Antifungal Activity of Kuchai *(Allium tuberosum)* Rottier ex Spreng Bulb Extract

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Abstract

This study was conducted to evaluate the antifungal activity of the kuchai bulb extract using ethyl alcohol and water as solvents against Candida albicans, Aspergillus niger, and Trichophyton mentagrophytes. The experiment was conducted at the Natural Products Chemistry and Biotechnology Laboratory, Science Complex I of the University of Northern Philippines, Tamag, Wigan City, Ilocos Surfrom October 2005 to January 2006.

Standard procedures and screening for antifungal activity by Ontengco, et al. (1992) and Guevarra (2005) were strictly followed in the conduct of the experiment.

Results showed that using water as extracting solvent, the antifungal activity in terms of the diameters of inhibition of the kuchai bulb extract was weak against C. albicans and A. niger and negative on T. mentagrophytes. Using ethyl alcohol, the kuchai bulb extract was strong against C. albicans and A. niger, and moderate on T mentagrophytes.

Furthermore, there was no significant difference between and among the diameters of inhibition of the three test organisms subjected to kuchai bulb extract using water as solvent, Likewise, the same result was obtained using ethyl alcohol as solvent.

Moreover, the t-test showed that the mean differences of the diameters of inhibition of the three test organisms subjected to kuchai bulb extract using ethyl alcohol and water as solvents were significant at .OJ level. This means that ethyl alcohol as solvent is better than water in testing the antifungal activity of kuchai bulb extract.

From the result of the study, it is evident that ethyl alcohol as extracting solvent is better than water in determining the antifungal activity of the kuchai bulb extract against C. albicans, A. niger, and T. mentagrophytes.

The following recommendations are forwarded: conduct the same study utilizing crude plant extract; determine the Minimal Inhibitory Concentration (MIC) and Minimal Fungicidal Concentration (MFC) of the plant extract; and compare the antifungal activity of the plant extract with a standard antibiotic.

Introduction

Background of the Study

There has been in more recent times an awakening towards the use of drugs and their preparations in a kind of "back-to-nature" movement, instead of the classical synthetic compounds manufactured in advanced countries.

While the use of synthetics is of undoubted value especially in the advanced stage of an illness, it is believed that the use of herbal medicines of properly-tested efficacy would be of great advantage in a developing country like ours which is still blessed with bountiful plant resources. The idea should be to keep people healthy by treating an illness at its early stage instead of resorting to treatment when it is already at an advanced stage. (Santos, 1985)

The medicinal values of the kuchai plant as mentioned in the book of Quisumbing and its practical application in wounds have led the researchers to conduct this study to test if the plant's bulb extract can be a cheaper but equally or more effective antifungal agent.

Objectives of the Study

This study was conducted to evaluate the antifungal activity of kuchai bulb extract using ethyl alcohol and water as extracting solvents on *Candida albicans, Aspergillus niger,* and *Trichophyton mentagrophytes.*

Specifically, it sought to answer the following questions:

1. What is the antifungal activity in terms of the diameters of inhibition of kuchai bulb extract using ethyl alcohol as solvent against C. *albicans,* A. *niger,* and T. *mentagrophytes7;*

2. Is there a significant difference between and among the diameters of inhibition of the three test organisms subjected to kuchai bulb extract using ethyl alcohol as solvent?;

3. What is the antifungal activity in terms of the diameters of inhibition of kuchai bulb extract using water as solvent against C. *abicans*, A. *niger*, T. *mentagrophytes?;*

4. Is there a significant difference among and between the diameters of inhibition of the three test organisms subjected to kuchai bulb extract, using water as solvent; and

5. Is there a significant difference in the antifungal activities of kuchai bulb extract using ethyl alcohol and water as solvents?

Scope and Limitation of the Study

This study was limited to the antifungal activity of kuchai bulb extract in terms of the diameters of inhibition against the test organisms used.

Determination of the Minimal Inhibitory Concentration (MIC) and Minimal Fungicidal Concentration (MFC) of the plant extract as well as comparison with the standard antibiotic was not conducted due to unavailability of materials to be used.

