Sensitivity of Moulds Isolated from Air to Antimycotic Drugs, Synthesized Metal Complexes and *Jatropha curcas* Seed oil

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Abstract: Antimold resistance is common due to drug abuse, mutation caused by genetic recombination and others. In a bid to contribute new antimold agents to the pharmaceutical and medical world molds were isolated from selected areas on the campus of the University of Ilorin. Their sensitivity profiles were evaluated against commercial antimold drugs, synthesized metal complexes and the seed oil of *Jatropha curcas*. The isolates were cultured and antibiotic sensitivity test was carried out on the isolates using the disc-diffusion and agar-well diffusion methods. Most of the molds showed susceptibility to the azoles (clotrimazole, fluconazole and ketoconazole) with inhibition zones ranging from 12mm to 25mm. *Geotrichum candidum* showed resistance to fluconazole. Griseofulvin had the least activity with inhibition zone as low as0mm. Fungusol had intermediate susceptibility with inhibition zone between 5mm to 21mm. Only *Mucor circinelloides* was resistant to the commercial antibiotics. The $[Ca(HBAB)](NO_3)_2.2H_2O$ complex, HBAB Schiff-base, and $[Mg(HBAB)](NO_3)_2.4H_2O$ complex synthesizedshowed minute level of antimicrobial activity against *Aspergillus terreus*, *Geotrichum candidum and Trichosporon mucoides*(2 mm -6mm). All the molds were resistant to *Jatropha* seed oil. In conclusion, the synthesized compounds if improved upon show promise of being good antimold agents.

Keywords: Sensitivity, Antimold, Synthesized metal complex, Disc, Agar-well, *Jatropha*.

INTRODUCTION

Antifungal drugs and antibiotics are types of antimicrobial agents. This implies that they are microstatic or microcidal in action. Antifungal drugs are life-saving. They are useful in the treatment of fungal infections. Unfortunately, fungi and bacteria can develop antimicrobial resistance (CDC, 2019).The increasing number of options for treating invasive mold disease, coupled with documented resistance to antimold agents among some strains and species, has confirmed the need for having standardised methods for determining the *in vitro* susceptibilities of both new and established antimold agents against clinical isolates of filamentous molds (Howard *et al*., 2011, Astvad *et al*., 2014).

Synthesized chemical compounds are produced by a deliberate engineering of chemical reactions to obtain a product, or many products (Vogel *et al*., 1996). This occurs by physical and chemical manipulations usually involving one or more reactions. The process like normal scientific procedures is reproducible, reliable, and established to work in multiple laboratories.Several scientists have produced new synthetic compounds with the aim of solving societal problems (Al-Nahary, 2009; Tella *et al*., 2017). One of such problems is that of antibiotic resistance. Drugs with antibiotic properties have transformed healthcare and made our modern way of life a possibility. Antibiotics are used in many non-medical applications such as livestock growth promotion, preservation of building materials from contamination and treatment of blight in plants. Nevertheless, overuse leads to abuse and threatens their potency due to the promotion and spread of antibiotic resistance (Richardson, 2017).

A study carried out in Mexico reported that methanolic extracts of *Jatropha curcas* L. shell possessed phenolic and antioxidant activity (Perea-Domínguez *et al*., 2016). In a related study it was suggested that all parts of *Jatropha* plant possess powerful antimicrobial activity (Sharma *et al*., 2016). Antimold resistance is becoming an emerging problem as a result of continuous availability of antibiotics over the counter, intrinsic resistance and development of secondary resistance among resistant strains. This paper reports the various sensitivity profile of some molds present in the air to selected antimold drugs, synthesized metal complexes and Jatropha seed oil.Therefore detection of resistant strains to available antibiotics and search for more novel antimicrobial is of great importance.

MATERIALS AND METHODS

The molds used were isolated from the air microflora within the campus of the University of Ilorin. Areas sampled included the CBT arena, block 1, block 4 and the Department of Microbiology area.

Isolation and Identification of Molds

Eight moldsused were isolated from the air microflora unto media using potato dextrose agar (PDA) with the addition of streptomycin to prevent growth of bacteria. The plates were incubated at room temperature for 3-5days. The organisms were subcultured to get pure cultures and were identified based on micro and macro morphology, reverse and surface coloration of colonies and microscopy with reference to Onions *et al.* (1981).

