

## *In vitro* Antifungal Activity of Camel's Urine against Dermatophytes

Mujeeb A. Kabbashi and Al Fadhil A. Omer

Department of Medical Microbiology, Faculty of Medical Laboratory Sciences, University of Sciences & Technology, Khartoum, Sudan

### Abstract

**Background:** Dermatophytes are fungi capable of invading keratinized tissues of humans and animals, causing dermatomycosis. Azoles antifungal drugs are often used in the treatment of dermatomycosis; however, azoles are expensive, toxic, and faced resistance by dermatophytes. Camel's urine proved its efficiency in treatment of skin diseases, and stands as an alternative anti-dermatophyte agent.

**Objective:** To investigate the *in vitro* antifungal activity of camel's urine against dermatophytes

**Materials and methods:** 85 isolates of dermatophytic fungi were examined for their interactions with camel's urine, and the antifungals ketoconazole and fluconazole. Following the protocol outlined by the National Committee for Clinical Laboratory Standards (NCCLS) susceptibility testing guidelines for filamentous fungi (M38-A). Assessment was reported after two incubation periods: 7 and 10 days. The efficacy was based on a minimum inhibition concentration (MIC) ranging from 0.098-25.0 µg/ml.

**Results:** The MIC's of camel's urine was found 0.312-2.5 µg/ml, ketoconazole 0.156-4.0 µg/ml, and fluconazole 0.625-64.0 µl/ml. Hence the order of efficacy was: fluconazole > camel urine > ketoconazole. The MIC response of dermatophytes attained to camel's urine was in the order: *Trichophyton rubrum* > *Trichophyton mentagrophytes* > *Trichophyton violaccum* > *Microsporum gypseum* > *Microsporum canis*, *Trichophyton tonsurans*, and *Epidermophyton floccosum*. Similar tendency was observed for ketoconazole and fluconazole. The order of efficacy was fluconazole > camel urine > ketoconazole.

**Conclusion:** Camel's urine showed a high potential as an antifungal agent against mycoses-causing dermatophytes; with a performance better than the synthetic drug fluconazole, and almost similar as ketoconazole.

**Keywords:** Camel's urine, antifungal drugs, dermatophytes

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## Introduction

Very little is known about the physical, biochemical and antimicrobial properties of camel's urine on various microbes infecting Man and animals. Data available show significant antimicrobial activities against some pathogenic microbes such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and other pathogenic microbes<sup>1</sup>.

The medicinal properties of the Arabian camel were known to Arab physicians. Arabs had been using boiled Arabian camel's urine in therapy. In his magisterial book (Ganon), Ibn Sina (Avicenna) mentioned that chronic imbalance of the liver producing jaundice, dropsy (istisqa) and swelling of the belly could be restored through a temporary diet of camel milk and male Arabian camel urine. Ibn Al -Azraq and Al Suwaydi stated that the cure of dropsy may be accomplished by drinking the camel's milk and urine. The action of camel's urine on human health was explained by Ibn Sayyid Al-Nas who specified that camels feed on warm wood herbs that are extremely useful in correcting human digestive disorders and help detoxification of the liver leading to treatment of hepatitis<sup>2</sup>. Thus Arabian camel's urine was an ancient prescription schedule in Arab medicine; and remained until now as a remedy and as a diuretic, snuff tool, and delousing hair wash<sup>3</sup>.

Al Awadi and Haikal reported that camel's urine had showed antifungal activity against *Aspergillus niger* and *Candida albicans*<sup>4</sup>. Other studies established the antifungal activity of camel's urine against *Rhizoctonia solani* and *Fusarium oxysporum*<sup>5</sup>. Now camel's urine has created a challenge as an antimicrobial agent<sup>6</sup> and was identified as one of the essential elements of primary health care<sup>7</sup>.

In the present study, *in vitro* antifungal activity of camel's urine, ketoconazole, and fluconazole against dermatophytic fungi was investigated. This study aimed to evaluate the micro-dilution

susceptibility testing for the determination of MICs of camel urine and these two antifungal medications.

