



## ***In vitro* Antifungal Activity of Combination of Miconazole, Salicylic Acid and Benzoic Acid in Two Different Bases against *Trichophyton mentagrophytes***

**Hassan Thoulfikar A. Almir<sup>1\*</sup>, M. E. L. A. Shayoub<sup>2</sup>, Wisal G. Abdalla<sup>3</sup> and Eltayeb Suliman Elamin<sup>1</sup>**

<sup>1</sup>Faculty of Pharmacy, Omdurman Islamic University, Sudan.

<sup>2</sup>Faculty of Pharmacy, Shayoub M. E. L. A Khartoum University, Sudan.

<sup>3</sup>Central Veterinary Research Laboratory, Department of Mycology, Animal Resources Research Corporation, P.O.Box 8067 (El Amarat), Khartoum, Sudan.

### **Authors' contributions**

This work was carried out in collaboration among all authors. Author HTAA designed the study, wrote the protocol and the first draft of the manuscript. Author MELAS, WGA and ESE managed the analyses of the study. All authors shared the laboratory work read and approved the final manuscript.

### **Article Information**

DOI: 10.9734/MRJI/2020/v30i930263

#### Editor(s):

(1) Dr. Marcin Lukaszewicz, University of Wroclaw, Poland.

(2) Dr. Laleh Naraghi, Iranian Research Institute of Plant Protection, Iran.

(3) Dr. Lachhman Das Singla, Guru Angad Dev Veterinary and Animal Sciences University, India.

#### Reviewers:

(1) D. A. Patil, Kavayitri Bahinabai Chaudhari North Maharashtra University Jalgaon, India.

(2) P. N. Kavitha., K. R. College of Pharmacy, India.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/62320>

**Original Research Article**

**Received 02 September 2020**

**Accepted 09 November 2020**

**Published 11 December 2020**

### **ABSTRACT**

*In vitro* activity of miconazole, salicylic acid and benzoic acid against *T. richophyton mentagrophytes* was done on Sabouraud's dextrose agar. The inhibition zones were measured in cm. 2.8 cm, 1.5 cm and no inhibition zone were reported for miconazole, salicylic acid and benzoic acid respectively. Synergistic effect of salicylic acid and benzoic acid against *T. mentagrophytes* was done using two different bases of cream lanette 20% and aqueous cream 30/70. Four different formula each one contain 2% miconazole with different concentrations of salicylic acid and benzoic acid were used. Biggest inhibition zone (5 cm) was observed of lanette cream which contains 2% miconazole, 3% salicylic acid and 6% benzoic acid.

\*Corresponding author: E-mail: wisalgafar8@gmail.com;

**Keywords:** Miconazole; benzoic acid; salicylic acid; antifungal activity.

## 1. INTRODUCTION

Dermatophytosis is a superficial fungi which causes of superficial skin infections. This fungi is adapted to parasitize keratin layer of the skin through the production of proteases, and is limited to the stratum corneum. Dermatophytosis is mostly caused by *Trichophyton*, *Microsporum* and *Epidermophyton* spp. They can be transmitted by contact with human, animal or soil and occurs in hot and humid climatic conditions [1].

Miconazole nitrate is one of azole class antifungal agent, lipophilic and characterized by relatively high molecular weight, and melting point. It is a weak base with pKa 6.7, high log octanol/water partition coefficient and exhibits poor aqueous solubility (1.03µg/mL).

2% w/w of miconazole is usually employed in topical drug preparations for treatment of dermatophytosis, superficial mycoses, mixed infection, and candida infections [2].

Modified Whitfield's ointment is used prepared by combining of benzoic acid and salicylic acid. The effect of this ointment is due to combination the fungi static action of benzoate with the keratolytic action of salicylate. The traditional half strength formulation contains benzoic acid and salicylic acid in a ratio 2:1 (usually 6%:3%). Benzoic acid beings a fungistatic agent, hence eradication of the infection occurs only after the infected stratum corneum is shed, and continuous medication is required for weeks to months. Salicylic acid is rapidly absorbed by the skin and causes the keratinocytes to swell, soften and desquamate. It causes mild irritation at the site of application [3]. World Health Organization (WHO) suggested a modified Whitfield ointment which containing 5% benzoic acid and 5% salicylic acid as dermatological preparations to be use in tropical countries [4].

