Assessing the Antifungal Activities of *Buchholzia 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 *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 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. audounii* (2), and *M. gypseum* (3) were the species recognized. 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 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 for this study.

Keywords: Dermatophytes, *Buccholzia 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.[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 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.[3] 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.[3]

In nail infections (onychomycosis), the nail may become thick, 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]

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.[6] Dermatophytoses are an example of a superficial fungal skin infection that can be zoonotic and pose a major health risk.[7] 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 11^0 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.[8] Hairs were pulled out and removed.[9] 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. 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.[10]

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.[11]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.[11]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.[12]

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 on Agar-based disk diffusion susceptibility for Dermatophytes.[13] 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.[14]

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. Table 1 displays the percentage of Dermatophytes isolated from horses with respect to the other fungi (Rhizopus, Mucor, Yeast, and Aspergillosis) also present. The Dermatophytes represented only 20% of the total fungi isolated.

Trichophyton (7) and Microsporum (5) were isolated and characterized (Table 2). The additional fungi that were isolated from the samples were Rhizopus, Mucor, Yeast, and Aspergillosis.

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

		Isolates			Total
		Other Fungi	*Microsporum*	*Trichophyton*	
Horses	Count	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 calculation. 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 (Tables 3, 4 and 5).

Dermatophytes were observed more in the 6-10 year old horses especially Trichophyton (Table 3). However, Microsporum were also isolated more from the same age group when compared to the other age groups (Table 3). Table 4 presented the anatomical site with the most isolates. The back of the horses were observed to offer more Dermatophytes (Trichophyton) when compared to other anatomical sites on the horse. Furthermore, Microsporum was found more on the neck, fewer on the back and none on the limbs of the horses (Table 4).

Dermatophytes isolates (Microsporum and Trichophyton) were recorded more in the males than females (Table 5). This might be due to Stallions being preferred for racing, cultural activities and for use as beast of burden. In addition, some Dermatophytes (Microsporum and Trichophyton) were also isolated from the females (Table 5).

The minimum inhibitory concentration (MIC) and the minimum fungicidal concentration (MFC) are represented in Table 7 for both Microsporum and Trichophyton species. These were used to determine the concentration in which visible growth was inhibited or completely eradicated. The different species of Dermatophytes presented unique MICs and MFCs, except for *Trichophyton equinum* isolate HF 57, *Trichophyton rubrum* isolate HM 58, *Trichophyton verrrucossum* isolates HF 39 and HM 39 which had the same concentrations for both MIC and MFC (Table 7).

Terbinafine recorded the most fungicidal action against the different isolates of Dermatophytes with respect to the different species. On the other hand, ketoconazole also presented remarkable action against the different species of Dermatophytes isolates (Table 8). Amphotericin B was not effective against any of the isolates (Table 8). Conversely, Nystatin was effective against all the species of Dermatophytes except for *Trichophyton verrucossum*, where it was resistant (Table 8).

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

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

Table 4: Anatomical Site and Dermatophytes Isolates

Anatomical site		Isolates	
		Microsporum	*Trichophyton*
Head	Counts	1	1
	% within isolates	20	14.3
Neck	Count	2	1
	% within isolates	40	14.3
Back	Count	2	4
	% within isolates	40	57.1
Limbs	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	
		Microsporum	*Trichophyton*
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

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)
Microsporum audounii			
A	HM10	125	250
B	HM13	125	250
Microsporum gypseum			
A	HM3	125	250
B	HM8	125	250
C	HF50	125	250
Trichophyton rubrum			
A	HM58	125	125
Trichophyton verrucossum			
A	HM39	125	125
B	HM45	125	250
C	HF39	125	125
Trichophyton equinum			

