

Short Research Article

***Buchholziacoriacea* and its Antifungal Activity on Dermatophytes Isolated from Donkeys in Katsina State, Nigeria**

Title

Antifungal Activity of *Buchholziacoriacea* on Dermatophytes Isolated from Donkeys 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 donkeys. Samples were initially cultivated on Sabouraud dextrose agar, and then on Potato dextrose agar (secondary culture). Sixty (60) clinical samples were collected out of which 13(21.7%) were positive for Dermatophytes. Species identified were; *T. rubrum* (3), *T. verrucosum* (1), *T. equinum* (3), *T. Mentagrophytes*(3) *M. audounii*(1), *M. gypseum* (2). 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, *Buchholziacoriacea*, Methanolic Extract, Antifungal drugs, Donkeys

Introduction

Skin diseases known as dermatophytosis are 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 military 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).

In Nigeria, donkeys are concentrated mainly in the northern states because of the savannah type of vegetation and fewer disease vectors such as tsetse flies (RIM, 1992). In south-eastern Nigeria, donkeys are used as meat animals (Mabayoje and Ademiluyi, 2004). Superficial fungal infections of the skin, including the dermatophytoses can be zoonotic, thereby posing a serious health hazard (Nweze *et al.*, 2007).

This research aims to provide data on the presence and species of Dermatophytes in Daura, Katsina State. With a growing concern for drug resistance, it further tries to evaluate the efficacy of plant extracts in the treatment of dermatophytosis.

Materials and Methods

Study Area

Daura is a local government in Katsina state, Northern Nigeria. Its GPS location is Latitude 11° 33' 14.76"N and Longitude 11° 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 donkey stables in the Daura Local Government area, sixty (60) skin scrapings and hair samples were collected from both clinical instances of Dermatophytoses in **horses** **donkeys** 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 (0.5 McFarland 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 0.5 ml 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**).

Data Presentation and 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

A total of 60 clinical samples from donkeys were cultured for Dermatophytes out of which 13(20%) were isolated and identified as Dermatophytes. *Microsporum* (3) and *Trichophyton* (10) were isolated and identified. *Rhizopus*, *Mucor*, *YeasandAspergillois* were the other fungi isolated from the samples.

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

Species	Frequency	Percent
Dermatophytes	13	21.7
Other fungi	47	78.3
Total	60	100.0

Table 2: Dermatophytes Isolated from Donkeys

		Isolates			Total
		Other Fungi	<i>Microsporum</i>	<i>Trichophyton</i>	
Donkeys	Count	47	3	10	60
	% within	78.3	5.0	16.7	100.0

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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		0	2
	% within isolates		0	20.0
6-10yrs	Count		3	6
	% within isolates		100.0	60.0
11-15yrs	Count		0	1
	% within isolates		0	10.0
16-20yrs	Count		0	1
	% within isolates		0	10.0
			3	10

Table 4: Anatomical Site and Dermatophytes Isolates

Anatomical site		Isolates	
		<i>Microsporum</i>	<i>Trichophyton</i>
Head	Counts	0	1
	% within isolates	0	10.0
	Count	1	1
Neck	% within isolates	33.3	10.0
	Count	2	7
	% within isolates	66.7	70.0
Back	Count	0	1
	% within isolates	0	10.0
	Count	3	10
Limbs	% within isolates	100.0%	100.0%
	Count	3	10
	% within isolates	100.0%	100.0%
Total		3	10
		100.0%	100.0%

Table 5: Sex and Dermatophytes Isolates Distribution

Sex		Isolates	
		<i>Microsporum</i>	<i>Trichophyton</i>
Male	Count	3	8
	% within isolates	100.0	80.0
Female	Count	0	2
	% within isolates	0	20.0
Total		3	10
		100.0	100.0

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 *Buchholziacoriacea* on the Dermatophytes Isolates

Dermatophytes	Isolates	MIC(mg/ml)	MFC(mg/ml)
<i>Microsporumaudounii</i>			
A	DM10	125	250
<i>Microsporum gypseum</i>			
A	DF4	125	250
B	DM60	125	250
<i>Trichophytonrubrum</i>			
A	DF21	125	125
B	DF33	125	125
C	DF39	125	125
<i>Trichophytonverrucossum</i>			
A	DM29	125	250
<i>Trichophyton mentagrophytes</i>			
	DF11	125	125
	DM15	125	125
	DM1	125	125

