

Efficacy of *Buchholzia coriacea* (Wonderful Kola) Extracts and Antifungal Agents on Caprine Dermatophytosis, in Gwagwalada FCT, Nigeria

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Abstract

The extracts of wonderful kola were prepared using the cold maceration technique using n-hexane, ethyl acetate, distilled water, and methanol as solvents. The extracts were then tested on each isolate using the broth macro dilution technique. Commercially available antifungal discs (Himedia®) containing Amphotericin B (100 units), Clotrimazole (10 µg), Fluconazole (25 µg), Itraconazole (10 µg), Ketoconazole (10 µg), and Nystatin (100 units) were used to assess the susceptibility pattern of the isolates to commonly available antifungal agents. Out of the 124 samples, 20 (16.13%) were positive for dermatophytes. The methanol extract of wonderful kola showed activity on all the isolates, while the hexane extract showed activity on only three isolates, and the ethyl acetate extract showed activity on only two isolates. The aqueous extract showed no activity on any of the isolates. For the six antifungal drugs used, Amphotericin B was the most effective agent against all the isolates followed by Nystatin, Itraconazole, Ketoconazole, Clotrimazole and finally Fluconazole which had little or no effect on majority of the isolates. All *Microsporum* species were resistant to fluconazole, and all but one were resistant to clotrimazole. *Trichophyton mentagrophytes* was resistant to all the antifungal agents. *Epidermophyton floccosum* was susceptible to all the agents except clotrimazole. Dermatophytosis is a health issue in goats from Gwagwalada, FCT, and

the methanol extract of wonderful kola has a significant effect on the dermatophytes isolated. There is a need to practice good hygiene and carry out proper diagnosis and treatment to prevent and control the spread of dermatophytes to humans and other animals. The antifungal activity of extracts of *Buchholzia coriacea* (Wonderful Kola) should be further explored and fully exploited.

Keywords

Wonderful Kola, Dermatophytes, Maceration, Methanol, n-Hexane, Ethyl Acetate

1. Introduction

It is a well-known fact that treating fungal infections can be challenging, and only a few classes of antifungal drugs, such as polyenes, azoles, echinocandins, allylamines, and flucytosine, are available to treat the myriad of fungal infections [1] [2]. Some topical antifungal agents that have been used with accorded success in various fungal conditions include iodine preparations (tincture of iodine, potassium iodide, iodophors), copper preparations (copper sulphate, copper naphthenate, cuprimyxin), sulfur preparations (monosulfiram, benzoyl disulfide), phenols (phenol, thymol), fatty acids and salts (propionates, undecylenates), organic acids (benzoic acid, salicylic acids), dyes (crystal [gentian] violet, carbolfuchsin), hydroxyquinolines (iodochlorhydroxyquin), nitrofurans (nitrofuraxine, nitrofurfurylmethyl ether), imidazoles (miconazole, ticonazole, clotrimazole, econazole, thia-bendazole), polyene antibiotics (amphotericin B, nystatin, pimarinic, candicidin, hachimycin), allylamines (naftifene, terbinafine), thiocarbamates (tolnaftate), and miscellaneous agents (acrisorcin, haloprogin, ciclopirox, olamine, dichlorophen, hexetidine, chlorphenesin, tiacetin, polynoxylin, amorolfine [3] [4]).

More than 80% of the population in developing countries depend on plants for their medical needs against various ailments [5] [6]. Although dermatophytes respond well to conventional antifungal agents [7] [8], many patients usually cannot afford the cost and instead use local medicinal plants to treat infections [6]. Some laboratory tests were carried out using some of these plant extracts found in Nigeria against dermatophytes recovered from patients, and some were found to have good *in vitro* antifungal activities against dermatophytes [9] [10].

Buchholzia coriacea, also known as “Wonderful kola”, belongs to the *Cappara-ceae* family [11]. The kola is recommended for the treatment of migraines and malaria in humans, and its leaves and seeds have been reported to have anthelmintic activity [12]. The ethanolic extract of *Buchholzia coriacea* seeds has been shown to have antitrypanosomal activity on mice experimentally infected with *Trypanosoma brucei* [13]. The seeds have also been shown to have antimicrobial properties in studies carried out by Nwachuckwu *et al.* [14]. The authors further stated that the fresh seed as well as hexane and methanolic extracts showed anti-

crobial activities against some food-borne bacteria like *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Trichoderma viridae*, and *Aspergillus niger*.