The air-drying and extraction processes as well as the screening of the anti- fungal activity was done at the Natural Products Chemistry and Biotechnology Laboratory, Science Complex I of the University of Northern Philippines, Tamag, Vigan City from October 2005 to January 2006.

Review of Literature

The continuing emergence of antibiotic- resistant bacterial and fungal pathogens demands the discovery of new antibiotics. The current market of antibiotics and anti-fungals are in billions of dollars worldwide. The Philippines, being a tropical archipelago with a rich biodiversity profile, can provide an abundant supply of biological materials that can be screened for medicinal activities. (Guevara, 2005)

Researchers at the National Institute of Science and Technology (NIST), the Philippine Council for Health Research and Development, and the University of the Philippines have proven the effectiveness of medicinal plants. (Banez, 2002)

Banez (2002) further mentioned that one of the funded programs of the Department of Science and Technology (DOST) is the intensified research of indigenous plant materials not only for drug manufacturers but also primarily for

providing the rural areas with adequate supply of medicines or drug preparations by the expanded utilization of plants in their raw and semi- processed forms.

Bonifacio et al. (2006) conducted a study on the anti-fungal activity of the kuchai leaf extract against *Candida albicans*, *Aspergillus niger* and *Tichophyton mentagrophytes* using water and ethyl alcohol as solvents. Findings revealed that ethyl alcohol is a better solvent against Trichophyton *mentagrophytes*.

The study of Bonifacio et al. is different from this study because although the same plant species was used, a different plant was utilized.

Definition of Terms

Antifungal Activity. It refers to the process of detecting the fungal activities of a particular plant extract by measuring the diameters of inhibition towards the growth of the test organisms.

Diameter of Inhibition. This refers to the extent through which the plant extract could inhibit the growth of test organisms. It is the observable clear zone around the filter paper disc which has been wet with the plant extract. The measurement is done from the outer edge of one end of the clear halo zone to the outer edge of the opposite end using a Vernier caliper expressed in millimeter. The greater the diameter, the greater is the antifungal activity of the plant extract.

Extract. This refers to a concentrated solution obtained by submerging finely-cut plant parts in extracting solvents. In this study, it is the concentrated solution obtained by submerging finely-cut air-dried bulbs of kuchai with ethyl alcohol and water as solvents.

Methodology

This study used the experimental method of research in an actual laboratory set-up.

Equipment and Materials

Alcohol Lamps
Autoclave
Beaker
Erlenmeyer flash
Filter paper discs
Forceps
Glass funnel
Graduated cylinder
Inoculating loops
Oven
Petri dishes
Pipettes
Spatula
Stirring rods
Test tubes
Test tubes racks
Vernier caliper

Test Organisms

Aspergillus niger
Candida albicans
Trichophyton mentagrophytes

Reagent /Culture Media/ Extracts

Distilled water
Ethyl alcohol
Kuchai bulb extract
Mc Farland Standard
Sabouraud Glucose Agar
Saline tween 80 solution

Procedure and Screening for Antifungal Property

A. Procurement of the Test Organisms

The test organisms were acquired from the Natural Science Research Institute (NSRI), University of the Philippines, Diliman, Quezon City.

B. Sterilization process

All laboratory equipment and materials needed were sterilized in the autoclave for thirty minutes. After the sterilization process, these were kept in the oven which was calibrated to 180°C for one hour to avoid contamination.

The following procedures were adopted from the Manual on Extraction and Microbial Assay of Medicinal Plants by Ontengco et al. (1992) and a Guidebook to Plant Screening: Phytochemical and Biological by Guevara (2005).

C. Preparation of the Plant Extract

Two hundred grams (200g) of kuchai bulb were air-dried and cut into fine pieces, one hundred grams (100g) of which were placed in separate Erlenmeyer flasks. Three hundred ml (300ml) of the 80% ethyl alcohol was poured into the flask to completely submerge the materials. Another 300ml of the distilled water was used in the second flask. The flasks were stoppered and allowed to stand for 24 hours after which the plant material were rinsed with fresh portions of alcohol, the washing of which were combined with the first filtrate.

The filtrates were concentrated in water bath to about 20ml. The exact volumes of the concentrated extract was measured. Then the concentration of the plant extract was computed, expressed as grams plant material per ml of the extract, tightly stoppered and stored inside the refrigerator. The plant extract was ready for the antifungal screening.

