

Assessing the Antifungal Activities of *Bucchozia coriacea* on Dermatophytes Isolated from Horses in Katsina State, Nigeria

Abstract

Dermatophytes are filamentous fungi that affect both human and animal skin, hair, and nails. There is a public health issue with it. In order to ascertain the effects of the methanolic extracts of *Bucchozia coriacea* on the isolates and the sensitivity level of the isolates to common antifungal drugs, this study was developed to explore the prevalence of Dermatophytes from clinical cases in horses. Samples were initially cultivated on Sabouraud dextrose agar, and then on Potato dextrose agar (secondary culture). Twelve (12%) of the sixty (60) clinical samples that were obtained were positive for Dermatophytes. *T. rubrum* (1), *T. verrucosum* (3), *T. equinum* (3), *M. audouinii* (2), and *M. gypseum* (3) were the species recognized. At concentrations between 125 and 250 mg/ml, the methanolic extract of *Bucchozia coriacea* demonstrated antifungal effects on every isolate with values for the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC). The isolates' susceptibility to six popular antifungal medications was assessed. The isolates were well inhibited by Ketoconazole and terbinafine, but none of them were susceptible to amphotericin B. This study was able to show that *Bucchozia coriacea*'s methanolic extract has antifungal properties. Additionally, two Dermatophytes species (Trichophyton and Microsporum) from Katsina state, Nigeria, were able to be isolated for this study.

Keywords: Dermatophytes, *Bucchozia coriacea*, Methanolic Extract, Antifungal drugs, Horses

Introduction

Skin disease known as dermatophytosis is brought on by a group of fungus that are morphologically and physiologically related (Mihali *et al.*, 2012). It is well recognized that dermatophytes can infect keratinized tissues like skin, hair, and nails with fungus. The three genera that these organisms fall under are Trichophyton, Epidermophyton, and Microsporum.

According to host preference and natural habitat, dermatophytes are further divided into three groups: zoophilic species, which typically infect non-human mammals, geophilic species, which are soil-based and may also infect both humans and animals, and anthropophilic species, which primarily infect humans (Gupta *et al.*, 2003).

The virulence of the infecting strain or species, the host's response to the metabolic byproducts of the fungus, the anatomic location of the infection, and local environmental conditions are some examples of the elements that affect the severity of the infection. Alopecia with erythema,

ranging from mild to severe, is typically one of the clinical symptoms (Chermette *et al.*, 2008). The majority of the time, lesions are not pruriginous. However, kerion and miliary dermatitis, which rapidly spread from the saddle and girth through the body, can also happen (Chermette *et al.*, 2008).

In nail infections (onychomycosis), the nail may become thick, develop white patches, or even become dystrophic and split from its bed (Degreef, 2008). Dermatophyte infections are often limited to the superficial epidermis, but in immunocompromised patients, these fungi can be invasive and result in a severe and widespread infection, leading to the development of dermatophytic granulomas (Rodwell *et al.*, 2008).

Stallions in particular play a significant role in Nigeria's sociocultural activities with regard to horses. They are also preserved by mounted police and the army for security operations, as well as being utilized for recreational riding, polo, racing, durbar, and traditional festivities (RIM, 1992). Dermatophytoses are an example of a superficial fungal skin infection that can be zoonotic and pose a major health risk (Nweze *et al.*, 2007). Data on the number and types of Dermatophytes in Daura, Katsina State, are to be provided by this study.

It further attempts to assess the effectiveness of plant extracts in the treatment of dermatophytosis in light of rising medication resistance concerns.

Materials and Methods

Study Area

Daura is a local government in Katsina state, Northern Nigeria. Its GPS location is Latitude 11^o 33' 14.76"N and Longitude 11^o 24' 21.60" E with an estimated population of 78,277.

Sampling and Sample Size

Purposive sampling was employed, with availability and sampling time taken into consideration. From several farms, residences, and horse stables in the Daura Local Government area, sixty (60) skin scrapings and hair samples were collected from both clinical instances of Dermatophytoses in horses between March and June.

Sample Collection

Using 70% alcohol to clean and disinfect the lesions, skin scrapings and swabs, as well as plucked hair, were gathered from the edges of the lesions (Elewski, 2000). Hairs were pulled out and removed (Quinn *et al.*, 1994). All acquired animal samples came with information about the animals' age, sex; anatomical sites where samples were taken, as well as the date the samples were taken. There was no previous antifungal therapy.