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

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

Drugs / Samples	KCA	TER	NY	PB	AMB	ITC	AGF
Microsporum gyseum							
HM3	S(10mm)	S(28mm)	S(10mm)	R	R	R	R
HM8	S(10mm)	S(30mm)	S(11mm)	R	R	R	R
Microsporum audounii							
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
Trichophyton rubrum							
HM58	S(22mm)	S(33mm)	S(15mm)	S(13mm)	R	R	R
Trichophyton verrucossum							
HM39	S(16mm)	S(28mm)	R	R	R	R	R
HM45	S(18mm)	S(30mm)	R	R	R	R	R
Trichophyton equinum							
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

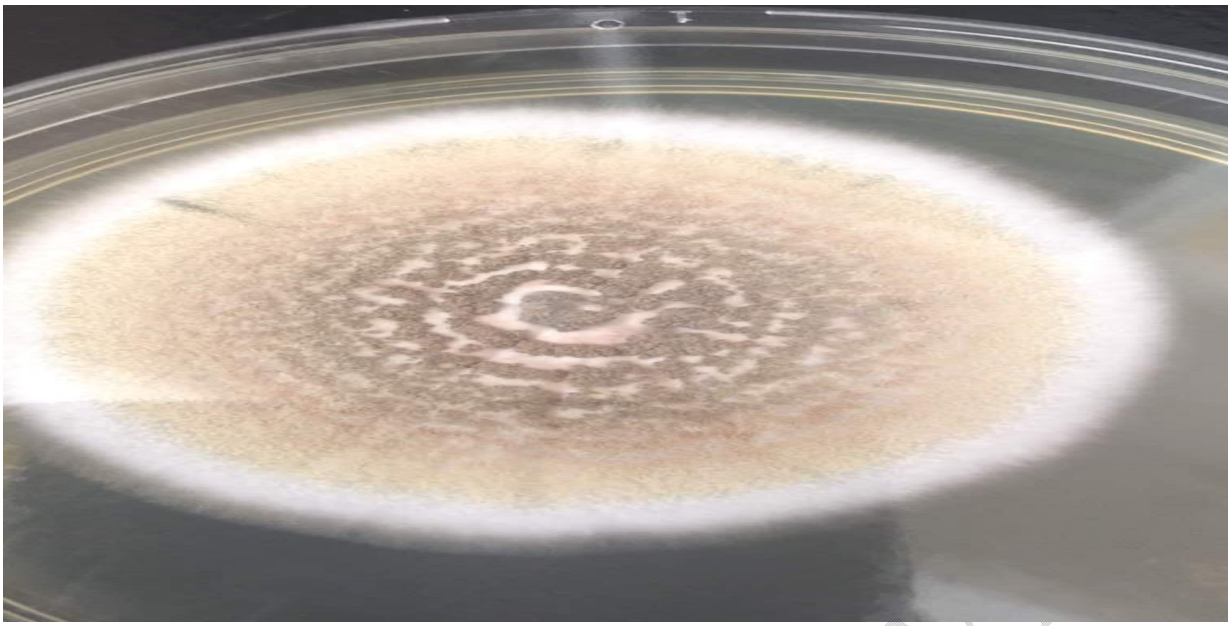

Plate 1; A colony of *Microsporum gypseum* on PDA having dark to a cinnamon brown appearance with granular texture after 10 days growth at room temperature of 25^0 c

