Asian Journal of Research in Animal and Veterinary Sciences

10(2): 24-33, 2022; Article no.AJRAVS.92528

Antifungal Activity of *Buchholzia coriacea*'s Methanolic Extract on Dermatophytes Isolated from Donkeys in Katsina State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/92528

Original Research Article

Received 27 July 2022
Accepted 30 September 2022
Published 07 October 2022

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 *Buccholzia 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. verrrucossum* (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 *Buccholzia 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

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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 *Buccholzia coriacea'*s methanolic extract has antifungal properties. Additionally, two Dermatophytes species (Trichophyton and Microsporum) from Katsina state, Nigeria, were able to be isolated in this study.

Keywords: Dermatophytes; Buchholzia coriacea; methanolic extract; antifungal drugs; Donkeys.

1. INTRODUCTION

Skin diseases known as dermatophytosis are caused by a group of fungus that are morphologically and physiologically related [1]. 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 [2].

The virulence of the infecting strain or species, the host's response to the metabolic by-products 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 [3]. The majority of the time, the lesions are not pruriginous. However, kerion and miliary dermatitis, which rapidly spread from the saddle and girth through the body, can also happen [3].

In nail infections (onychomycosis), the nail may become thicker, develop white patches, or even become dystrophic and split from its bed [4]. 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 [5].

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 [6]. In south-eastern Nigeria, donkeys are used as meat animals [7]. Superficial fungal infections of the skin, including the dermatophytoses can be zoonotic, thereby posing a serious health hazard [8].

Buchholzia coriacea has a variety of therapeutic uses. Due to its role in traditional medicine it has a widespread moniker that is known as wonderful kola. It is a member of the *Capparaceae* family. The seeds, which can be consumed either cooked or uncooked, are the plant parts most frequently consumed. It is helpful in Africa for the treatment of hypertension and for delaying the onset of premature aging. When applied on the forehead, the magnificent kola plant from Africa can stop migraine headaches. Back discomfort is treated by using an enema containing stem bark extract [9].

External applications of unidentified bark formulations are also made for pleurisy, rheumatism, conjunctivitis, smallpox, scabies, and other skin issues. Women who are sterile can be treated with leaf decoctions. Filarial nematodes are treated with leaf infusions administered to the eyes, and fever, ulcers, boils, and hemorrhoids are treated with powdered or pulped leaves [9] When compared to the common antibiotics ampicillin and tioconazole, a study conducted by Ajaiyeoba et al. [10] on fractions produced from the methanol extract of *Buchholzia coriacea* stem bark revealed a strong concentration-dependent antibacterial and antifungal activity of the fractions.

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.

2. MATERIALS AND METHODS

2.1 Study Area

Daura is a local government in Katsina state, Northern Nigeria. Its GPS location is Latitude 11^0 33'14.76"N and Longitude 11^o 24'21.60" E with an estimated population of 78,277.

2.2 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 donkeys between March and June.

2.3 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 [11]. Microscopic investigation of skin scrapings from dermatological lesions was used to confirm (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.

2.4 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 [12], 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.

2.5 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.

2.6 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 [13].

2.7 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).

2.8 Antifungal Activity of the Extracts

The method described in [14] was used to create the methanolic extract of *Buchholzia coriacea* seed. Fresh *Buchholzia coriacea* seeds were purchased from a local market and authenticated at the Botany department, Ahmadu Bello University Nigeria, where we had previously assigned the specimen voucher number 439971. To get rid of clinging materials, the seeds were thoroughly rinsed in distilled water. The seeds were then cut into pieces, shade dried, and ground. The 5kg of powdered seeds were defatted by soaking them in 15 L of hexane for 72 hours. The shaft was immersed in pure hexane for a further 72 hours before the hexane extract was extracted using a muslin bag.

The extract was concentrated in a rotary evaporator at 30°C after being filtered using Whatman paper (1 mm). The concentration of this extract was increased in a vacuum oven (30°C, 700 mmHg). The resulting extract was dried in the air for three hours before being extracted again with methanol. The extract was very water soluble. Until usage, the extract was kept in a refrigerator [14].

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).

2.9 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 [15].

2.10 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.

3. 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*, Y*east* and *Aspergillosis* were the other fungi isolated from the samples.