Direct Microscopic Examination of Samples

On a microscope slide, little amounts of each scraping were put, and 1–2 drops of 10% potassium hydroxide were added. According to Hainer's description (2003), a cover slip was put on and the slide was slowly heated over a flame. Each treated slide was meticulously inspected for the presence of diagnostic fungi characteristics using low (x10) and high (x40) power objectives.

Laboratory Culture of Dermatophytes

For primary isolation, Sabouraud dextrose agar (SDA) (Oxoid, UK), a selective media containing cycloheximide (500 mg/L), nicotinic acid (100 g/ml), and chloramphenicol (40 mg/L), was utilized. Most molds and yeasts are inhibited by cycloheximide, bacteria are killed by chloramphenicol, and *Trichophyton equinum* grows when nicotinic acid is present (Raymond and Piphet, 2008). The material was added to the SDA plates, which were then incubated for one to four weeks at room temperature.

Identification of Isolates

On Potato Dextrose Agar (PDA) (Oxoid, UK), suspected growths were sub-cultured in order to promote the synthesis of unique spores for identification and pigment production. For one to four weeks, the subcultures were incubated at room temperature (Raymond and Piphet, 2008). After staining with lactophenol cotton blue and utilizing the Fungal colour atlas, the colony (obverse and reverse morphology) and microscopic features were used to identify the species (Barros, 2007).

Preparation of Inoculums

To improve the formation of pure cultures, freshly grown cultures on the SDA were sub-cultured on Potato Dextrose Agar (PDA) plates for 4 days. A sterile loop was then used to harvest the growth. The suspension was then homogenized by shaking, allowed to settle for twenty minutes, and then its opacity was corrected with sterile distilled water to match a reference control (0.5McFarland standard).

Antifungal Activity of the Extracts

The extracts were diluted with distilled water to create a stock solution containing 1000 mg/ml of the extracts. For each set of labeled, sterile test tubes containing the different isolates, 4.5 ml of SDA broth was added. Using a sterile syringe and 0.5ml of the extracts drawn from the stock solution, a two-fold serial dilution was performed. A positive and negative control was set up, and both of them were cultured at room temperature for 24-48 hours before being monitored. Growth or cloudiness indicators were noted as negatives, while a lack of growth or cloudiness was noted as favorable. For the purpose of determining the minimum inhibitory concentration (MIC) and minimum fungicidal concentration, those lacking cloudiness or growth were cultivated on sterile SDA plates (MFC).

Antifungal Susceptibility Test Procedure

Seven antifungal medications were tested: Griseofulvin, 10 mg (Liofilchem, Italy), Ketoconazole, 50 mg (Liofilchem, Italy), Itraconazole, 50 mg (Liofilchem, Italy), Terbinafine, 100 mg (Novartis Research Institute, Vienna, Austria), and Amphotericin B, 20 mg (Liofilchem,

Italy) (Liofilchem, Italy). Based on the technique reported by Esteban *et al.*, (2005) on Agar-based disk diffusion susceptibility for Dermatophytes. It was applied to Petri dishes containing Mueller Hinton agar medium using the inoculums created for testing the extracts, distributed using a sterile swab, and allowed to air dry for five minutes in a safety cabinet. After being put to the plates with sterile forceps, the antifungal discs were incubated at room temperature for up to 5 days, at which point the zones of inhibition were visible. These were measured using a ruler for each antifungal agent and recorded (Penduka *et al.*, 2011).

Statistical Analysis

To provide a clear and accurate understanding of the outcomes, some statistical analysis was done on the data gathered from the field survey and laboratory study. The Chi-square test and the Descriptive Statistics of Cross-tabulation (Cross-tab) are examples of statistical techniques. The cross distributions of two separate outcomes were displayed using the cross-tab. The degree of independence between two groups was tested using the Chi-square. Additionally, some of the statistics were shown as graphs and tables. The statistical analysis was performed using the Statistical Package for Social Science (SPSS) version 20.0 software.

Results

60 clinical samples altogether were cultivated for dermatophytes, 12 (20%) were isolated and recognized as such. Trichophyton (7) and Microsporum (5) were isolated and characterized (Table 1). The additional fungi that were isolated from the samples were Rhizopus, Mucor, Yeast, and Aspergillus.

