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## Antifungal efficiency of three traditional medicinal plants against *Trichophyton rubrum*

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### ABSTRACT

Cutaneous fungal infection is a wide-spread public health concern affecting millions of people all across the world. Nearly half of the affected will experience multiple episodes of infection requiring numerous rounds of treatment. *Trichophyton rubrum* was the most common etiological agent of dermatophytes, and it is emerging as an important and significantly prevalent infection in an increasingly aging population and immune-compromised patients. Development of more effective and less toxic anti-fungal agents is required for the treatment of dermatophytosis. The aim of the study was to evaluate the anti-fungal activity of extracts of three plant species used in traditional medicine against *Trichophyton rubrum*. The ethanol and water extracts of *Eucalyptus globules*, *Croton macrostachyus*, and *Phytolacca dodecandra* leaves were evaluated in vitro for anti-fungal activity against *Trichophyton rubrum*, using the agar well diffusion technique. The mean inhibition zone of both extracts for *E. globulus*, *C. macrostachyus* and *P. dodecandra* were 19.8, 20, and 16.3 mm, respectively, and mean inhibition zone of the ethanol and water extracts were 23 and 14.4 mm, respectively. Generally, mean inhibition zone of plant extracts did not show statistically significant difference among plants and the mean inhibition zone of plant extracts did show statistically significant difference between extracts ( $P \leq 0.05$ ). On the basis of the current findings, *Eucalyptus globules* and *Croton macrostachyus* could be good candidates in the search for new antifungal agents from natural products against *Trichophyton rubrum*. Therefore, further studies are needed to study their toxicology and isolate the bio-active components from these plants.

**Keywords:** Dermatophytes, *Trichophyton rubrum*, *Eucalyptus globules*, *Croton macrostachyus*, *Phytolacca dodecandra*

## 1. INTRODUCTION

Dermatophytes are pathogenic fungi that have a high affinity for keratinized structures, like nails, skin or hair, causing superficial infections known as dermatophytoses in both, humans and animals (Luciene *et al.*, 2008). Dermatophytosis commonly accompanied by blister-like lesions are appearing along the webs of the toes (*Epidermophyton*), on the scalp or skin (*Microsporum*), or in the nail plate (*Trichophyton*) (Irene and Richard, 1995).

Dermatophytes secrete keratinase enzyme, is an enzyme that degrades keratin (a protein found in hair, skin and nails). This infection is transmitted from animal to animal by direct contact or by contact with infected hair, and epidermal cells (as from barber shop, clippers, or shower floors). The fungi that cause superficial mycosis are localized along the hair shafts and in superficial (surface) epidermal cells (Hogewoning *et al.*, 2006).

*Tinea pedis* is a fungal infection of the feet and usually related to sweating and warmth, and use of occlusion causative agents of the *tinea pedis* (athlete's foot) is *T. rubrum* (the most prevalent pathogenic fungus worldwide and it represents 80% of clinical isolate.), *E. floccosum* and *T. mentagrophyte*. Multiple dermatophytes are seen in the scales. This infection in most causes the epidermal dermatophytosis; the infection occurs initially on the feet (*T. rubrum*), and in time, spreads to sites, such as the inguinal area (*Tineacruris*), trunk, arms, legs (*tineacorporis*) and hands *tinea manuum* (Klaus *et al.*, 2013).

*Tinea pedis* (athlete's foot) infection is the major health problem that is one of the causes of economic, social, and physical crisis in a society. The highest rate of fungal infection, especially tinea pedis, affects all age of the people, but people of the age between 20-50 years old are highly vulnerable to this infection (Klaus and Richard, 2009). The other problem of this infection is its difficulty to treat and cure with chemical fungicides and these fungicides have side effects on skin.

Medicinal plants form the basis of traditional or indigenous health systems used, in the estimate of the World Health Organization, by the majority of the population of most developing countries. More than 35,000 plant species are being used in various human cultures around the world for medical purposes and many of them are subjected to uncontrolled local and external trade. People living in rural areas from their personal experience know that these traditional remedies are valuable source of natural products to maintain human health, but they may not understand the science behind these traditional medicines. Some medicinal plants are highly effective when it used at therapeutic doses. Traditional use of medicine is recognized as a way to learn about potential future medicines (Al-Bakri and Afifi, 2007).

