Asian Journal of Biology Education, 2018
https://doi.org/10.57443/ajbe.10.0_2

Practical Report

Antifungal Activity of *Capsicum frutescens* and *Allium cepa* against *Aspergillus* spp.: An Application of Scientific Process Skills by High School Students

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(Received: 23 December 2014; accepted: 27 May 2016)

Aspergillus niger and *Aspergillus flavus* are known to induce risks including aspergillosis in humans and common crop drought to plants. *Allium cepa* (white onion) and *Capsicum frutescens* (cayenne pepper) have been reported as having some antifungal potential. Thus, to practice scientific process skills, high school biology students investigated whether *A. cepa* and *C. frutescens* extracts are effective antifungal agents against these two pathogens. Sensitivity testing using Kirby-Bauer assay revealed that *C. frutescens* was more effective against *A. niger* and *A. flavus*. *C. frutescens* extract alone produced an inhibition zone of 19.29 *mm* for *A. niger* and 10.47 *mm* for *A. flavus*. Using *t*-test and repeated measures ANOVA (95% level of confidence), the results were comparable to an antifungal drug miconazole. It is therefore concluded that *C. frutescens* or the mixture of *C. frutescens* and *A. cepa* extracts (50-50 v/v) can be effectively used as antifungal agent against *A. niger*. This study possibly serves as a model for students to learn the scientific method practically and to experience different process skills essential in biological research tangibly.

Keywords: Allium cepa, *antifunal activity*, Aspergillus niger, Aspergillus flavus, Capsicum frutescens, *laboratory model for high school biology*

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INTRODUCTION

Aspergillus spp. are fungi commonly present in the air that people breathe (Yassin and Almouqatea, 2010; *cf.* CDC Website). There are more than 185 known species of *Aspergillus*, and at least 20 of them have been reported to cause human diseases, such as aspergillosis, pneumonia and fungus ball which attacks the lungs (Yassin and Almouqatea, 2010; Choudhury *et al*., 2011).

The most common species of *Aspergillus* are *A. niger* and *A. flavus* – both species cause diseases in human beings and plants. Moreover, *A. niger* is one of the top three causatives of human fungal diseases (Barker and Carrington, 1953; Choudhury *et al*., 2011). *A. niger* is very versatile and not fastidious, allowing itself to grow in different environments and microhabitats where the other fungi cannot. *A. niger* also infects plants, specifically ginger, onion, peanut, grapes and mangoes. It produces toxins that induce crop or fruit rotting rendering them unsafe for human consumption (U. S. EPA Website, 1997; Choudhury *et al*., 2011).

On the other hand, *A. flavus* produces aflatoxins that cause rotting in plants. It usually infects the seeds of plants like corn and peanut. However, manifestation of the infection becomes apparent only in the post-harvest and storage stages. As a result, the infected seeds would then not be useful any more (Montes-Belmont and Carvajal, 1998).

Corn is the second most important crop in the Philippines after rice. An annual report generated by the Bureau of Agriculture Statistics (BAS) in 2012 indicated that the crop sector grossed a total of 375.1 billion Philippine pesos, which is 51.79% of the total production of the agriculture sector, and 60% of which comes from the crop sector and 6% is attributable to corn (PSA Website, 2014). If an outbreak of these species of *Aspergillus* arises in the Philippines or even in neighboring Asian countries, the agricultural industry would certainly be paralyzed. With such unfortunate event, the poultry and livestock industries would also be affected because corn is one of the main sources of feeds in the country (Montes-Belmont and Carvajal, 1998). Sixty percent of corn produced in the Philippines (the average is around 3.21 metric tons per hectare) is used for feeds in the livestock and poultry sectors, while the remaining is used for human consumption. This would ultimately have a negative effect on the economy since the combined corn, livestock, and poultry industries are approximately 30% of the total agriculture sector of the Philippines (PSA Website, 2014).

Fortunately, natural products have been proven to inhibit the growth of these fungal species. For example, *Allium cepa* (white onion) has been reported to have some useful medicinal properties: It has anti-inflammatory, anti-asthmatic and antimicrobial properties and was even found to have a good effect on the cardiovascular system (Santas *et al.*, 2010). Moreover, onion has the potential to be a fungicidal agent. Species of *Aspergillus* and *Candida* were not able to reproduce when onion extracts were applied (Benkeblia, 2004). Also, Lanzotti *et al*. (2012) reported that three saponins in onion had a high antifungal activity. *Capsicum frutescens* (cayenne pepper) or locally known as "siling labuyo" in the Philippines, is usually used in food preparation and for homemade remedies (Cichewicz and Thorpe, 1996). De Lucca *et al.* (2006a) reported that it had an antifungal property due to a certain saponin called CAY-1.