The aim of this study was done to investigate *in vitro* synergistic effect of salicylic acid, benzoic acid and miconazole against *T richophyton mentagrophytes*

## 2. MATERIALS AND METHODS

### 2.1 Materials

Materials used for this study were Cetylalcohol (lanette 16), Polyquaternium, water, liquid

paraffin, wax, vaseline, benzoic acid, miconazole and salicylic acid.

### 2.2 Preparation of Formula 1 (F20%)

Cetylalcohol 20 g was weighed and melted by heat and 20 ml of Polyquaternium is added and stirred till base is formed. A total 60 ml of hot water was added gradually and the mixture was cooled till the homogenous cream is formed.

2 g miconazole was mixed with different ratios 6:3, 3:3, 3:1 and 6:1 of benzoic acid and salicylic acid respectively. Emulgel was added to make up 100g of each formula.

### 2.3 Preparation of Formula 2 (F30-70%)

20 ml Paraffin oil, 30 g wax and 50 g of vaseline were mixed and melted by heat. The mixture was cooled till homogeneous emulsifying ointment formed. A total 70g of ointment was taken and mixed with 30ml of hot water, stirred till homogenous aqueous cream formed.

2 g of miconazole was added to different ratios of salicylic acid and benzoic acid. Take sufficient quantity of cream to make up 100 g and stirred well.

Different ratios of two acids, benzoic acid and salicylic acid were used. In the two bases (lanette and aqueous cream) and the inhibition zones were detected. The activity detection was carried out by repeating the culture with the same ratios [5].

### 2.4 Disk Preparations

Under aseptic conditions, sterilized filter paper disks (6mm in diameter) were impregnated with 20µL of salicylic acid, benzoic acid, miconazole. The impregnated disks were dried in laminar flow for 15 minutes at room temperature. The dried disks were used afterwards for the disk diffusion assay [6]. 0.05 g of lanette 20% and aqueous cream 30/70 containing the four different formulas 6%:3%, 3%:3%, 3%:1% and 6%:1% of benzoic acid and salicylic acid respectively.

### 2.5 Preparation of *Trichophyton mentagrophytes*

The *T. mentagrophytes* was obtained from mycology department Central Veterinary

Research Laboratory Soba, Khartoum, Sudan. It was isolated from donkey.

*T. mentagrophytes* was subculture on Sabouraud's Dextrose Agar plates supplemented with 0.05 mg/ml chloramphenicol and 0.5 mg/ml cycloheximide and incubated aerobically at 27°C for 10 days. One drop of Tween 20 was added to the fungi colony then it was brushed with a sterile cotton swab and the mixture of conidia and hyphal fragments was suspended in 1 ml of sterile water. Heavy particles were allowed to settle after vortexing, and the homogenous suspension was adjusted to achieve a turbidity of 0.5 McFarland standards [7].

Duplicates Sabouraud's Dextrose Agar plates were seeded with 0.2 ml of fungal suspension. The plates were incubated for 10 days at 27°C.

The zone inhibition around each disc was measured in cm. The results are presented as mean  $\pm$ SD of zone of inhibition [8].

### 3. RESULTS

The results show that formulas containing lanette 20% gives zone inhibition more than aqueous cream formula, because lanette control without active ingredient produces 1 cm inhibition zone compared to 0 cm in aqueous cream control formula.

It is noted that as the salicylic acid increase, zone inhibition also increases due to its content. Salicylic acid control gave 1.5 cm inhibition zone (Fig. 1). No inhibition zone was observed when using benzoic acid (Fig. 2). Miconazole control gave 2.8 cm zone inhibition.

