first time.

**KEYWORDS:** Plants therapeutics, Anti-Fungal Cream, Jock Itch, Tinea Cruris.

## INTRODUCTION

Tinea cruris is disease known with many names like scrot rot, Dhobi itch, eczema marginatum, gym itch, crotch itch, crotch rot, jock rot and jock itch. (Rapini et al., 2007; James et al., 2006) This disease is caused by a dermatophyte fungi i.e., it is dermatophytic fungal infection of the groin areas of body in both male and female (Tinea Cruris in Women., 2005) but most commonly appears in male. Symptoms of this dermatophytic infection appears as ring like rash which are formed on groin regions. They appear as red or reddish-brown patches causing irritation with clearly made rings. This infection causes the burning sensation in the areas of infection. Most often this infection starts from under buttocks and other groin parts and could be transfer to any part of the skin as this infection is transmissible. It covers the inner parts of thighs and other genital areas, also extends to the perineum and the perianal areas. Colour of the affected skin areas become red, brown or tan, with flaking, rippling, peeling or cracking skin. (www.med.nyu.edu).

Acute form of infection starts on the area vicinity of groin fold half inch square which expands on all sides. Infected rings may get enlarge and lesions, sores or small wounds may develop. This itch or rash has very fine borders that may become blister and ooze. (www.medlineplus.gov). This Opportunistic disease is msot oftnly appeared due to diminishing of the imune system. An athelets's foot infections can also spread from infected feet areas to the groin regions via infected clothing. Tight and restrictive clothing which entraps heat and moisture such as jockstrap underwear is the best growing condition for *Trichophyton rubrum.* Its causative agent is dermatophytic fungi *Trichophyton rubrum.* (www.med.nyu.edu).

Tinea cruris which is caused by *Trichophyton rubrum* is best treated with topical antifungal medications of the allylamine or azole i.e., Fluconazole. (Nadalo et al., 2006) Plants possess many constituents that have anti-fungal activities. Most of the anti-fungal agents isolated from plants have no side effects (Onaran and Yilar, 2012). According to WHO about 80% of the world population depends directly on plants for their medicinal needs (WHO., 2014). In the current research, Plants and plants products had been selected as suggested by herbalists

and gypsies during ethnobotanical survey for Tinea cruris disease commonly known as jock itch.

## MATERIALS AND METHODS

### Fungal Strains

Tested strains of pathogenic fungus were obtained from Department of Biotechnology Mirpur University of Science and Technology (MUST) Mirpur, AJK, Pakistan, and sub-cultured on potato dextrose broth slants at 4 degree centigrade.

### Ethnobotanical survey

Ethnobotanical survey was made to consult local herbalist and gypsies to get acknowledge about plants species commonly used by them to treat jock itch. They were shown with jock itch symptoms in images and videos format, common names were used to ask about disease treatment. A list of suggested plants was made and further sections were made on the basis of plants suggestions frequency.

### Plants material

*Curcuma longa*, *Azadirachta indica*, *Allium sativum*, *Apple cider apple cider* apple cider vinegar, *Aloe vera* gel and *Lavandula Lavandula* were purchased from Mega Mart F-1 Mirpur Azad Kashmir.

### Preparation of ethanolic Extract

Selected plants species were shade dried for two weeks and then their twenty-gram samples of each plant and 20 ml in case of *apple cider apple cider* apple cider vinegar were soaked in 480 ml of 95% ethanol for three days. extracts are filtered with muslin cloth in 1000 ml beaker and stored at four degree centigrade. (Olayemi and Opaleye., 1999).

### Preparation of water Extract

Selected plants species were shade dried for two weeks same as for ethanolic extracts and then their twenty-gram samples of each plant and 20 ml in case of *apple cider apple cider* apple cider vinegar were soaked in 480 ml of water for three days. extracts are filtered with muslin cloth in 1000 ml beaker and stored at four degree centigrade. (Olayemi and Opaleye., 1999).

**Isolation of Fungus from Clinical survey**

Total of five patients were selected during isolation of clinical strains of *Trichophyton rubrum* from civil hospital DHQ Mirpur AJK Pakistan. Their infected hair and scalps were removed from Infected areas of patient's skin and grown on sabouraud dextrose agar after incubating at 30-33 degree centigrade for fifteen days. These strains isolated were identified using microscopic analysis and growth characteristics.

