Investigation of the anti-yeast activity of different fruit peel extracts (Pineapple, Tomato, Pear, Pomegranate) on fungus (Saccharomyces cerevisiae)

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Abstract

The epidemic served as the inspiration for my topic selection. In addition to the soaring COVID-19 death toll, many people were unable to get healthcare after the epidemic ended, regardless of whether they actually had the virus. In a rapidly evolving society, the reliance on synthetic medicine technologies has been on the rise.In contrast to contemporary medicine, Ayurveda and herbal remedies do not cause any adverse effects during treatment. The remarkable curative power of herbal medicine has been demonstrated on the dental plaque of Neanderthals as far back as 60,000 BCE. We may reduce our use of antibiotics and enhance our use of home cures for little problems by discovering alternative, safe medical approaches.The practice of Ayurveda is widespread among Indian residents. Before turning to allopathic medicine, my family always tries natural means. I decided to study pineapple, pear, tomato, and pomegranate fruit extracts because, as a fruit waste product, fruit peels contain a lot of bioactive compounds that could be put to good use.

Keywords: Pineapple, Tomato, Pear, Pomegranate, fungus, Antibiotics, Ayurveda, Anti-yeast Activity, Antifungal Drugs.

1. Introduction

Fungal diseases have been deeply neglected because of their minimal impact on human history. They are fundamentally distinct from other infections, but their heavy influence on immunocompromised patients has changed our outlook on the pathogen. Fungi usually share many similarities with their host because of their eukaryotic nature, due to this the manufacturing of anti-fungal medications has become extremely difficult. The Global Action Fund for Fungal Infections has estimated around 1 million eyes go blind from fungal keratitis, nearly one billion people experience skin mycoses, and over 300 million people suffer from fungal infections, with 1.5 million people dying from the severe consequences.[1]

Antifungal drugs help in treating fungal diseases that can affect multiple organs including the skin, lungs, blood and brain. The antifungals either work by targeting the overall growth of the fungus or destroying the existing fungus[2]. They targetthe cell membrane (preventing it from further growing)or the cell wall (causing the cell to burst resulting the leaking of the contents in the cell therefore killing the cell).[3]Fungus can grow resistant to antifungal drugs the same way bacteria does. Thisphenomena occurs when the pathogen growsresistant to the drug meant to defeat it because of various factors including improper usage of the drug (not taking the correct dosage or not completing the recommended time of usage by medical professionals),intake of antibiotics (killing the bacteria keeping the fungus in balance), the usage of fungicides, natural resistance (exhibited by the fungus) or transmitted resistance (spreading contagious drug resistant fungus).[4]Antifungal resistance is caused when the target mutates and reproduces, making the drug purposeless. Due to the existence oflimited number of antifungals in the market, this makes antifungal

[1]"Fungal Diseases As Neglected Pathogens: A Wake-up Call to Public Health Officials." *PLOS*, 20 Feb. 2020, journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0007964. Accessed 25 Nov. 2022.
[2]Seladi-Schulman, Jill. "Antifungal Medications: Types, How They Work, and More." *Healthline*, www.healthline.com/health/fungal-infection/antifungal. Accessed 11 Dec. 2022.
[3]Memon, Nazneen, and BHMSDCR. "How Do Topical Antifungals Work?" *RxList*, www.rxlist.com/how_do_topical_antifungals_work/drug-class.htm. Accessed 11 Dec. 2022.
[4]"Antifungal Resistance: What It Is, Causes, Treatment & Prevention." *Cleveland Clinic*, my.clevelandclinic.org/health/articles/21557-antifungal-resistance. Accessed 11 Dec. 2022.

resistance that much of problem, resulting in more damage than normal pathogenic resistance. It results in severely limited options in treating fungal diseases.[5]

These limited options are what motivates and makes it necessarytofind more methods and aspects in which we can approach the fungus.A field/method that researchers have looked further into is herbal medicine. Phytomedicine is the medicine of plants, it the use of plants or plant-parts for its therapeutic and herbal properties, it includes the analysis and characterization of biomolecules; with the raise in new infections/diseases and the development of new resistant pathogenic strains, it has started to become a field of focus. It is the most sought-after primary health-care option for around 3.5-4 billion humans, with a majority involving extraction from plants. Plant and fruit-based extracts have grown in popularity because of its effect on previously drug resistant strains of fungus.[6]The peels I have explored exhibit various properties including anti-fungal properties and anti-bacterial properties. Fruit peels are the protective layer of fruits, they are extraordinarily rich in essential oils and contain fibre, vitamins, minerals, and antioxidants[7]. The antifungal properties that these fruit peels exhibit can be due to numerous reasons, including the various compounds found in them like alkaloids, flavonoid, tannin, cardiac glycoside, saponin and phlobatannin[8]. The fruit peels I have moved forward with today are of Ananas comosus (pineapple), Solanum lycopersicum L. (tomato), Pyrus communis L. (pear), and Punica granatum (pomegranates).

Ananas comosus or **pineapple peel**contributes to 50%-65% of the waste produced by the fruit.[9] Itis rich in gallic acid, catechins, epicatechins, ferulic acids, these antioxidants contain compounds that exhibit antimicrobial properties known for reducing the rate of spoilage in food, such as flavonoids, saponins, and tannins[10]. Gallic acid is a polyphenol natural compound found in various plants, it is proven to have antibacterial and antifungal properties[11]. Research shows that gallic acid interferes with Zn accumulation which affects the development of the cell[12]. Catechin potent phytotoxin, present in tea, cocoa and berries and demonstrates antibacterial and antifungal properties. Research indicates thatCatechin EGCG inhibits the cytoplasmic enzyme dihydrofolate reductase by binding to the lipid membrane and thereby affecting the fungi's folic acid metabolism.[13]Epicatechin is known to prevent the decay of the ripening of the fruitby decreasing the degradationof the antifungal diene.[14]Ferulic acid is a natural organic compound found in various fruits and vegetables, it is said that a possible mechanism of its antifungal properties occurs through the cell wall, through molecular modelling it is seen that the compound interacts with enzymes essential for the development of the fungus.[15]

Solanum lycopersicum L. or Tomato peel is rich in various biologically active compounds such as phenolic compounds (phenolic acids and flavonoids), carotenoids, ascorbic acid and glycoalkaloids all of which help to protect the plant from microorganisms[16]. The major phenolic acids present in tomato peel are caffeic acid, p-coumaric acid, and ferulic acid. Caffeic acid interacts with membranes to show its antimicrobial action, while p-coumaric acid affects fungal cell membrane and growth, and ferulic acid targets fungal enzymes. The main flavonoids found in tomato are naringenin and chalcones[17], each targeting different aspects of fungal growth and metabolism. Research shows naringin's antifungal activity in fungus is due to its ability to induce

