Bioactive components of the methanolic extracts of *Terminalia* spp. against fungal pathogens in *Drosophila melanogaster* (*Diptericin-lacZ* II) as a Model

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ABSTRACT

Fungal pathogens are becoming prospective agents of bioterrorism; there is widespread emergence of fungi such as *Aspergillus* species being implicated in invasive diseases, leading to increased use of antifungal prophylaxis and resistance to standard antifungal drugs. *Drosophila melanogaster* has gained appreciation as a useful model organism of human diseases. Comparative genomic studies estimate that up to 75% of the human genes implicated in diseases are conserved in *Drosophila*. The research was designed to evaluate the antifungal potency of the different methanolic extracts of five *Terminalia* species on three *Aspergillus* species using *Drosophila melanogaster* (*Diptericin-lacZ* II) as a model. Extraction and phytochemical screening of the methanolic extracts were carried out. The ingestion method was used to infect the flies and was treated on a diet containing 60 mg/ml of the extracts from the leaves, stems and roots of the five plants respectively. The results of the phytochemical screening indicated that the different extracts contained the presence of secondary metabolites such as Tannins, Saponins, Flavonoids, Steroids, Terpenoids, Alkaloids, Saponins and Cardiac glycosides. The result of the infectious studies showed that *A. fumigatus* was the most lethal to the flies with a survival of 8.89%, followed by *A. terreus* at 17.78% and *A. flavus* was the least lethal with a survival of 22.22% of flies infected and fed 5% glucose (control group). At p≤0.05 there was a significant difference in the survival rate of the flies infected with different *Aspergillus* species and treated with 60 mg/ml of the methanolic extracts of the plants from the five *Terminalia* species. All five *Terminalia* species had a broad spectrum activity against the *Aspergillus* species with more than 50% survival of the flies infected and treated on the extracts. These showed that all the *Terminalia* species induced the production of fungal peptides in the flies and supported the use of these plants in folk medicine.

Keywords: *Terminalia* spp., *Aspergillus* spp., *Fusarium oxysporium*, *Drosophila melanogaster*, percentage survival, antifungal activities.

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INTRODUCTION

*Aspergillus* species are cosmopolitan filamentous fungi found in air, water and soils all over the world. As an opportunistic human pathogen, it causes localized infections, aspergillosis (fungus ball), allergic bronchopulmonary aspergillosis, and invasive aspergillosis in immune-compromised patients (Agarwal, 2009). The likelihood of serious *Aspergillus* infection, with accompanying high morbidity and mortality, is based on three factors: the status of immune-compromised patients, the degree of exposure, and fungal virulence. Individuals with hematological malignancies, hematopoietic stem cell transplant recipients, and recipients of solid organ transplants are at the highest risk of developing systemic aspergillosis (Abd El-Baky,
Combretaceae is a large family of trees, shrubs, vines and mangroves that consists of approximately 17 genera and 525 species (Maurin et al., 2010). The Combretaceae occur mainly in tropical and subtropical regions internationally, with the highest diversity in Asia and Africa (Mabberley, 2008). Two of the largest and most useful genera are Combretum, consisting of approximately 250 species of trees, shrubs and lianas, and Terminalia, consisting of approximately 150 species of trees and shrubs (Maurin et al., 2010). Due to the widespread distribution and biodiversity of the Combretaceae across Southern Africa, they are readily available and easily harvested for medicinal use. Possibly for this reason, they are widely used in diverse traditional healing systems for a wide variety of diseases and afflictions. The leaves and barks are predominantly used for these purposes (Van Wyk et al., 2009). Indeed, the fruit of many African species are considered toxic and are not used for internal consumption.

Model organisms have been extensively used to unravel basic and conserved biological processes. The fruit fly, Drosophila melanogaster (hereinafter Drosophila), is one of the most studied eukaryotic organisms and has made fundamental contributions to different areas of biology. Drosophila has also gained appreciation as a useful model organism for human diseases. Comparative genomic studies estimate that up to 75% of the human genes implicated in diseases are conserved in Drosophila (Reiter et al., 2001). The similarity between human and Drosophila genomes is not limited only to genetic elements, but also to the relationship between them, with numerous examples of conserved biological mechanisms. The Drosophila genome is smaller in size and has a smaller number of genes compared to the human genome, which facilitates genetic studies (Adams et al., 2000).

This study aimed to evaluate the antifungal activities of five Terminalia species against Aspergillus species using D. melanogaster as a research model. Some literature has studied the antifungal activity of the plants in the family Combretaceae mostly in vitro (Masoko and Eloff, 2006; Masoko et al., 2007; Eloff et al., 2008) but no in vivo studies on the plants. Therefore this research seeks to bridge the gap to study the host-pathogen interaction and how this extract helps the flies modulate against fungal infections.