3.1 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.

3.2 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 1. Dermatophytes and other fungi isolated 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	*Microsporum*	*Trichophyton*	
Donkeys	Count	47	3	10	60
	% within	78.3	5.0	16.7	100.0

Table 3. Age distribution in relation to isolation rate of dermatophytes

Age		Isolates	
		Microsporum	***Trichophyton***
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	
		Microsporum	***Trichophyton***
Head	Counts	0	1
	% within isolates	0	10.0
Neck	Count	1	1
	% within isolates	33.3	10.0
Back	Count	2	7
	% within isolates	66.7	70.0
Limbs	Count	0	1
	% within isolates	0	10.0
Total		**3**	**10**
		100.0%	**100.0%**

Table 5. Sex and dermatophytes isolates distribution

Sex			Isolates	
			Microsporum	***Trichophyton***
	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

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)
	Microsporum audounii		
A	DM10	125	250
	Microsporum gypseum		
A	DF4	125	250
B	DM60	125	250
	Trichophyton rubrum		
A	DF21	125	125
B	DF33	125	125

Dermatophytes	Isolates	MIC(mg/ml)	MFC(mg/ml)
C	DF39	125	125
Trichophyton verrucossum			
A	DM29	125	250
Trichophyton mentagrophytes			
	DF11	125	125
	DM15	125	125
	DM1	125	125
Trichophyton equinum			
A	DM42	125	125
B	DM48	125	125
C	DF57	125	125

Table 8. Results of commercially standardized antifungal agents on dermatophytes isolates (Donkeys)

Drugs Samples	KCA	TER	NY	PB	AMB	ITC	AGF
Microsporum gyseum							
DF4	S(11mm)	S(30mm)	S(12mm)	R	R	R	R
DM60	S(12mm)	S(28mm)	S(13mm)	R	R	R	R
Microsporum audounii							
DM10	S(22mm)	S(30mm)	S(18mm)	S(11mm)	R	S(23mm)	R
Trichophyton mentagrophtyes							
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
Trichophyton verrucossum							
DM29	S(18mm)	S(28mm)	R	R	R	R	R
Trichophyton equinum							
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
Trichophyton rubrum							
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

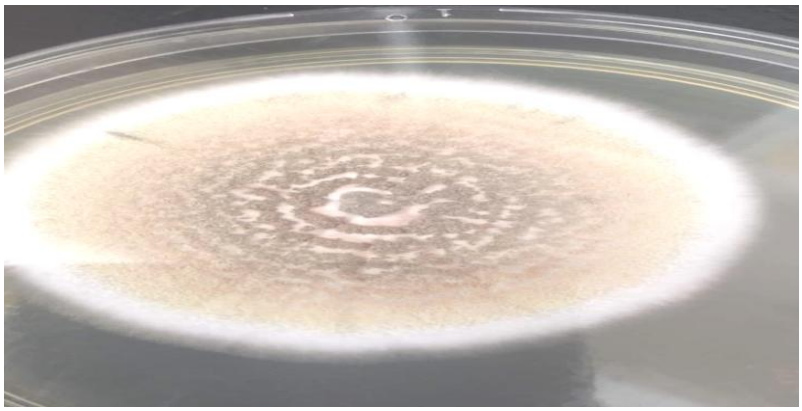

Plate I. A colony of *Microsporum gypseum* on PDA having dark to a cinnamon brown appearance with granular texture after 10 days growth at room temperature of 25^0c