[5]"Antifungal Resistance." *Centers for Disease Control and Prevention*, 8 July 2022, www.cdc.gov/fungal/antifungal-resistance.html. Accessed 11 Dec. 2022.
[6]www.sciencedirect.com/topics/nursing-and-health-professions/phytomedicine. Accessed 12 Dec. 2022.
[7](NL), Alina P. "Should You Peel Your Fruits and Vegetables?" *Healthline*, www.healthline.com/nutrition/peeling-fruits-veggies. Accessed 12 Dec. 2022.
[8]"Antifungal Activity and Phytochemical Analysis of Selected Fruit Peels." *International Open Access Journals | Peertechz Publications*, www.peertechzpublications.com/articles/JBM-3-113.php. Accessed 12 Dec. 2022.
[9]ajpp.in/uploaded/p382.pdf. Accessed 12 Dec. 2022
[10]"Assessment of Antioxidant and Antimicrobial Property of Polyphenol-Rich Chitosan-Pineapple Peel Film." *Publishing Open Access Research Journals & Papers | Hindawi*, 6 May 2022, www.hindawi.com/journals/jfq/2022/8064114/. Accessed 12 Dec. 2022.
[11]www.sciencedirect.com/topics/medicine-and-dentistry/gallic-acid. Accessed 12 Dec. 2022.
[12]"Interaction Between Polyphenolic Antioxidants and Saccharomyces Cerevisiae Cells Defective in Heavy Metal Transport Across the Plasma Membrane." *PubMed Central (PMC)*, www.ncbi.nlm.nih.gov/pmc/articles/PMC7694260/. Accessed 12 Dec. 2022.
[13]"Anti-infective Properties of Epigallocatechin-3-gallate (EGCG), a Component of Green Tea." *PubMed Central (PMC)*, www.ncbi.nlm.nih.gov/pmc/articles/PMC3594666/. Accessed 12 Dec. 2022.
[14]www.sciencedirect.com/topics/pharmacology-toxicology-and-pharmaceutical-science/epicatechin. Accessed 13 Dec. 2022.
[15]"Cytotoxic and Antifungal Amides Derived from Ferulic Acid: Molecular Docking and Mechanism of Action." *Publishing Open Access Research Journals & Papers | Hindawi*, 1 Nov. 2021, www.hindawi.com/journals/bmri/2021/3598000/. Accessed 6 Jan. 2023.
[16]"Bioactivities of Phytochemicals Present in Tomato." *PubMed Central (PMC)*, www.ncbi.nlm.nih.gov/pmc/articles/PMC6045986/#:~:text=Tomato%20is%20a%20good%20source,)%20and%20glycoalkaloids%20(tomatine). Accessed 17 Feb. 2023.
[17]*National Center for Biotechnology Information*, www.ncbi.nlm.nih.gov/pmc/articles/PMC4309898/sVbf8Mfd2LRh. Accessed 17 Feb. 2023.

apoptosis through mitochondrial dysfunction mediated by ROS[18]and chalcones may react with some proteins involved in cell separation[19].The major carotenoid in tomato is Lycopene[20], research shows that it causes significant membrane damage and inhibits budding process.[21] Additionally, ascorbic acid inhibits yeast-to-hypha transition and antagonizes Hsp90-induced morphogenetic changes in C. albicans.[22] Lastly, glycoalkaloids like alpha-tomatine and dehydrotomatine disrupt fungal cell membrane, metabolism, and cell growth.

Pyrus communis L. peel, also known as **pear peel**, has been shown to have potent anti-fungal properties due to the presence of various active compounds such as quercetin,chlorogenic acid, anthocyanins, catechins, and procyanidins.[23] Quercetin interferes with fungal cell metabolism by disrupting mitochondrial function, damaging cell membranes, and inhibiting protein and nucleic acid synthesis[24].Chlorogenic acid is shown to induce apoptosis through various mechanisms, including reduced cell viability, mitochondrial depolarization, reactive oxygen species production, and phosphatidylserine externalization.[25]Anthocyanin's mechanism of action is unclear, it is suggested that it interacts with the fungal cell membrane and disrupts its structure[26]. The peel also contains other active substances such as enzymes and essential oils with anti-fungal properties. These compounds work together to create a potent defense against fungal infections.Procyanidins have been shown to interact with the fungal cell membrane, causing changes in its fluidity and permeability, which can lead to leakage of intracellular contents and ultimately cell death[27], other studies have also suggested that it effects the Ras pathway[28].

In Punica granatum peel or pomegranate peel contains polyphenol substances include gallic acid, ellagic acid, punicalagin, and quercetin[29] which have been linked to a variety of pharmacological effects. Ellagic acid is said to act by modifying the fungal cell wall and eradicating and preventing the formation of the biofilm[30], other studies also suggest inhibition of ergosterol biosynthesis and reduced the activity of sterol 14α-demethylase P450 (CYP51) in the membrane.[31] Studies show punicalagin causes severe ultrastructural alterations in fungi, including cytoplasmic content disarray and/or thicker cell walls.Punicalagin triggers ergosterol biosynthesis disruption and cell cycle arrest in fungus.[32]

For the purpose of this investigation, 4 fruit extracts excluding the negative and positive control will be tested against Saccharomyces cerevisiae (yeast), to compare their zone of inhibition. Yeast, specifically, is a fungus usually found on the skin,digestive system and the vagina and various other organs, the overgrowth of this yeast can lead to multiple infections. Yeast can grow out of balance for various reasons, including a weak immune system, living in humid/topical areas, or by consuming antibiotics that kill the bacteria that usually keeping the yeast in balance.[33] Malassezia are lipophilic yeasts part of the skin microbiome, normally kept in control by the human immune system, the overgrowth of this pathogen can lead to the formation of diseases such as head and neck dermatitis, seborrheic dermatitis, Malassezia folliculitis and Tinea

[18]---. "Naringin-generated ROS Promotes Mitochondria-mediated Apoptosis in Candida Albicans." *PubMed*, Accessed 17 Feb. 2023.
[19]"Antifungal Activity of Chalcones: a Mechanistic Study Using Various Yeast Strains." *PubMed*, Accessed 17 Feb. 2023.
[20]"Antioxidative Properties of Lycopene and Other Carotenoids from Tomatoes: Synergistic Effects." *PubMed*, Accessed 20 Feb. 2023.
[21]"Damage to the Cytoplasmic Membrane and Cell Death Caused by Lycopene in Candida Albicans." *PubMed*, Accessed 20 Feb. 2023.
[22]"Ascorbic Acid Inhibition of Candida Albicans Hsp90-Mediated Morphogenesis Occurs Via the Transcriptional Regulator Upc2." *PubMed Central (PMC)*, www.ncbi.nlm.nih.gov/pmc/articles/PMC4187652/.

