Antifungal Activity of Crude Glycolated Extracts of *Solanum tuberosum* L. (White Potato) Peelings against *Candida* and *Aspergillus* Species

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**ABSTRACT**

**Background.** A rise in the number of immunocompromised patients has increased the risk of opportunistic fungal infections. Identifying novel sources of antifungal agents from commonly discarded materials (i.e. potato peelings) can provide a cheaper alternative for antifungal drugs.

**Objectives.** The aim of the study was to determine the antifungal activity of crude glycoalkaloid extract from *Solanum tuberosum* L. (white potato) peelings against opportunistic fungi *Candida albicans*, *Candida glabrata*, *Aspergillus niger*, *Aspergillus fumigatus*, and *Aspergillus flavus*.

**Methods.** The glycoalkaloid content from dried potato peelings were extracted using ethanol and confirmed using colorimetric tests. The extract had a concentration 833.33 microgram/mL. Determination of the minimum inhibitory concentrations (MICs) of the extract via two-fold broth dilution was performed for the five fungi with amphotericin B and fluconazole as the reference antifungal drugs.

**Results.** MICs of the crude extract for *C. albicans*, *C. glabrata*, *A. fumigatus*, and *A. niger* were not found within the concentration range of the studies and would thus need further experiments using a broader range of glycoalkaloid concentrations. The extract was found to have a MIC of 104.17 microgram/mL for *A. flavus*, thereby verifying the antifungal effect of glycoalkaloid against *A. flavus* at said concentration.

**Conclusion.** Glycoalkaloids from *Solanum tuberosum* are a potential source of antifungals against certain opportunistic fungi.

Key Words: Antifungal agent, opportunistic fungal infections, *Solanum tuberosum*, potato, glycoalkaloids, *Candida*, *Aspergillus*

**INTRODUCTION**

Healthcare-acquired infections (HAI) are common threats to patients resulting in increased morbidity and mortality. An increasing trend in morbidity has been presented by the World Health Organization across countries in Southeast Asia, Eastern Mediterranean, Europe, and Western Pacific from the years 2002 to 2011, and is continuing to rise worldwide due to a plurality of factors.1 Two of the most common fungi causing HAIs are *Candida* and *Aspergillus* species. In 2016, a total of 1,852,137 severe fungal infections were estimated to have occurred in the...
organisms) potatoes organically. It was ensured that all the potatoes used were sourced from the same farm and certified as *Solanum tuberosum* L. by the Philippine Department of Agriculture Bureau of Plant Industry.

**Glycoalkaloid Extraction**

The *Solanum tuberosum* L. tubers were mechanically separated from their peels via abrasion method after thorough washing with 10% sodium bicarbonate. The peels were then subjected to air drying for four weeks before maceration by a blender. The peel fragments were then stored in clean, airtight bottles away from sunlight.

The extraction method employed was adapted and modified from that of Bushway, Bureau, and Stickney using ethanol as the extracting solvent. Finely macerated peels weighing 75g were soaked with 300mL of analytical reagent-grade ethanol for 72 hours at room temperature away from sunlight. The mixture was then filtered and subjected to rotary evaporation until the volume had reached 25mL. Afterwards, 2mL of glacial acetic acid was added to the solution. After centrifugation at 38000g for 10 minutes, 25mL of concentrated ammonium hydroxide was added to precipitate the glycoalkaloids. The samples were then divided into separate tubes and subjected to water bath at 70°C for 30 minutes. These tubes were then refrigerated overnight.

After refrigeration, the tubes were again subjected to centrifugation at 38000g for 10 minutes. Pellets were pooled into a single container and sent for lyophilization. After drying, the extract was weighed before being stored in a freezer at -5°C.

**Confirmatory Tests for the Presence of Glycoalkaloids**

The extract was redissolved in dimethyl sulfoxide (DMSO) and was subjected to confirmatory tests to confirm the presence of alkaloids. The reagents used were the following: Mayer's reagent (potassium mercuric iodide solution), Wagner's reagent (iodine solution in potassium iodide), Hager's reagent (picric acid saturated solution), and Valser's reagent (mercuric iodide test solution). One milliliter (1mL) of each reagent was added to 0.5mL of the solution to check for the formation of colored precipitate that would verify the presence of alkaloid functional groups in the extract.

**Determination of Minimum Inhibitory Concentration (MIC)**

The minimum inhibitory concentration was investigated via two-fold broth dilution in accordance with the European Committee on Antimicrobial Susceptibility Testing (EUCAST) documents entitled, “Method for the determination of broth dilution minimum inhibitory concentration of antifungal agents for yeasts” and “Method for the determination of broth dilution minimum inhibitory concentration of antifungal agents for conidia-
forming moulds."6-7 These methods used fluconazole and amphotericin B as reference drugs for the various Candida and Aspergillus species.