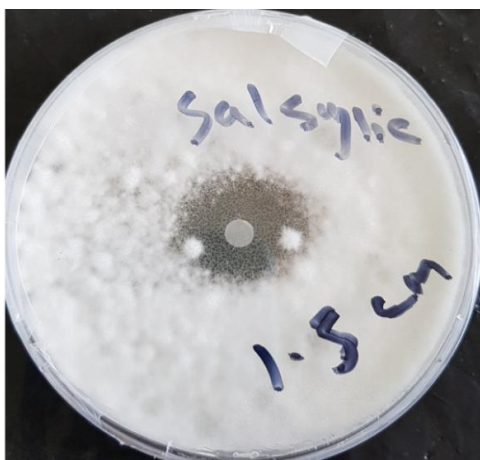


Fig. 1. Shows 1.5 cm zone inhibition salicylic acid

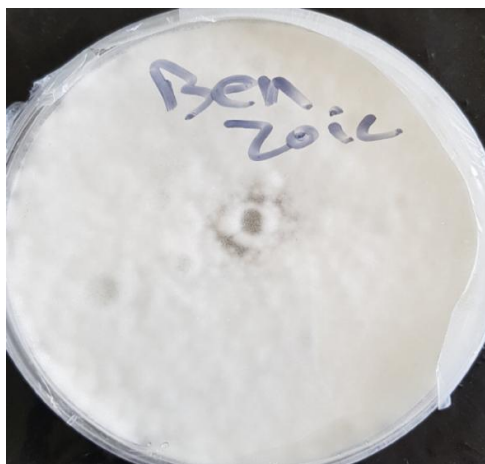
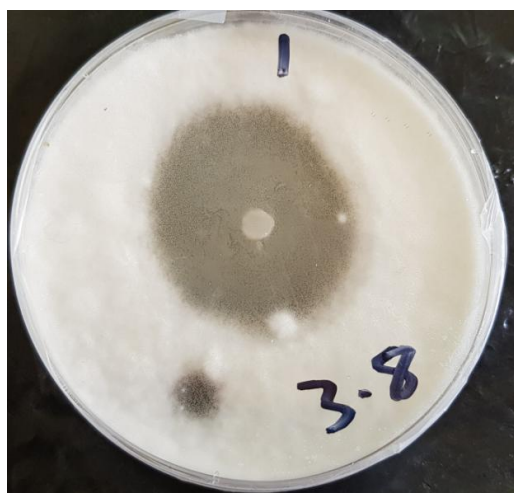


Fig. 2. Shows No zone inhibition of Benzoic acid alone

**Table 1. Mean  $\pm$ SD of zone of inhibition (cm), zones are the mean of three replicates**

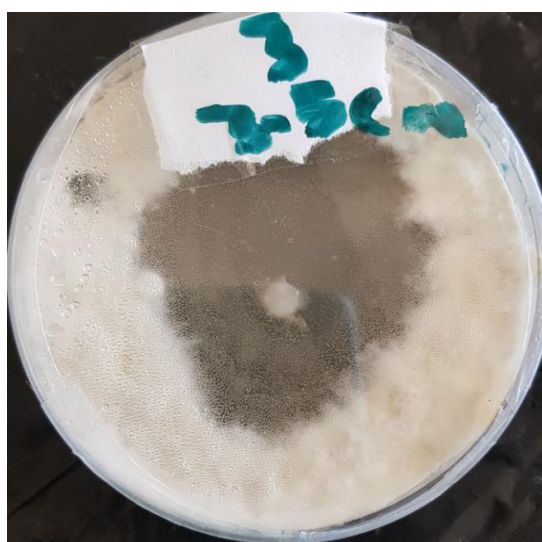
| No. | Formula | Benzoic Acid Concentration % | Salicylic Acid Concentration % | Inhibition Zone In Cm |
|-----|---------|------------------------------|--------------------------------|-----------------------|
| 1   | F2      | 6.00                         | 3.00                           | 3.85 $\pm$ 0.05       |
| 2   | F2      | 3.00                         | 3.00                           | 3.70 $\pm$ 0.1        |
| 3   | F2      | 3.00                         | 1.00                           | 3.55 $\pm$ 0.05       |
| 4   | F2      | 6.00                         | 1.00                           | 3.40 $\pm$ 0.1        |
| 5   | F1      | 3.00                         | 1.00                           | 3.95 $\pm$ 0.15       |
| 6   | F1      | 6.00                         | 1.00                           | 4.05 $\pm$ 0.5        |
| 7   | F1      | 6.00                         | 3.00                           | 4.95 $\pm$ 0.05       |
| 8   | F1      | 3.00                         | 3.00                           | 5.05 $\pm$ 0.05       |



