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Orange Peel (*Citrus sinensis)* and Rough Horsetail Plant (*Equisetum hyemale)* as Antifungal Foot Powder

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Abstract: The ultimate aim of this study was to investigate the efficiency of antifungal agents in various formulations of powdered orange peel and rough horsetail against a sample obtained from a participant's foot, which was suspected of having a fungal infection. The researchers prepared three sets of experimental setups with different formula. It was hypothesized that there are significant differences in inhibition zone measurement between the experimental setups. The study made use of experimental and comparative design to seek the effects of the two ingredients in different formula. The subject of the study was the collected sample from a participant in Liliw, Laguna. For a formal collection of samples, the researchers made a consent and confidential agreement with the participant. After the agreements, the process of interview and physical examination has been done with all positive results of the participant suffering in foot fungal infection, and proceeded on swabbing his foot with the use of required equipment. With the success of sample collection, researchers used observation and experimental approach as instruments in gathering the data. The study's findings revealed a substantial difference in the formula affecting the development of fungus in agar, as indicated by the computed analysis of variance values of 0, which are less than the tabular value of 0.05 at the 5% level of significance. Furthermore, it was recommended that between the two ingredients against foot fungal infection, the rough horsetail should have a higher percentage than Orange Peel in formula in terms of inhibiting a fungal growth.

KEYWORDS: *antifungal powder, foot fungi, orange peel, rough horsetail plant*

Introduction

Foot fungi are recurring unpleasant conditions afflicting millions globally. The symptoms experienced, such as irritation, inflammation, peeling, and discomfort, affect general health and could result in more severe issues. Treatment for fungal foot infections usually involves readily available antifungal drugs. Nevertheless, it is essential to acknowledge that medical interventions can cause adverse reactions.

Fungal infections are a common condition that affects the skin on the foot, especially in the spaces between the toes and the soles. These conditions are caused by fungus, such as molds, yeasts, and dermatophytes, which thrive in warm and humid settings Santhakumar (2021). The Philippines is a tropical country with warm temperatures and abundant rainfall. This condition contributes to the prevalence of a timely problem among Filipinos, which is foot fungi infection.

Many rely on commercial products that may cause skin irritation and fungal infections, especially in open wounds, to combat this issue. Fungi can cause varying infections, from allergic syndromes to life-threatening invasive fungal diseases based on Bongomin et al., (2017) in the study, “Global and Multi-National Prevalence of Fungal Diseases—Estimate Precision”.

Recently, a growing focus has been on investigating natural alternatives for managing fungal foot infections. Two promising sources of organic products are the orange peel (*Citrus sinensis*) and the rough horsetail plant (*Equisetum hyemale*), based on different studies.

The Philippines, currently ranking fourth among the world's largest citrus markets (Malapo, 2023), offers a readily available source of orange peels. Often discarded as waste, these peels hold potential as a natural antifungal agent.

Rough horsetail, on the other hand, is a spore-producing plant with potential medicinal properties. While uncontrolled populations can be invasive and toxic to livestock (Beaulieu, 2023), their antifungal properties suggest a valuable alternative use.

In connection with this, Orange (*Citrus sinensis*) comes from the genus Citrus. Citrus peel has high economic value due to its richness in bioactive compounds such as polyphenolics, carotenoids, dietary fiber, essential oils, ascorbic acid, and trace elements (Sharma et al., 2016). Its value lies not only in its efficacy but also in its availability and sustainability. As a byproduct of orange consumption, utilizing the peel contributes to waste reduction.

Moreover, according to Article 23 of the agricultural EU pesticide regulation (EC) No 1107/2009, horsetail has been recognized as a basic component with antifungal activities since 2014. It has also been authorized for use in organic production since 2016 (Marchand, 2016). High in silica and other minerals, rough horsetail (*Equisetum hyemale*) has low cultivation and harvesting costs, little processing needs, and the possibility of being used as a natural medicine by local communities—especially in regions where it grows prolifically—all contribute to its economic value.

To address the issue on fungal growth, researchers have explored natural alternatives that contain possible antifungal compounds that are easy to find, such as orange peel and rough horsetail. Making the powder, which involves dehydrating, oven toasting, and blending, is also easy. Integrating orange peel and rough horsetail into foot fungi, treatment regimens can reduce healthcare costs associated with pharmaceutical antifungals and encourage the use of renewable resources.