In these studies, the antifungal properties of both *A. cepa* and *C. frutescens* were characterized. However, the effectiveness mainly was examined on the dosage of each sample. Since *A. cepa* and *C. frutescens* have different types of saponins (De Lucca *et al.*, 2006a; Lanzotti *et al.,* 2012), it is presumed that the two would complement each other and become a stronger fungicidal agent. Therefore, in the present study, the combined antifungal effects of the extracts of *A. cepa* and *C. frutescens* on the growth of *A. niger* and *A. flavus* were investigated.

It should be noted that the present study came about after high school students were immersed to the different concerns about the safety of food crops and the development of antimicrobial agents through the works of Pandey *et al.* (1982) and De Lucca *et al.* (2006b) with further reinforcements from local news articles and stories in Philippine provinces. The students also learned from Yassin and Almouqatea (2010) that scientists had been producing much safer antifungal agents from plants in comparison to synthetic or artificial fungicides, which might be harmful to both plants and humans.

MATERIALS AND METHODS

Preparation of Test Organisms

Pure cultures of *A. niger* and *A. flavus* were obtained from the Microbial Culture Collection and Testing Laboratory of Department of Biological Sciences, Central Luzon State University, Philippines. All apparatus used were sterilised with heat. The pure cultures of *A. niger* and *A. flavus* were inoculated from a heated wire loop on the potato-dextrose-agar (PDA) slants and were kept in the refrigerator at 5°C until needed.

Acquisition and Extraction of Plant Materials

Plant materials, *A. cepa* bulbs and *C. frutescens* fruits, were purchased from a local market. They were identified by an agronomist at the Central Luzon State University.

Extraction procedure was carried out as adapted and modified from Abdou *et al.* (1972) and Benkeblia (2004). The cayenne pepper, alongside with onions were washed with clean water and allowed to air dry for 4 days. The outer coverings (tunic) of onion's bulb were manually peeled off. They were then separately cut into small pieces and underwent the process of maceration in which 20 *g* of each of the dried plants were soaked in 20 *ml* of 20% ethanol for 48 hours. They were then filtered using a filter paper.

Antimicrobial Sensitivity Testing

Sensitivity testing was carried out for *A. niger* and *A. flavus* using the Kirby-Bauer technique (Bauer *et al.*, 1966). A sterile cotton swab was used to spread the microorganisms all over the surface of the PDA plates. The plates were allowed to dry for about 5 minutes.

Whatman filter paper No. 2 disks, 6 *mm* in diameter, were immersed in the extracts of *A. cepa*, or *C. frutescens*, a 50-50 v/v mixture of *C. frutescens and A. cepa* extracts, chloramphenicol (30 *mg/ml*), or miconazole (30 *mg/ml*). The disks were placed on respective plates of test organisms which then were incubated at 37°C for 72 hours. Three replicates were made.

RESULTS

All the treatments showed positive results (Figure 1). The extracts inhibited the growth of *A. niger*. Chloramphenicol exhibited the widest zone of growth inhibition for *A. niger* (26.97 *mm*). The zones of growth inhibition of *C. frutescens* extract (19.29 *mm*) and the mixture of *A. cepa* and *C. frutescens* extracts (19.20 *mm*) were statistically comparable to that of miconazole (20.33 *mm*) using *t*-test and repeated measures ANOVA (95% level of confidence).

For *A. flavus*, *C. frutescens* extract inhibited the growth to a certain extent (10.47 *mm*) which was not comparable to chloramphenicol (25.50 *mm*) and miconazole (16.25 *mm*). However, this value was significant compared to the other treatments which did not significantly inhibit the growth of *A. flavus*.

DISCUSSION

Phytochemical testing in previous studies, such as Benkeblia (2004) and Kamilla *et al.* (2009), revealed that secondary metabolites present in cer-