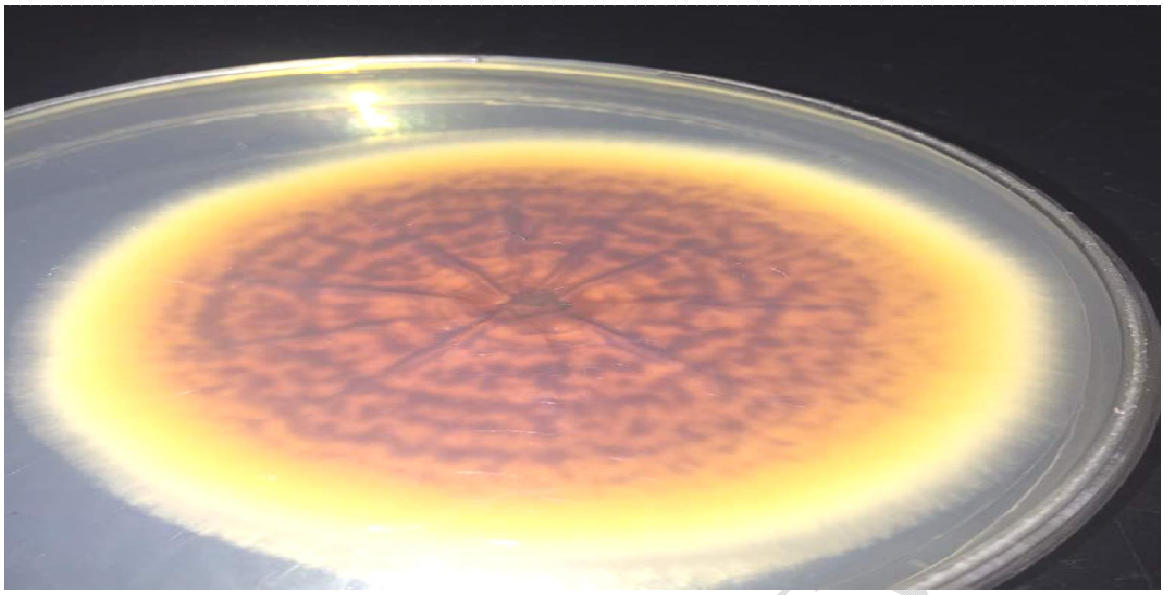

Plate 2: The reverse side of *Microsporum gypseum* with slight yellow to red coloration

