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Investigation on Anti-Fungal Properties of Cow Urine on Pathogenic Fungi.

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Abstract: This study was performed to investigate the anti-fungal properties of cow urine on pathogenic fungi. The investigation was done against *Aspergillus* species *Penicillium* species, *Mucor* species, and *Candida* species were isolated from human urine. Standard microbiological methods were adopted in the determination of the antifungal properties of the cow urine. Zone of inhibition ranged from 8mm to 18mm. The cow urine did not inhibit the growth of *Mucor* species. *Candida* species was inhibited at 8mm, *Penicilium* species and *Aspergillus* species were inhibited at 18mm. Minimum inhibitory concentration of the cow urine showed that cow urine inhibited the growth of *Aspergillus* and *Penicillium* species at 50%, while *Candida* species was inhibited at 100% concentration of cow urine and *Mucor* species was not inhibited at all. Fungicidal activity was recorded against *Aspergillus* species at 100% while other fungal isolates were resistant to the urine samples. Further studies should be carried out to justify the possible utilization of cow urine against fungal infection.

Keywords: Antifungal, Microbiological, concentration, properties, fungicidal, pathogenic, inhibitory.

I. INTRODUCTION

Antifungal resistance is the ability of fungi or fungal infections to spread and grow despite being exposed to the drugs designed to kill or inhibit them (Toda et al., 2019). And to combat the continuously increasing antifungal resistance, there is an increasing search for new antifungal agents. Panchagavya or Panchakavyam, a product of cow dung, milk, ghee, curd, and urine has been used by many people to treat various diseases (Hoh & Dhanashree, 2017). Research has shown that cow urine (gomutra) is capable of curing sicknesses such as blood pressure, arthritis, diabetes, heart attack, thyroid, blockage in arteries, cancer, asthma, psoriasis, eczema, prostrate, fits, AIDS, piles (Ghosh & Biswas, 2018). The rate at which invasive mycoses due to opportunistic fungal pathogens has increased over the past two decades is highly significant. This frequency of infections is associated with high morbidity and mortality rates and is directly related to increasing patient populations at risk for the development of serious fungal infections, and these include individuals undergoing solid-organ transplantation, blood and marrow transplantation (BMT), major surgery, and those with AIDS, immunosuppressive therapy, neoplastic disease, advanced age and premature birth (Brakhage, 2005).

Penicillium, one of the largest fungal genera, comprises some of the most commonly known filamentous fungi and are found on numerous substrates, also in very diverse habitats(Guevara-Suarez et al., 2016). *Penicillium* species play important roles in the decomposition of organic materials and also produce a wide range of mycotoxins, causing destructive rots in the food industry (Visagie et al., 2014). The fungus is ubiquitous in air and human habitats with some of its species considered factory enzymes (Guevara-Suarez et al., 2016; Visagie et al., 2014). The genus currently contains 354 species that have been accepted, including new combinations for *Aspergillus crystallinus*, *A. malodoratus* and *A. paradoxus*, which belong to *Penicillium* section *Paradoxa* (Visagie et al., 2014).

The genus *Aspergilus* contains more than 180 species including *Aspergillus fumigatus*, a saprobic fungus. *A. fumigates* can be regarded as a primary mold pathogen. Generally, diseases caused by *A. fumigatus* can be divided into three categories; allergic reactions and colonisation with restricted invasiveness which are observed in individuals that are immunocompetent, while systemic infections with high mortality rates are observed in patients with compromised immune systems (Brakhage & Langfelder, 2002).

Molds are widespread in buildings and homes and are known to cause a wide variety of human infections (Hardin et al., 2003). They thrive mostly in moisture laden places such as where leakages may have occurred in roofs, walls, pipes, plant vessels; they also grow in flood areas. Outdoor molds gain access indoor through open doorway, vents, windows, attachment to fabrics, and so on (Centre for Disease Control and Prevention, 2019). Allergy, infection and toxicity are three ways in which molds and other fungi affect humans very adversely. Fungal spores are generally abundant in the outdoor air, causing airway allergic diseases more than the indoor molds (Soldatova et al., 2014). Severe animal and human fungal infections have been recorded after ingestion of foods heavily contaminated with molds (Soldatova et al., 2014).