An increasing number of antifungal agents have been used for treating dermatophytosis [15] [16]. However, not all species have the same susceptibility patterns [17], and relative or absolute microbial resistance may occur in relation to some dermatophytes [18] [19]. The main objective of this research is to ascertain the antimicrobial effect of *Buchholzia coriacea* on dermatophytes isolated from goats in Gwagwalada, FCT, Nigeria.

2. Materials and Methods

2.1. Study Area

The study was conducted in the Gwagwalada area council, Abuja, Nigeria. Gwagwalada is the second-largest Area Council in the Federal Capital Territory (FCT), Abuja, and the major occupation of the local indigenes is subsistence farming, which includes the rearing of small and large ruminants, including goats and sheep [20]. It is one of the semi-urban settlements in the FCT and is located between latitude and longitude 8°56'29"N and 7°5'30"E, respectively [21].

2.2. Sample Collection and Processing

In the area council, skin samples were collected from any goat with clinically suggestive lesions in goat flocks in Fulani camps, live goat markets, and rural homesteads between the months of August 2015 and January 2016 after consent was received from the cattle owners. The purposive sampling technique was used and images of sampled goats taken using a digital camera.

Using protective gloves, a sterile scalpel blade was used to obtain skin scrapings from the margins of lesions suggestive of dermatophytosis after disinfecting the area with alcohol, according to the Elewski method as reported by Nagar *et al.* [22]. Samples were placed in clean paper envelopes and in separate polythene bags and correctly labeled. For each sample, a different scalpel was used to avoid contamination. Collected samples were transported as dry packs to the Microbiology Laboratory of the Department of Veterinary Microbiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria.

2.3. Culture for Dermatophytes on Media

The samples were then cultured on Sabouraud dextrose agar (Oxoid, U.K) with chloramphenicol (0.05 mg/ml) and cyclohexamide (5 mg/ml), which is a selective medium used for primary isolation of fungi [23]. The cultures were incubated at room temperature for one to four weeks.

2.4. Identification of Isolates

Suspected growths were subcultured on Potato Dextrose agar to facilitate distinctive spore formation for identification and pigment production. They were incubated at room temperature for one to four weeks [24]. Identification was based on

colonial and microscopic characteristics using the fungal color atlas [25] [26].

2.5. Preparation of n-Hexane, Ethyl Acetate, Aqueous, and Methanol Extracts of *B. coriacea* Seed

The seeds of *B. coriacea* were purchased, and a sample was sent to the Herbarium, Department of Biological Sciences of the Ahmadu Bello University, Zaria, for authentication and given the voucher number 6921. The seeds were cut into small pieces, dried in the shade, and ground into a coarse powder using a mortar and pestle. 600 grams of the powdered seeds of *B. coriacea* were extracted serially by cold maceration with n-hexane, ethyl acetate, distilled water, and methanol using the method of Goldfarb and Alani as reported by Hombach *et al.* [27], with modifications where necessary. For n-hexane, ethyl acetate, and methanol, the plant material (600 g) was soaked in each solvent for 72 hours each with intermittent shaking at 2-hour intervals. The extract was then filtered using filter paper and later concentrated using a rotary evaporator at reduced pressure. For the aqueous extract, the plant material (600 g) was soaked in one liter of distilled water for 24 hours. It was then sieved with a muslin cloth and the extract placed in a rotary evaporator at reduced pressure. The percentage yield for each extract was determined, and the extracts were kept for further use in a desiccator.

2.6. Preparation of the Inoculum

The isolated dermatophytes were subcultured from sterile distilled water in which they had been stored onto sterile Potato Dextrose Plates containing chloramphenicol (0.05 mg/ml) and cycloheximide (5 mg/ml). After 5 - 7 days of growth, the growths were harvested using a sterile loop and mixed in 10 mls of sterile saline. The tubes were agitated using a vortex mixer to break down the hyphae. The test tube was allowed to stand so larger hyphal segments could settle, and the suspension was adjusted to 0.5 MacFarland's standard.