*Eucalyptus globules*, *Croton macrostachyus* and *Phytolacca dodecandra* are medicinal plants that have been used traditionally for the treatment of different infectious diseases. The Aborigines (native Australians) have traditionally used eucalyptus leaves to heal wounds and fungal infections. The extracts exhibited various biological effects, such as antibacterial and anti-fungal activity (Takahashi *et al.*, 2004). *Croton macrostachyus* have significant role on the ring worm infection treatment in which its fresh leaf is applied for tropical treatment (Abyu *et al.*, 2014). It is discovered as *phytolacca dodecandra* has antifungal and antibacterial activity (Kjell *et al.*, 2008). However, these plants were used for treatment of different diseases, with the use of locally made medicines prepared as infusions in hot water or mixed with food to treat infection and the majority of the evidence on the antimicrobial activity of this plant was anecdotal and lacked scientific validity. Therefore, this study was aimed to evaluate antifungal efficiency of three traditional medicinal plants against *Trichophyton rubrum*.

## **2. MATERIAL AND METHODS**

### **2. 1. Study Design and Area**

Cross-sectional experimental study design was conducted to evaluate antifungal efficiency of three traditional medicinal plants against *Trichophyton rubrum* at Debre Tabor University, laboratories of Biology Department, Debre Tabor town, from April to June 2016.

### **2. 2. Plant Collection and Preparation**

Traditional medicinal plants (*E. globulus*, *P. dodecandra*, and *C. macrostachyus*) were collected around Debre Tabor Town. These plants were washed with a tap water, dried in an open air, separately powdered to suitable size and made ready for extraction (Sukhdev *et al.*, 2008).

#### **2. 2. 1. Extraction of plant materials**

Plant materials were extracted by maceration technique with ethanol and water solvents with occasional shaking at room temperature for three days. Grinded plant materials were soaked with each solvent separately at 10:1 solvent-to sample ratio (v/w). The extracts were separately filtered by Whatman No. 1 filter paper and concentrated with dry oven. Further, fresh solvents were added to the residue at the same ratio until required amount of extracts were obtained. The dry extracts were stored in sample bottles in refrigerator for further use (Sukhdev *et al.*, 2008).

#### **2. 3. Isolation of *Trichophyton rubrum***

A small portion of the infected person's skin was washed by using alcohol as disinfectant. This disinfected/washed portion of the skin was rushed using cotton swamp and small knife. The rushed sample was transferred/ spread over the Petri dish containing Potato Dextrose Agar (PDA) media containing streptomycin, and then it was incubated at 37 °C to induce the growth of the fungi for three days. Until pure culture was obtained, the isolated samples were transferred aseptically to fresh PDA agar plates. Identification of *Trichophyton rubrum* to the genus and species level was based on morphological characterization that places emphasis on colony characteristics, colony colour and shape for microscopic characteristics of their hyphae. The cultural features observed will be compared with those contained in the Fungal Colour Atlas (Haley *et al.*, 2000; Frey *et al.*, 1979).

#### **2. 4. Anti-fungal Efficiency of Traditional Medicinal Plants against *Trichophyton rubrum***

The antimicrobial activity tests of the plant extracts against *Trichophyton rubrum* were carried out by the agar well diffusion method, which is commonly used for screening of the anti-microbial activities of herbal drugs (Ramesh *et al.*, 2002). The standardized fungal cultures were swabbed on PDA (Himedia) plates using sterile cotton swabs. After thirty minutes, on each plate, five equidistant wells were made with a 6 mm diameter sterilized cork borer. Stock solution of each plant extract was prepared using sterilized distilled water at concentration 1 g/mL and 0.1 mL of each plant extracts were added into the wells aseptically. The plates were allowed to diffuse at room temperature for 2 hours and incubated at 37 °C for 3 days in triplicates. Sterile distilled water without plant extract was used as negative control. Zone of

inhibition was measured in mm (ruler) and value greater than the control was considered as active extract against fungus (Wayne, 2002).