Trichophytonequinum

A	DM42	125	125
B	DM48	125	125
C	DF57	125	125

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

Drugs	KCA	TER	NY	PB	AMB	ITC	AGF
Samples							
<i>Microsporungy seum</i>							
DF4	S(11mm)	S(30mm)	S(12mm)	R	R	R	R
DM60	S(12mm)	S(28mm)	S(13mm)	R	R	R	R
<i>Microsporumau dounii</i>							
DM10	S(22mm)	S(30mm)	S(18mm)	S(11mm)	R	S(23mm)	R
<i>Trichophytonm entagrophytes</i>							
DM1	S(18mm)	S(22mm)	S(9mm)	R	R	R	R
DF11	S(20mm)	S(24mm)	S(11mm)	R	R	R	R
DM15	S(18mm)	S(24mm)	S(11mm)	R	R	R	R
<i>Trichophytonve rrucossum</i>							
DM29	S(18mm)	S(28mm)	R	R	R	R	R
<i>Trichophytoneq uinum</i>							
DM42	S(30mm)	S(35mm)	S(16mm)	S(11mm)	R	R	R
HM48	S(26mm)	S(28mm)	S(12mm)	S(10mm)	R	S(15mm)	R
DM57	S(28mm)	S(32mm)	S(13mm)	S(10mm)	R	S(20mm)	R
<i>Trichophytonru brum</i>							
DF21	S(24mm)	S(35mm)	S(18mm)	S(15mm)	R	R	R
DM33	S(22mm)	S(33mm)	S(15mm)	S(13mm)	R	R	R
DF39	S(22mm)	S(31mm)	S(14)	S(12mm)	R	R	R

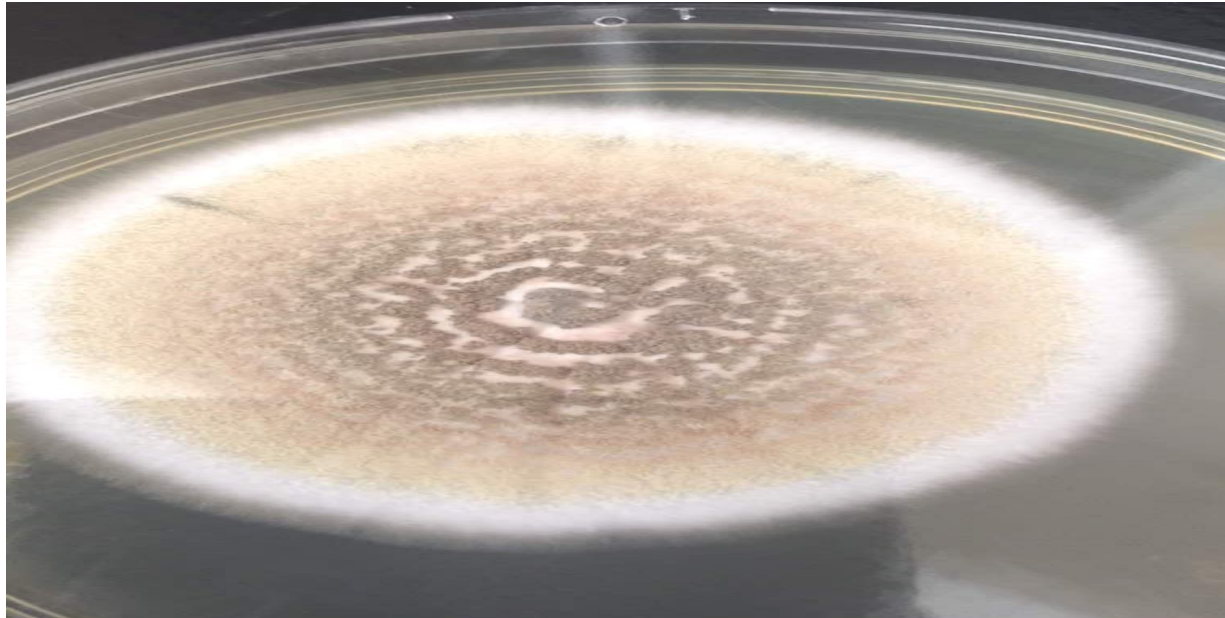


Plate I: A colony of *Microsporium gypseum* on PDA having dark to a cinnamon brown appearance with granular texture after 10 days growth at room temperature of 25⁰ c