2.7. Testing the Antifungal Activity of the Different Extracts of *B. coriacea* Seeds on the Isolates

The broth macro dilution method was used for in vitro testing of the antifungal activity of the extracts using Clinical and Laboratory Standards Institute (CLSI) M38-A standard for molds (CLSI document M38-A, 2002) as a guideline with modifications where necessary [28]. Briefly, a stock solution of 1000 mg/ml of each extract was prepared a day before using distilled water as a diluent. SDA broth (4.5 ml) was added to different sets of labeled sterile test tubes for the different isolates. From the stock solution of the different extracts, 0.5 ml of each extract was removed using a sterile syringe to perform a twofold dilution. After the dilution, a drop from the prepared inocula of the different isolates was put in each test tube. The tubes were then incubated at room temperature and observed for 24 - 48 hours. Negative and positive controls were set up. Any sign of cloudiness or growth was recorded as negative, and signs of no growth were recorded as

suspected positive. Test tubes that showed no growth were further plated on sterile plates of SDA to determine the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC). The MIC was defined as the lowest concentration of the extract that inhibited fungal growth after the period of incubation, while the MFC was defined as the lowest concentration of the extract that would kill the fungal growth.

2.8. Antifungal Susceptibility Tests

This was carried out according to the European Committee for Antimicrobial Susceptibility Testing (EUCAST) Definitive Documents E.DEF 9.1 (2008)/CLSI M-38A2 (2008) with modifications where necessary. Commercial antifungal susceptibility discs, HexaAntimyc-01 (Himedia®) were used. Each disc contained 6 standard antifungal agents, namely Amphotericin B (100 units), Clotrimazole (10 µg), Fluconazole (25 µg), Itraconazole (10 µg), Ketoconazole (10 µg), and Nystatin (100 units) [2].

The agar-based disk diffusion susceptibility method for dermatophytes was used as reported by Bongadpour *et al.* [29], with modifications where necessary. The inoculum prepared for testing the extracts was used. A sterile nontoxic swab stick was used to spread the standardized inoculum evenly on the surface of petri dishes containing Mueller Hinton agar medium with chloramphenicol and cycloheximide and exposed to air dry for 5 minutes in a safety cabinet. The antifungal discs were then applied to the plates using sterile forceps and incubated at room temperature for up to 5 days. When growth took place, the sizes of the zones of inhibition were measured using a ruler for each antifungal agent and recorded [30].

2.9. Statistical Analysis

The data obtained from the study were analyzed using the Statistical Package for the Social Sciences (SPSS) version 21.0, and the results obtained were presented in tables and charts. Statistical level of significance was fixed at $P < 0.05$ [31].

3. Result

3.1. Results of Antifungal Activity of *Buchholzia coriacea* Seed on the Isolates

The aqueous extract had the highest percentage yield (4.46%), followed by the methanol extract (3.54%), the hexane extract (0.64%), and finally the ethyl acetate extract (0.23%), as shown in **Table 1**. The methanol extract had 100% effect on all the isolates with MICs and MFCs ranging from 62.5 mg/ml to 500 mg/ml. The hexane extract had an effect on only 3 isolates, with MIC and MFC ranging from 62.5 mg/ml to 500 mg/ml. The ethyl acetate extract had an effect on only 2 isolates of *T. tonsurans*, with MIC and MFC ranging from 62.5 mg/ml to 500 mg/ml. The aqueous extract did not exhibit any inhibitory activity on any of the isolates. The one isolate of *Epidermophyton floccosum* only showed activity when the methanol extract was used.

Table 1. Percentage yield of extracts of *Buchholzia coriacea* seed.

Extract	Amount (g)	Percentage Yield (%)
n-Hexane	600	0.64
Ethyl Acetate	600	0.23
Aqueous	600	4.46
Methanol	600	3.54

Based on **Table 2**, which presents the distribution of dermatophytes based on anatomical location.

The table reports the number of samples collected and the positive cases of dermatophytes across various anatomical sites. The total number of samples collected is 124, with 20 positive cases, resulting in an overall positivity rate of 16.13%.

The ear had the highest positivity rate, with 8 positive samples out of 35 collected, equating to a percentage of 6.45%. The neck has the lowest positivity rate, with only 1 positive sample out of 10 collected, giving a percentage of 0.81%. This table further highlights the variability in dermatophyte prevalence based on the anatomical location of the samples. The chi-square (χ^2) test result and the associated p-value provide important statistical insights regarding the distribution of dermatophytes based on anatomical sites.