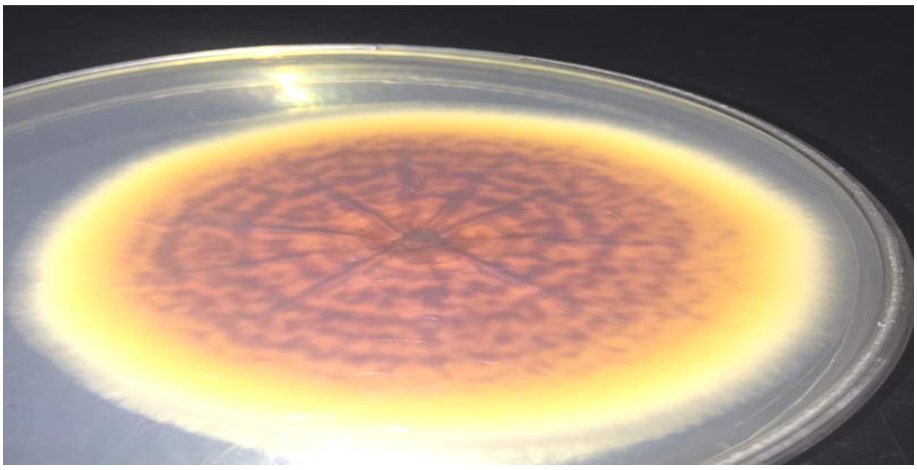

Plate II. The reverse side of *Microsporum gypseum* with slight yellow to red coloration

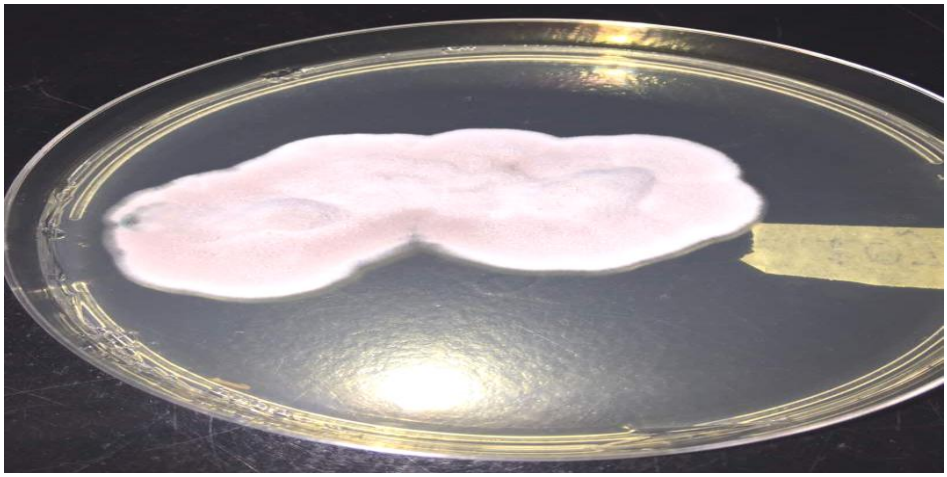

Plate III. Colony of *Trichophyton verrucossum* having a cream coloured glabrous growth after 14 days growth on PDA at 25^0c

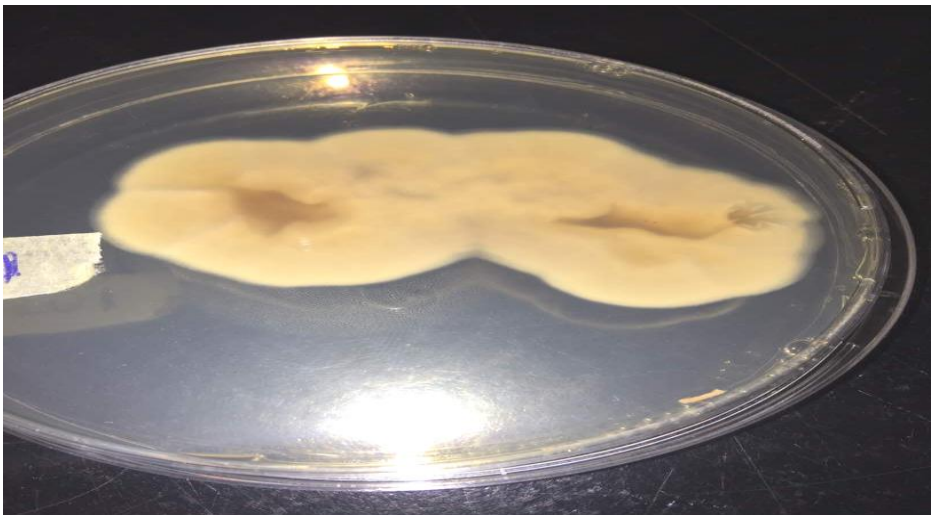

Plate IV. Reverse side of *Trichophyton verrucossum* colonial growth with slight appearance