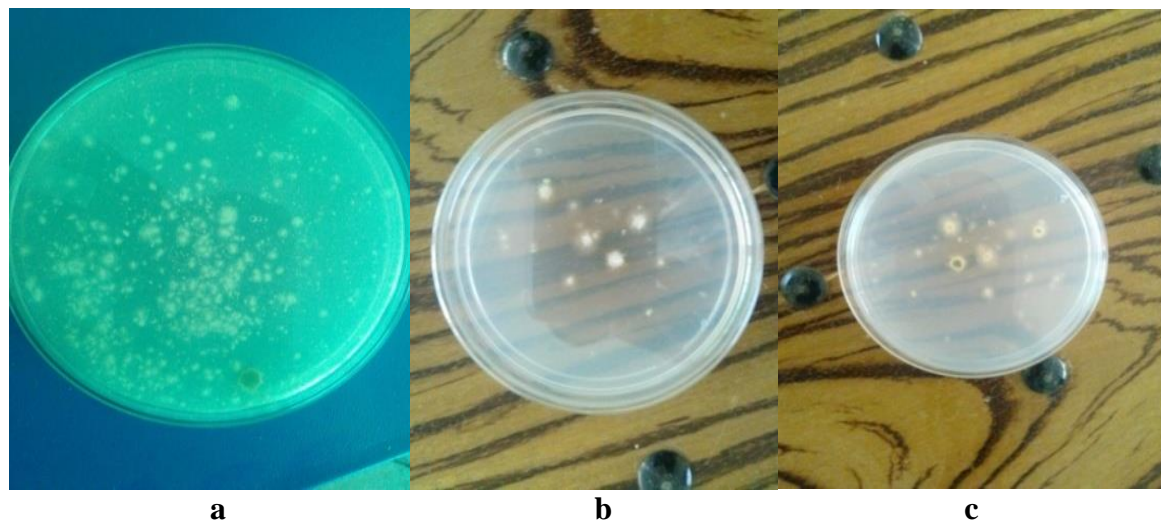
## 2. 5. Data Analysis

After completion of data collection, each measurements of the different variables were systematically organized into table and figures and subsequently subjected to statistical analysis. Data analysis was done using the SPSS computer software version 20.0. ANOVA was used to compare mean values among plant extracts. P-values less than 0.05 were considered statistically significant.

## 3. RESULTS AND DISCUSSIONS

### 3. 1. Isolation and identification of *Trichophyton rubrum* from infected person

As can be seen from **Fig. 1** (a), the fungi that isolated from infected leg of a patient were characterized based on colony morphology. As indicated in **Fig. 1** (b) and (c) the colony showed whitish and cottony morphology on the surface and deep brown colour on the reverse. From this characterization, the isolate was *T. rubrum* and the morphology of this isolate was similar to the isolate characterized by Haley *et al.* (2000) and Frey *et al.* (1979).



**Figure 1(a-c).** Colonies of *Trichophyton rubrum* on PDA media

### 3. 2. Antifungal activities of traditional medicinal plants extracts against *Trichophyton rubrum*

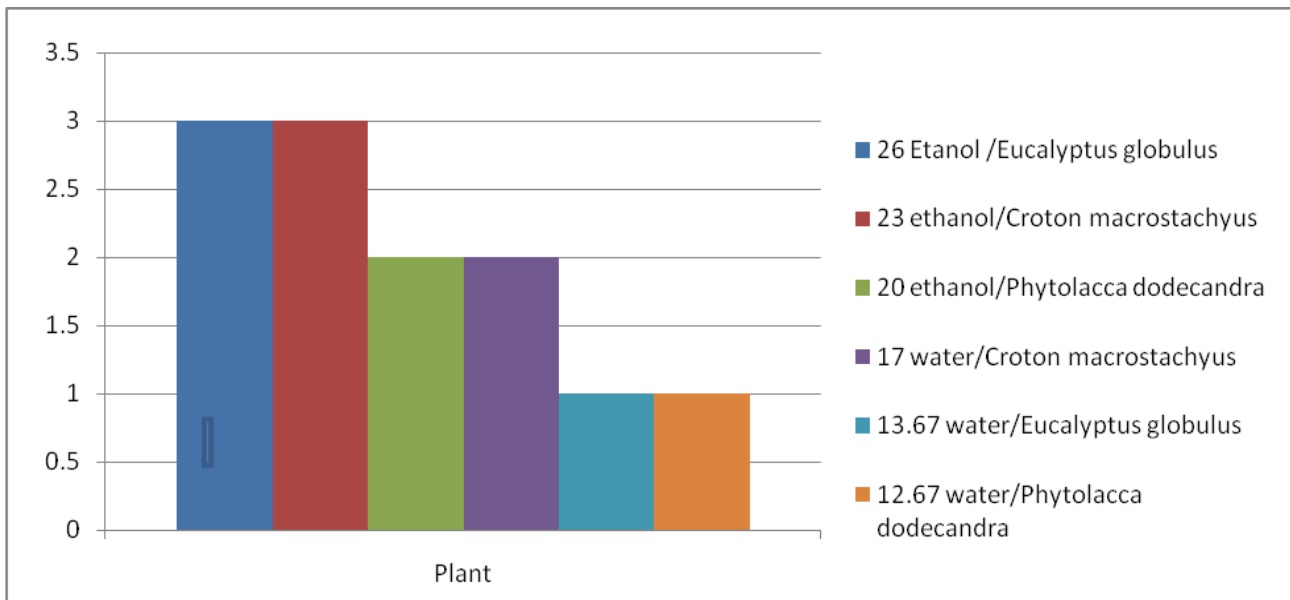
As the results indicated in the **Table 1** / **Fig. 2**, the mean inhibition zone of ethanol extracts for *E. globulus*, *C. macrostachyus* and *P. dodecandra* were (26, 23, and 20) mm, respectively. Water extracts of *E. globulus*, *C. macrostachyus* and *P. dodecandra* showed the mean inhibition zone of (13.67, 17, and 12.67) mm, respectively.