Antimold Drugs, Synthesized Metal Complexes and Jatropha seed oil

The antimold tablets were grinded separately into fine powders with the aid of mortar and pestle. Different quantities of the grinded tablets were weighed using a weighing balance and dissolved in absolute ethanol and further diluted with distilled water to make different concentrations of 0.2g/mL, 0.4g/mL and 0.6g/mL (Adetitun *et al*., 2015).

Synthesis of the Schiff-base and its metal complexes were carried out by a modification to the procedure reported by Tella *et al.* (2016). Sulphamethoxazole and salicylaldehyde used in the synthesis of the Schiff base (HBAB) were obtained from Sigma Aldrich Co., Germany and were of analytical grade. The Schiff base-metal complexes were prepared by reacting $Ca(NO_3)_2{\cdot}4H_2O$ and $Mg(NO_3)_2{\cdot}6H_2O$ separately with the Schiff-base (HBAB) formed in a 1:1 mole ratio in the presence of absolute ethanol under refluxing condition for 6 hours. Gallic acid (3,4,5-trihydroxybenzoicacid), $[Ca(HBAB)](NO_3)_2.2H_2O$ complex, HBAB Schiff-base, $[Mg(HBAB)](NO_3)_2.4H_2O$ complex, and sulphamethoxazole drug used are represented by T1, T4, T5, T6 and T7 respectively.Their preparation therefore was done by weighing the solutes and dissolving in appropriate solvents.Absolute ethanol was used to dissolve T1 while N,N-dimethylformamide was used to dissolve T4, T5, T6 and T7. Further dilution was done with distilled water to obtain a lower concentration.

Jatropha seed oil was obtained from the Department of Chemistry, University of Ilorin, Ilorin, Nigeria. The oil was extracted from *Jatropha curcas* seed using n-hexane as solvent. Three different concentrations of the oil were used (50%, 75% and 100%) (Shivani *et al*., 2011).

Preparation of Local Antibiotic Discs

The antibiotic discs used in this study were made by perforating Whatman filter paper with the aid of an ordinary office two-hole puncher and impregnating them separately with the commercial antimold drugs, the synthesized metal complexes and the *Jatropha* seed oil. This was done by soaking the perforated filter papers in the solvents for 2hours (Antonio-Velmonte *et al*., 1988) followed by air-drying and sterilization using ultraviolet light (UV light). The sterilized impregnated discs were placed in properly labelled sterile containers and stored at room temperature.

Antimold Sensitivity Testing

The disc diffusion method described by Kirby and Bauer (1966) was used to determine the antimold activity of the agents. Broth cultures of the molds were swabbed uniformly on sterile potato dextrose agar plates using sterile swab sticks. The antimold discs, synthesized metal complex impregnated discs and the *Jatropha* seed oil impregnated discs were placed aseptically on the agar plates using sterile forceps. The plates were properly labelled indicating the mold and the different antibiotic discs used. The plates were incubated for 3-5 days at 27^{o}C. After incubation, the diameter of the zone of inhibition around each disc was measured to the nearest diameter along two axis of 90^{o}C using a graduated transparent ruler.

The well method of agar diffusion technique as described by Hugo and Russel (1992) was used to determine the antimicrobial activity of the antimicrobial agents (commercial antimold drugs, synthesized metal complexes and *Jatropha curcas* seed oil). Broth cultures of the molds were swabbed uniformly on sterile potato dextrose agar plates using sterile cotton swab sticks. A sterile cork borer of size 5mm in diameter was used to make ditches on the plates. 0.1mL of the respective antimold drugs, synthesized metal complexes and *Jatropha* seed oil were then put into each appropriately labelled ditches with the aid of a pipette. The inoculated plates were then left on the workbench for 1hr to allow the various solutions to diffuse into the mediumbefore incubation at 25±2 ^{O}C. After incubation, the diameter of the zone of inhibition around each well was measured using a graduated transparent ruler. The presence of zones of inhibition indicates the antimold activity of the various compounds.