**Maintenance of Strains on culture**

Clinical pathogenic fungal strains were maintained on potato dextrose broth slants at 4 degree centigrade.

**Diagnostic Test for Isolated Fungus**

**Bromocresol purple (BCP) milk solid glucose agar test of Isolated Fungus**

Bromocresol purple (BCP) milk solid glucose agar test was used to identify *Trichophyton rubrum*, as different dermatophytic fungi release different amounts of ammonium and alter the PH of the medium hence change the colour of medium while *Trichophyton rubrum* don't alter PH hence the colour of medium remains unchanged sky blue indicating neutral PH. All the fungus strains which get identified through this test were maintained on potato dextrose agar for further experiments. (Kane., 1997; Weitzman., 1995).

**Morphology Test of Isolated Fungus**

It consisted on small and tear drop shaped microconidia and its blood red colony reversed the pigmentation on many of the growth media (Kane., 1997; Weitzman., 1995).

**Determination of antifungal activity**

The screening of the extracts for antifungal effect was carried out by determining the zone of inhibition using disc diffusion method. Sterile potato dextrose agar plates were prepared. Then 0.1 ml of spore's suspension of tested organism was taken from the stock (broth) and swabbed on the agar medium in aseptic condition. The filter paper disc of 2 mm diameter (Whatman's No.1 Filter paper) were prepared and sterilized. The plant extracts to be tested both ethanolic and water extracts were added to each disc of holding capacity of 10 µl [Soylu et al 2005]. The sterile impregnated disc with plant extracts were placed on the agar surface with framed forceps and gently pressed down to ensure complete contact of the disc with the agar surface. Positive control disc of Fluconazole (10 µl) were prepared and placed on the agar surface. The cultured plates were incubated at 37°C for 3 to 5 days. After incubation, the

antifungal activity area was measured the zone of inhibition by two directions at right angles to each other against test organisms. Experiments were carried out with three replicates per treatment and each treatment was repeated at least three times [Obagwu et al 2003].

**Water/Aqueous Phase**

Glycerine was taken 9%, Ethanolic plants extracts mix was taken 10% and water was taken 60% All these ingredients were taken in porcelain dish and heated at degree centigrade. Final product of this stage will act as Water/Aqueous Phase. (Ashwini et al., 2014; Chevalier., 1996.; Chevalier., 2016; Frawley., 2000).

**Oil Pase**

Cetyl alcohol (9%) propyl paraben (11%), potassium hydroxide (0.5%), sodium carbonate (0.5%) were taken in porcelain dish and this mixture was melted at 70 degree centigrade. This will act as Oil Phase. (Ashwini et al., 2014; Chevalier., 1996.; Chevalier., 2016; Frawley., 2000).

**Formulation of Cream**

Aqueous/Water phase was added drop by drop to Oil phase with constant stirring at 70 degree centigrade. It was continuous stirring until complete emulsification occurred and homogenous cream was formed. (Ashwini et al., 2014; Chevalier., 1996.; Chevalier., 2016; Frawley., 2000).

**Intense/Acute skin Irritation Test as Pre-Clinical Trial**

This test was performed on 12 months old four shaved skin rabbits of either sex. The 100 mg of the all the two plants ethanolic and water extracts mix were applied on one square centimetre area of entire shaved skin. 1$^{st}$ Rabbit was applied with ethanolic extracts while 2$^{nd}$ Rabbit with water extract, 3$^{rd}$ Rabbit was applied with prepared anti-fungal cream and 4$^{th}$ Rabbit was applied with 0.8% formalin a standard irritant and acted as control to notice any signs of allergy, oedema or erythema. This Pre-Clinical Trial study is used to test the safety of the medicine. (Marzulli and Maibach, 1997).

**Phase 1 Clinical Trial**

Two healthy skin Volunteers were applied with prepared anti-fungal cream to test the safety of developed medicine. Any signs of allergy will be led us to cease study and positive results

with led the research to proceed further on patient volunteers to test its medicinal efficacy. (DeMets *et al.*, 2010; Fisher *et al.*, 2015).

**Phase 2 Clinical Trial**

Normally this stage takes 100-300 patients in count but as referencing to scope of study, we took three patients to subject to this research. Patients were check up physician and then applied with this developed anti-fungal cream. (DeMets *et al.*, 2010; Fisher *et al.*, 2015).

**Statistical Analysis**

The resultant of clear zones around the discs were measured in mm. Data of all experiments were statistically analysed and expressed as Mean.