Candida albicans is a fungus that forms part of the normal flora of the human body. It commonly lives in areas of the mucous membrane such as the Gastrointestinal tract, mouth and vagina (Sumathi & Yogananth, 2016). *Candida* species has been implicated as the leading cause of nosocomial infections (Fridkin & Jarvis, 1996). *Candida* species cause both cutaneous and systemic mycosis and *C. albicans* is one of the causes of Candidiasis (St. Geme & Rempe, 2018)

Cow urine consumption helps in the maintenance of essential elements and minerals in the body. These essential elements and minerals include nitrogen, sulphur, sodium, iron, phosphate, manganese, silicon, chlorine, magnesium, maleic, citric, tartaric and calcium salts, vitamins A, B, C, D, E, minerals, lactose, enzymes, creatinine, hormones and gold acids (Ghosh & Biswas, 2018).

II. MATERIALS AND METHODS

Collection of Cow Urine

Fresh cow urine was collected in sterile containers from cows at Obinze Livestock Market in Owerri West Local Government Area of Imo State. The cow urine was filtered through Whatman No. 1 filter paper to get rid of debris and precipitated materials and was stored in airtight container at 4^0C before use.

Sterilization of Materials

All the glass wares to be used for the experiment were sterilized using the hot air oven at a temperature of 160^0 C for 2 hours. Wire loop was sterilized over bunsen flame and allowed till it was red-hot, while glass spreader was sterilized by dipping into 70 % ethanol and passing over Bunsen flame. The media used in this study which include; Nutrient agar, Eosin methylene blue agar, Sabouraud dextrose agar and Mueller-Hinton agar were prepared according to manufacturer's instructions and sterilized using the autoclave at a temperature of 121^0C at 15lb psi for 15minutes and was allowed to cool to a temperature of 45^0 C before pouring in sterile petri-dishes (Cheesebrough, 2006). Media were sterilized using laboratory autoclave at a temperature of 121^0C for 15 minutes at 15 lb psi (Cheesebrough, 2006).

Isolation of Fungal Pathogens

Penicillium species, *Aspergillus* species and *Mucor* species were isolated from spoilt food samples while *Candida* species was isolated from human urine properly collected from students diagnosed with recurrent Candidiasis by the physicians of the clinic of the Federal Polytechnic Nekede.

Preparation of fungal spore suspension

Spore suspensions of freshly grown fungal cultures were prepared in 0.84 % sterile saline water and the turbidity of homogeneous suspensions was adjusted to 0.5 McFarland's standards.

Antifungal Activity

The antifungal activity method described by (Bobbarala et al., 2009) was adopted in this study. Antifungal activity of cow urine was performed by agar well diffusion method as described previously with little modification. The homogeneous spores suspension of test fungal culture was swab inoculated on sterile Sabouraud dextrose agar plates. 6mm diameter well was made on the inoculated plate by using a cork borer. 100µl of filtered sterile cow urine was added to the well. Entire procedure was performed in triplicate manner for all test fungal culture. After diffusion, the plates were transferred to the incubator and incubated at 25° C for 3 days.

Determination of Minimum Inhibitory Concentration (MIC) of cow urine

The method described by (Rodríguez-Tudela et al., 2003) was adopted in the determination of the minimum inhibitory concentration (MIC) of sterile cow urine preparations on clinical isolates (Rodríguez-Tudela et al., 2003). Five different percentages (20%, 40%, 50%, 70% and 100% v/v) of cow urine were prepared. Having obtained the different concentrations, three (3) drops of overnight broth cultures of the test organisms were inoculated into the dilutions in each case of the test organisms. All plates were incubated at 27^0C for 24hours. The highest dilution of the cow's urine that did not show visible turbidity was taken as MIC.

Minimum Bactericidal Concentrations (MBC)

Tubes showing no visible growth from the MIC test was sub-cultured onto sterile nutrient agar and Sabouraud dextrose agar plates and incubated at 37^0C for 24 hours for the bacterial isolates and at room temperature for the fungal isolates. The lowest concentration of the extracts yielding no growth was recorded as the minimum bactericidal/fungicidal concentration as the case may be.

III. RESULTS AND DISCUSSION RESULTS

The results of this study are shown in table 1.1 to table 1.3. Table 1.1 shows the result of the antifungal screening properties of cow urine against the pathogenic fungi. Table 1.2 shows the result of the Minimum inhibitory concentrations (MIC) of the cow urine while Table 1.3 showed the result for minimum bactericidal concentrations of the cow urine against the pathogenic fungi.