RESULTS AND DISCUSSION

Themold isolated from air included *Geotrichum candidum, Mucor circinelloides, Trichosporon mucoides, Aspergillus niger, Aspergillus fumigatus, Rhodotorula* sp*, Aspergillus terreus* and *Trichoderma longibrachiatum.* Air is confirmed to constitute higher frequencies of some culturable molds including members of the *Aspergillus* genera (Adhikari*et al*., 2004). Of the two methods, the agar well diffusion method showed more antimicrobial activity of the antimold drugs in the study of the sensitivity of the mold isolates. Table 1 and 2 shows the result for the sensitivity profile of the mold isolates to the antimold drugs. Also, in both methods, the antimold drug 'griseofulvin' had the least activity of all the drugs against the mold isolates just as it was reported by El-Nakeeb*et al*. (1965) that the antibiotic action of griseofulvin on dermatophytes is highly sensitive and poorly sensitive on filamentous molds and insensitive for yeasts.

Geotrichumcandidum in this study was susceptible to clotrimazole and ketoconazole while moderately susceptible to fungusol and resistant to griseofulvin and fluconazole. Nenoff*et al*. (1999) in their study of invitro susceptibility of yeasts for fluconazole reported that *Geotrichum candidum* revealed very low MIC values to fluconazole. *Mucor circinelloides* showed resistance to all the antimold drugs; this agrees with a Mycology Proficiency Testing Program (2015) which reported that none of the triazoles which includes clotrimazole, fluconazole and ketoconazole in this study are active against *Mucor* spp. No recent study shows the susceptibility of *Mucor* to the azoles used in this study, griseofulvin and fungusol. Despite its resistance to the azoles and other antimolds used in this study, Galgoczy*et al*. (2009) reported the susceptibility of the mold to an antimold drug amphotericin B and also combination with suramin. Therefore, *Mucor* might show resistance to the azoles but are sensitive to other antimold drugs like amphotericin. *Trichosporon mucoides* was susceptible to clotrimazole and fluconazole at high concentrations; moderately susceptible to ketoconazole. Susceptibility of *Trichosporon mucoides* to the azoles especially fluconazole was reported by Raquel *et al*. (2010) in their study. Alastuey-Izquierdo *et al*. (2014) stated that azoles are

still the agents of choice to treat *Aspergillus* infections, in this study, the *Aspergillus* species which includes *Aspergillus fumigatus*, *Aspergillus niger* and *Aspergillus terreus* were all susceptible to clotrimazole, ketoconazole and fungusol while *Aspergillus niger* was susceptible to fluconazole.

Trichoderma longibrachiatum showed susceptibility to ketoconazole, fluconazole, griseofulvin and fungusol. The fungus susceptibility to fluconazole is in contradiction with the Mycology Proficiency Testing Program (2015) which reported resistance of *Trichoderma* to fluconazole. This contradiction may be because of the possibility of different strains of the fungus.

Rhodotorula spp was susceptible to clotrimazole, ketoconazole and fungusol. Seifi *et al.* (2013) reported in their study that *Rhodotorula* spp are dose dependent and sensitive to azoles. Although, they stated that *Rhodotorula* spp showed no activity against fluconazole. Their work tends to disagree with this current report as the *Rhodotorula* spp isolated in this study showed activities against fluconazole even at the lowest concentration of 0.2g/ml. This contradiction could be attributed to the fact that the *Rhodotorula* spp used in this study was isolated from the air within the university's campus and not a clinical isolate.

From the results, the azoles showed the most efficacy against most of the mold isolates which agrees with Sheehan *et al*. (1999) where it was reported that triazoles demonstrates a broad spectrum activity against both filamentous and yeast organisms, a good safety profile with increasing effectiveness at increasing concentrations.