Moreover, this study focuses on the susceptibility of the collected fungi to the three different ratios of combined orange peel and rough horsetail plants.

Conceptual Framework

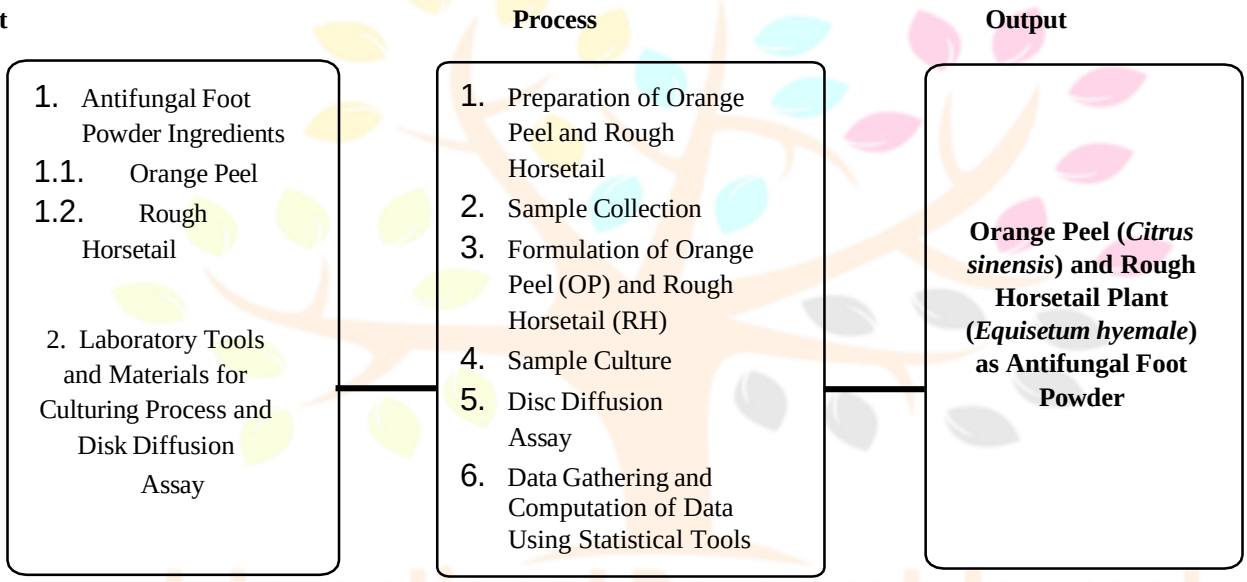

Figure 1. *Research Paradigm of the Study*

Figure 1 shows the input, process, and output of the study. The input is the foot powder formula, materials and tools for fungi culturing process, and for disc diffusion assay. The foot powder formula is composed of the orange peel and rough horsetail plant. The fungi culture tools and materials are composed of PDA, autoclave, sterile cotton swab, petri dish, inoculum, and alcohol lamp. The disc diffusion assay requires cultured plates, 6mm disc diffused in different formula, tweezer, flask, 95% ethanol alcohol, and tape.

Moreover, the process is divided into six parts; preparation of orange peel and rough horsetail plant, sample collection, ratio of orange peel and rough horsetail plant, sample culture, disc diffusion assay, and data gathering and computation of data using statistical tools. The preparation of ingredients is composed of washing, dehydration at 65º C for 8 hours, oven at 160°C for 2 minutes, thrice, crushing, sieving, and storing. The sample collection followed a procedure of cleaning, swabbing, and storing. Next, different formulations of orange peel and rough horsetail plant are made, respectively, according to different ratios on a 0.2 g foot powder: (a) 50:50 (0.1 g: 0.1 g), (b) 70:30 (0.14 g: 0.06 g), (c) 30:70 (0.06 g: 0.14 g). Then, the culturing process of the sample gathered proceeds with PDA media preparation, sample culturing, and incubation at room temperature.

Furthermore, the disc needed for disc diffusion assay was soaked in 95% ethanol for 5 minutes and autoclaved. It was then soaked in different formula of foot powder for 5 minutes. Place it at the center of the PDA plate and leave for 24 hours at room temperature. Lastly, the data gathering and computation of data using statistical tools such as mean, standard deviation, one-way ANOVA, and Waller-Duncan post- hoc analysis. On the other hand, the output is orange peel and rough horsetail plant as antifungal foot powder.