### Materials and methods

A total of 85 dermatophytes strains: *Trichophyton mentagrophytes* (n=11), *Trichophyton rubrum* (n=33), *Trichophyton violaceum* (n=15), *Epidermophyton floccosum* (n=7), *Microsporum canis* (n=10), *Microsporum gypseum* (n=5), and *Trichophyton tonsurans* (n=4) were investigated. All these fungi were clinical isolates obtained from hair, skin, and nail specimens recovered from patients attending Dermatology Teaching Hospital, Khartoum, Sudan. Quality control strains of *Trichophyton rubrum* (ATCC-10218) were included. These fungal strains were isolated and identified as per standard mycological procedures, and were maintained in sterile distilled water at 4°C until further tests were performed. The strains were sub-cultured on potato dextrose agar (PDA) at 28°C for 7-15 days to ensure the viability and purity of inocula<sup>8</sup>. The standard RPMI 1640 medium (NCCLS guidelines) used was prepared by adding 10.4 g of the dehydrated RPMI 1640 powder (Sigma) to 1000 ml distilled water. Then it was buffered by adding 34.54 g of 3, n-morpholino-propane sulfonic acid. Mixture was stirred by a magnetic stirrer until dissolved. It was adjusted to pH 7.0 at 25°C, sterilized by Millipore filter (0.22 µm) and stored at 4°C until use

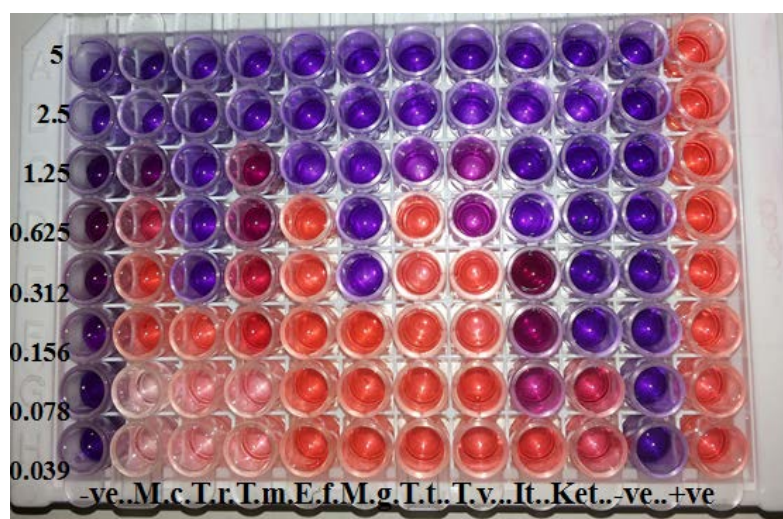
**Camel urine preparation:** Camel's urine was collected by natural urination. 200 ml of female and male camel urine were poured in pique bottles and freeze-dried by a freeze-drier machine. Two antifungal agents: fluconazole and ketoconazole (Janssen-Cilag) were used as controls. Each drug was dissolved in 100 % DMSO solution (Gibco) and prepared as stock solutions of 1000 mg/ ml. Serial two-fold dilutions were prepared according to M38-A at 100 times the strength of the final concentration, followed by further dilution (1 :50) in RPMI 1640 to yield twice the final strength required for the test. The resulting concentrations ranged from 0.062-2mg /ml for fluconazole and from 0.007-0.25 mg/ ml for ketoconazole.

**Preparation of inoculum suspensions:** Stock inoculum suspensions of the dermatophytes were prepared from 7 days-cultures grown on potato dextrose agar at 28° C to induce sporulation<sup>8</sup>. The colonies were covered with 5 ml sterile distilled water and the surface was scraped with a sterile loop. The mixture of conidia and hyphal fragments was transferred to a sterile syringe attached to a sterile filter holder and a sterile filter with pore diameter 8 mm (Whatman no. 40). Filtrate was

collected in a sterile tube <sup>9</sup>. This procedure removed the majority of hyphae and leaving an inoculum containing mainly spores. The turbidity of the final inoculum was adjusted to  $0.5 \times 10^6 - 5 \times 10^6$  spores per ml at a wavelength of 520 nm and a spectrophotometer transmission of 65-70 %. The inocula were quantified by inoculating Sabouraud glucose agar plates, using 0.01 ml adjusted inocula. The plates were incubated at 28° C and observed daily for growth. All inocula were adjusted to a final dilution of 1:50 in RPMI 1640.