Stock solutions containing fluconazole and amphotericin B were made taking into consideration the potency of the powdered drug using DMSO as the solvent for both drugs. The stock solutions contained 25,600 microgram/mL and 3,200 microgram/mL for fluconazole and amphotericin B, respectively. The crude extract had a stock concentration of 166.67 mg/mL.

Into the tubes designated as the tubes of largest concentration containing 3.96 mL of modified RPMI-1640 broth with 2% glucose and 3-(N-morpholino)propanesulfonic acid (MOPS) buffer, 40 microliters of the stock solutions were added to form 4mL, yielding a 100-fold dilution of the stock solutions. After mixing, 2-fold serial dilution was carried out by obtaining 2mL from the previous tube and adding these into new tubes containing 2mL of the same modified RPMI-1640 medium. This step was repeated until the last tube had been reached and the final 2mL volume disposed of.

For Candida species, fungi were directly transferred aseptically using an inoculating loop to sterile distilled water. For Aspergillus species, conidia were first dislodged using a wash of polysorbate 20 (Tween® 20) and sterile distilled water along with gentle scraping of the fungi. The fungal suspensions were added to sterile distilled water until turbidity had reached 0.5 McFarland standard, and was then diluted ten-fold to reach a concentration of 1 x 10⁵ colony forming units (CFU). A volume of 2mL was used as inoculum in each tube to reach the desired concentrations of the standard drugs and the extract.

After addition of inoculum, the final concentrations of the extract were from 833.33 microgram/mL to 1.63 microgram/mL. Final concentrations for the reference antifungals ranged from 128 microgram/mL to 0.25 microgram/mL for fluconazole and 16 microgram/mL to 0.0625 microgram/mL for amphotericin B.

Each organism had setups performed in triplicate. Tubes containing Candida were incubated for 24 hours while tubes containing Aspergillus were incubated for 48 hours.

Three control tubes were used in every run for each organism and were designated as follows: negative control (broth and fungal inoculum), positive control (broth and crude extract), and blank control (broth and crude extract).

The minimum inhibitory concentration was determined to be the smallest concentration wherein growth had been inhibited as determined visually using a turbidity comparison card.

Data Collection and Analysis

The minimum inhibitory concentrations were recorded for each trial. Results for Aspergillus spp. and Candida spp. for fluconazole and amphotericin B were compared to those of the breakpoints listed by the EUCAST for the specific organisms.

RESULTS

Confirmatory Tests

All of the qualitative tests verified the presence of alkaloid functional groups in the extract through the production of precipitates after mixing (Table 1).

Table 1. Qualitative test results for the presence of alkaloids in the extract

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Presence of Precipitates</th>
<th>Color of Precipitate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mayer’s</td>
<td>+</td>
<td>White</td>
</tr>
<tr>
<td>Wagner’s</td>
<td>+</td>
<td>Brick-red</td>
</tr>
<tr>
<td>Valser’s</td>
<td>+</td>
<td>Creamy white</td>
</tr>
<tr>
<td>Hager’s</td>
<td>+</td>
<td>Yellow</td>
</tr>
</tbody>
</table>

Determination of Minimum Inhibitory Concentration

Minimum inhibitory concentrations (MICs) for the reference drugs showed that none of the organisms tested were resistant strains according to EUCAST breakpoints although A. fumigates was found to have intermediate susceptibility to amphotericin B (Table 2). The results of A. fumigates were taken by the authors as still valid for use against the glycoalkaloid extract as the strain was not resistant to Amphotericin B.

Only A. flavus was inhibited by the extract through MIC determination (Table 3). In all other trials, each organism was able to grow in the tube of highest concentration; thus, their respective MICs were outside the range of the MIC concentrations tested for the extract.

Table 2. EUCAST reference antifungal mic breakpoints (microgram/ml) and actual results for Fluconazole and Amphotericin B.28-30