D. Screening for Antifungal Activity

The Kirby-Bauer Disk Diffusion Method was used for this specific part.

1. Preparation of 0.5 McFarland Standard

a. 0.5 mL of 0.048 MBaCl was mixed to 99.5 ml of 0.36N H_2SO_4
b. 5mL of the mixture was distributed into screw-capped tubes of the same dimension as those which were used in the preparation of the culture suspension;
c. The tubes were tightly sealed and stored in the dark at room temperature;
d. The turbidity standard was shaken vigorously prior to use.

2. Preparation of Sterile Isotonic saline-Tween 80 Solution

a. 0.85 g. NaCl was dissolved in 100 ml distilled water to prepare the isotonic saline solution;
b. 0.1 ml Tween 80 was added to the above 100 ml isotonic saline solution
c. The isotonic saline-tween 80 solution was sterilized in an autoclave at 121°C for 15 minutes, then cooled before use.

3. Preparation of Inoculum

a. A loopful of pure fungal culture was inoculated in 5 ml Sabouraud Glocuse Agar plate, then incubated for 5-7 days at 30°C until aerial hyphae have luxuriantly developed;

b. Five (5) loopfuls of conidia and hyphae were scrapped off and immersed in 5 ml of the sterile isotonic saline-Tween 80 solution contained in screw-capped tubes;
c. The tubes were shaken continuously for one (1) minute. This served as the spore suspension of which turbidity should match with the turbidity of McFarland;
d. 0.5 mL of the adjusted spore suspension was added to 20 ml saline- Tween 80 solution. This served as the inoculum.
e. The above suspensions were used within 15 minutes after the turbidity was adjusted.

4. Adjusting the Turbidity of the Inoculum

When the fungal inoculum did not appear to be of the same turbidity with McFarland standard, more of the spore scraping or the saline-Tween 80 solution was added to achieve identical turbidity.

5. Agar Plates

15 mL of Sabouraud glucose agar was poured into dry and sterile Petri dishes and then allowed to solidify before use.

6. Seeding of the Plates

A sterile cotton swab was dipped into the saline-tween 80 spore suspension. Excess inoculum was removed by rotating the swab several times against the wall of the test tube above the fluid level. The entire surface of the agar was streaked evenly in all directions. The swabbed plates stood for 5 minutes.

7. **Placement of Paper Discs**

Using sterile forceps, a sterile 6mm paper disc was dipped into the plant extract and laid and pressed gently to ensure maximum full contact of the disc with the agar medium on the estimated center of the quadrant of the Petri dish. Three quadrants of the Petri dishes were for the plant extract and the fourth for the control.

8. Incubation and Observation of Plates

The plates were inverted and incubated within 18-24 hours for the yeast and 2-3 days for molds at 27°C.

9, Reading and Interpretation

The diameter of each zone of inhibition was measured to the nearest tenth of the milliliter with a Vernier caliper. For purposes of standardization, the following interpretative range of standard zone was adopted from Otengco (1992).

Inhibitory Activity	Zone of Inhibition
+++, strong	>17
++, moderate	12-16
+, weak	7-11
-, negative	6 or 0

Results and Discussion

It can be seen from Table 1 that using ethyl alcohol as solvent, the *kuchai* bulb extract was strong against *Candida albicans* and *Aspergillus niger* with diameters of inhibition of 21.67 mm. and 17.25 mm., respectively. The plant extract was moderate against *Tricophyton mentagrophytes* with a diameter of inhibition of 12.50 mm.

Table 2. Summary of ANOVA on the differences between and among the diameters of inhibition of the test organisms using ethyl alcohol as solvent.

Source of Variation	**Sum of Squares**	df	**Mean** of **Squares**	F-ratio	**Interpretation**
Between Groups	504.39	2	252.20	3.40	Not Significant
Within Groups	2666.42	33	74.07		
Total	**3170.81**				

Table 2 shows that the computed F-ratio of 3.40 is not significant at 0.01 level. Therefore, the null hypothesis which states that there is no significant difference between and among the diameters of inhibition of the test organisms subjected to kuchai bulb extract using ethyl alcohol as solvent is accepted. This implies that the *kuchai* bulb extract had more or less the same inhibitory effect on the growth of the three test organisms using ethyl alcohol as solvent.