The resazurin solution was prepared by dissolving a 270 mg tablet in 40 mL sterile distilled water and vortexed to ensure a well-mixed and homogenous solution.

**Test procedure:** Polystyrene microtitre plates with 96 flat-bottomed wells were used. Aliquots of 100 µl of two-fold drug dilutions were inoculated into the wells with a multichannel pipette. The microtitre plates were stored at -70° C until use. Each microtitre plate was inoculated with 100 µl of the diluted inoculum suspensions to bring the final dilutions of the inoculum to  $0.5 \times 10^4 - 5 \times 10^4$  spores/ ml<sup>-1</sup>. Growth and sterility controls were included for each assay and tests were performed in triplicate, incubated at 28°C and read visually after 7 days of incubation <sup>3</sup>. 10 µl of resazurin indicator solution were added to each well and incubated for 2 days. The colour change was assessed visually. Color change from purple to pink or colorless was regarded as positive. The MIC was the lowest concentration at which there was a colour change. The average of 3 MIC's was calculated to give the mean MIC<sup>11</sup> (Fig. 1).



**Fig. (1): Determination of the MIC camel's urine activity against dermatophytes.**

## Results

The MIC's for fluconazole and ketoconazole drugs were found to have approximately 80 % growth inhibition; while camel's urine was found to have approximately 100 % growth inhibition. This efficacy of camel's urine was (0.312-2.5 µg/ml), ketoconazole (0.156-4.0 µg/ml), and fluconazole (0.625-64.0) µl/ml. The order of efficacy for the three was: fluconazole > camel's urine > ketoconazole. The efficacy of camel's urine activity against dermatophytes was in the order: *Trichophyton rubrum* > *Trichophyton mentagrophytes* > *Trichophyton violaceum* > *Microsporum gypseum* > *Microsporum canis* > *Trichophyton tonsurans* > *Epidermophyton floccosum*. Similar tendency was observed for ketoconazole and fluconazole at 28°C incubation (Table 1).

**Table (1): Activity of camel's urine, ketoconazole, and fluconazole against dermatophytes investigated**

Dermatophytes species	Camel's urine		Ketoconazole		Fluconazole	
	MIC <sub>50</sub> µg/ml	MIC <sub>90</sub> µg/ml	MIC <sub>50</sub> µg/ml	MIC <sub>90</sub> µg/ml	MIC <sub>50</sub> µg/ml	MIC <sub>90</sub> µg/ml
<i>Trichophyton rubrum</i> (ATCC -10218)	0.312	1.25	0.5	2.0	2.0	8.0
<i>Trichophyton rubrum</i>	0.312	1.25	0.5	2.0	2.0	4.0
<i>Trichophyton mentagrophytes</i>	1.25	2.5	1.0	4.0	4.0	64.0
<i>Microsporum gypseum</i>	0.312	2.5	0.156	0.625	2.0	4.0
<i>Microsporum canis</i>	1.25	2.5	0.625	1.25	1.0	4.0
<i>Epidermophyton floccosum</i>	.312	.625	0.625	1.25	0.5	2
<i>Trichophyton violaceum</i>	0.625	1.25	1.0	4.0	4.0	64.0
<i>Trichophyton tonsurans</i>	1.25	2.5	0.156	0.625	2.0	4.0

MIC<sub>50</sub> (µg/ml) is the MIC at which 50% of the isolates were inhibited  
 MIC<sub>90</sub> (µg/ml) is the MIC at which 90% of the isolates were inhibited