Table 1.1 Antifungal properties of the cow urine

	Zones of inhibition (mm)	
Test Organisms	**Cow urine**	**CLSI Guidelines**
Penicillium species	18	R =Resistant (0-12mm)
Candida species	8	
Mucor species	NI	S=Susceptible (16mm-above)
Aspergillus species	18	

Key: mm = Millimeter NI = No Inhibition
CLSI = Clinical Laboratory Standard Institute

The antifungal activity of the cow urine shows that the zones of inhibition ranged from 8mm to 18mm. The cow urine did not inhibit the growth of *Mucor* species. *Candida* species was inhibited at 8mm, *Penicillium* species and *Aspergillus* species were inhibited at 18mm.

Table 1.2 below shows that cow urine inhibited the growth of *Penicillium* and *Aspergillus* species at 50% concentration, *Candida* species at 100% and did not inhibit the growth of *Mucor* species.

Table 1.2 Minimum Inhibitory Concentration (MIC) of the Cow urine

Test Organisms	**Cow urine Concentration (%)**				
	100	**70**	**50**	**40**	**20**
Penicillium species	-	-	-	+	+
Candida species	-	+	+	+	+
Mucor species	+	+	+	+	+
Aspergillus species	-	-	-	+	+

Key: % = Percentage
\+ = Presence of turbidity
− = Absence of turbidity

The table 1.3 below shows that cow urine caused fungicidal activity against *Aspergillus* species at 100% while other fungal isolates were resistant to the cow urine samples.

Table 1.3: Minimum fungicidal concentration of the cow urine samples

Test Organisms	**Cow urine Concentration (%)**				
	100	**70**	**50**	**40**	**20**
Penicillium species	+	+	+	+	+
Candida species	+	+	+	+	+
Mucor species	+	+	+	+	+
Aspergillus species	-	+	+	+	+

Key: % = Percentage
\+ = Presence of turbidity
− = Absence of turbidity

IV. DISCUSSION

In this study, it was observed that *Aspergillus* and *Penicillium* species were susceptible to cow urine. The susceptibility test for both organisms showed zones of inhibition of 18mm. Candida species was inhibited mildly, showing a zone of inhibition of 8mm while *Mucor* species was not affected by cow urine. Table 1.2 shows the growth of *Aspergillus* and *Penicillium* species being inhibited at 50% concentration of cow urine. Candida species was inhibited at 100% concentration of cow urine while it had no visible effect on *Mucor* species. From Table 1.3, it is seen that cow urine caused fungicidal activity against Aspergillus species at 100% concentration while other fungal isolates, Penicillium, Candida and Mucor species were resistant to it. (Prashith et al, 2010) investigated the antimicrobial and antihelmintic activities of cow urine and marked a significant antifungal and inhibitory effect on *Aspergillus* species.

Cow urine distillate (CUD) was found to be effective in inhibiting the growth of *Aspergillus* species and *Penicillium* species. This suggests that CUD can be used as an alternative medicine which are on the rise across the world due to overuse or misuse of antifungal agents or antibiotics (Deshmukh et al., 2012). (Hoh & Dhanashree, 2017) reported that among the clinical *Candida* isolates that were sensitive to commonly used antifungal drugs, majority of them were also susceptible to 20 to 50% of cow urine. Thus the cow urine was proved to be effective against both antifungal resistant clinical *Candida* isolates and susceptible ones.

The antifungal property of cow urine can be explained by the presence of non-volatile active constituents (Kekuda et al., 2008; Kekuda et al., 2010) like phenolic acids which are found abundant in the chloroform fraction of cow urine when subjected to high performance liquid chromatographic (HPLC) analysis, the antioxidant property and the ability to reduce germination of spores (Vats & Miglani, 2011)

However, the exact mechanism of action of cow urine in inhibiting the growth of fungi is still not well known and needs to be explored. Cow urine is found to harbour a variety of bioactive compounds but as compared to plant derivatives, research on cow urine is less. The research works on cow urine in recent time support that cow urine has the ability to successfully fight many pathogenic agents. On the other hand, use of cow urine is cost effective and is also safe for man and the environment. The present qualitative analysis concluded that cow urine can be used in a controlled manner to minimize fungal infections. Future investigation is required to clarify role of actual lead compounds contained in cow urine against fungal pathogens.

V. CONCLUSION

The results of this study have shown that cow urine possesses antifungal properties against *Aspergillus* species. However, the cow urine showed inhibitory effect against *Penicillium* species, though the cow urine did not kill the fungus as seen in the case of *Aspergillus* species.

Therefore, cow urine can be incorporated to minimize fungal infections and future investigation is required to clarify role of actual lead compounds present in cow urine against fungal pathogens.

VI. RECOMMENDATIONS

Further field studies are to be carried out to justify the possible utilization of cow urine against fungal infections.

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