Table 1. Antifungal activity of *kuchai* bulb extract against the test organisms using ethyl alcohol as solvent.

Test Organisms	Diameter of Inhibition															Grand Mean	Antifungal Activity
	R		lication 1			Replication 2						Replication 3					
	1	2	3	C		1	2	3	C	X	1	2	3	C			
Candida albicans	24	29	26	6		37	25	7	6	18.75	30	29	35	6		21.67	+++
Aspergillus niger	26	2.5					<<										
Tricophyton mentagro-phytes	15	21		0				0	0						0		

Legend: +++ = strong
++ = moderate
C = control

Table 3. Antifungal activity of *kuchai* bulb extract against the test organisms using water as solvent

Test Organisms	Diameter of Inhibition																Grand Mean	Antifungal Activity
	Re					Replication 2						Replication 3						
	1	2				2	3	C				2	3	C				
Candida albicans	7.3	6.4				6.8	8.6	6				7.9	6.7	6			7.34	+
Aspergillus niger	8.3	8.8															7.19	+
Tricophyton mentagro-phytes	7.2	6																

Legend: + = weak
- = negative
C = control

It can be seen from Table 3 that using water as solvent, the kuchai bulb extract was weak against *Candida albicans* and *Aspergillus niger* with diameters of inhibition of 7.34 mm and 7.19 mm, respectively. The plant extract had no inhibitory effect against *Tricophyton mentagrophytes* with a diameter of inhibition of 6.30 mm.

Table 4 presents the result of the ANOVA on the difference among and between the diameters of inhibition of kuchai bulb extract using water as solvent.

Table 4. Summary of ANOVA on the differences between and among the diameters of inhibition of the test organisms using water as solvent

Source of Variation	Sum of Squares	df	Mean of Squares	F-ratio	Interpretation
Between Groups	7.56	2	3.78	2.82	Not Significant
Within Groups	44.07	33	1.34		
Total	**51.63**				

Table 4 shows that the computed F-ratio of 2.82 is not significant at .01 level. Therefore, the null hypothesis which states that there is no significant difference between and among the diameters of inhibition of the test organisms subjected to kuchai bulb extract using water as solvent is accepted. This implies that the *kuchai* bulb extract has more or less the same inhibitory effect on the growth of the three test organisms using water as solvent.

Table 5 presents the result of the t-test on the differences between ethyl alcohol and water as solvents.

Table 5, t-test on the difference between ethyl alcohol and water as solvents

Test Organisms	Mean		Mean Difference	df	t	Interpretation
	Ethyl alcohol	Water				
Candida albicans	21.67	7.34	14.33	22	4.12	Significant
Aspergillus niger	17.25	7.18	10.07	22	4.36	Significant
Tricophyton mentagrophytes	12.50	6.3	6.20	22	3.54	Significant

It can be seen from Table 5 that the mean differences of the diameters of inhibition of the three test organisms subjected to kuchai bulb extract using ethyl alcohol and water as solvents were significant at .01 level. Thus, the null hypothesis which states that there is no significant difference on the antifungal activity of kuchai bulb extract against the three test organisms using ethyl alcohol and water as solvents is rejected. This means that ethyl alcohol as solvent is better than water in testing the antifungal activity of kuchai bulb extract.

Conclusions and Recommendations

Using ethyl alcohol as solvent, the kuchai bulb extract has a strong antifungal activity against C. *albicans* and *A. niger*, and moderate on *T. mentagrophytes*.

Using water as solvent, the kuchai bulb extract has a weak antifungal activity against C. *albicans* and *A. niger*. The plant extract has a negative antifungal activity against *T. mentagrophytes*.

Ethyl alcohol as solvent is better than water in determining the antifungal activity of kuchai bulb extract against C. *albicans*, *A. niger*, and *T. mentagrophytes*.

The following recommendations are forwarded:

1. The same study utilizing crude extract be conducted;
2. Determine the Minimal Inhibitory Concentration (MIC) and Minimal Fungicidal Concentration (MFC) of the plant extract; and
3. Compare the antifungal activity of the plant extract with a standard antibiotic

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