Table 1: Number of Dermatophytes Isolates and Other Fungi from Horses

Species	Frequency	Percent
Dermatophytes	12	20.0
Other fungi	48	80.0
Total	60	100.0

Table 2: Dermatophytes Isolated from Horses

Horses	Count	Isolates			Total
		Other Fungi	<i>Microsporum</i>	<i>Trichophyton</i>	
		48	5	7	60
	% within	80.0	8.3	11.7	100.0

Cross Tabulation

The distribution of the isolates among the major sample-related parameters was ascertained using the cross-tab calculated. These variables include the samples' age, anatomical locations, and gender. This would allow the investigation to identify the areas with the highest concentrations of isolates among the aforementioned criteria.

Table 3: Age Distribution in Relation to Isolation Rate of Dermatophytes

Age		Isolates	
		<i>Microsporum</i>	<i>Trichophyton</i>
1-5yrs	Count	1	2
	% within isolates	20.0	28.6
6-10yrs	Count	3	4
	% within isolates	60.0	57.1
11-15yrs	Count	1	0
	% within isolates	20.0	0
16-20yrs	Count	0	1
	% within isolates	0	14.3
		5	7

Table 4: Anatomical Site and Dermatophytes Isolates

Anatomical site		Isolates	
		<i>Microsporum</i>	<i>Trichophyton</i>
Head	Counts	1	1
	% within isolates	20	14.3
	Count	2	1
Neck	% within isolates	40	14.3
	Count	2	4
	% within isolates	40	57.1
Back	Count	0	1
	% within isolates	0.0	14.3
	Total	5	7
		100.0%	100.0%

Table 5: Sex and Dermatophytes Isolates Distribution

Sex		Isolates	
		<i>Microsporum</i>	<i>Trichophyton</i>
Male	Count	3	5
	% within isolates	60.0	71.4
Female	Count	2	2
	% within isolates	40.0	28.6
Total		5	7
		100.0	100.0

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Chi-Square Tests

The distribution of anatomical sites, ages, and statistical differences between the isolates were examined using the chi-square test. Additionally, it is used to determine whether each of the components inside a factor, such as anatomical site, is independent of the other. For instance, it is used to determine whether the occurrence of isolates (Microsporum and Trichophyton) on the head is independent of the occurrence on the neck.

Table 6: Chi-Square Test Statistics

Relationships	Chi-square value	P value
Isolates and Anatomical sites	12.059	0.061
Isolates and Age	6.301	0.39
Isolates and Categories	1.542	0.463
Isolates and Sex	138	0.933

Table 7: Results of Antifungal Activity of *Buchholzia coriacea* on the Dermatophytes Isolates

Dermatophytes	Isolates	MIC(mg/ml)	MFC(mg/ml)
<i>Microsporium audounii</i>			
A	HM10	125	250
B	HM13	125	250
<i>Microsporium gypseum</i>			
A	HM3	125	250
B	HM8	125	250
C	HF50	125	250
<i>Trichophyton rubrum</i>			
A	HM58	125	125
<i>Trichophyton verrucosum</i>			
A	HM39	125	125
B	HM45	125	250
C	HF39	125	125
<i>Trichophyton equinum</i>			

A	HF42	125	250
B	HM44	125	250
C	HF57	125	125

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Table 8: Results of Commercially Standardized Antifungal Agents on Dermatophytes Isolates (Horses)

Drugs	KCA	TER	NY	PB	AMB	ITC	AGF
Samples							
<i>Microsporum gyseum</i>							
HM3	S(10mm)	S(28mm)	S(10mm)	R	R	R	R
HM8	S(10mm)	S(30mm)	S(11mm)	R	R	R	R
<i>Microsporum audounii</i>							
HM13	S(25mm)	S(35mm)	S(20mm)	S(10mm)	R	S(25mm)	R
HF50	S(11mm)	S(30mm)	S(18mm)	10(10mm)	R	S(20mm)	R
HF31	S(11mm)	S(28mm)	S(11mm)	R	R	R	R
<i>Trichophyton rubrum</i>							
HM58	S(22mm)	S(33mm)	S(15mm)	S(13mm)	R	R	R
<i>Trichophyton verrucosum</i>							
HM39	S(16mm)	S(28mm)	R	R	R	R	R
HM45	S(18mm)	S(30mm)	R	R	R	R	R
<i>Trichophyton equinum</i>							
HM42	S(28mm)	S(40mm)	S(15mm)	S(10mm)	R	S(25mm)	R
HM44	S(25mm)	S(28mm)	S(10mm)	R	R	S(20mm)	R
HF57	S(26mm)	S(32mm)	S(13mm)	S(10mm)	R	S(22mm)	R