Since the p-value (0.1264) is greater than the conventional alpha level of 0.05, this suggests that there is not a statistically significant association between the anatomical location and the occurrence of dermatophytes in this sample. In simpler terms, the differences in positive rates among the anatomical sites could be due to random variation rather than a true effect.

Although there are observed differences in dermatophyte prevalence across the anatomical sites, the statistical analysis indicates that these differences are not significant enough to suggest that anatomical location significantly influences the presence of dermatophytes within the sampled population.

Table 2. Distribution of dermatophytes based on anatomical location.

Anatomical Site	No. of Samples Collected	No. Positive (%)
Udder	15	4 (3.23)
Leg	20	4 (3.23)
Ear	35	8 (6.45)
Face	10	3 (2.42)
Neck	10	1 (0.81)
Others (Shoulders, Chest, Stomach)	34	0 (0)
TOTAL	124	20 (16.13)

P = 0.1264, df = 5, χ^2 = 8.593.

The Effect of Extracts of *Buchholzia coriacea* Seed on *Microsporum Spp* Isolated from Goats in Gwagwalada.

Extracts

Figure 1 illustrates the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of the methanol extract of *Buchholzia coriacea* seed against *Microsporum* species isolated from goats in Gwagwalada. The concentrations are expressed in mg/ml and indicate the effectiveness of the extract in inhibiting and killing the fungal isolates.

The results showed variations in the susceptibility of the *Microsporum* isolates to the methanol extract. Isolates MM22m and FC23F recorded low MIC and MFC values of approximately 60 mg/ml, indicating that the extract was highly effective against these isolates at relatively low concentrations. Similarly, isolate FC27f showed moderate susceptibility with both MIC and MFC values around 125 mg/ml.

On the other hand, isolate FC24f demonstrated higher MIC and MFC values of approximately 250 mg/ml, suggesting that a greater concentration of the extract was needed to inhibit and kill the organism. The isolate MM8m showed an MIC value of about 125 mg/ml and an MFC value of about 250 mg/ml, indicating that although fungal growth could be inhibited at a moderate concentration, a higher concentration was required to achieve fungicidal activity.

The isolate OMN5m showed the highest MIC and MFC values of approximately 500 mg/ml. This suggests that OMN5m was the most resistant isolate among the tested *Microsporum* species, requiring a very high concentration of the extract for both inhibition and destruction of the fungal cells.

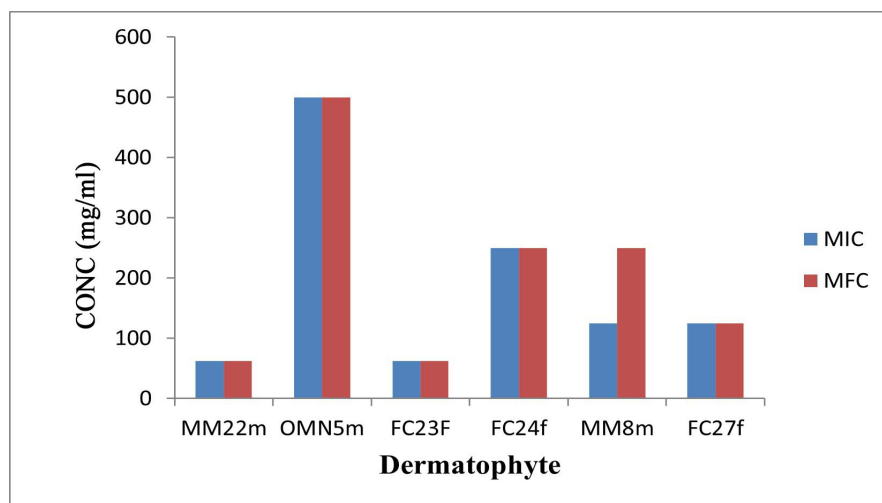


Figure 1. The minimum fungicidal concentration (MFC) and minimum inhibitory concentration (MIC) of the methanol extract of *Buchholzia coriacea* seed on *Microsporum* species isolated from goats in Gwagwalada.

Figure 2 presents the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of the methanol extract of *Buchholzia coriacea*

seed against different *Trichophyton* species isolated from goats in Gwagwalada. The values are expressed in mg/ml and indicate the concentration of the extract required to inhibit and kill the fungal isolates.