[23]"Bioactive Compounds and Health-Promoting Properties of Pear (Pyrus Communis L.) Fruits." *PubMed Central (PMC)*, www.ncbi.nlm.nih.gov/pmc/articles/PMC7582546/. Accessed 20 Feb. 2023.
[24]"Anti-Fungal Efficacy and Mechanisms of Flavonoids." *MDPI*, 26 Jan. 2020, www.mdpi.com/2079-6382/9/2/45. Accessed 20 Feb. 2023.
[25]"Evaluation of the Antifungal Effect of Chlorogenic Acid Against Strains of Candida Spp. Resistant to Fluconazole: Apoptosis Induction and in Silico Analysis of the Possible Mechanisms of Action." *PubMed*,
[26] Lai, Yu-Shan et al. "Antifungal activity of anthocyanins extracted from purple sweet potato." Journal of Food and Drug Analysis, vol. 24, no. 1, 2016, pp. 191-199. Accessed 20 Feb. 2023.
[27] E. Bayramoglu et al. "Procyanidins as a new antifungal agent against Candida albicans." Phytochemistry, vol. 132, 2017, pp. 50-59. Accessed 20 Feb. 2023.
[28] L. Ge et al. "Procyanidins from grape seeds inhibit the Ras/MAPK pathway and proliferation of Aspergillus fumigatus." International Journal of Medical Microbiology, vol. 299, no. 2, 2009, pp. 233-242. Accessed 20 Feb. 2023.
[29]www.nature.com/articles/s41598-022-11881-7. Accessed 20 Feb. 2023.
[30]"Antifungal Activity of the Phenolic Compounds Ellagic Acid (EA) and Caffeic Acid Phenethyl Ester (CAPE) Against Drug-Resistant Candida Auris." *PubMed Central (PMC)*, www.ncbi.nlm.nih.gov/pmc/articles/PMC8466507/. Accessed 20 Feb. 2023.
[31]onlinelibrary.wiley.com/doi/10.1002/ptr.5340. Accessed 20 Feb. 2023.
[32]"Punicalagin Triggers Ergosterol Biosynthesis Disruption and Cell Cycle Arrest in Cryptococcus Gattii and Candida Albicans: Action Mechanisms of Punicalagin Against Yeasts." *PubMed Central (PMC)*, www.ncbi.nlm.nih.gov/pmc/articles/PMC7688883/. Accessed 20 Feb. 2023.
[33]"Yeast Infection." *Johns Hopkins Medicine, Based in Baltimore, Maryland*, 2 Dec. 2019, www.hopkinsmedicine.org/health/conditions-and-diseases/candidiasis-yeast-infection. Accessed 11 Dec. 2022.

versicolor.[34]Tinea versicolor (also known asPityriasis Versicolor) is one of the most common skin disease found in tropical and subtropical areas, interfering with the pigmentation of the skin causing discoloration,and can result in excess sweating, itching and dried out patches near shoulders, back and upper chest.The disease is caused by the over growth of yeast usually found on the skin, this specific yeast thrives in warm, moist, and oily environments. It is most commonly found in young adults due to developing hormones and excess oil production.[35]

2. Hypothesis

Alternative Hypothesis 1: As the concentrations of Ananas comosus (pineapple), Solanum lycopersicum L. (tomato), Pyrus communis L. (pear), and Punica granatum (pomegranates) increase, the zone of inhibition increases along with the fungal growth.

Null Hypothesis 1: Increasing the concentrations of Ananas comosus (pineapple), Solanum lycopersicum L. (tomato), Pyrus communis L. (pear), and Punica granatum (pomegranates) have no effect on the fungus.

Alternative Hypothesis 2: Ananas comosus (pineapple), Solanum lycopersicum L. (tomato), Pyrus communis L. (pear), and Punica granatum (pomegranates) show significant inhibition on the fungus at all concentrations.

Null Hypothesis 2: Ananas comosus (pineapple), Solanum lycopersicum L. (tomato), Pyrus communis L. (pear), and Punica granatum (pomegranates) show no significant inhibition on the fungus at any concentrations.

Alternative Hypothesis3: Punica granatum (Pomegranate) shows more significant inhibition compared to other fruit peel extracts.

Null Hypothesis 3: There will be no significant differences in the inhibitoryeffect at different concentrations between the four fruit peel extracts.

Methodology

a. Objective of Investigation

1. Effectiveness of different fruit peels at 25%, 50% and 75%
2. Comparing the fruit peel extracts on the level inhibition
3. Comparing the inhibition of fruit peel extracts

b. Variables

Independent Variables:

1. Fruit peel extracts ofAnanas comosus (pineapple), Solanum lycopersicum L. (tomato), Pyrus communis L. (pear), and Punica granatum (pomegranates).

Dependent Variable:
The zone of inhibition measured in mm: antifungal disk diffusion methodby the Kirby-Bauer method is a standardized technique for testing rapidly growing pathogens.[36]

Controls:

1. Positive control: Fluconazole Powder
2. Negativecontrol: Distilled water

[34]"Malassezia-Associated Skin Diseases, the Use of Diagnostics and Treatment." *Frontiers*, www.frontiersin.org/articles/10.3389/fcimb.2020.00112/full. Accessed 11 Dec. 2022.

[35]"Tinea Versicolor: Symptoms, Causes & Treatments." *Cleveland Clinic*, my.clevelandclinic.org/health/diseases/17719-tinea-versicolor. Accessed 11 Dec. 2022.