<table>
<thead>
<tr>
<th>Fungus</th>
<th>MIC Breakpoints (microgram/mL)</th>
<th>Result of Study Organisms to Fluconazole or Amphotericin B</th>
<th>Susceptibility of Study Organisms to Control Antifungals</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans</td>
<td>≤2 Susceptible, &gt;4 Resistant</td>
<td></td>
<td>2 Susceptible to Fluconazole</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>-- Susceptible, -- Resistant</td>
<td>≤1 Susceptible, &gt;1 Resistant</td>
<td>0.0625 Susceptible to Amphotericin B</td>
</tr>
<tr>
<td>A. fumigatus</td>
<td>-- Susceptible, -- Resistant</td>
<td>≤1 Susceptible, &gt;2 Resistant</td>
<td>2 Intermediate Susceptibility to Amphotericin B*</td>
</tr>
<tr>
<td>A. flavus</td>
<td>-- Susceptible, -- Resistant</td>
<td>(The MIC breakpoints are higher than those for A. fumigatus.)</td>
<td>1 Susceptible to Amphotericin B</td>
</tr>
<tr>
<td>A. niger</td>
<td>-- Susceptible, -- Resistant</td>
<td>&lt;1 Susceptible, &gt;2 Resistant</td>
<td>0.5 Susceptible to Amphotericin B</td>
</tr>
</tbody>
</table>

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Table 3. Measured minimum inhibitory concentrations of extract and reference antifungals for Aspergillus and Candida species tested (microgram/mL) and EUCAST breakpoints for the respective antifungals used

<table>
<thead>
<tr>
<th>Fungus</th>
<th>Extract</th>
<th>MICs</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. flavus</td>
<td>Inhibited at 104.17 (2/3 trials)*</td>
<td></td>
</tr>
<tr>
<td>A. fumigatus</td>
<td>&gt; 833.33 (3/3 trials)</td>
<td></td>
</tr>
<tr>
<td>A. niger</td>
<td>&gt; 833.33 (3/3 trials)</td>
<td></td>
</tr>
<tr>
<td>C. albicans</td>
<td>&gt; 833.33 (3/3 trials)</td>
<td></td>
</tr>
<tr>
<td>C. glabrata</td>
<td>&gt; 833.33 (3/3 trials)</td>
<td></td>
</tr>
</tbody>
</table>

*Note: One trial had a MIC of > 833.33 microgram/mL.

**DISCUSSION**

_Candida_ are human pathogenic yeasts which are ubiquitous in the environment, with some species belonging to the normal human microbiota.11-13 These _Candida_ species are among the most common causes of fungal infections worldwide, representing nearly 96% of all opportunistic mycoses.14-16 In the Philippine setting, candidiasis caused by _Candida albicans_ ranks first among the common opportunistic infections due to the hot and humid weather coupled with the overgrowth of the diploid fungus.14 _Candida glabrata_, on the other hand, ranks second to third among other species in causing systemic or superficial candidal infection.15-16

_Aspерgillus_ is a mold commonly found both indoors (i.e. beddings), and outdoors (i.e. decomposing plant materials). It produces spores that are easily aerosolized, and causes infection when inhaled by susceptible hosts during extended antibiotic treatment and in instances of severe immunosuppression. _Aspergillus fumigatus_ is now considered as the most prevalent airborne fungal pathogen with an increasing number of cases of fatal invasive aspergillosis in the immunocompromised.17 _Aspergillus flavus_ is the second leading cause of human invasive aspergillosis and is the most common cause of superficial infection.18 _Aspergillus niger_ in contrast, is rarely reported as a causative agent of infection but is correlated with a 75% mortality rate for those infected.19

Extracts of _Solanum_ plants have been proven effective in inducing antimicrobial effect against _Staphylococcus aureus_ has been found to be significantly inhibited by the glycoalkaloid content of _Solanum nigrum_ at a concentration of 20 microgram/mL.20-22

Extracted solanine from _Solanum lycocarpum_ was shown to be inhibitory to the dermatopyle _Trichophyton rubrum_ at a concentration of 25 microgram/mL.23

The amount of glycoalkaloids in potato peels is closely associated with the production of chlorophyll, which causes the visible greening on the tuber surface. These processes are independent of each other but are both activated by light. Glycoalkaloid concentrations in the tuber decrease from the epidermis inwards with the highest amounts being found within the periderm and outer parenchymal tissue while having negligible concentrations in the pith.24 A level of 20 mg of glycoalkaloid is reported to be toxic to humans.25

Analysis of the specific glycoalkaloids reveals that α-solanine and α-chaconine are the two major glycoalkaloids found in commercial potatoes – comprising about 95% of the total glycoalkaloids in potato tubers.26 Solanine and chaconine have both fungicidal and pesticidal properties, having a synergistic reaction causing liposome lysis.27

A study by Zhao et al. looked into the antifungal activity of five solanaceous glycoalkaloids against _Cercospora brassicaceae_ and _Alternaria porri_, two phytopathogenic fungi. It was shown that a mixture of α-solanine and α-chaconine resulted in a marked synergistic antifungal activity. The combination of both glycoalkaloids produced a higher activity at lower concentrations than at higher concentrations.28