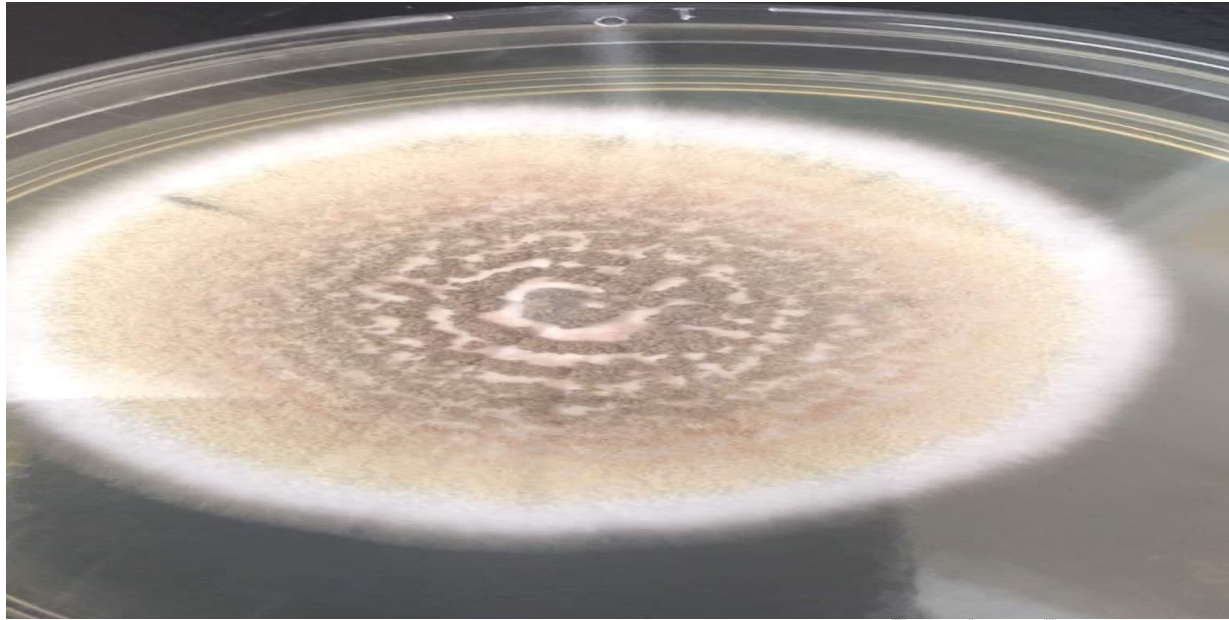


Plate I; A colony of *Microsporum gypseum* on PDA having dark to a cinnamon brown appearance with granular texture after 10 days growth at room temperature of 25⁰ c