[36]"www.sciencedirect.com/topics/immunology-and-microbiology/disk-diffusion. Accessed 12 Oct. 2022.

c. Controlled Variables:

Table 1. List of experimental control variables

Variable and Value	Method of control	Significance of control
Concentration of methanol used for preparing extract: 100%	100% methanol used for the extraction of all four fruit powders	The percentage concentration of methanol used affects the percentage extraction of the fruit peel extract
Mass of powdered fruit peels: 5g	Digital scale	To help maintain the concentration ratio between the distilled water and fruit extract
Concentration of agar produced:	Same ratio as suggested in the instructions from manufacturers	The concentration of the agar affects the diffusion rate of the extracts and the growth of the bacteria
Radius of the wells created:	One cork borer used to ensure all the wells are equal in diameter	For equal diffusion and distribution of the fruit extract
Amount of yeast spread on the plate:	Micropipette used to gather equal amounts of yeast for each petri dish	The amount spread can affect the amount of yeast formed which can alter the results
Time of fungal growth:	The yeast is cultured for 24hrs and after the extracts are added the petri dishes are incubated for 48hrs.	For equal reproduction and amounts of yeast
Volume of extract poured into the wells:	Micropipette used to gather equal amounts of extract for each petri dish	Having a fluctuating amounts of fruit extracts might affect the inhibition and alter the results making the values inaccurate.
Incubation temperature:	All petri dishes incubated at 27°C for 48 hours	Growth of yeast is dependent on the temperature.
Autoclave Conditions:	Apparatuses autoclave at 121°C at 15 psi for sterilization	To avoid any contamination

d. Requirements

Apparatus Required:

Apparatus	Quantity	Uncertainty
Measuring cylinder- 100ml	1	± 0.1 mL
Measuring cylinder- 10ml	4	± 0.02 mL
Weighing digital balance	1	±0.05 g
Spatula	1	-
Conical flask	1	-
Heating plate 10 x 12 1500W	1	-
Petri dish	54	-
Oven	1	
Laminar airflow chamber	1	-
Glass rod- stirrer	1	-
Cork borer	1	-
Autoclave	1	
Inoculation loop	1	-
Spirit lamp/candle	1	-
Spirit	20ml	-
Match box	1	-
Micropipette	1	± 0.2 µl
Micropipette Tips	12	-
Vernier calliper	1	±0.5 mm
Test Tubes	12	-
Whatman filter paperNo.1	4	-

Material Required:

Materials	Quantity
Potato Dextrose agar	52.65g
Distilled Water	2000 ml
Pineapple Peel	5g
Pear Peel	5g
Tomato Peel	5g
Pomegranate Peel	5g
Methanol	150ml
Gloves	1 Pair

Microorganism Required:

Microorganism	Saccharomyces cerevisiae (Yeast)

3. Experimental Procedure

Aseptic methods & safety precautions

- Clean Laminar airflow chamberand workplace with methanol prior to the procedure.
- When using the Laminar airflow chamber clean hands with spirit or methanol, wear gloves and mask to prevent contamination and contact with yeast.
- Light a candle in the Laminar airflow chamberwhile working to prevent contamination from airborne microbes.
- Lab coat used throughout the entire procedure.
- Autoclave all apparatus and materials at 121 °C and 15psi.
- After the investigation autoclave all apparatus with culture to kill the yeast
- Wear gloves when handling the oven with fruit peels.

Sub-culturing

- Prepare 100ml of nutrient broth by weighing 1.3g of nutrient broth
- Mix the broth powder in 100 ml and autoclave at 121°C and 15 psi.
- 1 g of dry yeast culture in 100 ml of nutrient broth and incubated for 24hrs at temperature of 37°C.

Preparing fruit extracts

- Peel Ananas comosus (pineapple), Solanum lycopersicum L. (tomato), Pyrus communis L. (pear), and Punica granatum (pomegranates).
- Dry the peels for 24 hours in an oven at 100°C.
- Take out the peels from the oven, and place for extra 3 hours in the oven if the peels are not completely dry.
- Grind the dried peels with a mortar and pestle until fine powder is formed.
- Using the strainer separate the fine powder from the rest of the powder and regrind the powder left in the strainer.
- Measure 5g of the fruit peel powder using a digital measure.
- Measure 31.25 ml of 100% methanol.[37]
- Mix the methanol and fruit peel powder in a beaker and cover with newspaper.
- Leave the solution for 24 hours in a dark area.
- Mixture was then filtered with using Whatman filter paper No.
- Distilled water was mixed into the fruit extract based on the concentration required and put into test tubes.For example,1ml of distilled water and 1ml of fruit extract was added to achieve 50% concentration.
- Mix the 5g fungal powder with 5ml of distilled water.

Inoculation

- 52.65g of PDA agar was added to 1350ml of distilled water.
- Pour the solution in a conical flask.
- Heat the solution at 100°F on a heating plate until bubbles reach the top for the agar to dissolve.
- Cover and seal the solution with aluminum foil and paper with a rubber band.
- Label the petri dishes based on the fruit extract, concentration,and trial, along with controls.
- Wrap petri dishes in newspaper.
- Autoclave the agar solution and petri dishes along with the glass spreader and cork borer.
- After disinfecting and sanitizing your hands place the petri dish in the Laminar airflow chamber.
- Pour the agar solution halfway through the petri dishes.
- Let the solution dry for 30min.
- Using the micropipette take 100µL of the yeast and pour it on top of the agar.
- Spread the yeast using the glass spreader.
- Using the cork borer make 3 holes to remove equally sized solidified agar.
- Pour 100µL of the labeled extract into the holes using the micropipette.
- Repeat the same for the positive and negative control.
- Leave the petri dishes for 10min for the extract to settle.
- Place the petri dishes in the incubation chamber at 27°C for 48 hours.

Measuring zone of inhibition

- After a period of 48 hours turn petri dish on its lid and mark the clear areas using a marker.
- Using a Vernier calliper measure the diameter of the clear area in mm.
- Measure the area of the well using the Vernier calliper.

[37]pdfs.semanticscholar.org/2816/83617bd361811ad47facf1e8960d261cb708.pdf. Accessed 19 Jan. 2023.

Calculating zone of inhibition (ZOI)

- Diameter of the marked zone – diameter of the well = ZOI in mm

Qualitative observations(Pictures in Appendix i)

- After 24 hours the yeast culture was milky white.
- After 12 hours of inoculation clear colonies of fungus could be seen.
- The fruit extracts are seen to have fully diffuse into the agar solution at 24 hours, with slight zone of inhibition.
- After 48 hours the wells are completely empty, and zones of inhibition can be seen.
- Pomegranate seems to have the greatest zone of inhibition from all the extracts.
- The positive control also shows clear zones of inhibition, and the negative control shows no signs of inhibition.

EthicalConsiderations

This experiment does not include any animal. As methanol is an irritant, the chemical was handled with gloves. All the petri dishes and apparatuses were disposed appropriately to avoid infection.