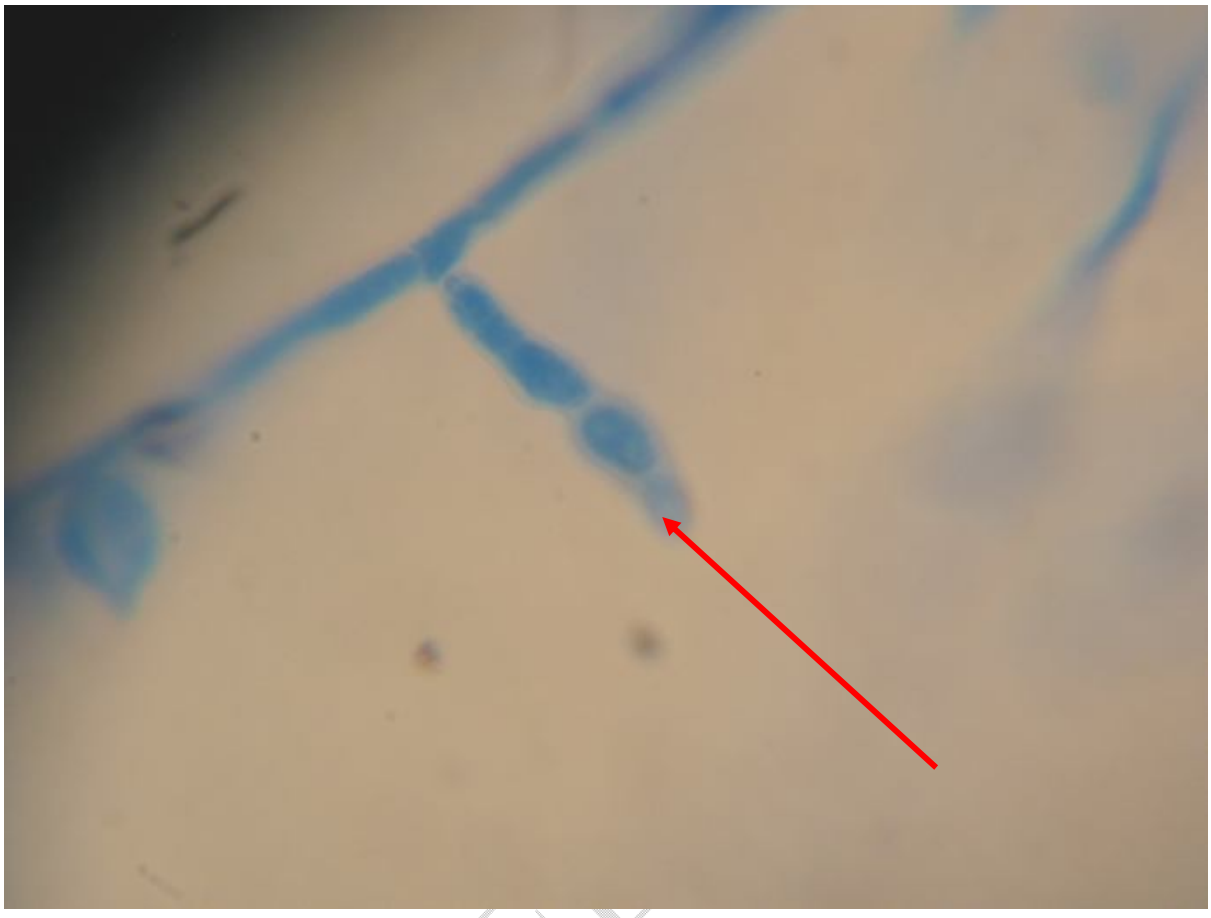

Plate 3: Microscopy of *Microsporum gypseum* showing barrel-shaped macro conidia (x400) (LCB stain)

Plate 4: Colony of *Trichophyton verrucossum* having a cream coloured glabrous growth after 14 days growth on PDA at 25^{o}c