## Discussion

Dermatophytes are a group of fungi able to invade keratinized tissues of human and animals, causing dermatomycosis<sup>4</sup>. The azole antifungal drugs such as ketoconazole and fluconazole are commonly used in the treatment of dermatomycosis. These antifungal agents are effective, but have increasing incidence of drug resistance. Also they generally have hepatotoxicity<sup>5</sup>. This fact had nictitated the search for natural safe products. Now camel's urine stands as a natural, pure, bioactive, and powerful medicinal compound for treatment of dermatophytes infections, based on the reports of several studies. Further, camel's urine has antimicrobial, antifungal, anticarcinogenic, antiparasitic, and hepatoprotective activities<sup>6</sup>.

In this study we investigated camel's urine to elucidate its antifungal activity against dermatophytes species in comparative with two antifungal medicinal drugs: ketoconazole and fluconazole. The mean MIC's of camel's urine against the dermatophytes species studied were 0.0625-1.0 µg/ml at MIC<sub>50</sub> and 0.125-4.0 µg/ml at MIC<sub>90</sub>. This was in agreement with some studies<sup>(7, 8)</sup>; but different from other research studies<sup>9</sup>. Besides, the MIC's of fluconazole at 28°C for 7 days incubation were 0.5 – 4.0 µg/ml at MIC<sub>50</sub> and 2.0– 64.0 µg/ml at MIC<sub>90</sub>. This result was in agreement with several studies<sup>(10, 11, 12, 13)</sup>; but different from other reports<sup>14</sup>. On the other hand, another study<sup>15</sup> reported that ketoconazole has a more effective activity than fluconazole. Meanwhile, our results found the efficacy order of the antifungals studied, based on the MIC<sub>50</sub> and MIC<sub>90</sub> at which 50% and 90% of the isolates were inhibited at 28°C, was fluconazole > camel's urine > ketoconazole.

In this context, the MIC's was estimated for the dermatophytes: *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Trichophyton violaceum*, *Microsporum canis*, and *Epidermophyton floccosum* at 28°C for 7 and 10 days incubation respectively. The MIC's of the camel's urine and fluconazole after 10 days-incubation were found increased from 1-2 dilutions; in comparison with the MIC's for 7 days-incubation. This was in agreement with another study<sup>16</sup> where the MIC's for 10 days-incubation compared with 7 days-incubation had increased from 1-2 dilutions.

Our study confirmed the reports of previous studies that camel's urine could inhibit the growth of dermatophytes. The study also provided the first evidence that there are some differences in

the dermatophytes growth-inhibitory properties and difference in the activity camel's urine as regard the breed, gender, virginity of camels. The female lactating camel's urine was known to have the highest degree of inhibition activity<sup>17</sup>. In the present context the inherited knowledge of traditional use of camel's urine for treating various diseases in Sudan could be a tool for the description of important biological activities of camel's urine. It was also reported that natural agents may play an important role in healthcare and offer a valuable source of potent compounds with a wide variety of biological activities and chemical structures that might help in drugs development<sup>18</sup>. Furthermore, the scientific evaluation of the mechanisms of action of camel's urine may accelerate its adopting in modern medicine<sup>19</sup>.

## Conclusion

It could be concluded that camel's urine may be potentially a good antifungal against dermatomycosis; and it may probably be a suitable alternative for other drugs used in the treatment of dermatomycosis. Also, camel's urine in this study was found to inhibit the growth of dermatophytes. This scientific information was not reported elsewhere, neither in Sudan nor in other countries.

## References

1. Hammer K, Carson C, Riley T. Antifungal activity of the components of *Melaleuca alternifolia* (tea tree) oil. *Journal of Applied Microbiology*. 2003;95(4):853-60.
2. Fontenelle R, Morais S, Brito E, Kerntopf M, Brillhante R, Cordeiro R, et al. Chemical composition, toxicological aspects and antifungal activity of essential oil from *Lippia sidoides* Cham. *Journal of Antimicrobial Chemotherapy*. 2007;59(5):934-40.
3. Kyle AA, Dahl MV. Topical therapy for fungal infections. *American journal of clinical dermatology*. 2004;5(6):443-51.
4. Gupta AK, Cooper EA. Update in antifungal therapy of dermatophytosis. *Mycopathologia*. 2008;166(5-6):353-67.