4. **Data processing and analysis**

The aim of this experiment was to find the most effective fruit extract out of Ananas comosus (pineapple), Solanum lycopersicum L. (tomato), Pyrus communis L. (pear), and Punica granatum (pomegranates) on the inhibition of yeast. This investigation will also be assessing the fruit extracts at different concentrations of 25%, 50% and 75%. The mean and standard deviation of the data will be taken to find accuracy of the data and give us insight into the effectiveness of the fruit peels. The mean of each concentration is calculated as the average of all the trials for that concentration. The standard deviation is a measure of the spread of the data around the mean. A lower standard deviation indicates that the data points are more tightly clustered around the mean, while a higher standard deviation indicates greater variability in the data.One-way ANOVA and Tukey's multiple comparisonstestwere used for the statistical analysis. 'One-way ANOVA ("analysis of variance") compares the means of two or more independent groups in order to determine whether there is statistical evidence that the associated population means are significantly different.'[38] 'Tukey's multiple comparison test is one of several tests that can be used to determine which means amongst a set of means differ from the rest.'[39]All statistical analysis and processing was done on Graph Pad Prism 8 software.

5. **Statistical Calculations**

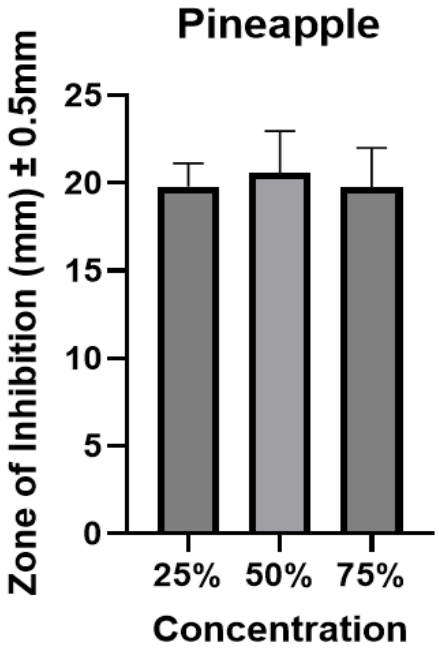
The average of the trial gives us the central tendency for the distributed data.
Standard deviation gives us insight into the distribution of the data.
(Please refer to **Appendix ii** for formulas and sample calculations)

Ananas comosus (pineapple)

Pineapple(mm) ±0.5mm			
Conc.	25%	50%	75%
Trial 1	17	22	23
Trial 2	20	21	20
Trial 3	18	20	18
Trial 4	20	22	22
Trial 5	20	26	22
Trial 6	22	20	19
Trial 7	21	16	17
Trial 8	19	20	15

[38]"SPSS Tutorials: One-Way ANOVA." *LibGuides at Kent State University*, Oct.-Nov. 1202, libguides.library.kent.edu/spss/onewayanova. Accessed 14 Feb. 2023.
[39]*Wiley-Blackwell*, www.blackwellpublishing.com/specialarticles/jcn_8_304.pdf. Accessed 14 Feb. 2023.

Trial 9	19.5	20	21
Trial 10	20	18	20
Trial 11	20	21	20
Trial 12	21	21	20
AVG	19.791667	20.583333	19.75
STDEV	1.3392388	2.3915888	2.2613351



In the graph above as the concentration increases the inhibition is not significantly affected. 50% pineapple extract shows x1.04 greater inhibition of yeast compared to 25% concentration. 50% pineapple extract shows x1.04 greater inhibition of yeast compared to 75% concentration. The error bars are small, but they overlap further confirming that increasing concentration does not affect inhibition.The standard deviation values are lowsuggesting data points are more tightly clustered around the mean.

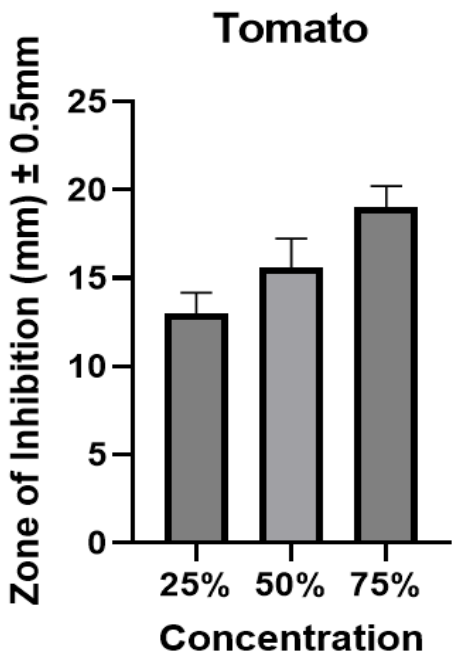
ANOVA summary Pineapple	
F	0.6286
P value	0.5396
P value summary	ns
Significant diff. among means (P < 0.05)?	No
R squared	0.0367

Tukey's multiple comparisons test Pineapple	Mean Diff.	95.00% CI of diff	Below threshold?	Summary	Adjusted P Value	Null Hypothesis
25% vs. 50%	-0.7917	-2.847 to 1.264	No	ns	0.616	Accept
25% vs. 75%	0.04167	-2.014 to 2.097	No	ns	0.9986	Accept
50% vs. 75%	0.8333	-1.222 to 2.889	No	ns	0.5852	Accept

The ANOVA results show that there is no significant difference in the mean of the pineapple samples at different concentrations (25%, 50%, and 75%). The F-value of 0.6286 and P-value of 0.5396 indicate that there is not enough evidence to reject the null hypothesis that the means are equal. The adjusted R-squared value of 0.0367 suggests that the differences in the means are not well explained by the predictor variable (concentration).The Tukey's multiple comparisons test confirms the lack of significant differences among the mean of the three concentrations. The mean differences between each pair of concentrations are not outside the 95% confidence interval of the differences and the adjusted P-values are all greater than 0.05, indicating that there is no evidence of significant differences at the 0.05 significance level.Thus, null hypothesis 1 is accepted and null hypothesis 2 is rejected.

Solanum lycopersicum L. (tomato)

Tomato (mm)±0.5mm			
Conc.	25%	50%	75%
Trial 1	12.5	14	20
Trial 2	11	16	21
Trial 3	12	17.5	18
Trial 4	15	17	19
Trial 5	14	19	19
Trial 6	13	15	19
Trial 7	14.5	16	18.5
Trial 8	14	15	19.5
Trial 9	12	15.5	17
Trial 10	13	13	17
Trial 11	12	15	20
Trial 12	13	14	20
AVG	13	15.583333	19
STDEV	1.1870514	1.6628745	1.2247449



As the concentration increases the inhibition of the yeast increases. 50% concentration of tomato extract shows a x1.199 greater inhibition level of yeast compared to 25% concentration of tomato extract. 75% concentration of tomato extract shows a x1.22 greater inhibition level of yeast compared to 50% concentration of tomato extract. Through this information we can conclude that increasing concentration plays a significant role in inhibition. The error bars are small and do not overlap showing that there is no anomaly in the data. The standard deviation values are low suggesting data points are more tightly clustered around the mean.