The results revealed varying levels of susceptibility among the *Trichophyton* isolates tested. Some isolates, such as FC11f, IB11Mfpc, IB11Mfpc, IB209m, and FC30f, showed low MIC and MFC values of approximately 60 mg/ml, indicating that the methanol extract was highly effective against these isolates even at low concentrations. Similarly, isolates MM14m, FC31f, OMN5m, and IB210m showed moderate MIC and MFC values of about 125 mg/ml, suggesting moderate sensitivity to the extract.

However, certain isolates demonstrated greater resistance to the methanol extract. Isolates OMJ21m, OMN4F, and FC13f recorded high MIC values of about 250 mg/ml and even higher MFC values of approximately 500 mg/ml. This indicates that larger concentrations of the extract were required not only to inhibit fungal growth but also to completely kill the organisms. The large difference between MIC and MFC values in these isolates suggests that the extract had more inhibitory than fungicidal effects at lower concentrations.

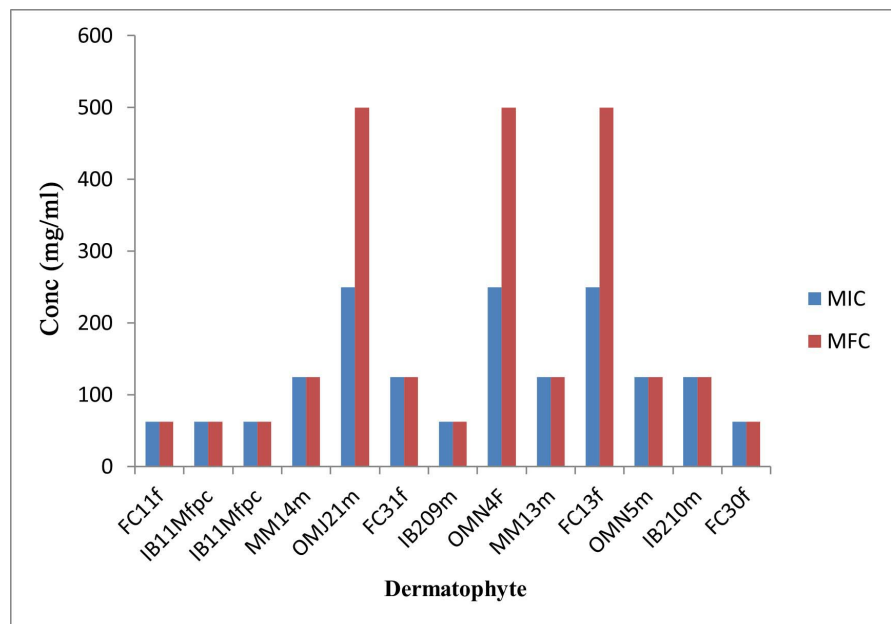


Figure 2. The minimum fungicidal concentration (MFC) and minimum inhibitory concentration (MIC) of the methanol extract of *Buchholzia coriacea* seed on *Trichophyton* species isolated from goats in Gwagwalada.

Figure 3 presents the Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) values of the hexane extract of *Buchholzia coriacea* seed against dermatophyte isolates obtained from goats in Gwagwalada. The results revealed varying levels of susceptibility of the dermatophyte isolates to the extract.

The isolate OMN5m recorded the highest MIC and MFC values of 500 mg/ml,

indicating that it was the most resistant isolate among those tested. This suggests that a high concentration of the hexane extract was required to inhibit and completely eliminate the fungal organism.

Similarly, OMJ21m showed an MIC value of approximately 250 mg/ml and an MFC value of 500 mg/ml. This indicates that although fungal growth could be inhibited at a lower concentration, a much higher concentration of the extract was necessary to achieve fungicidal activity. The difference between the MIC and MFC values suggests partial resistance of the isolate to the extract. In contrast, MM13m recorded the lowest MIC and MFC values of approximately 62.5 mg/ml, indicating that the hexane extract of Wonderful kola was highly effective against this isolate at relatively low concentrations.