**F2 (1) (Mic 2%, B.A 6%, S.A 3%)**



**F2 (2) ( Mic 2%, B.A 3 %, S,A 3 %)**

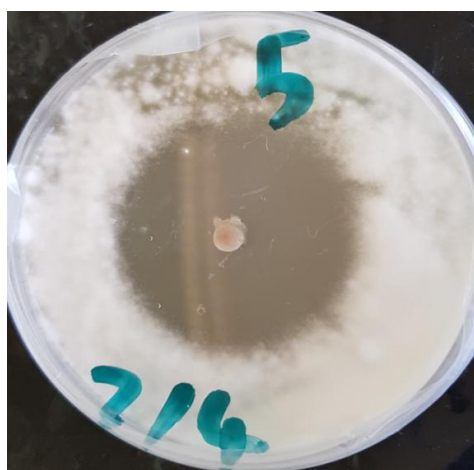


**F2 (3) (Mic 2%, B.A3%, S.A 1%)**

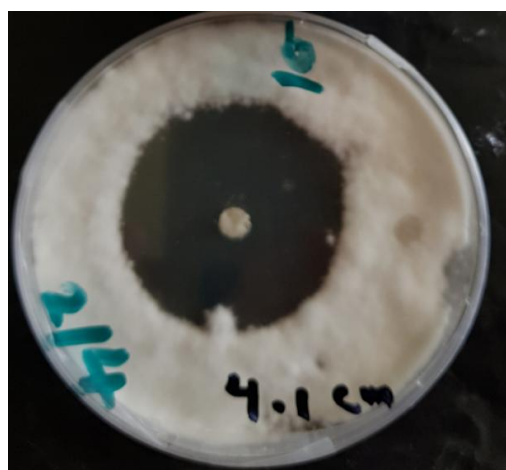


**F2 (4) (Mic 2%, B.A6 %, S.A 1%)**

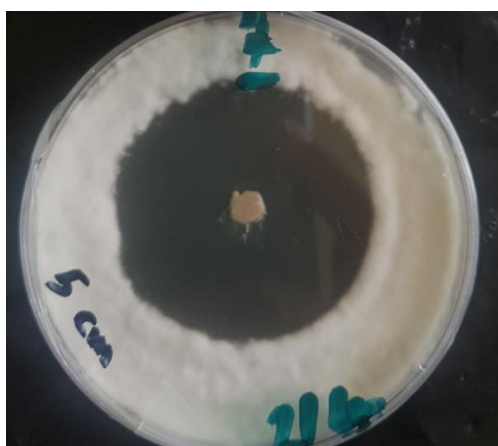
**Fig. 3. Shows zones inhibitions of different of aqueous cream containing 2% miconazole and different ratios of Benzoic acid and Salicylic acid**



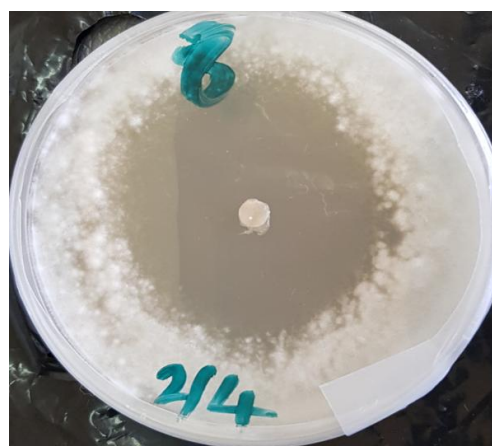
F1 (Mic 2%, B.A 3%, S.A 1%)