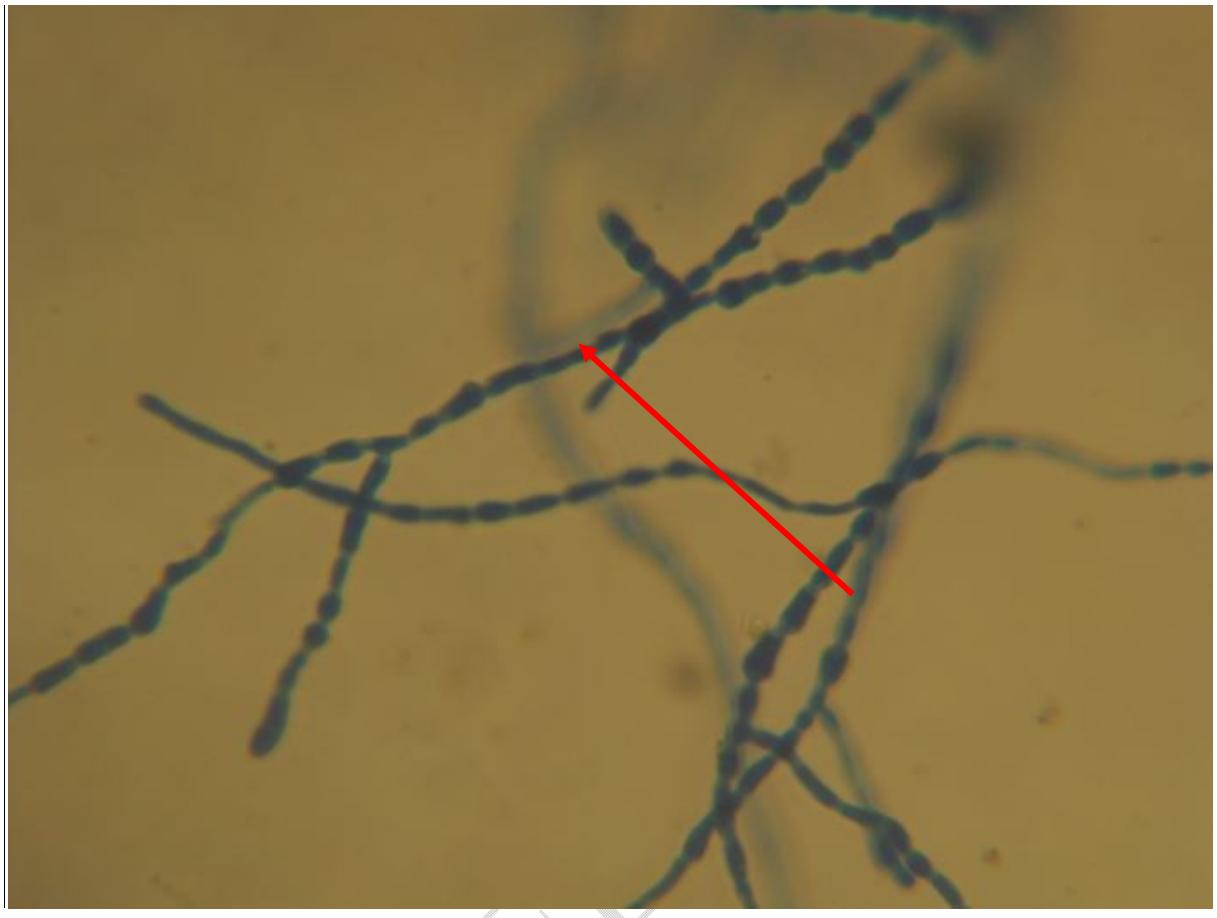

Plate 5: Microscopy of *Trichophyton verrucossum* with a arrow indicating the Chlamydospores (x400) (LCB stain)

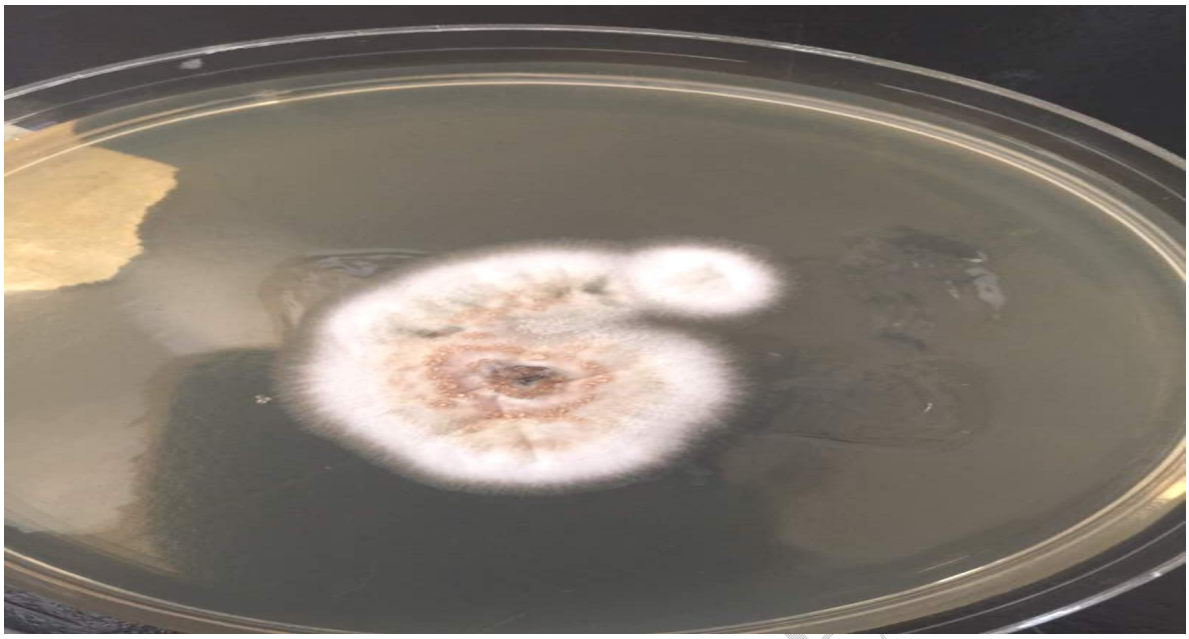

Plate 6: A colony of *Microsporum audouinii* on PDA exhibiting gray to white downy texture, after 14 days growth at room temperature of 25^{o}c