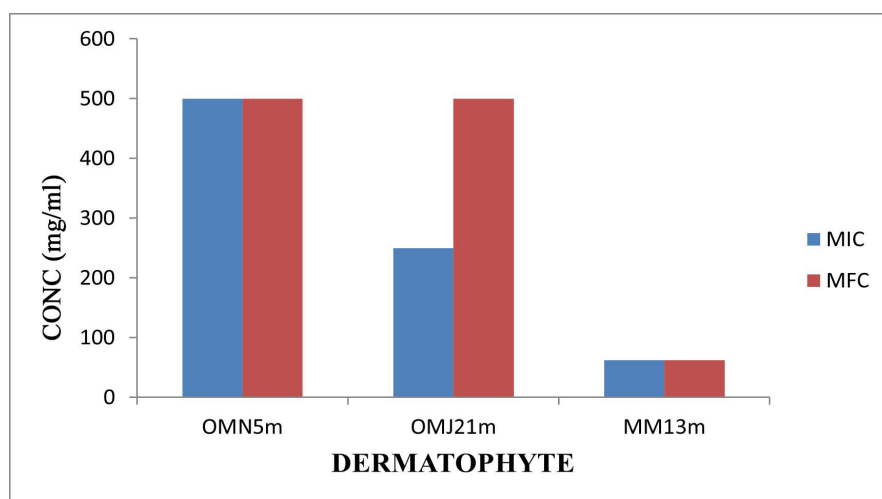


Figure 3. The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of the hexane extract of *Buchholzia coriacea* seed on dermatophyte isolates from goats in Gwagwalada.

Figure 4 shows the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of the ethyl acetate extract of *Buchholzia coriacea* seed against dermatophyte isolates obtained from goats in Gwagwalada. Two dermatophyte isolates were tested, namely IB209m and MM13m.

The result indicates that the MIC and MFC values differed between the two isolates, showing variations in their susceptibility to the plant extract. For isolate IB209m, both the MIC and MFC values were approximately 60 mg/ml. This suggests that a relatively low concentration of the extract was sufficient to inhibit and kill the fungal isolate. The similarity between the MIC and MFC values further indicates that the extract exhibited strong antifungal activity against IB209m, since the inhibitory concentration was nearly equal to the fungicidal concentration.

In contrast, isolate MM13m showed much higher MIC and MFC values. The MIC was approximately 250 mg/ml, while the MFC was about 500 mg/ml. This implies that a higher concentration of the extract was required to inhibit and com-

pletely kill this isolate. The higher values observed for MM13m suggest that it was more resistant or less susceptible to the antifungal effects of the ethyl acetate extract compared to IB209m.

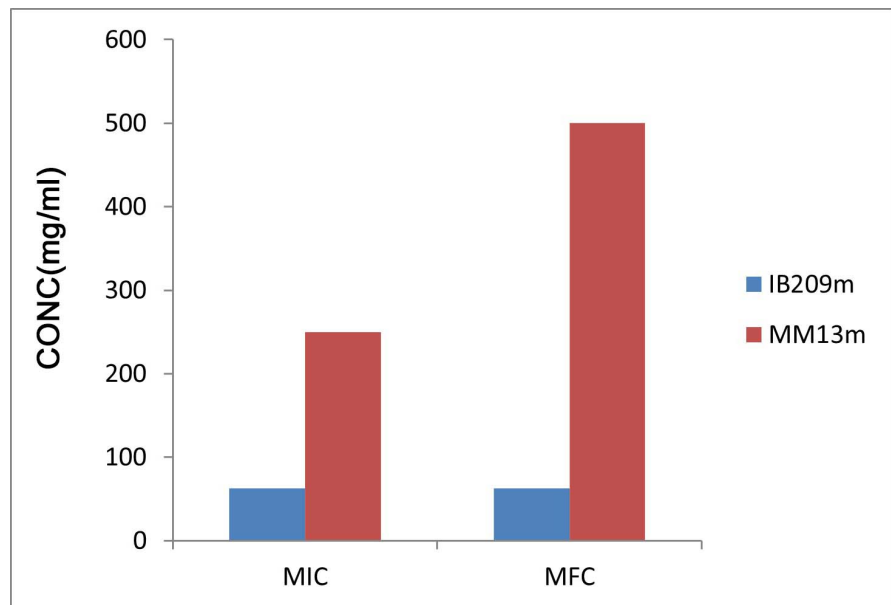


Figure 4. The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of the ethyl acetate extract of *Buchholzia coriacea* seed on dermatophyte isolates from goats in Gwagwalada.