Objectives

This study aims to test orange peel (*Citrus sinensis*) and rough horsetail plant (*Equisetum hyemale*) as antifungal foot powder. To achieve a better understanding of this study, it requires answers to the following questions:

1. What is the inhibition zone of the following formulations of orange peel and rough horsetail antifungal powder to the collected sample?
   - 1.1. 70% orange peel: 30% rough horsetail powder,
   - 1.2. 50% orange peel: 50% rough horsetail powder, and
   - 1.3. 30% orange peel: 70 % rough horsetail powder?

Hypotheses: There is no significant difference between the inhibition zone of cultured samples with three different orange peel and rough horsetail plant powder formulation.

Methodology

The researchers employed an observation and experimental approach. The observation method is simple and accurate since the observer directly interacts with the observed. It provides an exact description of the phenomenon in its natural research environment. This method complements the experimental approach, which is systematic and scientific.

Before conducting a sample collection, ethical consideration is first recognized. The researchers initially prepared a request for approval of sample collection on the College of Arts and Sciences Office and on the barangay where the participant is registered. After it is approved, the proponents, their research adviser, and the CAS Dean signed a confidentiality statement with regards to the participant's sensitive information that will be gathered. Then, the participant signed a letter of consent in exchange of his condition's confidentiality. Additionally, the researchers interviewed the participant about his background and current symptoms before conducting the experiment.

The sample collection was conducted in Liliw, Laguna. In this research, three different percentages of orange peel (OP) and rough horsetail (RH) powder are diluted in 2 ml distilled water: 70% (0.14 g OP) and 30% (0.06 g RH), 50% (0.1 g OP) and 50% (0.1 g RH), 30% (0.06 g OP) and 70% (0.14 mg RH). Each ratio has two soaked 6mm discs placed in six petri dishes. The researchers compared the significant effect of plates with each disc treated with foot powder after 24 hours at room temperature.

The study is conducted at the Molecular Biology Center of Laguna State Polytechnic University – San Pablo City Campus. The methodology is composed of five parts which includes: (1) Preparation of Foot Powder Ingredients (Orange Peel and Rough Horsetail Plant). (2) Sample Collection from Participant with Foot Fungal Symptoms. (3) Formulation of Different Ratios of Orange Peel and Horsetail Antifungal Powder. (4) Disc Preparation (5) Fungi Culturing and Disc Diffusion Process (6) Data Gathering and Computation of Data Using Statistical Tools.

Sample collection was conducted after gaining all the approval and proceeded to laboratory experimentation and data gathering followed. Lastly, the computation of raw data was prepared using the statistical tools; Mean, Standard Deviation, ANOVA, Waller-Duncan Post-hoc Analysis, providing the data for results and discussion.

Results and Discussion

In this chapter, it discussed the results of the 24-hour experiment and the zone of inhibition observed. The data presented below shows the observed zone of inhibition against the fungi cultured from the feet, for different ratios (50:50, 70:30, 30:70) of the mixture of powdered orange peel (OP) and rough horsetail (RH).

Table 1. *Inhibition Results Observed in the Experimental Setups of Orange Peel and Rough Horsetail Antifungal Foot Powder on PDA Agar Plates with the Collected Sample after 24 hours of incubation.*

	N	Mean	Standard Deviation
50% Orange Peel & 50% Rough Horsetail	2	6.2000	.00000
70% Orange Peel & 30% Rough Horsetail	2	.0000	.00000
30% Orange Peel & 70% Rough Horsetail	2	6.7500	.35355
Total	6	4.3167	3.35644

Table 1 depicts various setups that includes 6 experimental setups. Two setups with 50:50 formulation of Orange Peel to Rough

Horsetail, two setups with a 70:30 (OP:RH) formula, and two setups with a 30:70 (OP:RH) formula. The results indicate the inhibition performance over 24 hours of experiment of the 6 experimental setups.

The 70:30 (OP:RH) experimental setup exhibits zero standard deviation and mean. This formulation has growth of the collected sample fungi around its 6mm filter paper disk and has no any antifungal performance observed.