**Evaluation of Herbal Ointment**

**Colour and Odour**

Visionary and Smell sense was used to observe colour and odour of anti-fungal cream. (Sahu *et al.*, 2011).

**Consistency**

Anti-fungal cream was observed as smooth and no sign of voracity was appeared. (Sahu *et al.*, 2011).

**PH**

100 ml of water was added with 1 gram of anti-fungal cream and set at rest for two hours, then its PH was noted using digital PH meter. (Panigrahi *et al.*, 1997).

**Spread ability**

Spread ability of the cream was determined by experiment was described by Wood *et al.* (1963). Formula used was (S = M.L/T). where S = spread ability, M = Weight tied to upper slide, L = Length of glass slides and T = Time taken to separate the slides completely from each other. In this present experiment, M = 80 g, L = 10 cm and T was recorded in table (Ehrlich and Hunt, 1968).

**Extrudability**

Anti-fungal cream was filled in collapsible tube holder and its extrudability was found out by the weight required by anti-fungal cream to extrude 0.5 cm of ribbon of the ointment in 10 seconds. (Ravindra and Muslim., 2013).

**Diffusion study**

A gap board was placed on the ager medium filled with anti-fungal and diffusion time was noted for cream after 1h. (Sahu *et al.*, 2011).

**Loss on Dying**

Dish on water bath at 105 degree centigrade filled with anti-fungal cream and let it to dry to find LOD. (Kuchekar and Bhise., 2012).

**Solubility**

Dissolvable in boiling water to some extent, miscible with liquor, ether, chloroform. (Ashwini *et al*., 2014).

**Stability**

Stability test was applied for anti-fungal for one six weeks at 2°C, 25°C and 37°C. (Ashwini *et al*., 2014).

**RESULTS AND DISCUSSION**

*Trichophyton rubrum* was isolated from clinical survey from DHO Hospital patients identified through its morphology and Bromocresol purple (BCP) milk solid glucose agar test. Tear drop shaped and blood red colony were its top descriptive morphological characters while not changing of media colour from blue sky was result of its Bromocresol purple (BCP) milk solid glucose agar test. Those strains which have shown these results and maintained on potato dextrose agar. *Apple cider apple cider* apple cider vinegar and Five plants extracts antifungal activity is tested against *Trichophyton rubrum* fungus which cause jock itch. Fluconazole which is standard antifungal agent is used as positive control. Disc diffusion method is applied and Zone of Inhibition is measured in mm using Vernier callipers. Water extracts of plants appeared in ZOI as, Curcuma longa Extracts ZOI was 22mm, *Azadirachta indica* 26mm, *Allium sativum* 24mm, *Apple cider apple cider* apple cider vinegar 27mm, *Aloe vera* gel 29mm, *Lavandula Lavandula*30mm and Fluconazole which was positive control made 40 mm ZOI on the plate. As it was seen from results that Fluconazole which is positive control showed antifungal activity which is 0.65 times than that of plants extracts and *apple cider apple cider* apple cider vinegar. Ethanolic plant extracts anti-fungal activity were shown in ZOI as, Curcuma longa Extracts ZOI was 24mm, *Azadirachta indica* 27mm, *Allium sativum* 32mm, *Apple cider apple cider* apple cider vinegar

24mm, *Aloe vera* gel 33mm, *Lavandula Lavandula*35mm and Ctrl ZOI was 41. Results are given for individual plant extracts in water and ethanol in Table no.1 in terms of ZOI.

As it could be seen from results above that anti-fungal activity in terms of zone of inhibition of ethanolic plants extracts is way greater than that of water extracts so we took the ethanolic plants extracts for further study. We mixing all the ethanolic extracts and *apple cider apple cider* apple cider vinegar in equal proportion and then tested its activity and again against *Trichophyton rubrum* using fluconazole as control. This time results wondered us as positive control made 42 mm ZOI while water extracts made 38mm and ethanolic extract made 57 mm ZOI. Ethanolic extracts have shown antifungal activity which is 1.35 times higher than positive control which weighs more than previous values. These extracts used to make cream. Results are given in Table no. 2 for mixed plants water and ethanolic extracts.

Ethanolic extract was used because it had shown most powerful antifungal activity. Both water and ethanolic extracts along with anti-fungal cream was applied on the shaved skin of three rabbits individually with the fourth one rabbit acted as positive control and applied with 0.8% formalin. On all the 1 cm square area of shaved skin of rabbits no sign of irritation that oedema and erythema was observed for constant seven days except for ctrl which shown these signs. Pre-Clinical Trials came up with positive results so this cream was judged safe. Results are given in Table no.3 for pre-clinical Trials.