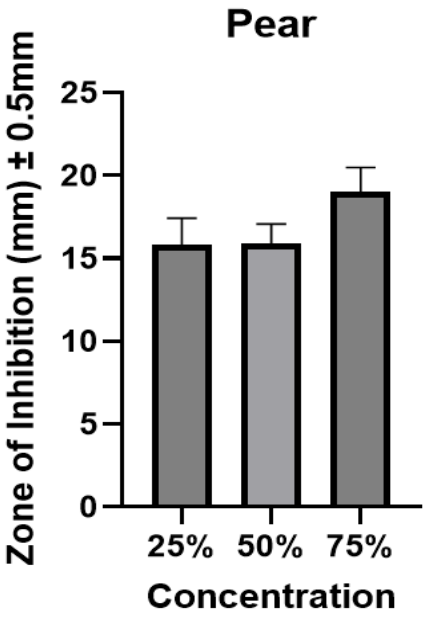
ANOVA summary Tomato	
F	57.47
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R squared	0.7769

Tukey's multiple comparisons test Tomato	Mean Diff.	95.00% CI of diff	Below threshold?	Summary	Adjusted P Value	Null Hypothesis
25% vs. 50%	-2.583	-3.961 to -1.206	Yes	***	0.0002	Accept
25% vs. 75%	-6	-7.378 to -4.622	Yes	****	<0.0001	Reject
50% vs. 75%	-3.417	-4.794 to -2.039	Yes	****	<0.0001	Reject

According to the analysis of variance (ANOVA), the tomato samples' means varied significantly at 25%, 50%, and 75% concentrations. The null hypothesis that the means are equal can be rejected due to the sufficient evidence provided by the F-value of 57.47 and P-value of <0.0001. The concentration predictor variable adequately explains the variances in the means, according to the adjusted R-squared value of 0.7769, which is trending towards one. The significance of the variations in the means of the three concentrations is further confirmed by Tukey's multiple comparisons test. With adjusted P-values below 0.05 and mean differences outside the 95% confidence interval, we can conclude that there are statistically significant differences between the concentration pairs in question. The results demonstrate that compared to the 25% and 50% concentrations, the 75% concentration has a much lower mean, and the 50% concentration has a significantly lower mean than the 25% concentration. This leads us to reject both of the null hypotheses.

Pyrus communis L. (pear)

Pear (mm) ±0.5mm			
Conc.	25%	50%	75%
Trial 1	21	14	15.5
Trial 2	20	15	14
Trial 3	19.5	18	16
Trial 4	20	13	16
Trial 5	21	17	16
Trial 6	16	15	15
Trial 7	18	17.5	15.5
Trial 8	18.5	16.5	15
Trial 9	17	15	17
Trial 10	19	18	15
Trial 11	19	16	17
Trial 12	19	15	18.5
AVG	19	15.833333	15.875
STDEV	1.492405	1.6001894	1.1894422



There seems to be no significant change from 25% to 50%, but as the concentration increases to 75% there is significant inhibition. 25% concentration of pear extract shows a x1.2 greater inhibitory effect on yeast compared to 50% concentration. 75% concentration of pear extract shows a x1.003 greater inhibition level of yeast compared to 50% concentration of pear extract. This shows that the inhibition is highest at 75%. The error bars are small suggesting little or no anomaly in the data;the error bars for 25% and 50% concentration overlap but the error bars between 75% and the other concentrations do not overlap. The standard deviation values are low suggesting data points are more tightly clustered around the mean.

ANOVA summary Pear	
F	19.15
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R squared	0.5371

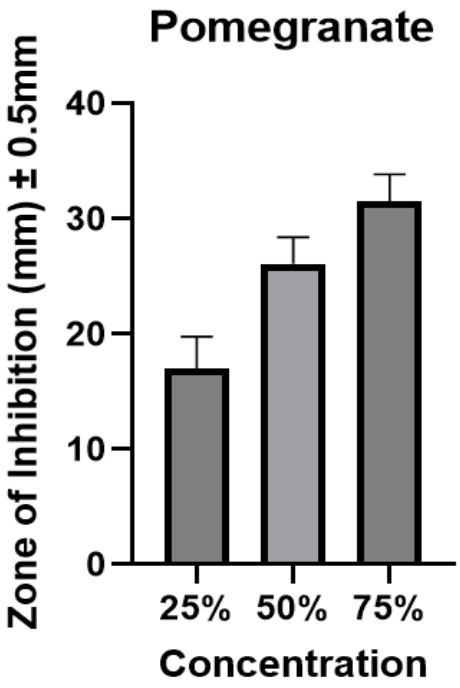
Tukey's multiple comparisons test Pear	Mean Diff.	95.00% CI of diff	Below threshold?	Summary	Adjusted P Value	Null Hypothesis
25% vs. 50%	-0.04167	-1.482 to 1.399	No	ns	0.9972	Accept
25% vs. 75%	-3.167	-4.607 to -1.726	Yes	****	<0.0001	Reject
50% vs. 75%	-3.125	-4.565 to -1.685	Yes	****	<0.0001	Reject

The ANOVA results show that there is a significant difference in the mean of the pear samples at different concentrations (25%, 50%, and 75%). The F-value of 19.15 and P-value of <0.0001 indicate that there is enough evidence to reject the null hypothesis 1 that the means are equal. The adjusted R-squared value of 0.5371 suggests that the differences in the means are partially explained by the predictor variable (concentration). The Tukey's multiple comparisons test further confirms the significant differences among the mean of the three concentrations. The mean difference between the 25% and 75% concentrations is outside the 95% confidence interval of the difference and the adjusted P-value is less than 0.05, indicating that there is evidence of a significant difference at the 0.05 significance level. The mean difference between the 50% and 75% concentrations is also outside the 95% confidence interval of the difference and the adjusted P-value is less than 0.05, indicating that there is evidence of a significant difference at the 0.05

significance level. However, the mean difference between the 25% and 50% concentrations is within the 95% confidence interval of the difference and the adjusted P-value is greater than 0.05, indicating that there is no evidence of a significant difference at the 0.05 significance level.Thus, null hypothesis 1 is partially accepted and null hypothesis 2 is rejected.