MATERIALS AND METHODS

Study area

The study was carried out from January 2022 to September 2023, at the Drosophila Laboratory: Fungal Pathogens and Plant Bioactive Compounds, Department of Plant Science and Biotechnology, University of Jos, Nigeria.

Collection of plant samples/parts and their identification

Leaves, stems and roots of the plant species tested in this study were collected from the Federal College of Forestry Jos, situated in Jos North local government area of Plateau state. All plants were identified with a number (UJH000296) and Voucher specimens were prepared and stored at the Department of Plant Science and Biotechnology, University of Jos. All plant materials were air-dried in the shade and ground into a fine powder.

Extraction of plants materials

Extraction was carried out using cold maceration by taking 50g and macerating in 500 ml of methanolic for 72 hours. The extracts were filtered through filter paper (Whatman No.1). The extracts were subsequently allowed to dry at room temperature (Parekh and Chanda, 2007).

Purity test of the extract

Purity tests of the different extracts were carried out by inoculating 0.1g of the dried extracts on a poured plate of Sabronuad dextrose agar and incubated for any growth. In the absence of growth in the media, this signifies that the extracts are pure.

Phytochemical determination

The plant fractions were screened for their phytochemical constituents to determine the presence of Alkaloids, Saponins, Tannins, Flavonoids, Carbohydrates, Steroids, Anthraquinones, Cardiac glycosides and Terpenoids using standard phytochemical screening procedures as described by (Sofowora, 2008).

Source of microorganisms

Standard isolates of the fungi Aspergillus flavus, Aspergillus fumigatus, and Aspergillus terrues were obtained from the Veterinary Research Institute Vom. The organisms were collected in a suspension of Sabronuad broth (SB).

Drosophila melanogaster fly stock selection

The fly Drosophila melanogaster (Diptericin-lacZ II) was
obtained from the National Species Stock Center (Switzerland). The flies were maintained and reared on cornmeal medium at a temperature of 23±1°C and 60% relative humidity under 12 h dark/light cycle conditions. All the experiments were carried out with the same D. melanogaster (Diptericin-lacZ II).

Preparation of fly food and plant extracts for treatments

Sixty mg/mL of the different plant extracts were prepared by dissolving 0.6g in 10 mL of distilled water. Basal fly food was prepared, 4 mL was placed in each feeding vial and allowed to dry for over 5 h. Burrows were made on its surface with a paintbrush. Then, 1 mL of each of 60 mg/ml of the extracts was introduced into the prepared food surface and allowed to stand for some time for optimal absorption into the meal. A small amount of yeast was spread over the surface of the food to absorb residual extract and to prevent moistening of the surface (Manasseh et al., 2020).

Sexing and sorting of the flies

While being anesthetized on ice, male and female flies were distinguished based on their genitalia, size, mark and shape of abdomen, stripes or bands, and bristle on forelegs. Virgin female flies were identified according to the dark mark on the ventral abdomen, which is an embryonic residue that is excreted from their gastrointestinal tract upon maturation 8–12 h after eclosion (Lionakis and Kontoyiannis, 2010). Two to three-day-old female flies were consistently used because they have significantly lesser mortality rates after Aspergillus infection than 10 to 15-day-old.

Establishment of Infections in the Flies

Infection was established in female flies using the ingestion methods of infection as described by Manasseh et al. (2020). Flies were harvested and starved for about 6 hours and then they were introduced into Yeast Agar Glucose media containing a 3-day-old culture of the 3 fungal isolates to feed for 6 hours. After this they were taken for various treatments on a diet prepared with the different extracts and survival rates were observed for seven days (Lionakis and Kontoyiannis, 2012). Two controls were used. These consisted of infected flies that were not treated and uninfected flies fed with 5% glucose containing fly meal.

Experimental groups

A = uninfected flies fed with normal diet (Vehicle).
B = infected flies that were not treated (control).
C = Infected flies with the Aspergillus species and treated on 60 mg/mL of Methanolic extract of Terminalia glaucescens.
D = Infected flies with the Aspergillus species and treated on 60 mg/mL of Methanolic extract of Terminalia cattapa.
E = Infected flies with the Aspergillus species and treated on 60 mg/mL of Methanolic extract of Terminalia superba.
F = Infected flies with the Aspergillus species and treated on 60 mg/mL of Methanolic extract of Terminalia avicinoides.

Data Analysis

The survival data were subjected to analysis of variance and results were presented as means ± standard error of means using Graph Pad Prism version 8 software. The results obtained were tested for significant differences at a 5% level.

RESULTS

The results of the phytochemical screening are presented in Table 1. It showed that different extracts contained the presence of secondary metabolites such as Tannins, Saponins, Flavonoids, Steroids, Terpenoids, Alkaloids, Saponins and Cardiac glycosides.