For phase 1 and 2 Clinical Trials we take Consent Forms from two healthy normal skin and three jock itch diseased patient who presented their selves voluntarily after reading and understanding the statements written on the Forms which clearly defined what worse can happen and questioning to their satisfaction.

Phase 1 results proved safety of anti-fungal cream as they appeared with no signs of irritation or allergy. Results are given in Table no.4 for phase 1 trial.

In Phase 2 Clinical Trials All the Three patient showed 100% positive result with totally cured skin. More patient showed with no side effects and signs of allergic reactions. Two of patients started showing positive results just after two days and it five days for third patient to show the positive sign of healing. Again, no negative signs were appeared. Results are given in Table no.5.

Results of various test and physical parameters applied on anti-fungal cream are given in Table no. 6, cream color was observed with eyes appear golden brown, Odor was smelled using nostrils was Characteristic, Its Consistency value is Very smooth, Ph was 5.4, Spreadable was measured in time was 5seconds , Acute Irritancy test have shown its as Non-irritant, its Extrudability is 0.6 gm and Diffusion study have given the results 0.9 cm measured after 1 hour, Loss on dying was 36%, its Solubility was characteristic i.e., its characteristic Soluble in boiling water to some extent, organic solvents, Wash ability was Very Good and Stability test have proven it as Stable at 2°C, 25°C and 37°C for six weeks.

**Table 1: Showing Zone of Inhibition made by Ethanolic and Water Plants Extracts individually.**

| S./N. | Botanical name | Common name | ZOI of Water Extracts | ZOI of Ethanolic Extracts |
|---|---|---|---|---|
| 1 | *Curcuma longa* | Turmeric | 22 | 24 |
| 2 | *Azadirachta indica* | Indian lilac | 26 | 27 |
| 3 | *Allium sativum* | Garlic | 24 | 32 |
| 4 | Apple cider vinegar | Vinegar | 27 | 24 |
| 5 | *Aloe vera* gel | Aloe vera | 29 | 33 |
| 6 | *Lavandula Lavandula* | lavender | 30 | 35 |
| 7 | Fluconazole | Anti-fungal agent | 40 | 41 |

**Table 2: Showing Zone of Inhibition made by Ethanolic and Water Extracts mix of all Plants.**

| Mixed plants Extracts in equal ratios | | |
|---|---|---|
| **S./N.** | **Extract** | **ZOI** |
| 1 | water | 38 |
| 2 | ethanol | 57 |
| 3 | Ctrl | 42 |

**Table 3: Showing results of irritation test on animals as Pre-Clinical Trials.**

| S./N. | Rabbits | Application | Irritation Signs |
|---|---|---|---|
| 1 | Rabbit 1 | Water Extracts mix | No |
| 2 | Rabbit 2 | Ethanolic Extracts mix | No |
| 3 | Rabbit 3 | Anti-fungal Cream | No |
| 4 | Rabbit 4 | 0.8 % Formalin | Yes |

**Table 4: Showing medicinal safety as Phase 1 Clinical Trials on two healthy skin volunteers.**

| Volunteers | Signs of irritation | Drug Safety |
|---|---|---|
| V1 | Nil | +ve |
| V2 | Nil | +ve |