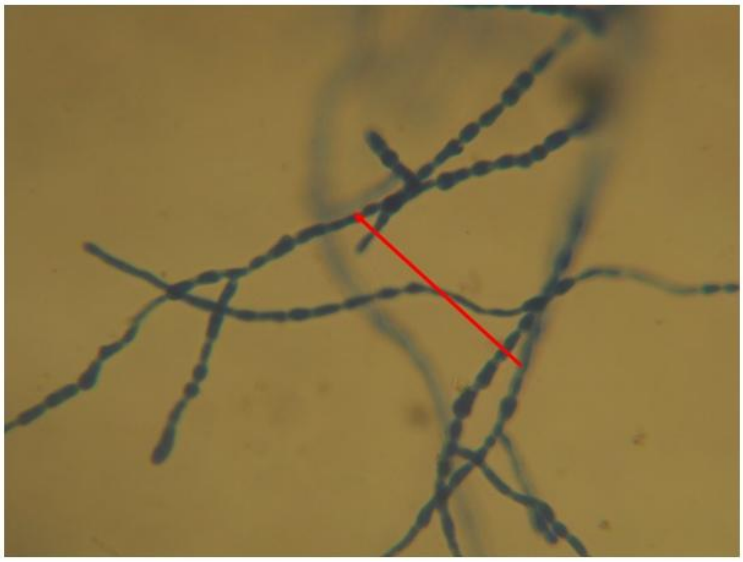

Plate V. Microscopy of *Trichophyton verrucossum* with a arrow indicating the Chlamydospores (x400) (LCB stain)

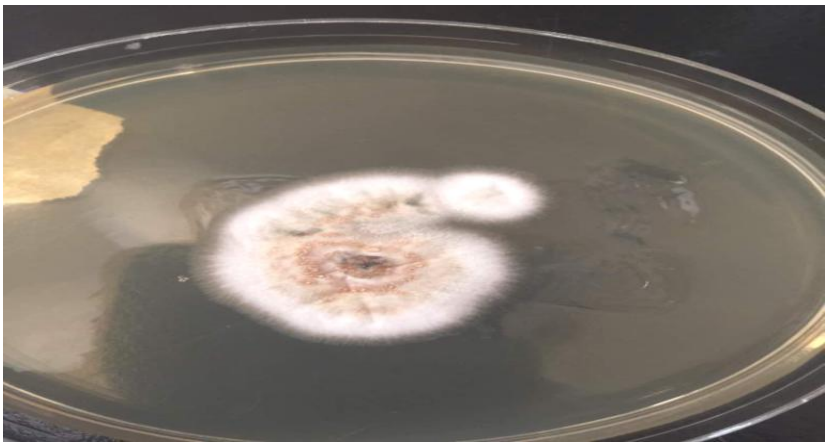

Plate VI. A colony of *Microsporum audouinii* on PDA exhibiting gray to white downy texture, after 14 days growth at room temperature of 25^0c

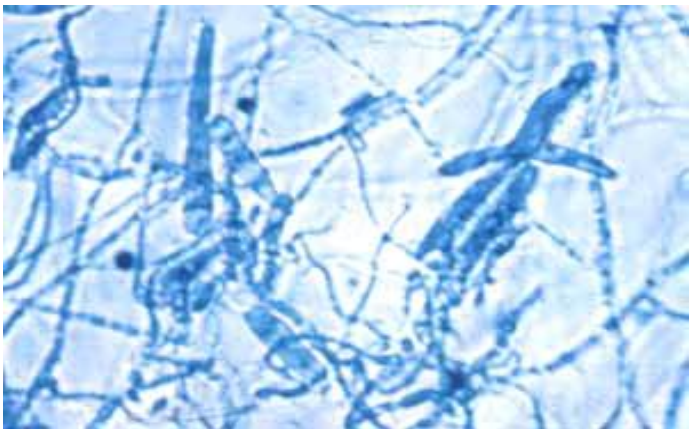

Plate VII. Microscopy of *Trichophyton equinum* showing club-shaped Macro conidia (x400) (LCB)

4. 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. [16], Nweze [17], 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. audounii*, are implicated in equine dermatophytoses in the study region. This is consistent with the writings of Nweze [17] and Popoola et al. [18].

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 [19].

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. [20] and Sitterle et al. [21] 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, [11], 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.

5. 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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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