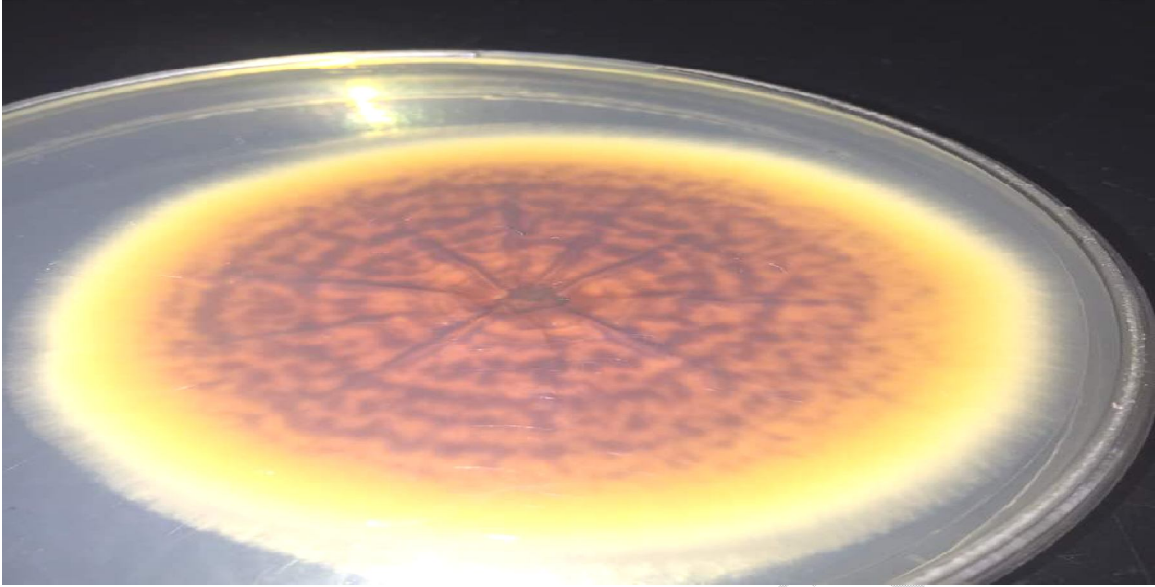


Plate II: The reverse side of *Microsporium gypseum* with slight yellow to red coloration

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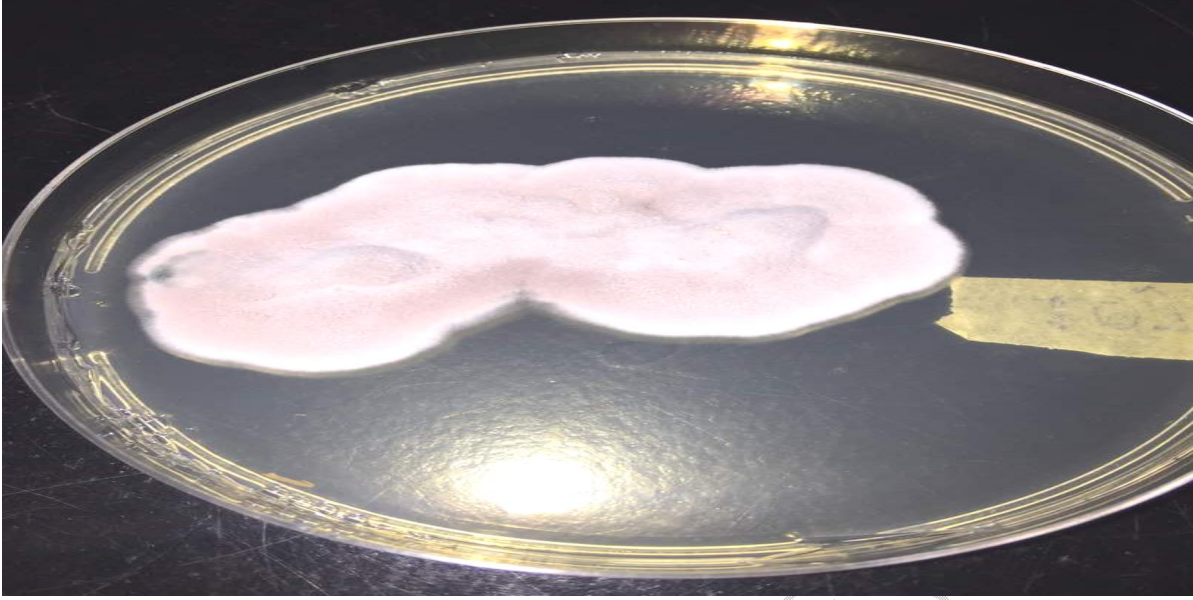