The results of antimicrobial activities of the synthesized metal complexes on the mold isolates using both the disc diffusion and agar well diffusion method showed little activity as shown in tables 3 and 4. The ligands, Schiff-base, and metal complexes used in this study constituted Gallic acid (3,4,5-trihydroxybenzoicacid), $[Ca(HBAB)](NO_3)_2.2H_2O$ complex, HBAB Schiff-base,$[Mg(HBAB)](NO_3)_2.4H_2O$ complex, and sulphamethoxazole. $[Ca(HBAB)](NO_3)_2.2H_2O$ complex, HBAB Schiff-base, and $[Mg(HBAB)](NO_3)_2.4H_2O$ complex were observed to have some level of antimicrobial activity against some of the mold isolates. $[Ca(HBAB)](NO_3)_2.2H_2O$ complex showed antimicrobial activity against *Aspergillus terreus* with inhibition zone of 6mm, The HBAB Schiff-base showed antimicrobial activity against *Trichosporon mucoides* with inhibition zone of 6mm, while $[Mg(HBAB)](NO_3)_2.4H_2O$ complex showed antimicrobial activity against *Geotrichum candidum* with inhibition zone of 4mm.

Although, there is no current literature that supports the antimicrobial activity of these particular set of metal complexes used in this study; some other works have been done on the antimicrobial activity of other metal complexes which demonstrated an increase in antimicrobial activity following the interaction of several compounds with metal ions (Antonio *et al*., 2014). The antimicrobial studies of metal (II) complexes of ciproflaxin was done by Mustapha *et al*. (2014) and tested against different strains of bacteria in which they reported that Large inhibition zones (21 mm - 40 mm) was shown by Ni(II) complex for most of the test strains. Obaleye and Lawal (2007) also assayed the antimold activities of some transition metal complexes of metronidazole on *Aspergillus niger*, *Aspergillus flavus* and *Rhizopus* sp and reported to have great inhibitory activity with zones of inhibition ranging between 14 mm and 69mm.

In another study by Chiericatti *et al*. (2014) in which they investigated and reported the action of silver mordenite against the growth of six mold that are problematic in the food industry. In their findings they found that the mold species studied including *Mucor circinelloides* and *Geotrichum candidum* were susceptible to the complex. Therefore, these compounds may qualify as suitable choice to the next step of drug fabrication.

The study done on the seed oil of *Jatropha curcas* for its antimicrobial activity against

the mold isolates showed that the mold isolates were resistant to the seed oil with no zone of inhibition recorded. This is in contradiction to previous studies on the plant's antimicrobial efficacy. In a study by Rampadarath *et al*. (2016), the bark, root, leaves and seed of *Jatropha curcas* plant was reported to have antimicrobial activity with several molds and bacteria showing significant susceptibility. Ethyl acetate and methanol were the solvents used for the extraction of the crude extracts in their study Egharevba *et al*. (2013) study of the broad spectrum antimicrobial activity of extracts of *Jatropha curcas* reported the good antimicrobial activity of the plant extracts against food pathogens. They also found that the solvent extract methanol was the most active and exhibited good activity in comparisons to other solvents used in their study.

Nevertheless, the contradiction between this study of the antimicrobial property of *Jatropha curcas* seed oil and other studies may be attributed to the inability of the solvent extract (n-hexane) used in this study to extract the active principles of the *Jatropha* seed. This reason is backed up by Srinivasan *et al*. (2001) who stated in their findings that different solvents have different extraction capacities and different spectrum of solubility for the phyto-constituents which are known to be biologically active. Another reason could be likely dependent on the concentration of the extract and the microorganisms tested as stated in the findings of Kalimuthu *et al*. (2010).

Table 1: Antibiotic Sensitivity Pattern of the Isolated Mold Isolates using Antimold Drugs (Disc Diffusion)