Conversely, both the 50:50 (OP: RH) and 30:70 (OP: RH) formula demonstrate inhibition against the sample collected. The 50:50 (OP: RH) setups exhibited 0 of standard deviation and a mean of 6.2000, suggesting that it has a very slight effect as antifungal foot powder. Moreover, the 30:70 (OP: RH) formula, containing a higher proportion of Rough Horsetail, proves more effective than the 50:50 (OP: RH) formula. Its mean inhibition zone measures 6.7500, with a standard deviation of 0.35355.

Gaining the results of all the setups of Orange Peel and Rough Horsetail antifungal foot powder, the 70:30 (OP:RH) formula was considered to be ineffective with zero antifungal performance within 24 hours experimentation. While the 50:50 (OP:RH) and 30:70 (OP:RH) data falls within the range of 6.2000 to 6.7500 mean inhibition, measuring with very close distance with the 6mm actual diameter size of the prepared filter paper disk, leads the researchers to a conclusion that the 50:50 (OP:RH) and 30:70 (OP:RH) are not that effective against the collected sample.

Overall, formulations with a significant amount of Rough Horsetail powder, equal to or greater than that of Orange Peel powder, indicates its antifungal activity against the sample collected since it still performs inhibition around 6 mm filter paper disk.

In the study entitled “Phytochemical Characterization, Antimicrobial Activity, and Antioxidant Potential of *Equisetum hyemale L*. (Equisetaceae) Extracts” by Rodrigues et al. (2015), the results verified that the rough horsetail, containing phenolic compounds, was observed to have a better antimicrobial activity against dermatophyte fungi. This supports that the Rough Horsetail plant can be used in inhibition of fungal growth.

Table 2. *Analyzation of Variance regarding the test of the Zone of Inhibition on the Three Experimental Setups based on its diameter in mm.*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	56.203	2	28.102	674.440	.000
Within Groups	.125	3	.042		
Total	56.328	5			

The p-value obtained (p=0.000) is less than the level of significance (a=0.05), then there is significant difference observed on the zone of inhibition between the 3 experimental setups. Disclaimer: However, this result may be caused by a low no. of samples (6 samples).

Table 2 shows the ANOVA for zone inhibition across the three experimental sets. The level of significance is set to a=0.05, and because the p-value attained is less than the level of significance, the zone of inhibition differs significantly between the three experimental setups. In this case, the null hypothesis indicates that there is no significant difference between the inhibitory zone of cultured samples with three different orange peel and rough horsetail plant powder formulations is rejected.

Although, this result may be caused by a low no. of samples (with only 6 samples used for the experiment). Still, there is a significant difference on the experimental setups that happened with the help of the properties that the Rough Horsetail and Orange peel possess, that created an effect on the results from the experiment. As described in the paper "Antimicrobial, Antitrypanosomal, and Antibiofilm activity of *Equisetum hyemale*" by Alves et al. (2016), the *E. hyemale* contains protective properties against infectious organisms. The *E. hyemale* has been shown to suppress bacterial growth at low concentrations. Therefore, both can be useful as effective agents, portraying an alternative treatment and a good prevention to several infections.

Furthermore, in another study entitled, "Green extraction of polyphenols from citrus peel by-products and their antifungal activity against *Aspergillus flavus*" by Liu et al. (2021), studied the citrus peel extracts antifungal performance which is prepared with ethanol or hot water. There were 12 polyphenols identified by the HPLC-DAD, shows that the most abundant compounds were narirutin and hesperidin. All in all, citrus peels appear to be a viable source of antifungal chemicals.

Table 3. *Waller-Duncan Post hoc Analysis between the Zone of Inhibition between 3 Experimental Setups*

	Setup Type	N	Subset for alpha = 0.05	
			1	2
Waller-Duncan[a,b]	70% Orange Peel & 30% Rough Horsetail	2	.0000	
	50% Orange Peel & 50% Rough Horsetail	2		6.2000
	30% Orange Peel & 70% Rough Horsetail	2		6.7500

It is shown that setups 1 & 3 have almost the same zone of inhibition observed while Setup 2 did not have a consistent zone of inhibition observed.

Table 3 presented the mean zone of inhibition of the different setups 70:30, 50:50, and 30:70 (70% OP with 30% RH, 50% OP with 70% RH, and 30% OP with 70% RH, respectively). The data stated that the 30:70 formula of foot fungi powder had the highest zone of inhibition and slight level of effectiveness, followed by the 50:50, which was also slightly effective, while the 70:30 exhibited no clear inhibition. This shows that there is a significant difference between 50:50 to 70:30, which displays less consistency between the two groups. Additionally, 30:70 also has the same condition with 50:50 on significant difference to 70:30.