The results of the infectious studies showed that there was a significant difference (P≤0.05) in pathogenicity of the fungal isolates to the flies after infection. A. fumigatus was the most lethal to the flies with a survival rate of 8.89% and a mortality rate of 91.11% after three days of exposure, followed by A. terrues with a survival rate of 17.78% and mortality rate of 82.22%. A. flavus was the least lethal with a survival rate of 22.22% and mortality rate of 77.78% respectively in flies control group infected and fed 5% glucose as observed in Figure 1-5.

DISCUSSION

The phytochemical screening results indicated that different extracts contained the presence of secondary metabolites such as Tannins, Saponins, Flavonoids, Steroids, Terpenoids, Alkaloids, Saponins and Cardiac glycosides. Tannins and Flavonoids were found in all the extracts whereas carbohydrate was not observed in the root extracts of Terminalia mantaly. Tannins were absent in Terminalia mantaly leaves extract and Terminalia cattapa roots extract (Table 1). The results of these findings concur with the work of others (Lawal et al., 2017; Adeeyo et al., 2018; Manasseh et al., 2020). Fahmy et al. (2015) reported in a review that several
Table 1. Phytochemical screening of the fractions of *Terminalia superba*.

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+ = presence, ++ = more present, +++ = Highly present, - = absence.

Figure 1. *Drosophila melanogaster* (Diptericin-lacZ II) infected with *Aspergillus* spp. and treated on Methanolic extract of *Terminalia glaucescens*. 

![Graph showing fungal inhibition](image)
Figure 2. *Drosophila melanogaster* (Diptericin-lacZ II) infected with *Aspergillus* spp. and treated on Methanolic extract of *Terminalia cattapa*.

Figure 3. *Drosophila melanogaster* (Diptericin-lacZ II) infected with *Aspergillus* spp. and treated with Methanolic extract of *Terminalia mantaly*.

*Terminalia* species contain secondary metabolites which are responsible for their bioactivities.

This result could be due to differences in the genetic makeup of the different fungal isolates. The completion of the *Aspergillus fumigatus* genome sequencing project (Nierman et al., 2005), along with significant strides in fungal genetics has led to a surge of genetic information about the contribution of individual genes to *Aspergillus* virulence. For instance, *Aspergillus* strains with defects in siderophore biosynthesis (ΔsidA, ΔsidC, ΔsidD, ΔsidF) (Schrettl et al., 2007; Schrettl et al., 2007), melanin (Δalb1) (Tsai et al., 1998) or gliotoxin production (ΔgliP) (Spikes et al., 2008), PABA metabolism (H515) (Brown et al., 2000), thermotolerance (ΔcgrA) (Bhabhra et al., 2004), Ras signalling (ΔrhbA) (Panepinto et al., 2003) or starvation stress response (ΔcpcA) (Krappmann et al., 2004) have shown to be hypovirulent in mammalian models of IA. Several other molecular factors that may be required for an *Aspergillus* strain to be an effective pathogen are likely to be discovered in the near future.
The results of the antifungal activity in vivo showed that *Terminalia glaucescens* root extract had the highest activity in the survival rate of 75.55%, 82.23% and 88.89% for flies infected with *A. fumigatus*, *A. terreus* and *A. flavus* respectively, followed by the stem extract and the leaves extracts had the least antifungal activity as seen in Figure 1. Similar results were observed in *T. cattapa, T. mantaly, T. superba* and *T. avecinoides* where the root extracts had the highest protective role against all the test fungi, followed by the stem and root extracts respectively as shown in Figure 2-5. In general at p≤0.05 there was a significant difference in the survival rate of the flies infected with different *Aspergillus* species and treated on 60 mg/ml of the extracts of the plants from the five *Terminalia* species as compared to flies that were infected and not treated with 100 % mortality after three days as seen in Plate 1, it was also observed that the *Aspergillus* species sporulated on the carcass of the infected and not treated flies. All the five *Terminalia* species had broad spectrum activities against the
Aspergillus species with more than 50% survival of the flies infected and treated by the extracts. This survival rate might be due to the trigger of fungicidal peptides because upon fungal challenge, Toll activation lead to downstream production of potent fungicidal peptides that protect flies against fungi (Manasseh et al., 2020). The antifungal effect observed is also attributed to the secondary metabolites present in the different extracts as reported by Lawal et al. (2017), Adeeyo et al. (2018) and Manasseh et al., (2020) that Terminalia species contain secondary metabolites which is responsible for their bioactivities.

CONCLUSION

In the course of this study, Terminalia glaucescens exhibited the highest antifungal properties as increased the survival rate of the flies infected and treated on the different extracts of root, stem and leaves. This supports the use of these plants in traditional medicine and for the treatment of various ailments. Therefore, further studies on the biochemical interaction should be evaluated.

REFERENCES


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