Plate III: Colony of *Trichophyton verrucosum* having a cream coloured glabrous growth after 14 days growth on PDA at 25°c

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Plate IV: Reverse side of *Trichophyton verrucosum* colonial growth with slight appearance

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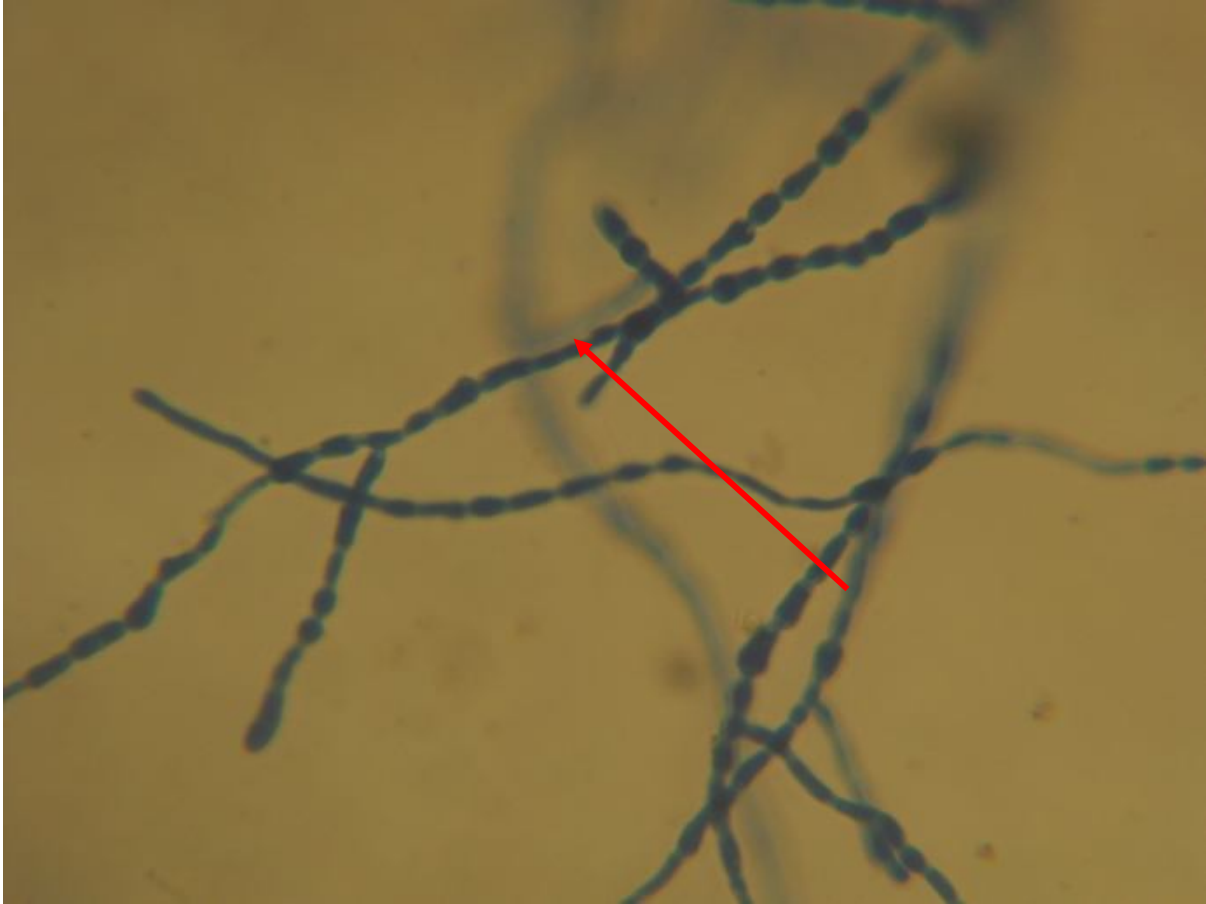