Punica granatum (pomegranates)

Pomegranate (mm) ±0.5mm			
Conc.	25%	50%	75%
Trial 1	15	25	29
Trial 2	16	27	33
Trial 3	20	26	30
Trial 4	18	24	32
Trial 5	24	31	29.5
Trial 6	16.5	27	35
Trial 7	15	25	28
Trial 8	14	26	29
Trial 9	14	25	33
Trial 10	17	29	31.5
Trial 11	17	26	34
Trial 12	17	22	34
AVG	16.958333	26.083333	31.5
STDEV	2.7998241	2.3143164	2.3452079



As the concentration increases the inhibition of the yeast increases. 50% concentration of pomegranate extract shows a x1.54 greater inhibition level of yeast compared to 25% concentration ofpomegranate extract. 75% concentration of pomegranate extract shows a x1.21 greater inhibition level of yeast compared to 50% concentration of pomegranate extract. Through this information we can conclude that concentration plays a significant role in inhibition. The error bars are small and do not overlap showing that there is no anomaly in the data. The standard deviation values are low suggesting data points are more tightly clustered around the mean.

ANOVA summary Pomegranate	
F	104
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R squared	0.8631

Tukey's multiple comparisons test Pomegranate	Mean Diff.	95.00% CI of diff	Below threshold?	Summary	Adjusted P Value	Null Hypothesis
25% vs. 50%	-9.125	-11.63 to -6.624	Yes	****	<0.0001	Reject
25% vs. 75%	-14.54	-17.04 to -12.04	Yes	****	<0.0001	Reject
50% vs. 75%	-5.417	-7.917 to -2.916	Yes	****	<0.0001	Reject

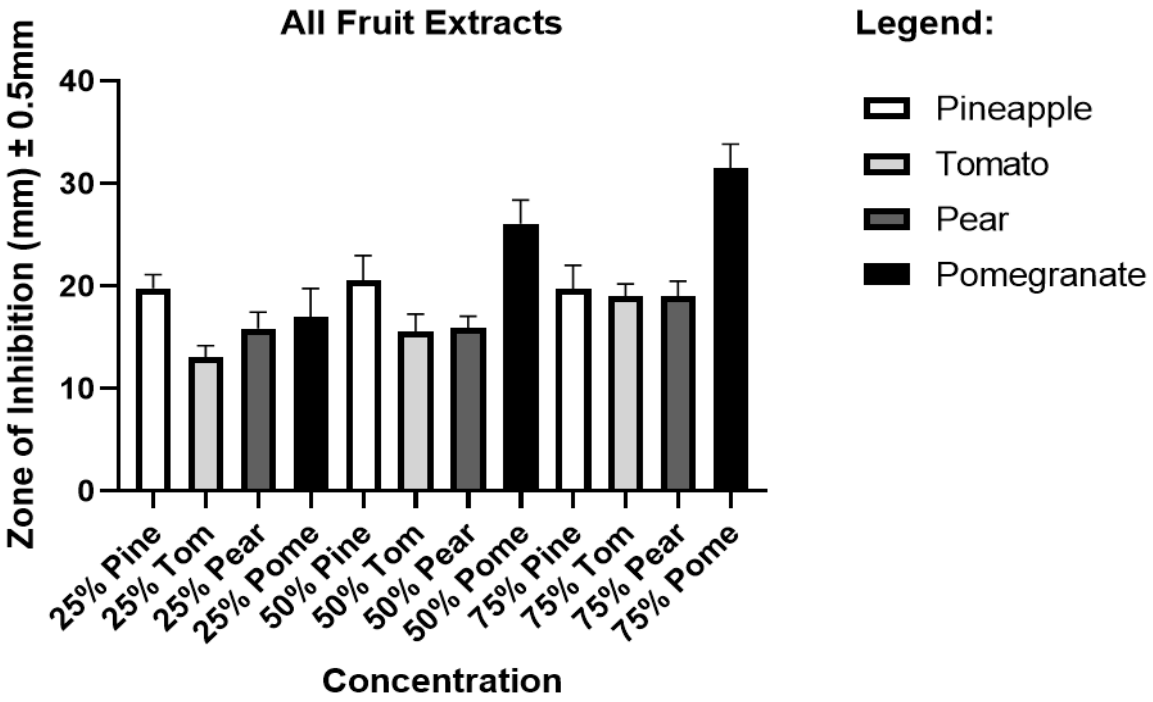
The ANOVA results show that there is a significant difference in the mean of the pomegranate samples at different concentrations (25%, 50%, and 75%). The F-value of 104 and P-value of <0.0001 indicate that there is enough evidence to reject the null hypothesis that the means are equal. The adjusted R-squared value of 0.8631 suggests that the differences in the means are well explained by the predictor variable (concentration).

The Tukey's multiple comparisons test further confirms the significant differences among the mean of the three concentrations. The mean difference between the 25% and 50% concentrations, 25% and 75% concentrations, and 50% and 75% concentrations are all outside the 95% confidence interval of the difference and the adjusted P-value is less than 0.05 in all cases, indicating that there is evidence of significant differences at the 0.05 significance level for all comparisons.Thus, null hypothesis 1 and null hypothesis 2 are rejected.

Control

Controls	Negative Control (Distilled Water) (mm) ±0.5mm	Positive control (Fungal Powder) ((mm) ±0.5mm
Trial 1	0	38
Trial 2	0	40
Trial 3	0	41
Trial 4	0	50
Trial 5	0	47
Trial 6	0	47
AVG	0	43.833333
STDEV	0	4.792355

The growth in the presence of the negative control (distilled water) is 0, with a standard deviation of 0. This suggests that the presence of the fungal powder significantly inhibits the growth of yeast, while distilled water does not have any effect.However, positive control at 43.83 mm shows more inhibitory effect than the pomegranate (75%) at 31.5mm, being 1.39 times higher. The standard deviation value of positive control is 4.79 which is small compared to the mean value suggesting no anomaly in the data.



The above graph shows the antifungal properties of all the fruit extracts in the present study. 25% pineapple extract shows the highest level of inhibition with a mean of 19.791667±0.5mm. This inhibition is x1.17 greater than 25% pomegranate extract, x1.25 greater than 25% pear extract and x1.52 greater than 25% tomato extract.50% pomegranateextract shows the highest level of inhibition with a mean of 26.083333±0.5mm. This inhibition is x1.27 greater than 50% pineapple extract, x1.64 greater than 50% pear extract and x1.67 greater than 50% tomato extract.75% pomegranate extract shows the highest level of inhibition with a mean of
31.5 ±0.5mm. This inhibition is x1.59 greater than 75% pineapple extract, x1.66 greater than 75% pear extract and 75% tomato extract.In 25% pineapple extract exhibited the maximum inhibition, while for 50% and 75% pomegranate extract exhibited the highest inhibition. The data presented have relativity low error bars, showing the significance and precision of the data. Thus, null hypothesis 3 is partially rejected, though pomegranate shows significantly greater inhibition at 50% and 75% than other fruit extracts at those concentrations, apart from 25% where pineapple shows more inhibitory effect than pomegranate.Small error bars obtained for all the variables tested shows the significance and precision in the data.