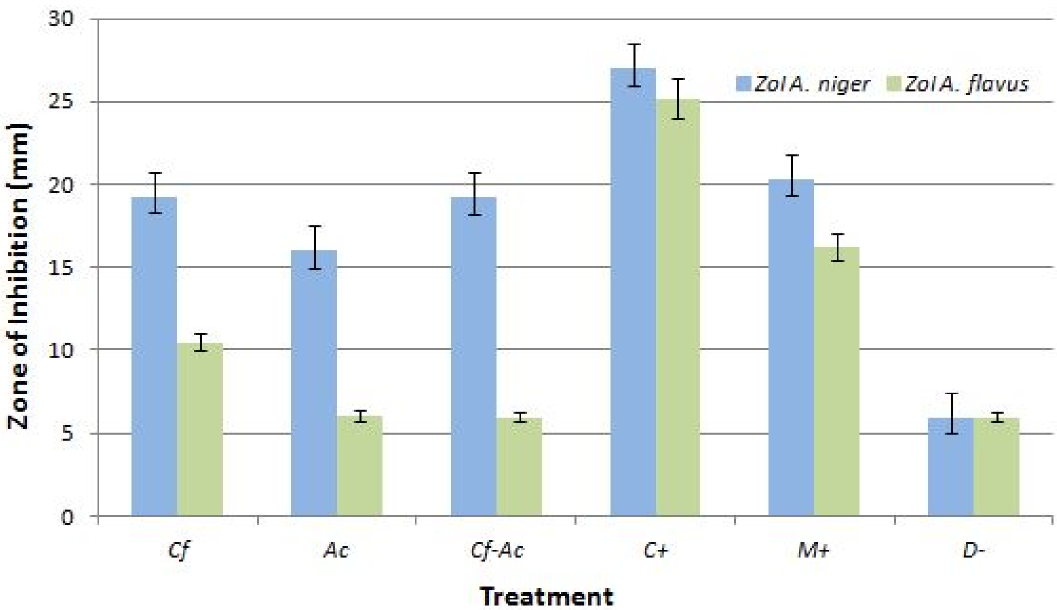

Figure 1 Inhibition zone (in mm) of <u>Capsicum frutescens</u> extract (Cf), <u>Allium cepa</u> extract (Ac), the 50-50 v/v mixture of <u>C. frutescens</u> and <u>A. cepa</u> extracts (Cf-Ac), chloramphenicol (C+), miconazole (M+), and distilled water (D-) against <u>Aspergillus niger</u> and <u>Aspergillus flavus</u>

tain plant extracts and commercially available medicines are responsible for antifungal activities against fungi from genera *Aspergillus* and *Candida* among others. The tested plants contain tannins, polyphenols, alkaloids and glycosides, which have natural antimicrobial properties (De Lucca *et al.*, 2006a; Lanzotti *et al.*, 2012).

Saponins are also a group of these secondary metabolites. They serve as important components in a wide range of plant species, for they function as a defending agent against microbial infections (Lanzotti *et al.,* 2012). They have detergent-like properties that are lethal to fungi due to their ability to combine with membrane sterols, which cause a loss of membrane integrity. Some plant species show compromised resistance to different fungal pathogens because of a deficiency in saponins (De Lucca *et al*., 2006a). Two saponins found in *C. frutescens* were tested amongst many strains of fungi, including some strains of *Aspergillus*, and were shown to be effective antifungal agents against most strains of fungi (De Lucca *et al.* 2006b). On the other hand, Ceposide A, B, and C are the saponins found in *A. cepa*, which have also been tested positively against different strains of fungi (Lanzotti *et al.,* 2012).

In the present study, *C. frutescens* extract showed the highest activity in all experiments. Antifungal results of *C. frutescens* were in line with that of Kamilla *et al.* (2009) who got 19.89 *mm* as the average zone of inhibition of *Clitoria ternatea* on *A. niger*. *A. cepa* extract showed an inhibitory activity against *A. niger*, but significantly less activity against *A. flavus*. Using repeated measures ANOVA, there was a significant difference between the results for *A. flavus* and for *A. niger*. This suggests that *A. flavus* might be resistant to *A. cepa* as affirmed by De Lucca *et al.* (2006a). The 50-50 v/v mixture of *A. cepa* and *C. frutescens* extracts showed an exemplary result for *A. niger*. On the other hand, the result for *A. flavus* was significantly lower. The result gap may be due to the synergism of the resistance of *A. flavus* to *A. cepa* and uncertain factors.

The description of methods and the presentation of findings in the present paper are derived from an attempt of high school students to apply the "scientific process research skills" necessary in biology. Onorato (2014) noted that one of the reasons why students have difficulty appreciating these research skills is the use of conceptual approach in teaching them, rather than the use of practical approach in the context of an actual scientific investigation problem. Thus, through the study they conducted as a class requirement, the first year high school students (grade 9, ages 14 - 15) were exposed to meaningful experiences to make theory meet practice while triggering curiosity to higher-level science for their age.

ACKNOWLEDGEMENT

The authors thank the Science Subject Area of Ateneo de Manila High School and the Department of Biological Science of the Central Luzon State University for all the support throughout the study.

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