Plate V: Microscopy of *Trichophyton verrucosum* with a arrow indicating the Chlamydospores (x400) (LCB stain)

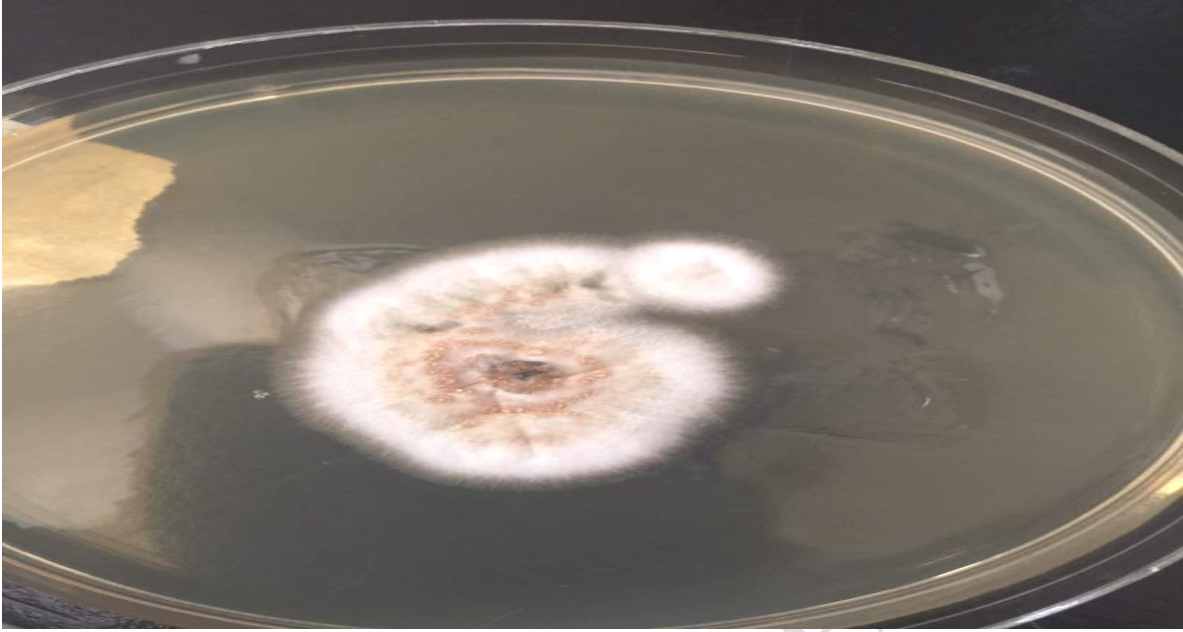


Plate VI: A colony of *Microsporium audouinii* on PDA exhibiting gray to white downy texture, after 14 days growth at room temperature of 25°C