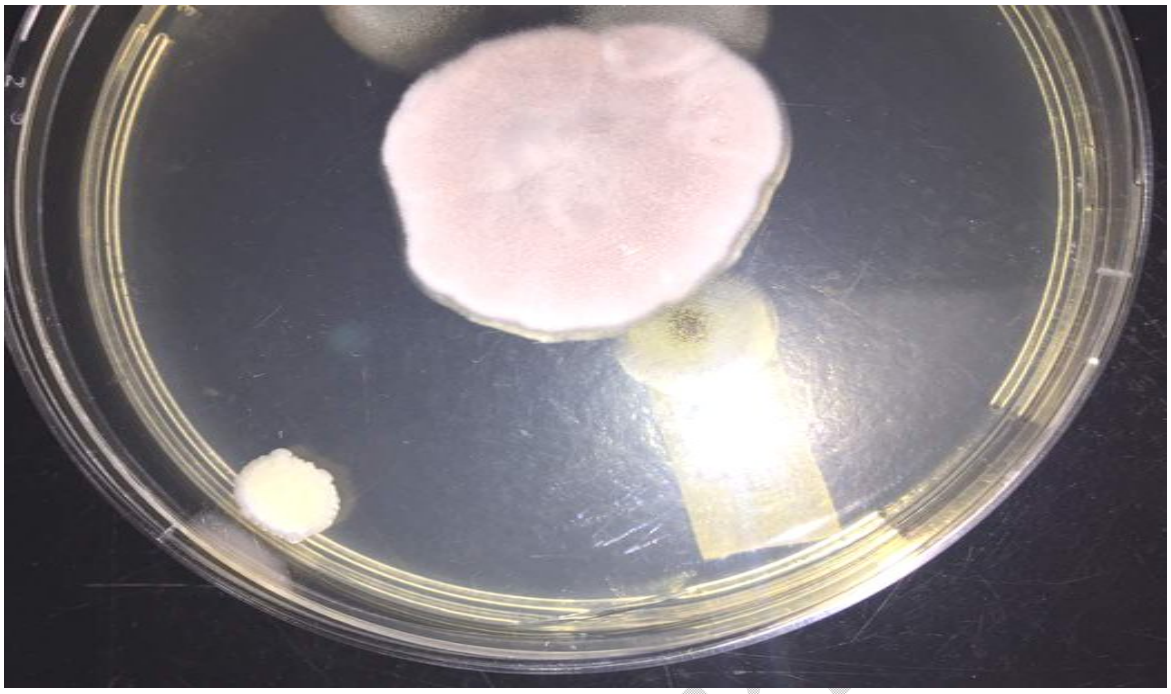

Plate 7: A colony of *Trichophyton equinum* on PDA with cream white to yellow appearance after 12 days growth

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 [15,16,17]. This investigation confirmed the presence of three Trichophyton species (*T. equinum, T. rubrum, T. verrucosum*) and two Microsporum species (*M. gypseum, and M. audounii*) in the study area implicated in equine dermatophytoses. This is consistent with the writings of [16,18].

The highest isolated Trichophyton etiological agent was *Trichophyton equinum*. The works which noted that *T. equinum* is the most frequent isolated Dermatophytes species from horses and *T. verrucossum* from cattle, concur with this finding.[12,19]The second-most isolated Dermatophyte in this investigation was *Microsporum audonii*. It is a fungus that infects animals that frequently come into contact with soil.[20]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.[21] 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 verrucossum* 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 that terbinafine can be used to treat the majority of Dermatophytes infections in horses.[22,23] Amphotericin B, one of the most widely used antifungal medications, was not effective against any of the isolates. This backed up the studies by that showed how amphotericin B was ineffective against dermatophytoses.[8]

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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