F1 (Mic 2%, B.A 6%, S.A 1%)



F1 (Mic 2%, B.A 6%, S.A 3%)



F1 (Mic 2%, B.A 3%, S.A 3%)

Fig. 4. Shows zones inhibitions of different of lanette 20% containing 2% miconazole and different ratios of Benzoic acid and Salicylic acid

#### 4. DISCUSSION

Antifungal activity of salicylic acid observed in this study is in harmony with that previously recorded by [9,10]. Management of dermatophytosis is becoming challenging for all dermatologists across the globe. Whitfield's ointment that contains salicylic acid 3% and benzoic acid 6% was found to be the best formula which gave the biggest inhibition zone. The use of combination of Whitfield's ointment with another topical antifungal drug for treatment of dermatophytosis was previously reported by [11] who used Whitfield's ointment (salicylic acid 3% and benzoic acid 6%) with 1% clotrimazole for treatment Tinea corporis.

#### 5. CONCLUSION

The antifungal activity of the two formulas (f1,f2) containing Miconazole, salicylic acid and benzoic acid was detected by inhibition zone using dermatophyte fungi as microorganism.

The synergistic action was detected and F1 formula gave higher activity containing salicylic acid 3% & benzoic acid 6%.

The F2 base was found to have antifungal activity due to its content of Poly Quaternium.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.



## REFERENCES

1. White JM. Tropical Diseases. 23<sup>rd</sup> Edition. Saunder's Ltd; 2013.
2. Rai VK, Yadav NP, Sinha P, Mishra N, Luqman S, Dwivedi H, et al. Development of cellulosic polymer based gel of novel ternary mixture of miconazole nitrate for buccal delivery. Carbohydr Polym. 2014; 3(103):126-33
3. Goodman G. The Pharmacological Basis of Therapies. 12 edition. McGraw-Hill; 2011.
4. Peter HW, Vincent G, Ben N, Rachel K, Nicolien W. Dermatological Preparations for the Tropics. 2nd edition. Beta Science Shop, University of Gronigen, Netherlands; 2012.
5. Vennat B, Gross D, Pourrat A. Procyanidin Gels Based on Cellulose and Carrageenan Derivatives. Drug Development and Industrial Pharmacy. 1992;18:1535-1548.
6. Nor Raihan M S, Zakaria I, Wan Ismahanisa I, Nurdiana Z, Nor Hafeeda R, Muhammad N F R, Nazar MZMA. Antimicrobial activity of cinnamon oil against bacteria that cause skin infections. Journal of Scientific Research and Development. 2016;3(2):1-6.
7. Ali AY, Rosdan S, Zeehaida M, Irfan M. Antifungal Activity of Malaysian Henna Leaves Extracts on Pathogenic Fungi of Otomycosis International Medical Journal. 2015;22(5):389–391.
8. Saadabi AMA. Evaluation of Lawsonia inermis Linn. (Sudanese Henna) leaf extract as an antimicrobial agent. Research Journal of Biological Sciences. 2007;2(4):419:423.
9. da Rocha Neto AC, Luiz C, Maraschin M, Di Piero RM. Efficacy of salicylic acid to reduce *Penicillium expansum* inoculum and preserve apple fruits. International Journal Food Microbiology. 2016;221:54–60.
10. Mandal S, Mallick N, Mitra A. Salicylic acid-induced resistance to *Fusarium oxysporum* f. sp. *lycopersici* in tomato. Plant Physiology Biochemistry. 2009;47: 642–649.
11. Rahangdale V, Deshmukh S, Vaishnavi I, Bhushan Madke B, Singh AL. Effect of Salicylic Acid 3% + Benzoic Acid 6%(Whitfield's Ointment), Clotrimazole 1% Cream, and Systemic Terbinafine on Mild-to-Moderate Tinea Corporis: A Randomized Comparative Study. International Journal of Recent Surgical and Medical Sciences. 2020;7:1-4.

© 2020 Alamir et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:

The peer review history for this paper can be accessed here:  
<http://www.sdiarticle4.com/review-history/62320>