Another study by Fewell and Roddick in 1997 found that α-solanine was more inhibitory than α-solanine on spore germination and on growth in liquid culture of _Alternaria brassicicola_ and _Phoma medicaginis_, as well as on the growth of _Ascochit solani_ and _Rhizoctonia solani_. At 50 micromoles or less, solanine caused little (≤ 20%) or no inhibition; but in combination with comparable concentrations of chaconine (which were sometimes also below the activity threshold), synergistic inhibitory effects were apparent by up to 100%.29

Generally, the effect of crude glycoalkaloid extract on _A. flavus_ may be attributed to the surfactant effects on the fungi’s sterol-containing cell membranes. Glycoalkaloid compounds destabilize the membrane forming complexes between the glycoside and membrane bound sterols such as those found in fungal hyphae.30

The study was able to successfully extract glycoalkaloids from _Solanum tuberosum_ peels with ethanol as the extracting solvent as shown by the presence of colored precipitates using the confirmatory reagents. The antifungal activity of these ethanolic glycoalkaloid-containing extracts of _S. tuberosum_ presented with inhibition of only _Aspergillus flavus_ among the five organisms tested. This fungus had an MIC of 104.17 microgram/mL while the MICs for _C. albicans_, _C. glabrata_, _A. fumigatus_, and _A. niger_ were outside the concentration range of the crude extract used in the experiment. It is thus highly possible that certain interspecies variations in cell wall composition make _A. flavus_ more susceptible to the action of the extract than any of the other fungi tested. However, it is noted that the antifungal activity of _Solanum tuberosum_ in these fungal species cannot be completely ruled out even though the MICs were not determined in the experiment.

The use of reference antifungal drugs was not meant to be a means of comparison of activity. Rather, amphotericin B and fluconazole were used in order to ensure that the fungi used in the experiment were not resistant strains that would otherwise lead to falsely negative results. Standardization was ensured through the usage of the same broth, inoculum size, solvent concentration (less than 1% DMSO), incubation time, and temperature throughout the experiment. The MICs for the respective drugs tested on each fungal species fell within the susceptible range, with the exception of _A. fumigatus_ which had intermediate susceptibility. As stated
in the EUCAST breakpoints for Candida and Aspergillus species, it is possible to conclude that the fungal species used were not resistant strains and would less likely provide falsely negative results in MIC determination.

Controls in the experiment were used in order to increase the validity of the results. A negative control was used in order to see how the growth of the specific fungus would appear, a positive control was used to see the appearance of the tubes that do not exhibit growth, and because the extract showed coarse flocculation that settled in the bottom of the tube, a blank control was used in order to avoid falsely reporting fungal growth. Also, the broth in the blank control did not become turbid and the color did not change from red to yellow which would have otherwise indicated growth of carbohydrate-fermenting organisms. In determining the MIC, it was ensured that the tubes were compared to these controls.

Possible reasons for the lack of positive results against the other fungi tested may be attributed to the inadequate concentration of extract applied during the assay to inhibit their growth. It is desired that nothing other than the glycoalkaloids and the drugs would be the sole producers of antifungal property. However, being an unpurified substance, the extract may have had unaccounted substances that may have affected the results.

CONCLUSION

The experiment showed that the crude glycoalkaloid extract from S. tuberosum peels has antifungal activity to a certain extent. The MIC of the extract for A. flavus was determined to be 104.17 microgram/mL. However, the experiment failed to demonstrate the antifungal activity within the concentrations tested for A. fumigatus, A. niger, C. albicans, and C. glabrata; hence, MICs for these fungi species were not determined. It should be noted that even though the MICs were not obtained, it does not necessitate that antifungal activity is absent. Further investigation is recommended to test antifungal activity at broader concentrations.

RECOMMENDATION

Several suggestions to further the study are recommended by its proponents. First, purification of the extract via absorption spectrophotometry, chromatography, or other quantitative confirmatory techniques is proposed in order to obtain a pure glycoalkaloid extract to further establish a stronger basis for the antifungal activity of glycoalkaloids.

Second, studies that use a broader range of concentrations of extracts are recommended in order to ascertain the MICs of the extract for the other organisms. Further studies may also probe further into other members of the Solanaceae family, which may reveal characteristics of the extract not previously seen in S. tuberosum that may prove helpful in improving its preparation. Third, applied researches investigating other clinical applications of the extract related to its antifungal property while considering the appropriate routes of administration will be most helpful in advancing the study’s practical use and formulation.

Statement of Authorship

All authors have approved the final version submitted.

Author Disclosure

All authors declared no conflicts of interest.

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