6. Discussion

The concerning increase in antifungal resistance is directly attributable to the scarcity of effective antifungal medications, which in turn is caused by the challenges associated with their research and production. Therefore, several studies are seeking for other ways to combat fungus.

Researchers found that the chlorophyll-containing pineapple crown leaf extract (aqueous extract) reduced the growth of the Saccharomyces cerevisiae (S. cerevisiae) laboratory strain by 70–95%. We found that the minimum inhibitory concentration (MIC) for this strain ranges from 1.65 to 4.95 mg/mL. The findings suggest that S. cerevisiae bacteria may be susceptible to crude bromelain. More research is needed to confirm these findings and clarify the antibacterial activities of crude bromelain. This study's conclusion that peels inhibit Saccharomyces cerevisiae is supported by both sets of observations.

Here, we observe that inhibition is dramatically reduced at 75% after being increased by pineapple extract from 25% to 50%. Yeast inhibition was also discovered in a 2020 investigation. Concentrations of 25%, 12.5%, 6.25%, 3.125%, and 1.5625% of an ethanol extract from pineapple peel were tested using the disc diffusion method. Malassezia furfur inhibition appears to be concentration dependent. With a total of 29.07±0.64, 24.90±0.27, 20.89±0.41, 6.96±0.52, 11.12±0.24, and 8.18±0.30 for each concentration, respectively.The results of this investigation might differ from the current study because Melzi Octaviani's article employed a lower dose. One possible reason is that there is a concentration at which the yeast inhibition becomes insignificant. Another potential explanation is offered by the inclusion of Malassezia furfur, or more precisely Saccharomyces cerevisiae, in this study.

F. Farooq et al. (2010) conducted a study to find out if the extract from tomatoes (Lycopersicon esculentum Mill.) could inhibit the growth of yeasts called Candida albicans and Saccharomyces cerevisiae. The authors employed the agar well diffusion method to ascertain the antifungal activity of the tomato extract in vitro. Tomato extract dramatically reduced the number of yeast cells used in the experiment, specifically Saccharomyces cerevisiae and Candida albicans. Researchers also found that tomato extract's antifungal activity was concentration dependant. Additionally, the researchers found that the antifungal activity of tomato extract was affected by the extraction process, with the most effective being ethanol extraction.The present investigation confirms previous findings that inhibition grows with concentration; moreover, the results are very exact, with very small error bars.

Drapes of guava leaves (Psidium guavava), pomegranate blooms (Punica grantum), tomato fruits (Solanum lycopersicum), and kaffir lime (Citrus hystrix) suppressed Malassezia, according to research on medical students' dandruff, its causes, and potential treatments. This research was similar to another that employed good diffusion with tomatoes and pomegranates. Scientists found that the tomato extract inhibited 12 mm and the pomegranate extract 11 mm, demonstrating robust inhibitory effects from both extracts. How well Pyrus communis L. inhibits yeast growth is an area where very little is known. However, studies conducted on the "Antimicrobial Activities of Fruits of Crataegus. and Pyrus Species" within the same genus have shown that yeast development is significantly suppressed. According to the study, Candida albicans had a 14 mm inhibition and Candida globrata a 10 mm inhibition. Using a different kind of yeast or an extract from ethyl acetate could have contributed to the lower quality of the results compared to the present investigation.

An investigation titled "Efficacy of Phytochemicals Present in Leaves of Punica granatum against Malassezia Species" discovered that several phytochemicals included in Punica granatum (P. granatum) are effective against Malassezia species. Wells filled with watery crude extracts of P. granatum suppressed the growth of every species of Malassezia, which is consistent with the present study. Yeast can't stand a chance against the polyphenols contained in pomegranate. These phytochemicals are also responsible for the large inhibitory zone seen in the current study. Both the positive control and extract groups showed significant inhibition, in line with the results of the current study, whereas the negative control group showed no inhibition at all. It should be noted that further research is needed to determine the practical effectiveness of fruit extracts as antifungal medications, however the studies listed above do indicate that they could be able to inhibit the growth of some fungi. Fruit peel extracts showed antifungal properties, despite the fact that the positive control had a 1.39-fold stronger inhibitory impact than the pomegranate (75%). As a result, this feature can be included into future studies employing state-of-the-art industrial technology to extract phytochemical components that can enhance anti-fungal medications for the treatment of diseases such as Tinea versicolor.

7. Evaluation

This section analyzes and evaluates the advantages and limitations of the inquiry so that the problems and their possible solutions can be understood. The agar well diffusion technique is one example of a benefit. Two of this method's strongest points are its ease of use and its ability to test multiple substances at once. Another way to find an antimicrobial agent's MIC is to use agar well diffusion. This is the minimum inhibitory concentration (MIC), which is the concentration at which the agent stops microorganisms from growing.Because of the meticulous cleanliness of the investigation site and the subsequent stringent waste management practices, no infectious diseases were spread. A total of twelve efforts were made to lessen the influence of luck. One limitation is the possibility that lab air pollution could affect the results. The bioactive components may have been lost when the fruit peels were heated to such high temperatures. You have other options besides drying; air drying is also a good one. It could be deceiving to compare fruit extracts from various fruits because fruit skins dry at different speeds. Because of its transparency and rather fuzzy edges, not all of them could be read. Notwithstanding these limitations, the research shows that natural fruit extracts have antifungal properties when compared to man-made, over-the-counter drugs.

8. Conclusion

This study examines the effects of fruit peel extracts from four different plants on yeast growth over a 48-hour period at 27^{oC}. The concentrations of the extracts were 25%, 50%, and 75%. The zone of inhibition was measured in millimeters. The plants tested were Ananas comosus (pineapple), Solanum lycopersicum L. (tomato), Pyrus communis L. (pear), and Punica granatum (pomegranates). The results reveal that all four extracts exhibit some degree of inhibition, and that the inhibition of most of the extracts increases with increasing concentration. The pomegranate fruit extract was the most inhibitory, followed by the pineapple, tomato, and pear fruit extracts. In sum, the study was fruitful and can help bring diverse alternatives to the current antifungals to market.

Further Scope

These fruit extracts could be tested on various fungi such as Candidiasis. to see if they produce similar results. The same can be said for bacteria, which contribute to the current antibiotic resistance crisis[40]. Apart from the antibacterial and antifungal properties, the anticancerous[41]and antiinflammation[42]properties can be tested.Finally, various other fruit extracts[43] such as orange peels or banana peels can be tested on Saccharomyces cerevisiae.

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Appendix- i (Images)