UNDER PEER REVIEW

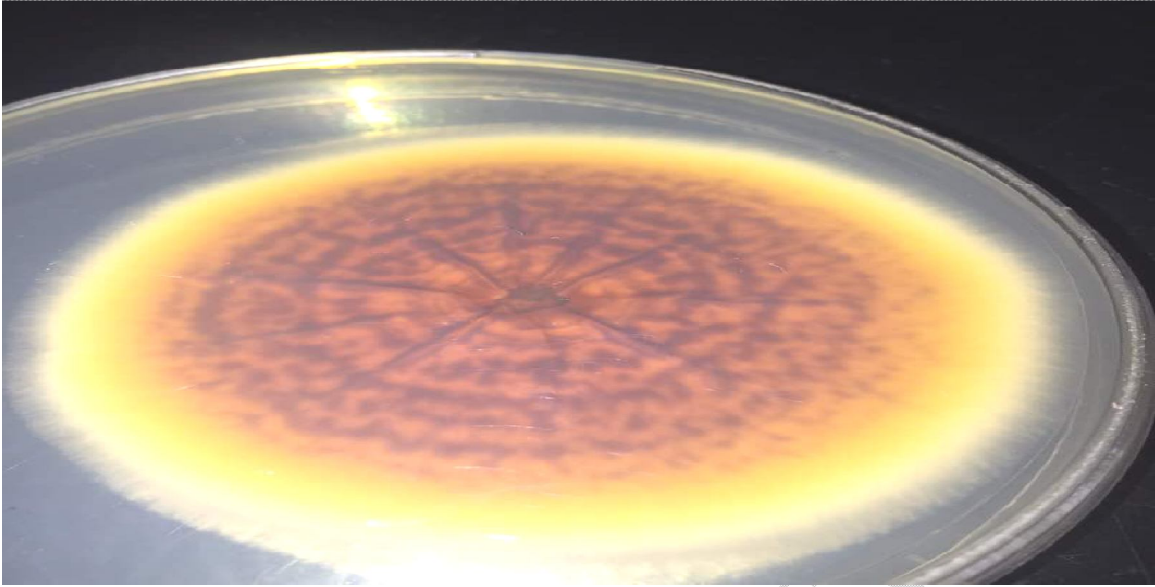


Plate II: The reverse side of *Microsporium gypseum* with slight yellow to red coloration

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Plate III: Microscopy of *Microsporium gypseum* showing barrel-shaped macro conidia (x400)
(LCB stain)

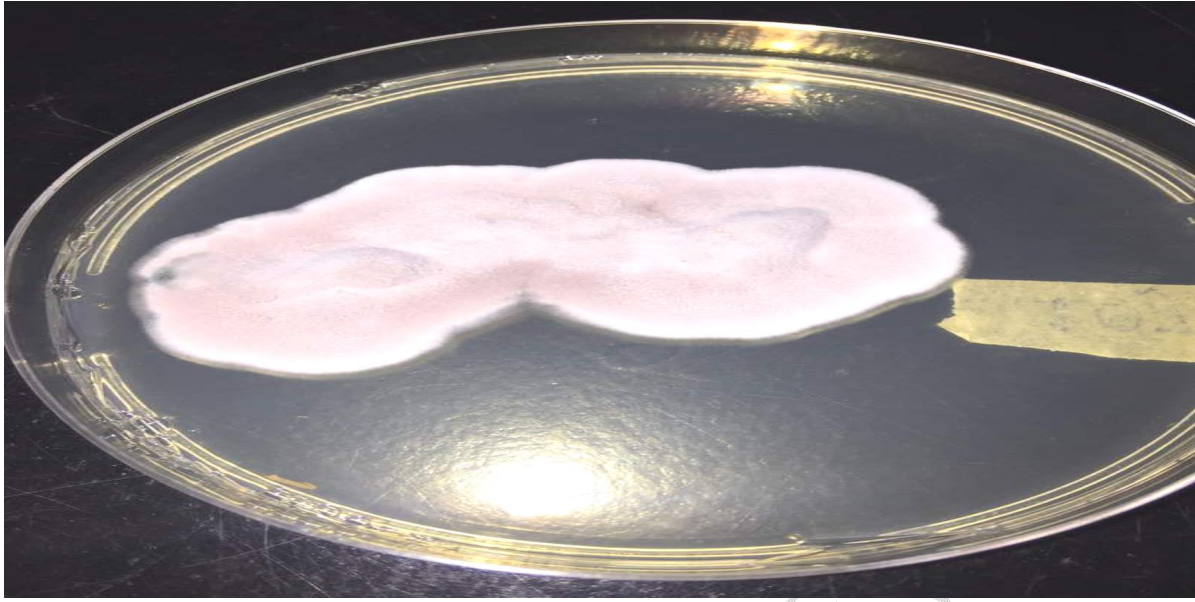


Plate IV: Colony of *Trichophyton verrucosum* having a cream coloured glabrous growth after 14 days growth on PDA at 25°c