5. Fernandez-Torres B, Cabanes FJ, Carrillo-Munoz AJ, Esteban A, Inza I, Abarca L, et al. Collaborative evaluation of optimal antifungal susceptibility testing conditions for dermatophytes. *Journal of clinical microbiology*. 2002;40(11):3999-4003.
6. Fernandez-Torres B, Carrillo AJ, Martin E, Del Palacio A, Moore MK, Valverde A, et al. In vitro activities of 10 antifungal drugs against 508 dermatophyte strains. *Antimicrobial agents and chemotherapy*. 2001;45(9):2524-8.
7. Ghannoum MA, Chaturvedi V, Espinel-Ingroff A, Pfaller MA, Rinaldi MG, Lee-Yang W, et al. Intra- and interlaboratory study of a method for testing the antifungal susceptibilities of dermatophytes. *Journal of clinical microbiology*. 2004;42(7):2977-9.
8. Fernandez-Torres B, Vazquez-Veiga H, Llovo X, Pereiro M, Jr., Guarro J. In vitro susceptibility to itraconazole, clotrimazole, ketoconazole and terbinafine of 100 isolates of *Trichophyton rubrum*. *Chemotherapy*. 2000;46(6):390-4.
9. Al-Awadi A, Al-Jedab.A. Antimicrobial agents in camel's urine in th international conference, . J union of Arab Biologist Cairo; Mansoura university 2000. p. 265-81.
10. Gupta A, Kohli Y. In vitro susceptibility testing of ciclopirox, terbinafine, ketoconazole and itraconazole against dermatophytes and nondermatophytes, and in vitro evaluation of combination antifungal activity. *British journal of dermatology*. 2003;149(2):296-305.
11. Pujol I, Guarro J, Llop C, Soler L, Fernández-Ballart J. Comparison study of broth macrodilution and microdilution antifungal susceptibility tests for the filamentous fungi. *Antimicrobial agents and chemotherapy*. 1996;40(9):2106-10.
12. Santos D, Hamdan J. Evaluation of broth microdilution antifungal susceptibility testing conditions for *Trichophyton rubrum*. *Journal of clinical microbiology*. 2005;43(4):1917-20.
13. Santos D, Barros M, Hamdan J. Establishing a method of inoculum preparation for susceptibility testing of *Trichophyton rubrum* and *Trichophyton mentagrophytes*. *Journal of clinical microbiology*. 2006;44(1):98-101.
14. Barros M, Santos D, Hamdan J. Evaluation of susceptibility of *Trichophyton mentagrophytes* and *Trichophyton rubrum* clinical isolates to antifungal drugs using a modified CLSI microdilution method (M38-A). *JMed Microbiol*. 2007;56:514–8.



15. Figueiredo VT, de Assis Santos D, Resende MA, Hamdan JS. Identification and in vitro antifungal susceptibility testing of 200 clinical isolates of *Candida* spp. responsible for fingernail infections. *Mycopathologia*. 2007;164(1):27-33.
16. Al-Mohsen I, Hughes WT. Systemic antifungal therapy: Past, present and future. *Annals of Saudi medicine*. 1998;18(1):28-38.
17. Alhaider AA, El Gendy MA, Korashy HM, El-Kadi AO. Camel urine inhibits the cytochrome P450 1a1 gene expression through an AhR-dependent mechanism in Hepa 1c1c7 cell line. *J Ethnopharmacol*. 2011;133(1):184-90.
18. Pezzuto JM. Plant-derived anticancer agents. *Biochemical pharmacology*. 1997;53(2):121-33.
19. Schwartzmann G. Marine organisms and other novel natural sources of new cancer drugs. *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO*. 2000;11 Suppl 3:235-43.