Furthermore, 50% OP with 50% RH and 30% OP with 70% RH had almost the same inhibition zone, 6.20 mm and 6.75 mm, respectively. This tells that the little difference between the mentioned two groups shows no significant difference.

It is possible that one of the reasons for the greater inhibition zone at 30:70 is due to the difference in the powdering process of rough horsetail and orange. Rough horsetail is tougher and therefore needed to undergo an additional 6 minutes (2 minutes, thrice exposure) in an oven at 160°C to make it easily breakable and powdery. On the other hand, orange peel is easier to break and only required one dehydration process to remove moisture. Thin strips of oranges from the dehydrator are not ideal for processing in an oven with a higher temperature, as it has a different texture that would cause it to harden and make it difficult to powderize. According to Santhakumar (2021), fungi thrive in warm, damp surroundings, making the feet an ideal locale for their propagation. Thus, due to excess moisture in the orange peel powder, it's still contributed to growth of the culture fungi and not be as efficient as the rough horsetail powder.

Further details of the probable reason for low inhibition zone of those with higher percentage of orange peel still needs to be investigated as there are few previous literature and studies that can support this theory.

Conclusions

The null hypotheses state the following: "There is no significant difference between the inhibition zone of cultured samples with three different orange peel and rough horsetail plant powder formulation".

The study's results showed that the average size of the inhibition zone, which indicates the effectiveness of the antifungal powder, varied from 0.00 - 6.75 mm in the experimental groups. Hence, showing significance in difference between inhibition zone of cultured samples with three formulations containing different percentages of orange peel and rough horsetail plant.

The statistical results revealed a significant difference (p-value < 0.000) between the experimental setups of antifungal powder with orange peel and rough horsetail. This is lower than the level of significance (0.05), therefore proving significant difference. This led to the rejection of the hypotheses. However, this may be a consequence of having only less samples (6 samples).

Overall, the study shows the potential of orange peel and rough horsetail as natural, easy-to-make, and eco-friendly ingredients in antifungal foot powder, with consideration to its availability in market and easy to reach areas.

Recommendations

Based upon the outcome and conclusion regarding orange peel (*Citrus sinensis)* and rough horsetail plant (*Equisetum hyemale*) as antifungal powder, the researchers recommend/suggests the following: The use of more experimental and control plates in the tests to obtain more data for comparison as evidence to verify efficiency and provide a strong foundation for your study's findings. Also, to look for additional specimens from various people to utilize for comparison. This will help to differentiate the study and provide further evidence to support it. And to further support the study, it is a good idea to visit a laboratory or science institute to identify the specimen used in the experiment, what type of fungi is growing, and what impacts may occur throughout the experiment. Another suggestion is to pair rough horsetail as an ingredient with fragrant fruits like orange peel to overwhelm the unpleasant odor from the rough horsetail plant. Also, consider the substance and amount of ingredients that will be used or added, as there may be an effect that causes harm to your health as the experiment progresses. In addition, by adding a substance that may help lengthen the specimen's life after the culture, to determine whether the specimen has made any significant progress throughout the investigation after a certain amount of time.

Addition of more setups for comparison and evidence in the experiment, such as a 100% Rough Horsetail plant and a 100% Orange peel, to compare with other setups and obtain much better results that will provide more efficiency of the used ingredients

in terms of anti-fungal properties. Moreover, when using an orange peel in an experiment, it is best to ensure that the component is completely dry, with no moisture seen or felt, so that it does not affect the growth of bacteria when combined with powdered or unpowdered materials. Furthermore, to extend the shelf life of powder, it is recommended that more techniques be found to prevent it from becoming stale or that molds be utilized to ensure that the powder's effectiveness is not compromised when used for experimentation. For example, to prevent contamination, store the powder in the refrigerator and carefully close the lid. Because the chilly temperature from the refrigerator helps the powder keep its stability and effectiveness. Lastly, another ideal suggestion is to combine the used ingredients (orange peel and rough horsetail plant) with other plants or fruits that are accessible to use for a new research and experimentation, or to use as a homemade medicine. Also, by doing a careful and detailed study on the composition of the selected materials, it will be helpful to combine perfectly with right ingredients. And with that, it may result in a successful or efficient product that benefits our society.

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