UNDER PEER REVIEW

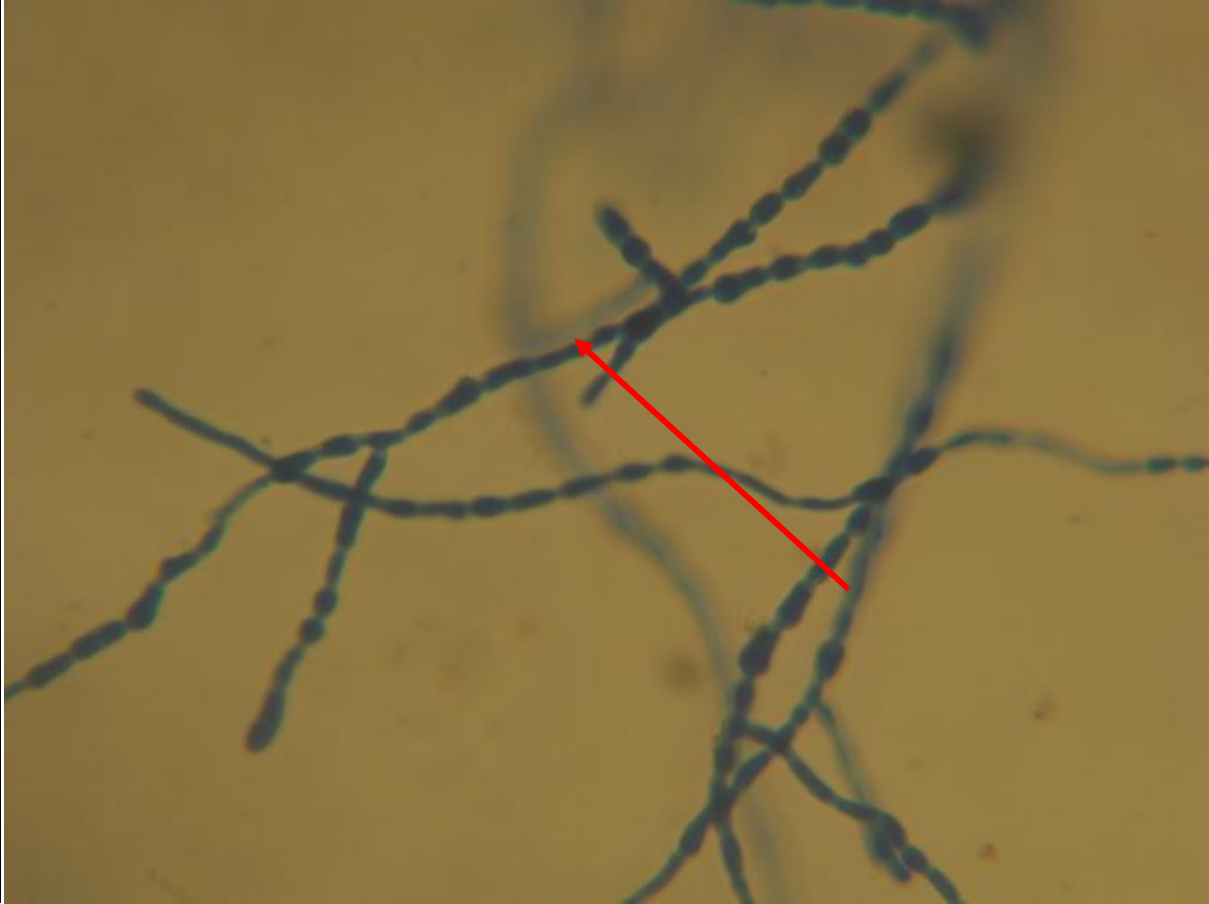


Plate V: Microscopy of *Trichophyton verrucosum* with a arrow indicating the Chlamydospores (x400) (LCB stain)