The mean inhibition zone of both extracts for *E. globulus*, *C. macrostachyus* and *P. dodecandra* were (19.8, 20, and 16.3) mm, respectively (Fig. 2). Mean inhibition zone of plant

extracts did not show statistically significant difference among plants ( $P \leq 0.05$ ). The mean inhibition zone of the ethanol and water extracts were 23 and 14.4 mm, respectively, and the mean inhibition zone of plant extracts did show statistically significant difference between extracts ( $P \leq 0.05$ ) (Table 1).

**Table 1.** Antifungal activity of traditional medicinal plants against *Trichophyton rubrum*

		Mean mm	N	Mean square	F value	P
Plants	<i>Eucalyptus globulus</i>	19.83	6	25.7	0.81	0.46
	<i>Croton macrostachyus</i>	20	6			
	<i>Phytolacca dodecandra</i>	16.3	6			
Extracts	Ethanol	23	9	329.4	10.41	0.006
	Water	14.4	9			



**Fig. 2.** Antifungal activities of traditional medicinal plants extracts against *Trichophyton rubrum*

Ethanol extracts of all plants have shown higher anti-fungal activities on *T. rubrum*, while water extracts of these plants showed the least anti-fungal activity which were in agreement with the works of Adejamo and Bamidele (2009) and Ke-Qiang *et al.* (2001), who reported that, ethanol extracts of *C. alata* and *J. gossypifolia* have shown higher anti-fungal activities than

water extracts on *T. rubrum*. All ethanol extracts were very effective on *T. rubrum*, more than the water extract.

The mean inhibition zone of plant extracts did show statistically significant difference between extracts ( $P \leq 0.05$ ) (Table 1). Many reports have indicated that the ethanol extracts of plants parts were more inhibitory than the aqueous extracts, which suggest that ethanol may be a better extracting solvent (Ke-Qiang *et al.*, 2001).

Among the three traditional medicinal plants, ethanol extract of *E. globulus* had the greatest effect with the mean inhibition zone of 26 mm on *T. rubrum*, while *P. dodecandra* had the least effect on *T. rubrum*, with mean inhibition zone of 20 mm. Water extract of *C. macrostachyus* had the greatest effect with mean inhibition zone of 17 mm on *T. rubrum*, while *P. dodecandra* had the least effect on *T. rubrum* with the mean inhibition zone of 12.67 mm.

#### 4. CONCLUSIONS

The results in the present study clearly indicate that ethanol extract of the tested plants had good effect on *T. rubrum*. Ethno-medicinal uses of these traditional medicinal plants, used by communities to treat athlete's foot were effective. On the basis of the current findings, *Eucalyptus globules* and *Croton macrostachyus* could be good candidates in the search for new antifungal agents from natural products against *Trichophyton rubrum*. Therefore, further studies are needed to study their toxicology and isolate the bio-active components from these plants.

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