**Table 5: Showing Response time, irritation and remarks as Phase 2 Clinical Trials.**

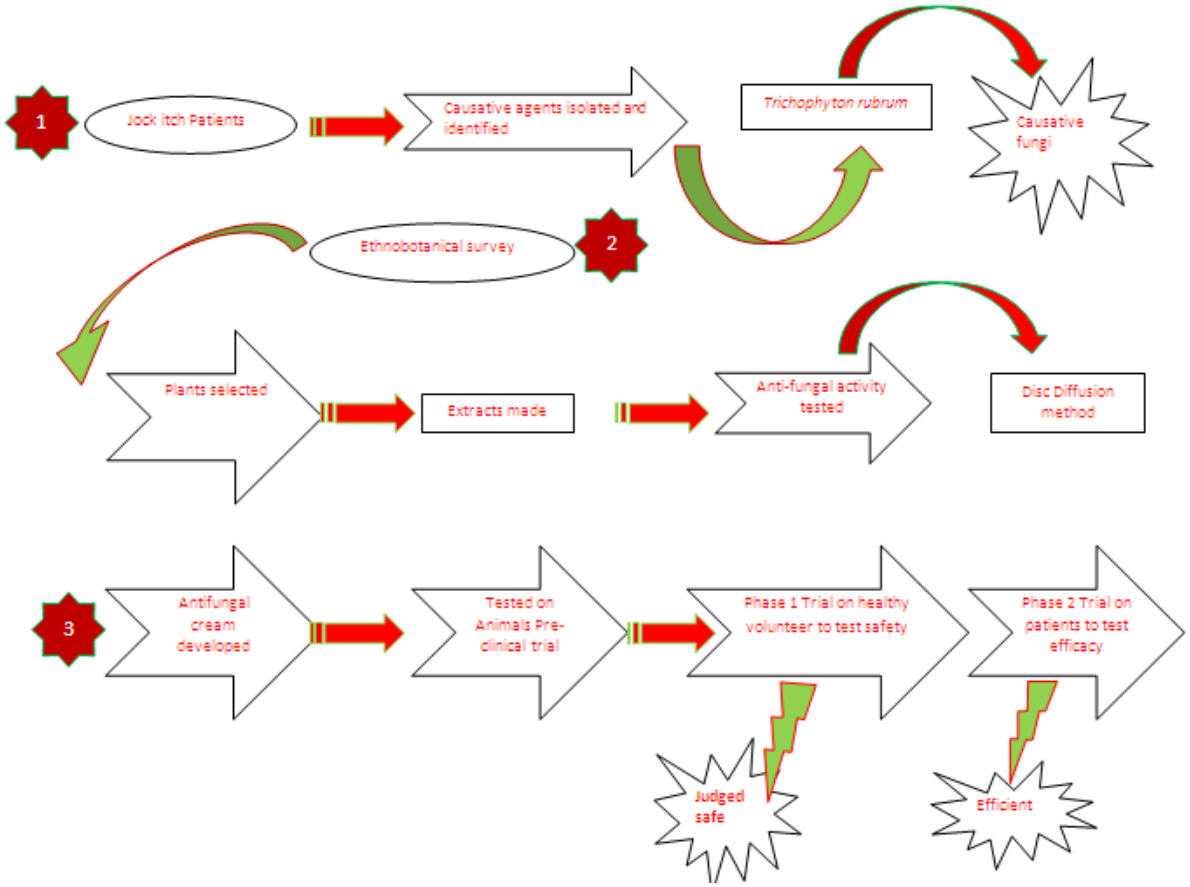
| Patients | No. Days after start functioning | Remarks | Signs of irritation |
|---|---|---|---|
| P1 | 3 | +ve | Nil |
| P2 | 3 | +ve | Nil |
| P3 | 5 | +ve | Nil |

**Table 6: Showing results of various physical parameters and test applied on cream.**

| S./N. | Physical Parameters | Results |
|---|---|---|
| 1 | Color | Golden Brown |
| 2 | Odor | Characteristic |
| 3 | Consistency | Smooth |
| 4 | Ph | 5.4 |
| 5 | Spread ability | 5 seconds |
| 6 | Acute Irritancy | Non-irritant |
| 7 | Extrudability | 0.6 gm |
| 8 | Diffusion study | 0.9 cm after 1 hour |
| 9 | Loss on dying | 36% |
| 10 | Solubility | Characteristic Soluble in boiling water to some extent, organic solvents |
| 12 | Stability | Stable at 2 °C, 25 °C and 37 °C for 6 weeks |

**Figure 1: Physical appearance of Muhammad Abdullah Rashid Anti-fungal Cream.**

**Graphical Abstract**




## CONCLUSION

Our results conclude that isolated fungus strains were *Trichophyton rubrum* strains. Ethanolic extracts are more powerful anti-fungal than water extracts. Both water and ethanolic extracts including formulated cream are safe and non-irritant proved from both Pre-Clinical and Phase 1 Clinical Trials. Cream is efficient and effective in treatment of *Trichophyton rubrum* caused disease jock itch. More this anti-fungal cream has intensive market and industrial values also. This type of study was performed by the first time, so it could be concluded as novel.

### Dedication

The author would like to dedicate this research to Mr. Muhammad Abdullah Rashid for his support and name this product after him as Muhammad Abdullah Rashid Anti-fungal Cream.

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