UNDER PELL

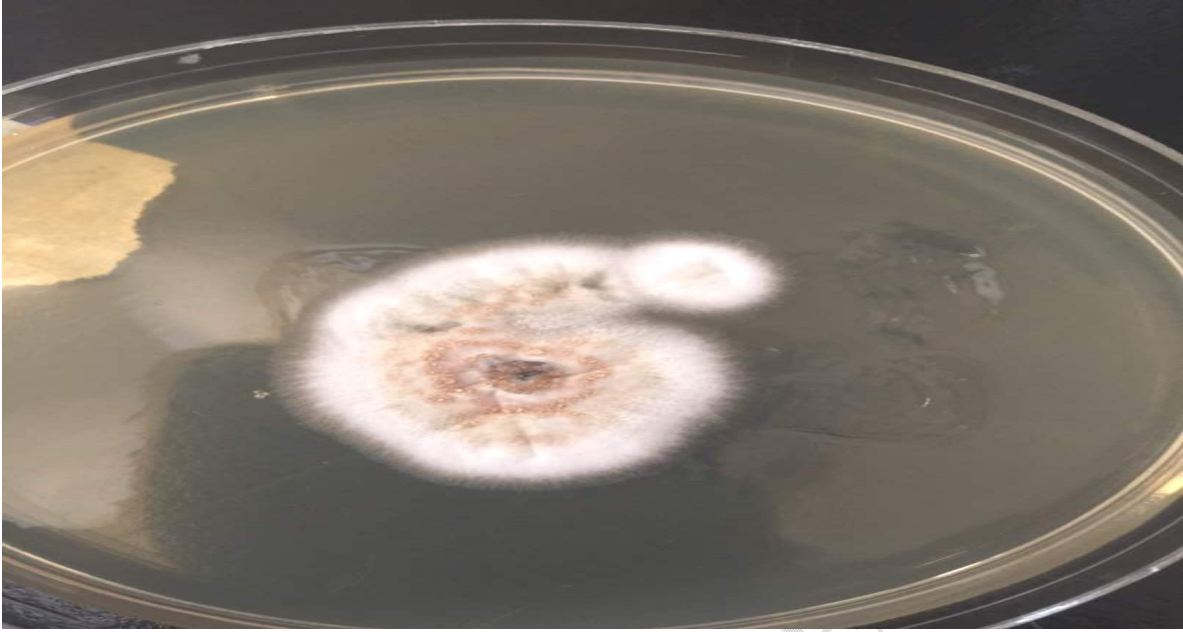


Plate VI: A colony of *Microsporium audouinii* on PDA exhibiting gray to white downy texture, after 14 days growth at room temperature of 25°C

UNDER PEER REVIEW

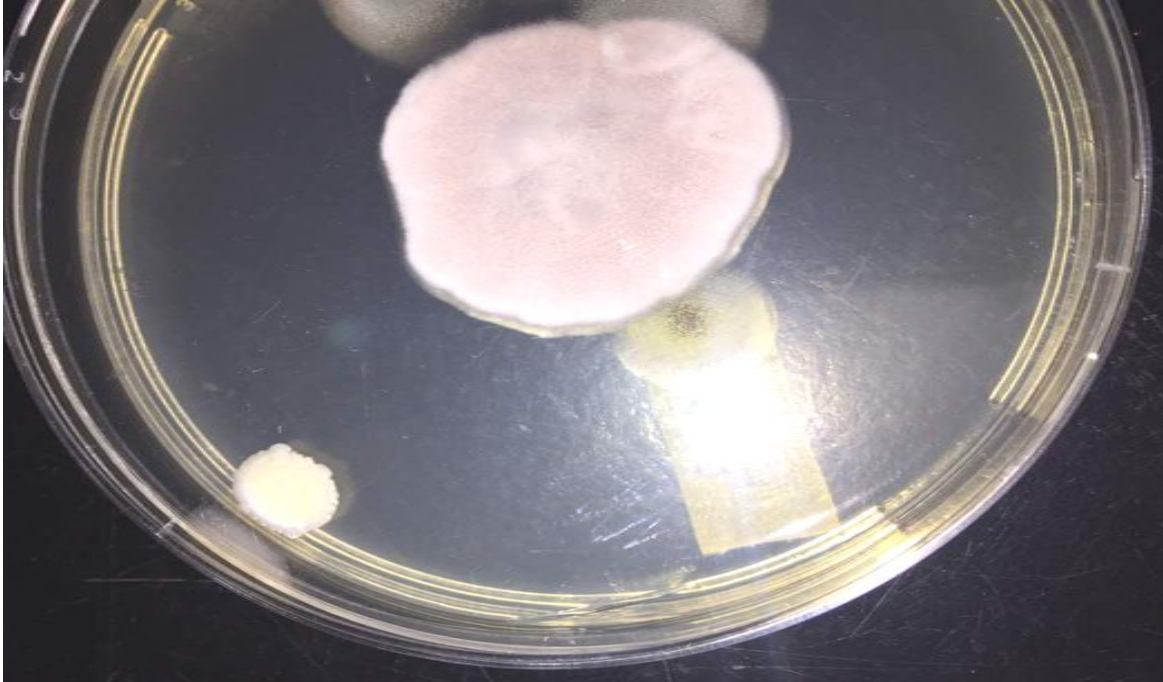


Plate VII: A colony of *Trichophyton equinum* on PDA with cream white to yellow appearance after 12 days growth