3.2. Results of the Antifungal Activity of Commercially Standard Antifungal Agents on the Isolates

Amphotericin B was the most effective agent against all the isolates, followed by Nystatin, Itraconazole, Ketoconazole, Clotrimazole, and finally Fluconazole, which had little or no effect on a majority of the isolates. The isolates were most susceptible to Amphotericin B (70%), followed by Nystatin (60%), Ketoconazole (60%), Itraconazole (55%), Clotrimazole (30%), and finally Fluconazole (15%). All *Microsporum* species were resistant to Fluconazole, and all but one were resistant to Clotrimazole. *Trichophyton mentagrophytes* was resistant to all the antifungal agents. *Epidermophyton floccosum* was susceptible to all the agents except Clotrimazole.

4. Discussion

From the antifungal susceptibility test results, the methanol extract was the only extract that possessed an antifungal effect on all the isolates at the concentrations tested. The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of the extract ranged from 62.5 mg/ml to 500 mg/ml. Most of the isolates had the same MICs and MFCs, with the exception of four isolates. This shows that the same concentration of an extract that can inhibit a dermatophyte can also kill it, which is a sign of a potentially effective antifungal agent. The

aqueous extract had a higher percentage yield than the methanol, hexane, and ethyl acetate extracts. [32] observed that the more polar the solvent, the better its extraction power. The seeds of *B. coriacea* are rich in phytonutrients such as alkaloids, glycosides, saponins, flavonoids, tannins, and phenols both quantitatively and qualitatively [33].

The hexane extract of *B. coriacea* seed had an MIC and MFC that ranged from 62.5 to 500 mg/ml on *Microsporum audouinii*, *Trichophyton verrucosum*, and *Trichophyton tonsurans*, which were all isolated from goat markets. The ethyl acetate extract of *B. coriacea* seed had an effect on two isolates of *T. tonsurans* with MICs and MFCs ranging from 62.5 to 500 mg/ml. This indicates that activity against the isolated dermatophytes decreased with decreasing polarity in extracts of *B. coriacea*. Despite the polarity and high percentage yield of the aqueous extract, it did not have any effect on the isolates. The variations in performance of the extracts on each test organism may be a result of possible synergistic interactions between the active components in the extracts, as well as the biodiversity of the isolates.

In this study, *Microsporum species* were resistant to fluconazole but were susceptible to amphotericin B, nystatin, itraconazole, and ketoconazole. Amphotericin B and nystatin are polyenes, a class of antifungal drugs usually indicated for superficial and systemic infections. It is well documented that amphotericin B is not effective against dermatophytosis [34]-[36].

The resistance seen in fluconazole and the other azoles, which are more commonly used to treat dermatophytosis, could be due to widespread use and misuse since they are relatively cheaper and more available than the polyenes [37].

The one isolate of *Trichophyton mentagrophytes* was resistant to all the drugs tested. Some isolates of *T. verrucosum* and *T. tonsurans* also showed resistance to the conventional antifungal drugs. However, most of the *Trichophyton* isolates were still resistant to fluconazole and clotrimazole. It's interesting to note that the same species of dermatophytes isolated from different sources showed different results with the different extracts used and also with the conventional antifungal drugs. This agrees with Fernández-Torres *et al.* [28], who stated that not all species have the same susceptibility patterns, and relative or absolute microbial resistance may occur in relation to some dermatophytes. This study has shown that differences in strains of the same species of dermatophytes affects the choice of drug used for treatment.

5. Conclusions

The methanol extract of *Buchholzia coriacea* seed showed in vitro antifungal activity against all the dermatophytes isolated, while the hexane and ethyl acetate extracts showed in vitro antifungal activity against only a few of the isolates. Thus, the crude extracts of *Buchholzia coriacea* seed have a moderate effect against dermatophytes except the aqueous extract. Most of the dermatophytes isolated are susceptible to commercially available antifungal agents.

All the isolates were susceptible to the methanol extract of *Buchholzia coriacea* with MICs and MFCs ranging from 62.5 mg/ml to 500 mg/ml. The hexane extract had an effect on only 3 isolates, namely *Microsporum audouinii*, *Trichophyton verrucosum*, and *Trichophyton tonsurans*, with the MICs and MFCs ranging from 62.5 mg/ml to 500 mg/ml. The ethyl acetate extract has an effect on only 2 isolates of *Trichophyton tonsurans* with MICs and MFCs within the same range. The aqueous extract had no effect on the isolates. Most of the isolates were susceptible to the range of conventional antifungal drugs used, but there were cases of resistance to fluconazole and clotrimazole.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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