	Molds	Concentrations (g/ml)	Diameter of zones of inhibition (mm)				
			Clo	Flu	Gri	Fun	Keto
1	*Geotrichum candidum*	0.2	14±1.5	9±0	14±2.0	0±0	8±6.5
		0.4	12±1.5	0±0	7±3.0	10±1.0	10±9.5
		0.6	12±2.5	0±0	0±0	10±3.5	14±5.5
2	*Mucor circinelloides*	0.2	0±0	0±0	0±0	0±0	0±0
		0.4	0±0	0±0	0±0	0±0	0±0
		0.6	0±0	0±0	0±0	0±0	0±0
3	*Trichosporon mucoides*	0.2	9±0	12±0.5	10±2.5	0±0	6±6.0
		0.4	9±4.0	5±5.0	7±3.0	0±0	6±6.0
		0.6	8±7.5	7±7.0	10±1.0	6±5.5	8±8.5
4	*Aspergillus niger*	0.2	14±1.5	8±3.5	14±0	9±1.0	12±1.0
		0.4	15±1.0	11±1.0	8±0.5	8±3.0	12±2.5
		0.6	15±2.5	13±1.0	11±2.0	9±5.0	14±2.0
5	*Aspergillus fumigatus*	0.2	4±4.0	10±1.0	4±4.5	3±2.5	0±0
		0.4	10±1.0	0±0	6±5.5	8±7.5	0±0
		0.6	5±5.0	0±0	4±4.5	6±6.5	0±0
6	*Rhodotorula* sp	0.2	7±2.0	0±0	0±0	8±1.0	16±3.5
		0.4	12±1.0	5±5.0	6±6.5	11±2.0	20±5.5
		0.6	16±0.5	16±7.5	6±5.5	13±2.5	22±0.5
7	*Aspergillus terreus*	0.2	5±5.0	2±2.5	2±2.5	4±3.5	6±0
		0.4	4±4.5	4±0	3±3.5	4±3.5	7±0
		0.6	0±0	6±0	0±0	3±3.0	12±0
8	*Trichoderma longibrachiatum*	0.2	12±2.5	10±1.0	12±0.5	8±1.0	15±3.0
		0.4	14±2.5	12±3.0	10±1.5	10±0	14±0
		0.6	13±3.0	9±1.5	12±0.5	10±1.5	14±1.5

Legend: Each value is a mean of three determinations ± standard error.
<13mm =resistant ; 14 mm-16mm = intermediate; >17mm = susceptible
Clo = Clotrimazole, Flu = Fluconazole, Gri = Griseofulvin, Fung = Fungusol, Ket = Ketoconazole

Table 2: Antibiotic Sensitivity Pattern of the Isolated Molds using Antimold Drugs (Agar-Well Diffusion)

	Molds	Concentrations (g/ml)	Diameter of zones of inhibition measured in mm				
			Clo	Flu	Gri	Fun	Keto
1	*Geotrichum candidum*	0.2	14±1.0	0±0	3±3.0	14±0.5	14±1.0
		0.4	18±5.0	0±0	0±0	14±0	16±0
		0.6	22±2.5	0±0	4±4.5	18±1.5	5±5.0
2	*Mucor circinelloides*	0.2	0±0	0±0	0±0	3±3.0	0±0
		0.4	0±0	0±0	0±0	10±0.5	0±0
		0.6	0±0	0±0	0±0	10±0	0±0
3	*Trichosporon mucoides*	0.2	11±6.5	15±0	0±0	15±1.0	9±1.0
		0.4	14±1.5	18±0.5	0±0	16±1.5	14±
		0.6	18±6.5	12±12.0	0±0	18±7.5	17±1.0
4	*Aspergillus niger*	0.2	16±1.0	15±0	0±0	20±0	16±0
		0.4	18±5.0	14±1.0	0±0	20±3.0	16±2.0
		0.6	20±3.0	23±2.0	7±1.0	20±5.0	17±3.0
5	*Aspergillus fumigatus*	0.2	16±0	15±0	15±0	16±0	17±0
		0.4	20±0	10±0	13±0	15±0	14±1.0
		0.6	24±0	0±0	0±0	20±0	13±0
6	*Rhodotorula* sp	0.2	18±2.0	12±2.5	15±1.0	17±2.0	15±0
		0.4	20±0	14±2.5	15±0	17±1.0	17±1.0
		0.6	23±1.0	17±1.5	12±7.5	25±3.0	21±1.0
7	*Aspergillus terreus*	0.2	17±2.0	12±2.5	14±1.0	18±4.0	18±6.0
		0.4	16±1.0	12 ±2.5	14±1.0	18±7.5	15±5.0
		0.6	24±6.5	13±2.6	0±0	21±11.0	20±10.0
8	*Trichoderma longibrachiatum*	0.2	15±2.0	15±5.0	19±1.0	16±0.0	15±0.0
		0.4	17±3.0	19±5.0	20±0	17±1.0	18±1.0
		0.6	16±6.0	20±6.0	20±0	21±1.0	20±0