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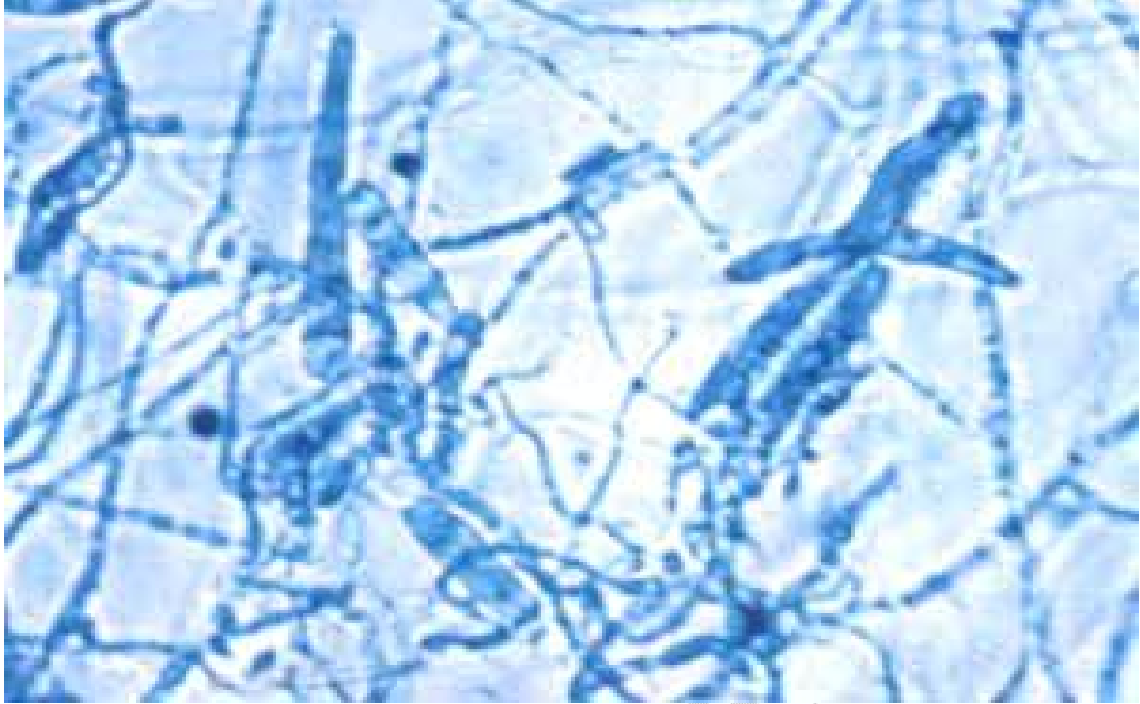


Plate VII: Microscopy of *Trichophytonequinum* showing club-shaped Macro conidia (x400)
(LCB)

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Discussion

Dermatophytes of the genera *Microsporum* and *Trichophyton* from donkeys were isolated and identified as a result of the investigation. The most frequent species were *Trichophyton* species, which was consistent with the findings of **Collise *et al.*, (2011)**, **Nweze (2010)**, and **Hassan (2011)**. This investigation confirms that three species of *Trichophyton* and two species of *Microsporum*, namely *T. equinum* (3), *T. rubrum* (3), *T. verrucosum*(1), *T. mentagrophytes* (3), *M. gypseum* (2), and *M. audouinii*, are implicated in equine dermatophytoses in the study region. This is consistent with the writings of **Nweze (2010)** and **Popoola *et al.*, (2006)**.

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)**.

This study has shown that there is a significant relationship between anatomical distribution and the Dermatophytes isolated. The back yielded the highest distribution rate of Dermatophytes isolates in donkeys; out of which *Trichophyton* have the highest number of isolates. This is also in agreement with **OIE (2005)** report which stated that most Dermatophytes lesions are found on the back of donkeys because they are used for carrying loads as a form of transportation.

The highest incidence of Dermatophytes fell in the age bracket of 6-10. This can be attributed to the fact that this age bracket is used more for farm works, transportation etc. The study showed a higher incidence of Dermatophytoses in males than females. 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. Terbinafine's 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 donkeys.

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, \(2000\)](#), Hay and Ashbee (2010), and others 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 study was able to demonstrate the antifungal efficacy of the methanolic extract of *Buchholzia coriacea*. *Buchholzia coriacea* methanolic extract exhibited antifungal activities on all the isolates at varying MICs and MFCs. Terbinafine and Ketoconazole are the drugs of choice as they displayed antifungal activity on all the isolates. The study was able to isolate two Dermatophytes species (*Trichophyton* and *Microsporum*) from Katsina state Nigeria, Thereby, establishing its presence in the study area. It provides a foundation on which further research can be carried out in the study area.

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