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Discussion

Dermatophytes of the genera *Microsporum* and *Trichophyton* from horses were isolated and identified as a result of the investigation. The most frequent species were *Trichophyton*, which is consistent with the findings of Collise *et al.*, (2011), Nweze (2010), and Hassan (2011). This investigation confirmed the presence of three *Trichophyton* species (*T. equinum*, *T. rubrum*, *T. verrucosum*) and two *Microsporum* species (*M. gypseum*, and *M. audouinii*) in the study area implicated in equine dermatophytoses. This is consistent with the writings of Nweze (2010) and Popoola *et al.*, (2006).

The highest isolated *Trichophyton* etiological agent was *Trichophyton equinum*. The works of Barros, (2007) and Kharna and Bharti, (2014), which noted that *T. equinum* is the most frequent isolated Dermatophytes species from horses and *T. verrucosum* from cattle, concur with this finding. The second-most isolated Dermatophyte in this investigation was *Microsporum audouinii*. It is a fungus that infects animals that frequently come into contact with soil (Haria and Bryson, 1995). The location where the study was conducted may have contributed to the high abundance of this particular species.

One of the most typical ringworm causes in the globe is *Trichophyton rubrum*, which was isolated for this investigation. It is primarily blamed for nail and finger Dermatophytes infections (Seanego and Ndip, 2012). The most isolated were in the head and the back. All of the isolated *Trichophyton verrucosum* in this study came from horses' backs. The proximity of the cattle, horses, and donkeys may have contributed to the high number of positive cases discovered in horses.

This study has established a strong link between anatomical distribution and the isolated Dermatophytes. Horses' backs showed the highest distribution rate for Dermatophytes isolates, with *Trichophyton* having the most isolates. This is also in line with the OIE data from 2005, which suggested that the bulk of Dermatophytes lesions are seen on horses' backs that have come into contact with saddles. The age range from 6 to 10 years old saw the highest prevalence of Dermatophytes. This is explained by the fact that activities like sports and farm work are more common among people in this age group. Males had a higher incidence of Dermatophytoses than females, according to the study.

Chi-square tests were used to analyze the link between the isolates and the test parameters, and the results revealed a significant difference between the distribution of the isolates and anatomical sites. In terms of the occurrence of the isolates, the anatomical sites are also distinct from one another. The p value of 0.061 at the 10% threshold of significance led to this conclusion. All of the isolates were subjected to the antifungal effects of the *Buchholzia coriacea* methanolic extract. *Trichophyton verrucosum* had MIC of 125 mg/ml and MFC of 250 mg/ml, indicating susceptibility to the extract. At MICs of 125 mg/ml and 250 mg/ml, *Microsporum gypseum* demonstrated susceptibility. The MICs and MFCs were the same for all other isolates.

Terbinafine results demonstrated the greatest level of effectiveness against all isolated species; supporting the claims made by Favre *et al.*, (2003) and Sitterle *et al.*, (2012) that terbinafine can be used to treat the majority of Dermatophytes infections in horses. Amphotericin B, one of the most widely used antifungal medications, was not effective against any of the isolates. This backed up the studies by Elewski (2000) that showed how amphotericin B was ineffective against dermatophytoses.

Against each isolate, Ketoconazole and Terbinafine demonstrated a high level of antifungal activity. The two medications that did not exhibit any antifungal action against the Dermatophytes isolated from the study area were Amphotericin B and Griseofulvin.

Conclusion

This investigation was successful in proving *Buchholzia coriacea*'s methanolic extract's antifungal effectiveness. All of the isolates were susceptible to the *Buchholzia coriacea* methanolic extract's antifungal effects at various MICs and MFCs. The recommended medications include Terbinafine and Ketoconazole because they all shown antifungal efficacy against all isolates. The study was able to identify two Dermatophytes species; *Trichophyton* and *Microsporum* in Nigeria's Katsina state, proving their presence there. It offers a base upon which subsequent study in the research area can be conducted.

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