Legend: Each value is a mean of three determinations ± standard error.
<13mm = resistant ; 14 mm – 16mm = intermediate; >17mm = susceptible
Clo = Clotrimazole, Flu = Fluconazole, Gri = Griseofulvin, Fung = Fungusol, Ket = Ketoconazole

Table 3: Antibiotic Sensitivity Pattern of the Isolated Mold Isolates using Synthesized Metal Complexes (Disc Diffusion)

	MOLD ISOLATES	DIAMETER OF ZONES OF INHIBITION (mm)								
		T1	T4	T5	T6	T7	T8	T9	DMF	ETHANOL
1	*Geotrichum candidum*	0	0	0	2	0	0	0	0	0
2	*Mucor circinelloides*	0	0	0	0	0	0	0	0	0
3	*Trichosporon mucoides*	0	0	2	0	0	0	0	0	0
4	*Aspergillus niger*	0	0	0	0	0	0	0	0	0
5	*Aspergillus fumigates*	0	0	0	0	0	0	0	0	0
6	*Rhodotorula* sp	0	0	0	0	0	0	0	0	0
7	*Aspergillus terreus*	0	6	0	0	0	0	0	0	0
8	*Trichoderma longibrachiatum*	0	0	0	0	0	0	0	0	0

Key: T1- 3,4,5-trihydroxybenzoicacid **(THB)** (0.67g), T4- $[Ca(HBAB)](NO_3)_2.2H_2O$ complex(0.001g), T5- 4-((2-hydroxybenzylidene)amino)-N-(5-methylisoxazol-3-yl)benzenesulfonamide **(HBAB)** (0.002g), T6- $[Mg(HBAB)](NO_3)_2.4H_2O$ complex (0.002g), T7- sulphamethoxazole (0.028g), DMF**-** N,N-dimethylformamide, T8- $[Cu(THB)](CH_3COO)_2$ complex (0.020g); stirred in solvent at room temperature, T9- $[Cu(THB)](CH_3COO)_2$ complex (0.010g); by grinding.

Table 4: Antibiotic Sensitivity Pattern of the Isolated Mold Isolates using Synthesized Metal Complexes (Agar-Well Diffusion)

	MOLD ISOLATES	DIAMETER OF ZONES OF INHIBITION (mm)								
		T1	T4	T5	T6	T7	T8	T9	DMF	ETHANOL
1	*Geotrichum candidum*	0	0	0	4	0	0	0	0	8
2	*Mucor circinelloides*	0	0	0	0	0	0	0	0	8
3	*Trichosporon mucoides*	0	0	6	0	0	0	0	0	0
4	*Aspergillus niger*	0	0	0	0	0	0	0	0	8
5	*Aspergillus fumigates*	0	0	0	0	0	0	0	0	12
6	*Rhodotorula sp*	0	0	0	0	0	0	0	0	0
7	*Aspergillus terreus*	0	6	0	0	0	0	0	0	13
8	*Trichoderma sp*	0	0	0	0	0	0	0	0	10

Key:
T1- 3,4,5-trihydroxybenzoicacid **(THB)** (0.67g)
T4- [Ca(HBAB)]$(NO_3)_2.2H_2O$ complex(0.001g)
T5- 4-((2-hydroxybenzylidene)amino)-N-(5-methylisoxazol-3-yl)benzenesulfonamide **(HBAB)**(0.002g)
T6- [Mg(HBAB)]$(NO_3)_2.4H_2O$ complex (0.002g)
T7- sulphamethoxazole (0.028g), DMF**-** N,N-dimethylformamide
T8- [Cu(THB)]$(CH_3COO)_2$ complex (0.020g); stirred in solvent at room temperature
T9- [Cu(THB)]$(CH_3COO)_2$ complex (0.010g); by grinding.

CONCLUSION

It is concluded that if the synthesized compounds (commercial antimold drugs, synthesized metal complexes and J*atropha curcas* seed oil) used in this study are improved upon better results would be obtained. They all show promise of being good antimold agents.

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